

A trait-based perspective on seagrass ecology: from plant to ecosystem level processes



Dissertation by
Agustín Moreira Saporiti
In partial fulfillment of the requirements for the degree of
Doctor of Natural Sciences
- Dr. rer. nat. -

Faculty 2 Biology and Chemistry
Universität Bremen

May 2021, Bremen

The present work was developed between February 2017 and May 2020 in the Leibniz Centre for Tropical Marine Research (ZMT, Bremen, Germany) and the Institute of Marine Sciences (IMS, University of Dar es Salaam, Zanzibar Archipelago, Tanzania).

Funding for this project was provided by the ZMT internal project funding and the German Research Foundation (DFG) under the project “Seagrass and Macroalgal Community Dynamics and Performance under Environmental Change (SEAMAC)” (TE 1046/3-1), both awarded to Dr. Mirta Teichberg.

Scientific supervisor	Dr. Mirta Teichberg
Official supervisor	Prof. Dr. Kai Bischof
Panel members	Dr. Mirta Teichberg Prof. Dr. Achim Schlüter Prof. Dr. Agostino Merico
Dissertation examination committee	Prof. Dr. Kai Bischof Prof. Dr. Agostino Merico Prof. Dr. Martin Gullström (External examiner)
Doctoral colloquium examination committee	Prof. Dr. Christian Wild (Chair) Dr. Mirta Teichberg Prof. Dr. Martin Gullström Prof. Dr. Kai Bischof Sara Doolittle Llanos (PhD student) Tatiana Torres (Master student)
Date of Doctoral colloquium	12.07.2021

Acknowledgments

There are a million people I would like to thank, without whom the completion of this thesis would have been impossible.

I would first like to thank my family: my parents María Marta and Enrique and my sister Camila. Thank you for supporting me even when I told you that I was going to study tropical ecology. I am really thankful that you did not look at me completely puzzled and wonder where you went wrong. Thank you for your unconditional love and support. Despite my grandparents having left us, a couple some time ago and a couple very recently, I would like to thank them for their strong support, and for always believing in your grandchildren.

Very importantly, I would like to thank my friends and chosen family. Thank you to my friends in Spain for always checking on me despite the distance, and for pretending to be interested when I talk about my work. Thank you Amara, Arantza, Cristina, Juan, Lara, Laura, Marta, Nerea, Olalla and Tamara. Thank you to the friends I made in Bremen, starting from the time I was studying my masters. Thank you Ryan, Emma and Stefan, for really believing I could make it despite my complete lack of skills. I would like to thank the friends I also made in Bremen after my masters, which became my new family. I would like to thank Ana, Rafa, Julia, Ilemi and Carlos for being there for me all this time. Last but not least, I would like to thank all the friends I was fortunate to make at work. Thank you Dani, Pilar, Inés, Fay, Gui, the two Saras, Marie and their significant others which I will not mention to not to make this longer. Thank you for being there in the both horrible and amazing time that is the PhD.

Very importantly, I would like to thank all the members of the Algae and Seagrass Ecology Group in the ZMT. Unfortunately the group ended this year, but I am happy to know that all of you went forward to do better and bigger things. I would like to thank Ana, Suci, Merlin, Aoife, Fay, Inés, and all the students and guests we had along the years. I truly learnt so much from all of you. A fundamental part of the seagrass team is its leader, and I cannot do the acknowledgements without thanking her. I would like to thank my supervisor Mirta Teichberg. You are really the person who made possible me being now in this position, becoming a doctor. You were the first person who ever hired me for a scientific work, and I cannot believe that we have worked together for as many years as we did. Despite us butting heads sometimes, I am forever grateful for all the opportunities that you provided me with. Thank you very much.

Finally, I would like to thank my significant other, Maziar, for hanging in there with me all this time. I am sure it was not easy to stand by my side during the stressful times of deadlines, papers

being rejected, then the pandemic...I am forever thankful to you for your unconditional love and support. Thank you.

Abstract

Seagrasses are marine flowering plants that inhabit the coastal area forming important ecosystems due to a number of ecosystem services they provide. However, they are subjected to both global and local impacts, including warming water temperatures and eutrophication, which threaten their survival. Despite the fact that the most diverse seagrass meadows are found in the tropical Indo-Pacific Region, there is less information about tropical species than their temperate counterparts. There are, therefore, knowledge gaps in the response of tropical seagrass meadows to environmental drivers and their links to ecosystem functions and services. In the last three decades, trait-based frameworks (TBFs) have advanced different fields of ecological research through establishing novel links between functional traits, environmental drivers and ecosystem functions. A number of concepts have been proposed in order to answer different ecological questions using a functional trait-based perspective. This field of research has been widely developed in terrestrial plants. However, the use of TBFs in seagrass research is currently in its infancy.

The goal of this dissertation is the incorporation of TBFs into seagrass ecological research, by establishing novel links between seagrass traits, environmental drivers and ecosystem functions and services. The study site chosen for this work was Unguja Island (Zanzibar Archipelago, Tanzania). Unguja Island is located in the tropical Indo-Pacific region, and is considered one of the hotspots of seagrass biodiversity worldwide. The seagrass meadows in Unguja Island are subjected to a wide range of conditions, from pristine and oligotrophic to heavily impacted and eutrophic. Due to its high seagrass diversity and the variety of conditions under which seagrass survive, Unguja Island is a perfect laboratory for the study of seagrass communities using a TBF. The research questions selected for this dissertation have the goal of understanding the importance of traits at different organizational levels, from their individual responses to environmental drivers, to the effect of traits on the interspecific competition of seagrass species and, lastly, their effect on ecosystem functioning.

First, to assess the knowledge gaps in seagrass trait-based research, I carried out a systematic review of the seagrass literature. The analysis showed that seagrass trait research has mostly focused on the effect of environmental drivers on traits (65%), whereas links between traits and functions are less common (33%). Despite the richness of trait-based data available, concepts related to TBFs are rare in the seagrass literature (7% of studies). These knowledge gaps in seagrasses indicate ample potential for further research. In order to address these knowledge gaps, I propose a TBF that can help guide future seagrass research.

Secondly, the responses of traits of individual seagrass plants of tropical seagrass species (*Halophila stipulacea*, *Cymodocea serrulata*, *Thalassia hemprichii* and seedlings of *Enhalus acoroides*) were assessed to two environmental drivers: temperature (global) and nutrient enrichment (local). To achieve this aim, a 1-month experiment under laboratory conditions combining two temperature (maximum ambient temperature and current average temperature) and two nutrient (high and low nitrogen and phosphorus concentrations) treatments was conducted. The results of this experiment showed that trait responses are species-specific, and that temperature was a much more significant driver than nutrient enrichment. In the case of the seedlings of *E. acoroides*, they rely energetically in the reserves within the seedling and increasing temperature resulted in faster seedling development. *T. hemprichii* and *C. serrulata* showed an enhanced morphology, while the contrary was true for *H. stipulacea*. These results highlight the different effects and strategies that co-inhabiting seagrasses have in response to environmental changes. Thirdly, an experiment was developed in the field to test the effects of light shading and trampling due to the farming of *Euchema denticulatum* on seagrass meadows, an environmental driver endemic to the tropical region. Areas covered by *T. hemprichii*, *H. stipulacea* were selected for the building of seaweed farms for 3 months. Light was reduced in the seaweed farm plots by 75 to 90% by the end of a seaweed growth cycle. The responses of seagrass were, again, species-specific. *H. stipulacea*, despite its capacity for rapid growth, was significantly affected by the combination of shading and trampling under the seaweed farm treatment, while the climax seagrass species *T. hemprichii* was unaffected.

Fourthly, to link individual plant traits to seagrass community level processes, I carried out an observational study in Unguja Island. The goal was to understand how seagrass traits linked to light and nutrient competition affected space preemption among seagrass species under different trophic scenarios. Traits determining the functional strategy of the seagrass showed that there was a size gradient in the seagrass species. When tested the effect of the difference in the functional strategy of species pairs, the probability of preemption was highest for the bigger species, increased when their size difference was higher and was not affected by the eutrophication. This indicated that the competitive interactions among seagrass species were asymmetrical, i.e. a species had a negative effect on another species, while the effect was not reciprocal and the driver behind space preemption was determined by traits related to the size of the seagrass plants.

Fifthly, to study the link between seagrass traits and ecosystem functions, sediment cores were collected and compared within seagrass meadows of varying communities across sites of

Unguja Island. The goal was to find out which seagrass traits are relevant indicators of carbon storage, and which environmental conditions constrain the storage of carbon in the sediments. Very fine sediments ($<125 \mu\text{m}$) were negatively correlated to organic carbon in the sediment. Leaf area index of seagrass was positively correlated to organic carbon content in the sediment, indicating an effect of particle trapping and retention. Root maximum length was the most important functional trait driving carbon storage, suggesting that rooting depth is of fundamental importance for carbon accumulation.

To conclude, TBFs can help to push seagrass research forward by the study of traits from the individual plant level, scaling up their effects on the seagrass community, interspecific competition and, lastly, ecosystem functioning. The individual trait responses of seagrass to environmental drivers, through adaptive processes, have fundamental consequences for interspecific competition and, ecosystem function. Changes in seagrass morphology can determine the outcome of interspecific competition for nutrients and light and, therefore, the final configuration of seagrass meadows. These traits of the species in the meadow ultimately determine the capacity of the meadow for carbon storage, which shows a prime example of how traits can affect important seagrass ecosystem functions.

Zusammenfassung

Seegräser sind marine Blütenpflanzen, die im Küstenbereich leben und aufgrund einer Reihe von Leistungen, die sie erbringen, wichtige Ökosysteme bilden. Allerdings sind sie sowohl globalen als auch lokalen Einflüssen ausgesetzt, darunter die Erwärmung der Wassertemperaturen und die Eutrophierung, die ihr Überleben bedrohen. Obwohl die vielfältigsten Seegraswiesen in der tropischen Region des Indopazifiks zu finden sind, gibt es weniger Informationen über die tropischen Arten als über ihre Gegenstücke in den gemäßigten Breiten. Daher gibt es Wissenslücken in Bezug auf die Reaktion tropischer Seegraswiesen auf Umweltfaktoren und ihre Verbindungen zu Ökosystemfunktionen und -dienstleistungen. In den letzten drei Jahrzehnten haben trait-based frameworks (TBFs) verschiedene Bereiche der ökologischen Forschung vorangebracht, indem sie neue Verbindungen zwischen funktionalen Merkmalen, Umwelttreibern und Ökosystemfunktionen hergestellt haben. Es wurde eine Reihe von Konzepten vorgeschlagen, um verschiedene ökologische Fragen mit Hilfe einer auf funktionalen Merkmalen basierenden Perspektive zu beantworten. Dieses Forschungsgebiet ist bei terrestrischen Pflanzen weit entwickelt worden. Die Anwendung von TBFs in der Seegrasforschung steckt jedoch noch in den Anfangsstadien.

Das Ziel dieser Dissertation ist die Einbeziehung von TBFs in die ökologische Seegrasforschung, indem neue Verbindungen zwischen Seegrasmerkmalen, Umwelttreibern und Ökosystemfunktionen und -dienstleistungen hergestellt werden. Als Untersuchungsort für diese Arbeit wurde die Insel Unguja (Sansibar-Archipel, Tansania) gewählt. Unguja Island liegt in der tropischen Indo-Pazifik-Region und gilt als einer der Hotspots der Seegras-Biodiversität weltweit. Die Seegraswiesen auf Unguja Island sind einem breiten Spektrum von Bedingungen ausgesetzt, von unberührt und oligotroph bis stark belastet und eutroph. Aufgrund der hohen Seegrasvielfalt und der unterschiedlichen Bedingungen, unter denen Seegras überlebt, ist Unguja Island ein perfektes Labor für die Untersuchung von Seegrasgemeinschaften mit Hilfe einer TBF. Die Forschungsfragen, die für diese Dissertation ausgewählt wurden, haben das Ziel, die Bedeutung von Merkmalen auf verschiedenen Organisationsebenen zu verstehen, von der individuellen Reaktion auf Umwelteinflüsse über die Wirkung von Merkmalen auf die interspezifische Konkurrenz von Seegrasarten bis hin zu ihrer Wirkung auf das Funktionieren des Ökosystems.

Um die Wissenslücken in der auf Seegrasmerkmalen basierenden Forschung zu bewerten, habe ich zunächst eine systematische Überprüfung der Literatur zu Seegras durchgeführt. Die Analyse zeigte, dass sich die Forschung zu Seegrasmerkmalen meist auf die Auswirkungen von

Umweltfaktoren auf die Merkmale konzentriert hat (65 %), während Verbindungen zwischen Merkmalen und Funktionen weniger häufig sind (33 %). Trotz der Fülle an verfügbaren Merkmalsdaten sind Konzepte, die sich auf TBFs beziehen, in der Seegras-Literatur selten (7% der Studien). Diese Wissenslücken bei Seegräsern weisen auf reichlich Potenzial für weitere Forschung hin. Um diese Wissenslücken zu schließen, schlage ich ein TBF vor, das als Leitfaden für zukünftige Seegrasforschung dienen kann.

Zweitens wurden die Reaktionen von Merkmalen einzelner Seegraspflanzen tropischer Seegrasarten (*Halophila stipulacea*, *Cymodocea serrulata*, *Thalassia hemprichii* und Keimlinge von *Enhalus acoroides*) auf zwei Umweltfaktoren untersucht: Temperatur (global) und Nährstoffanreicherung (lokal). Um dieses Ziel zu erreichen, wurde ein einmonatiges Experiment unter Laborbedingungen mit zwei Temperatur- (maximale Umgebungstemperatur und aktuelle Durchschnittstemperatur) und zwei Nährstoffbehandlungen (hohe und niedrige Stickstoff- und Phosphorkonzentrationen) durchgeführt. Die Ergebnisse dieses Experiments zeigten, dass die Merkmalsreaktionen artspezifisch sind und dass die Temperatur ein wesentlich signifikanterer Einflussfaktor war als die Nährstoffanreicherung. Im Fall der Keimlinge von *E. acoroides* sind sie energetisch auf die Reserven innerhalb des Keimlings angewiesen und eine Erhöhung der Temperatur führte zu einer schnelleren Keimlingsentwicklung. *T. hemprichii* und *C. serrulata* zeigten eine verbesserte Morphologie, während für *H. stipulacea* das Gegenteil der Fall war. Diese Ergebnisse verdeutlichen die unterschiedlichen Effekte und Strategien, mit denen koexistierende Seegräser auf Umweltveränderungen reagieren. Drittens wurde ein Experiment im Feld entwickelt, um die Auswirkungen von Lichtbeschattung und Zertrampelung durch die Bewirtschaftung von *Euchema denticulatum* auf Seegraswiesen zu testen, ein Umweltfaktor, der in der tropischen Region endemisch ist. Flächen, die von *T. hemprichii*, *H. stipulacea* bewachsen waren, wurden für den Bau von Seegrasfarmen für 3 Monate ausgewählt. Das Licht wurde in den Seegrasfarm-Parzellen bis zum Ende eines Seegras-Wachstumszyklus um 75 bis 90% reduziert. Die Reaktionen des Seegrases waren wiederum artspezifisch. *H. stipulacea* wurde trotz seiner Fähigkeit zu schnellem Wachstum durch die Kombination von Beschattung und Zertrampelung unter der Seegrasfarm-Behandlung signifikant beeinträchtigt, während die Klimax-Seegrasart *T. hemprichii* unbeeinflusst blieb.

Viertens habe ich eine Beobachtungsstudie auf der Insel Unguja durchgeführt, um individuelle Pflanzeigenschaften mit Prozessen auf Seegras-Gemeinschaftsebene zu verbinden. Ziel war es, zu verstehen, wie Seegrasmerkmale, die mit Licht- und Nährstoffkonkurrenz verbunden

sind, die Raumübernahme zwischen Seegrasarten unter verschiedenen trophischen Szenarien beeinflussen. Die Merkmale, die die funktionale Strategie des Seegrases bestimmen, zeigten, dass es einen Größengradienten bei den Seegrasarten gab. Als der Effekt des Unterschieds in der funktionalen Strategie von Artenpaaren getestet wurde, war die Wahrscheinlichkeit der Raumübernahme für die größeren Arten am höchsten, nahm zu, wenn der Größenunterschied größer war und wurde durch die Eutrophierung nicht beeinflusst. Dies deutete darauf hin, dass die konkurrierenden Interaktionen zwischen den Seegrasarten asymmetrisch waren, d.h. eine Art hatte einen negativen Effekt auf eine andere Art, während der Effekt nicht wechselseitig war und die Triebkraft hinter der Raumvorwegnahme durch Merkmale bestimmt wurde, die mit der Größe der Seegraspflanzen zusammenhängen.

Fünftens: Um den Zusammenhang zwischen Seegrasmerkmalen und Ökosystemfunktionen zu untersuchen, wurden Sedimentkerne innerhalb von Seegraswiesen unterschiedlicher Gemeinschaften an verschiedenen Stellen der Insel Unguja gesammelt und verglichen. Ziel war es, herauszufinden, welche Seegras-Eigenschaften relevante Indikatoren für die Kohlenstoffspeicherung sind und welche Umweltbedingungen die Speicherung von Kohlenstoff in den Sedimenten einschränken. Sehr feine Sedimente ($<125 \mu\text{m}$) waren negativ mit dem organischen Kohlenstoff im Sediment korreliert. Der Blattflächenindex von Seegras war positiv mit dem Gehalt an organischem Kohlenstoff im Sediment korreliert, was auf einen Effekt des Einfangens und Zurückhaltens von Partikeln hinweist. Die maximale Wurzellänge war das wichtigste funktionale Merkmal für die Kohlenstoffspeicherung, was darauf hindeutet, dass die Wurzeltiefe für die Kohlenstoffakkumulation von grundlegender Bedeutung ist.

Zusammenfassend lässt sich sagen, dass TBFs dazu beitragen können, die Seegrasforschung voranzutreiben, indem sie Merkmale auf der Ebene der einzelnen Pflanze untersuchen und ihre Auswirkungen auf die Seegrasgemeinschaft, die interspezifische Konkurrenz und schließlich auf die Funktion des Ökosystems hochskalieren. Die individuellen Reaktionen von Seegras auf Umwelteinflüsse durch adaptive Prozesse haben grundlegende Konsequenzen für die interspezifische Konkurrenz und die Funktion des Ökosystems. Veränderungen in der Seegrasmorphologie können das Ergebnis der interspezifischen Konkurrenz um Nährstoffe und Licht und damit die endgültige Konfiguration der Seegraswiesen bestimmen. Diese Eigenschaften der Arten in der Wiese bestimmen letztendlich die Kapazität der Wiese zur Kohlenstoffspeicherung, was ein Paradebeispiel dafür ist, wie Eigenschaften wichtige Funktionen des Seegras-Ökosystems beeinflussen können.

Table of Contents

CHAPTER 1. General introduction	1
I. Seagrass ecosystems	3
(1) Evolutionary origin of marine plants and their characteristics	3
(2) Threats to seagrass ecosystems	6
(3) Perspective on the use of classical approaches to seagrass ecology	8
II. Trait-based frameworks (TBFs).....	9
(1) A short introduction to the origin, concepts and importance of TBFs.....	9
(2) History of the trait-based framework, examples and evolution of the concept	12
III. Dissertation: Goals and outline	13
IV. Author’s contributions	15
V. References	16
CHAPTER 2. A trait-based framework to seagrass ecology: trends and prospects	29
Abstract	29
I. Trait-based frameworks (TBFs): traits as answer to complex ecological questions	31
II. A novel TBF: from individual traits to ecosystem services.....	31
(1) Community assembly processes and response traits: dispersal, abiotic and biotic filters	32
(2) Phylogenetic and genotypic control of trait diversity	34
(3) The role of intraspecific and interspecific trait diversity	34
(4) Ecosystem function provision and effect traits	35
(5) Ecosystem service provision, vulnerability and service traits	35
III. Applying the TBF to seagrass communities.....	36
(1) Seagrass ecosystems: Origin, threats and idiosyncrasy of marine plants	36
(2) Literature review of seagrass trait research	38
IV. TBF in seagrass communities: knowledge gaps, challenges and opportunities	39
(1) Seagrass TBF studies and studies including the word trait.....	39
(2) Community assembly processes in seagrass communities	39
(3) Phylogenetic and genotypic control of seagrass traits	43
(4) Intraspecific trait variability in seagrasses is key to their survival	43
(5) Effect traits linked to seagrass ecosystem function provision: challenges in the understanding of complementarity, dominance and environmental control of ecosystem functions	45
(6) Vulnerability of seagrass ecosystem function and service provision under global change	46
V. Conclusions	48
VI. Acknowledgments.....	50
VII. References.....	50
VIII. Supplementary material.....	63
(1) Supplementary material 1	63
(2) Supplementary material 2	65

CHAPTER 3. Morphological and physiological responses of <i>Enhalus acoroides</i> seedlings under varying temperature and nutrient treatment	69
Abstract	69
I. Introduction.....	71
II. Materials and Methods	74
(1) Collection and Maintenance of Seagrass Seeds.....	74
(2) Experimental Design and Setup.....	75
(3) Water Sampling	76
(4) Seedlings Morphological and Physiological Traits	77
(5) Statistical analysis.....	80
III. Results	82
(1) Experimental water chemistry and trophic conditions within the aquaria.....	82
(2) Seedling traits	82
IV. Discussion	86
(1) Seedling responses to increased temperature.....	86
(2) Seedling response to increased nutrients	91
(3) Ecological implications.....	96
V. Acknowledgments	98
VI. References.....	98
CHAPTER 4. Species-Specific trait responses of three tropical seagrasses to multiple stressors: The case of increasing temperature and nutrient enrichment	111
Abstract	111
I. Introduction.....	113
II. Materials and Methods	116
(1) Collection and Maintenance of Seagrasses.....	116
(2) Experimental Design and Setup.....	117
(3) Water Monitoring and Sampling	119
(4) Measurement of Seagrass Traits	120
(5) Statistical Analysis.....	123
III. Results	124
(1) Experimental Conditions	124
(2) Seagrass Biochemical Traits.....	128
(3) Seagrass Morphological Traits	129
(4) Seagrass physiological traits.....	131
(5) Relationships between Biochemical, Morphological, and Physiological Seagrass Traits.....	131
IV. Discussion	133
(1) Seagrass Responses to an Extended Exposure to the Maximum Ambient Temperature.....	133
(2) Seagrass Responses to Water Column Nutrient Enrichment.....	137

(3) Combined Effect of Different Drivers: Stressors Do Not Act Alone	138
(4) Ecological Implications	141
V. Conclusions	143
VI. Acknowledgments.....	143
VII. References.....	144
VIII. Supplementary Material	156
CHAPTER 5. Seaweed farming pressure affects seagrass and benthic macroalgae dynamics in Chwaka Bay (Zanzibar, Tanzania).....	163
Abstract	163
I. Introduction.....	165
II. Materials and Methods	167
(1) Study site	167
(2) Seaweed farm experimental plots	167
(3) Seagrass and benthic macroalgae variables	169
(4) Light reduction.....	170
(5) Statistical analysis.....	170
III. Results	171
(1) Light reduction.....	171
(2) Shoot density of <i>T. hemprichii</i> and <i>H. stipulacea</i>	171
(3) Cover.....	173
IV. Discussion	173
(1) The effect of seaweed farming due to shading and trampling on seagrass meadows and its associated benthic macroalgae	173
(2) The effect of trampling	175
(3) Management recommendations, current state of seaweed farming in Zanzibar and its future under climate change.....	177
V. Conclusions	179
VI. Acknowledgments.....	179
VII. References.....	180
VIII. Supplementary Material	185
CHAPTER 6. Local victory: Assessing interspecific competition in seagrass from a trait-based perspective.....	189
Abstract	189
I. Introduction.....	191
II. Materials and Methods	195
(1) Study area	195
(2) Characterization of the trophic states.....	195
(3) Characterization of the seagrass species and their traits	197
(4) Data analysis.....	201

III. Results	205
(1) Trophic states and indicators	205
(2) Seagrass cover and probability of preemption across trophic states.....	207
(3) Functional strategy of seagrass species as defined by their traits	207
(4) Effect of the trophic state on the functional strategy of seagrass species	209
(5) Difference in the seagrass functional strategy and the probability of space preemption.....	210
(6) Probability of space preemption of seagrass species as determined by PC1	212
IV. Discussion	212
(1) A trait-based approach to differentiate the functional strategy of seagrass species.....	212
(2) Response of seagrass functional strategy to different trophic states.....	214
(3) Seagrass functional strategy and its control on space preemption.....	218
V. Conclusions	222
VI. Acknowledgements	223
VII. References.....	223
VIII. Supplementary Material	234
(1) Supplementary material 1. Raw trait data.....	234
(2) Supplementary material 2. Site clustering in trophic states	234
(3) Supplementary material 3. Ordination diagnostics	235
(4) Supplementary material 4. Effect of difference in the functional strategy of seagrass on space preemption.....	236
(5) Supplementary material 5. Cover and preemption: pairwise comparison	237
(6) Supplementary material 6. Results of PCA	241
(7) Supplementary material 7. Trophic state effect on species centroids in all PCs.....	247
(8) Supplementary material 8. Probability of space preemption in PC2, PC3, PC4 and PC6.....	248
CHAPTER 7. Sediment properties and seagrass functional traits control carbon storage in tropical seagrass meadows.....	251
Abstract	251
I. Introduction.....	253
II. Materials and Methods	256
(1) Description of study sites.....	256
(2) Sampling design.....	256
(3) Sediment control: grain size distribution	257
(4) Functional trait control.....	257
(5) Sediment organic carbon	258
(6) Data analysis	259
III. Results	260
(1) Sediment grain size distribution	260
(2) Seagrass functional traits at the community level.....	261

(3) Carbon stocks in seagrass communities of Unguja Island.....	262
(4) Influence of site, parent material and functional traits on carbon stocks in seagrass meadows	263
IV. Discussion	263
V. Conclusions	267
VI. Acknowledgments.....	267
VII. References.....	268
CHAPTER 8. Final Synthesis and Conclusions	277
I. Trait-based ecology in seagrass: A final synthesis.....	279
II. TBFs in seagrasses: new possibilities for classical ecological questions	279
III. From individual plants to ecosystem level processes: a trait-based perspective in seagrass ecology	282
(1) Abiotic filter: individual plants responses to environmental drivers	282
(2) Biotic filter: Community level interspecific competition among seagrasses.....	285
(3) Ecosystem level: Carbon storage in seagrass ecosystems	286
IV. Limitations, knowledge gaps and future research	287
V. Final conclusions	288
VI. References.....	289

CHAPTER 1.

General introduction



I. Seagrass ecosystems

(1) Evolutionary origin of marine plants and their characteristics

Seagrasses are a polyphyletic assemblage of basal monocotyledonous angiosperms belonging to four families in the Alismatales, namely Posidoniaceae, Zosteraceae, Cymodoceaceae and Hydrocharitaceae. Most species and populations feature a mix of sexual reproduction and clonal growth. The recolonization of marine habitats from terrestrial wetland habitats occurred exclusively from this monocot order and took place at least three times independently through parallel evolutionary events (Figure 1). Despite their ecological importance, seagrasses exhibit a low taxonomic diversity (60-70 recognized species) when compared to other freshwater and terrestrial groups in the Alismatales (Figure 2). Their distribution is limited to shallow coastal areas, with a global distribution of around 177 000 km² (<0.2% of the oceans' surface; Figure 3) (Spalding et al. 2003).

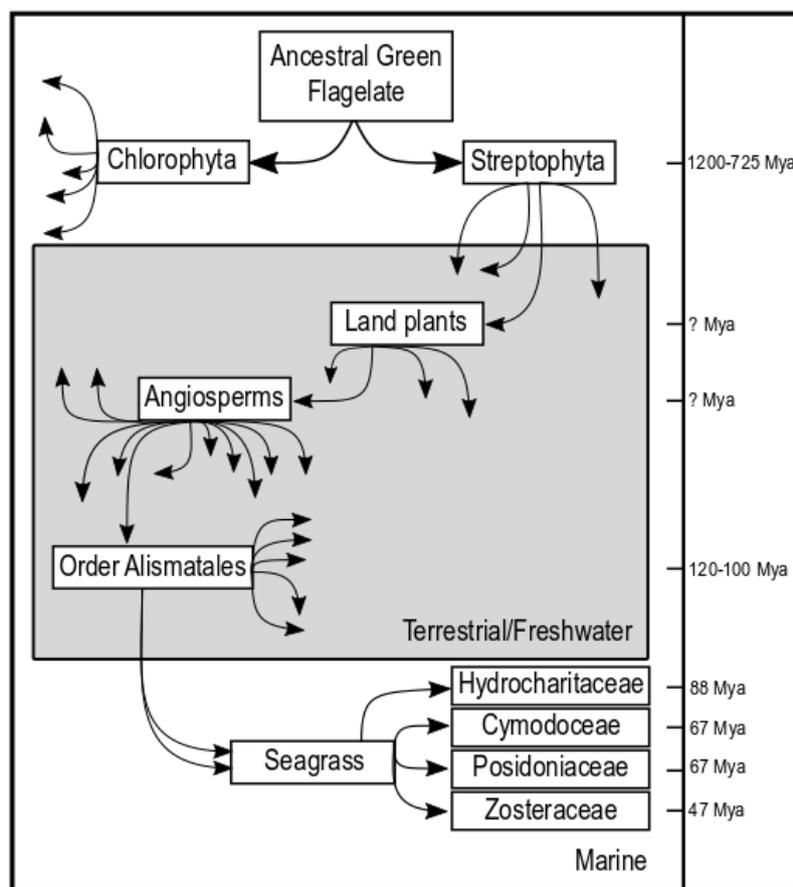


Figure 1. Simplified phylogeny of seagrasses and their timeline of evolution. The figure shows that the colonization of the ocean happened in four independent events. Timeline of seagrass colonization of the ocean from Janssen and Bremer (2004); Les et al. (1997) and Waycott et al. (2006). Mya = Million of years.

They are clonal plants, with most species possessing strap-like leaves grouped in shoots connected by rooted underground rhizomes for anchoring and mechanical support. These shoots are generally formed by a vertical rhizome, sheath and a number of leaves, which are variable length and size depending on the species (for a schematic representation of seagrass morphology, see Figure 4). Additionally, this morphology shows a certain level of plasticity with species undergoing adaptive processes depending on the environmental conditions (Fourqurean et al. 1995). The low morphological diversity of seagrasses is possibly the result of a convergent evolution to the submerged lifestyle in a highly hydrodynamic environment. At the molecular level, these adaptations are reflected in specific genomic losses and gains (Golicz et al. 2015; Lee et al. 2016; Olsen et al. 2016), with adaptive changes in sets of genes associated with central biological pathways (Wissler et al. 2011).

Some of the most important challenges of the marine lifestyle are the rapid attenuation of the irradiance level through the water column along with alterations in its spectral composition, the reduced CO₂ availability (but high bicarbonate concentration, Beer and Rehnberg 1997; Invers et al. 1999), the high and often fluctuating salinity regime and Na⁺ toxicity (Touchette 2007), as well as the presence of sulphide-rich anoxic sediments, wave action and tidal currents. Key seagrass innovations to carry out photosynthesis underwater include the concentration of chloroplasts in epidermal cells, the enlargement of gene sets encoding for light-harvesting proteins (Olsen et al. 2016), and special mechanisms relying on carbonic acid and bicarbonate for inorganic carbon acquisition (Larkum et al. 2017). In contrast, genes for UV protection and phytochromes for red/far-red sensing are reduced due to the low penetration of UV-B and red/far-red wavelengths in the aquatic medium. Seagrasses have also regained physiological functions enabling them to adjust to full salinity and possess algal-like cell walls for leaf flexibility and ion exchange (Olsen et al. 2016). They have lost the entire repertoire of genes required for stomatal differentiation, and have drastically reduced genes involved in volatile synthesis and sensing (Olsen et al. 2016). Seagrasses complete their entire life cycle totally submerged, including flowering, pollination and seed germination (Kuo and Den Hartog 2007). Changes in pollen (e.g. exine-less) and flower structure are reflected in the disappearance of some of their associated genes (Olsen et al. 2016; Entrambasaguas et al. 2017). Thanks to all these adaptations, they have colonized the coasts of both temperate and tropical areas (Short et al. 2007), which are divided in six bioregions. Among those, the tropical Indo-Pacific region hosts the highest diversity of seagrasses, with approximately 24 coexisting species.

Seagrasses are, therefore, a special group of Angiosperms. They occupy a thin strip of coastline as a consequence of the adaptations that they have undergone for the marine life. Unfortunately, this same strip is also under high and increasing anthropogenic pressure.

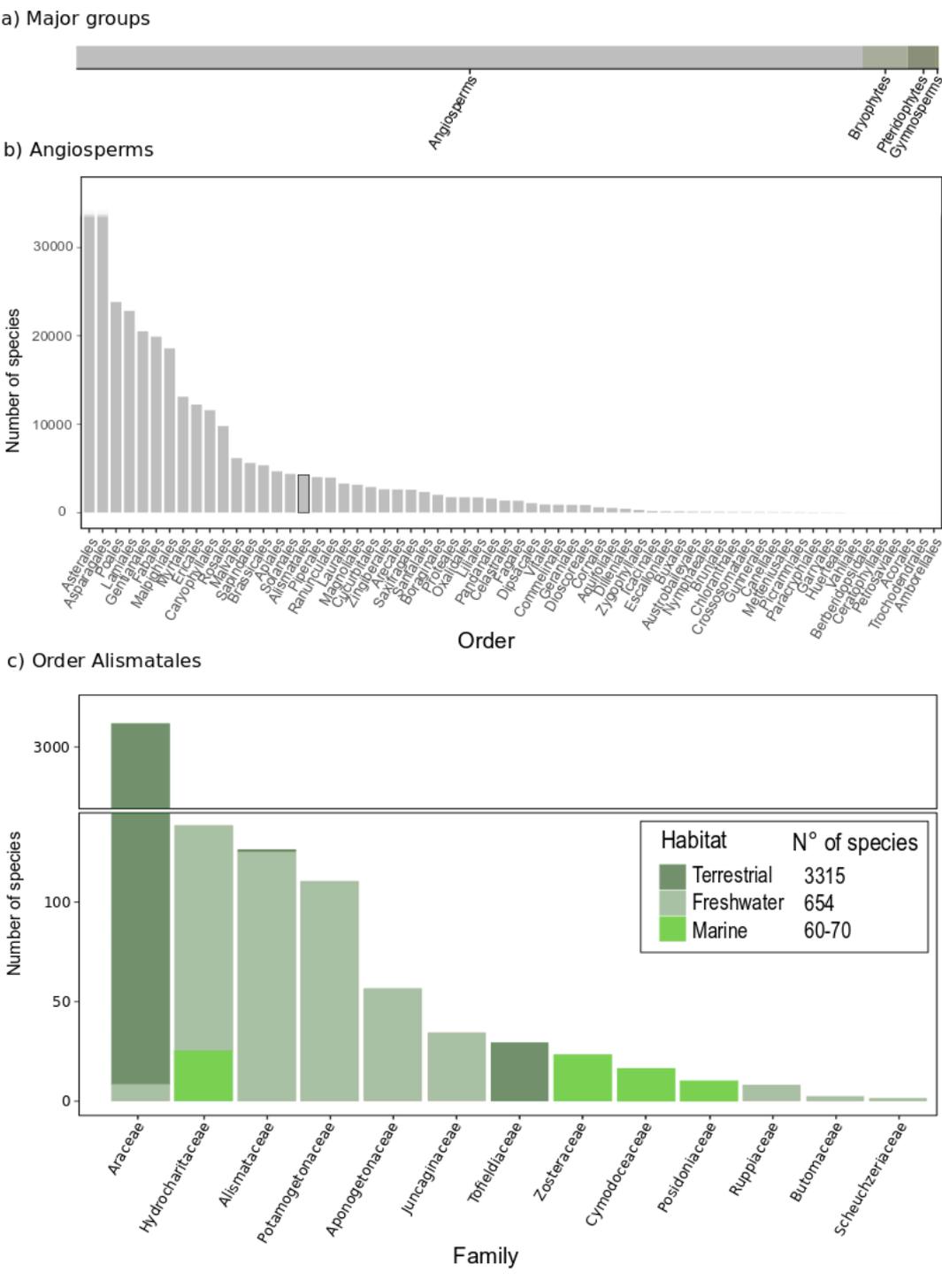


Figure 2. a) Major plant groups, Angiosperms being the most dominant in number of extant species. b) Number of species of each of the orders forming the Angiosperms group. c) Detail of number of species per family belonging to the order Alismatales, order to which all seagrasses belong. Data obtained from theplantlist.org

(2) Threats to seagrass ecosystems

Seagrasses are under global decline worldwide (Orth et al. 2006; Waycott et al. 2009), and face multiple local and global pressures by largely unknown synergistic and/or antagonistic drivers.

Climate change models predict increased frequency and severity of destructive storms, with subsequent increases in wave force and occasionally more frequent discharge of fresh water from land to sea (Holland and Webster 2007; Izaguirre et al. 2011). Global change impacts include ocean acidification (OA) with increasing CO₂ levels. While available data on OA effects on seagrasses remains inconclusive, many findings suggest a slight increase in productivity (Guinotte and Fabry 2008; Koch et al. 2013; Alexandre et al. 2012), while some studies have reported developmental effects, such as increase in below-ground biomass and increases in reproductive output (Palacios and Zimmerman 2007); or increases in the belowground carbohydrate reserves (Campbell and Fourqurean 2013). High temperatures negatively affect performance and growth of several seagrasses (Bulthuis 1987; Campbell et al. 2006; Collier and Waycott 2014; Lee et al. 2007). The frequency and intensity of temperature stress events are anticipated to increase (Bernstein et al. 2008; Pachauri et al. 2014), which will amplify the negative impacts of climate warming on seagrasses, causing that certain species may decline drastically while others may become replaced by more temperature-tolerant ones (McMillan 1984). Finally, as a result of global change, species' spatial distributions and ranges (in latitude and elevation) are changing, with some species being pushed towards extinction (Short et al. 2011; Poloczanska et al. 2013; Bates et al. 2014; Chefaoui et al. 2018).

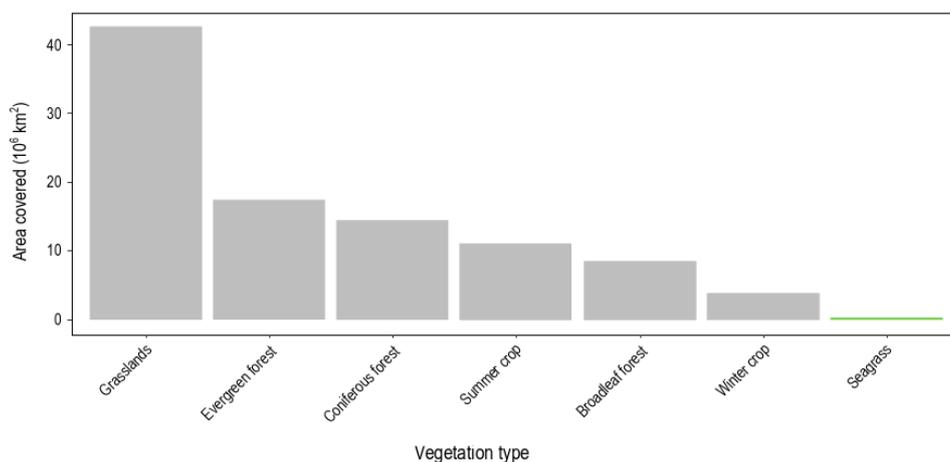


Figure 3. Area covered by each vegetation type. Aquatic (freshwater) plants are not included as no data regarding their cover was found. Terrestrial plants data obtained from Munier et al. (2018); seagrass cover data obtained from Spalding et al. (2003).

In the case of local anthropogenic pressure, eutrophication and overfishing are the main threats to seagrass ecosystems. Eutrophication can lead to a phase shift from seagrass, to macroalgae dominated systems (Duarte 1995; Orth et al. 2006; Burkholder et al. 2007; Waycott et al. 2009). These bottom-up mechanisms drive competition among primary producers and cause seagrass loss or degradation due to indirect (e.g. sediment anoxia), or direct effects such as shading by macroalgae or by epiphytes (Erftemeijer and Lewis 2006; Burkholder et al. 2007; van Katwijk et al. 2010). Top-down manipulation of food webs through overfishing may have massive negative consequences for seagrass survival and provisioning of ecosystem services (Jackson et al. 2001; Duffy 2006; Baden et al. 2012; Heithaus et al. 2014). Grazing activity can control the physical structure and dominance in seagrass meadows by reducing the epiphyte growth on seagrass leaves (Poore et al. 2012), thereby mitigating some of the negative effects of eutrophication. In turn, nutrient enrichment can increase the palatability of seagrasses thereby increasing their susceptibility to herbivores (Jimenez-Ramos et al. 2018). Eutrophication, climate warming and hypersalinity can lead to a reduction in seagrass vitality and in their resistance and resilience to disease outbreaks (Sullivan et al. 2018).

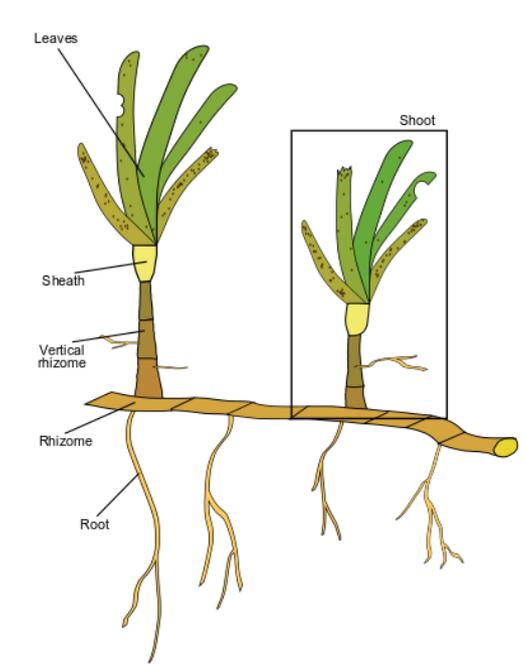


Figure 4. Schematic representation of the seagrass morphology.

Ultimately, the traits of seagrasses will determine their responses to these stressors and thereby the size of their populations and the future of the seagrass ecosystems. However, we lack a

comprehensive picture and predictive framework of how key seagrass traits underpin the resistance and resilience of seagrass plants and populations to current and future pressures.

(3) Perspective on the use of classical approaches to seagrass ecology

To determine the causes and consequences of seagrass decline, most seagrass research to date has followed a classical ecology approach carried out at the organism, population, community, and ecosystem levels. These studies have included measurements of response variables for understanding their basic biology and function in combination with measurements from their surrounding environment to better protect seagrass meadows from decline (Harlin and Thorne-Miller 1981; McGlathery 1995; Duffy 2006; Fernandes et al. 2019), restore degraded ecosystems (Fonseca et al. 2000; van Katwijk et al. 2009; Paulo et al. 2019), and predict their fate under future global change scenarios (Marbà and Duarte 2010; Koch et al. 2013; Valle et al. 2014; Hyndes et al. 2016).

Research on positive and negative feedbacks within seagrass ecosystems have also helped us to understand the role they play as ecosystem engineers and has led to a greater understanding of the valuable ecosystem functions and services they provide (Barbier et al. 2011; Nordlund et al. 2016), like their role in biogeochemical processes in the sediment and water column (Hemminga et al. 1991; Enríquez et al. 2001; Unsworth et al. 2012) or in food webs (Moksnes et al. 2008; Best et al. 2012). Synthesis of the existing data on seagrass responses to the environment has been extensively used to identify potential environmental indicators and for assessing the health of the seagrass ecosystem (Martínez-Crego et al. 2008; Marbà et al. 2013; Roca et al. 2016). Despite the plethora of data on response variables, questions still remain as to the future of these coastal habitats and to the impact on the ecosystem services that they provide.

If there is an elusive link in ecological studies it is the one mechanistically connecting organisms to ecosystem functions and, consequently, to services. This is also the case in seagrasses where, despite the generalized assumption that seagrasses significantly contribute to ecosystem functions, quantitative examples and empirical functions are scarce in the literature. There is a clear understanding that the solely presence of seagrass ecosystems is enough for the performance of ecosystem functions when compared to its absence, e.g. carbon sequestration and storage (Greiner et al. 2013; Oreska et al. 2017; Belshe et al. 2018), invertebrate habitat provisioning (Virnstein et al. 1983) and modification of the inorganic carbon system (Unsworth et al. 2012). This elusive link is resolved in only a handful of examples of direct effects of trait

on functions. The modification of the inorganic carbon system was related with leaf area index (LAI) in Mediterranean meadows (Hendricks et al. 2014). Leaf length and shoot density can be directly related with the percentage of wave energy attenuation per meter of seagrass bed (Fonseca and Callahan 1992). Similarly, it has been demonstrated that leaf length, LAI and leaf density in *Posidonia oceanica* can control particle trapping and hydrodynamics (Hendricks et al. 2008).

Under this scope, we are still scratching the surface in relation to which seagrass traits affect the ecosystems functions, and which methods are available for establishing the missing links between relevant metrics for seagrass fitness and ecosystem functions that, ultimately, deliver ecosystem services. It is for this reason that trait-based approaches and frameworks may help to uncover unknown relations between seagrass and ecosystem functions and services, as trait metrics underpin both seagrass response to environmental change and their delivery of ecosystem functions.

II. Trait-based frameworks (TBFs)

(1) A short introduction to the origin, concepts and importance of TBFs

The classical approach to assess ecosystem health and functioning has been focused on species richness and abundance as an important explanatory mechanism, known as biodiversity-ecosystem functioning (BEF) hypothesis (Naeem et al. 1994). This theory builds upon the idea that groups of organisms differing in species composition will differentially impact higher levels of biological organization. Nevertheless, in the last two decades, many ecologists started to consider that the specific traits that species have are largely responsible for determining the properties and dynamics of ecological systems (Eviner and Chapin 2003; Lavorel and Garnier 2002; McGill et al. 2006; Mlambo 2014; Violle et al. 2007).

Variation in individual traits can change during the course of evolution and can affect the strength of selection (Dobzhansky 1937), but the potential effects of this individual trait variation on ecological processes are less understood (Bolnick et al. 2011). To fully understand how individual variation in traits can affect ecological dynamics and processes, it is necessary to develop a mechanistic understanding of how trait variation scales up from individuals, through species interactions to ecosystem processes and functions (Pawar et al. 2015).

The most general definition of trait is any characteristic of an organism that affects its fitness. Nevertheless, the interest in traits comes not only for their influence in organismal performance, but also in ecosystem functioning, as the second is a direct consequence of the first. For this reason, a functional trait can be defined as any morpho-physio-phenological characteristic which impacts fitness indirectly via their effects on growth, reproduction and survival (Violle et al. 2007), and/or may directly influence processes at higher organizational levels (i.e. ecosystem functioning). The study of functional traits can inform us about important aspects of ecosystem functioning and community responses to environmental change that species richness does not (Diaz and Cabido 2001; Lavorel and Garnier 2002). Measuring traits can better reveal the mechanisms and forces that ultimately structure biological diversity (Grime 2006; Stegen et al. 2009) and increase the generality and predictability of ecological models (Diaz et al. 2004; Kattge et al. 2011; Webb et al. 2010).

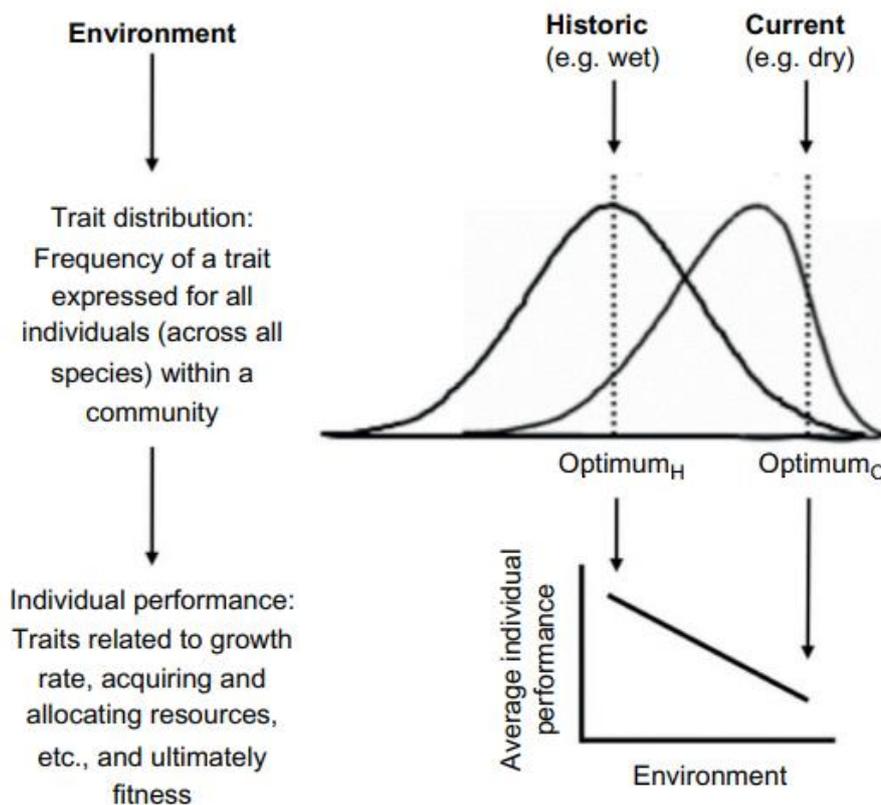


Figure 5. Diagram linking changes in optimal traits in changing environments with the frequency distributions for that trait in historic versus current environments (from Enquist et al. 2015).

The Trait Driver Theory (TDT, Enquist et al. 2015) links how the dynamics of the biotic and abiotic environment drive the performance of individuals and ecosystems via their traits. A key

assumption of TDT is that traits of individuals can be used to predict individual performance and that can be effectively summed or scaled up to the functioning of ecosystems (Lavorel and Garnier 2002; Suding et al. 2008). The core concept is to use functional traits, instead of species identities, to better predict community and ecosystem dynamics (Lavorel and Garnier 2002; Lavorel et al. 2007; Suding and Goldstein 2008).

Another core concept of TDT is that shifts in the environment will cause shifts in trait distribution. As a consequence of this concept, the difference between the optimal trait value (which generates a better organismal performance) and the observed mean trait, as well as the trait variance, provides a measure of the capacity of a community to respond to environmental change (Enquist et al. 2015) (Figure 5).

Functional traits are traditionally divided in response traits, which emerge in response to environmental factors which constrain individuals (Lavorel and Garnier 2002; Violle et al. 2007), and effect traits, which determine the impact of the organism in ecosystem functioning. The examination of response traits in different environmental contexts allows us to understand how the adaptive changes in traits can lead to a shift in the structure and functioning of ecosystems (Schmitz et al. 2015). In other words, traits that are acted on by adaptive processes may also indirectly alter ecosystem processes via their changes to ecosystem processes and functions (Figure 6).

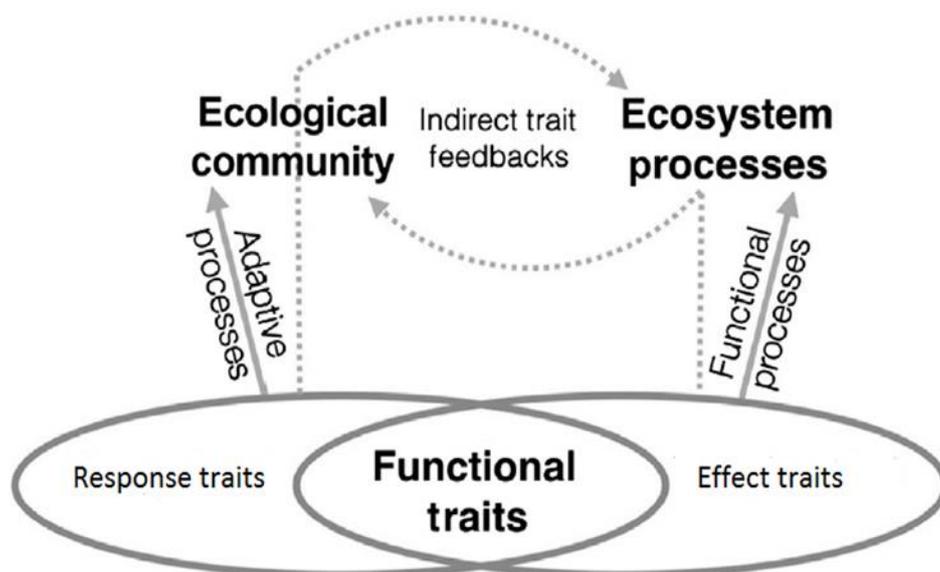


Figure 6. Interaction between response traits (which define the ecological community) and the effect traits (which control the ecosystem processes). The interaction between the ecosystem processes and the community creates a loop in which effect and response affect each other (adapted from Schmitz et al. 2015).

The study of the interplay between response and effect traits can give us a comprehensive image to both predict the fitness of an organism under an adaptive process and how this will affect the functioning of the ecosystem at a higher organizational level.

(2) History of the trait-based framework, examples and evolution of the concept

Although a trait-based framework can be applied to all kinds of organisms, it is currently most developed in terrestrial plants for two reasons (Lavorel et al. 2007; Garnier et al. 2016). First, since plants make up more than 99% of living material in terrestrial ecosystems, their functioning determines the quantity and quality of habitats utilized by other organisms, as well as playing an important role in controlling the Earth's atmospheric composition and climate (Keddy 2007). Second, there is a need to simplify the complexity of the plant world, with the aim of understanding and predicting the effects of global change and management on plant distributions and the properties of ecosystems, locally and at global scales (Lavorel et al. 2007).

In other words, to understand the ecology of plants, we have to move away from a taxonomic perspective and to a non-taxonomic one, focusing on the classification of plants according to their life-forms, i.e. “trees”, “shrubs”, “subshrubs” and “herbs” (Schimper 1903). This classifications paved the way for the development of non-taxonomic approaches to plant diversity that developed during the 20th century (Duckworth et al. 2000; Grime and Pierce 2012). Traits are the means by which form and function are captured in organisms (Garnier et al. 2016).

The development of trait-based frameworks to study plant diversity and functioning triggered its propagation to other fields of ecology, including the marine realm. Particularly in pelagic ecosystems—e.g. in phytoplankton (Litchman et al. 2010), zooplankton (Lichtmann et al. 2013) or microbial communities (Brown et al. 2014)—the use of trait-based frameworks has been discussed in depth. Thus, modelling marine ecosystems using traits has grown in appeal ever since (Kiørboe et al. 2018 and references herein). TBFs have been applied to predict the distribution of marine microbial communities on a global scale (Follows et al. 2007), to explain phytoplankton community response to seasonal fluctuations (Edwards et al. 2013), the comparison of functional niches of native and invasive fish species (Elleouet et al. 2014) or the exploration of trophic cascades as a result of fishing pressure (Andersen and Pedersen 2009).

TBFs have been also used in benthic marine ecosystems. Trait analysis can be used to describe ecosystem functioning of subtidal invertebrate communities (Bremner et al. 2006). Since

ecosystem properties in coastal systems are highly variable already at small scales, traits such as feeding mode or mobility have proved useful to predict shifts along environmental gradients (van der Linden et al. 2012; van der Wal et al. 2017). The use of easy to measure ‘supertraits’ that function as a proxy to capture community variability has been suggested as a valuable tool to quantify fundamental ecological processes in coral communities (Madin et al. 2016). Life-history strategies of corals can help to assess adaptability and response to environmental and anthropogenic disturbance (Darling et al. 2012). The benefit of trait-based methods for seagrass ecology becomes particularly apparent when looking at its use to describe ecological processes in aquatic macrophytes. In freshwater plants responses to environmental stressors and impacts on ecosystem properties have been linked to morphological traits (Engelhardt 2006). The broad applicability of TBFs can go as far as revealing generalities across ecosystems such as wetland mangroves and upland forests (Ellison 2002).

It is for these reasons that the wider use of trait-based frameworks into seagrass ecosystems is of fundamental importance to understand the ecology of seagrass, their responses to environmental drivers and the links between seagrass traits and the ecosystem functions and services they provide.

III. Dissertation: Goals and outline

The goal of this dissertation is the incorporation of TBFs into seagrass ecological research. TBFs have shown their value in other fields of research, specifically terrestrial plant ecology, and can therefore help push seagrass research forward by establishing novel links between seagrass traits, environmental drivers and ecosystem functions and processes.

The study site chosen for this work was Unguja Island (Zanzibar Archipelago, Tanzania). Unguja Island is surrounded by extensive seagrass meadows, mainly formed by nine seagrass species: *Cymodocea rotundata*, *Cymodocea serrulata*, *Enhalus acoroides*, *Halophila ovalis*, *Halophila stipulacea*, *Halodule uninervis*, *Syringodium isoetifolium*, *Thalassia hemprichii* and *Thalassodendron ciliatum*. The seagrass meadows are generally mixed, with several species coexisting at the local level, despite signs of zonation according to depth and other physical factors, like meadow exposure to air. For example, species like *H. uninervis* or *S. isoetifolium* can be found in the intertidal area, while *T. ciliatum* can form monospecific meadows in the deep subtidal area. The geomorphological coastal landscape is typical of the Indo-Pacific region, formed by reef platforms, which are generally shallow and surrounded by a fringing reef (Short et al. 2007).

The coastal communities in Unguja Island heavily depend on seagrass meadows for their survival, as they are their preferred fishing grounds (de la Torre-Castro et al. 2004), and are also the preferred areas for the location of seaweed farms (Hedberg et al. 2018). This leads to anthropogenic pressure on the seagrass, due to the removal of species exerting top-down control through fishing (Rehren 2017) and light deprivation due to seaweed farming (Eklöf et al. 2006). Additionally, coastal communities can threaten seagrass ecosystems through eutrophication by runoff of sewage water and fertilizers (Unsworth et al. 2018). For these reasons, the locations that seagrass meadows around Unguja Island inhabit can range from generally pristine and oligotrophic, to heavily impacted and/or eutrophic.

Due to its high seagrass diversity and the variety of conditions under which seagrass survive, Unguja Island is a perfect laboratory for the study of seagrass communities using a trait-based framework. The research questions selected for this dissertation have the goal of understanding the importance of traits at different organizational levels, from their individual responses to environmental drivers, to the effect of traits on the relative abundance of species and, lastly, their effect on ecosystem functioning. The research questions (RQs) for this study were:

- RQ1. What do we know about seagrass trait-based research? What are the knowledge gaps?
- RQ2. What are the responses of seagrass to global and local stressors? Are responses common across species, or species-specific? Which traits are important indicators of response?
- RQ3. How do traits affect the configuration of seagrass meadows through interspecific competition?
- RQ4. What are the links between traits and ecosystem functions?

In order to answer these research questions, the thesis has been divided in six chapters. Firstly, to answer RQ1, I carried out a systematic review of the seagrass literature, which corresponds to Chapter 2 of the dissertation. The goal was to identify any works in which seagrass traits were studied, and which studies additionally make use of concepts developed under a trait-based theory. With this review, I identified the traits and concepts that have been under- and over-studied in seagrass research and present a comprehensive trait-based framework (TBF) to push seagrass research forward. Secondly, to answer RQ2, three different experiments were performed. In two experiments (Chapters 3 and 4) tropical seagrass species (*Enhalus acoroides*, *Halophila stipulacea*, *Cymodocea serrulata* and *Thalassia hemprichii*) were subjected to four treatments with varying levels of temperature and nutrient enrichment. The goal of these

experiments were to identify the trait responses of seagrass to both a global (temperature) and local (nutrient enrichment) stressors individually and in combination. In the third experiment (Chapter 5), I studied how two seagrass species (*T. hemprichii* and *H. stipulacea*) and the associated benthic macroalgae community respond to a local stressor endemic to tropical areas, seaweed farming. Thirdly, to answer RQ3, I carried out an observational study in Unguja Island to understand how seagrass traits linked to light and nutrient preemption affected competitive outcomes among seagrass species (Chapter 6). Fourthly, to answer RQ4, sediment cores were collected in seagrass meadows of Unguja Island to find out which seagrass traits are relevant indicators of carbon storage (Chapter 7), and which environmental conditions constrain the storage of carbon in the sediments. Lastly, in Chapter 8, I comprehensively revise the findings from all the previous chapters to better understand how the study of seagrass traits reveals which characteristics of seagrass are important drivers of the ecosystem processes, and make a case for the introduction of TBFs in seagrass research.

IV. Author's contributions

The author contributions varied among the chapters according to the categories in Table 1.

Table 1. Contribution of the author of this dissertation to the chapters included. Chapters corresponding to manuscripts in which I was first author are indicated in “State of publication”. Numbers are percentages.

Chapters	Concept/ design	Data acquisition	Data analysis/ interpretation	Figures/ tables	Drafting of manuscript	State of publication
2	50	95	90	100	75	First author. Under review in <i>Biological Reviews</i>
3	5	30	75	10	25	Published in <i>Frontiers in Marine Science</i> https://doi.org/10.3389/fmars.2020.00325
4	5	30	50	0	30	Published in <i>Frontiers in Plant Science</i> https://doi.org/10.3389/fpls.2020.571363
5	90	90	90	100	90	First author. Published in <i>Regional Environmental Change</i> https://doi.org/10.1007/s10113-020-01742-2
6	90	30	80	100	90	First author. Under review in <i>Frontiers in Plant Science</i>
7	80	50	90	100	90	First author. In preparation

V. References

- Alexandre, A., Silva, J., Buapet, P., Björk, M., and Santos, R. (2012). Effects of CO₂ enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*. *Ecology and Evolution*, 2, 2625-2635. <https://doi.org/10.1002/ece3.333>
- Andersen, K. H., and Pedersen, M. (2009). Damped trophic cascades driven by fishing in model marine ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 277, 795-802. <https://doi.org/10.1098/rspb.2009.1512>
- Baden, S., Emanuelsson, A., Pihl, L., Svensson, C. J., and Åberg, P. (2012). Shift in seagrass food web structure over decades is linked to overfishing. *Marine Ecology Progress Series*, 451, 61-73. <https://doi.org/10.3354/meps09585>
- Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., and Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological monographs*, 81, 169-193. <https://doi.org/10.1890/10-1510.1>
- Bates, A. E., Pecl, G. T., Frusher, S., Hobday, A. J., Wernberg, T., Smale, D. A., ... and Watson, R. A. (2014). Defining and observing stages of climate-mediated range shifts in marine systems. *Global Environmental Change*, 26, 27-38. <https://doi.org/10.1016/j.gloenvcha.2014.03.009>
- Beer, S., and Rehnberg, J. (1997). The acquisition of inorganic carbon by the seagrass *Zostera marina*. *Aquatic Botany*, 56, 277-283. [https://doi.org/10.1016/s0304-3770\(96\)01109-6](https://doi.org/10.1016/s0304-3770(96)01109-6)
- Belshe, E. F., Hoeijmakers, D., Herran, N., Mtolera, M., and Teichberg, M. (2018). Seagrass community-level controls over organic carbon storage are constrained by geophysical attributes within meadows of Zanzibar, Tanzania. *Biogeosciences*, 15, 4609-4626. <https://doi.org/10.5194/bg-15-4609-2018>
- Bernstein, L., Bosch, P., Canziani, O., Chen, Z., Christ, R., Davidson, O., ... and Yobe, G. (2008). *Climate Change 2007 Synthesis Report*. Intergovernmental Panel on Climate Change. <https://doi.org/10.1017/cbo9780511546013>
- Best, R. J., and Stachowicz, J. J. (2012). Trophic cascades in seagrass meadows depend on mesograzers: variation in feeding rates, predation susceptibility, and abundance. *Marine Ecology Progress Series*, 456, 29-42. <https://doi.org/10.3354/meps09678>
- Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., ... and Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in ecology and evolution*, 26, 183-192. <https://doi.org/10.1016/j.tree.2011.01.009>
- Bremner, J., Rogers, S. I., and Frid, C. L. J. (2006). Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). *Ecological Indicators*, 6, 609-622. <https://doi.org/10.1016/j.ecolind.2005.08.026>
- Brown, M. V., Ostrowski, M., Grzymalski, J. J., and Lauro, F. M. (2014). A trait based perspective on the biogeography of common and abundant marine bacterioplankton clades. *Marine Genomics*, 15, 17-28. <https://doi.org/10.1016/j.margen.2014.03.002>

- Bulthuis, D. A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany*, 2, 27–40. [https://doi.org/10.1016/0304-3770\(87\)90084-2](https://doi.org/10.1016/0304-3770(87)90084-2)
- Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of experimental marine biology and ecology*, 350, 46-72. <https://doi.org/10.1016/j.jembe.2007.06.024>
- Campbell, S. J., McKenzie, L. J., and Kerville, S. P. (2006). Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *Journal of Experimental Marine Biology and Ecology*, 330, 455-468. <https://doi.org/10.1016/j.jembe.2005.09.017>
- Campbell, J. E., and Fourqurean, J. W. (2013). Effects of in situ CO₂ enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. *Marine Biology*, 160, 1465-1475. <https://doi.org/10.1007/s00227-013-2199-3>
- Chefaoui, R. M., Duarte, C. M., and Serrão, E. A. (2018). Dramatic loss of seagrass habitat under projected climate change in the Mediterranean Sea. *Global change biology*, 24, 4919-4928. <https://doi.org/10.1111/gcb.14401>
- Collier, C. J., and Waycott, M. (2014). Temperature extremes reduce seagrass growth and induce mortality. *Marine pollution bulletin*, 83, 483 - 490. <https://doi.org/10.1016/j.marpolbul.2014.03.050>
- Darling, E. S., Alvarez-Filip, L., Oliver, T. A., McClanahan, T. R., and Côté, I. M. (2012). Evaluating life-history strategies of reef corals from species traits. *Ecology Letters*, 15, 1378-1386. <https://doi.org/10.1111/j.1461-0248.2012.01861.x>
- de la Torre-Castro, M., and Rönnbäck, P. (2004). Links between humans and seagrasses—an example from tropical East Africa. *Ocean and Coastal Management*, 47, 361-387. <https://doi.org/10.1016/j.ocecoaman.2004.07.005>
- Diaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, A., ... and Band, S. R. (2004). The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation science*, 15, 295-304. <https://doi.org/10.1111/j.1654-1103.2004.tb02266.x>
- Díaz, S., and Cabido, M. (2001). Vive la différence: plant functional diversity matters to ecosystem processes. *Trends in ecology and evolution*, 16, 646-655. [https://doi.org/10.1016/s0169-5347\(01\)02283-2](https://doi.org/10.1016/s0169-5347(01)02283-2)
- Dobzhansky, T. (1937). Genetic nature of species differences. *The American Naturalist*, 71, 404-420. <https://doi.org/10.1086/280726>
- Duarte, C. M. (1995). Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, 41, 87-112. <https://doi.org/10.1080/00785236.1995.10422039>
- Duckworth, J. C., Kent, M., and Ramsay, P. M. (2000). Plant functional types: an alternative to taxonomic plant community description in biogeography?. *Progress in Physical Geography*, 24, 515-542. <https://doi.org/10.1191/030913300701542778>

- Duffy JE (2006) Biodiversity and the functioning of seagrass ecosystems. *Marine Ecology Progress Series*, 311, 233-250. <https://doi.org/10.3354/meps311233>
- Edwards, K. F., Litchman, E., and Klausmeier, C. A. (2013). Functional traits explain phytoplankton community structure and seasonal dynamics in a marine ecosystem. *Ecology letters*, 16, 56-63. <https://doi.org/10.1111/ele.12012>
- Eklöf, J. S., Henriksson, R., and Kautsky, N. (2006). Effects of tropical open-water seaweed farming on seagrass ecosystem structure and function. *Marine Ecology Progress Series*, 325, 73-84. <https://doi.org/10.3354/meps325073>
- Elleouet, J., Albouy, C., Ben Rais Lasram, F., Mouillot, D., and Leprieur, F. (2014). A trait-based approach for assessing and mapping niche overlap between native and exotic species: the Mediterranean coastal fish fauna as a case study. *Diversity and Distributions*, 20(11), 1333-1344. <https://doi.org/10.1111/ddi.12235>
- Ellison, A. M. (2002). Macroecology of mangroves: large-scale patterns and processes in tropical coastal forests. *Trees*, 16, 181-194. <https://doi.org/10.1007/s00468-001-0133-7>
- Engelhardt, K. A. (2006). Relating effect and response traits in submersed aquatic macrophytes. *Ecological Applications*, 16, 1808-1820. [https://doi.org/10.1890/1051-0761\(2006\)016\[1808:rearti\]2.0.co;2](https://doi.org/10.1890/1051-0761(2006)016[1808:rearti]2.0.co;2)
- Enríquez, S., Marbà, N., Duarte, C. M., Van Tussenbroek, B. I., and Reyes-Zavala, G. (2001). Effects of seagrass *Thalassia testudinum* on sediment redox. *Marine Ecology Progress Series*, 219, 149-158. <https://doi.org/10.3354/meps219149>
- Entrambasaguas, L., Jahnke, M., Biffali, E., Borra, M., Sanges, R., Marín-Guirao, L., and Procaccini, G. (2017). Tissue-specific transcriptomic profiling provides new insights into the reproductive ecology and biology of the iconic seagrass species *Posidonia oceanica*. *Marine genomics*, 35, 51-61. <https://doi.org/10.1016/j.margen.2017.05.006>
- Enquist, B. J., Norberg, J., Bonser, S. P., Violle, C., Webb, C. T., Henderson, A., ... and Savage, V. M. (2015). Scaling from traits to ecosystems: developing a general trait driver theory via integrating trait-based and metabolic scaling theories. *Advances in ecological research*, 52, 249-318. <https://doi.org/10.1016/bs.aecr.2015.02.001>
- Erfteimeijer, P. L., and Lewis III, R. R. R. (2006). Environmental impacts of dredging on seagrasses: a review. *Marine pollution bulletin*, 52, 1553-1572. <https://doi.org/10.1016/j.marpolbul.2006.09.006>
- Eviner, V. T., and Chapin III, F. S. (2003). Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. *Annual Review of Ecology, Evolution, and Systematics*, 34, 455-485. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132342>
- Fernandes, M. B., van Gils, J., Erfteimeijer, P. L., Daly, R., Gonzalez, D., and Rouse, K. (2019). A novel approach to determining dynamic nitrogen thresholds for seagrass conservation. *Journal of applied ecology*, 56, 253-261. <https://doi.org/10.1111/1365-2664.13252>

- Follows, M. J., Dutkiewicz, S., Grant, S., and Chisholm, S. W. (2007). Emergent biogeography of microbial communities in a model ocean. *Science*, *315*, 1843-1846. <https://doi.org/10.1126/science.1138544>
- Fonseca, M. S., and Callahan, J. A. (1992). A preliminary evaluation of wave attenuation for four species of seagrasses. *Estuarine Coastal Shelf Science*, *35*. [https://doi.org/10.1016/s0272-7714\(05\)80039-3](https://doi.org/10.1016/s0272-7714(05)80039-3)
- Fonseca, M. S., Julius, B. E., and Kenworthy, W. J. (2000). Integrating biology and economics in seagrass restoration: How much is enough and why?. *Ecological Engineering*, *15*, 227-237. [https://doi.org/10.1016/s0925-8574\(00\)00078-1](https://doi.org/10.1016/s0925-8574(00)00078-1)
- Fourqurean, J. W., Powell, G. V., Kenworthy, W. J., and Zieman, J. C. (1995). The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. *Oikos*, 349-358. <https://doi.org/10.2307/3546120>
- Garnier, E., Navas, M. L., and Grigulis, K. (2016). *Plant functional diversity: organism traits, community structure, and ecosystem properties*. Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780198757368.001.0001>
- Golicz AA, Schliep M, Lee HT, Larkum AWD, Dolferus R, Batley J, Chan CKK, Sablok G, Ralph PJ, Edwards D (2015) Genome-wide survey of the seagrass *Zostera muelleri* suggests modification of the ethylene signaling network. *Journal of experimental botany*, *66*, 1489-1498. <https://doi.org/10.1093/jxb/eru510>
- Greiner, J. T., McGlathery, K. J., Gunnell, J., and McKee, B. A. (2013). Seagrass restoration enhances “blue carbon” sequestration in coastal waters. *PloS one*, *8*, e72469. <https://doi.org/10.1371/journal.pone.0072469>
- Grime, J. P. (2006). Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *Journal of Vegetation Science*, *17*, 255-260. <https://doi.org/10.1111/j.1654-1103.2006.tb02444.x>
- Grime, J. P., and Pierce, S. (2012). *The evolutionary strategies that shape ecosystems*. John Wiley and Sons. <https://doi.org/10.1002/9781118223246>
- Guinotte, J. M., and Fabry, V. J. (2008). Ocean acidification and its potential effects on marine ecosystems. *Annals of the New York Academy of Sciences*, *1134*, 320-342. <https://doi.org/10.1196/annals.1439.013>
- Harlin, M. M., and Thorne-Miller, B. (1981). Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. *Marine Biology*, *65*, 221-229. <https://doi.org/10.1007/bf00397115>
- Hedberg, N., von Schreeb, K., Charisiadou, S., Jiddawi, N. S., Tedengren, M., and Nordlund, L. M. (2018). Habitat preference for seaweed farming—A case study from Zanzibar, Tanzania. *Ocean and Coastal Management*, *154*, 186-195. <https://doi.org/10.1016/j.ocecoaman.2018.01.016>

- Heithaus, M. R., Alcoverro, T., Arthur, R., Burkholder, D. A., Coates, K. A., Christianen, M. J., ... and Fourqurean, J. W. (2014). Seagrasses in the age of sea turtle conservation and shark overfishing. *Frontiers in Marine Science*, 1, 28. <https://doi.org/10.3389/fmars.2014.00028>
- Hemminga, M. A., Harrison, P. G., and Van Lent, F. (1991). The balance of nutrient losses and gains in seagrass meadows. *Marine Ecology Progress Series*, 85-96. <https://doi.org/10.3354/meps071085>
- Hendriks, I. E., Sintes, T., Bouma, T. J., and Duarte, C. M. (2008). Experimental assessment and modeling evaluation of the effects of the seagrass *Posidonia oceanica* on flow and particle trapping. *Marine Ecology Progress Series*, 356, 163-173. <https://doi.org/10.3354/meps07316>
- Hendriks, I. E., Olsen, Y. S., Ramajo, L., Basso, L., Steckbauer, A., Moore, T. S., ... and Duarte, C. M. (2014). Photosynthetic activity buffers ocean acidification in seagrass meadows. *Biogeosciences*, 11, 333-346. <https://doi.org/10.5194/bg-11-333-2014>
- Holland, G. J., and Webster, P. J. (2007). Heightened tropical cyclone activity in the North Atlantic: natural variability or climate trend?. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 365, 2695-2716. <https://doi.org/10.1098/rsta.2007.2083>
- Hyndes, G. A., Heck Jr, K. L., Vergés, A., Harvey, E. S., Kendrick, G. A., Lavery, P. S., ... and Wilson, S. (2017). Accelerating tropicalization and the transformation of temperate seagrass meadows. *Bioscience*, 66, 938-948. <https://doi.org/10.1093/biosci/biw111>
- Invers, O., Pérez, M., and Romero, J. (1999). Bicarbonate utilization in seagrass photosynthesis: role of carbonic anhydrase in *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson. *Journal of Experimental Marine Biology and Ecology*, 235, 125-133. [https://doi.org/10.1016/s0022-0981\(98\)00172-5](https://doi.org/10.1016/s0022-0981(98)00172-5)
- Izaguirre, C., Méndez, F. J., Menéndez, M., and Losada, I. J. (2011). Global extreme wave height variability based on satellite data. *Geophysical Research Letters*, 38. <https://doi.org/10.1029/2011gl047302>
- Jackson, E. L., Rowden, A. A., Attrill, M. J., Bossey, S. J., and Jones, M. B. (2001). The importance of seagrass beds as a habitat for fishery species. *Oceanography and marine biology*, 39, 269-304. <https://doi.org/10.1111/cobi.12436>
- Janssen, T., and Bremer, K. (2004). The age of major monocot groups inferred from 800+ rbcL sequences. *Botanical Journal of the Linnean Society*, 146, 385-398. <https://doi.org/10.1111/j.1095-8339.2004.00345.x>
- Jiménez-Ramos, R., Egea, L. G., Vergara, J. J., and Brun, F. G. (2018). Nutrient load and epiphytes are drivers of increased herbivory in seagrass communities. *Marine Ecology Progress Series*, 599, 49-64. <https://doi.org/10.3354/meps12622>
- Kattge, J., Diaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Bönisch, G., ... and Wirth, C. (2011). TRY—a global database of plant traits. *Global change biology*, 17, 2905-2935. <https://doi.org/10.5194/egusphere-egu2020-20191>

- Keddy, P. (2007). *Plants and vegetation: origins, processes, consequences*. Cambridge University Press. <https://doi.org/10.1017/cbo9780511812989>
- Kjørboe, T., Visser, A., and Andersen, K. H. (2018). A trait-based approach to ocean ecology. *ICES Journal of Marine Science*, 75, 1849 - 1863. <https://doi.org/10.1093/icesjms/fsy090>
- Koch, M., Bowes, G., Ross, C., and Zhang, X. H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global change biology*, 19(1), 103-132. <https://doi.org/10.1111/j.1365-2486.2012.02791.x>
- Kuo, J., and Den Hartog, C. (2007). Seagrass morphology, anatomy, and ultrastructure. In *Seagrasses: biology, ecology and conservation* (pp. 51-87). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-2983-7_3
- Larkum, A. W. D., Davey, P. A., Kuo, J., Ralph, P. J., and Raven, J. A. (2017). Carbon-concentrating mechanisms in seagrasses. *Journal of experimental botany*, 68, 3773-3784. <https://doi.org/10.1093/jxb/erx206>
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional ecology*, 16, 545-556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Lavorel, S., Díaz, S., Cornelissen, J. H. C., Garnier, E., Harrison, S. P., McIntyre, S., ... and Urcelay, C. (2007). Plant functional types: are we getting any closer to the Holy Grail?. In *Terrestrial ecosystems in a changing world* (pp. 149-164). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-32730-1_13
- Lee, K. S., Park, S. R., and Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology*, 350, 144-175. <https://doi.org/10.1016/j.jembe.2007.06.016>
- Lee, H., Golicz, A. A., Bayer, P. E., Jiao, Y., Tang, H., Paterson, A. H., ... and Edwards, D. (2016). The genome of a southern hemisphere seagrass species (*Zostera muelleri*). *Plant physiology*, 172, 272-283. <https://doi.org/10.1104/pp.16.00868>
- Les, D. H., Cleland, M. A., and Waycott, M. (1997). Phylogenetic studies in Alismatidae, II: evolution of marine angiosperms (seagrasses) and hydrophily. *Systematic Botany*, 443-463. <https://doi.org/10.2307/2419820>
- Litchman, E., de Tezanos Pinto, P., Klausmeier, C. A., Thomas, M. K., and Yoshiyama, K. (2010). Linking traits to species diversity and community structure in phytoplankton. In *Fifty years after the 'Homage to Santa Rosalia': Old and new paradigms on biodiversity in aquatic ecosystems* (pp. 15-28). Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9908-2_3
- Litchman, E., Ohman, M. D., and Kjørboe, T. (2013). Trait-based approaches to zooplankton communities. *Journal of Plankton Research*, 35, 473-484. <https://doi.org/10.1093/plankt/fbt019>

- Madin, J. S., Anderson, K. D., Andreasen, M. H., Bridge, T. C., Cairns, S. D., Connolly, S. R., ... and Baird, A. H. (2016). The Coral Trait Database, a curated database of trait information for coral species from the global oceans. *Scientific Data*, 3, 1-22. <https://doi.org/10.1038/sdata.2016.17>
- Marbà, N., and Duarte, C. M. (2010). Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Global Change Biology*, 16, 2366-2375. <https://doi.org/10.1111/j.1365-2486.2009.02130.x>
- Marbà, N., Krause-Jensen, D., Alcoverro, T., Birk, S., Pedersen, A., Neto, J. M., ... and Duarte, C. M. (2013). Diversity of European seagrass indicators: patterns within and across regions. *Hydrobiologia*, 704, 265-278. <https://doi.org/10.1007/s10750-012-1403-7>
- Martínez-Crego, B., Vergés, A., Alcoverro, T., and Romero, J. (2008). Selection of multiple seagrass indicators for environmental biomonitoring. *Marine Ecology Progress Series*, 361, 93-109. <https://doi.org/10.3354/meps07358>
- McGlathery, K. J. (1995). Nutrient and grazing influences on a subtropical seagrass community. *Marine Ecology Progress Series*, 122, 239 - 252. <https://doi.org/10.3354/meps122239>
- McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in ecology and evolution*, 21, 178-185. <https://doi.org/10.1016/j.tree.2006.02.002>
- McMillan, C. (1984). The distribution of tropical seagrasses with relation to their tolerance of high temperatures. *Aquatic Botany*, 19, 369-379. [https://doi.org/10.1016/0304-3770\(84\)90049-4](https://doi.org/10.1016/0304-3770(84)90049-4)
- Mlambo, M. C. (2014). Not all traits are ‘functional’: insights from taxonomy and biodiversity-ecosystem functioning research. *Biodiversity and Conservation*, 23, 781-790. <https://doi.org/10.1007/s10531-014-0618-5>
- Moksnes, P. O., Gullström, M., Tryman, K., and Baden, S. (2008). Trophic cascades in a temperate seagrass community. *Oikos*, 117, 763-777. <https://doi.org/10.1111/j.0030-1299.2008.16521.x>
- Mtwana Nordlund, L., Koch, E. W., Barbier, E. B., and Creed, J. C. (2016). Seagrass ecosystem services and their variability across genera and geographical regions. *PLoS One*, 11, e0163091. <https://doi.org/10.1371/journal.pone.0163091>
- Munier, S., Carrer, D., Planque, C., Camacho, F., Albergel, C., and Calvet, J. C. (2018). Satellite Leaf Area Index: global scale analysis of the tendencies per vegetation type over the last 17 years. *Remote Sensing*, 10, 424. <https://doi.org/10.3390/rs10030424>
- Naeem, S., Thompson, L. J., Lawler, S. P., Lawton, J. H., and Woodfin, R. M. (1994). Declining biodiversity can alter the performance of ecosystems. *Nature*, 368, 734-737. <https://doi.org/10.1038/368734a0>

- Olsen, J. L., Rouzé, P., Verhelst, B., Lin, Y. C., Bayer, T., Collen, J., ... and Van de Peer, Y. (2016). The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature*, 530, 331-335. <https://doi.org/10.1038/nature16548>
- Oreska, M. P., McGlathery, K. J., and Porter, J. H. (2017). Seagrass blue carbon spatial patterns at the meadow-scale. *PloS one*, 12, e0176630. <https://doi.org/10.1371/journal.pone.0176630>
- Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., ... and Williams, S. L. (2006). A global crisis for seagrass ecosystems. *Bioscience*, 56, 987-996. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:agcfse\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2)
- Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., ... and van Ypserle, J. P. (2014). *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change* (p. 151). IPCC. <https://doi.org/10.1017/cbo9781107415416>
- Palacios, S. L., and Zimmerman, R. C. (2007). Response of eelgrass *Zostera marina* to CO₂ enrichment: possible impacts of climate change and potential for remediation of coastal habitats. *Marine Ecology Progress Series*, 344, 1-13. <https://doi.org/10.3354/meps07084>
- Paulo, D., Cunha, A. H., Boavida, J., Serrão, E. A., Gonçalves, E. J., and Fonseca, M. (2019). Open coast seagrass restoration. Can we do it? Large scale seagrass transplants. *Frontiers in Marine Science*, 6, 52. <https://doi.org/10.3389/fmars.2019.00052>
- Pawar, S., Woodward, G., and Dell, A. I. (2015). *Trait-Based Ecology-from structure to function*. Academic Press. [https://doi.org/10.1016/s0065-2504\(15\)x0002-x](https://doi.org/10.1016/s0065-2504(15)x0002-x)
- Poloczanska, E. S., Brown, C. J., Sydeman, W. J., Kiessling, W., Schoeman, D. S., Moore, P. J., ... and Richardson, A. J. (2013). Global imprint of climate change on marine life. *Nature Climate Change*, 3, 919-925. <https://doi.org/10.1038/nclimate1958>
- Poore, A. G., Campbell, A. H., Coleman, R. A., Edgar, G. J., Jormalainen, V., Reynolds, P. L., ... and Emmett Duffy, J. (2012). Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology letters*, 15, 912-922. <https://doi.org/10.1111/j.1461-0248.2012.01804.x>
- Rehren, J. (2017). *Modelling the Multispecies Fishery of Chwaka Bay, Zanzibar-Basis for Exploration of Use and Conservation Scenarios* (Doctoral dissertation, Universität Bremen).
- Roca, G., Alcoverro, T., Krause-Jensen, D., Balsby, T. J., Van Katwijk, M. M., Marbà, N., ... and Romero, J. (2016). Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis. *Ecological Indicators*, 63, 310-323. <https://doi.org/10.1016/j.ecolind.2015.12.007>
- Schmitz, O. J., Buchkowski, R. W., Burghardt, K. T., and Donihue, C. M. (2015). Functional traits and trait-mediated interactions: connecting community-level interactions with ecosystem functioning. *Advances in Ecological Research*, 52, 319-343. <https://doi.org/10.1016/bs.aecr.2015.01.003>

- Schimper, A. F. W. (1903). *Plant-geography Upon a Physiological Basis...* Clarendon Press. <https://doi.org/10.5962/bhl.title.8099>
- Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350, 3-20. <https://doi.org/10.1016/j.jembe.2007.06.012>
- Short, F. T., Polidoro, B., Livingstone, S. R., Carpenter, K. E., Bandeira, S., Bujang, J. S., ... and Zieman, J. C. (2011). Extinction risk assessment of the world's seagrass species. *Biological Conservation*, 144, 1961-1971. <https://doi.org/10.1016/j.biocon.2011.04.010>
- Spalding, M. (2003). The distribution and status of seagrasses. *World atlas of seagrasses*, (pp. 5-27). <https://doi.org/10.1515/bot.2004.029>
- Stegen, J. C., Enquist, B. J., and Ferriere, R. (2009). Advancing the metabolic theory of biodiversity. *Ecology letters*, 12, 1001 - 1015. <https://doi.org/10.1111/j.1461-0248.2009.01358.x>
- Suding, K. N., and Goldstein, L. J. (2008). Testing the Holy Grail framework: using functional traits to predict ecosystem change. *New Phytologist*, 180, 559 - 562. <https://doi.org/10.1111/j.1469-8137.2008.02650.x>
- Sullivan, B. K., Trevathan-Tackett, S. M., Neuhauser, S., and Govers, L. L. (2018). Host-pathogen dynamics of seagrass diseases under future global change. *Marine pollution bulletin*, 134, 75-88. <https://doi.org/10.1016/j.marpolbul.2017.09.030>
- Touchette, B. W. (2007). Seagrass-salinity interactions: physiological mechanisms used by submersed marine angiosperms for a life at sea. *Journal of Experimental Marine Biology and Ecology*, 350, 194-215. <https://doi.org/10.1016/j.jembe.2007.05.037>
- Unsworth, R. K., Collier, C. J., Henderson, G. M., and McKenzie, L. J. (2012). Tropical seagrass meadows modify seawater carbon chemistry: implications for coral reefs impacted by ocean acidification. *Environmental Research Letters*, 7, 024026. <https://doi.org/10.1088/1748-9326/7/2/024026>
- Unsworth, R. K., Ambo-Rappe, R., Jones, B. L., La Nafie, Y. A., Irawan, A., Hernawan, U. E., ... and Cullen-Unsworth, L. C. (2018). Indonesia's globally significant seagrass meadows are under widespread threat. *Science of the Total Environment*, 634, 279-286. <https://doi.org/10.1016/j.scitotenv.2018.03.315>
- Valle, M., Chust, G., del Campo, A., Wisz, M. S., Olsen, S. M., Garmendia, J. M., and Borja, Á. (2014). Projecting future distribution of the seagrass *Zostera noltii* under global warming and sea level rise. *Biological Conservation*, 170, 74 - 85. <https://doi.org/10.1016/j.biocon.2013.12.017>
- van der Linden, P., Patrício, J., Marchini, A., Cid, N., Neto, J. M., and Marques, J. C. (2012). A biological trait approach to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. *Ecological Indicators*, 20, 121 - 133. <https://doi.org/10.1016/j.ecolind.2012.02.004>

- van der Wal, D., Lambert, G. I., Ysebaert, T., Plancke, Y. M., and Herman, P. M. (2017). Hydrodynamic conditioning of diversity and functional traits in subtidal estuarine macrozoobenthic communities. *Estuarine, Coastal and Shelf Science*, 197, 80-92. <https://doi.org/10.1016/j.ecss.2017.08.012>
- Van Katwijk, M. M., Bos, A. R., De Jonge, V. N., Hanssen, L. S. A. M., Hermus, D. C. R., and De Jong, D. J. (2009). Guidelines for seagrass restoration: importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Marine pollution bulletin*, 58, 179-188. <https://doi.org/10.1016/j.marpolbul.2008.09.028>
- van Katwijk, M. M., Bos, A. R., Kennis, P., and de Vries, R. (2010). Vulnerability to eutrophication of a semi-annual life history: A lesson learnt from an extinct eelgrass (*Zostera marina*) population. *Biological Conservation*, 143, 248 - 254. <https://doi.org/10.1016/j.biocon.2009.08.014>
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. (2007). Let the concept of trait be functional!. *Oikos*, 116, 882-892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- Virnstein, R. W., Mikkelsen, P. S., Cairns, K. D., and Capone, M. A. (1983). Seagrass beds versus sand bottoms: the trophic importance of their associated benthic invertebrates. *Florida Scientist*, 363-381.
- Waycott, M., Procaccini, G., Les, D. H., and Reusch, T. B. (2006). *Seagrasses: Biology, Ecology and Conservation*. Springer. https://doi.org/10.1007/1-4020-2983-7_2
- Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., ... and Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the national academy of sciences*, 106, 12377-12381. <https://doi.org/10.1073/pnas.0905620106>
- Webb, C. T., Hoeting, J. A., Ames, G. M., Pyne, M. I., and LeRoy Poff, N. (2010). A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecology letters*, 13, 267-283. <https://doi.org/10.1111/j.1461-0248.2010.01444.x>
- Wissler, L., Codoñer, F. M., Gu, J., Reusch, T. B., Olsen, J. L., Procaccini, G., and Bornberg-Bauer, E. (2011). Back to the sea twice: identifying candidate plant genes for molecular evolution to marine life. *BMC evolutionary biology*, 11, 1-13. <https://doi.org/10.1186/1471-2148-11-8>



CHAPTER 2.

A trait-based framework to seagrass ecology: trends and prospects

Agustín Moreira-Saporiti^{1,2}, *Mirta Teichberg*¹, *Eric Garnier*³, *Johannes H.C. Cornelissen*⁴, *Teresa Alcoverro*⁵, *Mats Björk*⁶, *Christoffer Boström*⁷, *Emanuela Dattolo*⁸, *Johan Eklöf*⁶, *Harald Hasler-Sheetal*⁹, *Marianne Holmer*⁹, *Nuria Marbà*¹⁰, *Lázaro Marín-Guirao*^{8,11}, *Lukas Meysick*^{7,12,13}, *Irene Olivé*^{8,12,14}, *Thorsten B.H.Reusch*¹⁵, *Miriam Ruocco*⁸, *João Silva*¹⁶, *Ana I. Sousa*¹⁷, *Gabriele Procaccini*^{8,*}, *Rui Santos*¹⁶

¹ Leibniz Zentrum für Marine Tropenforschung, Fahrenheitstraße 6, 28359 Bremen. ² Universität Bremen, Bibliothekstraße 1, 28359 Bremen. ³ CEFÉ, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France. ⁴ Vrije Universiteit Amsterdam, De Boelelaan 1105, 1081 HV Amsterdam, The Netherlands. ⁵ Centre d'Estudis Avançats de Blanes, C/Acc. Cala Sant Francesc 14, 17300 Blanes, Spain. ⁶ Department of Ecology, Environment and Plant Sciences (DEEP) Stockholm University, 106 91 Stockholm, Sweden. ⁷ Åbo Akademi University, Environmental and Marine Biology, Tykistökatu 6, 20520 Turku, Finland. ⁸ Stazione Zoologica Anton Dohrn, 80121 Naples, Italy. ⁹ University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark. ¹⁰ Mediterranean Institute for Advanced Studies, C/Miquel Marquès, 21 07190 Esporles Illes Balears, Spain. ¹¹ Oceanographic Center of Murcia, Spanish Institute of Oceanography, 30740 Murcia, Spain. ¹² Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), 26129 Oldenburg, Germany. ¹³ Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, 27570 Bremerhaven, Germany. ¹⁴ University of Glasgow, Glasgow G12 8QQ, United Kingdom. ¹⁵ Marine Evolutionary Ecology, Division of Marine Ecology, GEOMAR Helmholtz Center for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany. ¹⁶ Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. ¹⁷ CESAM – Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

Abstract

In the last three decades, trait-based approaches have advanced different fields of ecological research through establishing novel links between functional traits, environmental drivers and ecosystem functions. A number of concepts have been proposed in order to answer different ecological questions using a functional trait-based perspective. Here, we present a novel trait-based response-effect framework (TBF) which builds on previous concepts and ideas, including the use of traits for the study of community assembly processes, from dispersal and response to abiotic and biotic factors, to ecosystem function and service provision. We used seagrasses, marine vascular plants under strong anthropogenic pressure, to test the concepts and hypotheses presented in the TBF and to investigate research gaps and opportunities in seagrass trait-based research. For this purpose, we compiled a systematic review of the seagrass trait literature. The analysis showed that seagrass trait research has mostly focused on the effect of environmental drivers on traits (65%), whereas links between traits and functions are less common (33%). Despite the richness of trait-based data available, concepts related to TBFs are rare in the seagrass literature (7% of studies), including the relative importance of neutral and niche

assembly processes or the influence of trait dominance or complementarity in ecosystem function provision. These knowledge gaps in seagrasses indicate ample potential for further research, highlighting the links between the unique traits of seagrass and the ecosystem services they provide.

Keywords: Functional ecology, trait-based approach, seagrass traits database, ecosystem service vulnerability, response-effect framework.

I. Trait-based frameworks (TBFs): traits as answer to complex ecological questions

Trait-based response-effect frameworks (hereafter TBFs) have been extensively used in terrestrial plant ecology (Suding et al. 2008; Diaz et al. 2013). TBFs are based on the study of traits, which capture the form and function of organisms, and are defined as “any morphological, physiological or phenological heritable feature measurable at the individual level, from the cell to the whole organism, without reference to the environment or any other level of organization” (Violle et al. 2007 as modified by Garnier et al. 2016).

Traits are divided into response and effect traits. Hence, the structure of a plant community is the result of the environmental filters and biotic interactions that exclude phenotypes that do not possess appropriate response trait values (Weiher and Keddy 1995; Diaz et al. 1998; Belyea and Lancaster 1999). Effect traits, on the other hand, influence how the organism affects ecosystem functions and they are therefore controlled by the distribution of trait values shaping the community (Garnier et al. 2016). There are many examples of the use of TBFs in terrestrial plant ecology. Functional traits and their diversity improved our mechanistic understanding of plant biomass production (Roscher et al. 2012). For instance, in the case of plant digestibility, leaf dry matter is one of the main drivers of biomass production (Gardarin et al. 2014) while litter decomposition is not only controlled by the abiotic environment, but also by species-level plant traits (Cornwell et al. 2008; Tardif et al. 2014).

The general relevance of the TBF to the study of terrestrial plant ecology has triggered its development in marine ecology (Solan et al. 2004; Follows et al. 2007; Edwards et al. 2013; Elleouet et al. 2014; Andersen and Pedersen 2009). This is because a TBF enables generalized prediction of community composition and function of any type of ecosystem across organizational and spatial scales, independent of taxonomy (Shipley et al. 2016), which allows for the testing of a variety of ecological hypotheses.

II. A novel TBF: from individual traits to ecosystem services

We propose a novel conceptual TBF, based on the seminal works by Lavorel and Garnier (2002) and Suding et al. (2008), which also takes into account phylogeny (Diaz et al. 2013) and intraspecific variability (Violle et al. 2012) using modern statistical methods (Mouillot et al. 2013) (Figure 1). The goal of this TBF is to (i) put together previous key TBFs in a comprehensive paradigm, (ii) link organismal traits to ecosystem service provision and (iii) present the potential of the framework to be applied to any organism, community or ecosystem.

First, in section II, we explain the main parts of the framework presented in Figure 1. Secondly, in section III, we use seagrasses as our model example for the adaptation of the aforementioned TBF, to indicate not only the advantages of using this comprehensive framework, but also the specialty and particularity of seagrasses. Finally, in section IV, we implement the TBF to seagrasses and discuss how this framework can guide future trait-based research on seagrasses and other groups of organisms by introducing concepts, methods and hypotheses that can push forward our knowledge in this field.

(1) Community assembly processes and response traits: dispersal, abiotic and biotic filters

Community assembly processes encompass the mechanisms underlying the composition and structure of communities in response to environmental variation (McGill et al. 2006; Weiher et al. 2011; Grime and Pierce 2012), from dispersal to the influence of abiotic and biotic factors.

