The importance of carbon dioxide and dinitrogen fixation for seasonal coral reef metabolism: from organism to ecosystem functioning

A dissertation by

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To my family, old and new

"Every time I slip into the ocean, it's like going home."
Sylvia Earle

"We still have much to learn."
Callum Roberts

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Table of contents

Abstract

The exceptional productivity of coral reefs is maintained through efficient recycling of nutrients, the capture of allochthonous materials, and de novo synthesis of nutrients through e.g. microbial dinitrogen (N_2) fixation. These processes are performed on the organism level by multipartite "holobionts" such as scleractinian corals with symbiotic photosynthetic dinoflagellates (i.e. zooxanthellae), and N_2 fixing microbes (i.e. diazotrophs), and on the ecosystem level by reef ecosystem engineers such as corals and sponges in interplay with microbial communities. Studies that provide comparative data on the contribution of functional groups to total reef primary production and link photosynthetic primary production with other carbon (C) cycling processes or N_2 fixation under environmental change are lacking. This thesis therefore presents a series of interconnected studies performed under seasonally changing environmental conditions (e.g. light, temperature, nutrient availability) in a high latitude coral reef in the northern Red Sea to address the following key questions:

> What are the rates of primary production through photosynthesis and N_2 fixation of the water column and dominant benthic functional groups over the seasonal variation?
>
> How much do key functional groups contribute to total reef primary production, and how much does N_2 fixation contribute to the potential nitrogen (N) demand of photosynthetic primary production in the reef?
>
> How does reef photosynthetic primary production compare to other C cycling processes?

Findings revealed that water column photosynthetic primary production was relatively low compared to other seas and lowest in the oligotrophic summer. Water column N_2 fixation was stable throughout the year and may have contributed substantially (~21 %) to the N demand of planktonic primary producers during the nutrient-depleted summer period. Benthic photosynthetic primary production was dominated by hard and soft corals (59 - 71 % of primary production), while highest benthic N_2 fixation was performed by microbial mats, the coral rock reef structure, the carbonate sediment community, and turf algae (summed ~ 95 % of benthic N_2 fixation). The reef community acted net autotrophic in winter and spring, balanced in summer, and net heterotrophic in fall. Community N_2 fixation peaked in summer, coinciding with low nutrient availability and high temperatures. This was also the season when N_2 fixation could have contributed most to the N demand of reef-wide photosynthetic primary production (~18 %). Uptake of dissolved organic C (DOC) via sponges from the surrounding water was comparable to reef primary production, outranking it in summer and autumn when DOC uptake rates by the sponges were 3-fold higher than in winter and spring. Sponge DOC uptake was an order of

magnitude higher than DOC released by the benthic community, indicating that this sponge-mediated process goes beyond recycling and actually captures allochthonous C from surrounding waters. While the extensively researched hard and soft corals are the dominant photosynthetic primary producers, less investigated communities inhabiting the coral rock reef structure, its cavities, and sediments obviously play key roles in elemental cycles throughout coral reefs. This thesis presents quantitative insights into the major C cycles of a coral reef under variable seasonal conditions. The C cycling model presented herein should be seen as an initial step, to be expanded with other elemental cycles to gain a more cohesive perspective on coral reef functioning.

Zusammenfassung

Korallenriffe zeichnen sich durch eine außergewöhnlich hohe Produktivität aus, die vor allem durch ein effizientes Recycling von Nährstoffen, die Aufnahme von allochthonen Stoffen und die de novo Synthese von Nährstoffen (z.B. *via* mikrobielle Distickstoff (N_2)- Fixierung) gewährleistet wird. Diese Prozesse finden einerseits auf Organismus-Ebene in Multipartner Holobionten statt, wie beispielsweise in der Symbiose zwischen Steinkorallen, phototrophen Dinoflagellaten (Zooxanthellen) und N_2-fixierenden Mikroben (Diazotrophe), und andererseits auf Ökosystem-Ebene durch das Zusammenspiel zwischen Ökosystem-Ingenieuren (z.B. Korallen, Schwämme) und mikrobiellen Gemeinschaften.

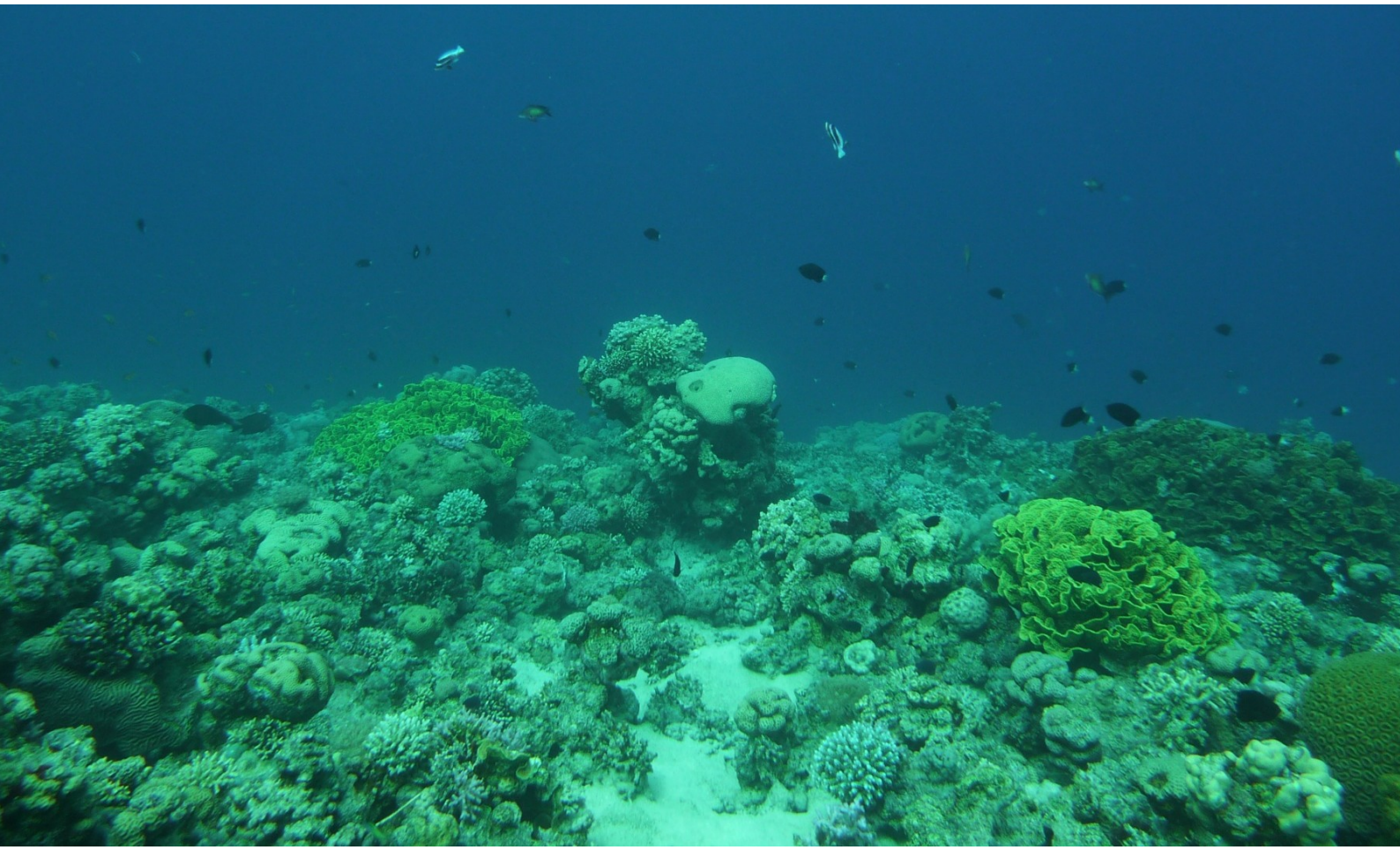
Bislang gibt es kaum Studien, die den relativen Beitrag von funktionellen Gruppen zur gesamten Primärproduktion des Korallenriffes vergleichend darstellen, und die die photosynthetische Primärproduktion mit anderen relevanten Prozessen des Kohlenstoffkreislaufs oder mit dem Prozess der N_2 Fixierung unter sich verändernden Umweltbedingungen in Verbindung bringen. In dieser Dissertation werden daher eine Reihe von miteinander verbundenen Studien vorgestellt, die in einem in hohem Breitengraden gelegenen Korallenriff des nördlichen Roten Meeres unter saisonal schwankenden Umweltbedingungen (z.B. Licht, Temperatur, Nährstoffverfügbarkeit) durchgeführt wurden um folgende Schlüsselfragestellungen zu beantworten:

> Wie hoch ist die photosynthetische Primärproduktion und N_2 Fixierung von funktionellen Gruppen des Riffbenthos und der darüber liegenden Wassersäule im jahreszeitlichen Wandel?
>
> Wie hoch tragen die einzelnen funktionellen Gruppen des Riffbenthos zur gesamten Primärproduktion des Korallenriffes bei, und inwieweit deckt die N_2 Fixierung den potentiellen Stickstoff-Bedarf der photosynthetisch aktiven Primärproduzenten im Riff?
>
> Wie vergleichbar ist die photosynthetische Primärproduktion mit anderen Prozessen des Kohlenstoffkreislaufes im Korallenriff?

Die Ergebnisse zeigen, dass die photosynthetische Primärproduktion im Riffwasser des nördlichen Roten Meeres im Vergleich zu anderen Gewässern relativ niedrig ist und dass die niedrigsten Raten während der oligotrophen Sommermonate gemessen wurden. Dahingegen wies die N_2 Fixierung im Riffwasser im saisonalen Vergleich konstante Raten auf, wodurch sie vor allem während der nährstoffarmen Sommermonate wesentlich (~21 %) zum Stickstoff-Bedarf der Primärproduktion beitragen könnte. Insgesamt machten Steinkorallen und Weichkorallen den Hauptanteil der gesamten benthischen Primärproduktion aus (59 - 71 % der Primärproduktion),

während mikrobielle Matten, Korallengestein, Karbonatsande und filamentöse Algen mit ~ 95 % zur gesamten benthischen N_2 Fixierung beitrugen. Der Metabolismus der gesamten Riffgemeinschaft war im Winter und Frühling netto autotroph, ausgeglichen im Sommer, und netto heterotroph im Herbst. Die N_2 Fixierung der gesamten Riffgemeinschaft zeigte ein eindeutiges Maximum in den Sommermonaten, in denen zeitgleich die niedrigsten Nährstoffgehalte und höchsten Wassertemperaturen im Riff gemessen wurden. Zusätzlich war der prozentuale Beitrag der N_2 Fixierung zum Stickstoff-Bedarf der gesamten Primärproduktion im Riff mit ~18 % während der Sommermonate am höchsten. Die Aufnahme von gelöstem organischen Kohlenstoff (DOC) aus dem Umgebungswasser durch Schwämme war vergleichbar mit der Primärproduktion der Riffgemeinschaft, vor allem im Sommer und Herbst, wenn die DOC Aufnahmerate durch Schwämme 3-fach höher war verglichen mit Winter und Frühling. Insgesamt lag die DOC Aufnahme durch Schwämme eine Größenordnung über der DOC Abgabe des Riffbenthos, was darauf hindeutet, dass Schwämme nicht nur DOC wiederverwerten sondern darüber hinaus auch allochthonen Kohlenstoff aus dem Riffwasser einfangen. Steinkorallen und Weichkorallen sind die dominanten photosynthetisch aktiven Primärproduzenten und waren bereits Gegenstand von umfangreichen Studien. Darüberhinaus wird deutlich, dass auch die Riffgemeinschaften, welche Korallengesteine, Hohlräume und Sande in Korallenriffen besiedeln und in Studien bislang weniger Beachtung fanden, vermutlich eine entscheidende Schlüsselfunktion innerhalb der Stoffkreisläufe von Korallenriffen einnehmen. Die vorliegende Dissertation präsentiert daher quantitative Einblicke in die wichtigsten Kohlenstoffkreisläufe in Korallenriffen unter sich saisonal verändernden Umweltbedingungen. Das dafür entworfene und vorgestellte Kohlenstoffkreislaufmodel, in welches zusätzliche Stoffkreisläufe integriert werden können, dient dabei als Ausgangspunkt um die Funktionsweise von Korallenriffen zusammenfassend und übergreifend abzubilden.

General Introduction



Coral reefs, oases in a marine desert

Coral reefs rank among the world's most productive ecosystems which makes them highly important for surrounding oceans, as well as human populations living in their vicinity (Moberg and Folke 1999; Burke et al. 2011). They occur in shallow seas between 30 °N and 30 °S (Fig. I-1), and are generally found in strongly oligotrophic environments (Lewis 1977; Kleypas et al. 1999; Veron 2000). This seeming paradox of high productivity under oligotrophic conditions is resolved by tight recycling loops of nutrients and organic matter within coral reef communities (Naumann et al. 2012; de Goeij et al. 2013), the assimilation of allochthonous material through benthic-pelagic coupling (Wild et al. 2004; Houlbreque and Ferrier-Pages 2009), and the production of new nutrients through microbial dinitrogen (N_2) fixation (Cardini et al. 2014). The above mentioned processes all come together in the organisms which give coral reefs their name. Corals are multipartite symbioses (i.e. holobionts) of cnidarian hosts and microbial symbionts (Knowlton and Rohwer 2003). The most researched symbionts are dinoflagellate algae of the genus *Symbiodinium* (i.e. zooxanthellae) which live in the host's tissues and provide it with carbohydrates from photosynthesis (Stambler 2011). In addition, corals have been found to host a wide range of bacteria and other microorganisms which play a role in the survival of the host in its environment. For example, microbial N_2 fixation to ammonia by diazotrophic symbionts may provide the host and zooxanthellae with a much needed nitrogen (N) source for protein production (Shashar et al. 1994a; Lesser et al. 2007). However, microbial symbionts may also become pathogenic when environmental conditions change detrimentally (Knowlton and Rohwer 2003). In addition, the coral host is able to obtain nutrients and carbon (C) heterotrophically by feeding on a wide range of particulate and dissolved sources of nutrients and C, allowing energy and nutrients to be harvested from the surrounding ocean as waters wash through the reef (Grottoli et al. 2006; Houlbreque and Ferrier-Pages 2009). A fraction of the C assimilated by corals through daily photosynthesis is released through their outer surface as mucus (e.g. 40-50% for the genus *Acropora*; Crossland et al. 1980), which protects the coral from sedimentation and pathogens (Bythell and Wild 2011). The mucus quickly sloughs off from the coral and the majority of it dissolves into the water column (Wild et al. 2004). As the particulate mucus fraction enters the water column, it can pick up pelagic particles and form aggregates which over time become negatively buoyant and sink back down to the reef. These aggregates are remineralized in substrates such as carbonate sediments, and the captured nutrients and organic C become available to the wider coral reef community (Wild et al. 2004; Mayer and Wild 2010). The fraction of coral mucus which dissolves (together with dissolved organic carbon (DOC) released by other reef organisms) forms a major pathway in coral reef communities (Haas et al. 2010; Naumann et al. 2010). In the open ocean, this DOC is made re-available to higher trophic levels

via the "microbial loop" (Azam and Malfatti 2007). This bacterioplankton mediated process was, however, insufficient for observed DOC removal in Caribbean and Indo-Pacific reefs (de Goeij and van Duyl 2007). Cavities which riddle the solid calcium carbonate structure of reefs have been found to harbor high densities of sponges (Richter et al. 2001). These cavity sponges potentially take up DOC at rates far surpassing the bacterioplankton in the water column above (de Goeij et al. 2013). Simultaneously, the sponges expel vast amounts of choanocyte and mesohyl cells from their filtration apparatus (Alexander et al. 2014; Maldonado 2015), and in doing so, they transform water column DOC into particulate organic carbon (POC) which is available to the wider reef community (de Goeij et al. 2013). Ultimately, the foundation of all this biological activity is formed by primary production through photosynthesis.

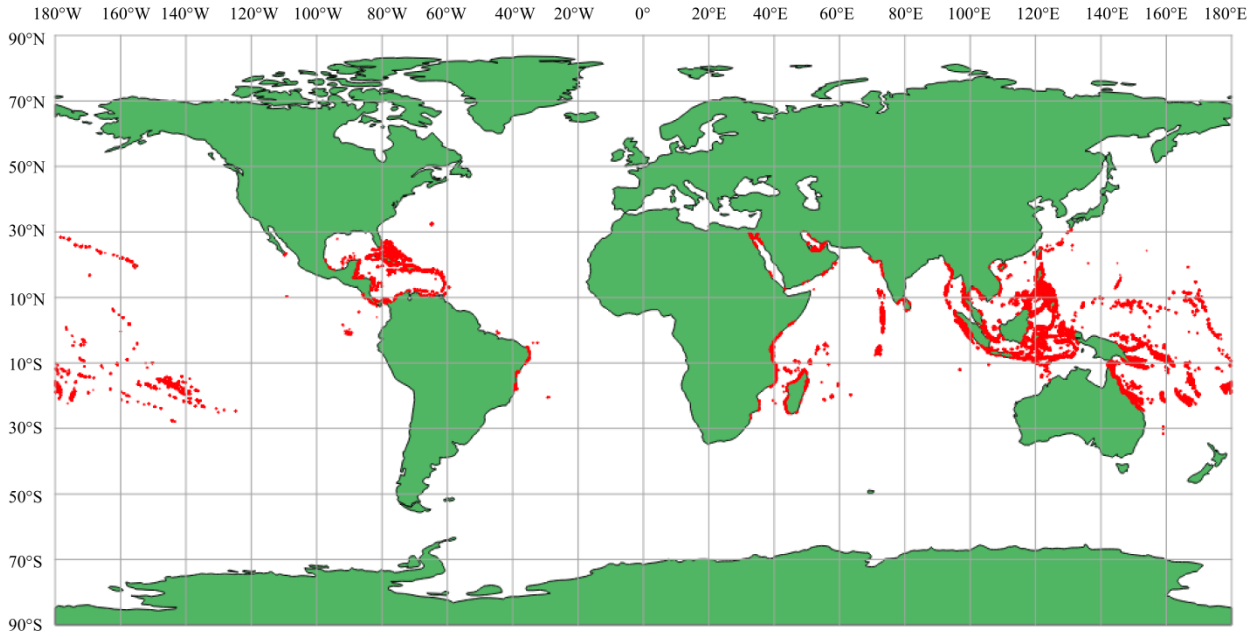


Fig. I-1. Coral reef distribution throughout the world's oceans. Coral reefs in red, data source: UNEP-WCMC 2010.

Primary production in coral reefs

Coral reefs harbor many different organisms that are capable of primary production (Venn et al. 2008; Fong and Paul 2011; Charpy et al. 2012; see further below). The heterogeneous reef structure provides habitats which differ substantially in their environmental conditions (Titlyanov and Titlyanova 2012). While the reef crest is subjected to fierce wave action, back reef lagoons have a much more docile water regime. Organisms which live near the water surface receive maximum irradiance which lessens further down the reef slope, causing shallow reef flats to generally have higher levels of gross primary production (GPP) than deeper reef slopes (Long et al. 2013). Daytime GPP is offset by respiration (R) during the full diurnal cycle. GPP on coral reefs ranges between 100 and 2000 mmol C m^{-2} day^{-1} (Atkinson 2011). Although there is a

positive correlation between GPP and R in coral reef habitats, there are differences between them with regard to the balance between the two processes (GPP/R). This production to respiration (PR) ratio indicates whether a specific habitat is net autotrophic (PR > 1), or net heterotrophic (PR < 1). When a habitat is net autotrophic, there is a higher rate of production than consumption of organic material, and when it is net heterotrophic, more organic material is consumed than produced. Although PR ratios differ between reefs with their variety of topological and environmental conditions, the shallow reef crest fringe with its rugose structure and community of corals and algae is generally net autotrophic, while inner reef areas such as lagoons or sand flat areas are generally more net heterotrophic (Hatcher 1997). Excess organic material is transported from the reef fringe to the inner reef areas where it is decomposed by the net heterotrophic community. After decomposition and remineralization by this community, nutrients are transported back to the reef fringe where they help to sustain the high levels of primary production. Due to this tight internal recycling, reefs as a whole tend to balance around unity (PR = 1; e.g. Hatcher 1988; Silverman et al. 2007)

Photosynthetic organisms

Primary producing organisms in coral reefs form a group that consists of members of several phyla including cyanobacteria, dinoflagellates, Chlorophyta (green algae), Rhodophyta (red algae), and Heterokontophyta (brown macroalgae and diatoms) (Fong and Paul 2011). There is a wide diversity of forms of these groups ranging from single celled cyanobacteria and dinoflagellates to plaques of crustose coralline algae and thickets of calcifying macroalgae such as *Halimeda* that can cover wide areas of the reef (Fig. I-2). Cyanobacteria occur in many different habitats on coral reefs as single-celled, symbiotic, and colonial organisms (Titlyanov and Titlyanova 2012). Microbial mats on sandy substrates consist of associations of microorganisms dominated by cyanobacteria. These several mm thick mats can extend for several meters across the substrate (Charpy et al. 2012). Cyanobacteria also grow on (epilithic), or within (endolithic) the coral reef framework. Certain species of cyanobacteria are capable of degrading dead coral branches, and thereby contribute to the dissolution of the calcium carbonate structure (Glynn 1997). The endolithic cyanobacteria, together with endolithic chlorophytes, are some of the most active primary producers in areas of coral rubble (Tribollet et al. 2006). Other cyanobacteria occur as epiphytes, attaching themselves to macroalgae. Sponges and at least one coral species are known to have cyanobacterial endosymbionts which may contribute to their host's energy and nutrient demands through photosynthesis and N_2 fixation (Erwin and Thacker 2007; Lesser et al.

2007; Charpy et al. 2012). Besides all these benthic habitats, cyanobacteria also constitute a large part of the plankton.

The single celled diatom algae are abundant in similar benthic habitats, and also form a common group in the phytoplankton (Mann and Droop 1996).The protist dinoflagellates also occur both in pelagic and benthic environments and can have population blooms which severely impact the coral community (Guzman et al. 1990). Zooxanthellae are the most studied dinoflagellate group in regard to coral reefs, and are found in symbiosis with a wide range of animals (e.g. scleractinian corals, the jellyfish genus *Cassiopea*, sponges, mollusks, and tunicates; Venn et al. 2008). Their photosynthesis and the subsequent exchange of the photosynthates with nutrients from the animal host form an important component of coral reef metabolism (Stambler 2011).

Macroalgae is a polyphyletic group which includes species of the green algae, brown algae, and red algae. There is high structural diversity within this group with forms ranging from structurally simple filiformous turf algae to encrusting or upright articulated kinds and large leathery robust forms with complex internal structures (Fig. I-2). Green algae such as *Caulerpa* and *Halimeda* can be highly abundant on coral reefs and can have detrimental effects on coral colonies through overgrowth, or physical damage due to contact which might lead to pathogenic infection (Nugues et al. 2004; Smith et al. 2010). As mentioned above, green algae also occur endolithically, i.e. in coral skeletons where their primary production may support the coral tissue when its endosymbiotic zooxanthellae are absent due to bleaching (Odum and Odum 1955; Fine and Loya 2002). Brown algae occur as robust forms (e.g. *Sargassum*), but there are also fast growing groups such as the genus *Dictyota* that have seasonal blooms where they cover substantial areas of the reef structure (Lirman and Biber 2000). Many red algal species belong to either the crustose coralline algae that enforce the reef framework by depositing calcite crusts, or filamentous forms which are part of the algal turfs (Scott and Russ 1987; Fabricius and De'ath 2001). These turf assemblages, also consisting of cyanobacteria, brown algae, and green algae, are opportunistic consortiums that quickly colonize any open spot on the reef and show some of the highest primary production within the reef community (Hatcher 1988; Smith et al. 2013; Connell et al. 2014). Turf consortiums are subjected to high levels of herbivory due to a lack of physical or chemical defenses, and seem to cope with this high pressure by growing at least as fast as they are grazed (Fong and Paul 2011).

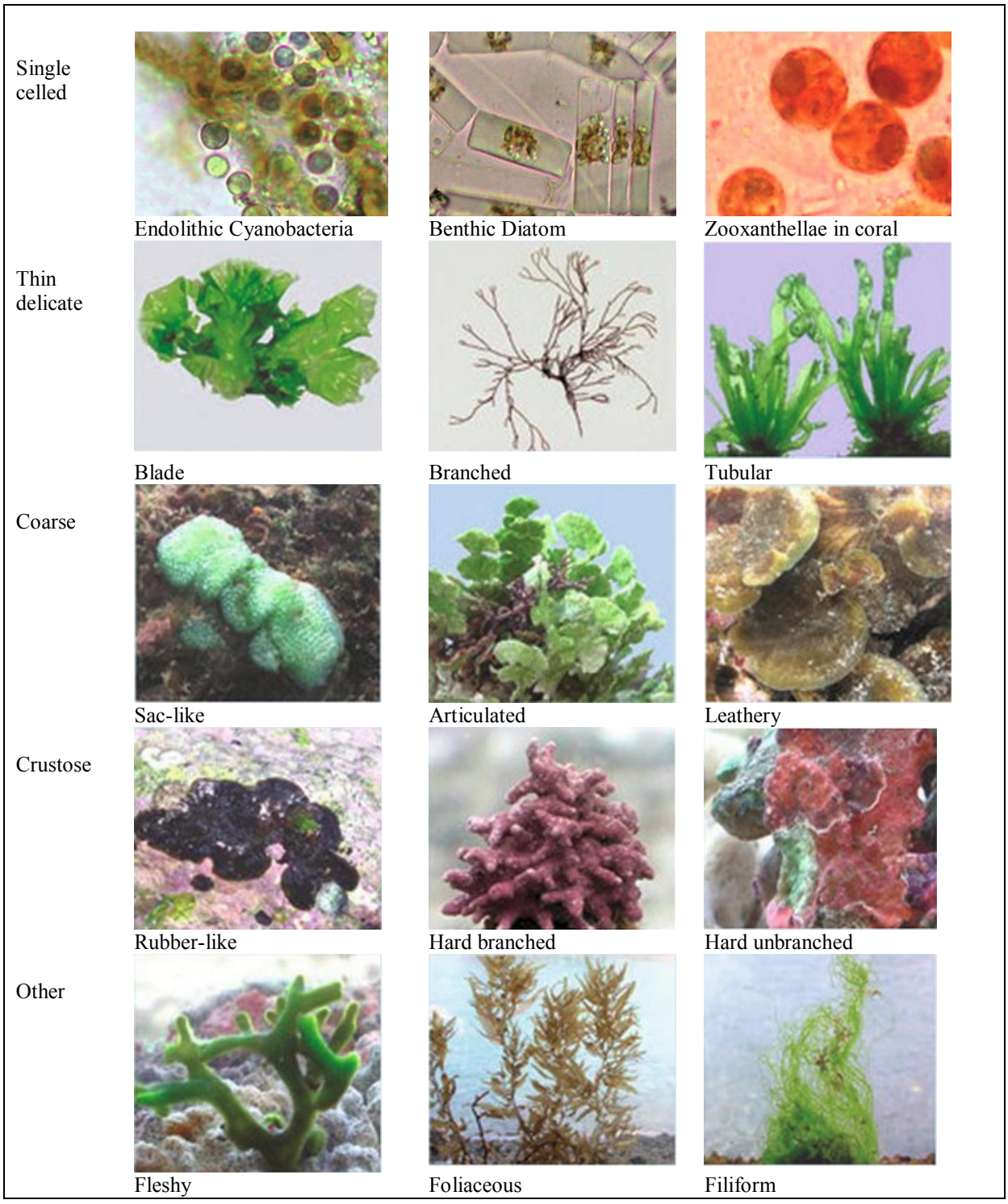


Fig. I-2. Coral reef single celled and multicellular carbon fixing organisms: structural diversity, modified from (Titlyanov and Titlyanova 2012).

Factors influencing primary production

Light

As light is the energy source fueling photosynthesis, the level of irradiance primary producers receive is vital (Falkowski and Raven 2007). Primary producers use light with a wavelength between 400 and 700 nm, i.e. photosynthetically active radiation (PAR) (Osinga et al. 2012). Irradiance decreases exponentially as light travels down from the water surface, with the additional effect that red light is absorbed first with blue and green light reaching deeper water (Fong and Paul 2011). Marine primary producers are adapted to these differences in absorption between wavelengths by harboring different pigments. All primary producers use the pigment chlorophyll a which absorbs red and blue wavelengths most efficiently. Green algae have chlorophyll b as a second pigment, but this does not differ substantially from chlorophyll a in the range of wavelengths it can use. Brown algae have the pigment fucoxanthin which can absorb a wider range of blue and green wavelengths. Red algae have phycobilin pigments that are even more efficient at absorbing blue and green light, making it possible for red algae to photosynthesize in even deeper waters down to 268 m water depth in the Bahamas (Littler et al. 1986). Primary producers can adapt to changes in irradiance; decreases in irradiance can lead to increased pigment concentrations, or photosynthetic symbiont numbers in holobionts (Brown et al. 1999), and increased irradiance levels may cause photoinhibition in which part of the photosynthetic system is deactivated. When irradiance levels become too high, this deactivation may become permanent; leading to photodamage (Franklin et al. 1996).

Temperature

The temperature on coral reefs can range from 14°C to 32°C with wider temperature ranges in more temperate reefs, for example in Japan and around the Arabian peninsula (Brown and Cossins 2011). Primary producers differ in their sensitivity to changes in water temperature. Most macroalgae and cyanobacteria are quite resistant to changes in water temperature (Fong and Paul 2011). While they do have a maximum and minimum temperature which delineates their global distribution, a potential increase over the next 100 years in seawater temperature as predicted in climate change scenarios (1.1 – 6.4°C; IPCC 2007) may not severely impact most macroalgal species, which might actually benefit from increased production rates in warmer waters (Diaz-Pulido et al. 2007). The opposite is expected for the animal-zooxanthellae symbioses, where severe reductions in photosynthetic symbiont density (i.e. bleaching) have been observed in scleractinian corals, soft corals, and sponges in response to increased water temperatures (Brown 1997; Fromont and Garson 1999; Goulet et al. 2008). This reaction leaves the animal without its

symbiotic source of C and/or N compounds, making it more susceptible to additional detrimental impacts (e.g. overgrowth, disease, sedimentation); which can lead to the demise of the animal host (Knowlton and Rohwer 2003). Recovery is possible over time, but the increase in bleaching occurrences in recent years has led to substantial mortality of symbiotic animals on coral reefs (Hughes et al. 2003; Donner et al. 2005; Hoegh-Guldberg et al. 2007).

Nutrients

Nutrients are compounds that support C metabolism in organisms (Atkinson 2011). The most important nutrients are those containing nitrogen and phosphorus (e.g. ammonium, nitrate, phosphate; Hatcher 1997; Kleypas et al. 1999). Odum and Odum (1955) concluded in their seminal paper on primary productivity in Eniwetok Atoll that the influx of dissolved nutrients from adjacent waters was insufficient to support the high levels of productivity on the reef. This was the start of decades of research on processes that could maintain high productivity under such nutrient-limited conditions (Barile 2004). These processes include the inflow of external sources of nutrients in planktonic, particulate, or dissolved form, the efficient storing and recycling of nutrients already present in the coral reef community, and de novo production through microbial N_2 fixation (Shashar et al. 1994b; Suzuki et al. 1995; Wyatt et al. 2010). N_2 fixation is performed by cyanobacteria and other types of bacteria on the reef (Casareto et al. 2008; Cardini et al. 2014). This process is energetically costly since it requires the breaking of the triple bond in N_2 and is therefore often inactive in diazotrophic organisms in the presence of other N sources (Koop et al. 2001; Kneip et al. 2007; Holmes and Johnstone 2010). The contribution of this process to the reef's bio-available N pool may be substantial (Casareto et al. 2008). While N_2 fixation is reliant on energy produced by photosynthesis; synthesis of nitrogenase, the enzyme which performs the fixation, is inhibited by the presence of oxygen (O_2) produced by that photosynthesis (Stal 2009). This complication is overcome by phototrophic diazotrophs such as cyanobacteria through separating the process either spatially (separating the two processes with specialized cells for N_2 fixation: heterocysts), or temporally (only performing N_2 fixation during twilight and night when O_2 production is inactive; Stal 2009).

The Red Sea environment

Situated between Africa and the Arabian Peninsula, and formed as a rift valley by those two landmasses moving apart, the Red Sea is a unique body of water (Fig. I-3a). It is a narrow deep channel: 2250 km long, 355 km wide and 2211 m deep. There is only limited water input from the Indian Ocean, consisting of relatively warm surface waters due to shallow depths at the interface

between the two water bodies: the Strait of Bab el Mandeb (Hanish sill: 160 m water depth) (Murray and Johns 1997; Fengchao et al. 2014). This limited oceanic input, combined with high air temperatures resulting in high evaporation and little to no freshwater input due to the surrounding deserts, result in highly saline waters (> 40 psu) (Bower et al. 2000). The northern Red Sea splits into two gulfs: the western Gulf of Suez and the eastern Gulf of Aqaba. These gulfs are some of the most northern coral habitats worldwide (Fig. I-1), leading to strong seasonality in light availability (Veron 2000; Winters et al. 2009). While the Gulf of Suez is a shallow basin with depths not exceeding 100 m, the Gulf of Aqaba is a miniature version of the Red Sea as a whole with a max. depth of 1800 m and a shallow sill (260 m) connecting it to the general Red Sea at the Strait of Tiran (Fig. I-3b) (Fengchao et al. 2014).

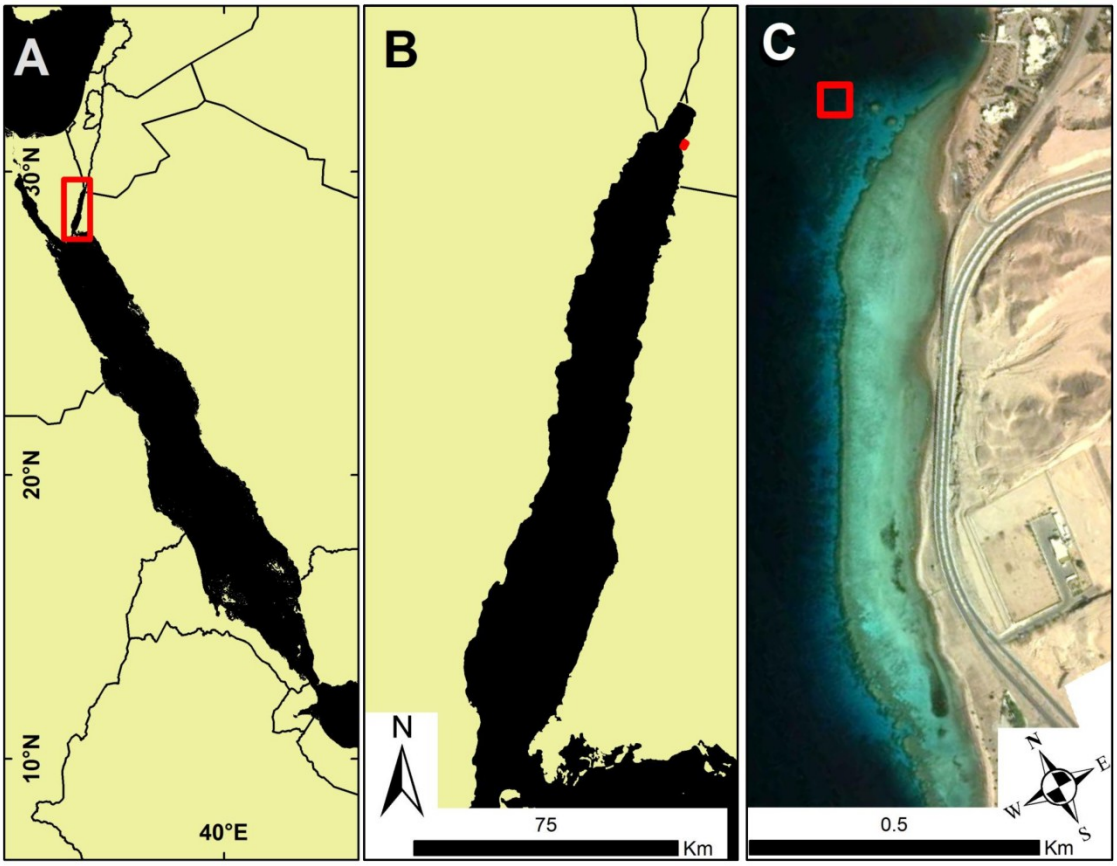


Fig. I-3. Maps of the research area. A) the Red Sea, projection WGS 1984; B) the Gulf of Aqaba; C) studied fringing reef with Marine Science Station in the top right, red square in C indicates the study site known as "U7" (Mergner and Schuhmacher 1974), satellite image map C: Google GeoEye.

The bathymetry, together with a relatively warm water column due to sole inflow of warm waters from the south, creates an annual cycle in the Gulf of Aqaba. In winter, the entire water column approaches 21 °C, causing deep water mixing which brings nutrients to surface waters. In late spring and summer, increasing surface water temperatures cause stratification down to 200 m water depth which traps nutrients in deeper water layers and leads to strongly

oligotrophic conditions in surface waters and coral reefs (Carlson et al. 2014). This cycle can have significant effects on the coral reef ecosystems of the Gulf. For example, during a cold temperature anomaly in the winter of 1992 due to the eruption of Mount Pinatubo, exceptionally strong upwelling of nutrients led to an outbreak of filamentous algae which caused extensive coral death (Genin et al. 1995). The seasonal variation in light availability, water temperature, and nutrient concentrations make the Gulf of Aqaba a "natural laboratory" to investigate how coral reef processes such as primary production, other C cycling processes, and N_2 fixation are influenced by changing environmental conditions. The Red Sea is, however, under-investigated compared to the Caribbean, or Great Barrier Reef (Berumen et al. 2013; Loya et al. 2014), while this unique habitat may provide a window into understanding how reef processes in currently more stable environments may respond as they experience more variable conditions due to predicted anthropogenic stressors (Hoegh-Guldberg 2014).

Objectives

The study of coral reef primary production has primarily been divided between either in-situ full reef metabolism studies using methods such as flow respirometry, control volume approach, or eddy correlation (Atkinson and Grigg 1984; Falter et al. 2008; Long et al. 2013), and incubations of specimens either in-situ, or in laboratory conditions (Reynaud et al. 2003; Wild et al. 2009; Kremien et al. 2013). The full reef in-situ methods allow precise quantification of primary production in a certain reef area as a whole. However, it is not possible with these methods to determine the contribution of separate functional groups to primary production to investigate their relative importance. The incubation of specimens allows the quantification of photosynthetic activity by individual species or substrates. So far studies using these methods have, for the majority, only investigated single species, genera, or functional groups (Fabricius and Klumpp 1995; Ateweberhan et al. 2006). Studies which collected a simultaneous overarching ecosystem-scale view of the contribution of all dominant functional groups to reef primary production are rare. In addition, little is known on the further distribution of C fixed by primary production throughout the reef community (Naumann et al. 2012), especially concerning the recently established sponge-mediated recycling loop mentioned above (de Goeij et al. 2013). Activity of this "sponge loop" was estimated as comparable to total reef GPP, but actual simultaneous quantification is lacking. The role of N_2 fixation in coral reefs has also been studied by focusing primarily on single functional groups and by using various quantification methods (acetylene reduction vs. $^{15}N_2$ tracer incubations; Mohr et al. 2010) making an overarching comparison throughout the ecosystem difficult (Shashar et al. 1994a; Charpy et al. 2010; Cardini et al. 2014). N_2 fixing diazotrophs have been found throughout coral reefs and quantifying their combined

contribution to N demand by primary production will help determine the importance of this process in coral reef metabolism. As mentioned above, the Red Sea is relatively under-studied compared to other coral reef areas around the world, while this natural laboratory would allow the investigation of the effect of variation in environmental factors on ecosystem processes such as N_2 fixation and C cycling. To address these gaps of current knowledge, this thesis has the following objectives:

1. To quantify the seasonal variation in environmental conditions in the water column over a Red Sea fringing coral reef and its effect on planktonic photosynthetic primary production and N_2 fixation.

2. To comparatively quantify photosynthetic primary production by the dominant benthic functional groups under seasonal variation in environmental conditions.