For plants, dispersal into a local community (Figure 1: Filter 1) is partly controlled by stochastic processes occurring at the regional scale, which are poorly related to the traits of organisms (Weiher et al. 2011; but see section 2a below). The abiotic filter (Figure 1: Filter 2) determines which species can establish due to the influence of local environmental conditions, the availability of resources, and the disturbance regime (Wilson 2011). This defines the fundamental niche of a species. The biotic filter (Figure 1: Filter 3) corresponds to the positive and negative interactions between living organisms within communities and determines the set of neighboring species coexisting within a community (Tilman 1985). It is the realized niche of the species along the range of possibilities from competitive exclusion (Gause 1937) to facilitation (Maxwell et al. 2017). These niche assembly processes define how local communities assemble from the regional species pool through the filtering of abiotic and biotic factors (Keddy 1992) that, together with stochastic processes, explain the characteristics of local communities (Vellend 2010). To understand which metrics might be useful for detecting which assembly process predominates in shaping a community, it is helpful to envisage species trait values as coordinates (e.g. along axes of variation in multivariate analysis such as principal component analysis) locating species in the functional space (see Mouillot et al. 2013). Studying whether the functional coordinates of a species are sorted out from the local pool is random or the consequence of their response to the environmental drivers provides the grounds to test the niche and neutral assembly theories (Garnier et al. 2016) (Figure 1: Hypothesis #1).

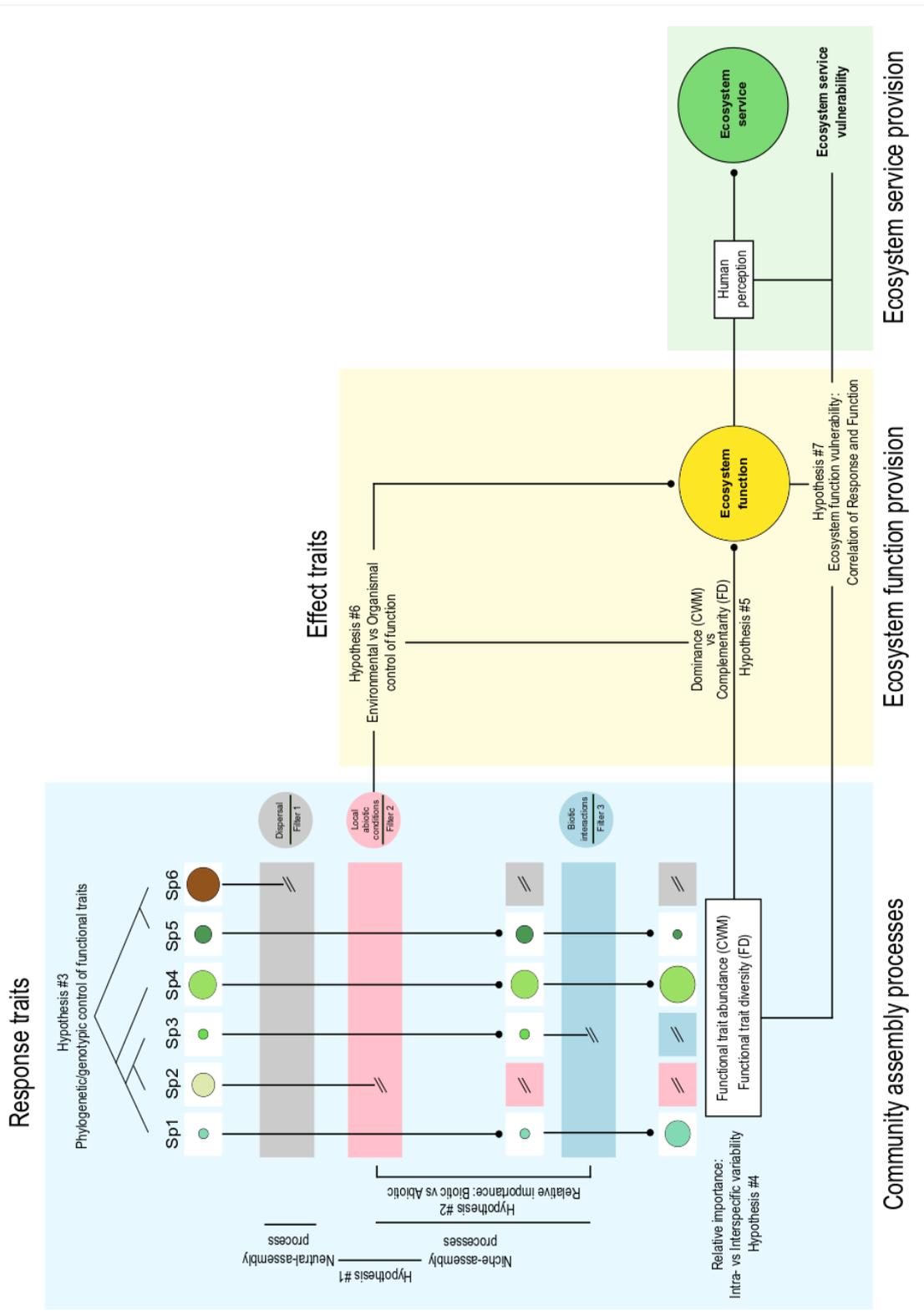


Figure 1. Conceptual TBF (trait-based response-effect framework). Traits are used to assess dispersal and the influence of abiotic and biotic filters (Hypothesis #1 and #2). Functional diversity can be controlled by phylogenetic/genotypic diversity (Hypothesis #3), and the role of intra- vs interspecific variability should be studied (Hypothesis #4). Ecosystem function provision depends on traits (Hypothesis #5) but also on the environmental context (Hypothesis #6). The correlation of the ecosystem function and response determines the function vulnerability (Hypothesis #7). Ecosystem functions and their classification in ecosystem services by humankind will determine their vulnerability.

Lastly, the functional trait structure of the local community can be convergent (showing high similarity among functional traits in co-existing species) or divergent (showing dissimilarity among functional traits in co-existing species) (Grime 2006; Bernard-Verdier et al. 2012; Cornwell and Ackerly 2009; Gross et al. 2013) depending on the relative importance of the abiotic and biotic filtering on the community (Figure 1: Hypothesis #2). Abiotic factors tend to dominate the trait distributions when they set major physico-chemical constraints on the ecosystem, which then leads to a convergent distribution, whereas biotic factors dominate when there are few or weak abiotic constraints and there is room for increasing competition (Grime 2006; Weiher et al. 1998), which tends to lead to competitive exclusion and thereby a divergent distribution (but see discussion in Mayfield and Levine 2010).

(2) Phylogenetic and genotypic control of trait diversity

Importantly, functional traits can be phylogenetically conserved or they can diverge strongly at the tips of the phylogeny, thereby reflecting relatively recent evolutionary trait change (Figure 1: Hypothesis #3). Therefore, the measurement of phylogenetic diversity (PD) can be an indicator of functional trait diversity (FD) (Forest et al. 2007), even though there is a considerable debate on this topic (Garnier et al. 2016). Indeed, the correlation between PD and FD is not universal, and high PD can generate many assemblages that have a lower FD than randomly chosen sets of species (Mazel et al. 2018).

(3) The role of intraspecific and interspecific trait diversity

Intraspecific trait variability can constitute a relatively large part of overall community-level trait variability (Violle et al. 2012). In terrestrial plants, some traits show non-negligible amounts of intraspecific variability (Albert et al. 2010; Garnier et al. 2001; Kazakou et al. 2014) and among seagrasses, marine flowering plants, it can even replace species-level biodiversity effects (Reusch et al. 2005). Therefore, it is fundamental to determine the relative importance of inter- vs intraspecific variability (Figure 1: Hypothesis #4). Violle et al. (2012) show the importance of including intraspecific variability in order to get a better understanding of the environmental filters acting on the vegetated community in spite of the use of mean trait values per species present in the community. This is a revision of the concepts of alpha and beta niches (Pickett and Bazzaz 1978), which allows for the understanding of the effects of environmental filters on intraspecific variability. For detailed calculations consider Ackerly and Cornwell (2007) and the references in this paragraph.

(4) Ecosystem function provision and effect traits

Effect traits allow to scale up from the functioning of an individual to that of ecosystems (Grime 1998; Chapin et al. 2000; Lavorel and Garnier 2002; Diaz et al. 2007). Two different and non-exclusive hypotheses have been formulated to relate the functional structure of communities to ecosystem properties: dominance (mass-ratio effect) and niche complementarity.

The dominance hypothesis stipulates that the functional traits of the dominant species will be the predominant influence on the ecosystem function (Grime 1998; Smith and Knapp 2003), this being proportional to its abundance in the community (Diaz et al. 2007; Garnier et al. 2004; Violle et al. 2007). The metric used to test this hypothesis is the community weighted mean (CWM). By contrast, the niche complementarity hypothesis stipulates that the presence of functionally different species, which use environmental resources in a complementary manner, will positively influence ecosystem functioning (Diaz et al. 2006; Eviner and Chapin 2003; Loreau and Hector 2001; Petchey and Gaston 2006; Tilman 2001). It is therefore hypothesized that positive relations exist between ecosystem functions and functional diversity (FD) indices. These two hypotheses are not mutually exclusive, and it is possible that both are important in influencing ecosystem functions (Diaz et al. 2007, Figure 1: Hypothesis #5). More evidence has been found, however, for a relation between dominance and function (Garnier et al. 2016). The last drawback in the study of function provision is that some functions may not be correlated with traits under constraining environmental factors, not allowing for the determination of causality between trait and function. Environmental factors should be, therefore, controlled for in a “common garden” or statistically with structured equation models (Grace et al. 2007; Shipley 2010) (Figure 1: Hypothesis #6) to disentangle the links between environment, trait and function.

(5) Ecosystem service provision, vulnerability and service traits

Ecosystem services are defined as the capacity of natural processes and components to provide goods and services that satisfy human needs, directly or indirectly (de Groot et al. 2002). The definition of an ecosystem service is contingent upon human perception and needs, and therefore each ecosystem service has underlying functions that are biologically measurable. The importance of the concept of ecosystem service is the possibility to integrate ecosystem functions in management and policy. Díaz et al. (2013) introduced the concept of ecosystem service vulnerability, based on the idea that the security of ecosystem functions depends on how the effects and tolerances of organisms (which both depend on combinations of functional

traits) correlate across species. Therefore, the correlation of the response and effect traits of organisms can determine the vulnerability of an ecosystem function (Figure 1: Hypothesis #7). The final step in the TBF proposed in this work is the translation of effect traits from ecosystem functions to ecosystem services. Effect traits driving ecosystem service provision are, therefore, a tool to understand the link between organism, function and service, and the vulnerability of the service provision under a changing environment.

III. Applying the TBF to seagrass communities

(1) Seagrass ecosystems: Origin, threats and idiosyncrasy of marine plants

Marine plants, seagrasses, are a polyphyletic assemblage of basal monocotyledonous angiosperms belonging to four families in the Alismatales: Posidoniaceae, Zosteraceae, Cymodoceaceae and Hydrocharitaceae. Limited to coastal areas, they occupy a global surface of about 177 000 km² (<0.2% of the ocean's surface) (conservative calculation by Spalding et al. 2003). Most species and populations feature a mix of sexual reproduction and clonal growth via rhizome extension. The recolonization of marine habitats from terrestrial wetland habitats occurred exclusively from this monocotyledonous order and took place in four independent and parallel evolutionary events (Janssen and Bremer 2004; Les et al. 1997; Waycott et al. 2006). The successful colonization of flowering plants from terrestrial wetlands into the marine environment on an evolutionary time scale was contingent upon a number of critical adaptations, which partially reverted many of the original key adaptations of flowering plants to terrestrial life. These adaptations are reflected in specific genomic losses and gains (Golicz et al. 2015; Lee et al. 2016; Olsen et al. 2016; Lee et al. 2018), with adaptive changes in sets of genes associated with central biological pathways (Wissler et al. 2011). Despite their ecological importance, seagrasses exhibit very low species richness (60-70 species) compared to other groups in the Alismatales, which is possibly partially compensated by pronounced local adaptation (or intraspecific variability) within species (e.g. Jüterbock et al. 2016; Jahnke et al. 2019; Dattolo et al. 2017).

Seagrasses, share a similar morphology with basal meristems that form strap-like leaves grouped in shoots connected by rooted rhizomes in the sediment. Their low morphological diversity is possibly the result of a convergent evolution to the submerged lifestyle in a highly hydrodynamic and saline environment (Arber 1920; Les et al. 1997). Some of the most important challenges of adapting to marine environments are the rapid attenuation of the

irradiance level through the water column, along with alterations in its spectral composition and the reduced CO₂ availability (Beer and Rehnberg 1997; Invers et al. 1999). Unfortunately, the coastal habitat colonized by seagrasses is under high and increasing anthropogenic pressure. As a consequence, seagrasses are under decline worldwide due to multiple local and global pressures (Orth et al. 2006; Waycott et al. 2009). Reversal of this negative trend, however, is possible, as observed in certain cases in the U.S. and Europe (Lefcheck et al. 2018; de los Santos et al. 2019; Sousa et al. 2019), if appropriate management and conservation actions are implemented.

Most seagrass research to date has included measurements of response variables to environmental variation in order to understand basic seagrass biology and ecological functions, to prevent their decline (Fernandes et al. 2019), to restore degraded ecosystems (Paulo et al. 2019), or to predict their fate under future global change scenarios (Hyndes et al. 2016). Synthesis of the existing data on seagrass response to the environment has been used to identify potential indicators for assessing the health of seagrass ecosystems (Roca et al. 2016). In contrast to this body of literature on seagrass response to environmental drivers, quantitative empirical data on ecosystem functions of seagrasses are scarce, even though such information is key to managing seagrass ecosystems sustainably. There is an understanding that the sole presence of seagrass is enough for the provisioning of functions like invertebrate habitat (Virnstein et al. 1983), the modification of the inorganic carbon system (Unsworth et al. 2012) or ability to foster associated species to face environmental changes and enhance response plasticity (Bulleri et al. 2018). The provisioning of these functions, and variation therein related to seagrass bed composition, must be underpinned by the traits of the component species or genotypes, but the link between seagrass traits and functions has been resolved in only a handful of examples (e.g. Fonseca and Callahan 1992; Hendricks et al. 2008; Gustafsson and Boström 2011; Hendricks et al. 2014). At present, we lack a comprehensive picture and predictive framework of how key seagrass traits underpin the resistance and resilience of seagrass species to current and future pressures, and their relation to ecosystem functions and services.

In order to push seagrass research forward, we used seagrass communities as an example for the application of the TBF proposed in this manuscript. We carried out a systematic review of the seagrass literature with the goal of quantifying the use of TBFs in the assessment of seagrass responses, ecosystem functions and services and to identify the gaps of knowledge in this field, including (1) how frequently trait-based research has been adopted in seagrass ecological research and how many of these studies could be classified as TBFs (as defined by the seminal

work from Lavorel and Garnier 2002), (2) which of the theories and hypotheses introduced in our TBF have been already studied in seagrass communities in relation to their traits (as presented in Figure 1) and (3) identify under- and over-studied traits, drivers and functions in seagrass research, with examples from the literature. The conceptualization of the results of the literature review under the TBF proposed in this manuscript will allow the exploration of the aforementioned research gaps and indicate future research pathways in seagrass ecology, specifically focusing on the ecosystem function and service provision and vulnerability.

(2) Literature review of seagrass trait research

We followed the ROSES protocol (Haddaway et al. 2018) for a literature review (metadata of the review can be found in the Supplementary Material 1). We identified 18185 publications of potential relevance within the Google Scholar database using the query “Seagrass trait” and “*Seagrass species* trait” (“*Seagrass species*” being the names of seagrass species accepted in theplantlist.org). To guarantee that the focus of the publication was in the study of trait-based research, the word trait had to be present in the title, abstract and/or keywords of the publication, otherwise the publication was not included in the review process. The number of publications was limited to those in which at least title, abstract and keywords were in English. The temporal range of the sample was restricted to the limitations of the database itself. Publications included the range from 1988 through August 2019. Using the above screening criteria, the initial number of publications was reduced to 325. From these 325 publications, 137 were discarded as they referred to the study of associated seagrass fauna, benthic macroalgae within seagrass meadows and seagrass epiphytes; 12 duplicates and 19 misclassified publications were also discarded. The final database was sized down to 157 relevant publications.

For goal (1), we counted the number of studies including the word “trait” and the number of studies in which an existing TBF (as defined by Lavorel and Garnier 2002) was used for the testing of a hypothesis or research question. For goal (2), we categorized the studies in *a priori* categories derived from the conceptual TBF presented in section II (Supplementary material 2: Table 1), and extract examples for the literature to illustrate their use. Lastly, for goal (3), we created an *a priori* classification of seagrass traits (Supplementary material 2: Table 2) and a *posteriori* classification with the environmental drivers and ecosystems functions found in the literature (Supplementary material 2: Table 3). Additionally, we counted the connections between the defined categories as the number of times that the categories were studied together, i.e. number of entries in the review database. The goal of the count was to highlight which

categories were studied in connection with others and which ones were studied in isolation. We made the final figures using the software InkScape (v 0.92).

IV. TBF in seagrass communities: knowledge gaps, challenges and opportunities

(1) Seagrass TBF studies and studies including the word trait

Trait-based response-effect frameworks, TBFs, are currently underexplored in seagrass research. The number of studies including the word “trait” when studying seagrasses increased steadily since the first study from the year 1988, reaching a maximum in 2018 (n=32). However, we only found 13 studies in the database that used existing TBFs, accounting for only 7% of the total. The “trait-based approach” was first developed in 2002 (Lavorel and Garnier 2002) for terrestrial plants, and it does not appear in the seagrass literature until 2012. This indicates that the body of knowledge available from terrestrial plant ecology has been under-utilized by seagrass researchers, probably due to the lack of awareness of the methods and concepts that have already been developed in other research fields. However, we can re-evaluate and analyze existing data collected from classical approaches and identify gaps to be filled through future research activities.

A bias in the number of studies exists towards the widespread species *Zostera marina*, which accounts for 61% of the TBF studies in the seagrass literature, whereas for the rest of the seagrass species the number decreased to two (*Halophila stipulacea*) and one publications (*Cymodocea serrulata*, *Halophila ovalis*, *Thalassia hemprichii*, *Cymodocea rotundata*, *Syringodium isoetifolium*, *Posidonia australis*, *Halodule uninervis*, *Thalassodendron ciliatum*, *Ruppia cirrhosa*). This bias highlights the problem of inference from a unique species to the others, and the necessity to investigate other seagrass species under TBFs.

(2) Community assembly processes in seagrass communities

(a) Dispersal and settlement in seagrass communities: Challenging the neutral assembly theory

We found three studies that linked seagrass dispersal and settlement to traits (1.9% of the total, Figure 2), which suggests that dispersal can be predicted by the traits of the seagrass (Figure 3). First, the life stage of the seagrass plant (seed or vegetative fragment) determines which traits to study (Orth et al. 2007; van Dijk et al. 2014; Bryan-Brown et al. 2017). In the case of seeds, traits including their flotation capacity and digestibility determine the distance they can

disperse and the effectiveness of using animal vectors respectively (Wu et al. 2016). In the case of vegetative fragments, the plant morphology can partially control the dislodgement resistance of plant fragments, whereas the age and rooting rate determine their capacity for settlement (Lai et al. 2018).

The identification of traits controlling dispersal and settlement challenges the perception of dispersal as a stochastic and unpredictable process. However, this hypothesis (Figure 1: Hypothesis #1) has not been formally tested in seagrass, while it has been successfully tested in fish communities (Ford and Roberts 2018), which assemble neutrally at the regional scale but not at the local scale, and woody plants, whose dispersal and settlement are primarily trait-driven (Duarte et al. 2010). The relative importance of dispersal and settlement traits, together with response traits under the abiotic and biotic filters, is a research pathway currently underexplored in seagrass research.

(b)The abiotic and biotic filters in seagrass communities

Much research has been performed on the responses of seagrass traits to environmental drivers, making a total of 72% of the studies found in the literature review. There was, however, a large imbalance between the study of seagrass traits under abiotic (81%) vs biotic (19%) factors (Figure 2). The three most frequent abiotic drivers studied were nutrients (25%), temperature (23%) and light (21%), whereas the least studied include grain size or substrate type (3.5% each). The traits used to assess the effect of the abiotic filter are countless and specific to the environmental driver, and it is out of the scope of this review to discuss each of them (Figure 3). However, the prevalence of the use of morphological traits (60%) among all other trait categories is worth mentioning. This is likely explained by their relatively easy and inexpensive measurement compared to physiological measurements of e.g. photosynthetic efficiency (Hernán et al. 2016; Llagostera et al. 2016) or enzymatic activity (Alexandre et al. 2004, 2010), which require specialized equipment, technical staff and laboratories. There are, therefore, extrinsic economical and technical reasons that constrain scientific questions in seagrass research. This trait type imbalance may impede a deeper understanding of responses of seagrasses to abiotic drivers, as physiological and biochemical indicators are recommended over morphological ones for early stress detection in seagrasses (Roca et al. 2016).

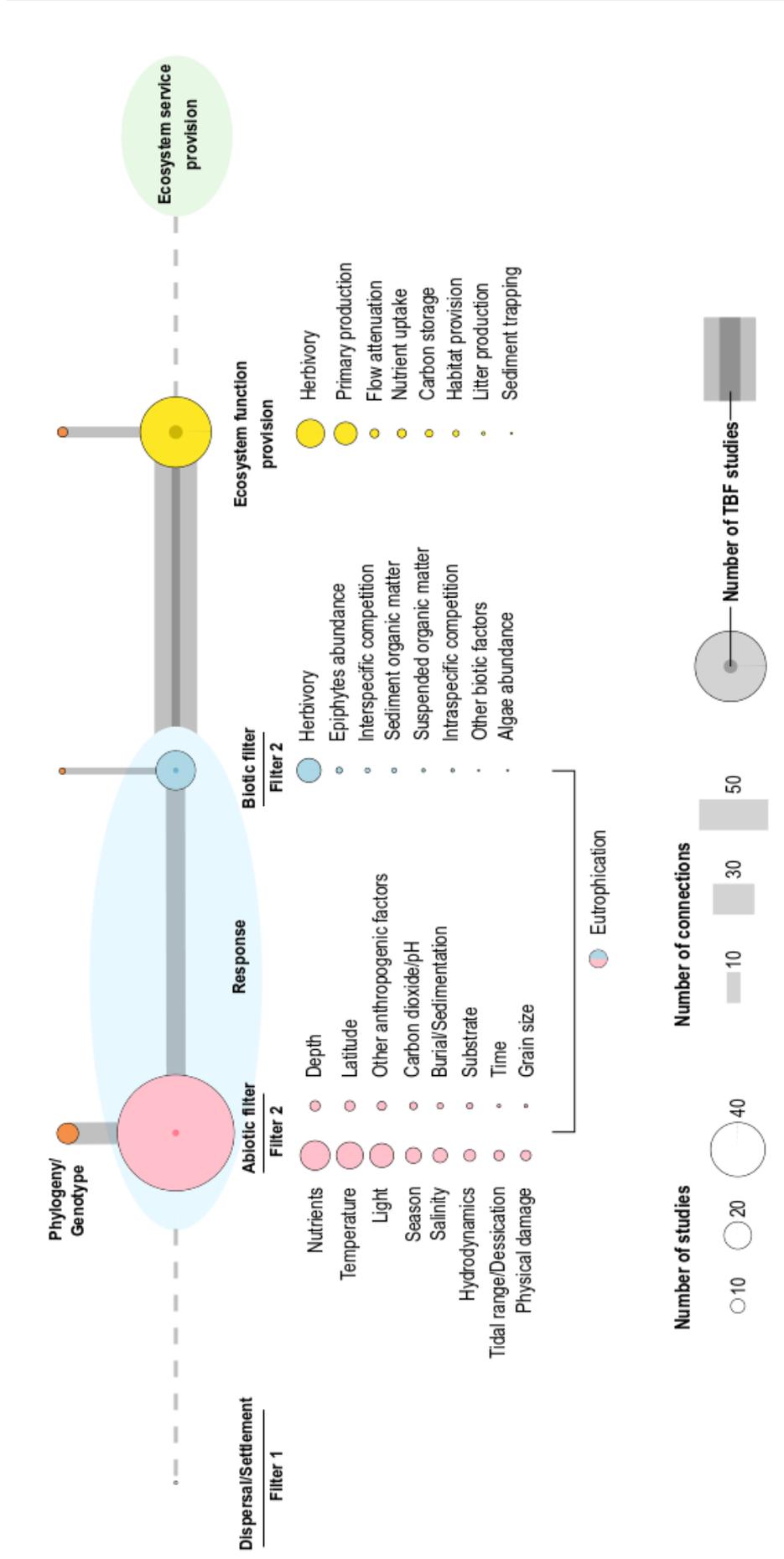


Figure 2. Results of the systematic literature review adapted to the conceptual TBF proposed by this manuscript.

The study of morphological (41%), biochemical (38%) and growth traits (28%) under the biotic filter was more balanced compared to the abiotic filter. However, most of the traits were uniquely studied in the herbivory context (62%), showing that seagrass plants respond to this driver using a comprehensive array of traits, including growth compensation, changes in their nitrogen content or mobilization of carbohydrates (Sanmartí et al. 2014).

One of the main questions posed in the conceptual TBF is the relative importance of the abiotic vs the biotic filters (Figures 1: Hypothesis 2) to better understand the fundamental and realized niches of species. We found, however, that only 13% of the studies included both biotic and abiotic factors simultaneously, the most representative example being eutrophication (12% of response studies). Eutrophication is one of the main threats to seagrasses, as it can lead to a phase shift in primary producers from seagrass to macroalgal dominance (Duarte 1995; Orth et al. 2006; Burkholder et al. 2007; Waycott et al. 2009). Eutrophication affects different compartments of the ecosystem (seagrass, micro- and macroalgae, epiphytic organisms), changing their relative abundances and causing changes in the light penetration or the redox potential in the sediment. The disentanglement of the relative effects of the two filters becomes, therefore, complicated. Trait-based approaches suggest the study of the convergence and divergence of trait distributions to unravel the relative effects of the abiotic and biotic filters.

However, the available trait-based studies have tended to assess the fundamental and realized niches of seagrass species separately. For example, the vertical zonation of tropical seagrasses was explained by physiological traits controlling their ability to tolerate high irradiances and nutrient inputs (Björk et al. 1999). Other examples focus on the assessment of the fundamental niche of individual species. *Halophila decipiens* can, due to its phenotypic plasticity, occupy a wide range of irradiances and temperatures (Gorman et al. 2016). *Zostera marina* has a low niche specialization in the Baltic Sea, allowing this species to exist under variable environmental conditions in comparison to other macrophytes (Herkül et al. 2018). In regard to the realized niche of seagrasses, it has been shown that primary producer traits, specifically their morphology and growth form, predict grazing impacts on a global scale (Poore et al. 2012).

The large body of literature on response traits under abiotic and biotic factors shows that there is a wealth of data that can be reassessed to answer questions in the context of the TBF presented in this manuscript. The disentanglement of fundamental and realized niches under a TBF is currently unexplored in seagrasses, providing an opportunity to answer fundamental research questions under global change that includes both abiotic and biotic drivers.

(3) Phylogenetic and genotypic control of seagrass traits

The study of the phylogenetic and genotypic control of traits is quite prevalent in the seagrass literature (23% of studies). Specifically, 18% of response trait studies and 15% of effect traits studies (Figure 2) include phylogenetic metrics.

There is ample evidence that genotypic richness covaries with phenotypic variation in functionally relevant traits, such as leaf morphology and shoot productivity within *Posidonia australis* (Evans et al. 2016). In contrast, genetic diversity is a poor proxy for trait differentiation in *Zostera marina* (Abbott et al. 2018). In *Posidonia oceanica* a reproductive trait like flower abundance was negatively correlated to genotypic diversity and positively correlated to heterozygosity (Jahnke et al. 2015a), while there was a correlation of genetic indices and its response to environmental conditions (Jahnke et al. 2015b). Ecosystem functions like the accumulation of biomass and susceptibility to herbivory are also genotypically controlled in *Z. marina* (Tomas et al. 2011), with genotypes differing in key traits related to these processes. Similarly, nutrient uptake rates differed among genotypes of *Z. marina* (Hughes et al. 2009).

These studies confirm that there is genetic control of seagrass traits (Figure 1, 2: Hypothesis #3) and, as a consequence, of ecosystem functions and services (Diaz et al. 2013). However, this control has been shown to be species specific. Knowledge gaps in this area lay in the lack of information from most of the seagrass species, as the genus *Zostera* and *Posidonia* accumulate 67% of the studies.

(4) Intraspecific trait variability in seagrasses is key to their survival

In seagrass ecosystems, characterized by low plant species richness, intraspecific variation is likely to play a more important role in response and community assembly than in terrestrial ecosystems. In comparison to terrestrial plant lineages, the taxonomic diversity of seagrass is low, with all species belonging to four Alismatales families. Indeed, many temperate meadows are monospecific, and most tropical meadows consist of only a handful of co-occurring species (Short et al., 2007).

Several studies (9% of studies in this review) have indicated that the intraspecific trait variability of seagrass species is key for their survival. For example, the plant size of *Z. marina* (one of the predominant seagrasses in the northern hemisphere) spans more than two orders of magnitude across its distribution range (Ruesink 2018), while different genotypes also show large differences in nutrient uptake capacity and key photosynthetic parameters when grown in

a “common garden” (Hughes et al. 2009). Even putatively less plastic species such as *P. oceanica* display a large variation in acclimation to environmental factors (e.g. heat, Marín-Guirao et al. 2018). This feature, potentially supported by high intra-specific and intra-clonal (epi-)genetic diversity, enables seagrasses to cope with major environmental changes (Maxwell et al. 2014) and has most likely contributed to their successful colonization of shallow coastal zones along five continents, despite their low taxonomic diversity.

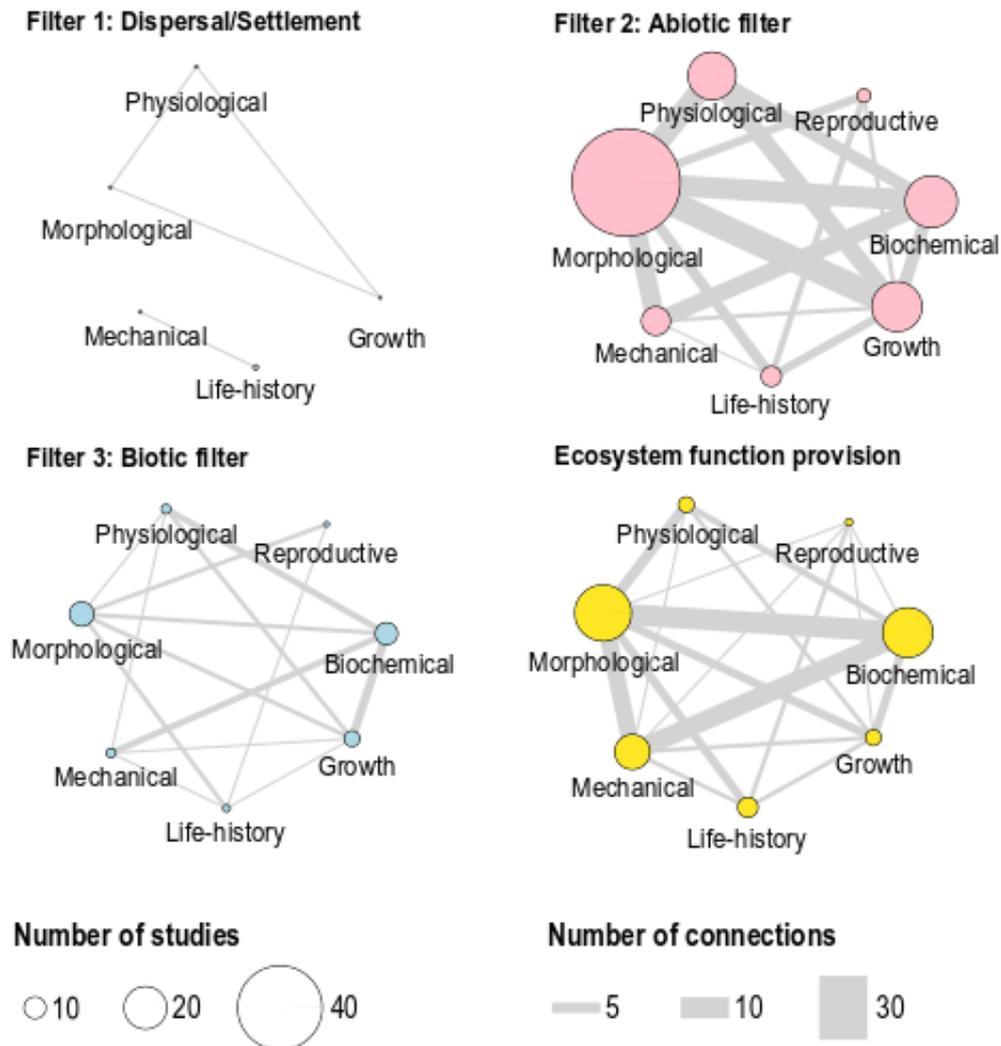


Figure 3. Trait categories encountered in the literature review classified by the a) Traits related to dispersal, b) Trait studied under the abiotic filter, c) Traits studied under the biotic filter and d) Traits studied in connection to ecosystem functions.

Additionally, intraspecific variability in traits does not only occur at the species level, but also at the shoot, rhizome and clone levels. Epigenetic differences are even present within the same rhizome, and has been shown to foster clonal persistence (Ruocco et al. 2020), within the same shoot, and between different portions of the same leaf (Ruocco et al., 2019 a, b). Recent

evidence even points out that within single clones, somatic mutations lead to differentiation of ramets (= clone mates), with the potential to result in phenotypic differences within clones (Yu et al. 2020).

This body of literature highlights the importance of intraspecific trait variability in the response of seagrasses to disturbances, their resilience and capacity for ecosystem functions provision. However, we found only one example of the simultaneous study of intra- and interspecific variability of structural and nutritional traits, which drive palatability and herbivory in seagrasses (Jiménez-Ramos et al. 2018). Future research assessing the relative importance of inter- vs intraspecific variability in both response and effect traits (Figure 1: Hypothesis #4) will be necessary to understand whether intraspecific diversity plays a role as important as interspecific diversity in seagrass ecosystems.

(5) Effect traits linked to seagrass ecosystem function provision: challenges in the understanding of complementarity, dominance and environmental control of ecosystem functions

The links between effect traits and ecosystem functions were tested in 33% of the studies (Figure 2), of which only 13% included multiple functions simultaneously. Herbivory (40%) and primary production (32%) were the most studied functions. Morphological (52%) and biochemical (46%) traits were the most used in the assessment of functions (Figure 3). Examples include wave attenuation, which is explained by a combination of morphological and mechanical traits including blade stiffness, shoot density and leaf length (Bouma et al. 2005; Paul et al. 2012) or herbivory of *Z. noltei*, which is mediated by both structural and nutritional leaf traits (Martínez-Crego et al. 2016). Examples of more nuanced, indirect interactions between traits and functions include the reduction of the canopy height in *Posidonia oceanica* by grazing, thereby increasing the predation risk on associated sea urchins (Pagès et al. 2012). There are, therefore, clear mechanistic links between seagrass effect traits and ecosystem functions. However, at the community level, there is the question of whether effect traits control ecosystem functions through dominance (CWM) or complementarity (FD) (Figure 1: Hypothesis #5).

The hypotheses of the control of ecosystem functions by functional complementarity (FD) versus dominance have been barely tested in seagrass ecosystems, with examples unique for *Z. marina*. Primary production is influenced by genotypic and FD of *Z. marina* at the plot level (Abbott et al. 2017). Additionally, trait diversity was positively correlated with abundance of

invertebrate grazers (Abbott et al. 2017). There is also evidence of intraspecific niche complementarity in the partitioned nutrient uptake of genotypes of *Z. marina* (Hughes et al. 2009). Regarding the dominance hypothesis, CWM has also been found as a reliable predictor of primary production in plant communities, including the aforementioned *Z. marina* (Gustafsson and Norkko 2016). Dominance and complementarity hypotheses have been tested simultaneously in one study in *Z. marina* (Hughes and Stachowicz 2011). Biomass production was higher in polycultures (i.e. higher complementarity) at high disturbance levels, whereas under no disturbance, monocultures (i.e. dominance) outperformed polycultures. Additionally, polycultures outperformed monocultures in shoot and biomass production under a macroalgal bloom. The results of this study display the complexity in the trait complementarity and dominance on ecosystem functions, and their differential importance under a changing environment.

The link between traits and ecosystem functions can be environmentally constrained (Figure 1: Hypothesis #6), however, only 59% of the studies on ecosystem functions included environmental metrics. The literature indicates that the relative control of environmental drivers and traits is function-dependent. Functions like organic carbon storage can also be independent of seagrass functional traits under constraining geophysical conditions (Belshe et al. 2018), while nutrient uptake seems to be directly affected by traits like root length (Angove et al. 2018), and primary production can be predicted by functional traits regardless of the environmental conditions (Jänes et al. 2017).

In summary, in order to test the dominance and complementarity hypotheses, it is of fundamental importance to find effect traits with proven mechanistic relationships with ecosystem functions. Additionally, these relationships may be environmentally controlled, and it is therefore necessary to add relevant environmental metrics in the study of ecosystem functions (van der Plas et al. 2020). Lastly, the literature review shows that these hypotheses have been nearly uniquely tested on *Z. marina*. The study of other seagrass species is thus needed for the comprehensive understanding of the ecology of effect traits and ecosystem function provision.

(6) Vulnerability of seagrass ecosystem function and service provision under global change

The rate of seagrass loss worldwide and the numerous threats to seagrass ecosystems (Orth et al 2006; Waycott et al. 2009) call for the assessment of the vulnerability of the ecosystem

functions provided by seagrasses. Therefore, it is necessary to study the correlation between response and effect in seagrass ecosystems (Figure 1: Hypothesis #7).

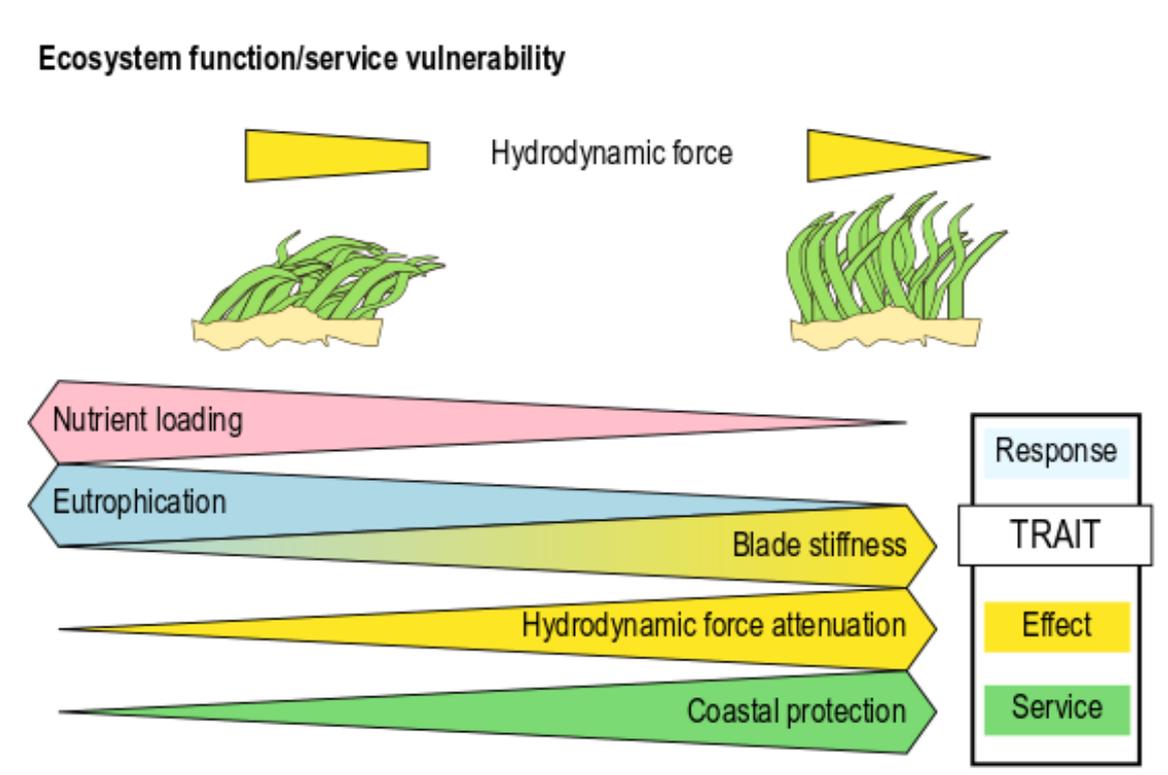


Figure 4. Example of the assessment of ecosystem function and service vulnerability by the use of a trait, i.e. blade stiffness, that is simultaneously response, effect and service. The concept for this figure is based on the studies of Soissons et al. (2018) for the response part, and Bouma et al. (2005), Peralta et al. (2008) and Paul et al. (2012) for the effect part.

As stated in previous sections, the study of the response of traits to environmental drivers is common, stating the adaptive effects that traits undergo due to changes in e.g. temperature or light (Mota et al. 2018; Tanaka and Nakaoka 2006). Traits are sensitive indicators of plant stress under environmental change (Roca et al. 2016). When it comes to ecosystem functions, vulnerability of functions is discussed in terms of seagrass loss, i.e. the loss of the seagrass meadows would mean the end of ecosystem function provision (Trevathan-Tackett et al. 2018). However, one important missing link is the identification of traits that drive both response and effect. The rationale is that, despite the presence of seagrasses, a change in their trait values or the replacement by another species or taxa with different traits would alter ecosystem function provision. This hypothesis has not been explicitly tested in seagrass ecosystems (Figure 1: Hypothesis #7).

To illustrate this concept, we build an example around seagrass mechanical traits, specifically blade stiffness. Blade stiffness changes with the latitude and season, being lower in northern

European latitudes and particularly at the end of the growing season (Soissons et al. 2018). Additionally, blade stiffness tends to weaken in eutrophic conditions compared to oligotrophic ones. It is, therefore, a response trait that can be used as indicator of eutrophication. Several studies, however, have shown that blade stiffness (among other morphometric traits) is also directly responsible for the capacity of seagrasses and halophytes to attenuate hydrodynamic forces, including waves and currents (Bouma et al. 2005; Peralta et al. 2008; Paul et al. 2012), making it an effect trait. Combining these studies, reveals that an increase in nutrient input in the coastline leading to eutrophied conditions can cause lower hydrodynamic force attenuation. The loss of the ecosystem function can, therefore, happen before the loss of seagrass vegetation if the trait values change. This example highlights how the correlation of response and effect trait can be assessed to study the vulnerability of an ecosystem function (Figure 4, Suding et al. 2008; Diaz et al. 2013).

Lastly, the concept of ecosystem service has gained increasing importance in the last two decades, as a tool to couple science and environmental policy-making (Constanza et al. 1997, 2017). The identification of effect traits responsible for the provision of ecosystem services is of fundamental importance in order to have a taxon-independent metric that could be incorporated into policy making. For seagrass meadows, Ruiz-Frau et al. (2017) classified ecosystem functions performed by seagrass in ecosystem services based on the TEEB - The Economics of Ecosystems and Biodiversity categorization created by Brink et al. (2009). For example, fisheries are classified as food provision or carbon storage classified as gas and climate regulation. Using the same example as for the assessment of the vulnerability of the ecosystem function, the same trait, blade stiffness, can be used as the effect trait to assess the vulnerability of the ecosystem service provision by seagrasses, in this case, coastal protection (Figure 4).

With the knowledge of which functions underlie each ecosystem service, and the possibility to assess ecosystem function and service vulnerability, we can have a holistic view of seagrass response, function provision and service provision in a changing environment with the use of simple trait metrics.

V. Conclusions

TBFs (trait-based response-effect frameworks) have proven to be a powerful tool to address ecological questions in all fields of study, both terrestrial and marine. The synthesis of a novel TBF comprehensively including previous ideas allows for a holistic view of traits, from their

role in response to environmental drivers to ecosystem service provision. The proposal to apply a TBF to seagrass ecology acknowledges the importance of considering the scientific advances of other research fields in order to push marine research forward. The application of a TBF to seagrasses appears as a powerful avenue to unveil new insights on the functioning of ecosystems, due to their special evolutionary history and narrow phylogenetic origin. Our literature review demonstrated that there is a wealth of data on seagrass response and effect traits and on seagrass ecosystem functions. There is, therefore, a great potential to re-analyze these data under a TBF lens in order to test new research questions and hypotheses; and to collect new data identified as critical but missing by this TBF approach.

The review of the seagrass literature has demonstrated that most of the hypotheses proposed in the TBF have not yet been formally tested. There is much evidence that stochastic processes (Hypothesis #1) have a lower relative importance than niche based processes, both in the dispersal and vegetative stages of community assembly (Hypothesis #2). Additionally, traits are under a certain level of genotypic control (Hypothesis #3), specifically within species. Intraspecific diversity seems to be important in seagrasses (Hypothesis #4), as there is a level of complementarity for ecosystem function provision (Hypothesis #5). However, there is evidence that both dominance and complementarity are responsible for function provision, and this may be environmentally controlled (Hypothesis #6). Despite the positive signs of seagrass recovery in Europe, we cannot ignore the fact that the world is experiencing fast and unprecedented changes. The use of a TBF that assesses the vulnerability of ecosystem function and service provision (Hypothesis #7) can help to understand which ecosystem services may be compromised by the changes in species traits or species abundances. Therefore, the translation of biological and ecological seagrass research into a framework explicitly considering ecosystem services will prove fundamental for the development of comprehensive policies and for the informed management of seagrass meadows.

In an era in which global open data storage and sharing is becoming a central part of research, there is real need for a seagrass trait database. Currently, a seagrass trait database is being developed at the Centro de Ciências do Mar (CCMAR, Portugal) in collaboration with the Portuguese national bioinformatics research infrastructure (<http://biodata.pt/Elixir.pt>). This tool will prove fundamental for the development of holistic and global research on seagrasses and a great opportunity for the application of the proposed TBF while also providing important input to global plant trait databases such as TRY (Kattge et al. 2020). We believe that the inclusion of the TBF presented in this manuscript in seagrass research will aid the assessment of

ecosystem services provided and, as a consequence, will improve the awareness of humankind on the importance of seagrass meadows worldwide. Moreover, we believe that our novel and integrative adaptations to the trait-based response-effect framework can help to better understand the relationships between traits, functioning and services of other marine, freshwater or terrestrial ecosystems.

VI. Acknowledgments

This review and conceptual TBF was initiated as part of the Euromarine workshop "TRAITGRASS" led by Gabriele Procaccini and Rui Santos. A. Moreira-Saporiti was funded by the Leibniz Association as part of the project SEATRAIT and M. Teichberg was supported by the German Research Foundation (DFG) within the individual Grants Program, project SEAMAC (TE 1046/3-1). AI Sousa was funded by Portuguese national funds through the FCT - Foundation for Science and Technology, I.P., under the project CEECIND/00962/2017 and by FCT/MCTES through the financial support to CESAM (UIDB/50017/2020+UIDP/50017/2020). I Olivé was funded by the EU-H2020-MSCA grant n° 752250 (SEAMET). C. Boström was funded by the Åbo Akademi University Foundation Sr and L. Meysick was funded by the doctoral network Functional Marine Biodiversity (FunMarBio) at Åbo Akademi University.

VII. References

- Abbott, J. M., Grosberg, R. K., Williams, S. L., and Stachowicz, J. J. (2017). Multiple dimensions of intraspecific diversity affect biomass of eelgrass and its associated community. *Ecology*, 98(12), 3152-3164. <https://doi.org/10.1002/ecy.2037>
- Abbott, J. M., DuBois, K., Grosberg, R. K., Williams, S. L., and Stachowicz, J. J. (2018). Genetic distance predicts trait differentiation at the subpopulation but not the individual level in eelgrass, *Zostera marina*. *Ecology and evolution*, 8 (15), 7476 - 7489. <https://doi.org/10.1002/ece3.4260>
- Ackerly, D. D., and Cornwell, W. K. (2007). A trait-based approach to community assembly: partitioning of species trait values into within-and among-community components. *Ecology letters*, 10(2), 135-145. <https://doi.org/10.1111/j.1461-0248.2006.01006.x>
- Albert, C. H., Thuiller, W., Yoccoz, N. G., Soudant, A., Boucher, F., Saccone, P., and Lavorel, S. (2010). Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology*, 98(3), 604-613. <https://doi.org/10.1111/j.1365-2745.2010.01651.x>
- Alexandre, A., Silva, J., and Santos, R. (2004). The maximum nitrate reductase activity of the seagrass *Zostera noltii* (Hornem.) varies along its vertical distribution. *Journal of Experimental Marine Biology and Ecology*, 307(1), 127-135. <https://doi.org/10.1016/j.jembe.2004.02.002>

- Alexandre, A., Silva, J., and Santos, R. (2010). Inorganic nitrogen uptake and related enzymatic activity in the seagrass *Zostera noltii*. *Marine Ecology*, 31 (4), 539 - 545. <https://doi.org/10.1111/j.1439-0485.2010.00378.x>
- Andersen, K. H., and Pedersen, M. (2009). Damped trophic cascades driven by fishing in model marine ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 277(1682), 795-802. <https://doi.org/10.1098/rspb.2009.1512>
- Angove, C., Norkko, A., and Gustafsson, C. (2018). Assessing the efficiencies and challenges for nutrient uptake by aquatic plants. *Journal of Experimental Marine Biology and Ecology*, 507, 23-30. <https://doi.org/10.1016/j.jembe.2018.07.005>
- Arber, M. A. (1920). *Water-plants, a study of aquatic angiosperms*. Cambridge University. <https://doi.org/10.5962/bhl.title.17150>
- Belshe, E. F., Hoeijmakers, D., Herran, N., Mtolera, M., and Teichberg, M. (2018). Seagrass community-level controls over organic carbon storage are constrained by geophysical attributes within meadows of Zanzibar, Tanzania. *Biogeosciences*, 15(14), 4609-4626. <https://doi.org/10.5194/bg-15-4609-2018>
- Belyea, L. R., and Lancaster, J. (1999). Assembly rules within a contingent ecology. *Oikos*, 402-416. <https://doi.org/10.2307/3546646>
- Beer, S., and Rehnberg, J. (1997). The acquisition of inorganic carbon by the seagrass *Zostera marina*. *Aquatic Botany*, 56(3-4), 277-283. [https://doi.org/10.1016/s0304-3770\(96\)01109-6](https://doi.org/10.1016/s0304-3770(96)01109-6)
- Bernard-Verdier, M., Navas, M. L., Vellend, M., Violle, C., Fayolle, A., and Garnier, E. (2012). Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. *Journal of Ecology*, 100(6), 1422-1433. <https://doi.org/10.1111/1365-2745.12003>
- Björk, M., Uku, J., Weil, A., and Beer, S. (1999). Photosynthetic tolerances to desiccation of tropical intertidal seagrasses. *Marine Ecology Progress Series*, 191, 121-126. <https://doi.org/10.3354/meps191121>
- Bouma, T. J., De Vries, M. B., Low, E., Peralta, G., Táncoz, I. V., van de Koppel, J., and Herman, P. M. J. (2005). Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. *Ecology*, 86(8), 2187-2199. <https://doi.org/10.1890/04-1588>
- Brink, P., Berghöfer, A., Schröter-Schlaack, C., Sukhdev, P., Vakrou, A., White, S., and Wittmer, H. (2009). TEEB-The Economics of Ecosystems and Biodiversity for National and International Policy Makers 2009. *TEEB - The Economics of Ecosystems and Biodiversity for National and International Policy Makers 2009*. <https://doi.org/10.1093/acprof:oso/9780199676880.003.0007>
- Bryan-Brown, D. N., Brown, C. J., Hughes, J. M., and Connolly, R. M. (2017). Patterns and trends in marine population connectivity research. *Marine Ecology Progress Series*, 585, 243-256. <https://doi.org/10.3354/meps12418>

- Bulleri F., Eriksson B.K., Queirós A., Airoidi L., Arenas F., Arvanitidis K., Bouma T.J., Crowe T.P., Davoult D., Guizien K., Iveša L., Jenkins S.R, Michalet R., Olabarria C., Procaccini G., Serrão E.A., Wahl M., Benedetti-Cecchi L. (2018). Harnessing positive species interactions as a tool against climate-driven loss of coastal biodiversity. *Plos Biology* 16(9): e2006852. <https://doi.org/10.1371/journal.pbio.2006852>
- Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, 350 (1-2), 46-72. <https://doi.org/10.1016/j.jembe.2007.06.024>
- Chapin, F.S., III, Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. and Díaz, S. (2000) Consequences of changing biodiversity. *Nature*, 405, 234-242. <https://doi.org/10.1038/35012241>
- Cornwell, W. K., and Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs*, 79(1), 109-126. <https://doi.org/10.1890/07-1134.1>
- Cornwell, W. K., Cornelissen, J. H., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., ... and Westoby, M. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology letters*, 11(10), 1065-1071. <https://doi.org/10.1111/j.1461-0248.2008.01219.x>
- Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260. <https://doi.org/10.1038/387253a0>
- Costanza, R., de Groot, R., Braat, L., Kubiszewski, I., Fioramonti, L., Sutton, P., Farber, S., Grasso, M. (2017) Twenty years of ecosystem services: how far have we come and how far do we still need to go? *Ecosystem Services* 28, 1–16. <https://doi.org/10.1016/j.ecoser.2017.09.008>
- Dattolo, E., Marín-Guirao, L., Ruiz, J. M., and Procaccini, G. (2017). Long-term acclimation to reciprocal light conditions suggests depth-related selection in the marine foundation species *Posidonia oceanica*. *Ecology and Evolution*, 7(4), 1148-1164. <https://doi.org/10.1002/ece3.2731>
- de Groot R. S., Wilson M. A., Boumans R. M. (2002) A typology for the classification, description and valuation of ecosystems functions, goods and services. *Ecological Economics* 41, 393–408. [https://doi.org/10.1016/s0921-8009\(02\)00089-7](https://doi.org/10.1016/s0921-8009(02)00089-7)
- de los Santos, C.B., Krause-Jensen, D., Alcoverro, T., Marbà, N., Duarte, C. M., Van Katwijk, M. M., ... and Jankowska, E. (2019). Recent trend reversal for declining European seagrass meadows. *Nature communications*, 10(1), 1-8. <https://doi.org/10.1038/s41467-019-11340-4>
- Díaz, S., Cabido, M., and Casanoves, F. (1998). Plant functional traits and environmental filters at a regional scale. *Journal of vegetation science*, 9 (1), 113 - 122. <https://doi.org/10.2307/3237229>

- Díaz, S., Fargione, J., Chapin III, F. S., and Tilman, D. (2006). Biodiversity loss threatens human well-being. *PLoS Biol*, 4(8), e277. <https://doi.org/10.1371/journal.pbio.0040277>
- Díaz, S., Lavorel, S., de Bello, F., Quétier, F., Grigulis, K. and Robson, M. (2007) Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 20684-20689. <https://doi.org/10.1073/pnas.0704716104>
- Díaz, S., Purvis, A., Cornelissen, J. H. C., Mace, G. M., Donoghue, M. J., Ewers, R. M., ... and Pearse, W. D. (2013). Functional traits, the phylogeny of function, and ecosystem service vulnerability. *Ecology and evolution*, 3(9), 2958-2975. <https://doi.org/10.1002/ece3.601>
- Duarte, C. M. (1995). Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, 41(1), 87-112. <https://doi.org/10.1080/00785236.1995.10422039>
- Duarte, L. D. S., Hofmann, G. S., Dos Santos, M. M., Hartz, S. M., and Pillar, V. D. (2010). Testing for the influence of niche and neutral factors on sapling community assembly beneath isolated woody plants in grasslands. *Journal of Vegetation Science*, 21(3), 462-471. <https://doi.org/10.1111/j.1654-1103.2009.01153.x>
- Edwards, K. F., Litchman, E., and Klausmeier, C. A. (2013). Functional traits explain phytoplankton community structure and seasonal dynamics in a marine ecosystem. *Ecology letters*, 16(1), 56-63. <https://doi.org/10.1111/ele.12012>
- Elleouet, J., Albouy, C., Ben Rais Lasram, F., Mouillot, D., and Leprieux, F. (2014). A trait-based approach for assessing and mapping niche overlap between native and exotic species: the Mediterranean coastal fish fauna as a case study. *Diversity and Distributions*, 20(11), 1333-1344. <https://doi.org/10.1111/ddi.12235>
- Evans, S. M., Sinclair, E. A., Poore, A. G., Bain, K. F., and Vergés, A. (2016). Genotypic richness predicts phenotypic variation in an endangered clonal plant. *PeerJ*, 4, e1633. <https://doi.org/10.7717/peerj.1633>
- Eviner, V. T., and Chapin III, F. S. (2003). Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), 455-485. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132342>
- Fernandes, M. B., van Gils, J., Erftemeijer, P. L., Daly, R., Gonzalez, D., and Rouse, K. (2019). A novel approach to determining dynamic nitrogen thresholds for seagrass conservation. *Journal of applied ecology*, 56(1), 253-261. <https://doi.org/10.1111/1365-2664.13252>
- Follows, M. J., Dutkiewicz, S., Grant, S., and Chisholm, S. W. (2007). Emergent biogeography of microbial communities in a model ocean. *Science*, 315(5820), 1843-1846. <https://doi.org/10.1126/science.1138544>
- Fonseca, M. S., and Callahan, J. A. (1992). A preliminary evaluation of wave attenuation for four species of seagrasses. *Estuarine Coastal Shelf Science*, 35. [https://doi.org/10.1016/s0272-7714\(05\)80039-3](https://doi.org/10.1016/s0272-7714(05)80039-3)

- Ford, B. M., and Roberts, J. D. (2018). Latitudinal gradients of dispersal and niche processes mediating neutral assembly of marine fish communities. *Marine Biology*, 165(5), 1-13. <https://doi.org/10.1007/s00227-018-3356-5>
- Forest, F., Grenyer, R., Rouget, M., Davies, T. J., Cowling, R. M., Faith, D. P., ... and Reeves, G. (2007). Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*, 445(7129), 757-760. <https://doi.org/10.1038/nature05587>
- Gardarin, A., Garnier, É., Carrère, P., Cruz, P., Andueza, D., Bonis, A., ... and Kazakou, E. (2014). Plant trait–digestibility relationships across management and climate gradients in permanent grasslands. *Journal of Applied Ecology*, 51(5), 1207-1217. <https://doi.org/10.1111/1365-2664.12293>
- Garnier, E., Laurent, G., Bellmann, A., Debain, S., Berthelie, P., Ducout, B., ... and Navas, M. L. (2001). Consistency of species ranking based on functional leaf traits. *New phytologist*, 152(1), 69-83. <https://doi.org/10.1046/j.0028-646x.2001.00239.x>
- Garnier, E., Cortez, J., Billès, G., Navas, M. L., Roumet, C., Debussche, M., ... and Neill, C. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, 85(9), 2630-2637. <https://doi.org/10.1890/03-0799>
- Garnier, E., Navas, M.-L. and Grigulis, K. (2016) *Plant Functional Diversity - Organism Traits, Community Structure, and Ecosystem Properties*. Oxford University Press, Oxford. <https://doi.org/10.1093/acprof:oso/9780198757368.001.0001>
- Gause, G. F. (1937). Experimental populations of microscopic organisms. *Ecology*, 18(2), 173-179. <https://doi.org/10.1086/280702>
- Golicz A.A., Schliep M., Lee H.T., Larkum A.W.D., Dolferus R., Batley J., Chan C.K.K., Sablok G., Ralph P.J., Edwards, D. (2015) Genome-wide survey of the seagrass *Zostera muelleri* suggests modification of the ethylene signaling network. *Journal of experimental botany* 66, 1489-1498. <https://doi.org/10.1093/jxb/eru510>
- Gorman, D., Turra, A., Bergstrom, E. R., and Horta, P. A. (2016). Population expansion of a tropical seagrass (*Halophila decipiens*) in the southwest Atlantic (Brazil). *Aquatic Botany*, 132, 30-36. <https://doi.org/10.1016/j.aquabot.2016.04.002>
- Grace, J. B., Michael Anderson, T., Smith, M. D., Seabloom, E., Andelman, S. J., Meche, G., ... and Knops, J. (2007). Does species diversity limit productivity in natural grassland communities?. *Ecology Letters*, 10 (8), 680 - 689. <https://doi.org/10.1111/j.1461-0248.2007.01058.x>
- Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, 86, 902-910. <https://doi.org/10.1046/j.1365-2745.1998.00306.x>
- Grime, J. P. (2006). Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *Journal of Vegetation Science*, 17(2), 255-260. <https://doi.org/10.1111/j.1654-1103.2006.tb02444.x>

- Grime, J.P. and Pierce, S. (2012) *The Evolutionary Strategies that Shape Ecosystems*. Wiley-Blackwell, Oxford. <https://doi.org/10.1002/9781118223246>
- Gross, N., Börger, L., Soriano-Morales, S. I., Le Bagousse-Pinguet, Y., Quero, J. L., García-Gómez, M., ... and Maestre, F. T. (2013). Uncovering multiscale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands. *Journal of Ecology*, 101(3), 637-649. <https://doi.org/10.1111/1365-2745.12063>
- Gustafsson, C., and Boström, C. (2011). Biodiversity influences ecosystem functioning in aquatic angiosperm communities. *Oikos*, 120(7), 1037-1046. <https://doi.org/10.1111/j.1600-0706.2010.19008.x>
- Gustafsson, C., and Norkko, A. (2016). Not all plants are the same: Exploring metabolism and nitrogen fluxes in a benthic community composed of different aquatic plant species. *Limnology and Oceanography*, 61(5), 1787-1799. <https://doi.org/10.1002/lno.10334>
- Haddaway, N. R., Macura, B., Whaley, P., and Pullin, A. S. (2018). ROSES RepOrting standards for Systematic Evidence Syntheses: pro forma, flow-diagram and descriptive summary of the plan and conduct of environmental systematic reviews and systematic maps. *Environmental Evidence*, 7(1), 7. <https://doi.org/10.1186/s13750-018-0121-7>
- Hendriks, I. E., Sintès, T., Bouma, T. J., and Duarte, C. M. (2008). Experimental assessment and modeling evaluation of the effects of the seagrass *Posidonia oceanica* on flow and particle trapping. *Marine Ecology Progress Series*, 356, 163-173. <https://doi.org/10.3354/meps07316>
- Hendriks, I. E., Olsen, Y. S., Ramajo, L., Basso, L., Moore, T. S., Howard, J., and Duarte, C. M. (2014). Photosynthetic activity buffers ocean acidification in seagrass meadows. *Biogeosciences*, 11(333-346). <https://doi.org/10.5194/bg-11-333-2014>
- Herkül, K., Torn, K., and Möller, T. (2018). The environmental niche separation between charophytes and angiosperms in the northern Baltic Sea. *Botany Letters*, 165(1), 115-127. <https://doi.org/10.1080/23818107.2017.1399824>
- Hernán, G., Ramajo, L., Basso, L., Delgado, A., Terrados, J., Duarte, C. M., and Tomas, F. (2016). Seagrass (*Posidonia oceanica*) seedlings in a high-CO₂ world: from physiology to herbivory. *Scientific reports*, 6(1), 1-12. <https://doi.org/10.1038/srep38017>
- Hughes, A. R., Stachowicz, J. J., and Williams, S. L. (2009). Morphological and physiological variation among seagrass (*Zostera marina*) genotypes. *Oecologia*, 159(4), 725-733. <https://doi.org/10.1007/s00442-008-1251-3>
- Randall Hughes, A., and Stachowicz, J. J. (2011). Seagrass genotypic diversity increases disturbance response via complementarity and dominance. *Journal of Ecology*, 99(2), 445-453. <https://doi.org/10.1111/j.1365-2745.2010.01767.x>
- Hyndes, G. A., Heck Jr, K. L., Vergés, A., Harvey, E. S., Kendrick, G. A., Lavery, P. S., ... and Wernberg, T. (2016). Accelerating tropicalization and the transformation of temperate seagrass meadows. *Bioscience*, 66(11), 938-948. <https://doi.org/10.1093/biosci/biw111>

- Invers, O., Perez, M., and Romero, J. (1999). Bicarbonate utilization in seagrass photosynthesis: role of carbonic anhydrase in *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson. *Journal of experimental marine biology and ecology*, 235(1), 125-133. [https://doi.org/10.1016/s0022-0981\(98\)00172-5](https://doi.org/10.1016/s0022-0981(98)00172-5)
- Jahnke M., Pagès J.F., Alcoverro T., Lavery P.S., McMahon K.M., Procaccini G. (2015a) Should we sync? Seascape-level genetic and ecological factors determine seagrass flowering patterns. *Journal of Ecology*, 103, 1464-1474. <https://doi.org/10.1111/1365-2745.12470>
- Jahnke M., Olsen J.L., Procaccini G. (2015b) A meta-analysis reveals a temporal mismatch between genetic diversity metrics and environmental status in the long-lived seagrass *Posidonia oceanica*. *Molecular Ecology*, 24(10), 2336-2348. <https://doi.org/10.1111/mec.13174>
- Jahnke, M., D'Esposito, D., Orrù, L., Lamontanara, A., Dattolo, E., Badalamenti, F., ... and Orsini, L. (2019). Adaptive responses along a depth and a latitudinal gradient in the endemic seagrass *Posidonia oceanica*. *Heredity*, 122(2), 233-243. <https://doi.org/10.1038/s41437-018-0103-0>
- Jänes, H., Kotta, J., Pärnoja, M., Crowe, T. P., Rindi, F., and Orav-Kotta, H. (2017). Functional traits of marine macrophytes predict primary production. *Functional Ecology*, 31(4), 975-986. <https://doi.org/10.1111/1365-2435.12798>
- Janssen, T., and Bremer, K. (2004). The age of major monocot groups inferred from 800+ rbcL sequences. *Botanical Journal of the Linnean Society*, 146(4), 385-398. <https://doi.org/10.1111/j.1095-8339.2004.00345.x>
- Jiménez-Ramos, R., Brun, F. G., Egea, L. G., and Vergara, J. J. (2018). Food choice effects on herbivory: Intra-specific seagrass palatability and inter-specific macrophyte palatability in seagrass communities. *Estuarine, Coastal and Shelf Science*, 204, 31-39. <https://doi.org/10.1016/j.ecss.2018.02.016>
- Jueterbock, A., Franssen, S. U., Bergmann, N., Gu, J., Coyer, J. A., Reusch, T. B., ... and Olsen, J. L. (2016). Phylogeographic differentiation versus transcriptomic adaptation to warm temperatures in *Zostera marina*, a globally important seagrass. *Molecular Ecology*, 25(21), 5396-5411. <https://doi.org/10.1111/mec.13829>
- Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., ... and Cuntz, M. (2020). TRY plant trait database—enhanced coverage and open access. *Global change biology*, 26(1), 119-188. <https://doi.org/10.5194/egusphere-egu2020-20191>
- Kazakou, E., Violle, C., Roumet, C., Navas, M. L., Vile, D., Kattge, J., and Garnier, E. (2014). Are trait-based species rankings consistent across data sets and spatial scales?. *Journal of Vegetation Science*, 25(1), 235-247. <https://doi.org/10.1111/jvs.12066>
- Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *Journal of vegetation science*, 3(2), 157-164. <https://doi.org/10.2307/3235676>
- Lai, S., Yaakub, S. M., Poh, T. S., Bouma, T. J., and Todd, P. A. (2018). Unlikely nomads: settlement, establishment, and dislodgement processes of vegetative seagrass fragments. *Frontiers in plant science*, 9, 160. <https://doi.org/10.3389/fpls.2018.00160>

- Lavorel, S. and Garnier, E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16, 545-556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Lee, H., Golicz, A. A., Bayer, P. E., Jiao, Y., Tang, H., Paterson, A. H., ... and Kendrick, G. A. (2016). The genome of a southern hemisphere seagrass species (*Zostera muelleri*). *Plant physiology*, 172(1), 272-283. <https://doi.org/10.1104/pp.16.00868>
- Lee, H., Golicz, A. A., Bayer, P. E., Severn-Ellis, A. A., Chan, C. K. K., Batley, J., ... and Edwards, D. (2018). Genomic comparison of two independent seagrass lineages reveals habitat-driven convergent evolution. *Journal of experimental botany*, 69(15), 3689-3702. <https://doi.org/10.1093/jxb/ery147>
- Lefcheck, J. S., Orth, R. J., Dennison, W. C., Wilcox, D. J., Murphy, R. R., Keisman, J., ... and Patrick, C. J. (2018). Long-term nutrient reductions lead to the unprecedented recovery of a temperate coastal region. *Proceedings of the National Academy of Sciences*, 115(14), 3658-3662. <https://doi.org/10.1073/pnas.1715798115>
- Les DH, Cleland MA, Waycott M (1997) Phylogenetic Studies in Alismatidae, II: Evolution of Marine Angiosperms (Seagrasses) and Hydrophily. *Systematic botany*, 22, 443-463. <https://doi.org/10.2307/2419820>
- Llagostera, I., Cervantes, D., Sanmartí, N., Romero, J., and Pérez, M. (2016). Effects of copper exposure on photosynthesis and growth of the seagrass *Cymodocea nodosa*: an experimental assessment. *Bulletin of environmental contamination and toxicology*, 97(3), 374-379. <https://doi.org/10.1007/s00128-016-1863-y>
- Loreau, M., and Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412(6842), 72-76. <https://doi.org/10.1038/35083573>
- Marín-Guirao, L., Bernardeau-Esteller, J., García-Muñoz, R., Ramos, A., Ontoria, Y., Romero, J., ... and Procaccini, G. (2018). Carbon economy of Mediterranean seagrasses in response to thermal stress. *Marine pollution bulletin*, 135, 617 - 629. <https://doi.org/10.1016/j.marpolbul.2018.07.050>
- Martínez-Crego, B., Arteaga, P., Tomas, F., and Santos, R. (2016). The role of seagrass traits in mediating *Zostera noltei* vulnerability to mesograzers. *PloS one*, 11(6), e0156848. <https://doi.org/10.1371/journal.pone.0156848>
- Maxwell, P. S., Pitt, K. A., Burfeind, D. D., Olds, A. D., Babcock, R. C., and Connolly, R. M. (2014). Phenotypic plasticity promotes persistence following severe events: physiological and morphological responses of seagrass to flooding. *Journal of Ecology*, 102(1), 54-64. <https://doi.org/10.1111/1365-2745.12167>
- Maxwell, P. S., Eklöf, J. S., van Katwijk, M. M., O'Brien, K. R., de la Torre-Castro, M., Boström, C., ... and van der Heide, T. (2017). The fundamental role of ecological feedback mechanisms for the adaptive management of seagrass ecosystems—a review. *Biological Reviews*, 92(3), 1521-1538. <https://doi.org/10.1111/brv.12294>

- Mayfield MM, Levine JM. (2010). Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters*, 13, 1085 – 1093. <https://doi.org/10.1111/j.1461-0248.2010.01509.x>
- Mazel, F., Pennell, M. W., Cadotte, M. W., Diaz, S., Dalla Riva, G. V., Grenyer, R., ... and Pearse, W. D. (2018). Prioritizing phylogenetic diversity captures functional diversity unreliably. *Nature communications*, 9(1), 2888. <https://doi.org/10.1038/s41467-018-05126-3>
- McGill, B.J., Enquist, B.J., Weiher, E. and Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, 21, 178 - 185. <https://doi.org/10.1016/j.tree.2006.02.002>
- Mota, C. F., Engelen, A. H., Serrao, E. A., Coelho, M. A., Marbà, N., Krause-Jensen, D., and Pearson, G. A. (2018). Differentiation in fitness-related traits in response to elevated temperatures between leading and trailing edge populations of marine macrophytes. *PLoS One*, 13(9), e0203666. <https://doi.org/10.1371/journal.pone.0203666>
- Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends in ecology and evolution*, 28(3), 167-177. <https://doi.org/10.1016/j.tree.2012.10.004>
- Olsen, J. L., Rouzé, P., Verhelst, B., Lin, Y. C., Bayer, T., Collen, J., ... and Michel, G. (2016). The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature*, 530(7590), 331. <https://doi.org/10.1038/nature16548>
- Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., ... and Short, F. T. (2006). A global crisis for seagrass ecosystems. *Bioscience*, 56(12), 987-996. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:agcfse\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2)
- Orth, R. J., Harwell, M. C., and Inglis, G. J. (2007). Ecology of seagrass seeds and seagrass dispersal processes. In *Seagrasses: Biology, Ecology and Conservation* (pp. 111-133). Springer, Dordrecht. https://doi.org/10.1007/1-4020-2983-7_5
- Pagès, J. F., Farina, S., Gera, A., Arthur, R., Romero, J., and Alcoverro, T. (2012). Indirect interactions in seagrasses: fish herbivores increase predation risk to sea urchins by modifying plant traits. *Functional Ecology*, 26 (5), 1015 - 1023. <https://doi.org/10.1111/j.1365-2435.2012.02038.x>
- Paul, M., Bouma, T. J., and Amos, C. L. (2012). Wave attenuation by submerged vegetation: combining the effect of organism traits and tidal current. *Marine Ecology Progress Series*, 444, 31-41. <https://doi.org/10.3354/meps09489>
- Paulo, D., Cunha, A. H., Boavida, J., Serrao, E., Gonçalves, E. J., and Fonseca, M. (2019). Open coast seagrass restoration. Can we do it? Large scale seagrass transplants. *Frontiers in Marine Science*, 6, UNSP-52. <https://doi.org/10.3389/fmars.2019.00052>
- Peralta, G., Van Duren, L. A., Morris, E. P., and Bouma, T. J. (2008). Consequences of shoot density and stiffness for ecosystem engineering by benthic macrophytes in flow dominated areas: a hydrodynamic flume study. *Marine Ecology Progress Series*, 368, 103-115. <https://doi.org/10.3354/meps07574>

- Petchey, O. L., and Gaston, K. J. (2006). Functional diversity: back to basics and looking forward. *Ecology letters*, 9(6), 741-758. <https://doi.org/10.1111/j.1461-0248.2006.00924.x>
- Pickett, S. T. A., and Bazzaz, F. A. (1978). Organization of an assemblage of early successional species on a soil moisture gradient. *Ecology*, 59 (6), 1248 - 1255. <https://doi.org/10.2307/1938238>
- Poore, A. G., Campbell, A. H., Coleman, R. A., Edgar, G. J., Jormalainen, V., Reynolds, P. L., ... and Emmett Duffy, J. (2012). Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology letters*, 15(8), 912-922. <https://doi.org/10.1111/j.1461-0248.2012.01804.x>
- Reusch, T. B.H., Ehlers, A., Hämmerli, A., and Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences*, 102(8), 2826-2831. <https://doi.org/10.1073/pnas.0500008102>
- Roca, G., Alcoverro, T., Krause-Jensen, D., Balsby, T. J. S., van Katwijk, M. M., Marbà, N., ... and Pérez, M. (2016). Response of seagrass indicators to shifts in environmental stressors: A global review and management synthesis. *Ecological Indicators*, 63, 310-323. <https://doi.org/10.1016/j.ecolind.2015.12.007>
- Roscher, C., Schumacher, J., Gubsch, M., Lipowsky, A., Weigelt, A., Buchmann, N., ... and Schulze, E. D. (2012). Using plant functional traits to explain diversity–productivity relationships. *PloS one*, 7(5), e36760. <https://doi.org/10.1371/journal.pone.0036760>
- Ruesink, J. L. (2018). Size and fitness responses of eelgrass (*Zostera marina* L.) following reciprocal transplant along an estuarine gradient. *Aquatic Botany*, 146, 31-38. <https://doi.org/10.1016/j.aquabot.2018.01.005>
- Ruiz-Frau, A., Gelcich, S., Hendriks, I. E., Duarte, C. M., and Marbà, N. (2017). Current state of seagrass ecosystem services: research and policy integration. *Ocean and Coastal Management*, 149, 107-115. <https://doi.org/10.1016/j.ocecoaman.2017.10.004>
- Ruocco, M., Marín-Guirao, L., and Procaccini, G. (2019a). Within-and among-leaf variations in photo-physiological functions, gene expression and DNA methylation patterns in the large-sized seagrass *Posidonia oceanica*. *Marine Biology*, 166(3), 24. <https://doi.org/10.1007/s00227-019-3482-8>
- Ruocco, M., De Luca, P., Marín-Guirao, L., and Procaccini, G. (2019b). Differential Leaf Age-Dependent Thermal Plasticity in the Keystone Seagrass *Posidonia oceanica*. *Frontiers in Plant Science*, 10, 1556. <https://doi.org/10.3389/fpls.2019.01556>
- Ruocco M., Entrambasaguas L., Dattolo E., Milito A., Marín-Guirao L, Procaccini G. (2020) The king and vassals tale: molecular signatures of clonal integration in seagrasses under chronic light shortage. *Journal of Ecology*, 109(1), 294 - 312. <https://doi.org/10.1111/1365-2745.13479/v2/response1>
- Sanmartí, N., Saiz, L., Llagostera, I., Pérez, M., and Romero, J. (2014). Tolerance responses to simulated herbivory in the seagrass *Cymodocea nodosa*. *Marine Ecology Progress Series*, 517, 159-169. <https://doi.org/10.3354/meps11084>

Shipley, B. (2010). *From plant traits to vegetation structure: chance and selection in the assembly of ecological communities*. Cambridge University Press. <https://doi.org/10.1017/cbo9780511806971>

Shipley, B., De Bello, F., Cornelissen, J.H.C., Laliberté, E., Laughlin, D.C. and Reich, P.B. (2016) Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*, 180, 923-931. <https://doi.org/10.1007/s00442-016-3549-x>

Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350(1-2), 3-20. <https://doi.org/10.1016/j.jembe.2007.06.012>

Smith, M. D., and Knapp, A. K. (2003). Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters*, 6(6), 509-517. <https://doi.org/10.1046/j.1461-0248.2003.00454.x>

Soissons, L. M., Van Katwijk, M. M., Peralta, G., Brun, F. G., Cardoso, P. G., Grilo, T. F., ... and Bouma, T. J. (2018). Seasonal and latitudinal variation in seagrass mechanical traits across Europe: the influence of local nutrient status and morphometric plasticity. *Limnology and Oceanography*, 63(1), 37-46. <https://doi.org/10.1002/lno.10611>

Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A., Ruesink, J. L., and Srivastava, D. S. (2004). Extinction and ecosystem function in the marine benthos. *Science*, 306(5699), 1177-1180. <https://doi.org/10.1126/science.1103960>

Sousa, A. I., Silva J. F., Azevedo A., Lillebø A. I. (2019) Blue Carbon stock in *Zostera noltei* meadows at Ria de Aveiro coastal lagoon (Portugal) over a decade. *Scientific Reports*, 9, 14387. <https://doi.org/10.1038/s41598-019-50425-4>

Spalding, M., Taylor, M., and Ravilious, C. (2003). Global Overview–The Distribution and Status of Seagrass In: Green EP, Short FT, Spalding MD (eds) *The World Atlas of Seagrasses: present status and future conservation*. [https://doi.org/10.1016/s0025-326x\(01\)00310-1](https://doi.org/10.1016/s0025-326x(01)00310-1)

Suding, K. N., Lavorel, S., Chapin Iii, F. S., Cornelissen, J. H., Diaz, S., Garnier, E., ... and Navas, M. L. (2008). Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology*, 14(5), 1125-1140. <https://doi.org/10.1111/j.1365-2486.2008.01557.x>

Tanaka, Y., and Nakaoka, M. (2006). Morphological variation in the tropical seagrasses, *Cymodocea serrulata* and *C. rotundata*, in response to sediment conditions and light attenuation. *Botanica Marina*, 49. <https://doi.org/10.1515/bot.2006.047>

Tardif, A., Shipley, B., Bloor, J. M., and Soussana, J. F. (2014). Can the biomass-ratio hypothesis predict mixed-species litter decomposition along a climatic gradient? *Annals of Botany*, 113(5), 843-850. <https://doi.org/10.1093/aob/mct304>

Tilman, D. (1985). The resource-ratio hypothesis of plant succession. *The American Naturalist*, 125(6), 827-852. <https://doi.org/10.1086/284382>

- Tilman, D. (2001). An evolutionary approach to ecosystem functioning. *Proceedings of the National Academy of Sciences*, 98(20), 10979-10980. <https://doi.org/10.1073/pnas.211430798>
- Tomas, F., Abbott, J. M., Steinberg, C., Balk, M., Williams, S. L., and Stachowicz, J. J. (2011). Plant genotype and nitrogen loading influence seagrass productivity, biochemistry, and plant–herbivore interactions. *Ecology*, 92(9), 1807-1817. <https://doi.org/10.1890/10-2095.1>
- Trevathan-Tackett, S. M., Wessel, C., Cebrián, J., Ralph, P. J., Masqué, P., and Macreadie, P. I. (2018). Effects of small-scale, shading-induced seagrass loss on blue carbon storage: Implications for management of degraded seagrass ecosystems. *Journal of applied ecology*, 55(3), 1351-1359. <https://doi.org/10.1111/1365-2664.13081>
- Unsworth, R. K., Collier, C. J., Henderson, G. M., and McKenzie, L. J. (2012). Tropical seagrass meadows modify seawater carbon chemistry: implications for coral reefs impacted by ocean acidification. *Environmental Research Letters*, 7(2), 024026. <https://doi.org/10.1088/1748-9326/7/2/024026>
- van der Plas, F., Schröder-Georgi, T., Weigelt, A., Barry, K., Meyer, S., Alzate, A., ... and Wirth, C. (2020). Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nature ecology and evolution*, 4 (12), 1602 - 1611. <https://doi.org/10.1038/s41559-020-01316-9>
- McMahon, K., van Dijk, K. J., Ruiz-Montoya, L., Kendrick, G. A., Krauss, S. L., Waycott, M., ... and Duarte, C. (2014). The movement ecology of seagrasses. *Proceedings of the Royal Society B: Biological Sciences*, 281(1795), 20140878. <https://doi.org/10.1098/rspb.2014.0878>
- Vellend, M. (2010). Conceptual synthesis in community ecology. *The Quarterly review of biology*, 85(2), 183-206. <https://doi.org/10.1086/652373>
- Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. and Garnier, E. (2007) Let the concept of trait be functional! *Oikos*, 116, 882-892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- Violle, C., Enquist, B. J., McGill, B. J., Jiang, L. I. N., Albert, C. H., Hulshof, C., ... and Messier, J. (2012). The return of the variance: intraspecific variability in community ecology. *Trends in ecology and evolution*, 27(4), 244-252. <https://doi.org/10.1016/j.tree.2011.11.014>
- Virnstein, R. W., Mikkelsen, P. S., Cairns, K. D., and Capone, M. A. (1983). Seagrass beds versus sand bottoms: the trophic importance of their associated benthic invertebrates. *Florida Scientist*, 363-381.
- Waycott M, Procaccini G, Les D, Reusch TBH (2006) Seagrass evolution, ecology and conservation: a genetic perspective. In *Seagrasses: biology, ecology and conservation*. Springer, (pp. 25-50). https://doi.org/10.1007/1-4020-2983-7_2
- Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., ... and Kendrick, G. A. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, 106(30), 12377-12381. <https://doi.org/10.1073/pnas.0905620106>

- Weihner, E., and Keddy, P. A. (1995). Assembly rules, null models, and trait dispersion: new questions from old patterns. *Oikos*, 159-164. <https://doi.org/10.2307/3545686>
- Weihner, E., Clarke, G. P., and Keddy, P. A. (1998). Community assembly rules, morphological dispersion, and the coexistence of plant species. *Oikos*, 309-322. <https://doi.org/10.2307/3547051>
- Weihner, E., Freund, D., Bunton, T., Stefanski, A., Lee, T. and Bentivenga, S. (2011) Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 366, 2403-2413. <https://doi.org/10.1098/rstb.2011.0056>
- Wilson, J. B. (2011). The twelve theories of co-existence in plant communities: the doubtful, the important, and the unexplored. *Journal of Vegetation Science*, 22(1), 184–195. <https://doi.org/10.1111/j.1654-1103.2010.01226.x>
- Wissler, L., Codoñer, F. M., Gu, J., Reusch, T. B. H., Olsen, J. L., Procaccini, G., and Bornberg-Bauer, E. (2011). Back to the sea twice: identifying candidate plant genes for molecular evolution to marine life. *BMC evolutionary biology*, 11(1), 8. <https://doi.org/10.1186/1471-2148-11-8>
- Wu, K., Chen, C. N. N., and Soong, K. (2016). Long distance dispersal potential of two seagrasses *Thalassia hemprichii* and *Halophila ovalis*. *PloS one*, 11(6), e0156585. <https://doi.org/10.1371/journal.pone.0156585>
- Yu, L., Boström, C., Franzenburg, S., Bayer, T., Dagan, T., and Reusch, T. B. H. (2020). Somatic genetic drift and multilevel selection in a clonal seagrass. *Nature Ecology and Evolution*, 4, 952–962. <https://doi.org/10.1038/s41559-020-1196-4>

VIII. Supplementary material

(1) Supplementary material 1

Metadata of the systematic review process following the ROSES protocol (Haddaway et al. 2018).

Protocol		ROSES Haddaway et al. (2018) https://link.springer.com/article/10.1186/s13750-018-0121-7
Title	Title	A trait-based framework to seagrass ecology: trends and prospects
Type of review	Type of review	Systematic review
Authors contacts	Author contacts	Agustín Moreira Saporiti (am.saporiti@gmail.com)
Abstract	Structured summary	See abstract in manuscript
Background	Background	See manuscript
Objective of the review		Screen methods and concepts used in the study of seagrass traits, with a specific focus on trait-based approaches and functional ecology.
Methods		
Searches	Search strategy	Web-search
	Search string	“Seagrass”+”trait” and “ <i>seagrass species</i> ”+”trait”
	Languages	English
	Languages-grey literature	No grey literature included.
	Web-based search engines	Google Scholar
	Estimating the comprehensiveness of the search	All articles published subjected to the inclusion criteria.
Article screening and study inclusion criteria	Screening strategy	Screening of title, abstract and keywords
	Inclusion criteria	“trait” has to be present in title, abstract and/or keywords in reference to seagrass
	Reasons for exclusion	“trait” is used for the study of seagrass associated fauna, epiphytes, macroalgae or environment.

Screening process	Number of search results from web-based search engines.	18185
	Number of articles retained following title and abstract screening.	325
	Number of articles retained after applying exclusion criteria	188
	Number of articles retained after removing duplicates	175
	Number of articles retained after removing misclassified articles	157
	Final number of articles included in the review process	157
	Data extraction	No data extraction
Data synthesis and presentation	Type of synthesis	Narrative and quantitative
	Narrative synthesis strategy	Summary of trait-based and functional ecology concepts used in seagrass research
	Quantitative synthesis strategy	Network analysis of study types, traits study method, environmental drivers and functions studied in seagrass, both with and without trait-based approaches.
	Assessment of risk of publication bias	The inclusion of articles that include the word “trait” ignores a lot of publications in which traits are being studied, but the word “trait” is not used.

(2) Supplementary material 2

Table 1. Categorization of the studies encountered in the review process according to which step in the TBF was tested. Each category has a definition and criteria of inclusion and the number of studies included.

TBF part	Definition and criteria	Number of studies
Dispersal	Study of dispersal mechanisms of seagrass	3
Abiotic filter	Study of the response traits of seagrass under abiotic environmental drivers	85
Biotic filter	Study of response traits of seagrass under biotic interaction, among seagrass species or between seagrass and other compartments of the ecosystem (herbivores, epiphytes, macroalgae...)	29
Ecosystem function provision	Study of the provision of ecosystem functions by seagrass related to seagrass traits.	52
Phylogeny	Study of seagrass genotypes, genes and gene expression in connection to seagrass traits.	37
Intraspecific variability	Studies assessing trait variability at the intraspecific level (i.e. within a species)	14

Table 2. Categorization of the studies encountered in the review process according to the trait category included in the study. Each category has a definition and criteria of inclusion and the number of studies included.

Trait category	Definition and criteria	Number of studies
Biochemical	Study of the biochemical composition of seagrass e.g. nitrogen, carbohydrates, fatty acids, secondary metabolites...	40
Growth	Study of growth of seagrass e.g. shoot production, leaf growth, rhizome elongation...	38
Life-history	Studies that categorize seagrass attending to traditional classifications e.g. climax species, opportunistic species...	24
Mechanical	Study of sturdiness or robustness of seagrass e.g. flexibility, structural carbon content, force-to-break...	24
Morphological	Study of any morphological characteristic of seagrass e.g. leaf area, rhizome diameter, root length...	75
Physiological	Study of physiological processes e.g. photosynthetic parameters.	25
Reproductive	Study of reproductive structures of seagrass e.g. number of flowers, seed production...	17

Table 3. *A posteriori* classification of abiotic and biotic filters, as well as ecosystem functions found in the systematic literature review.

Main category	Factor or function	Definition	Number of studies
Abiotic factors	Burial/Sedimentation		5
	CO ₂ /pH	Carbon dioxide enrichment and/or pH	7
	Depth		7
	Hydrodynamics	Currents, flow or wave energy.	8
	Latitude		8
	Light		20
	Nutrients	Nutrient conditions and enrichment.	23
	Other anthropogenic	Anthropogenic factors recorded once and clustered.	9
	Physical damage	Damage due to mooring, trampling...	11
	Salinity		11
	Season	Seasonal patterns.	12
	Grain size		4
	Site	Site comparisons.	9
	Sediment organic matter	Sediment organic matter.	3
	Suspended organic matter		2
	Substrate type	Rocky or sedimentary.	4
	Temperature	Local or global.	22
	Tidal range/Desiccation	Air exposure due to the tide.	7
Time	Longitudinal studies.	2	
Biotic factors	Algae abundance	Macroalgae abundance.	2
	Epiphytes abundance		3
	Herbivory (Response)	Effects of herbivores on seagrass.	16
	Interspecific competition		4
	Intraspecific competition		2
	Other biotic	Biotic factors recorded once and clustered.	4
Ecosystem functions	Carbon storage	Carbon storage in the sediment.	5
	Flow attenuation	Flow attenuation due to the seagrass canopy.	6
	Habitat provision	Habitat provision for fauna.	3
	Herbivory (Function)	Food provision to herbivores.	20
	Litter production	Production of seagrass litter.	2
	Nutrient uptake	Uptake of inorganic nutrients.	4
	Primary production	Seagrass primary production.	11
	Sediment trapping	Trapping of sediment particles.	1



CHAPTER 3.

Morphological and physiological responses of *Enhalus acoroides* seedlings under varying temperature and nutrient treatment

Suci Rahmadani Artika¹, Rohani Ambo-Rappe^{1*}, Mirta Teichberg², Agustín Moreira-Saporiti^{2,3}, Inés G. Viana^{2,4}

¹ Department of Marine Science, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia, ² Leibniz Centre for Tropical Marine Research GmbH (ZMT), Bremen, Germany, ³ Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany, ⁴ Department of Ecology and Animal Biology, University of Vigo, Vigo, Spain

Abstract

Seagrass meadows are declining globally. In Indonesia, 75% loss has been reported in the last 5 years. The decrease of the seagrass area is influenced by the simultaneous occurrence of many factors at the local and global scale, including nutrient enrichment and climate change. This study aims to find out how increasing temperature and nutrient enrichment affect the morphological, biochemical and physiological responses of *Enhalus acoroides* in the seedling phase, which has not previously been studied. To achieve these aims, a laboratory experiment of combined temperature and nutrient treatments was conducted using recently-germinated seedlings of *E. acoroides*. The results showed that the seedlings were tolerant to an extended exposure to the current ambient maximum temperature. Under higher temperature treatment, the seedlings were observed to increase in aboveground size traits (e.g., number of leaves, leaf length, biomass, and leaf area), as well as in belowground traits, such as root length. The results in this study also showed that the initial seed size matters for morphological responses. On the contrary, nutrient responses of seedlings were practically absent, suggesting they could rely on internal reserves. Interaction between both factors was limited, with the exception of low temperature and high nutrient treatment, in which the AG:BG ratio and leaf elongation rate increased. Fluorescence parameters were not influenced by any of the water treatments. The results in this study suggest that *E. acoroides* seedlings rely energetically in the reserves within the seedling and that increasing temperature might result in faster seedling development, although no interactions with other organisms were tested. This is of importance when studying the resilience capacity of this species and when restoration attempts are planned, as a faster root development would provide a faster stabilization in the sediment and the survival of the whole plant.

Keywords: tropical seagrass, Indo-Pacific, traits, growth, nutrient content, photosynthesis, carbohydrates, Indonesia

I. Introduction

Seagrasses are marine flowering plants that are globally distributed and can form dense meadows in shallow water coastal environments (Duarte 1991, 2001; Duarte and Cebrián 1996; Short et al. 2007). They are key ecosystem engineers and the only submerged marine species with both above- (AG) and below-ground (BG) tissues that sustain multiple ecosystem services and functions. Seagrasses fuel food webs by supplying a combination of food and shelter to various macro flora and fauna, including commercially important fish species, sustain high rates of primary production, filter coastal waters by capturing particles and dissolved nutrients, participate in nutrient cycling, and provide coastal protection by attenuating waves and stabilizing sediments by their singular BG tissues (Hemminga et al. 1991; Duarte and Cebrián 1996; Hemminga and Duarte 2000; Duarte, 2001; Cullen-Unsworth and Unsworth 2013; Tuya et al. 2014; Weitzman et al. 2015). Seagrass meadows also help to mitigate climate change by the capture and storage of organic carbon (“blue carbon”), reducing CO₂ concentrations in seawater (Fourqurean et al. 2012; Macreadie et al. 2019). Despite their global significance the attention paid to seagrass meadows is much lower than other coastal ecosystems (Cullen-Unsworth et al. 2014).

Seagrasses are also one of the most threatened ecosystems due to their presence in coastal areas (Orth et al. 2006; Burkholder et al. 2007), where they are declining globally, with a loss of 29% from 1876 to 2006 (Waycott et al. 2009). Many factors have been identified as the reason for this decrease, both at the local and global scale, including nutrient enrichment and climate change (Orth et al. 2006; Waycott et al. 2009). Regional losses may be even higher in the tropics, such as in Indonesia, where 75% loss has been reported in the last 5 years (Unsworth et al. 2018). Tropical ecosystems are biodiversity hotspots, with the tropical Indo-Pacific bioregion hosting 24 of the approximately 60 seagrass species (Short et al. 2007). As seagrass traits and functions sustaining different ecosystem services and the stressors that affect them are species-specific, multi-specific assemblages have a greater probability of containing a greater functional diversity, but also greater losses (Duarte 2000).

Human development and associated activities (e.g., agriculture, fish and seaweed aquaculture) have triggered land use changes and urbanization which have led to an increase in the concentration of nutrients and sediments into the coastal waters. This nutrient increment exceeds the nutrient cycling capacity of the system, increasing the organic carbon supply, and ultimately leading to eutrophication processes that are characterized by algal blooms, epiphyte growth, anoxic conditions in the sediments and, eventually, biodiversity loss and seagrass death

(Lee et al. 2006; Khan and Mohammad 2014). The effects of nutrient enrichment depend on species-specific features, such as nutrient uptake capacity, the level of nutrient surplus that can vary from moderate to severe, and on local physical conditions, such as currents and tides. Eutrophication has been identified as one of the most important factors affecting productivity, community carbon dynamics, and seagrass growth, and is one of the major threats confronting coastal ecosystems. Direct effects occur through stability of physiological mechanisms (Burkholder et al. 1992; Bird et al. 1998; Brun et al. 2002; Invers et al. 2004; Touchette and Burkholder 2007) causing increased nutrient uptake ability (Viana et al. 2019), nutrient imbalance (Li et al. 2019), changes in morphological indices (Mvungi and Pillay 2019), changes in growth (Terrados et al. 1999a), changes in sexual reproduction (Duarte et al. 1997), or direct ammonium toxicity (Van Katwijk et al. 1997). Indirect effects of nutrient inputs occur through blooming algae which cause light depletion or nutrient competition (Duarte 1995; Short et al. 1995; Moore and Wetzel 2000; Nixon et al. 2001; Burkholder et al. 2007), through the ecological role of herbivores due to modifications in palatability and plant defenses against herbivory (Tomás et al. 2015; Jiménez-Ramos et al. 2017; Marco-Méndez et al. 2017; Campbell et al. 2018; Hernán et al. 2019), or through oxygen depletion in sediments (Terrados et al. 1999b). Therefore, the effects of nutrients on seagrass responses range from no impacts to positive or negative impacts. Tropical systems are oligotrophic and naturally nutrient limited; therefore, even small amounts of nutrient additions might rapidly increase competence among primary producers.

Climate change may also impact seagrasses due to rising mean water temperatures and more frequent and lengthy heat waves (Marbà et al. 1996; Short and Wyllie-Echeverria 1996). Impacts of temperature increase can be detected at the plant level and have been found to benefit the growth, biomass (Masini et al. 2001), flowering (Diaz-Almela et al. 2007), or photosynthetic rates (Campbell et al. 2006) of several seagrass species. But if elevated temperature rises above a threshold, or persist for longer periods of time, effects could be detrimental and result in community structure damages by causing impacts on seagrass metabolism and nutrient uptake ability (Lee et al. 2007; Moore and Short, 2007; Collier and Waycott 2014). This can eventually lead to die off if extreme temperatures persist (Cambridge et al. 1986; Bulthuis 1987; Short and Neckles 1999; York et al. 2013). Recent experiments suggest that heat waves may enhance the autotrophic metabolism of seagrass communities in contrast to other previous research suggesting solely negative effects (Egea et al. 2019). Therefore, temperature is of crucial importance in determining seagrass metabolism, growth and survival. While climate change has not yet significantly impacted seagrass areas in Indonesia (Unsworth et al. 2018), it is

essential to take in consideration that tropical seagrasses are growing closer to their photosynthetic and physiological limits in comparison to temperate seagrasses, making them highly vulnerable to rising temperature thresholds caused by climate warming (Tewksbury et al. 2008).

The ecosystem functions performed by seagrasses are consequences of their physiological, biochemical and morphological traits. Therefore, even though changes in seagrass traits could be seen as positive for individual seagrass plants (i.e., increasing growth or photosynthetic rate), it could also lead to changes in their functions and imbalances in their biotic and abiotic interactions, negatively affecting the ecosystem services they perform. For example, it could lead to changes in hydrodynamic conditions or sedimentation rates (Fonseca et al. 2019) which affect the distribution of organisms within the canopies, and therefore, biodiversity (González-Ortiz et al. 2014; Jiménez et al. 2019; Meysick et al. 2019). Seagrass responses to changes in nutrient and temperature conditions can be measured by changes in their trait values which are often used as indicators of environmental stress in coastal management. Different traits have been identified as indicators of seagrass stress such as nutrient inputs, temperature or shading (Lee et al. 2007; Martínez-Crego et al. 2008; De los Santos et al. 2016; Roca et al. 2016). But seagrass plants are rarely affected by just one variable and identifying the effect of single stressors is a challenge in natural seagrass meadows. The interaction between stressors is now viewed as a crucial issue, and it is suggested that single factor experiments are not adequate for assessing the effects of several disturbances on coastal marine ecosystems (Wernberg et al. 2012; Todgham and Stillman 2013; Ontoria et al. 2019). In this way, laboratory experiments under controlled conditions might help to isolate the effects on plant trait variability of single and multiple stressors. These physiological experiments are also needed in order to make predictions about seagrass resilience or tolerance to future climate scenarios. The combined impacts of rising temperature and increased nutrient loading has been studied in adult species of *Zostera spp.* and *Cymodocea nodosa* (Touchette and Burkholder 2002; Touchette et al. 2003; Kaldy 2014; Jiménez-Ramos et al. 2017; Mvungi and Pillay 2019; Ontoria et al. 2019), but, as far as we know, no studies were carried out in seagrass early life stages. There are few works on the combined effects of other stressors in seagrass seedlings but none in tropical species (Hernán et al. 2016; Alexandre et al. 2018; Pereda-Briones et al. 2018, 2019; Yue et al. 2019).

Enhalus acoroides is a tropical seagrass with a high tolerance to environmental changes such as temperature and nutrients (McMillan 1984; Terrados et al. 1999a), and therefore, changes in its morphological, biochemical or physiological traits can be used as indicators to increasing

temperature and varying nutrient fluctuations. *E. acoroides* is also an ecosystem engineer which, by altering the physical and chemical properties of the environment, can facilitate the presence of species that otherwise would be absent. The opportunity of colonizing new habitats and the genetic diversity provided by sexual reproduction could make seagrass populations more resistant to the current changing scenario. In addition, due to the highly variable flower production and low success of seedling establishment, sexual reproduction and seedling stages are critical phases in the life of seagrasses (Bewley and Black 1994; Schupp 1995; Peterson and Baldwin 2004). However, there is a lack of research conducted on *E. acoroides* seeds and seedlings in order to understand their response to various environmental changes. This is of importance as seagrass restoration programs could be based on adult seagrass transplantation or on generative techniques. Planting seedlings is a cost-efficient method for large-scale seagrass meadow restoration. However, the main limitation of seedling establishment programs is the low seedling survival rate observed due to unsuitability of environmental conditions (Ambo-Rappe et al. 2019). Therefore, the study of seedling trait responses under different environmental conditions, such as temperature or nutrient enrichment, on this early-life phase may enhance future restoration and conservation management plans of these threatened ecosystems.

This study aims to assess the morphological, physiological and biochemical trait responses of the seedling stage of *E. acoroides* to increased temperature and nutrient enrichment. Furthermore, the results of this study will provide important information and serve as a reference to predict the effects of temperature changes, as a proxy for climate change conditions, and nutrient enhancement on seagrass survival. To achieve these aims, a laboratory experiment was conducted using seedlings of *E. acoroides* under the combination of increased temperature and nutrient enrichment. We hypothesized that seedlings of *E. acoroides* might be tolerant to rising temperatures and that nutrient enrichment would increase their growth performance, causing synergetic effects under higher temperature.

II. Materials and Methods

(1) Collection and Maintenance of Seagrass Seeds

Fruits of *E. acoroides* were collected on mid-January 2017 on the southwest side of Barrang Lompo Island, South Sulawesi, Indonesia (S 5°03'05, E 119°19'37), where *E. acoroides* is abundant at a depth range of 1-3m. Highest nitrate concentrations in the area range between 0.1 and 0.6 μM , while phosphate concentrations range between 0.12 and 0.14 μM (Kegler et al.

2018). Annual temperature range in the dry season varies in this area between 26 and 32°C (Teichberg et al. 2018). During seed collection, we measured 28-32°C during mid-day. The ripe seagrass fruit was opened, packed in a Styrofoam box with wet breathable polyester fiber sheets, and then transported to the Marine Experimental facilities (MAREE) at the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen (Germany) in <24 h. Once at the MAREE, seeds were planted directly in polypropylene trays previously filled with silicate sediment of at least 10 cm depth.

All trays with seeds were kept in 250 l aquaria filled with low nutrient artificial sea water (ASW) (Red Sea Salt, Red Sea Europe Company) under controlled conditions of light irradiance ($200 \pm 30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), temperature ($26 \pm 1^\circ\text{C}$) and salinity (35 PSU) for a 1 week acclimation phase until the root and some leaf growth was observed (at which point they entered the seedling stage). The photoperiod of the fluorescent lights was 12:12 h light:dark cycle.

(2) Experimental Design and Setup

We conducted a full-factorial experiment combining two water temperatures (26 and 31°C) representing the minimum and maximum temperatures within the home region that seagrasses are currently exposed to, and two nutrient treatments (low nutrient concentrations of 2 μM of NH_4NO_3 and 0.1 μM KH_2PO_4 and high nutrient concentrations of 20 μM of and 1 μM of NH_4NO_3 and KH_2PO_4). This yielded in 4 experimental treatments: low temperature and low nutrient concentrations, low temperature and high nutrient concentrations, high temperature and low nutrient concentrations, and high temperature and high nutrient concentrations. The experiment was conducted under laboratory conditions in an indoor flow-through system at the MAREE (ZMT, Bremen) with 24 individual aquaria with $29 \times 13 \times 30$ cm dimensions and 10 l volume (Figure 1A). Seedlings (consisting of visible cotyledon, seeds and no roots at the beginning of the experiment) were categorized as small (diameter between 0.6 and 1.0 cm), medium (diameter between 1.1 and 1.5 cm) and large (diameter between 1.6 and 1.7 cm). One seedling of each size was distributed in each aquarium, making a total of three seedlings per aquarium. The size categories were included to avoid confounding effects due to the amount of nutrient reserves available to the seedling and were decided according to the size distribution of the seedlings in order to obtain equal amount of seeds per category. The aquaria additionally contained adult seagrasses from 3 different species (Viana et al. in prep).

The target temperature values were obtained by placing aquaria in larger experimental tanks (ETs) of 250 l that acted as water baths maintaining a constant water temperature. Six aquaria

were placed in 4 different ETs following a split-plot experimental design with nutrient treatments nested within the 4 ETs set at the two temperatures (Figure 1A). There was no interaction between aquaria that acted as replicates ($n = 6$). Water temperature was controlled in each ET by using heaters (EHEIM) connected to an individual electronic system that was continuously regulating the temperature of the water bath by digital controllers and individual temperature probes ($\pm 0.2^\circ\text{C}$). Air pumps were also placed in each ET to ensure water movement of the water bath. The light was provided by LED lamps (Hydra Fifty-two HD, AquaIllumination, Iowa), 2 lamps were placed at the same height at the top part of each ET, providing $200 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$ of light (measured at each aquarium to ensure homogeneous irradiance). Light was set on a 12:12 h light:dark photoperiod with sunrise and sunset simulation. Transparent PVC lids were placed on each ET to reduce water evaporation.

High and low nutrient ASW solutions were individually supplied to each of the 24 aquaria from two different water reservoirs of ~ 115 l each, with either high or low nutrient concentrations using a 24-channel peristaltic pump (ISMATEC, Germany). The flow from both water reservoirs was maintained at a constant rate at $\sim 5.8 \text{ l d}^{-1}$ ensuring total water renovation inside each aquarium every ~ 1.5 days. Water reservoirs were manually emptied from any remaining water and refilled with fresh ASW every other day. Nutrients to the water reservoirs were added in a previously dissolved form from stock solutions of NH_4NO_3 and KH_2PO_4 (Merck, Germany). Once in the water reservoir, ASW was gently mixed and an air pump was placed in each water reservoir to ensure further aeration and mixing. Air pumps were placed in each aquarium to ensure water aeration and mixing by moving water from the bottom to the top. Water constantly overflowed from the aquaria to the water bath of the ETs ensuring water renewal. At the same time, ETs were drained of the surplus water flowing out of the aquaria. Algae were removed from the blades of the plants throughout the experiment but not from the rest of the aquaria.

For the experiment to begin, the temperature was increased in 2 random ETs from 26 to 31°C at 1°C d^{-1} while the other 2 ETs remained at the initial temperature of 26°C . Once the desired temperatures were stable in all ETs, the nutrient enrichment began. From that moment, the experiment lasted for approximately 1 month (January 20th to February 22nd, 2017).

(3) Water Sampling

Water parameters, including pH, temperature and salinity, were monitored three times per week during the treatment phase with a multi parameter probe (WTW Multiprobe). During the

experiment the temperature inside aquaria was also continuously monitored by Hobo loggers (Onset, MA, USA) placed in one random aquarium of each ET ($n = 4$). Water samples were taken every week from the two water reservoirs and random aquaria of each treatment ($n = 4$ each week) for DIN (dissolved inorganic nitrogen, NH_4^+ , NO_x^- and NO_2^-); silicate and phosphate. Water samples were sampled with a syringe, immediately filtered (0.45 μm pore size) in pre-rinsed polyethylene bottles and frozen (-20°C). Analysis was performed using a continuous flow injection analyzing system (Skalar SAN++ -System) following Grasshoff et al. (1999). At the end of the experiment, water samples from all aquaria were collected and immediately filtered for chlorophyll a and b (Chl-*a* and -*b*) measurement. Water was filtered under constant pressure onto pre-combusted (5 h, 450°C) Whatman GF/F filters. Filters for Chl-*a* and -*b* analysis were stored at -20°C . Pigments were extracted from the filters in 8ml of 96% ethanol in glass vials placed for 5 min at 80°C and subsequently placed in a rotor at room temperature in the dark for approximately 24 h. Extracts were subsequently centrifuged at 5,000 rpm for 20 min. Chl-*a* and -*b* samples were determined in a photometer Shimadzu UV-1700.

(4) Seedlings Morphological and Physiological Traits

At the end of the experiment, seedlings were removed from the aquaria and the morphological measurements on each plant were first performed. Afterwards, plants were carefully separated with a glass slide into the different parts: leaves (for fluorescence measurements and nutrient content), seeds and roots (for nutrient content). While morphological and fluorescence measurements were individually performed on the three seedlings, the nutrient content was analyzed in the pooled material of the three seedlings from each aquarium. Samples of the separated plant were gently cleaned with distilled water to remove any sediment or epiphytes and subsequently frozen at -80°C until analysis.

(a) Morphological Traits

Seagrass morphological traits were determined by measuring the length, width and number of leaves per seedling, the length and number of roots, the height and diameter of seeds and the biomass of leaves, seeds and roots. AG (blades) and BG (roots) biomass was also determined (± 0.01 g).

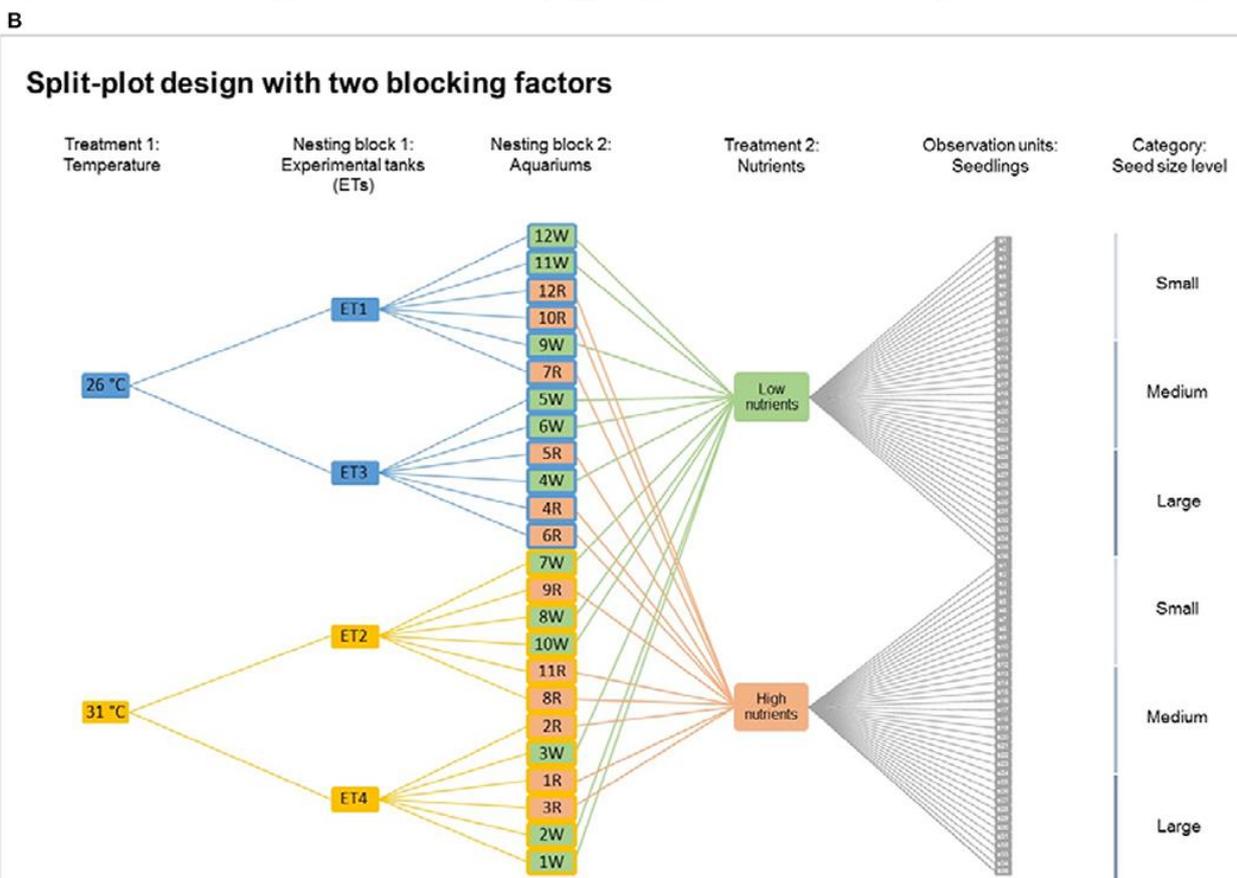
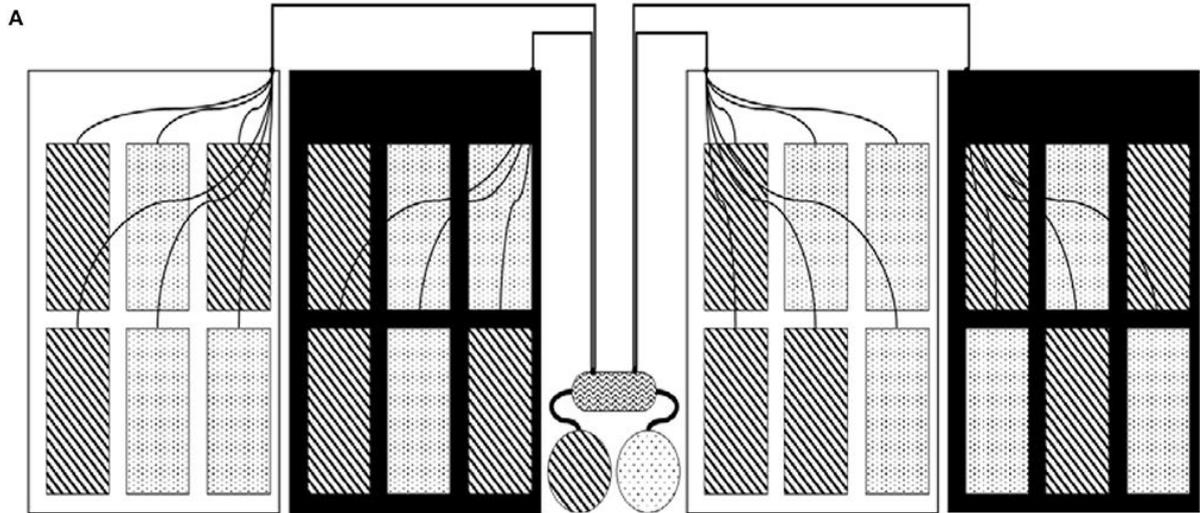


Figure 1. (a) Nutrient and temperature split-plot experimental design. Artificial seawater was individually supplied to aquaria ($n = 24$) from reservoirs with either high nutrient (dot boxes/circle) or low nutrient artificial seawater (strip boxes/circle) using a peristaltic pump. Six aquaria were placed in each experimental tank (ET) that acted as a water bath maintaining a constant temperature of either 26°C (white boxes) or 31°C (black boxes), with no interaction between aquaria that acted as replicates ($n = 6$). (b) Experimental split-plot design with two blocking factors. Temperature and nutrient treatments are the fixed effects (explanatory variables) in the model, and they are fully crossed (nutrient treatments are not nested by temperature treatments). Temperature, as it groups ETs and aquaria, is a group level predictor. Nutrients, as it groups the observation units (seedlings) is a data level predictor. ET and aquaria are nesting variables, this means that ET nests the aquaria and the aquaria nests the observation units (seedlings). The seedlings are also further classified as large, medium and small.

(b) Growth Rates

Seedling growth rate measurements were done using the leaf marking method (Short and Duarte 2001). At the beginning of the experiment, leaves were perforated close to the seed using a pin. At the end of the experiment, the length from the seed to the mark of each leaf was measured. Leaf growth rates were obtained by dividing the elongation (distance from the base to the mark) with the number of days since the seagrass leaves were marked. Surface area (SA) was calculated with the following Equation (1).

$$SA \text{ Growth rate } (cm^2d^{-1}) = \frac{\text{distance from the base to the mark} \times \text{leaf width}}{\text{number of days}} \quad (1)$$

(c) Photosynthetic Performance

A PAM-2500 (Walz, Germany) was used for the measurement of the fluorescence of the seagrass through rapid light curves (RLC). The optical cable of the PAM was attached with leaf clips to the second leaf of the seedling, above the meristem, and at 3mm distance from the tissue. The leaves were dark adapted for 5min before measurement.

The RLC consisted of 12 saturating light pulses (separated by 30 s intervals), increasing the photosynthetic active radiation (PAR) between pulses until 2,001 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. From the data of the RLC, several parameters were calculated. Light saturation coefficient (E_k) and the slope of the light limited part of the curve (α) were calculated using the package Phytotools (Silsbe and Malkin 2015) with the R software (R Core Team, 2019) following the model of Jassby and Platt (1976). The maximum light utilization efficiency or maximum quantum yield was calculated following Equation (2) (Genty et al. 1989).

$$\text{Maximum quantum yield} = \frac{(F_m - F_o)}{F_m} \quad (2)$$

Where F_m is the maximum dark-adapted fluorescence and F_o is the minimum dark-adapted fluorescence. The relative electron transport rate (rETR) was calculated for each step of the curve following Equation (3) (Sakshaug et al., 1997).

$$rETR = \frac{F_m' - F'}{F_m'} \times \frac{PAR}{2} \quad (3)$$

Where F_m' is the light adapted maximum fluorescence and F' the fluorescence yield at a particular light level. From the rETR values, maximum rETR (rETR_{max}) was calculated as the inflection point of the fitted rETR curve.

(d) Nutrient content

Leaf, seed and root nitrogen (N) and carbon (C) content (%N and %C, respectively) was analyzed on previously dried (60°C, 48 h) and powdered seagrass tissue samples. Aliquots of the samples were weighed into tin capsules using an analytical scale prior to analysis (Euro EA3000 Elemental Analyzer).

(e) Non-structural carbohydrate (NSC) content

The concentrations of soluble sugars (sucrose) and starch were measured on leaf and seed material that was previously freeze dried (48 h) and ground to a fine powder. Sucrose was extracted from plant tissue by heating (80°C) in 95% EtOH. The ethanol extracts were subsequently evaporated bubbling the samples with N₂, and the remaining residues were dissolved in deionized water for sucrose analysis. Starch was extracted during 24 h from the ethanol-insoluble residue in 0.1N NaOH. The sucrose and starch concentrations were determined spectrophotometrically (486 and 640 nm, respectively) using an F200-Pro TECAN plate reader. Resorcinol and anthrone assays were used for sucrose and starch determination, respectively, and sucrose was used as the standard for the calibration curve (Yemm and Willis, 1954; Huber and Israel, 1982). Results were reported in glucose equivalents g⁻¹ DW. Current testing of this method has shown that NaOH extracts both starch and cellulose which can confound the results. Regarding the sucrose determination, this method only determines ketoses (as fructose) so we are ignoring the other component of sucrose, glucose, underestimating the final concentrations (M. Birkicht, personal communication).

(5) Statistical analysis

The experiment followed a split-plot design with three nesting factors (Schielzeth and Nakagawa 2013). The two main factors (temperature and nutrient treatments) had two levels each (26 and 31°C, and low and high nutrient concentrations, respectively), which were fully crossed.

We used permutational multivariate analysis of variance (PERMANOVA) (Anderson et al. 2008) to analyze the data. The statistic to test the null hypothesis of no differences in the position of the group centroids in the space of the chosen dissimilarity measure is the pseudo F-statistic. The fixed effects in the model were temperature and nutrient treatments, the seedling

size and their interactions, together with the nesting factors temperature, ET and Aquarium. Three seedlings were nested in each aquarium; six aquaria were nested in four ETs, and two ETs were nested within each temperature treatment. Seedlings, with 3 levels of sizes, were evenly distributed between aquaria (Figure 1B). Seedling size and Aquarium were not included as factors in the model for biochemical trait analysis because seedling samples within the same aquaria were pooled. Data was scouted for outliers, which were identified as data exceeding 1.5 times the interquartile range of variation of the dataset. Outliers were eliminated from the model when they did not allow for meeting the model assumptions. Afterwards, we calculated the dissimilarity matrix using the Euclidean dissimilarity measure for all continuous variables and the Bray-Curtis dissimilarity measure for the count variable (number of leaves).

The assumptions of exchangeability of permutable units and homogeneity of multivariate dispersion were tested before analysis. When the homogeneity of multivariate dispersions was not met, the data were transformed (square root, log or inverse) and the dissimilarity matrix was recalculated. Once the assumptions were met, the model selection was performed with the Akaike Information Criterion (AIC). The model with the lowest AIC was chosen with three extra rules: The nesting variables and seedling size, and the interaction Temperature*Nutrient treatment was never dropped from the model to avoid pseudo-replication and because it was part of the hypothesis, respectively. If the final model had no significant interaction with seedling size, these interactions were dropped for simplification. The statistical analysis in the variable number of roots could not be performed due to presence of zeros in the data, which does not allow for the calculation of the Bray- Curtis dissimilarity matrix. Starch concentration in seeds was not analyzed as homogeneity of multivariate dispersion assumptions could not be met. Water parameters (DIN, phosphate, and Chl-*a* and -*b*) and temperature in aquaria were compared using PERMANOVA, while DIN and phosphate concentrations in water reservoirs were analyzed using a two-way ANOVA.

We used R software to perform the analysis (R Core Team, 2019) with the `adonis2` function of the package “vegan” (Oksanen et al. 2019). Temperature, nutrient, seed size, their interactions and the nesting structure (Temperature:ET:Aquarium) were the fixed effects in the model. The permutational unit for the model was the aquarium with 999 permutations, which is the recommended minimum number to test at an alpha-level of 0.05 (Manly 1997).

III. Results

(1) Experimental water chemistry and trophic conditions within the aquaria

Water temperature was nearly constant throughout the experiment and within the target temperatures (PERMANOVA, $P < 0.01$) (Table 1). Nutrient concentrations in the two main water reservoirs that continuously provided ASW to the aquaria were within the target concentrations throughout the experiment and were significantly different between the nutrient treatments (two-way ANOVA, $P < 0.001$). Once in the aquaria, however, nutrients were rapidly taken up resulting in low inorganic nutrient concentrations in all treatments regardless of the inputs (PERMANOVA, $P > 0.05$). In fact, some of the concentrations measured were not included in the analysis as they were below the quantification limit. Chl-*a* concentrations in the water column were higher in the high nutrient treatments (2.6-5.9 $\mu\text{g l}^{-1}$) than in the low nutrient treatments (2-2.1 $\mu\text{g l}^{-1}$) and significantly higher in the high temperature and high nutrient treatment (5.91 $\mu\text{g l}^{-1}$); Chl-*b* concentrations also increased in the high temperature and high nutrient treatment although no significant concentrations were observed among treatments (PERMANOVA, $P > 0.05$). Therefore, the different treatments effectively changed trophic conditions within aquaria, as indicated by the increased Chl-*a* and Chl-*b* concentrations, as well as other algal blooms which were observed in the high nutrient treatments.

Although the abundance of these other microorganisms could not be quantified, they were observable by naked eye and could be felt as a slimy layer on the aquaria and some seagrass leaves. They also formed fluffy masses with a slimy feel which disintegrated when attempts were made to capture them. Therefore, even though nutrient concentration parameters in aquaria were low, other observable parameters suggested eutrophic conditions were occurring in the high nutrient treatments. These symptoms were especially noticeable when high nutrients were combined with high temperature, leading to a greater growth of epiphytic algae. The salinity, pH and silicate values were constant across all treatments.

(2) Seedling traits

Seagrass seedling morphological traits and biochemical and physiological traits are shown in Tables 2, 3. Results of the PERMANOVA analyses are shown in Tables 4, 5 for morphological and biochemical and physiological traits respectively.

(a) Seedling morphological traits

Seagrass seedling morphological traits showed a greater response to temperature changes with fewer traits affected by nutrient enrichment (Table 2). Leaf traits showed the greatest differences relative to root traits, especially under different temperature treatments (Table 4). The high temperature and low nutrient treatment had 5 leaves, while all other treatments had 4 leaves per seedling. Maximum leaf length was the highest in the high temperature treatments, particularly when combined with high nutrients. This also had an impact on leaf SA and AG biomass, although significant interactions were not observed. The only seed trait that was significantly influenced by temperature was the diameter. Seed height and biomass were not significantly affected by any of the treatments (Tables 2, 4). All seedlings had two roots except for the low temperature and high nutrient treatment that just had one root (Table 2).

Table 1. Experimental water quality parameters.

	n	Treatments			
		26°C + Low nutrient	31°C + Low nutrient	26°C + High nutrient	31°C + High nutrient
DIN (μM) ^R	4	5.66 \pm 0.51		22.40 \pm 0.98	
PO ₄ ⁻ (μM) ^R	4	0.19 \pm 0.01		1.01 \pm 0.04	
Water temperature (°C)	25*	26.25 \pm 0.05	31.01 \pm 0.07	26.28 \pm 0.03	31.01 \pm 0.05
Salinity	3735 ϕ	26.47 \pm 0.00	31.13 \pm 0.00	26.22 \pm 0.00	31.48 \pm 0.00
pH	25	35.43 \pm 0.12	35.39 \pm 0.10	35.33 \pm 0.07	35.39 \pm 0.11
DIN (μM)	12	8.47 \pm 0.02	8.39 \pm 0.02	8.67 \pm 0.03	8.59 \pm 0.02
PO ₄ ⁻ (μM)	5-10	0.62 \pm 0.33	0.88 \pm 0.44	0.64 \pm 0.31	1.25 \pm 0.01
Si (μM)	5-10	0.15 \pm 0.01	0.14 \pm 0.02	0.14 \pm 0.00	0.14 \pm 0.01
Chl- <i>a</i> ($\mu\text{g l}^{-1}$)	5-10	0.71 \pm 0.07	1.15 \pm 0.22	0.91 \pm 0.20	0.85 \pm 0.06
Chl- <i>b</i> ($\mu\text{g l}^{-1}$)	24	2.07 \pm 0.53	2.07 \pm 0.53	2.59 \pm 0.98	5.91 \pm 2.41
	24	0.36 \pm 0.07	0.36 \pm 0.07	0.42 \pm 0.18	0.88 \pm 0.43

Water temperature (°C) data correspond to the multiprobe (*) and Hobo loggers (ϕ) measurements. DIN (Dissolved inorganic nitrogen as the sum of NH₄⁺, NO_x⁻ and NO₂⁻) and phosphate (PO₄⁻) concentrations in water reservoirs (R) and random aquaria are shown. Values are given as means (\pm SE).

Maximum root length and root biomass were significantly higher in the high temperature treatments (Table 4). Overall, the total seedling biomass was the highest in the high temperature treatments (Figure 2), and there was an effect of the initial seed size (Table 4). Seed biomass was the highest, with no differences among treatments, followed by AG, and lowest in BG biomass, which showed a significant decrease in low temperature treatments (Table 4). Seed size also had an effect on almost all biomass traits, BG:seed ratio and number of leaves (Table 4). The AG:BG biomass was the only morphological trait that significantly varied both with temperature and nutrients, with the highest ratio observed in the low temperature and high nutrient treatment, and the lowest ratio with high temperature and low nutrient treatment (Tables 2, 4). Also, significant differences were found in the ratio between AG:seed biomass and BG:seed biomass with highest ratios under the high temperature treatments. This trait was

also the only morphological measurement that showed an interaction between temperature and nutrient treatments.

Table 2. Morphological traits (mean \pm SE, n = 6) of *Enhalus acoroides* seedlings in the four different temperature (Temp) and nutrient treatments at the end of the experiment.

Traits	Treatments		
	Temp	Low nutrient	High nutrient
N° of leaves seedling ⁻¹	26°C	4 \pm 0.13	4 \pm 0.31
	31°C	5 \pm 0.18	4 \pm 0.35
Maximum leaf length (cm)	26°C	1.79 \pm 0.17	1.97 \pm 0.25
	31°C	3.45 \pm 0.33	4.19 \pm 0.33
Leaf width (cm)	26°C	0.35 \pm 0.01	0.33 \pm 0.01
	31°C	0.38 \pm 0.03	0.37 \pm 0.01
Leaf SA (cm ²)	26°C	3.40 \pm 0.30	3.44 \pm 0.32
	31°C	7.86 \pm 0.98	9.34 \pm 0.78
AG biomass (g FW)	26°C	0.19 \pm 0.02	0.18 \pm 0.02
	31°C	0.28 \pm 0.03	0.31 \pm 0.02
Seed diameter (cm)	26°C	1.03 \pm 0.03	1.05 \pm 0.04
	31°C	1.13 \pm 0.07	1.17 \pm 0.07
Seed height (cm)	26°C	1.14 \pm 0.03	1.11 \pm 0.03
	31°C	1.14 \pm 0.07	1.21 \pm 0.09
Seed biomass (g FW)	26°C	0.63 \pm 0.04	0.68 \pm 0.04
	31°C	0.73 \pm 0.05	0.76 \pm 0.07
N° of roots	26°C	2 \pm 0.25	1 \pm 0.06
	31°C	2 \pm 0.21	2 \pm 0.15
Maximum root length (cm)	26°C	2.92 \pm 0.42	2.43 \pm 0.21
	31°C	5.9 \pm 0.20	5.62 \pm 0.27
BG biomass (g FW)	26°C	0.12 \pm 0.02	0.05 \pm 0.00
	31°C	0.25 \pm 0.04	0.21 \pm 0.04
Ratio AG:BG	26°C	2.48 \pm 0.68	7.53 \pm 3.16
	31°C	1.30 \pm 0.16	2.11 \pm 0.28
Ratio BG:Seed	26°C	0.16 \pm 0.00	0.08 \pm 0.02
	31°C	0.33 \pm 0.05	0.27 \pm 0.04
Ratio AG:Seed	26°C	0.30 \pm 0.02	0.25 \pm 0.03
	31°C	0.40 \pm 0.02	0.43 \pm 0.03
Total biomass (g FW)	26°C	0.95 \pm 0.08	0.88 \pm 0.07
	31°C	1.23 \pm 0.13	1.28 \pm 0.13

SA, Surface area; AG, above-ground tissues; BG, below-ground tissues.

(b) Seedling physiological and biochemical traits

Leaf elongation rate was significantly higher in the high nutrient low temperature treatment (0.02 cm d⁻¹, Tables 3, 5). With the exception of biomass traits, we found that initial seed size did not matter in seedling physiological responses, growth and photosynthetic parameters.

Photosynthetic parameters used to measure the relative photosynthetic performance of the seedlings showed little differences across treatments (Figure 3, Tables 3, 5). No photoinhibition was observed under any of the treatments as shown by the RLCs (Figure 3). From the curve fit parameters, only maximum quantum yield values were significantly higher under high temperature treatments (Table 5). The photosynthetic performance of the seedlings was affected by the nested blocking variables, as reflected by the significant effect they have on rETR_{max},

alpha and Ek. Due to the high variability between enclosures, any effect of the temperature and nutrient treatments may have been confounded, and therefore we cannot draw any conclusion about the influence of these factors on *Enhalus seedlings*.

Table 3. Biochemical and physiological traits (mean \pm SE, n = 6) of *Enhalus acoroides* seedlings in the four different temperature (Temp) and nutrient treatments at the end of the experiment.

Traits	Treatments		
	Temp	Low nutrient	High nutrient
Leaf elongation rate (cm d ⁻¹)	26°C	0.01 \pm 0.00	0.02 \pm 0.00
	31°C	0.03 \pm 0.00	0.03 \pm 0.00
SA growth rate (cm ² d ⁻¹)	26°C	0.11 \pm 0.01	0.11 \pm 0.01
	31°C	0.26 \pm 0.03	0.31 \pm 0.03
Maximum quantum yield	26°C	0.73 \pm 0.01	0.70 \pm 0.03
	31°C	0.75 \pm 0.01	0.67 \pm 0.05
rETRmax	26°C	16.11 \pm 2.28	15.43 \pm 2.28
	31°C	14.54 \pm 0.77	15.10 \pm 2.38
Alpha	26°C	0.70 \pm 0.01	0.66 \pm 0.03
	31°C	0.72 \pm 0.01	0.66 \pm 0.04
Ek	26°C	8.83 \pm 1.91	8.54 \pm 3.06
	31°C	8.41 \pm 1.23	8.89 \pm 1.13
Leaf C (%DW)	26°C	31.84 \pm 0.37	30.96 \pm 0.32
	31°C	30.90 \pm 0.53	30.62 \pm 0.32
Seed C (%DW)	26°C	35.00 \pm 0.21	33.98 \pm 0.68
	31°C	33.11 \pm 1.16	33.65 \pm 0.36
Root C (%DW)	26°C	23.12 \pm 1.74	27.26 \pm 0.56
	31°C	24.40 \pm 2.23	23.62 \pm 2.81
Leaf N (%DW)	26°C	2.29 \pm 0.02	2.2 \pm 0.04
	31°C	2.26 \pm 0.05	2.26 \pm 0.07
Seed N (%DW)	26°C	1.32 \pm 0.11	1.28 \pm 0.06
	31°C	1.03 \pm 0.07	1.15 \pm 0.07
Root N (%DW)	26°C	1.84 \pm 0.17	2.08 \pm 0.2
	31°C	1.38 \pm 0.15	1.16 \pm 0.12
Leaf C:N ratio	26°C	13.9 \pm 0.18	14.1 \pm 0.21
	31°C	13.71 \pm 0.15	13.59 \pm 0.34
Seed C:N ratio	26°C	27.35 \pm 2.14	26.67 \pm 1.22
	31°C	32.73 \pm 1.88	29.79 \pm 2.02
Root C:N ratio	26°C	12.69 \pm 0.59	13.55 \pm 1.14
	31°C	17.90 \pm 0.67	20.24 \pm 1.46
Leaf sucrose (sucrose eq g ⁻¹ DW)	26°C	35.85 \pm 4.41	45.39 \pm 2.84
	31°C	49.13 \pm 2.77	48.30 \pm 2.50
Seed sucrose (sucrose eq g ⁻¹ DW)	26°C	88.42 \pm 10.60	69.09 \pm 14.40
	31°C	33.46 \pm 5.47	43.22 \pm 3.62
Leaf starch (sucrose eq g ⁻¹ DW)	26°C	7.23 \pm 1.63	7.83 \pm 0.78
	31°C	11.93 \pm 1.91	12.66 \pm 1.45
Seed starch (sucrose eq g ⁻¹ DW)	26°C	11.56 \pm 1.03	15.95 \pm 2.70
	31°C	13.06 \pm 1.27	9.63 \pm 1.23

Values of %C did not show any significant difference within any of the seedlings parts, leaves, seeds or roots (Tables 3, 5). In contrast, %N of the seeds and roots was significantly lower in the high-temperature treatments, but did not change in leaves. Therefore, C:N ratio of leaves showed no significant differences, while C:N ratio in seeds and roots were the highest under the high temperature and low nutrient treatment (Table 3). Concentrations of NSC in the leaves were significantly higher in high temperature treatments (Table 5). Although both types of

NSC, sucrose and starch, showed significant differences in the leaves among treatments ($P < 0.05$), concentrations of sucrose in the seeds showed the opposite trends. The lowest concentrations of sucrose in seeds were observed in the high temperature treatments, while starch concentration in the seeds was the lowest in the high temperature and high nutrient treatment and the highest in the low temperature and high nutrient treatment.

IV. Discussion

Overall, we found that *E. acoroides* seedlings were highly tolerant to an extended exposure to high temperature within the reported tolerance limits for adult individuals of this species with enhanced development of new tissue. There were no signs of seedling mortality nor stress response during the experiment under any of the treatments. This indicated that the initial development of the seedling from a seed of this species showed resilience under future average temperature increases expected to occur under climate change impacts. Additionally, increased temperature stimulated rapid development and growth of both AG and BG tissues compared to ambient temperature. In contrast, nutrient enrichment did not increase growth performance of *E. acoroides* seedlings, suggesting that they rely on internal nutrient and energy stores during this stage. Multiple stressor experiments are now highlighted as the necessary steps for predicting the consequences under future scenarios, as interactions between stressors could be synergistic, additive or antagonistic (Gunderson et al. 2016). In our experiment, however, the effects on most traits showed no strong interactions, indicating a lack of synergistic or antagonistic effects under combined stressors. The combined treatments, therefore, can be considered additive, in which nutrient effects were generally lacking and did not add further to the temperature effect.

(1) Seedling responses to increased temperature

The increase in size and quantity of the majority of morphological and physiological traits of the leaves and roots under increasing temperature indicates that this abiotic factor may be an important driver of seedling development of tropical species at the seedling phase.

This is in contrast to previous studies on subtropical and temperate seagrass species that showed negative effects on seedling performance under increasing temperatures (Abe et al. 2009; Niu et al. 2012; Guerrero-Meseguer et al. 2017; Pereda-Briones et al. 2019). The seedlings of *Zostera japonica*, for example, survived up to 29°C, but died at temperatures above 30°C in a temperature tolerance study (Abe et al. 2009). In *Zostera marina* seedlings, growth was inhibited at temperatures higher than 30°C, and photochemical pigments were negatively

affected at 25°C, a temperature that is 8 to 9°C higher than the optimum temperature for this species (Niu et al. 2012). The opposite effect of rising temperatures between our study and other studies could be due to different reasons, such as the inter-species differences to responses to temperature changes. Unlike *Z. marina*, which is a temperate species, *E. acoroides* is tropical, with a higher temperature range in its native distribution. Meanwhile, positive effects of temperature have also been observed during germination processes in some subtropical seagrass species, such as *Ruppia sinensis* collected from northern China (Gu et al. 2018). Such effects could also be positive in recently germinated seeds, such as the ones used in our study. On the other hand, selected target temperatures of the different experimental studies could draw different conclusions. As long as the temperature is not increased above the thermal tolerance of the species in question, higher physiological performance in terms of growth and photosynthesis are more probable. It is possible that seedlings in this study were still within their thermal niche, as adult *E. acoroides* plants grow naturally in seawater within a temperature range of 24 to 33°C (Agawin et al. 2001). More specifically, average seawater temperature at mid-day recorded in Barrang Lompo, Spermonde Archipelago (*E. acoroides* fruit collecting site) during December to January was $29.3 \pm 0.3^\circ\text{C}$ (Artika et al. 2019), and $31.5 \pm 0.1^\circ\text{C}$ during April to May (Ambo-Rappe 2014). Our study did not intend to find the optimal temperature for growth of *E. acoroides* seedlings, but rather to test the effect of increasing temperature under different nutrient regimes. Further research is required to determine the thermal niche and optimum thermal regime for these seedlings, as well as temperatures in which thermal stress is observed.

The combined enhancement of the various morphological leaf trait responses, including the increase in number of leaves, maximum leaf length, leaf SA and AG biomass, confirmed the positive growth response of AG tissue of the seedlings observed under increasing temperature, specifically the higher leaf elongation rates under high temperature. Root trait responses including the increase in number of roots per seedling, maximum root length, and BG biomass, additionally supported the positive growth response of BG tissue to increasing temperature. In combination, we suggest that these morphological traits can be used as indicators of either healthy or suboptimal *E. acoroides* seedling development under climate related effects.

Table 4. Results of the permutational analysis of variance (PERMANOVA) of the effects of temperature and nutrient treatments on the morphological traits of *Enhalus acoroides* seedlings included in Table 2, with the exception of number of roots.

Traits		df	SS	R-squared	Pseudo-F	p-value
N° leaves seedling ⁻¹	Temperature	1	0.164	17.202	18.028	0.001
	Nutrient	1	0.004	0.467	0.490	0.510
	Seed size	2	0.015	1.573	0.824	0.455
	Temperature*Nutrient	1	0.002	0.187	0.196	0.695
	Nutrient*Seed size	2	0.053	5.604	2.937	0.049
	Temperature:ET:Aquarium	20	0.313	32.982	1.728	0.058
	Residual variability	44	0.399	41.985		
Max leaf length	Temperature	1	33.970	47.845	68.991	0.001
	Nutrient	1	1.393	1.962	2.829	0.109
	Seed size	2	1.835	2.584	1.863	0.180
	Temperature*Nutrient	1	0.700	0.986	1.421	0.249
	Temperature:ET:Aquarium	20	10.453	14.723	1.062	0.444
	Residual variability	46	22.649	31.900		
Leaf width	Temperature	1	6.527	9.194	6.300	0.015
	Nutrient	1	0.299	0.422	0.289	0.594
	Seed size	2	5.077	7.150	2.450	0.091
	Temperature*Nutrient	1	0.076	0.107	0.073	0.777
	Temperature:ET:Aquarium	20	11.359	15.998	0.548	0.929
	Residual variability	46	47.662	67.129		
Leaf SA	Temperature	1	29.332	41.313	44.792	0.001
	Nutrient	1	0.473	0.667	0.723	0.418
	Seed size	2	2.699	3.802	2.061	0.140
	Temperature*Nutrient	1	0.867	1.222	1.325	0.241
	Temperature:ET:Aquarium	20	7.505	10.570	0.573	0.921
	Residual variability	46	30.123	42.247		
AG biomass	Temperature	1	14.839	22.148	17.626	0.002
	Nutrient	1	0.084	0.125	0.100	0.758
	Seed size	2	4.904	7.319	2.912	0.050
	Temperature*Nutrient	1	1.076	1.605	1.278	0.272
	Temperature:ET:Aquarium	20	9.896	14.770	0.619	0.899
	Residual variability	46	36.202	54.032		
Seed diameter	Temperature	1	4.511	6.353	4.904	0.026
	Nutrient	1	0.251	0.353	0.272	0.586
	Seed size	2	4.827	6.798	2.624	0.085
	Temperature*Nutrient	1	0.032	0.046	0.035	0.846
	Temperature:ET:Aquarium	20	19.069	26.857	1.037	0.454
	Residual variability	46	42.311	59.593		
Seed height	Temperature	1	0.416	0.586	0.487	0.490
	Nutrient	1	0.055	0.077	0.064	0.805
	Seed size	2	2.395	3.373	1.399	0.271
	Temperature*Nutrient	1	0.897	1.264	1.049	0.310
	Temperature:ET:Aquarium	20	27.876	39.263	1.629	0.072
	Residual variability	46	39.360	55.437		

Table 4 continued.

Traits		df	SS	R-squared	Pseudo-F	p-value
Seed biomass	Temperature	1	1.771	2.644	1.611	0.207
	Nutrient	1	0.382	0.571	0.348	0.566
	Seed size	2	6.238	9.310	2.837	0.075
	Temperature*Nutrient	1	0.021	0.031	0.019	0.892
	Temperature:ET:Aquarium	20	11.315	16.888	0.542	0.928
	Residual variability	46	47.273	70.556		
Max root length	Temperature	1	29.864	42.663	45.703	0.001
	Nutrient	1	0.111	0.159	0.171	0.672
	Seed size	2	3.155	4.508	2.414	0.115
	Temperature*Nutrient	1	0.356	0.508	0.544	0.458
	Temperature:ET:Aquarium	20	7.110	10.157	0.544	0.926
	Residual variability	46	29.404	42.006		
BG biomass	Temperature	1	18.659	26.280	27.083	0.001
	Nutrient	1	0.678	0.955	0.984	0.349
	Seed size	2	7.049	3.929	5.116	0.011
	Temperature*Nutrient	1	0.606	0.854	0.880	0.373
	Temperature:ET:Aquarium	20	12.316	17.347	0.894	0.590
	Residual variability	46	31.692	44.636		
Ratio AG:BG	Temperature	1	7.837	13.750	10.418	0.003
	Nutrient	1	4.448	7.804	5.912	0.018
	Seed size	2	0.789	1.383	0.524	0.628
	Temperature*Nutrient	1	0.203	0.356	0.270	0.605
	Temperature:ET:Aquarium	20	18.897	33.152	1.322	0.233
	Residual variability	46	24.826	43.555		
Ratio BG:Seed	Temperature	1	20.217	30.174	31.709	0.001
	Nutrient	1	0.861	1.286	1.351	0.246
	Seed size	2	7.188	10.728	5.637	0.008
	Temperature*Nutrient	1	1.277	1.906	2.003	0.147
	Temperature:ET:Aquarium	20	10.041	14.987	0.829	0.665
	Residual variability	46	27.416	40.919		
Ratio AG:Seed	Temperature	1	20.678	30.863	31.457	0.001
	Nutrient	1	0.107	0.160	0.163	0.678
	Seed size	2	0.926	1.382	0.704	0.502
	Temperature*Nutrient	1	2.973	4.437	4.523	0.032
	Temperature:ET:Aquarium	20	14.049	20.696	1.125	0.370
	Residual variability	46	28.266	42.188		
Total biomass	Temperature	1	8.619	12.864	10.194	0.003
	Nutrient	1	0.085	0.127	0.100	0.752
	Seed size	2	8.243	12.303	4.875	0.014
	Temperature*Nutrient	1	0.110	0.164	0.130	0.711
	Nutrient*Seed size	2	6.267	9.353	3.706	0.040
	Temperature:ET:Aquarium	20	9.011	13.449	0.561	0.905
	Residual variability	46	34.666	51.740		

P-values are in bold when significant differences were observed (≤ 0.05) SA, Surface area; AG, above-ground tissues; BG, below-ground tissues.

Overall, the strategy of seedlings also showed that under lower temperature (which is suboptimal for the tropical species), more energy goes for development of AG biomass first and less biomass allocation to BG tissues (see Figure 2). This was contrary to biomass accumulation under high temperature treatments, where AG and BG tissues were equally supported, suggesting that temperature plays a role in determining energy resource allocation in tropical seagrass seedling development. This finding is interesting in the context of climate change, specifically with respect to possible pole-wards migration. Many tropical species ranges are moving to higher latitudes as average water temperatures rise. These include animals and plants (Doney et al. 2011), and *E. acoroides* could be expected to migrate in a similar manner to mangroves (Osland et al. 2016). However, this energy budget allocation pattern might be a limiting factor because root development is key when establishing and maintaining a seagrass meadow. Less BG development under temperatures at the lower range of tolerance (even seasonally) could make otherwise suitable habitat difficult to colonize, especially as hydrodynamic forces (e.g., wave action) tend to increase in the subtropics and temperate regions compared to the tropical/equatorial region. The slow root development under high nutrient levels could further impede seedling establishment in cooler waters with natural or manmade eutrophic conditions.

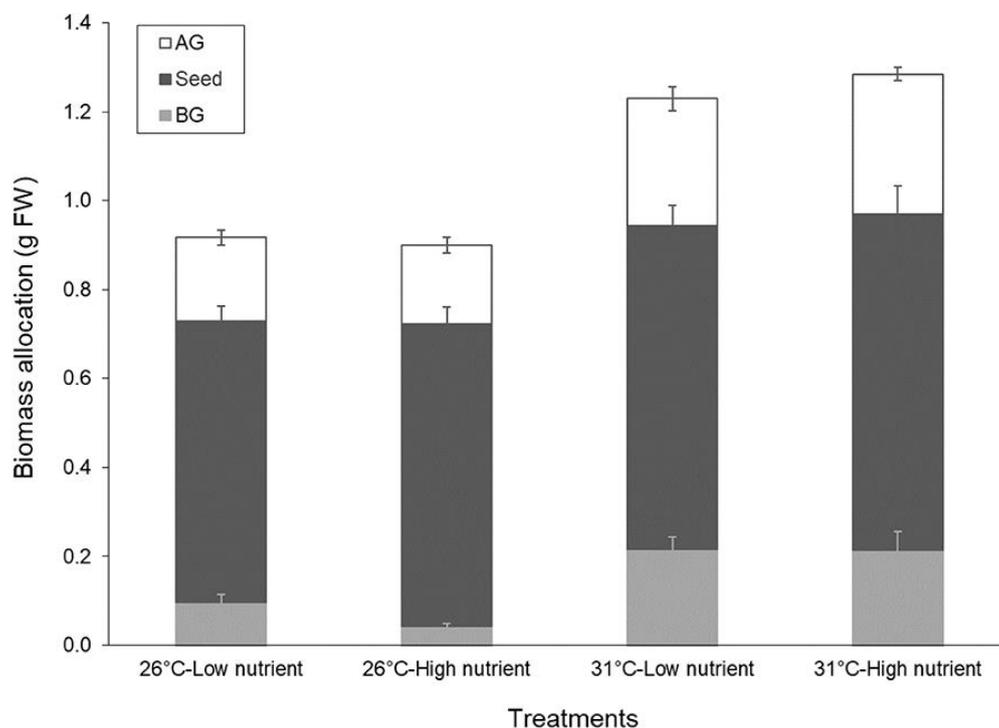


Figure 2. Allocation of above-ground (blades, AG), seed, and below-ground (roots, BG) biomass in the seedlings of *Enhalus acoroides* in the four different temperature and nutrient treatments at the end of the experiment. Values are mean \pm SE (n = 6). Statistical results are shown in Table 4.

Additionally, seed size of the seedlings matters for morphological responses; this has been widely observed in terrestrial plants (e.g., Kennedy et al. 2004) and just in *Posidonia australis* seagrass seedlings (Glasby et al. 2014). This could be related with the positive relation found between starch and nutrient contents and seedling size (Delefosse et al. 2016). In our study, the decrease in %N and sucrose content of the seed also indicates that growth and biomass allocation was supported by internal nutrient and energy stores found in the seed. This has been previously observed in temperate seagrass seedlings under different stressors, such as CO₂ enrichment, invasive algae or temperature (Hernán et al. 2016; Guerrero-Meseguer et al. 2017; Pereda-Briones et al. 2019).

(2) Seedling response to increased nutrients

Our results show that nutrient affected the AG:BG ratio and leaf elongation rate of *E. acoroides* seedlings only at the low temperature treatment. This suggests that the initial development of the seedling phase of *E. acoroides* does not depend much on the availability of external nutrients, at least on these newly germinated seedlings. While it seems that seedlings of some opportunistic species, namely *C. nodosa* or *Amphibolis antarctica*, take nutrients from the water (Paling and McComb 1994; Alexandre et al. 2018), other studies show that seagrass seedlings in some other species, including *Posidonia oceanica*, use internal resources on the early stages of development (Balestri et al. 2009). Moreover, despite this capacity for nutrient uptake, the responses in terms of growth and enhanced morphological features are variable, even within the same species (Zarranz et al. 2010; Pereda-Briones et al. 2018). Different factors including environment, availability of phosphate and its balance with nitrogen, seedling's age, or the seed size have been discussed as the reason for the variability of responses (Balestri et al. 2009; Delefosse et al. 2016; Alexandre et al. 2018).

This last feature, seed size, is related with the absolute quantity of nutrients in the seeds (Delefosse et al. 2016). In this way, *E. acoroides* seedlings have common features with other persistent species such as *Posidonia sp.*, as seeds are bigger in size compared to other fast-growing species (Orth et al. 2007). Therefore, the ability of *E. acoroides* to produce big nutrient-rich seeds, as also observed in other persistent species like *P. oceanica*, may be a strategy to allow prolonged seedling development in oligotrophic environments (Balestri et al. 2009). In this way, studies with persistent seagrasses showed that seedling growth was more dependent on seed nutrient reserves rather than the external nutrient additions (Statton et al. 2014).

In this study, the eutrophic conditions in the high nutrient treatment aquaria are confirmed by the enhanced microalgae growth and the significant effect on some adult seagrass biochemical traits, including higher leaf nitrogen, free amino acid content or enhanced leaf SA (Viana et al. in prep). These adult seagrasses, namely *Thalassia hemprichii* and *Cymodocea serrulata*, have greater SA and potentially higher absolute uptake rates than seedlings, therefore differences between them could be expected. Moreover, the former rely mainly on external nutrient concentrations for growth (Viana et al. 2019), contrary to recently germinated seedlings which can use resources stored in the seed (Balestri et al. 2009). Competition for nutrients among microalgae, seagrass adults and seedlings, however, cannot be discarded, but was not directly measured in this study. Nevertheless, enhanced leaf elongation and AG:BG ratio, plus evidence of former studies for persistent seagrasses and the seed features of *E. acoroides* suggest that it is very likely that the seedlings in this study rely primarily on nutrient reserves in the seeds. The findings of this study are the first data available on *Enhalus* seedling response to nutrients, and, as far as we know, is the first study among tropical seagrass seedlings. With the exception of a few studies on seedlings from temperate seagrasses in which no positive effects on physiology, growth, survival or photosynthetic potential were observed with nutrients (Glasby et al. 2014; Alexandre et al. 2018), most studies on nutrient enrichment effects have been done on adult stages of seagrasses with mixed results. For example, while nutrient enrichment often has an effect on seagrass tissue nutrient content (e.g. Ontoria et al. 2019; Viana et al. in prep), it does not always have a significant effect on leaf length, leaf width and seagrass production, as has been shown in *Thalassia testudinum* (Heck et al. 2000). *Z. marina*, on the other hand, has been found to survive and stay healthy under high nutrient enrichment for 2 weeks, as under natural conditions this species is acclimated and often exposed to high nutrient concentrations (Kaldy 2014). Other studies that have reviewed seagrass responses to nutrients show that a number of environmental factors, such as sedimentary characteristics or water velocity, and intraspecific characteristics, such as seagrass leaf SA, influence nutrient limitation in seagrasses (Short 1987; Lee et al. 2007).

Our results are in line with previous studies which did not observe interactive effects of both factors on adult temperate seagrass plants (Touchette and Burkholder 2002; Kaldy 2014; Moreno-Marín et al. 2018; Mvungi and Pillay 2019; Ontoria et al. 2019) and tropical seagrass plants (Viana et al. in prep). In these studies, the combination of effects of nutrient enrichment of the water column and increasing temperature did not show clear effects on seagrass plants. In contrast, other previous studies showed that morphological traits, such as leaf length, growth

Table 5. Results of the permutational analysis of variance (PERMANOVA) of the effects of temperature and nutrient treatments on the biochemical and physiological traits of *Enhalus acoroides* seedlings included in Table 3, with the exception of starch concentration in seeds.

Traits		df	SS	R-squared	Pseudo-F	p-value
Leaf elongation rate	Temperature	1	27.285	38.429	51.351	0.001
	Nutrient	1	2.942	4.144	5.537	0.024
	Seed size	2	0.911	1.283	0.857	0.406
	Temperature*Nutrient	1	0.827	1.164	1.556	0.214
	Temperature:ET:Aquarium	19	14.594	20.555	1.373	0.178
	Residual variability	43	24.441	34.425		
SA growth rate	Temperature	1	15.632	22.656	18.276	0.001
	Nutrient	1	1.608	2.331	1.880	0.205
	Seed size	2	0.749	1.085	0.438	0.647
	Temperature*Nutrient	1	0.170	0.246	0.199	0.681
	Temperature:ET:Aquarium	20	13.205	19.137	0.772	0.734
	Residual variability	44	37.636	54.545		
Max quantum yield	Temperature	1	7.554	12.589	11.683	0.003
	Nutrient	1	0.088	0.146	0.136	0.689
	Seed size	2	1.104	1.840	0.854	0.434
	Temperature*Nutrient	1	0.013	0.022	0.021	0.889
	Temperature:ET:Aquarium	17	26.673	44.455	2.427	0.018
	Residual variability	38	24.569	40.948		
rETRmax	Temperature	1	0.001	0.002	0.001	0.969
	Nutrient	1	0.000	0.000	0.000	0.989
	Seed size	2	1.377	2.295	0.639	0.546
	Temperature*Nutrient	1	1.491	2.486	1.384	0.255
	Temperature:ET:Aquarium	17	16.188	26.981	0.884	0.589
	Residual variability	38	40.941	68.236		
Alpha	Temperature	1	1.739	2.898	2.605	0.136
	Nutrient	1	1.071	1.786	1.605	0.210
	Seed size	2	2.331	3.886	1.747	0.184
	Temperature*Nutrient	1	0.029	0.048	0.044	0.832
	Temperature:ET:Aquarium	17	29.466	49.110	2.597	0.012
	Residual variability	38	25.363	42.272		
Ek	Temperature	1	0.004	0.007	0.005	0.966
	Nutrient	1	0.015	0.025	0.020	0.890
	Seed size	2	2.173	3.621	1.393	0.237
	Temperature*Nutrient	1	0.053	0.088	0.068	0.800
	Temperature:ET:Aquarium	17	28.129	46.882	2.122	0.035
	Residual variability	38	29.626	49.376		
Leaf C	Temperature	1	2.726	11.855	3.971	0.059
	Nutrient	1	2.277	9.9	3.316	0.078
	Temperature*Nutrient	1	0.605	2.634	0.882	0.351
	Temperature:ET	2	5.031	21.876	3.664	0.050
	Residual variability	18	12.359	53.735		
Seed C	Temperature	1	1.806	8.212	2.592	0.121
	Nutrient	1	1.692	7.692	2.427	0.132
	Temperature*Nutrient	1	0.467	2.125	0.671	0.425
	Temperature:ET	2	6.182	28.101	4.434	0.039
	Residual variability	16	11.851	53.869		

Table 5 continued.

Traits		df	SS	R-squared	Pseudo-F	p-value
Root C	Temperature	1	0.200	1.004	0.228	0.663
	Nutrient	1	2.838	14.194	3.217	0.080
	Temperature*Nutrient	2	3.710	18.552	4.205	0.058
	Temperature:ET	2	0.015	0.077	0.009	0.995
	Residual variability	15	13.234	66.174		
Leaf N	Temperature	1	0.103	0.448	0.114	0.741
	Nutrient	1	1.064	4.628	1.177	0.284
	Temperature*Nutrient	1	1.269	5.520	1.404	0.274
	Temperature:ET	2	4.282	18.620	2.368	0.121
	Residual variability	18	16.280	70.784		
Seed N	Temperature	1	6.341	27.571	12.495	0.005
	Nutrient	1	0.307	1.335	0.605	0.459
	Temperature*Nutrient	1	0.849	3.694	1.674	0.205
	Temperature:ET	2	6.366	27.681	6.272	0.008
	Residual variability	18	9.135	39.719		
Root N	Temperature	1	11.476	49.896	21.096	0.001
	Nutrient	1	0.001	0.005	0.002	0.976
	Temperature*Nutrient	1	1.250	5.435	2.298	0.118
	Temperature:ET	2	0.480	2.091	0.442	0.660
	Residual variability	18	9.791	42.573		
Leaf C:N ratio	Temperature	1	2.754	11.976	2.624	0.124
	Nutrient	1	0.047	0.208	0.046	0.830
	Temperature*Nutrient	1	0.574	2.496	0.547	0.471
	Temperature:ET	2	0.729	3.171	0.347	0.690
	Residual variability	18	18.894	82.148		
Seed C:N ratio	Temperature	1	5.227	22.729	8.125	0.009
	Nutrient	1	0.953	4.145	1.482	0.257
	Temperature*Nutrient	1	0.369	1.607	0.574	0.466
	Temperature:ET	2	4.868	21.165	3.783	0.042
	Residual variability	18	11.581	50.354		
Root C:N ratio	Temperature	1	14.495	63.026	41.555	0.001
	Nutrient	1	1.054	4.584	3.023	0.098
	Temperature*Nutrient	1	0.224	0.977	0.644	0.415
	Temperature:ET	2	0.945	4.113	1.356	0.296
	Residual variability	18	6.279	27.301		
Leaf sucrose	Temperature	1	9.590	20.404	11.396	0.004
	Nutrient	1	1.423	3.028	1.691	0.210
	Temperature*Nutrient	1	0.001	0.001	0.001	0.980
	Temperature:ET	2	0.644	1.371	0.383	0.684
	Residual variability	18	35.342	75.196		

Table 5 continued.						
Traits		df	SS	R-squared	Pseudo-F	p-value
Seed sucrose	Temperature	1	10.062	21.408	11.757	0.003
	Nutrient	1	0.000	0.000	0.000	0.992
	Temperature*Nutrient	2	0.022	0.047	0.026	0.876
	Temperature:ET	2	0.972	2.068	0.568	0.581
	Residual variability	15	35.944	76.477		
Leaf starch	Temperature	1	17.129	36.446	24.453	0.001
	Nutrient	1	0.096	0.204	0.137	0.696
	Temperature*Nutrient	1	0.157	0.334	0.224	0.630
	Temperature:ET	2	0.197	0.419	0.140	0.864
	Residual variability	18	29.421	62.598		

P-values are in bold when significant differences were observed (≤ 0.05).

or number of leaves per shoot, were the most variable traits under the influence of both factors (Bintz et al. 2003; Mvungi and Pillay 2019). Interactive effects on the response in the %N of the leaves were only observed in *Z. marina* (Moreno-Marín et al. 2018). Otherwise, interactive effects were observed when temperature was combined with other nutrient sources such as labile organic C in the sediment in *C. nodosa* (Ontoria et al. 2019). Therefore, there is still a limited interaction between temperature and nutrient enrichment.

Although nutrient effects may be considered as positive on seagrass individual traits, many studies of nutrient enrichment on seagrasses address the negative indirect effects of eutrophication. This is supported by the fact that enrichment of nutrients can cause algae blooms which will reduce light and contribute to the decline of seagrass (McGlathery 2001). In our experiment, nutrient treatments led to higher chlorophyll concentrations in the water. However, no significant negative effects of the eutrophication in our tanks were found on seedling morphology and physiology, indicating that light was not limiting seedling growth. This is furthermore supported by the lack of an effect on the photosynthetic performance across treatments, as shown by the fluorescence data (see Table 3). This implies that initial seedling development is not light or nutrient limited. Actually, while $rETR_{max}$ values for adult *E. acoroides* individuals fall within 45–200 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ (Jiang et al. 2014) values in our study are 3- to 8-fold lower (Figure 3). This suggests that photosynthesis in *E. acoroides* seedlings might develop later, as observed in experiments with *C. nodosa* (Alexandre et al. 2018).

(3) Ecological implications

In addition to being a critical stage (Ambo-Rappe and Yasir 2015) for further seagrass development, the seedling stage response to climate change and nutrient enrichment has been little studied compared to adult seagrasses (Touchette and Burkholder 2002; Kaldy 2014; Moreno-Marín et al. 2018; Mvungi and Pillay 2019; Ontoria et al. 2019). Overall, as far as we know, even less data is available on seagrass seedling responses in tropical species. This is the first study to suggest that the seedling stage of a tropical seagrass may be tolerant to and even positively affected by an extended exposure to the current ambient maximum temperature. This implies that extended exposure to warmer temperatures such as those close to the maximum as that expected under climate change, will not affect seedling survival. To further test temperature tolerance of tropical seagrass seedlings, however, we would need to carry out experiments under a higher range of temperatures (above 32°C).

Additionally, we showed that increased nutrient inputs may play a less important role in seedling growth response during its initial growth phase, due to internal nutrient reserves in the seed. As nutrient enrichment has direct and indirect effects, the seedling performance under more persistent eutrophication processes, during which organic matter concentration increases in the sediment or light deprivation happens, should be addressed. We should also consider further studies on the effect of increasing temperature and nutrients on reproductive outputs of the adult plants, including number of fruits, number of seed per fruit, and the size on the fruit and the seed, as well as energy reserves of the seed. All these factors combined will influence long term seagrass resilience to climate related and local stressors, as well as restoration programs based on seedling establishment.

This experiment provides interesting results but there are still a large number of gaps and the need to continue researching how factors related to global change affects the success in seedlings of seagrass. This information would help in future management plans and recovery.

Moreover, this is the first step in studying the combined impact of temperature and nutrients on seagrass seedlings. In the natural environment, biotic and abiotic interactions with the other elements in the ecosystem might also change (Brodeur et al. 2015; Hernán et al. 2017; Pereda-Briones et al. 2019) and, therefore, need to be considered in combination with these stressors. Variations in seedling traits due to temperature and nutrients might have an influence in their functions, and therefore in a number of seagrass ecosystem services, as has been observed to happen with other stressors (Hernán et al. 2016, 2017). Additionally, climate change not only

will bring higher average temperatures but also more frequent and adverse events, such as heat waves, that might affect seagrass seedlings differently than smaller increments of higher constant temperature exposure (Guerrero-Meseguer et al. 2017).

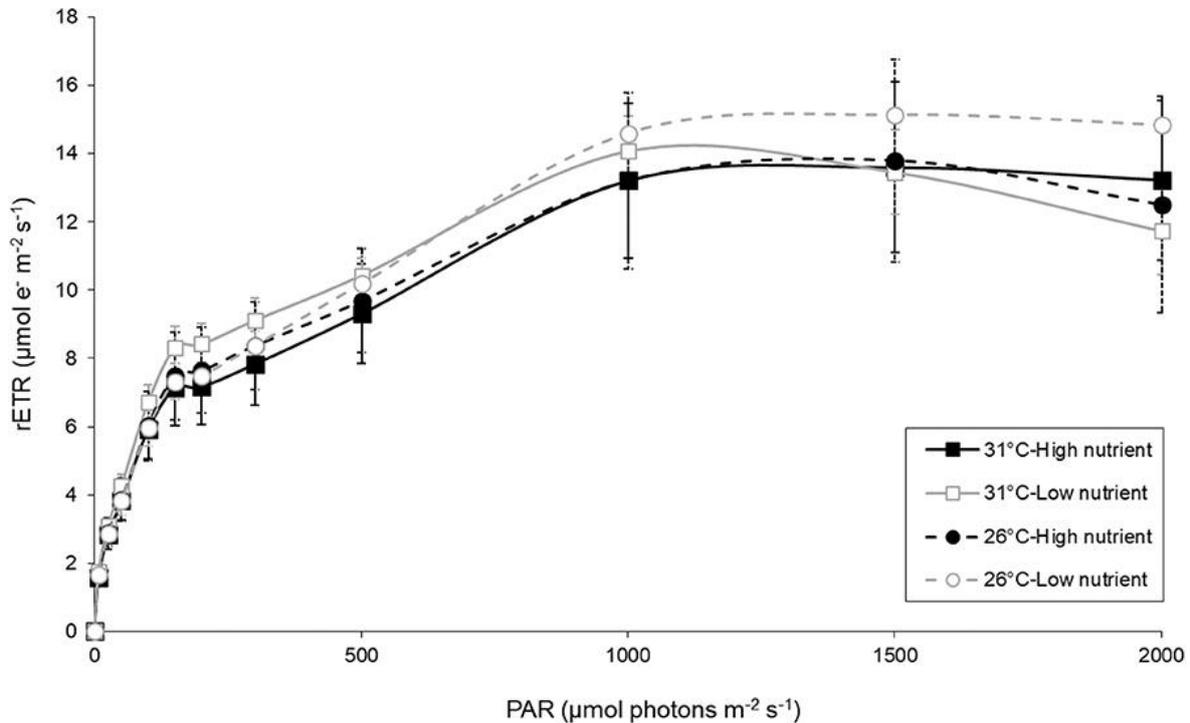


Figure 3. Rapid light curves. Relative electron transport rate (rETR) as a function of photosynthetic active radiation (PAR) on leaves of *Enhalus acoroides* seedlings in the four different temperature and nutrient treatments at the end of the experiment. Values are mean \pm SE (n = 5-6).

Enhanced growth under sub-lethal temperatures at the seedling phase could improve the resilience capacity of *E. acoroides* under natural heat stress processes or in restoration attempts. Higher temperatures will enhance a faster AG and BG development providing further root development. This is critical for stabilization into sediment, and the survival of the whole plant in the following life stages. This step, when the anchoring capacity of the seedling happens, is the bottleneck to seagrass further development (Ambo-Rappe and Yasir 2015). The ability of seeds to germinate and remain attached to ever-changing sediments within the marine environment is a critical factor in the establishment of new populations and the ongoing survival of pre-existing genetically diverse populations.

Combining morphological, biochemical, physiological, and life history traits allowed us to better understand the effects of environmental stressors on ecological functioning and survival of seedlings, and the influence on future seagrass community dynamics. Further study of seedling and adult plant traits of other tropical seagrass species is important to filling gaps of

knowledge of tropical seagrass ecology and future consequences of environmental change on this critical ecosystem.

V. Acknowledgments

We would like to thank to the Chemistry laboratory and MAREE facilities at the ZMT for the technical support during the experiment and the sample analysis. We are grateful to the two reviewers for their useful comments that have helped to improve the first version of the manuscript.

SA was supported by PMDSU (Pendidikan Magister Menuju Doktor untuk Sarjana Unggul) scholarship (SP DIPA- 042.06.1.401516/2018) from the Ministry of Research and Technology of Higher Education (Indonesia) in collaboration with Hasanuddin University. The present paper is part of the project SEAMAC [Seagrass and macroalgal community dynamics and performance under environmental change funded by the German Research Foundation (DFG; TE 1046/3-1)] awarded to MT. IV was awarded with a postdoctoral fellowship of the Leibniz-DAAD postdoctoral programme (Germany) and Xunta de Galicia (Consellería de Educación, Universidad e Formación Profesional) postdoctoral programme (ED481B-2016/189-0).

VI. References

- Abe, M., Yokota, K., Kurashima, A., and Maegawa, M. (2009). High water temperature tolerance in photosynthetic activity of *Zostera japonica* ascherson and graebner seedlings from ago bay, mie prefecture, central Japan. *Fisheries Science*, 75, 1117–1123. <https://doi.org/10.1007/s12562-009-0141-x>
- Agawin, N. S., Duarte, C. M., Fortes, M. D., Uri, J. S., and Vermaat, J. E. (2001). Temporal changes in the abundance, leaf growth and photosynthesis of three co-occurring Philippine seagrasses. *Journal of Experimental Marine Biology and Ecology*, 260, 217–239. [https://doi.org/10.1016/S0022-0981\(01\)00253-2](https://doi.org/10.1016/S0022-0981(01)00253-2)
- Alexandre, A., Silva, J., and Santos, R. (2018). Light is more important than nutrient ratios of fertilization for *Cymodocea nodosa* seedling development. *Frontiers in Plant Science*, 9, 768. <https://doi.org/10.3389/fpls.2018.00768>
- Ambo-Rappe, R. (2014). Developing a methodology of bioindication of human-induced effects using seagrass morphological variation in Spermonde Archipelago, South Sulawesi, Indonesia. *Marine Pollution Bulletin* 86, 298–303. <https://doi.org/10.1016/j.marpolbul.2014.07.002>
- Ambo-Rappe, R., La Nafie, Y. A., Syafiuddin., Limbong, S. R., Asriani, N., Handayani, N. T., et al. (2019). Short communication: restoration of seagrass *Enhalus acoroides* using a combination of generative and vegetative techniques. *Biodiversitas* 20, 3358–3363. <https://doi.org/10.13057/biodiv/d201132>

- Ambo-Rappe, R., and Yasir, I. (2015). The effect of storage condition on viability of *Enhalus acoroides* seedlings. *Aquatic Botany* 127, 57 – 61. <https://doi.org/10.1016/j.aquabot.2015.07.004>
- Anderson, M. J., Gorley, R. N., and Clarke, K. R. (2008). *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods*. Plymouth: PRIMER-E.
- Artika, S. R., Kneer, D., Ambo-Rappe, R., Syahid, S., and Teichberg, M. (2019). Decreasing pH affects seagrass epiphyte communities. *IOP Conference Series: Earth and Environmental Science*, 253, 012024. <https://doi.org/10.1088/1755-1315/253/1/012024>
- Balestri, E., Gobert, S., Lepoint, G., and Lardicci, C. (2009). Seed nutrient content and nutritional status of *Posidonia oceanica* seedlings in the northwestern Mediterranean Sea. *Marine Ecology Progress Series*, 388, 99–109. <https://doi.org/10.3354/meps08104>
- Bewley, J. D., and Black, M. (1994). *Seeds: Physiology of Development and Germination*. Boston, MA: Springer. <https://doi.org/10.1007/978-1-4899-1002-8>
- Bintz, J. C., Nixon, S. W., Buckley, B. A., and Granger, S. L. (2003). Impacts of temperature and nutrients on coastal lagoon plant communities. *Estuaries* 26, 765. <https://doi.org/10.1007/BF02711987>
- Bird, K. T., Johnson, J. R., and Jewett-Smith, J. (1998). In vitro culture of the seagrass *Halophila decipiens*. *Aquatic Botany*, 60, 377–387. [https://doi.org/10.1016/S0304-3770\(97\)00093-4](https://doi.org/10.1016/S0304-3770(97)00093-4)
- Brodeur, M. C., Piehler, M. F., and Fodrie, F. J. (2015). Consumers mitigate heat stress and nutrient enrichment effects on eelgrass *Zostera marina* communities at its southern range limit. *Marine Ecology Progress Series*, 525, 53 – 64. <https://doi.org/10.3354/meps11186>
- Brun, F. G., Hernández, I., Vergara, J. J., Peralta, G., and Pérez-Lloréns, J. L. (2002). Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. *Marine Ecology Progress Series*, 225, 177 – 187. <https://doi.org/10.3354/meps225177>
- Bulthuis, D. A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany*, 2, 27–40. [https://doi.org/10.1016/0304-3770\(87\)90084-2](https://doi.org/10.1016/0304-3770(87)90084-2)
- Burkholder, J. M., Mason, K. M., and Glasgow, H. B. Jr. (1992). Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. *Marine Ecology Progress Series*, 81, 163 – 178. <https://doi.org/10.3354/meps081163>
- Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, 350, 46 – 72. <https://doi.org/10.1016/j.jembe.2007.06.024>
- Cambridge, M. L., Chiffings, A. W., Brittan, C., Moore, L., and McComb, A. J. (1986). The loss of seagrass in cockburn sound, Western Australia. II. Possible causes of seagrass decline. *Aquatic Botany*, 24, 269–285. [https://doi.org/10.1016/0304-3770\(86\)90062-8](https://doi.org/10.1016/0304-3770(86)90062-8)

- Campbell, J. E., Altieri, A. H., Johnston, L. N., Kuempel, C. D., Paperno, R., Paul, V. J., et al. (2018). Herbivore community determines the magnitude and mechanism of nutrient effects on subtropical and tropical seagrasses. *Journal of Ecology*, 106, 401–412. <https://doi.org/10.1111/1365-2745.12862>
- Campbell, S. J., McKenzie, L. J., and Kerville, S. P. (2006). Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *Journal of Experimental Marine Biology and Ecology*, 330, 455–468. <https://doi.org/10.1016/j.jembe.2005.09.017>
- Collier, C. J., and Waycott, M. (2014). Temperature extremes reduce seagrass growth and induce mortality. *Marine Pollution Bulletin*, 83, 483–490. <https://doi.org/10.1016/j.marpolbul.2014.03.050>
- Cullen-Unsworth, L., and Unsworth, R. (2013). Seagrass meadows, ecosystem services, and sustainability. *Environment: Science and Policy for Sustainable Development*, 55, 14–28. <https://doi.org/10.1080/00139157.2013.785864>
- Cullen-Unsworth, L. C., Nordlund, L. M., Paddock, J., Baker, S., McKenzie, L. J., and Unsworth, R. K. (2014). Seagrass meadows globally as a coupled social–ecological system: implications for human wellbeing. *Marine Pollution Bulletin*, 83, 387–397. <https://doi.org/10.1016/j.marpolbul.2013.06.001>
- De los Santos, C. B., Onoda, Y., Vergara, J. J., Pérez-Lloréns, J. L., Bouma, T. J., La Nafie, Y. A., et al. (2016). A comprehensive analysis of mechanical and morphological traits in temperate and tropical seagrass species. *Marine Ecology Progress Series*, 551, 81–94. <https://doi.org/10.3354/meps11717>
- Delefosse, M., Povidisa, K., Poncet, D., Kristensen, E., and Olesen, B. (2016). Variation in size and chemical composition of seeds from the seagrass *Zostera marina*—ecological implications. *Aquatic Botany*, 131, 7–14. <https://doi.org/10.1016/j.aquabot.2016.02.003>
- Diaz-Almela, E., Marbà, N., and Duarte, C. M. (2007). Consequences of Mediterranean warming events in seagrass (*Posidonia oceanica*) flowering records. *Global Change Biology*, 13, 224–235. <https://doi.org/10.1111/j.1365-2486.2006.01260.x>
- Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, P. B., Chan, F., English, C. A., et al. (2011). Climate change impacts on marine ecosystems. *Annual Review in Marine Science*, 4, 11–37. <https://doi.org/10.1146/annurev-marine-041911-111611>
- Duarte, C. M. (1991). Seagrass depth limits. *Aquatic Botany*, 40, 363–377. [https://doi.org/10.1016/0304-3770\(91\)90081-F](https://doi.org/10.1016/0304-3770(91)90081-F)
- Duarte, C. M. (1995). Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, 41, 87–112. <https://doi.org/10.1080/00785236.1995.10422039>
- Duarte, C. M. (2000). Marine biodiversity and ecosystem services: an elusive link. *Journal of Experimental Marine Biology and Ecology*, 250, 117–131. [https://doi.org/10.1016/S0022-0981\(00\)00194-5](https://doi.org/10.1016/S0022-0981(00)00194-5)

- Duarte, C.M. (2001). *Encyclopedia of Biodiversity Vol. 1: Seagrasses*. SanDiego, CA: Academic Press. <https://doi.org/10.1016/b0-12-226865-2/00241-8>
- Duarte, C. M., and Cebrián, J. (1996). The fate of marine autotrophic production. *Limnology and Oceanography*, 41, 1758–1766. <https://doi.org/10.4319/lo.1996.41.8.1758>
- Duarte, C.M., Uri, J. S., Agawin, N. S. R., Fortes, M. D., Vermaat, J. E., and Marbà, N. (1997). Flowering frequency of Philippine seagrasses. *Botanica. Marina*, 40, 497–500. <https://doi.org/10.1515/botm.1997.40.1-6.497>
- Egea, L. G., Jiménez–Ramos, R., Hernández, I., and Brun, F. G. (2019). Effect of in situ short–term temperature increase on carbon metabolism and dissolved organic carbon (DOC) fluxes in a community dominated by the seagrass *Cymodocea nodosa*. *PLoS ONE* 14, e0210386. <https://doi.org/10.1371/journal.pone.0210386>
- Fonseca, M. S., Fourqurean, J.W., and Hoehl, M. A. R. (2019). Effect of seagrass on current speed: importance of flexibility vs. shoot density. *Frontiers in Marine Science*, 6, 376. <https://doi.org/10.3389/fmars.2019.00376>
- Fourqurean, J.W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., et al. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, 5, 505–509. <https://doi.org/10.1038/ngeo1477>
- Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *BBA Gen. Subjects*, 990, 87–92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9)
- Glasby, T. M., Taylor, S. L., and Housefield, G. P. (2014). Factors influencing the growth of seagrass seedlings: a case study of *Posidonia australis*. *Aquatic Botany*, 120, 251–259. <https://doi.org/10.1016/j.aquabot.2014.09.003>
- González-Ortiz, V., Egea, L. G., Jiménez-Ramos, R., Moreno-Marín, F., Pérez-Lloréns, J. L., Bouma, T. J., et al. (2014). Interactions between seagrass complexity, hydrodynamic flow and biomixing alter food availability for associated filter-feeding organisms. *PLoS ONE*, 9, e104949. <https://doi.org/10.1371/journal.pone.0104949>
- Grasshoff, K., Kremling, K., and Ehrhardt, M. (1999). *Methods of Seawater Analysis*. New York; Chichester; Brisbane; Singapore; Toronto: Wiley-VCH. <https://doi.org/10.1002/9783527613984>
- Gu, R., Zhou, Y., Song, X., Xu, S., Zhang, X., Lin, H., et al. (2018). Effects of temperature and salinity on *Ruppia sinensis* seed germination, seedling establishment, and seedling growth. *Marine Pollution Bulletin*, 134, 177–185. <https://doi.org/10.1016/j.marpolbul.2017.08.013>
- Guerrero-Meseguer, L., Marín, A., and Sanz-Lázaro, C. (2017). Future heat waves due to climate change threaten the survival of *Posidonia oceanica* seedlings. *Environmental Pollution*, 230, 40–45. <https://doi.org/10.1016/j.envpol.2017.06.039>
- Gunderson, A. R., Armstrong, E. J., and Stillman, J. H. (2016). Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine

environment. *Annual Review of Marine Science*, 8, 357–378. <https://doi.org/10.1146/annurev-marine-122414-033953>

Heck K. L. Jr., Pennock, J. R., Valentine, J. F., Coen, L. D., and Sklenar, S. A. (2000). Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnology and Oceanography*, 45, 1041–1057. <https://doi.org/10.4319/lo.2000.45.5.1041>

Hemminga, M. A., and Duarte, C. M. (2000). *Seagrass Ecology*. Cambridge, UK: Cambridge University Press. <https://doi.org/10.4319/lo.2002.47.2.0611>

Hemminga, M. A., Harrison, P. G., and van Lent, F. (1991). The balance of nutrient losses and gains in seagrass meadows. *Marine Ecology Progress Series*, 71, 85–96. <https://doi.org/10.3354/meps071085>

Hernán, G., Castejón, I., Terrados, J., and Tomas, F. (2019). Herbivory and resource availability shift plant defense and herbivore feeding choice in a seagrass system. *Oecologia*, 189, 719–732. <https://doi.org/10.1007/s00442-019-04364-6>

Hernán, G., Ortega, M. J., Gándara, A. M., Castejón, I., Terrados, J., and Tomas, F. (2017). Future warmer seas: increased stress and susceptibility to grazing in seedlings of a marine habitat-forming species. *Global Change Biology*, 23, 4530–4543. <https://doi.org/10.1111/gcb.13768>

Hernán, G., Ramajo, L., Basso, L., Delgado, A., Terrados, J., Duarte, C. M., et al. (2016). Seagrass (*Posidonia oceanica*) seedlings in a high-CO₂ world: from physiology to herbivory. *Scientific Reports*, 6, 38017. <https://doi.org/10.1038/srep38017>

Huber, S. C., and Israel, D. W. (1982). Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max* Merr.) leaves. *Plant Physiology*, 69, 691–696. <https://doi.org/10.1104/pp.69.3.691>

Invers, O., Kraemer, G. P., Pérez, M., and Romero, J. (2004). Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. *Journal of Experimental Marine Biology and Ecology*, 303, 97–114. <https://doi.org/10.1016/j.jembe.2003.11.005>

Jassby, A. D., and Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography*, 21, 540–547. <https://doi.org/10.4319/lo.1976.21.4.0540>

Jiang, Z., Huang, X., Zhang, J., Zhou, C., Lian, Z., and Ni, Z. (2014). The effects of air exposure on the desiccation rate and photosynthetic activity of *Thalassia hemprichii* and *Enhalus acoroides*. *Marine Biology*, 161, 1051–1061. <https://doi.org/10.1007/s00227-014-2398-6>

Jiang, Z., Liu, S., Zhang, J., Wu, Y., Zhao, C., Lian, Z., and Huang, X. (2018). Eutrophication indirectly reduced carbon sequestration in a tropical seagrass bed. *Plant and Soil*, 426(1), 135–152. <https://doi.org/10.1007/s11104-018-3604-y>

- Jiménez, R., Egea, L. G., Vergara, J. J., Bouma, T. J., and Brun, F. G. (2019). The role of flow velocity combined with habitat complexity as a top–down regulator in seagrass meadows. *Oikos* 128, 64–76. <https://doi.org/10.1111/oik.05452>
- Jiménez-Ramos, R., Egea, L. G., Ortega, M. J., Hernández, I., Vergara, J. J., and Brun, F. G. (2017). Global and local disturbances interact to modify seagrass palatability. *PLoS ONE*, 12, e0183256. <https://doi.org/10.1371/journal.pone.0183256>
- Kaldy, J. E. (2014). Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. *Journal of Experimental Marine Biology and Ecology*, 453, 108–115. <https://doi.org/10.1016/j.jembe.2013.12.020>
- Kegler, H. F., Hassenrück, C., Kegler, P., Jennerjahn, T. C., Lukman, M., Jompa, J., et al. (2018). Small tropical islands with dense human population: differences in water quality of near-shore waters are associated with distinct bacterial communities. *PeerJ*, 6, e4555. <https://doi.org/10.7717/peerj.4555>
- Kennedy, P. G., Hausmann, N. J., Wenk, E. H., and Dawson, T. E. (2004). The importance of seed reserves for seedling performance: an integrated approach using morphological, physiological, and stable isotope techniques. *Oecologia*, 141, 547–554. <https://doi.org/10.1007/s00442-004-1686-0>
- Khan, M. N., and Mohammad, F. (2014). Eutrophication: challenges and solutions. In *Eutrophication: Causes, Consequences And Control* (pp. 1–15), eds A. Ansari and S. Gill (Dordrecht: Springer. https://doi.org/10.1007/978-94-007-7814-6_1
- Lee, K. S., Park, S. R., and Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology*, 350, 144–175. <https://doi.org/10.1016/j.jembe.2007.06.016>
- Lee, S. Y., Dunn, R. J. K., Young, R. A., Connolly, R. M., Dale, P. E. R., Dehayr, R., et al. (2006). Impact of urbanization on coastal wetland structure and function. *Austral Ecology*, 31, 149–163. <https://doi.org/10.1111/j.1442-9993.2006.01581.x>
- Li, M., Lundquist, C. J., Pilditch, C. A., Rees, T. A. V., and Ellis, J. (2019). Implications of nutrient enrichment for the conservation and management of seagrass *Zostera muelleri* meadows. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 1484–1502. <https://doi.org/10.1002/aqc.3141>
- Macreadie, P. I., Anton, A., Raven, J. A., Beaumont, N., Connolly, R. M., Fries, E. A., et al. (2019). The future of blue carbon science. *Nature Communications*, 10, 3998. <https://doi.org/10.1038/s41467-019-11693-w>
- Manly, B. F. J. (1997). *Randomization, Bootstrap and Monte Carlo Methods in Biology*. London: Chapman and Hall. <https://doi.org/10.1201/9781315273075>
- Marbà, N., Duarte, C. M., Cebrián, J., Gallegos, M. E., Olesen, B., and Sand-Jensen, K. (1996). Growth and population dynamics of *Posidonia oceanica* on the Spanish Mediterranean coast: elucidating seagrass decline. *Marine Ecology Progress Series*, 137, 203–213. <https://doi.org/10.3354/meps137203>

- Marco-Méndez, C., Prado, P., and Sánchez-Lizaso, J. L. (2017). Epiphytes and nutrient contents influence *Sarpa salpa* herbivory on *Caulerpa* spp vs. seagrass species in Mediterranean meadows. *Estuarine, Coastal and Shelf Science*, 184, 54–66. <https://doi.org/10.1016/j.ecss.2016.11.005>
- Martínez-Crego, B., Vergés, A., Alcoverro, T., and Romero, J. (2008). Selection of multiple seagrass indicators for environmental biomonitoring. *Marine Ecology Progress Series*, 361, 93–109. <https://doi.org/10.3354/meps07358>
- Masini, R. J., Anderson, P. K., and McComb, A. J. (2001). A *Halodule* dominated community in a subtropical embayment: physical environment, productivity, biomass, and impact of dugong grazing. *Aquatic Botany*, 71, 179–197. [https://doi.org/10.1016/S0304-3770\(01\)00181-4](https://doi.org/10.1016/S0304-3770(01)00181-4)
- McGlathery, K. J. (2001). Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology*, 37, 453–456. <https://doi.org/10.1046/j.1529-8817.2001.037004453.x>
- McMillan, C. (1984). The distribution of tropical seagrasses with relation to their tolerance of high temperatures. *Aquatic Botany*, 19, 369–379. [https://doi.org/10.1016/0304-3770\(84\)90049-4](https://doi.org/10.1016/0304-3770(84)90049-4)
- Meysick, L., Infantes, E., and Boström, C. (2019). The influence of hydrodynamics and ecosystem engineers on eelgrass seed trapping. *PLoS ONE*, 14, e0222020. <https://doi.org/10.1371/journal.pone.0222020>
- Moore, K. A., and Short, F. T. (2007). “*Zostera*: biology, ecology, and management,” in *Seagrasses: Biology, Ecology and Conservation* (pp. 361–386), eds A. W. D. Larkum, R. J. Orth, and C. M. Duarte (Dordrecht: Springer). https://doi.org/10.1007/1-4020-2983-7_16
- Moore, K. A., and Wetzel, R. L. (2000). Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems. *Journal of Experimental Marine Biology and Ecology*, 244, 1–28. [https://doi.org/10.1016/S0022-0981\(99\)00135-5](https://doi.org/10.1016/S0022-0981(99)00135-5)
- Moreno-Marín, F., Brun, F. G., and Pedersen, M. F. (2018). Additive responses to multiple environmental stressors in the seagrass *Zostera marina*. *Limnology and Oceanography*, 63, 1528–1544. <https://doi.org/10.1002/lno.10789>
- Mvungi, E. F., and Pillay, D. (2019). Eutrophication overrides warming as a stressor for a temperate African seagrass (*Zostera capensis*). *PloS ONE*, 14, e0215129. <https://doi.org/10.1371/journal.pone.0215129>
- Niu, S., Zhang, P., Liu, J., Guo, D., and Zhang, X. (2012). The effect of temperature on the survival, growth, photosynthesis, and respiration of young seedlings of eelgrass *Zostera marina* L. *Aquaculture*, 350, 98–108. <https://doi.org/10.1016/j.aquaculture.2012.04.010>
- Nixon, S., Buckley, B., Granger, S., and Bintz, J. (2001). Responses of very shallow marine ecosystems to nutrient enrichment. *Human and Ecological Risk Assessment: An International Journal*, 7, 1457–1481. <https://doi.org/10.1080/20018091095131>

- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). Vegan: Community Ecology Package. R package version 2.5- 4. Available online at: <https://CRAN.R-project.org/package=vegan> (accessed February 25, 2020).
- Ontoria, Y., Gonzalez-Guedes, E., Sanmarti, N., Bernardeau-Esteller, J., Ruiz, J. M., Romero, J., et al. (2019). Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. *Marine Environmental Research*, 145, 27–38. <https://doi.org/10.1016/j.marenvres.2019.02.002>
- Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C.M., Fourqurean, J.W., Heck, K. L., et al. (2006). A global crisis of seagrass ecosystems. *BioScience*, 56, 987–996. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:AGCFSE\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2)
- Orth, R. J., Harwell, M. C., and Inglis, G. J. (2007). Ecology of seagrass seeds and seagrass dispersal processes. In *Seagrasses: Biology, Ecology and Conservation* (pp. 111–133), eds A. W. D. Larkum, R. J. Orth, and C. M. Duarte (Dordrecht: Springer). https://doi.org/10.1007/1-4020-2983-7_5
- Osland M. J., Day, R. H., Hall, C. T., Brumfield, M. D., Dugas, J. L., and Jones, W. R. (2016). Mangrove expansion and contraction at a poleward range limit: climate extremes and land-ocean temperature gradients. *Ecology*, 98, 125–137. <https://doi.org/10.1002/ecy.1625>
- Paling, E. I., and McComb, A. J. (1994). Nitrogen and phosphorus uptake in seedlings of the seagrass *Amphibolis antarctica* in Western Australia. *Hydrobiologia*, 294, 1–4. <https://doi.org/10.1007/BF00017618>
- Pereda-Briones, L., Terrados, J., and Tomas, F. (2019). Negative effects of warming on seagrass seedlings are not exacerbated by invasive algae. *Marine Pollution Bulletin*, 141, 36–45. <https://doi.org/10.1016/j.marpolbul.2019.01.049>
- Pereda-Briones, L., Tomas, F., and Terrados, J. (2018). Field transplantation of seagrass (*Posidonia oceanica*) seedlings: effects of invasive algae and nutrients. *Marine Pollution Bulletin*, 134, 160–165. <https://doi.org/10.1016/j.marpolbul.2017.09.034>
- Peterson, J. E., and Baldwin, A. H. (2004). Seedling emergence from seed banks of tidal freshwater wetlands: response to inundation and sedimentation. *Aquatic Botany*, 78, 243–254. <https://doi.org/10.1016/j.aquabot.2003.10.005>
- R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available online at: <http://www.R-project.org/>
- Roca, G., Alcoverro, T., Krause-Jensen, D., Balsby, T. J. S., van Katwijk, M. M., Marbà, N., et al. (2016). Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis. *Ecological Indicators*, 63, 310–323. <https://doi.org/10.1016/j.ecolind.2015.12.007>
- Sakshaug, E., Bricaud, A., Dandonneau, Y., Falkowski, P. G., Kiefer, D. A., Legendre, L., et al. (1997). Parameters of photosynthesis: definitions, theory and interpretation of results. *Journal of Plankton Research*, 19, 1637–1670. <https://doi.org/10.1093/plankt/19.11.1637>

- Schielzeth, H., and Nakagawa, S. (2013). Nested by design: model fitting and interpretation in a mixed model era. *Methods in Ecology and Evolution*, 4, 14–24. <https://doi.org/10.1111/j.2041-210x.2012.00251.x>
- Schupp, E. W. (1995). Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany*, 82, 399–409. <https://doi.org/10.1002/j.1537-2197.1995.tb12645.x>
- Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350, 3–20. <https://doi.org/10.1016/j.jembe.2007.06.012>
- Short, F. T. (1987). Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. *Aquatic Botany*, 27, 41–57. [https://doi.org/10.1016/0304-3770\(87\)90085-4](https://doi.org/10.1016/0304-3770(87)90085-4)
- Short, F. T., Burdick, D. M., and Kaldy, III, J. E. (1995). Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography*, 40, 740–749. <https://doi.org/10.4319/lo.1995.40.4.0740>
- Short, F. T., and Duarte, C. M. (2001). Methods for the measurement of seagrass growth and production. In *Global Seagrass Research Methods* (pp. 155–198), eds F. T. Short and R. G. Coles (Amsterdam: Elsevier Science SV). <https://doi.org/10.1016/b978-044450891-1/50009-8>
- Short, F. T., and Neckles, H. A. (1999). The effects of global climate change on seagrasses. *Aquatic Botany*, 63, 169–196. [https://doi.org/10.1016/S0304-3770\(98\)00117-X](https://doi.org/10.1016/S0304-3770(98)00117-X)
- Short, F. T., and Wyllie-Echeverria, S. (1996). Natural and human induced disturbance of seagrasses. *Environmental Conservation*, 23, 17–27. <https://doi.org/10.1017/S0376892900038212>
- Silsbe, G., and Malkin, S. Y. (2015). Phytotools: Phytoplankton Production Tools. An R package available on CRAN.
- Statton, J., Kendrick, G. A., Dixon, K. W., and Cambridge, M. L. (2014). Inorganic nutrient supplements constrain restoration potential of seedlings of the seagrass, *Posidonia australis*. *Restoration Ecology*, 22, 196–203. <https://doi.org/10.1111/rec.12072>
- Teichberg, M., Wild, C., Bednarz, V. N., Kegler, H. F., Lukman, M., Gärdes, A. A., et al. (2018). Spatio-temporal patterns in coral reef communities of the Spermonde Archipelago, 2012–2014, I: comprehensive reef monitoring of water and benthic indicators reflect changes in reef health. *Frontiers in Marine Science*, 5, 33. <https://doi.org/10.3389/fmars.2018.00033>
- Terrados, J., Agawin, N. S., Duarte, C. M., Fortes, M. D., Kamp-Nielsen, L., and Borum, J. (1999a). Nutrient limitation of the tropical seagrass *Enhalus acoroides* (L.) royle in cape Bolinao, NW Philippines. *Aquatic Botany*, 65, 123–139. [https://doi.org/10.1016/S0304-3770\(99\)00036-4](https://doi.org/10.1016/S0304-3770(99)00036-4)
- Terrados, J., Duarte, C. M., Kamp-Nielsen, L., Agawin, N. S. R., Gacia, E., Lacap, D., et al. (1999b). Are seagrass growth and survival constrained by the reducing conditions of the sediment? *Aquatic Botany*, 65, 175–197. [https://doi.org/10.1016/S0304-3770\(99\)00039-X](https://doi.org/10.1016/S0304-3770(99)00039-X)

- Tewksbury, J. J., Huey, R. B., and Deutsch, C. A. (2008). Putting the heat on tropical animals. *Science*, *320*, 1296–1297. <https://doi.org/10.1126/science.1159328>
- Todgham, A. E., and Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integrative and Comparative Biology*, *53*, 539–544. <https://doi.org/10.1093/icb/ict086>
- Tomás, F., Martínez-Crego, B., and Santos, R. (2015). Responses of seagrass to anthropogenic and natural disturbances do not equally translate to its consumers. *Global Change Biology*, *21*, 4021–4030. <https://doi.org/10.1111/gcb.13024>
- Touchette, B. W., and Burkholder, J. M. (2002). Seasonal variations in carbon and nitrogen constituents in eelgrass (*Zostera marina* L.) as influenced by increased temperature and water-column nitrate. *Botanica Marina*, *45*, 23–34. <https://doi.org/10.1515/BOT.2002.004>
- Touchette, B. W., and Burkholder, J. M. (2007). Carbon and nitrogen metabolism in the seagrass, *Zostera marina* L.: environmental control of enzymes involved in carbon allocation and nitrogen assimilation. *Journal of Experimental Marine Biology and Ecology*, *350*, 216–233. <https://doi.org/10.1016/j.jembe.2007.05.034>
- Touchette, B. W., Burkholder, J. M., and Glasgow, H. B. Jr. (2003). Variations in eelgrass (*Zostera marina* L.) morphology and internal nutrient composition as influenced by increased temperature and water column nitrate. *Estuaries*, *26*, 142–155. <https://doi.org/10.1007/BF02691701>
- Tuya, F., Haroun, R., and Espino, F. (2014). Economic assessment of ecosystem services: monetary value of seagrass meadows for coastal fisheries. *Ocean and Coastal Management*, *96*, 181–187. <https://doi.org/10.1016/j.ocecoaman.2014.04.032>
- Unsworth, R. K., Ambo-Rappe, R., Jones, B. L., La Nafie, Y. A., Irawan, A., Hernawan, U. E., et al. (2018). Indonesia's globally significant seagrass meadows are under widespread threat. *Science of the Total Environment*, *634*, 279–286. <https://doi.org/10.1016/j.scitotenv.2018.03.315>
- Van Katwijk, M. M., Vergeer, L. H. T., Schmitz, G. H. W., and Roelofs, J. G. M. (1997). Ammonium toxicity in eelgrass *Zostera marina*. *Marine Ecology Progress Series*, *157*, 159–173. <https://doi.org/10.3354/meps157159>
- Viana, I. G., Saavedra-Hortúa, D. A., Mtolera, M., and Teichberg, M. (2019). Different strategies of nitrogen acquisition in two tropical seagrasses from an oligotrophic environment. *New Phytologist*, *223*, 1217–1229. <https://doi.org/10.1111/nph.15885>
- Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, *106*, 12377–12381. <https://doi.org/10.1073/pnas.0905620106>
- Weitzman, J. S., Zeller, R. B., Thomas, F. I. M., and Koseff, J. R. (2015). The attenuation of current- and wave-driven flow within submerged multispecific vegetative canopies. *Limnology and Oceanography*, *60*, 1855–1874. <https://doi.org/10.1002/lno.10121>

Wernberg, T., Smale, D. A., and Thomsen, M. S. (2012). A decade of climate change experiments on marine organisms: procedure, patterns and problems. *Global Change Biology*, 18, 1491–1498. <https://doi.org/10.1111/j.1365-2486.2012.02656.x>

Yemm, E. W., and Willis, A. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemistry Journal*, 57, 508–514. <https://doi.org/10.1042/bj0570508>

York, P. H., Gruber, R. K., Hill, R., Ralph, P. J., Booth, D. J., and Macreadie, P. I. (2013). Physiological and morphological responses of the temperate seagrass *Zostera muelleri* to multiple stressors: investigating the interactive effects of light and temperature. *PLoS ONE*, 8, e76377. <https://doi.org/10.1371/journal.pone.0076377>

Yue, S., Zhou, Y., Zhang, Y., Xu, S., Gu, R., Xu, S., et al. (2019). Effects of salinity and temperature on seed germination and seedling establishment in the endangered seagrass *Zostera japonica* Asch. and Graebn. in northern China. *Marine Pollution Bulletin*, 146, 848–856. <https://doi.org/10.1016/j.marpolbul.2019.07.037>

Zarranz, M. E., González-Henríquez, N., García-Jiménez, P., and Robaina, R. R. (2010). Restoration of *Cymodocea nodosa* (Uchria) Ascherson seagrass meadows through seed propagation: seed storage and influences of plant hormones and mineral nutrients on seedling growth in vitro. *Botanica Marina*, 53, 439–448. <https://doi.org/10.1515/BOT.2010.051>



CHAPTER 4.