3. To quantify N_2 fixation by the dominant benthic functional groups to determine their relative contribution to total N_2 fixation and the potential contribution by N_2 fixation to N demand by primary production under seasonal variation in environmental conditions.

4. To combine the data on all functional groups into an overarching estimate of primary production, further carbon cycling processes, and N_2 fixation in the coral reef to quantitatively compare the magnitudes of these processes.

Approach

The research for this thesis was conducted at the Leibniz Center for Tropical Marine Ecology in Bremen, Germany. The fieldwork was performed at the Marine Science Station (MSS) of the University of Jordan and Yarmouk University, just south of the city of Aqaba, Jordan (Fig. I-3c) in collaboration with Professor Fuad Al-Horani and Professor Mamoon M. D. Al-Rhaidat of the MSS and University of Jordan. The MSS borders a marine protected area which encompasses a crescent shaped fringing coral reef of ca. 1 km long. All monitoring and sampling was performed within this fringing reef near a location known as "U7" (29° 27' 31" N, 34° 58' 26" E; Mergner and Schuhmacher 1974). Standardized metabolic rates of organisms were quantified during laboratory incubations of specimens collected in the reef. These rates were later combined with data on benthic cover of functional groups within the reef into a N_2 fixation budget and a trophic C cycling model to gain an overarching view of these processes in the coral reef community. Further details on the methods are provided in the respective chapters.

Chapter and publication outline

This thesis consists of this general introduction, eight chapters presenting the performed research, and a general discussion in which the key findings of the chapters were brought together and discussed. Chapter 1 sets the stage with an investigation into the seasonal environmental conditions (e.g. temperature, and light and nutrient availability) in the waters surrounding the fringing coral reef and how these affect water column photosynthetic primary production and N_2 fixation. Following this are four chapters (chapter 2 – chapter 5) which investigate separate dominant benthic functional groups: hard corals, soft corals, reef framework substrates (i.e. turf algae, coral rock reef structure, and an abundant sponge species), and permeable sediments respectively. In these chapters, findings on photosynthetic primary production, N_2 fixation and potential linkages between these processes are presented. The N_2 fixation rates for the benthic functional groups and water column are combined in chapter 6 into a reef-wide budget. The benthic primary production rates are bought together in chapter 7 to compare how the different functional groups respond to the seasonal variation. Finally, chapter 8 presents a trophic food web model in which measurements of photosynthetic primary production, inflows of allochthonous C and further C cycling processes through the water column, benthos and cavity sponge communities were combined over the seasons. This chapter provides an overarching view of C cycling in this high latitude fringing reef as it experiences the strong seasonality in environmental conditions.

The chapters described above are based on the following scientific publications:

Chapter 1) **van Hoytema N**, Cardini U, Bednarz VN, Rix L, Naumann MS, Al-Horani FA, Wild C. Effects of seasonality on planktonic primary production and dinitrogen fixation in a Red Sea coral reef. In preparation for *Marine Environmental Research*.

This study was designed by N van Hoytema, U Cardini, VN Bednarz, and C Wild. Fieldwork and sample analyses were performed by N van Hoytema, U Cardini, VN Bednarz, and L Rix with support from MS Naumann and FA Al-Horani. Data analyses were performed by N van Hoytema and U Cardini. N van Hoytema wrote the manuscript with critical revision by all authors.

Chapter 2) Cardini U, Bednarz VN, Naumann MS, **van Hoytema N**, Rix L, Foster RA, Al-Rshaidat MMD, Wild C. Microbial dinitrogen fixation sustains high coral productivity in oligotrophic reef ecosystems. In preparation for *Scientific Reports*.

The concept of this study was developed by U Cardini, VN Bednarz, N van Hoytema, and C Wild. Fieldwork was performed by U Cardini, VN Bednarz, N van Hoytema, and L Rix. The C and N flux model was designed by U Cardini and MS Naumann. RA Foster helped design the acetylene reduction incubations. U Cardini analyzed the data and wrote the manuscript with revision by all authors.

Chapter 3) Bednarz VN, Cardini U, **van Hoytema N**, Al-Rshaidat MMD, Wild C. (2015) Seasonal variation of N_2 fixation and O_2 fluxes associated with two dominant zooxanthellate soft corals from the Northern Red Sea. *Marine Ecology Progress Series 519: 141-152.*

This study was designed by VN Bednarz, U Cardini, N van Hoytema, and C Wild. Field work and sample analyses were performed by VN Bednarz, U Cardini, and N van Hoytema with support from MMD Al-Rshaidat. Data analyses were performed by VN Bednarz and U Cardini. The manuscript was written by VN Bednarz with input from all authors.

Chapter 4) Rix L, Bednarz VN, Cardini U, **van Hoytema N**, Al-Horani FA, Wild C, Naumann MS. Seasonality in dinitrogen fixation and primary productivity by coral reef framework substrates from the northern Red Sea. In revision at *Marine Ecology Progress Series*.

This study was developed by VN Bednarz, U Cardini, N van Hoytema, and C Wild. Fieldwork and sample analyses were performed by L Rix, VN Bednarz, U Cardini, and N van Hoytema with support from FA A-Horani and MS Naumann. L Rix analyzed the data and wrote the manuscript with revision by all authors.

Chapter 5) Bednarz VN, **van Hoytema N**, Cardini U, Naumann MS, Al-Rshaidat MMD, Wild C. (2015) Dinitrogen fixation and primary productivity by carbonate and silicate reef sand communities of the Northern Red Sea. Accepted by *Marine Ecology Progress Series DOI: 10.3354/meps11224*.

The concept of this study was developed by VN Bednarz, N van Hoytema, U Cardini, and C Wild. VN Bednarz, N van Hoytema, and U Cardini performed the fieldwork and sample analyses, supported by MMD Al-Rshaidat and MS Naumann. VN Bednarz wrote the manuscript with input from all authors.

Chapter 6) Cardini U, Bednarz VN, **van Hoytema N**, Rovere A, Naumann MS, Al-Rshaidat MMD, Wild C. Revising the role of biological dinitrogen fixation in biogeochemical cycling of coral reef ecosystems. In preparation for *Ecosystems*.

This study was designed by U Cardini, VN Bednarz, N van Hoytema, and C Wild. Field work and sample analyses were performed by U Cardini, VN Bednarz, and N van Hoytema. Geographic Information System analyses were conducted by U Cardini and A Rovere. U Cardini analyzed the data and wrote the manuscript with critical input from all authors.

Chapter 7) **van Hoytema N**, Bednarz VN, Cardini U, Naumann MS, Al-Horani FA, Wild C. The influence of seasonality on primary production by dominant benthic organisms in a Red Sea coral reef. Under review at *Marine Biology*.

This study was initiated by N van Hoytema, VN Bednarz, U Cardini, and C Wild. Fieldwork was conducted by N van Hoytema, VN Bednarz, and U Cardini with support from MS Naumann, and FA Al-Horani. N van Hoytema analyzed the data and wrote the manuscript with critical feedback from all authors.

Chapter 8) **van Hoytema N**, van Oevelen D, Rix L, Cardini U, Bednarz VN, Naumann MS, Al-Horani FA, Wild C. Ecosystem-engineered seasonal carbon cycling in a Red Sea coral reef. In preparation for *Coral Reefs*.

This study was designed by N van Hoytema, L Rix, U Cardini, VN Bednarz, and C Wild. Fieldwork and sample analyses were performed by N van Hoytema, L Rix, U Cardini, and VN Bednarz with support from MS Naumann and FA Al-Horani. The linear inverse trophic food web model was designed by N van Hoytema and D van Oevelen. The manuscript was written by N van Hoytema with critical revision by all authors.

Related publications

These publications are related to the work presented here, but not included in this thesis:

Plass-johnson JG, Cardini U, **van Hoytema N**, Bayraktarov E, Burghardt I, Naumann MS, Wild C (2015) Coral bleaching. In: Armon RH, Hänninen O (eds) Environmental Indicators. Springer Netherlands, pp 117 - 146

Bednarz VN, Rix L, Cardini U, **van Hoytema N**, Naumann MS, Al-Rshaidat MMD, Wild C Dinitrogen fixation and nitrogen fractionation in scleractinian corals along a depth-gradient in the northern Red Sea. In preparation for *The Journal of Experimental Biology*.

Cardini U, **van Hoytema N**, Al-Rshaidat MMD, Schuhmacher H, Wild C, Naumann MS 37 years later: Revisiting a Red Sea long-term monitoring site. In review at *Coral Reefs*.

Cardini U, **van Hoytema N**, Bednarz VN, Rix L, Foster RA, Al-Rshaidat MMD, Wild C Diazotrophs contribute towards the resilience of the coral holobiont to ocean warming. In review at *The Journal of Experimental Biology*.

Ford A, **van Hoytema N**, Moore B, Wild C, Ferse S Sedimentary oxygen consumption as an effective process indicator of organic matter stress on coral reefs. In preparation for *Marine Pollution Bulletin*.

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Chapter 1
Seasonality of water column environmental parameters, photosynthetic primary production, and microbial dinitrogen fixation

Abstract

The northern Red Sea, because of its relatively high-latitude location, experiences strong seasonality in environmental conditions. This allows the study of regulatory effects by key status parameters (temperature, inorganic nutrient and organic matter concentrations) on process parameters (photosynthetic primary production and dinitrogen (N_2) fixation) and picoplanktonic abundance in the water column above coral reefs (10 m water depth). Knowledge on interactions between these parameters is lacking. Therefore, this study measured status and process parameters, and picoplankton abundance using a comparative approach between mixed (January – April) and stratified (September – November) water column scenarios in 2013. Findings revealed that inorganic nutrient concentrations were significantly higher in the mixed compared to the stratified season. Concurrently, daily gross primary production decreased 4-fold from the mixed to stratified season, while N_2 fixation did not change significantly. The phytoplanktonic community changed from dominance by picoeukaryotes to that by *Prochlorococcus* sp. and indications were found for a diazotrophic community shift from its autotrophic to its heterotrophic component. Primary production was primarily regulated by inorganic N concentrations, while dissolved organic carbon concentrations affected both primary production and N_2 fixation, emphasizing the importance of the microbial loop in planktonic tropho-dynamics of Red Sea coral reefs. N_2 fixation could potentially contribute 3.4 % of N needed for primary production in the mixed season. However, this contribution increased drastically to 20.8 % in the stratified season, indicating planktonic N_2 fixation as a potentially important source of N to phytoplankton during very oligotrophic summer conditions.

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Chapter 1 Planktonic primary production and N_2 fixation

Introduction

The Gulf of Aqaba forms one of the northern tips of the Red Sea. Its desert coasts are fronted by fringing coral reefs that experience relatively strong variation in light availability and water temperature for warm water coral reefs due to their high latitude location. The annual fluctuation in sea surface temperature (21 – 29 °C) combined with relatively warm deeper water layers (year round ~ 21 °C, water depth > 200 m) result in an annual cycle of deep water mixing from December until May and stratification down to 200 m water depth from June until November (Carlson et al. 2014). Inorganic nutrients are brought up to surface water layers during deep water mixing, while they are trapped in deeper waters during stratification, creating extreme oligotrophic conditions in coral reef surrounding surface waters (Rasheed et al. 2002; Silverman et al. 2007; Rasheed et al. 2012). These local conditions offer the rare opportunity to study the effects of seasonal variation in key environmental factors on important processes such as primary production within coral reefs.

In addition to receiving allochthonous energy and nutrients in the form of plankton, and particulate and dissolved matter from offshore, coral reefs thrive in oligotrophic conditions particularly due to autochthonous creation of organic carbon (C) and nitrogen (N) containing nutrients through photosynthetic primary production and prokaryotic dinitrogen (N_2) fixation and efficient internal recycling of those materials within the ecosystem (Hatcher 1997). This recycling can occur through strong benthic-pelagic coupling of dissolved and particulate organic matter and nutrients (Yahel et al. 1998; Wild et al. 2004; Chipman et al. 2012). Benthic-pelagic coupling is mediated by benthic organisms such as corals, algae, and sponges (Haas et al. 2010; Naumann et al. 2010; de Goeij et al. 2013), but also by reef sediment and framework microbiota (Rasheed et al. 2002; Wild et al. 2009). Planktonic photosynthesis rates in reef-surrounding waters can be 1 to 2 orders of magnitude higher than in oceanic waters offshore (D'Elia and Wiebe 1990; Adey 1998), and N_2 fixation by so-called "diazotrophs" (i.e. prokaryotes capable of N_2 fixation) can facilitate primary production under oligotrophic conditions as found in the Gulf of Aqaba (Capone et al. 1997; Karl et al. 2002; Furnas et al. 2011).

Planktonic primary production in the Gulf of Aqaba and northern Red Sea is dominated by photoautotrophic nano- and picoplanktonic organisms. The plankton fraction < 20 µm performs on average 81 % of photosynthesis at 10 m water depth (Al-Najjar et al. 2007; Qurban et al. 2014). Primary production rates range from 0.02 to 3.38 µmol C L^{-1} d^{-1} (Levanon-Spanier et al. 1979; Qurban et al. 2014), assuming a 12 h period of daylight. The phytoplankton community in the Gulf of Aqaba is characterized by a strong seasonal shift in composition. During the mixed season, eukaryotic algae account for up to 95 % of phytoplankton biomass, while during

stratification > 60% of the biomass consists of prokaryotes, in particular *Prochlorococcus* sp. which then may comprise up to 50% of the biomass (Al-Najjar et al. 2007). The main groups of planktonic diazotrophs in the Gulf of Aqaba are cyanobacteria and proteobacteria, responsible for water column N_2 fixation rates ranging from 0.01 to 1.9 nmol N L^{-1} d^{-1} (Foster et al. 2009; Rahav et al. 2013; Rahav et al. 2015). Primary production and N_2 fixation can interact synergistically within or between planktonic organisms: while photosynthesis (and subsequent organic carbon release to the surrounding water) may fuel the energy-demanding process of N_2 fixation, primary production is supported by N_2 fixation providing bio-available N required for proteins needed in photosynthesis (e.g. Foster et al. 2011).

Information on planktonic primary production in the Gulf of Aqaba, or even the Red Sea as a whole, is scarce, especially for waters overlying coral reefs (e.g. Levanon-Spanier et al. (1979) for the Gulf of Aqaba). In addition, there are few studies on planktonic N_2 fixation in the Gulf of Aqaba (Foster et al. 2009; Rahav et al. 2015), and only Rahav et al. (2015) provide a first look at conditional interactions between primary production and N_2 fixation. These authors reported a pelagic community shift from autotrophic to heterotrophic diazotrophs from the mixed to the stratified season. To increase our understanding of the regulating factors for planktonic production of organic C and inorganic N-nutrients, which are in high demand by the planktonic community as well as the coral reef below, the objectives of this study were 1) to measure status parameters in a Gulf of Aqaba fringing coral reef over the two distinct seasons of mixing and stratification, 2) to measure abundances of the dominant picoplanktonic groups, 3) to quantify the process parameters primary production and N_2 fixation in the reef-surrounding water over the seasons and 4) to investigate conditional relationships between the measured status and process parameters.

Materials and methods

Research area

The fieldwork for this study was conducted at the Marine Science Station (MSS) of the University of Jordan, located 10 km south of Aqaba, Jordan. The MSS is situated adjacent to a marine protected area encompassing a crescent shaped fringing coral reef with a length of ca. 1 km. All water column sampling was performed at 10 m water depth in the fore reef section of this reef (29° 27' 31" N, 34° 58' 26" E). Two extensive fieldwork campaigns were performed in 2013: one during the mixed season (January – April; 12 weeks), and one during the stratified season (September – November; 13 weeks). The studied reef consists of a shallow reef flat (< 1 m water

depth) surrounded by a carbonate sediment belt at ca. 5 m water depth and a coral dominated middle-fore reef facing the open sea (see Mergner and Schuhmacher (1974) for an extensive description of the studied reef).

Status parameters

Light (lux) and water temperature at 10 m water depth were measured over both campaigns at 1 min frequency with Onset HOBO data loggers (Pendant UA-002-64; temperature accuracy: ± 0.53 °C, spectral detection range: 150 – 1200 nm). The lux measurements were converted to photosynthetically active radiation (PAR) by a conversion factor calculated from a simultaneous minute-by-minute measurement of lux and PAR (08:00 to 14:00 on one day n = 353) using a HOBO pendant logger and a LI-COR LI192SA underwater quantum sensor: lux = PAR x 52.0, R^2 = 0.83. This value is comparable to the conversion factor given by Valiela (1984): 51.2.

Water samples were collected from 10 m water depth on a weekly basis during both campaigns for measurement of the status parameters, described in detail below, (n = 4) and primary production (n = 6) in clean high density poly-ethylene (HDPE) containers (volume: 5 L) using SCUBA. All samples were collected between 08:00 and 10:00 within a 10 min time-frame. Sampling was performed 1 m above the seafloor without disturbing the benthos. The samples were transported back to the MSS, and subsamples were taken from the containers within 20 min to measure concentrations of inorganic nutrients (NH_4^+, PO_4^{3-}, and NO_x ($NO_x = NO_2^- + NO_3^-$)), particulate organic carbon (POC), particulate nitrogen (PN), dissolved organic carbon (DOC), and chlorophyll a (Chl a). Simultaneously, glass incubation chambers (volume: 0.5 L) were filled for measurements of net primary production (Pnet), and biological oxygen demand (BOD) (as described below). Subsamples for inorganic nutrients (50 mL) were filtered through sample pre-rinsed cellulose acetate filters (nominal pore size 0.45 µm), and nutrient concentrations were measured according to Murphy and Riley (1962), Strickland and Parsons (1968), and Holmes et al. (1999). NH_4^+ was measured with a Trilogy Fluorometer (Turner Designs), while PO_4^{3-} and NO_x were measured with a JASCO-V630 photometer. Detection limits for NH_4^+, PO_4^{3-}, and NO_x were 0.09, 0.01, and 0.02 µmol L^{-1}, respectively. Subsamples for POC (1L) and PN (2L) were taken from the containers after homogenisation and filtered onto pre-combusted (450 °C, 5 h) GF/F filters (nominal pore size 0.7 µm), which were dried (40 °C for 48 h) and sealed in Eppendorf vials pending analysis. POC and PN filters were placed in silver cups and measured using a EuroVector elemental analyzer (EURO EA 3000; analytical precision ≤ 0.1 % (C) and ≤ 0.03 % (N)) POC filters were acidified (0.1 N HCl) prior to measurement to remove any inorganic carbon. Subsamples for DOC (50 mL) were vacuum filtered (max. pressure 20kPa) through pre-combusted GF/F filters straight into 30 mL HDPE sample bottles. All components of the filtration

apparatus and the sample bottles were acid-washed in 0.4 mol L^{-1} HCl for 24 h, before being rinsed with MQ water prior to sampling. The first 2 x 10 mL of the DOC samples were used to rinse the sample bottles twice and discarded, after which the remaining 30 mL were filtered and collected. DOC samples were directly acidified with 80 µL of 18.5 % HCl and stored at 4 °C in the dark until analysis by high-temperature catalytic oxidation on a Shimadzu TOC-V_{CPH} total organic C analyser. The analyser was calibrated with a 10 point calibration using serial dilutions of a potassium hydrogen phthalate certified stock solution (1000 ppm standard, Fluka 76067-500ML-F). Reference water samples (CRM program, Hansell Research Lab, USA, DA Hansell and W Chan; Batch 13, Lot #08-13, 41-45 µmol C L^{-1}) were applied as a positive control after every 10 samples. Each sample was measured five times and analytical precision was < 3 % of the certified value. Chl a subsamples (1L) were filtered onto pre-combusted GF/F filters and stored in the dark at -80 °C. Chl a was later extracted in 90 % acetone for 12 h at 4 °C in the dark and measured on a Trilogy fluorometer using the non-acidification module (CHL NA #046, Turner Designs).

Picoplankton community

Picoplankton samples (2 mL) were collected in March (n = 4, 1 sampling day) and November (total n = 53, 14 sampling days over the period) for quantification of picoeukaryote, *Prochlorococcus* sp., *Synechococcus* sp., and heterotrophic bacteria abundance. Samples were fixed with 0.1 % paraformaldehyde (final concentration) for 30 min at room temperature, frozen with liquid N, and stored at – 80 °C until analysis. All groups were sorted with flow cytometry (FACSCalibur, Becton Dickinson, 488 nm excitation laser). Picoeukaryotes, *Prochlorococcus* sp., and *Synechococcus* sp. were quantified at a flow rate of ~ 0.06 µL min^{-1} for two minutes and gated on an orange versus red fluorescence dot plot using CellQuestPro (BD and Co. New Jersey, USA). Subsamples for heterotrophic bacteria quantification were stained with SYBR Green 1 (conc. 1 per 1000) for 30 min prior to analysis. Heterotrophic bacteria were also sorted at a flowrate of ~ 0.06 µL min^{-1} but for 1 min and subsequently gated on a side scatter versus green fluorescence density plot. The cytometer flow rate was gravimetrically calibrated according to Current Protocols in Cytometry (Robinson et al. 1997).

Process parameters

Primary production

The water samples for measurements of Pnet and BOD were carefully transferred from the sampling containers into 0.5 L glass incubation chambers. Two samples were taken per container, one sample for Pnet and one for BOD measurement. The salinity and temperature corrected

dissolved O_2 concentration (mg L^{-1}) was measured with an O_2 optode and a conductivity probe (MultiLine® IDS 3430, WTW GmbH, Weilheim, Germany, accuracy: ± 0.5 % of measured value), after which the chambers were directly sealed with gastight glass lids, ensuring no air remained in the chambers. Chambers for BOD measurements were immediately placed in bags made of dense opaque plastic (volume: 10 L) for incubation in the dark. All chambers were incubated in an outside flow-through aquarium (800 L, flow-through 4000 L h^{-1}) fuelled with water pumped from 10 m water depth on the reef to simulate in-situ temperatures. PAR in the flow-through aquarium was modified to in-situ 10 m water depth levels with layers of plastic mesh following in-situ measurements with a LI-COR LI192SA underwater quantum sensor. Pnet incubations were performed from 10:00 until sunset (17:00 - 18:00; depending on the season), while BOD incubations ran for 24 h. At the end of the incubations, chambers were removed from the aquarium, and the O_2 concentration was immediately measured for each chamber under light stirring to ensure a homogenous O_2 concentration.

N_2 fixation

N_2 fixation in the water was measured for seven weeks in the mixed season (four weeks in February and three weeks in April), and seven weeks in the stratified season (three weeks in September, and four weeks in November). N_2 fixation rates were quantified using a modified acetylene (C_2H_2) reduction technique (Capone 1993; Wilson et al. 2012). C_2H_2 enriched seawater was produced by bubbling it with C_2H_2 gas freshly produced from calcium carbide. Incubations were performed in 1 L chambers (n = 8) containing 800 mL fresh seawater taken from the flow-through aquarium described above. Of this 800 mL, 10 % was replaced by C_2H_2 enriched seawater. Chambers were directly sealed with gastight glass lids, after which 10 % of the air headspace was replaced with C_2H_2 gas through a rubber injection port in the lid which was also used for subsequent sampling. Chambers were then incubated under in-situ conditions in the flow-through aquarium while being stirred at 600 rpm by Cimarec™ i Telesystem Multipoint Stirrers (Thermo Scientific™) for the duration of the incubation to promote equalizing of gas concentrations between the water and the head space. Control incubations were performed without C_2H_2 addition to measure biological ethylene (C_2H_4) production, as well as with sterile filtered seawater (0.2 µm) to measure inorganic C_2H_4 production from C_2H_2, but no significant C_2H_4 production was found in these control incubations. The incubations lasted for 24 h, and 1 mL gas samples were extracted from a port in the lid at 0 h, 4 h, and 24 h with a gastight syringe. These samples were injected into gas-tight 2 mL vials, previously filled with distilled water, by displacing 1 mL of the water. Vials were frozen upside down pending analysis. Prior to measurements, vials were thawed completely, after which a gas subsample was injected into a reducing compound photometer (RCP) (Peak Laboratories) with a detection limit of 100 ppb for

measurement of C_2H_4. The RCP was calibrated with a serial dilution of a C_2H_4 standard (200 ± 4 ppm; Restek, Bellefonte, PA, USA).

Data treatment

PAR values measured per minute were summed for each weekly water sampling day: values in µmol quanta m^{-2} s^{-1} were multiplied x 60 for the minute following each measurement. These 60 sec measurements were then summed for the entire day resulting in mol quanta m^{-2} d^{-1}. Water temperature measured per minute on the water sampling day was averaged over the 24 h period. The O_2 concentration changes over the Pnet and BOD incubation periods were calculated by subtracting start O_2 concentrations from end O_2 concentrations. This concentration difference was corrected for incubation duration, chamber volume, and recalculated to molar equivalents resulting in O_2 fluxes in µmol O_2 L^{-1} h^{-1}. BOD is presented here as a positive flux. Estimates of gross primary production (Pgross) were derived from addition of every BOD measurement to its corresponding Pnet measurement (Pgross = Pnet + BOD). C_2H_4 measurements were recalculated to nmol C_2H_4 in the whole chamber water volume. It was determined in pilot experiments that the C_2H_2 concentration equilibrated between headspace and incubation water in the first 4 h, the C_2H_4 production over 4 h to 24 h were therefore used for flux calculations. Changes in C_2H_4 concentration over time were corrected for incubation duration and volume of water in the chamber, resulting in fluxes of nmol C_2H_4 L^{-1} h^{-1}.

Statistical analyses

The results of replicate measurements for all status and process parameters for each week were averaged prior to statistical analyses. All statistics were performed in SPSS 14.0 for Windows (SPSS Inc. Chicago, USA). Status and process parameters, as well as picoplankton abundances in the two seasons were tested for normality with the Shapiro-Wilk test. Seasonal comparisons were performed with independent samples t-tests if data were normally distributed and with Mann-Whitney U tests if data lacked normality. Differences were deemed significant at $p < 0.05$. To investigate correlations between all status and process parameters, Spearman Rank correlation tests were performed on the dataset as a whole and on each season separately.

Results

Status parameters

Mean weekly measurements of status parameters were variable over time (Fig. 1-1). Daily PAR increased from January to April (i.e. mixed season), and decreased again from September to November (i.e. stratified season) (Fig. 1-1 a, b). Temperature was stable throughout the mixed season (22.1 – 22.9 °C), but had increased to a maximum of 27.5 °C in early September followed by a decrease to 24.7 °C until the end of November. Inorganic nutrient and Chl a concentrations were all significantly lower during the stratified than during the mixed season (Fig. 1-1 c, d; Table 1-1, all $p < 0.003$), while DOC was significantly higher during the stratified season than during the mixed season (Table 1-1). POC concentrations showed no significant differences between seasons, while PN was significantly lower in the stratified season (Fig. 1-1 e, f), causing a significantly higher POC:PN ratio. The DIN:PO_4^{3-} ratio was not significantly different between seasons (Table 1-1).

Table 1-1. Status and process parameters in the mixed and stratified seasons. Values are given as mean ± SD. Differences were tested with independent sample t-tests, except for parameters with *, these were tested with Mann-Whitney U tests due to lack of normality. Bold p-values indicate $p < 0.05$. Chl a = chlorophyll a, DOC = dissolved organic carbon, POC = particulate organic carbon, PN = particulate nitrogen, $NO_x = NO_2^- + NO_3^-$, DIN = $NH_4^+ + NO_x$, Pnet = net primary production, BOD = biological oxygen demand, Pgross = gross primary production = Pnet + BOD.

Parameters	Mixed	Stratified	p
Temperature (°C)*	22.34 ± 0.24	25.84 ± 1.00	**<0.001**
Irradiance (mol m^{-2} d^{-1})	4.71 ± 1.27	5.39 ± 1.58	0.264
Chl a µg L^{-1}	0.20 ± 0.03	0.14 ± 0.05	**0.002**
DOC µmol L^{-1}	75.51 ± 6.96	87.36 ± 4.20	**<0.001**
POC µmol L^{-1}	7.90 ± 2.98	7.74 ± 1.79	0.865
PN µmol L^{-1}*	1.08 ± 0.27	0.88 ± 0.17	**0.022**
NH_4^+ µmol L^{-1}	0.52 ± 0.19	0.25 ± 0.12	**<0.001**
PO_4^{3-} µmol L^{-1}	0.11 ± 0.02	0.04 ± 0.02	**<0.001**
NO_x µmol L^{-1}	0.63 ± 0.29	0.19 ± 0.12	**<0.001**
DIN:PO_4^{3-}	10.42 ± 2.32	16.52 ± 11.31	0.122
POC:PN	7.16 ± 1.34	8.77 ± 1.24	**0.005**
Pnet (µmol O_2 L^{-1} h^{-1})*	-0.16 ± 0.14	-0.26 ± 0.07	**0.003**
BOD (µmol O_2 L^{-1} h^{-1})	0.25 ± 0.10	0.29 ± 0.08	0.369
Pgross (µmol O_2 L^{-1} h^{-1})	0.09 ± 0.10	0.02 ± 0.09	0.094
N_2 fixation (nmol C_2H_4 L^{-1} h^{-1})*	0.34 ± 0.18	0.50 ± 0.48	1.000

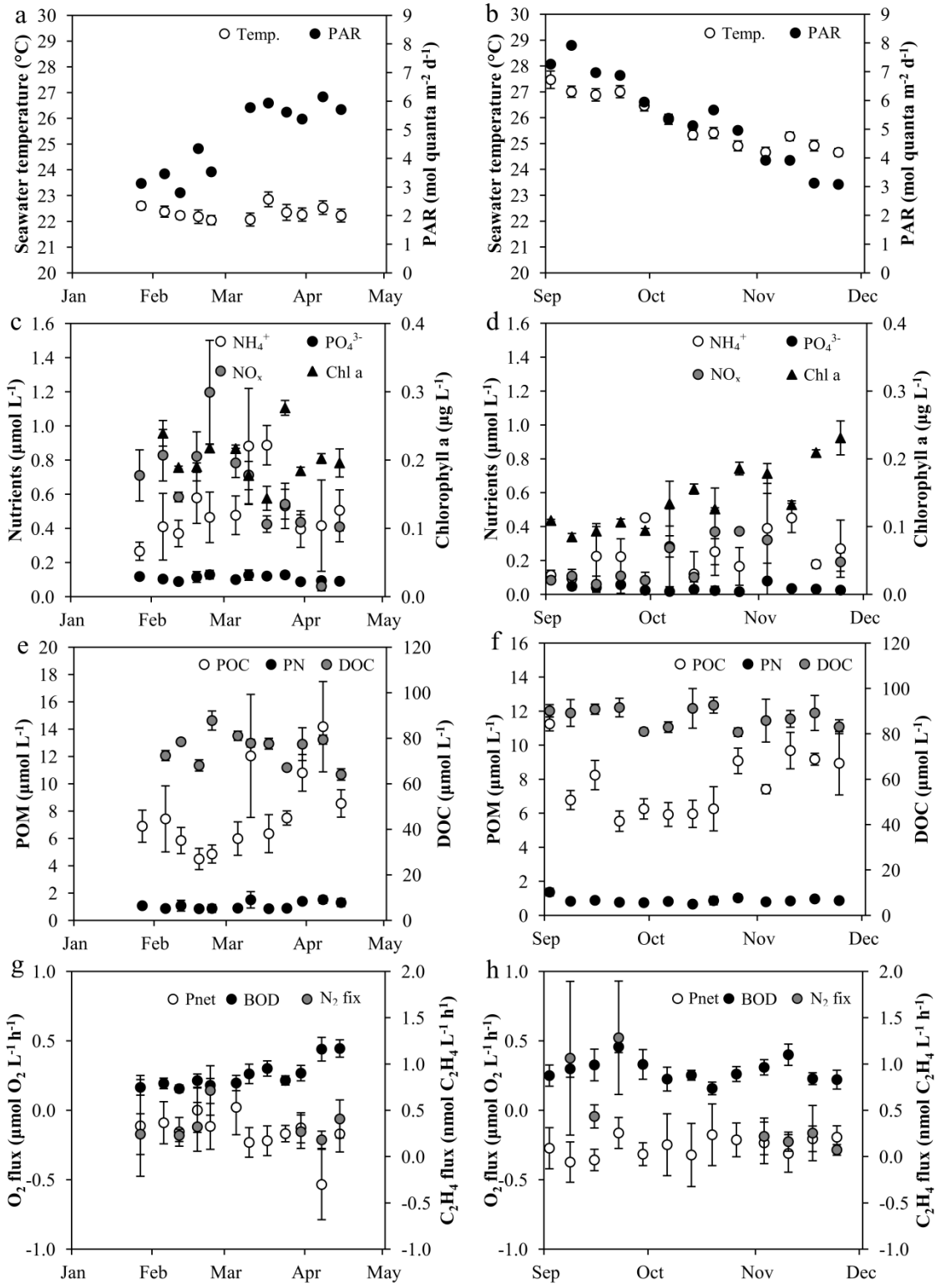

Fig. 1-1. Mean weekly status and process parameters during mixed (a, c, e, g) and stratified (b, d, f, h) seasons as measured at 10 m water depth, 1 m over coral reef benthos. Values given as mean ± SD. Temp. = water temperature, PAR = photosynthetically active radiation measured at 10 m water depth, $NO_x = NO_2^- + NO_3^-$, POM = particulate organic matter, POC = particulate organic carbon, PN = particulate nitrogen, DOC = dissolved organic carbon, Pnet = net primary production, BOD = biological oxygen demand, N_2 fix = N_2 fixation.

Picoplankton community composition

There was a clear seasonal shift in the photosynthetic picoplankton community from the mixed to the stratified season (Fig. 1-2). *Prochlorococcus* mean abundance increased significantly 3-fold, while *Synechococcus* and picoeukaryote mean abundances decreased significantly 2- and 3-fold respectively. Mean heterotrophic bacteria abundance was very similar between seasons with just a 1.9% increase from the mixed to the stratified season.

Process parameters

Mean water column Pnet was negative in all weeks (-0.54 to -0.0001 μmol O_2 L^{-1} h^{-1}), except on 18 Feb.: 0.02 μmol O_2 L^{-1} h^{-1}, and significantly lower during the stratified season than the mixed season (Fig. 1-1 g, h; Table 1-1). BOD ranged from 0.15 to 0.46 μmol O_2 L^{-1} h^{-1} and was not significantly different between seasons. Estimates of Pgross were similar for the mixed and stratified seasons with averages of 0.09 and 0.02 μmol O_2 L^{-1} h^{-1} respectively, which were not significantly different. Weekly mean N_2 fixation ranged from 0.18 to 0.71 and 0.07 to 1.28 nmol C_2H_4 L^{-1} h^{-1} in the mixed and stratified seasons respectively (Fig. 1-1 g, h). Although mean N_2 fixation on 8 and 22 Sep., during stratification, was substantially higher than during the mixed season, overall there was no significant seasonal difference.

Correlation analyses

Analyses for both seasons combined identified strong negative correlations between temperature and each of the inorganic nutrient concentrations (Appendix 1 - Table 1); showing the expected effect of the mixing-stratification cycle. Chl a, Pnet, and Pgross were all negatively correlated with temperature and positively correlated with two of the inorganic nutrients (Chl a: PO_4^{3-} and NO_x; Pnet: PO_4^{3-} and NO_x; Pgross: NH_4^+ and NO_x). Interestingly, daily PAR was negatively correlated with both Chl a, and Pnet. During the mixed season, correlation analyses revealed that Pnet was positively correlated with NO_x, while lacking correlations with the other nutrients (Appendix 1 - Table 2). BOD was positively correlated with POC and N_2 fixation was positively correlated with Pgross. In the stratified season, both Chl a and Pnet were positively correlated with NO_x, while the other nutrients were not correlated to either Pnet, or Pgross (Appendix 1 - Table 3). Finally, N_2 fixation was positively correlated to both temperature and DOC during stratification.

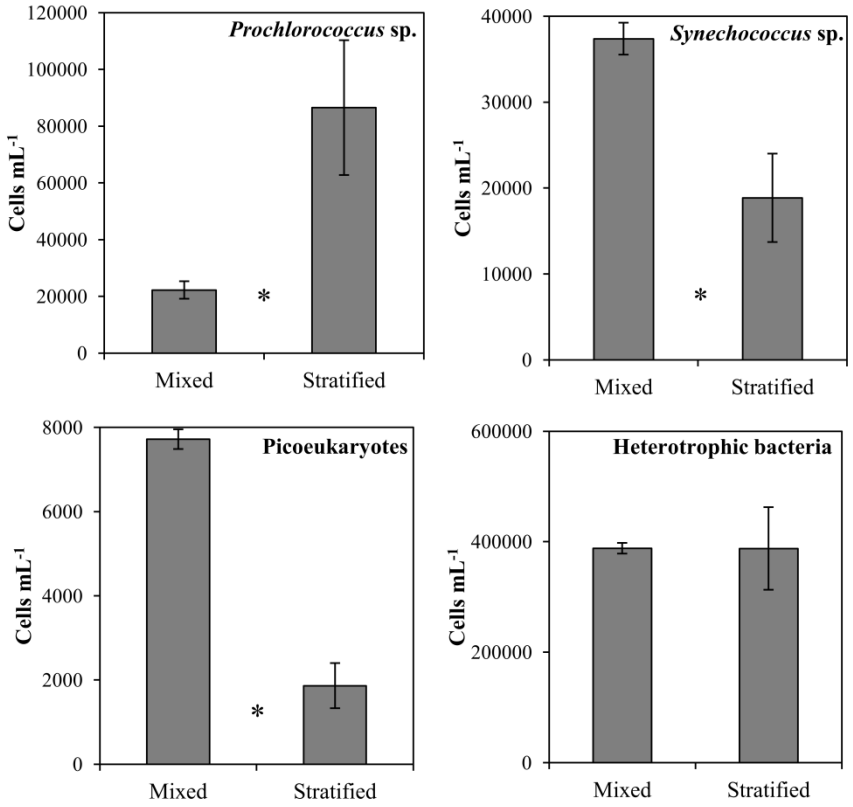

Fig. 1-2. Mean seasonal picoplankton group abundances in the mixed and stratified seasons, March: n = 4, November: n = 53. Error bars = SD. * indicates a significant difference between seasons (Mann-Whitney U tests $p < 0.001$).

Discussion

Status parameters

PAR, water temperature, and inorganic nutrient concentrations during the mixed and stratified seasons were comparable to previous research at the study site and surrounding waters (Rasheed et al. 2002; Naumann et al. 2010; Rasheed et al. 2012). The mixed season average DIN:PO_4^{3-} ratio (10.42) was clearly lower than the Redfield ratio for N:P (16:1; Redfield 1958), suggesting N as the limiting nutrient. The average ratio in the stratified season was similar to the Redfield N:P ratio with maximum values between 31 and 33 in 3 nonconsecutive weeks in October and November, suggesting that inorganic N and P availability in that season was on average balanced, but there were times when P may have been limiting. Previous studies have found both N and P as limiting factors for primary production in the northern Red Sea (Stihl et al. 2001; Mackey et al. 2007; Suggett et al. 2009).

Average POC:PN ratios in both seasons were higher than Redfield (106:16 = 6.625), suggesting that the particulate organic matter (POM) in the water column was impoverished in N throughout the year, particularly so during the stratified season when PN concentrations were significantly reduced. The dominant source of POM in coral reef-surrounding waters is often mucus released by hard corals (Johannes 1967; Naumann et al. 2012b). Mucus POC and PN release by the dominant scleractinian corals in the studied reef is constant over the year (Naumann et al. 2010). However, the average POC:PN ratio of coral mucus (12 ± 1; Naumann et al. 2010), is far higher than the ratio found for the water column here (7.16, 8.77), indicating that a large fraction of water column PN originated from a different source which may be affected by seasonality. This is confirmed by (Hadas et al. 2009) who found the majority of water column PN in a Gulf of Aqaba reef to consist of, or be produced by, pelagic prokaryotes. DOC may be more abundantly released by benthic coral reef algae than by co-occurring hard corals (Haas et al. 2013; Mueller et al. 2014). DOC release by turf algae and the algal genus *Peyssonnelia* in the studied reef is higher during the stratified than during the mixed season (Haas et al. 2010), potentially due to increased temperature. A positive relation between DOC release and temperature is common in marine macrophytes (e.g. Maher and Eyre 2010). Increased benthic release could explain the increased water column DOC concentration found during the stratified season.