Species-Specific trait responses of three tropical seagrasses to multiple stressors: The case of increasing temperature and nutrient enrichment

Inés G. Viana^{1,2}, Agustín Moreira-Saporiti^{2,3}, Mirta Teichberg²

¹ Department of Ecology and Animal Biology, University of Vigo, Vigo, Spain, ² Leibniz Centre for Tropical Marine Research GmbH, Bremen, Germany, ³ Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany

Abstract

Seagrass meadows are declining globally. The decrease of seagrass area is influenced by the simultaneous occurrence of many factors at the local and global scale, including nutrient enrichment and climate change. This study aims to find out how increasing temperature and nutrient enrichment affect the morphological, biochemical and physiological responses of three coexisting tropical species, *Thalassia hemprichii*, *Cymodocea serrulata* and *Halophila stipulacea*. To achieve these aims, a 1-month experiment under laboratory conditions combining two temperature (maximum ambient temperature and current average temperature) and two nutrient (high and low N and P concentrations) treatments was conducted. The results showed that the seagrasses were differentially affected by all treatments depending on their life-history strategies. Under higher temperature treatments, *C. serrulata* showed photoacclimation strategies, while *T. hemprichii* showed decreased photo-physiological performance. In contrast, *T. hemprichii* was resistant to nutrient over-enrichment, showing enhanced nutrient content and physiological changes, but *C. serrulata* suffered BG nutrient loss. The limited response of *H. stipulacea* to nutrient enrichment or high temperature suggests that this seagrass is a tolerant species that may have a dormancy state with lower photosynthetic performance and smaller-size individuals. Interaction between both factors was limited and generally showed antagonistic effects only on morphological and biochemical traits, but not on physiological traits. These results highlight the different effects and strategies co-inhabiting seagrasses have in response to environmental changes, showing winners and losers of a climate change scenario that may eventually cause biodiversity loss. Trait responses to these stressors could potentially make the seagrasses weaker to cope with following events, due to BG biomass or nutrient loss. This is of importance as biodiversity loss in tropical seagrass ecosystems could change the overall effectiveness of ecosystem functions and services provided by the seagrass meadows.

Keywords: *Cymodocea serrulata*, *Thalassia hemprichii*, *Halophila stipulacea*, morphology, storage, photophysiology, nutrient allocation, interactive effect

I. Introduction

Seagrass meadows are valued for the ecosystem services they provide that have been recorded in an increasing number of studies (Costanza et al. 1997; Kirsch et al. 2002; Romero et al. 2006; Fourqurean et al. 2012; Ondiviela et al. 2014; Dewsbury et al. 2016; Nordlund et al. 2016). Despite their global significance and them being threatened by multiple anthropogenic stressors that affect their biodiversity and functioning (Lu et al. 2018) the attention paid to seagrass meadows is much lower than other coastal ecosystems, such as coral reefs (Unsworth et al. 2019).

The most prominent stressor of seagrasses generated locally is cultural eutrophication caused by increased loading of nutrients from human activities (Beiras 2018). During the last decades, losses in seagrass meadows have been documented worldwide, especially in quiet, and poorly flushed estuaries where nutrient loads are intense and frequent (Burkholder et al. 2007). Contrary to temperate ecosystems, in the oligotrophic tropical environments, seagrass productivity is mainly limited by nutrients and not by light irradiance (Short 1987); therefore, nutrient inputs could result in drastic changes in diversity loss (Kamermans et al. 2002). As seagrasses are the only submerged marine angiosperms, they can access both nutrients from water column and pore water. Thus, below-ground (BG) tissues play a key role in taking up different nutrient sources to meet in situ demands and translocating them along the plant (Viana et al. 2019a). This is especially important in the tropics as nitrogen (N) is constantly available in the pore water at higher concentrations than in the water column, and leaf turnover is constant throughout the year, whereas BG tissues have lower turnover rates (Duarte 1991). Seagrasses are able to take advantage of nutrient pulses by increasing the enzymatic activity in response to uptake and assimilation and storing the incorporated nutrients (Viana et al. 2019a). In this way, nutrient additions can result in higher growth rates, primary productivity or nutrient content (Duarte 1990; Agawin et al. 1996; Terrados et al. 1999; Ferdie and Fourqurean 2004). However, if eutrophication persists, changes in biotic or abiotic interactions might occur, including algal blooms that increase nutrient competition and light deprivation, alterations in top-down regulation because of changes in leaf palatability, or increasing organic matter in the sediments that might create anoxic environments for benthic organisms (Burkholder et al. 2007). All these changes may ultimately affect species fitness and survival.

Although nutrient over-enrichment has been one of the main drivers of seagrass mortality worldwide, other abiotic factors, including temperature, play an important role in species distribution and survival. Temperature is one of the main drivers of biochemical reactions,

significantly affecting growth, photosynthesis, sexual reproduction and survival (Durako and Moffler 1985; Bulthuis 1987; Lee et al. 2007; Xu et al. 2016; Zayas-Santiago et al. 2020). Temperature also affects seagrass nutrient content, as lower carbon (C) and N concentrations and higher C:N ratios have been observed at increasing temperatures (Kaldy 2014; Mvungi and Pillay 2019; Ontoria et al. 2019b). While moderate elevations in temperature might be positive for seagrass performance (Artika et al. 2020), the individual fitness is lost once the thermal optimum range is exceeded often leading to increasing mortality rates (Collier and Waycott 2014). Tropical seagrasses usually grow at their upper optimal temperature limit, therefore changes in mean seawater temperatures might be critical (Koch et al. 2013). Changes in seagrass population and community structures have already been observed in areas affected by climate change (Jordà et al. 2012; Kendrick et al. 2019; Beca-Carretero et al. accepted) and will become more relevant under future climate change. Therefore, the knowledge accumulated on the effects of these individual stressors through field and laboratory experiments on temperate and tropical seagrasses is very extensive (see as example Bulthuis 1987; Burkholder et al. 1994; Campbell et al. 2006; Winters et al. 2011; Collier and Waycott 2014; Li et al. 2019).

In coastal systems, stressors rarely occur alone in the environment and, when acting together, their effects can be synergistic, additive or antagonistic (Todgham and Stillman 2013; Gunderson et al. 2016; Stockbridge et al. 2020), although synergistic effects are most likely to occur when the stress events happen simultaneously or in quick succession (Gunderson et al. 2016). Accordingly, local anthropogenic impacts of human development, such as eutrophication, can combine with indirect consequences of climate change, including sea surface temperature or CO₂ enrichment, causing even more dramatic consequences of future scenarios than initially predicted by single-factor experiments. As a result, the rates of change in seagrass ecosystems are faster than those experienced in their evolutionary history and may occur too fast to allow seagrasses to adapt (Orth et al. 2006; Waycott et al. 2009). Therefore, the interaction between stressors is now viewed as a critical issue, and it is suggested that single-factor experiments are not adequate for assessing the effects of several disturbances on coastal marine ecosystems (Wernberg et al. 2012; Todgham and Stillman 2013; Ontoria et al. 2019b). In the last years, an increasing number of papers aiming to understand cumulative impacts of stressors have exponentially increased (Gunderson et al. 2016; Adams et al. 2020; Stockbridge et al. 2020), and more empirical data on the effects of the interaction of increasing temperature and nutrient over-enrichment at an individual level has been obtained (Touchette and Burkholder 2002; Bintz et al. 2003; Touchette et al. 2003; Burnell et al. 2013; Kaldy 2014; Kaldy et al. 2017; Egea et al. 2018; Moreno-Marín et al. 2018; Mvungi and Pillay 2019; Ontoria

et al. 2019b). Nevertheless, responses depend on their local adaptation and life history traits (Tuya et al. 2019; Anton et al. 2020) are species-specific, and to our knowledge, there is very limited information about the combined effects of these two stressors in any tropical seagrasses species (Artika et al. 2020).

The study of the response of seagrass individual traits, namely biochemical, morphological, and physiological traits, serve as early indicators of environmental change (Roca et al. 2016) before population level responses, such as changes in shoot density, biomass and species composition or biodiversity loss, are detected. This approach has been used in a wide number of studies to detect rapid responses (within weeks) to different stressors (Lee et al. 2007; Roca et al. 2016; Bertelli and Unsworth 2018, and previously cited references). One important limitation of trait-based responses is that different stressors could cause the same effect, for instance, lower rhizome carbohydrate content is both observed after nutrient over-enrichment or reduced light exposures (Roca et al. 2016). More recent studies that simultaneously assess different plant individual traits have highlighted the importance of testing the responses at different levels of organization in the plants (Bertelli and Unsworth 2018; Mvungi and Pillay 2019). Moreover, a deeper understanding of the species-specific responses to stressors and their interaction is important as different combinations of seagrass traits may sustain different ecological functions that upscale to the ecosystem level (Barbier et al. 2011). Therefore, even though changes in seagrass traits could be seen as positive at an individual plant level (i.e., increasing photosynthetic rate) they could potentially change their related functions, negatively affecting the sustained ecosystem services (Burnell et al. 2013; Jiménez-Ramos et al. 2017; Soissons et al. 2018).

Tropical areas are seagrass biodiversity hotspots, gathering most of the 60 seagrass species that exist worldwide (Short et al. 2007). *Thalassia hemprichii* and *Cymodocea serrulata* are widely distributed in the Indo-Pacific bioregion. These two species have large blades and slow shoot turnover, especially *T. hemprichii* (Duarte 1991). They both form persistent mixed or monospecific meadows that sustain food webs, including commercially important species (de la Torre-Castro et al. 2014). *Halophila stipulacea* is a tolerant species native to the Indo-Pacific bioregion that has colonized both the Mediterranean and Caribbean Seas (Winters et al. 2020). It has a smaller size than most seagrasses, so it frequently grows in sand patches or in the edges of bigger seagrass meadows, but it can also grow mixed with other macrophytes or form large monospecific meadows (Boudouresque et al. 2009; Sghaier et al. 2011). It presents faster shoot turnover and growth than the two other species (Duarte 1991). All three species have been

observed to tolerate different trophic conditions (Van Tussenbroek et al. 2016; Mwaura et al. 2017; Thomsen et al. 2020; Teichberg et al. in preparation) and adverse maximum temperatures (Campbell et al. 2006; Georgiou et al. 2016; Pedersen et al. 2016; Collier et al. 2017; George et al. 2018; Anton et al. 2020; Nguyen et al. 2020; Winters et al. 2020; Beca-Carretero et al. accepted). Furthermore, *T. hemprichii*, *C. serrulata* and *H. stipulacea* show differences in their life-history traits representing permanent, opportunistic and colonizing strategies, therefore different resistance and responses to stressors might be expected (O'Brien et al. 2018).

This study aims to find out how increasing temperature, nutrient over-enrichment, and the combination of both factors affect the morphological, biochemical and physiological responses of three common tropical Indo-Pacific seagrasses. To achieve this aim, combined temperature and nutrient enrichment laboratory experiments were conducted, and the responses in trait values of the three selected species were measured after 1 month under four different treatment combinations. We hypothesized that the combination of both factors will cause interactive (synergistic or antagonistic) responses in the three species, and these responses will be species-specific according to their life-history traits. The results of this study will provide important information on how the combined effects of climate change and nutrient enrichment will shape tropical seagrass meadows and their responses.

II. Materials and Methods

(1) Collection and Maintenance of Seagrasses

Thalassia hemprichii, *C. serrulata* and *H. stipulacea* were collected in the dry season 2016 from different areas of a seagrass meadow located in the western coast of Zanzibar, Tanzania (6° 7' 43'' S, 39° 10' 47'' E). The selected meadows are within a shallow area (0.5-4 m depth) situated in the East coast of Changuu Island, located approximately 5 km northwest of Stone Town. This island is an uninhabited coral rock outcrop, although is a touristic spot due to its turtle zoo and snorkeling trips to the fringing reef surrounding the seagrass meadows. Low nutrient concentrations were observed in both the water column ($\text{NO}_3^- + \text{NO}_2^-$: $0.13 \pm 0.01 \mu\text{M}$, NH_4^+ : $0.67 \pm 0.05 \mu\text{M}$, PO_4^{3-} : $0.42 \pm 0.07 \mu\text{M}$), and in pore water at 5 cm below the sediment surface ($\text{NO}_3^- + \text{NO}_2^-$: $0.42 \pm 0.07 \mu\text{M}$, NH_4^+ : $1.36 \pm 0.37 \mu\text{M}$, PO_4^{3-} : $0.74 \pm 0.11 \mu\text{M}$). Therefore, this area is relatively pristine with the highest relative cover of seagrasses and the highest water quality within the sites included in our study in Zanzibar Archipelago (Teichberg et al. in preparation). Temperature in the seagrass meadows varied between 26.4 and 28.4 °C with mean values of 27.30 ± 0.08 °C during daytime (Teichberg et al. in preparation). Rhizomes

with approximately 5 to 6 shoots were collected for each species from different areas around the meadow to avoid collecting shoots from the same individual plant. Seagrasses were packed in paper tissues dampened with seawater, placed inside plastic bags and transported within 48 h to the Marine Experimental facilities (MAREE) at the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen (Germany). Once at the MAREE, seagrasses were replanted in polypropylene trays filled with marine carbonate substrate of at least 10 cm depth. Acclimation took place in 300-l aquaria with a recirculation system mimicking as best as possible the original natural conditions of the seagrass plants. The collected seagrasses co-inhabited the aquaria with fish, hermit crabs, sponges and natural rocks providing nutrient recycling to low nutrient (NO_3^- : $2.8 \pm 1.5 \mu\text{M}$, NH_4^+ : $<0.3 \mu\text{M}$, PO_4^{3-} : $< 0.002 \mu\text{M}$) artificial seawater (ASW), and, therefore, avoiding any extra nutrient addition. The fluorescent lights ($200 \pm 30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were regulated to a photoperiod of 12:12 h, and temperature ($26 \pm 1^\circ\text{C}$) and salinity (35) acclimation conditions were similar to the average values observed in native meadows, providing the seagrasses time to recuperate and acclimate to the aquaria, which based on our experience, is approximately 3 months.

(2) Experimental Design and Setup

We conducted an experiment with a nested split-plot design (Schielzeth and Nakagawa 2013) to study the effects of nutrient over-enrichment, elevated temperature and their interaction on the performance of adult individuals of the three tropical seagrass species *T. hemprichii*, *C. serrulata* and *H. stipulacea*.

The experiment was carried out in the same experimental setup as Artika et al. (2020). We applied low and high levels of temperature, 26°C (LT) and 31°C (HT), respectively, and low and high levels of nutrients by adding $2 \mu\text{M NH}_4\text{NO}_3 + 0.1 \mu\text{M KH}_2\text{PO}_4$ (LN) and $20 \mu\text{M NH}_4\text{NO}_3 + 2 \mu\text{M KH}_2\text{PO}_4$ (HN), respectively. This resulted in 4 experimental treatments: low temperature and low nutrient concentrations (LT + LN) considered as the control, low temperature and high nutrient concentrations (LT + HN), high temperature and low nutrient concentrations (HT + LN), and high nutrient concentration and high temperature (HT + HN). Experimental temperature treatments were selected based on lower and higher average temperatures in the area. Nutrient concentrations were selected on nutrient concentrations in impacted seagrass meadows in the area, as well as in the sewage effluent in Stone Town (Zanzibar) (Teichberg et al. in preparation) and the nutrient concentrations found in Changuu Island, the relatively pristine site where seagrasses were collected.

The experiment was conducted under laboratory conditions in an indoor system in the MAREE (ZMT, Bremen) with a total of 24 glass aquaria (29 x 13 x 30 cm dimensions) of 10 l volume. Each aquarium was considered as a replicate, and each of the 4 treatments had 6 replicate aquaria (see experimental design in Artika et al. 2020).

The experimental temperatures were obtained by placing aquaria in larger (250 l) experimental tanks (ETs) that acted as water baths maintaining a constant experimental water temperature. Six aquaria were placed in 4 different ETs with nutrient treatments nested within the 4 ETs set at the two temperatures and with no interactions among aquaria. Water bath temperature was controlled in each ET by heaters (EHEIM, Germany) connected to an individual electronic system that was continuously regulating the temperature (± 0.2 °C) by digital controllers and individual temperature probes. Air pumps were placed in each ET to ensure water movement of the water bath. The light (200 ± 20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was provided by 2 LED lamps (Hydra Fifty-two HD, Aqualllumination, Iowa) at the top of each ET and placed at the same height. A photoperiod of 12:12 h light:dark was set with sunrise and sunset simulation. Transparent PVC lids were placed on each ET to lower water evaporation.

The experimental setup was a flow through system that took water from two water reservoirs (~115 l each) with either high or low nutrient ASW solutions. To achieve the experimental nutrient concentrations, previously dissolved stock solutions of NH_4NO_3 and KH_2PO_4 (Merck, Germany) were added to each water reservoir. Once in the water reservoir, the solution was gently mixed with fresh ASW and an air pump was placed in each water reservoir to ensure further aeration and mixing. Water reservoirs were manually emptied from any remaining water and refilled with fresh ASW every other day. Equal water flow was assured to all aquaria by using a 24-channel peristaltic pump (ISMATEC, Germany) that maintained a constant flow rate (4 ml min^{-1}) to each aquarium. Water constantly overflowed from the aquaria to the water bath of the ETs ensuring constant water renewal inside the aquaria and, at the same time, ETs drained the surplus water. In each aquarium, an air pump ensured water aeration and mixing by moving water from the bottom to the top.

After the 3-month acclimation period, seagrasses with several ramets (i.e., iterating modular plant units) and no apparent damage or epiphyte cover were selected. Three selected ramets (here forward referred to as plant) of each species were cut and carefully planted in silicate sediment (~7 cm depth) together with 3 seedlings of *Enhalus acoroides* (Artika et al. 2020) in the experimental setup previously described, where they were acclimated one more month before the start of the treatments. *T. hemprichii* and *C. serrulata* plants consisted of one shoot

of 2-4 leaves, roots and a small portion of rhizome (3.6 ± 0.2 cm long). Plants of the same rhizome were randomly distributed along the treatments. *H. stipulacea* was divided in fragments of rhizome with 3-5 shoots and 8.8 ± 0.6 cm long.

After the acclimation period, temperature was elevated by increasing $1 \text{ }^\circ\text{C day}^{-1}$ until reaching the $31 \text{ }^\circ\text{C}$ in two random ETs. The other two ETs remained at the acclimation temperature of $26 \text{ }^\circ\text{C}$. Once the target temperatures were stable, nutrient addition started, and from that moment the experiment started. Plants were exposed to the selected experimental treatments for approximately 1 month (January 20th to February 22nd 2017) to ensure a response of the selected individual plant traits (Lee et al. 2007; McMahon et al. 2013; Roca et al. 2016).

(3) Water Monitoring and Sampling

Water pH, temperature and salinity were monitored every other day during the acclimation and experimental phase with a multi-parameter probe (WTW Multiprobe). Salinity was adjusted by adding distilled water when necessary to aquaria and water reservoirs. Hobo loggers continuously monitored water temperature inside one of the aquaria in each ET ($n = 4$). The electronic system within the ETs continuously measured the temperature of all the water baths. Water was sampled every week from the two water reservoirs and random aquaria of each treatment ($n = 4$ each week) for silicate, phosphate, and dissolved inorganic N (DIN), as the sum of NH_4^+ , NO_3^- and NO_2^- . Water was sampled with a syringe, immediately filtered ($0.45 \text{ }\mu\text{m}$ pore size, Whatman GF/F filters) in prerinsed polyethylene bottles and frozen ($-20 \text{ }^\circ\text{C}$). Analysis was performed using a continuous flow injection analyzing system (Skalar SAN⁺⁺-System) following Grasshoff et al. (1999). The measuring procedure had a relative standard deviation $< 3.5\%$ with reference to the linear regression of an equidistant 10-point calibration line from NIST standards.

Detached leaves of the seagrasses were removed from the aquaria every day, and epiphytes growing on the blades of the seagrasses were removed weekly. Microalgae growing in the chamber walls, however, were not removed. At the end of the experiment, the sediment N and C content were analyzed by drying the homogenized samples in a forced air oven at $60 \text{ }^\circ\text{C}$ until constant dry weight (DW), ground to a fine powder with mortar and pestle, and weighed into tin capsules prior to analysis using Euro EA3000 Elemental Analyzer. Water samples from all aquaria were sampled for chlorophyll *a* (Chl-*a*) and *b* (Chl-*b*) and suspended particulate matter (SPM) measurements. Water was immediately filtered under constant pressure onto pre-combusted (5 h, $450 \text{ }^\circ\text{C}$) and pre-weighed Whatman GF/F filters ($0.45 \text{ }\mu\text{m}$ pore size). Filters

for SPM analysis were dried at 50 °C and filters for Chl-*a* and *b* analysis were stored at -20 °C. Concentrations of SPM were determined by weighing the dried filter, subtracting the weight of the empty filter and dividing it by the respective volume of water filtered. Pigments were extracted from the filters in 8 ml of 96% ethanol in glass vials heated for 5 min at 80 °C, covered with aluminum foil, and placed in a rotor at room temperature for approximately 24 h. Extracts were subsequently centrifuged at 5000 rpm for 20 min. Chl-*a* and *b* samples were determined in a photometer Shimadzu UV-1700.

(4) Measurement of Seagrass Traits

Different biochemical, morphological and physiological individual-level traits were selected based on their quick response times observed under other single effect experiments of nutrient over-enrichment or temperature in order to better understand the combined effect of these factors (Campbell et al. 2006; Lee et al. 2007; Leoni et al. 2008; Martínez-Crego et al. 2008; Pedersen et al. 2016; Roca et al. 2016). At the end of the experiments, seagrasses were removed from the aquaria and the morphological measurements on each plant were performed. Afterward, plants were carefully separated with a glass spatula into the different parts: leaves (for fluorescence measurements, nutrient content, and free amino acids content, FAAs), rhizome (for nutrient content, and nonstructural carbohydrate content, NSC), and roots (for nutrient content) in *T. hemprichii* and *C. serrulata* plants. Plant tissues of the three plants of each species within each aquarium were pooled together for nutrient analysis. In *H. stipulacea* plants, due to the limited plant material, rhizome and root material were pooled, and FAAs in leaves were not measured. Moreover, samples from two aquaria of this latter species had to be pooled for fluorescence measurements and nutrient content analysis.

(a) Biochemical Traits

i. Nitrogen and carbon content

To assess whether the experimental water column N enrichment affected internal nutrient storage and allocation, we measured the final N and C contents of different plant tissues. *T. hemprichii* and *C. serrulata* plants were divided into leaves, rhizome and roots; while *H. stipulacea* individuals were divided into leaves (representing the AG compartment) and rhizome and roots (representing the BG compartment). Samples were dried at 50 °C in a forced air oven until constant DW, ground to a fine powder with mortar and pestle, and weighed (1.42 ± 0.02 mg) into tin capsules using an analytical scale prior to analysis using Euro EA3000 Elemental Analyzer.

ii. Free amino acid content in leaves

The FAAs were extracted from ~50 mg fresh weight (FW) of leaves of *T. hemprichii* and *C. serrulata* grounded material (FastPrep-24 Instrument) during 60 min at room temperature by adding 4 ml of 0.05 N HCl. The supernatant (5 min, 10,000 g) was filtered through 0.2 µm CA-filters into glass vials and stored at -20 °C for posterior FAAs composition and concentration analysis using an ion-exchange liquid chromatography for hydrolyzed samples (Biochrom 30). For simplicity, only the total FAA concentration (sum of 14 FAAs for these samples: ALA, ARG, ASP, GLU, GLY, HIS, ILE, LEU, MET, PHE, THR, TYR, SER and VAL) were considered in this study. However, relative differences among FAAs can also be identified (Supplementary Table 1).

iii. Non-structural carbohydrate content in rhizomes

The concentration of sucrose and starch were measured on rhizome material of *T. hemprichii* and *C. serrulata* and in the BG compartment (rhizome and roots) of *H. stipulacea*. We followed a modified protocol from Salo and Pedersen (2014). The samples were frozen (-80 °C) and freeze-dried for 48 h. Soluble sugars, namely sucrose, were extracted from ground plant tissue by boiling in 96% ethanol. The ethanol extracts were evaporated and the residues were dissolved in deionized water for sucrose analysis. Starch was extracted from the ethanol-insoluble residue in 1 N NaOH for 24 h. The sucrose and starch concentrations of the extracts were determined spectrophotometrically (wavelengths 486 and 640 nm, respectively) using resorcinol and anthrone assays, respectively, with sucrose as a standard (Yemm and Willis 1954; Huber and Israel 1982). Results were reported in sucrose equivalents g⁻¹ DW. Current testing of this method has shown that NaOH extracts not only starch, but also cellulose, which can confound the results. Regarding the sucrose determination, this method only determines ketoses (as fructose) so we are ignoring glucose, the other component of sucrose, therefore underestimating the final concentrations (M. Birkicht, personal communication). Despite its drawbacks, this method has been frequently used in other studies and allows for direct comparisons of the data.

(b) Morphological Traits

Morphological measurements were individually performed in each of the three plants of each species within the different aquaria. The measurements included leaf morphometrics (length, width and surface area, SA), sheath length (or petiole for *H. stipulacea*), root length, and internode length (IL) (just for *H. stipulacea*).

(c) Physiological Traits

i. Growth rates

Leaves of *T. hemprichii* and *C. serrulata* were double-pinned in parallel just above the sheath at the beginning of the experiment for leaf growth measurements following the method by Short and Duarte (2001). However, leaf growth and turnover were higher than expected during the experiment, and no leaves with pinning remained at the end of the experiment. Therefore, the growth was estimated by collecting the detached leaves found daily in each aquaria. Leaf growth (cm d^{-1}) was measured as the distance from the base of the detached leaf to the place where the pins were, divided by the days when the leaf was sampled. Only leaves detached during the last week of the experiment were considered. We tried to measure *H. stipulacea* growth by marking the rhizome, however, no label was found by the end of the experiment, and no optional method of growth estimation was possible with this species.

ii. Photosynthetic variables

The photosynthetic performance of the seagrasses was measured through pulse amplitude modulated (PAM) chlorophyll fluorescence using rapid light response curves (RLCs) generated by the PAM-2500 chlorophyll fluorometer (Walz, Germany). RLCs were performed above the meristem of the second leaf of the three plants of each aquarium for *T. hemprichii* and *C. serrulata*. For *H. stipulacea*, 3 measurements were performed at the base of the leaf, close to the petiole, and plants of two aquaria of each treatment had to be pooled. The basal portion of the leaf was chosen since it represents similar distances from the surface (and thus from the light source) among plants with different leaf lengths, thus minimizing variability within plants and species (Winters et al. 2011). A clip was attached to the leaf and helped to hold the optical cable of the PAM at 3 mm distance from the tissue and to dark adapt the tissue during 5 min. Leaves were maintained in a petri dish with some ASW during the dark adaptation and the measurements.

The first quantum yield measurement was performed in the absence of actinic light (dark-adapted effective quantum yield, Y_0 ; Saroussi and Beer 2007), after which the RLC consisted of 12 saturating light pulses (separated by 30s intervals), increasing the photosynthetic active radiation (PAR) between pulses until $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Each step lasted 10 s and was followed by a measurement of the effective quantum yield ($\Delta F/F_m'$) (Ralph and Gademann 2005). From the data of the RLC, light saturation coefficient (E_k) and the slope of the light limited part of the curve (Alpha) were calculated using the package Phytotools (Silsbe and

Malkin, 2015) following the model of Jassby and Platt (1976) under the R software (R Core Team, 2019).

The maximum light utilization efficiency or maximum quantum yield of PSII was calculated following equation by Genty et al. (1989) [$F_v/F_m = (F_m - F_o)/F_m$], where F_m is the maximum dark-adapted fluorescence and F_o is the minimal fluorescence from a dark-adapted sample. The relative electron transport rate (rETR) was calculated for each step of the curve following equation by Sakshaug et al. (1997), [$rETR = (F_m' - F')/F_m' * (PAR/2)$], where F_m' is the light adapted maximum fluorescence and F' the fluorescence yield at a particular light level. From the rETR values, maximum rETR (rETR_{max}) was estimated as the inflection point of the fitted rETR curve.

(5) Statistical Analysis

The experiment followed a split-plot design with three nesting factors (Schielzeth and Nakagawa 2013). The two main factors (temperature and nutrient) had two fully crossed levels each (LT and HT, and LN and HN respectively). Two ETs were nested within each temperature treatments, six aquaria were nested within each ET and three plants of each species within each aquaria.

We used permutational multivariate analysis of variance (PERMANOVA) (Anderson et al. 2008) to analyze the data of each species. The Pseudo F-statistic was used to test the null hypothesis of no differences in the position of the group centroids in the space of the chosen dissimilarity measure. The fixed effects in the model were temperature and nutrient treatments, and their interactions, together with the nesting structure of temperature, ET and Aquarium. The factor Aquarium was not included in biochemical trait analysis because tissues from the different plants within aquaria were pooled. For *H. stipulacea* data, the factors Aquarium or ET were not included in biochemical measurements analysis because tissues from different aquaria were pooled. Data were scouted for outliers, which were identified as data exceeding 1.5 times the interquartile range of variation of the dataset. Outliers were only eliminated from the model when they did not allow to meet the model assumptions. We calculated the Euclidean dissimilarity matrix for all variables, as they were continuous. The assumptions of exchangeability of permutable units and homogeneity of multivariate dispersion were tested before analysis. When the homogeneity of multivariate dispersions was not met, the data were transformed (square root, or log) and the dissimilarity matrix recalculated. The homogeneity of

multivariate dispersion assumption for ET grouping could not be met for leaf C:N ratio and Alpha values in *T. hemprichii*.

Water parameters in aquaria (DIN, phosphate, SPM, and Chl-*a* and *b*) were compared using PERMANOVA while DIN and phosphate concentrations in water reservoirs were analyzed using a two-way ANOVA. Temperature, nutrient, their interactions and the nesting structure (Temperature: ET: Aquarium) were the fixed effects in the model. The permutational unit for the model was the aquarium with 999 permutations, which is the recommended minimum number to test at an alpha-level of 0.05 (Manly, 1997). Comparisons were considered significant when $P \leq 0.05$. We used R software to perform the analysis (R Core Team, 2019) with the `adonis2` function of the package “vegan” (Oksanen et al. 2019).

Pearson correlation analysis was applied in order to investigate potential relationships between biochemical, morphological and physiological traits. Data from aquaria of all treatments were combined to generate statistically independent means for each aquarium (without error), resulting in statistically independent replicate measurements ($n = 24$, except of *H. stipulacea*, $n = 12$). This statistical analysis was performed with SPSS (IBM SPSS Statistics for Windows v.24, Armonk, NW, United States).

III. Results

(1) Experimental Conditions

The four different treatments, combining two nutrient and temperature levels, changed the conditions under which the seagrasses were grown. Water temperature in the aquaria was shown to be constant both during the day, with no sharp variations when light was absent (data not shown), and through the experimental period, while showing significant differences between temperature treatments (PERMANOVA, $P < 0.01$) (Table 1). DIN and phosphate concentrations in the two main water reservoirs were within the target concentrations throughout the experiment and were significantly different between the HN and LN treatments (two-way ANOVA, $P < 0.001$). However, once in the aquaria, nutrients were rapidly taken up, resulting in low inorganic nutrient concentrations in all treatments regardless of the treatment (PERMANOVA, $P > 0.05$). In fact, some of the concentrations measured were not included in the analysis as they were below the quantification limit. However, even though nutrient concentration parameters in aquaria were low, other observable parameters suggested eutrophic conditions were occurring in the HN treatments. Algal blooms were observed in the HN treatments, showing different trophic conditions in the LN and HN treatments, especially in the

HT + HN treatment. Algae were observable by naked eye on the glass of the aquaria and seagrasses. However the abundance of these microorganisms could not be quantified, as they formed fluffy layers that disintegrated when tried to sample them. SPM concentrations in the water column were also higher in the HN treatments (PERMANOVA, $P < 0.05$) with the highest concentrations in the HT + HN treatment (Table 1).

Table 1. Experimental seawater parameters (mean \pm SE) and number of measurements taken (n) during the experimental period in each of the four treatments (LT, low temperature; LN, low nutrient; HT, high temperature; HN, high nutrient).

	n	Treatments			
		LT + LN	HT + LN	LT + HN	HT + HN
DIN (μM) ^R	4	5.66 \pm 0.51		22.40 \pm 0.98	
PO ₄ ⁻ (μM) ^R	4	0.19 \pm 0.01		1.01 \pm 0.04	
Water temperature (°C)	25* 3735 ^ϕ	26.25 \pm 0.05	31.01 \pm 0.07	26.28 \pm 0.03	31.01 \pm 0.05
Salinity	25	26.47 \pm 0.003	31.13 \pm 0.004	26.22 \pm 0.004	31.48 \pm 0.002
pH	12	35.43 \pm 0.12	35.39 \pm 0.10	35.33 \pm 0.07	35.39 \pm 0.11
DIN (μM)	5	8.47 \pm 0.02	8.39 \pm 0.02	8.67 \pm 0.03	8.59 \pm 0.02
PO ₄ ⁻ (μM)	5	0.62 \pm 0.33	0.88 \pm 0.44	0.64 \pm 0.31	1.25 \pm 0.01
Si (μM)	5	0.15 \pm 0.01	0.14 \pm 0.02	0.14 \pm 0.00	0.14 \pm 0.01
SPM (mg l ⁻¹)	5	0.71 \pm 0.07	1.15 \pm 0.22	0.91 \pm 0.20	0.85 \pm 0.06
Chl- <i>a</i> ($\mu\text{g l}^{-1}$)	24	27.62 \pm 4.65	24.04 \pm 1.99	38.21 \pm 3.69	74.91 \pm 11.75
Chl- <i>b</i> ($\mu\text{g l}^{-1}$)	24	2.07 \pm 0.53	2.07 \pm 0.53	2.59 \pm 0.98	5.91 \pm 2.41
		0.36 \pm 0.07	0.36 \pm 0.07	0.42 \pm 0.18	0.88 \pm 0.43

DIN, dissolved inorganic nitrogen (the sum of NH₄⁺, NO₃⁻ and NO₂⁻); SPM, suspended particulate matter. ^R Measurements taken in the water reservoirs. * Water temperature provided by multiprobe measurements, ^ϕ Water temperature provided by the continuous measurement of Hobo loggers.

Water column Chl-*a* concentrations were also higher in the HN treatments (2.6-5.9 $\mu\text{g l}^{-1}$) than in the LN treatments (2-2.1 $\mu\text{g l}^{-1}$) showing the highest mean value in the HT + HN treatment (5.91 $\mu\text{g l}^{-1}$). Even though Chl-*b* concentrations also increased in the HT + HN treatment, no significant differences were observed among treatments (PERMANOVA, $P > 0.05$). Therefore, eutrophic conditions were especially noticeable in the HT + HN treatment. The water column nutrient enrichment did not significantly change the sediment nutrient conditions, and sediment N concentrations were below the detection limits in all aquaria (data not shown). The other variables measured in the water column, including salinity, pH and Si concentrations, were constant throughout the experiment and did not show significant differences among treatments (Table 1).

Table 2. Permutational analysis of variance (PERMANOVA) of the effects of temperature (T) and nutrient (N) treatments on the biochemical traits of *T. hemprichii*, *C. serrulata*, and *H. stipulacea* in Figure 1.

Trait	Source	<i>T. hemprichii</i>					<i>C. serrulata</i>					<i>H. stipulacea</i>				
		df	SS	<i>R</i> ²	Pseudo-F	<i>P</i>	df	SS	<i>R</i> ²	Pseudo-F	<i>P</i>	df	SS	<i>R</i> ²	Pseudo-F	<i>P</i>
Leaf N	T	1	2.37	0.1	3.08	0.11	1	2.45	0.11	3.58	0.07	1	1.36	0.12	2.15	0.197
	N	1	4.86	0.21	6.32	0.03	1	2.78	0.12	4.07	0.05	1	0.39	0.04	0.61	0.437
	T:N	1	0.84	0.04	1.09	0.29	1	1.18	0.05	1.72	0.20	1	4.22	0.38	6.69	0.045
	T:ET	2	1.07	0.05	0.70	0.52	2	4.29	0.19	3.14	0.06	-	-	-	-	-
	Res	18	13.8	0.6			18	12.3	0.53			8	5.04	0.46		
Rhizome N ^ϕ	T	1	4.42	0.19	4.75	0.04	1	0.07	0.00	0.07	0.78	1	0.09	0.01	0.07	0.789
	N	1	0.09	0.00	0.10	0.75	1	2.27	0.10	2.19	0.17	1	0.08	0.01	0.06	0.806
	T:N	1	1.44	0.06	1.55	0.22	1	1.53	0.07	1.48	0.25	1	0.04	0.00	0.03	0.852
	T:ET	2	0.29	0.01	0.15	0.84	2	0.51	0.02	0.25	0.81	-	-	-	-	-
	Res	18	16.7	0.73			17	17.6	0.8			8	10.7	0.98		
Roots N	T	1	8.12	0.35	16.86	0.00	1	2.25	0.10	2.61	0.13	-	-	-	-	-
	N	1	0.83	0.04	1.72	0.22	1	0.15	0.01	0.17	0.70	-	-	-	-	-
	T:N	1	0.03	0.00	0.07	0.79	1	1.92	0.09	2.22	0.14	-	-	-	-	-
	T:ET	2	5.35	0.23	5.55	0.01	2	3.02	0.14	1.75	0.20	-	-	-	-	-
	Res	18	8.67	0.38			17	14.6	0.67			-	-	-	-	-
Leaf C:N	T	1	1.41	0.06	2.05	0.18	1	3.07	0.13	4.78	0.04	1	2.58	0.23	4.18	0.084
	N	1	6.48	0.28	9.40	0.01	1	2.84	0.12	4.43	0.06	1	0.78	0.07	1.26	0.303
	T:N	1	0.87	0.04	1.27	0.27	1	2.81	0.12	4.39	0.03	1	2.72	0.25	4.42	0.054
	T:ET	2	1.83	0.08	1.33	0.30	2	2.74	0.12	2.13	0.15	-	-	-	-	-
	Res	18	12.4	0.54			18	11.5	0.50			8	4.93	0.45		
Leaf C	T	1	4.44	0.19	5.21	0.02	1	8.77	0.40	14.67	0.00	1	0.04	0.00	0.04	0.838
	N	1	0.34	0.01	0.40	0.52	1	0.02	0.00	0.04	0.85	1	0.03	0.00	0.02	0.864
	T:N	1	2.37	0.10	2.78	0.12	1	0.47	0.02	0.78	0.35	1	2.76	0.25	2.70	0.126
	T:ET	2	0.5	0.02	0.30	0.72	2	2.58	0.12	2.16	0.13	-	-	-	-	-
	Res	18	15.3	0.67			17	10.1	0.46			8	8.17	0.74		
Rhizome C ^ϕ	T	1	0.11	0.01	0.10	0.79	1	0.14	0.01	0.58	0.47	1	3.96	0.36	5.30	0.045
	N	1	0.03	0.00	0.02	0.90	1	15.01	0.68	60.44	0.00	1	0.03	0.00	0.04	0.859
	T:N	1	0.22	0.01	0.20	0.69	1	0.36	0.02	1.44	0.25	1	1.04	0.09	1.39	0.265
	T:ET	2	2.7	0.12	1.22	0.33	2	2.26	0.10	4.55	0.02	-	-	-	-	-
	Res	18	19.9	0.87			17	4.22	0.19			8	5.97	0.54		
Roots C	T	1	5.04	0.22	8.97	0.01	1	3.12	0.14	4.70	0.03	-	-	-	-	-
	N	1	0.67	0.03	1.20	0.28	1	0.65	0.03	0.99	0.38	-	-	-	-	-
	T:N	1	0.01	0.00	0.01	0.90	1	3.01	0.14	4.53	0.04	-	-	-	-	-
	T:ET	2	7.18	0.31	6.40	0.01	2	3.93	0.18	2.96	0.06	-	-	-	-	-
	Res	18	10.1	0.44			17	11.2	0.51			-	-	-	-	-
Rhizome sucrose ^ϕ	T	1	2.21	0.10	2.01	0.16	1	0.32	0.01	0.28	0.60	1	0.34	0.03	0.31	0.578
	N	1	0.25	0.01	0.23	0.68	1	0.79	0.04	0.71	0.39	1	0.09	0.01	0.08	0.774
	T:N	1	0.34	0.02	0.31	0.60	1	1.46	0.07	1.30	0.23	1	1.78	0.18	1.60	0.263
	T:ET	2	0.54	0.02	0.25	0.79	2	0.46	0.02	0.21	0.80	-	-	-	-	-
	Res	17	18.6	0.85			17	18.9	0.86			7	7.79	0.78		
Rhizome starch ^ϕ	T	1	0.69	0.03	0.81	0.38	1	0.26	0.01	0.34	0.56	1	0.54	0.05	0.58	0.475
	N	1	0.31	0.01	0.36	0.56	1	5.23	0.23	6.81	0.02	1	2.39	0.24	2.58	0.145
	T:N	1	0.67	0.03	0.79	0.38	1	1.05	0.05	1.36	0.25	1	0.57	0.06	0.62	0.454
	T:ET	2	6.10	0.27	3.61	0.05	2	2.64	0.11	1.72	0.22	-	-	-	-	-
	Res	18	15.2	0.66			18	13.8	0.60			7	6.49	0.65		
Leaf FAA	T	1	2.13	0.09	2.24	0.16	1	2.15	0.09	2.30	0.16	-	-	-	-	-
	N	1	0.61	0.03	0.64	0.45	1	3.8	0.17	4.07	0.06	-	-	-	-	-
	T:N	1	0.48	0.02	0.5	0.47	1	0.01	0.00	0.01	0.91	-	-	-	-	-
	T:ET	2	2.65	0.12	1.39	0.28	2	0.22	0.01	0.12	0.89	-	-	-	-	-
	Res	18	17.1	0.74			18	16.8	0.73			-	-	-	-	-

Significant differences are in boldface (*: < 0.05; **: < 0.01; ***: < 0.001). (ET: experimental tank, FAA: free amino acids).

^ϕ Below-ground tissues (rhizome and roots) for *H. stipulacea*.

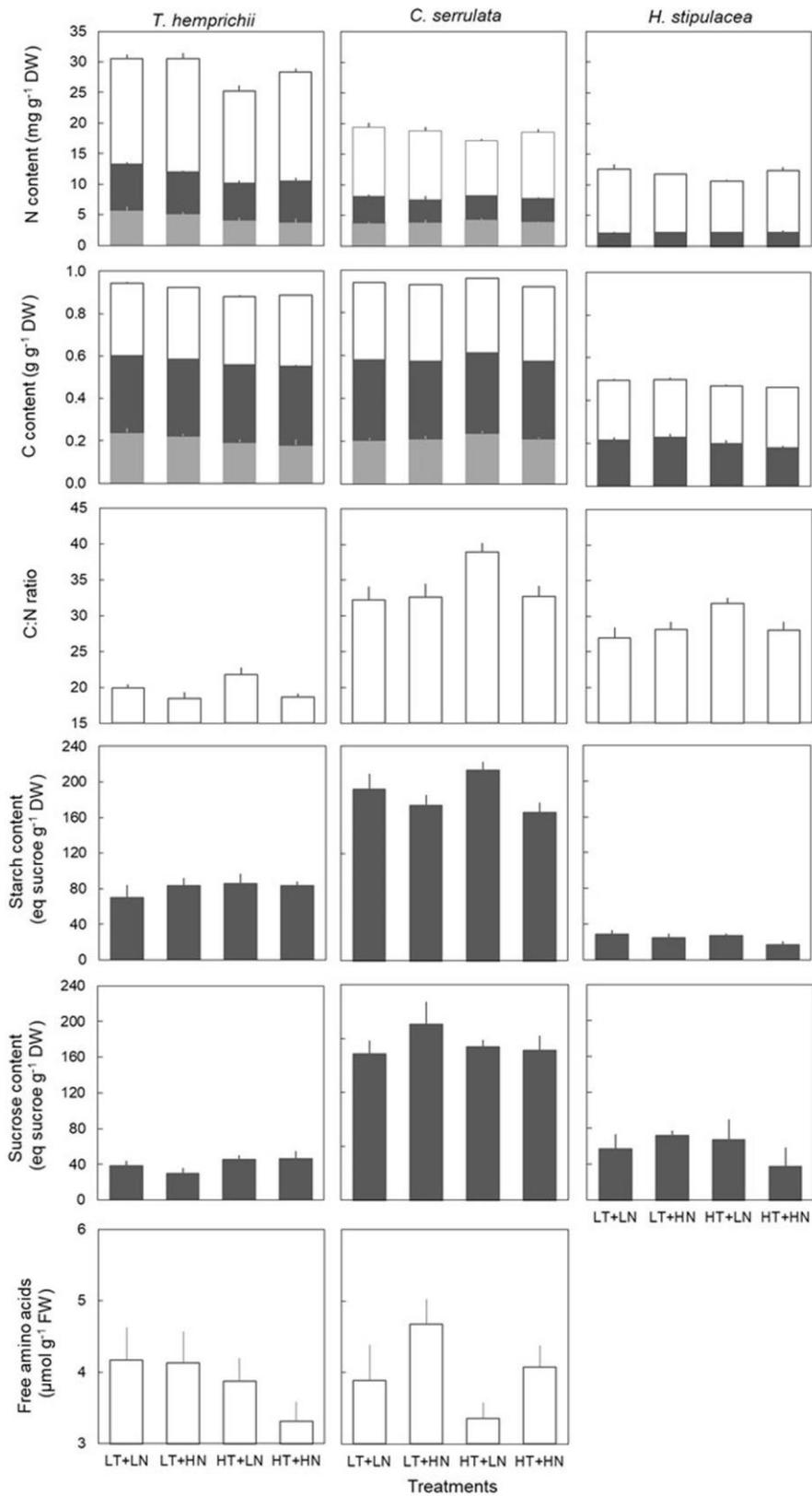


Figure 1. Biochemical traits (mean \pm SE, $n = 6$, except of *H. stipulacea*, $n = 3$) of *T. hemprichii*, *C. serrulata* and *H. stipulacea* in leaves (open bars), rhizome (dark gray bars) and roots (light gray bars) in the four treatments (LT, low temperature; LN, low nutrient; HT, high temperature; HN, high nutrient).

(2) Seagrass Biochemical Traits

Thalassia hemprichii showed the highest N content in all three tissues compared to that of the other species, followed by *C. serrulata* and *H. stipulacea* (Figure 1). Total C content was similar in all species, although allocation changed. While *T. hemprichii* showed higher C content in rhizome compared to leaves, *C. serrulata* showed similar content in these two tissues. On the other hand, *H. stipulacea* had slightly higher C content in AG than BG tissues (Figure 1).

Temperature treatments changed the biochemical contents and the nutrient allocation in the three species (Table 2). *C. serrulata* was the species most influenced by temperature in terms of its leaf biochemical traits, as leaf N and C content decreased, and C:N ratio significantly increased under HT treatments (Figure 1). Leaf C:N ratio also increased in *H. stipulacea*, although no significant effect was detected. *T. hemprichii* BG tissues were the most influenced by HT treatment, with significantly lower N content in rhizome and roots under HT treatments (Table 2). Root C content was also significantly lower under HT treatments in this species and *H. stipulacea*, while in *C. serrulata*, significantly higher C content was observed under HT treatments (Figure 1).

Nutrient treatments significantly influenced the nutrient contents of *T. hemprichii* and *C. serrulata* but not of *H. stipulacea* (Table 2). Both *T. hemprichii* and *C. serrulata* showed significantly higher leaf N content under HN treatments, and *T. hemprichii* also showed significantly lower C:N ratio ($P < 0.05$), while non-significant decreasing values were observed in *C. serrulata* ($P = 0.06$). Leaf FAAs content only positively responded to nutrient treatment in *C. serrulata* ($P = 0.06$). This species also showed the highest concentrations of NSC in the rhizome of the three species. In *C. serrulata* and *H. stipulacea*, the main NSC form was sucrose, while in *T. hemprichii*, starch concentrations were higher than sucrose under all treatments. The BG tissues of *C. serrulata* were the only ones that changed in response to nutrient enrichment, with lower starch and C content in rhizomes, and lower C content in roots in HN treatments (Figure 1 and Table 2). The effects of warming on *C. serrulata* and *H. stipulacea* biochemical traits, namely leaf C:N ratio in both species and root C content in *C. serrulata*, were significantly mediated by nutrient over-enrichment. This resulted in an antagonistic effect of both factors, as values were lower than expected for effects to be additive. Contrary, N content in AG tissues in *H. stipulacea* showed the only synergistic effect observed in this study. There were no interactive effects in the biochemical traits of *T. hemprichii* (Table 2).

(3) Seagrass Morphological Traits

Temperature had an overall significant effect on seagrass morphological traits (Figure 2 and Table 3). Leaf morphology was enhanced under HT treatments in *T. hemprichii* and *C. serrulata*, with longer leaves, bigger leaf SA, and longer sheaths. Root length did not respond to HT treatment in any of these two species. However, HT negatively influenced *H. stipulacea* leaf traits, with shorter leaves and smaller leaf SA, and also shorter roots and IL (Figure 2 and Supplementary Figure 1). Overall, nutrient addition did not show significant effects on morphological trait responses with the exception of lower leaf SA of *T. hemprichii* under HN treatments (Figure 2). *C. serrulata* morphological traits were unaffected by single nutrient treatments (Table 3).

Table 3. Permutational analysis of variance (PERMANOVA) of the effects of temperature (T) and nutrient (N) treatments on the morphological traits of *T. hemprichii*, *C. serrulata*, and *H. stipulacea* in Figure 2 and Supplementary Figure 1.

Trait	Source	<i>T. hemprichii</i>					<i>C. serrulata</i>					<i>H. stipulacea</i>				
		df	SS	<i>R</i> ²	Pseudo-F	<i>P</i>	df	SS	<i>R</i> ²	Pseudo-F	<i>P</i>	df	SS	<i>R</i> ²	Pseudo-F	<i>P</i>
Leaf length	T	1	9.12	0.13	12.9	0.00	1	16.1	0.23	18.3	0.00	1	7.97	0.13	13.1	0.00
	N	1	0.63	0.01	0.90	0.34	1	0.60	0.01	0.68	0.40	1	0.92	0.01	1.51	0.24
	T:N	1	0.00	0.00	0.00	0.96	1	0.33	0.00	0.37	0.53	1	0.07	0.00	0.11	0.75
	T:ET:Aq	20	27.5	0.39	1.96	0.02	20	11.6	0.16	0.66	0.86	20	29.6	0.47	2.43	0.01
	Res	48	33.7	0.48			48	42.2	0.59			40	24.4	0.39		
Leaf SA	T	1	11.0	0.16	33.3	0.00	1	12.1	0.17	13.1	0.00	1	5.78	0.09	7.22	0.01
	N	1	1.54	0.02	4.66	0.03	1	0.46	0.01	0.49	0.47	1	0.08	0.00	0.10	0.75
	T:N	1	5.24	0.07	15.7	0.00	1	0.81	0.01	0.87	0.36	1	0.07	0.00	0.09	0.79
	T:ET:Aq	20	37.2	0.52	5.61	0.00	20	12.9	0.18	0.70	0.79	20	25.1	0.40	1.57	0.13
	Res	48	15.9	0.22			48	44.6	0.63			40	32.0	0.51		
Sheath length	T	1	17.9	0.25	27.4	0.00	1	5.43	0.08	6.54	0.01	1	2.26	0.04	2.44	0.13
	N	1	0.08	0.00	0.12	0.71	1	1.00	0.01	1.20	0.26	1	1.40	0.02	1.51	0.25
	T:N	1	0.24	0.00	0.37	0.55	1	0.27	0.00	0.33	0.57	1	2.97	0.05	3.20	0.07
	T:ET:Aq	20	21.4	0.30	1.64	0.08	20	24.4	0.34	1.48	0.13	20	19.3	0.31	1.04	0.32
	Res	48	31.3	0.44			48	39.8	0.56			40	37.1	0.59		
Root length	T	1	1.49	0.02	2.39	0.13	1	0.00	0.00	0.00	0.99	1	23.5	0.37	46.4	0.00
	N	1	0.48	0.01	0.77	0.37	1	1.21	0.02	1.30	0.25	1	0.59	0.01	1.16	0.31
	T:N	1	7.19	0.10	11.5	0.00	1	0.73	0.01	0.78	0.38	1	0.04	0.00	0.09	0.77
	T:ET:Aq	20	31.8	0.45	2.55	0.01	20	24.3	0.34	0.130	0.24	20	18.6	0.30	1.83	0.05
	Res	48	29.9	0.42			48	44.7	0.63			40	20.2	0.32		
IL	T	-	-	-	-	-	-	-	-	-	-	1	3.44	0.05	3.85	0.05
	N	-	-	-	-	-	-	-	-	-	-	1	3.29	0.05	3.69	0.06
	T:N	-	-	-	-	-	-	-	-	-	-	1	1.07	0.02	1.20	0.26
	T:ET:Aq	-	-	-	-	-	-	-	-	-	-	20	19.4	0.31	1.09	0.39
	Res	-	-	-	-	-	-	-	-	-	-	40	35.7	0.57		

Significant differences are in boldface (*: < 0.05; **: < 0.01; ***: < 0.001). (ET: experimental tank, Aq: aquarium, SA: surface area, IL: internode length). Petiole length for *H. stipulacea*.

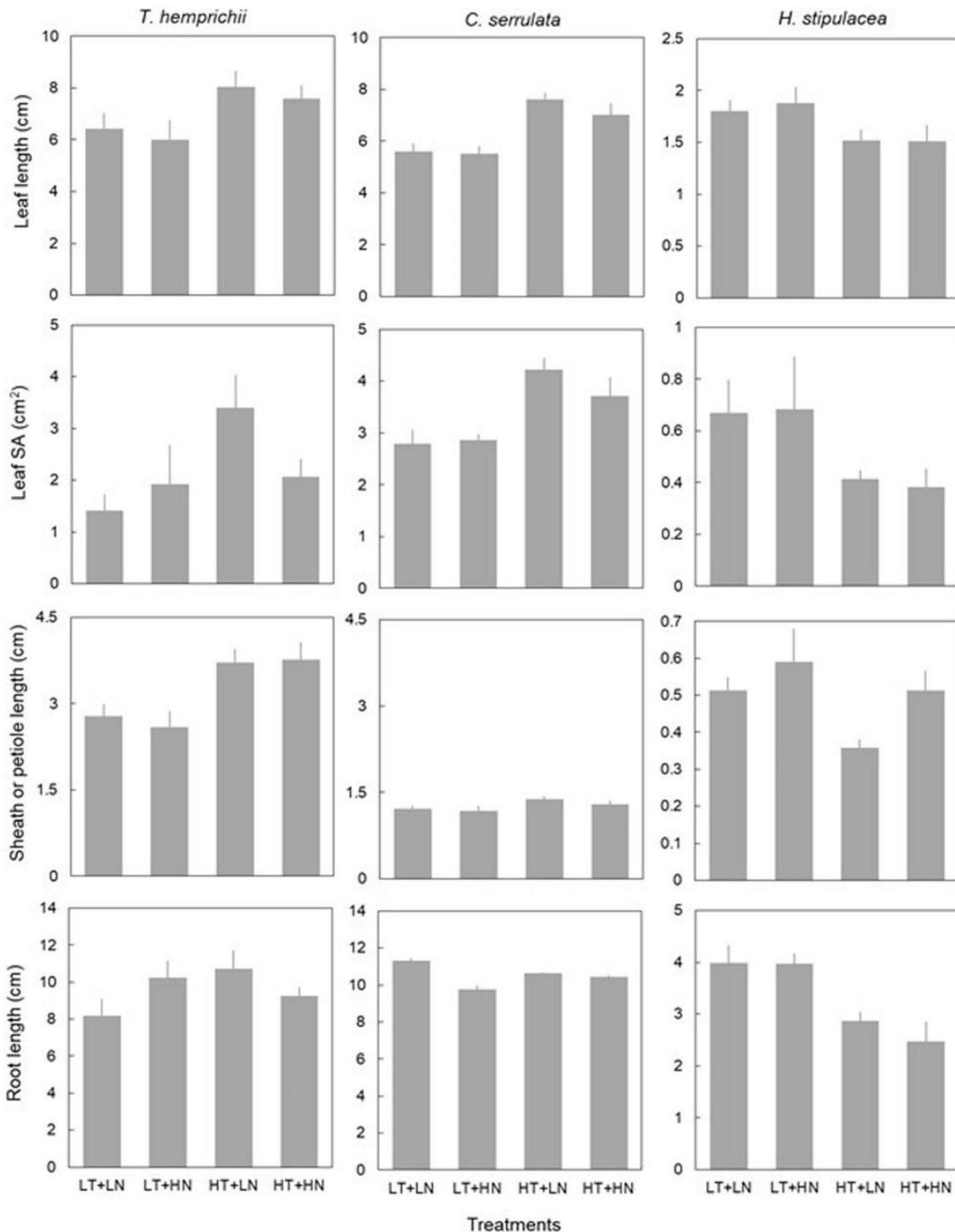


Figure 2. Morphological traits (mean \pm SE, n = 6) of *T. hemprichii*, *C. serrulata* and *H. stipulacea* in the four treatments (LT, low temperature; LN, low nutrient; HT, high temperature; HN, high nutrient). Note that Y-axis of *H. stipulacea* panels differ.

Leaf SA and root length in *T. hemprichii* showed a significant interaction of temperature and nutrients, in which both traits showed an antagonistic effect, i.e., a cancelation of the enhanced effects of nutrients when also exposed to higher temperatures (Figure 2 and Table 3). Petiole

length in *H. stipulacea* showed some additive interaction of both factors, although the effect was not significant (Table 3). *C. serrulata* showed no interactive effects of both factors in the morphological traits considered (Table 3). Morphological variables were also significantly affected by the nested blocking variables in *T. hemprichii* and *H. stipulacea*, but not in *C. serrulata* (Table 3). Therefore, the high variability between enclosures may have confounded some effects of the temperature and nutrient treatments.

(4) Seagrass physiological traits

Growth rate responded both to temperature and nutrient treatments in *T. hemprichii* but showed no significant effects in *C. serrulata* (Figure 3 and Table 4). On the contrary, the three species in this study significantly responded to both nutrient and temperature in terms of their photosynthetic efficiency (Figure 3 and Table 4). Temperature was the main driver of these responses, and higher Fv/Fm and Alpha in *C. serrulata*, and Alpha in *H. stipulacea* were observed under HT treatments. In contrast, rETRmax was negatively affected by HT treatments in the three species, and Ek in *C. serrulata* and *H. stipulacea* (Figure 3).

A single nutrient effect on photosynthetic performance was more limited than temperature alone. A significant effect of HN treatments was only detected by Alpha values in *T. hemprichii*, which was more evident in the combined HN + HT treatment (Figure 3). In *C. serrulata*, no trait showed a response to nutrients. The absence of responses of photosynthetic traits to nutrients was more evident when considering its interaction with temperature, as no significant effects were detected (Table 4). In the same way, no interaction between factors was observed in the other species in any of the physiological traits considered (Table 4). Some photosynthetic variables, Fv/Fm, Alpha and Ek, were significantly affected by the nested blocking variables in *T. hemprichii* and *C. serrulata* (Table 4). Therefore, the high variability between enclosures may have confounded some effects of the temperature and nutrient treatments.

(5) Relationships between Biochemical, Morphological, and Physiological Seagrass Traits

We explored all correlations among biochemical, morphological and physiological traits within each of the three studied species (Supplementary Tables 2–4). After examining the data we recognize some correlations that were interesting to understand how trait responses differed among the three species (Figure 4). *Thalassia hemprichii* showed no correlations between photosynthetic traits and growth, while leaf nutrient content was positively correlated with those traits (Supplementary Table 2). Specifically, leaf C content was positively correlated with

rETR_{max} (Figure 4A). *T. hemprichii* was also the only species showing positive correlations between AG and BG traits, both through its nutrient content and photo-physiological traits (Supplementary Table 2), such as, for instance, between FAAs in leaves and the root N (Figure 4E). *Cymodocea serrulata* showed positive correlations among physiological traits, while negative correlations between the AG and BG nutrient contents were observed (Supplementary Table 3). Particularly, leaf nutrient contents were negatively correlated with leaf morphological traits (Figure 4C), but in terms of photosynthesis or growth, no relations with leaf physiology were detected (Figure 4A). On the contrary, photosynthetic traits were positively correlated with leaf morphology and rhizome nutrient content. For example, rETR_{max} and rhizome C content were positively correlated (Figure 4B).

Table 4. Permutational analysis of variance (PERMANOVA) of the effects of temperature (T) and nutrient (N) treatments on the physiological traits of *T. hemprichii*, *C. serrulata*, and *H. stipulacea* in Figure 3.

Trait	Source	<i>T. hemprichii</i>					<i>C. serrulata</i>					<i>H. stipulacea</i>				
		df	SS	R ²	Pseudo-F	P	df	SS	R ²	Pseudo-F	P	df	SS	R ²	Pseudo-F	P
F _v /F _m	T	1	1.00	0.01	1.42	0.25	1	3.00	0.04	3.68	0.05	1	1.12	0.03	1.08	0.31
	N	1	0.91	0.01	1.28	0.29	1	0.93	0.01	1.14	0.28	1	0.01	0.00	0.01	0.91
	T:N	1	0.00	0.00	0.00	0.99	1	0.05	0.00	0.06	0.79	1	1.48	0.04	1.43	0.23
	T:ET:Aq	20	34.5	0.50	2.45	0.02	20	27.5	0.40	1.69	0.06	7	6.45	0.19	0.89	0.55
	Res	46	32.5	0.47			46	37.4	0.54			24	24.9	0.73		
rETR _{max}	T	1	12.2	0.18	17.2	0.00	1	7.61	0.11	9.78	0.00	1	7.56	0.22	8.46	0.01
	N	1	1.93	0.03	2.72	0.10	1	0.58	0.01	0.74	0.41	1	0.19	0.01	0.21	0.64
	T:N	1	1.25	0.02	1.75	0.19	1	1.09	0.02	1.40	0.25	1	0.01	0.00	0.01	0.95
	T:ET:Aq	20	20.8	0.03	1.46	0.16	20	23.9	0.35	1.54	0.10	7	4.80	0.14	0.77	0.62
	Res	46	32.7	0.47			46	35.7	0.52			24	21.4	0.63		
Alpha	T	1	0.37	0.01	0.76	0.38	1	4.56	0.07	7.81	0.00	1	17.7	0.52	36.4	0.00
	N	1	2.48	0.04	5.11	0.03	1	0.02	0.00	0.03	0.84	1	0.29	0.01	0.60	0.47
	T:N	1	0.21	0.00	0.44	0.50	1	1.62	0.02	2.77	0.09	1	0.59	0.02	1.21	0.30
	T:ET:Aq	20	43.6	0.63	4.50	0.00	20	35.9	0.52	3.07	0.00	7	3.74	0.11	1.10	0.38
	Res	46	22.3	0.32			46	26.8	0.39			24	11.6	0.34		
E _k	T	1	1.13	0.02	1.68	0.18	1	6.35	0.09	8.48	0.00	1	19.7	0.58	46.8	0.00
	N	1	0.97	0.01	1.45	0.27	1	2.29	0.03	3.05	0.08	1	1.01	0.03	2.41	0.13
	T:N	1	0.05	0.00	0.07	0.80	1	1.37	0.02	1.83	0.18	1	0.21	0.01	0.51	0.47
	T:ET:Aq	20	35.9	0.52	2.68	0.01	20	24.5	0.36	1.64	0.09	7	2.89	0.08	0.98	0.46
	Res	46	30.9	0.45			46	34.4	0.50			24	10.1	0.30		
Growth	T	1	4.70	0.20	7.19	0.01	1	0.35	0.02	0.31	0.56	-	-	-	-	-
	N	1	3.19	0.14	4.87	0.04	1	0.02	0.00	0.02	0.90	-	-	-	-	-
	T:N	1	0.21	0.01	0.32	0.59	1	0.17	0.01	0.15	0.67	-	-	-	-	-
	T:ET:Aq	2	3.15	0.14	2.41	0.12	2	2.56	0.11	1.16	0.32	-	-	-	-	-
	Res	18	11.7	0.51			18	19.9	0.87			-	-	-		

Significant differences are in boldface (*: < 0.05; **: < 0.01; ***: < 0.001). (ET: experimental tank, Aq: aquarium).

Contrary to the other two species, all *H. stipulacea* correlations, when observed, were positive (Supplementary Table 4). Correlations between leaf length and BG C content, including sucrose, and E_k and root length were detected, linking AG and BG traits (Supplementary Table 4). Correlations between the same plant part were frequent, such as between leaf N and C contents (Figure 4D), or root C content and length (Figure 4F). But no correlations between

photosynthetic performance and leaf morphometrics or nutrient content were detected (Figures 4 A, C).

IV. Discussion

The present study aimed to examine the interactive and single effects on an extended exposure to the current ambient maximum temperature and nutrient enrichment in three tropical seagrass species. During the 5-week period, the two factors and their interaction differentially affected the biochemical, morphological and physiological traits considered, highlighting the varying strategies and tolerances among species to the treatments (Figure 5). Therefore, this study shows that *T. hemprichii*, *C. serrulata* and *H. stipulacea* have distinctly different responses to increasing temperature and nutrient enrichment despite their overlap in their distribution and the co-inhabitation of seagrass meadows. This suggests that ecosystem functioning and seagrass survivorship will be differently affected by changing environments.

(1) Seagrass Responses to an Extended Exposure to the Maximum Ambient Temperature

Temperature was the main driver of seagrass responses during this study, as all trait categories showed some response to the single effect of this factor, and these responses showed overall bigger differences than responses to nutrients (Figure 5). In accordance with previous studies we did not observe any variation in F_v/F_m (maximum quantum yield) from reference values in any of the three species, showing that the highest target temperature (31 °C) is lower than the maximum tolerance limits of the studied species (Campbell et al. 2006; Pedersen et al. 2016; Collier et al. 2017; Anton et al. 2020). However, the photosynthetic capacity ($rETR_{max}$), related with the investment in biochemical mechanisms for CO₂ fixation, is lower under HT treatments in the three species studied, showing some down-regulating mechanisms, and suggesting that all individuals were above their thermal optima.

In this study, *C. serrulata* and *H. stipulacea* showed photoacclimation mechanisms to maximize C fixation and obtain extra energy, with lower saturating irradiance (E_k) and maximal photosynthetic efficiency (Alpha), as shown in congeneric species (Torquemada et al. 2005; Campbell et al. 2007). Contrary to this, and in accordance with previous studies, no physiological acclimation was observed in *T. hemprichii* under HT treatments (Campbell et al. 2006; Pollard and Greenway 2013; George et al. 2018). Instead, this latter species showed a high morphological plasticity (longer leaves and sheaths and faster growth rate) that might maximize exposure of photosynthetic material to irradiance and minimize boundary layer

thickness for gas, lowering its thermal tolerance. The ability of *T. hemprichii* to change its morphology without enhancing its photosynthetic performance, suggests this species may rely on its high N storage capacity (Viana et al. 2019a) shown by the high plant N content in comparison to the other two species (Figure 1). Similar to *T. hemprichii*, the opportunistic species *C. serrulata*, also enhanced its morphology during the study period. But contrary to the former species, *C. serrulata* was able to obtain greater energy from photosynthesis suggesting that the thermal limit of this species is higher than for *T. hemprichii*. While thermal optima values in the literature are highly variable even within species, our results are in accordance with previous findings in *T. hemprichii* from the Red Sea in which the optimum temperature for the metabolic rates was 30.4 °C (Anton et al. 2020). Instead, experiments with *C. serrulata* showed large declines at 35 °C or lower temperatures at an individual and community level (Collier et al. 2018; George et al. 2018; Burkholz et al. 2019).

The results in this study showed that the tolerant species *H. stipulacea* also acclimated to HT treatments similar to *C. serrulata*. This acclimation was performed both by increasing its Alpha values and, contrary to *C. serrulata*, with smaller-sized plants (lower leaf and root length). The decrease in BG growth is a common strategy in seagrasses plants to adjust their productivity to environmental resources (Alcoverro et al. 2001). Shorter IL, as a proxy of higher shoot and root density, is a common feature under stressful environmental factors, suggesting that *H. stipulacea* invests excess energy (increased Alpha values) in increasing number of shoots (Jensen and Bell 2001; Kilminster et al. 2008) rather than on leaf length. In the literature, *H. stipulacea* thermal optima and limits showed differences between populations (Nguyen et al. 2020; Wesselmann et al. 2020) but thermal optima at the Red Sea was set at 30 C (Anton et al. 2020; Wesselmann et al. 2020). It is possible that these differences highlight the importance of the different population responses in coping with stressors (McMillan and Phillips 1979; Winters et al. 2011; Nguyen et al. 2020; Wesselmann et al. 2020) where other environmental factors, such as light irradiance, or population genetics influence the responses (Collier et al. 2016).

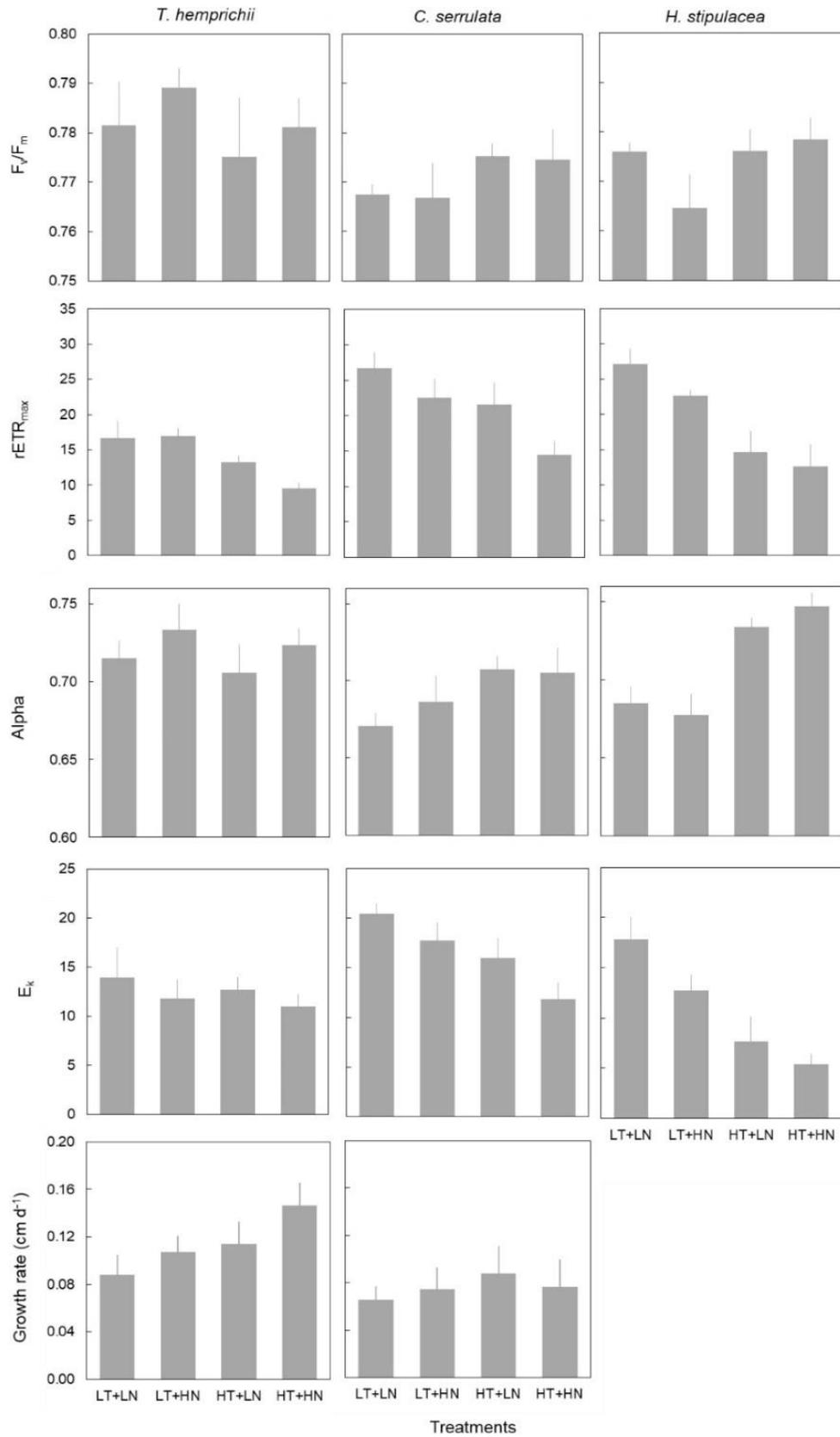


Figure 3. Physiological traits (mean \pm SE, $n = 6$, except of *H. stipulacea*, $n = 3$) of *T. hemprichii*, *C. serrulata* and *H. stipulacea* in the four treatments (LT, low temperature; LN, low nutrient; HT, high temperature; HN, high nutrient).

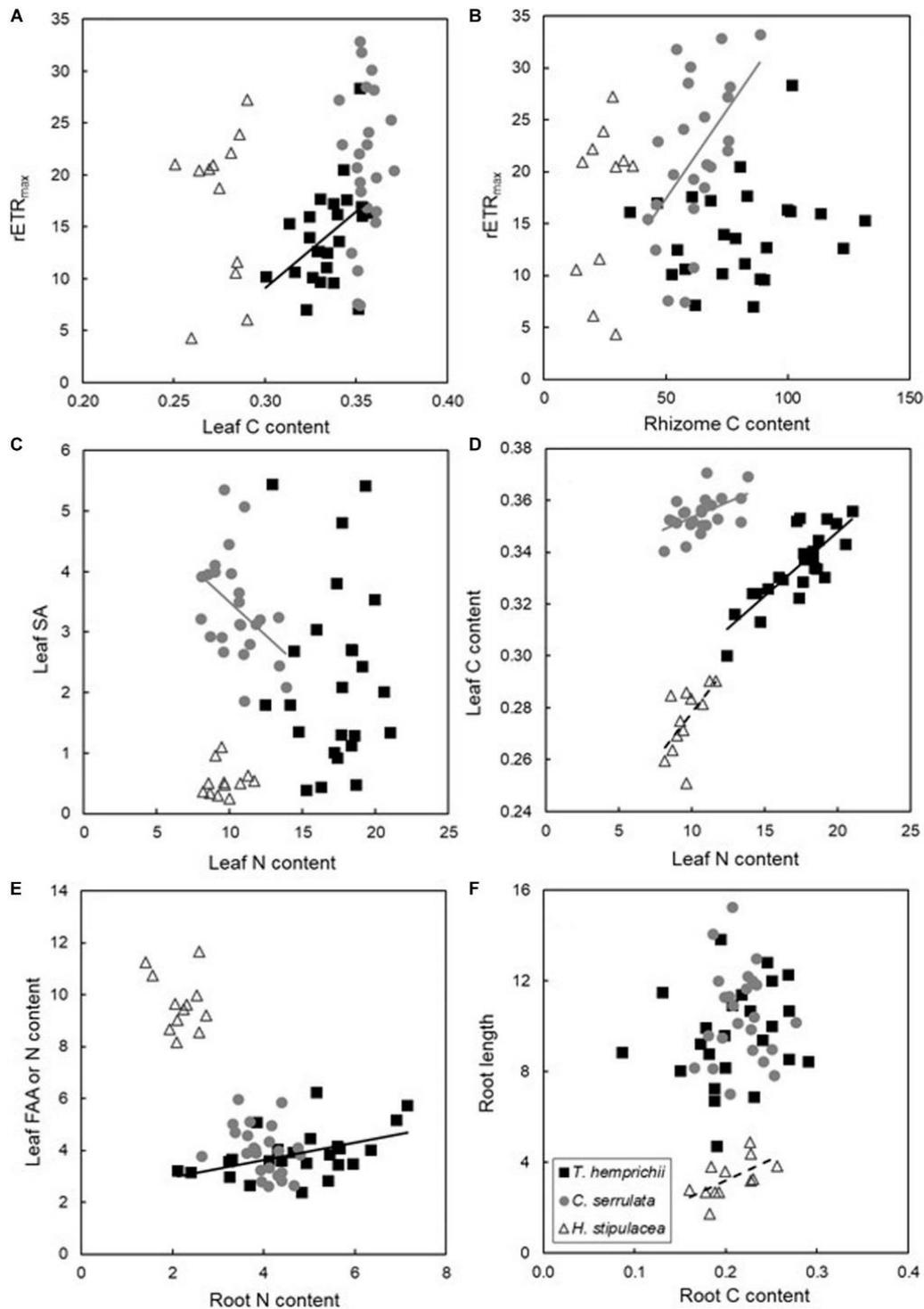


Figure 4. Pearson r correlations among selected biochemical (A–F), morphological (C,F) and physiological (A,B) traits (Supplementary Tables 2–4). Correlation lines are shown only when significant as black, gray and dashed lines for *T. hemprichii*, *C. serrulata* and *H. stipulacea* data respectively. Panel (E) show leaf FAA content excepting for *H. stipulacea* that shows leaf N content.

Therefore, projections under future climate change scenarios might vary among co-habiting species, and within the same species, depending on the local adaptations and acclimation of seagrasses across geographical ranges. Morphological plasticity was a key acclimation response

of the three species, but *H. stipulacea* and *C. serrulata* were the species that better acclimated to HT temperatures by primarily changing a combination of their biochemical, physiological and morphological traits. *T. hemprichii*, a climax species, which is expected to show slower acclimation times, surprisingly acclimatized to new environmental conditions by varying primarily its morphology, in the same time frame as *H. stipulacea*, a colonizing species. Further studies on *T. hemprichii* physiology are needed to understand its acclimation strategy and high plasticity.

(2) Seagrass Responses to Water Column Nutrient Enrichment

In tropical environments, nutrients, as N, P or even Fe, are usually the limiting factor to growth (Pérez et al. 1991; Duarte et al. 1995; Agawin et al. 1996). Even though native species from these areas are adapted to survive in extremely low nutrient habitats, they usually respond to in situ or experimental nutrient additions by increasing their nutrient content, but also in terms of physiology and morphology, as shown in this study (Figure 5).

Although there were no changes in N content in the BG tissues, the enhanced leaf N content in *T. hemprichii* and *C. serrulata* suggests that they were nutrient limited. These differences between tissues might be explained because we enriched the water column but not the sediment, so it would take more time for the sediment to become nutrient enriched and to see changes in N storage in BG tissues. In contrast, the absence of biochemical and other trait responses both in AG and BG tissues of *H. stipulacea* suggests that this species was not N-limited. This interesting result might be related to the smaller size of *H. stipulacea*, which is related to the lower N demands, with lower N uptake and assimilation rates compared to the other two species, even under high N treatments (Alexandre et al. 2014; Viana et al. 2019a). But, surprisingly, *H. stipulacea* has been spotted in highly eutrophic areas where native species are not able to grow (Van Tussenbroek et al. 2016; Winters et al. 2020) showing significantly higher leaf N content and enhanced morphology compared to control sites (Mejia et al. 2016; Beca-Carretero et al. 2020). These differential trait responses between natural populations and this study suggests that trait's plasticity under nutrient-enrichment might be an adaptative response of the population to long-term changes rather than an acclimation response at an individual level. Although interspecific competition was not purposely studied, due to the experimental design in which all seagrasses were planted together, we cannot rule out competition among the primary producers in the aquaria for nutrient resources (Artika et al. 2020). *H. stipulacea* may be outcompeted for resources due to its smaller size when compared to the leaf and sheath length of *T. hemprichii* and *C. serrulata*. However, there are several

occasions where the species forms mixed meadows with other seagrass species or macroalgae (Boudouresque et al. 2009; Sghaier et al. 2011; Gambi et al. 2018; Apostolaki et al. 2019).

As N and P are normally the limiting nutrients in the tropics, C could potentially become the limiting factor under HN scenarios (Buapet et al. 2013; Apostolaki et al. 2014). Therefore, species with large C storage pools, such as *T. hemprichii*, might have their C demands already covered in all tissues (including leaves), and nutrient over-enrichment might first increase its photosynthetic efficiency (α) and afterward enhance growth rate without C being limiting. Contrary to *T. hemprichii*, the opportunistic species *C. serrulata* followed a common strategy that mobilizes the rhizome carbohydrate reserves to perform photosynthesis (Figure 4B) and synthesize amino acids leading to lower levels of starch in BG tissues (Invers et al. 2004; Leoni et al. 2008). The correlation between $rETR_{max}$ and leaf C in *T. hemprichii* (Figure 4A) also supports the hypothesis that this species does not depend on storage tissues. Together with C storage depletion, *C. serrulata* showed no enhanced growth, and lower saturating irradiance (E_k), which are all typical responses to nutrient inputs in both tropical and temperate seagrasses (Martínez-Crego et al. 2008; Jiang et al. 2013). Overall, *T. hemprichii* performed better than *C. serrulata* under short-term nutrient enrichment (> 5 weeks), while *H. stipulacea* showed a lack of response, which indicates a dormancy state typical for this tolerant species (Apostolaki et al. 2018; Hernández-Delgado et al. 2020).

(3) Combined Effect of Different Drivers: Stressors Do Not Act Alone

Our results show that the interaction between both factors is limited to responses in morphological and biochemical traits, as no changes were observed in the physiological performance of the seagrasses, at least during the short-term experimental period (Figure 5). This study provides the first insights of the interactive effects of nutrient over-enrichment and increasing temperature in tropical species, therefore comparisons with other tropical species of other geographic areas is still not possible. While synergistic effects are frequent in coastal ecosystems, including seagrasses, in this study we mainly detected antagonistic interactive effects of the studied stressors (Gunderson et al. 2016; Stockbridge et al. 2020). Therefore, the results within this study show that the impact of increasing temperature average values will potentially be less detrimental to plant traits in tropical nutrient over-enrichment seagrass meadows in the short-term. However, this study does not take into account other biogeochemical processes taken place during the eutrophication process, as sediment anoxia or light deprivation (Burkholder et al. 2007).

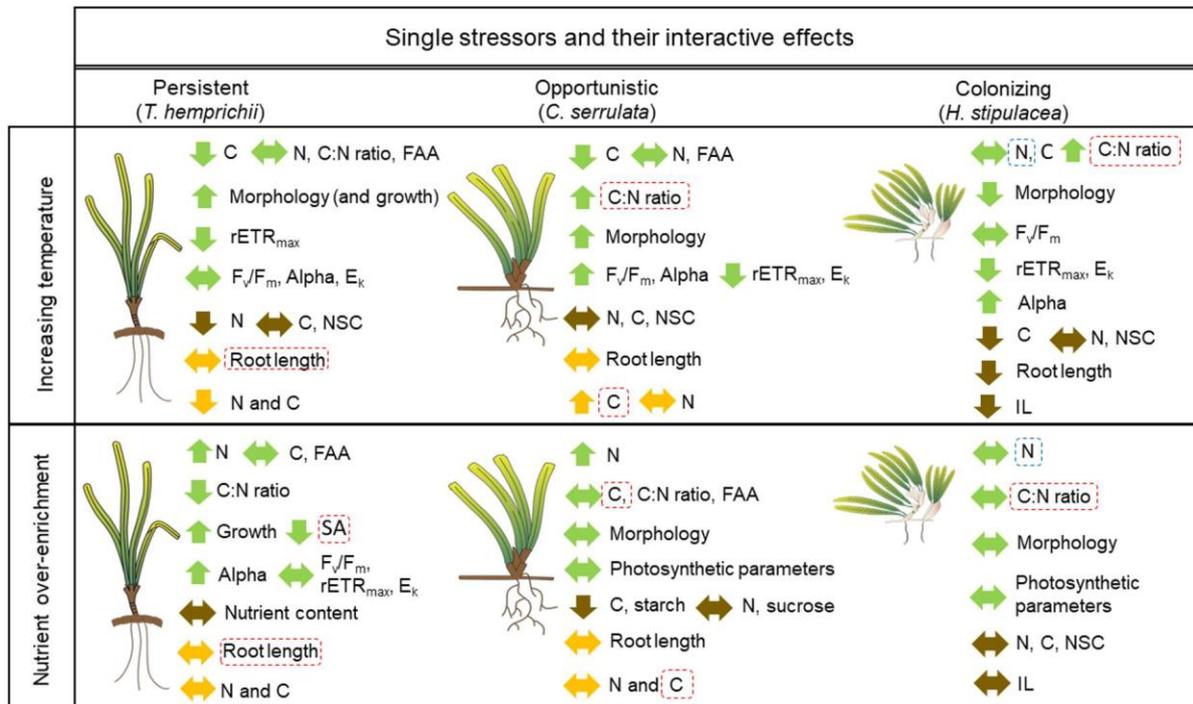


Figure 5. Schematic representation of the significant responses ($P < 0.05$) to increasing temperature (31C), nutrient over-enrichment and their antagonistic (dashed red lines) and synergistic (dashed blue line) interactive effects of leaves (green arrows), rhizome (brown arrows) and roots (orange arrows) in *T. hemprichii*, *C. serrulata* and *H. stipulacea*. Upward arrows show enhanced, downward arrows show depressed and horizontal arrows show no changes in the responses under single factor effects. (FAA, free amino acids; SA, surface area; NSC, non-structural carbohydrates; IL, internode length) Seagrass drawings are courtesy of the Integration and Application Network (www.ian.umces.edu/symbols/).

Interactions between both factors were observed in both AG and BG tissues. The effects of warming on *C. serrulata* and *H. stipulacea* leaf C:N ratio were significantly mediated by nutrient over-enrichment, showing lower values than expected if effects would be additive (Figure 1). The enhanced productivity caused by higher temperatures in these species suggests that C limitation may occur in both AG and BG tissues when other nutrients, namely N and P, are not limiting in the water column. This would not be deleterious for these species, as C:N ratio values observed under the HT + HN treatment were, overall, closer to values in the control (LT + LN treatment) showing less unbalances among tissues than it would be expected under synergistic interactions. While leaf N content in *H. stipulacea* did not respond to any single-factor treatment, it showed the only synergistic interactive effect in this study. This shows that temperature might mediate responses to N over-enrichment through enhancing N metabolism, as shown for the temperate species *Z. marina* (Alexandre et al. 2020). In the literature, biochemical responses to the interactive effect of temperature and nutrients are variable, and

have also been observed in leaves and BG nutrient content of *Z. marina* (Touchette and Burkholder 2002; Moreno-Marín et al. 2018), but not in *C. nodosa*, or other *Zostera* species (Kaldy 2014; Brodeur et al. 2015; Egea et al. 2018; Mvungi and Pillay 2019; Ontoria et al. 2019b).

The interactive effects of both factors in *T. hemprichii*, contrary to the results of the other two species, were related to morphological traits. Leaf SA and root length values under the HT + HN treatment decreased drastically, showing an antagonistic effect of both factors. Morphological plasticity has been suggested to have adaptive advantages in heterogeneous environments, allowing organisms to maximize resource acquisition under unpredictable or changing levels of resource availability (Houston and McNamara 1992). In this sense, the high plasticity shown by this species, both under single and cumulative stressors, is highly remarkable, especially when compared to *C. serrulata*, indicating that *T. hemprichii* can adopt a functional role closer to opportunistic or climax species when necessary. Interactive effects on morphology have been observed in leaf SA of *Z. marina*; leaf length of *Z. capensis* (Bintz et al. 2003; Mvungi and Pillay 2019), and more frequently under natural conditions in *T. hemprichii* and other tropical species (Udy et al. 1999; Ali et al. 2018). Even though the mechanisms behind this interaction cannot be elucidated within the current study, high physiological activity in terms of photosynthesis and nutrient uptake and assimilation under enhanced temperature and nutrient over-enrichment (Touchette and Burkholder 2007; Kaldy 2014; Alexandre et al. 2020) may cause this limitation of energy to enhance growth, and therefore, morphology. Also, increasing organic matter in sediment has been related with lower BG growth (Ontoria et al. 2019b), although the low nutrient concentrations in sediment in this experiment does not sustain this hypothesis as the main reason. The interaction of both factors might be positive for the species survival, as no large differences in morphological traits might infer smaller differences between AG and BG biomass, one of the main reasons behind the increasing mortalities in seagrass meadows (Collier and Waycott 2014).

In this study, physiological traits showed little variance under combined stressors in the three species studied. These results are in accordance with other studies with temperate species that showed no interactive effects on fluorescence parameters or growth (Bintz et al. 2003; Moreno-Marín et al. 2018; Ontoria et al. 2019b). However, in some studies significant interactions were found for yield, growth (Kaldy 2014; Mvungi and Pillay 2019; Ontoria et al. 2019a), or photosynthetic rate, as well as gross production rates (Egea et al. 2018; Moreno-Marín et al. 2018). Comparison among studies is difficult due to the variable traits measured; and care must

be taken when comparing results that differ in exposure period, the intensity of the stressors (e.g., nutrient concentration used), or even the different geographical areas of the species, as all these factors, among others, might affect the plasticity of seagrass traits.

It is generally accepted that plant acclimation follows a sequence of ordered changes that starts in the photosynthetic apparatus, followed by biochemical changes, as nutrient content, morphological changes and finally the population and community changes (McMahon et al. 2013; Roca et al. 2016). Such sequence will be also slower in foundation or persistent species, which are recognized to show higher physiological resistance than opportunistic or tolerant species. The simultaneous study of species with different life-history traits and trait categories have shown that different species might show different response and acclimation strategies. Therefore, despite the increasing number of studies on interactive effects of stressors, this study shows the need of further research to extract further conclusions on how seagrass meadows will respond to multiple stressors.

(4) Ecological Implications

The varying responses of the three tropical species studied might imply that there are winners and losers under the changing scenarios studied. For instance, changes in biochemical traits have been observed under single-factor and cumulative treatments, especially in the large-blade species in this study. AG nutrient changes have been related with changes in palatability and herbivory preferences which might affect the food web structure and top-down pressure, affecting the resilience capacity of seagrass meadows (Jiménez-Ramos et al. 2017). Also, although C concentrations in AG tissues could be unaffected by changes in environmental nutrient concentrations (Brodeur et al. 2015), increases in C content with increasing dissolved N or P can occur (Lee and Dunton 1999; Ali et al. 2018; Mvungi and Pillay 2019) (Figure 4D). In fact, the ability of *T. hemprichii* to take advantage of the excess nutrients by increasing its photosynthetic efficiency suggests a dependence between nutrient limitation and photosynthetic C incorporation related to the synthesis of photosynthetically involved molecules such as chlorophyll, on nutrient supply (Agawin et al. 1996). This can result in a biochemical unbalance caused by the allocation of photosynthetically fixed C to leaf production at the expense of nutrient-deplete BG tissues.

The diminishing C concentrations in rhizome, as observed in *C. serrulata* under HN treatments, may ultimately affect C storage and nutrient retention, which are also important ecosystem services provided by seagrasses (Nielsen et al. 2004; Fourqurean et al. 2012), as C storage plays

a key role in mitigating anthropogenic CO₂ emissions under the current climate change scenario. The biochemical disproportion caused by these stressors might eventually cause a biomass imbalance, increasing the AG:BG biomass ratios that will reduce the sediment stabilization or oxygenation capacity (Pedersen et al. 1998; Christianen et al. 2013), which are essential ecosystem services provided by seagrass meadows. Moreover, internal C reserves, as sugar and starch concentrations in the rhizome, are important energy suppliers under stressful events such as physical perturbations, shading or nutrient limitation (Soissons et al. 2018). Therefore, the diminishing C storage products would also be expected to exacerbate seagrass decline and to depress the ability of plants to survive dehiscence and dormancy periods (Burkholder et al. 2007). In fact, *T. hemprichii*, and not *C. serrulata*, was present in a highly eutrophic seagrass meadow in the Stone Town area (Zanzibar Archipelago, Tanzania), confirming that the former species have higher tolerance to these conditions even under long-term natural conditions (Teichberg et al. in preparation). Similarly, *T. hemprichii* was pointed as the most persistent species in other highly impacted seagrass meadows in China (Thomsen et al. 2020). Therefore, the unequal effect of eutrophication in different species might cause a biodiversity loss in tropical seagrass meadows.

Enhanced leaf morphological traits, primarily observed in *T. hemprichii*, may limit the biomechanical properties and the exposed surface area of the blades, which directly affect survival under high water dynamics during storms (La Nafie et al. 2012). *H. stipulacea*, contrary to the large blades species, did not appear to be negatively affected by the studied factors, showing potentially a dormancy strategy under the different treatments. Even though performance differences have been observed between individuals from its native and invasive ranges (Nguyen et al. 2020; Wesselmann et al. 2020) this species is generally recognized to be tolerant to a wide range of trophic conditions and temperatures (Winters et al., 2020). Although *H. stipulacea* is considered as invasive in the Mediterranean and Caribbean Seas, there is no evidence of competition and displacement of native species in these areas (Boudouresque et al. 2009; Al-Rousan et al. 2011; Gambi et al. 2018; Apostolaki et al. 2019). In addition, the provision of essential ecosystems services by this little seagrass is still under debate and needs further research (Apostolaki et al. 2019; Viana et al. 2019b; Muthukrishnan et al. 2020).

As the duration and frequency of heat waves has increased worldwide in the last century and is expected to continue increasing (Oliver et al. 2018), *C. serrulata* may show some advantages over *T. hemprichii* under these increasing temperature scenarios, at least under the target temperature of this study. On the other side, *T. hemprichii* may perform better under nutrient

over-enrichment scenarios, even at higher temperature. If conditions are too detrimental for these species to grow, in terms of light deprivation or anoxic conditions in the sediment, *H. stipulacea* could potentially colonize the area, as it has been shown to be the most plastic and tolerant species in this study and, in general, among other seagrass species.

Overall, the tropical seagrasses in this study showed different tolerances and strategies to cope with stressors, but these responses could be critical in the long run for the seagrass survival and meadow persistence, compromising the maintenance of functions and services provided by the seagrass meadows. Seagrass individual trait responses to stressors are important as a first step to understand the upscaling of ecological consequences of climate change and eutrophication on ecosystem functioning and services. How the individual responses affect their functioning and their abiotic and biotic interactions, however, is an issue that cannot be answered with this experiment and needs further study.

V. Conclusions

The holistic picture of seagrass responses highlights that the acclimation and resistance mechanisms behind photosynthesis are closely related with the whole-plant physiology, affecting the performance of the species in the long run. Seagrass species sharing the same original geographic area, or even the same meadow, may respond differently to temperature and nutrient conditions due to unique life history traits. *T. hemprichii* was positively influenced by nutrient overenrichment conditions, while *H. stipulacea* was tolerant to these conditions, and *C. serrulata* was negatively affected. On the contrary, *C. serrulata* showed a better acclimation under HT scenarios than *H. stipulacea* and *T. hemprichii*. Interaction of both factors negatively affected some important traits of the three species.

Therefore, different scenarios might show winners and losers, but there is no trait that makes a winner under all circumstances, suggesting that if conditions change, some species survivorship might be endangered. Tropical seagrass meadows are characterized by high species diversity, but their functional roles are not always clearly interchangeable, as within the species in this study, and displacement of any of the three species may cause a functional or service loss of the seagrass community as a whole (Duarte, 2000).

VI. Acknowledgments

We would like to thank Suci R. Artika for her help during the experiment, the Chemistry laboratory, BioLab and MAREE facilities at the ZMT for the technical support during the experiment and the sample analysis. The present study is under the framework of the project

SEAMAC (Seagrass and macroalgal community dynamics and performance under environmental change funded by the German Research Foundation (DFG; TE 1046/3-1) awarded to MT. IV was awarded with a postdoctoral fellowship of the Leibniz-DAAD postdoctoral program (Germany) and Xunta de Galicia (Consellería de Educación, Universidade e Formación Profesional) postdoctoral programme (ED481B- 2016/189-0).

VII. References

- Adams, M. P., Koh, E. J. Y., Vilas, M. P., Collier, C. J., Lambert, V. M., Sisson, S. A., et al. (2020). Predicting seagrass decline due to cumulative stressors. *Environmental Modelling and Software*, 130, 104717. <https://doi.org/10.1016/j.envsoft.2020.104717>
- Agawin, N. S. R., Duarte, C. M., and Fortes, M. D. (1996). Nutrient limitation of Philippine seagrasses (Cape Bolinao, NW Philippines): in situ experimental evidence. *Marine Ecology Progress Series*, 138, 233–243. <https://doi.org/10.3354/meps138233>
- Alcoverro, T., Manzanera, M., and Romero, J. (2001). Annual metabolic carbon balance of the seagrass *Posidonia oceanica*: the importance of carbohydrate reserves. *Marine Ecology Progress Series*, 211, 105–116. <https://doi.org/10.3354/meps211105>
- Alexandre, A., Georgiou, D., and Santos, R. (2014). Inorganic nitrogen acquisition by the tropical seagrass *Halophila stipulacea*. *Marine Ecology*, 35, 387–394. <https://doi.org/10.1111/maec.12128>
- Alexandre, A., Quintã, R., Hill, P. W., Jones, D. L., and Santos, R. (2020). Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass *Zostera marina*. *Functional Ecology*, 34, 1325–1335. <https://doi.org/10.1111/1365-2435.13576>
- Ali, S. M., He, J., Goh, B. P. L., and Sin, T. M. (2018). Environmental correlates of *Thalassia hemprichii* status descriptors: an evaluation of tools for diagnostic monitoring. *Botanica Marina*, 61, 321–335. <https://doi.org/10.1515/bot-2017-0083>
- Al-Rousan, S., Al-Horani, F., Eid, E., and Khalaf, M. (2011). Assessment of seagrass communities along the Jordanian coast of the Gulf of Aqaba, Red Sea. *Marine Biology Research*, 7, 93–99. <https://doi.org/10.1080/17451001003660319>
- Anderson, M. J., Gorley, R. N., and Clarke, K. R. (2008). *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods*. Plymouth: PRIMER-E.
- Anton, A., Baldry, K., Coker, D. J., and Duarte, C. M. (2020). Drivers of the low metabolic rates of seagrass meadows in the Red Sea. *Frontiers in Marine Science*, 7, 69. <https://doi.org/10.3389/fmars.2020.00069>
- Apostolaki, E. T., Holmer, M., Santinelli, V., and Karakassis, I. (2018). Species specific response to sulfide intrusion in native and exotic Mediterranean seagrasses under stress. *Marine Environmental Research*, 134, 85–95. <https://doi.org/10.1016/j.marenvres.2017.12.006>

- Apostolaki, E. T., Vizzini, S., Hendriks, I. E., and Olsen, Y. S. (2014). Seagrass ecosystem response to long-term high CO₂ in a Mediterranean volcanic vent. *Marine Environmental Research*, 99, 9–15. <https://doi.org/10.1016/j.marenvres.2014.05.008>
- Apostolaki, E. T., Vizzini, S., Santinelli, V., Kaberi, H., Andolina, C., and Papatthanassiou, E. (2019). Exotic *Halophila stipulacea* is an introduced carbon sink for the Eastern Mediterranean Sea. *Scientific Reports*, 9, 9643. <https://doi.org/10.1038/s41598-019-45046-w>
- Artika, S. R., Ambo-Rappe, R., Teichberg, M., Moreira-Saporiti, A., and Viana, I. G. (2020). Morphological and physiological responses of *Enhalus acoroides* seedlings under varying temperature and nutrient treatment. *Frontiers in Marine Science*, 7, 325. <https://doi.org/10.3389/fmars.2020.00325>
- Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., and Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81, 169–193. <https://doi.org/10.1890/10-1510.1>
- Beca-Carretero, P., Rotini, A., Mejia, A., Migliore, L., Vizzini, S., and Winters, G. (2020). *Halophila stipulacea* descriptors in the native area (Red Sea): a baseline for future comparisons with native and non-native populations. *Marine Environmental Research*, 153, 104828. <https://doi.org/10.1016/j.marenvres.2019.104828>
- Beiras, R. (2018). *Marine Pollution. Sources, Fate and Effects of Pollutants in Coastal Ecosystems*. Amsterdam: Elsevier. <https://doi.org/10.1016/b978-0-12-813736-9.00015-5>
- Bertelli, C. M., and Unsworth, R. K. F. (2018). Light stress responses by the eelgrass, *Zostera marina* (L). *Frontiers in Environmental Science*, 6, 39. <https://doi.org/10.3389/fenvs.2018.00039>
- Bintz, J. C., Nixon, S. W., Buckley, B. A., and Granger, S. L. (2003). Impacts of temperature and nutrients on coastal lagoon plant communities. *Estuaries*, 26, 765–776. <https://doi.org/10.1007/BF02711987>
- Boudouresque, C. F., Bernard, G., Pergent, G., Shili, A., and Verlaque, M. (2009). Regression of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review. *Botanica Marina*, 52, 395–410. <https://doi.org/10.1515/BOT.2009.057>
- Brodeur, M. C., Piehler, M. F., and Fodrie, F. J. (2015). Consumers mitigate heat stress and nutrient enrichment effects on eelgrass *Zostera marina* communities at its southern range limit. *Marine Ecology Progress Series*, 525, 53–64. <https://doi.org/10.3354/meps11186>
- Buapet, P., Rasmusson, L. M., Gullström, M., and Björk, M. (2013). Photorespiration and carbon limitation determine productivity in temperate seagrasses. *PLoS One*, 8, e83804. <https://doi.org/10.1371/journal.pone.0083804>
- Bulthuis, D. A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquatic Botany*, 2, 27–40. [https://doi.org/10.1016/0304-3770\(87\)90084-2](https://doi.org/10.1016/0304-3770(87)90084-2)
- Burkholder, J. M., Glasgow, H. B. Jr., and Cooke, J. E. (1994). Comparative effects of water-column nitrate enrichment on eelgrass *Zostera marina*, shoalgrass *Halodule wrightii*, and

- widgeongrass *Ruppia maritima*. *Marine Ecology Progress Series*, 105, 121–138. <https://doi.org/10.3354/meps105121>
- Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, 350, 46–72. <https://doi.org/10.1016/j.jembe.2007.06.024>
- Burkholz, C., Duarte, C. M., and Garcias-Bonet, N. (2019). Thermal dependence of seagrass ecosystem metabolism in the Red Sea. *Marine Ecology Progress Series*, 614, 79–90. <https://doi.org/10.3354/meps12912>
- Burnell, O. W., Russell, B. D., Irving, A. D., and Connell, S. D. (2013). Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification. *Marine Ecology Progress Series*, 485, 37–46. <https://doi.org/10.3354/meps10323>
- Campbell, S. J., McKenzie, L. J., and Kerville, S. P. (2006). Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *Journal of Experimental Marine Biology and Ecology*, 330, 455–468. <https://doi.org/10.1016/j.jembe.2005.09.017>
- Campbell, S. J., McKenzie, L. J., Kerville, S. P., and Bite, J. S. (2007). Patterns in tropical seagrass photosynthesis in relation to light, depth and habitat. *Estuarine, Coastal and Shelf Science*, 73, 551–562. <https://doi.org/10.1016/j.ecss.2007.02.014>
- Christianen, M. J., van Belzen, J., Herman, P. M., van Katwijk, M. M., Lamers, L. P., van Leent, P. J., et al. (2013). Low-canopy seagrass beds still provide important coastal protection services. *PLoS One*, 8, e62413. <https://doi.org/10.1371/journal.pone.0062413>
- Collier, C. J., Adams, M. P., Langlois, L., Waycott, M., O'Brien, K. R., Maxwell, P. S., et al. (2016). Thresholds for morphological response to light reduction for four tropical seagrass species. *Ecological Indicators*, 67, 358–366. <https://doi.org/10.1016/j.ecolind.2016.02.050>
- Collier, C. J., Langlois, L., Yan Ow, Y., Johansson, C., Giammusso, M., Adams, M. P., et al. (2018). Losing a winner: thermal stress and local pressures outweigh the positive effects of ocean acidification for tropical seagrasses. *New Phytologist*, 219, 1005–1017. <https://doi.org/10.1111/nph.15234>
- Collier, C. J., Ow, Y. X., Langlois, L., Uthicke, S., Johansson, C. L., O'Brien, K. R., et al. (2017). Optimum temperatures for net primary productivity of three tropical seagrass species. *Frontiers in Plant Science*, 8, 1446. <https://doi.org/10.3389/fpls.2017.01446>
- Collier, C. J., and Waycott, M. (2014). Temperature extremes reduce seagrass growth and induce mortality. *Marine Pollution Bulletin*, 83, 483–490. <https://doi.org/10.1016/j.marpolbul.2014.03.050>
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., and Hannon, B. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387, 253–260. <https://doi.org/10.1038/387253a0>

- de la Torre-Castro, M., Di Castro, G., and Jiddawi, N. S. (2014). Seagrass importance for a small-scale fishery in the tropics: the need for seascape management. *Marine Pollution Bulletin*, 83, 398–407. <https://doi.org/10.1016/j.marpolbul.2014.03.034>
- Dewsbury, B. M., Bhat, M., and Fourqurean, J. W. (2016). A review of seagrass economic valuations: gaps and progress in valuation approaches. *Ecosystem Services*, 18, 68–77. <https://doi.org/10.1016/j.ecoser.2016.02.010>
- Duarte, C. M. (1990). Seagrass nutrient content. *Marine Ecology Progress Series*, 67, 201–207. <https://doi.org/10.3354/meps067201>
- Duarte, C. M. (1991). Allometric scaling of seagrass form and productivity. *Marine Ecology Progress Series*, 77, 289–300. <https://doi.org/10.3354/meps077289>
- Duarte, C. M. (2000). Marine biodiversity and ecosystem services: an elusive link. *Journal of Experimental Marine Biology and Ecology*, 250, 117–131. [https://doi.org/10.1016/S0022-0981\(00\)00194-5](https://doi.org/10.1016/S0022-0981(00)00194-5)
- Duarte, C. M., Merino, M., and Gallegos, M. (1995). Evidence of iron deficiency in seagrasses growing above carbonate sediments. *Limnology and Oceanography*, 40, 1153–1158. <https://doi.org/10.4319/lo.1995.40.6.1153>
- Durako, M. J., and Moffler, M. D. (1985). Observations on the reproductive ecology of *Thalassia testudinum* (Hydrocharitaceae). III. Spatial and temporal variations in reproductive patterns within a seagrass bed. *Aquatic Botany*, 22, 265–276. [https://doi.org/10.1016/0304-3770\(85\)90004-X](https://doi.org/10.1016/0304-3770(85)90004-X)
- Egea, L. G., Jiménez-Ramos, R., Vergara, J. J., Hernández, I., and Brun, F. G. (2018). Interactive effect of temperature, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa*. *Marine Pollution Bulletin*, 134, 14–26. <https://doi.org/10.1016/j.marpolbul.2018.02.029>
- Ferdie, M., and Fourqurean, J. W. (2004). Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology and Oceanography*, 49, 2082–2094. <https://doi.org/10.4319/lo.2004.49.6.2082>
- Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., et al. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, 5, 505–509. <https://doi.org/10.1038/ngeo1477>
- Gambi, M. C., Gaglioti, M., and Barbieri, F. (2018). Sometimes they come back: the recolonization of the alien seagrass *Halophila stipulacea* (Forsskål) Ascherson, 1867 (Hydrocharitaceae) in the Palinuro Harbor (Tyrrhenian Sea, Italy). *BioInvasions Records*, 7, 215–221. <https://doi.org/10.3391/bir.2018.7.3.01>
- Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *BBA Gen. Subj.* 990, 87–92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9)

- George, R., Gullström, M., Mangora, M. M., Mtolera, M. S. P., and Björk, M. (2018). High midday temperature stress has stronger effects on biomass than on photosynthesis: a mesocosm experiment on four tropical seagrass species. *Ecology and Evolution*, 8, 4508–4517. <https://doi.org/10.1002/ece3.3952>
- Georgiou, D., Alexandre, A., Luis, J., and Santos, R. (2016). Temperature is not a limiting factor for the expansion of *Halophila stipulacea* throughout the Mediterranean Sea. *Marine Ecology Progress Series*, 544, 159–167. <https://doi.org/10.3354/meps11582>
- Grasshoff, K., Kremling, K., and Ehrhardt, M. (1999). *Methods of Seawater Analysis*. New York, NY: Wiley-VCH. <https://doi.org/10.1002/9783527613984>
- Gunderson, A. R., Armstrong, E. J., and Stillman, J. H. (2016). Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Annual Review of Marine Science*, 8, 357–378. <https://doi.org/10.1146/annurev-marine-122414-033953>
- Hernández-Delgado, E. A., Toledo-Hernández, C., Ruíz-Díaz, C. P., Gómez-Andújar, N., Medina-Muñiz, J. L., Canals-Silander, M. F., et al. (2020). Hurricane impacts and the resilience of the invasive Sea Vine, *Halophila stipulacea*: a case study from Puerto Rico. *Estuaries and Coasts*, 43, 1263–1283. <https://doi.org/10.1007/s12237-019-00673-4>
- Houston, A. I., and McNamara, J. M. (1992). Phenotypic plasticity as a state-dependent life-history decision. *Ecology and Evolution*, 6, 243–253. <https://doi.org/10.1007/BF02214164>
- Huber, S. C., and Israel, D. W. (1982). Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max* Merr.) leaves. *Plant Physiology*, 69, 691–696. <https://doi.org/10.1104/pp.69.3.691>
- Invers, O., Kraemer, G. P., Pérez, M., and Romero, J. (2004). Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. *Journal of Experimental Marine Biology and Ecology*, 303, 97–114. <https://doi.org/10.1016/j.jembe.2003.11.005>
- Jassby, A. D., and Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography*, 21, 540–547. <https://doi.org/10.4319/lo.1976.21.4.0540>
- Jensen, S., and Bell, S. (2001). Seagrass growth and patch dynamics: crossscale morphological plasticity. *Plant Ecology*, 155, 201–217. <https://doi.org/10.1023/A:1013286731345>
- Jiang, Z., Huang, X., and Zhang, J. (2013). Effect of nitrate enrichment and salinity reduction on the seagrass *Thalassia hemprichii* previously grown in low light. *Journal of Experimental Marine Biology and Ecology*, 443, 114–122. <https://doi.org/10.1016/j.jembe.2013.02.034>
- Jiménez-Ramos, R., Egea, L. G., Ortega, M. J., Hernández, I., Vergara, J. J., and Brun, F. G. (2017). Global and local disturbances interact to modify seagrass palatability. *PLoS One*, 12, e0183256. <https://doi.org/10.1371/journal.pone.0183256>

- Jordà, G., Marbà, N., and Duarte, C. M. (2012). Mediterranean seagrass vulnerable to regional climate warming. *Nature Climate Change*, 2, 821–824. <https://doi.org/10.1038/NCLIMATE1533>
- Kaldy, J. E. (2014). Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. *Journal of Experimental Marine Biology and Ecology*, 453, 108–115. <https://doi.org/10.1016/j.jembe.2013.12.020>
- Kaldy, J. E., Brown, C. A., Nelson, N. G., and Frazier, M. (2017). Macrophyte community response to nitrogen loading and thermal stressors in rapidly flushed mesocosm systems. *Journal of Experimental Marine Biology and Ecology*, 497, 107–119. <https://doi.org/10.1016/j.jembe.2017.09.022>
- Kamermans, P., Hemminga, M. A., Tack, J. F., Mateo, M. A., Marbà, N., Mtolera, M., et al. (2002). Groundwater effects on diversity and abundance of lagoonal seagrasses in Kenya and on Zanzibar Island (East Africa). *Marine Ecology Progress Series*, 231, 75–83. <https://doi.org/10.3354/meps231075>
- Kendrick, G. A., Nowicki, R. J., Olsen, Y. S., Strydom, S., Fraser, M. W., Sinclair, E. A., et al. (2019). A systematic review of how multiple stressors from an extreme event drove ecosystem-wide loss of resilience in an iconic seagrass community. *Frontiers in Marine Science*, 6, 455. <https://doi.org/10.3389/fmars.2019.00455>
- Kilminster, K. L., Thompson, P. A., and Raven, J. A. (2008). Changes in growth, internode distance and nutrient concentrations of the seagrass *Halophila ovalis* with exposure to sediment sulphide. *Marine Ecology Progress Series*, 361, 83–91. <https://doi.org/10.3354/meps07479>
- Kirsch, K. D., Valentine, J. F., and Heck, K. L. Jr. (2002). Parrotfish grazing on turtlegrass *Thalassia testudinum*: evidence for the importance of seagrass consumption in food web dynamics of the Florida Keys National Marine Sanctuary. *Marine Ecology Progress Series*, 227, 71–85. <https://doi.org/10.3354/meps227071>
- Koch, M., Bowes, G., Ross, C., and Zhang, X.-H. (2013). Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology*, 19, 103–132. <https://doi.org/10.1111/j.1365-2486.2012.02791.x>
- La Nafie, Y. A., de los Santos, C., Brun, F. G., van Katwijk, M. M., and Bouma, T. J. (2012). Waves and high nutrient loads jointly decrease survival and separately affect morphological and biomechanical properties in the seagrass *Zostera noltii*. *Limnology and Oceanography*, 57, 1664–1672. <https://doi.org/10.4319/lo.2012.57.6.1664>
- Lee, K.-S., and Dunton, K. H. (1999). Inorganic nitrogen acquisition in the seagrass *Thalassia testudinum*: development of a whole-plant nitrogen budget. *Limnology and Oceanography*, 44, 1204–1215. <https://doi.org/10.4319/lo.1999.44.5.1204>
- Lee, K.-S., Park, S. R., and Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology*, 350, 144–175. <https://doi.org/10.1016/j.jembe.2007.06.016>

- Leoni, V., Vela, A., Pasqualini, V., Pergent-Martini, C., and Pergent, G. (2008). Effects of experimental reduction of light and nutrient enrichments (N and P) on seagrasses: a review. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18, 202–220. <https://doi.org/10.1002/aqc.842>
- Li, M., Lundquist, C. J., Pilditch, C. A., Rees, T. A. V., and Ellis, J. (2019). Implications of nutrient enrichment for the conservation and management of seagrass *Zostera muelleri* meadows. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 1484–1502. <https://doi.org/10.1002/aqc.3141>
- Lu, Y., Yuan, J., Lu, X., Su, C., Zhang, Y., and Wang, C. (2018). Major threats of pollution and climate change to global coastal ecosystems and enhanced management for sustainability. *Environmental Pollution*, 239, 670–680. <https://doi.org/10.1016/j.envpol.2018.04.016>
- Manly, B. F. J. (1997). *Randomization, Bootstrap and Monte Carlo Methods in Biology*. London: Chapman and Hall. <https://doi.org/10.1201/9781315273075>
- Martínez-Crego, B., Vergés, A., Alcoverro, T., and Romero, J. (2008). Selection of multiple seagrass indicators for environmental monitoring. *Marine Ecology Progress Series*, 361, 93–109. <https://doi.org/10.3354/meps07358>
- McMahon, K., Collier, C. J., and Lavery, P. S. (2013). Identifying robust bioindicators of light stress in seagrasses: a meta-analysis. *Ecological Indicators*, 30, 7–15. <https://doi.org/10.1016/j.ecolind.2013.01.030>
- McMillan, C., and Phillips, R. C. (1979). Differentiation in habitat response among populations of New World seagrasses. *Aquatic Botany*, 7, 185–196. [https://doi.org/10.1016/0304-3770\(79\)90021-4](https://doi.org/10.1016/0304-3770(79)90021-4)
- Mejia, A. Y., Rotini, A., Lacasella, F., Bookman, R., Thaller, M. C., Shem-Tov, R., et al. (2016). Assessing the ecological status of seagrasses using morphology, biochemical descriptors and microbial community analyses. A study in *Halophila stipulacea* (Forsk.) Aschers meadows in the northern Red Sea. *Ecological Indicators*, 60, 1150–1163. <https://doi.org/10.1016/j.ecolind.2015.09.014>
- Moreno-Marín, F., Brun, F. G., and Pedersen, M. F. (2018). Additive responses to multiple environmental stressors in the seagrass *Zostera marina*. *Limnology and Oceanography*, 63, 1528–1544. <https://doi.org/10.1002/lno.10789>
- Muthukrishnan, R., Chiquillo, K. L., Cross, C., Fong, P., Kelley, T., Toline, C. A., et al. (2020). Little giants: a rapidly invading seagrass alters ecosystem functioning relative to native foundation species. *Marine Biology*, 167, 81. <https://doi.org/10.1007/s00227-020-03689-8>
- Mvungi, E. F., and Pillay, D. (2019). Eutrophication overrides warming as a stressor for a temperate African seagrass (*Zostera capensis*). *PLoS One*, 14, e0215129. <https://doi.org/10.1371/journal.pone.0215129>
- Mwaura, J., Umezawa, Y., Nakamura, T., and Kamau, J. (2017). Evidence of chronic anthropogenic nutrient within coastal lagoon reefs adjacent to urban and tourism centers,

- Kenya: a stable isotope approach. *Marine Pollution Bulletin*, 119, 74–86. <https://doi.org/10.1016/j.marpolbul.2017.04.028>
- Nguyen, H. M., Yadav, N. S., Barak, S., Lima, F. P., Sapir, Y., and Winters, G. (2020). Responses of invasive and native populations of the seagrass *Halophila stipulacea* to simulated climate change. *Frontiers in Marine Science*, 6, 812. <https://doi.org/10.3389/fmars.2019.00812>
- Nielsen, S. L., Banta, G. T., and Pedersen, M. F. (2004). Decomposition of marine primary producers: consequences for nutrient recycling and retention in coastal ecosystems. In *Estuarine Nutrient Cycling: The Influence of Primary Producers* (pp. 187–216), eds S. L. Nielsen, G. T. Banta, and M. F. Pedersen (Dordrecht: Springer). <https://doi.org/10.1007/978-1-4020-3021-5>
- Nordlund, L. M., Koch, E. W., Barbier, E. B., and Creed, J. C. (2016). Seagrass ecosystem services and their variability across genera and geographical regions. *PLoS One*, 11, e0163091. <https://doi.org/10.1371/journal.pone.0163091>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). Vegan: Community Ecology Package. R package version 2.5-4. Available online at: <https://CRAN.R-project.org/package=vegan> (accessed March 30, 2020).
- Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Lisa, V., et al. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, 9, 1324. <https://doi.org/10.1038/s41467-018-03732-9>
- Ondiviela, B., Losada, I. J., Lara, J. L., Maza, M., Galván, C., Bouma, T. J., et al. (2014). The role of seagrasses in coastal protection in a changing climate. *Coastal Engineering*, 87, 158–168. <https://doi.org/10.1016/j.coastaleng.2013.11.005>
- Ontoria, Y., Cuesta-Gracia, A., Ruiz, J. M., Romero, J., and Pérez, M. (2019a). The negative effects of short-term extreme thermal events on the seagrass *Posidonia oceanica* are exacerbated by ammonium additions. *PLoS One*, 14, e0222798. <https://doi.org/10.1371/journal.pone.0222798>
- Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J. M., Romero, J., et al. (2019b). Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. *Marine Environmental Research*, 145, 27–38. <https://doi.org/10.1016/j.marenvres.2019.02.002>
- Orth, R. J., Carruthers, T. J. B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., et al. (2006). A global crisis of seagrass ecosystems. *Bioscience*, 56, 987–996. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:agcfse\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2)
- O’Brien, K. R., Waycott, M., Maxwell, P., Kendrick, G. A., Udy, J. W., Ferguson, A. J. P., et al. (2018). Seagrass ecosystem trajectory depends on the relative timescales of resistance, recovery and disturbance. *Marine Pollution Bulletin*, 134, 166–176. <https://doi.org/10.1016/j.marpolbul.2017.09.006>

- Pedersen, O., Borum, J., Duarte, C. M., and Fortes, M. D. (1998). Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. *Marine Ecology Progress Series*, 169, 283–288. <https://doi.org/10.3354/meps169283>
- Pedersen, O., Colmer, T. D., Borum, J., Zavala-Perez, A., and Kendrick, G. A. (2016). Heat stress of two tropical seagrass species during low tides-impact on underwater net photosynthesis, dark respiration and diel in situ internal aeration. *New Phytologist*, 210, 1207–1218. <https://doi.org/10.1111/nph.13900>
- Pérez, M., Romero, J., Duarte, C. M., and Sand-Jensen, K. (1991). Phosphorus limitation of *Cymodocea nodosa* growth. *Marine Biology*, 109, 129–133. <https://doi.org/10.1007/BF01320239>
- Pollard, P. C., and Greenway, M. (2013). Seagrasses in tropical Australia, productive and abundant for decades decimated overnight. *Journal of Biosciences*, 38, 157–166. <https://doi.org/10.1007/s12038-013-9299-6>
- R Core Team (2019). R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing.
- Ralph, P. J., and Gademann, R. (2005). Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquatic Botany*, 82, 222–237. <https://doi.org/10.1016/j.aquabot.2005.02.006>
- Roca, G., Alcoverro, T., Krause-Jensen, D., Balsby, T. J. S., van Katwijk, M. M., Marbà, N., et al. (2016). Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis. *Ecological Indicators*, 63, 310–323. <https://doi.org/10.1016/j.ecolind.2015.12.007>
- Romero, J., Lee, K.-S., Pérez, M., Mateo, M. A., and Alcoverro, T. (2006). Nutrient dynamics in seagrass ecosystems. In *Seagrasses: Biology, Ecology and Conservation* (pp. 227–254), eds A. W. D. Larkum, R. J. Orth, and C. M. Duarte (Dordrecht: Springer). <https://doi.org/10.1007/1-4020-2983-79>
- Sakshaug, E., Bricaud, A., Dandonneau, Y., Falkowski, P. G., Kiefer, D. A., Legendre, L., et al. (1997). Parameters of photosynthesis: definitions, theory and interpretation of results. *Journal of Plankton Research*, 19, 1637–1670. <https://doi.org/10.1093/plankt/19.11.1637>
- Salo, T., and Pedersen, M. F. (2014). Synergistic effects of altered salinity and temperature on estuarine eelgrass (*Zostera marina*) seedlings and clonal shoots. *Journal of Experimental Marine Biology and Ecology*, 457, 143–150. <https://doi.org/10.1016/j.jembe.2014.04.008>
- Saroussi, S., and Beer, S. (2007). Alpha and quantum yield of aquatic plants derived from PAM fluorometry: uses and misuses. *Aquatic Botany*, 86, 89–92. <https://doi.org/10.1016/j.aquabot.2006.09.003>
- Schielzeth, H., and Nakagawa, S. (2013). Nested by design: model fitting and interpretation in a mixed model era. *Methods in Ecology and Evolution*, 4, 14–24. <https://doi.org/10.1111/j.2041-210x.2012.00251.x>

- Sghaier, Y. R., Zakhama-Sraieb, R., Bernamer, I., and Charfi-Cheikhrouha, F. (2011). Occurrence of the seagrass *Halophila stipulacea* (Hydrocharitaceae) in the southern Mediterranean Sea. *Botanica Marina*, 54, 575–582. <https://doi.org/10.1515/BOT.2011.061>
- Short, F. T. (1987). Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. *Aquatic Botany*, 27, 41–57. [https://doi.org/10.1016/0304-3770\(87\)90085-4](https://doi.org/10.1016/0304-3770(87)90085-4)
- Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350, 3–20. <https://doi.org/10.1016/j.jembe.2007.06.012>
- Short, F. T., and Duarte, C. M. (2001). Methods for the measurement of seagrass growth and production. In *Global Seagrass Research Methods*, eds F. T. Short and R. G. Coles (Amsterdam: Elsevier), (pp. 155–198). <https://doi.org/10.1016/B978-044450891-1/50009-8>
- Silsbe, G., and Malkin, S. Y. (2015). Phytotools: Phytoplankton Production Tools. Available online at: <https://CRAN.R-project.org/package=phytotools> (accessed March 30, 2020).
- Soissons, L. M., Haanstra, E. P., van Katwijk, M. M., Asmus, R., Auby, I., Barillé, L., et al. (2018). Latitudinal patterns in European seagrass carbon reserves: Influence of seasonal fluctuations versus short-term stress and disturbance events. *Frontiers in Plant Science*, 9, 88. <https://doi.org/10.3389/fpls.2018.00088>
- Stockbridge, J., Jones, A. R., and Gillanders, B. M. (2020). A meta-analysis of multiple stressors on seagrasses in the context of marine spatial cumulative impacts assessment. *Scientific Reports*, 10, 11934. <https://doi.org/10.1038/s41598-020-68801-w>
- Terrados, J., Agawin, N. S., Duarte, C. M., Fortes, M. D., Kamp-Nielsen, L., and Borum, J. (1999). Nutrient limitation of the tropical seagrass *Enhalus acoroides* (L.) royle in cape Bolinao, NW Philippines. *Aquatic Botany*, 65, 123–139. [https://doi.org/10.1016/S0304-3770\(99\)00036-4](https://doi.org/10.1016/S0304-3770(99)00036-4)
- Thomsen, E., Herbeck, L. S., and Jennerjähnet, T. C. (2020). The end of resilience: surpassed nitrogen thresholds in coastal waters led to severe seagrass loss after decades of exposure to aquaculture effluents. *Marine Environmental Research*, 160, 104986. <https://doi.org/10.1016/j.marenvres.2020.104986>
- Todgham, A. E., and Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integrative and Comparative Biology*, 53, 539–544. <https://doi.org/10.1093/icb/ict086>
- Torquemada, Y. F., Durako, M. J., and Sánchez-Lizaso, J. L. (2005). Effects of salinity and possible interactions with temperature and pH on growth and photosynthesis of *Halophila johnsonii* Eiseman. *Marine Biology*, 148, 251–260. <https://doi.org/10.1007/s00227-005-0075-5>
- Touchette, B. W., and Burkholder, J. M. (2002). Seasonal variations in carbon and nitrogen constituents in eelgrass (*Zostera marina* L.) as influenced by increased temperature and water-column nitrate. *Botanica Marina*, 45, 23–34. <https://doi.org/10.1515/BOT.2002.004>

- Touchette, B. W., and Burkholder, J. M. (2007). Carbon and nitrogen metabolism in the seagrass, *Zostera marina* L.: environmental control of enzymes involved in carbon allocation and nitrogen assimilation. *Journal of Experimental Marine Biology and Ecology*, 350, 216–233. <https://doi.org/10.1016/j.jembe.2007.05.034>
- Touchette, B. W., Burkholder, J. M., and Glasgow, H. B. Jr. (2003). Variations in eelgrass (*Zostera marina* L.) morphology and internal nutrient composition as influenced by increased temperature and water column nitrate. *Estuaries*, 26, 142–155. <https://doi.org/10.1007/BF02691701>
- Tuya, F., Fernández-Torquemada, Y., Zarcero, J., del Pilar-Ruso, Y., Csenteri, I., Espino, F., et al. (2019). Biogeographical scenarios modulate seagrass resistance to small-scale perturbations. *Journal of Ecology*, 107, 1263–1275. <https://doi.org/10.1111/1365-2745.13114>
- Udy, J. W., Dennison, W. C., Long, W. J. L., and McKenzie, L. J. (1999). Responses of seagrass to nutrients in the Great Barrier Reef, Australia. *Marine Ecology Progress Series*, 185, 257–271. <https://doi.org/10.3354/meps185257>
- Unsworth, R. K. F., McKenzie, L. J., Collier, C. J., Cullen-Unsworth, L. C., Duarte, C. M., Eklöf, J. S., et al. (2019). Global challenges for seagrass conservation. *Ambio*, 48, 801–815. <https://doi.org/10.1007/s13280-018-1115-y>
- Van Tussenbroek, B. I., van Katwijk, M. M., Bouma, T. J., van der Heide, T., and Leuven, R. S. E. W. (2016). Non-native seagrass *Halophila stipulacea* forms dense mats under eutrophic conditions in the Caribbean. *Journal of Sea Research*, 115, 1–5. <https://doi.org/10.1016/j.seares.2016.05.005>
- Viana, I. G., Saavedra-Hortúa, D. A., Mtolera, M., and Teichberg, M. (2019a). Different strategies of nitrogen acquisition in two tropical seagrasses from an oligotrophic environment. *New Phytologist*, 223, 1217–1229. <https://doi.org/10.1111/nph.15885>
- Viana, I. G., Siriwardane-de Zoysa, R., Willette, D. A., and Gillis, L. G. (2019b). Exploring how non-native seagrass species could provide essential ecosystems services: A perspective on the highly invasive seagrass *Halophila stipulacea* in the Caribbean Sea. *Biological Invasions*, 21, 1461–1472. <https://doi.org/10.1007/s10530-019-01924-y>
- Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, 106, 12377–12381. <https://doi.org/10.1073/pnas.0905620106>
- Wernberg, T., Smale, D. A., and Thomsen, M. S. (2012). A decade of climate change experiments on marine organisms: procedure, patterns and problems. *Global Change Biology*, 18, 1491–1498. <https://doi.org/10.1111/j.1365-2486.2012.02656.x>
- Wesselmann, M., Anton, A., Duarte, C. M., Hendriks, I. E., Agustí, S., Savva, I., et al. (2020). Tropical seagrass *Halophila stipulacea* shifts thermal tolerance during Mediterranean invasion. *Proceedings of the Royal Society B*, 287, 20193001. <https://doi.org/10.1098/rspb.2019.3001>

Winters, G., Beer, S., Willette, D. A., Viana, I. G., Chiquillo, K. L., Beca-Carretero, P., et al. (2020). The invasive tropical seagrass *Halophila stipulacea*: a review of what we know alongside identifying gaps in our knowledge. *Frontiers in Marine Science*, 7, 300. <https://doi.org/10.3389/fmars.2020.00300>

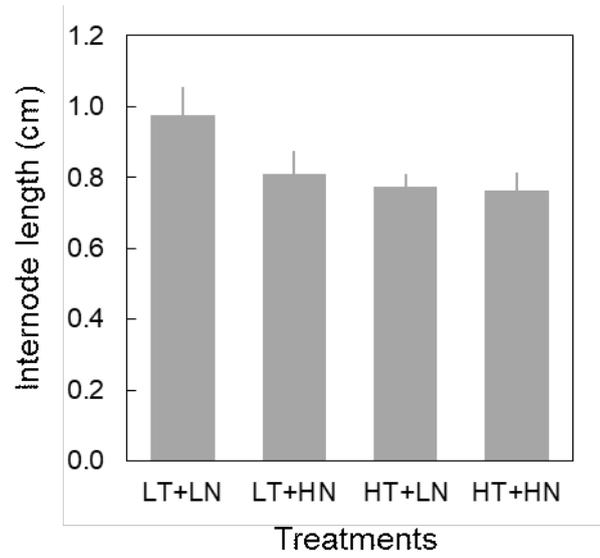
Winters, G., Nelle, P., Fricke, B., Rauch, G., and Reusch, T. B. H. (2011). Effects of a simulated heat wave on photophysiology and gene expression of high- and low-latitude populations of *Zostera marina*. *Marine Ecology Progress Series*, 435, 83–95. <https://doi.org/10.3354/meps09213>

Xu, S., Zhou, Y., Wang, P., Wang, F., Zhang, X., and Gu, R. (2016). Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass *Zostera marina* L. *PeerJ*, 4, e2697. <https://doi.org/10.7717/peerj.2697>

Yemm, E. W., and Willis, A. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemistry Journal*, 57, 508–514. <https://doi.org/10.1042/bj0570508>

Zayas-Santiago, C., Rivas-Ubach, A., Kuo, L.-J., Ward, N. D., and Zimmerman, R. C. (2020). Metabolic profiling reveals biochemical pathways responsible for eelgrass response to elevated CO₂ and temperature. *Scientific Reports*, 10, 4693. <https://doi.org/10.1038/s41598-020-61684-x>

VIII. Supplementary Material



Supplementary Figure 1. *Halophila stipulacea* internode length (mean \pm SE, n=6) in the four treatments (LT: low temperature; LN: low nutrient; HT: high temperature; HN: high nutrient). Statistical results are shown in Table 3.

Supplementary Table 1. Free amino acids concentrations in the four treatments (LT: low temperature; LN: low nutrient; HT: high temperature; HN: high nutrient).

	<i>T. hemprichii</i>				<i>C. serrulata</i>			
	LT+LN	LT+HN	HT+LN	HT+HN	LT+LN	LT+HN	HT+LN	HT+HN
ALA	0.38±0.07	0.33±0.04	0.43±0.07	0.35±0.05	0.39±0.07	0.48±0.06	0.28±0.04	0.39±0.07
ARG	0.05±0.004	0.05±0.001	0.06±0.002	0.05±0.005	0.07±0.004	0.07±0.004	0.06±0.002	0.07±0.004
ASP	1.03±0.15	1.19±0.14	1.16±0.18	0.99±0.2	0.77±0.1	0.81±0.08	0.54±0.07	0.77±0.1
GLU	0.78±0.14	0.85±0.1	0.74±0.07	0.63±0.08	0.71±0.12	0.85±0.13	0.56±0.06	0.86±0.12
GLY	0.10±0.01	0.11±0.01	0.13±0.01	0.09±0.01	0.18±0.02	0.21±0.03	0.17±0.01	0.17±0.02
HIS	0.13±0.01	0.11±0.01	0.12±0.01	0.11±0.01	0.13±0.02	0.15±0.01	0.16±0.02	0.17±0.02
ILE	0.09±0.01	0.08±0.01	0.1±0.01	0.07±0.005	0.12±0.02	0.13±0.02	0.14±0.01	0.13±0.02
LEU	0.08±0.01	0.07±0.002	0.08±0.004	0.07±0.001	0.1±0.01	0.13±0.02	0.12±0.02	0.1±0.01
MET	0.06±0.005	0.06±0.002	0.06±0.003	0.05±0.004	0.06±0.003	0.06±0.003	0.05±0.01	0.06±0.003
PHE	0.11±0.01	0.10±0.01	0.11±0.01	0.09±0.005	0.12±0.01	0.16±0.03	0.14±0.01	0.14±0.01
THR	0.82±0.09	0.74±0.29	0.39±0.1	0.43±0.09	0.53±0.13	0.88±0.27	0.41±0.08	0.43±0.13
TYR	0.06±0.005	0.05±0.002	0.06±0.003	0.05±0.002	0.06±0.004	0.08±0.01	0.07±0.005	0.06±0.004
SER	0.35±0.06	0.29±0.04	0.32±0.03	0.23±0.03	0.47±0.1	0.47±0.05	0.48±0.08	0.56±0.1
VAL	0.13±0.02	0.10±0.01	0.12±0.01	0.1±0.01	0.16±0.02	0.19±0.03	0.17±0.01	0.16±0.02

Supplementary Table 2. *Thalassia hemprichii* Pearson correlation coefficients (lower semimatrix) and significance (upper semimatrix) between biochemical, morphological and physiological traits. Green cells indicate significantly positive correlations while red cells indicate significantly inverse correlations (*P*-value: *: <0.05, **: <0.01, ***: <0.001). Values in bold represent statistical significance at the 10% level of significance (FAA: free amino acids).

	Leaf C:N	Leaf N	Leaf C	Leaf FAA	Rhizome N	Rhizome C	Sucrose	Starch	Root N	Root C	Leaf length	Leaf SA	Sheath length	Root length	Growth	F _v /F _m	rETR _{max}	Alpha	E _k
Leaf C:N	-	<0.001	<0.001	0.34	0.75	0.32	0.75	0.87	0.95	0.88	0.60	0.81	0.25	0.15	0.20	0.30	0.49	0.17	0.36
Leaf N	-0.97***	-	<0.001	0.37	0.95	0.19	0.75	0.57	0.94	0.86	0.50	0.91	0.25	0.23	0.11	0.23	0.26	0.21	0.59
Leaf C	-0.68***	0.81***	-	0.38	0.55	0.09	0.82	0.24	0.52	0.61	0.27	0.85	0.29	0.75	0.08	0.44	0.03	0.90	0.51
Leaf FAA	-0.20	0.19	0.19	-	0.61	0.42	0.42	0.38	0.02	0.02	0.31	0.37	0.13	0.83	0.04	0.63	0.03	0.27	0.05
Rhizome N	0.07	-0.01	0.13	0.11	-	0.08	0.06	0.14	0.005	0.03	0.39	0.99	0.38	0.05	0.47	0.22	0.46	0.45	0.96
Rhizome C	0.21	-0.28	-0.35	0.17	0.36	-	0.68	0.02	0.04	0.03	0.22	0.20	0.33	0.56	0.86	0.95	0.74	0.95	0.95
Sucrose	0.07	-0.07	-0.05	-0.17	-0.40	-0.09	-	0.54	0.13	0.02	0.49	0.73	0.35	0.08	0.81	0.27	0.24	0.03	0.08
Starch	0.03	-0.12	-0.25	0.19	-0.31	0.46*	0.13	-	0.52	0.33	0.10	0.13	0.09	0.10	0.39	0.60	0.45	0.48	0.56
Root N	0.01	0.02	0.14	0.47*	0.55**	0.42*	-0.32	0.14	-	<0.001	0.53	0.39	0.48	0.34	0.12	0.82	0.02	0.90	0.32
Root C	-0.03	0.04	0.11	0.48*	0.44*	0.45*	-0.48*	0.21	0.93***	-	0.45	0.21	0.52	0.35	0.15	0.73	0.16	0.73	0.99
Leaf length	-0.11	0.14	0.24	-0.22	0.19	-0.26	-0.15	-0.34	-0.13	-0.16	-	0.001	<0.001	0.30	0.14	0.22	0.75	0.82	0.87
Leaf SA	0.05	-0.02	-0.04	-0.19	0.00	-0.27	-0.07	-0.31	-0.18	-0.27	0.63***	-	0.01	0.54	0.62	0.62	0.43	0.86	0.67
Sheath length	-0.24	0.24	0.23	-0.32	0.19	-0.21	-0.20	-0.35	-0.15	-0.14	0.92***	0.51*	-	0.23	0.06	0.17	0.58	0.36	0.54
Root length	-0.30	0.25	0.07	0.05	0.40*	-0.13	-0.37	-0.34	0.20	0.20	0.22	0.13	0.26	-	0.84	0.78	0.07	0.26	0.15
Growth	-0.27	0.34	0.37	-0.43*	0.15	-0.04	0.05	-0.18	-0.33	-0.31	0.31	-0.11	0.40	0.04	-	0.31	0.52	0.32	0.28
F _v /F _m	-0.22	0.25	0.16	-0.10	0.26	0.01	-0.24	-0.11	-0.05	-0.08	0.26	0.11	0.29	-0.06	0.22	-	0.53	<0.001	0.74
rETR _{max}	-0.15	0.24	0.44*	0.44*	0.16	0.07	0.25	0.16	0.46*	0.30	-0.07	-0.17	-0.12	-0.38	-0.14	0.14	-	0.36	0.002
Alpha	-0.29	0.26	0.03	-0.24	0.16	-0.01	-0.45*	-0.15	-0.03	0.07	0.05	0.04	0.19	0.24	0.21	0.73**	-0.20	-	0.001
E _k	0.19	-0.11	0.14	0.41*	0.01	0.01	0.36	0.12	0.21	0.00	0.03	-0.09	-0.13	-0.31	-0.23	-0.07	0.61**	-0.64***	-

Supplementary Table 3. *Cymodocea serrulata* Pearson correlation coefficients (lower semimatrix) and significance (upper semimatrix) between biochemical, morphological and physiological traits. Green cells indicate significantly positive correlations while red cells indicate significantly inverse correlations (*P-value*: *: <0.05, **: <0.01, ***: <0.001). Values in bold represent statistical significance at the 10% level of significance (FAA: free amino acids).

	Leaf C:N	Leaf N	Leaf C	Leaf FAA	Rhizome N	Rhizome C	Sucrose	Starch	Root N	Root C	Leaf length	Leaf SA	Sheath length	Root length	Growth	F _v /F _m	rETR _{max}	Alpha	E _k
Leaf C:N	-	<0.001	0.05	0.56	0.35	0.24	0.81	0.06	0.01	0.003	0.12	0.08	0.13	0.57	0.19	0.62	0.42	0.41	0.64
Leaf N	-0.93***	-	0.01	0.76	0.76	0.12	0.45	0.02	0.03	0.003	0.07	0.04	0.04	0.25	0.17	0.71	0.34	0.31	0.50
Leaf C	-0.41*	0.52**	-	0.75	0.52	0.88	0.45	0.45	0.10	0.08	<0.001	<0.001	0.01	0.33	0.83	0.41	0.72	0.24	0.58
Leaf FAA	-0.13	0.06	0.07	-	0.42	0.83	0.93	0.36	0.11	0.09	0.10	0.10	0.39	0.04	0.74	0.45	0.75	0.75	0.59
Rhizome N	0.20	-0.07	0.14	0.17	-	0.91	0.96	0.81	0.04	0.25	0.21	0.24	0.14	0.89	0.98	0.37	0.27	0.58	0.48
Rhizome C	0.25	-0.33	0.03	0.04	0.02	-	0.49	0.01	0.78	0.08	0.86	1.00	0.28	1.00	0.06	0.49	0.02	0.40	0.01
Sucrose	0.05	-0.17	-0.17	0.02	0.01	0.15	-	-	0.11	0.01	0.89	0.90	0.43	0.48	0.18	0.88	0.22	0.84	0.27
Starch	0.39	-0.47*	-0.17	-0.20	-0.05	0.55**	0.17	-	0.63	0.35	0.25	0.39	0.36	0.61	0.17	0.35	0.01	0.69	0.01
Root N	0.49*	-0.45*	-0.36	-0.33	0.42*	0.06	0.34	0.10	-	<0.001	0.48	0.29	0.64	1.00	0.59	0.73	0.83	0.77	0.66
Root C	0.59**	-0.57**	-0.38	-0.35	0.24	0.36	0.52*	0.20	0.82**	-	0.27	0.29	0.34	0.77	0.58	0.89	0.59	0.71	0.83
Leaf length	0.33	-0.38	-0.71***	-0.35	-0.27	0.04	0.03	0.25	0.15	0.23	-	<0.001	<0.001	0.53	0.71	0.08	0.18	0.03	0.11
Leaf SA	0.36	-0.42*	-0.73***	-0.34	-0.25	0.00	-0.03	0.18	0.23	0.23	0.95***	-	<0.001	0.46	0.84	0.03	0.12	0.004	0.08
Sheath length	0.32	-0.43*	-0.55**	-0.19	-0.31	0.23	0.17	0.20	-0.10	0.20	0.82***	0.78**	-	0.26	0.68	0.06	0.58	0.05	0.69
Root length	0.12	-0.25	-0.21	0.42*	-0.03	0.00	-0.16	-0.11	0.00	-0.06	0.14	0.16	0.24	-	0.50	0.47	0.92	0.37	0.78
Growth	-0.28	0.29	0.05	-0.07	0.00	-0.38	-0.29	-0.29	-0.11	-0.12	0.08	0.04	-0.09	-0.14	-	0.43	0.01	0.12	0.002
F _v /F _m	0.11	-0.08	-0.18	0.16	0.19	-0.15	0.03	-0.20	-0.07	-0.03	0.37	0.45*	0.40	-0.15	0.17	-	0.03	<0.001	0.07
rETR _{max}	0.17	-0.21	0.08	0.07	0.24	0.46*	0.26	0.53**	0.05	0.11	-0.28	-0.33	-0.12	-0.02	-0.52**	-0.45*	-	<0.001	<0.001
Alpha	0.17	-0.22	-0.26	-0.07	-0.12	-0.18	0.04	-0.09	0.06	0.08	0.45*	0.57**	0.40*	-0.19	0.33	0.80***	-0.62**	-	0.01
E _k	0.10	-0.14	0.12	0.12	0.15	0.54**	0.24	0.53**	-0.09	0.05	-0.33	-0.37	-0.09	-0.06	-0.60**	-0.37	0.90**	-0.55**	-

Supplementary Table 4. *Halophila stipulacea* Pearson correlation coefficients (lower semimatrix) and significance (upper semimatrix) between biochemical, morphological and physiological traits. Green cells indicate significantly positive correlations while red cells indicate significantly inverse correlations (*P-value*: *: <0.05, **: <0.01, ***: <0.001). Values in bold represent statistical significance at the 10% level of significance (AG: above-ground tissues, BG: below-ground tissues, SA: surface area, IL: internode length).

	AG C:N	AG N	AG C	BG N	BG C	Sucrose	Starch	Leaf length	Leaf SA	Petiole length	Root length	IL	F _v /F _m	rETR _{max}	Alpha	E _k
AG C:N	-	<0.001	0.52	0.35	0.88	0.18	0.75	0.23	0.68	0.12	0.72	0.59	0.31	0.25	0.92	0.16
AG N	-0.90***	-	0.04	0.41	0.73	0.18	0.37	0.39	0.81	0.07	0.97	0.69	0.15	0.38	0.54	0.34
AG C	-0.21	0.61*	-	0.87	0.26	0.54	0.08	0.81	0.85	0.24	0.48	0.87	0.11	0.99	0.12	0.79
BG N	0.30	-0.26	-0.05	-	0.08	0.90	0.91	0.59	0.54	0.52	0.60	0.36	0.81	0.58	0.89	0.69
BG C	-0.05	-0.11	-0.35	0.52	-	0.13	0.21	0.05	0.16	0.89	0.03	0.83	0.94	0.11	0.33	0.09
Sucrose	0.44	-0.44	-0.21	0.04	0.48	-	0.48	0.04	0.07	0.42	0.04	0.41	0.33	0.69	0.32	0.63
Starch	0.11	-0.30	-0.55	0.04	0.41	0.24	-	0.63	0.85	0.17	0.23	0.96	0.45	0.47	0.16	0.19
Leaf length	-0.37	0.27	-0.08	-0.18	0.58*	0.62*	0.16	-	0.002	0.02	0.004	0.19	0.92	0.39	0.55	0.15
Leaf SA	-0.13	0.08	-0.06	-0.20	0.43	0.56	0.06	0.80**	-	0.06	0.001	0.05	0.89	0.43	0.62	0.19
Petiole length	-0.48	0.54	0.37	-0.21	0.05	0.27	-0.44	0.67*	0.57	-	0.21	0.01	0.42	0.62	0.66	0.50
Root length	-0.12	-0.01	-0.23	-0.17	0.61*	0.62*	0.39	0.77**	0.83***	0.39	-	0.02	0.95	0.07	0.41	0.004
IL	-0.17	0.13	-0.05	-0.29	0.07	0.28	0.02	0.28	0.40*	0.53**	0.49*	-	0.78	0.34	0.43	0.07
F _v /F _m	-0.32	0.44	0.49	0.08	-0.03	-0.32	-0.26	0.03	-0.04	0.26	0.02	-0.09	-	0.07	<0.001	0.64
rETR _{max}	-0.36	0.28	0.01	0.18	0.48	-0.14	0.25	0.27	0.25	0.16	0.54	0.30	0.53	-	0.67	0.01
Alpha	-0.03	0.20	0.48	0.04	-0.31	-0.33	-0.46	-0.19	-0.16	0.14	-0.26	-0.25	0.88***	0.14	-	0.36
E _k	-0.43	0.30	-0.09	-0.13	0.50	0.17	0.43	0.45	0.40	0.21	0.77**	0.54	0.15	0.74**	-0.29	-



CHAPTER 5.

Seaweed farming pressure affects seagrass and benthic macroalgae dynamics in Chwaka Bay (Zanzibar, Tanzania)

Agustín Moreira-Saporiti^{1,2}, Dieuwke Hoeijmakers¹, Flower E. Msuya³, Hauke Reuter¹, Mirta Teichberg¹

¹Leibniz Centre for Tropical Marine Research GmbH, Bremen, Germany, ²Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany, ³University of Dar es Salaam, University Road, P.O Box 35060, Dar es Salaam, Tanzania

Abstract

Farming of *Eucheuma denticulatum* is a major activity in Zanzibar affecting seagrass ecosystems primarily through shading and trampling. The aim of this study was to test the impacts of shading and trampling during seaweed farming on seagrass meadows composed by *Halophila stipulacea* and *Thalassia hemprichii* and their associated benthic macroalgae. Areas covered by these species were selected for the building of seaweed farms in three treatments: seaweed farm plots (with shading and trampling effects), trampling plots (with trampling effects only) and control plots (with no shading or trampling effects). Reduction of light within the plots was recorded over 9 weeks. Percentage cover of seagrasses and macroalgae and shoot density of seagrasses were measured over 12 weeks to assess the impact of shading and trampling by seaweed farming activities. Light was significantly reduced in the seaweed farm plots by 75 to 90% by the end of a seaweed growth cycle. *H. stipulacea*, despite its capacity for rapid growth, was significantly affected by the combination of shading and trampling under the seaweed farm treatment, while the climax seagrass species *T. hemprichii* was unaffected. Due to the decline in *H. stipulacea*, benthic macroalgae cover increased in the seaweed farm treatment, suggesting a change in seagrass community dynamics. In contrast, trampling had a negative effect on the benthic macroalgae as an isolated disturbance, which suggests that seagrasses are more resistant to trampling than macroalgae and would likely dominate the benthic macrophyte community under these conditions.

Keywords: *Thalassia hemprichii*, *Halophila stipulacea*, *Eucheuma denticulatum*, shading, trampling

I. Introduction

Farming of red macroalgae of the genus *Eucheuma* is a major economic activity in Asian countries including the Philippines and Indonesia (Hurtado et al. 2014). In 2016, the total production of *Eucheuma* worldwide was around 10519 thousand tons of live weight (FAO 2018), of which 214 thousand tons were from the species *Eucheuma denticulatum*. The value of this seaweed comes from its carrageenan, a substance used for its gelling, thickening, emulsifying and water holding properties (Juanich 1988).

The success of seaweed farming stems from its low cost and simple infrastructure needs relative to its high economic benefit. Seaweed farming introduction has been promoted in coastal communities of developing countries as an alternative livelihood to fishing (Smith 1979). In Zanzibar, Tanzania, the adoption of seaweed farming was highly successful (Crawford et al. 2002), improving the household economy in comparison to previous situations in which seaweed farming was not practiced (Msuya 2006). Several villages in Zanzibar adopted the farming of the red macroalgae *Eucheuma denticulatum* around 1989 (Pettersson-Löfquist 1995; Msuya 2011), with a production of 12000 metric tons of dry weight of this seaweed in 2010 (de San 2012). By 2008, seaweed became a leading contributor to the economy of Zanzibar as the largest marine export product from Tanzania (Eklöf et al. 2012).

The most popular cultivation method in Zanzibar is the off-bottom monoline deployed in the intertidal area. Most of the farms are located on sandy bottoms or seagrass meadows following the recommendations from the Food and Agriculture Organization of the United Nations (Juanich 1988), in which it is encouraged to choose sites where marine plants are already growing. Seaweed farming is considered a sustainable and environmentally friendly aquaculture activity as no chemicals or fertilizers are used and no waste is produced (Bryceson 2002). Nevertheless, the expansion of farming along the coasts of tropical countries combined with weak or non-existent regulations (de la Torre-Castro 2012; Hedberg et al. 2018) make it necessary to assess more closely the potential environmental impacts it may have on natural coastal habitats. Seagrass meadows, in particular, occupy the same physical space as seaweed farms due to the FAO recommendations and convenience for the seaweed farmers (de la Torre-Castro and Rönnbäck 2004; Hedberg et al. 2018).

Seagrass are angiosperms that grow in the marine environment. They are one of the most important coastal ecosystems due to a number of ecosystem services they provide (Costanza et al. 1997), such as climate regulation (Duarte and Chiscano 1999), nutrient filtering (Hemminga et al. 1991) and habitat provision (Orth et al. 1984). Effects of seaweed farming in seagrass

ecosystems include lower seagrass shoot density, cover, biomass and canopy height and indirect effects on macrofauna biomass relative to unfarmed areas (Eklöf et al. 2005). More specifically, the biomass of the seagrass species *Thalassia hemprichii* was shown to be lower in areas with seaweed farms than without seaweed farms (Lyimo et al. 2006). Another study in Zanzibar (Eklöf et al. 2006) showed that a larger climax species like *Enhalus acoroides* is more negatively affected than the smaller climax species *T. hemprichii* with greater impacts on above-ground biomass, shoot density and length. Seaweed farming was also recently reported to be one of the leading causes for the decrease in seagrass cover in Indonesia (Unsworth et al. 2018). Apart from the effects of shading, trampling has been reported to negatively impact seagrass meadows, algae dominated communities and macrobenthos (Eckrich and Holmquist 2000; Milazzo et al. 2004; Alexandre et al. 2005). A study testing the effects of trampling in combination with shading in seaweed farms on seagrass ecosystems showed that shading was mainly responsible for negative effects on climax seagrass species, while trampling had inconsistent effects (Blankenhorn 2007).

These studies challenge the notion that seaweed farming activities are sustainable and harmless to the natural seagrass habitat and lead to further questions as to the main driver of seagrass decline under extensive seaweed farming. The studies so far have focused uniquely on the effects of seaweed farming on climax species. Less research, however, has been done on seagrass community dynamics, which may also shift under seaweed farming pressure. In particular, changes in abundance of opportunistic and ephemeral species, such as *Halophila stipulacea*, may lead to changes in ecosystem function, as has been shown in bioregions where it is invasive and has successfully displaced native species (Ruiz and Ballantine 2004; Willette and Ambrose 2009; Willette and Ambrose 2012; Viana et al. 2019). *H. stipulacea* is native in East Africa (Lipkin 1975), it is therefore necessary to study if *H. stipulacea* could displace climax species where they are growing together under intense seaweed farming pressure.

For these reasons we tested the effects of seaweed farming on a climax seagrass species (*T. hemprichii*), an opportunistic seagrass species (*H. stipulacea*) and their associated benthic macroalgae. We focused on the impacts of seaweed farms through their combined effects of shading and trampling, and on the impact of trampling alone as an isolated stressor. Additionally, we were interested in studying the temporal trend in relative abundance of these organisms under seaweed farming. To test these effects, we built a seaweed farm plot (shading and trampling effect), a trampling plot (only trampling) and a control plot (no disturbances) in three replicate experimental blocks within an extended area dominated by *T. hemprichii*, but

also with the presence of *H. stipulacea* and benthic algae in Chwaka Bay (Zanzibar, Tanzania). To measure the effects of the treatments on these organisms across time, we sampled the abundance of seagrass and total macroalgae approximately every 15 days for a total experimental duration of 96 days (25.11.2015-29.02.2016). We also sampled light reduction under seaweed farm plots relative to unfarmed plots to measure the magnitude of the shading by the farms.

We hypothesized that *T. hemprichii* would show a negative trend under seaweed farming and trampling pressure, while benthic macroalgae and *H. stipulacea* would be less affected. This could, therefore, lead to a shift from a seagrass community dominated by a climax seagrass species to opportunistic seagrass and benthic macroalgae species, which may radically changing the ecosystem structure and function.

II. Materials and Methods

(1) Study site

Chwaka Bay (Fig.1a) is located in the east coast of Zanzibar. It is surrounded by mangroves in the south and sandy beaches and coral rubble on the west and east sides. Several channels run from the mangrove area to the mouth of the bay. Extensive seagrass meadows mixed with macroalgae cover the bay, together with bare carbonate sand and coral rock and rubble. The seagrass meadows in the selected site (-6.1489°, 39.4528°) were dominated by *Thalassia hemprichii* (Ehrenberg) Ascherson 1871, mixed with *Halophila stipulacea* (Forsskål) Ascherson in Anon. 1868. The macroalgae *Valonia sp.* C.Agardh, 1823, *Halimeda sp.* J.V.Lamouroux, 1812, nom. et typ. cons. and *Caulerpa sp.* J.V.Lamouroux, 1809 were also present in the study site mixed with the seagrass.

Chwaka Bay is surrounded by several villages which depend on fishing as their main economic activity, with seaweed farming as a secondary source of income. Seaweed farms are present in the west coast of the bay, in shallow waters and close to the coastal areas, mainly over seagrass meadows but also on sandy bottoms. The selected meadows were within a shallow area (0.5-4 m depth). During the experimental time, the temperature of the seawater was of 31.9 ± 0.3 °C, the conductivity 52.9 ± 0.3 μ S/m and the pH 8.46 ± 0.1 (averages \pm standard errors).

(2) Seaweed farm experimental plots

Two local seaweed farmers provided the material and instructed on how to build the farms in the same method as they do in Chwaka Bay. We delimited three blocks (A, B and C, separated

by approximately 50 meters) in an area dominated by *T. hemprichii* with presence of *H stipulacea* and a mixed macroalgae community. We built three treatment plots (each 3 meters wide and 4 meters long, with an area of 12 m²) nested within each block: one seaweed farm plot, one trampling plot and one control plot (each separated by 3-5 meters within a block, Fig.1b), resulting in a total of three replicate plots per treatment.

The seaweed farm consisted of four seaweed lines (ropes) tied to eight wooden pegs (two for each line), as is done in the off-bottom method. In each of the ropes, twelve *E. denticulatum* seedlings were attached by using the “tie-tie” method with a total of 48 seedlings per farm. The seedling size was standardized based on typical farming practices for *E. denticulatum* used at the beginning of the harvesting cycle. The trampling plot consisted of the same line structure as the seaweed farm plot, but without *E. denticulatum* attached to the ropes. The control plot consisted of four wooden pegs pressed into the sediment within the seagrass meadow, delimiting an area of the same size as the seaweed farm plot.

The experiment ran for 96 days (25.11.2015-29.02.2016) and was sampled approximately every 15 days for a total of seven sampling times (Days 0, 15, 34, 49, 63, 81 and 96). The experiment was carried out during the *Kaskazi* season, which is generally considered unfavorable for seaweed growth due to warmer temperatures (Hassan and Othman 2019). Nevertheless, the seaweeds grew uniformly and no die-off or problems with seaweed growth were detected (Personal observation). The seaweed was not harvested until the end of the experiment. In every sampling, the seaweed and the seaweed farm ropes were cleaned from epiphytes, debris and sediment by carefully shaking the algae to remove sediment and loose epiphytes and hand picking any epiphytic algae and anemones growing on the algae or on the ropes. Loose epiphytes were then flushed by the tidal current or directly thrown out of the plots.

Trampling pressure was exerted by one scientist walking between the lines in the seaweed farm and trampling plots during the sampling and cleaning time. The total time in which trampling was exerted in each seaweed farm and trampling plot was of about 40 minutes per sampling campaign. There was no trampling between samplings in order to reproduce the same effect that actual seaweed farmers would have on their farms. At the end of the experiment, a total of four hours and 40 minutes of trampling was exerted in each seaweed farm and trampling plot by one person. With this design, shading and trampling were applied in the seaweed farm plots, trampling on the trampling plots, and no-disturbance on the control plots.

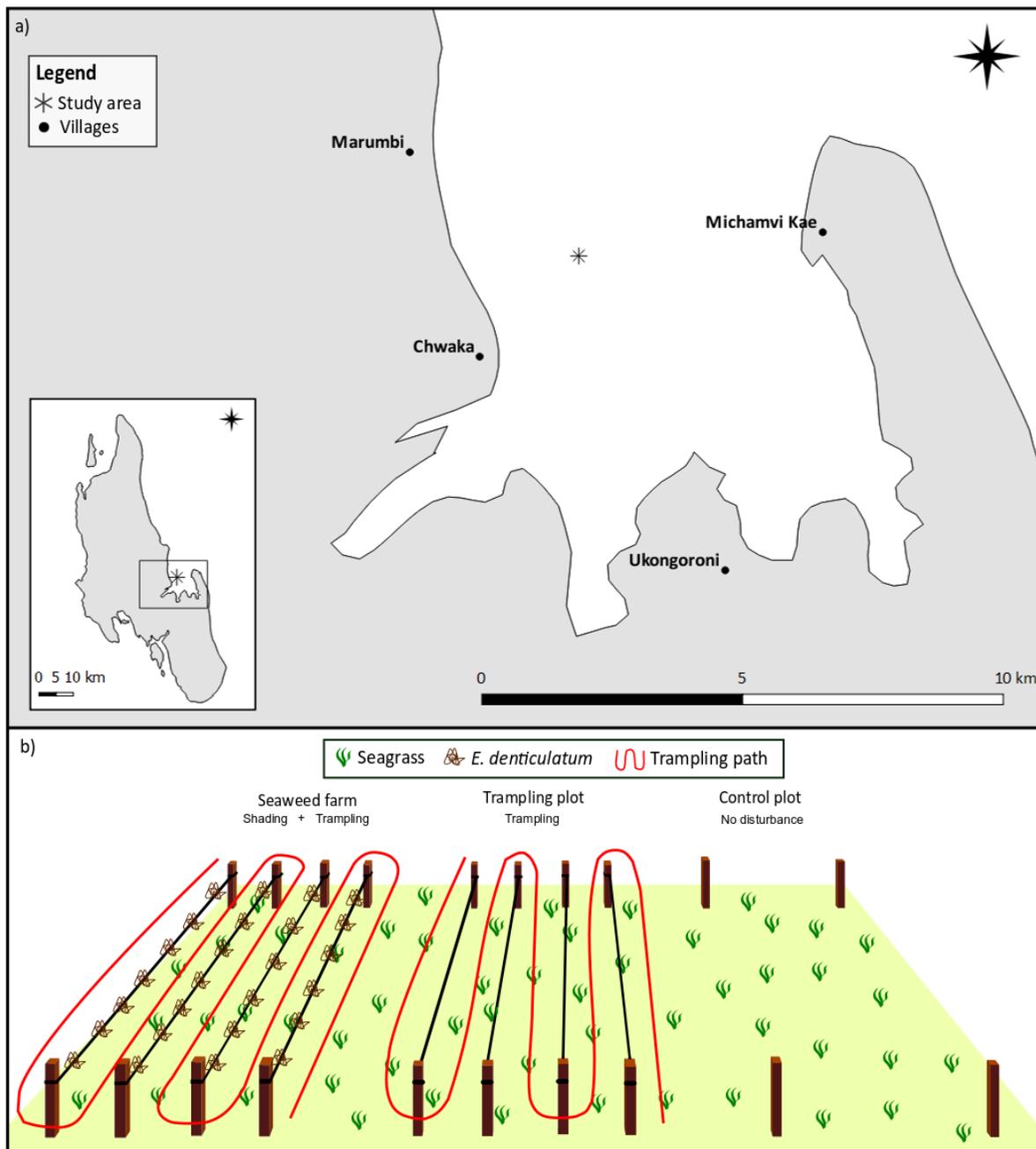


Figure 1. a) Map of the study area along the east coast of Unguja Island, Zanzibar (small box with inset of Chwaka Bay blown up). Chwaka Bay is surrounded by several villages, Chwaka being the biggest one in the area. Several hotels are present between Chwaka and Marumbi, the two villages closest to the study area where the seaweed farm experimental plots were built. Map generated with Qatum GIS (Version 2.18.3). b) Schematic representation of the treatment plots in the experiment. Figure produced with InkScape (Version 0.92.2)

(3) Seagrass and benthic macroalgae variables

Percentage cover and shoot density of seagrasses were measured taking three random points in each plot at each sampling time with 0.25 m² and 0.01 m² quadrats, respectively. Percentage cover was included as the only variable for the measurement of benthic macroalgae as

macroalgae could not be assessed by shoot density. The Braun-Blanquet scale was used for the cover measurements (Mueller-Dombois and Ellenberg 1974), consisting of a scale of 8 numbers each one referring to an interval of percentage cover. The scale goes as follows: 0.1 = <5% solitary, 0.5 = <5% sparse, 1 = <5% numerous, 2 = cover $\geq 5\%$ - $\leq 25\%$, 3 = cover $> 25\%$ - $\leq 50\%$, 4 = cover $> 50\%$ - $\leq 75\%$, and 5 = cover $> 75\%$. The cover categories were transformed into the midpoint cover range (Braun-Blanquet 1964). The percentage cover was then divided by 100, to obtain proportions between 0 and 1. Shoot density was measured counting the individual shoots of the two seagrass species within the 0.01 m² quadrat.

(4) Light reduction

A Li-Cor 1500 with a PAR (photosynthetically active radiation) sensor was used to measure the degree of shading by the seaweed growing over the seagrass meadow at each sampling time. Three random points were chosen within the seaweed farm plots and outside the farmed plots. The underwater PAR sensor ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was placed just below the water surface and at the seagrass canopy, while recording also the water depth at the canopy. Light reduction was calculated as the percent difference in surface and bottom PAR irradiance for the seaweed farm plots and the control plots. The worksheets with PAR data in three samplings were unfortunately lost (Days 49, 81 and 96); therefore, we present only the data from the days 0, 15, 24 and 63 (up to 9 weeks of the experiment). Nevertheless, as the typical harvesting cycle in Zanzibar is between 35-45 days (Hurtado et al. 2017), these values are representative of the light reduction experienced by seagrass and its associated community due to seaweed farming.

(5) Statistical analysis

Statistical software R (R Core Team 2016) was used for the statistical analysis. Graphs were plotted in R using the package “ggplot2” (Wickham 2016) and “ggpubr” (Kassambara 2020). Aesthetical editing of the figures was done using InkScape (Version 0.92.2).

To test whether there was a change in shoot density and cover in time due to shading and/or trampling, the slopes of regression models were compared. Time, treatment and their interaction (slope) were tested as fixed effects. Due to the use of the experimental block design, block and its interaction with both sampling time and treatment were included as fixed effects. Due to the added complication of including extra interactions with block, analysis of deviance tests and likelihood ratio tests were used to simplify the models if the interactions with block did not add explanatory power. Both treatment and sampling and their interaction were never dropped from

the model as they were part of the hypothesis testing. Block was never dropped from the model to avoid pseudoreplication (see Supplementary Material for further details).

The differences in light reduction inside and outside of the seaweed farms (introduced in the model as “area”) at each sampling time were analyzed using a linear model. Block and its interaction with the sampling area (outside or inside the seaweed farms) were added as a covariate to avoid pseudoreplication. Analysis of deviance test was used for model comparison and simplification. The significance in the differences in light reduction inside and outside the seaweed farms was obtained with analysis of variance (Type II test). Another linear model was used for the analysis of the differences in light reduction within an area (inside or outside seaweed farms) among the sampling times. Block and its interaction with sampling area were included in the model as fixed effects. The significance in the differences in light reduction within a sampling area in time was obtained with analysis of variance (Type II test). Post hoc pairwise comparisons were tested by using permutations in the models.

III. Results

(1) Light reduction

In seaweed farm plots, the range of light reaching the seagrass canopy was between 31 and 1117 μmol of photons m^{-2} second^{-1} with a mean of 237 μmol of photons m^{-2} second^{-1} . Outside the farmed plots the range was between 437 and 1674 μmol of photons m^{-2} second^{-1} , with a mean of 951 μmol of photons m^{-2} second^{-1} . Although there was a small reduction in light outside the farmed plots, 15-25%, light reduction was significantly greater inside the seaweed farm plots at every sampling time, stabilizing between 75-90% from day 15 onwards (Fig.2, Table 1). Block was a significant factor affecting light levels on day zero and 63, and its interaction with the sampling area was significant on days 34 and 63 (Table 1). These results suggest that the light reduction, despite being always greater inside seaweed farm plots, was also dependent on the block in the experimental design, and therefore not completely uniform within seaweed farms and outside of the seaweed farms. Overall, light reduction within seaweed farm plots significantly increased during the sampling time ($F(3, 24) = 72.854, p = 3.382 \cdot 10^{-12}$) in which every sampling time was significantly higher than day zero (Day 0 – Day 15: $t = 8.800, p = 5.61 \cdot 10^{-9}$; Day 0 – Day 34: $t = 8.191, p = 2.07 \cdot 10^{-8}$; Day 0- Day 63: $t = 9.531, p = 1.25 \cdot 10^{-9}$).

(2) Shoot density of *T. hemprichii* and *H. stipulacea*

In general, shoot density of *T. hemprichii* was higher in all plots compared to *H. stipulacea* and differed in its response to seaweed farming treatment (Fig.3a-c, g-I, Table 2). As shown by the

negative slopes of the regressions over time, shoot density of *T. hemprichii* and *H. stipulacea* decreased in the seaweed farm treatment ($\beta = -0.0004 \pm 0.002$; $\beta = -0.006 \pm 0.006$). In contrast the slopes of the regressions were positive for both the control ($\beta = 0.0034 \pm 0.001$; $\beta = 0.0072 \pm 0.004$) and the trampling treatments ($\beta = 0.0014 \pm 0.002$; $\beta = 0.0191 \pm 0.010$). These differences were not significantly different for *T. hemprichii* ($\chi^2 = 3.1402$, $df = 2$, $p = 0.208$), while they were for *H. stipulacea* ($\chi^2 = 7.1823$, $df = 2$, $p = 0.027$). The slopes of the regression of the control and trampling treatments for *H. stipulacea* were significantly higher in comparison to the slope of the seaweed farm treatment ($z = -2.11$, $p = 0.034$; $z = -2.35$, $p = 0.018$). There were no differences between control and trampling plots ($z = 1.16$, $p = 0.243$).

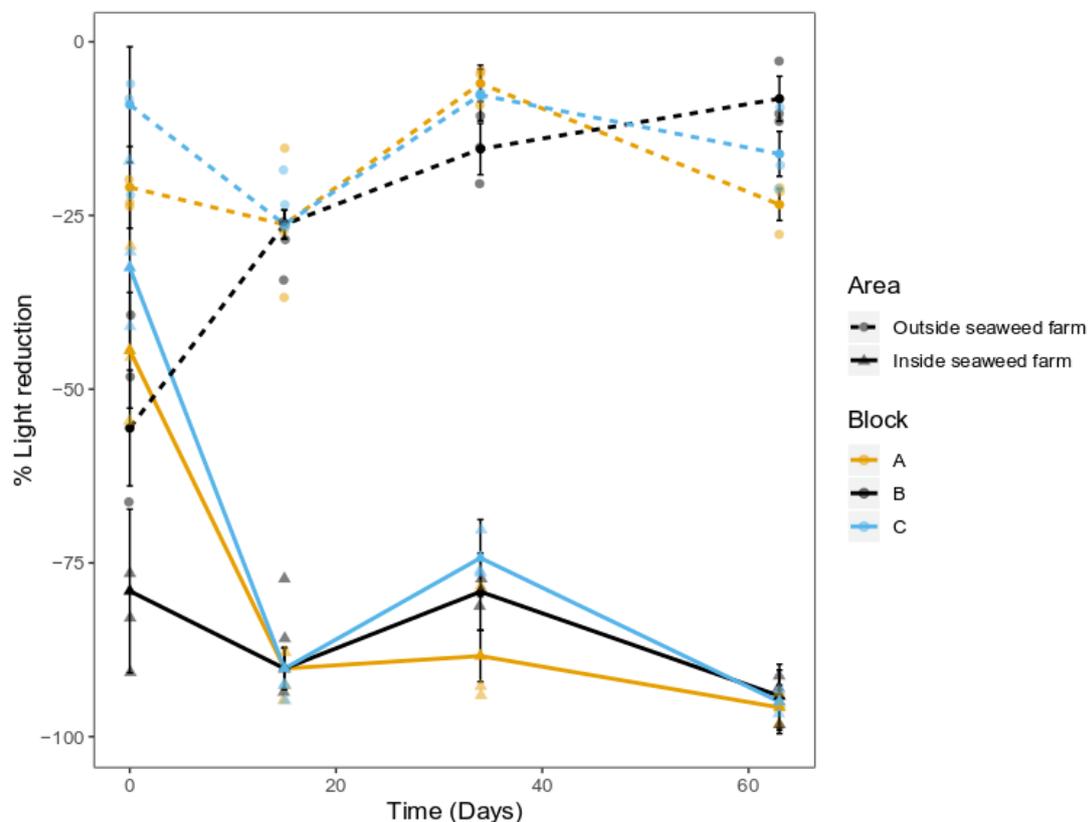


Figure 2. Light reduction (%) inside (circles) and outside (triangles) the seaweed farms during the experiment. The colors represent the different blocks A, B, and C.

For *H. stipulacea*, the interaction between block and treatment were significant when tested as fixed effects, showing that the response was variable between the replicates (Table 2). Treatment as a categorical fixed effect was significant for the two variables, indicating that the response was dependent on treatment overall, but variable in time, resulting in small differences between slopes of the regressions.

(3) Cover

Variability was high in the response of seagrass cover across the treatments over time (Fig.3 d-f, j-l, Table 2). Treatment as a fixed effect was significant for all the variables, and block was significant for all except *H. stipulacea* (Table 2). This result showed that, despite differences in responses between treatments, the variability over time translated into small differences in slopes overall.

Benthic macroalgae cover showed significant differences between slopes (Fig.3 m-o; $\chi^2 = 8.622$, $df = 2$, $p = 0.012$). The trampling treatment had a negative slope ($\beta = -0.007 \pm 0.004$), while both control and seaweed farm treatments showed a positive slope ($\beta = 0.0009 \pm 0.003$; $\beta = 0.005 \pm 0.004$). This slope difference was only significant between the seaweed farm and the trampling treatment ($z = -2.903$, $p = 0.003$). These trends resulted in an increase of macroalgae cover from 22.1% to 31.4% in seaweed farms and a decrease from 47.5% to 25.9% in the trampling treatment.

IV. Discussion

(1) The effect of seaweed farming due to shading and trampling on seagrass meadows and its associated benthic macroalgae

The effect of seaweed farming resulted in a loss of shoots of *H. stipulacea*. However, with the exception of the negative slope in shoot density of *T. hemprichii*, no clear effect of seaweed farming on this species was found. Our initial hypothesis was, therefore, partially rejected. Our results show that *H. stipulacea* was more sensitive to seaweed farming than *T. hemprichii* in the short term. Our findings are in agreement with an earlier study from Eklöf et al. (2006), in which *T. hemprichii* biomass and shoot density were unaffected after 11 weeks of seaweed farming. Nevertheless, in permanent seaweed farms, shoot density of *T. hemprichii* has been shown to be lower than in seagrass beds without seaweed farms (Eklöf et al. 2005; Lyimo et al. 2006). This suggests that over a longer period of time, *T. hemprichii* can be negatively affected by seaweed farming if conditions persist. In the case of *H. stipulacea*, there was no previous literature addressing its response to seaweed farming. The shading levels measured in this study inside and outside of seaweed farms were within the range of light irradiance of survival for both *H. stipulacea* (Sharon et al. 2011; Winters et al. 2020) and *T. hemprichii* (Collier et al. 2012), without showing negative effects on their physiology, morphology or density. This indicates that it is the additional stress of trampling combined with shading that is likely responsible for the response of *H. stipulacea*. This is also supported by Blankenhorn (2007)

who showed that trampling had negative effects on *E. acoroides* only in combination with shading and unfavorable seasonality, but had no impact as an isolated effect. The higher resistance of *T. hemprichii* was probably due to its higher carbohydrate reserve in the rhizome (Alcoverro et al. 2001; Longstaff and Dennison 1999) that are often lacking in opportunistic species like *H. stipulacea*. The higher energy reserves of *T. hemprichii* may be explained by this species investment in below-ground tissues, which also makes it more robust in structure and resistant to physical damage. *H. stipulacea*, in contrast, has a very fragile morphology (small and thin leaves, thin rhizome and short roots) that can be easily damaged and uprooted. The combination of the lack of energy reserves and its fragility may explain the loss of this species over time (a decrease in shoot density by half during the experiment), suggesting that this species could be displaced in the long term.

Cover is a visual indicator measured in a coarse scale, which was less accurate a measure than shoot counts and was not able to capture a change in a small quantity of shoots over time. Despite the proved usefulness of cover as a tool to assess seagrass abundance over larger spatial scales, we would not recommend cover as a measurement to capture small changes in seagrass densities over shorter temporal scales. Benthic macroalgae abundance was measured only as cover, and its slope in the seaweed farm treatment was positive and did not differ from the control. Taking into account that the shoot density of *H. stipulacea* was reduced in the seaweed farm treatments, macroalgae may be able out-compete seagrass under seaweed farming. This result partially agrees with our initial hypothesis, suggesting that benthic macroalgae can outcompete *H. stipulacea* under seaweed farms, but not *T. hemprichii*. It is important to point out the limitations of this result as well. As we did not identify all the components of the benthic macroalgae community, this shift cannot be extrapolated, as the change in the community is contingent on the algae species present in the area. Importantly, the loss of seagrass in the long term may also negatively affect macroalgae, as it has been reported that the cover of macroalgae is lower in seaweed farms compared to non-seaweed farm beds (Eklöf et al. 2005). The loss of seagrass may lead to a lower chance of macroalgae settling in the carbonate sand substrate and, therefore, not allow for the formation of a permanent community. Ultimately, there was no shift in the dominant macrophyte in the seagrass and macroalgae community. This indicates that, in the short term, there is no concern with the permanent loss of climax seagrass species. However, the loss of seagrass biodiversity due to the displacement of *H. stipulacea* can have unexpected consequences in ecosystem functioning (Sommerfield et al. 2002; Duffy 2006).

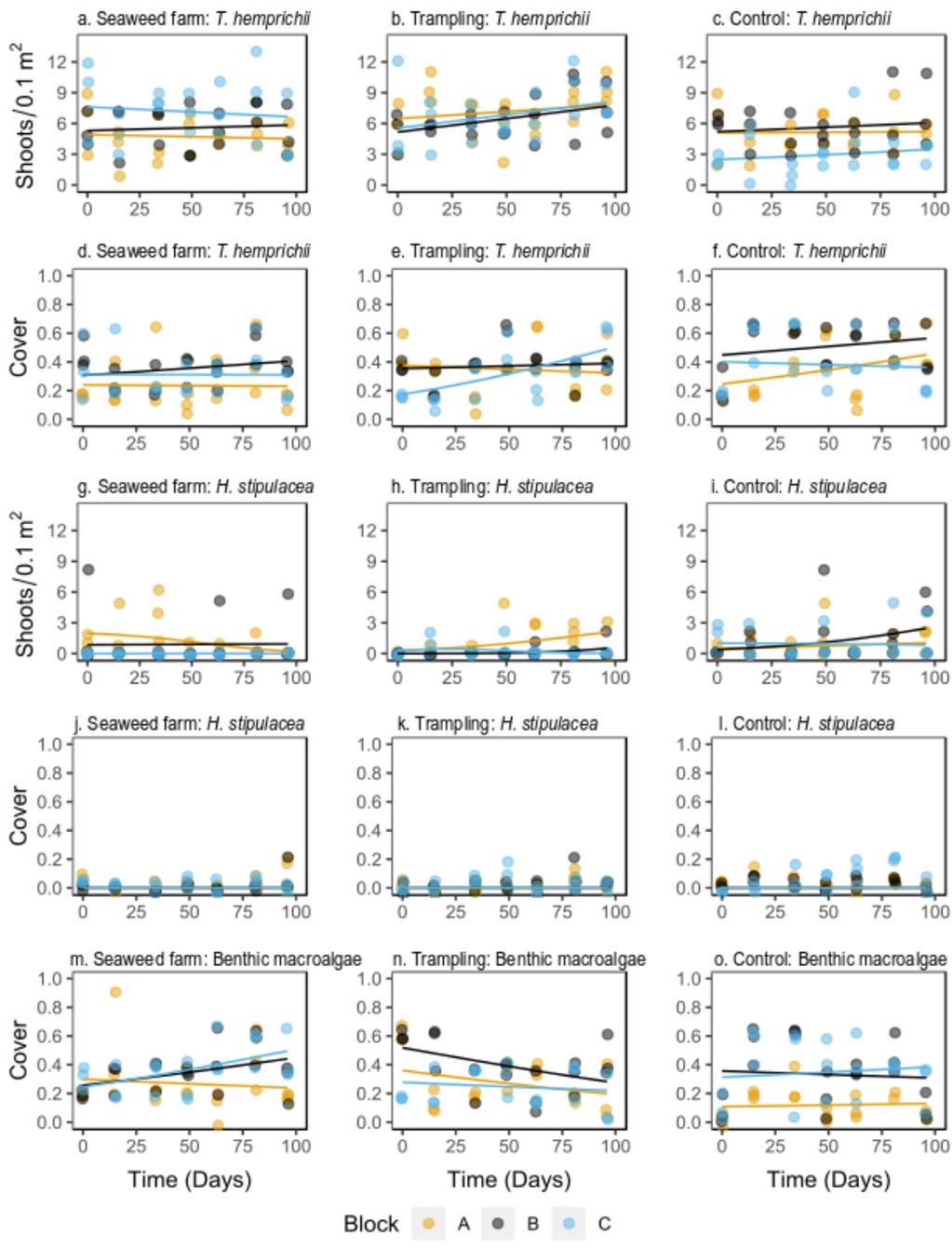


Figure 3. Shoot density and cover regression plots for the three treatments and the three blocks for all the variables. For shoot density plots, the y axis is the number of shoots in a 0.1m² quadrat. For cover plots, the y axis is the cover between 0 and 1, 0 meaning absence and 1 meaning complete cover, or 100%. The x axis is the time (days) that the experiment was running. Each of the columns represents one treatment, and each row represents the macrophyte tested. The colors represent the blocks and the regression line for each of the replicates.

(2) The effect of trampling

The trampling treatment had similar results as the control treatment for the seagrass shoot density, showing that low intensity trampling as an isolated disturbance had no effects on

seagrass. On the other hand, the cover of benthic macroalgae decreased. The pattern that macroalgae followed in the trampling treatment differed from that of the seaweed farm plots where it increased. The reason behind the loss of macroalgae could be that, as there is no loss of seagrass due to the lack of shading, it cannot out-compete seagrass for space. This is further confirmed by the fact that the slope of the regression of benthic macroalgae cover is only significantly lower in the trampling treatment when compared to the seaweed farm treatment. This agrees with a similar experiment (Blankenhorn 2007), which showed that trampling had no effects on seagrass as an isolated disturbance. We therefore reject the initial hypothesis that trampling as a disturbance could change the dominant macrophyte in the area.

Table 1. Results of the ANOVA comparisons of light reduction inside and outside the seaweed farms. Light reduction was analyzed at all the sampling times using a linear model with an identity function. The covariates column specifies the fixed effects included in the models after model simplification and selection. F = F-value; df = degrees of freedom; * = Significant explanatory variable in the model (in p-value column).

Sampling time	Variable	F	df	p-value
Day 0	Area	24.305	1, 14	0.000*
	Block	34.458	2, 14	0.000*
Day 15	Area	465.680	1, 16	0.000*
Day 34	Area	1101.624	1, 12	0.000*
	Block	3.839	2, 12	0.051
	Interaction Area*Block	7.371	2, 12	0.008*
Day 63	Area	1796.931	1, 12	0.000*
	Block	6.835	2, 12	0.010*
	Interaction Area*Block	4.427	2, 12	0.036*

The interpretation of these results regarding seagrass shoot density should be, however, conservative. The opposite result is normally reported: increased trampling results in the loss of seagrass biomass (Eckrich and Holmquist 2000), cover and reduction of blade length (Travaille et al. 2015). Trampling may also affect seagrass associated fauna. Other studies showed that epifauna were affected by intense trampling of seagrass in the past (Eckrich and Holmquist 2000; Skilleter et al. 2006). In other ecosystems like mudflats, the diversity and abundance of sessile organisms changed (Rossi et al. 2007). Apart from the direct physical effect on the benthos, trampling has the side effect of increasing the suspended sediment in the water column. This effect was considered of little importance due to fast flushing of the sediment by tidal currents in the area (Personal observation). Reports in the literature show that suspended sediment inputs over decadal timescales can reduce benthic macroalgae species richness, (Shepherd et al. 2009), while their composition and abundance depend more on algae reproductive traits in the short term (Eriksson and Johansson 2005). Nevertheless, this is not

the case for this experiment and for the practice of seaweed farming, in general, as sediment input is not continuous or persistent. We can make a conservative assumption that sediment suspension and turbidity are not driving factors affecting seagrass and benthic macroalgae communities under seaweed farming.

Even though we found no negative effects of trampling on seagrass during the experiment, this response may be subjected to the frequency of the trampling. The frequency at which the experimental seaweed farm and trampling plots were sampled was low, and therefore, did not negatively affect the seagrass. As farmers tend to work also in the low spring tide due to the lack of swimming training (Farmers of Chwaka Bay, personal communication), trampling frequency and the associated impact on seagrass would likely also be minimized. Nevertheless, if the trampling frequency or the number of people trampling on the plot increased, this result could lead to higher impacts on the seagrass ecosystem.

(3) Management recommendations, current state of seaweed farming in Zanzibar and its future under climate change

To date, the scientific literature on the effects of seaweed farms on seagrass meadows generally concludes with a similar message that despite the recorded negative effects of farming on seagrass meadows, it is generally a preferable aquaculture activity when compared with more destructive ones, like shrimp farming (Eklöf et al. 2006). Here we argue the contrary that, as any activity developed in the natural environment, it should be managed in order to minimize its negative effects. These management recommendations should take into account the social and economic context of this activity in the region. First, seaweed farming has decreased in the last years in Chwaka Bay (seaweed farmers, personal communication) due to the low revenue obtained from this activity. The commercial viability of seaweed in Zanzibar is threatened by several key points, including low price of the seaweed, low organization among farmers and low institutional support. A comprehensive work by Songwe et al. (2016) explains in detail each of these problems. As a consequence, there is little incentive for the professionalization and proper management of seaweed farming. Management recommendations such as a shift from the off-bottom method to the floating long-line, which does not disturb seagrass but requires more equipment and investment, may not be adopted if the activity does not also bring economic revenue.

Table 2. Results of the regression models for all the variables. They are grouped in shoot density and cover. The model column specifies the distribution and link function used, and the covariates specify the fixed effects included in the model after model simplification. χ^2 = Chi-squared; df = degrees of freedom; *=Significant covariate in the model (in p-value column).

Variable	Model	Covariate	χ^2	df	p-value	
Shoot density	<i>T. hemprichii</i>	GLM Poisson distribution (Log)	Time	2.7977	1	0.094
			Treatment	27.6695	2	0.000*
			Block	0.3664	2	0.832
			Time*Treatment	3.1402	2	0.208
			Treatment*Block	31.1763	4	0.000*
	<i>H. stipulacea</i>	Zero-inflated Poisson (log/logit)	Time	1.416	1	0.234
			Treatment	7.0381	2	0.296
			Block	10.6069	2	0.004*
			Time*Treatment	7.1823	2	0.027*
			Treatment*Block	10.4552	4	0.033*
Cover	<i>T. hemprichii</i>	Beta regression (Logit)	Time	5.1418	1	0.023*
			Treatment	15.6702	2	0.000*
			Block	17.5702	2	0.000*
			Time*Treatment	1.0112	2	0.603
	<i>H. stipulacea</i>	Zero-One-Inflated Beta regression (LogLog/Log /Logit)	Time	0.082	1	0.685
			Treatment	10.554	2	0.000*
			Block	0.549	2	0.577
			Time*Treatment	1.097	2	0.334
	Benthic macroalgae	Beta regression (Logit)	Time	0.0173	1	0.895
			Treatment	8.6024	2	0.013*
			Block	28.0228	2	0.000*
			Time*Treatment	8.6226	2	0.012*
			Treatment*Block	16.3136	4	0.002*

In the last policy briefs produced by The Revolutionary Government of Zanzibar (2014) and the Ministry of Livestock and Fisheries Development of the United Republic of the Tanzania (2014), the promotion of seaweed farming by value addition techniques and the implementation of coastal management for seaweed farming was highlighted as being necessary to professionalize seaweed farming and reduce environmental impacts. From our findings there are two main recommendations that could avoid negative long-lasting effects of seaweed farming on seagrass meadows: 1) rotating crops and 2) targeting of bare sediment areas for seaweed farms. The first recommendation was suggested by Eklöf et al. (2006) and Blankenhorn (2007), and we agree that it would decrease the potential impacts of long term seaweed farming on seagrass ecosystems. The second recommendation contradicts the

recommendations of the FAO (Juanich 1988) for the site selection. Although we agree that farmers should locate their farms in areas where macrophytes already grow as a proof that environmental conditions are suitable for photosynthetic organisms, they should avoid the placing of farms directly on top of seagrass meadows. Another potential solution is to locate the lines further apart from each other to allow for more light reaching the seagrass canopy. These recommendations could be easily implemented, do not require of extra investment, and would also protect other livelihoods such as fishing, which is tightly linked to the health of seagrass ecosystems.

V. Conclusions

This study shows that seaweed farming, through the combined effects of trampling and shading, can negatively affect the ephemeral species *H. stipulacea* despite its capacity for rapid growth. On the other hand, *T. hemprichii* was mainly unaffected in the short term. Due to the decline in *H. stipulacea*, benthic macroalgae cover increased in the seaweed farm treatment, suggesting a possible change in the seagrass and macroalgae community under the impact of seaweed farming. Trampling as an isolated disturbance had only a negative effect on the benthic macroalgae, which suggests that, under this isolated disturbance, seagrass can out-compete the benthic macroalgae community.

This study further contributes to the analysis of the effects of seaweed farms, raising awareness about possible long term effects over the seagrass and benthic macroalgae communities if seaweed farming is not properly managed. The question of the sustainability of seaweed farming should not only be addressed from an ecological point of view, but jointly with social and economic considerations under a climate change scenario. Efforts in the professionalization and organization of seaweed farming would help the farmers to bring extra value to their activity, together with a stronger capacity for the negotiation and bargain of the price of their product, while supporting coastal efforts to make it more sustainable.

VI. Acknowledgments

The authors thank the staff of the Institute of Marine Sciences (IMS) in Stone Town for their support both administratively and scientifically, specifically Mtumwa Mwadini for his advice and help.

We want to thank the people from Chwaka Village for their help. Specifically to Hakim Machano for his help in the building of the experimental plots. Also we want to thank his son

Hassan Machano for his help in the sampling activities. We would like to thank Stephanie Helber for her help in building the seaweed farms.