Primary production

The Pnet and BOD measurements can be recalculated to metabolic carbon fluxes per day by the following equations, assuming photosynthetic and respiratory quotients of 1.4 and 1.1, respectively (McKinnon et al. 2013). Daily gross primary production (GPP) = (Pnet + BOD) x h of daylight; daily respiration (RESP) = BOD x 24 h; daily metabolic balance (MET) = GPP – RESP. All carbon fluxes are then in µmol C L-1 d-1. GPP was relatively stable within each season, but significantly higher in the mixed than in the stratified season, while mean RESP was not significantly different between seasons (Fig. 1-3; Fig. 1-4). MET was always negative and significantly lower in the stratified than the mixed season (Fig. 1-4), indicating that the water column as a whole acted net heterotrophically, particularly so during the stratified season. Coral reef planktonic communities are often net heterotroph and are likely fueled by a steady supply of organic matter released from the reef benthos (Naumann et al. 2012a; Haas et al. 2013). The planktonic GPP measured in the mixed and stratified seasons falls in the range of other reef-surrounding waters worldwide (Table 1-2). Mean seasonal water column total organic C (TOC = POC + DOC) was 83.5 and 95.1 µmol L^{-1} for the mixed and stratified seasons respectively. Relating seasonal TOC to MET resulted in comparable net daily planktonic C turnover of 7.3 ± 3.2, and 7.7 ± 1.8 % d^{-1} during the mixed and stratified seasons respectively (Fig. 1-4).

The picoeukaryote to *Prochlorococcus* sp. change in the phytoplankton community from the mixed to the stratified season was similar to that reported in previous study (Al-Najjar et al. 2007). This change in phytoplankton composition may explain the negative correlations found between PAR and Chl a, and Pnet. While PAR reached maximum values in the beginning of the stratified season, picoeukaryote abundance was substantially decreased compared to the mixed season. Since picoeukaryotes contain up to two orders of magnitude more Chl a per cell than *Prochlorococcus* sp. (Davey et al. 2008), and are capable of primary production rates up to two orders of magnitude higher than *Prochlorococcus* sp. per cell (Worden et al. 2004), both Chl a and Pnet were reduced during highest PAR. The picoplankton sample size was unequal between seasons due to a more extensive sampling effort during the stratified season. However, the differences between seasons are highly significant (Fig. 2, all differences $p < 0.001$), and the Mann Whitney U test is not influenced by unequal sample size.

Table 1-2. Comparison of GPP (daily gross primary production) rates from this study and literature.

Location	GPP (μmol C L^{-1} d^{-1})	Reference
Gulf of Aqaba	0.00 - 2.54†	This study
Gulf of Aqaba	0.05 - 3.38*	Levanon-Spanier et al. (1979)
Northern Red Sea	0.02 - 3.00*	Qurban et al. (2014)
Great Barrier Reef, Australia	0.80 - 3.33	Sorokin (1995)
Vietnam	0.15 - 3.00	Nguyen et al. (2013)
Hawaii	2.01 ± 0.17† (mean ± SE)	Johnson and Wiegner (2014)
New Caledonia	0.47 ± 0.05 (mean ± SE)	Torreton et al. (2010)

† recalculated from O_2 values using a photosynthetic quotient of 1.4 (McKinnon et al. 2013). * extrapolated to per day assuming 12 hours of daylight

Pgross and PAR showed no correlation, while Pgross and NH_4^+, and NO_x were positively correlated, indicating that inorganic N, rather than PAR, was limiting photosynthesis in these well-lit, but oligotrophic, waters. This is supported by similar findings for the northern Red Sea (Qurban et al. 2014). Although DIN:PO_4^{3-} ratios in the stratified season often exceeded Redfield with values up to 33, no correlations were found between Pgross and PO_4^{3-}. This may be explained by the smaller size of *Prochlorococcus* sp. compared to picoeukaryotes. Their smaller size with theoretically increased uptake efficiency due to allometrically higher surface area to volume ratio, may have allowed them to maintain photosynthesis under extremely low phosphorus (P) availability (Finkel et al. 2010). However, Maranon et al. (2013) found this surface area to volume relation to be lacking over a wide range of phytoplankton sizes (0.1 - 10^6 μm^3). In addition, primary production by various kinds of phytoplankton may have a wide range of optimal N:P ratios, often strongly exceeding the classic Redfield ratio of 16:1 (Klausmeier et al. 2004).

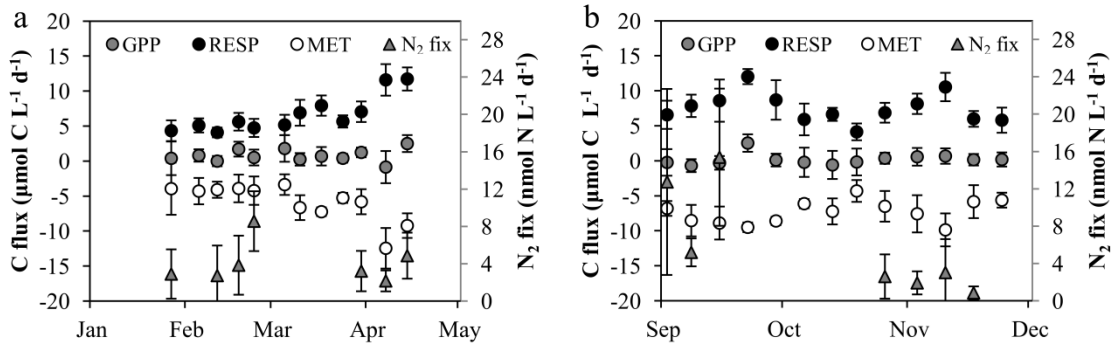

Fig. 1-3. Mean daily metabolic carbon fluxes and N_2 fixation during mixed (a) and stratified (b) seasons. Values given as mean ± SD. GPP = gross primary production, RESP = community respiration, MET = metabolic balance over the day = GPP - RESP, N_2 fix = N_2 fixation

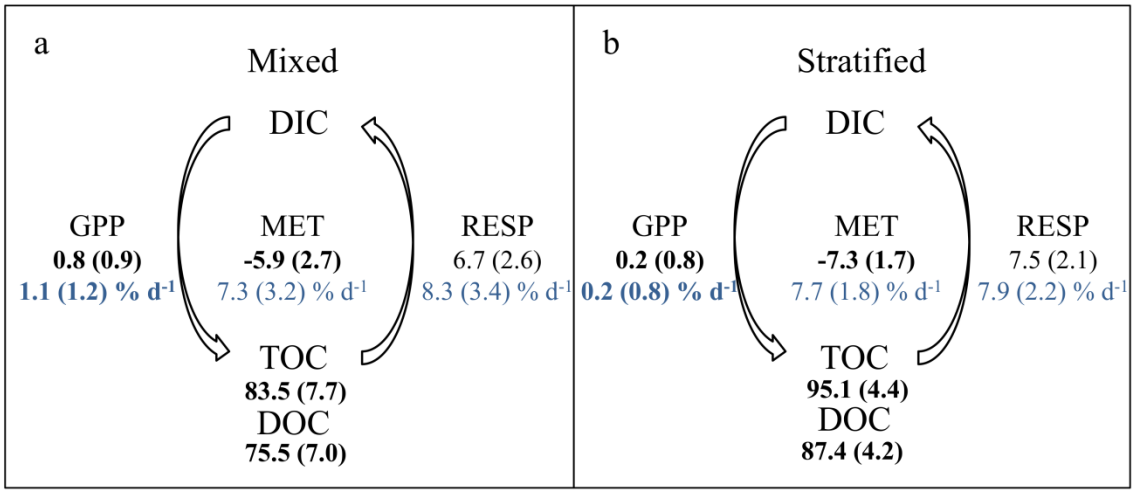

Fig. 1-4. Mean seasonal daily planktonic carbon balance in mixed (a) and stratified (b) seasons. GPP = gross primary production, RESP = community respiration, MET = daily metabolic balance = GPP - RESP. Values given as mean (SD). GPP, RESP, MET in µmol C L^{-1} d^{-1}. DOC = dissolved organic carbon, TOC = total organic carbon in the water column = POC (particulate organic carbon) + DOC, TOC and DOC in µmol C L^{-1} DIC = dissolved inorganic carbon. Below each C flux in blue is the percentage d^{-1} (mean (SD)) it constitutes of TOC. Bold indicates a significant difference between seasons (Mann-Whitney U tests $p < 0.05$).

N_2 fixation

The hourly C_2H_4 fluxes can be converted into daily N fluxes by multiplying by 24 h, dividing according to the conservative theoretical C_2H_4:N_2 ratio of 4:1 (Mulholland et al. 2004), and multiplying by 2 to convert N_2 to N, resulting in fluxes of nmol N L^{-1} d^{-1}. Daily N_2 fixation rates ranged from 0.86 to 15.39 nmol N L^{-1} d^{-1} and average N_2 fixation was similar between seasons: 4.04 ± 2.17 and 5.96 ± 5.74 nmol N L^{-1} d^{-1} (mean ± SD) for mixed and stratified seasons, respectively (Fig. 1-3). Assuming the Redfield C:N ratio (6.625), planktonic water column N_2 fixation had, on average, the potential to contribute 3.42 % of N needed for GPP during the mixed season. The average potential contribution during stratification was substantially higher as N_2 fixation produced 20.84 % of the potential N demand by GPP.

N_2 fixation rates measured in this study were within the range found for planktonic communities from different locations worldwide (Table 1-3). Our maximum rates appear high compared to most literature values, but this is mainly due to two high values measured in September (12.74, 15.39 nmol N L^{-1} d^{-1}). The other weekly mean values in the present study have an average of 3.43 ± 1.88 nmol N L^{-1} d^{-1} which falls within the range of most values from literature (0.01 - 6.24 nmol N L^{-1} d^{-1}). The measurements from the tropical Atlantic Ocean (up to 12.44 nmol N L^{-1} d^{-1}, Großkopf et al. 2012) indicate that high rates as found in September are possible in comparable oligotrophic conditions. Moreover, the high September N_2 fixation rates coincided with lowest DIN concentrations of all weeks measured (0.20 - 0.29 µmol L^{-1}). N_2 fixation is energy-costly, and many diazotrophs can increase their N_2 fixation in times of inorganic/organic N scarcity (Mulholland et al. 2001).

Table 1-3. Comparison of daily dinitrogen (N_2) fixation rates from this study and literature.

Location	N_2 fixation (nmol N L^{-1} d^{-1})	Reference
Gulf of Aqaba	0.86 – 15.39†	This study
Gulf of Aqaba	0.01 - 1.90	Foster et al. (2009)
Gulf of Aqaba	~ 0.40	Rahav et al. (2015)
Hawaii	0.70 - 2.21	Dore et al. (2002)
Hawaii	0.96 - 6.24	Wilson et al. (2012)
North East Pacific Ocean	0.15 - 0.31	Needoba et al. (2007)
Tropical Atlantic Ocean	~ 0.96	Falcon et al. (2004)
Tropical Atlantic Ocean	0.02 - 12.44	Großkopf et al. (2012)

† recalculated from C_2H_4 production rates using a C_2H_4:N_2 ratio of 4:1 (Mulholland et al. 2004).

Rahav et al. (2015) found PO_4^{3-} amendments to planktonic N_2 fixation incubations in the Gulf of Aqaba to enhance the N_2 fixation. However, (Foster et al. 2009) did not detect P limitation of N_2 fixation and attributed this to the relatively small size of the N_2 fixing microbes in the Gulf of Aqaba, allowing maintenance of N_2 fixation at very low P availability due to the same allometric relationship as discussed above. We did not find correlations between N_2 fixation and PO_4^{3-} which could indicate such a limitation. However, nutrient limitation of planktonic processes may well be a more complicated subject than indicated by the frequent use of the canonical Redfield ratio (Klausmeier et al. 2004).

(Rahav et al. 2015) also found evidence for a potential transition from autotrophic to heterotrophic diazotrophs between the mixed and stratified seasons. The positive correlations found in the present study between N_2 fixation and Pgross in the mixed season, and N_2 fixation and DOC concentration during stratification lend additional support to such a transition. The potential N contribution by N_2 fixation to primary production was more than six times higher in the stratified season than in the mixed season, indicating N_2 fixation as a possible substantial source of N-nutrients to the autotrophic community as a whole during extremely oligotrophic times in the Gulf of Aqaba. The contribution percentages calculated in the present study are comparable to those under comparable oligotrophic scenarios in other regions (Montoya et al. 2004; White et al. 2007).

Ecological implications

Seasonal primary production in the water column of the Gulf of Aqaba appears to be primarily regulated by inorganic N availability. As inorganic N concentrations decline due to stratification, GPP decreases substantially resulting in a more heterotrophic daily net C balance coinciding with a shift in the pico-phytoplankton community. While MET was significantly more heterotrophic during stratification, daily net C turnover was similar between seasons because of increased DOC concentrations. The maintenance of biological activity in the water column due to increased DOC availability in times of reduced GPP underlines the importance of the microbial loop in planktonic tropho-dynamics in these waters (Azam and Malfatti 2007; Nelson et al. 2011).

In addition to this change in the C budget, indications of a potential shift in the N_2 fixation community from its autotrophic to its heterotrophic component, as posited by Rahav et al. (2015), were found. Causes for this shift could be the decline in inorganic nutrients, as well as, again, the increased DOC concentration in the water, giving a competitive advantage to the heterotrophic fraction (Suggett et al. 2009). This increase in DOC may be primarily attributable to increased release by benthic turf- and macroalgae (Haas et al. 2013; Mueller et al. 2014). Algal-

derived organic matter has also been indicated in promoting a more heterotrophic planktonic community than that derived from scleractinian corals (Haas et al. 2011; Nelson et al. 2013). During stratification, when GPP is strongly nutrient limited and significantly reduced, N_2 fixation has the potential to contribute a substantial fraction of the N needed to maintain the reduced primary production in the water column.

This study is a first investigation of regulatory effects by key status parameters on both primary production and N_2 fixation, as well as phytoplankton community composition, in waters surrounding a high latitude coral reef and shows that the seasonality in status parameters coincided with strong changes in the phytoplanktonic community composition over the year with subsequent consequences for planktonic primary production. While indications were found for a similar community change in diazotrophs, N_2 fixation was maintained at comparable levels in both seasons, potentially being an important source of N to planktonic primary production. DOC appears to play an important role in dynamics of planktonic C and N fluxes. Further investigation into DOC fluxes through coral reefs is warranted to unravel its effect on energy and nutrient cycles in coral reefs and their surrounding waters. The findings presented here on which status parameters play a controlling role in the production of organic C and N-nutrients in reef surrounding waters can be applied to lower latitude coral reefs where the more stable environmental conditions make the disentanglement of driving status parameters more complicated.

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Chapter 2
Seasonal photosynthetic primary production and microbial dinitrogen fixation in hard corals

Abstract

In nutrient-depleted waters, hard corals act as primary ecosystem engineers of tropical reefs by providing the structural complexity, biomass, and organic matter on which the entire reef ecosystem is sustained. The exceptionally high gross primary production of corals is achieved by efficient recycling of nutrients through coordinated metabolic exchanges with their endosymbiotic dinoflagellate algae (zooxanthellae). However, additional inputs of nitrogen are required for net production and growth. Here we show that photoautotrophic inorganic carbon (CO_2) fixation by zooxanthellae is sustained by dinitrogen (N_2) fixation performed by coral-associated N_2-fixing prokaryotes if other external sources of inorganic nutrients are scarce. The functioning of this host-microbe ecosystem enables the nutritional success of hard corals and explains their high gross productivity and organic matter release, which in turn set the basis for the functioning of reef ecosystems and the services that they provide.

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Main text

Scleractinian hard corals fulfill important functions as key ecosystem engineers of tropical coral reefs, providing the habitat for some of the most productive, diverse and economically important biological systems on Earth (Moberg and Folke 1999). They do so by controlling a wide range of biogeochemical processes important to coral reef functioning and by intensively generating and transforming inorganic and organic materials (Wild et al. 2011). These sessile cnidarians owe their evolutionary success to an endosymbiosis with photosynthetic dinoflagellate microalgae of the genus *Symbiodinium* (commonly referred to as zooxanthellae) (Dubinsky and Jokiel 1994; Stambler 2011) that contribute a substantial fraction of the total gross primary productivity (i.e., the amount of inorganic carbon (CO_2) photosynthetically fixed per unit of time) in coral reef ecosystems. Up to 50% of the net fixed CO_2 in the coral is subsequently released as organic carbon (C) in the form of mucus, and provides a nutritious food source to other reef organisms via the microbial and sponge loops (Wild et al. 2004; de Goeij et al. 2013).

Hard corals show a remarkable degree of nutritional plasticity, being able to fix CO_2 at high rates, but also to adapt and rapidly assimilate organic C when zooxanthellae are lost (e.g. during coral bleaching; Grottoli et al. 2006). Additionally, corals have evolved strategies to exploit any source of inorganic and organic nitrogen (N) (Cardini et al. 2014), enabling them to survive in oligotrophic tropical waters where N is the most limiting nutrient to primary productivity (D'Elia and Wiebe 1990; Eyre et al. 2008). However, the abundance of symbiotic zooxanthellae within the coral host is limited by N concentrations (Falkowski et al. 1993; Dubinsky and Jokiel 1994). N deficiency and starvation of zooxanthellae cause severe depletion of the main N-containing photopigment (Hoegh-Guldberg and Smith 1989; Jiang et al. 2014), chlorophyll *a* (chl *a*), and result in a decline in productivity. Although very efficient internal recycling of N prevents disintegration of the symbiosis and loss of zooxanthellae, recycled N cannot account for new biomass production and growth if external sources are in short supply (Szmant-Froelich 1983).

Besides endosymbiotic eukaryotic algae, hard corals are also associated with a diverse array of prokaryotes (Rohwer et al. 2002). The prokaryote-dinoflagellate-coral assemblage is often referred to as the coral holobiont (Rohwer et al. 2002). In the resulting host-microbe ecosystem, the interactions among the partners determine the functioning and ecological success of the whole group of organisms (McFall-Ngai et al. 2013). Evidence is accumulating that among these prokaryotic communities dinitrogen (N_2) fixing microorganisms (termed diazotrophs) form highly specific associations with their cnidarian hosts (Shashar et al. 1994; Lesser et al. 2004; Lesser et al. 2007; Olson et al. 2009; Lema et al. 2012; Lema et al. 2014b). However, the

ecological significance of N_2 fixation in sustaining the high gross primary productivity of hard corals has not yet been evaluated.

Here, we present results of a year-long study examining the relationship between N_2 fixation and CO_2 fixation in hard coral holobionts at a fringing coral reef in the northern Red Sea (Gulf of Aqaba). We made use of the characteristic seasonality of the sampling location, which harbors some of the northernmost warm water coral reefs on Earth. At our study location, water column mixing takes place during spring and strong thermal stratification occurs in summer (Fig. 2-1, Appendix 2 - Fig. 1). This leads to inorganic N concentrations which are one order of magnitude lower in summer compared to spring (Appendix 2 - Table 1). We thus repeated our measurements of the four locally dominant hard coral genera during all seasons. This strategy allowed us to assess the contribution of diazotrophs to changing N requirements of the coral holobiont in response to pronounced seasonal environmental variations.

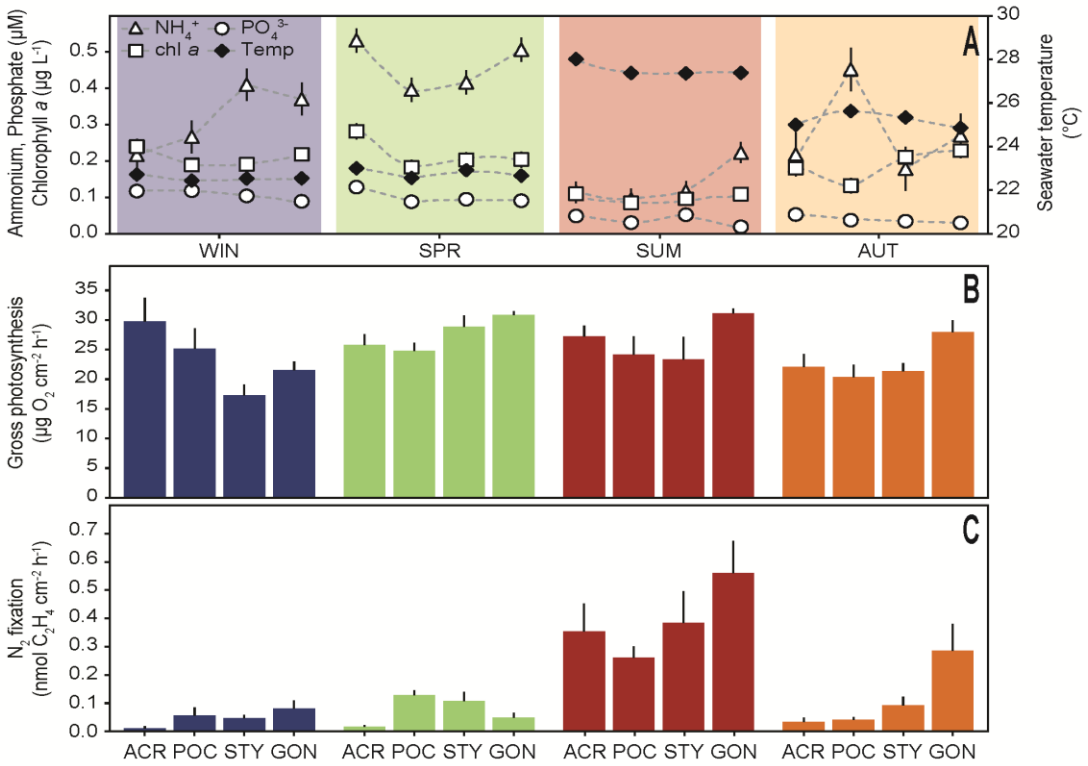

Fig. 2-1. Seasonal environmental conditions with corresponding primary productivity and dinitrogen fixation in corals. (A) Seasonal weekly averages of the main environmental variables measured at the sampling location are shown. (B) Gross photosynthesis and (C) N_2 fixation of *Acropora* (ACR), *Pocillopora* (POC), *Stylophora* (STY) and *Goniastrea* (GON) are presented here as means (n = 8) ± s.e.m.. See Appendix 2 - Table 1 - 6 for the complete set of environmental variables and for statistical results. Colours represent winter (WIN, blue), spring (SPR, green), summer (SUM, red) and autumn (AUT, orange).

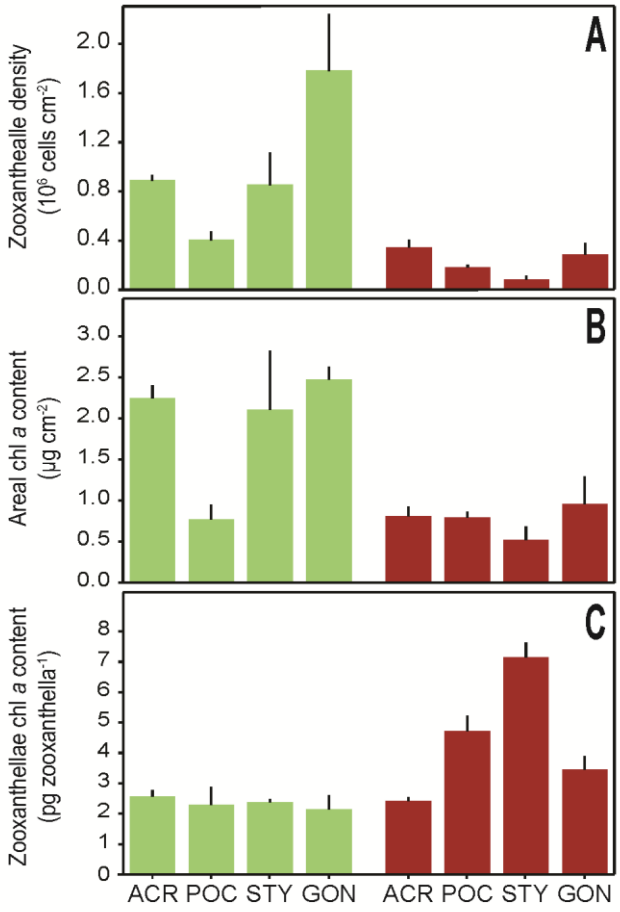

Fig. 2-2. Seasonal zooxanthellae and photopigment content in corals. Shown are (A) zooxanthellae density, (B) areal chl a content, and (C) zooxanthellae chl a content. Colours represent spring (green) and summer (red). See Appendix 2 - Table 3 - 6 for the statistical results. Data are presented as means (n = 4) ± s.e.m..

Our findings revealed that diazotrophs are consistently active and associated with all dominant hard coral genera (*Acropora*, *Pocillopora*, *Stylophora* and *Goniastrea*) throughout the year, despite the pronounced seasonality (Fig. 2-1). This association has previously been described in physiological and molecular studies (Shashar et al. 1994; Lesser et al. 2004; Lesser et al. 2007; Davey et al. 2008; Olson et al. 2009; Lema et al. 2012; Lema et al. 2014b), and N_2 fixation rates measured here are in the range of those reported for corals in the literature (Cardini et al. 2014). This highlights diazotrophs as constant members of hard coral holobionts. N_2 fixation significantly increased in all corals during summer (Fig. 2-1, Appendix 2 - Table 3, 4). At the same time, gross photosynthesis did not show any consistent trend with regard to coral genus or season (Fig. 2-1). In summer, all corals exhibited a significant decrease in zooxanthellae density and areal chl a content, while a significant increase in zooxanthellae chl a content was detected (Fig. 2-2, Appendix 2 - Table 3 - 6). The observed pattern of seasonal variability in zooxanthellae abundance (also known as physiological bleaching) represents an established phenomenon in

tropical and subtropical scleractinian corals caused by an increase in photosynthetically active radiation, which enhances zooxanthellae loss presumably due to concomitant warmer temperatures (Fitt et al. 2000; Fitt et al. 2001). Moreover, growth efficiency and mitotic index of the symbiotic algae are temperature-dependent and decrease if temperature exceeds 25°C (Wooldridge 2013). Despite the observed decreases in zooxanthellae population density, all corals maintained high gross productivity throughout the year (Fig. 2-1) indicating high metabolic plasticity to seasonal variation in environmental conditions.

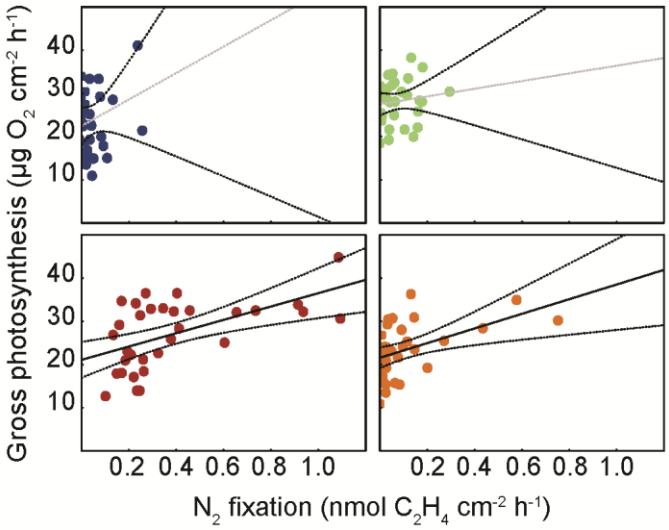

Fig. 2-3. Seasonal relationships between N_2 fixation and gross photosynthesis in corals. Data points for each season (n = 32) are colour coded in blue (winter), green (spring), red (summer) and orange (autumn). Best-fit linear regression lines (±95% confidence intervals) are solid black if the relationship is significant (winter: Pg = 22.488+30.553x; r = 0.205; p = 0.261; spring: Pg = 27.517+8.890x; r = 0.128; p = 0.484; summer: Pg = 21.076+15.417x; r = 0.551; p = 0.001; autumn: Pg = 21.565+16.940x; r = 0.475; p = 0.006).

Gross photosynthesis was positively and linearly correlated with N_2 fixation in summer, and to a minor extent in autumn (Fig. 2-3). The high sensitivity of nitrogenase (the enzyme responsible for N_2 fixation) to molecular and reactive oxygen species accumulating during photosynthesis requires a mechanism protecting nitrogenase from inactivation. The mechanism remains enigmatic, and may involve quenching via respiration and/or the Mehler reaction (Lesser et al. 2004). A possible explanation for the significant correlation between gross photosynthesis and N_2 fixation is that active diazotrophs provide the limiting N required for sustaining zooxanthellae photosynthesis in the nutrient-depleted waters of the Gulf of Aqaba, while obtaining energy in the form of C from the zooxanthellae within the coral host. Similar scenarios are well documented in land plants where heterotrophic diazotrophs in roots, such as rhizobia (also common partners in the coral holobiont; Lema et al. 2012; Lema et al. 2014a; Lema et al. 2014b), fuel the plant with bioavailable N, while relying on plant photosynthesis to carry out the

energetically demanding process of N_2 fixation. Unique to the coral system is that photosynthetic eukaryotic algae (zooxanthellae), prokaryotes (N_2 fixers) and the animal host (coral) cooperate, facilitating the nutritional success of the entire group of partners.

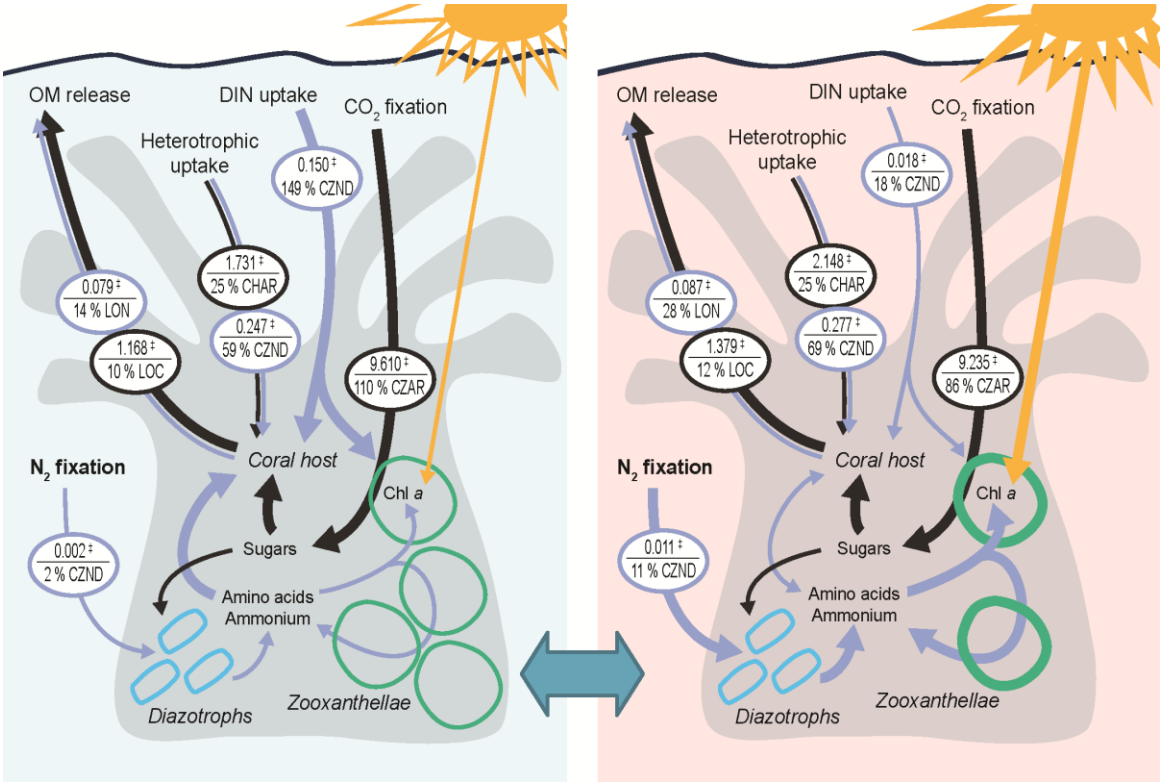

Fig. 2-4. C and N flux model of the diazotroph-dinoflagellate-coral symbiosis. Shown are models for spring (left panel) and summer (right panel). Colours represent C (black) and N (blue) fluxes (‡ µmol cm^{-2} d^{-1}), and the width of arrows highlights seasonal differences. Percentages are: contribution of zooxanthellae-acquired N to zooxanthellae N demands (CZND); contribution of zooxanthellae-acquired C to animal respiration (CZAR); contribution of heterotrophically-acquired C to animal respiration (CHAR); loss by organic C release of the total acquired C (LOC); loss by organic N release of the total acquired N (LON); Parameters presented in the model (± standard deviation) and the respective calculations are located in appendix 2.

For tropical coral reefs, it is widely accepted that N is the most limiting nutrient to coral primary productivity (Eyre et al. 2008). Phosphorus (P) may also be limiting to coral metabolism (Godinot et al. 2011), particularly in the case of an imbalanced nutrient supply with excess inorganic N (e.g., of anthropogenic origin) (Wiedenmann et al. 2013). However, at our study site, the reef-surrounding seawater exhibits particulate organic C to N (POC:PN) and dissolved inorganic N to phosphate ($DIN:PO_4^{3-}$) ratios that are respectively higher and lower than the Redfield ratio (106:16:1) during all seasons (Appendix 2 - Table 1), indicating that N rather than P is the limiting nutrient to coral primary productivity. It is well-known that N limitation causes

decreased pigment content in zooxanthellae (Hoegh-Guldberg and Smith 1989), with N deficiency resulting in up to an 86% reduction in zooxanthellae chl *a* content after only one week (Jiang et al. 2014). Conversely, corals investigated here showed higher zooxanthellae chl *a* content (Fig. 2-2) and a positive linear relationship between N_2 and CO_2 fixation during the highly N-depleted summer period (Fig. 2-3). Overall, these findings suggest that microbial N_2 fixation is metabolically connected to photosynthetic algal CO_2 fixation and have important implications for our current understanding of the coral holobiont as a prominent example of a host-microbe ecosystem.

C and N flux modelling (see Appendix 2) was applied to investigate the relative importance of each N source to the zooxanthellae N demands (Fig. 2-4). In spring, when ambient DIN concentrations and uptake rates are high, but irradiance and water temperature are low, the coral host is replenished with N (Fig. 2-4, left panel) and actively limits algal population growth by removing excess nutrients from the intracellular milieu surrounding the zooxanthellae (Falkowski et al. 1993; Wooldridge 2013). Conversely, during the warm, high irradiance, nutrient-depleted summer (Fig. 2-4, right panel), environmental conditions trigger a dynamic expulsion of symbiotic algae leading to smaller zooxanthellae populations (Fitt et al. 2000; Wooldridge 2013). However, N fixed and transferred by the thriving diazotrophic community to the zooxanthellae prevents N deficiency within the holobiont. Zooxanthellae are thus provided with sufficient N to sustain biosynthesis of the chlorin ring of chl *a*, the light-capturing engine of photosynthesis. In summer, the contribution of N_2 fixation to zooxanthellae N demands (CZND) is comparable with the contribution of DIN uptake from reef-surrounding waters (Fig. 2-4, right panel). These two N sources together with heterotrophic N uptake facilitate 98 % of the total CZND, implying an almost complete N sufficiency of zooxanthellae (Fig. 2-4, Appendix 2 - Table 7).

These results contribute importantly to our understanding of coral metabolism; the dynamic equilibrium between the associated eukaryotic and prokaryotic communities in the coral holobiont (Fig. 2-4) sets the basis for high functional stability, sustaining high gross productivity despite changes in environmental conditions. Corals achieve this functional stability by adjusting population densities of symbiotic zooxanthellae and by benefiting from N fixed by symbiotic diazotrophs (Fig. 2-4). As a consequence of stable year-round primary productivity, hard corals are able to sustain high production and regeneration of their mucus surface layer and its concomitant release as organic matter (OM) (Fig. 2-4, Appendix 2 - Fig. 2). On a daily basis, we estimate that 10-12% of the total C and 14-28% of the total N acquired by the holobiont are lost via OM release (LOC and LON, respectively; Fig. 2-4). This released organic matter provides a

food source for other reef organisms, thereby contributing to the functioning and productivity of the entire coral reef ecosystem (Wild et al. 2004; de Goeij et al. 2013).

Recent research from various reef locations has identified diazotrophic assemblages in different coral species to be spatially and temporally consistent (Lema et al. 2012; Lema et al. 2014a; Lema et al. 2014b). Concurrently, tropical reef systems worldwide typically exhibit extremely low seawater N concentrations, comparable to those measured in summer in the Gulf of Aqaba (Kleypas et al. 1999). Further, no lower thresholds for nutrients considered limiting to coral reef growth have been found (Kleypas et al. 1999), implicating the widespread importance of N_2 fixation. Therefore, our results are contributing to the resolution of Darwin's paradox (by which coral reefs thrive in nutrient-depleted waters). We predict that the association with diazotrophs is fundamental to the functioning and high gross productivity of oligotrophic coral reefs worldwide and consequently to the essential goods and services (e.g., food and economic security, biodiversity; Moberg and Folke 1999) that reef ecosystems provide.

Materials and methods

Collection and maintenance of corals

All research described was conducted in the northern Red Sea (Gulf of Aqaba) during 2013. Sample collection was carried out along the slope of the fringing reef in front of the Marine Science Station (MSS) Aqaba, Jordan (location: 29° 27′ N, 34° 58′ E) using SCUBA. In February (winter), April (spring), September (summer) and November (autumn), 8 coral fragments (5-6 cm in height) of each of the hard coral genera *Acropora*, *Pocillopora*, *Stylophora* and *Goniastrea*, identified morphologically, were sampled haphazardly at 10 m water depth from individual colonies that were at least 5 m apart to avoid sampling clones. Fragments were cut underwater with bone cutters and placed in plastic bags, taking care not to cause any abrasion of the tissues. In the laboratory, fragments were glued on ceramic tiles and maintained in a 800 L flow-through aquarium directly supplied with untreated reef water (from 10 m water depth) at a rate of approximately 4000 L h^{-1}. Natural levels of light intensity inside the maintenance aquarium were adjusted to seasonal in-situ conditions at 10 m water depth using layers of black mesh. Monthly measurements of light intensity and inorganic nutrient concentrations in the flow-through aquarium were performed and differences from in-situ conditions were undetectable.