Leibniz Centre for Tropical Marine Research Master Thesis grant and the PROMOS scholarship (Programm zur Steigerung der Mobilität von Studierenden deutscher Hochschulen, University of Bremen) awarded to Agustín Moreira Saporiti partially funded the field work. This project was developed under the SUTAS program (Sustainable Use of Tropical Aquatic Systems- funding to Dieuwke Hoeijmakers). The Seagrass and Macroalgal Community Dynamics and Performance under Environmental Change (SEAMAC) project (Deutsche Forschungsgemeinschaft, DFG, TE 1046/3-1) awarded to Mirta Teichberg partially funded this project.

VII. References

- Agardh, C.A. (1823) Species algarum rite cognitae: cum synonymis, differentiis specificis et descriptionibus succinctis. sumtibus E. Mavritii. Vol 1
- Alcoverro, T., Manzanera, M., Romero, J. (2001) Annual metabolic carbon balance of the seagrass *Posidonia oceanica*: the importance of carbohydrate reserves. *Marine Ecology Progress Series*, 211, 105-116. <https://doi.org/10.3354/meps211105>
- Alexandre, A., Santos, R., Serrão, E. (2005) Effects of clam harvesting on sexual reproduction of the seagrass *Zostera noltii*. *Marine Ecology Progress Series*, 298, 115-122. <https://doi.org/10.3354/meps298115>
- Ascherson, P. (1868) Vorarbeiten zu einer Uebersicht der phanerogamen Meergewächse. *Linnaea*, 35, 152-208.
- Ascherson, P. (1871) Die geographische verbreitung der seegräser. *Petermans Geographische Mittheilungen*, 17, 241-248.
- Blankenhorn, S.U. (2007) Seaweed farming and artisanal fisheries in an Indonesian seagrass bed. Dissertation, University of Bremen.
- Braun-Blanquet, J. (1964) *Pflanzensoziologie, Grundzüge der Vegetationskunde*. 3rd edn. Springer.
- Bryceson, I. (2002) Coastal aquaculture developments in Tanzania: sustainable and non-sustainable experiences. *Western Indian Ocean Journal of Marine Science*, 1, 1-10.
- Collier, C.J., Waycott, M., Ospina, A.G. (2012) Responses of four Indo-West Pacific seagrass species to shading. *Marine Pollution Bulletin*, 65, 342-354. <https://doi.org/10.1016/j.marpolbul.2011.06.017>
- Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M. et al (1997) The value of the world's ecosystem services and natural capital. *Nature*, 387, 253. <https://doi.org/10.1038/387253a0>

- Crawford, B.R., Pollnac, R.B., Sukmara, A., Tulungen, J.J. (2002) Community-based coastal resources management: an interim assessment of the Proyek Pesisir field site in Bentenan and Tumbak villages, North Sulawesi, Indonesia. Narragansett, RI: University of Rhode Island, Coastal Resources Center. Technical report TE-02/01-E. https://www.crc.uri.edu/download/2000_Crawford_Tech_R_Community_Based_CRM.pdf Accessed 22 October 2020
- de la Torre-Castro, M. D., Rönnbäck, P. (2004) Links between humans and seagrasses—an example from tropical East Africa. *Ocean and Coastal Management*, 47, 361-387. <https://doi.org/10.1016/j.ocecoaman.2004.07.005>
- de la Torre-Castro, M. (2012) Governance for sustainability: Insights from marine resource use in a tropical setting in the Western Indian Ocean. *Coastal Management*, 40, 612-633. <https://doi.org/10.1080/08920753.2012.727738>
- de San, M. (2012) The farming of seaweeds. FAO Report SF/2012/28. <http://www.fao.org/3/a-bl759e.pdf> Accessed 22 October 2020
- Duffy, J.E. (2006) Biodiversity and the functioning of seagrass ecosystems. *Marine Ecology Progress Series*, 311, 233-250. <https://doi.org/10.3354/meps311233>
- Duarte, C.M., Chiscano, C.L. (1999) Seagrass biomass and production: a reassessment. *Aquatic botany*, 65, 159-174. [https://doi.org/10.1016/s0304-3770\(99\)00038-8](https://doi.org/10.1016/s0304-3770(99)00038-8)
- Eckrich, C.E., Holmquist, J.G. (2000) Trampling in a seagrass assemblage: direct effects, response of associated fauna, and the role of substrate characteristics. *Marine ecology progress series*, 201, 199-209. <https://doi.org/10.3354/meps201199>
- Eklöf, J.S., de la Torre-Castro, M., Adelsköld, L., Jiddawi, N.S., Kautsky, N. (2005) Differences in macrofaunal and seagrass assemblages in seagrass beds with and without seaweed farms. *Estuarine, Coastal and Shelf Science*, 63, 385-396. <https://doi.org/10.1016/j.ecss.2004.11.014>
- Eklöf, J.S., Henriksson, R., Kautsky, N. (2006) Effects of tropical open-water seaweed farming on seagrass ecosystem structure and function. *Marine Ecology Progress Series*, 325, 73-84. <https://doi.org/10.3354/meps325073>
- Eklöf, J.S., Msuya, F.E., Lyimo, T.J., Buriyo, A.S. (2012) Seaweed farming in Chwaka Bay: a sustainable alternative in aquaculture. In *People, nature and research in Chwaka Bay* (pp. 213-233). WIOMSA, Zanzibar.
- Eriksson, B.K., Johansson, G. (2005) Effects of sedimentation on macroalgae: species-specific responses are related to reproductive traits. *Oecologia* 143, 438-448. <https://doi.org/10.1007/s00442-004-1810-1>
- FAO (2018) The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. <http://www.fao.org/3/i9540en/i9540en.pdf> Accessed 22 October 2020

- Hassan, I.H., Othman, W.J. (2019) Seaweed (Mwani) Farming as an Adaptation Strategy to Impacts of Climate Change and Variability in Zanzibar. In *Climate Change and Coastal Resources in Tanzania* (pp. 53-68). Springer, Cham.
- Hedberg, N., von Schreeb, K., Charisiadou, S., Jiddawi, N.S., Tedengren, M. et al (2018) Habitat preference for seaweed farming—A case study from Zanzibar, Tanzania. *Ocean and Coastal Management*, 154, 186-195. <https://doi.org/10.1016/j.ocecoaman.2018.01.016>
- Hemminga, M.A., Harrison, P.G., Van Lent, F. (1991) The balance of nutrient losses and gains in seagrass meadows. *Marine Ecology Progress Series*, 28, 85-96. <https://doi.org/10.3354/meps071085>
- Hurtado, A.Q., Gerung, G.S., Yasir, S., Critchley, A.T. (2014) Cultivation of tropical red seaweeds in the BIMP-EAGA region. *Journal of Applied Phycology*, 26, 707-718. <https://doi.org/10.1007/s10811-013-0116-2>
- Hurtado, A.Q., Critchley, A.T., Neish, I. (2017) *Tropical seaweed farming trends, problems and opportunities*. Springer Publ. AG., New York
- Juanich, G.L. (1988) Manual on seaweed farming 1. *Eucheuma spp.* FAO publications. <http://www.fao.org/3/ac416e/ac416e00.htm> Accessed 22 October 2020
- Kassambara, A. (2020) ggpubr: ‘ggplot2’ Based Publication Ready Plots. R package version 0.4.0. <https://CRAN.R-project.org/package=ggpubr> Accessed 02 November 2020
- Lamouroux, J.V.F. (1809). Exposition des caractères du genre Dictyota, et tableau des espèces qu’il renferme. *Journal de botanique* (Desvaux), 2, 38-44
- Lamouroux, J.V.F. (1812). Sur la classification des Polypiers coralligènes non entièrement pierreux. *Nouv. Bull. Sci. Soc. Philom. Paris*, 3, 181-188
- Lipkin, Y. (1975) *Halophila stipulacea*, a review of a successful immigration. *Aquatic Botany*, 1, 203-215. [https://doi.org/10.1016/0304-3770\(75\)90023-6](https://doi.org/10.1016/0304-3770(75)90023-6)
- Longstaff, B.J., Dennison, W.C. (1999) Seagrass survival during pulsed turbidity events: the effects of light deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*. *Aquatic Botany*, 65, 105-121. [https://doi.org/10.1016/s0304-3770\(99\)00035-2](https://doi.org/10.1016/s0304-3770(99)00035-2)
- Lyimo, T.J., Mvungi, E.F., Lugomela, C., Björk, M. (2006) Seagrass biomass and productivity in Seaweed and Non-Seaweed Farming areas in the East Coast of Zanzibar. *Western Indian Ocean Journal of Marine Science*, 5, 141-152. <https://doi.org/10.4314/wiojms.v5i2.28506>
- Milazzo, M., Badalamenti, F., Riggio, S., Chemello, R. (2004) Patterns of algal recovery and small-scale effects of canopy removal as a result of human trampling on a Mediterranean rocky shallow community. *Biological Conservation*, 117, 191-202. [https://doi.org/10.1016/s0006-3207\(03\)00292-1](https://doi.org/10.1016/s0006-3207(03)00292-1)
- Ministry of Livestock and Fisheries Development United Republic of Tanzania (2014) First South West Indian Ocean Fisheries Governance and shared growth project-SWIOfish, Environmental and Social Assessment (ESA) and Environmental and Social Management Framework (ESMF).

<http://documents1.worldbank.org/curated/ar/921011468003576936/pdf/E46250V30P132100Box385306B00PUBLIC0.pdf> Accessed 23 October 2020

Msuya, F.E. (2006) The impact of seaweed farming on the social and economic structure of seaweed farming communities in Zanzibar, Tanzania. In Critchley AT, Ohno M, Largo DB (eds.) World seaweed resources: an authoritative reference system. Amsterdam, ETI BioInformatics. <http://196.44.162.10:8080/xmlui/handle/123456789/653> Accessed 23 October 2020

Msuya, F.E. (2011) The impact of seaweed farming on the socioeconomic status of coastal communities in Zanzibar, Tanzania. *World Aquaculture*, 42, 45-48

Mueller-Dombois, D., Ellenberg, H. (1974) *Aims and methods of vegetation ecology*. John Wiley and Sons. Inc., New York, USA

Orth, R.J., Heck, K.L., van Montfrans, J. (1984) Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries*, 7, 339-350. <https://doi.org/10.2307/1351618>

Pettersson-Löfquist, P. (1995) The development of open-water algae farming in Zanzibar: reflections on the socioeconomic impact. *Ambio*, 24, 487-491. JSTOR www.jstor.org/stable/4314394 Accessed 22 October 2020

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/> Accessed 02 November 2020

Rossi, F., Forster, R.M., Montserrat, F., Ponti, M., Terlizzi, A. et al (2007) Human trampling as short-term disturbance on intertidal mudflats: effects on macrofauna biodiversity and population dynamics of bivalves. *Marine Biology*, 151, 2077-2090. <https://doi.org/10.1007/s00227-007-0641-0>

Ruiz, H., Ballantine, D.L. (2004) Occurrence of the seagrass *Halophila stipulacea* in the tropical west Atlantic. *Bulletin of Marine Science*, 75, 131-135

Sharon, Y., Levitan, O., Spungin, D., Berman-Frank, I., Beer, S. (2011) Photoacclimation of the seagrass *Halophila stipulacea* to the dim irradiance at its 48-meter depth limit. *Limnology and Oceanography*, 56, 357-362. <https://doi.org/10.4319/lo.2011.56.1.0357>

Shepherd, S.A., Watson, J.E., Womersley, H.B.S., Carey, J.M. (2009) Long-term changes in macroalgal assemblages after increased sedimentation and turbidity in Western Port, Victoria, Australia. *Botanica Marina*, 52, 195-206. <https://doi.org/10.1515/bot.2009.036>

Skilleter, G.A., Cameron, B., Zharikov, Y., Boland, D., McPhee, D.P. (2006) Effects of physical disturbance on infaunal and epifaunal assemblages in subtropical, intertidal seagrass beds. *Marine Ecology Progress Series*, 308, 61-78. <https://doi.org/10.3354/meps308061>

Smith, I.R. (1979) Traditional fisheries development in the Philippines. ICLARM Newsletter. http://pubs.iclarm.net/resource_centre/WF_1282.pdf Accessed 22 October 2020

- Somerfield, P.J., Yodnarasri, S., Aryuthaka, C. (2002) Relationships between seagrass biodiversity and infaunal communities: implications for studies of biodiversity effects. *Marine Ecology Progress Series*, 237, 97-109. <https://doi.org/10.3354/meps237097>
- Songwe, B.A., Khamis, S.M., Khalfan, M.H., Msuya, F.E. (2016) Commercial Seaweed Farming in Zanzibar Coastal Villages: Potential for Innovative and Competitive Economic Growth. *Journal of the Open University of Tanzania*, 22, 1153-173
- The Revolutionary Government of Zanzibar (2014) Non-Governmental NGOs (NGOs) Policy <https://tile.loc.gov/storage-services/service/gdc/gdcovop/2019352552/2019352552.pdf>
Accessed 22 October 2020
- Travaille, K.L., Salinas-de-León, P., Bell, J.J. (2015) Indication of visitor trampling impacts on intertidal seagrass beds in a New Zealand marine reserve. *Ocean and Coastal Management*, 114, 145-150. <https://doi.org/10.1016/j.ocecoaman.2015.06.002>
- Unsworth, R.K., Ambo-Rappe, R., Jones, B.L., La Nafie, Y.A., Irawan, A. et al (2018) Indonesia's globally significant seagrass meadows are under widespread threat. *Science of the Total Environment*, 634, 279-286. <https://doi.org/10.1016/j.scitotenv.2018.03.315>
- Viana, I.G., Siriwardane-de Zoysa, R., Willette, D.A., Gillis, L.G. (2019) Exploring how non-native seagrass species could provide essential ecosystems services: a perspective on the highly invasive seagrass *Halophila stipulacea* in the Caribbean Sea. *Biological Invasions*, 21, 1461-1472. <https://doi.org/10.1007/s10530-019-01924-y>
- Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. <https://CRAN.R-project.org/package=ggplot2> Accessed 02 November 2020
- Willette, D.A., Ambrose, R.F. (2009) The distribution and expansion of the invasive seagrass *Halophila stipulacea* in Dominica, West Indies, with a preliminary report from St. Lucia. *Aquatic Botany*, 91, 137-142. <https://doi.org/10.1016/j.aquabot.2009.04.001>
- Willette, D.A., Ambrose, R.F. (2012) Effects of the invasive seagrass *Halophila stipulacea* on the native seagrass, *Syringodium filiforme*, and associated fish and epibiota communities in the Eastern Caribbean. *Aquatic botany*, 103, 74-82. <https://doi.org/10.1016/j.aquabot.2012.06.007>
- Winters, G., Beer, S., Willette, D.A., Viana, I.G., Chiquillo, K.L. et al (2020). The tropical seagrass *Halophila stipulacea*: reviewing what we know from its native and invasive habitats, alongside identifying knowledge gaps. *Frontiers in Marine Science*, 7, 300. <https://doi.org/10.3389/fmars.2020.00300>

VIII. Supplementary Material

The shoot density of *T. hemprichii* was analyzed with a regression using a generalized linear model with a Poisson distribution and a logarithmic link function. Shoot density of *H. stipulacea* was analyzed using a zero-inflated model with a Poisson distribution (ZIP). This model was selected due to zero-inflation. The data was analyzed in two steps: it modelled the zero and non-zero values with a Binomial distribution, while it modelled the count data via a Poisson distribution. For the construction of the ZIP and ZINB models the R package “pscl” was used (Jackman et al. 2007; Jackman 2010), and for the likelihood ratio test comparisons, the package “lmtree” was used (Zeileis and Hothorn 2002).

The cover of benthic macroalgae and *T. hemprichii* were analyzed using a Beta regression with a beta distribution. This model was used due to the proportional nature of the data, which was between 0 and 1 (excluding these two values). The dataset presented “1” values in several instances and was therefore transformed following Smithson and Verkuilen (2006): $(y * (n-1) + 0.5) / n$. This equation transformed the 0 and 1 values into slightly higher than zero and lower than one. The R package “betareg” (Zeileis et al. 2010) was used for the construction of the beta regression. The significance in the differences between the regression slopes and the categorical variables were obtained with analysis of variance (Type II test) using the R package “car” (Fox and Weisberg 2011). Post hoc pairwise comparisons were tested by using permutations in the models.

The cover of *H. stipulacea* was analyzed using a zero-one-inflated beta regression (BEZI) due to zero-inflation. The likelihood ratio test was used for model comparison and simplification. The significance for each covariate in the final model was obtained by stepwise adding of each fixed effect in the model and model comparison with a likelihood ratio test.

References for supplementary information:

Fox, J., Weisberg, S. (2011) An {R} Companion to Applied Regression. Sage Publications, Thousand Oaks CA

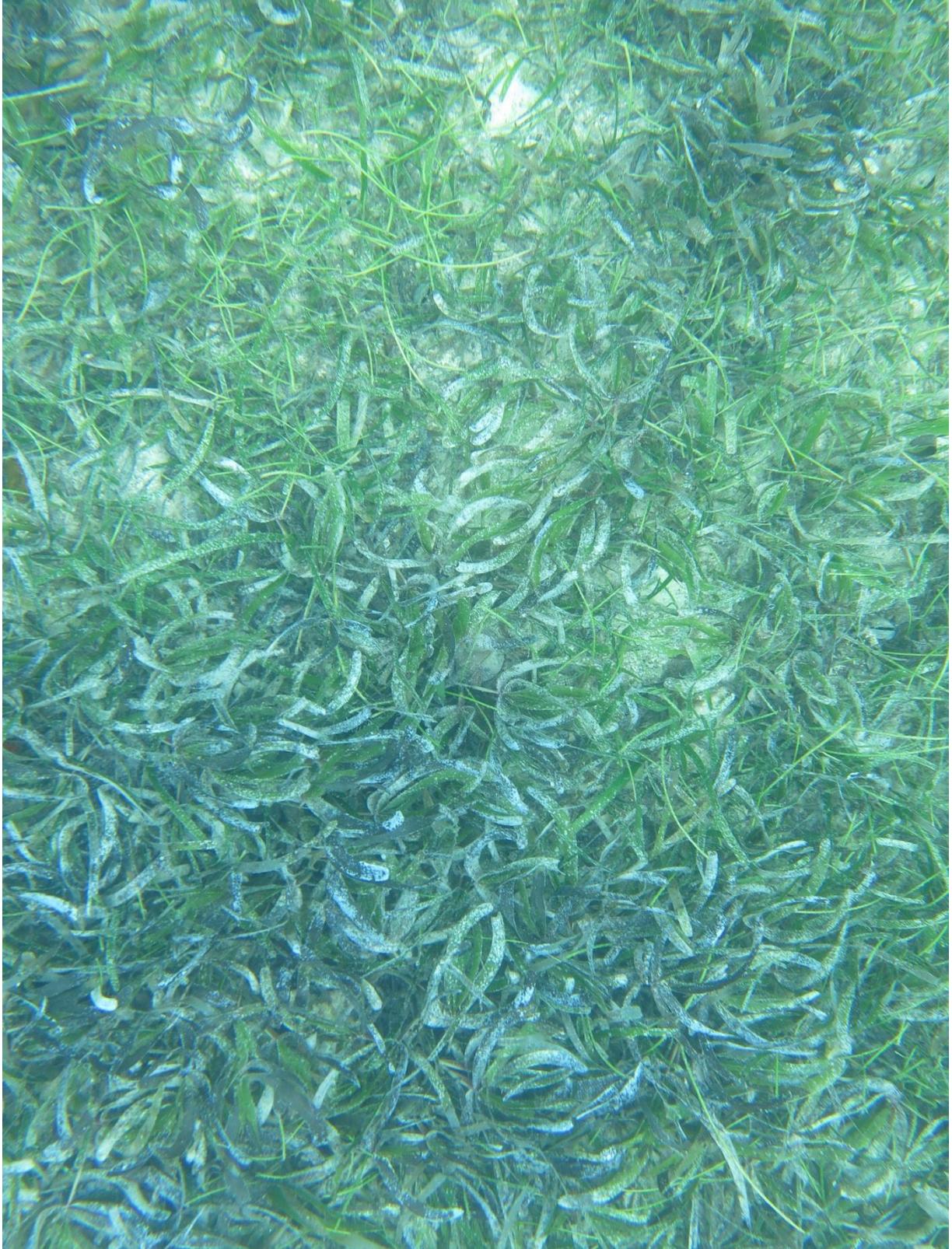
Jackman, S., Kleiber, C., Zeileis, A. (2007) *Regression models for count data in R*. Working papers, Faculty of Business and Economics, University of Basel. <https://doi.org/10.18637/jss.v027.i08>

Jackman, S. (2010) pscl: Classes and methods for R. Developed in the Political Science Computational Laboratory, Stanford University. Department of Political Science, Stanford University, Stanford, CA. R package version 1.03.5. <https://cran.r-project.org/web/packages/pscl/index.html> Accessed 02 November 2020

Smithson, M., Verkuilen, J. (2006) A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. *Psychological methods*, 11, 54. <https://doi.org/10.1037/1082-989x.11.1.54>

Zeileis, A., Cribari-Neto, F., Grün, B., Kos-midis, I. (2010) Beta regression in R. *Journal of statistical software*, 34, 1-24. <https://doi.org/10.18637/jss.v034.i02>

Zeileis, A., Hothorn, T. (2002) Diagnostic checking in regression relationships. *R News*, 2, 7-10



CHAPTER 6.

Local victory: Assessing interspecific competition in seagrass from a trait-based perspective

Agustín Moreira-Saporiti^{1,2}, Sonia Bejarano¹, Inés G. Viana³, E. Fay Belshe¹, Matern Mtolera⁴, Mirta Teichberg¹

¹Leibniz Centre for Tropical Marine Research GmbH, Bremen, Germany, ²Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany, ³Department of Ecology and Animal Biology, University of Vigo, 36310 Vigo, Galicia, Spain, ⁴University of Dar es Salaam, University Road, P.O. Box 35060, Dar es Salaam, Tanzania

Abstract

Tropical seagrass meadows are formed by an array of seagrass species that share the same space. Species sharing the same plot are competing for resources, namely light and inorganic nutrients, which results in the capacity of some species to preempt space from others. However, the drivers behind seagrass species competition are not completely understood. In this work, we studied the competitive interactions among tropical seagrass species of Unguja Island (Zanzibar Archipelago, Tanzania) using a trait-based approach. For this purpose, we quantified the abundance of 8 seagrass species under an eutrophication gradient, and selected 9 different traits related to light and inorganic nutrient preemption (maximum leaf length and width, leaves/shoot, leaf mass area, vertical rhizome length, shoots/meter, rhizome diameter, roots/meter and root maximum length), what we named the functional strategy of the seagrass species. From the seagrass abundance we calculated the probability of space preemption between seagrass pairs and for each seagrass species in the eutrophication gradient. The results showed that the species had different probabilities of space preemption, with *Thalassodendron ciliatum*, *Enhalus acoroides*, *Thalassia hemprichii* and *Cymodocea serrulata* generally having the highest preemptions, while *Halophila ovalis*, *Syringodium isoetifolium*, *Halodule uninervis* and *Cymodocea rotundata*, the lowest. Traits determining the functional strategy of the seagrass showed that there was a size gradient in the seagrass species. When tested the effect of the difference in the functional strategy of species pairs, the probability of preemption was highest for the bigger species, increased when their size difference was higher and was not affected by the eutrophication. This indicated that the competitive interactions among seagrass species were asymmetrical, i.e. a species had a negative effect on another species, while the effect was not reciprocal and the driver behind space preemption was determined by traits related to the size of the seagrass plants. This study disentangled how seagrass compete for resources and allowed for the calculation of the probability that a species would exert preemption using a trait-based approach.

Keywords: functional ecology, trait-based approach, traits ordination, resource preemption, space preemption

I. Introduction

Seagrasses are a polyphyletic assemblage of angiosperm plants that inhabit coastal areas and undergo their entire life cycle in the water. Their ecosystems are highly productive habitats that support considerable biomass of associated species diversity (Orth et al. 1984) and offer highly valued ecosystem services (Constanza and Folke 1997), including climate regulation (Duarte and Chiscano 1999) and nutrient filtering (Hemminga et al. 1991). Despite successfully adapting to the marine realm, their habitats are confined to a thin strip of shallow nearshore waters (Olsen et al. 2016) that presently are susceptible to increasingly high anthropogenic pressures, both at a local and global scale. Leading anthropogenic factors include eutrophication that reduce light by the increase in phytoplankton and opportunistic macroalgae biomass (Cardoso et al. 2004, Schmidt et al. 2012). Global warming directly affects seagrasses through thermal stress (Ontoria et al. 2019), reducing their biomass and eventually causing mortality (Rasheed and Unsworth 2011). This results in a decline in the coverage of seagrass worldwide (Orth et al. 2006, Waycott et al. 2009, Unsworth et al. 2018). Although this trend may be reversed in temperate and sub-tropical areas (de los Santos et al. 2019, Schäfer et al. 2021), the situation in tropical areas is largely underreported.

The seagrass communities present in a specific habitat are the product of several filters that generally act in a hierarchical fashion (Garnier et al. 2016). Firstly, seagrass dispersion methods control the potential of a species to colonize a new habitat (Orth et al. 2007, McMahon et al. 2014). Secondly, abiotic filters determine which seagrass species can establish given the local environmental conditions, availability of resources and disturbance regime (Wilson 2011). Lastly, the subset of species that have successfully colonized an area will interact and compete with each other for resources (Tilman 1985). All these processes filter the regional species pool and ultimately assemble local seagrass communities (Keddy 1992).

Seagrasses, similarly to terrestrial plant communities, undergo successional states from pioneer to climax species (Young and Kirkman 1975, Birch and Birch 1984, Williams 1987, 1990; Fourqurean et al. 1995, Davis and Fourqurean 2001). Pioneer species within seagrass meadows are generally small and fast growing, whereas climax species are large and slow-growing. If conditions are not extrinsically disturbed, succession shall follow a direction, which has been long believed to end in a monospecific meadow formed by a climax seagrass species (Moliner and Picard 1952, Aleem 1955, den Hartog 1971, 1977; Zieman 1982). Competitive interactions between pioneer and climax seagrass species are the mechanisms driving the course of the succession (Connell and Slatyer 1977, Tilman 1994). In plants, interspecific competition can

be broadly defined as the limiting effect that species may have on each other, directly or indirectly reducing or preventing growth and survival (Connell 1990, Vilà and Sardans 1999).

Since the first descriptions of seagrass meadows, there were reports of potential interspecific competition between species. Competition for space between *Posidonia oceanica* and *Cymodocea nodosa* was early reported in the Mediterranean Sea (Moliner and Picard 1952, Aleem 1955), as well as the dominance of *Thalassia testudinum* over other seagrasses present in the coasts of Florida (USA, Phillips 1960), or the competition between *Ruppia* sp. and other seagrasses (den Hartog 1970). *Zostera marina* suppresses shoot production of *Z. japonica* (Nomme and Harrison 1991a, b) and *Halophila stipulacea* can displace *Syringodium filiforme* from its native habitat (Willette and Ambrose 2012, Viana et al. 2019a). The observation of competition prompted the classification of seagrass species according to their life-history strategies. For instance, Harrison (1979) classified *Z. marina* as a k-strategist and *Z. japonica* as an r-strategist due to their differential investment on maintenance of belowground and reproductive structures, respectively. Birch and Birch (1984) provided a detailed description of succession in a seagrass meadow after a hurricane, providing insight into how the successional states are dominated by seagrass with differential life-history strategies, suggesting that succession is directional and not probabilistic.

Competition happens when there are resources that are preemtable and limited (Tilman 1985). Preemtable resources are those that, once taken by an organism, they are not available for the others (Underwood and Denley 1984). Light is, therefore, a preemtable resource for plants (Schwinning and Weiner 1998). Particularly, in the case of seagrasses, shading has been proposed as one of the main underlying mechanisms for space competition (Turner 1983, 1985) also common in giant kelp forests (Rosenthal et al. 1974). The canopy of *T. testudinum* blocks up to 75% of the light reaching it (Zieman et al. 1984), and *Z. marina* can competitively exclude *Ruppia maritima* by light shading (Orth 1977), among other examples (Fourqurean et al. 1995, Duarte et al. 1998). Shading is one of the mechanisms through which *T. testudinum* displaces *S. filiforme*, with the larger leaves of *T. testudinum* commonly intercepting light that otherwise would reach *S. filiforme* (Williams, 1987). These reports indicate that the preemption of light is heavily influenced by morphological aboveground traits related to plant size.

Inorganic nutrients are also a preemtable resource for seagrasses (Williams 1987). Seagrasses obtain inorganic nutrients both from the pore water in the sediments and the water column (Iizumi and Hattori 1982, Thursby and Harlin 1982, Short and McRoy 1984, Williams and Ruckelshaus 1993, Viana et al. 2019b) and can often be nutrient-limited in tropical areas (Orth

1977, Bulthuis and Woelkerling 1981, Short et al. 1985, Powell et al. 1989, Duarte et al. 1995, Agawin et al. 1996). Therefore, the characteristics of aboveground and rhizomatic structures can influence seagrass competition for nutrients in the water column and in the sediments respectively. In competition between *Ruppia maritima* and *Halodule wrightii*, for example, involvement of belowground nutrients has been shown (Pulich 1985). Additionally, other studies suggest that nutrient competition among seagrasses may be happening and is affected by the characteristics of their rhizomes and roots (Fourqurean et al. 1995, Duarte et al. 2000, Bando 2006).

Interspecific seagrass competition for light and inorganic nutrients appears to be asymmetric, whereby a species affects another but with no reciprocity in the effect (Connell 1983, Schoener 1983, Schwinning and Weiner 1998, Davis and Fourqurean 2001). Duarte et al. (2000) suggests that differences in plant size among species may be partly responsible for this phenomenon (see also Vermaat et al. 1995, Terrados et al. 1999). More recent studies show that competition for light and nutrients happen simultaneously, limiting the possibility to separate their effects in the field (Fourqurean et al. 1995, Duarte et al. 1997, 2000; Nakaoka and Iizumi 2000, Davis and Fourqurean 2001, Taplin et al. 2005). Additionally, species may exploit resources differently. Seagrasses show considerable vertical stratification within the sediment with a tendency of larger species to extend deeper into the sediments than smaller ones (Duarte et al. 1998). Therefore, although these plants co-occur in aboveground space, they do not share the same belowground space (Williams 1990, Duarte et al. 2000, Ooi et al. 2011), suggesting the possibility of belowground niche differentiation among seagrass species (Meilhac et al. 2020). Competition for resources should therefore be fundamentally determined by the traits of coexisting plants (Hofman and Ennik 1980, Firbank and Watkinson 1987, Schwinning and Weiner 1998), yet this remains to be tested in seagrass communities.

Mixed seagrass meadows are a common feature in tropical seascapes, challenging the general hypothesis that final successional stages could be monopolized by a single species out-competing the rest (Young and Kirkman 1975). Unlike other terrestrial and marine assemblages, colonizing and middle-successional seagrass species are not confined to patch mosaics within mature seagrass assemblages, but occur as individuals scattered throughout, posing the question as to how a multispecies meadow can be maintained (Williams 1990). The environmental conditions in which seagrass meadows develop can, however, favor specific species when it comes to interspecific competition. In the field, *Z. japonica* dominates the intertidal area, much more prone to disturbances, whereas *Z. marina* is more abundant in the

subtidal zone (Harrison 1979). Nutrient enrichment changes the dominance of seagrass communities from *T. testudinum* to *H. wrightii* (Fourqurean et al. 1995). Seaweed farming can, through trampling, favor the dominance of seagrass over benthic macroalgae (Moreira-Saporiti et al. 2021). Seagrass plants respond to varying environmental conditions and levels of stress through their traits (Roca et al. 2016). This is the case for tropical seagrass species in controlled experiments, in which temperature and nutrient enrichment affected and changed their morphological, biochemical and photo-physiological traits (Mvungi and Pillay 2019, Artika et al. 2020), with a majority of these responses being species-specific (Viana et al. 2020). It is therefore expected that a change in the traits of seagrass species under different levels of anthropogenic pressure (e.g. eutrophication) may affect the outcome of interspecific competition.

Traits are defined as “any morphological, physiological or phenological heritable feature measurable at the individual level, from the cell to the whole organism, without reference to the environment or any other level of organization” (Violle et al. 2007 as modified by Garnier et al. 2016). The study of traits, therefore, allows us to understand the relationships between organisms from a functional perspective. Trait-based ecology in fact assumes that structures at higher organizational scales are largely a result of the composite traits of the individuals (Grime 1998, Shipley et al. 2016). One of the main tools used in functional trait-based studies is the construction of a multidimensional space where axes are ecologically relevant traits or linear combinations of a set of traits of a species or a community of species (Mouillot et al. 2013). The coordinates of the species in the multidimensional space are, therefore, determined by its traits. Although trait-based approaches can be applied to all kinds of organisms, these are currently most developed for terrestrial plants (Lavorel and Garnier 2002, Garnier et al. 2016), but are also used for marine organisms (Litchman and Klausmeier 2008, Andersen and Pedersen 2009, Litchman et al. 2010, 2013; Elleouet et al. 2014). Trait-based approaches have been rarely, but successfully, applied in seagrass communities, with results suggesting that functional traits underpin community-level primary production (Jänes et al. 2017, Gustafsson and Norkko 2019) or mediate herbivory and predation in seagrass ecosystems (Pagès et al. 2012, Lewis and Boyer 2014).

This study addresses our incipient understanding of competition among tropical seagrass species (Ooi et al. 2011) and the role traits play in competitive outcomes. Specifically, we aim to quantify the extent to which seagrass traits (known to correlate with their ability to compete for light and nutrients) affect the probability of space preemption by seagrass species under

different trophic states. For this purpose, we i) quantified the abundance of the seagrass species off Unguja Island (Zanzibar Archipelago, Tanzania) in sites subject to varying trophic states and examined pairwise space preemption of seagrass at the local level, ii) ordered the species according to nine traits informative of their functional strategy during interspecific competition for inorganic nutrients and light, iii) examined the effect of the trophic states on seagrass traits, and iv) tested the relationship between the difference between the functional strategies of pairs of seagrass species and their probability of space preemption. We hypothesized that a species' functional strategy will have an effect in its preemptive ability, and this effect may change under different trophic states.

II. Materials and Methods

(1) Study area

Unguja Island is the most populated island in the tropical archipelago of Zanzibar (~900.000 inhabitants), off the coast of Tanzania in the Western Indian Ocean (Fig. 1), and one of the main hotspots of seagrass biodiversity in the world (Short et al. 2007). Seagrass communities are mainly formed by 8 species: *Cymodocea rotundata*, *Cymodocea serrulata*, *Enhalus acoroides*, *Halophila ovalis*, *Halodule uninervis*, *Syringodium isoetifolium*, *Thalassodendron ciliatum* and *Thalassia hemprichii*. We surveyed seven different sites (Fig. 1) expected to show different trophic states. Each of the seven study sites comprised a subtidal seagrass meadow of ~ 10000 m² generally bounded by a coastal rocky or sandy area and a fringing coral reef. The water depth ranged in all sites between 0.5 and 4 meters due to the tidal regime. All seven study sites were surveyed in November 2016.

(2) Characterization of the trophic states

We surveyed different environmental variables in order to determine the trophic states of the sampling sites (Burkholder et al. 2007). The indicators selected were: macroalgae biomass (g DW m⁻²), chlorophyll-*a* in the water column (μg l⁻¹), sediment δ¹⁵N (‰) and concentrations of dissolved inorganic nitrogen (DIN) and PO₄³⁻ in pore water (μM).

(a) Macroalgae biomass

Macroalgae biomass was quantified along five 50-m transects per site, set perpendicular to the coast and parallel to each other, separated by ~50 meters. We collected the macroalgae present in three random 0.25x0.25 meters quadrats per transect. The macroalgae samples were cleaned of sediments and rinsed with water. They were then dried at 50°C in a forced air oven until

constant dry weight (g DW). The macroalgae biomass was calculated as the g DW divided by the area of the quadrat (g DW m⁻²).

(b) Chlorophyll-*a* in the water column

In the proximities of each transect, we collected five ~3-liter seawater samples from each site and kept them in a cooler box until filtration. Seawater was immediately filtered upon arrival in the Institute of Marine Sciences (IMS, Stone Town, Zanzibar) under constant pressure onto pre-combusted (5 h, 450°C) and pre-weighed Whatman GF/F filters (0.45 µm pore size). The filters were stored at -20°C and transported frozen to the Leibniz Centre for Tropical Marine Research in Bremen (ZMT) (Germany). Chlorophyll-*a* was extracted from the filters in 8 ml of 96% ethanol in glass vials heated for 5 min at 80°C, covered with aluminum foil, and placed in a rotor at room temperature for approximately 24 h. Extracts were subsequently centrifuged at 5000 rpm for 20 min. Chlorophyll-*a* samples were determined in a photometer Shimadzu UV-1700, and calculated as µg l⁻¹.

(c) DIN and PO₄³⁻ concentrations in pore water

We took one sediment pore water sample per transect from each site using 30 cm PVC cores. The cores were pushed into the sediments, and after extraction an Eijkelkamp rhizon connected to a 20 ml syringe was placed in a hole corresponding to a depth of 5 cm below the sediment surface. Making vacuum with the syringe, the water was pulled out of the sediment cores. These samples were immediately filtered (0.45 µm pore size, Whatman GF/F filters) in pre-rinsed polyethylene bottles, frozen (-20°C) and transported to the ZMT. Analysis was performed using a continuous flow injection analyzing system (Skalar SAN++-System) following Grasshoff et al. (2009). The measuring procedure had a relative standard deviation < 3.5% with reference to the linear regression of an equidistant 10-point calibration line from NIST standards.

(d) δ¹⁵N in the sediment

We took one 50ml surface sediment sample per transect for δ¹⁵N analysis. The samples were stored at -20°C and transported frozen to the ZMT. They were then dried at 50°C in a forced air oven until constant DW, ground to a fine powder with mortar and pestle, and weighed into tin capsules prior to analysis for nitrogen stable isotope composition (δ¹⁵N) with a gas isotope ratio mass spectrometer (Thermo Finnigan Delta Plus). Results are expressed in δ notation (‰) where the standard for δ¹⁵N is atmospheric N₂.

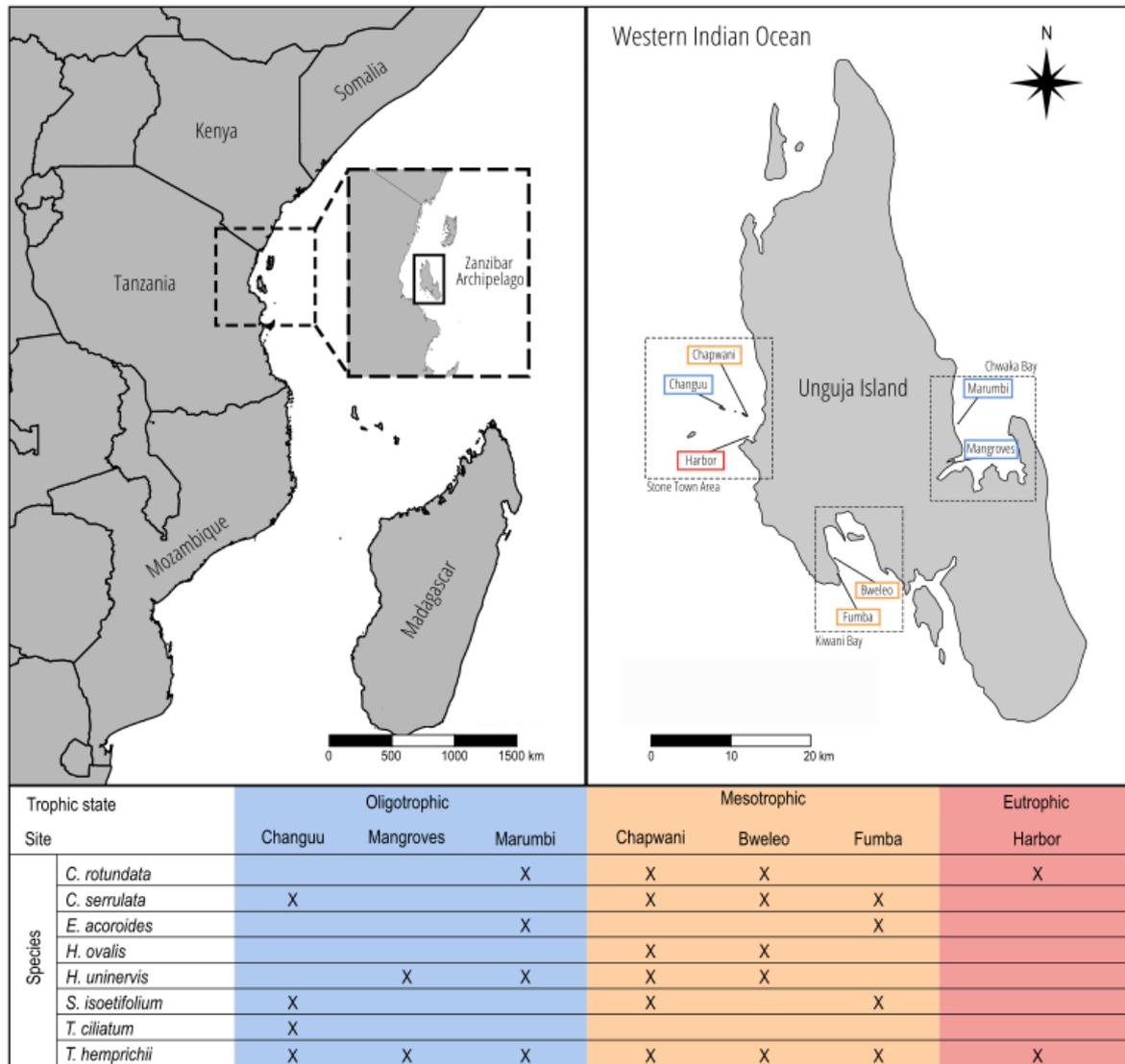


Figure 1. Map of the study area. On the top left, an overview of the Eastern African coast, where the Zanzibar Archipelago is located. On the top right, Unguja Island and the seven sites selected in three different areas around the island. The colors indicate the trophic state to which they were assigned. The bottom table indicates which seagrass species were present in which sites.

(3) Characterization of the seagrass species and their traits

(a) Measurement of seagrass cover and pairwise space preemption per species

Seagrass cover was quantified along five 50-m transects per site, set perpendicular to the coast and parallel to each other, separated by ~50 meters. Seagrass cover was quantified as the percent area occupied per species within seagrass plots of 0.5 x 0.5 m marked by PVC quadrats, randomly placed along each transect (n = 9 per transect, 45 per site). In order to assess the abundance of seagrass and the competitive outcome between species, we selected only the plots in which at least 2 seagrass species were present (Fig. 2). We discarded any plot in which

seagrass species were absent to eliminate the confounding effect of presence/absence of seagrass, which is also governed by factors other than competition (e.g. colonization success) (see Tilman 1985, Keddy 1992, Garnier et al. 2016).

We also discarded any plot in which only one species was present in order to measure abundance only in plots where competitive interactions were occurring (Fig. 2). The goal of this step is to ensure that the seagrass abundance in the resulting plots can only be attributed to the outcome of interspecific interactions. The resulting number of plots differed between species: *C. rotundata* (n=35), *C. serrulata* (n=58), *E. acoroides* (n=18), *H. ovalis* (n=24), *H. uninervis* (n=64), *S. isoetifolium* (n=45), *T. ciliatum* (n=19) and *T. hemprichii* (n=127).

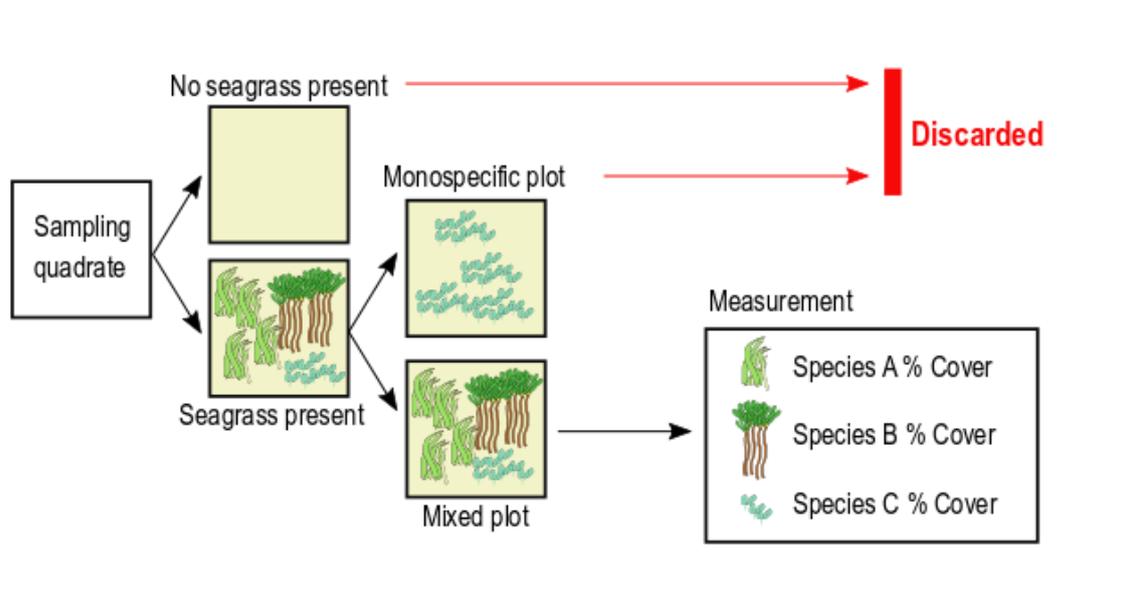


Figure 2. Schematic representation of the sampling design to measure seagrass percent cover.

Per plot and species, we calculated percent cover and pairwise space preemption. We calculated the pairwise space preemption following equation 1:

$$\begin{aligned}
 \text{Space preemption}_{\text{Species A}} &= \% \text{ Cover}_{\text{SpA}} - \% \text{ Cover}_{\text{SpB}} = \\
 &\begin{cases} "1" & \text{if } \% \text{ Cover}_{\text{SpA}} > \% \text{ Cover}_{\text{SpB}} \\ "0" & \text{if } \% \text{ Cover}_{\text{SpA}} \leq \% \text{ Cover}_{\text{SpB}} \end{cases} \quad (1)
 \end{aligned}$$

Pairwise space preemption was computed here as the difference between the percent cover of a given seagrass species (A) minus the percent cover of a second species (B) sharing the plot (Fig. 2). Pairwise space preemption was then converted into a binary variable taking a value of “1” where the percent cover of species A was higher than percent cover of species B (preemption by A), and “0” where percent cover of species A was equal or lower than percent

cover of species B (no preemption by A). This calculation was repeated with each species sharing the plot with species A. Lastly, the probability of preemption by species A was calculated as the number of successes (preemption) divided by the total number of plots in which species A was present (Equation 2)

$$\text{Probability of space preemption}_{\text{Species A}} = \frac{\text{Number of successes}_{\text{Species A}}}{\text{Total number of plots}_{\text{Species A}}} \quad (2)$$

(b) Sampling and measurement of traits linked to resource competition

Finding explicit links between traits and interspecific competition for resources proved a difficult task due to the lack of studies addressing this question. We considered nine traits reportedly correlated to light and inorganic nutrients preemption (Fig. 3, Table 1), namely, leaf maximum length (Leaf ML, cm), leaf maximum width (Leaf MW, cm), vertical rhizome length (VR length, cm), leaves per shoot (Leaves/Sh, leaves shoot⁻¹), rhizome diameter (RhD, cm), roots per meter (Roots/M, roots meter⁻¹), root maximum length (Root ML, cm), leaf mass area (LMA, g cm⁻²), and shoots per meter (Shoots/M, shoots m⁻¹). For further clarification, traits were classified into two groups: canopy forming and belowground structure traits.

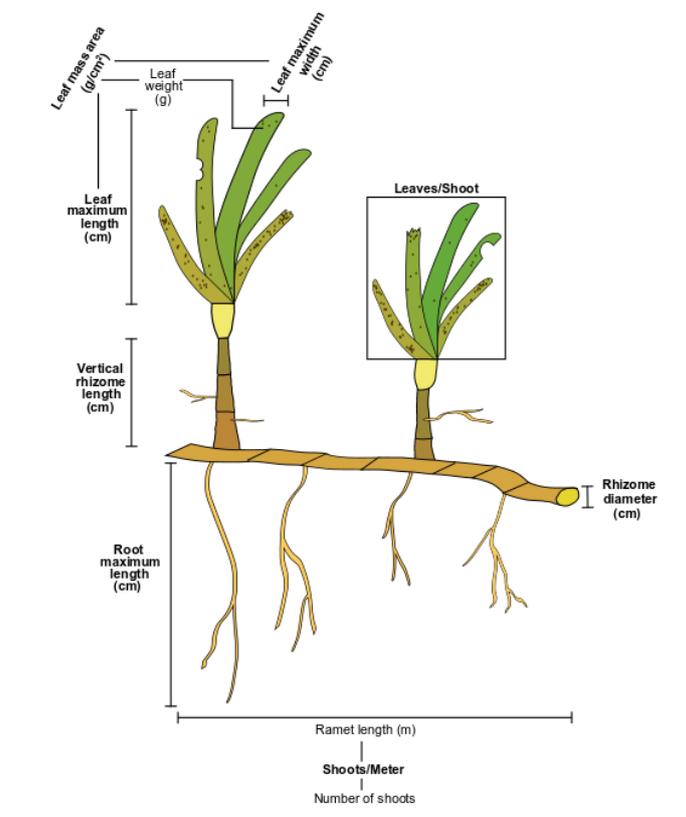


Figure 3. Generic representation of a seagrass ramet and the traits measured. Traits included in the analysis are highlighted in bold. Traits that are not in bold were necessary in order to calculate the functional traits included in the analysis.

The references cited in Table 1 indicate competition for resources, and they propose traits that could be responsible for the competition for said resource. We did not find references addressing competition for inorganic nutrients in the water column, despite the capacity of seagrass to uptake nutrients by their leaves (Viana et al. 2019b). For this reason, we state that competition is suspected but not stated in the literature.

Table 1. Measured traits in relation to the preemtable resource they are correlated to. All traits are continuous. References show seagrass studies suggesting a preemption effect of traits over the resources.

Trait category	Traits	Acronym	Scale	Unit	Description	Resource and references	
						Light	Inorganic nutrients
Canopy forming traits	Leaf maximum length	Leaf ML	Leaf	cm	Distance between the end of the sheath and the tip of the longest leaf.		
	Leaf maximum width	Leaf MW	Leaf	cm	Width of the broadest part of the leaf of the broadest leaf in a shoot.		
	Leaves/Shoot	Leaves/Sh	Shoot	leaves shoot ⁻¹	Number of leaves in a shoot		
	Leaf mass area	LMA	Leaf	g cm ⁻²	Grams of leaf tissue per unit of surface. See “Materials and methods” for further details.	1, 2, 3, 4, 5, 6, 7, 8, 9	Suspected, not stated in the literature
	Vertical rhizome length	VR Length	Shoot	cm	Distance between the base of the shoot and the base of the sheath.		
	Shoots/Meter	Shoots/M	Ramet	shoots m ⁻¹	Number of shoots of the ramet divided by the length of the ramet.		
Belowground structural traits	Rhizome diameter	RhD	Ramet	cm	Diameter of the cross section of the rhizome.		
	Roots/Meter	Roots/M	Ramet	roots m ⁻¹	Number of roots of the ramet divided by the length of the ramet.	-	8, 9, 10, 11
	Root maximum length	Root ML	Ramet	cm	Vertical length of the longest root in the ramet.		

References: ¹ Aleem (1955), ² Orth (1977), ³ Zieman et al. (1984), ⁴ Williams (1987), ⁵ Williams (1990), ⁶ Nomme and Harrison (1991b), ⁷ Fourqurean et al. (1995), ⁸ Vermaat et al. (1995), ⁹ Duarte et al. (1998), ¹⁰ Duarte et al. (2000), ¹¹ Ooi et al. (2011).

To quantify the seagrass traits at each site, we defined four zones of ~2500 m² per site, within which we sampled five ramets (defined as a train of at least two shoots) per species, amounting

to a total of approximately 20 ramets per species and site. The ramets were transported frozen at -4°C to the ZMT for trait measuring. We measured leaf ML, leaf MW, VR length, RhD and root ML with a ruler to the nearest millimeter and leaves/sh, shoots/M and roots/M were visually counted (as described in Table 1). For the measurement of the LMA (g cm^{-2}), we took a subsample of the second leaf of one shoot per ramet. The subsample was cleaned of epiphytes and rinsed with distilled water and cut squared for easier measurement of its surface with a ruler (cm^2). It was then dried at 50°C in a forced air oven until constant DW. Finally, the LMA was calculated as the DW of the leaf subsample divided by its area (g DW cm^{-2}).

The traits measured at the leaf and shoot levels (i.e. leaf ML, leaf MW, LMA, Leaves/Sh, VR length) were averaged per ramet. This made a total of 573 ramets, distributed across eight seagrass species. The number of ramets per species were: *C. rotundata* ($n = 61$), *C. serrulata* ($n = 74$), *E. acoroides* ($n = 44$), *H. ovalis* ($n = 83$), *H. uninervis* ($n = 81$), *S. isoetifolium* ($n = 50$), *T. ciliatum* ($n = 41$) and *T. hemprichii* ($n = 139$) (See Supplementary Material 1 for the mean values of the measured traits per species per site).

(4) Data analysis

We used R statistical software (R Core Team 2020) for the analysis of the data.

(a) Clustering of sites in trophic states

To identify different trophic states, we classified the seven sampling sites according to macroalgal biomass, chlorophyll-*a* concentration in the seawater, DIN and PO_4^{3-} concentrations in the pore water and $\delta^{15}\text{N}$ in the sediment. Ward hierarchical clustering based on a Euclidean distance matrix was used (Murtagh and Legendre 2014), and the linear model criterion (i.e. minimization the within-group sum of squares) applied to build the clusters. We validated the membership of each site to their cluster by the calculation of the silhouette width. This measurement is based on the average dissimilarity between one site and all the other sites of the clusters to which it belongs, compared to the same measure computed for the next closest cluster (see silhouette plot in Supplementary Material 2).

(b) Differences in seagrass cover within and among trophic states

In order to investigate whether the percent cover of the seagrass species varied among coastal areas subjected to different trophic states, we built two different models. First, we studied the seagrass community structure within the trophic states by assessing the differences in percent cover among species (Model 1). Secondly, we studied if the percent cover of each seagrass species varied across the trophic states (Model 2). For both models, we used a generalized linear

mixed-effects model with a beta distribution and logit link function, with seagrass percent cover as the dependent variable.

In Model 1 we used seagrass species as the categorical explanatory variable, and sampling site and transect as random effects. This was not the case for the eutrophic state, because it is composed of a single site, and therefore only transect was used as a random effect in the model. In Model 2 we used the trophic state as the categorical explanatory variable and sampling site and transect as random effects.

Significance of explanatory variables in both models was tested by analysis of variance (Type II test). The models were validated for homoscedasticity, normality and independence of the residuals. Model 1 showed problems with heteroscedasticity and residual dependence to transects. Model 2 showed some degree of heteroscedasticity and dependence to sampling transect for all species. In both cases we used a square root transformation to correct these validation problems. We used the package “glmmTMB” (Brooks et al. 2017) for this analysis.

(c) Differences in probability of space preemption within and among trophic states

In order to investigate whether the probability of space preemption of the seagrass species varied among seagrass meadows subjected to different trophic states, we built two different models. First, we studied the differences in probability of space preemption among seagrass species coexisting within the same trophic state (Model 3). Secondly, we studied if the probability of space preemption of each seagrass species varied across the trophic states (Model 4). For both models, we used a generalized mixed-effects model with a binomial distribution and a logit link function, with probability of space preemption as the dependent variable.

For Model 3, we used seagrass species as the categorical explanatory variable and sampling site and transect as random effects. This was not the case for the eutrophic state, because it is composed of a single site, and therefore only transect was used as a random effect in the model. For Model 4, we used the trophic state as the categorical explanatory variable, and sampling site and transect as random effects.

Significance of explanatory variables was tested with an analysis of variance (Type II test). The models were validated for homoscedasticity, normality and independence of the residuals. Unfortunately, when Model 4 was applied to the species *C. rotundata*, *H. uninervis*, *S. isoetifolium* and *T. hemprichii*, some level of heteroscedasticity and residual dependence to sampling site was shown and could not be resolved. The results of these models should,

therefore, be conservatively interpreted. We used the package “glmmTMB” (Brooks et al. 2017) for this analysis.

(d) Characterization of the functional strategy of seagrass species for interspecific competition and resource preemption

To characterize seagrass species in terms of their resource preemption capacity we ordered them based on their dissimilarity (Euclidean distance) in standardized traits (Table 1) using a Principal Components Analysis (PCA). Species trait values were obtained by averaging the corresponding ramet values per site (Supplementary Material 1), thus obtaining a single PCA where dots correspond to species and the number of dots per species represents the number of sites where the species occurred ($n = 27$). The scores of each dot in the principal components (PCs) of the PCA are, therefore, informative of the functional strategy of the seagrass species at the sampling sites. The first six PCs were retained for further analyses because these ensured a faithful representation of the initial functional dissimilarity among ramets within the ordination space (mean squared deviation = 0.000151) (Maire et al. 2015) (See Supplementary Material 3 for ordination diagnostics).

Additionally, we characterized the functional strategy of each seagrass species i) across the study area and ii) within each trophic state. To determine the functional strategy of a species across the study area, we calculated the centroid of the hypervolume enclosing all the species occurrences across sites in a six-dimensional space. Similarly, to identify the functional strategy of a species per trophic state, we calculated the centroid of the hypervolume enclosing all species occurrences in all sites within a trophic state in a six-dimensional space.

The correlations of each trait with the PCs, informative of the amount of the variability that is correlated to a given trait per PC, were then computed. We tested whether such correlations were significant calculating their t-statistic (Yamamoto et al. 2014). We performed the PCA with the R packages “FactoMineR” (Le et al. 2008) and “factoextra” (Kassambara and Mundt 2020).

(e) Effect of the trophic states on the functional strategy of seagrass species

To determine whether a given seagrass species adopted different functional strategies under different trophic states, we compared the centroids of the trophic states of each species in the PCA. We used the coordinates of species’ centroids in the oligotrophic state as a reference value. From this value, we subtracted the value of the coordinates of the centroids in the mesotrophic and eutrophic states. The difference in the species centroids in each PC indicates

the difference in the species' functional strategy when it occurs under mesotrophic and eutrophic conditions.

(f) Effect of the seagrass functional strategy on probability of space preemption

In order to test whether the functional strategy of a species has any effect on its probability of space preemption, we calculated the difference in the functional strategy of every species pair found together within plots (as seen in Fig. 2). For this purpose, we subtracted the species scores for every species pair. The scores of each dot in the PCs are informative of the functional strategy of the seagrass species at the sampling sites, and the difference indicates how similar or different their functional strategies are. We followed equation 3:

$$\Delta FS_{PC\ n} = Score_{Species\ B} - Score_{Species\ A} \quad (3)$$

$\Delta FS_{PC\ n}$ is the difference in the functional strategy of a pair of species in PC n (n adopting a number between 1 and 6). $Score_{Species\ B}$ and $Score_{Species\ A}$ are the scores of each species in the PC n. $\Delta FS_{PC\ n}$ was then matched per species with the space preemption result as calculated in equation 1.

To test whether the effect of the difference in the functional strategies of a seagrass pair ($\Delta FS_{PC\ n}$) on the probability of pairwise space preemption, we fitted a generalized linear mixed-effects model with a binomial distribution and a logit link function. Due to collinearity among several $\Delta FS_{PC\ n}$, we could not include them in the same model, and decided to create one model per PC conforming the functional strategy. We used $\Delta FS_{PC\ n}$, trophic state and their interaction as the explanatory variables in each model. Site was included as a random effect to avoid confounding effects from other species present in the meadow, and Species A and B to comply with the model validation assumptions.

In order to avoid spurious outcomes in the model we took three precautionary steps. First, we eliminated the eutrophic state from the dataset due to the presence of a unique species pair. Second, we did not use sampling transect as random effect, as the species scores were calculated at the scale of site and not at the scale of transect. Third, we did not include any species pair present in less than 5 plots per site (for the final number of pairwise interactions per species pair and site, see Supplementary Material 4). Final model selection and significance of explanatory variables were tested through model comparison (Likelihood ratio test). All models were validated for homoscedasticity, normality and independence of the residuals. We used the

packages “lme4” for the construction of the generalized linear mixed-effects model (Bates et al. 2015) and “lmerTest” for the model selection (Zeileis and Hothorn 2002).

(g) Prediction of probability of preemption by PC 1 of the functional strategy of seagrass

PC1 was identified as the driver for space preemption in the previous test and it was used as a predictor for the probability of space preemption of a species as calculated in equation 2.

We used a generalized linear mixed-effects model with a binomial distribution and a logit link function to predict the probability of space preemption by a seagrass species due to its score on PC1. Species' scores on PC1 were used as fixed effect in the model. Site and seagrass species were included as a random effect to avoid confounding effects from other species present in the meadow and to comply with the model validation assumptions respectively. Significance of explanatory variables was tested through model comparison (Likelihood ratio test). All models were validated for homoscedasticity, normality and independence of the residuals. We used the packages “lme4” for the construction of the generalized linear mixed model (Bates et al. 2015) and “lmerTest” for the model selection (Zeileis and Hothorn 2002).

We made the plots with the package “ggplot2” (Wickham 2016), with aesthetical changes done with the software InkScape (v. 2.0).

III. Results

(1) Trophic states and indicators

Sites clustered in two groups marking areas subject to oligotrophic and mesotrophic conditions, whereas a single site was markedly different from all others given its high eutrophication (Figs. 1 and 4a). $\delta^{15}\text{N}$ in the sediment was double in the eutrophic site than in the oligo- and mesotrophic areas (Fig. 4b), indicating discharge of human waste water in the eutrophic site. These differences were also reflected in the macroalgae biomass. While the oligotrophic sites had macroalgae biomass close to zero (Mean \pm SE: 1.87 ± 3.07 g DW m^{-2}), the mesotrophic (12.61 ± 4.35 g DW m^{-2}) and eutrophic (33.78 ± 6.15 g DW m^{-2}) sites had nearly 7 and 18 times the biomass of the oligotrophic site, respectively (Fig. 4c). Similarly, the concentration of chlorophyll-*a* in the water column (Oligotrophic: 0.53 ± 0.21 < Mesotrophic: 0.88 ± 0.29 < Eutrophic: 1.41 ± 0.41 $\mu\text{g l}^{-1}$) and DIN in pore water (Oligotrophic: 7.28 ± 2.68 < Mesotrophic: 8.96 ± 3.79 < Eutrophic: 14.82 ± 5.36 μM) steadily increased along the trophic states (Fig. 4d, e). Lastly, the concentration of PO_4^{3-} in the pore water reached its maximum in the mesotrophic

sites ($1.85 \pm 0.60 \mu\text{M}$), being slightly lower in the eutrophic site ($1.63 \pm 0.51 \mu\text{M}$) and approximately half in the oligotrophic sites ($0.86 \pm 0.60 \mu\text{M}$) (Fig. 4f).

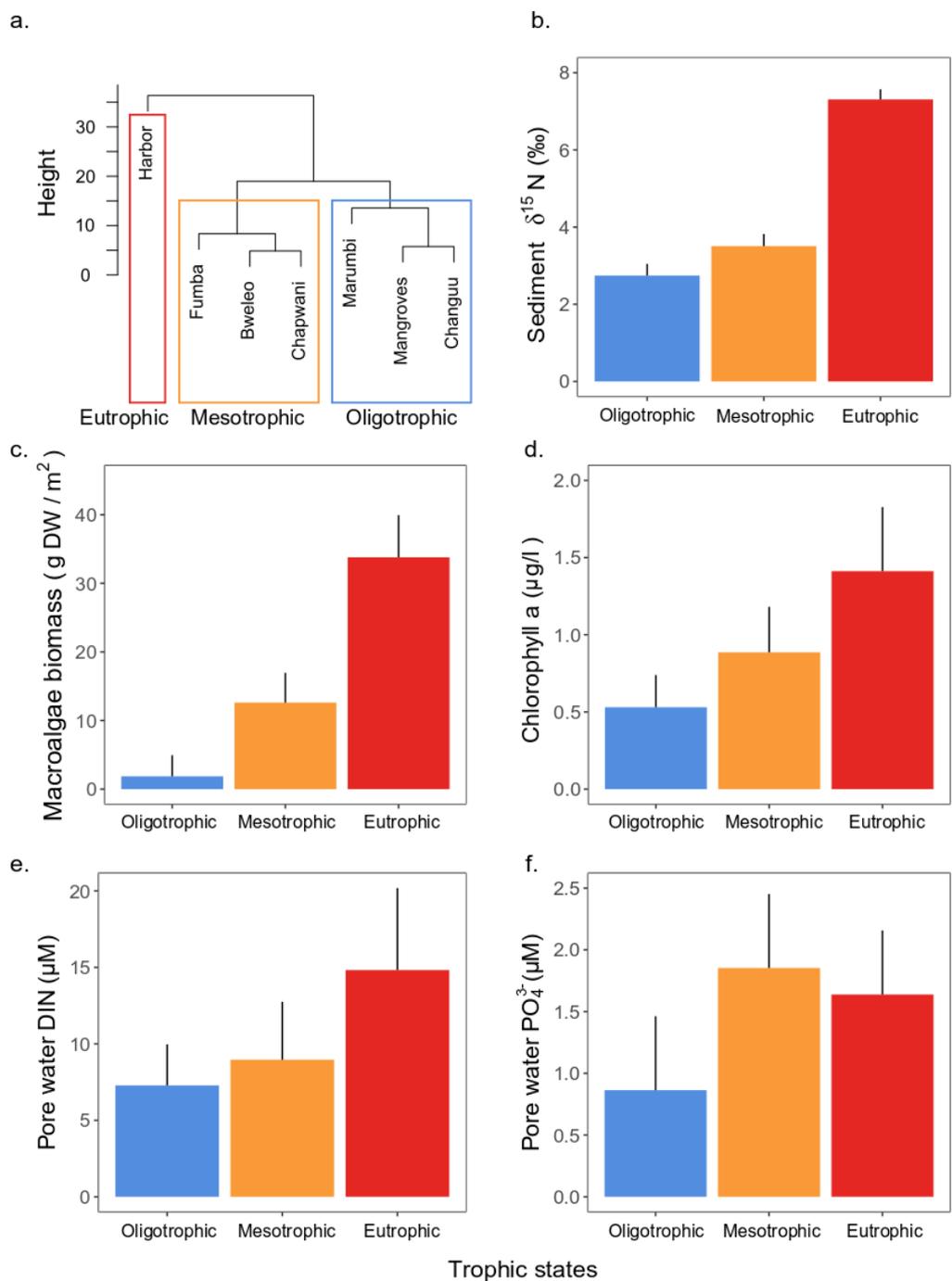


Figure 4. a) Ward clustering of sites according to trophic state. Mean (\pm SE) b) sediment $\delta^{15}\text{N}$, c) Macroalgae biomass, d) Chlorophyll-*a* in the water column, e) Pore water DIN, and f) Pore water PO_4^{3-} , taken as proxies for eutrophication indicators. Means were calculated among samples taken in the sites (three sites for the oligotrophic and mesotrophic states, one site for the eutrophic state). For macroalgae biomass, $n = 45$ for the oligo- and mesotrophic states, and $n = 15$ for the eutrophic state. For $\delta^{15}\text{N}$, chlorophyll-*a*, pore water DIN and pore water PO_4^{3-} , $n = 15$ for the oligo- and mesotrophic states, and $n = 5$ for the eutrophic state.

(2) Seagrass cover and probability of preemption across trophic states

When present, the opportunistic *C. serrulata* and the climax species *E. acoroides*, *T. ciliatum* and *T. hemprichii* were dominant in coverage (> 27%) regardless of the trophic state (Fig. 5a). The pioneer *C. rotundata*, *H. uninervis* and *H. ovalis* were the least abundant (8-30%) throughout the study area. *S. isoetifolium* was among the least abundant species under oligotrophic conditions (Mean \pm SE: $15.77 \pm 3.43\%$), yet equally abundant to climax species under a mesotrophic regime (Fig. 5a, Table 2). The opposite occurred for *H. uninervis*, which was more abundant in the oligotrophic ($22.50 \pm 5.65\%$) than the mesotrophic sites ($12.16 \pm 1.83\%$). The seagrass meadows in the eutrophic site were composed by only two seagrass species, *T. hemprichii* and *C. rotundata*. The latter was the only species showing significant differences in coverage among the three trophic states (Table 2), reaching at the eutrophic site twice the coverage it reached in areas of oligo- and mesotrophic conditions (Eutrophic: $30.00 \pm 12.14\%$ > Mesotrophic: $9.31 \pm 1.91\% \approx$ Oligotrophic: $11.75 \pm 4.02\%$), making its coverage comparable to that of *T. hemprichii*.

The probability of preemption of a given seagrass species (Table 3, Fig. 5b) across trophic states mirrored the spatial patterns of percent cover. Climax species, together with *C. serrulata*, tended to have the highest probability of preemption regardless of the trophic state. *S. isoetifolium* was the only pioneer species to be more likely to preempt space under mesotrophic conditions (0.47 ± 0.08) in comparison to oligotrophic conditions (0.20 ± 0.05).

(3) Functional strategy of seagrass species as defined by their traits

The functional traits included in the ordination reflected the differential strategies that seagrass take regarding interspecific competition across sites as reflected by the species centroids across the study area. PC1 explained 43.46% of the unconstrained variability in the PCA and it was determined primarily by their RhD (0.88) and Leaf MW (0.85) (Fig. 6a-d, Tables 4 and 5). Along PC1 the distribution of seagrass species centroids marked a size gradient, from the smallest ephemeral species to the two climax, big species: *H. ovalis*, *S. isoetifolium*, *H. uninervis*, *C. rotundata*, *C. serrulata*, *T. hemprichii*, *T. ciliatum* and *E. acoroides*. This PC also reflects the correlation among traits indicative of size of plant structures (aforementioned traits, together with Leaf ML, leaves/Sh, LMA, Root ML), indicating that they all increase and decrease in size collectively, both for canopy forming and belowground traits. Interestingly, the trait defining the density of shoots (Shoots/M) was inversely correlated to PC1. This suggests a trade-off between size of the seagrass plant and shoot density of seagrass.

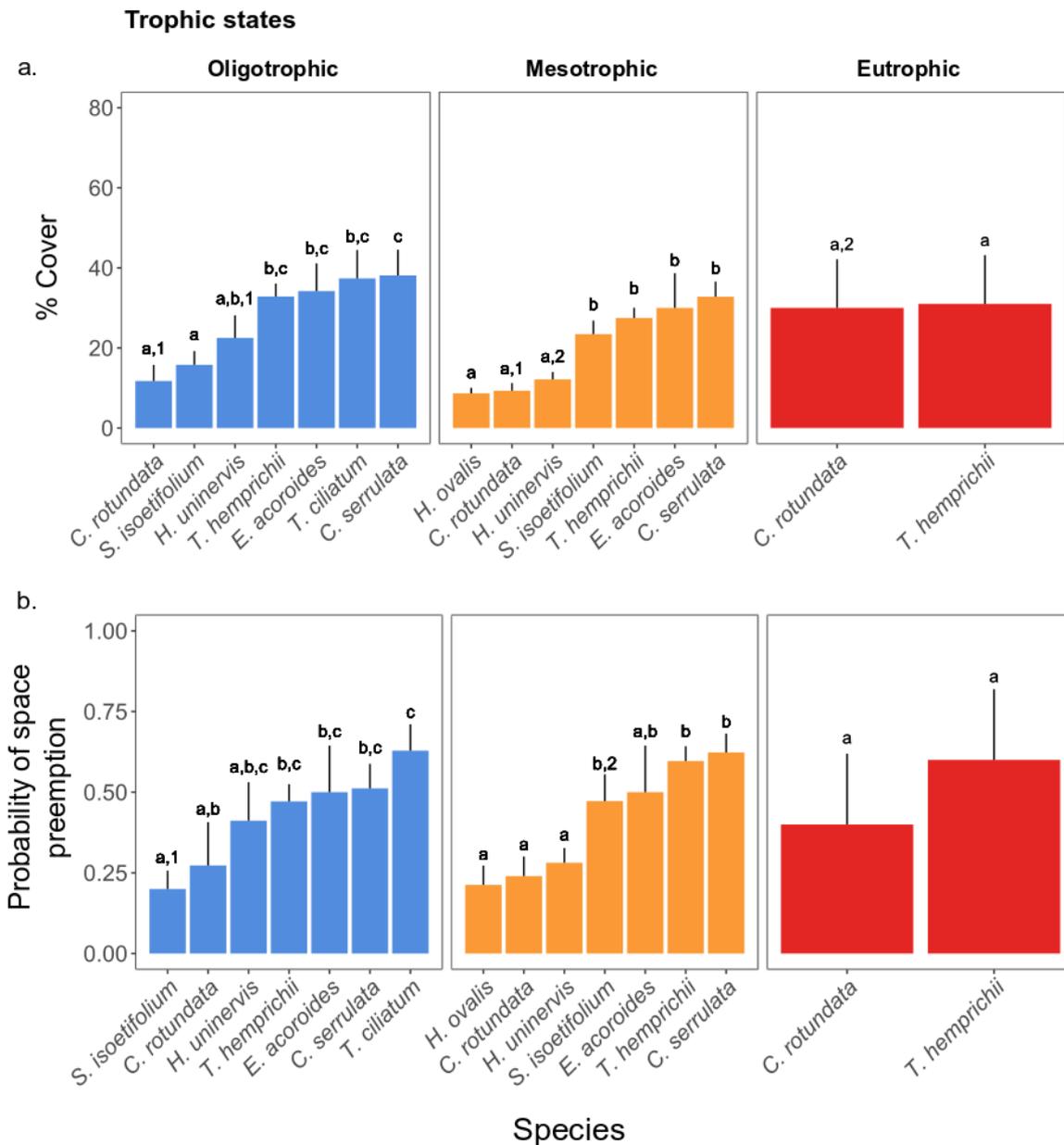


Figure 5. a) Seagrass % cover of each species in the trophic states. b) Probability of preemption of the seagrass species in the trophic states. The letters indicate significant differences within the states and among species, whereas the numbers indicate significant differences within a species among states. For the statistical output, see Tables 2 and 3. For the statistical output of pairwise differences, see Supplementary Material 5.

In PC2 (20.22%), all the seagrass species except *T. ciliatum* and *E. acoroides* were somewhat grouped, showing that this dimension separated the two climax species (Tables 4 and 5, Fig. 6a-d). Despite both the species showing a similar centroid coordinates in PC1, this was achieved

with different traits according to PC2. *T. ciliatum* showed higher VR length and leaves/Sh, whereas *E. acoroides* showed higher Leaf ML, RhD and density of roots (Roots/M) and shoots (Shoots/M). In PC3 (12.42%) (Tables 4 and 5), species' scores mainly reflected variability in Roots/M. In this dimension, *H. ovalis* showed an inversed correlation to this and other traits (VR length, Shoots/M, Root ML), indicating that it had a different functional strategy than the rest of the pioneer seagrass species, with not only small plant size, but also with sparse root and shoot density. For the report on unconstrained variability explained by all PCs, trait correlations and seagrass species centroids of PCs 4, 5 and 6, please see Supplementary Material 6.

(4) Effect of the trophic state on the functional strategy of seagrass species

The trophic state had different effects for each seagrass species and dimension (PC) of their functional strategy. This was reflected by the difference in the species centroid in the oligotrophic, mesotrophic and eutrophic states (Figs. 6c and 7). Between the oligotrophic and mesotrophic states, we saw both general and species-specific responses in the functional strategy of seagrass.

Species' centroids on PC1 were lower under mesotrophic than under oligotrophic conditions (Fig. 7). This indicated a general decrease in seagrass plants size (leaf ML and MW, RhD, root ML, Leaves/Sh) and an increase in Shoots/M, suggesting that a functional strategy more typical of opportunistic/pioneer species was selected for mesotrophic conditions. The only exception was *S. isoetifolium*, whose centroid was higher in mesotrophic sites. The same trend was true in PC1 in the differences in the centroids of *C. rotundata* and *T. hemprichii* between the oligotrophic and eutrophic states. In PC2 (Fig. 7), all species except *T. hemprichii* showed very low or positive differences in their centroids between oligotrophic and mesotrophic conditions. VR length was inversely correlated to this PC (Table 5, Fig. 6c), suggesting that an increase in vertical rhizome length was not a selected functional strategy under the mesotrophic state.

Due to the low amount of unconstrained variability explained by the rest of the PCs (PC3=12.42%, PC4=11.29%, PC5=5.62%, PC6=4.62%) the centroid migrations among trophic states were not reported in depth (See Supplementary Material 6 for the results).

Table 2. Complete output of models 1 and 2 assessing a) Model 1: Differences in % cover of the seagrass species within trophic states and b) Model 2: Differences in % cover of each seagrass species across trophic states. Significance is highlighted in boldface ($p < 0.05$). Std deviation = Standard deviation.

Response variable:	Explanatory variables	χ^2	df	p-value
% Seagrass cover	Random effects		Variance	Std deviation
a) Model 1: Differences in % cover of the seagrass species within trophic states				
	Species	24.085	6	0.0005
Oligotrophic	Random effects	Site	$7.06 \cdot 10^{-13}$	$8.40 \cdot 10^{-7}$
		Transect	$3.34 \cdot 10^{-11}$	$5.78 \cdot 10^{-6}$
	Species	62.871	6	1.173 · 10⁻¹¹
Mesotrophic	Random effects	Site	0.0015	0.1244
		Transect	$2.51 \cdot 10^{-9}$	$5.02 \cdot 10^{-5}$
Eutrophic	Species	0.0014	1	0.9699
	Random effects	Transect	$4.59 \cdot 10^{-11}$	$6.78 \cdot 10^{-6}$
b) Model 2: Differences in % cover of each seagrass species across trophic states				
	Trophic state	9.511	2	0.0086
<i>C. rotundata</i>	Random effects	Site	$8.60 \cdot 10^{-13}$	$9.27 \cdot 10^{-7}$
		Transect	$1.73 \cdot 10^{-10}$	$1.31 \cdot 10^{-5}$
<i>C. serrulata</i>	Trophic state	0.014	1	0.9044
	Random effects	Site	$5.23 \cdot 10^{-10}$	$2.28 \cdot 10^{-5}$
		Transect	0.2879	0.5366
<i>E. acoroides</i>	Trophic state	0.1749	1	0.6758
	Random effects	Site	$1.26 \cdot 10^{-14}$	$1.12 \cdot 10^{-7}$
		Transect	$2.24 \cdot 10^{-10}$	$1.49 \cdot 10^{-5}$
<i>H. uninervis</i>	Trophic state	3.928	1	0.0474
	Random effects	Site	$1.91 \cdot 10^{-10}$	$1.38 \cdot 10^{-5}$
		Transect	0.1030	0.3209
<i>S. isoetifolium</i>	Trophic state	0.377	1	0.5389
	Random effects	Site	$3.33 \cdot 10^{-10}$	$1.82 \cdot 10^{-5}$
		Transect	0.3182	0.5641
<i>T. hemprichii</i>	Trophic state	0.562	2	0.755
	Random effects	Site	0.1066	0.3265
		Transect	0.0134	0.1158

(5) Difference in the seagrass functional strategy and the probability of space preemption

The probability of space preemption was only significantly explained by ΔFS_{PC1} (Table 6, Fig. 8a). The effect of this dimension of the functional strategy on the probability of space preemption did not differ between trophic states, indicating that regardless of the trophic state, the same traits were responsible for preemption. PC1 correlated positively with traits related to plant size and negatively with Shoots/M (Table 5). The probability of species A preempting

space from species B was highest when the score of species A on PC1 was higher than the score of species B on PC1 (i.e. $\Delta FS_{PC1} < 0$). Conversely, the probability of species 2 preempting space from species 1 was highest when species 2 scored higher on PC1 than species 1 ($\Delta FS_{PC1} > 0$). When species A and B scored similarly on PC1 ($\Delta FS_{PC1} \approx 0$), both species had equal probability (50%) of preempting space from the other (Fig. 8a).

Table 3. Complete output of models assessing a) Model 3: Differences in probability of space preemption of the seagrass species within trophic states and b) Model 4: Tested if the probability of space preemption of each seagrass species varied along the trophic states. Significance is highlighted in boldface ($p < 0.05$). Std deviation = Standard deviation.

Response variable:	Explanatory variables	χ^2	df	p-value
Probability of preemption	Random effects		Variance	Std deviation
a) Model 3: Differences in probability of space preemption of the seagrass species within trophic states				
	Species	17.916	6	0.0064
Oligotrophic	Random effects	Site	$7.87 \cdot 10^{-13}$	$8.87 \cdot 10^{-7}$
		Transect	$5.17 \cdot 10^{-10}$	$2.27 \cdot 10^{-5}$
	Species	46.224	6	$2.67 \cdot 10^{-8}$
Mesotrophic	Random effects	Site	$9.93 \cdot 10^{-11}$	$9.96 \cdot 10^{-6}$
		Transect	$4.54 \cdot 10^{-10}$	$2.13 \cdot 10^{-5}$
Eutrophic	Species	0.3946	1	0.5299
	Random effects	Transect	$2.37 \cdot 10^{-10}$	$1.54 \cdot 10^{-5}$
b) Model 4: Differences in the probability of space preemption of each seagrass species across the trophic states				
<i>C. rotundata</i>	Trophic state	0.605	2	0.7389
	Random effect	Site	$2.64 \cdot 10^{-9}$	$5.14 \cdot 10^{-5}$
<i>C. serrulata</i>	Trophic state	1.346	1	0.2458
	Random effect	Site	$1.89 \cdot 10^{-10}$	$1.37 \cdot 10^{-5}$
<i>E. acoroides</i>	Trophic state	0.000	1	1.0000
	Random effect	Site	$2.72 \cdot 10^{-10}$	$1.65 \cdot 10^{-5}$
<i>H. uninervis</i>	Trophic state	1.149	1	0.2838
	Random effect	Site	$2.038 \cdot 10^{-10}$	$1.42 \cdot 10^{-5}$
<i>S. isoetifolium</i>	Trophic state	6.875	1	0.0087
	Random effect	Site	$6.64 \cdot 10^{-10}$	$2.57 \cdot 10^{-5}$
<i>T. hemprichii</i>	Trophic state	0.393	2	0.8214
	Random effect	Site	0.4266	0.6531

Differences in species scores on PC2, PC3, PC4, and PC6 played a negligible role in determining the probability of space preemption. Interestingly, the role of ΔFS on PC5 in driving space preemption was contingent on the trophic state (Table 5). ΔFS_{PC5} was inversely related to the probability of pairwise space preemption under oligotrophic, yet positively related to the probability of preemption under mesotrophic conditions (Fig. 8b). However, the lack of

significance of PC5 as a fixed effect ($\chi^2 = 0.005$, $df = 1$, $p = 0.939$) and the high confidence intervals (approximately double of the fitted values) makes difficult to make inferences from this result.

(6) Probability of space preemption of seagrass species as determined by PC1

As previously explained, ΔFS_{PC1} was identified as the driver behind space preemption in seagrass species pairs and we used it to predict the probability of preemption of each seagrass species as a result of their scores in this dimension (Fig. 9). The probability of space preemption by a given seagrass species significantly increases with its score on PC1 ($\chi^2 = 7.796$, $df = 1$, $p = 0.005$). In other words, the greater the size of a seagrass species (as defined by leaf MW, ML, RhD, Root ML, LMA), the higher the likelihood it would preempt space from other species.

IV. Discussion

Tropical seagrass meadows offer unique opportunities for the study of species interactions due to the high diversity of species sharing the same habitat (Short et al. 2007). This is the case of Unguja Island, located in the Western Indian Ocean, one of the hotspots of seagrass biodiversity, in which we found meadows occupied by an array of different seagrasses with a variety of life-history strategies. Competition for space is an ecological process common for all sessile organisms, and has been widely reported both in terrestrial plants (meta-analysis on the topic by Kinlock 2019) and seagrass (Aleem 1955, Phillips 1960, den Hartog 1970, Birch and Birch 1984, Williams 1990, Duarte et al. 2000, Willette and Ambrose 2012). However, a description of the potential mechanisms behind the preemption for space has been generally suggested and not tested in seagrass (Orth 1977, Zieman et al. 1984, Williams 1987, Nomme and Harrison 1991b, Fourqurean et al. 1995, Vermaat et al. 1995, Duarte et al. 1998, Ooi et al. 2011). This study related, for the first time, seagrass morphological traits with their capacity to exert space preemption on other seagrass species. Specifically, we identified a positive correlation between bigger structures (leaf ML, leaf MW, RhD, root ML, among others) and probability of space preemption.

(1) A trait-based approach to differentiate the functional strategy of seagrass species

Seagrass species have been classified according to different criteria, generally in a categorical fashion differentiating pioneer, opportunistic and climax species. These classifications prove

useful when discussing succession in the development of a meadow (Williams 1990) and in the differentiation between transitory and persistent meadows (Kilminster et al. 2015). However, they generally provide little insight in other ecological processes and functions.

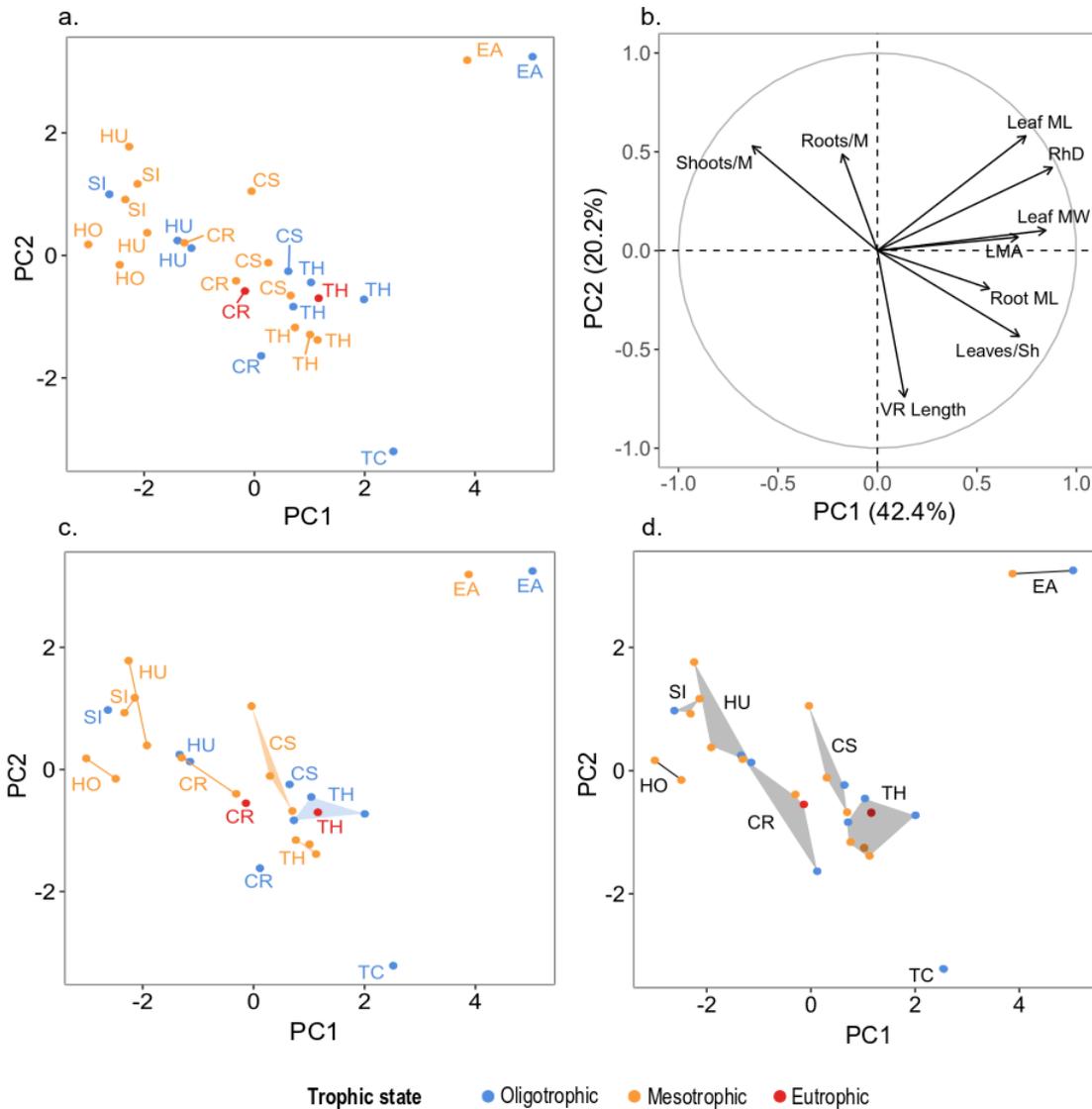


Figure 6. Principal components analysis, a) dots correspond to seagrass species per site plotted on PC1 and PC2 (which collectively explained 62.6% of the unconstrained variability), b) traits as vectors and their correlation with PC1 and PC2 (Table 5 for trait nomenclature and PCA output), c) species' hypervolumes per trophic state projected on PC1 and PC2, d) species' hypervolumes across all study sites projected on PC1 and PC2. For the graphical representation of PCs 3-6 see Supplementary Material 6: Section 4. Colors represent trophic states and acronyms in a), c), and d) represent species' names abbreviated as follows: CR = *C. rotundata*, CS = *C. serrulata*, EA = *E. acoroides*, HO = *H. ovalis*, HU = *H. uninervis*, SI = *S. isoetifolium*, TC = *T. ciliatum*, TH = *T. hemprichii*.

It is for this reason that trait-based approaches (TBAs) are considered fundamental tools that may be used to better understand how traits of organisms affect their response to environmental

drivers (Viana et al. 2020) and in turn how these traits affect ecosystem functions and processes (Gustafsson and Norkko 2016, Jänes et al. 2017). With TBAs the existing body of knowledge can be directly used to select biologically relevant functional traits linked to the research question at hand (Violle et al. 2007, Mouillot et al. 2013). In the present study, we were able to order seagrass species in a continuous fashion using traits related to resource preemption. What this allows is the creation of a scores per species in the multidimensional space that has a quantifiable biological meaning, an approach that sets it apart from traditional categorical classifications. In PC1, species centroids were ordered in a density to size gradient. Smaller species, like *H. ovalis*, *S. isoetifolium*, *H. uninervis* and *C. rotundata* had negative scores, while *T. hemprichii*, *T. ciliatum* and *E. acoroides* had positive scores. Interestingly, *C. serrulata* had a positive centroid near zero, showing a mixed strategy between the two groups. This showed a trade-off from density to size that had important implications when it came to space preemption as discussed in the next sections.

TBAs, therefore, offer the opportunity to directly link seagrass functional traits to the ecological process of interest for the study. It is, however, of fundamental importance to use traits that have been reported to affect the process at hand to obtain a multidimensional space of biological meaning.

Table 4. Centroids of the species hypervolumes in the six dimensions of the multidimensional space created by the ordination of the functional traits (Fig. 6).

Species	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
<i>C. rotundata</i> (CR)	-0.40	-0.60	-0.04	-0.40	-0.86	0.43
<i>C. serrulata</i> (CS)	0.40	0.00	0.79	-0.98	-0.14	-0.90
<i>E. acoroides</i> (EA)	4.45	3.22	-0.32	-0.54	0.25	0.45
<i>H. ovalis</i> (HO)	-2.75	0.01	-1.34	-1.27	1.59	0.21
<i>H. uninervis</i> (HU)	-1.67	0.64	0.71	1.17	-0.04	0.05
<i>S. isoetifolium</i> (SI)	-2.36	1.03	0.16	-0.40	-0.45	0.29
<i>T. ciliatum</i> (TC)	2.52	-3.21	2.74	-0.97	1.07	0.13
<i>T. hemprichii</i> (TH)	1.12	-0.93	-0.82	0.95	0.11	-0.09

(2) Response of seagrass functional strategy to different trophic states

Seagrass meadows in Unguja Island were subjected to varying trophic states ranging from oligotrophic to eutrophic, albeit only in one site. While seagrass meadows were composed of seven species in the oligotrophic and mesotrophic sites, only two species were found in the eutrophic site, *C. rotundata* and *T. hemprichii*. The final species arrangement for each site was controlled not only by species competition but also by two other filters, namely species

dispersion and the abiotic filter (Tilman 1985, Keddy 1992, Garnier et al. 2016). Although we did not study the effect of these two filters on the presence/absence of seagrass in different sites, it was possible to detect changes in the functional traits among the trophic states.

Seagrass traits are good indicators of change and stress (Roca et al. 2016), and can give insights on the effect of the environmental conditions on the seagrass species. The traits selected for this study were not specifically chosen as response traits, but rather due to their functional relationship with resource preemption. However, the traits selected were morphological, which have been reported to change under varying environmental conditions (Mvungi and Pillay 2019, Artika et al. 2020, Viana et al. 2020). This was also the case in this study, in which seagrass species revealed differences in their centroids in PC1 under different trophic states. All species with the exception of *S. isoetifolium* showed a decrease in the value of the coordinates of their centroids in PC1, which translates in a decrease in size and an increase in shoot density.

Table 5. Correlations of the functional traits to the dimensions of the multidimensional space (Fig. 6). Numbers in boldface and underlined indicate a trait with a significant correlation to the dimension ($p < 0.05$). For statistical output of the trait correlation, see Supplementary Material 6: Section 2.

	PC 1 43.36 %	PC 2 20.22 %	PC 3 12.42 %	PC 4 11.29 %	PC 5 5.62 %	PC 6 4.62 %
Canopy forming traits						
Leaf maximum length (Leaf ML)	<u>0.75</u>	<u>0.58</u>	-0.02	-0.10	-0.04	0.29
Leaf maximum width (Leaf MW)	<u>0.85</u>	0.10	-0.01	-0.28	0.36	-0.13
Leaves/Shoot (Leaves/Sh)	<u>0.71</u>	<u>-0.43</u>	0.19	0.28	0.27	-0.25
Leaf mass area (LMA)	<u>0.71</u>	0.07	-0.14	<u>0.61</u>	-0.25	0.09
Vertical rhizome length (VR length)	0.14	<u>-0.74</u>	<u>0.49</u>	-0.02	0.10	<u>0.43</u>
Shoots/Meter (Shoots/M)	<u>-0.63</u>	<u>0.53</u>	<u>0.41</u>	-0.20	0.13	0.05
Belowground structure traits						
Rhizome diameter (RhD)	<u>0.88</u>	<u>0.42</u>	0.03	-0.14	0.06	0.09
Roots/Meter (Roots/M)	-0.18	<u>0.49</u>	<u>0.67</u>	<u>0.48</u>	0.03	-0.10
Root maximum length (Root ML)	<u>0.60</u>	-0.19	<u>0.46</u>	<u>0.42</u>	<u>-0.46</u>	-0.21

Generally, in nutrient limited systems, a higher concentration of inorganic nutrients (both nitrogen and phosphorus) in the environment correlates with an increase in seagrass growth. *T. hemprichii* and *C. rotundata* have increased leaf growth and biomass under nutrient enrichment (Agawin et al. 1996, Terrados et al. 1999). Similarly, *H. uninervis* and *S. isoetifolium* have been found to increase their growth rate under nutrient enrichment, in addition to changes in biochemical traits (Udy et al. 1999). However, eutrophication encompasses other environmental conditions apart from an increase in nutrients (Burkholder et al. 2007). Indirect consequences of eutrophication include light attenuation due to the growth of epiphytes, macroalgae and phytoplankton, and anoxic sediments, with associated negative consequences for seagrasses. *T. hemprichii* has responded to shading by showing morphological stress

symptoms, like reduced shoot growth and lower belowground biomass (Browne et al. 2017), and the production of new, altered leaves with reduced length, width and thickness (Collier et al. 2012). This response is shared by *C. serrulata* and *H. uninervis* (Collier et al. 2012), despite other reports indicating an increase in vertical rhizome length for *C. serrulata* under shading (Lam et al. 2004). These results agree with the general trend of seagrass in the present study showing lower values for their centroids in PC1 and, consequently, a reduction in size under mesotrophic conditions. A similar trend is shared in the difference in the centroids of *T. hemprichii* and *C. rotundata* between oligotrophic and eutrophic conditions. The only species in this study increasing the value of its centroid under mesotrophic conditions, *S. isoetifolium*, has also been reported to increase its leaf growth and elongation under shading (Fokeera-Wahedally and Bhikajee 2005)

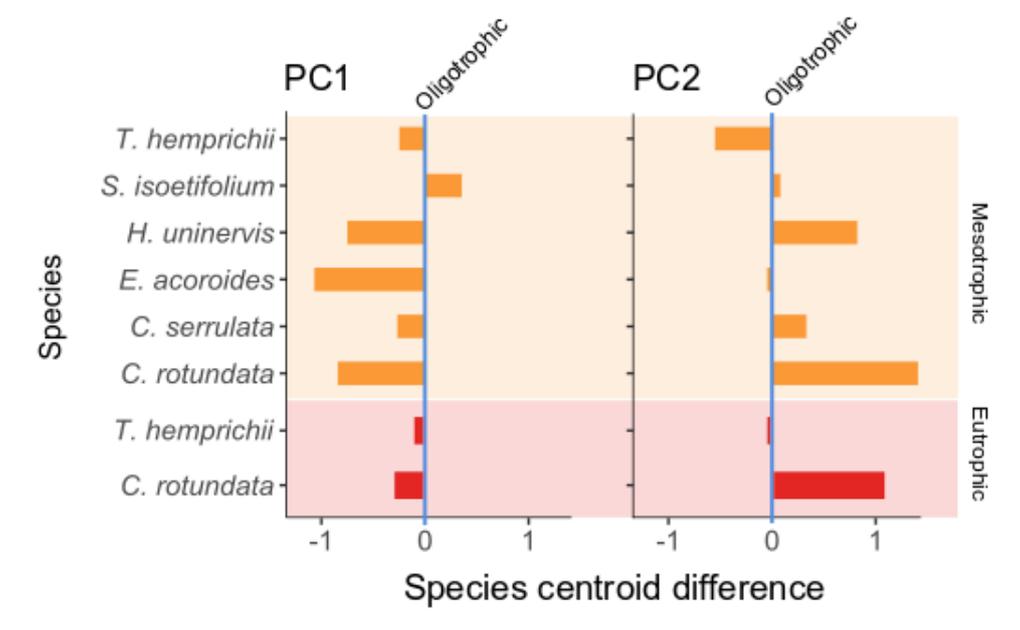


Figure 7. Difference in the species centroids from the oligotrophic (vertical zero axis) to the mesotrophic (orange bar, light orange background) and eutrophic (red bars, light red background) states. *T. ciliatum* and *H. ovalis* are not present in these plots due to their presence uniquely under a single trophic state, oligotrophic and mesotrophic respectively. For the differences in species centroids in the rest of the PCs see Supplementary Material 7.

These results indicate a certain amount of species-level plasticity, allowing morphological traits to undergo changes potentially aiding species survival under different environmental conditions. Additionally, as it will be discussed in the next section, the change in species morphological traits has consequences in the probability of space preemption among seagrass species.

Table 6. Complete output of generalized linear mixed-effects model testing the effect of the difference in the functional strategies (Δ FS) of a seagrass pair on the probability of space preemption under different trophic states. Significance is highlighted in boldface ($p < 0.05$). Std deviation = Standard deviation.

Response variable: Probability of space preemption	Explanatory variables	χ^2	df	p-value	
	Random effects		Variance	Std deviation	
PC 1	Δ FS _{PC1}	19.7010	1	9.06·10⁻⁶	
	Trophic state	0.5040	1	0.4778	
	Δ FS _{PC1} : Trophic state	0.0017	1	0.9675	
	Random effects	Site	0.0061		0.0777
		Species A	0.1290		0.3592
		Species B	0.0140		0.1183
PC 2	Δ FS _{PC2}	2.5085	1	0.1132	
	Trophic state	0.3826	1	0.5362	
	Δ FS _{PC2} : Trophic state	0.7890	1	0.3744	
	Random effects	Site	0.0122		0.1105
		Species A	0.6443		0.8027
		Species B	0.3682		0.6068
PC 3	Δ FS _{PC3}	0.1641	1	0.6854	
	Trophic state	0.4836	1	0.4867	
	Δ FS _{PC3} : Trophic state	3.5475	1	0.0596	
	Random effects	Site	0.0138		0.1177
		Species A	0.7500		0.8660
		Species B	0.4712		0.6865
PC 4	Δ FS _{PC4}	0.3407	1	0.5595	
	Trophic state	0.4594	1	0.4979	
	Δ FS _{PC4} : Trophic state	0.5307	1	0.4663	
	Random effects	Site	0.0134		0.6448
		Species A	0.6758		0.8221
		Species B	0.4157		0.6448
PC 5	Δ FS _{PC5}	0.0058	1	0.9394	
	Trophic state	0.4687	1	0.4935	
	Δ FS _{PC5} : Trophic state	9.6665	1	0.0018	
	Random effects	Site	0.0067		0.0820
		Species A	0.4857		0.6969
		Species B	0.2829		0.5319
PC 6	Δ FS _{PC6}	2.1849	1	0.1394	
	Trophic state	0.7199	1	0.3962	
	Δ FS _{PC6} : Trophic state	1.7076	1	0.1913	
	Random effects	Site	0.0123		0.1109
		Species A	0.6953		0.8338
		Species B	0.4147		0.6440

(3) Seagrass functional strategy and its control on space preemption

Competition between plants has been described as an asymmetric phenomenon (Schwinning and Weiner 1998). This means that, while one plant can exert a negative effect on a second plant, there is usually not a reciprocal effect (Davis and Fourqurean 2001). The reason behind this asymmetry has been generally related to plant size, but it depends on the resource type. In the case of light, as a directional resource, preemption is directly related to size of plant structures (Weiner and Fishman 1994, Horvitz and Schemske 2002), i.e. how much light a plant can block from smaller plants. However, the case of inorganic nutrients is more complicated (Schwinning and Weiner 1998) and depends on the plant nutritional demands (Fourqurean et al. 1995), uptake capacity and the nutrient distribution in the sediment.

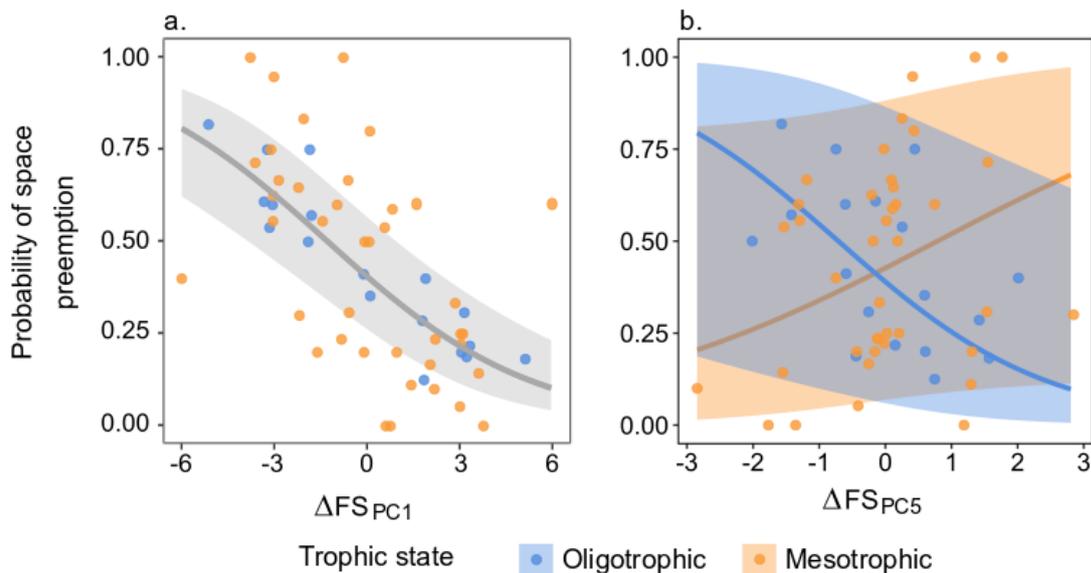


Figure 8. Relationship between the difference in the functional strategy of a pair of species on PCs 1 (a) and 5 (b) ($\Delta FS_{PC1,5}$) on the probability of space preemption of species A on species B. The points represent the empirical space preemption for each seagrass species pair. a) The gray line represents the predicted preemption according to ΔFS_{PC1} and the light gray ribbons the confidence interval of the prediction. Model equation for ΔFS_{PC1} : $\ln\left(\frac{p_i}{1-p_i}\right) = -0.391 - 0.301 \cdot \Delta FS_{PC1}$. b) The blue and orange lines represent the predicted preemption per trophic state according to ΔFS_{PC5} , and the light blue and orange ribbons the confidence intervals of the prediction. See Supplementary Material 8 for the regressions in the other PCs.

Additionally, submerged marine plants can obtain nutrients from the water column (Viana et al. 2019b), thus complicating the identification of traits related to nutrient preemption. This is, likely, one of the reasons why we could not find studies directly addressing the relation between

aboveground seagrass traits and inorganic nutrients preemption (Table 1). Our study showed that the same asymmetry principle described in terrestrial plants applied to seagrasses. When a species pair is sharing a plot, the difference between their scores in PC1 (ΔFS_{PC1}) showed that the higher the difference in size, the higher the probability that the bigger plant will exert space preemption. Applied to the actual species scores, the species with a higher score in PC1 does also have the highest probability of preemption. The traits correlated to PC1 can give fundamental insight on what were the mechanisms behind the preemption.

First, the only trait negatively correlated to PC1 was Shoots/M. This indicates that traditionally classified ephemeral and pioneer species, namely *H. ovalis*, *H. uninervis*, *S. isoetifolium* and *C. rotundata*, tend to show lower probability of space preemption. This is clear when this result is compared to previous observations on competition or successional stages in seagrass meadows. In one of the first reports describing Mediterranean seagrass meadows, Aleem (1955) observed that when *P. oceanica* and *C. nodosa* shared the same space, the former would dominate the meadow. This idea was further developed by Young and Kirkman (1975), who described that seagrass meadows are in a state leading to bigger climax species. A similar work on succession is presented by Birch and Birch (1984), describing the development of a meadow from pioneer species (genus *Halophila*) to climax ones (in this case, *C. serrulata*). Secondly, four of the traits that were positively correlated to PC1 (leaf ML and MW, RhD and root ML) are a proxy for the size of the plant and, supporting the assumption that plant size tends to be the controlling factor in space preemption. Therefore, these traits could influence both light and inorganic nutrients preemption.

The case of light is relatively simple: seagrass tend to shade the understory of their canopy (Zieman et al. 1984), inhibiting the growth of plants that have higher light requirements than the light reaching them. As a directional resource, higher leaf ML and MW would proportionally block more light (Williams 1987). Additionally, greater leaves/Sh indicate a denser canopy, and greater LMA indicates thicker leaves, further reducing the amount of light reaching the canopy understory. The case of inorganic nutrients is more complicated due to the non-directional nature of the resource. Intuitively, greater plant size translates into more tissue area for resource acquisition. Additionally, plants with longer root length would have access to deeper sediments and potentially new nutrient pools, inaccessible to smaller plants, which share the shallow sediment layer (Williams 1990, Duarte et al. 1998). This establishes an extra advantage to bigger plants in addition to light preemption. This differentiation in belowground resource access also indicates niche differentiation, as it allows plants to access different

resource pools, avoiding competition (Wilson 1988, McConnaughay and Bazzaz 1991, Duarte et al. 2000). However, the question of whether the selected traits indicate inorganic nutrient preemption remains unanswered, as the traits could indicate access to other nutrient pools, but not an interference of one seagrass species in the nutrient acquisition of a second seagrass species. Controlled experiments disentangling the confounding effects of light and inorganic preemption will prove fundamental to better understand which preemptable resource is more important for the meadow final configuration, or if the same preemptable resource is the driver of meadow configuration under changing environmental conditions.

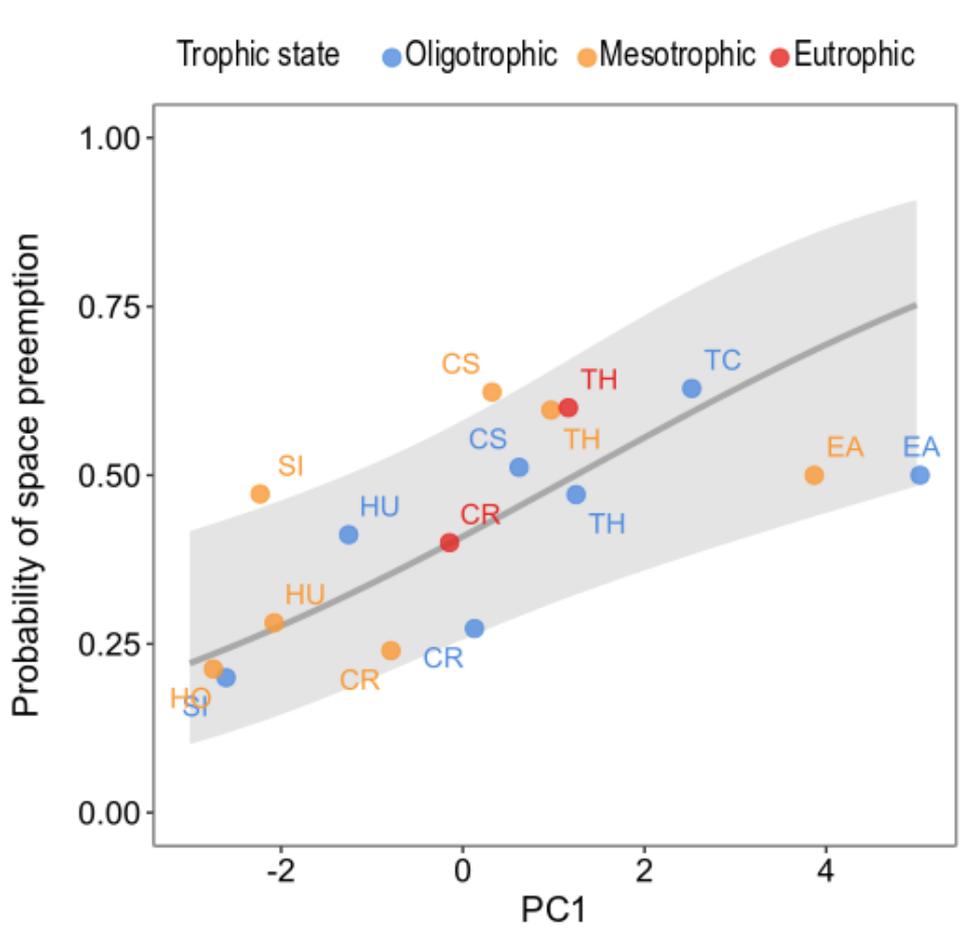


Figure 9. Probability of preemption of all seagrass species present in the studied meadows in Unguja Island as explained by their scores in PC1. Each data point is the score of the seagrass species in a trophic state, coded by their corresponding colors, and the letters are the seagrass species. The gray line is the fitted line and the gray ribbons the confidence intervals of the prediction of preemption. Model equation: $\ln\left(\frac{p_i}{1-p_i}\right) = -0.367 + 0.296PC1$. Seagrass species: CR = *C. rotundata*, CS = *C. serrulata*, EA = *E. acoroides*, HO = *H. ovalis*, HU = *H. uninervis*, SI = *S. isoetifolium*, TC = *T. ciliatum*, TH = *T. hemprichii*.

The trophic states drove changes in the seagrass species traits. We showed how all species except *S. isoetifolium* decreased their size in the mesotrophic and eutrophic states in PC1. For a small species like *S. isoetifolium*, the increase in size relative to the other species translated in an increase in the probability of space preemption (Fig. 9). Therefore, despite the same traits being responsible for competition, it is the increase in the size of *S. isoetifolium* that drove the increase in its capacity for space preemption. Similarly, this has been reported for the competition between *T. testudinum* and *H. wrightii* (Fourqurean et al. 1995). When the nutrient supply increased, equating the nutrient demands of *H. wrightii*, this species developed higher aboveground structures exerting light preemption on *T. testudinum*, resulting in the replacement of this species in the long term. It is therefore apparent that the traits driving competition for resources and, as a consequence, space preemption, remain the same even under inorganic nutrient excess. The change in the species that outcompetes another is a consequence of the species-specific trait response to the nutrient excess. This highlights the importance of including environmental conditions together with trait metrics for the prediction of ecological processes (van der Plas et al. 2020).

It is however worth discussing which traits determine space preemption in radically different environments e.g. subtidal and intertidal areas. Citing Aleem (1955) again, he described that, towards the intertidal area, *P. oceanica* is “at a minimum” and *C. nodosa* outcompetes the former. Zonation due to the tidal level is also mentioned in Phillips (1960). Harrison (1979) suggested that the intertidal area was dominated by *Zostera americana* due to being an r-strategist compared to the k-strategist *Z. marina*. Similarly, den Hartog (1970) reports that *Ruppia* sp. dominates in brackish waters, while other seagrass species preempts it from penetrating in waters with higher salinity. In the present study we found an effect of eutrophication on seagrass traits, but not in competition for space, likely because despite this effect, the traits determining preemption remained the same. However, other environmental factors can change which traits are important for species competition i.e. traits that determine space preemption in subtidal seagrass meadows are not the same in intertidal meadows. The selection of traits for the research question at hand is therefore fundamental.

Despite the higher probability of space preemption for bigger seagrass plants, seagrass meadows in Unguja Island are generally mixed. This begs the question of how a mixed meadows are maintained, despite the capacity of bigger species to preempt space from smaller species. Habitats are subjected to a disturbance regime that, potentially, can promote the seasonal or periodic growth of a species that generally would not exert space preemption

(Turner 1985). It is therefore apparent that seagrass meadows are able to maintain an array of species (Williams 1990), in a successional series that is dynamic and does not end in the formation of a completely monospecific meadow. Additionally, other traits may be responsible for the persistence of a species despite its incapacity for space resource preemption. The study of more complex traits and physiological processes in seagrass could disentangle remaining questions in the competition for light and inorganic nutrients. In the case of competition for inorganic nutrients, the study of the nutrient demands and nutrient uptake rates (Angove et al. 2018) would give insight on seagrass competition under nutrient excess and limitation. In the case of the study of light preemption, the knowledge of the photosynthetic performance and pigment composition of seagrass plants would give insight into which species can withstand higher exposure to light or, inversely, shading.

Lastly, there are some relevant concepts that were not deeply discussed in this study that could open new and interesting lines of research in seagrass competition. First, plasticity and niche differentiation have been shown to be important drivers for the competition of terrestrial plants (Meilhac et al. 2020), showing how the trait differentiation in terrestrial grasslands influence competition. Due to the parallelism between terrestrial plant and seagrass competition presented in this study, it is expected that these phenomena will have an effect on competition among seagrass species as well. Second, competition with other primary producers was not included in this study, but there are a great number of reports showing that seagrass and benthic macroalgae compete for resources and space (Dethier 1984, Turner 1985, Davis and Fourqurean 2001, Taplin et al. 2005, Moreira-Saporiti et al. 2021). While traits important for interspecific competition with benthic algae may be the same as for competition with seagrass, this may not be the case. This question is of great importance for the understanding of invasive algae colonization in seagrass meadows (de Villèle and Verlaque 1995, Ceccherelli and Cinelli 1997).

V. Conclusions

This study advances our understanding of the ecological processes that shape the configuration of seagrass meadows by describing competition using a trait-based approach. The traits linked to light and inorganic nutrients preemption that were used here (leaf length and width, rhizome diameter, shoots meter⁻¹, among others) define the functional strategy of seagrass species by showing a trade-off between size and density of shoots. We found that the probability of space preemption was positively correlated with the traits' indicative of larger plant size. This indicates that competitive interactions in seagrass are asymmetrical and favor larger seagrass species, which exert a negative effect on smaller species without a reciprocal negative response.

VI. Acknowledgements

The authors thank the staff of the Institute of Marine Sciences (IMS) in Stone Town for their support both administratively and scientifically, specifically Mtumwa Mwadini for his advice and help, and Flower Msuya and Jiddawi Narriman for their logistical support. We would like to thank Daniel Arturo Saavedra Hortua, Imke Podbielski, Dieuwke Hoeijmakers, Mondy Muhando and Ulrich Pint for their help in the fieldwork campaign. We want to thank the staff of the Chemistry and Biology laboratories in the Leibniz Centre for Marine Tropical Research (ZMT) for their assistance.

Inés G. Viana was awarded with a postdoctoral contract of Xunta de Galicia (Consellería de Educación, Universidad e Formación Profesional) postdoctoral program (ED481B-2016/189-0) and Juan de la Cierva-Incorporación postdoctoral program (IJC2019-040554-I). This study was part of the project Seagrass and Macroalgal Community Dynamics and Performance under Environmental Change (SEAMAC) (Deutsche Forschungsgemeinschaft, DFG, TE 1046/3-1) awarded to Mirta Teichberg.

VII. References

- Agawin, N. S., Duarte, C. M., and Fortes, M. D. (1996). Nutrient limitation of Philippine seagrasses (Cape Bolinao, NW Philippines): in situ experimental evidence. *Marine Ecology Progress Series*, 138, 233-243. <https://doi.org/10.3354/meps138233>
- Aleem, A. A. (1955). Structure and evolution of the seagrass communities *Posidonia* and *Cymodocea* in the southeastern Mediterranean. *Essays in the natural sciences in honor of Captain Allan Hancock, on the occasion of his birthday, July, 26*, 279-298. <https://doi.org/10.5962/bhl.title.6088>
- Andersen, K. H., and Pedersen, M. (2009). Damped trophic cascades driven by fishing in model marine ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 277, 795-802. <https://doi.org/10.1098/rspb.2009.1512>
- Angove, C., Norkko, A., and Gustafsson, C. (2018). Assessing the efficiencies and challenges for nutrient uptake by aquatic plants. *Journal of Experimental Marine Biology and Ecology*, 507, 23-30. <https://doi.org/10.1016/j.jembe.2018.07.005>
- Artika, S. R., Ambo-Rappe, R., Teichberg, M., Moreira-Saporiti, A., and Viana, I. G. (2020). Morphological and physiological responses of *Enhalus acoroides* seedlings under varying temperature and nutrient treatment. *Frontiers in Marine Science*, 7, 325. <https://doi.org/10.3389/fmars.2020.00325>
- Bando, K. J. (2006). The roles of competition and disturbance in a marine invasion. *Biological Invasions*, 8, 755-763. <https://doi.org/10.1007/s10530-005-3543-4>

- Birch, W. R., and Birch, M. (1984). Succession and pattern of tropical intertidal seagrasses in Cockle Bay, Queensland, Australia: a decade of observations. *Aquatic Botany*, 19, 343-367. [https://doi.org/10.1016/0304-3770\(84\)90048-2](https://doi.org/10.1016/0304-3770(84)90048-2)
- Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1-48. <https://doi.org/10.18637/jss.v067.i01>
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., et al. (2017). glmmTMB Balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9, 378-400. <https://doi.org/10.32614/rj-2017-066>
- Browne, N. K., Yaakub, S. M., Tay, J. K., and Todd, P. A. (2017). Recreating the shading effects of ship wake induced turbidity to test acclimation responses in the seagrass *Thalassia hemprichii*. *Estuarine, Coastal and Shelf Science*, 199, 87-95. <https://doi.org/10.1016/j.ecss.2017.09.034>
- Bulthuis, D. A., and Woelkerling, W. J. (1981). Effects of in situ nitrogen and phosphorus enrichment of the sediments on the seagrass *Heterozostera tasmanica* (Martens ex Aschers.) den Hartog in Western Port, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology*, 53, 193-207. [https://doi.org/10.1016/0022-0981\(81\)90019-8](https://doi.org/10.1016/0022-0981(81)90019-8)
- Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology*, 350, 46-72. <https://doi.org/10.1016/j.jembe.2007.06.024>
- Cardoso, P. G., Pardal, M. A., Lillebø, A. I., Ferreira, S. M., Raffaelli, D., and Marques, J. C. (2004). Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. *Journal of Experimental Marine Biology and Ecology*, 302, 233-248. <https://doi.org/10.1016/j.jembe.2003.10.014>
- Ceccherelli, G., and Cinelli, F. (1997). Short-term effects of nutrient enrichment of the sediment and interactions between the seagrass *Cymodocea nodosa* and the introduced green alga *Caulerpa taxifolia* in a Mediterranean bay. *Journal of Experimental Marine Biology and Ecology*, 217, 165-177. [https://doi.org/10.1016/s0022-0981\(97\)00050-6](https://doi.org/10.1016/s0022-0981(97)00050-6)
- Collier, C. J., Waycott, M., and Ospina, A. G. (2012). Responses of four Indo-West Pacific seagrass species to shading. *Marine Pollution Bulletin*, 65, 342-354. <https://doi.org/10.1016/j.marpolbul.2011.06.017>
- Connell, J. H. (1983). On the prevalence and relative importance of interspecific competition: evidence from field experiments. *The American Naturalist*, 122, 661-696. <https://doi.org/10.1086/284165>
- Connell, J. H. (1990). Apparent versus “real” competition in plants. *Perspectives on plant competition*, 9-26. <https://doi.org/10.1016/b978-0-12-294452-9.50006-0>

- Connell, J. H., and Slatyer, R. O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist*, *111*, 1119-1144. <https://doi.org/10.1086/283241>
- Costanza, R., and Folke, C. (1997). Valuing ecosystem services with efficiency, fairness and sustainability as goals. *Nature's services: Societal dependence on natural ecosystems*, 49-70.
- Davis, B. C., and Fourqurean, J. W. (2001). Competition between the tropical alga, *Halimeda incrassata*, and the seagrass, *Thalassia testudinum*. *Aquatic Botany*, *71*, 217-232. [https://doi.org/10.1016/s0304-3770\(01\)00179-6](https://doi.org/10.1016/s0304-3770(01)00179-6)
- de los Santos, C.B., Krause-Jensen, D., Alcoverro, T., Marbà, N., Duarte, C. M., Van Katwijk, M. M., et al. (2019). Recent trend reversal for declining European seagrass meadows. *Nature Communications*, *10*, 3356. <https://doi.org/10.1038/s41467-019-11340-4>
- De Villèle, X., and Verlaque, M. (1995). Changes and degradation in a *Posidonia oceanica* bed invaded by the introduced tropical alga *Caulerpa taxifolia* in the north western Mediterranean. *Botanica Marina*, *38*, 79-88. <https://doi.org/10.1515/botm.1995.38.1-6.79>
- den Hartog, C. (1970). The sea-grasses of the world. *North-Holland, Amsterdam*. <https://doi.org/10.1002/iroh.19710560139>
- den Hartog, C. (1971). The dynamic aspect in the ecology of seagrass communities. *Thalassia Jugoslavica*, *7*, 101-112.
- den Hartog, C. (1977). Structure, function, and classification in seagrass communities. In C. P. McRoy and C. Helffferich, editors. *Seagrass ecosystems: a scientific perspective* (pp. 89-121). Marcel Dekker, New York, New York, USA.
- Dethier, M. N. (1984). Disturbance and recovery in intertidal pools: maintenance of mosaic patterns. *Ecological Monographs*, *54*, 99-118. <https://doi.org/10.2307/1942457>
- Duarte, C. M., and Chiscano, C. L. (1999). Seagrass biomass and production: a reassessment. *Aquatic Botany*, *65*, 159-174. [https://doi.org/10.1016/s0304-3770\(99\)00038-8](https://doi.org/10.1016/s0304-3770(99)00038-8)
- Duarte, C. M., Merino, M., and Gallegos, M. (1995). Evidence of iron deficiency in seagrasses growing above carbonate sediments. *Limnology and Oceanography*, *40*, 1153-1158. <https://doi.org/10.4319/lo.1995.40.6.1153>
- Duarte, C. M., Terrados, J., Agawin, N. S., Fortes, M. D., Bach, S., and Kenworthy, W. J. (1997). Response of a mixed Philippine seagrass meadow to experimental burial. *Marine Ecology Progress Series*, *147*, 285-294. <https://doi.org/10.3354/meps147285>
- Duarte, C. M., Merino, M., Agawin, N. S., Uri, J., Fortes, M. D., Gallegos, M. E., et al. (1998). Root production and belowground seagrass biomass. *Marine Ecology Progress Series*, *171*, 97-108. <https://doi.org/10.3354/meps171097>
- Duarte, C. M., Terrados, J., Agawin, N. S., and Fortes, M. D. (2000). An experimental test of the occurrence of competitive interactions among SE Asian seagrasses. *Marine Ecology Progress Series*, *197*, 231-240. <https://doi.org/10.3354/meps197231>

- Elleouet, J., Albouy, C., Ben Rais Lasram, F., Mouillot, D., and Leprieur, F. (2014). A trait-based approach for assessing and mapping niche overlap between native and exotic species: the Mediterranean coastal fish fauna as a case study. *Diversity and Distributions*, 20, 1333-1344. <https://doi.org/10.1111/ddi.12235>
- Firbank, L. G., and Watkinson, A. R. (1987). On the analysis of competition at the level of the individual plant. *Oecologia*, 71, 308-317. <https://doi.org/10.1007/bf00377300>
- Fokeera-Wahedally, S. B. M., and Bhikajee, M. (2005). The effects of in situ shading on the growth of a seagrass, *Syringodium isoetifolium*. *Estuarine, Coastal and Shelf Science*, 64, 149-155. <https://doi.org/10.1016/j.ecss.2005.01.006>
- Fourqurean, J. W., Powell, G. V., Kenworthy, W. J., and Zieman, J. C. (1995). The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. *Oikos*, 72, 349-358. <https://doi.org/10.2307/3546120>
- Garnier, E., Navas, M. L., and Grigulis, K. (2016). *Plant functional diversity: organism traits, community structure, and ecosystem properties*. Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780198757368.001>
- Grasshoff, K., Kremling, K., and Ehrhardt, M. (Eds.). (2009). *Methods of seawater analysis*. John Wiley and Sons. <https://doi.org/10.1002/9783527613984>
- Grime, J. P. (1979). Plant strategies and vegetation processes. *Plant strategies and vegetation processes*. <https://doi.org/10.2307/3898436>
- Grime, J. P. (1998). Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, 86, 902-910. <https://doi.org/10.1046/j.1365-2745.1998.00306.x>
- Gustafsson, C., and Norkko, A. (2016). Not all plants are the same: Exploring metabolism and nitrogen fluxes in a benthic community composed of different aquatic plant species. *Limnology and Oceanography*, 61, 1787-1799. <https://doi.org/10.1002/lno.10334>
- Gustafsson, C., and Norkko, A. (2019). Quantifying the importance of functional traits for primary production in aquatic plant communities. *Journal of Ecology*, 107, 154-166. <https://doi.org/10.1111/1365-2745.13011>
- Harrison, P. G. (1979). Reproductive strategies in intertidal populations of two co-occurring seagrasses (*Zostera* spp.). *Canadian Journal of Botany*, 57, 2635-2638. <https://doi.org/10.1139/b79-312>
- Hemminga, M. A., Harrison, P. G., and Van Lent, F. (1991). The balance of nutrient losses and gains in seagrass meadows. *Marine Ecology Progress Series*, 71, 85-96. <https://doi.org/10.3354/meps071085>
- Hofman, T. B., and Ennik, G. C. (1980). Investigation into plant characters affecting the competitive ability of perennial ryegrass (*Lolium perenne* L.). *NJAS Wageningen Journal of Life Sciences*, 28, 97-109. <https://doi.org/10.18174/njas.v28i2.17034>

- Horvitz, C. C., and Schemske, D. W. (2002). Effects of plant size, leaf herbivory, local competition and fruit production on survival, growth and future reproduction of a neotropical herb. *Journal of Ecology*, *90*, 279-290. <https://doi.org/10.1046/j.1365-2745.2001.00660.x>
- Iizumi, H., and Hattori, A. (1982). Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. III. The kinetics of nitrogen uptake. *Aquatic Botany*, *12*, 245-256. [https://doi.org/10.1016/0304-3770\(82\)90020-1](https://doi.org/10.1016/0304-3770(82)90020-1)
- Jänes, H., Kotta, J., Pärnoja, M., Crowe, T. P., Rindi, F., and Orav-Kotta, H. (2017). Functional traits of marine macrophytes predict primary production. *Functional Ecology*, *31*, 975-986. <https://doi.org/10.1111/1365-2435.12798>
- Kassambara, A., and Mundt, F. (2020). factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra>
- Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, *3*, 157-164. <https://doi.org/10.2307/3235676>
- Kilminster, K., McMahon, K., Waycott, M., Kendrick, G. A., Scanes, P., McKenzie, L., et al. (2015). Unravelling complexity in seagrass systems for management: Australia as a microcosm. *Science of the Total Environment*, *534*, 97-109. <https://doi.org/10.1016/j.scitotenv.2015.04.061>
- Kinlock, N. L. (2019). A meta-analysis of plant interaction networks reveals competitive hierarchies as well as facilitation and intransitivity. *The American Naturalist*, *194*, 640-653. <https://doi.org/10.1086/705293>
- Lam, S. L., Bujang, J. S., Arshad, A., and Muta Harah, Z. (2004). Adaptability of *Cymodocea serrulata* (R. Br.) Aschers. and magnus to different environment.
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, *16*, 545-556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Le, S., Josse, J., and Husson, F. (2008). FactoMineR: An R Package for multivariate analysis. *Journal of Statistical Software*, *25*. <https://doi.org/10.18637/jss.v025.i01>
- Lewis, J. T., and Boyer, K. E. (2014). Grazer functional roles, induced defenses, and indirect interactions: implications for eelgrass restoration in San Francisco Bay. *Diversity*, *6*, 751-770. <https://doi.org/10.3390/d6040751>
- Litchman, E., and Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 615-639. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173549>
- Litchman, E., de Tezanos Pinto, P., Klausmeier, C. A., Thomas, M. K., and Yoshiyama, K. (2010). Linking traits to species diversity and community structure in phytoplankton. In *Fifty years after the ‘Homage to Santa Rosalia’: Old and new paradigms on biodiversity in aquatic ecosystems* (pp. 15-28). Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9908-2_3

- Litchman, E., Ohman, M. D., and Kiørboe, T. (2013). Trait-based approaches to zooplankton communities. *Journal of Plankton Research*, 35, 473-484. <https://doi.org/10.1093/plankt/fbt019>
- Maire, E., Grenouillet, G., Brosse, S., and Villéger, S. (2015). How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. *Global Ecology and Biogeography*, 24, 728-740. <https://doi.org/10.1111/geb.12299>
- McConnaughay, K. D. M., and Bazzaz, F. A. (1991). Is physical space a soil resource? *Ecology*, 72, 94-103. <https://doi.org/10.2307/1938905>
- McMahon, K., van Dijk, K. J., Ruiz-Montoya, L., Kendrick, G. A., Krauss, S. L., Waycott, M., et al. (2014). The movement ecology of seagrasses. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20140878 . <https://doi.org/10.1098/rspb.2014.0878>
- Meilhac, J., Deschamps, L., Maire, V., Flajoulot, S., and Litrico, I. (2020). Both selection and plasticity drive niche differentiation in experimental grasslands. *Nature Plants*, 6, 28-33. <https://doi.org/10.1038/s41477-019-0569-7>
- Moliner, R., and Picard, J. (1952). Recherches sur les herbiers des phanerogames marines du littoral méditerranéen française. *Annales de l'Inst. Oceanographique*, 27, 157-235.
- Moreira-Saporiti, A., Hoeijmakers, D., Msuya, F.E., Reuter H., and Teichberg, M. (2021). Seaweed farming pressure affects seagrass and benthic macroalgae dynamics in Chwaka Bay (Zanzibar, Tanzania). *Regional Environmental Change* 21, 11. <https://doi.org/10.1007/s10113-020-01742-2>
- Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends in Ecology and Evolution*, 28, 167-177. <https://doi.org/10.1016/j.tree.2012.10.004>
- Murtagh, F., and Legendre, P. (2014). Ward's hierarchical agglomerative clustering method: which algorithms implement Ward's criterion? *Journal of Classification*, 31, 274-295. <https://doi.org/10.1007/s00357-014-9161-z>
- Mvungi, E. F., and Pillay, D. (2019). Eutrophication overrides warming as a stressor for a temperate African seagrass (*Zostera capensis*). *PloS ONE*, 14, e0215129. <https://doi.org/10.1371/journal.pone.0215129>
- Nakaoka, M., and Iizumi, H. (2000). Magnitude of within-patch variation in seagrass *Halophila ovalis* growth affected by adjacent *Thalassia hemprichii* vegetation. *Ecological Research*, 15, 415-424. <https://doi.org/10.1046/j.1440-1703.2000.00362.x>
- Nomme, K. M., and Harrison, P. G. (1991a). A multivariate comparison of the seagrasses *Zostera marina* and *Zostera japonica* in monospecific versus mixed populations. *Canadian Journal of Botany*, 69, 1984-1990. <https://doi.org/10.1139/b91-249>

- Nomme, K. M., and Harrison, P. G. (1991b). Evidence for interaction between the seagrasses *Zostera marina* and *Zostera japonica* on the Pacific coast of Canada. *Canadian Journal of Botany*, 69, 2004-2010. <https://doi.org/10.1139/b91-252>
- Olsen, J. L., Rouzé, P., Verhelst, B., Lin, Y. C., Bayer, T., et al. (2016). The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature*, 530, 331-335. <https://doi.org/10.1038/nature16548>
- Ooi, J. L. S., Kendrick, G. A., Van Niel, K. P., and Affendi, Y. A. (2011). Knowledge gaps in tropical Southeast Asian seagrass systems. *Estuarine, Coastal and Shelf Science*, 92, 118-131. <https://doi.org/10.1016/j.ecss.2010.12.021>
- Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J. M., Romero, J., and Pérez, M. (2019). Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. *Marine Environmental Research*, 145, 27-38. <https://doi.org/10.1016/j.marenvres.2019.02.002>
- Orth, R. J. (1977). Effect of nutrient enrichment on growth of the eelgrass *Zostera marina* in the Chesapeake Bay, Virginia, USA. *Marine Biology*, 44, 187-194. <https://doi.org/10.1007/bf00386958>
- Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., et al. (2006). A global crisis for seagrass ecosystems. *BioScience*, 56, 987-996. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:agcfse\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2)
- Orth, R. J., Harwell, M. C., and Inglis, G. J. (2007). Ecology of seagrass seeds and seagrass dispersal processes. In *Seagrasses: Biology, Ecology and Conservation* (pp. 111-133). Springer, Dordrecht. https://doi.org/10.1007/1-4020-2983-7_5
- Orth, R. J., Heck, K. L., and van Montfrans, J. (1984). Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries*, 7, 339-350. <https://doi.org/10.2307/1351618>
- Pagès, J. F., Farina, S., Gera, A., Arthur, R., Romero, J., and Alcoverro, T. (2012). Indirect interactions in seagrasses: fish herbivores increase predation risk to sea urchins by modifying plant traits. *Functional Ecology*, 26, 1015-1023. <https://doi.org/10.1111/j.1365-2435.2012.02038.x>
- Phillips, R. C. (1960). *Observations on the ecology and distribution of the Florida seagrasses* (No. 44). Florida State Board of Conservation, Marine Laboratory.
- Powell, G. V., Kenworthy, J. W., and Fourqurean, J. W. (1989). Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. *Bulletin of Marine Science*, 44, 324-340.
- Pulich Jr, W. M. (1985). Seasonal growth dynamics of *Ruppia maritima* Lsl and *Halodule wrightii* Aschers. in southern Texas and evaluation of sediment fertility status. *Aquatic Botany*, 23, 53-66. [https://doi.org/10.1016/0304-3770\(85\)90020-8](https://doi.org/10.1016/0304-3770(85)90020-8)

- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rasheed, M. A., and Unsworth, R. K. (2011). Long-term climate-associated dynamics of a tropical seagrass meadow: implications for the future. *Marine Ecology Progress Series*, 422, 93-103. <https://doi.org/10.3354/meps08925>
- Roca, G., Alcoverro, T., Krause-Jensen, D., Balsby, T. J. S., van Katwijk, M. M., Marbà, N., et al. (2016). Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis. *Ecological Indicators*, 63, 310-323. <https://doi.org/10.1016/j.ecolind.2015.12.007>
- Rosenthal, R. J., Clarke, W. D., and Dayton, P. K. (1974). Ecology and natural history of a stand of giant kelp, *Macrocystis pyrifera*, off Del Mar, California. *Fishing Bulletin*, 72, e684.
- Schäfer, S., Monteiro, J., Castro, N., Gizzi, F., Henriques, F., Ramalhosa, P., et al. (2021). Lost and found: A new hope for the seagrass *Cymodocea nodosa* in the marine ecosystem of a subtropical Atlantic Island. *Regional Studies in Marine Science*, 41, 101575. <https://doi.org/10.1016/j.rsma.2020.101575>
- Shipley, B., De Bello, F., Cornelissen, J. H. C., Laliberté, E., Laughlin, D. C., and Reich, P. B. (2016). Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*, 180, 923-931. <https://doi.org/10.1007/s00442-016-3549-x>
- Schmidt, A. L., Wysmyk, J. K., Craig, S. E., and Lotze, H. K. (2012). Regional-scale effects of eutrophication on ecosystem structure and services of seagrass beds. *Limnology and Oceanography*, 57, 1389-1402. <https://doi.org/10.4319/lo.2012.57.5.1389>
- Schoener, T. W. (1983). Field experiments on interspecific competition. *The American Naturalist*, 122, 240-285. <https://doi.org/10.1086/284133>
- Schwinning, S., and Weiner, J. (1998). Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia*, 113, 447-455. <https://doi.org/10.1007/s004420050397>
- Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350, 3-20. <https://doi.org/10.1016/j.jembe.2007.06.012>
- Short, F. T., Davis, M. W., Gibson, R. A., and Zimmermann, C. F. (1985). Evidence for phosphorus limitation in carbonate sediments of the seagrass *Syringodium filiforme*. *Estuarine, Coastal and Shelf Science*, 20, 419-430. [https://doi.org/10.1016/0272-7714\(85\)90086-1](https://doi.org/10.1016/0272-7714(85)90086-1)
- Short, F. T., and McRoy, C. P. (1984). Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. *Botanica Marina*, 27, 547-556. <https://doi.org/10.1515/botm.1984.27.12.547>
- Taplin, K. A., Irlandi, E. A., and Raves, R. (2005). Interference between the macroalga *Caulerpa prolifera* and the seagrass *Halodule wrightii*. *Aquatic Botany*, 83, 175-186. <https://doi.org/10.1016/j.aquabot.2005.06.003>

- Terrados, J., Borum, J., Duarte, C. M., Fortes, M. D., Kamp-Nielsen, L., Agawin, N. S. R., and Kenworthy, W. J. (1999). Nutrient and mass allocation of South-east Asian seagrasses. *Aquatic Botany*, 63, 203-217. [https://doi.org/10.1016/s0304-3770\(99\)00004-2](https://doi.org/10.1016/s0304-3770(99)00004-2)
- Thursby, G. B., and Harlin, M. M. (1982). Leaf-root interaction in the uptake of ammonia by *Zostera marina*. *Marine Biology*, 72, 109-112. <https://doi.org/10.1007/bf00396910>
- Tilman, D. (1985). The resource-ratio hypothesis of plant succession. *The American Naturalist*, 125, 827-852. <https://doi.org/10.1086/284382>
- Tilman, D. (1994). Competition and biodiversity in spatially structured habitats. *Ecology*, 75, 2-16. <https://doi.org/10.2307/1939377>
- Turner, T. (1983). Facilitation as a successional mechanism in a rocky intertidal community. *The American Naturalist*, 121, 729-738. <https://doi.org/10.1086/284098>
- Turner, T. (1985). Stability of rocky intertidal surfgrass beds: Persistence, preemption, and recovery. *Ecology*, 66, 83-92. <https://doi.org/10.2307/1941308>
- Udy, J. W., Dennison, W. C., Long, W. J. L., and McKenzie, L. J. (1999). Responses of seagrass to nutrients in the Great Barrier Reef, Australia. *Marine Ecology Progress Series*, 185, 257-271. <https://doi.org/10.3354/meps185257>
- Underwood, A. J., and Denley, E. J. (1984). Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shore. *Ecological communities: conceptual issues and the evidence*, 151-180. <https://doi.org/10.1515/9781400857081.151>
- Unsworth, R. K., Ambo-Rappe, R., Jones, B. L., La Nafie, Y. A., Irawan, A., Hernawan, U. E., et al. (2018). Indonesia's globally significant seagrass meadows are under widespread threat. *Science of the Total Environment*, 634, 279-286. <https://doi.org/10.1016/j.scitotenv.2018.03.315>
- van der Plas, F., Schröder-Georgi, T., Weigelt, A., Barry, K., Meyer, S., Alzate, A., et al. (2020). Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nature Ecology and Evolution*, 4, 1602-1611. <https://doi.org/10.1038/s41559-020-01316-9>
- Vermaat, J. E., Agawin, N. S., Duarte, C. M., Fortes, M. D., Marbà, N., and Uri, J. S. (1995). Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. *Marine Ecology Progress Series*, 124, 215-225. <https://doi.org/10.3354/meps124215>
- Viana, I. G., Siriwardane-de Zoysa, R., Willette, D. A., and Gillis, L. G. (2019a). Exploring how non-native seagrass species could provide essential ecosystems services: a perspective on the highly invasive seagrass *Halophila stipulacea* in the Caribbean Sea. *Biological Invasions*, 21, 1461-1472. <https://doi.org/10.1007/s10530-019-01924-y>
- Viana, I. G., Saavedra-Hortúa, D. A., Mtolera, M., and Teichberg, M. (2019b). Different strategies of nitrogen acquisition in two tropical seagrasses under nitrogen enrichment. *New Phytologist*, 223, 1217-1229. <https://doi.org/10.1111/nph.15885>

- Viana, I.G., Moreira-Saporiti, A., and Teichberg, M. (2020). Species-specific trait responses of three tropical seagrasses to multiple stressors: the case of increasing temperature and nutrient enrichment. *Frontiers in Plant Science*, 11, 571363. <https://doi.org/10.3389/fpls.2020.571363>
- Vilà, M., and Sardans, J. (1999). Plant competition in Mediterranean-type vegetation. *Journal of Vegetation Science*, 10, 281-294. <https://doi.org/10.2307/3237150>
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116, 882-892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., et al. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, 106, 12377-12381. <https://doi.org/10.1073/pnas.0905620106>
- Weiner, J., and Fishman, L. (1994). Competition and allometry in *Kochia scoparia*. *Annals of Botany*, 73, 263-271. <https://doi.org/10.1006/anbo.1994.1031>
- Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer-Verlag New York. <https://doi.org/10.1111/j.1541-0420.2011.01616.x>
- Willette, D. A., and Ambrose, R. F. (2012). Effects of the invasive seagrass *Halophila stipulacea* on the native seagrass, *Syringodium filiforme*, and associated fish and epibiota communities in the Eastern Caribbean. *Aquatic Botany*, 103, 74-82. <https://doi.org/10.1016/j.aquabot.2012.06.007>
- Williams, S. L. (1987). Competition between the seagrasses *Thalassia testudinum* and *Syringodium filiforme* in a Caribbean lagoon. *Marine Ecology Progress Series*, 35, 91-98. <https://doi.org/10.3354/meps035091>
- Williams, S. L. (1990). Experimental studies of Caribbean seagrass bed development. *Ecological Monographs*, 60, 449-469. <https://doi.org/10.2307/1943015>
- Williams, S. L., and Ruckelshaus, M. H. (1993). Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology*, 74, 904-918. <https://doi.org/10.2307/1940815>
- Wilson, J. B. (1988). Shoot competition and root competition. *Journal of Applied Ecology*, 25, 279-296. <https://doi.org/10.2307/2403626>
- Wilson, J. B. (2011). The twelve theories of co-existence in plant communities: the doubtful, the important, and the unexplored. *Journal of Vegetation Science*, 22, 184-195. <https://doi.org/10.1111/j.1654-1103.2010.01226.x>
- Yamamoto, H., Fujimori, T., Sato, H., Ishikawa, G., Kami, K., and Ohashi, Y. (2014). Statistical hypothesis testing of factor loading in principal component analysis and its application to metabolite set enrichment analysis. *BMC Bioinformatics*, 15, 51. <https://doi.org/10.1186/1471-2105-15-51>

Young, P. C., and Kirkman, H. (1975). The seagrass communities of Moreton Bay, Queensland. *Aquatic Botany*, 1, 191-202. [https://doi.org/10.1016/0304-3770\(75\)90022-4](https://doi.org/10.1016/0304-3770(75)90022-4)

Zeileis, A., and Hothorn, T. (2002). Diagnostic checking in regression relationships. *R News* 2, 7-10. URL: <https://CRAN.R-project.org/doc/Rnews/>

Zieman, J. C. (1982). *The ecology of the seagrasses of south Florida: a community profile*. Department of the Interior, US Fish and Wildlife Service.

Zieman, J. C., Iverson, R. L., and Ogden, J. C. (1984). Herbivory effects on *Thalassia testudinum* leaf growth and nitrogen content. *Marine Ecology Progress Series*, 15, 151-158. <https://doi.org/10.3354/meps015151>

VIII. Supplementary Material

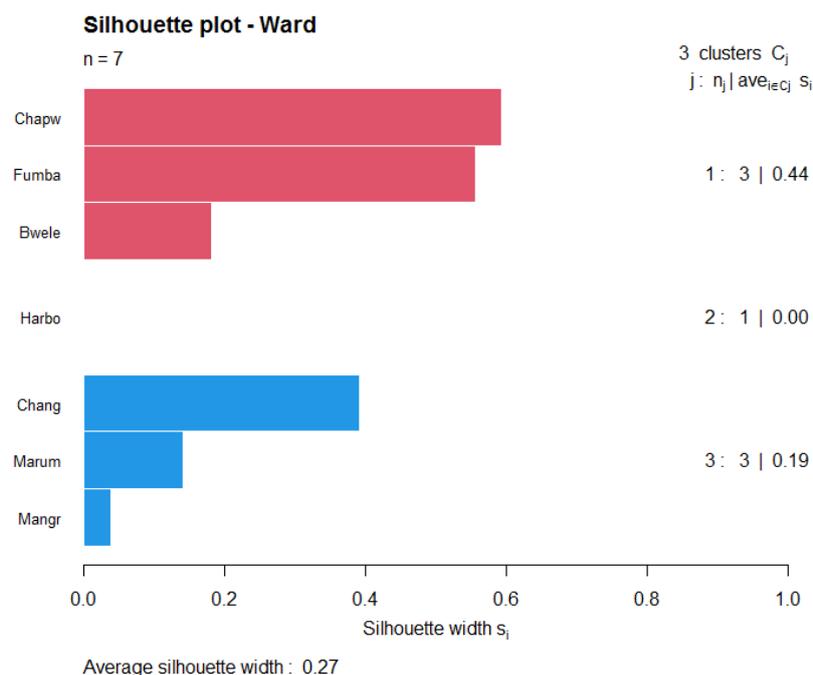
(1) Supplementary material 1. Raw trait data

All data is presented as Mean \pm Standard Error

Species	Sites	Sample size (n)	Belowground structure traits				Canopy forming traits				
			RhD (cm)	Roots/M	Root ML (cm)	Shoots/M	VR length (cm)	Leaves/Sh	Leaf ML (cm)	Leaf MW (cm)	LMA (g DW/cm ²)
CR	Marumbi	2	0.28 \pm 0.03	76.58 \pm 0.34	11.45 \pm 2.45	26.73 \pm 0.63	3.90 \pm 1.7	2.00 \pm 0.00	13.05 \pm 0.45	0.45 \pm 0.00	3.30 \pm 0.25
	Chapwani	10	0.25 \pm 0.01	244.63 \pm 64.07	8.14 \pm 0.51	55.76 \pm 4.28	1.59 \pm 0.24	2.94 \pm 0.14	6.30 \pm 0.45	0.27 \pm 0.01	3.39 \pm 0.15
	Bweleo	20	0.28 \pm 0.01	88.30 \pm 9.96	10.34 \pm 1.01	29.54 \pm 2.18	0.83 \pm 0.32	2.62 \pm 0.10	8.93 \pm 0.51	0.39 \pm 0.01	3.16 \pm 0.09
	Harbor	19	0.27 \pm 0.01	122.06 \pm 18.61	10.57 \pm 0.78	37.79 \pm 2.08	1.87 \pm 0.25	2.75 \pm 0.12	10.63 \pm 0.66	0.35 \pm 0.01	3.80 \pm 0.26
CS	Changuu	15	0.34 \pm 0.02	107.61 \pm 26.13	14.21 \pm 0.96	37.10 \pm 3.69	0.57 \pm 0.12	3.09 \pm 0.05	7.82 \pm 0.59	0.87 \pm 0.02	2.97 \pm 0.15
	Chapwani	20	0.35 \pm 0.02	245.27 \pm 56.38	12.20 \pm 1.09	32.47 \pm 2.72	2.28 \pm 0.36	3.23 \pm 0.08	8.84 \pm 0.50	0.90 \pm 0.02	3.05 \pm 0.09
	Bweleo	19	0.34 \pm 0.01	174.59 \pm 33.51	10.51 \pm 0.80	35.91 \pm 5.12	1.17 \pm 0.33	3.08 \pm 0.11	10.18 \pm 0.70	0.93 \pm 0.03	2.70 \pm 0.08
	Fumba	20	0.38 \pm 0.01	504.18 \pm 107.43	11.40 \pm 0.85	49.90 \pm 5.92	1.32 \pm 0.39	3.18 \pm 0.08	10.38 \pm 0.66	0.96 \pm 0.02	2.52 \pm 0.06
EA	Marumbi	19	1.09 \pm 0.04	236.37 \pm 4.66	10.96 \pm 1.02	37.81 \pm 0.21	0.00 \pm 0.00	3.77 \pm 0.12	44.48 \pm 2.47	1.57 \pm 0.06	5.21 \pm 0.09
	Fumba	20	1.00 \pm 0.03	236.37 \pm 0.00	9.57 \pm 0.76	37.81 \pm 0.00	0.00 \pm 0.00	3.30 \pm 0.14	43.44 \pm 2.32	1.32 \pm 0.04	4.33 \pm 0.12
HO	Chapwani	13	0.11 \pm 0.01	50.39 \pm 4.63	3.14 \pm 0.37	54.81 \pm 3.92	0.79 \pm 0.09	1.72 \pm 0.08	1.08 \pm 0.04	0.50 \pm 0.03	1.67 \pm 0.12
	Bweleo	20	0.12 \pm 0.01	50.21 \pm 4.54	3.39 \pm 0.29	51.41 \pm 5.36	1.34 \pm 0.10	1.93 \pm 0.04	1.78 \pm 0.11	0.78 \pm 0.03	1.54 \pm 0.07
HU	Mangroves	10	0.17 \pm 0.01	230.79 \pm 26.08	8.78 \pm 0.35	42.49 \pm 2.57	0.50 \pm 0.09	2.91 \pm 0.16	5.26 \pm 0.57	0.23 \pm 0.03	3.68 \pm 0.16
	Marumbi	15	0.18 \pm 0.01	439.94 \pm 52.64	7.19 \pm 0.32	50.01 \pm 2.79	3.05 \pm 0.49	2.62 \pm 0.13	10.97 \pm 0.71	0.25 \pm 0.01	3.92 \pm 0.24
	Chapwani	20	0.15 \pm 0.01	616.92 \pm 75.54	6.29 \pm 0.57	58.68 \pm 4.62	0.71 \pm 0.21	2.46 \pm 0.13	6.95 \pm 0.58	0.20 \pm 0.01	3.43 \pm 0.17
	Bweleo	20	0.16 \pm 0.01	357.01 \pm 59.31	6.10 \pm 0.62	43.31 \pm 2.76	1.12 \pm 0.26	2.77 \pm 0.17	7.41 \pm 0.35	0.22 \pm 0.01	2.54 \pm 0.08
SI	Changuu	20	0.20 \pm 0.01	217.73 \pm 20.92	7.23 \pm 0.37	53.98 \pm 2.94	0.43 \pm 0.04	1.36 \pm 0.07	6.23 \pm 0.44	0.13 \pm 0.00	2.36 \pm 0.07
	Chapwani	10	0.22 \pm 0.01	298.94 \pm 45.43	7.23 \pm 0.41	59.27 \pm 7.05	1.36 \pm 0.46	1.52 \pm 0.08	6.84 \pm 0.59	0.14 \pm 0.01	2.96 \pm 0.19
	Fumba	20	0.25 \pm 0.01	246.78 \pm 22.74	8.13 \pm 0.42	63.07 \pm 3.61	0.88 \pm 0.13	1.82 \pm 0.09	10.15 \pm 0.78	0.17 \pm 0.01	2.67 \pm 0.10
TC	Changuu	20	0.50 \pm 0.02	140.30 \pm 10.55	14.30 \pm 0.80	37.55 \pm 2.20	5.54 \pm 0.59	6.41 \pm 0.20	7.47 \pm 0.36	1.02 \pm 0.04	3.00 \pm 0.27
TH	Changuu	20	0.30 \pm 0.01	84.03 \pm 9.76	7.75 \pm 0.39	19.06 \pm 1.10	0.67 \pm 0.15	3.85 \pm 0.19	7.15 \pm 0.49	0.57 \pm 0.03	4.39 \pm 0.33
	Mangroves	20	0.36 \pm 0.02	317.85 \pm 27.38	5.45 \pm 0.40	29.53 \pm 2.48	1.56 \pm 0.26	4.98 \pm 0.27	6.10 \pm 0.45	0.71 \pm 0.04	5.05 \pm 0.50
	Marumbi	20	0.41 \pm 0.01	194.89 \pm 39.07	9.72 \pm 0.75	17.38 \pm 1.42	1.92 \pm 0.33	3.83 \pm 0.14	13.20 \pm 0.89	0.87 \pm 0.05	4.89 \pm 0.11
	Chapwani	19	0.31 \pm 0.02	68.01 \pm 9.97	8.43 \pm 0.82	17.57 \pm 1.23	0.91 \pm 0.16	4.01 \pm 0.19	5.89 \pm 0.47	0.54 \pm 0.02	4.21 \pm 0.13
	Bweleo	20	0.36 \pm 0.02	127.01 \pm 16.50	7.82 \pm 0.58	13.88 \pm 0.96	2.71 \pm 0.48	3.49 \pm 0.14	11.82 \pm 0.57	0.80 \pm 0.02	3.72 \pm 0.10
	Fumba	20	0.31 \pm 0.01	160.88 \pm 46.55	8.01 \pm 0.64	16.88 \pm 1.65	2.08 \pm 0.34	4.13 \pm 0.24	9.92 \pm 0.71	0.77 \pm 0.03	3.65 \pm 0.15
	Harbor	20	0.35 \pm 0.01	138.06 \pm 29.23	6.32 \pm 0.46	19.53 \pm 1.82	2.16 \pm 0.28	3.58 \pm 0.15	16.36 \pm 0.64	0.76 \pm 0.02	4.25 \pm 0.26

(2) Supplementary material 2. Site clustering in trophic states

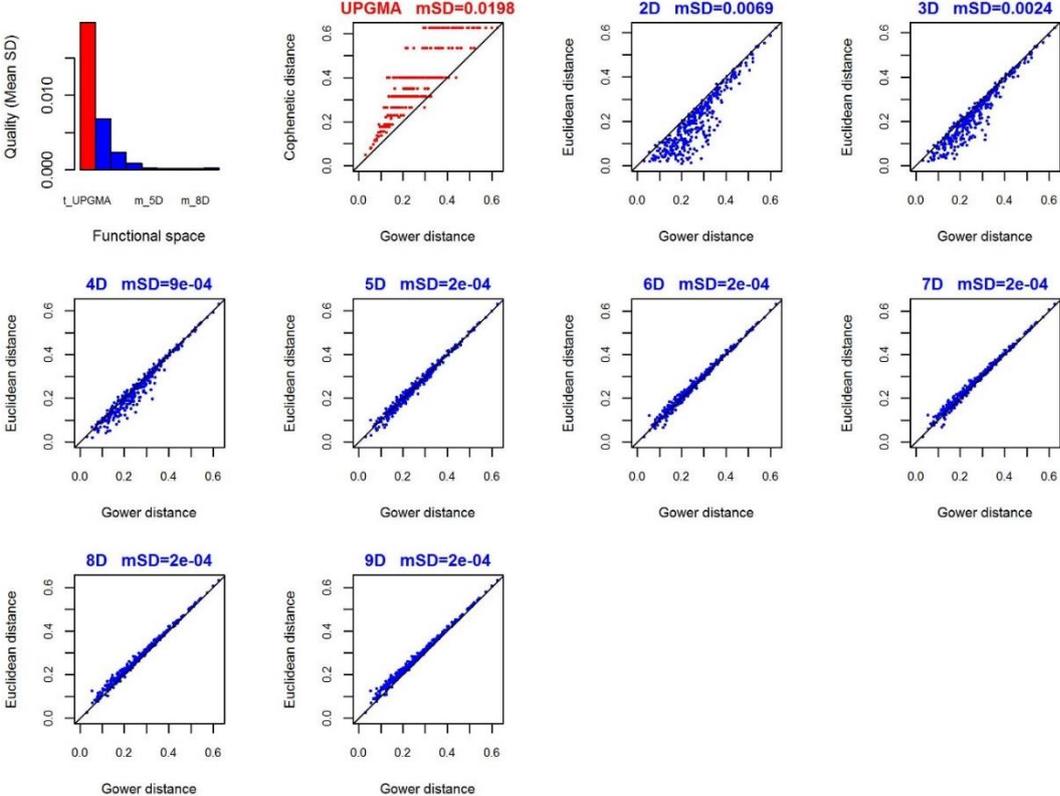
Silhouette width of the sites in the trophic clusters



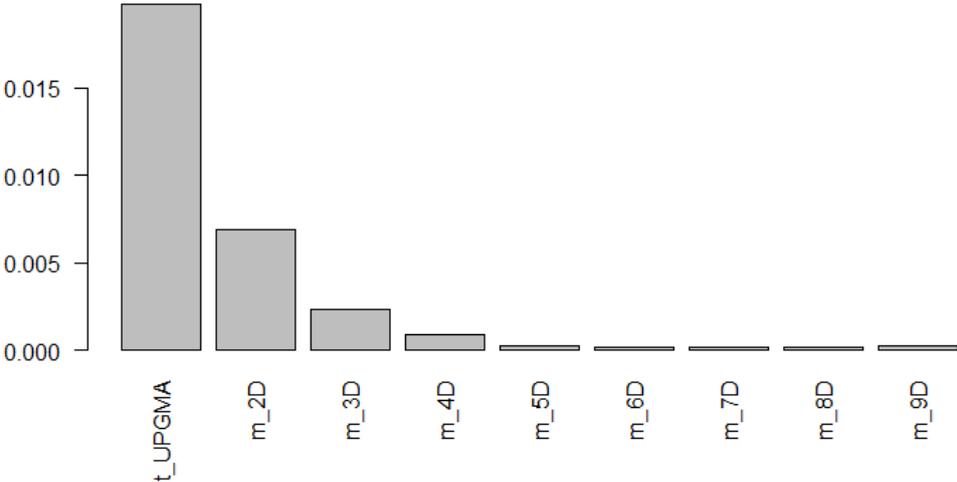
(3) Supplementary material 3. Ordination diagnostics

(a) Quality of representation of the PCA

i. mSD calculation:



ii. Comparison of mSDs for principal components retention:



(4) Supplementary material 4. Effect of difference in the functional strategy of seagrass on space preemption

Species pairs: Number of plots per species pair and site

Trophic state	Site	Species pair		Number of plots	Preemption	No preemption	
Oligotrophic	Changuu	<i>C. serrulata</i>	<i>S. isoetifolium</i>	16	12	4	
			<i>T. ciliatum</i>	10	4	6	
			<i>T. hemprichii</i>	17	6	11	
		<i>S. isoetifolium</i>	<i>C. serrulata</i>	16	3	13	
			<i>T. ciliatum</i>	11	2	9	
			<i>T. hemprichii</i>	23	5	18	
		<i>T. ciliatum</i>	<i>C. serrulata</i>	10	5	5	
			<i>S. isoetifolium</i>	11	9	2	
			<i>T. hemprichii</i>	14	8	6	
		<i>T. hemprichii</i>	<i>C. serrulata</i>	17	7	10	
			<i>S. isoetifolium</i>	23	14	9	
			<i>T. ciliatum</i>	14	4	10	
	Marumbi		<i>C. rotundata</i>	<i>T. hemprichii</i>	8	1	7
			<i>E. acoroides</i>	<i>T. hemprichii</i>	10	6	4
			<i>H. uninervis</i>	<i>T. hemprichii</i>	13	4	9
			<i>T. hemprichii</i>	<i>C. rotundata</i>	8	6	2
				<i>E. acoroides</i>	10	2	8
				<i>H. uninervis</i>	13	7	6
Mesotrophic	Bweleo	<i>C. rotundata</i>	<i>C. serrulata</i>	6	0	6	
			<i>H. ovalis</i>	10	3	7	
			<i>H. uninervis</i>	10	2	8	
			<i>T. hemprichii</i>	9	1	8	
		<i>C. serrulata</i>	<i>C. rotundata</i>	6	4	2	
			<i>H. uninervis</i>	17	11	6	
			<i>T. hemprichii</i>	17	10	7	
		<i>H. ovalis</i>	<i>C. rotundata</i>	10	1	9	
			<i>H. uninervis</i>	13	7	6	
			<i>T. hemprichii</i>	7	1	6	
		<i>H. uninervis</i>	<i>C. rotundata</i>	10	6	4	
			<i>C. serrulata</i>	17	4	13	
			<i>H. ovalis</i>	13	4	9	
			<i>T. hemprichii</i>	18	4	14	
			<i>T. hemprichii</i>	<i>C. rotundata</i>	9	5	4
				<i>C. serrulata</i>	187	4	13
		<i>H. ovalis</i>		7	5	2	
		<i>H. uninervis</i>		18	10	8	
	Chapwani	<i>C. rotundata</i>	<i>H. uninervis</i>	5	3	2	
			<i>T. hemprichii</i>	6	1	5	
		<i>C. serrulata</i>	<i>S. isoetifolium</i>	8	5	3	
			<i>T. hemprichii</i>	10	5	5	
		<i>H. ovalis</i>	<i>H. uninervis</i>	6	0	6	
			<i>T. hemprichii</i>	5	0	5	
		<i>H. uninervis</i>	<i>C. rotundata</i>	5	1	4	
			<i>H. ovalis</i>	6	6	0	
			<i>S. isoetifolium</i>	5	1	4	
			<i>T. hemprichii</i>	19	1	18	
		<i>S. isoetifolium</i>	<i>C. serrulata</i>	8	2	6	
			<i>H. uninervis</i>	5	4	1	
<i>T. hemprichii</i>	12		3	9			
<i>T. hemprichii</i>	<i>C. rotundata</i>	6	5	1			
	<i>C. serrulata</i>	10	5	5			
	<i>H. ovalis</i>	5	5	0			
	<i>H. uninervis</i>	19	18	1			
	<i>S. isoetifolium</i>	12	9	3			
Fumba	<i>E. acoroides</i>	<i>S. isoetifolium</i>	5	2	3		
		<i>T. hemprichii</i>	6	4	2		
	<i>S. isoetifolium</i>	<i>E. acoroides</i>	5	3	2		
	<i>T. hemprichii</i>	<i>E. acoroides</i>	6	2	4		
Eutrophic	Harbor	<i>C. rotundata</i>	<i>T. hemprichii</i>	5	2	3	
		<i>T. hemprichii</i>	<i>C. rotundata</i>	5	3	2	

(5) Supplementary material 5. Cover and preemption: pairwise comparison

(a) Model 1: Pairwise differences in the % cover species within trophic state

Response variable	Trophic state	Species pairs	z-value	p-value
% Seagrass cover	Oligotrophic	CR-CS	2.884	0.0039**
		CR-EA	2.092	0.0364*
		CR-HU	1.134	0.2570
		CR-SI	0.483	0.6289
		CR-TC	2.783	0.0053**
		CR-TH	2.519	0.0117*
		CS-EA	0.567	0.5706
		CS-HU	2.063	0.0391*
		CS-SI	3.485	0.0004***
		CS-TC	0.085	0.9324
		CS-TH	1.004	0.3152
		EA-HU	1.198	0.2307
		EA-SI	2.175	0.0297*
		EA-TC	0.489	0.6249
		EA-TH	0.108	0.9138
		HU-SI	0.942	0.3461
		HU-TC	1.945	0.0518
		HU-TH	1.548	0.1216
		SI-TC	3.301	0.0009***
	SI-TH	3.297	0.0009***	
	TC-TH	0.866	0.3867	
	Mesotrophic	CR-CS	4.931	0.0000***
		CR-EA	2.619	0.0088**
		CR-HO	0.548	0.5834
		CR-HU	0.471	0.6375
		CR-SI	2.717	0.0065**
		CR-TH	4.054	0.0000***
		CS-EA	0.063	0.9490
		CS-HO	5.620	0.0000***
CS-HU		5.602	0.0000***	
CS-SI		1.513	0.1300	
CS-TH		1.510	0.1310	
EA-HO		2.933	0.0033**	
EA-HU		2.467	0.0136*	
EA-SI		0.932	0.3512	
EA-TH		0.712	0.4763	
HO-HU		1.129	0.2588	
HO-SI		3.264	0.0011**	
HO-TH		4.790	0.0000***	
HU-SI		2.737	0.0062**	
HU-TH	4.690	0.0000***		
SI-TH	0.498	0.6183		

(b) Model 2: Pairwise differences in the % cover within a seagrass species among trophic states

Response variable	Species	Trophic states	z-value	p-value
%Seagrass cover	<i>Cymodocea rotundata</i>	Oligotrophic-Mesotrophic	0.243	0.8082
		Oligotrophic-Eutrophic	2.437	0.0148*
		Mesotrophic-Eutrophic	3.044	0.0023**
		<hr/>		
	<i>Halodule uninervis</i>	Oligotrophic-Mesotrophic	1.982	0.0475*

(c) Model 3: Pairwise differences in the probability of preemption of species within trophic state

Response variable	Trophic state	Species pairs	z-value	p-value
Probability of preemption	Oligotrophic	CR-CS	1.384	0.167
		CR-EA	1.102	0.270
		CR-HU	0.745	0.456
		CR-SI	0.531	0.596
		CR-TC	1.978	0.048*
		CR-TH	1.219	0.223
		CS-EA	0.071	0.943
		CS-HU	0.696	0.486
		CS-SI	3.068	0.002**
		CS-TC	1.033	0.301
		CS-TH	0.433	0.664
		EA-HU	0.470	0.638
		EA-SI	2.048	0.040*
		EA-TC	0.779	0.435
		EA-TH	0.187	0.851
		HU-SI	1.698	0.089
		HU-TC	1.461	0.144
		HU-TH	0.449	0.653
		SI-TC	3.845	0.000***
		SI-TH	3.073	0.002**
	TC-TH	1.562	0.118	
	Mesotrophic	CR-CS	4.000	0.000***
		CR-EA	1.732	0.083
		CR-HO	0.320	0.749
		CR-HU	0.534	0.593
		CR-SI	2.215	0.026*
		CR-TH	4.038	0.000***
		CS-EA	0.800	0.423
		CS-HO	4.170	0.000***
		CS-HU	4.283	0.000***
		CS-SI	1.476	0.139
		CS-TH	0.358	0.720
EA-HO		1.928	0.053	
EA-HU		1.512	0.130	
EA-SI		0.167	0.867	
EA-TH		0.643	0.520	
HO-HU		0.876	0.381	
HO-SI		2.451	0.014*	
HO-TH		4.203	0.000***	
HU-SI	2.049	0.040*		
HU-TH	4.481	0.000***		
SI-TH	1.306	0.191		

(d) Model 4: Pairwise differences in the probability of preemption within a seagrass species among trophic states

Response variable	Species	Trophic states	z-value	p-value
Probability of preemption	<i>Syringodium isoetifolium</i>	Oligotrophic-Mesotrophic	2.622	0.0087**

(6) Supplementary material 6. Results of PCA

(a) Eigenvalues of the PCs of the multidimensional space:

Principal component	Eigenvalue
PC 1	3.81
PC 2	1.82
PC 3	1.11
PC 4	1.01
PC 5	0.50
PC 6	0.41

(b) Functional traits: Test of significant correlations to the six PCs of the multidimensional space

Following Yamamoto et al. (2014), the t-statistic of the correlation can be calculated as:

$$t = \frac{FT_{corr} * \sqrt{n - 2}}{\sqrt{1 - (FT_{corr})^2}}$$

Where FT_{corr} is the correlation value of the functional traits to the multidimensional space d and n is the number of replicates. The t-values for each functional trait and PC can be found in the following table. Significance was set at $p < 0.05$ and is marked in boldface and underlined.

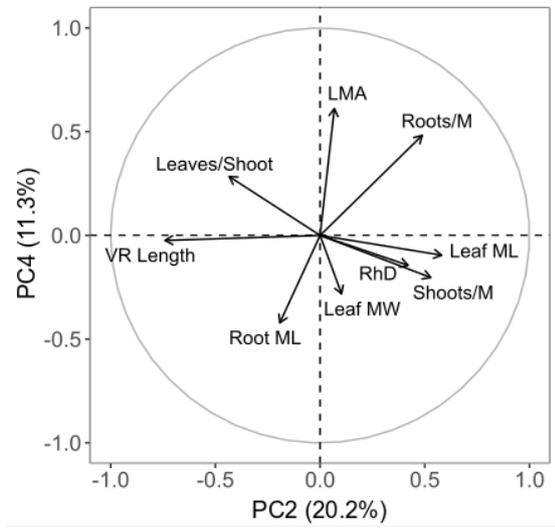
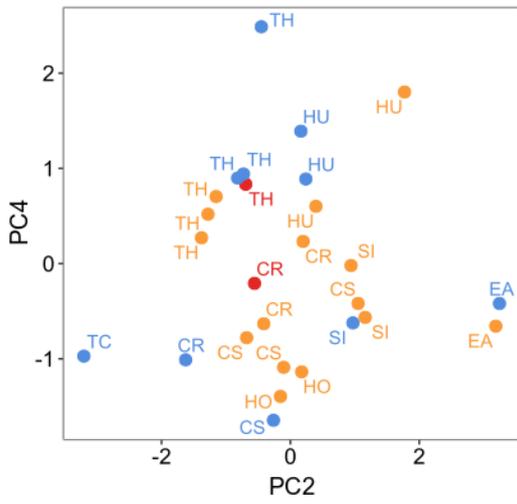
Functional trait	t – value (df = 26, t-critic = 2.056)					
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Rhizome diameter	<u>9.389</u>	<u>2.361</u>	0.174	-0.744	0.287	0.446
Root maximum length	<u>3.468</u>	-1.001	<u>2.656</u>	<u>-2.372</u>	<u>-2.649</u>	-1.072
Shoots/Meter	<u>-4.121</u>	<u>3.177</u>	<u>2.293</u>	-1.059	0.689	0.249
Roots/Meter	-0.914	<u>2.847</u>	<u>4.541</u>	<u>2.806</u>	0.161	-0.536
Leaves/Shoot	<u>5.181</u>	<u>-2.458</u>	1.011	1.509	1.406	-1.306
Vertical rhizome length	0.703	<u>-5.621</u>	<u>2.830</u>	-0.123	0.537	<u>2.421</u>
Leaf maximum length	<u>5.709</u>	<u>3.636</u>	-0.110	-0.490	-0.224	1.518
Leaf maximum width	<u>8.093</u>	0.526	-0.033	-1.500	1.952	-0.666
Leaf mass area	<u>5.095</u>	0.348	-0.714	<u>3.944</u>	-1.294	0.482

(c) Species centroids across the study area in the rest of the PCs:

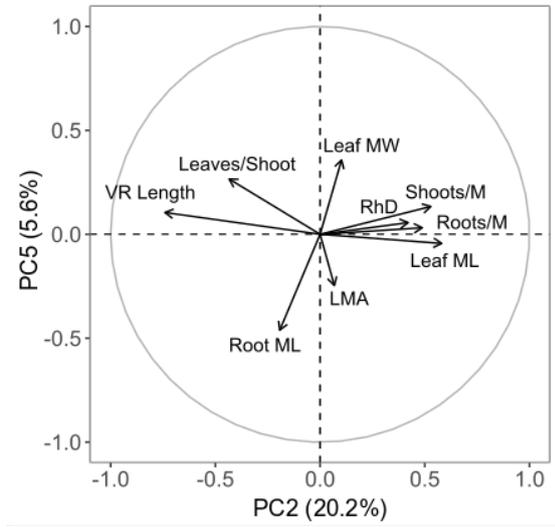
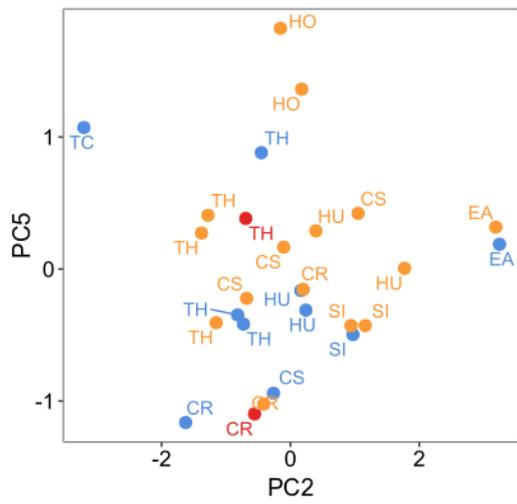
PC4 (Tables 4 and 5) indicated that only *H. uninervis* and *T. hemprichii* showed a certain correlation between root density and length, which in previous PCs were inversely correlated. In the last two PCs (PC 5 and 6), there were only unique trait correlations and the portion of

Trophic state ● Low ● Intermediate ● High

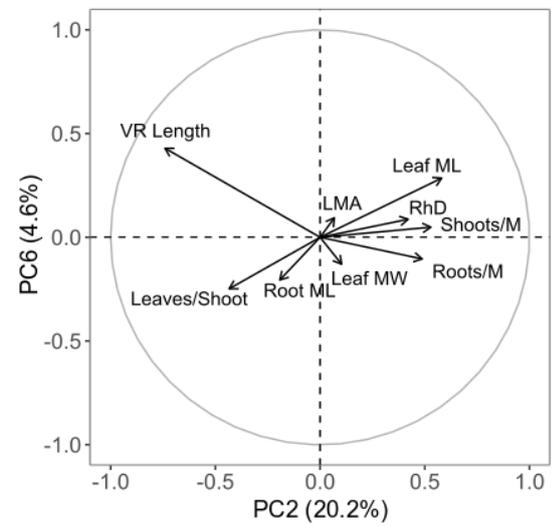
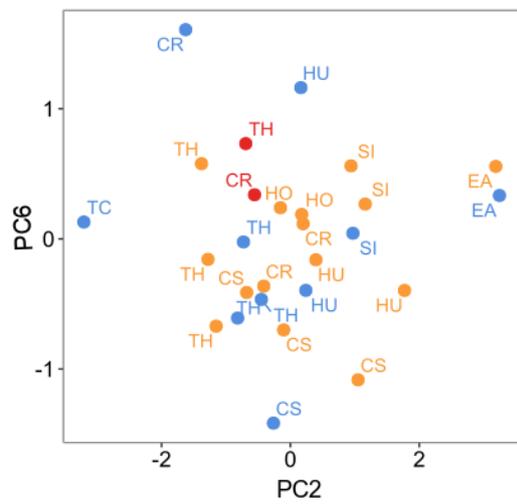
f)



g)

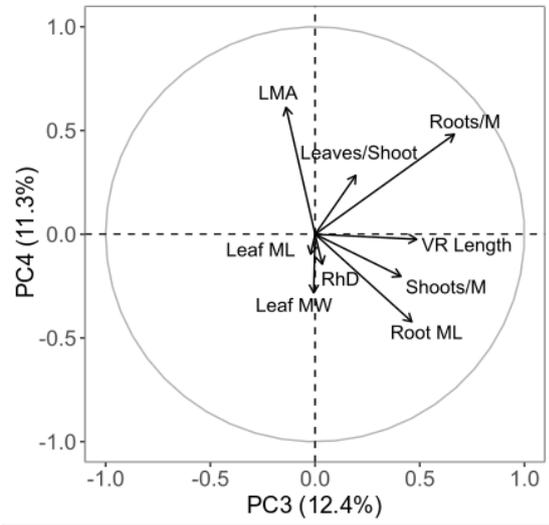
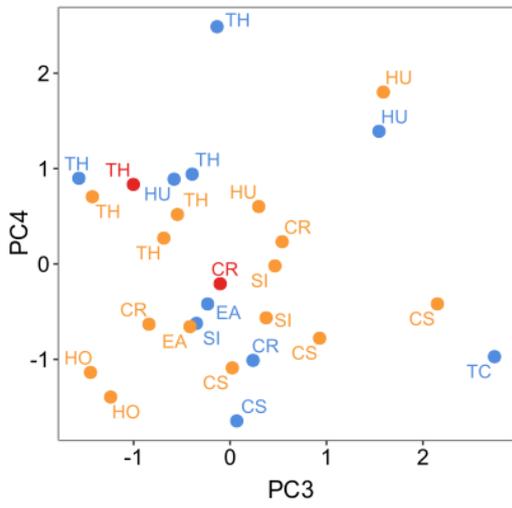


h)

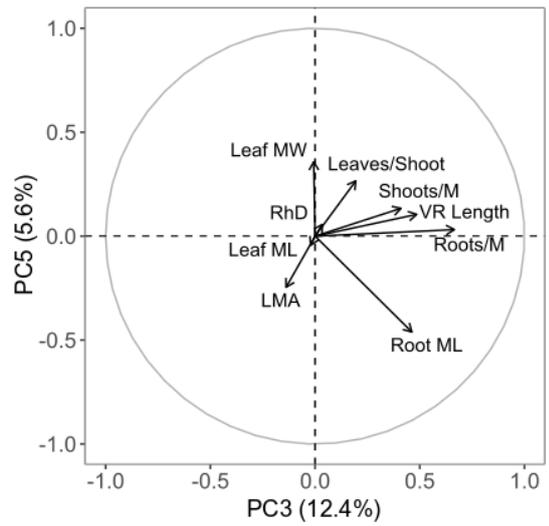
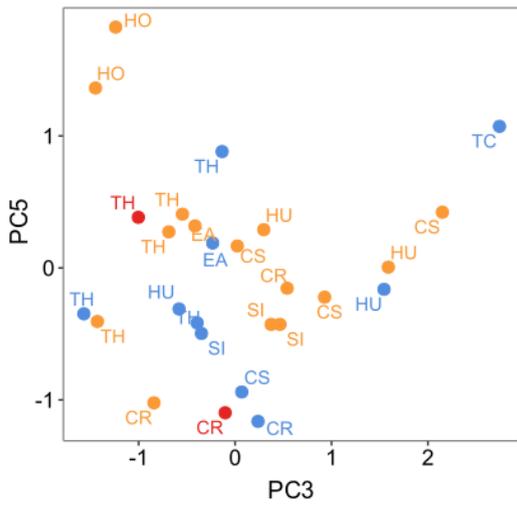


Trophic state ● Low ● Intermediate ● High

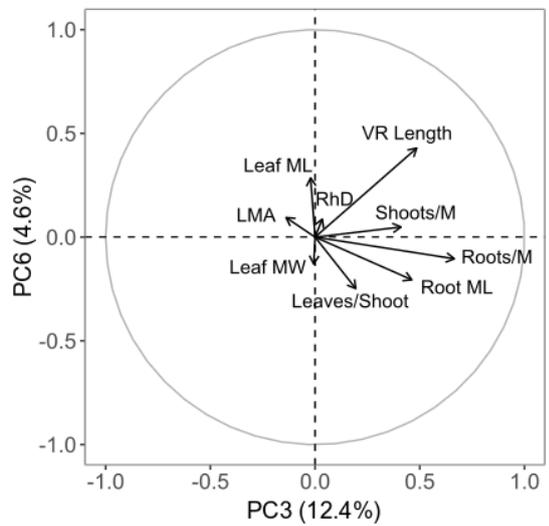
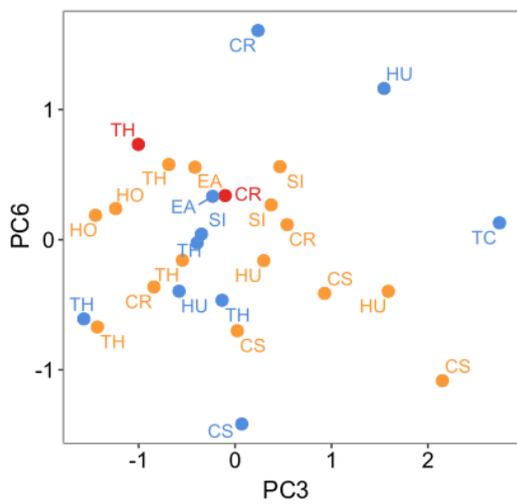
i)



j)

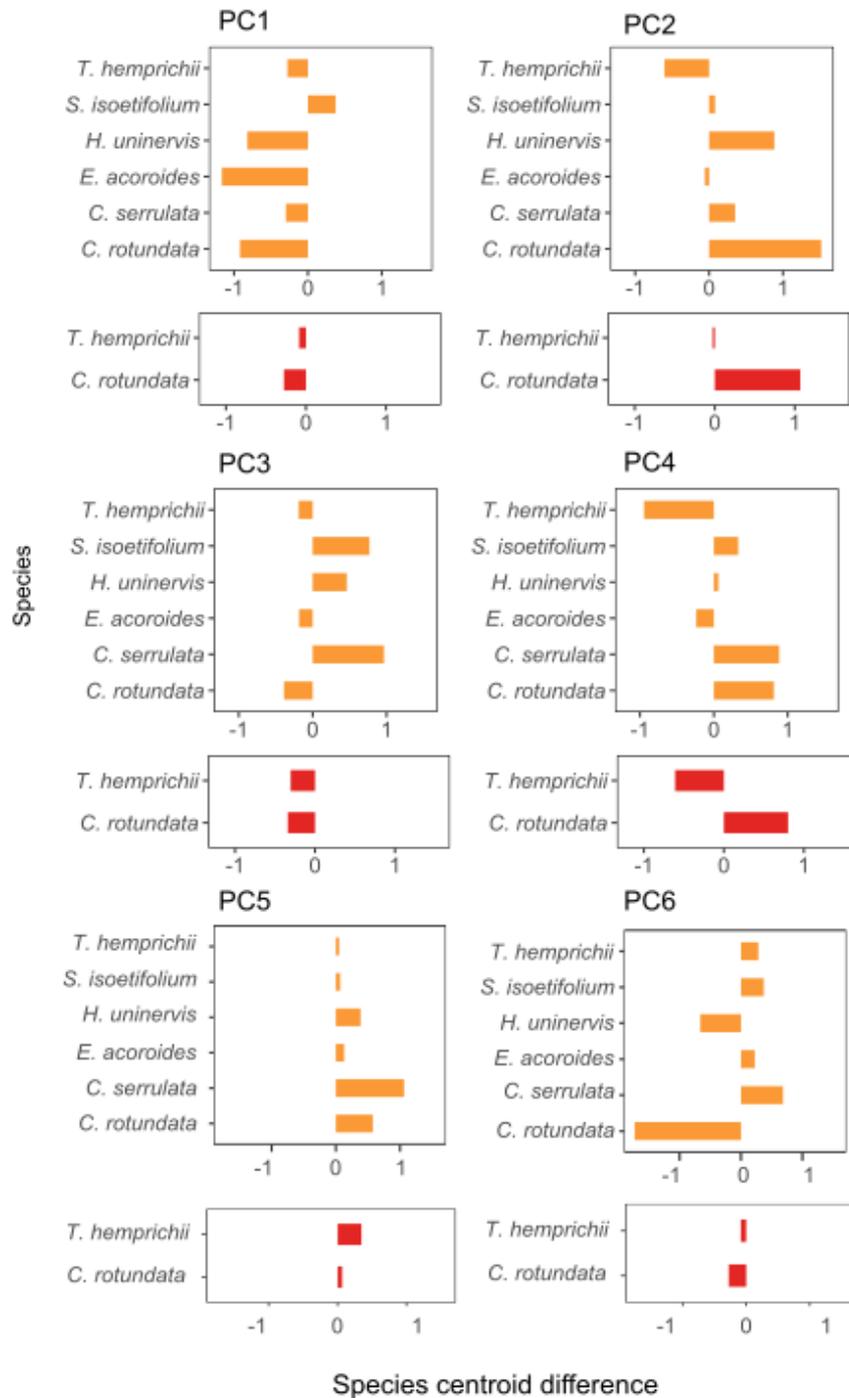


k)



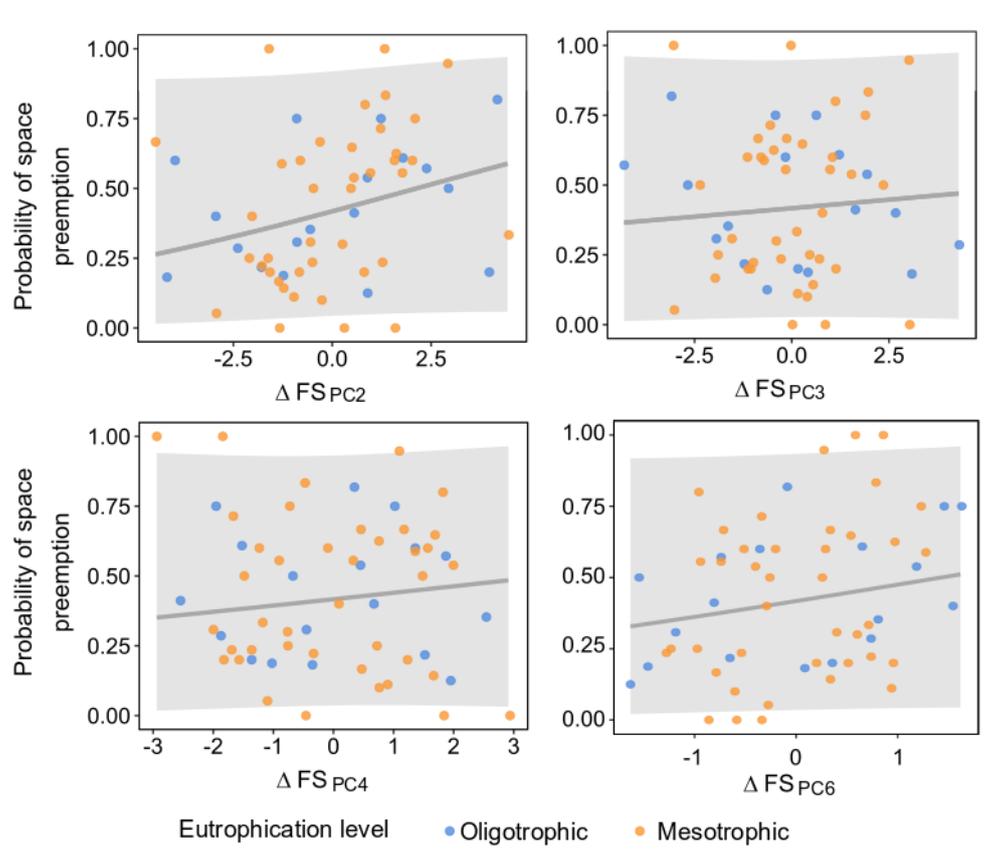
(7) Supplementary material 7. Trophic state effect on species centroids in all PCs

Orange bars represent the difference between the species centroids in the oligotrophic and mesotrophic states, and the red bars the difference between the oligotrophic and eutrophic states.



(8) Supplementary material 8. Probability of space preemption in PC2, PC3, PC4 and PC6

Results of effect of ΔFS of PC2, PC3, PC4 and PC6





CHAPTER 7.

Sediment properties and seagrass functional traits control carbon storage in tropical seagrass meadows

Agustín Moreira-Saporiti^{1,2}, Elizabeth Fay Belshe¹, Inés G. Viana³, Aoife O'Sullivan⁴, Matern Mtolera⁵, Mirta Teichberg¹

¹Leibniz Centre for Tropical Marine Research GmbH, Bremen, Germany, ²Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany, ³ Instituto Español de Oceanografía, Centro Oceanográfico de A Coruña, Galicia, Spain, ⁴University of Groningen, 9712 CP Groningen, Netherlands, ⁵Institute of Marine Sciences, University of Dar es Salaam, University Road, P.O Box 35060, Dar es Salaam, Tanzania

Abstract

Seagrass meadows are important carbon sinks worldwide, as these ecosystems accumulate organic carbon in their sediments that is stored for long periods of time. Seagrass sediment carbon accumulation and storage are the result of a variety of interacting factors such as seagrass trait-level controls on both sediment trapping and organic carbon inputs and properties of the sediment that mediate the decomposition of carbon inputs. In this study, we aimed to determine which factor(s) influence carbon storage within the sediment from seagrass meadows of Unguja Island (Zanzibar Archipelago, Tanzania). For this reason, we selected 6 different seagrass meadows and collected 18 sediment cores to measure organic carbon stocks and grain size within seagrass. In tandem, we measured the seagrass abundance and functional traits linked to particle trapping and flow reduction (leaf area index, LAI, and plant height), and traits linked to belowground carbon deposition and depth distribution (belowground biomass, rhizome diameter, roots m⁻¹ and root maximum length). We show that the sampling site was of minor importance, while sediment grain size was the most important driver. Very fine sediments (<125 µm) were negatively correlated to organic carbon in the sediment, while medium-fine sands were positively correlated. In the case of seagrass functional traits, LAI was positively correlated to organic carbon content in the sediment, indicating an effect of particle trapping and retention. Root maximum length was the most important functional trait driving carbon storage, suggesting that rooting depth is of fundamental importance for carbon accumulation. These results indicate that the estimation of the carbon stocks in tropical seagrass meadows in Zanzibar depend on factors at both the organismal and sediment level, while the site where the meadow was located played a minor role.

Keywords: rooting depth, trait-based approach, particle trapping, blue carbon, grain size

I. Introduction

Seagrass are a polyphyletic assemblage of angiosperms plants that inhabit the coastal area and develop their whole life cycle in the ocean. They are one of the most important coastal ecosystems due to a number of services they provide (Constanza et al. 1997), like nutrient filtering (Hemminga et al. 1991) and habitat provision (Orth et al. 1984). They have undergone adaptations to the marine realm, inhabiting a thin strip of coastal sea (Olsen et al. 2016). Unfortunately, this same habitat is also under high and increasing anthropogenic pressure. As a consequence, seagrasses are under decline worldwide due to multiple local and global pressures (Orth et al 2006, Waycott et al. 2009), even though this trend may be reversed in some areas (de los Santos et al. 2019).

The study of how matter circulates in the biosphere constitutes one of the fundamental research objectives for ecologists: stocks of materials, their distribution and fluxes between ecological compartments are basic parameters that need to be characterized to explain the functioning of any ecosystem (Mateo et al. 2006). This fundamental research question has been brought into the forefront due to the concept of blue carbon, which highlights the potential of different marine ecosystems to capture and store carbon, aiding climate regulation (Nellemann and Corcoran 2009). In the last decade, seagrass meadows received renewed attention under the blue carbon umbrella due to their potential to store organic carbon in their sediments (e.g. Fourqurean et al. 2012; Greiner et al. 2013; Alongi et al. 2016; Oreska et al. 2017). The carbon stocks in seagrass meadows result from a budget over space and time, which is the balance between production and decay rates of organic material (Romero et al. 1992). Organic carbon within seagrass meadows is made up of the living biomass of the plants, and the non-living organic matter and microbial biomass within the sediment. For this work, our focus is on the organic carbon within the sediment and not the living seagrass biomass.

The organic carbon stocks in seagrass sediments are highly variable, as shown by the blue carbon literature, with stocks ranging from 115.3 to 829.2 Mg C_{org} ha⁻¹ (Fourqurean et al. 2012). While a climax species like *Enhalus acoroides* can store up to 239.2 Mg C ha⁻¹ (Prirosambodo 2006), mixed meadows of the species with smaller sizes such as *Halodule uninervis*, *Halophila stipulacea* and *Halophila ovalis* have been reported to store a mean of 49.1 Mg C ha⁻¹ (Campbell et al. 2015). This demonstrates that sediment carbon storage can be species dependent, and these species-specific differences could be attributed to the traits that determine the characteristics of the seagrass plants.

Traits are defined as “any morphological, physiological or phenological heritable feature measurable at the individual level, from the cell to the whole organism, without reference to the environment or any other level of organization” (Violle et al. 2007 as modified by Garnier et al. 2016). The study of traits, therefore, allows us to understand the relationships between organisms from a functional perspective. Additionally, trait-based ecology assumes that structure at higher organizational scales is largely a result of the composite traits of the individuals (Grime 1998; Shipley et al. 2016). Although a trait-based approach can be applied to all kinds of organisms, it is currently most developed in terrestrial plants (Lavorel and Garnier 2002; Garnier et al. 2016), but it is used also in marine organisms (Litchmann and Klausmeier 2008; Andersen and Pedersen 2009; Litchman et al. 2010; Lichtmann et al. 2013; Elleouet et al. 2014). Seagrass functional traits have been scarcely related to ecosystem functions, with a few remarkable exceptions, finding that functional traits underpin primary production in marine plant communities (Gustafsson and Norkko 2016; Jänes et al. 2017) or mediate herbivory and predation in seagrass ecosystems (Pagès et al. 2012; Lewis and Boyer 2014). When a trait-based approach was applied to the study of carbon storage, some of the traits selected (i.e. shoot density, biomass and C:N ratio) were unrelated with organic carbon in the sediment (Belshe et al. 2018), while others, such as canopy structural complexity (Samper-Villarreal et al. 2016) and belowground biomass (Dahl et al. 2016) had a significant effect.