Environmental monitoring

Temperature and light intensity were continuously monitored at the coral sampling location on the reef (10 m depth) during all seasons by means of data loggers (Onset HOBO Pendant UA-002-64; Bourne, MA, USA). Monthly measurements of light intensity were also performed using a quantum sensor (Model LI-192SA; Li-Cor, Lincoln, Nebraska, USA) parallel to data loggers to allow conversion from light intensity (LUX) to photosynthetically active radiation (PAR, µmol photons m^{-2} s^{-1}, wavelength 400 to 700 nm) (Long et al. 2012). Seawater inorganic nutrients, chlorophyll *a* (chl *a*), particulate organic C (POC), particulate N (PN) and dissolved organic C (DOC) concentrations were monitored weekly in-situ at the coral sampling location. Seawater samples (n = 4) were collected by SCUBA approximately 1 m above the substrate, using high-density polyethylene canisters (5 L). In the laboratory, subsamples were drawn from each canister for analyses of inorganic nutrients, chl *a*, POC, PN and DOC as detailed in the following. For each inorganic nutrient analysis, one subsample of 50 mL was collected from each canister (n = 4) and gently filtered through cellulose acetate membrane filters (nominal pore size: 0.45 µm). Immediately after filtration, inorganic nutrient concentrations (ammonium - NH_4^+, phosphate - PO_4^{3-}, nitrate - NO_3^-, nitrite - NO_2^-) were measured using established methods (Murphy and Riley 1962; Strickland and Parsons 1972; Holmes et al. 1999). NH_4^+ was determined fluorometrically using a Trilogy fluorometer (Turner Designs), while all other inorganic nutrients were measured spectrophotometrically with a V-630 UV-Vis Spectrophotometer (Jasco Analytical Instruments). Detection limits for NH_4^+, PO_4^{3-}, NO_x and NO_2^- were 0.09, 0.01, 0.02 and 0.005 µM, respectively. For chl *a*, one subsample of 1 L was collected from each canister (n = 4), filtered onto a pre-combusted (450˚C, 4h) GF/F filter (VWR, diameter: 25 mm, nominal pore size 0.7 µm), and frozen at -80˚C in the dark until analysis. Prior to analysis, chl *a* was extracted with 90% acetone for 12 h in the dark at 4˚C, and analyzed on the Trilogy fluorometer fitted with the non-acidification chl module (CHL NA #046). For POC and PN analyses, subsamples of 1 and 2 L, respectively, were collected from each canister (n = 4), filtered onto pre-combusted GF/F filters, dried for 48 h at 40˚C, and stored dry until analysis. POC filters were acidified (0.1N HCl) prior to analysis, and POC and PN content of dried filters were measured using a EuroVector elemental analyser (EURO EA 3000). C and N content were derived from calculations using elemental standards (OAS 187560; analytical precision ≤0.1% (C) and ≤0.03% (N) of the standard value). For DOC analyses, one 50 mL subsample was collected from each canister (n = 4), gently vacuum filtered (max. suction pressure 20 kPa) through a pre-combusted GF/F filter directly into acid-washed 30 mL HDPE sample bottles using a custom set-up with an in-line polycarbonate filter holder. Prior to filtration, the syringes, the HDPE sample bottles and the filtration set-up were soaked in 0.4M HCl for 24 h and then washed with MQ water. Upon collection, the first 20 mL of

sample was used to wash the filtration set-up and the sample bottle with 2 x 10 mL sample water and discarded before collecting the remaining 30 mL. DOC samples were immediately acidified with 80 µl of 18.5% HCl and stored in the dark at 4˚C until analysis. Samples were analyzed by the high-temperature catalytic oxidation (HTCO) method on a Shimadzu TOC-V_{CPH} total organic carbon analyser. The instrument was calibrated with a 10-point calibration curve of serial dilutions from a potassium hydrogen phthalate certified stock solution (1000 ppm Standard Fluka 76067-500ML-F). Consensus reference material provided by DA Hansell and W Chan of the University of Miami (Batch 13, Lot #08-13, 41-45 µmol C L^{-1}) was used a positive control between every 10 samples. Each sample was measured with 5 replicate injections, and analytical precision was <3% of the certified value.

Physiological measurements

Coral fragments (n = 8 for each coral genus during each season) were maintained in the flow-through aquarium described above during measurements of net photosynthesis (Pn), dark respiration (R), N_2 fixation and POC and DOC release to ensure in-situ temperature and light intensity in the chambers. Fragments were allowed to recover from fragmentation for at least one week prior to the measurements. Ceramic tiles were cleaned of sediment and epibionts with a fine brush immediately prior to the start of the incubations. Only visually healthy and entirely healed fragments were used for incubation experiments. Incubation chambers were filled with the seawater from the aquarium and corals were transferred into the chambers, taking particular care to prevent any air exposure. Before closing the chamber for incubation, each fragment was allowed to adjust to chamber conditions for at least 15 min. Each chamber contained a stir bar powered by a submersible magnetic stirrer (600 rpm, Cimarec i Telesystem Multipoint Stirrers, Thermo Scientific) to ensure water mixing (and gas equilibration with the headspace, if applicable). Temperature and light intensity were monitored during all measurements by placing a data logger in one additional chamber. Following each incubation, the dissolved oxygen (O_2) concentration in the incubation water was measured to ensure that oxic conditions in the chambers were maintained. Rates of Pn, R, N_2 fixation, organic matter (OM) release as well as zooxanthellae density and chlorophyll *a* (chl *a*) content were normalized to skeletal surface area of the coral fragments, measured using the Advanced Geometry protocol (Naumann et al. 2009).

Primary productivity

Pn and R of the coral fragments were assessed by their O_2 fluxes in closed-cell respirometric glass chambers (1 L). Gross photosynthesis (Pg) was calculated (Pg=Pn+R) for each specimen. R was measured in the dark, one hour after sunset, to allow for specimens to reach a stable dark

respiration rate. The following day, Pn was measured between 12:00 and 14:00, the most stable and maximum light conditions of the day as confirmed by light intensity monitoring. The Pg rates presented here should therefore be considered a conservative estimate. Coral O_2 fluxes were determined as differences between salinity corrected O_2 start and end concentrations (incubation time ≈ 90 min) measured with an O2 optode sensor (MultiLine® IDS 3430, WTW GmbH, Weilheim, Germany). Two different sets of controls were incubated in parallel to determine O_2 fluxes in the same unfiltered seawater and unfiltered seawater plus the mounting tile. Differences in O_2 concentration were corrected for control signals (absolute blank/biological ratios < 20%), normalized to incubation period and fragment surface area and reported as μg O_2 cm^{-2} h^{-1}. All coral fragments were returned to the maintenance aquarium for approximately 4 h before starting subsequent incubation measurements for N_2 fixation rates.

N_2 fixation

N_2 fixation was quantified by using an adapted acetylene (C_2H_2) reduction technique (Capone 1993; Wilson et al. 2012). For this purpose, 1 L glass chambers were filled with 800 mL of natural seawater, whereof 10% was replaced with C_2H_2-saturated seawater. The chamber was immediately sealed gas-tight and 10% of the 200 mL headspace was replaced with C_2H_2 gas through a gas-tight rubber stopper equipped with a push-button syringe valve with Luer lock (SGE Analytical Science). To generate the C_2H_2 gas and the C_2H_2-saturated seawater, calcium carbide (CaC_2; Sigma) was used following a modified procedure described in Wilson et al. (2012). Briefly, C_2H_2 was bubbled in an additional flask with 300 mL of deionized water to reduce background ethylene (C_2H_4) contamination, before being bubbled in a flask filled with 800 mL of natural seawater to be enriched. From there, the C_2H_2 was finally collected in a Tedlar® gas sampling bag. C_2H_2 and the C_2H_2-saturated seawater were freshly prepared ≈10 min prior to use. N_2 fixation incubations lasted for 24 h, starting and ending before sunset (approximately at 17:00). The incubation time was kept shorter than in previous studies on corals (Shashar et al. 1994), in order to minimize stress for the organisms. Gas samples (1 mL) were withdrawn from the headspace after time intervals of 0, 4, 12, 16 and 24 h and collected in glass vials with grey butyl stoppers (CS-Chromatographie Service GmbH) previously filled with deionized water. Vials were stored frozen upside down until analysis to prevent any leakage via the septa. Prior to analysis, vials were thawed and water and gas phases in the vials were equilibrated at room temperature by vigorous shaking. C_2H_4 concentration in the vials' headspace was measured using a reducing compound photometer (RCP; Peak Laboratories) (Wilson et al. 2012). The instrument was customized with a sample loop of 10 μL which resulted in an optimal compromise between high sensitivity (± 100 ppb) and wide linear range. Calibration of the RCP was routinely conducted using serial dilutions of a 200 ppm (± 2 %) C_2H_4 standard in air (Restek, Bellefonte,

PA, USA). Here, differences in C_2H_4 concentration between the time intervals of the incubation period were converted into daily C_2H_4 evolution rates according to Breitbarth et al. (2004). Four sets of controls were also tested for C_2H_4 production: 1) 0.2 µm-filtered seawater, 2) unfiltered seawater, 3) unfiltered seawater plus the tile and 4) unfiltered seawater with coral fragments, but without addition of C_2H_2 (natural C_2H_4 production). Filtered seawater and coral fragments without addition of C_2H_2 showed negligible C_2H_4 production. C_2H_4 evolution rates of the biological samples were corrected for control signals (blank/biological ratios < 50%) and normalized to the surface area of the fragments. N_2 fixation rates are reported here as nmol C_2H_4 cm^{-2} h^{-1}. All coral fragments were returned to the maintenance aquarium for approximately one week before continuing with incubation measurements for organic matter release.

Organic matter release

OM release by the four coral genera was quantified during each of the four seasons using the established beaker incubation method (Herndl and Velimirov 1986; Wild et al. 2005; Naumann et al. 2010). Incubation chambers with a subset of the identical coral fragments (n = 6, ca. 700 mL) and seawater controls (n = 6, ca. 900 mL) were incubated for six hours during the day (10:00 – 16:00 h) in open chambers covered with transparent cellophane to prevent the input of airborne particles, leaving small side openings for air exchange. Chambers were not stirred to allow for comparisons with previous studies (Naumann et al. 2010) and to rule out the influence of water currents on OM release and fractionation. Initial samples (50 mL) for DOC were immediately taken from the incubation water of each chamber at the start of the incubation with an acid-washed 50 mL polycarbonate syringe. Initial samples (1.5 L) for POC (n = 3) were collected at the same time from the ambient aquarium water used to fill the chambers. At the end of the 6-hour incubation period, coral fragments were carefully removed from the incubation chambers with clean tweezers and 50 mL samples for DOC were immediately taken and processed using the same protocol as described for the in-situ environmental monitoring. The remaining volume of incubation water (ca. 600 mL for coral chambers and ca. 800 mL for controls) was first measured and then vacuum-filtered onto pre-combusted GF/F filters for POC analyses following the protocol described above for in-situ environmental monitoring. For the calculation of OM release rates (POC and DOC), concentration differences measured between the incubation start and end of each coral chamber were corrected by the average control signal and normalized by the filtered volume, incubation period and coral surface area. Total organic C (TOC) release was calculated as the sum of POC and DOC release for each coral fragment. OM release rates are reported here as µg C cm^{-2} h^{-1}.

Zooxanthellae density and chlorophyll a

For zooxanthellae density and chl *a* content analysis of the corals, a subset of the incubated coral fragments (n = 4) was stored at -20˚C prior to analysis in spring and in summer. Coral tissue was removed from the skeleton using a jet of pressurized air and 0.2 µm-filtered seawater. The resulting tissue slurry volume was brought to 50 mL with 0.2 µm-filtered seawater and homogenised using a vortex. One aliquot of 9 mL was subsequently subsampled, fixed in 3 mL 16% formaldehyde solution (final concentration 4%) and preserved at 4˚C until zooxanthellae density analysis. Subsequently, the homogenate was centrifuged at 5000 rpm for 5 min, the supernatant removed and the pellet resuspended with a known volume of 0.2 µm-filtered seawater. The number of zooxanthellae was counted using an improved Neubauer haemocytometer (Pillay et al. 2005). The total number of zooxanthellae in the initial 50 mL slurry was calculated and normalized to coral surface area (cells cm^{-2}). Another aliquot of 5 mL was taken from each homogenate and immediately centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the pellet frozen at -20˚C overnight to break the cells. The following day the pellet was resuspended in 10 mL 90% acetone and chl *a* was extracted for 24 h in the dark at 4˚C. After another centrifugation run, chl *a* was measured using the non-acidification fluorometric method (Welschmeyer 1994) on a Trilogy fluorometer equipped with the non-acidification chl module (CHL NA #046). Chl *a* content was normalized to coral surface area (µg chl *a* cm^{-2}) and to the total number of zooxanthellae of each fragment (pg chl *a* $zooxanthella^{-1}$).

Statistical analyses

To identify differences in Pn, R, Pg and N_2 fixation for the different coral genera during the four seasons we used a Two-way ANOVA design with the factor “Season” (fixed and orthogonal, 4 levels) and the factor “Genus” (fixed and orthogonal, 4 levels) and n = 8. POC, DOC and TOC release rates were tested for differences with the same design but with n = 6. Zooxanthellae density, areal chl *a* content and zooxanthellae chl *a* content were tested for differences with the same design but with the factor “Season” having 2 levels only (spring and summer) and n = 4. Data were visually inspected for normality using q-q plots, tested for homogeneity of variances using the Cochran’s C-test and transformed if necessary. In case of a significant interaction term, SNK (Student-Newman-Keuls) comparison tests were applied a posteriori to identify significant differences. To visualize multivariate changes in environmental variables among the four seasons, the unconstrained ordination of Principal Component Analysis (PCA) was performed on previously normalized environmental data resulting from weekly averages. Differences in response to seasonality were tested using a PERMANOVA test (Anderson 2005). The analysis was conducted using the Euclidean distance as coefficient of dissimilarity on previously

normalized data. Type 3 (partial) sum of squares was used with unrestricted permutation of raw data (9999 permutations). All multivariate analyses were run using the PERMANOVA tool included in the Primer 6+ package. To determine whether the process of inorganic CO_2 fixation (Pg) was linked to the process of N_2 fixation in the corals over the four different seasons, linear models were fitted to the data and the significance of relationships was tested using the Pearson r-test in Statistica 12 software. CO_2 and N_2 fixation rates were established using proxies (O_2 for CO_2 and C_2H_4 for N_2). However, there is a direct relationship between these measures and the actual CO_2 and N_2 fixation rates (Capone 1993; Montoya et al. 1996; Falkowski and Raven 2007), and the proxies chosen are routinely considered accurate surrogates for measurements of the latter rates in hard corals.

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Chapter 3
Seasonal photosynthetic primary production and microbial dinitrogen fixation in soft corals

Abstract

Dinitrogen (N_2) fixation by specialized prokaryotes (diazotrophs) represents an important source of bioavailable nitrogen (N) in the ocean. In coral reefs, several substrates and organisms are associated with diazotrophs, but potential N_2 fixation activity by zooxanthellate soft corals has not yet been investigated. Such soft corals may contribute importantly to the input of new N into the reef ecosystem, as they can cover substantial benthic areas in today's coral reefs. Therefore, this study investigated N_2 fixation of 2 dominant zooxanthellate soft coral groups (*Sarcophyton* sp. and Xeniidae) in a northern Red Sea fringing reef during all 4 seasons of 1 yr. This was supplemented by respirometry incubations and in-situ monitoring of key environmental parameters. Findings revealed detectable N_2 fixation for both soft corals during all seasons. Annual N_2 fixation by *Sarcophyton* sp. was 3 times higher than that by Xeniidae, but both soft corals exhibited similar seasonal patterns. N_2 fixation significantly increased during summer, when water temperature and light intensity were highest and inorganic nutrient availability was lowest. Coral respiration also peaked during summer and was positively correlated to N_2 fixation, while photosynthesis revealed maximum rates during the nutrient-enriched spring season. Given the importance of N for reproduction and growth, N_2 fixation may be a key component of soft coral nutrition during summer, when inorganic nutrient availability in the water column is lowest and likely not sufficient to sustain the high metabolic demand of soft corals.

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Chapter 3 Soft coral primary production and N_2 fixation

Introduction

Nitrogen (N) is a major limiting nutrient for primary productivity in oligotrophic coral reefs, with dissolved inorganic nitrogen (DIN) concentrations often below 1 μM (Hatcher 1990). Therefore, several reef organisms have evolved physiological mechanisms to conserve, recycle and collect the essential N (Szmant et al. 1990; Tanaka et al. 2006). Many symbiotic cnidarians (e.g. scleractinian corals) show a bidirectional translocation of N compounds between the zooxanthellae and the host. There, the zooxanthellae use the waste N compounds of their host (mostly in the form of ammonium), assimilate it into amino acids and then translocate a portion of them back to the animal (Muscatine and Porter 1977; Rahav et al. 1989). In addition to this inner recycling loop, several benthic organisms (e.g. scleractinian corals, sponges) have evolved a mutualistic symbiosis with dinitrogen (N_2)-fixing microbes (diazotrophs) that are able to convert atmospheric N_2 into bioavailable N (Fiore et al. 2010; Cardini et al. 2014). Up to 60% of N fixed by diazotrophs can be released as dissolved organic N, thereby making it available for organisms unable to fix N_2 (Williams and Carpenter 1997). A recent study on scleractinian corals associated with diazotrophs demonstrated that zooxanthellae are the primary users of the N_2 fixation products, implying that this process is important for coral photosynthesis and primary production (Lesser et al. 2007). Therefore, the ability of corals to overcome N limitation through N_2 fixation may determine their success in oligotrophic waters, ultimately influencing their ecological distribution and abundance on coral reefs (Fiore et al. 2010).

N_2 fixation in scleractinian corals has been described in several studies (Williams et al. 1987; Shashar et al. 1994a; Lesser et al. 2007), but there is a paucity of data available for soft corals, despite their common occurrence in tropical coral reef habitats (Benayahu and Loya 1981; Fabricius 1997). At present, N_2 fixation rates have been quantified for only 2 octocoral species (*Tubipora musica* and *Parerythropodium f. fulvum*), including 1 azooxanthellate soft coral (*P. f. fulvum*; accepted name: *Rhytisma fulvum*; Shashar et al. 1994b), while no data are available for zooxanthellate soft corals.

Some soft corals display opportunistic life history features such as fast growth rates, high fecundity and asexual reproduction. These traits can give soft corals an ecological advantage compared to other benthic organisms that allows them to rapidly colonize large areas of a reef. Combined with environmental disturbance, this could result in benthic community shifts from hard coral- to soft coral-dominated reefs, which have been observed at several reef locations worldwide (Tilot et al. 2008; Norstrom et al. 2009). In the northern Egyptian Red Sea, hard coral cover declined by 5 to 25% between 1996 and 2002, with a concurrent increase in zooxanthellate soft corals of the families Xeniidae, Nephteidae and Alcyoniidae (Tilot et al. 2008). Thus, as soft

corals in reefs of the northern Red Sea are becoming an increasingly dominant benthic functional group, they may contribute significantly to N_2 fixation, an important biogeochemical process within coral reef functioning. However, little is known about the capacity and contribution of soft corals to N_2 fixation within the reef and, furthermore, about the relationship of their N_2 fixation with photosynthesis and environmental factors.

Several environmental factors including light, temperature, oxygen (O_2) concentrations and nutrient availability can affect marine N_2 fixation (Sohm et al. 2011; Knapp 2012; Cardini et al. 2014). However, thus far the effects of environmental variables on N_2 fixation have mostly been studied for free-living diazotrophs, while N_2 fixation associated with living corals has received much less attention. Lesser et al. (2007) found that N_2 fixation activity in the scleractinian coral *Montastrea cavernosa* follows a diurnal pattern, with maximum rates during twilight, and Davey et al. (2008) reported no effect of seasonal water temperature changes (22 vs. 28 °C) on N_2 fixation associated with *Acropora aspera*. The coral reefs in the Gulf of Aqaba (northern Red Sea) represent a natural laboratory for studying the effect of seasonally changing environmental conditions on coral physiology. Their high-latitude location and the annual stratification cycle of the water column result in pronounced seasonal fluctuations in water temperature, light and inorganic nutrient availability (Silverman et al. 2007; Carlson et al. 2014).

In this study, we thus investigated N_2 fixation rates associated with 2 of the most dominant zooxanthellate soft corals, Xeniidae and *Sarcophyton* sp. (family: Alcyoniidae), from a fringing reef of the northern Gulf of Aqaba (Red Sea). Furthermore, responses of N_2 fixation, gross photosynthesis (P_{gross}) and dark respiration (R) rates (in terms of O_2 fluxes) to seasonally changing environmental conditions (e.g. inorganic nutrient concentrations, light intensity, water temperature) were studied over all 4 seasons within the year 2013 to identify how these 2 soft corals react to varying environmental conditions. Lastly, N_2 fixation was related to the P_{gross} and R rates of the corals to detect a potential linkage between diazotrophic activity and the physiology of the corals.

Materials and methods

Description of study site

This study was carried out during 2 expeditions (January to April and August to December 2013) to the Marine Science Station (MSS) at the northern Gulf of Aqaba, Jordan (29° 27′ N, 34° 58′ E). The MSS is situated at the Jordanian Red Sea coast approximately 10 km south of Aqaba city,

with access to a fringing coral reef inside a marine reserve. To study the effect of seasonally changing environmental conditions on soft coral physiology, all experiments described below were conducted once in each of the months to represent all 4 seasons during the year 2013: February (winter), April (spring), September (summer), and November (fall).

Soft coral distribution

Line point intercept (LPI) transects were carried out to determine relative soft coral cover in the study area. Three replicate 50 m LPI transects were conducted during each season at 1, 5, 10 and 20 m water depth. Benthic cover was recorded at 0.5 m intervals directly below the transect line (100 recorded data points per LPI transect). The relative abundance of soft corals was calculated as their percentage of benthic coverage.

Soft coral collection and maintenance

Individual colonies of the genus *Sarcophyton* sp. (n = 8, average polyp number = 459 ± 41) and the family Xeniidae (n = 8, average polyp number = 64 ± 4) were collected during each season from the reef slope at 10 m water depth using SCUBA. To prevent any tissue damage, all soft corals were collected along with a small piece of the anchoring rock (<0.5 cm diameter) to which they were attached using a hammer and chisel. Subsequently, individual coral colonies, with their attachment rock, were fixed onto ceramic tiles using a 2-part epoxy putty (Reef Construct, Aqua Medic). The putty was used to cover the anchoring rock to ensure that encrusting communities on the rock did not affect rate measurements during subsequent incubations. All corals were transferred to an outdoor 800 L flow-through aquarium supplied with seawater pumped directly from the reef at 10 m water depth (exchange rate: 4000 L h^{-1}), thereby providing in-situ water temperature and nutrient levels. Layers of netting were positioned above the tank to adjust light levels to those measured in-situ at 10 m water depth with data loggers (HOBO Pendant UA-002-64, spectral detection range: 150 to 1200 nm, temperature accuracy: ±0.53°C, Onset). The corals were allowed to acclimate for 1 wk before further experimentation. All incubations took place in the outdoor 800 L flow-through aquarium to ensure the same water temperature, nutrient and light conditions and to avoid any stress to the coral colonies.

Quantification of O_2 fluxes

The tiles with the attached corals were carefully cleaned of algal turf using a toothbrush. Each coral colony (n = 8) was transferred, without exposure to air, to individual 1 L glass chambers. In addition, 8 chambers filled only with seawater served as controls to measure planktonic background metabolism. The start O_2 concentration in each chamber was measured using a

salinity-corrected O_2 optode sensor (FDO®925 Optical Dissolved Oxygen Sensor, range: 0.00 to 20.00 mg O_2 l^{-1}, accuracy: ±0.5% of the value, MultiLine® IDS 3430, WTW). All chambers were sealed gas tight (without any air bubbles inside) and incubated twice with constant stirring (600 rpm) on magnetic stirring plates for 1 to 2 h, respectively (Cimarec^{TM} i Telesystem Multipoint Stirrers, Thermo Scientific). After that, each chamber was opened to measure end O_2 concentrations. The first incubation was carried out 1 to 2 h after sunset to measure R in complete darkness, while the second incubation started the following day between 12:00 and 13:00 h to determine net photosynthesis (P_{net}). To calculate O_2 fluxes (P_{net} and R) from dark and light incubations, O_2 start concentrations were subtracted from end concentrations, and the results were normalized by incubation time. Finally, O_2 fluxes were corrected for the seawater control signal, related to incubation volume and normalized to the coral surface area. P_{gross} rates were subsequently calculated according to $P_{gross} = P_{net} + R$. As P_{net} was measured during the highest daily irradiance levels, and dark R rates were shown to be significantly lower than light R rates for corals during active photosynthesis (Fabricius and Klumpp 1995; Al-Horani et al. 2003), the presented P_{gross} rates are conservative estimates of daily maximum gross O_2 production.

Quantification of N_2 fixation

N_2 fixation rates of the same soft coral colonies were measured 3 to 4 h after the light incubation for quantification of P_{net} ended. An adapted acetylene (C_2H_2) reduction assay (ARA) was applied, as it was recently confirmed to be applicable for N_2 fixation quantification in oligotrophic waters (Capone 1993; Wilson et al. 2012). C_2H_2 gas was freshly generated from calcium carbide and bubbled through seawater to produce C_2H_2-enriched seawater. Without air exposure, each coral colony (n = 8) was placed individually in a 1 L glass chamber containing 800 mL of unfiltered seawater and 200 mL of air headspace. Then, 10% of the seawater (80 mL) was replaced with C_2H_2-enriched seawater before the chambers were closed gas tight. Immediately after, 10% of the headspace (20 mL) was replaced by C_2H_2 gas. The addition of C_2H_2 to the seawater minimizes the lag phase of the ARA because of a faster equilibration of C_2H_2 between the gas and liquid phase and an immediate C_2H_2 saturation of the nitrogenase enzyme. In addition, 4 sets of controls were also tested for ethylene (C_2H_4) production from C_2H_2 reduction: (1) unfiltered seawater control (without coral fragments, n = 8); (2) 0.2 µm filtered seawater control (without coral fragments, n = 6); (3) tiles (without coral fragments) in unfiltered seawater (n = 6); and (4) coral fragments in unfiltered seawater without C_2H_2 addition (natural C_2H_4 production, n = 6). Over the whole incubation period (24 h), all chambers were constantly stirred (600 rpm), and gas samples were taken at the incubation start and after 4, 12, 16 and 24 h. At each of these time intervals, 1 mL of gas sample was collected with a gastight syringe from each chamber, transferred into gastight 2 mL vials previously filled with distilled water and stored frozen and upside down until analysis.

C_2H_4 concentrations in the gas samples were measured in the field laboratory using a customized reducing compound photometer (RCP) (Peak Laboratories) with a detection limit of 100 ppb. The higher sensitivity of the RCP compared to the commonly used gas chromatograph equipped with a flame ionization detector allows best accurate estimations of C_2H_4 reduction rates. Calibration of the RCP was conducted using serial dilutions of a 200 ± 4 ppm C_2H_4 standard in air (Restek). To calculate C_2H_4 production rates of the coral fragments, C_2H_4 signals of the biological samples were corrected for seawater control signals (blank/biological ratios: 0.15 to 0.57) and subsequently normalized to incubation time and coral surface area. All rates are reported as nmol C_2H_4 cm^{-2} h^{-1}, since no parallel ^{15}N incubations were conducted, and the use of a theoretical conversion factor is controversial, as the ^{15}N method may have largely underestimated N_2 fixation until recently (Mohr et al. 2010). Additionally, it allows best comparability with the current literature, as the C_2H_2 reduction method has been most widely applied for benthic N_2 fixation quantification measurements in coral reefs (see Table 3-6).

Surface area determination of soft corals

Activity rates (N_2 fixation, P_{gross} and R) for each colony were related to coral surface area that was quantified using an advanced geometry approach (Naumann et al. 2009). This approach has already been applied for soft corals by Bednarz et al. (2012) and has been commonly used in physiological coral reef studies, thereby allowing best comparability of the present data to previous studies. Briefly, the number of polyps of each coral colony was counted, and each of these polyps was mathematically handled as the area of a circle. The radius of 50 completely expanded polyps randomly distributed over all colonies was measured separately for Xeniidae and *Sarcophyton* sp. using the image analysis software ImageJ (National Institutes of Health, USA). Subsequently, the average circular surface area of a polyp was calculated ($r^2 \times \pi$, where r = radius) and multiplied by the number of polyps per colony. In addition, the surface area of the body foot of each colony was approximated to a cylinder ($2 \times \pi \times r \times h$, where h = height) by measuring its diameter ($2 \times r$) and height using a caliper (accuracy ±0.01 cm). To generate the total surface area of each incubated Xeniidae and *Sarcophyton* sp. colony, the total number of polyps of each colony was multiplied with the average surface area of an expanded polyp and subsequently added to the surface area of the body foot.

Monitoring of environmental parameters

During each season, water temperature and light intensity (lux) at 10 m water depth were continuously recorded over 4 wk by data loggers (HOBO Pendant UA-002-64, temperature accuracy: ± 0.53°C, spectral detection range: 150 to 1200 nm, Onset). The presented light

intensities were standardized to the time of day with maximum light intensities (11:00 to 13:00 h), and lux readings were converted to photosynthetically active radiation (PAR, µmol quanta m^{-2} s^{-1}, wavelength 400 to 700 nm) using the following approximation: 1 µmol quanta m^{-2} s^{-1} = 52.0 lux. This conversion factor was obtained by intercalibrating the lux readings with data obtained from a PAR sensor (LI-COR LI-192SA underwater quantum sensor) during a simultaneous minute-by-minute measurement over 5 h. Both readings correlated well (r^2 = 0.83), and the obtained conversion factor of 52.0 was very similar to 51.2 reported by Valiela (1984). Once a week, seawater samples (50 mL, n = 4) were taken from the place of coral collection. After filtering the seawater through sample-washed cellulose acetate membrane filters (nominal pore size: 0.45 µm), inorganic nutrient (ammonium, phosphate) concentrations were immediately measured following methods described by Murphy and Riley (1962) and Holmes et al. (1999). Ammonium was determined fluorimetrically using a Trilogy fluorometer (Turner Designs) with a detection limit of 0.09 µM, while phosphate was measured photometrically with a JASCO-V630 spectrophotometer and a detection limit of 0.01 µM.

Statistical analysis

All statistical analyses were carried out using PRIMER-E version 6 software (Clarke and Gorley 2006) with the PERMANOVA+ add-on (Anderson 2001). Analyses were based on Euclidean distance of environmental data (normalized) and Bray-Curtis similarities of physiological parameters (square root transformed). A principal coordinate analysis and a 1-factor PERMANOVA with type III (partial) sum of squares and unrestricted permutation of raw data (999 permutations) was used to test for seasonal differences of the environmental variables (water temperature, light intensity, inorganic nutrients; co-linear variables ammonium and phosphate are summarized). Two-factor PERMANOVAs were performed to test for differences of physiological parameters (N_2 fixation, P_{gross}, R) between soft corals and seasons. Therefore, type I (sequential) sum of squares was used with permutation of residuals under a reduced model (999 permutations), and pairwise tests were carried out when significant differences occurred. In addition, a nonmetric multidimensional scaling ordination of the physiological parameters (N_2 fixation, P_{gross} and R) in relation to soft coral and season was conducted. Finally, correlations between the physiological variables as well as between the physiological variables and the environmental factors were determined via linear regression.

Results

Environmental conditions and soft coral cover

The 4 seasons differed significantly from each other with respect to the environmental water parameters (PERMANOVA, df = 3, SS = 51.138, MS = 17.046, pseudo-*F* = 23.083, p = 0.001). The winter and spring season were most similar to each other, followed by fall, with lower inorganic nutrient concentrations and higher water temperatures. The summer season was most distinct from the other 3 seasons and exhibited the highest water temperatures and light intensities but the lowest inorganic nutrient concentrations (Fig. 3-1, Table 3-1).

Table 3-1. Summary of environmental water parameters monitored over 4 wks during each of the 4 different seasons at 10 m water depth. During each season, water temperature was continuously recorded and is averaged over the 4 wk period, while light intensity represents maximum values measured daily between 11:00 and 13:00 h. Ammonium and phosphate concentrations were measured once a week from seawater samples (n = 4) taken in the early morning. Values are means, with SE in parentheses. PAR: photosynthetically active radiation (µmol quanta m^{-2} s^{-1})

Month (season)	Temperature (°C)	Light intensity (PAR)	Ammonium (µM)	Phosphate (µM)
Jan/Feb (winter)	22.97 (0.11)	180 (15)	0.32 (0.04)	0.11 (0.01)
Mar/Apr (spring)	22.78 (0.10)	257 (9)	0.46 (0.03)	0.10 (0.01)
Sep (summer)	27.52 (0.16)	317 (17)	0.14 (0.03)	0.04 (0.01)
Nov (fall)	25.19 (0.17)	159 (18)	0.28 (0.06)	0.04 (0.01)

Despite these strong seasonal changes in environmental water parameters, the soft coral cover remained constant throughout the year at each water depth (Table 3-2). Over all depths, soft coral cover was approximately 8.6 ± 0.8%, whereby the highest cover was recorded at 10 m water depth with a percentage cover of 21.6 ± 1.3%. Most of the soft coral cover was represented by Xeniidae that was approximately 10 times more abundant than *Sarcophyton* sp.

Table 3-2. Benthic soft coral cover in different water depths and relative contribution by *Sarcophyton* sp., Xeniidae and other soft corals. Values are annual means (n = 12), with SE in parentheses

	Benthic soft	Soft coral composition (%)		
Depth (m)	coral cover (%)	*Sarcophyton* sp.	Xeniidae	Others
1	3.2 (0.6)	10.9 (8.7)	2.3 (2.2)	86.8 (8.7)
5	3.0 (0.7)	3.2 (1.9)	57.4 (12.1)	45.4 (11.9)
10	21.6 (1.3)	5.4 (2.3)	91.0 (2.2)	3.5 (1.3)
20	6.6 (0.6)	6.7 (2.7	88.8 (3.5)	4.5 (2.3)
Average	8.6 (0.8)	6.6 (3.9)	59.9 (5.0)	35.1 (6.1)

N_2 fixation and O_2 fluxes

Active N_2 fixation rates occurred throughout the year for both soft corals, indicated by the up to 75-fold higher C_2H_4 production in coral-containing incubation chambers compared to the seawater controls (Table 3-3). Soft coral-associated N_2 fixation revealed significant effects of soft coral, season and their interaction (Fig. 3-2, Table 3-4). The corals exhibited similar rates during the winter season, but *Sarcophyton* sp. showed significantly higher rates in the other 3 seasons compared to Xeniidae. Rates ranged during the year from 0.004 to 0.205 nmol C_2H_4 cm^{-2} h^{-1} for *Sarcophyton* sp. and from 0.001 to 0.096 nmol C_2H_4 cm^{-2} h^{-1} for Xeniidae, with annual averages of 0.055 ± 0.011 nmol C_2H_4 cm^{-2} h^{-1} and 0.019 ± 0.005 nmol N_2 cm^{-2} h^{-1}, respectively. Besides these soft coral-specific differences, both soft corals showed the same seasonal pattern of N_2 fixation activity. While no differences occurred between winter, spring and fall, N_2 fixation rates significantly increased during the summer season for both investigated soft corals. Overall, summer rates were 3 to 6 (*Sarcophyton* sp.) and 6 to 14 times (Xeniidae) higher than N_2 fixation during the other seasons.

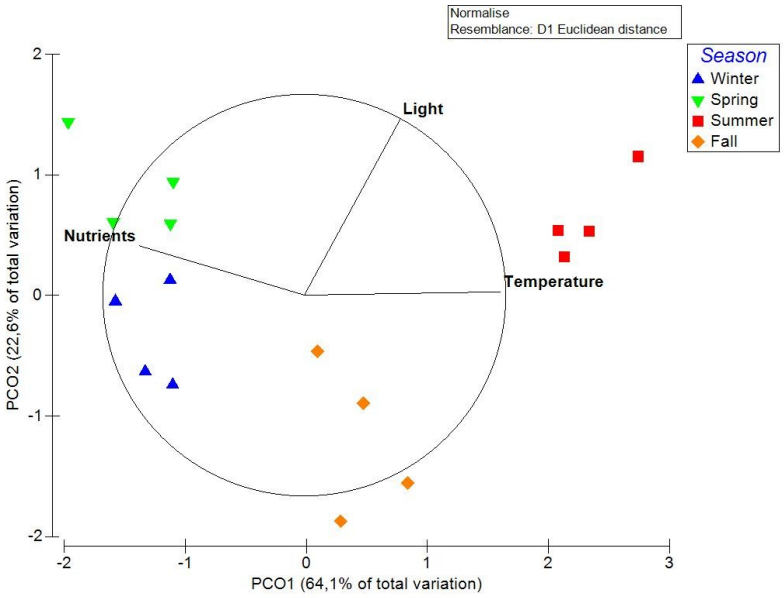

Fig. 3-1. Principal coordinate analysis performed on Euclidean distance matrix and normalized data for the different environmental variables (temperature, light, inorganic nutrients) measured in seawater samples from 10 m water depth once a week during 4 different seasons (winter, spring, summer, fall). PCO1: first principal coordinate; PCO2: second principal coordinate

O_2 fluxes (P_{gross} and R) also exhibited soft coral-specific and seasonal differences (Fig. 3-2, Table 3-4). Averaged among all seasons, Xeniidae (15.6 ± 0.8 µg O_2 cm^{-2} h^{-1}) revealed significantly higher P_{gross} rates, approximately 1.5-fold higher than *Sarcophyton* sp. (11.7 ± 0.8 µg O_2 cm^{-2} h^{-1}). On a seasonal scale, Xeniidae displayed the highest P_{gross} rates during spring, while

Sarcophyton sp. had maximum rates during both spring and summer. In contrast, R rates were constantly lower in Xeniidae (2.8 ± 0.2 µg O_2 cm^{-2} h^{-1}) compared to *Sarcophyton* sp. (4.9 ± 0.3 µg O_2 cm^{-2} h^{-1}) averaged among all seasons. Xeniidae exhibited highest R rates in both spring and summer, while R rates of *Sarcophyton* sp. peaked during the summer season, thereby following the seasonal pattern of N_2 fixation rates.

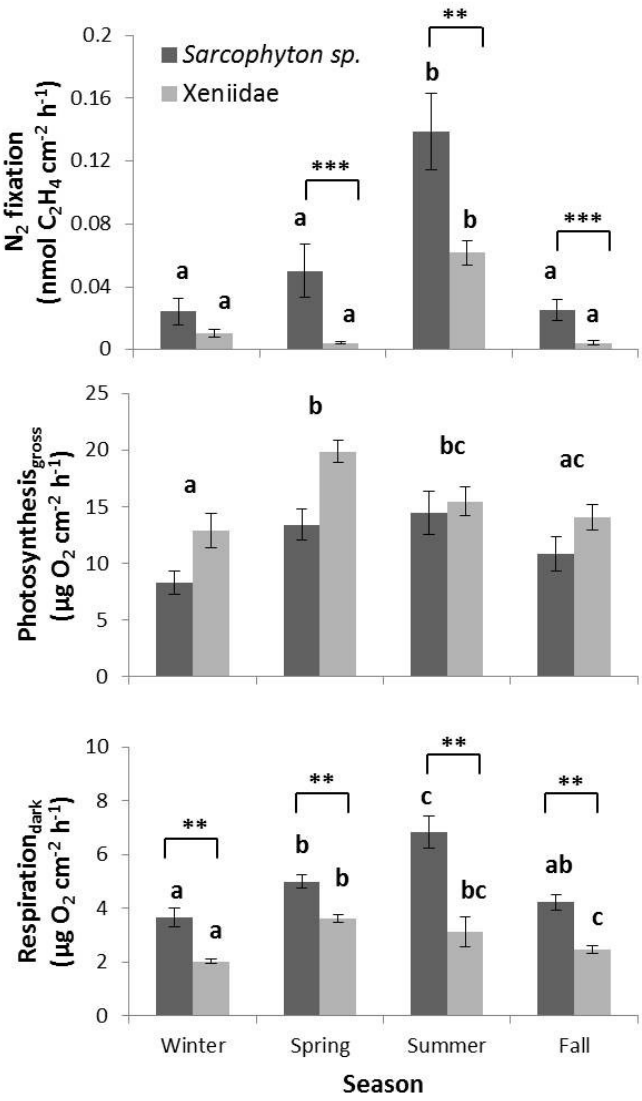

Fig. 3-2. Rates of dinitrogen (N_2) fixation, gross photosynthesis (P_{gross}) and dark respiration (R) for *Sarcophyton* sp. and Xeniidae measured during 4 different seasons (winter, spring, summer, fall) in 2013. Values are given as mean ± SE (n = 8). Asterisks indicate significant differences between the 2 soft corals during each season (**p < 0.005, ***p < 0.001); different letters indicate significant differences between the seasons for *Sarcophyton* sp. and Xeniidae, respectively, based on pair-wise PERMANOVA analysis

Table 3-3. Dinitrogen (N_2) fixation (nmol C_2H_4 l^{-1} h^{-1}) measured in soft coral and seawater control (without corals) incubation chambers. Values are normalized to 1 l incubation water and presented as means of n = 8 replicates, with SE in parentheses

Incubation	Winter	Spring	Summer	Fall
Sarcophyton sp.	2.07 (0.83)	4.05 (0.92)	14.26 (1.77)	3.57 (1.47)
Xeniidae	1.20 (0.25)	0.68 (0.08)	2.13 (0.57)	0.61 (0.12)
Seawater control	0.06 (0.04)	0.36 (0.05)	0.19 (0.06)	0.15 (0.08)

Table 3-4. Results of 2-factorial PERMANOVAs for dinitrogen (N_2) fixation (nmol C_2H_4 cm^{-2} h^{-1}), gross photosynthesis (P_{gross}; µg O_2 cm^{-2} h^{-1}) and dark respiration (R; µg O_2 cm^{-2} h^{-1}) rates for 2 soft corals (*Sarcophyton* sp. and Xeniidae) and 4 different seasons (winter, spring, summer, fall). Soft coral (Sc) and season (Se) were fixed effects. PERMANOVA was based on Bray-Curtis similarity after square root transformation. Type 1 (sequential) sum of squares was used with permutation of residuals under a reduced model (999 permutations). Significant p-values are in **bold**

Variable	Effect	df	SS	MS	Pseudo-F	p
N_2 fixation	Sc	1	10144	10144	31.34	**0.001**
	Se	3	19555	6519	20.14	**0.001**
	Sc × Se	3	5208	1736	5.36	**0.001**
P_{gross}	Sc	1	918	918	16.66	**0.002**
	Se	3	1350	450	8.17	**0.001**
	Sc × Se	3	129	43	0.78	0.513
R	Sc	1	2534	2534	77.52	**0.001**
	Se	3	1503	501	15.33	**0.001**
	Sc × Se	3	332	111	3.38	**0.032**

Table 3-5. Linear regression analysis (r^2 values) between dinitrogen (N_2) fixation (nmol C_2H_4 cm^{-2} h^{-1}), gross photosynthesis (P_{gross}; µg O_2 cm^{-2} h^{-1}) and dark respiration (R; µg O_2 cm^{-2} h^{-1}) rates of 2 soft corals (*Sarcophyton* sp. and Xeniidae) and 4 different environmental water parameters (ammonium concentration, phosphate concentration, water temperature and light intensity). **Bold** characters indicate significant positive relationships, and *italicized* characters indicate significant negative relationships (*p < 0.05, **p < 0.005, ***p < 0.001)

	Ammonium	Phosphate	Water temp.	Light intensity
Sarcophyton sp.				
N_2 fixation	*0.217**	*0.137**	**0.344*****	**0.478*****
P_{gross}	0.001	0.047	0.060	**0.180***
R	*0.142**	*0.158**	**0.326****	
Xeniidae				
N_2 fixation	*0.561*****	*0.222****	**0.592*****	**0.513*****
P_{gross}	**0.144***	0.022	0.021	0.130
R	0.040	0.000	0.002	

Relationships between N_2 fixation, O_2 fluxes and environmental factors

N_2 fixation and O_2 fluxes (P_{gross} and R) clearly showed a separation between the 2 soft corals as well as a distinct difference in summer compared to the other 3 seasons (Fig. 3-3). Separation of summer from the other seasons was mainly driven by changes in N_2 fixation and R rather than by changes in P_{gross}, suggesting a potential linkage between N_2 fixation and R. Indeed, linear regression analysis revealed a significant positive relationship between R and N_2 fixation for *Sarcophyton* sp. ($F = 16.070$, $r^2 = 0.373$, $p < 0.001$) but not for Xeniidae ($F = 1.249$, $r^2 = 0.043$, $p = 0.273$). In contrast, no significant relationship between P_{gross} and N_2 fixation was found, neither for *Sarcophyton* sp. ($F = 2.784$, $r^2 = 0.093$, $p = 0.107$) nor for Xeniidae ($F = 0.047$, $r^2 = 0.002$, $p = 0.8297$).

N_2 fixation of both soft corals showed similar relationships with each water parameter, with negative correlations to ammonium and phosphate concentrations and positive correlations to water temperature and light intensity (Table 3-5). R rates of *Sarcophyton* sp. showed the same correlation to the water parameters as N_2 fixation. In contrast, no correlations between R rates of Xeniidae and any water parameters were found. P_{gross} of Xeniidae was positively correlated to ammonium concentration, while P_{gross} of *Sarcophyton* sp. was positively correlated to light intensity.

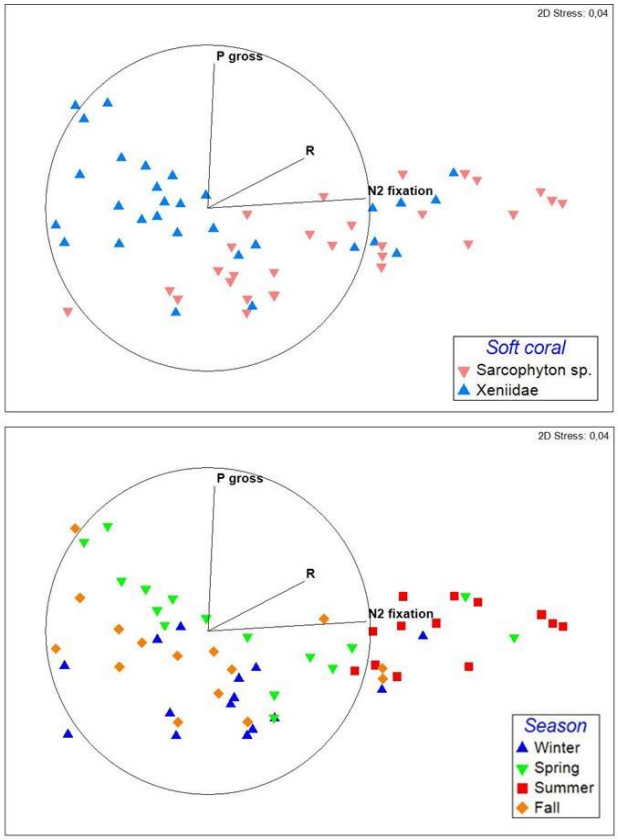

Fig. 3-3. Multidimensional scaling plot of dinitrogen (N_2) fixation, gross photosynthesis (P_{gross}) and dark respiration (R) rates for 2 soft corals (*Sarcophyton* sp. and Xeniidae) and 4 different seasons (winter, spring, summer, fall). Analysis was performed on Bray-Curtis similarities of square root-transformed data

Discussion

Soft coral-specific N_2 fixation and O_2 fluxes

This study for the first time identified N_2 fixation rates associated with 2 dominant zooxanthellate soft corals from an oligotrophic, subtropical coral reef environment. Previous studies, mainly focused on hard corals, identified their different hard coral-associated N_2-fixing bacteria (Rohwer et al. 2002; Wegley et al. 2007; Olson et al. 2009; Lema et al. 2012), or quantified their N_2 fixation rates (Shashar et al. 1994a; Lesser et al. 2007; Davey et al. 2008). These rates are 1 to 2 orders of magnitude higher than the rates detected for *Sarcophyton* sp. and Xeniidae in the present study (Table 3-6). These apparent differences in N_2 fixation rates may be due to abundance and composition differences of the diazotrophic communities associated with hard and soft corals. Lema et al. (2012) demonstrated that hard coral species form specific associations with diazotrophs which may further result in species-specific N_2-fixing activities. Likely, hard and soft corals may harbor a very distinct diazotrophic community because of the presence of endolithic algae and endolithic bacteria in the former. Endolithic, heterotrophic bacteria are most likely responsible for the majority of N_2 fixation activity in scleractinian corals (Shashar et al. 1994a), which was supported by measurements of endolithic ammonium excretion rates (0.8 to 1.4 nmol NH_4^+ cm^{-2} h^{-1}) that matched the rates of N_2 fixation (Ferrer and Szmant 1988). The main energy source for N_2 fixation is likely provided via the excretion of organic photosynthates by the coral into the coralline skeleton, thereby establishing a suitable microhabitat for a diazotrophic community that is absent in soft corals. Therefore, soft corals may harbor fewer diazotrophs compared to scleractinian corals that may explain their lower N_2 fixation rates.

So far, N_2 fixation has only been investigated for 2 octocoral species (*Tubipora musica* and *Rhytisma fulvum*) using the ARA (Shashar et al. 1994b). They measured rates of 35.7 ± 14.2 nmol C_2H_4 cm^{-2} h^{-1}, thus exceeding rates quantified during the present study for *Sarcophyton* sp. and Xeniidae by 2 to 3 orders of magnitude. The octocoral species *T. musica* contains an endoskeleton-providing habitat for endolithic diazotrophs comparable to scleractinian corals, while *R. fulvum* is an azooxanthellate, encrusting soft coral species. Because of its encrusting morphology, *R. fulvum* is extremely difficult to separate from its attaching substrate, and Shashar et al. (1994b) did not remove epilithic algae from the soft corals. This may result in biased N_2 fixation rates, as the activity of diazotrophs which are not associated with the coral is also taken into account. Shashar et al. (1994b) also present N_2 fixation rates of other unidentified cnidarians and sponges, which are similar to the rates from the present study. Overall, this demonstrates that there is a wide range of N_2 fixation activity among different benthic coral reef organisms.

Table 3-6. Reported dinitrogen (N_2) fixation rates (nmol C_2H_4 cm^{-2} h^{-1}) for benthic reef organisms and substrates in comparison with annual averages of the soft corals *Sarcophyton* sp. and Xeniidae from the present study, GBR = Great Barrier Reef.

Organism/Substrate	N_2 fixation	Location	Reference
Octocorals			
Xeniidae	0.001-0.096	Aqaba, Red Sea	This study
Sarcophyton sp.	0.004-0.205	Aqaba, Red Sea	This study
T. musica and *R. fulvum*	35.7 ± 14.2	Eilat, Red Sea	Shashar et al. (1994b)
Scleractinian corals			
Acropora aspera	0.56-1.16	Australia, GBR	Davey et al. (2008)
Acropora sp.	8.7 ± 7.3	Eilat, Red Sea	Shashar et al. (1994a)
Stylophora pistillata	6.4 ± 2.4	Eilat, Red Sea	Shashar et al. (1994a)
Pocillopora damicornis	0.6 ± 0.4	Eilat, Red Sea	Shashar et al. (1994a)
Other cnidarians	0.1 ± 0.3	Eilat, Red Sea	Shashar et al. (1994b)
Sponges	0.2 ± 0.4	Eilat, Red Sea	Shashar et al. (1994b)
Dead coral skeleton	55.45 ± 28.5	Eilat, Red Sea	Shashar et al. (1994b)
Dead coral skeleton	0.15-12.77	Australia, GBR	Davey et al. (2008)
Algal substrate	9.25 ± 0.5	Eilat, Red Sea	Shashar et al. (1994b)
Microbial mats	0.59-2.97	Indian Ocean	Charpy et al. (2012)
Carbonate sand	0.18 ± 1.02	French Polynesia	Charpy-Roubaud et al. (2001)
Carbonate sand	19.5 ± 17.5	Eilat, Red Sea	Shashar et al. (1994b)

Although N_2 fixation rates associated with *Sarcophyton* sp. and Xeniidae are in the lower range among benthic reef organisms, the rates are up to 75-fold higher compared to N_2 fixation in the seawater controls, thus confirming that the 2 soft corals are associated with active N_2-fixing bacteria. N_2 fixation in the seawater controls (0.8 to 4.3 nmol N l^{-1} d^{-1}, obtained using 4:1 as the C_2H_4:N_2 conversion factor) is slightly higher but within the range of seawater N_2 fixation previously measured in the Gulf of Aqaba using the $^{15}N_2$ method (0.1 to 1.9 nmol N l^{-1} d^{-1}; Foster et al. 2009). The classical $^{15}N_2$ method underestimates N_2 fixation rates (Mohr et al. 2010) when the $^{15}N_2$ tracer is only introduced as a gas bubble, as in Foster et al. (2009), while the addition of $^{15}N_2$-enriched seawater accelerates the gas equilibration process and improves the accuracy of the method (Wilson et al. 2012). Similarly, the ARA can reliably quantify N_2 fixation in oligotrophic waters if C_2H_2-enriched seawater is used, as in the present study (Wilson et al. 2012). These methodological differences provide reasoning for the slightly higher rates obtained in our seawater controls and confirm the validity of the soft coral-associated N_2 fixation rates.

The two zooxanthellate soft corals investigated in the present study also revealed differences in their specific N_2 fixation activity, with significantly higher rates in *Sarcophyton* sp. compared to Xeniidae on an annual average. Lower N_2 fixation rates in Xeniidae may be caused by highly oxygenated areas due to higher P_{gross} and lower R rates, which indicate a more autotrophic nutrition by Xeniidae compared to *Sarcophyton* sp. This is confirmed by (Schlichter

et al. 1983), who characterized soft corals of the family Xeniidae as functionally autotrophic plant animals. Also, compared to *Sarcophyton* sp., Xeniidae exhibit nonretractile, pumping polyps, thereby creating conditions which facilitate photosynthesis. Pumping increases water exchange between the boundary layer of the organism and the water body (Mass et al. 2010; Kremien et al. 2013), while polyp expansion increases the surface area for potential gas exchange through the epidermal tissue (Fabricius and Klumpp 1995). Furthermore, the polyp's tip hosts most of the organism's zooxanthellae and represents the photosynthetically most active tissue. Polyp retraction reduces light exposure for zooxanthellae and can decrease photosynthesis in several soft corals (Fabricius and Klumpp 1995). This may help explain the higher photosynthesis rates measured in Xeniidae during each season, which likely lead to hyperoxic conditions in the tissue during daytime (Shashar et al. 1993; Kuhl et al. 1995). As the nitrogenase enzyme is highly sensitive to O_2 (Postgate 1982), it may explain lower N_2 fixation rates in Xeniidae compared to *Sarcophyton* sp.

Seasonal variation in N_2 fixation and O_2 fluxes

In the present study, we measured for the first time the effect of seasonally changing environmental conditions on N_2 fixation associated with soft corals. Both of the 2 investigated soft corals showed the highest N_2 fixation rates during summer, when ammonium availability in the ambient seawater was low, but water temperature and irradiance were high. This is supported by the linear regression analysis revealing for N_2 fixation a significant negative relationship to inorganic nutrients and positive correlations to water temperature and light intensity (Table 3-5). This seasonal pattern is in accordance with 2 recent studies on pelagic diazotrophs in the Gulf of Aqaba (Foster et al. 2009; Rahav et al. 2013). Both measured up to 6 times higher N_2 fixation rates in the photic water layer during the stratified summer and early fall months compared to the well-mixed conditions in winter and spring. High water temperature can stimulate the enzymatic activity of nitrogenase (Capone et al. 1997; Capone et al. 2005), while high ammonium concentrations in the water can clearly inhibit N_2 fixation, as demonstrated in laboratory studies on diazotroph cultures (reviewed in Sohm et al. 2011). Roughly 25% more energy is required to reduce N_2 (87 kcal) than NO_3^- (69 kcal) to ammonium; therefore, it is energetically inefficient to fix N_2 in marine environments with DIN concentrations above a certain threshold (~1 μM). High irradiance can inhibit the process of N_2 fixation indirectly because of enhanced photosynthetic O_2 production. However, P_{gross} of the 2 investigated soft corals showed only a slight increase during summer, which started already during spring, when inorganic nutrient availability in the water column was highest. Thus, the strong increase in N_2 fixation during summer is most likely caused by a combination of these different environmental factors but may be more strongly influenced by

the direct effects of high water temperature and low nutrient availability than by the indirect effects of light.

Corals have developed several seasonal adaptations to protect the photosystem from harmful photons during the summer months. This includes, for example, the down-regulation of photosynthesis by the zooxanthellae (Warner et al. 2002), the decrease of zooxanthellae abundance (Fitt et al. 2000) or the enhanced production of photoprotecting mycosporine-like amino acids (MAAs) (Michalek-Wagner 2001). The present study thus suggests that the high N_2 fixation during summer may provide soft corals with the N compounds needed to produce MAAs during the brightest periods of the year or to regain their zooxanthellae density during the following recovery phase.

In both soft corals, R revealed a trend similar to N_2 fixation, with maximum rates during summer. This is also supported by the positive relationship between N_2 fixation and R rates, suggesting linkage between both processes. During summer, the respiratory metabolism of corals generally increases as they build up biomass for reproduction (Shlesinger et al. 1998; Fitt et al. 2000). For example, in the soft coral *Heteroxenia fuscescens* (Xeniidae) from the northern Red Sea, the biochemical tissue composition changed over the year, with the highest energy content during summer, followed by spring, fall and finally winter (Ben-David-Zaslow and Benayahu 1999). This seasonal pattern reflects the high coral fecundity and reproduction in summer (Ben-David-Zaslow et al. 1999). Thus, reproduction during summer may be supported by the increased availability, and use, of N_2 fixation products. Given the low DIN availability and the low N_2 fixation activity in the water column of the Gulf of Aqaba (Foster et al. 2009; Rahav et al. 2013), as well as the importance of N for cell maintenance, growth and functioning, the association with N_2-fixing bacteria may be a key component of soft coral nutrition during summer.

Ecological implications

Corals profit from the association with several symbionts including N_2-fixing bacteria. Although the present study could not determine whether the diazotrophs are internally (i.e. as endosymbionts in the coral tissue; Lesser et al. 2004) or externally associated with the coral (i.e. in the mucus layer; Lema et al. 2012), both associations are likely to benefit the coral holobiont by providing bioavailable N. The zooxanthellae may also internally harbor diazotrophs, thereby directly receiving fixed N, similar to what has been shown for a different diazotrophs–eukaryotic algae symbiosis (Foster et al. 2011). Overall, the ability of corals to acquire N both via diazotrophy and via uptake of DIN from the surrounding seawater is advantageous in an environment where the availability of dissolved nutrients is generally low and episodic. Recently,

enzymes enabling ammonium assimilation were detected in endosymbiotic algae and the coral host, suggesting that both could benefit from the products of N_2 fixation (Leggat et al. 2007; Yellowlees et al. 2008; Stambler 2011). The present study suggests that uptake of DIN from the seawater may fuel coral metabolism during the nutrient-enriched spring period, while N_2 fixation products may be a key component of coral nutrition during the nutrient-depleted summer months.

Given the usually low rates of N_2 fixation in the water column of nutrient-poor coral reefs (Foster et al. 2009; Rahav et al. 2013), benthic reef organisms, including soft corals, may provide habitat for diazotrophs, thereby playing a key role for the input of new N into the reef ecosystem. In many reef locations worldwide, soft corals represent the second most dominant benthic group after scleractinian corals (Benayahu and Loya 1977; Fabricius and De'ath 2001; Inoue et al. 2013), and in the investigated study area, soft coral cover has increased by 50% since 2007 (C. Wild unpublished data), now reaching up to 21.6% of the total benthic cover. Therefore, the present study suggests that soft corals may contribute importantly to the overall input of fixed N within the reef, although N_2 fixation rates per unit surface area are low compared to scleractinian corals. Soft corals also represent major space competitors for hard corals, and shifts from hard to soft coral dominance has been observed in several reef locations worldwide (Tilot et al. 2008; Norstrom et al. 2009). The data in the present manuscript thus indicate that input of new N via N_2 fixation may be reduced in soft coral reefs compared to those dominated by hard corals, with potential implications on biogeochemical element cycles and reef ecosystem functioning.

Acknowledgments

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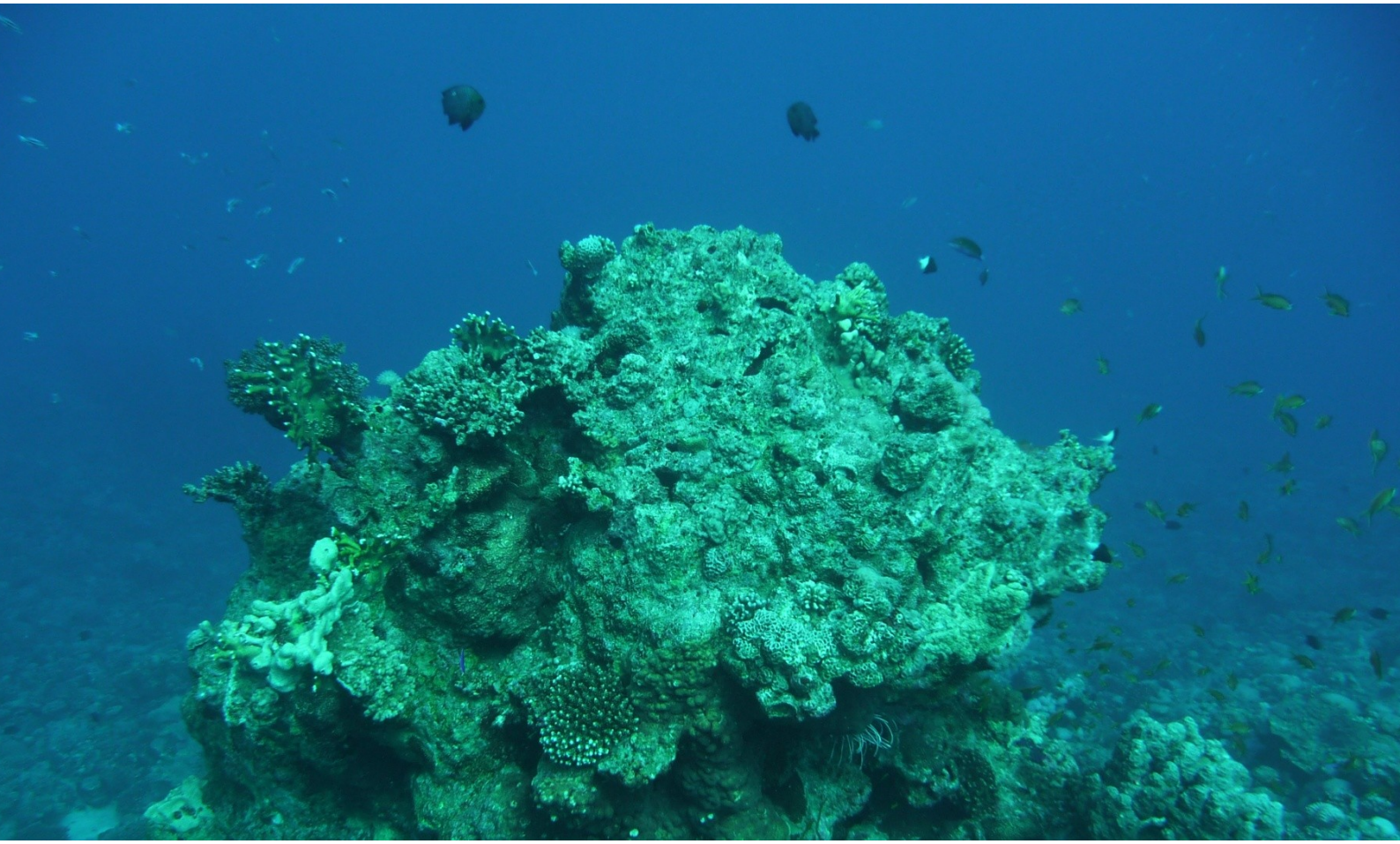
Chapter 4
Seasonal photosynthetic primary production and microbial dinitrogen fixation by coral reef framework substrate communities



Abstract

N_2 fixation by coral reef benthic substrates may support primary productivity on oligotrophic coral reefs. However, little is known regarding the influence of environmental parameters on coral reef benthic N_2 fixation. This study quantified N_2 fixation and photosynthesis by three abundant reef framework substrates: turf algae, coral rock, and the abundant sponge *Mycale fistulifera*, over four seasons in the northern Gulf of Aqaba. N_2 fixation activity was detected during day and night for all substrates, but on an annual average was significantly higher for turf algae (4.4 ± 3.9 nmol C_2H_4 cm^{-2} h^{-1}) and coral rock (3.5 ± 2.8 nmol C_2H_4 cm^{-2} h^{-1}) compared to *M. fistulifera* (0.2 ± 0.2 nmol C_2H_4 cm^{-2} h^{-1}). There was strong seasonality in N_2 fixation, with rates one order of magnitude higher in summer when temperature and irradiance were highest but inorganic nutrient concentrations lowest. During summer and fall when nutrients were low we found a significant positive linear relationship between gross photosynthesis (P_{gross}) and N_2 fixation in turf algae and coral rock. Further, we estimate N_2 fixation can supply up to 20 and 27% of the N demand for net photosynthesis (P_{net}) in coral rock and turf algae, respectively. By contrast there was no significant relationship between N_2 fixation and photosynthesis in *M. fistulifera,* which displayed negative P_{net} and heterotrophic metabolism (P_{gross}:Respiration < 1). These findings highlight the role of environmental parameters in regulating benthic substrate-associated N_2 fixation and the potential importance of fixed N in supporting primary production, particularly during nutrient-depleted conditions.

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Chapter 4 Framework primary production and N_2 fixation

Introduction

Coral reefs are characterized by high productivity but are typically surrounded by oligotrophic waters (Odum and Odum 1955; Hatcher 1988), where nitrogen (N) is a key limiting nutrient for growth (Delgado and Lapointe 1994; Eyre et al. 2008). Efficient internal nutrient recycling in the benthos contributes to this high productivity, but input of new N is essential to sustain net ecosystem production and growth. Though energetically costly, numerous studies demonstrate that dinitrogen (N_2) fixation represents a substantial source of new N on coral reefs (Webb et al. 1975; Larkum et al. 1988; O'Neil and Capone 1989; Charpy et al. 2007).

Biological N_2 fixation is carried out by a diverse group of heterotrophic and photoautotrophic bacteria (Zehr et al. 2003), but cyanobacteria are a key contributor to benthic N_2 fixation on coral reefs (Casareto et al. 2008; Charpy et al. 2012). Cyanobacterial mats have attracted much research focus due to their high N_2 fixation rates (e.g. Charpy et al. 2007; Diez et al. 2007; Bauer et al. 2008; Casareto et al. 2008). However, cyanobacteria are also important components of the various reef framework substrates that are ubiquitous on coral reefs; including algal turfs, endolithic algal communities associated with calcium carbonate structures, and endosymbiotic communities of sponges (Charpy et al. 2012). In coral reefs in the northern Gulf of Aqaba, these reef framework substrates are a dominant component of the benthos. Turf algae make up 72% of the benthic community on reefs in Eilat (Israel), while on the Jordanian side of the Gulf, biogenic reef framework with only sparse epilithic overgrowth (hereafter: coral rock) can account for up to 58% of the benthic cover (Bahartan et al. 2010). Sponges meanwhile dominate the cryptic reef habitat (Richter et al. 2001). High rates of N_2 fixation have been measured in both turf algae and coral rock (eg. Larkum et al. 1988; Williams and Carpenter 1998), therefore these reef framework substrates may contribute importantly to fixed N on reefs in the Gulf of Aqaba. Evidence for active N_2 fixation in sponges is scarce (Wilkinson and Fay 1979; Shashar et al. 1994b; Shieh and Lin 1994), but many species harbor microbial symbionts capable of fixing N (Taylor et al. 2007; Mohamed et al. 2008) suggesting N_2 fixation in sponges may be widespread.

Due to their association with photosynthetic cyanobacteria and algae, reef framework substrates also contribute to reef photoautotrophic primary production. Turf algae are dominant primary producers on many reefs (Adey and Goertemiller 1987; Carpenter and Williams 2007) and more than one third of sponges in the Caribbean, Great Barrier Reef (GBR) and West Indian Ocean harbor photosynthetic symbionts (Wilkinson 1987; Steindler et al. 2002; Erwin and Thacker 2007). Due to the oxygen (O_2) sensitivity of nitrogenase, the enzyme responsible for N_2 fixation, photosynthesizing diazotrophs have evolved strategies to allow photosynthesis and N_2

fixation to co-occur (Berman-Frank et al. 2003). Spatial separation in heterocystous cyanobacteria allows the fixation of N_2 during the day (Gallon 2001), while non-heterocystous cyanobacteria typically fix N_2 at night, relying on energy derived from the carbon (C) fixed during the previous daylight period (Bergman et al. 1997; Charpy et al. 2007). In marine sponges, hypoxic zones may facilitate O_2-sensitive processes such as N_2 fixation (Hoffmann et al. 2005). N_2 fixation may provide an additional source of N to support benthic primary production, however, little is known regarding the interaction between N_2 fixation and photosynthesis in benthic substrates, and few studies have quantified both processes in parallel (Williams and Carpenter 1997; Charpy et al. 2007; Lesser et al. 2007; Casareto et al. 2008).

Fringing reefs in the Gulf of Aqaba experience strong seasonal variation in key environmental parameters as a result of the annual stratification cycle in the water column that occurs due to their northern latitude. Winter and early spring are characterized by low temperature and irradiance but high inorganic nutrient concentrations as deep convective mixing of the water column transports nutrient-enriched deep water into the photic zone (Carlson et al. 2014). Increasing irradiance followed by warming sea surface temperatures throughout spring and summer lead to the development of a thermocline > 100 m deep (Carlson et al. 2014) with a nutrient-depleted surface layer (Silverman et al. 2007), resulting in summer conditions of high temperature and irradiance but low inorganic nutrient concentrations. Near-surface temperatures range from 21 – 28 °C throughout the year while inorganic nutrient concentrations can vary by an order of magnitude (Silverman et al. 2007; Carlson et al. 2014). Such environmental parameters are known to influence planktonic N_2 fixation (Sohm et al. 2011), but their effect on benthic reef diazotrophs is largely unknown (Cardini et al. 2014).

The objectives of this study, therefore, were 1) to quantify rates of N_2 fixation and primary productivity (i.e. photosynthesis) in three dominant reef framework substrates; turf algae, coral rock, and an abundant encrusting sponge in seasonal resolution in order to evaluate the effect of seasonally variable key environmental parameters on these processes, and 2) to estimate the contribution of N_2 fixation to the N requirements for primary production in the three investigated substrates.

Materials and methods

Study site

This study was conducted in the northern Gulf of Aqaba at the Marine Science Station (MSS) Aqaba, Jordan (29°27' N, 34°58' E). Sampling was carried out on the 1 km long fringing reef in front of the MSS, which is designated as a marine reserve, and experimental work was carried out in the MSS laboratories. In order to examine the effect of seasonality, all experiments were repeated over four seasonal periods in 2013: winter (February), spring (April), summer (September), and fall (November).

The benthic reef community was dominated by hard and soft corals (58.1 ± 13.8%), while coral rock represented the third most abundant benthic substrate type at 10 m water depth, covering on average 14.2 ± 5.0% of the available substrate. The percent cover of turf algae (annual average: 4.4 ± 4.5%) was seasonally variable reaching a maximum of 10.3 ± 4.2% in winter and decreasing to a minimum of 1.0 ± 1.0% in fall. Sponge cover was constant throughout the year averaging 1.2 ± 0.9%. The non-cryptic sponge community was dominated by the abundant encrusting sponge *Mycale fistulifera*, which accounted for 65% of the visible sponge cover at 10 m water depth. Together the three investigated substrates accounted for 19.8 ± 10.3% of the total benthic coverage.

Environmental monitoring

During each season in-situ water temperature and irradiance were continuously monitored at one minute intervals over four weeks at the sampling site (10 m water depth) using data loggers (Onset HOBO Pendant UA-002-64; temperature accuracy: ± 0.53 °C, spectral detection range: 150 – 1200 nm). One data logger was placed in an unshaded position on the reef at the sampling location at 1, 5, 10, and 20 m water depth respectively. Parallel irradiance measurements with a quantum sensor (Model LI-192SA; Li-Cor) allowed the conversion of lux measurements to photosynthetically active radiation (PAR µmol photons m^{-2} s^{-1}, wavelength 400 – 700 nm) using a conversion factor of 1 µmol quanta m^{-2} s^{-1} = 52 lux (Long et al. 2012). Irradiance data are presented as seasonal means (± SD) of daily maximum values (µmol quanta m^{-2} s^{-1}) and integrated diurnal values (mol quanta m^{-2} d^{-1}; Table 1). Weekly seawater samples (n = 4 per season) were collected by SCUBA using acid-washed high-density polyethylene canisters (5 L, n = 4) at 10 m water depth (~1 m above the bottom) and immediately transferred to the laboratory for further processing. Subsamples were taken from each canister for quantification of inorganic nutrients, particulate organic carbon (POC), particulate nitrogen (PN), and chlorophyll *a* (Chl *a*). Inorganic

nutrient subsamples were syringe-filtered through cellulose acetate filters (nominal pore size 0.45 µm) for determination of ammonium (NH_4^+), nitrate (NO_3^{2-}), nitrite (NO_2^-), and phosphate (PO_4^{3-}) concentrations using standard methods, although a modification of the cadmium column method for NO_3^{2-} determination using a cadmium sponge was used to enable field analysis (Murphy and Riley 1962; Strickland and Parsons 1968; Holmes et al. 1999). NH_4^+ was determined fluorometrically using a Trilogy Fluorometer (Turner Designs), while all other nutrients were measured photometrically with a JASCO-V630 spectrophotometer (Jasco Analytical Instruments). The detection limits for NH_4^+, PO_4^{3-}, and NO_3^{2-} + NO_2^- were 0.09, 0.01, and 0.02 µM, respectively. Subsamples for Chl *a* determination (n = 4, 1 L) were filtered onto pre-combusted (450 ˚C, 4 h) GF/F filters (VWR: nominal pore size 0.7 µm) and stored frozen at -80 °C in the dark until further processing. Chl *a* was extracted with 90% acetone (12 h in the dark at 4˚C) and analyzed fluorometrically using a Trilogy fluorometer fitted with the non-acidification module (CHL NA #046, Turner Designs). Subsamples for POC (1 L) and PN (2 L) were filtered onto pre-combusted GF/F filters and dried in the oven (40 °C, 48 h). Prior to analysis POC filters were decalcified with 0.1 N HCl. POC and PN filter contents were measured on a EuroVector elemental analyser (EURO EA 3000) with analytical precision of ≤0.1% (C) and ≤0.03% (N) using the elemental standard Acetanilide OAS (certificate 187560).

Substrate collection and maintenance

Samples of the three investigated reef framework substrates; turf algae, coral rock, and the encrusting sponge *M. fistulifera*, were collected from the reef at 10 m water depth by SCUBA and immediately transferred to the aquarium facility without air exposure. Turf algae were defined as a thick mat consisting of a heterogeneous assemblage of filamentous algae, crustose coralline algae (CCA), and filamentous cyanobacteria. In the Gulf of Aqaba, turf algae are composed predominately of Phaeophyta and Rhodophyta of the order Ceramiales as well as green algae of the genus C*ladophora* and cyanobacteria (Bahartan et al. 2010; Haas et al. 2010). Coral rock was considered biogenic reef framework lacking coverage by a single dominant visible epilithic group with the carbonate structure clearly visible and open for settling organisms. In the Gulf of Aqaba this hard substrate can cover large areas of the reef and is commonly referred to as “bare rock” or “bare substrate” (e.g. Shashar et al. 1994b; Bahartan et al. 2010), although it is associated with endolithic algae, epilithic microbial biofilms, and sparse patches of CCA, cyanobacteria and filamentous algae (Bahartan et al. 2010; Charpy et al. 2012). *M. fistulifera* is an encrusting sponge approximately 0.2 – 0.5 cm thick, typically found encrusting coral skeletons. Turf algae and *M. fistulifera* were collected by chiseling small pieces of dead branching corals overgrown by either turf or *M. fistulifera*, and were attached to ceramic tiles with coral glue (Reef Construct, Aqua Medic®) to minimize stress during experimental handling. Coral rock was sampled by chiseling

pieces of reef framework. For each substrate, 8 replicates per season were collected with a mean height of 5-6 cm and mean surface area of 33.5 ± 18.9 cm^2. Specimens were maintained in an outdoor 800 L flow-through tank supplied with seawater pumped directly from the reef at 10 m water depth at approximately 4000 L h^{-1}, ensuring key environmental parameters (e.g. temperature and inorganic nutrient concentrations) corresponded to seasonal in-situ conditions. Irradiance (PAR) was adjusted to in-situ levels at 10 m water depth with layers of black mesh. Parallel irradiance measurements with a quantum PAR sensor (Model LI-192SA; Li-Cor) in-situ at 10 m and in the maintenance tank ensured irradiance corresponded to seasonal in-situ conditions as presented in Table 4-1 with the following mean deviations from in-situ: 21.8, 56.4, 26.4, 19.6 µmol photons m^{-2} s^{-1} for winter, spring, summer and fall, respectively. All incubation measurements were conducted in the same maintenance tank to ensure consistent temperature and irradiance values. *M. fistulifera* specimens were allowed to heal and acclimate for 1 wk prior to experiments and only healthy specimens were incubated. Turf algae and coral rock were collected within 24 h before incubations were conducted.

Quantification of dinitrogen fixation

N_2 fixation rates were quantified using the acetylene (C_2H_2) reduction assay method (Capone 1993; Wilson et al. 2012). Specimens (n = 8 per substrate) were incubated in individual 1000 mL transparent glass chambers containing 800 mL of natural seawater and 200 mL headspace. Organisms were transferred into chambers without air exposure and positioned to ensure comparable irradiances in all chambers. Immediately prior to the start of the incubations 10% of the seawater was replaced with C_2H_2-saturated seawater. Chambers were then sealed gas-tight and 10% of the 200 mL headspace was replaced with freshly generated C_2H_2 gas. Sealed chambers were stirred with magnetic stirrers (600 rpm) and positioned in the flow-through tank to ensure in-situ temperature and irradiance throughout the 24 h incubation period. Parallel measurements of irradiance inside the chambers and in the flow-through tank revealed no significant differences ensuring irradiances inside the chambers corresponded to the seasonal values provided in Table 4-1. Incubations started and ended just prior to sunset (approximately 17:00) and gas samples were taken at 0, 4, 12, 16 and 24 h, except during spring when samples were taken only at 0 and 24 h. These sampling intervals were selected to capture the periods of dusk, night, dawn, and full daylight as N_2 fixation during low light conditions (dawn and dusk) is a strategy by some diazotrophs to manage the oxygen inhibition of nitrogenase (Lesser et al. 2007). At each time interval 1 mL of gas sample was collected from the headspace of each chamber with a gastight syringe and transferred into gas-tight 2 mL glass vials fitted with butyl septa and filled with distilled water. Vials were stored frozen upside down until analysis.

Table 4-1. Environmental parameters monitored at 10 m water depth over four seasonal periods in 2013; winter (February), spring (April), summer (September), fall (November). Parameters include photosynthetically active radiation (PAR), measured as both the mean daily maximum (µmol photons m^{-2} s^{-1}) and integrated diurnal mean (mol photons m^{-2} d^{-1}), temperature (°C), ammonium (NH_4^+), nitrate + nitrite (NO_3^{2-} + NO_2^-), total dissolved inorganic nitrogen (DIN), phosphate (PO_4^{3-}), particulate organic carbon (POC), particulate nitrogen (PN), and chlorophyll a (Chl *a*). Temperature and irradiance were measured continuously during each seasonal period while other parameters were measured once weekly (n = 4 per season). Values are presented as mean ± SD.

Parameter	Winter	Spring	Summer	Fall
Daily maximum PAR (µmol photons m^{-2} s^{-1})	180 ± 43	252 ± 38	307 ± 25	171 ± 20
Integrated diurnal PAR (mol photons m^{-2} d^{-1})	3.43 ± 0.66	5.71 ± 0.32	7.25 ± 0.47	3.51 ± 0.47
Temperature (°C)	22.5 ± 0.1	22.8 ± 0.3	27.5 ± 0.2	25.2 ± 0.2
NH_4^+ (µM)	0.32 ± 0.09	0.46 ± 0.11	0.14 ± 0.07	0.28 ± 0.07
NO_3^{2-} + NO_2^- (µM)	0.79 ± 0.16	0.49 ± 0.19	0.09 ± 0.21	0.18 ± 0.05
DIN (µM)	1.11 ± 0.19	0.96 ± 0.08	0.23 ± 0.07	0.46 ± 0.10
PO_4^{3-} (µM)	0.11 ± 0.01	0.10 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
DIN:PO_4^{3-}	10.50 ± 1.09	9.68 ± 0.43	8.10 ± 3.40	12.93 ± 2.22
POC (µM)	6.33 ± 0.70	10.25 ± 0.72	7.96 ± 1.35	8.81 ± 2.10
PN (µM)	0.85 ± 0.07	1.27 ± 0.05	0.96 ± 0.28	0.87 ± 0.37
POC:PN	7.34 ± 1.15	8.18 ± 1.29	8.34 ± 1.17	10.20 ± 1.62
Chl *a* (µg L^{-1})	0.21 ± 0.02	0.22 ± 0.04	0.10 ± 0.04	0.19 ± 0.04

Ethylene (C_2H_4) concentrations in the gas samples were measured using a reducing compound photometer (RCP) (Peak Laboratories) with a detection limit of 100 ppb. Calibration of the RCP was routinely conducted using serial dilutions of a 200 ppm (± 2%) C_2H_4 standard in air (Restek, USA). Differences in C_2H_4 concentration between the time intervals of the incubation period were converted into C_2H_4 evolution rates according to Breitbarth et al. (2004). The C_2H_4 concentrations of the samples were corrected for the signal of unfiltered seawater controls (n = 8) and normalized to incubation time and surface area of the specimen in order to calculate C_2H_4 evolution rates (nmol C_2H_4 cm^{-2} h^{-1}). Additional controls for 0.2 µm filtered seawater (n = 6), unfiltered seawater and ceramic tile (n = 6), and unfiltered seawater with specimens but no addition of C_2H_2 (natural C_2H_4 production, n = 6), showed negligible C_2H_4 evolution. Surface areas were measured using geometric techniques (Advanced Geometry) as described by Naumann et al. (2009). To convert C_2H_4 evolution rates to N_2 fixation rates, a conservative theoretical ratio of 4:1 (C_2H_4:N_2) was used, which assumes that 4 mol of C_2H_4 are reduced per 1 mol of N_2. This is more conservative than the theoretical stoichiometric ratio of 3:1 as it accounts for the inhibition of the hydrogenase reaction of nitrogenase under C_2H_4-reducing conditions (Capone 1993; Mulholland et al. 2004).