However, other functional traits can be potentially related to carbon storage. The traits that configure the aboveground morphology of seagrass have been directly linked to particle trapping (Wilkie et al. 2012) and flow reduction. In the case of particle trapping, a developed seagrass canopy can trap large amounts of particles (Hasegawa et al. 2008), while the stiffness of seagrass shoots and their density affect their capacity to accrete sediment from the water column (Peralta et al. 2008). In the case of flow reduction, blade stiffness, shoot density and leaf length are important drivers of wave attenuation (Bouma et al. 2005, Paul et al. 2012). Particles trapped by seagrass are an important contributor to belowground carbon stocks (Marbà et al. 2015), and should be taken into consideration when estimating carbon storage. Belowground structures (rhizomes, roots and, additionally, sheaths) are directly deposited into the sediment and, therefore, represent a major autochthonous source of organic matter. This biomass compartment is less susceptible to transport, and completes the cycle of production and decay at the site of its formation (Kenworthy and Thayer 1984). In one of the first works studying belowground biomass decay, Kenworthy and Thayer (1984) showed that both temperate (*Zostera marina*) and the subtropical (*Thalassia testudinum*) belowground biomass decomposes slowly and there is a higher proportion of refractory material in the belowground

structures than in the leaves. *Posidonia oceanica* is one of the seagrass species that has been shown to store a high amount of carbon in the sediment due to the formation of a “matte” (Romero et al. 1994), which has a turnover rate of the order of centuries (Aloisi et al. 1975; Romero et al. 1992). In addition to the higher refractory nature of the belowground materials (Zeikus 1981), most of the seagrass plants show higher biomass belowground than aboveground (Brouns 1987), albeit the biomass allocation is species specific. Traits related to the amount of belowground seagrass biomass, rhizomatic and root structures and rooting depth will likely have an impact on the organic carbon stocks.

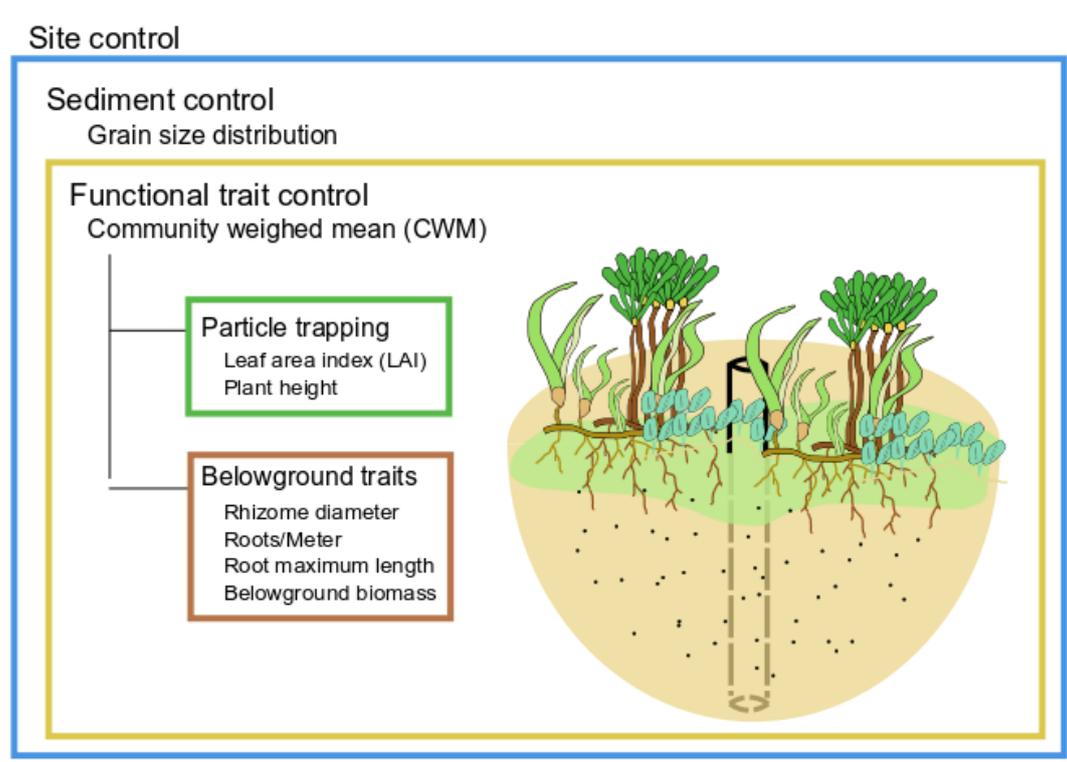


Figure 1. Conceptual figure illustrating the possible causes of variation in the carbon stocks in the sediments of seagrass meadows. The site and sediment establish the boundary conditions for the organic carbon, whereas the functional traits of the seagrass can control the organic carbon stored in the sediments at the patch level.

Despite the links that could be established between seagrass traits and carbon storage, the environmental conditions of the seagrass habitat determine the boundary conditions for the development of a carbon stock (Belshe et al. 2017; Mazarrasa et al. 2018). The sediment has a strong influence on organic carbon stocks, specifically grain size and type (Dahl et al. 2016; Röhr et al. 2016; Serrano et al. 2016). Additionally, landscape conditions like the flow regime and the bathymetry, can also affect carbon stocks (Belshe et al. 2018), adding more layers of complexity to the correct estimation of carbon stocks by using seagrass traits (Gullström et al. 2018). Based on these studies, we hypothesize that seagrass functional traits will have an

influence on organic carbon stocks in the sediments only under certain site and parent material constraints (Fig. 1).

For this purpose, in this work, we quantified the relative importance of site conditions, sediment grain size, and seagrass functional traits for the storage of organic carbon in seagrass meadows. We collected sediment cores in six seagrass meadows in Unguja Island (Zanzibar Archipelago, Tanzania), and characterized the sediment by analyzing its grain size distribution. To study the links between seagrass functional traits and carbon storage, we calculated the community weighed mean of relevant traits for particle trapping (leaf area index, plant height) and belowground carbon deposition (belowground biomass, rhizome diameter, roots/meter and root length).

II. Materials and Methods

(1) Description of study sites

Unguja Island is the most populated island in the tropical archipelago of Zanzibar (~900.000 inhabitants), off the coast of Tanzania in the Western Indian Ocean (Fig. 2), and one of the main hotspots of seagrass biodiversity in the world (Short et al. 2007). Seagrass communities are mainly formed by 8 species: *Cymodocea rotundata*, *Cymodocea serrulata*, *Enhalus acoroides*, *Halophila ovalis*, *Halodule uninervis*, *Syringodium isoetifolium*, *Thalassodendron ciliatum* and *Thalassia hemprichii*. We surveyed six different sites (Fig. 2) in which we found mixed seagrass communities. Each of the six study sites comprised a shallow subtidal seagrass meadow (up to ~4 meters depth) of ~10000 m² generally bounded by a coastal rocky or sandy area and a fringing coral reef. The water depth ranged in all sites between 0.5 and 4 meters due to the tidal regime. All six study sites were surveyed between July and October 2017.

(2) Sampling design

In each site, we deployed five 50 meters transects perpendicular to the coast and parallel to each other, separated by ~50 meters. Seagrass shoot density was measured in nine 10x10cm quadrats per transect. We collected one surface sediment sample (n=5) and one biomass core per transect (n=5) to determine the biomass of the seagrass species.

As a result of the deployment of the transects, we enclosed four sampling areas between them. We took one sediment core in three out of the four areas enclosed by the transects. In the location where the sediment core was taken, we characterized the abundance of the seagrass

species and we sampled five ramets (i.e. train of at least two shoots) of the seagrass species present to measure their functional traits (n=5 per species and sampling area).

(3) Sediment control: grain size distribution

For the characterization of the sediment grain size distribution, the surface sediment samples were stored at -20°C and transported frozen to the Leibniz Centre for Tropical Marine Research (ZMT) in Bremen (Germany). They were then dried at 50°C until constant dry weight. The samples were sieved in a stack-shaker sieve for 10 min, passing through 2mm, 1mm, 500 μm , 250 μm , 125 μm and 63 μm sieves. The amount of sediment trapped in each sieve was then weighed in a scale to the nearest milligram, and the percentage contribution of each grain size calculated.

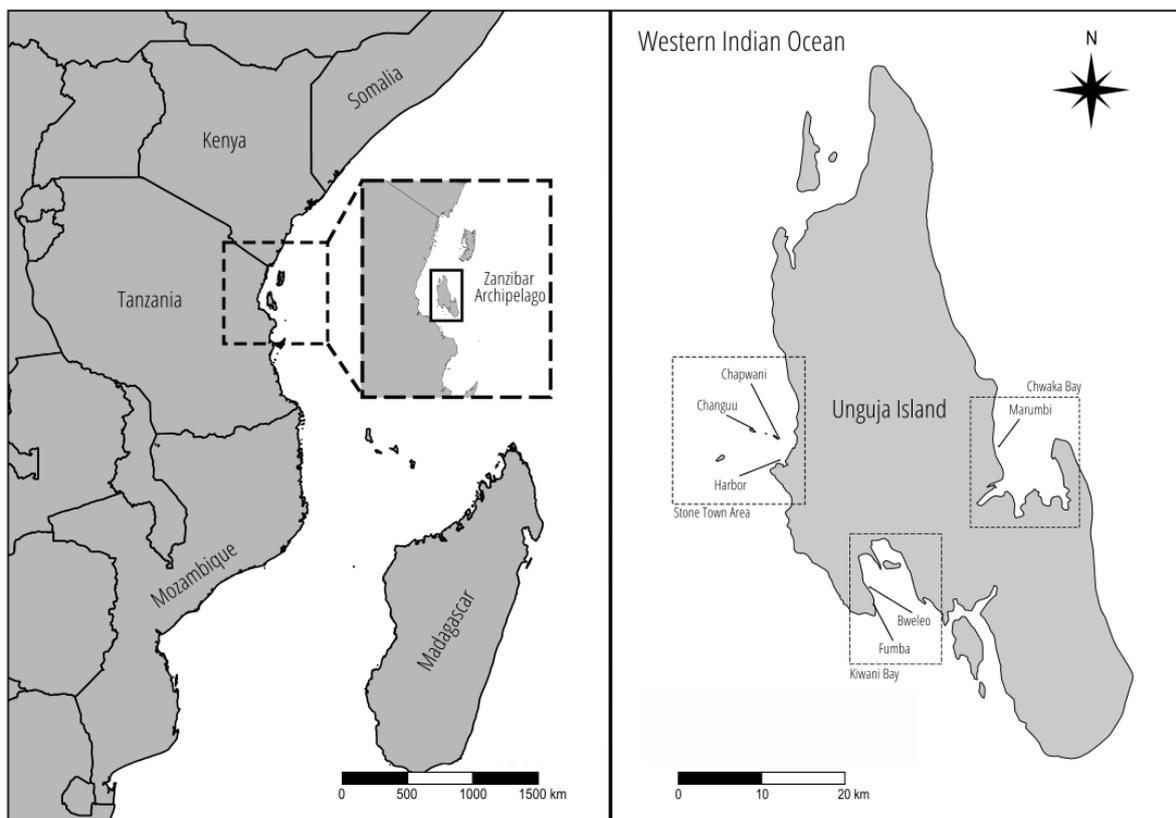


Figure 2. Study area for this work. Unguja Island is located in the Western Indian Ocean, part of the Zanzibar Archipelago in the coast of Tanzania (Left). The sampling sites were located in three main areas, which are the coast of Stone Town, the biggest city in Unguja Island. Kiwani Bay is located in the south of the island and Chwaka Bay is located in the east coast.

(4) Functional trait control

To characterize the functional traits of the seagrass species, the ramets were stored at -20°C and transported frozen to the ZMT for their measurement.

(a) Flow attenuation and particle trapping

The traits measured for flow attenuation and particle trapping were leaf area index (LAI) and plant height. Plant height (cm) was measured to the nearest millimeter with a ruler as the distance between the base of the vertical rhizome to the tip of the longest leaf of the seagrass shoot. Plant height was measured for all the shoots in a ramet and then averaged per ramet. LAI was calculated as the multiplication between shoot density, leaves per shoot and leaf area. For this purpose, we calculated shoot density of a species as the number of shoots counted in the 10x10 cm quadrats, standardized by the area of the quadrat (shoots hectare⁻¹). We counted the leaves per shoot in each shoot of the ramet. Finally, we calculated the leaf area as the multiplication of the maximum leaf length and width per shoot, which were measured with a ruler to the nearest millimeter. The shoot-level measurements were averaged per ramet and multiplied by the shoot density, resulting in leaf area m² hectare⁻¹.

(b) Belowground traits

The traits measured to characterize the belowground characteristics of the seagrass species were belowground biomass, rhizome diameter, root maximum length and roots/meter. Rhizome diameter (cm) and root maximum length (cm) were measured with a ruler to the nearest millimeter. For the calculation of roots meter⁻¹, we counted the number of roots per ramet and then divided the number by the ramet length. For the measurement of belowground biomass, biomass cores were pushed into the sediment (diameter = 20 cm), and the material trapped was scooped into a sampling bag. The biomass cores were cleaned with freshwater to separate the seagrass biomass from the sediment and the aboveground (leaves) and belowground biomass (sheath, rhizome, roots) were separated. Lastly, the biomass was dried at 50°C until constant dry weight (DW), and divided by the core area to obtain kg DW hectare⁻¹.

(5) Sediment organic carbon

In order to quantify the organic carbon in the seagrass sediment, used a 4.8 cm diameter core that was 60 cm long that was hammered 50 cm down into the sediment. Secondly, the length from the top of the core to the sediment surface on the outside and inside the sediment was measured to estimate sediment compression. Before extraction, we measured the percentage seagrass cover on top of each core using a 0.5 x 0.5 meters quadrat to quantify the relative abundance of seagrass species. Lastly, the core was pulled out of the sediment, stored in a cool dry location and transported to the ZMT, where all cores were frozen until being processed in the lab.

In the lab, cores were opened by cutting the core's pvc tubes on each side with a Dremel, and then split longitudinally into two halves using a knife. Opened cores were photographed with a high-resolution camera, and then one hemi-core was subdivided into 2.5-5 cm slices depending on the heterogeneity of the sediment. Sediment subsections (slices) were weighed before and after drying at 40°C until constant weight, and sediment dry bulk density (DBD; g cm⁻³) was calculated as the dry weight of sediment per volume of each sample. The volume of each core slice was calculated for each hemi-core's specific dimensions, to account for any uneven longitudinal cutting of the cores. Dried sediments were homogenized in a ball mill and percentage of OC was determined, after acidification with 1 M HCL to remove carbonates, on an elemental analyzer (Euro EX 3000; Eurovector). The OC density (CC; g C cm³) of each slice was calculated from measured %OC and the DBD of the slice following Eq. (1):

$$CC_{\text{slice}} = z_{\text{slice}} \times DBD_{\text{slice}} \times OC_{\text{slice}}/100, (1)$$

where z_{slice} is the slice thickness (cm), and the %OC content of the slice is multiplied by 100 to convert % to grams of OC per gram of dry weight. To more correctly represent the thickness of each sediment slice (z_{slice}) an exponential decompression function was applied to correct for the gradual shortening (compression) of the cores during coring (Morton and White 1997). The amount of carbon stored in each core was calculated by summing the OC content in each depth increment (slice). Because the total core length varied among sites (from 43-50 cm after decompression correction), total core carbon storage was estimated in the upper 50 cm of sediment by gap-filling missing data down to 50 cm using an exponential decay function from the 'drc' package (Ritz et al. 2015) in R. The carbon storage was lastly standardized per unit of area as MgC hectare⁻¹.

(6) Data analysis

(a) Calculation of community weighed means (CWM) of the functional traits

The CWM represents the most probable attribute that an individual would have if drawn at random from the community (Garnier et al. 2016). We calculated the CWMs for the traits measured in this study for the seagrass communities encountered where each carbon core was extracted. CWM was calculated following Eq. (2):

$$CWM_{\text{trait}} = \sum_{i=1}^n \% \text{Cover}_i \times \text{Trait}_i (2)$$

Where n is the number of species, $\% \text{Cover}_i$ is the cover of a species i and Trait_i is the trait value of the species i .

(b) Partial least squares modelling (PLS) to investigate the influence of factors on carbon stocks

We performed a partial least squares (PLS) regression in order to investigate the influence of site, sediment grain size, and functional traits on carbon stocks of seagrass meadows. PLS regression is a technique that reduces the predictors to a smaller set of uncorrelated components and performs least squares regression on these components instead of on the original data. PLS regression is particularly suited for this study due to the high number of predictor variables in relation to the number of observations, and due to the potential multicollinearity among predictors. In this study we used the site, sediment grain size distribution and functional traits as predictor variables for the carbon storage in the upper 50 cm of sediment (MgC h^{-1}). The explanatory variables were standardized before the analysis.

The model was validated to determine the number of components for the PLS regression using the root mean squared error of prediction (RMSEP). We extracted the coefficients of the PLS regression for each predictor to assess if they had a positive or negative effect on the carbon stocks. Additionally, we calculated the variable influence on projection (VIP) as measure of a variable's importance in the PLS model. It summarizes the contribution a variable makes to the model. The VIP score of a variable is calculated as a weighted sum of the squared correlations between the PLS components and the original variable. The weights correspond to the percentage variation explained by the PLS component in the model. A $\text{VIP} > 1$ indicates that a variable has an above average influence on the response variable. We used the package "pls" (Mevik et al. 2020) to perform the PLS regression and the package "plsVarSel" (Mehmood et al. 2012) to calculate the VIP.

III. Results

(1) Sediment grain size distribution

The sediment grain size distribution around Unguja Island showed general trends across sites, but also site-specific differences (Fig. 3). Across sites, the silt sediment fraction ($< 63 \mu\text{m}$) represented one of the smallest portions of the sediment (between 0.64 and 4.26%), while the rest of the grain sizes were highly variable among sites. The sediment at the Harbor site mainly consisted of fine to coarse sand ($250 \mu\text{m}$ - 2 mm , 83% of the total), with a very small proportion of gravel ($> 2 \text{ mm}$) and very fine sand ($< 125 \mu\text{m}$). Chapwani and Fumba, on the other hand, had a very balanced grain size distribution among most of the grain size categories. Changuu Island had the coarsest sediments among all sites, with a proportion of less than 3% for very fine sand and silt. Bweleo, inversely from Changuu Island, had the finest sediments, with 78% of the

grains being smaller than 250 μm . Lastly, sediments in Marumbi were mainly formed by grains between the 125 μm and 500 μm sizes, encompassing a total of 73% of the sediment.

(2) Seagrass functional traits at the community level

We quantified the CWM of functional traits linked with particle trapping (plant height, leaf area index) and belowground carbon inputs (belowground biomass, rhizome diameter, root maximum length and roots m^{-1} ; Table 1).

Plant height was similar across all seagrass communities except Fumba, which was formed uniquely by the species *E. acoroides*. While all the other seagrass communities had a height of between 15 cm and 24 cm, the community formed by *E. acoroides* was twice as tall with blades reaching 55 cm. This was not the case for the LAI. First, LAI was much more variable than plant height. The lowest LAI (5107 $\text{m}^2 \text{h}^{-1}$) was found at Harbor, which had a seagrass community formed by two species, *T. hemprichii* and *C. rotundata*. The *T. hemprichii* community in Marumbi, on the other hand, had the highest LAI with (32966.93 $\text{m}^2 \text{h}^{-1}$). The rest of the seagrass communities varied between an LAI of 8052.08 $\text{m}^2 \text{h}^{-1}$ and 21628.50 $\text{m}^2 \text{h}^{-1}$.

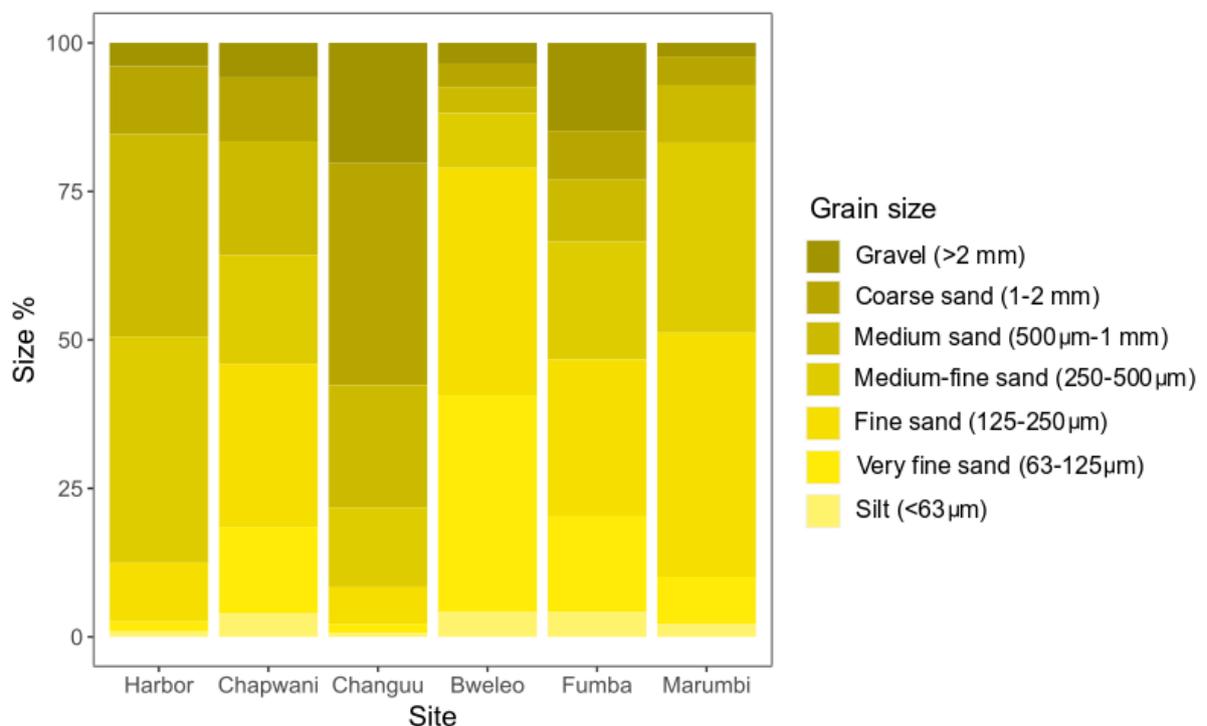


Figure 3. Sediment grain size distribution in the sampling sites.

Regarding belowground biomass, Harbor (499.46 kg DW h^{-1}) and Bweleo (444.39-616.28 kg DW h^{-1}) had the lowest belowground biomass regardless of the seagrass community. On the

other hand, Changuu had the highest belowground biomass among all sites, with values between 3474.74 and 4636.74 kg DW h⁻¹. Rhizome diameter was similar among all seagrass communities and sites, with the exception of the *E. acoroides* in Fumba, which had a rhizome diameter of 1.01 cm. In the case of root maximum length, the monospecific *T. hemprichii* meadow in Changuu Island had the lowest length (7.41 cm), while monospecific meadows of *C. serrulata* (11.70 cm) and *E. acoroides* (11.88 cm) had the highest. Lastly, roots m⁻¹ was highest in two monospecific meadows in Chapwani (*C. serrulata*, 358.13 roots m⁻¹) and Fumba (*E. acoroides*, 350.53 roots m⁻¹). Mixed meadows in the other sites had, generally, lower number of roots m⁻¹.

Table 1. Community weighed mean (CWM) of functional traits linked to particle trapping and belowground carbon deposition and distribution.

Site	Seagrass species	Cores		Particle trapping		Belowground traits		
		n	Plant height (cm)	Leaf area index (m ² h ⁻¹)	Belowground biomass (kg DW h ⁻¹)	Rhizome diameter (cm)	Root maximum length (cm)	Roots m ⁻¹
Harbor	<i>T. hemprichii</i> , <i>C. rotundata</i>	3	18.24	5107.71	499.46	0.31	9.04	109.81
Chapwani	<i>C. serrulata</i>	1	15.70	10690.36	1287.79	0.33	11.70	358.13
	<i>T. hemprichii</i> , <i>C. serrulata</i> , <i>S. isoetifolium</i>	2	16.83	21628.50	2253.04	0.29	9.67	290.63
Changuu	<i>T. hemprichii</i> , <i>T. ciliatum</i>	1	17.33	14193.95	4636.74	0.41	9.48	158.30
	<i>T. hemprichii</i>	1	17.49	8052.08	3474.74	0.31	7.41	148.64
	<i>T. hemprichii</i> , <i>C. serrulata</i>	1	15.06	17081.31	3605.51	0.33	9.40	274.11
Bweleo	<i>T. hemprichii</i> , <i>C. rotundata</i> , <i>H. ovalis</i> , <i>H. uninervis</i>	1	16.52	8199.38	578.72	0.27	9.45	227.42
	<i>T. hemprichii</i> , <i>S. isoetifolium</i>	1	21.82	6295.99	444.39	0.32	9.38	254.09
	<i>T. hemprichii</i> , <i>C. serrulata</i> , <i>C. rotundata</i>	1	18.90	10146.03	616.28	0.32	11.69	223.96
Fumba	<i>E. acoroides</i>	3	54.86	14473.04	3524.74	1.01	11.88	350.53
Marumbi	<i>T. hemprichii</i>	3	23.83	32966.93	2913.97	0.40	8.93	199.35

(3) Carbon stocks in seagrass communities of Unguja Island

The sites located in Kiwani Bay (Bweleo and Fumba) consistently showed the lowest carbon stocks among all sites (Fig. 4). Within these two sites, the seagrass community formed by *T. hemprichii* and *S. isoetifolium*, and the community formed by *T. hemprichii*, *C. rotundata*, *H.*

ovalis and *H. uninervis* had the lowest carbon stocks, with 20.64 MgC h⁻¹ and 29.11 MgC h⁻¹ respectively. The highest carbon stock was found in Marumbi, Chwaka Bay, with a maximum value of 81.61 MgC h⁻¹, quadrupling and doubling the stocks of Bweleo and Fumba, respectively.

Chapwani had carbon stocks of 29.21 - 36.60 MgC h⁻¹ in a mixed seagrass community formed by *T. hemprichii*, *C. serrulata* and *S. isoetifolium* and 38.70 MgC h⁻¹ in a monospecific community formed by *C. serrulata*. Changuu had consistently higher stocks of ~48 MgC h⁻¹. Lastly, the harbor site had the second highest carbon stock among all sites, with a maximum of 69.62 MgC h⁻¹.

(4) Influence of site, parent material and functional traits on carbon stocks in seagrass meadows

Seagrass carbon stocks were mostly influenced by the sediment grain size (Fig. 5). Particularly, silt sized sediments (grain smaller than 125 μm) were strongly negatively correlated to organic carbon stocks (Model coefficient = -0.14, VIP = 1.49). This was not the case for larger grain sizes (between 250 and 500 μm), which were positively correlated (VIP = 1.53). The rest of the sizes did not have a strong correlation with carbon stocks. In the case of the functional traits of the seagrass, they had generally lower model coefficients than sediment grain size (all below 0.1), but three traits had an above average contribution to the prediction of sediment carbon stocks. For traits linked with particle trapping, only LAI was positively correlated with carbon (VIP = 1.62). For belowground traits, roots m⁻¹ (VIP = 1.27) and root maximum length (VIP = 1.33) were strong predictors of carbon storage. Lastly, site was not strongly correlated with the carbon stocks in seagrasses.

IV. Discussion

In this work, we studied the confounding effects of the control exerted by site, sediment grain size and functional traits on the carbon stocks in seagrass communities. The carbon stocks observed in Unguja Island were mainly controlled by grain size distribution of the sediment, with locations with finer sediments (<125 μm) having lower carbon stocks than locations with predominately coarser sediment fractions of 250 and 500 μm. Additionally, there is a certain level of control by seagrass functional traits, with LAI and root maximum length positively correlated with MgC h⁻¹, whereas roots m⁻¹ was negatively correlated with this variable. This indicated that both traits related with particle trapping and configuring the belowground structure of seagrass play a role in the storage of organic carbon in the sediment.

The carbon stocks within the top 50 cm (20.64-81.616 MgC h⁻¹) of the locations we sampled were within the range previously reported for Zanzibar, the coast of Tanzania and Mozambique (21.3-73.8 MgC h⁻¹, Gullström et al. 2018). They doubled, however, the stocks previously reported for sites off the coast of Stone Town (top 1 m: 33.9 ± 7.7 MgC h⁻¹, Belshe et al. 2018), showing that there are large spatial differences in sites even within the same coastal area. In comparison to the global average (top 1 m: 194.2 ± 20.2 MgC h⁻¹, Fourqurean et al. 2012), the carbon stocks fall lower. However, this was to be expected due to the global average being calculated with very few observations from the Indo Pacific region.

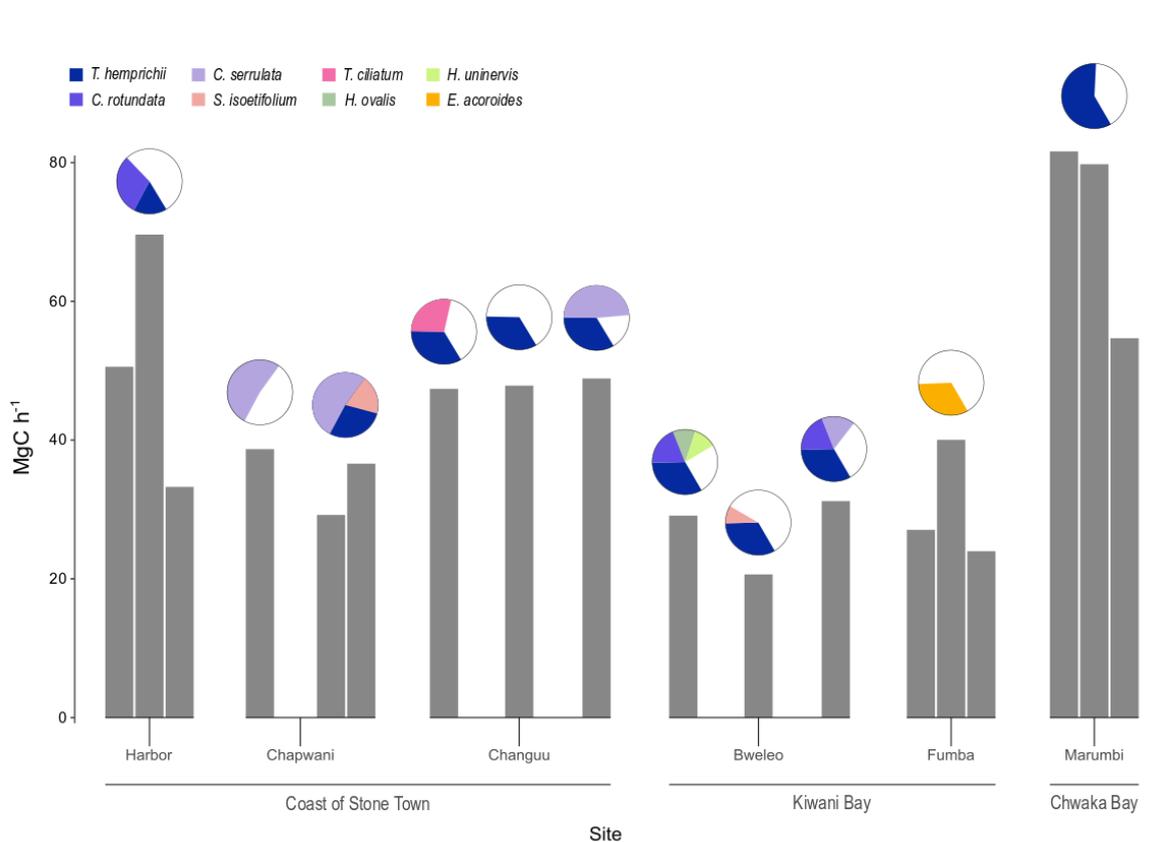


Figure 4. Carbon stocks as measured in the sediment cores in each site and seagrass community encountered. The grey bars are the carbon stock quantified in the upper 50 cm of sediment. The pie charts represent the percentage seagrass cover on top of the cores, with the colors representing each seagrass species. Bars adjacent to each other represent cores taken in communities with the same species within the same site.

In our study, site was of lower importance than sediment grain size and seagrass functional traits. However, there were some trends worth discussing. Chwaka Bay is known for its extensive seagrass meadows (Gullström et al. 2006) and total seagrass area at the landscape level has been positively correlated to carbon stocks in seagrass sediments (Gullström et al. 2018). Marumbi, located in Chwaka Bay, showed a positive correlation with carbon stocks,

agreeing with this finding. Another landscape effect on carbon stocks is the level of exposure of seagrasses to waves and currents. Sheltered seagrass meadows tend to accumulate more carbon in the sediments (Samper-Villarreal et al. 2016), which is the case of the seagrass meadows sampled in Changuu, completely surrounded by a fringing reef. On the other hand, a site like Chapwani has lower carbon stocks and lacks the protection of a fringing reef, leaving it exposed to wave action and currents. The content of organic matter have been reported to decrease in seagrass sediments at higher current speeds and wave exposure (Fonseca and Bell 1998). Additionally, other site characteristics can drive the carbon stocks in seagrass meadows, like connectivity with other ecosystems (Saavedra-Hortua, 2020) or the trophic state of the system (Juang et al. 2018).

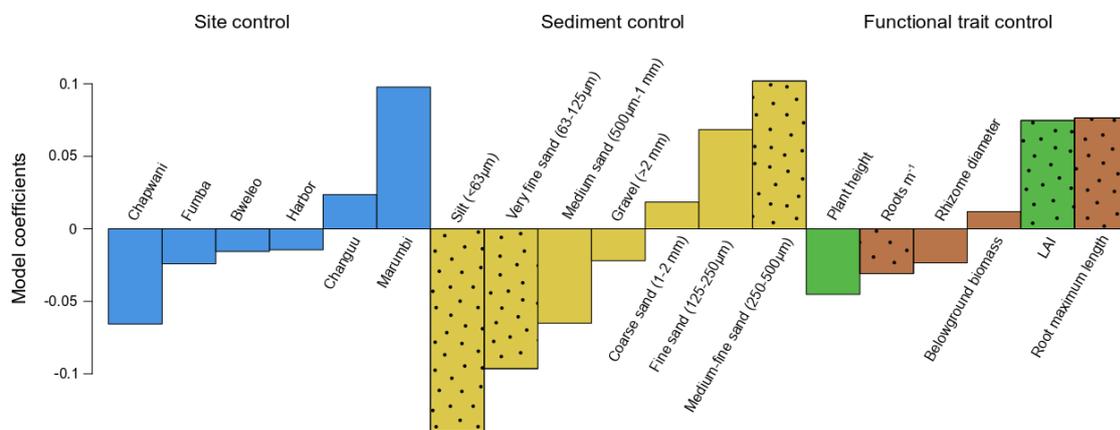


Figure 5. Model coefficients of the PLS regression predicting carbon storage in seagrass meadows. The coefficients were divided in three sections: a) Site control (blue), b) Sediment control (yellow), and c) Functional trait control (green for traits linked to particle trapping and brown for belowground traits). The dotted bars indicate a VIP>1, i.e. the explanatory variable has an above average influence on the response variable, in this case, carbon storage.

The composition of the sediment has been proposed as one of the main constraints for carbon accumulation in seagrass (Belshe et al. 2017), due to its control on biotic suppression and physical stabilization of carbon (Dahl et al. 2016; Röhr et al. 2016; Serrano et al. 2016). In previous studies, porosity and density of the sediment have been found responsible for positive and negative effects on the carbon respectively (Dahl et al. 2016; Samper-Villarreal et al. 2016). In this study, the finer sediments (<125 µm) had a negative effect on the carbon stocks of seagrass, which could be indicative of a higher density of the sediments and lower organic carbon. However, there have been reports in temperate areas indicating that silt content is positively correlated to carbon storage in the sediments (Dahl et al. 2016; Röhr et al. 2016). This correlation is, nevertheless, not universal (Serrano et al. 2016), being important when most

of the carbon accumulated in the sediments is allochthonous instead of autochthonous. It has been shown that carbon has mainly an allochthonous origin in meadows formed by small fast-growing species, but not in meadows formed by long-living species. In this study, most of the communities sampled were dominated by species forming permanent meadows, such as *T. hemprichii*, *T. ciliatum*, *E. acoroides* and *C. serrulata*, which agrees with the results from Serrano et al. (2016). As a consequence, this indicates that, potentially, most of the carbon accumulated in these sites is autochthonous. There was also a strong positive correlation between medium-fine sand sediments (250-500 μm) and organic carbon in the sediment, which suggests that this grain size would be optimal for growth of the seagrass and therefore the deposition of autochthonous carbon in the sediment. Further research in the isotopic content of the carbon and seagrass growth conditions would be needed to confirm this result.

Trait-based approaches have shown to be useful to relate seagrass traits and some ecosystem functions, such as primary production (Jänes et al. 2017), or on the maintenance of fish assemblages (Jones et al. 2021). In the case of carbon storage, both aboveground and belowground seagrass traits have been previously related to carbon storage in the sediments (Dahl et al. 2016; Röhr et al. 2016; Gullström et al. 2018). In our study, we show that LAI and root maximum length are both strong predictors of carbon stocks, and are positively correlated to organic carbon storage. However, roots m^{-1} showed a negative correlation to organic carbon in the sediment. LAI is correlated to particle trapping and flow reduction (Paul et al. 2012). The importance of the particle retention (and their associated carbon) by seagrass for the accumulation of sediment carbon has been previously reported (Kennedy et al. 2010; Duarte et al. 2013), and is directly related to the structural complexity of the seagrass canopy (Koch et al. 2006; Hendriks et al. 2008; Samper-Villarreal et al. 2016). Our study provides further proof that the seagrass structural complexity, as indicated by LAI, is a fundamental driver of carbon accumulation in the sediment in tropical ecosystems. However, plant height did not have a strong correlation with carbon, indicating that the complexity of the seagrass canopy is more important than its height for carbon retention. Additionally, higher LAI can be indicative of a higher pool of autochthonous carbon available to accumulate in the sediments, which simultaneously is more easily trapped due to the high LAI, establishing a positive feedback loop.

Belowground seagrass structures are important sources of carbon in the sediment (Dahl et al. 2016; Röhr et al. 2016; Gullström et al. 2018), because they are already trapped below the surface (Kenworthy and Thayer 1984), are composed of highly refractory material (Zeikus

1981) and add carbon by their turnover and root exudates (Duarte et al. 1998, 2005). For these reasons, the positive correlation between carbon stocks and root maximum length agrees with previous reports, and indicates that rooting depth is an important factor to increase carbon storage. There is, however, a negative correlation between root density (roots m^{-1}) and carbon. There is no simple explanation for this result. Intuitively, a higher density of roots would indicate a higher amount of carbon stored in the sediments. On the other hand, it could indicate that communities with higher root density have more decaying roots that are more easily remineralized by the microbiota in the sediments. There is, however, no previous reports of this result, and warrants further investigation. Rhizome diameter and belowground biomass were not strong predictors of carbon, indicating that the morphology and density of the roots may be more important for the accumulation of carbon in the sediments than the morphology of the rhizome and the total amount of belowground biomass.

V. Conclusions

In this work we studied the effects of site, sediment grain size and functional traits of seagrass on carbon storage. We found out that, for the sampling sites selected, attributes of the sites were of minor importance despite a certain effect of exposure to waves and currents and seagrass total area. Sediment grain size was the most important driver, indicating that fine sediments had a negative effect on the carbon storage, while an abundance of medium-fine sands have a positive effect. This may be due to a positive seagrass response to growing in larger grain sediments favoring higher autochthonous over allochthonous carbon associated with finer sediments. Lastly, LAI had a positive effect on carbon accumulation in the sediment, indicating that this seagrass trait aids carbon capture and retention. Root maximum length was the most important functional trait driving carbon storage, showing that rooting depth has a positive effect on the organic carbon content in the sediment. The importance of both of biological and sedimentological factors shows that the quantification and prediction of carbon storage in seagrass meadows is multifaceted and needs to be tackled using a variety of approaches.

VI. Acknowledgments

The authors thank the staff of the Institute of Marine Sciences (IMS) in Stone Town for their support both administratively and scientifically, specifically Mtumwa Mwadini for his advice and help, and Flower Msuya and Jiddawi Narriman for their logistical support. We would like to thank Merlin Helle for his help in the fieldwork campaign. We want to thank the staff of the Chemistry and Biology laboratories in the Leibniz Centre for Marine Tropical Research (ZMT) for their assistance. Inés G. Viana was awarded with a postdoctoral contract of Xunta de Galicia

(Consellería de Educación, Universidad e Formación Profesional) postdoctoral program (ED481B-2016/189-0) and Juan de la Cierva-Incorporación postdoctoral program (IJC2019-040554-I). This study was part of the project Seagrass and Macroalgal Community Dynamics and Performance under Environmental Change (SEAMAC) (Deutsche Forschungsgemeinschaft, DFG, TE 1046/3-1) awarded to Mirta Teichberg.

VII. References

Aloisi, J. C. (1975). Evolution paleogeographique du plateau continental languedocien dans le cadre du Golfe du Lyon. Analyse comparee des donnees sismiques, sedimentologiques et radiometriques concernant le quaternaire recent.

Alongi, D. M., Murdiyarso, D., Fourqurean, J. W., Kauffman, J. B., Hutahaean, A., Crooks, S., ... and Wagey, T. (2016). Indonesia's blue carbon: a globally significant and vulnerable sink for seagrass and mangrove carbon. *Wetlands Ecology and Management*, 24, 3-13. <https://doi.org/10.1007/s11273-015-9446-y>

Andersen, K. H., and Pedersen, M. (2009). Damped trophic cascades driven by fishing in model marine ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 277, 795-802. <https://doi.org/10.1098/rspb.2009.1512>

Belshe, E. F., Mateo, M. A., Gillis, L., Zimmer, M., and Teichberg, M. (2017). Muddy waters: unintentional consequences of blue carbon research obscure our understanding of organic carbon dynamics in seagrass ecosystems. *Frontiers in Marine Science*, 4, 125. <https://doi.org/10.3389/fmars.2017.00125>

Belshe, E. F., Hoeijmakers, D., Herran, N., Mtolera, M., and Teichberg, M. (2018). Seagrass community-level controls over organic carbon storage are constrained by geophysical attributes within meadows of Zanzibar, Tanzania. *Biogeosciences*, 15, 4609-4626. <https://doi.org/10.5194/bg-15-4609-2018>

Bouma, T. J., De Vries, M. B., Low, E., Peralta, G., Tánčzos, I. V., van de Koppel, J., and Herman, P. M. J. (2005). Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. *Ecology*, 86(8), 2187-2199. <https://doi.org/10.1890/04-1588>

Brouns, J. J. (1987). Aspects of production and biomass of four seagrass species (Cymodoceoideae) from Papua New Guinea. *Aquatic Botany*, 27, 333-362. [https://doi.org/10.1016/0304-3770\(87\)90073-8](https://doi.org/10.1016/0304-3770(87)90073-8)

Campbell, J. E., Lacey, E. A., Decker, R. A., Crooks, S., and Fourqurean, J. W. (2015). Carbon storage in seagrass beds of Abu Dhabi, United Arab Emirates. *Estuaries and Coasts*, 38, 242-251. <https://doi.org/10.1007/s12237-014-9802-9>

- Costanza, R., and Folke, C. (1997). Valuing ecosystem services with efficiency, fairness and sustainability as goals. *Nature's services: Societal dependence on natural ecosystems*, 49-70. <https://doi.org/10.1016/j.ecoser.2020.101096>
- Dahl, M., Deyanova, D., Gütschow, S., Asplund, M. E., Lyimo, L. D., Karamfilov, V., ... and Gullström, M. (2016). Sediment properties as important predictors of carbon storage in *Zostera marina* meadows: a comparison of four European areas. *PLoS One*, 11, e0167493. <https://doi.org/10.1371/journal.pone.0167493>
- De los Santos, C.B., Krause-Jensen, D., Alcoverro, T., Marbà, N., Duarte, C. M., Van Katwijk, M. M., ... and Santos, R. (2019). Recent trend reversal for declining European seagrass meadows. *Nature communications*, 10, 1-8. <https://doi.org/10.1038/s41467-019-11340-4>
- Dahl, M., Deyanova, D., Gütschow, S., Asplund, M. E., Lyimo, L. D., Karamfilov, V., et al. (2016). Sediment properties as important predictors of carbon storage in *Zostera marina* meadows: a comparison of four European areas. *PLoS ONE*, 11, e0167493. <https://doi.org/10.1371/journal.pone.0167493>
- Duarte, C. M., Merino, M., Agawin, N. S. R., Uri, J., Fortes, M. D., Gallegos, M. E., et al. (1998). Root production and belowground seagrass biomass. *Marine Ecology Progress Series*, 171, 97–108. <https://doi.org/10.3354/meps171097>
- Duarte, C. M., and Chiscano, C. L. (1999). Seagrass biomass and production: a reassessment. *Aquatic botany*, 65, 159-174. [https://doi.org/10.1016/s0304-3770\(99\)00038-8](https://doi.org/10.1016/s0304-3770(99)00038-8)
- Duarte, C. M., Holmer, M., and Marba, N. (2005). Plant-Microbe Interactions in Seagrass Meadows in Macroand Microorganisms. In *Interactions Between Macro- and Microorganisms in Marine Sediments* (pp. 1–30). American Geophysical Union. <https://doi.org/10.1029/ce060p0031>
- Duarte, C. M., H. Kennedy, N. Marbà, and I. Hendriks. 2013. Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean and Coastal Management*, 83, 32–38. <https://doi.org/10.1016/j.ocecoaman.2011.09.001>
- Elleouet, J., Albouy, C., Ben Rais Lasram, F., Mouillot, D., and Leprieur, F. (2014). A trait-based approach for assessing and mapping niche overlap between native and exotic species: the Mediterranean coastal fish fauna as a case study. *Diversity and Distributions*, 20, 1333-1344. <https://doi.org/10.1111/ddi.12235>
- Fonseca, M., and S. Bell. 1998. Influence of physical setting on seagrass landscapes near Beaufort, North Carolina, USA. *Marine Ecology Progress Series*, 171, 109. <https://doi.org/10.3354/meps171109>
- Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., ... and Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature geoscience*, 5, 505-509. <https://doi.org/10.1038/ngeo1477>
- Garnier, E., Navas, M. L., and Grigulis, K. (2016). *Plant functional diversity: organism traits, community structure, and ecosystem properties*. Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780198757368.001.0001>

- Grasshoff, K., Kremling, K., and Ehrhardt, M. (Eds.). (2009). *Methods of seawater analysis*. John Wiley and Sons. <https://doi.org/10.1002/9783527613984>
- Greiner, J. T., McGlathery, K. J., Gunnell, J., and McKee, B. A. (2013). Seagrass restoration enhances “blue carbon” sequestration in coastal waters. *PloS one*, 8, e72469. <https://doi.org/10.1371/journal.pone.0072469>
- Grime, J. P. (1998). Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, 86, 902-910. <https://doi.org/10.1046/j.1365-2745.1998.00306.x>
- Gullström, M., Lundén, B., Bodin, M., Kangwe, J., Öhman, M. C., Mtolera, M. S., and Björk, M. (2006). Assessment of changes in the seagrass-dominated submerged vegetation of tropical Chwaka Bay (Zanzibar) using satellite remote sensing. *Estuarine, Coastal and Shelf Science*, 67, 399-408. <https://doi.org/10.1016/j.ecss.2005.11.020>
- Gullström, M., Lyimo, L. D., Dahl, M., Samuelsson, G. S., Eggertsen, M., Anderberg, E., ... & Björk, M. (2018). Blue carbon storage in tropical seagrass meadows relates to carbonate stock dynamics, plant–sediment processes, and landscape context: insights from the western Indian Ocean. *Ecosystems*, 21, 551-566. <https://doi.org/10.1007/s10021-017-0170-8>
- Gustafsson, C., and Norkko, A. (2016). Not all plants are the same: Exploring metabolism and nitrogen fluxes in a benthic community composed of different aquatic plant species. *Limnology and Oceanography*, 61, 1787-1799. <https://doi.org/10.1002/lno.10334>
- Hasegawa, N., Hori, M., and Mukai, H. (2008). Seasonal changes in eelgrass functions: current velocity reduction, prevention of sediment resuspension, and control of sediment–water column nutrient flux in relation to eelgrass dynamics. *Hydrobiologia*, 596, 387-399. <https://doi.org/10.1007/s10750-007-9111-4>
- Hemminga, M. A., Harrison, P. G., and Van Lent, F. (1991). The balance of nutrient losses and gains in seagrass meadows. *Marine Ecology Progress Series*, 85-96. <https://doi.org/10.3354/meps071085>
- Hendriks, I. E., T. Sintes, T. J. Bouma, and C. M. Duarte. 2008. Experimental assessment and modeling evaluation of the effects of seagrass *Posidonia oceanica* on flow and particle trapping. *Marine Ecology Progress Series*, 356, 163–173. <https://doi.org/10.3354/meps07316>
- Jänes, H., Kotta, J., Pärnoja, M., Crowe, T. P., Rindi, F., and Orav-Kotta, H. (2017). Functional traits of marine macrophytes predict primary production. *Functional Ecology*, 31, 975-986. <https://doi.org/10.1111/1365-2435.12798>
- Jones, B. L., Nordlund, L. M., Unsworth, R. K., Jiddawi, N. S., and Eklöf, J. S. (2021). Seagrass structural traits drive fish assemblages in small-scale fisheries. *Frontiers in Marine Science*, 8, 354. <https://doi.org/10.3389/fmars.2021.640528>
- Kennedy, H., J. Beggins, C. M. Duarte, J. W. Fourqurean, M. Holmer, N. Marbà, J. J. Middelburg (2010). Seagrass sediments as a global carbon sink: Isotopic constraints. *Global Biogeochemical Cycles*, 24, GB4026. <https://doi.org/10.1029/2010GB003848>

- Kenworthy, J. W., and Thayer, G. W. (1984). Production and decomposition of the roots and rhizomes of seagrasses, *Zostera marina* and *Thalassia testudinum*, in temperate and subtropical marine ecosystems. *Bulletin of Marine Science*, 35, 364-379. [https://doi.org/10.1016/0304-3770\(87\)90088-x](https://doi.org/10.1016/0304-3770(87)90088-x)
- Koch, E., J. Ackerman, J. Verduin, and M. Keulen. 2006. Fluid dynamics in seagrass ecology: From molecules to ecosystems. In A. W. D. Larkum, R. J. Orth, and C. M. Duarte [eds.], *Biology, ecology and conservation* (pp. 193–225). Springer. https://doi.org/10.1007/1-4020-2983-7_8
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional ecology*, 16, 545-556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Lewis, J. T., and Boyer, K. E. (2014). Grazer functional roles, induced defenses, and indirect interactions: implications for eelgrass restoration in San Francisco Bay. *Diversity*, 6, 751-770. <https://doi.org/10.3390/d6040751>
- Litchman, E., and Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Annual review of ecology, evolution, and systematics*, 39, 615-639. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173549>
- Litchman, E., de Tezanos Pinto, P., Klausmeier, C. A., Thomas, M. K., and Yoshiyama, K. (2010). Linking traits to species diversity and community structure in phytoplankton. In *Fifty years after the ‘Homage to Santa Rosalia’: Old and new paradigms on biodiversity in aquatic ecosystems* (pp. 15-28). Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9908-2_3
- Litchman, E., Ohman, M. D., and Kiørboe, T. (2013). Trait-based approaches to zooplankton communities. *Journal of Plankton Research*, 35, 473-484. <https://doi.org/10.1093/plankt/fbt019>
- Marbà, N., Arias-Ortiz, A., Masqué, P., Kendrick, G. A., Mazarrasa, I., Bastyan, G. R., ... and Duarte, C. M. (2015). Impact of seagrass loss and subsequent revegetation on carbon sequestration and stocks. *Journal of ecology*, 103, 296-302. <https://doi.org/10.1111/1365-2745.12370>
- Mazarrasa, I., Samper-Villarreal, J., Serrano, O., Lavery, P. S., Lovelock, C. E., Marbà, N., ... and Cortés, J. (2018). Habitat characteristics provide insights of carbon storage in seagrass meadows. *Marine pollution bulletin*, 134, 106-117. <https://doi.org/10.1016/j.marpolbul.2018.01.059>
- Mehmood, T., Liland, K. H., Snipen, L. and Sæbø, S. (2012). A review of variable selection methods. In *Partial Least Squares Regression. Chemometrics and Intelligent Laboratory Systems* (pp. 62-69). <https://doi.org/10.1016/j.chemolab.2012.07.010>

- Mevik, B., Wehrens, R., and Liland, K. H. (2020). pls: Partial Least Squares and Principal Component Regression. R package version 2.7-3. <https://CRAN.R-project.org/package=pls>
- Morton, R. A., and White, W. (1997). Characteristics of and corrections for core shortening in unconsolidated sediments. *Journal of Coastal Research*, 13, 761–769.
- Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends in ecology and evolution*, 28, 167-177. <https://doi.org/10.1016/j.tree.2012.10.004>
- Nellemann, C., and Corcoran, E. (Eds.). (2009). *Blue carbon: the role of healthy oceans in binding carbon: a rapid response assessment*. UNEP/Earthprint.
- Olsen, J. L., Rouzé, P., Verhelst, B., Lin, Y. C., Bayer, T., Collen, J., ... and Van de Peer, Y. (2016). The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. *Nature*, 530, 331-335. <https://doi.org/10.1038/nature16548>
- Oreska, M. P., McGlathery, K. J., and Porter, J. H. (2017). Seagrass blue carbon spatial patterns at the meadow-scale. *PloS one*, 12, e0176630. <https://doi.org/10.1371/journal.pone.0176630>
- Orth, R. J., Heck, K. L., and van Montfrans, J. (1984). Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries*, 7, 339-350. <https://doi.org/10.2307/1351618>
- Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., ... and Williams, S. L. (2006). A global crisis for seagrass ecosystems. *Bioscience*, 56, 987-996. [https://doi.org/10.1641/0006-3568\(2006\)56\[987:agcfse\]2.0.co;2](https://doi.org/10.1641/0006-3568(2006)56[987:agcfse]2.0.co;2)
- Pagès, J. F., Farina, S., Gera, A., Arthur, R., Romero, J., and Alcoverro, T. (2012). Indirect interactions in seagrasses: fish herbivores increase predation risk to sea urchins by modifying plant traits. *Functional Ecology*, 26, 1015-1023. <https://doi.org/10.1111/j.1365-2435.2012.02038.x>
- Paul, M., Bouma, T. J., and Amos, C. L. (2012). Wave attenuation by submerged vegetation: combining the effect of organism traits and tidal current. *Marine Ecology Progress Series*, 444, 31-41. <https://doi.org/10.3354/meps09489>
- Peralta, G., Van Duren, L. A., Morris, E. P., and Bouma, T. J. (2008). Consequences of shoot density and stiffness for ecosystem engineering by benthic macrophytes in flow dominated areas: a hydrodynamic flume study. *Marine Ecology Progress Series*, 368, 103-115. <https://doi.org/10.3354/meps07574>
- Priosambodo D (2006) Growth rate and production of tropical seagrass *Enhalus acoroides* (L.) f. Royle in Awerange and Labuange Bays, Barru Regency, south Sulawesi. *Bulletin of Marine Science*, 16, 334–345. <https://doi.org/10.33086/ijmlst.v2i1.1463>
- Ritz, C., Baty, F., Streibig, J. C., Gerhard, D. (2015) Dose-Response Analysis Using R. *PLOS ONE*, 10, e0146021. <https://doi.org/10.1371/journal.pone.0146021>

- Röhr, M. E., Boström, C., Canal-Vergés, P., and Holmer, M. (2016). Blue carbon stocks in Baltic Sea eelgrass (*Zostera marina*) meadows. *Biogeosciences*, *13*, 6139–6153. <https://doi.org/10.5194/bg-13-6139-2016>
- Romero, J., Pergent, G., Pergent-Martini, C., Mateo, M. A., and Regnier, C. (1992). The detritic compartment in a *Posidonia oceanica* meadow: litter features, decomposition rates, and mineral stocks. *Marine Ecology*, *13*, 69-83. <https://doi.org/10.1111/j.1439-0485.1992.tb00341.x>
- Romero, J., Pérez, M., Mateo, M. A., and Sala, E. (1994). The belowground organs of the Mediterranean seagrass *Posidonia oceanica* as a biogeochemical sink. *Aquatic Botany*, *47*, 13-19. [https://doi.org/10.1016/0304-3770\(94\)90044-2](https://doi.org/10.1016/0304-3770(94)90044-2)
- Saavedra Hortua, D. A. (2020). *Exploring carbon dynamics in connected mangrove forests and seagrass beds: How important is it?* (Doctoral dissertation, Universität Bremen).
- Samper-Villarreal, J., Lovelock, C. E., Saunders, M. I., Roelfsema, C., and Mumby, P. J. (2016). Organic carbon in seagrass sediments is influenced by seagrass canopy complexity, turbidity, wave height, and water depth. *Limnology and Oceanography*, *61*, 938–952. <https://doi.org/10.1002/lno.10262>
- Serrano, O., Lavery, P. S., Duarte, C. M., Kendrick, G. A., Calafat, A., York, P. H., et al. (2016). Can mud (silt and clay) concentration be used to predict soil organic carbon content within seagrass ecosystems? *Biogeosciences*, *13*, 4915–4926. <https://doi.org/10.5194/bg-13-4915-2016>
- Shiple, B., De Bello, F., Cornelissen, J. H. C., Laliberté, E., Laughlin, D. C., and Reich, P. B. (2016). Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*, *180*, 923-931. <https://doi.org/10.1007/s00442-016-3549-x>
- Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, *350*, 3-20. <https://doi.org/10.1016/j.jembe.2007.06.012>
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*, 882-892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., ... and Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the national academy of sciences*, *106*, 12377-12381. <https://doi.org/10.1073/pnas.0905620106>
- Wilkie, L., O'Hare, M. T., Davidson, I., Dudley, B., and Paterson, D. M. (2012). Particle trapping and retention by *Zostera noltii*: A flume and field study. *Aquatic Botany*, *102*, 15-22. <https://doi.org/10.1016/j.aquabot.2012.04.004>
- Zeikus, J. G. (1981). Lignin metabolism and the carbon cycle. *Advances in microbial ecology*, 211-243.



CHAPTER 8.

Final Synthesis and Conclusions



I. Trait-based ecology in seagrass: A final synthesis

With the advancement of numerical ecology in the 1970s (Legendre and Legendre 1979), natural scientists became more aware of the potential to use numerical metrics in research. It is for this reason that trait-based approaches (trait-based frameworks in this dissertation, henceforth TBF) were created 20 years ago (Lavorel and Garnier 2002), to take a deeper look at classical ecological questions and theories. The potential of TBFs lies in using the power of measurable organismal characteristics to make predictions that can be scaled up from the individual to the ecosystem level (Shipley et al. 2016). More than a novelty at the conceptual level, it is a novelty at the perspective that we take for answering these classical problems. In this section, I will first discuss the state of the art in TBFs in seagrass ecology. Secondly, I will synthesize the findings of the chapters presented in this thesis under a comprehensive picture, with special emphasis on the implications of the results for tropical seagrass meadows in the Anthropocene. Thirdly, I will explore the limitations of the results and which knowledge gaps were noticed after the development of the work presented, as well as future research possibilities.

II. TBFs in seagrasses: new possibilities for classical ecological questions

There is an intimidating large number of scientific publications proposing, assessing and testing different concepts and theories linked to TBFs (see Garnier et al. 2016 and citations therein). However, in the case of seagrass ecology, the first attempts in the introduction of TBFs have been very recent (first study found in 2012, see Chapter 2), and there has been no previous publication to comprehensively explain TBFs and their usefulness for seagrasses.

In order to answer the first research question (RQ1) in this thesis, I studied what was known in seagrass research through a review of existing literature. In the literature review process on seagrass trait research for Chapter 2, it was apparent that, despite not using the word “trait”, there is a wealth of studies using continuous trait metrics to answer ecological questions. Therefore, the division between trait-based and not trait-based research was more complicated than expected. However, it was also apparent that the concepts developed within functional ecology and TBFs were absent in seagrass studies, with only 7% of the studies found in the literature review incorporating them. This leads to interesting conclusions about the divide between terrestrial and marine ecology, and the negative effects of the specialization in a single field. Seagrasses are angiosperm plants, and it is therefore expected that the same principles applied to terrestrial plant ecology should also apply in seagrasses, despite inhabiting a very

different habitat. With the development of TBFs in terrestrial plant ecology since 2002 (Lavorel and Garnier 2002), it is clear that there is a lag of a decade between the incorporation of concepts from terrestrial ecology to marine ecology. The proposal to apply a TBF to seagrass ecology in Chapter 2 acknowledges the importance of considering the scientific advances of other research fields in order to push marine research forward, as trait-based research appears as a powerful avenue to unveil new insights into the functioning of ecosystems.

As an example to understand the evolution of knowledge in seagrass ecosystems, we can take a look at seagrass as habitat providers (Orth et al. 1984). Seagrass ecosystems are categorized as ecosystem engineers, which indicates that their sole presence is enough for the provision of certain ecosystem functions (Jones et al. 1994), e.g. habitat provision. First descriptions of this ecological process described seagrass as hotspots of invertebrates and fish diversity and abundance, especially in comparison to unvegetated sediments (e.g. Virnstein et al., 1983; Summerson and Peterson, 1984; Williams et al., 1990). However, further research showed that not all seagrass communities perform this function equally. Meadows formed by different seagrass communities showed also differences in their fish abundance (Stoner 1983). Other characteristics including meadow patchiness (Salita et al. 2003) or structural complexity (Güllstrom et al. 2008) also played a role determining the abundance of associated fishes. These studies refined our understanding of habitat provision for fishes, establishing clear links between meadow characteristics and habitat provision. These reported links have been reassessed under a TBF (Jones et al. 2021), showing a positive correlation between seagrass structural traits and fish abundance. The application of a TBFs was a final step only possible with the ecological knowledge previously developed, and adds the possibility of modelling the direct influence of traits on fish abundance. There is, therefore, a great potential to re-analyze data from previous seagrass research and reassess it under a TBF. Online databases with seagrass trait data will, therefore, prove to be of fundamental importance in future seagrass research (see Chapter 2 and Kattge et al. 2020).

Lastly, as the last step in the TBF in Chapter 2, there is a need for the translation of scientific data into effective and clear information for policy makers. This is one of the reasons why the concept of ecosystem service was developed (Brink et al. 2009), which puts ecosystem functions into a categorization that highlights their importance for humankind survival. In the case of seagrass, it is generally accepted that they provide a wide variety of ecosystem services (Constanza et al. 1997). However, there is a simultaneous lack of quantification of the

ecosystem services and the characteristics of seagrass which provide these services (Mtwana Nordlund et al. 2016).

This is where TBFs can aid to build the bridge between science and communication. In this dissertation, I try to make a case for the use of effect traits (i.e. traits that have an effect on ecosystem functions and processes) to quantify the potential for a seagrass meadow to provide ecosystem services. The understanding that seagrass traits can be indicators of services is not new (Mtwana Nordlund et al. 2016, Viana et al. 2019), showing that seagrass species with greater leaf area are perceived to provide more services and that invasive seagrass with similar traits as native seagrass could provide the same services.

There is, however, a lack of literature numerically assessing the direct relation between effect traits, functions and services in seagrasses. This is probably due to two main reasons. Firstly, mechanistic links between traits and functions are difficult to establish and are, often, environmentally dependent (Grace et al. 2007). Therefore, the strength of the causality between trait and function can be weaker than expected and variable in time (Hughes and Stachowicz 2011, Belshe et al. 2018). Despite these difficulties, there is the potential to identify effect traits that are directly related to ecosystem functions (Bouma et al. 2005; Peralta et al. 2008; Paul et al. 2012), as long as the temporal and environmental variability of the function are acknowledged (van der Plas et al. 2020). Secondly, the translation of functions to services in a quantitative manner is not universally clear and there are several steps to be taken to include them in policy. Ecosystem functions provided by seagrass are already well classified in services (Ruiz-Frau et al. 2017), namely provision, regulation, supporting and cultural. Unfortunately, the next critical steps to include a service in policy are underdeveloped for most services (Ruiz-Frau et al. 2017), except for the case of blue carbon. Blue carbon is one of the few ecosystem services that succeeded in being included in the local and national financial markets and coastal management plans to ensure its long term protection (Herr et al. 2012). The success of its incorporation in the international conservation agenda stems from a general acceptance of the importance of climate regulation, which pushed the research of carbon storage by primary producers in the forefront of the climate change debate.

In summary, the unraveling of the link between traits and functions is only a first step in the evaluation of ecosystem services. Strong categorizations of ecosystem functions in services for management use and their incorporation in the international debate are fundamental to their inclusion in conservation and management decisions. In this context, seagrass effect traits could

help to easily communicate the importance of seagrass with simple measurable metrics, which could be incorporated in management and conservation plans

III. From individual plants to ecosystem level processes: a trait-based perspective in seagrass ecology

Tropical seagrass meadows, due to their high diversity in coexisting species (Short et al. 2007), offer a perfect laboratory for the study of seagrass responses to environmental change, both at the local and at the global scale. In the case of this dissertation, I studied temperature as the global stressor, and nutrient enrichment, eutrophication and seaweed farming as local stressors. In this section, I will comprehensively speculate about the implications of the individual responses of seagrasses to these environmental drivers (RQ2) in relation to interspecific competition (RQ3) and carbon storage (RQ4), putting together the results presented in chapters 3, 4, 6 and 7. In this way, I will explore the effect of the abiotic filter in the response traits of seagrass, and what are the implications of these responses for the biotic filter, as represented by interspecific competition. Finally, I will discuss how this final configuration can affect the function of carbon storage in seagrass ecosystems. For the sake of simplicity in this discussion section, I will not include seaweed farming. This section is speculative and presented as an example of how the effects of individual traits can be scaled up to the ecosystem level.

(1) Abiotic filter: individual plants responses to environmental drivers

For the tropical seagrass species included in this dissertation, an increase in temperature generally translated into enhanced morphological traits in the experiments described in chapters 3 and 4 (Figure 1.1.a). *E. acoroides* increased in number of leaves and leaf length, and in the number of roots and their length. Similarly, *T. hemprichii* and *C. serrulata* showed longer leaves and sheaths, and faster growth rates. The only exception was *H. stipulacea*, which decreased in size, with lower leaf and root length and higher shoot density. These results imply that tropical climax seagrass species benefited from being exposed to the upper limits of their thermal range. These results contrast with previous research both on seedlings (Abe et al., 2009; Niu et al., 2012; Guerrero-Meseguer et al., 2017; Pereda-Briones et al., 2019) and adult plants (Durako and Moffler, 1985; Bulthuis, 1987; Lee et al., 2007; Xu et al., 2016; Zayas-Santiago et al., 2020), which generally indicate negative effects on seagrass fitness. The only species which showed negative effects on its morphology was *H. stipulacea*.

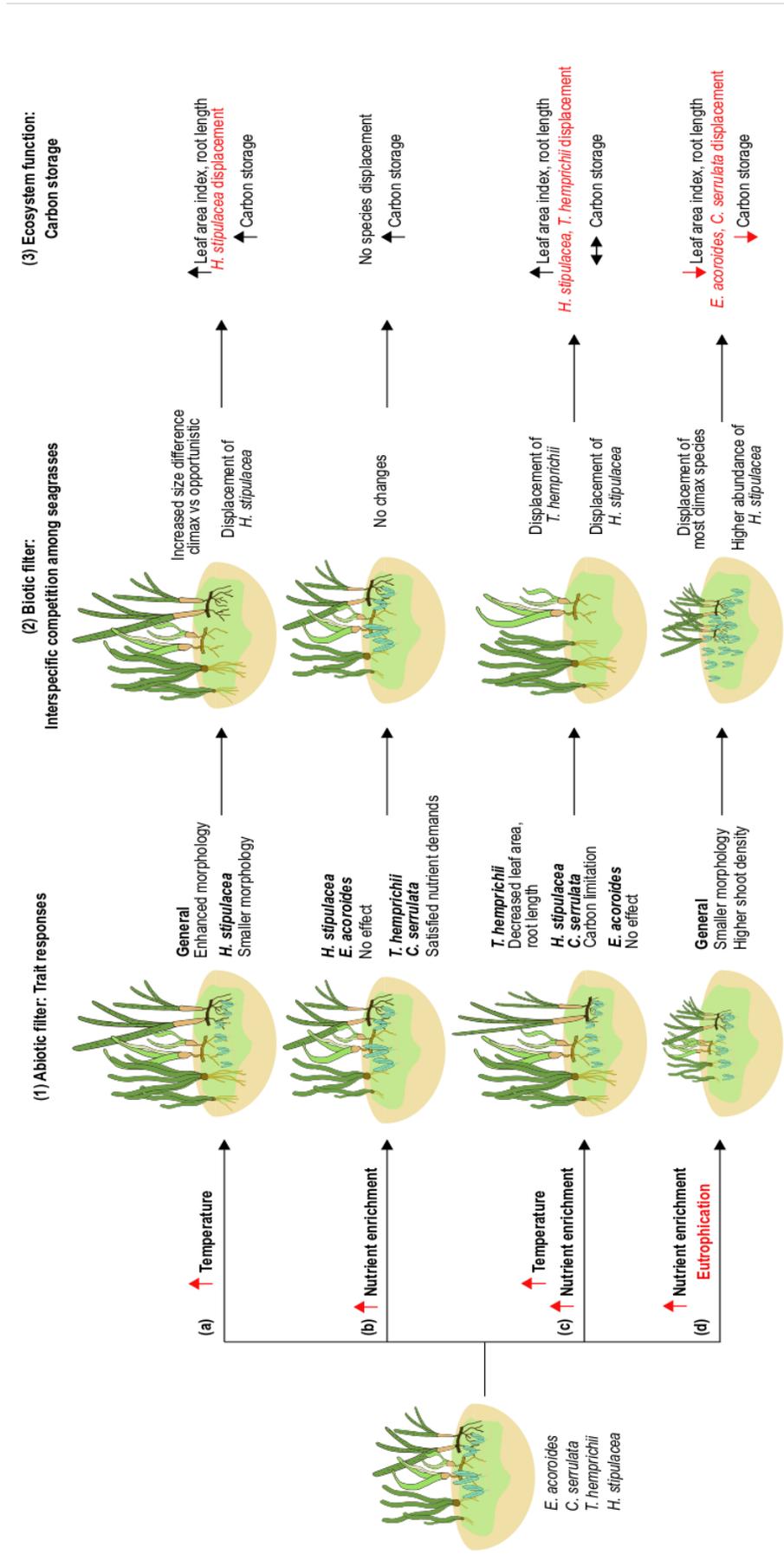


Figure 1. Potential scenarios for seagrass meadows under (a) Temperature increase, (b) Nutrient enrichment, (c) Combined effect of temperature and nutrient enrichment and (d) Eutrophication. First the trait responses are presented (1), secondly the interspecific competition among seagrass (2) and finally the potential carbon storage scenarios (3). Upwards arrows indicate increase, downwards decrease and double sideways arrows indicate no change.

Nutrient enrichment had fewer effects on seagrass than temperature (Figure 1.1.b). Seedlings of *E. acoroides* were not affected by the nutrient enrichment, indicating that they rely on their internal nutrient reserves. In the case of *T. hemprichii* and *C. serrulata*, both showed an increase in nitrogen in the leaves, indicating that they were nutrient limited. Tropical seagrass meadows are often present in oligotrophic habitats, which makes them nutrient limited (Pérez et al., 1991; Duarte et al., 1995; Agawin et al., 1996). It is therefore expected that increases in nutrient availability aid the growth of seagrass plants by satisfying their nutrient demands.

However, when temperature and nutrient enrichment are studied together, they do not act independently (Figure 1.1.c). While the interaction of both factors had no effects on *E. acoroides*, *T. hemprichii* drastically decreased its leaf area and root length under high temperature and nutrients. In the case of *C. serrulata* and *H. stipulacea*, they showed negative effects on biochemical traits related to their carbon content. These results show the importance of the development of experiments with interactive stressors to understand responses of organisms to environmental change.

Nutrient enrichment does not only affect seagrass plants, but also other compartments of the ecosystems leading to eutrophication, which has additional effects on seagrass species (Burkholder et al. 2007) (Figure 1.1.d). As presented in chapter 6, we observed different trophic states along the coast of Unguja Island. *E. acoroides*, *T. hemprichii* and *C. serrulata* showed a decrease in trait values related to their size in mesotrophic conditions, which include leaf length and width, leaves per shoot, rhizome diameter, root length and leaf mass area. However, they increased in shoot density. This indicates that despite their nutrient demands being likely satisfied, they adopted a functional strategy more similar to opportunistic than to climax species. These results agree with previous reports indicating similar consequences in seagrass morphology and growth for acclimatization to eutrophic conditions (Collier et al. 2012, Browne et al. 2017). There is no information for *H. stipulacea* as it was not found in the sampling sites.

Unfortunately, we have no results of the interactive effects of eutrophication and temperature. It has been reported that increased temperature can intensify negative effects by eutrophication, compromising the survival of seagrass plants (Ontoria et al. 2019). Additionally, warming can lead to exacerbated epiphytic fouling on seagrass leaves (Mvungi and Pillay 2019). This indicates that the combined effects of warming and eutrophication can be synergistic and detrimental for the individual response of seagrass plants. However, the single effects of temperature and eutrophication on seagrass traits can be offset when in combination (Pazzaglia et al. 2020), suggesting that there is a compensation in the effects. Due to the lack of universality

in the state of the art in the combined effects of eutrophication and warming, I will consider the effect of eutrophication separately from temperature in this discussion.

(2) Biotic filter: Community level interspecific competition among seagrasses

As concluded in chapter 6, interspecific competition in seagrass is an asymmetrical process, in which species with larger structures are favored and exert space preemption on smaller species. The traits related to interspecific competition were leaf length and width, rhizome diameter, shoots meter⁻¹ and root maximum length, among others. Several of these traits changed under increases in temperature, nutrient enrichment and eutrophication. Changes in response traits have, therefore, fundamental consequences for interspecific competition in seagrass meadows, as they determine the winners and losers in the outcome of space competition. In this section, we will discuss the potential scenarios under which interspecific competition would happen, and the resulting seagrass community.

The enhanced morphology under increased temperature for *T. hemprichii*, *C. serrulata* and *E. acoroides* indicates that most of the climax seagrass species will see their probability of space preemption increased (Figure 1.2.a). For the ephemeral species *H. stipulacea*, this probability would decrease along with its size. The climax species would therefore be able to outcompete *H. stipulacea* due to the increasing size difference. However, there is one consideration to be taken into account. Under increased temperature, other traits apart from the size of the seagrass structures may be important for competition, opening new ways in which species could outcompete each other.

In a nutrient enrichment scenario, *T. hemprichii* and *C. serrulata* have their nutrient demands satisfied, yet they are normally nutrient limited in oligotrophic habitats. An increase in nutrients can enhance their growth (Udy and Dennison 1997). Even though *H. stipulacea* and seedlings of *E. acoroides* did not show signs of nutrient limitation, this principle could also be true for these species in the longer term. This scenario does not change traits related to interspecific competition and, therefore, would not change the final configuration of the meadow (Figure 1.2.b).

Interspecific competition under the combined effects of increased temperature and nutrient enrichment would potentially mean the displacement of *T. hemprichii* and *H. stipulacea* (Figure 1.2.c). The reduction of *T. hemprichii*'s leaf area indicates a lower capacity for light preemption (Williams, 1987), and the reduction of root length has a double negative effect, as this species

loses its capacity to access deeper nutrient pools in the sediment and it must compete in the shallower sediment layer with roots of other species (Williams 1990, Duarte et al. 1998). For *H. stipulacea*, its reduced size due to the increase in temperature also means a potential competitive displacement. The final scenario sees an increase in the abundance of *E. acoroides*, which shows a bigger size due to the warmer temperature and the presence of *C. serrulata*, which could show future loss of performance due to carbon limitation.

Lastly, eutrophic conditions are generally negative for seagrass meadows (Burkholder et al. 2007). In Unguja Island, meadows under eutrophic conditions were formed only by two species, *T. hemprichii* and *C. rotundata*. These meadows, additionally, had lower percentage cover than meadows in oligotrophic and mesotrophic sites (Teichberg et al., *in prep*). Therefore, the first clear effect of eutrophication is the disappearance of some species in the community (Figure 1.2.d). Additionally, generally ephemeral and opportunistic species tend to thrive under these conditions (Fourqurean et al. 1995), displacing other climax seagrass species. For this reason, this scenario sees the disappearance of two seagrass species (*E. acoroides* and *C. serrulata*), and only the presence of *T. hemprichii* (with a reduced site and higher shoot density) and *H. stipulacea*, a species present under array of trophic conditions (Winters et al. 2020).

(3) Ecosystem level: Carbon storage in seagrass ecosystems

In this section, I will discuss how the final configuration of the meadows and their trait characteristics will affect carbon storage in tropical seagrasses. As presented in chapter 7, two functional traits were positively correlated with carbon storage: the leaf area index (LAI) and rooting depth. Higher leaf area index indicated higher particle trapping, while rooting depth indicated a deeper source of carbon.

There were two scenarios in which a potential increase in carbon storage was predicted, and these were under the single effect of temperature and nutrient enrichment (Figure 1.a.3 and 1.b.3). For higher temperatures, bigger leaves and root length are indicative of higher LAI and rooting depth respectively, increasing carbon storage. For the nutrient enrichment scenario, the lack of displacement of seagrass species indicated, at least, no changes in the carbon storage and the potential for a variety of rooting depths and canopy complexity. The functional diversity of traits could, potentially, be linked to higher carbon storage (Garnier et al. 2016).

In the case of the combined increase in temperatures and nutrients (Figure 1.c.3), there is an offset between the effects of both stressors. There is an increase in leaves and root sizes in *E. acoroides* and *C. serrulata*. However, the displacement of two other species could translate in

the loss of potential carbon sources. Therefore, no increase nor decrease in carbon storage was predicted.

Lastly, eutrophic conditions could indicate a loss of potential carbon storage. First, with eutrophication comes the displacement of a variety of seagrass species (Burkholder et al. 2007). Secondly, the decrease in leaves size and rooting length is negatively correlated with LAI and rooting depth, and therefore, with carbon storage. As a consequence, eutrophic conditions could translate in the loss of carbon storage capacity in seagrass meadows.

IV. Limitations, knowledge gaps and future research

When discussing limitations in the study of seagrasses, there are extrinsic reasons that constrain the length and scope of seagrass research. First, the experiments presented in chapters 3 and 4 are of a length of ~6 weeks. Similarly, the experiment on the effect of seaweed farming on seagrass took place for 9 weeks. Therefore, the exposure time to experimental conditions is short, which makes long term predictions difficult. Longer experimental times are needed in order to make long term predictions on the effects of environmental drivers on seagrasses, which are generally not possible due to logistical and economical reasons.

A similar problem occurs with the limited approach taken to the study of ecological processes in seagrasses in the field. In order to scale up results from individual plants to ecosystem level processes, we need of a comprehensive scope, which is rarely the case. In this last issue is where the TBF presented in this thesis can aid researchers, as it presents ecological hypotheses that can be answered using simple trait metrics. With only 7% of seagrass studies being performed using concepts developed under a trait-based theory, there are big research opportunities to further push seagrass research.

More specifically in relation to TBFs, there is a concept that was not deeply discussed in this dissertation and is currently under-explored in seagrass research, namely seagrass trait plasticity. Trait plasticity has several definitions, but it is generally considered the as adaptive process that traits undergo under different environmental conditions at the plant level. In other words, it reflects the interaction between the plant genotype and the environment. In the last years, plasticity in plant traits has been brought into the forefront in trait-based research, due to its implications for species coexistence (Meilhac et al. 2020). Additionally, the role of phenotypic plasticity has also been highlighted in seagrasses (Pazzaglia et al. 2021). The relation between response traits and phenotypic plasticity is one currently underexplored and

will prove fundamental for conservation and restoration efforts under environmental change (Pazzaglia et al. 2021).

Finally, there are two topics that were previously mentioned but beg repetition, which are the inclusion of ecosystem services in seagrass research and the adoption of concepts from terrestrial plant ecology. Science communication is an area of fundamental importance for the future of research, not only to highlight important results, but also for the accurate communication of scientific data. In this field, the use of the concept of ecosystem services is necessary to include seagrasses into management and policy (Ruiz-Frau et al. 2017), and to communicate their importance to the general public.

Lastly, up-to-date information from the terrestrial plant ecology field could aid the development of research in seagrasses. The TBF developed in this dissertation could not have been possible without all the research done in terrestrial plant ecology (see Garnier et al. 2016) and, therefore, the exploration of this literature can show future areas of research for marine plants.

V. Final conclusions

This dissertation fosters the inclusion of a trait-based perspective into seagrass research, which allows for the answering of classical ecological questions using simple trait metrics. The change from a species identity focus to a trait one shows the evolution to a more mathematical and numerical approach to seagrass ecology.

The proposal to apply a TBF to seagrass ecology acknowledges the importance of considering the scientific advances of other research fields in order to push marine research forward. My literature review demonstrated that there is a wealth of data on seagrass response and effect traits and on seagrass ecosystem functions. There is, therefore, a great potential to re-analyze these data under a TBF lens in order to test new research questions and hypotheses; and to collect new data identified as critical but missing by this TBF approach. The review of the seagrass literature has demonstrated that most of the hypotheses proposed in the TBF have not yet been formally tested, including the relative importance of stochastic vs niche based processes in seagrasses, phylogenetic and genotypic control of traits, or the importance of trait dominance or complementarity for ecosystem function provision. The use of a TBF that assesses the vulnerability of ecosystem function and service provision can help to understand which ecosystem services may be compromised by the changes in species traits or species abundances. Therefore, the translation of biological and ecological seagrass research into a framework explicitly considering ecosystem services will prove fundamental for the

development of comprehensive policies and for the informed management of seagrass meadows.

When it comes to response traits, seagrass species sharing the same original geographic area, or even the same meadow, may respond differently to temperature and nutrient conditions. Different scenarios might show winners and losers, but there is no trait that makes a winner under all circumstances, suggesting that if conditions change, some species survivorship might be endangered. Worth highlighting is the importance of multifactorial experiments, which unveiled the combined effects of stressors on seagrasses, endangering a climax species like *T. hemprichii*. In addition to global stressors like temperature and extensive local stressors like nutrient enrichment, there are endemic environmental impacts like seaweed farming that can drive the evolution of seagrass meadows and the disappearance of species like *H. stipulacea*. The study of all of these environmental drivers gives information important for developing effective management strategies in the future. The trait responses of seagrasses to environmental drivers have fundamental implications for interspecific competition among seagrasses and, lastly, ecosystem function provision. The finding of asymmetrical competition among seagrasses determined that size difference is an important driver for space preemption. This final configuration of the seagrass meadows determines the capacity for carbon storage, with the structural complexity of the canopy and rooting depth as two of its main drivers under no environmental constraints.

In conclusion, the comprehensive synthesis of the main results of this dissertation highlights the value of using a trait-based perspective on seagrass research, which allows to scale up individual plant responses to community processes and ecosystem functions. The use of traits for ecological research can, therefore, provide new insights in classical ecological questions to further understand the biology and ecology of seagrasses.

VI. References

- Abe, M., Yokota, K., Kurashima, A., and Maegawa, M. (2009). High water temperature tolerance in photosynthetic activity of *Zostera japonica* ascherson and graebner seedlings from ago bay, mie prefecture, central Japan. *Fisheries Science*, 75, 1117–1123. <https://doi.org/10.1007/s12562-009-0141-x>
- Agawin, N. S., Duarte, C. M., and Fortes, M. D. (1996). Nutrient limitation of Philippine seagrasses (Cape Bolinao, NW Philippines): in situ experimental evidence. *Marine Ecology Progress Series*, 138, 233-243. <https://doi.org/10.3354/meps138233>
- Belshe, E. F., Hoeijmakers, D., Herran, N., Mtolera, M., and Teichberg, M. (2018). Seagrass community-level controls over organic carbon storage are constrained by geophysical attributes

- within meadows of Zanzibar, Tanzania. *Biogeosciences*, 15, 4609-4626. <https://doi.org/10.5194/bg-15-4609-2018>
- Bouma, T. J., De Vries, M. B., Low, E., Peralta, G., Táncoz, I. V., van de Koppel, J., and Herman, P. M. J. (2005). Trade-offs related to ecosystem engineering: A case study on stiffness of emerging macrophytes. *Ecology*, 86, 2187-2199. <https://doi.org/10.1890/04-1588>
- Brink, P., Berghöfer, A., Schröter-Schlaack, C., Sukhdev, P., Vakrou, A., White, S., and Wittmer, H. (2009). TEEB-The Economics of Ecosystems and Biodiversity for National and International Policy Makers 2009. *TEEB-The Economics of Ecosystems and Biodiversity for National and International Policy Makers 2009*. <https://doi.org/10.1093/acprof:oso/9780199676880.003.0007>
- Browne, N. K., Yaakub, S. M., Tay, J. K., and Todd, P. A. (2017). Recreating the shading effects of ship wake induced turbidity to test acclimation responses in the seagrass *Thalassia hemprichii*. *Estuarine, Coastal and Shelf Science*, 199, 87 - 95. <https://doi.org/10.1016/j.ecss.2017.09.034>
- Bulthuis, D. A. (1987). Effects of temperature on photosynthesis and growth of seagrasses. *Aquat. Bot.* 2, 27–40. [https://doi.org/10.1016/0304-3770\(87\)90084-2](https://doi.org/10.1016/0304-3770(87)90084-2)
- Burkholder, J. M., Tomasko, D. A., and Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of experimental marine biology and ecology*, 350, 46-72. <https://doi.org/10.1016/j.jembe.2007.06.024>
- Collier, C.J., Waycott, M., Ospina, A.G. (2012) Responses of four Indo-West Pacific seagrass species to shading. *Marine Pollution Bulletin*, 65, 342-354. <https://doi.org/10.1016/j.marpolbul.2011.06.017>
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., and Hannon, B. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387, 253–260. <https://doi.org/10.1038/387253a0>
- Duarte, C. M., Martín, M., & Margarita, G. (1995). Evidence of iron deficiency in seagrasses growing above carbonate sediments. *Limnology and Oceanography*, 40, 1153-1158. <https://doi.org/10.4319/lo.1995.40.6.1153>
- Duarte, C. M., Merino, M., Agawin, N. S. R., Uri, J., Fortes, M. D., Gallegos, M. E., et al. (1998). Root production and belowground seagrass biomass. *Marine Ecology Progress Series*, 171, 97–108. <https://doi.org/10.3354/meps171097>
- Durako, M. J., and Moffler, M. D. (1985). Observations on the reproductive ecology of *Thalassia testudinum* (Hydrocharitaceae). III. Spatial and temporal variations in reproductive patterns within a seagrass bed. *Aquatic Botany*, 22, 265–276. [https://doi.org/10.1016/0304-3770\(85\)90004-X](https://doi.org/10.1016/0304-3770(85)90004-X)
- Fourqurean, J. W., Powell, G. V., Kenworthy, W. J., and Zieman, J. C. (1995). The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. *Oikos*, 349-358. <https://doi.org/10.2307/3546120>

- Garnier, E., Navas, M. L., and Grigulis, K. (2016). *Plant functional diversity: organism traits, community structure, and ecosystem properties*. Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780198757368.001.0001>
- Grace, J. B., Michael Anderson, T., Smith, M. D., Seabloom, E., Andelman, S. J., Meche, G., ... and Knops, J. (2007). Does species diversity limit productivity in natural grassland communities?. *Ecology Letters*, 10, 680-689. <https://doi.org/10.1111/j.1461-0248.2007.01058.x>
- Guerrero-Meseguer, L., Marín, A., and Sanz-Lázaro, C. (2017). Future heat waves due to climate change threaten the survival of *Posidonia oceanica* seedlings. *Environmental Pollution* 230, 40–45. <https://doi.org/10.1016/j.envpol.2017.06.039>
- Gullström, M., Bodin, M., Nilsson, P. G., and Öhman, M. C. (2008). Seagrass structural complexity and landscape configuration as determinants of tropical fish assemblage composition. *Marine Ecology Progress Series*, 363, 241 - 255. <https://doi.org/10.3354/meps07427>
- Herr, D., Pidgeon, E., and Laffoley, D. D. A. (2012). *Blue carbon policy framework 2.0: based on the discussion of the International Blue Carbon Policy Working Group*. IUCN. <https://doi.org/10.1201/9780429435362-15>
- Hughes, A.R., and Stachowicz, J. J. (2011). Seagrass genotypic diversity increases disturbance response via complementarity and dominance. *Journal of Ecology*, 99, 445-453. <https://doi.org/10.1111/j.1365-2745.2010.01767.x>
- Jones, C. G., Lawton, J. H., and Shachak, M. (1994). Organisms as ecosystem engineers. In *Ecosystem management* (pp. 130-147). Springer, New York, NY. https://doi.org/10.1007/978-1-4612-4018-1_14
- Jones, B. L., Nordlund, L. M., Unsworth, R. K., Jiddawi, N. S., and Eklöf, J. S. (2021). Seagrass structural traits drive fish assemblages in small-scale fisheries. *Frontiers in Marine Science*, 8, 354. <https://doi.org/10.3389/fmars.2021.640528>
- Kaldy, J. E. (2014). Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. *Journal of Experimental Marine Biology and Ecology*, 453, 108–115. <https://doi.org/10.1016/j.jembe.2013.12.020>
- Kattge, J., Diaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Bönisch, G., ... and Wirth, C. (2011). TRY—a global database of plant traits. *Global change biology*, 17, 2905-2935. <https://doi.org/10.5194/egusphere-egu2020-20191>
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional ecology*, 16, 545-556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Lee, K. S., Park, S. R., and Kim, Y. K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology*, 350, 144-175. <https://doi.org/10.1016/j.jembe.2007.06.016>

- Legendre, L., Legendre, P.: *Écologie Numérique*. Masson, Paris and Les Presses de l'Université du Québec, Québec (1979). <https://doi.org/10.1002/iroh.19810660515>
- Meilhac, J., Deschamps, L., Maire, V., Flajoulot, S., and Litrico, I. (2020). Both selection and plasticity drive niche differentiation in experimental grasslands. *Nature Plants*, 6, 28-33. <https://doi.org/10.1038/s41477-019-0569-7>
- Mtwana Nordlund, L., Koch, E. W., Barbier, E. B., and Creed, J. C. (2016). Seagrass ecosystem services and their variability across genera and geographical regions. *PLoS One*, 11, e0163091. <https://doi.org/10.1371/journal.pone.0163091>
- Mvungi, E. F., and Pillay, D. (2019). Eutrophication overrides warming as a stressor for a temperate African seagrass (*Zostera capensis*). *PloS one*, 14, e0215129. <https://doi.org/10.1371/journal.pone.0215129>
- Niu, S., Zhang, P., Liu, J., Guo, D., and Zhang, X. (2012). The effect of temperature on the survival, growth, photosynthesis, and respiration of young seedlings of eelgrass *Zostera marina* L. *Aquaculture*, 98–108. <https://doi.org/10.1016/j.aquaculture.2012.04.010>
- Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J. M., Romero, J., and Pérez, M. (2019). Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. *Marine environmental research*, 145, 27-38. <https://doi.org/10.1016/j.marenvres.2019.02.002>
- Orth, R. J., Heck, K. L., and van Montfrans, J. (1984). Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries*, 7, 339-350. <https://doi.org/10.2307/1351618>
- Paul, M., Bouma, T. J., and Amos, C. L. (2012). Wave attenuation by submerged vegetation: combining the effect of organism traits and tidal current. *Marine Ecology Progress Series*, 444, 31-41. <https://doi.org/10.3354/meps09489>
- Pazzaglia, J., Santillán-Sarmiento, A., Helber, S. B., Ruocco, M., Terlizzi, A., Marín-Guirao, L., and Procaccini, G. (2020). Does Warming Enhance the Effects of Eutrophication in the Seagrass *Posidonia oceanica*?. *Frontiers in Marine Science*, 7, 1067. <https://doi.org/10.3389/fmars.2020.564805>
- Pazzaglia, J., Reusch, T. B., Terlizzi, A., Marín-Guirao, L., & Procaccini, G. (2021). Phenotypic plasticity under rapid global changes: The intrinsic force for future seagrasses survival. *Evolutionary Applications*, 14, 1181-1201. <https://doi.org/10.1111/eva.13212>
- Peralta, G., Van Duren, L. A., Morris, E. P., and Bouma, T. J. (2008). Consequences of shoot density and stiffness for ecosystem engineering by benthic macrophytes in flow dominated areas: a hydrodynamic flume study. *Marine Ecology Progress Series*, 368, 103-115. <https://doi.org/10.3354/meps07574>
- Pereda-Briones, L., Tomas, F., and Terrados, J. (2018). Field transplantation of seagrass (*Posidonia oceanica*) seedlings: effects of invasive algae and nutrients. *Marine Pollution Bulletin* 134, 160–165. <https://doi.org/10.1016/j.marpolbul.2017.09.034>

- Pérez, M., Romero, J., Duarte, C. M., and Sand-Jensen, K. (1991). Phosphorus limitation of *Cymodocea nodosa* growth. *Marine Biology*, 109, 129–133. <https://doi.org/10.1007/BF01320239>
- Ruiz-Frau, A., Gelcich, S., Hendriks, I. E., Duarte, C. M., and Marbà, N. (2017). Current state of seagrass ecosystem services: research and policy integration. *Ocean and Coastal Management*, 149, 107-115. <https://doi.org/10.1016/j.ocecoaman.2017.10.004>
- Salita, J. T., Ekau, W., and Saint-Paul, U. (2003). Field evidence on the influence of seagrass landscapes on fish abundance in Bolinao, northern Philippines. *Marine Ecology Progress Series*, 247, 183-195. <https://doi.org/10.3354/meps247183>
- Shipley, B., De Bello, F., Cornelissen, J.H.C., Laliberté, E., Laughlin, D.C. and Reich, P.B. (2016) Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*, 180, 923-931. <https://doi.org/10.1007/s00442-016-3549-x>
- Short, F., Carruthers, T., Dennison, W., and Waycott, M. (2007). Global seagrass distribution and diversity: a bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350, 3-20. <https://doi.org/10.1016/j.jembe.2007.06.012>
- Stoner, A. W. (1983). Distribution of fishes in seagrass meadows: role of macrophyte biomass and species composition. *Fish Bulletin*, 81, 837-845.
- Summerson, H. C., and Peterson, C. H. (1984). Role of predation in organizing benthic communities of a temperate-zone seagrass bed. *Marine ecology progress series*, 15, 63-77. <https://doi.org/10.3354/meps015063>
- Udy, J. W., and Dennison, W. C. (1997). Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. *Journal of Experimental Marine Biology and Ecology*, 217, 253-277. [https://doi.org/10.1016/s0022-0981\(97\)00060-9](https://doi.org/10.1016/s0022-0981(97)00060-9)
- van der Plas, F., Schröder-Georgi, T., Weigelt, A., Barry, K., Meyer, S., Alzate, A., ... and Wirth, C. (2020). Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nature ecology and evolution*, 4, 1602-1611. <https://doi.org/10.1038/s41559-020-01316-9>
- Viana, I. G., Siriwardane-de Zoysa, R., Willette, D. A., and Gillis, L. G. (2019). Exploring how non-native seagrass species could provide essential ecosystems services: a perspective on the highly invasive seagrass *Halophila stipulacea* in the Caribbean Sea. *Biological Invasions*, 21, 1461-1472. <https://doi.org/10.1007/s10530-019-01924-y>
- Virnstein, R. W., Mikkelsen, P. S., Cairns, K. D., and Capone, M. A. (1983). Seagrass beds versus sand bottoms: the trophic importance of their associated benthic invertebrates. *Florida Scientist*, 363-381.
- Williams, S. L. (1987). Competition between the seagrasses *Thalassia testudinum* and *Syringodium filiforme* in a Caribbean lagoon. *Marine Ecology Progress Series*, 35, 91-98. <https://doi.org/10.3354/meps035091>

- Williams, S. L. (1990). Experimental studies of Caribbean seagrass bed development. *Ecological Monographs*, 60, 449-469. <https://doi.org/10.2307/1943015>
- Williams, A. H., Coen, L. D., and Stoelting, M. S. (1990). Seasonal abundance, distribution, and habitat selection of juvenile *Callinectes sapidus* (Rathbun) in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology*, 137, 165-183. [https://doi.org/10.1016/0022-0981\(90\)90183-d](https://doi.org/10.1016/0022-0981(90)90183-d)
- Winters, G., Beer, S., Willette, D. A., Viana, I. G., Chiquillo, K. L., Beca-Carretero, P., et al. (2020). The invasive tropical seagrass *Halophila stipulacea*: a review of what we know alongside identifying gaps in our knowledge. *Frontiers in Marine Science*, 7, 300. <https://doi.org/10.3389/fmars.2020.00300>
- Xu, S., Zhou, Y., Wang, P., Wang, F., Zhang, X., and Gu, R. (2016). Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass *Zostera marina* L. *PeerJ*, 4, e2697. <https://doi.org/10.7717/peerj.2697>
- Zayas-Santiago, C., Rivas-Ubach, A., Kuo, L.-J., Ward, N. D., and Zimmerman, R. C. (2020). Metabolic profiling reveals biochemical pathways responsible for eelgrass response to elevated CO₂ and temperature. *Scientific Reports*, 10, 4693. <https://doi.org/10.1038/s41598-020-61684-x>

Ort, Datum: Bremen, 28.05.2021

Versicherung an Eides Statt

Ich, Agustina Moreira Seporati (Vorname, Name, Anschrift, Matr.-Nr.)

versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.

Ich versichere an Eides Statt, dass ich die vorgenannten Angaben nach bestem Wissen und Gewissen gemacht habe und dass die Angaben der Wahrheit entsprechen und ich nichts verschwiegen habe.

Die Strafbarkeit einer falschen eidesstattlichen Versicherung ist mir bekannt, namentlich die Strafandrohung gemäß § 156 StGB bis zu drei Jahren Freiheitsstrafe oder Geldstrafe bei vorsätzlicher Begehung der Tat bzw. gemäß § 161 Abs. 1 StGB bis zu einem Jahr Freiheitsstrafe oder Geldstrafe bei fahrlässiger Begehung.

Ort, Datum Unterschrift