Quantification of primary productivity

Primary productivity (i.e. photosynthesis) was quantified via dissolved O_2 fluxes. Substrates and seawater controls (n = 8 replicates each) were incubated in individual 1 L airtight transparent glass chambers filled with natural seawater and sealed with a transparent glass lid. The sealed chambers were incubated under identical conditions as described above for N_2 fixation measurements. Incubations for respiration (R) were conducted 1-2 h after sunset in complete darkness for 90 – 120 min. Incubations for net photosynthesis (P_{net}) were carried out at between 12:00 – 14:00 the following day during maximum light intensity for 60 – 90 min. Dissolved O_2 concentrations were measured at the start and end of each incubation period using a salinity and temperature corrected O_2 optode sensor (MultiLine ® IDS 3430, WTW GmbH). Start O_2 concentrations were subtracted from end O_2 concentrations to quantify P_{net} and R. O_2 fluxes were corrected for the mean O_2 difference found in the seawater controls and normalized to incubation time and surface area of the respective specimen. R is presented as a positive rate and gross photosynthesis (P_{gross}) rates were calculated as: $P_{gross} = P_{net} + R$.

To calculate the contribution of fixed N to the N demand for primary production, O_2 fluxes were converted into dissolved inorganic C fluxes using a photosynthetic quotient (PQ) of 1.04 and respiratory quotient (RQ) of 0.96 for turf algae and coral rock (Carpenter and Williams 2007). Since no literature values were available for marine sponges, PQ and RQ of 1 were used for *M. fistulifera*. It was assumed that turf algae and *M. fistulifera* assimilate biomass with C:N ratios of 13.7 ± 1.3 and 6.2 ± 0.3, respectively, based on C and N elemental analyses of macroalgae and *M. fistulifera* from the study site (L. Rix unpublished). Since no data were available for coral rock from the Gulf of Aqaba, C:N ratios of epi- and endolithic algae associated with coral rubble from Le Reunion and Sesoko Islands were used (9.7 ± 1.5; Casareto et al. 2008). These reefs also belong to the Indo-Pacific and display comparable inorganic nutrient concentrations (Casareto et al. 2008), and were therefore deemed representative. However, variations in community assemblages may result in corresponding variations in tissue C:N ratios. Nevertheless, our intention is to provide a mainly qualitative estimate of the importance of N_2 fixation for primary production by demonstrating how much new N is made available by N_2 fixation that could potentially be used to meet the demand for net primary production and biomass generation.

Statistical analysis

The influence of "season" and "substrate" on all physiological parameters was estimated using fully crossed general linear models fitted in R version 3.1.1 (R Development Core Team, 2013). A second model was run examining the effect of "season" and "substrate" and "time of day" (day or night) on N_2 fixation. Season was used as a fixed factor encompassing the combined effects of all environmental parameters. The influence of individual environmental parameters was further examined using linear regressions. To confirm the assumptions of normally distributed and homogenous residuals, qqplots and scatter plots of residuals against fitted values were visually inspected (Quinn and Keough 2002), and data were log-transformed where necessary. Model stability was checked by examining leverage and Cook's distance as well as dffits and dfbetas, and all values were deemed acceptable. Model significance was tested using likelihood ratio tests, comparing the deviances of full models with those of the null models comprising only the intercept. The significance of individual factors was tested by removing the factor of interest and comparing the deviance to the respective full models. If factors were found to be significant, pairwise post-hoc comparisons (*t* –tests) were used to check the comparisons of interest.

Results

Environmental monitoring

All environmental parameters monitored showed marked seasonal variability over the study period, with the most pronounced differences occurring between winter and summer (Table 4-1). Both the daily maximum irradiance and integrated diurnal irradiance were higher in spring and summer compared to winter and fall (Table 4-1). In-situ temperature at 10 m water depth ranged from 22.4 to 28.0 °C over the year, remaining low throughout winter and spring then reaching a maximum in summer before decreasing again in fall (Table 4-1). Concentrations of NH_4^+, NO_x and PO_4^{3-} exhibited a negative correlation with temperature (lm: all $p < 0.001$), with concentrations more than twice as high in winter and spring compared to summer and fall, reflecting the deep winter mixing and summer stratification of the water column (Silverman et al. 2007). The ratio of dissolved inorganic nitrogen to phosphate (DIN:PO_4^{3-}) ranged from 3.4 to 15.6 over the year but was consistently lower than the Redfield ratio (16:1), while POC:PN ratios always exceeded the Redfield ratio (106:16), indicating a deficiency of N compared to Redfield proportions. Chl *a* decreased by half in summer compared to all other seasons, while POC and PN were highest in spring during the seasonal plankton bloom (Table 4-1).

Dinitrogen fixation

N_2 fixation activity varied significantly by substrate, season, and an interaction between the two factors (Fig. 4-1, Appendix 3 - Table 1). On annual average, N_2 fixation was significantly higher in turf algae (4.4 ± 3.9 nmol C_2H_4 cm^{-2} h^{-1}) and coral rock (3.5 ± 2.8 nmol C_2H_4 cm^{-2} h^{-1}) compared to *M. fistulifera* (0.2 ± 0.2 nmol C_2H_4 cm^{-2} h^{-1}) (post hoc paired *t*-test: both $p < 0.001$). N_2 fixation of turf algae and coral rock were similar on annual average but significantly higher for turf algae in winter and summer, although in summer this was due to higher nighttime N_2 fixation (post hoc paired *t*-test: all $p < 0.001$). N_2 fixation rates for all substrates were significantly higher in summer compared to all other seasons (post hoc paired *t*-test: all $p < 0.001$) (Fig. 4-1). Coral rock and *M. fistulifera* also displayed significantly lower N_2 fixation activity in winter (post hoc paired *t*-test: all $p < 0.05$), with the winter N_2 fixation activity in *M. fistulifera* not significantly different from seawater controls. There were no significant differences in N_2 fixation between spring and fall for any substrate (Fig. 4-1). Across all substrates, N_2 fixation was one order of magnitude higher in the summer compared to the lowest rates in winter. Irradiance and temperature had a positive effect on N_2 fixation, while inorganic nutrient concentrations had a negative effect (Table 4-2). Irradiance explained more variation in N_2 fixation in turf algae and *M. fistulifera* than in coral rock, and for all substrates DIN explained more variation in N_2 fixation than PO_4^{3-} concentrations (Table 4-2).

N_2 fixation activity also varied significantly by time of day (Fig. 4-2, Appendix 3 - Table 2). However, no significant differences were observed during dawn and dusk, and therefore we present only the day and night rates (Fig. 4-2). *M. fistulifera* exhibited significantly higher N_2 fixation during the day compared to the night in all three seasons examined (i.e. winter, summer, and fall) (post hoc paired *t*-test: all $p < 0.01$). N_2 fixation in turf algae showed a seasonally variable response to time of day, with significantly higher daytime N_2 fixation in winter but significantly higher nighttime N_2 fixation in summer (Fig. 4-2). This was the only instance of significantly higher N_2 fixation at night but it was also the highest N_2 fixation rate measured over all substrates and seasons, with a rate of 17.8 ± 5.5 nmol C_2H_4 cm^{-2} h^{-1}. Coral rock displayed no significant differences in N_2 fixation between day and night (Fig. 4-2).

Table 4-2. Linear regression analysis of the influence of key environmental parameters on the P_{gross} and N_2 fixation rates of the three investigated substrates. Data are presented as R-squared values at significance levels of <0.05*, <0.01**, <0.001***. Italicized values indicate a significant positive linear relationship and bold values indicate a significant negative linear relationship. Abbreviations: P_{gross} = gross photosynthesis, DIN = dissolved inorganic nitrogen, PO_4^{3-} = phosphate.

		Irradiance	Temperature	DIN	PO_4^{3-}
N_2 fixation	Turf algae	*0.542***	*0.696***	**0.586*****	**0.399*****
	Coral rock	*0.415***	*0.458***	**0.511*****	**0.396*****
	Mycale fistulifera	*0.503***	*0.714***	**0.696*****	**0.584*****
P_{gross}	Turf algae	*0.505***	*0.424***	**0.305****	**0.163 ***
	Coral rock	0.028 NS	0.122 NS	0.096 NS	0.088 NS
	Mycale fistulifera	*0.403***	*0.212***	**0.244****	**0.162***

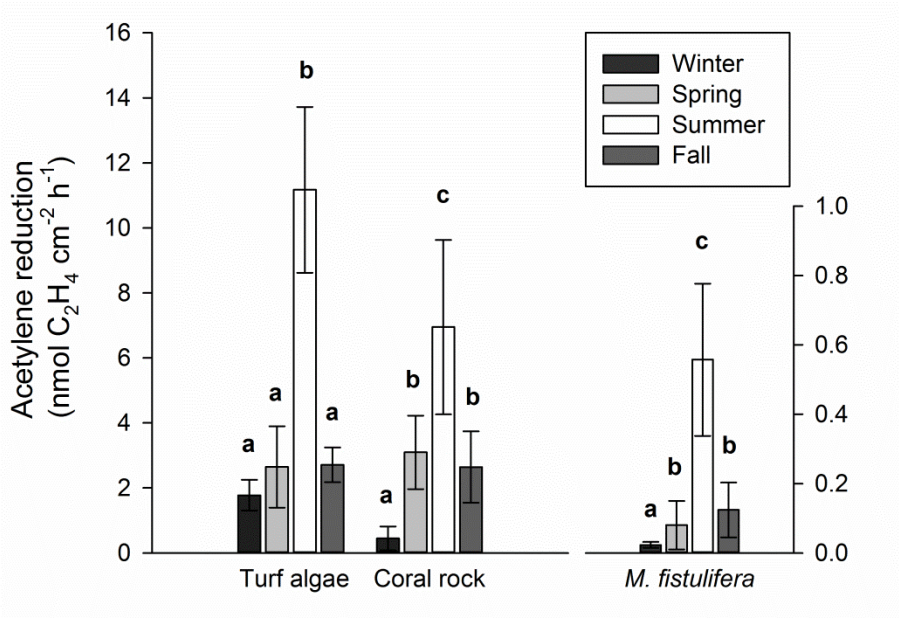

Fig. 4-1. Mean N_2 fixation measured as acetylene reduction rates of the three investigated benthic substrates over the four seasonal periods in 2013; winter (February), spring (April), summer (September), fall (November). Values (acetylene reduction nmol C_2H_4 cm^{-2} h^{-1}) are presented as mean (n = 8) ± SD. Different letters indicate statistical differences within each substrate. Note the different y-axis scale for *Mycale fistulifera*.

Primary productivity

There were significant effects of substrate and season as well as an interaction between the two factors for all physiological parameters measured (Appendix 3 - Table 1). Over all seasons, rates of P_{gross} were significantly higher in turf algae compared to coral rock (post hoc paired *t*-test: p <

0.001) and significantly lower in *M. fistulifera* compared to both other substrates (post hoc paired *t*-test: both $p < 0.001$). Despite low positive P_{gross} rates, *M. fistulifera* exhibited negative P_{net} rates (Table 4-3). This was due to high R rates, which were significantly higher than for turf algae and coral rock (post hoc paired *t*-test: both $p < 0.001$) and resulted in low P_{gross}:R ratios (< 1) indicating heterotrophic metabolism (Wilkinson 1987). Coral rock exhibited significantly higher R and lower P_{gross}:R ratios than turf algae (post hoc paired *t*-test: both $p < 0.001$) (Table 4-3).

Seasonal variations in P_{gross} were less pronounced than for N_2 fixation (Table 4-3). P_{gross} was significantly higher in turf algae in summer (post hoc paired *t*-test: all $p < 0.001$) and significantly lower in coral rock in spring (post hoc paired *t*-test: $p < 0.01$) (Table 4-3). It should be noted that P_{gross} and P_{net} rates for turf algae in spring could represent the lower end of typical spring values due to unseasonably low irradiances during the turf algae photosynthesis incubations (73.8 ± 26.3 µmol photons m^{-2} s^{-1} compared to the seasonal mean of 252 ± 38 µmol photons m^{-2} s^{-1}). P_{gross} in *M. fistulifera* was significantly higher in spring compared to winter and in summer compared at all other seasons (post hoc paired *t*-test: all $p < 0.001$). Irradiance and temperature were positively correlated with P_{gross} in turf algae and *M. fistulifera* and appeared to explain more of the seasonal variation in P_{gross} than DIN and PO_4^{3-}, which were negatively correlated (Table 4-2). There was little seasonal variation in P_{gross} of coral rock with no significant effect of any of the monitored environmental parameters (Table 4-2).

Table 4-3. Metabolic parameters measured in the three investigated substrates over four seasonal periods. Rates are presented as nmol O_2 cm^{-2} h^{-1} (mean ± SD, n = 8). Abbreviations: P_{gross} = gross photosynthesis, R = respiration, P_{net} = net photosynthesis.

Substrate	Season	P_{gross}	R	P_{net}	P_{gross}:R
Turf algae	Winter	528 ± 85	95 ± 12	433 ± 77	5.6 ± 0.7
	Spring	550 ± 107	78 ± 19	473 ± 95	7.2 ± 1.1
	Summer	894 ± 162	119 ± 27	775 ± 155	7.8 ± 2.1
	Fall	509 ± 84	95 ± 19	415 ± 68	5.4 ± 0.6
	Mean	**620 ± 195**	**97 ± 24**	**524 ± 179**	**6.5 ± 1.6**
Coral rock	Winter	472 ± 237	96 ± 55	379 ± 186	4.7 ± 0.4
	Spring	354 ± 165	69 ± 38	271 ± 136	3.4 ± 1.9
	Summer	553 ± 152	179 ± 95	374 ± 124	3.5 ± 1.2
	Fall	446 ± 134	144 ±35	302 ± 105	3.1 ± 0.5
	Mean	**438 ± 189**	**122 ± 72**	**316 ± 158**	**3.7 ± 1.9**
Mycale fistulifera	Winter	64 ± 49	238 ± 38	-174 ± 27	0.3 ± 0.1
	Spring	220 ± 42	348 ± 103	-139 ± 109	0.7 ± 0.2
	Summer	307 ± 108	563 ± 55	-256 ± 80	0.5 ± 0.2
	Fall	139 ± 68	564 ± 106	-393 ± 190	0.3 ± 0.2
	Mean	**190 ± 121**	**431 ± 164**	**-240 ± 149**	**0.5 ± 0.3**

Contribution of dinitrogen fixation to primary productivity

There was a significant positive linear relationship between P_{gross} and N_2 fixation for turf algae and coral rock only during summer and fall (Table 4-5). N_2 fixation explained 65 – 76% of the variation in P_{gross} in turf algae and 62 – 74% in coral rock. In contrast there was no significant relationship between N_2 fixation and P_{gross} in *M. fistulifera* during any of the four seasons (Table 4-5). The potential contributions of N_2 fixation to the N demand for P_{gross} in turf algae, coral rock, and *M. fistulifera* were estimated to be 8.9, 7.1, and 0.6%, respectively, on annual average (Table 4-4). The contributions of N_2 fixation to P_{net}, which represents the new production available for growth after accounting for respiration, were higher; on average 10.5% and 14.5%, for turf algae and coral respectively, but non-calculable for *M. fistulifera,* which displayed negative P_{net}. This contribution was seasonally variable with N_2 fixation having the potential to supply the highest amounts of N in summer (up to 19.8 and 26.8 % of the N required to meet the demand for P_{net} in turf algae and coral rock, respectively), while the contributions in winter were estimated to be less than 6% for all substrates (Table. 4-4).

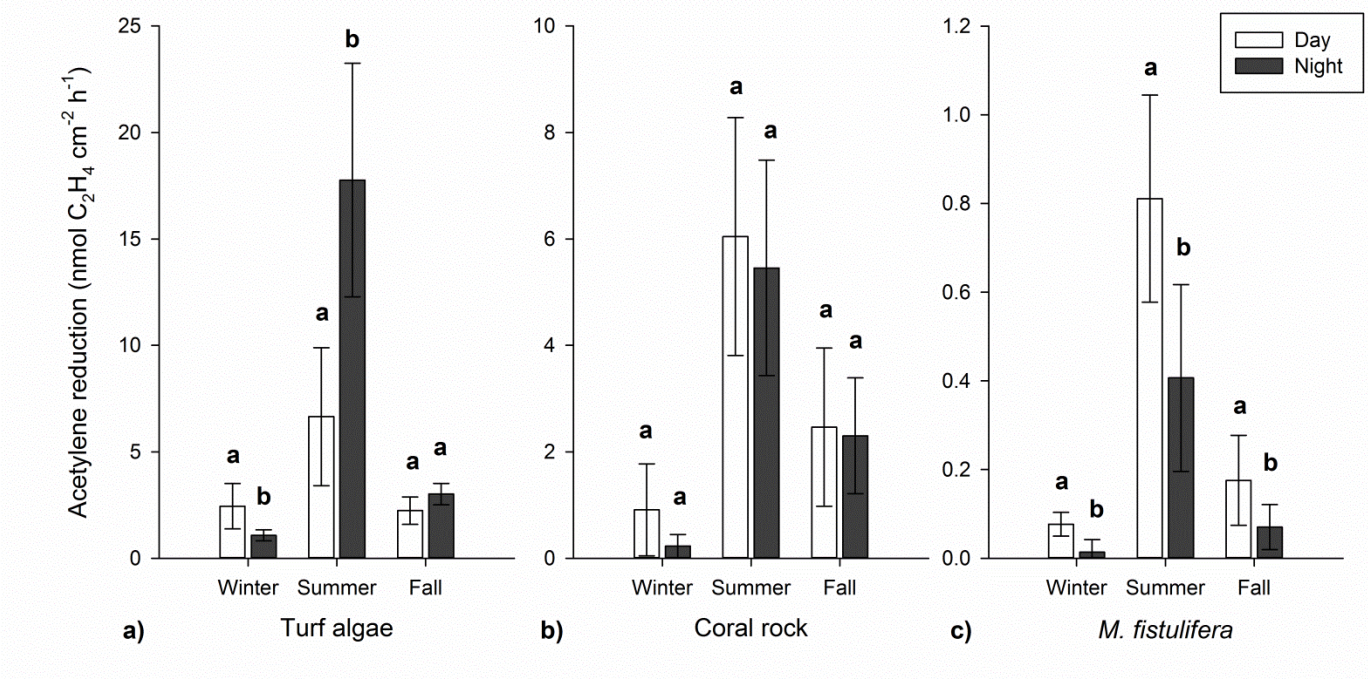

Fig. 4-2. Mean day (light bars) and night (dark bars) N_2 fixation measured as acetylene reduction rates of a) turf algae, b) coral rock, and c) *Mycale fistulifera* over three seasons in 2013 (winter, summer, and fall). Values (acetylene reduction nmol C_2H_4 cm^{-2} h^{-1}) are presented as mean (n = 8) ± SD. Different letters indicate statistical differences within each substrate. Note the changes in scale of the y-axis.

Table 4-4. Nitrogen (N) requirement for gross production and net production in the three investigated substrates and the percentage of N requirement met by N_2 fixation. Values are reported as mean ± SD (n = 8).

Substrate	Season	N requirement for gross production (μmol N cm^{-2} d^{-1})	% N requirement for gross production met by N_2 fixation	N requirement for net production (μmol N cm^{-2} d^{-1})	% N requirement for net production met by N_2 fixation
Turf algae	Winter	0.44 ± 0.07	4.8 ± 1.1	0.36 ± 0.07	5.9 ± 1.4
	Spring	0.46 ± 0.09	7.0 ± 2.9	0.40 ± 0.08	8.1 ± 3.6
	Summer	0.75 ± 0.13	17.2 ± 2.5	0.68 ± 0.12	19.8 ± 3.2
	Fall	0.43 ± 0.07	7.6 ± 0.8	0.35 ± 0.06	9.3 ± 0.9
	Mean	**0.52 ± 0.17**	**8.9 ± 5.1**	**0.44 ± 0.15**	**10.5 ± 5.8**
Coral rock	Winter	0.56 ± 0.30	1.2 ± 1.0	0.32 ± 0.16	2.2 ± 1.9
	Spring	0.42 ± 0.20	6.9 ± 1.0	0.25 ± 0.11	12.6 ± 1.7
	Summer	0.66 ± 0.18	12.8 ± 3.5	0.31 ± 0.10	26.8 ± 6.4
	Fall	0.53 ± 0.16	5.9 ± 1.3	0.25 ± 0.09	12.5 ± 2.8
	Mean	**0.55 ± 0.22**	**7.1 ± 4.9**	**0.28 ± 0.15**	**14.5 ± 10.0**
Mycale	Winter	0.12 ± 0.10	0.3 ± 0.1	0	0
fistulifera	Spring	0.39 ± 0.38	0.2 ± 0.6	0	0
	Summer	0.57 ± 0.20	1.2 ± 0.6	0	0
	Fall	0.32 ± 0.21	0.6 ± 0.5	0	0
	Mean	**0.34 ± 0.28**	**0.6 ± 0.6**	**0**	**0**

Discussion

Dinitrogen fixation in coral reef framework substrates

N_2 fixation rates presented here are comparable to those reported for turf algae, coral rock, and sponges on coral reefs worldwide (Table 4-6). The relatively high variability in N_2 fixation reported for turf algae likely results from regional differences in turf community composition (Bauer et al. 2008) or responses to local environmental conditions (Williams and Carpenter 1998). While N_2 fixation rates previously reported for turf algae are typically higher than those of coral rock (Table 4-6), our rates for both substrates were similar, with significantly higher N_2 fixation in turf algae only during winter and summer at night. However, studies reporting higher rates of N_2 fixation in "bare" rock compared to rock with epilithic algal, suggest endolithic N_2 fixation can exceed that of some epilithic communities (Wilkinson et al. 1984; Casareto et al. 2008). This demonstrates the importance of apparently "bare" substrate in generating new N on coral reefs.

N_2 fixation rates for *M. fistulifera* were an order of magnitude lower than those for turf algae and coral rock, but are in the range typically reported for other animal-microbe symbioses such as scleractinian corals (Shashar et al. 1994a; Davey et al. 2008) and other cnidarians (Shashar et al. 1994b; Bednarz et al. 2015). There are very few reports of active N_2 fixation in marine sponges, but the rates for *M. fistulifera* are consistent with those reported for other Red Sea sponges in the only other study presenting sponge N_2 fixation rates normalized to organism surface area (Table 4-6; Shashar et al. 1994b). It should be noted that both studies used the acetylene reduction method, which has reportedly proven problematic for some sponges (Wilkinson 1999). However, all sponges were actively pumping post-incubation and the high pumping rate would ensure rapid flushing of the tissue, excluding the likelihood of acetylene toxicity or insufficient acetylene and ethylene transport. We cannot exclude metabolism of ethylene by microbial symbionts, however this would affect all three substrates, not only the sponge. It may rather be that this apparent difficulty in measuring N_2 fixation in sponges is due to low N_2 fixation activity, as observed here (particularly in winter) and in other studies (Wilkinson and Fay 1979; Shashar et al. 1994b; Shieh and Lin 1994; Wilkinson 1999). Despite the low rates, we observed consistent and measurable N_2 fixation with low variability and clear seasonal trends. Further, the low $\delta^{15}N$ tissue values of *M. fistulifera* (0.05 ± 0.2 ‰ , L. Rix unpublished) are indicative of biological ^{15}N fixation (Yamamuro et al. 1995; Montoya et al. 2002). The lower N_2 fixation activity in *M. fistulifera* is likely due to low diazotroph abundances in the sponge-associated microbial community compared to turf algae and coral rock, which can be composed largely of N_2 fixing cyanobacteria (Charpy et al. 2012). Given that the DIN release rates reported for sponges exceed reported rates of N_2 fixation by orders of magnitude (Diaz and Ward 1997), N_2 fixation may not be functionally important for the nutrition of the sponge host. As efficient filter feeders, sponges may meet their N demand primarily via heterotrophic feeding (Pile et al. 2003).

Table 4-5. Linear regression analysis between gross photosynthesis (P_{gross}) rates and N_2 fixation rates of each of the three substrates during each of the four seasonal periods. Data are presented as R-squared values at significance levels of <0.05*, <0.01**, and NS which indicates no significance. Bold values indicate a significant positive linear relationship.

	Turf algae	Coral rock	*Mycale fistulifera*
Winter	0.245 NS	0.216 NS	0.022 NS
Spring	0.064 NS	0.318 NS	0.205 NS
Summer	**0.653***	**0.626***	0.199 NS
Fall	**0.741****	**0.704****	0.374 NS

Table 4-6. Comparison of known N_2 fixation rates of turf algae, coral rock, and sponges reported from coral reefs worldwide. Values are presented as nmol N cm^{-2} h^{-1}. Original C_2H_4:N_2 conversion rates were used to calculate the N_2 fixation rates from acetylene reduction rates if reported in the original study. If no conversion rate was available the conservative ratio of 4:1 was used (Mulholland et al. 2004).

Substrate	N fixation	Region	Reference
Turf algae	0.9 – 5.6	Red Sea	Present study
Turf algae	4.6 ± 0.3	Red Sea	Shashar et al. (1994b)
Turf algae	0.3 – 29.7[a]	Great Barrier Reef	Larkum et al. (1988)
Turf algae	8.3 – 36.7	Great Barrier Reef	Wilkinson and Sammarco (1983)
Turf algae	13.5 ± 5.5	Hawaiian Islands	Williams and Carpenter (1998)
Turf algae	3.7 ± 5.4	Caribbean	Williams and Carpenter (1997)
Turf algae	6.0 ± 0.9	Caribbean	den Haan et al. (2014)
Coral rock	0.2 – 3.5	Red Sea	Present study
Coral rock	0.3 ± 0.2	Red Sea	Shashar et al. (1994b)
Coral rock	0.2 – 1.9	Great Barrier Reef	Wilkinson et al. (1984)
Coral rock	0.1 – 6.4	Great Barrier Reef	Davey et al. (2008)
Coral rock	6.4 ± 1.8[a]	Great Barrier Reef	Larkum et al. (1988)
Coral rock	0.6[b]	French Polynesia	Charpy-Roubaud et al. (2001)
Sponge (*M. fistulifera*)	0.01 – 0.3	Red Sea	Present study
Sponges	0.1 ± 0.2	Red Sea	Shashar et al. (1994b)

[a] Conversion factor 3.45, [b] Conversion factor 3.3

Diel dinitrogen fixation pattern

While many studies have found substantially higher daytime N_2 fixation activity in coral rock (Wilkinson et al. 1984; Charpy-Roubaud et al. 2001; Holmes and Johnstone 2010) and turf algae (Williams and Carpenter 1997; den Haan et al. 2014), our results show consistent and substantial nighttime N_2 fixation by both substrates. Turf algae exhibited significantly higher daytime N_2 fixation in winter but significantly (3-times) higher nighttime N_2 fixation in summer. This could indicate a shift in the turf community towards more non-heterocystous cyanobacteria or heterotrophic diazotrophs in summer, as typically only heterocystous cyanobacteria can fix N_2 in the presence of O_2 generated by photosynthesis (Bergman et al. 1997). While cyanobacteria have long been considered the primary diazotrophs responsible for benthic marine N_2 fixation, the role of heterotrophic bacteria is increasingly being recognized (Zehr et al. 1995; Bauer et al. 2008) and identification of the diazotroph community would provide further insight into the patterns of N_2 fixation observed here. Similar day and night N_2 fixation rates by coral rock suggest a diazotroph community equally adapted to light and dark conditions. Only *M. fistulifera* consistently exhibited significantly higher N_2 fixation activity in the day compared to night (2-times), suggesting either the role of phototrophic diazotrophs or heterotrophic diazotrophs energetically dependent on photosynthetic products. Symbiotic cyanobacteria are widespread in coral reef sponges (Wilkinson and Fay 1979; Erwin and Thacker 2007) and the expression of cyanobacterial *nifH*

genes has been detected in the congeneric *Mycale laxissima* (Mohamed et al. 2008), although heterotrophic diazotrophs have also been found to fix N_2 in sponges (Shieh and Lin 1994). The presence of cyanobacteria could also explain our findings of P_{gross} in *M. fistulifera* and future studies should investigate the symbionts responsible for photosynthesis and N_2 fixation in the sponge. The lack of peaks in N_2 fixation during low light levels as observed for the coral *Montastrea cavernosa* (Lesser et al. 2007) and the co-occurrence of N_2 fixation during the day with photosynthesis indicate that the diazotroph communities of the three investigated substrates are equipped with other strategies to overcome O_2 inhibition of nitrogenase.

Seasonality in dinitrogen fixation and primary productivity

This is the first study examining seasonal N_2 fixation in a diverse group of reef framework substrates, and our findings highlight the importance of environmental parameters in regulating benthic N_2 fixation activity. Temperature and irradiance positively affected N_2 fixation, while inorganic nutrients had a negative influence, resulting in the highest rates in summer when irradiance and temperature were highest but inorganic nutrients lowest. This seasonal pattern showed remarkable consistency across the three substrates despite their differing trophic strategies, and is consistent with reports of higher summer benthic N_2 fixation in the GBR (Larkum et al. 1988) as well as higher summer N_2 fixation in soft corals (Bednarz et al. 2015) and pelagic communities in the Red Sea (Rahav et al. 2015). High temperatures can directly stimulate the enzyme activity of nitrogenase and are associated with increased growth and N_2 fixation in free-living cyanobacteria (Breitbarth et al. 2007). Conversely, lower temperatures can increase respiratory costs associated with N_2 fixation in unicellular cyanobacteria (Brauer et al. 2013). However, temperature alone likely cannot explain the observed seasonality, as there were no significant differences in N_2 fixation between spring and fall, despite a 2.5 ºC temperature difference. By enhancing photosynthesis, irradiance may stimulate the energetically costly process of N_2 fixation through the provision of larger quantities of energy-rich photosynthates (Bebout et al. 1993), if the responsible diazotrophs are protected from the corresponding increase in O_2 production. Although this largely appears to be the case for the substrates investigated here, given only turf algae and *M. fistulifera* exhibited significantly higher summer photosynthesis but all three substrates displayed an increase in N_2 fixation, irradiance was unlikely the primary driving factor. The effect of decreased DIN concentrations during summer likely played a key role and can be explained by the higher energetic costs of N_2 fixation compared to DIN assimilation (Gallon 2001), making it a seasonal strategy when external nutrients are scarce. This is supported by observations of increased nitrogenase activity in N-starved cultured filamentous cyanobacteria (Ramos et al. 1985) and inhibition of N_2 fixation in coral skeletons and reef sediments under elevated NH_4^+ concentrations (Koop et al. 2001; Holmes and Johnstone 2010). These findings

suggest diazotrophs are capable of altering their N_2 fixation activity to adjust to the availability of external N sources. While elevated N_2 fixation also increases iron (Fe) demand compared to NH_4^+ assimilation (Kustka et al. 2003), Fe limitation is unlikely to be a limiting factor in the Gulf of Aqaba due to high dust inputs (Ying et al. 2007; Foster et al. 2009). Alternatively, seasonal variability in N_2 fixation activity may be influenced by seasonal changes in the diazotroph communities associated with the three substrates. Overall the combination of key environmental parameters in summer (i.e. high irradiance, high temperature, and low inorganic nutrients), appear to interact to cause substantially higher N_2 fixation rates. Importantly, this results in the highest N_2 fixation when the inorganic N supply is lowest.

Contribution of dinitrogen fixation to primary productivity

Interestingly, high photosynthesis rates were sustained in summer when temperature and irradiance were highest, despite low inorganic nutrient availability. This suggests that either primary production was not nutrient (DIN) limited or that additional nutrient sources contributed to supporting photosynthesis. Given the significant positive linear relationship between P_{gross} and N_2 fixation for both turf algae and coral rock only during summer and fall when inorganic nutrient concentrations were low, this suggests fixed N may play a role in supporting primary production, at least when other sources of nutrients are scarce. For turf algae, we estimated that while fixed N could only supply 6% of the N demand for P_{net} in winter, this increased to 20% in summer, suggesting N_2 fixation has the potential to be an important N supply for photosynthesis. Williams and Carpenter (1997) found that N_2 fixation contributed less than 2% to the N demand for P_{net} in turf algae in the Caribbean, with the estimated contribution by NH_4^+ assimilation an order of magnitude higher. While comparable to our winter estimates, this is substantially lower than our summer values. However, the N_2 fixation rates measured by Williams and Carpenter (1997) were lower than those measured during our study in summer and NH_4^+ concentrations may have differed. While DIN assimilation would also represent an important process at our study site, increased summer N_2 fixation may compensate for the decrease in DIN concentrations, sustaining high summer P_{gross} rates. For coral rock we estimate that N_2 fixation could supply 2% of the N demand for P_{gross} in winter and 27% in summer. This is remarkably consistent to the 2 – 28% estimated for coral rock from Sesoko Island (Pacific Ocean) and Le Reunion (Indian Ocean) (Casareto et al. 2008). However, it should be noted that these calculations are highly dependent on the C:N of the substrates, which may vary spatially and temporally. Further, photosynthetic rates change over the day but here were measured only during periods of maximum irradiance, which may also influence these calculations. Future studies should investigate the utilization of fixed N by the turf algae and coral rock consortia to confirm a relationship between N_2 fixation and primary production. In contrast to turf algae and coral rock, we found no significant relationship

between N_2 fixation and P_{gross} in *M. fistulifera* and estimate that fixed N can only supply < 1.2% of the N required to support P_{gross} in *M. fistulifera*. Sponges release large quantities of DIN (Diaz and Ward 1997; Southwell et al. 2008), which may provide another source of N for their photosynthetic symbionts and could explain the lack of correlation between the two processes found here. While photosynthesis supplies a significant portion of the energy demand of some sponge species (Wilkinson 1987; Erwin and Thacker 2007), given *M. fistulifera* exhibited negative P_{net} and overall heterotrophic metabolism (P_{gross}:R < 1), photosynthesis is unlikely to be important to its overall nutrition. Nevertheless, these findings highlight the potential for N_2 fixation to contribute to sustaining high rates of P_{net} in coral rock and turf algae during the period of water column stratification in the Gulf of Aqaba.

Ecological implications

In addition to directly supporting their own growth, N fixed by turf algae and coral rock may substantially contribute to new N on coral reefs via N release from cyanobacterial cells (Mulholland et al. 2006), mechanical disturbances such as grazing (Williams and Carpenter 1997), or by the recycling of diazotroph biomass. Using the estimated 3D-surface area of each substrate per m^2 of reef, we calculate that N_2 fixation by the three substrates contributes 47 µmol N_2 m^{-2} d^{-1} of fixed N in winter and up to 185 µmol N m^{-2} d^{-1} in summer. These values are comparable to the benthic reef community N_2 fixation estimated for One Tree Island (GBR) of 78 – 156 µmol N_2 m^{-2} d^{-1} (Larkum et al. 1988), but lower than those calculated for Eilat (Red Sea) of 576 – 960 µmol N_2 m^{-2} d^{-1} (Shashar et al. 1994b). However, it is likely that N_2 fixation by other benthic substrates not accounted for here, such as reef sands (Charpy-Roubaud et al. 2001; Casareto et al. 2008), cyanobacterial mats (Charpy et al. 2007), and hard corals (Lesser et al. 2007), also substantially contribute to reef N generation. Due to the low abundance of *M. fistulifera* on the studied reef and the comparatively low rates of N_2 fixation associated with the microbial community of *M. fistulifera*, it contributed < 1% of the total new N fixed. Unless the abundant cryptic sponge community fixes N at higher rates, sponges likely do not contribute substantially to reef N generation, at least via N_2 fixation. They do produce large quantities of inorganic nutrients through remineralization of particulate organic matter and association with nitrifying bacteria (Diaz and Ward 1997; Richter et al. 2001; Southwell et al. 2008). While turf algae are increasingly a dominant component on many coral reefs (e.g. Bahartan et al. 2010), their low and seasonally variable abundance at the present study site resulted in a substantial contribution to reef new N generation only in winter (72%). Low abundances in other seasons resulted in a contribution of 10 – 31% of the total N fixed by the three investigated substrates during the rest of the year. Given their high potential for N_2 fixation, turf algae are likely an important source of new N on turf-dominated reefs (e.g. den Haan et al. 2014). High N_2 fixation

and high benthic coverage (11 – 20%) characterize coral rock as the major year-round contributor of newly fixed N among the three investigated substrates, accounting for 28% in winter but 69 – 90% of the N fixed during the other seasons. Overall two- to four-times more N was fixed in summer compared to the other seasons. This fixed N appears to be of greater ecological importance during the low nutrient summer season in the Gulf of Aqaba, particularly for supporting primary production. This highlights the potential significance of N_2 fixation by coral rock and turf algae in coral reef ecosystems with more constant oligotrophic conditions.

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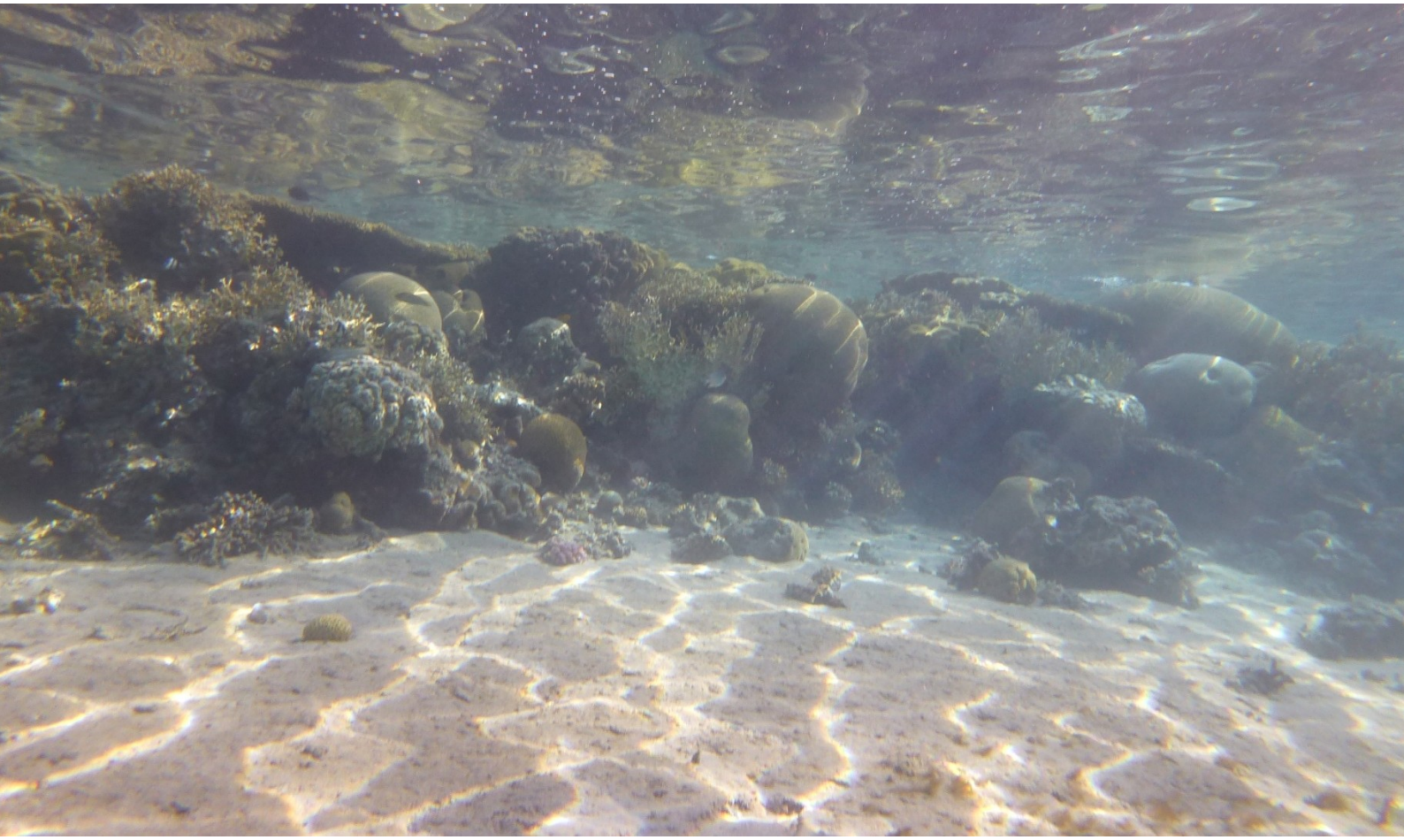
Chapter 5
Seasonal photosynthetic primary production and microbial dinitrogen fixation by permeable reef sand communities



Abstract

Permeable sediments are highly bioactive compartments in coral reefs. The associated dense microbial communities sustain fast degradation of organic matter, thereby playing a key role in nutrient recycling within the reef. Besides nutrient recycling, new nutrients (i.e. nitrogen) are acquired by dinitrogen (N_2) fixing microbial communities, but knowledge about the influence of sand mineralogy and key environmental factors on this process is scarce. Therefore, this study quantified seasonal N_2 fixation (via acetylene reduction) along with gross photosynthesis (via O_2 fluxes) by adjacent carbonate and silicate sands in a Northern Red Sea coral reef. Findings revealed significantly higher N_2 fixation in carbonate than in silicate sands (2.88 and 1.52 nmol C_2H_4 cm^{-2} h^{-1}) and a more pronounced seasonal response in the former, likely caused by its higher permeability, grain size and microbial abundance. Ambient light and organic matter availability were the main controlling environmental factors for sand-associated N_2 fixation. Carbonate and silicate sands showed similar gross photosynthesis rates (270 and 233 nmol O_2 cm^{-2} h^{-1}) that positively (carbonate sands) or negatively (silicate sands) correlated with N_2 fixation, likely due to different diazotrophic communities. Seasonal appearance of microbial mats on carbonate sands increased N_2 fixation and gross photosynthesis by up to one order of magnitude. On an annual average, carbonate and silicate sands obtain ~8% and microbial mat communities obtain ~13% of their photo-metabolic N demand via N_2 fixation.

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Chapter 5 Sediment primary production and N_2 fixation

Introduction

Coral reefs are characterized by high benthic community biomass and primary production despite being surrounded by oligotrophic waters (e.g. Odum and Odum 1955; Gattuso et al. 1998). Up to 90% of total carbon (C) fixation on coral reefs is derived from benthic photosynthetic primary production, where the highest production is often associated with corals (zooxanthellae), turf algae or macroalgae (Kinsey 1985; Gattuso et al. 1998). In comparison, the sand-associated microphytobenthos displays lower primary productivity rates per unit surface area, but given the often large areal extent of unconsolidated sandy sediments in reefs, net microphytobenthic primary productivity may on the reef scale be on the same order of magnitude and equally important as coral or macroalgal production (Kinsey 1985; Clavier and Garrigue 1999; Werner et al. 2006; Garren and Azam 2012).

Besides primary productivity, reef sands represent an important biocatalytical filter system for organic matter (Wild et al. 2004a; Wild et al. 2004b; Werner et al. 2006). The relatively large grain size of reef sands ensures high permeability ($> 10^{-12}$ m^2) for water exchange and provides settling space for microphytobenthic communities, which represent key factors for both efficient organic matter degradation and concomitant nutrient recycling (Rasheed et al. 2003a; Wild et al. 2004a; Wild et al. 2004b; Werner et al. 2006). Reef sands generally contain 10^3 times more bacteria and up to 80 times higher nutrient concentrations than the surrounding seawater (Rasheed et al. 2002), highlighting the importance of this reef compartment for nutrient recycling in oligotrophic reef environments (Garren and Azam 2012). As oligotrophic reefs receive low amounts of allochthonous nutrient input, they strongly rely on the efficient recycling and new generation of nutrients (Howarth 1988). In particular, nitrogen (N) is mostly the limiting nutrient for primary productivity in coral reefs (Eyre et al. 2008).

Besides recycling of essential nutrients, measurements of dinitrogen (N_2) fixation indicate that reef sands also play an important role for the generation of new bioavailable N (Shashar et al. 1994b; Charpy-Roubaud et al. 2001). Capone et al. (1992) found that N_2 fixation in the top layers (0 to 2 cm) of reef sediments accounted for more than 50% of the total sedimentary ammonium production. Biological N_2 fixation is a physiological process unique to diazotrophic prokaryotes and, despite being energy-costly, can represent an alternative nutrient supply if growing under N-limited ambient conditions typical for coral reef environments (Charpy-Roubaud et al. 2001; Scanlon and Post 2008). In coral reefs several benthic substrates (e.g. sand, coral rubble, cyanobacterial mats and living corals) are actively fixing N_2 (Cardini et al. 2014). Since reef sands can cover large areas on a reef, previous studies have highlighted the magnitude of sedimentary N_2 fixation and its importance for the N requirement of the total reef benthos (Shashar et al.

1994b; Charpy-Roubaud et al. 2001; Casareto et al. 2008). Shashar et al. (1994b) calculated for a lagoon in the Northern Red Sea that reef sands contribute ~70% to the total N_2 fixation within the reef, while (Charpy-Roubaud et al. 2001) estimated that sedimentary N_2 fixation covers ~24% of the annual N requirements for the total benthic primary productivity in the Tikehau Lagoon (French Polynesia).

The dominant sand type in reef environments is biogenic carbonate sand, while in some regions terrigenous silicate sands co-occur. At the Northern Red Sea, the rare occurrence of flood events through otherwise desiccated river mouths lead to the deposition of silicate sands in many fringing reefs of the area. These 2 sand types are exposed to identical, seasonally variable environmental conditions but exhibit different physico-chemical characteristics in grain size, surface structure and area, permeability and transparency to light (see Table 5-1). Together these factors define 2 different habitats, which in turn select sand-specific microbial communities (Schottner et al. 2011) subsequently affecting sedimentary primary productivity and N_2 fixation rates. Previous studies have demonstrated the importance of microphytobenthic photosynthesis and N_2 fixation for total benthic primary productivity and biogeochemical nutrient cycles within the reef ecosystem (Charpy-Roubaud et al. 2001; Werner et al. 2008). Nonetheless, to our best knowledge, no study has investigated both processes with particularly focus on the effect of sand mineralogy and environmental key parameters (e.g. temperature, light intensity, nutrient concentrations).

Therefore, the main objectives of the present study were (1) to quantify N_2 fixation and microphytobenthic photosynthesis of 3 different reef sand communities (bare carbonate sands, silicate sands and microbial mats on carbonate sands) in a seasonal resolution in order to investigate the effects of sand type along with seasonally changing environmental key parameters, and (2) to calculate the respective contribution of fixed N to the N requirements for microphytobenthic primary productivity.

Materials and methods

Study site

This study was conducted at the Marine Science Station (MSS) Aqaba in the Northern Gulf of Aqaba, Jordan (29° 27`N, 34° 58`E). The MSS is situated ~10 km south of Aqaba City with access to a Red Sea fringing coral reef inside a marine reserve. Strong regional seasonality is reflected by substantial variability of environmental key parameters throughout the year due to the annual water column stratification cycle in the Gulf of Aqaba (Silverman et al. 2007; Carlson et

al. 2014). The hard coral dominated (38.6 ± 2.6%) fringing reef site reveals an average bare carbonate sand cover of 18.5 ± 2.8% with highest coverage at 5 m water depth (50.7 ± 6.3%) followed by the reef flat (19.0 ± 3.9%) and 10 m water depth (16.2 ± 1.4%). At 1 and 20 m depth, the bare carbonate sand coverage is < 4.0%, respectively. Overall for the site, < 1% of the total bare carbonate sand area is covered by microbial mat communities throughout the year, with the highest abundance (~3%) at 5 m water depth and a seasonal development ranging from < 1% in winter and summer to 5% in fall and 7% in spring. The fringing reef is interrupted by a ~100 m long area completely covered by silicate sand from the shore down to at least 40 m. This area is almost free of hard coral structures but covered 10 to 20% by seagrass beds. In order to study the effect of seasonality on N_2 fixation and primary productivity by microbial communities of the different reef sands, all experiments described below were conducted once in each of the following months representing a respective season: February (winter), April (spring), September (summer) and November (autumn) during the year 2013. Thermal stratification in the Gulf of Aqaba develops from May to November with a maximum during August/September, while deep-water mixing occurs from January to April reaching the maximum mixing depth in March/April (Manasrah et al. 2006).

Table 5-1. Sediment properties of carbonate and silicate sand in the Gulf of Aqaba previously measured at the study site. OC: organic carbon, DIN: dissolved inorganic nitrogen, DIP: dissolved inorganic phosphate, Chl a: chlorophyll a.

Parameter	Carbonate sand	Silicate sand	Reference
$CaCO_3$ content (%)	75-87	4-19	Rasheed et al. (2003b), Schottner et al. (2011)
Grain size (µm)	553-559	229-326	Rasheed et al. (2003b), Schottner et al. (2011)
Sorting coefficient	1.3	0.9	Rasheed et al. (2003b)
Permeability (m^{-2} x 10^{-12})	116-143	19-27	Rasheed et al. (2003b), Wild et al. (2005)
Porosity (%)	47	33	Rasheed et al. (2003b)
OC content (%)	0.36	0.24	Rasheed et al. (2003b)
OC decomposition (mg m^{-2} d^{-1})	3.0	2.0	Rasheed et al. (2003a)
DIN content (µmol L^{-1})	17-20	6-7	Rasheed et al. (2003b)
DIP content (µmol L^{-1})	1.4-1.9	0.5-0.6	Rasheed et al. (2003b)
Ammonium efflux (mmol m^{-2} d^{-1})	3.41 ± 0.32	2.15 ± 0.26	Rasheed et al. (2003a)
DIP efflux (mmol m^{-2} d^{-1})	0.03 ± 0.002	0.02 ± 0.001	Rasheed et al. (2003a)
Chl *a* (µg g^{-1})	0.72 ± 0.16	0.63 ± 0.12	Rasheed et al. (2003b)
Bacterial cell number (cm^{-3})	3.1 ± 0.9 x 10^9	1.5 ± 0.5 x 10^9	Schottner et a. (2011)

Monitoring of environmental parameters

In-situ water temperature (°C) and light intensity (lux) were continuously recorded at the sampling locations (10 m depth) using data loggers (Onset HOBO Pendant UA-002-64; temperature accuracy: ± 0.53°C, spectral detection range: 150 to 1200 nm). The presented light data are seasonal means of maximum irradiance measured during 11:00 and 13:00 h (see Table 5-2), and lux readings were converted to photosynthetically active radiation (PAR; µmol quanta m^{-2} s^{-1}; 400 to 700 nm wavelengths) using the following approximation: 1 µmol quanta m^{-2} s^{-1} = 52.0 lux. This conversion factor was obtained by inter-calibrating the lux readings with data obtained from a parallel deployed PAR sensor (LI-COR LI-192SA underwater quantum sensor) during a simultaneous minute-by-minute measurement over 5 h. Both readings correlated well (r^2 = 0.83) and the obtained conversion factor of 52.0 was similar to 51.2 reported by Valiela (1984). Weekly seawater samples were collected at 10 m water depth (~1 m above the sand) using high-density polyethylene canisters (5 L, n = 4) and transported back to the laboratory within 30 min. There, subsamples for inorganic nutrients, chlorophyll *a* (chl *a*), particulate organic carbon (POC) and particulate nitrogen (PN) were collected. Inorganic nutrient subsamples (50 ml) were filtered through cellulose acetate membrane filters (nominal pore size: 0.45 µm) for determination of dissolved inorganic nitrogen (DIN: ammonium, nitrate and nitrite) and dissolved inorganic phosphate (DIP) following standard methods (Murphy and Riley 1962; Strickland and Parsons 1972; Holmes et al. 1999). Ammonium was determined fluorometrically using a Trilogy Fluorometer (Turner Designs), while all other nutrients were measured photometrically with a JASCO-V630 spectrophotometer (Jasco Analytical Instruments). Detection limits for ammonium, DIP and nitrogen oxides (nitrate and nitrite) were 0.09, 0.01 and 0.02 µM, respectively. Chl *a* subsamples (1 L) were filtered onto pre-combusted GF/F filters (nominal pore size: 0.7 µm) and stored frozen at −80°C in the dark until analysis. Chl *a* was extracted with 90% acetone (12 h in the dark at 4°C) and measured using a Trilogy Fluorometer fitted with a non-acidification module (CHL NA #046, Turner Designs). Additional subsamples for POC (1 L) and PN (2 L) were filtered onto pre-combusted GF/F filters, dried in the oven (40°C, 48 h) and stored dry pending analysis. Prior to analysis dried filters were wrapped in silver foil and POC filters were acidified with 0.1 N HCl to remove any inorganic carbon. POC and PN filter contents were measured on a EuroVector elemental analyser (EURO EA 3000) with analytical precision of ≤0.1% C and ≤0.03% N.

Substrate sampling

Two neighboring back reef sites at 10 m water depth in front of the MSS covered by either carbonate or silicate sand were chosen for substrate sampling using SCUBA. The lateral distance

between the 2 sites was ~150 to 200 m, and both sites were in close vicinity (5 m distance) to the adjacent coral reef framework. Both sand types revealed distinct mineralogical, physical and biological characteristics as repeatedly measured by several previous studies (Table 5-1). Once during each season, carbonate sand (n = 8) and silicate sand (n = 8) samples were taken using custom-made PVC sediment corers (inner diameter: 4.3 cm). Additional carbonate sand samples (n = 8) showing dark-brown microbial mats (~1 to 2 mm thick) on top were collected within 100 m distance from the bare carbonate sand sampling site. Cores were immediately transported back to the MSS where the top 1 cm surface layer of each core was individually transferred into a petri-dish of equal diameter (planar surface: 14.52 cm^2) before being placed into individual incubation glass chambers (500 ml chamber for carbonate and silicate sands, 1000 ml chamber for microbial mats). During all handling, special care was taken to keep the sediment stratification and minimize the exposure time to air (<30 s). All chambers were kept in an outdoor 800 L flow-through aquarium during subsequent measurements of sedimentary O_2 fluxes and N_2 fixation over the next 2 d.

Table 5-2. Summary of key environmental water parameters monitored at 10 m water depth during 4 seasons. DIN: dissolved inorganic nitrogen, DIP: dissolved inorganic phosphate, POC: particulate organic carbon, PN: particulate nitrogen, POM (POC+PN): particulate organic matter. Values are means (n = 4) (±SE)

Environmental variable	Winter	Spring	Summer	Autumn
Irradiance (PAR)	180 (15)	257 (9)	317 (17)	159 (18)
Temperature (°C)	23.0 (0.1)	22.8 (0.1)	27.5 (0.2)	25.2 (0.2)
DIN (µM)	1.03 (0.02)	1.02 (0.11)	0.20 (0.04)	0.43 (0.08)
Ammonium (µM)	0.32 (0.04)	0.46 (0.03)	0.14 (0.03)	0.28 (0.06)
Nitrate (µM)	0.34 (0.03)	0.44 (0.04)	0.04 (0.01)	0.13 (0.05)
Nitrite (µM)	0.37 (0.06)	0.12 (0.04)	0.02 (0.01)	0.02 (0.01)
DIP (µM)	0.11 (0.01)	0.10 (0.01)	0.04 (0.01)	0.04 (0.01)
DIN:DIP	9.59 (1.09)	10.21 (0.43)	5.31 (3.40)	11.25 (2.22)
POM (µM)	7.18 (0.70)	11.52 (1.48)	8.92 (1.23)	9.68 (0.49)
POC:PN	7.34 (0.57)	8.18 (0.59)	8.34 (0.44)	10.20 (0.51)
Chl *a* (µg l^{-1})	0.21 (0.01)	0.22 (0.02)	0.10 (0.01)	0.19 (0.02)

Quantification of O_2 fluxes

All following incubations took place in the outdoor 800 L flow-through aquarium supplied with seawater pumped directly from the reef at the 10 m sampling depth (exchange rate: 4000 L h^{-1}) to ensure in-situ water temperature and nutrient concentrations. Light intensity was monitored with

lux and PAR data loggers (see above) and adjusted with black netting to those measured in-situ at 10 m water depth. O_2 fluxes of the sand samples as a proxy for primary productivity were quantified in 2 individual incubations. The first incubation was carried out on the sample collection day 1 to 2 h after sunset to measure dark respiration (R), while the second incubation was started the following day at 12:00 h for net photosynthesis (P_{net}) determination. Each sand substrate (n = 8) was incubated individually and additional chambers (500 ml, n = 8) only filled with seawater served as controls to measure planktonic background metabolism. Chambers were sealed and incubated under constant stirring (600 rpm) for 2 to 6 h (Cimarec™ i Telesystem Multipoint Stirrers, Thermo Scientific™). O_2 concentrations were measured at the beginning and end of each incubation period using a salinity- and temperature-corrected O_2 optode sensor (MultiLine® IDS 3430, WTW). End concentrations never exceeded 8.3 mg O_2 l^{-1} during P_{net} nor did they fall below 5.4 mg O_2 l^{-1} during R incubations. To calculate O_2 fluxes, O_2 start concentrations were subtracted from end concentrations, and the results were normalized by incubation time. Finally, O_2 fluxes were corrected for the seawater control signal related to the chamber volume and normalized to the sand surface area (nmol O_2 cm^{-2} h^{-1}). Gross photosynthesis (P_{gross}) rates were calculated according to $P_{gross} = P_{net} - R$. In order to calculate the N requirement for P_{gross}, the daily O_2 production was calculated assuming a daily 12 h photoperiod and values were converted into C fluxes using a community photosynthetic (PQ) and respiratory quotient (RQ) of 1.0 (1 mol O_2 = 1 mol C) according to (Taddei et al. 2008) who experimentally determined similar PQ and RQ values for coral reef sands.

Quantification of N_2 fixation

N_2 fixation rates were quantified 3 to 4 h after the P_{net} incubation ended by applying a modified acetylene (C_2H_2) reduction technique (Capone 1993; Wilson et al. 2012). C_2H_2 gas was freshly generated from calcium carbide and bubbled through fresh seawater in order to produce C_2H_2-enriched seawater. Incubations were conducted in 500 mL glass chambers containing 400 mL natural seawater of which 10% were replaced with C_2H_2-enriched seawater. Chambers were immediately sealed gas-tight with a spring-loaded glass lid equipped with a rubber injection port on top for gas sampling, and 10% of the air headspace was replaced by freshly generated C_2H_2 gas. In addition, 4 different sets of controls were tested for the reduction of C_2H_2 to ethylene (C_2H_4) production: (1) unfiltered seawater control (without sand samples, n = 8); (2) 0.2 µm-filtered seawater control (without sand samples, n = 6); (3) petri-dish in unfiltered seawater (without sand sample, n = 6); (4) sand sample in unfiltered seawater without C_2H_2 addition (natural C_2H_4 production, n = 6). Over the entire incubation period (24 h), all chambers were magnetically stirred as described above, and gas samples were taken at 0, 4, 12, 16 and 24 h. At each of these time intervals, 1 mL of gas sample was collected with a gastight syringe from each

chamber, transferred into gastight 2 mL vials previously filled with distilled water, and stored frozen upside down until analysis. C_2H_4 concentrations of gas samples were measured in the field laboratory using a reducing compound photometer (RCP; Peak Laboratories) with a detection limit of 100 ppb. Calibration of the RCP was conducted using serial dilutions of a 200 ± 4 ppm C_2H_4 standard in air (Restek). The C_2H_4 evolution in each incubation chamber was calculated according to Breitbarth et al. (2004). Values were finally corrected for the unfiltered seawater control signal related to the chamber volume and normalized to incubation time and sand planar surface area. All rates are reported as means ± SE and in C_2H_4 production rates (nmol C_2H_4 cm^{-2} h^{-1}) to allow good comparison to previous studies using the C_2H_2 reduction assay. C_2H_4 rates were only converted to N_2 fixation rates in order to calculate the percentage contribution by N_2 fixation to the N requirements for microphytobenthic primary production. Since no parallel ^{15}N calibration was applied, a theoretical ratio of 3 mol C_2H_2 reduced to 1 mol N_2 fixed was used, which has been previously found for white coral reef sands dominated by diatoms and dinoflagellates (Charpy-Roubaud et al. 2001).

Statistical analysis

All statistical analyses were carried out using Primer-E version 6 software (Clarke and Gorley 2006) with the PERMANOVA+ add on (Anderson 2001). Analyses were based on Bray Curtis similarities of the physiological parameters (square root transformed). Two-factor PERMANOVAs were performed to test for differences of the parameters N_2 fixation, P_{gross} and R rates between substrate type and season. Therefore, type I (sequential) sum of squares was used with permutation of residuals under a reduced model (999 permutations), and pairwise-tests were carried out if significant differences occurred. Finally, correlations between N_2 fixation rates and the environmental water parameters as well as between N_2 fixation and sedimentary O_2 fluxes (P_{gross} and R) were determined via linear regression.

Results

Environmental key parameters

All monitored environmental key parameters exhibited strong seasonal patterns (Table 5-2) with the most distinct differences between the stratified (summer and fall) and the deep-water mixed (winter and spring) season. Highest irradiance (PAR) was measured in spring and summer compared to winter and fall (Table 5-2). Summer also revealed the highest water temperature before it decreased during fall until it reached annual minimum values during winter and spring.

Inorganic nutrients (DIN and DIP) were negatively correlated to water temperature with at least twice as high concentrations during winter and spring compared to summer and fall, thereby clearly reflecting the seasonal change between stratification and deep-water mixing of the water column. The calculated DIN:DIP ratio ranged from 5.31 to 11.25 throughout the year but was consistently lower than the Redfield ratio (16:1), indicating N limited conditions in the water column, particularly during summer. N limitation is further suggested by the ratio of POC:PN in the water column that always exceeded the Redfield ratio (106:16). POC and PN revealed highest concentrations during spring together with highest Chl *a* concentrations in the water, thereby indicating the seasonal plankton bloom and the increased production of biomass during this period of the year.

O_2 fluxes by reef sand communities

P_{gross} rates averaged 270 ± 25 and 233 ± 17 nmol O_2 cm^{-2} h^{-1} for carbonate sand and silicate sand, respectively, across all seasons. Both bare sands exhibited similar P_{gross} rates during each season except during spring when carbonate sand exhibited significantly higher rates compared to silicate sand. The seasonal pattern was similar with significantly increased P_{gross} rates during spring and summer for both sands (Fig. 5-1, Table 5-3). Microbial mats showed no seasonal variation of P_{gross} rates but the annual average of 809 ± 43 nmol O_2 cm^{-2} h^{-1} was 3 times higher compared to carbonate and silicate sand. *R* was on annual average almost twice as low in carbonate (–70 ± 3 nmol O_2 cm^{-2} h^{-1}) compared to silicate sand (–126 ± 12 nmol O_2 cm^{-2} h^{-1}).

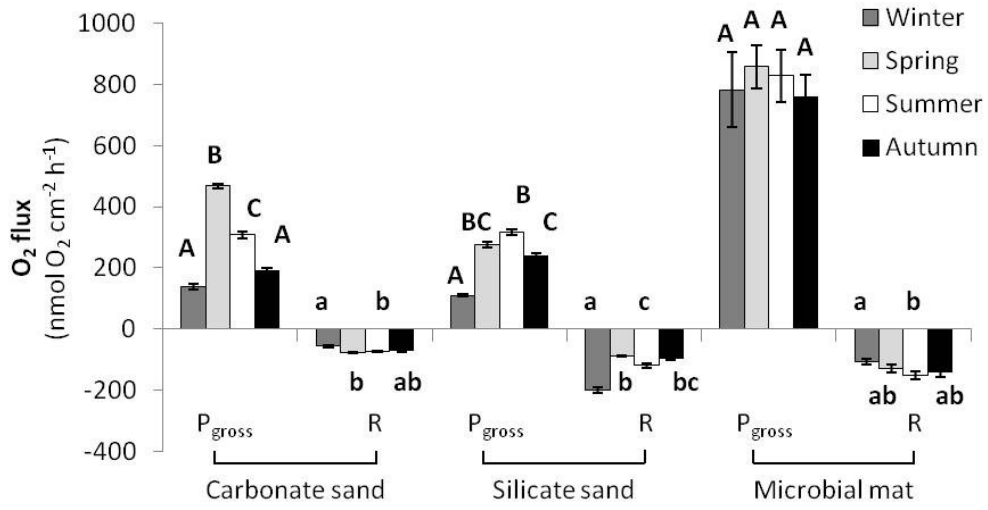

Fig. 5-1. Gross photosynthesis (P_{gross}) and dark respiration (R) rates measured as O_2 fluxes in the substrates carbonate sand, silicate sand and microbial mat during all seasons. Values: mean (n = 8) ± SE. Different letters = significant differences for P_{gross} (A–C) and R (a–c) rates between the 4 seasons for each substrate type, respectively, based on pair-wise PERMANOVA analysis

While carbonate sand showed significantly higher *R* rates during spring and summer, *R* in silicate sand peaked during winter and summer. *R* rates of microbial mats were significantly the highest during summer and averaged –135 ± 7 nmol O_2 cm^{-2} h^{-1} over all seasons, thus being in the range of *R* measured for silicate sands.

N_2 fixation by reef sand communities

On annual average, N_2 fixation by carbonate sand communities (2.88 ± 0.41 nmol C_2H_4 cm^{-2} h^{-1}) was significantly higher when compared to silicate sand (1.52 ± 0.15 nmol C_2H_4 cm^{-2} h^{-1}). The 2 sands revealed a specific seasonal variability in N_2 fixation rates (Fig. 5-2, Table 5-3). Carbonate sand was significantly more active during spring and summer thereby following the seasonal pattern of P_{gross}. This is supported by a significant positive linear relationship with N_2 fixation explaining 69% of the variation in P_{gross} (Table 5-4). In contrast, silicate sand revealed significantly the highest N_2 fixation activity during winter and summer similar to seasonal maxima of *R* rates. Correlation revealed a significant positive linear relationship between the 2 processes with 38% of the variation in *R* being explained by N_2 fixation (Table 5-4). Overall, seasonal N_2 fixation variability was more pronounced in carbonate (1.14 to 5.25 nmol C_2H_4 cm^{-2} h^{-1}) compared to silicate sand (0.81 to 2.42 nmol C_2H_4 cm^{-2} h^{-1}). Correlations to the key environmental parameters for N_2 fixation of carbonate sand revealed a significant positive linear relationship to light intensity and POM content in the water, while N_2 fixation of silicate sand was negatively correlated to POM content but not to light intensity (Table 5-4). Additionally, N_2 fixation of both sands showed a significant negative relationship to the DIN:DIP ratio in the water column.

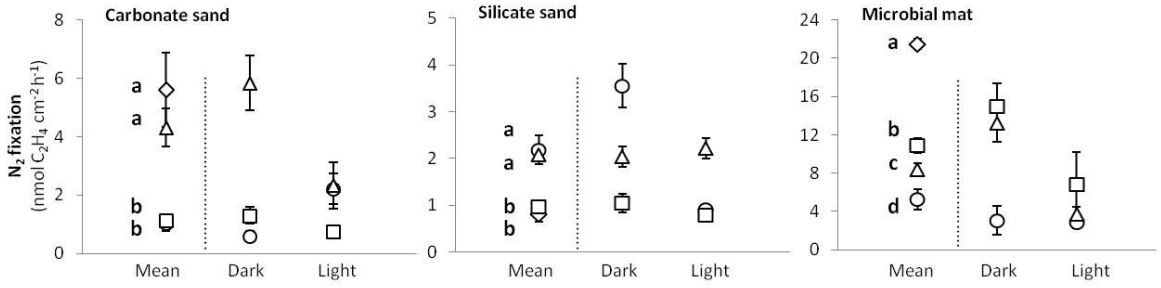

Fig. 5-2. Mean N_2 fixation (C_2H_4 production) rates of the different substrates (carbonate sand, silicate sand, microbial mat) measured during winter (circles), spring (diamonds), summer (triangles) and autumn (quadrates) over a 24 h incubation period. N_2 fixation rates for the dark and light periods are separately presented, except for spring. Values: mean (n = 8) ± SE. Different letters (a–d) = significant differences between the 4 seasons for each substrate type, respectively, based on pair-wise PERMANOVA analysis

Table 5-3. Results of 2-factorial PERMANOVAs for N_2 fixation, gross photosynthesis (P_{gross}) and dark respiration (R) rates for the substrate types (carbonate sand, silicate sand and microbial mat) during the 4 investigated seasons (winter, spring, summer and autumn) in 2013. Substrate and season were fixed effects. PERMANOVA was based on Bray Curtis similarity after square root transformation. Type I (sequential) sum of squares was used with permutation of residuals under a reduced model (999 permutations). Significant p-values are **in bold**

Variables	Effect	df	SS	MS	Pseudo-F	p-value
N_2 fixation (nmol C_2H_4 cm^{-2} h^{-1})	Substrate (Su)	2	31607	15804	140.95	**< 0.001**
	Season (Se)	3	5384	1795	16.01	**< 0.001**
	Su × Se	6	11707	1951	17.40	**< 0.001**
	Residuals	76	8521	112		
	Total	87	57219			
P_{gross} (nmol O_2 cm^{-2} h^{-1})	Substrate (Su)	2	15933	7967	150.12	**< 0.001**
	Season (Se)	3	3990	1330	25.06	**< 0.001**
	Su × Se	6	2264	377	7.11	**< 0.001**
	Residuals	76	4033	53		
	Total	87	26221			
R (nmol O_2 cm^{-2} h^{-1})	Substrate (Su)	2	4204	2102	44.89	**< 0.001**
	Season (Se)	3	358	119	2.55	0.052
	Su × Se	6	2385	398	8.49	**< 0.001**
	Residuals	76	3559	47		
	Total	87	10507			

Compared to the 2 bare reef sands, N_2 fixation activity associated with microbial mats was always almost one order of magnitude higher (seasonal average: 11.95 ± 1.16 nmol C_2H_4 cm^{-2} h^{-1}). N_2 fixation in microbial mats was significantly different between each season, with the highest rates in spring, followed by fall, summer and winter (Fig. 5-2). However, no significant relationship was found between N_2 fixation activity and the key environmental water parameters (Table 5-4). Correlation analysis between N_2 fixation and O_2 fluxes in microbial mats revealed significant positive relationships to both P_{gross} and R (Table 5-4).

Besides the seasonal variability of N_2 fixation averaged over 24 h, all 3 substrates revealed specific dark and light N_2 fixation rates with either similar dark and light N_2 fixation or relatively higher dark N_2 fixation on a 24 h basis (Fig. 5-2). Higher dark N_2 fixation was measured for carbonate sand during summer, for silicate sand during winter and for microbial mat communities during summer and autumn.

Table 5-4. Linear regression analysis between N_2 fixation rates of the 3 sand substrates (carbonate sand, silicate sand, microbial mat) and both the key environmental water parameters (DIN: dissolved inorganic nitrogen, DIP: dissolved inorganic phosphate, POM: particulate organic matter) and the O_2 fluxes (P_{gross}: gross photosynthesis, R: dark respiration) of the sand substrates. Data presented as R^2 values at significant levels of *p < 0.05, **p < 0.005, ***p < 0.001. Significant positive relationships **in bold;** significant negative relationships in *italics*

Parameter	Carbonate sand	Silicate sand	Microbial mat
Environmental factor			
Irradiance	**0.491*** **	0.023	0.057
Temperature	0.045	0.048	0.052
DIN	0.017	0.009	0.043
DIP	0.003	0.002	0.033
DIN:DIP	*0.189**	*0.259***	0.048
POM	**0.212***	*0.467****	0.006
Sedimentary O_2 fluxes			
P_{gross}	**0.690*****	*0.153**	**0.568*****
R	**0.215***	**0.375*****	**0.610*****

Discussion

Primary productivity and N_2 fixation by reef sand communities

This is the first study comparatively describing primary productivity and N_2 fixation activity of carbonate and silicate reef sand communities. We investigated the top sediment layer where highest diazotrophic activity occurs (Werner et al. 2008). The top sediment layer of both sands can be characterized as net-autotrophic and largely independent from allochthonous C input, as P_{gross} rates largely exceeded R rates. N_2 fixation rates for carbonate and silicate sands presented here agree well with values previously measured at different reef locations (Table 5-5). Shashar et al. (1994) measured higher, yet variable, N_2 fixation rates in reef sediments from a close site in the Gulf of Aqaba (Eilat). These differences may be explained by the use of mixed grain sizes ranging from gravel (5 mm) to fine (0.1 mm) and a higher proportion of large grain sized sands, while the present study measured N_2 fixation exclusively in fine grained sands (0.2 to 0.6 mm; Table 5-1).

The present study measured significantly higher N_2 fixation rates in carbonate sand than in silicate sand, and this may be explained by sediment type-specific characteristics. The coarser carbonate sand was less well sorted but had a much higher permeability and porosity than the silicate sand (Rasheed et al. 2003a; Wild et al. 2005). High permeability generates advective driven fluid fluxes between the sediment and the overlying water. This enhances solute exchange

and the flux of suspended organic matter (Rasheed et al. 2003a), while a highly porous grain structure increases the specific surface area and thus the available substrate for microbial community growth. These characteristics support microbial abundance in carbonate sands that largely exceeds cell numbers in silicate sands (Wild et al. 2004a; Wild et al. 2006; Schottner et al. 2011). Furthermore, significantly higher organic matter degradation and C turnover rates in carbonate sand occur (Rasheed et al. 2003a; Wild et al. 2005), which increase organic substrate availability (Table 5-1; Rasheed et al. 2003b). This has previously been described as a main factor controlling N_2 fixation activity in shallow carbonate sediments (O'Neil and Capone 1989). Since N_2 fixation represents an energetically costly process (due to breakage of the N_2 triple-bond), diazotrophs have a high need for energy-rich organic substrates, and thus may benefit from the higher organic C content in carbonate compared to silicate sands (Table 5-1).

Table 5-5. Acetylene reduction (AR; nmol C_2H_4 cm^{-2} h^{-1}) and inferred N_2 fixation rates (NF; mmol N m^{-2} d^{-1}) of the different reef sand communities investigated in the present study in comparison with values reported from other coral reef areas worldwide (GBR: Great Barrier Reef). AR:NF is the respective C_2H_2:N_2 conversion ratio used to calculate NF from AR.

Substrate	AR	AR:NF	NF	Location	Method	Reference
Carbonate sands	2.88 ± 0.41[a]	3	0.46 ± 0.07	Red Sea	C_2H_2	Present study
	0.04-2.32	4	0.01-0.28	Caribbean	C_2H_2	O'Neil and Capone (1989)
	0.75-1.95	3	0.12-0.31	GBR, Australia	C_2H_2	Capone et al. (1992)
	19.52 ± 17.50[b]	4	2.34 ± 2.10	Red Sea	C_2H_2	Shashar et al. (1994)
	0.18-1.02	1.8-4.8[c]	0.03-0.28	French Polynesia	C_2H_2; $^{15}N_2$	Charpy-Roubaud et al. (2001)
	-	-	0.10-0.16	Ishigaki Island	$^{15}N_2$	Miyajima et al. (2001)
	0.32	1.6[c]	0.34	French Polynesia	C_2H_2; $^{15}N_2$	Charpy-Roubaud and Larkum (2005)
	9.76 ± 3.21[a]	4	1.17 ± 0.39	New Caledonia	C_2H_2	Charpy et al. (2007)
	0.03-0.12	3	0.004-0.019	GBR, Australia	C_2H_2	Werner et al. (2008)
Silicate sands	1.52 ± 0.15[a]	3	0.24 ± 0.02	Red Sea	C_2H_2	Present study
Microbial mats	11.95 ± 1.16[a]	3	1.91 ± 0.19	Red Sea	C_2H_2	Present study
	2.7-47.8	4	0.3-5.7	California	C_2H_2	Paerl et al. (1993)
	0.96	1.6[c]	0.57	French Polynesia	C_2H_2; $^{15}N_2$	Charpy-Roubaud and Larkum (2005)
	0.59-2.97	4	0.07-0.36	Indian Ocean	C_2H_2	Charpy et al.(2012)

[a] mean ± SE [b] mean ± SD [c] Conversion factor was empirically determined

Furthermore, Schottner et al. (2011) investigated microbial communities of carbonate and silicate sand in the same area and identified sand type as a main factor structuring sediment-associated microbial assemblages. Similarly, diazotrophic assemblages likely differ between the two sands. Overall, the present findings highlight the influential role of sediment-specific characteristics (e.g. grain size, permeability, diazotrophic composition) in controlling sediment-associated N_2 fixation activities.

Unconsolidated reef sands also provide open space for the development of microbial mats which are often dominated by cyanobacteria communities and represent important contributors to benthic primary productivity and N supply in coral reefs (Charpy et al. 2010; Charpy et al. 2012; Cardini et al. 2014). The presented values for N_2 fixation compare well with values previously reported for benthic microbial mats in other coral reef ecosystems (Table 5-5). Compared to bare carbonate sand, N_2 fixation and P_{gross} rates of microbial mats were ~4.5 and ~3 times higher, respectively, thus indicating a higher de novo input of N relative to photosynthetically fixed C. This increased N availability may enable rapid accumulation of biomass and the formation of dense mats in an extremely oligotrophic environment. This is supported by the present study displaying highest microbial mat development and abundance during spring, the season also showing the highest year-round N_2 fixation activity by the mats. Nevertheless, all microbial mats in the study site were of small size and overall covered <1% of the bare carbonate sand area on the reef. Considering such low coverage by microbial mats compared to bare carbonate sand (18% of total reef area), the contribution of bare reef sand areas to total benthic N_2 fixation is likely much higher despite the lower fixation rates per unit of surface area.

Seasonal variability of primary productivity and N_2 fixation

This study investigated the response of sediment-associated primary productivity and N_2 fixation to seasonally changing environmental conditions. Carbonate and silicate sands were exposed to similarly changing environmental conditions, thus differences in the biological variables reflect a sand type specific response. Overall, seasonal variability was more pronounced in carbonate than in silicate sands. This is most likely due to sand-specific differences in permeability, specific surface area, microbial community and mineralogy leading to tighter benthic–pelagic coupling between the water column and sediment pore-water in carbonate sand. Therefore, seasonal variation in water column nutrient availability will more directly affect the nutrient inventory in the upper sediment layer (0 to 2 cm) of carbonate than silicate sand (Rasheed et al. 2003b). Schottner et al. (2011) investigated the effects of season, sediment depth and location on microbial community structure in reef sediments in the Gulf of Aqaba and found that season was the most significant structuring factor in carbonate sands, while sediment depth was more

influential in silicate sands. Seasonality and sediment depth may also determine the diazotrophic community structure, thus explaining the stronger seasonal variation in N_2 fixation activity observed for carbonate compared to silicate sand in the present study.

N_2 fixation in carbonate sand was primarily stimulated during spring and summer by seasonally increased ambient light and POM availability. This agrees with previous studies describing light as a main factor influencing sedimentary N_2 fixation (Charpy-Roubaud et al. 2001; Charpy et al. 2007; Werner et al. 2008) and suggests the dominance of phototrophic diazotrophs. The increased N_2 fixation rates in carbonate sand are mainly due to elevated diazotrophic activity during night, indicating a shift towards a more non-heterocystous bacterial community. Non-heterocystous diazotrophs separate the O_2-sensitive N_2 fixing nitrogenase enzyme complex temporally from O_2 producing photosynthesis, whereas heterocystous diazotrophs can fix N_2 also during daylight in specialized O_2-free cells (heterocyst). Night-time N_2 fixation activity also depends on a photosynthetic energy supply and correlates positively to the intensity of the previous daylight period (Charpy et al. 2007). Furthermore, N_2 fixation activity heterotrophically profits from available organic C sources. Thus, the 2-fold higher POM supply via sedimentation during spring and summer (Wild et al. 2009) likely provides additional energy for sediment-associated N_2 fixation. Despite seasonal changes in POM availability, carbonate sand communities revealed little seasonal variation in *R* rates, while primary productivity responded similarly as N_2 fixation to seasonality. This is in line with previous studies (Rasheed et al. 2002; Rasheed et al. 2003b; Wild et al. 2009) and suggests that the microphytobenthos is largely independent from allochthonous C input and likely sustains its primary productivity via N_2 fixation.

N_2 fixation in silicate sand was negatively correlated to P_{gross}, positively to *R* and was not influenced by ambient light availability; thus it strongly indicates the dominance of heterotrophic diazotrophs. Although activity of heterotrophic diazotrophs completely relies on external organic C sources, N_2 fixation in silicate sand was negatively related to POM concentrations in the water column. This implies a minor organic C supply and trophic link between the sediment and the overlaying water and is further supported by a slower transport of organic substrates through the rather diffusion-limited silicate sands compared to the highly advection-driven carbonate sands (Rasheed et al. 2003b). Despite a sand-specific seasonal response, N_2 fixation of both sands correlate negatively to the DIN:DIP water column ratio. The low DIN:DIP ratio over the year indicates N limited conditions and suggest N_2 fixation as an advantageous strategy for sedimentary primary productivity.

Contribution of N_2 fixation to primary productivity

The significant linear correlation between N_2 fixation and P_{gross} suggests a tight coupling between the 2 processes. Averaged over all seasons, daily P_{gross} in carbonate sand, silicate sand and microbial mats was calculated to require 4.89, 4.21 and 14.66 mmol N m^{-2} d^{-1}, respectively, assuming the Redfield ratio (106:16) for primary productivity applicable to microphytobenthic communities of reef sands (Delesalle et al. 1998; Charpy-Roubaud et al. 2001; Werner et al. 2008). Thus, on annual average, N_2 fixation rates measured here would supply 8.4, 8.1 and 13.3% of the total N needed for microphytobenthic primary productivity in carbonate sand, silicate sand and microbial mats, respectively. These estimates are similar to a New Caledonian reef lagoon, where N_2 fixation in reef sands and microbial mat communities contributed between 5 and 21% of the N required for primary productivity (Charpy et al. 2007; Charpy et al. 2010). Also at Sesoko, Japan, similar contributions of 5.7% for sandy bottoms and 10.0 to 26.5% for microbial mats were calculated (Casareto et al. 2008). However, these estimates likely underestimate the contribution of N_2 fixation, as a substantial quantity of N is recycled (autochthonous N-input) within the reef sediments (Crossland et al. 1991; Charpy-Roubaud et al. 2001) thereby largely reducing the photometabolic demand for 'new' N (allochthonous N-input) but increasing the relative N input via N_2 fixation.

DIN fluxes from the sediment to the overlaying waters were shown to importantly fuel primary productivity of the whole reef benthos (Charpy-Roubaud et al. 1996; Charpy-Roubaud et al. 2001; Rasheed et al. 2002). At the study site, carbonate sand shows a 2.8 higher DIN content and 1.6 higher ammonium efflux to the overlaying water compared to silicate sand, which may be explained by its generally higher N_2 fixation and organic matter degradation rates (Table 5-1, Rasheed et al. 2003a; Rasheed et al. 2003b). Overall, the present findings highlight the significant role of N_2 fixation as an important N source for sedimentary primary productivity. By releasing large quantities of fixed N to the overlaying water, reef sediments, particularly carbonate sands, may significantly support primary productivity of other benthic organisms and of the entire coral reef ecosystem.

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Chapter 6
A seasonal dinitrogen fixation budget for a northern Red Sea coral reef

Abstract

Biological dinitrogen (N_2) fixation (diazotrophy) relieves marine primary producers of nitrogen (N) limitation in a large part of the world oceans. N concentrations are particularly low in tropical regions where coral reefs are located, and N is therefore a key limiting nutrient in these highly productive ecosystems. However, the role and importance of diazotrophy for primary productivity of coral reef ecosystems is still not resolved. Here we present N_2 and carbon (C) fixation rates in a high spatiotemporal resolution for a fringing reef in the northern Red Sea, based on a combination of physiological and benthic cover measurements. We found that diazotrophy was omnipresent in space and time. Contribution of N by planktonic diazotrophs was ca. 20-fold lower compared to the contribution by the benthos. Benthic N_2 fixation rates were 0.16 to 0.92 mmol N m^{-2} d^{-1}. These results imply that approximately 10% of the overall net reef production was supported by autochthonous input of N from N_2 fixation. If our findings can be extrapolated to other reef areas, this suggests reef-wide N_2 fixation rates from 1.60 to 2.58 Tg N yr^{-1}, placing coral reef ecosystems among the benthic communities contributing most to marine fixed N inputs.

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Chapter 6 Reef-wide N_2 fixation budget

Introduction

Nitrogen (N) is a fundamental component of all living organisms. In particular, N is needed in primary producers in capturing energy through photosynthesis and in building biomass, leading to a tight coupling of the N and the carbon (C) cycle (Gruber and Galloway 2008) and dictating constrains to the flexibility of the ecosystem C:N stoichiometry (Geider and La Roche 2002). However, bioavailable forms of N are scarce in unperturbed marine ecosystems due to low atmospheric inputs and N losses, and primary productivity is often N limited (Vitousek and Howarth 1991). This limitation is of particular significance in coral reef ecosystems, as these are among the most productive ecosystems on earth while experiencing very low ambient concentrations of dissolved nutrients. Here, biological dinitrogen (N_2) fixation (diazotrophy) is thought to play an important role in replenishing the N reservoir, maintaining the ecosystem productivity and its biological storage of carbon (D'Elia and Wiebe 1990; Capone 1996; O'Neil and Capone 2008). Since the early work by Wiebe et al. (1975) many studies found high rates of N_2 fixation associated with several benthic substrates, ranging from sediments and cyanobacterial mats to macroalgae and scleractinian corals (see reviews by O'Neil and Capone (2008) and Cardini et al. (2014)). Moreover, after first evidence of diazotroph-coral interactions by Shashar et al. (1994a) and the subsequent discovery of diazotrophic cyanobacteria in the tissue of scleractinian corals of the genus Montastrea (Lesser et al. 2004), there has been emergent recognition of the potential contribution of N_2-fixing symbioses in corals. While reef sediments and cyanobacterial mats show high rates of N_2 fixation activity (O'Neil and Capone 1989; Capone et al. 1992; Shashar et al. 1994b; Charpy-Roubaud et al. 2001), N_2-fixing coral symbioses may also be responsible of significant inputs of N on an ecosystem level, particularly in high coral coverage areas of the reef. However, rates of N_2 fixation in coral reef habitats are highly variable (O'Neil and Capone 2008) and are potentially affected by global and local anthropogenic disturbances (Cardini et al. 2014). Therefore, it is increasingly important to quantify N_2 fixation at the reef scale, and under changing environmental conditions, if we want to understand how diazotrophy is contributing to the functioning of reef ecosystems. However, because of the uncertainty in (i) the distribution of N_2 fixation in coral reefs and (ii) the tightness of the coupling between N_2 fixation and primary production, the ecological significance of N_2 fixation in coral reef ecosystems is still not resolved. Thus, the objective of this paper is to provide an assessment of the quantitative importance of the process of N_2 fixation for primary production in coral reef ecosystems. While some studies attempted to do this before (Capone and Carpenter 1982; Capone 1996), no systematic and comprehensive analyses considering the multifarious diversity of coral reef substrates have been performed so far that would allow for a synthesis. Using results from a year-long study at a highly seasonal fringing reef in the northern Red sea, we evaluate here the

contribution of all key benthic players to the production of fixed C and N in the reef environment. Moreover, geographic information system (GIS) analyses allow us to discuss the relative importance of all typical reef-associated habitats to primary production and N_2 fixation of the entire ecosystem. Finally, we indicate the most probable environmental factors driving N_2 fixation in coral reef habitats, with consequent implications for predicting the potential effects of climate change on this important biogeochemical process.

Materials and methods

Study site

This study was carried out at a northern Red Sea fringing reef (Aqaba, Jordan) during two expeditions (January - April 2013 and August - December 2013). Four sampling campaigns, each encompassing three weeks, were performed in February, April, September, and November, respectively. The timing of these periods (hereinafter called winter, spring, summer, and autumn respectively) was determined from literature to best cover the annual diversity in environmental conditions in this area of the Red Sea, characterized by an annual cycle of deep water mixing from December until May and stratification down to 200 m water depth from June until November (Silverman et al. 2007; Carlson et al. 2014). All measurements were conducted on site in the aquarium and laboratory facilities of the Marine Science Station (MSS, University of Jordan; location: 29° 27′ N, 34° 58′ E) and monitoring and sample collection took place on the adjacent fore reef slope at 10 m water depth. The fringing reef system extends approximately 1.1 km along its reef crest bordering the coastline. This reef has the typical morphology and zonation of Red sea fringing reefs, and can be partitioned into a reef flat, a reef crest and slope (hereafter: reef crest), and a forereef facing the open sea (Mergner and Schuhmacher 1974; Naumann et al. 2012). The fore reef consists of an upper, middle and lower part characterized by distinctive morphological features and species composition (hereafter: sand belt, transition zone and forereef). The present study focuses on the reef area framed by the reef flat and the fore reef slope at the maximum investigated depth (depth range: 0.5 - 20 m).

Environmental monitoring

Monitoring of the key environmental parameters was carried out as described in chapter 1. Briefly, light availability (lux) and water temperature were measured at 1 min frequency with Onset HOBO data loggers (Pendant UA-002-64; temperature accuracy: ± 0.53 °C, spectral detection range: 150 – 1200 nm), and lux measurements were subsequently converted to photosynthetically active radiation (PAR) using a conversion factor obtained from parallel

measurements with a LI-COR LI192SA underwater quantum sensor. Sampling for dissolved inorganic nitrogen (DIN = NH_4^+ + NO_x), dissolved inorganic phosphorous (DIP), chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), particulate organic carbon (POC) and particulate nitrogen (PN) was carried out once a week by SCUBA using high density poly-ethylene (HDPE) containers. Subsamples for inorganic nutrients (50 mL) were filtered through cellulose acetate filters (nominal pore size = 0.45 µm), and nutrient concentrations were measured according to Holmes et al. (1999), Murphy and Riley (1962), and Strickland and Parsons (1972). NH_4^+ was measured with a Trilogy Fluorometer (Turner Designs), while PO_4^{3-} and NO_x were measured with a JASCO-V630 photometer. Detection limits for NH_4^+, PO_4^{3-}, and NO_x were 0.09, 0.01, and 0.02 µmol L^{-1}, respectively. Subsamples for Chl *a* (1 L) were filtered onto pre-combusted GF/F filters and stored in the dark at -80 °C. Chl *a* was later extracted with 90 % acetone for 12 h in the dark at 4 °C and measured on the Trilogy Fluorometer using the non-acidification module (CHL NA #046, Turner Designs). Subsamples for DOC (50 mL) were vacuum filtered (max. pressure 20kPa) through a pre-combusted GF/F filter into 30 mL HDPE bottles using an acid-washed and MQ and sample-rinsed filtration apparatus, acidified with 80 µL of 18.5 % HCl and stored at 4 °C in the dark until analysis. Samples were measured using the high-temperature catalytic oxidation (HTCO) method on a Shimadzu TOC-V_{CPH} TOC analyser with analytical precision of < 3 %. Subsamples for POC (1 L) and PN (2 L) were filtered onto pre-combusted GF/F filters (nominal pore size = 0.7 µm), which were subsequently dried at 40 °C for 48 h and sealed in Eppendorf tubes pending further analysis. Prior to their measurement, POC filters were acidified with 0.1 N HCl to remove any inorganic carbon. POC and PN samples were measured using a EuroVector elemental analyser (EURO EA 3000) with an analytical precision of ≤ 0.1 % (C) and ≤ 0.03 % (N).

Benthic community composition

Line point intercept surveys (LPI) were conducted to quantify benthic community composition and identify the dominant benthic categories. In each season, three LPI, each of 50 m length, were carried out at 0.5, 1.0, 5.0, 10.0 and 20.0 m water depth, at increasing distance from land along transects parallel to the shore-line, for a total of 120 LPI. The transect depths were selected as in previous studies (Naumann et al. 2012) to include all major reef habitats and to be representative of the seafloor coverage by all dominant benthic categories. Intervals of 0.5 m between intercepts resulted in 101 data points per LPI. Results obtained from all LPI in each depth were used to calculate the percentage coverage for each reef habitat (i.e., reef flat, reef crest, sand belt, transition zone and forereef) in each season (Appendix 4 - Table 1), and to select the benthic categories that together accounted for ca. 90% of the 2D reef seafloor coverage on average (Appendix 4 - Table 2 - 3) to be subsequently incubated.

Reef bathymetry and zonation

Bathymetric contours were obtained merging data from the Aqaba Special Economic Zone Authority (ASEZA) GIS Unit and original bathymetric data obtained in the field, overlaid onto a high-resolution satellite image (© Digital Globe). Bathymetric contours were subsequently processed into a continuous raster using the ArcGis Topo to Raster tool (ESRI ArcGis®), with the final raster having a cell size of ~ 2.9 m. The habitat map was produced based on prior knowledge of the reef zonation (Mergner and Schuhmacher 1974), on the interpretation of the high-resolution satellite image, and on ground truthing during the line point intercept surveys. Finally, zonal statistics referring to the bathymetric raster was calculated for each habitat using ArcGis 3D Analyst tools. The slope 2D area (i.e., considering the depth profile) for each habitat was obtained from the planar 2D area using the bathymetric raster as base layer. The bathymetric and habitat maps are presented in Fig. 6-1, and zonal statistics for each habitat are reported in Appendix 4 - Table 2 and were used in our subsequent ecological analyses.

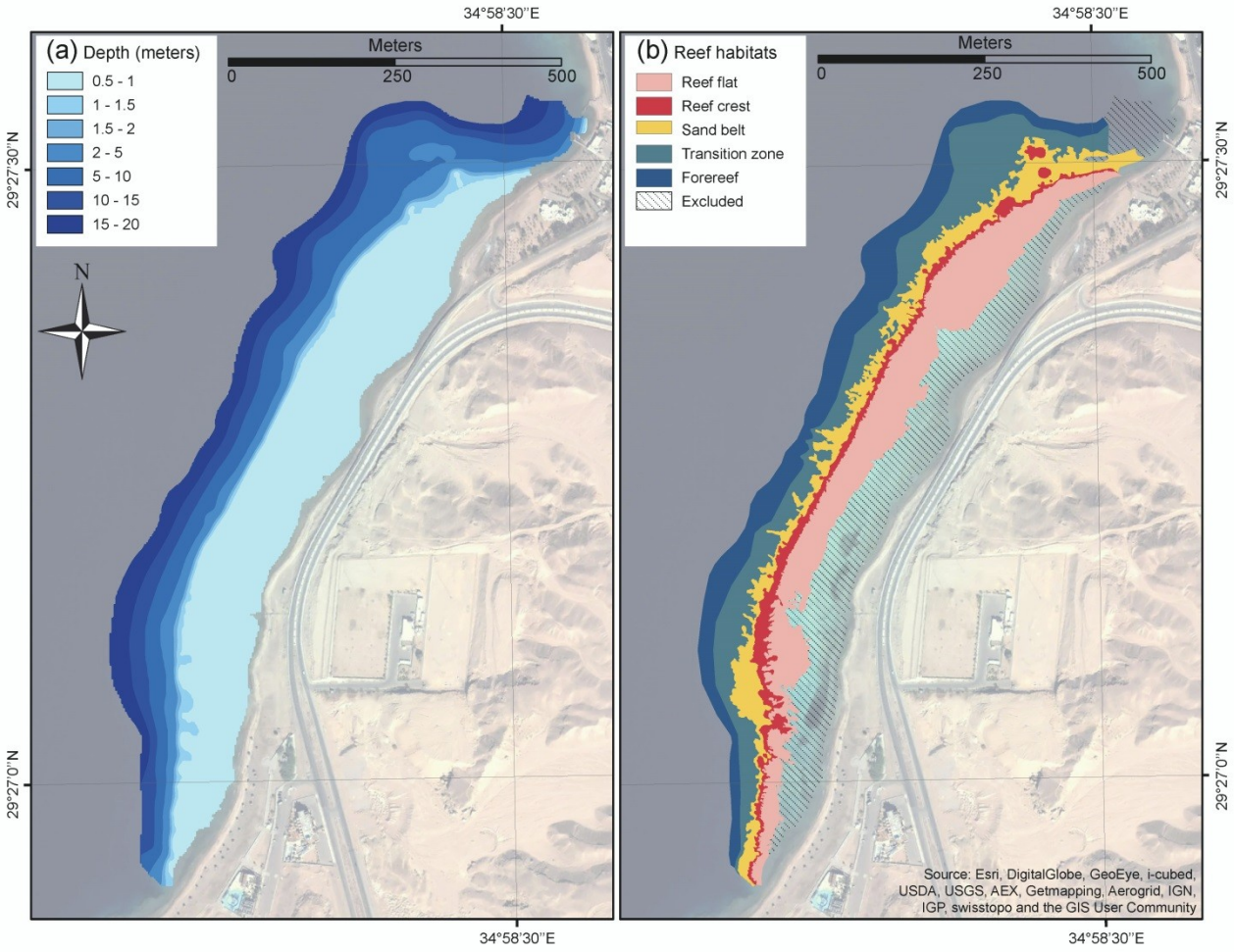

Fig. 6-1. Bathymetry (a) and habitat (b) maps of the reef site.

Sample collection and surface area quantification

In each season, specimens of the key benthic categories were collected for measurement of primary production and dinitrogen fixation. For an overview and description of the benthic categories incubated and respective replication see Appendix 4 - Table 3. All benthic categories were sampled on the reef and transported back to the MSS facilities within 60 min of sampling. Hard and soft corals, as well as turf algae covered branches, were fixed to ceramic tiles with Reef Construct. Coral rock fragments were stable without fixation. Macroalgae were fixed to ceramic tiles or Petri dishes with rubber bands while protecting the algal tissue from crushing. Sediment and cyanobacterial mat samples were collected with round PVC sediment corers (internal diameter 43 mm) and transported back to the MSS laboratory where the top 1 cm of the core was extruded and placed in a Petri dish with the same internal diameter as the sediment corer. Organisms were maintained in an outside flow-through aquarium of 800 L with an exchange rate of 4000 L h^{-1} of water which was pumped straight from the coral reef at 10 m water depth, thus resembling in-situ conditions of temperature and inorganic nutrients. In-situ light conditions from 10 m water depth were generated using layers of black mesh and monitored with data loggers (Onset HOBO Pendant UA-002-64). Hard and soft corals were maintained for at least 7 d, and macroalgae for at least 24 h, to recover from extraction. Sediment, turf algae, coral rock, and cyanobacterial mats were incubated on the day of collection to minimize any changes in their communities before incubation. The 3D surface areas of all benthic categories were also quantified. 3D surface areas of hard and soft corals, turf algae, and coral rock were measured using advanced geometry (Naumann et al. 2009; Bednarz et al. 2012). Macroalgae surface areas were measured by planar photograph analysis in ImageJ v.1.48 (Schneider et al. 2012), doubling the one-sided surface area to obtain the total 3D specimen surface area (Haas et al. 2010). Sediment and cyanobacterial mat surface areas were calculated from the circular surface area sampled by the sediment corer (πr^2). Specimen 3D surface area was related to the respective reef slope 2D area at the water-bottom interface determined during LPI surveys to generate the 2D to benthos 3D area conversion factor for each taxon. The specific resulting 2D to 3D conversion factors are summarized in Appendix 4 - Table 3.

Physiological measurements

Specimens were incubated for measurements of net photosynthesis, dark respiration and dinitrogen fixation. Briefly, incubations were conducted under in-situ conditions in the flow-through aquarium in closed glass chambers (0.5-1 L). Magnetic stirrer plates (Cimarec™ i Telesystem Multipoint Stirrers, Thermo Scientific™) and Teflon-coated stir bars in the chambers provided water movement during the incubations at 600 rpm. All material used to fix organisms

was thoroughly cleaned before each incubation with a fine brush to remove potential epi-biota. Net photosynthesis and dark respiration of each specimen were quantified by their respective oxygen (O_2) fluxes in the light (i.e., net photosynthesis) and in the dark (i.e., dark respiration) with an O_2 optode and a conductivity probe (MultiLine® IDS 3430, WTW GmbH, Weilheim, Germany, accuracy: ± 0.5% of measured value). In addition, seawater controls with and without fixation tiles were run in parallel to correct for metabolic activity in the incubation seawater or potential microbiota on the tiles. Specimen dinitrogen fixation was quantified using the acetylene (C_2H_2) reduction assay during a full dark-light cycle incubation (20 hours), replacing 10% of the water volume with C_2H_2-enriched seawater and 10% of the headspace with C_2H_2 gas, both produced using calcium carbide. 1 mL gas samples were collected from each chamber at time intervals (0, 4, 12, 16 and 24 h) and transferred into gastight 2 mL vials previously filled with distilled water, subsequently stored frozen upside down until analysis. Ethylene (C_2H_4) concentrations were measured using a customized reducing compound photometer (RCP) (Peak Laboratories, detection limit = 100 ppb), calibrated with serial dilutions of a 200 ± 4 ppm C_2H_4 standard in air (Restek, Bellefonte, PA, USA). In addition, 4 sets of controls were also tested for ethylene (C_2H_4) production: 1. unfiltered seawater control (without fragments, n = 8); 2. 0.2 µm-filtered seawater control (without fragments, n = 6); 3. tiles in unfiltered seawater (without fragments, n = 6); 4. fragments in unfiltered seawater without C_2H_2 addition (natural C_2H_4 production, n = 6). Only unfiltered seawater showed measurable ethylene (C_2H_4) production. C_2H_4 fluxes were calculated following the endpoint assays equations of Capone (1993). Finally, O_2 and C_2H_4 fluxes of the biological samples were corrected for unfiltered seawater control signals and normalized to incubation time and specimen 3D surface area.

Data treatment

O_2 and C_2H_4 fluxes were converted into carbon (C) and nitrogen (N) fluxes assuming theoretical molar ratios of CO_2:O_2 = 1 and C_2H_4:N_2 = 4. Whilst we acknowledge that these ratios may vary depending on the biological system under study (Larkum 1988; Larkum et al. 1988; Falkowski and Raven 2007), many systems display ratios not substantially different from these theoretical ratios (Capone 1988; Clavier et al. 1994), and the use of the conservative theoretical ratios does allow here to compare C and N_2 fixation rates by different reef organisms. To estimate daily gross primary production (GPP), every respiration measurement was added to its corresponding net photosynthesis measurement and extrapolated to per day estimates assuming 12 hours of daylight, while daily respiration (R) and dinitrogen fixation (BNF) were calculated on a 24 hours diel cycle. The daily net primary production (NPP) was obtained by subtracting each R measurement from its respective GPP. Daily C and N fluxes obtained were then scaled to the reef benthos 3D area considering the 2D:3D factors calculated for each benthic category (i.e., taking into account their

respective three-dimensional structure) and are reported in Appendix 4 - Table 3 expressed as mmol (C or N) m^{-2} benthos 3D area d^{-1}. To extrapolate our data to the ecosystem level, individual rates were used to calculate the contribution of each investigated benthic category to benthic GPP, NPP and BNF taking into account the specific percentage cover related to the slope 2D area of each reef habitat in each season (Appendix 4 – Fig. 1,Appendix 4 - Table 1-2). Here, rates measured under light conditions resembling 10 m water depth were extrapolated to different reef habitats (i.e., depths, range = 0.5 - 20 m). While this assumption may underestimate or overestimate the relative contribution of each reef habitat to the whole reef area, we believe it does provide a solid basis for assessing the overall significance and range of C and N_2 fixation that may occur at the ecosystem level. To allow comparison with other systems, resulting contributions in mmol (C or N) d^{-1} were subsequently normalized to the planar 2D reef area and are expressed as mmol (C or N) m^{-2} planar 2D area d^{-1}. To estimate the percentage contribution of BNF to NPP, a C:N:P ratio of 550:30:1 was assumed for reef benthic autotrophs (Atkinson and Smith 1983; Atkinson and Falter 2003).

Statistical analysis

Analyses were conducted using Primer-E v6 (Clarke and Gorley 2006) with the PERMANOVA extension (Anderson et al. 2008). Differences in the benthic community cover among seasons (fixed, four levels) and reef habitats (fixed, five levels) were tested for significance using multivariate analysis of variance (PERMANOVA) on a Bray-Curtis similarity matrix of square root transformed data. Type I (sequential) sum of squares was used with permutation of residuals under a reduced model (9999 permutations). Differences among habitats were visualized using principal coordinate analysis (PCO). Vector plots using Pearson ranking based on correlations > 0.4 were overlaid on the PCO plot to visualize relationships between variables and the ordination axes. Canonical analysis of principal coordinates, CAP (Anderson et al. 2008) was used to identify specific groups in the benthic community responsible for differences among seasons, and benthic categories with correlations > 0.4 were overlaid on the plot as vectors. Differences in BNF, GPP, and R among seasons (four levels) were tested for significance using PERMANOVA on a Bray-Curtis similarity matrix of square root transformed data. Type I (sequential) sum of squares was used with unrestricted permutation of raw data (9999 permutations). If data did not conform to assumptions of homogeneity after transformation, tested with PERMDISP (Anderson et al. 2008), a conservative α value of 0.01 was used (Underwood 1997). Differences among seasons were visualized using PCOs, with benthic categories correlation vectors overlaid on the plots.

Results

Seasonal environmental conditions

Table 6-1. Environmental conditions during the four different seasons at the study site, and comparison with typical coral reef values as reported by Atkinson and Falter (2003) and Kleypas et al. (1999). Planktonic N_2 fixation rates are compared with rates obtained by Großkopf et al. (2012) for oligotrophic ocean waters using the ^{15}N dissolution method. Values are means with SD or range of values in parentheses.

Parameter	Winter	Spring	Summer	Autumn	Typical conditions
Temperature (°C)	22.3 (0.1)	22.4 (0.1)	27.0 (0.2)	24.9 (0.1)	27.6 (1.1)
Light (mol quanta m^{-2} d^{-1})	3.45 (0.26)	5.76 (0.13)	6.99 (0.32)	3.80 (0.34)	-
DIN (µM)	1.25 (0.13)	1.11 (0.07)	0.31 (0.06)	0.47 (0.09)	(0.1 - 1.1)
DIP (µM)	0.11 (0.01)	0.10 (0.01)	0.04 (0.01)	0.04 (0.01)	(0.05 - 0.3)
Chl *a* (µg L^{-1})	0.21 (0.01)	0.20 (0.02)	0.10 (0.00)	0.19 (0.02)	-
DOC (µM)	76.62 (3.82)	73.07 (3.13)	88.60 (1.93)	85.15 (1.46)	~100
POC (µM)	5.90 (0.56)	9.47 (1.39)	7.62 (1.01)	8.86 (0.38)	~10
PN (µM)	0.94 (0.05)	1.18 (0.14)	0.92 (0.11)	0.90 (0.04)	<1.0
POC:PN	6.81 (0.59)	7.95 (0.47)	8.25 (0.34)	9.86 (0.45)	~10
BNF (nmol N L^{-1} d^{-1})	3.1 (4.8)	3.3 (5.6)	8.1 (9.2)	2.6 (4.0)	2.5 (0.0 - 12.4)

Mean weekly measurements of environmental parameters were variable over time (Table 6-1) reflecting differences between the mixed and stratified seasons. Seawater temperature was low and stable throughout winter and spring but increased to 27 °C in summer and decreased again in autumn. Daily light availability increased from winter to spring and summer, while decreased in autumn. Inorganic nutrient concentrations and Chl *a* were significantly lower in summer and autumn than in winter and spring. Conversely, DOC showed a reversed pattern, with higher concentrations in summer and autumn. POC concentrations did not differ between the seasons, while PN was lower in summer and autumn, with subsequent increase of the POC:PN ratio. Planktonic N_2 fixation was highly variable within seasons (Table 6-1), with no significant seasonal differences despite an average increase in summer. Overall, the stratified season resembled warm and highly oligotrophic conditions, typical of tropical coral reefs worldwide, while the mixed season had environmental conditions which were typical of marginal reef communities (Table 6-1; Kleypas et al. 1999).

Benthic community composition

Analysis of LPI data yielded the percentage coverage by all benthic categories as reported in Appendix 4 - Table 1. Multivariate analysis of the benthic community (Appendix 4 - Table 5)

showed a significant effect of the factors 'Season' and 'Reef Habitat' (PERMANOVA: P < 0.001), but not of their interaction (PERMANOVA: P>0.1). The different reef habitats are characterized by distinctive communities (Fig. 6-2). The reef flat was correlated with coral rock, turf algae on rock, crustose coralline algae (CCA) and zoanthids. The reef crest was highly correlated with coral rock, *Millepora*, turf algae on rock and CCA. Conversely, the sand belt was correlated with bare sediment, coral rubble and turf algae on sediment, whereas the fore reef was correlated with a high hard and soft coral coverage. The transition zone had an intermediate community between the sand belt and the fore reef, correlated with the presence of sponges, sediment and microbial mats on sediment, but also hard and soft corals (Fig. 6-2). Differences among seasons were less noticeable in the cloud of data, but the CAP analysis showed that seasonal changes were mainly attributable to macroalgae, microbial mats and turf algae cover (Appendix 4 - Fig. 1), which were higher in winter and spring (turf algae); only in spring (macroalgae and microbial mats on rock) or in spring and summer (microbial mats on sediment).

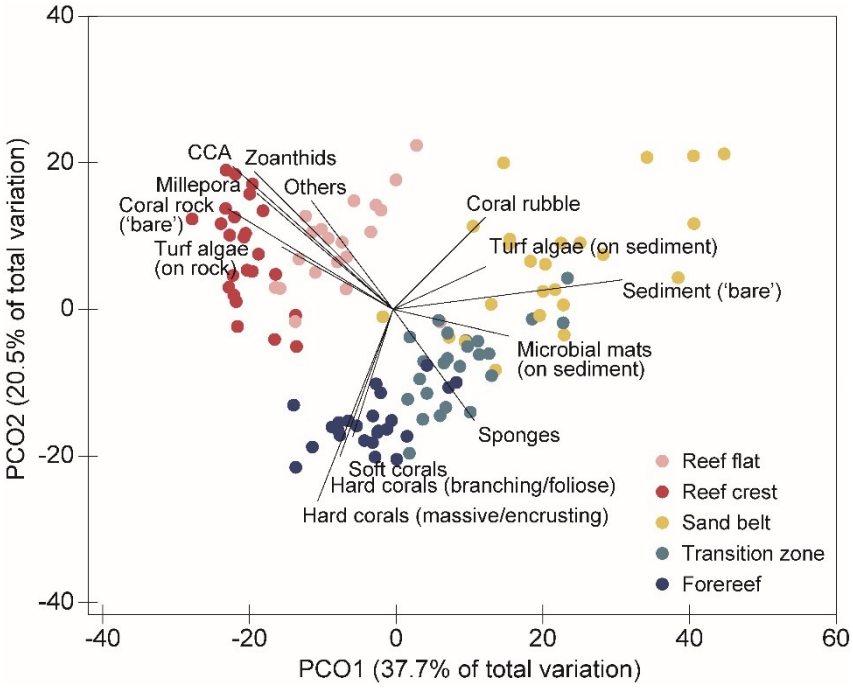

Fig. 6-2. Principle coordinate analysis (PCO) of benthic community cover at the different reef habitats. Different colors represent different habitats as in the habitat map in Fig.1. Vector overlay represents correlations > 0.4 based on Pearson ranking.

Metabolic rates of benthic primary producers

Benthic community metabolic rates showed significant differences between seasons (PERMANOVA: p < 0.001 and pair-wise comparisons, Appendix 4 - Table 6). GPP displayed a weak seasonal pattern, but generally increased in spring and summer compared to the other

seasons for all benthic primary producers (Appendix 4 - Fig. 2a). Hard corals, soft corals, microbial mats and turf algae on rock displayed the highest rates of benthos 3D area-related primary production, while sediment the lowest (appendix 4 - Table 4). Respiration increased in summer for all benthic categories but for macroalgae and sediment which displayed higher respiration in spring compared to the other seasons (Appendix 4 - Fig. 2b). The highest benthos 3D area-related respiration rates were associated with sponges and hard and soft corals, the lowest with sediment (Appendix 4 - Table 4). N_2 fixation rates associated with benthic primary producers showed the clearest seasonal pattern, with highest rates recorded in summer compared to all other seasons, except for sand-associated categories (macroalgae, sediment and microbial mats) which showed the highest associated N_2 fixation activity in spring compared to the other seasons (Appendix 4 - Fig. 2c). The highest benthos 3D area-related N_2 fixation rates were associated with microbial mats on rock, the lowest with soft corals and sponges (Appendix 4 - Table 4).

Contribution of the benthic community to fixed C and N inputs

Scleractinian hard corals (all growth forms) contributed the largest share of benthic GPP (41 - 76 %) in all reef habitats and in all seasons (Fig. 6-3a). Their contribution to GPP was unrivalled even in the sand belt and the reef flat, where their benthic cover was generally less than 20 % (Appendix 4 - Table 1). Soft corals importantly contributed to GPP in the forereef (8 - 11 %), in the transition zone (13 - 20 %) and on the reef flat (7 - 16 %) but their contribution was low in the other reef habitats. The contribution of 'bare' sediment to GPP was only relevant in the sand belt (4 - 20 %). 'Bare' hard substrates contributed importantly to GPP in the shallow reef habitats (i.e., sand belt, reef crest and reef flat) in winter, summer and autumn (11 - 26 %) but their contribution decreased significantly in spring (5 - 8 %) as a result of lower rates (Appendix 4 - Fig. 2, Appendix 4 - Table 4) and lower benthic coverage (Appendix 4 - Table 1). Turf algae provided a large share of benthic GPP in shallow reef habitats (i.e., reef flat, reef crest) in winter and spring (9 - 20 %) but less in summer and autumn. Turf algae also importantly contributed in winter in the sand belt (11 %) because of a sharp increase in their benthic coverage (Appendix 4 - Table 1, turf algae on sediment). Macroalgae were less important to the overall reef GPP, but their contribution increased in shallow and sandy reef habitats (i.e., reef flat, reef crest, sand belt) in spring (6 - 10 %). Microbial mats contributed only minor fractions to reef GPP, but their contribution was highly variable and depended on their benthic coverage, increasing up to 11 % on the reef flat in summer (Fig. 6-3a). Conversely, sponges showed negligible contributions to benthic GPP in all reef habitats and in all seasons. Benthic categories contributing most to BNF (Fig. 6-3b) were microbial mats, turf algae, 'bare' hard substrates and 'bare' sediment, thus drawing a very different picture when compared with the categories contributing most to GPP (Fig. 6-3a). Despite very low benthic coverage (0 - 1.7 %) microbial mats provided a large fraction of BNF in spring

in all reef habitats (27 - 64 %), but their contribution to BNF was highly variable in the other seasons. Turf algae contributed the largest share of BNF in winter in all reef habitats (41 - 66 %), but were also important in the other seasons, particularly in the reef crest and reef flat (12 - 41 %). 'Bare' hard substrates importantly contributed to BNF in all reef habitats and seasons (11 - 69 %), but particularly in summer and autumn in the reef crest and reef flat (38 - 69 %). 'Bare' sediment contributed a large fraction of BNF in the sand belt in all seasons (31 - 41 %), and was also important for BNF in the transition zone (17 - 34 %). The contribution of hard corals was low but occasionally relevant particularly in the forereef, increasing up to 18 % in winter and 17 % in summer. Macroalgae showed significant individual BNF rates (Appendix 4 - Table 4) but very low benthic coverage (Appendix 4 - Table 1), consequently contributing a low fraction of BNF to the reef (Fig. 6-3b). Sponges and soft corals showed negligible contributions to BNF in all habitats in all seasons (Fig. 6-3b).

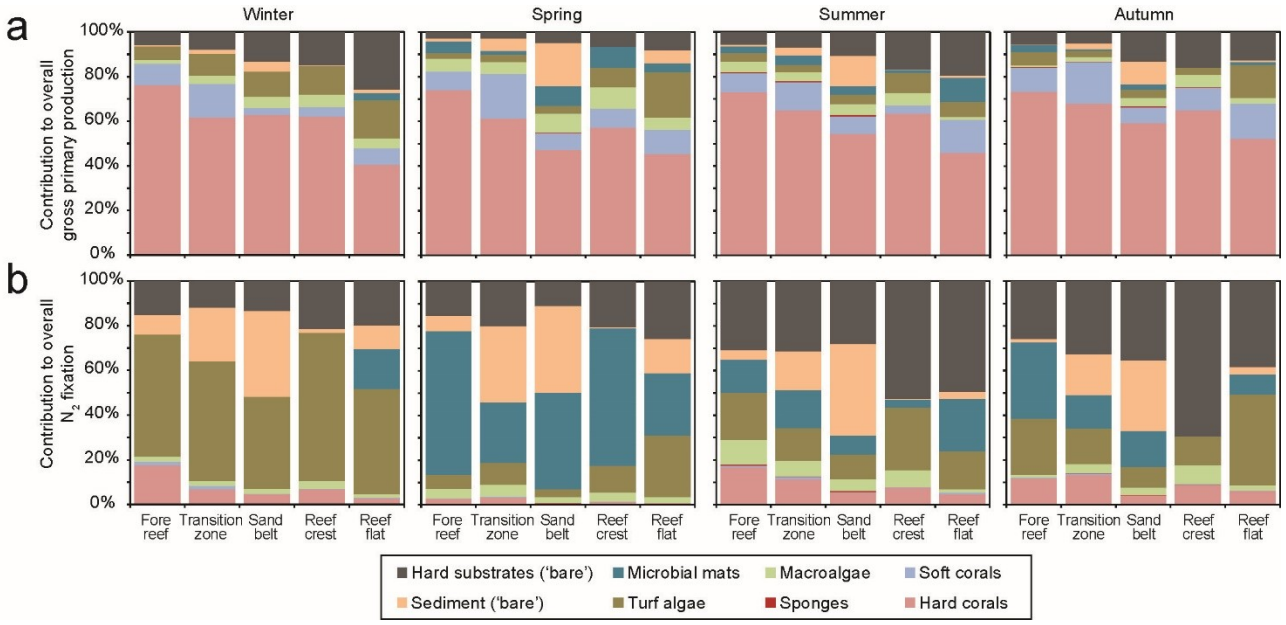

Fig. 6-3. Contribution of the main benthic components to GPP (a) and BNF (b) during the four seasons, in each reef habitat. All hard coral morphologies are classified as one category; coral rock and dead corals are grouped into hard substrates ('bare'); macroalgae, microbial mats and turf algae include categories 'on rock' and 'on sediment'.

Productivity and dinitrogen fixation in the different reef habitats

Overall, the contribution of the water column to GPP and BNF to the entire reef system was negligible (Fig. 6-4a,b). Areal GPP increased in spring and summer compared to the other seasons, but remained high all year around (Fig. 6-4a). Reef habitats that showed the highest areal rates were the ones with the highest hard coral cover (i.e., reef crest, transition zone and forereef).

The lowest GPP was associated with the sand belt in all seasons. GPP was highest in summer in shallow reef habitats (i.e. reef crest and reef flat), while it was highest in spring in the other reef habitats. Areal BNF displayed a strong seasonal pattern with 4-fold higher rates in spring and summer compared to winter and autumn (Fig. 6-4b). The highest areal BNF rates were associated with the reef flat and the reef crest. BNF in the sand belt was highest in spring, while BNF in all the other reef habitats was highest in summer. On an annual average, the contribution of BNF to NPP was highest in the sand belt, and lowest in the forereef (Table 6-2).

Table 6-2. Community annual mean dinitrogen fixation (BNF), gross primary production (GPP), respiration (R), net primary production (NPP) in mmol (C or N) m^{-2} planar 2D area d^{-1} and % contribution of BNF to NPP in the different reef habitats. Values are means followed by the range in parentheses.

Habitat	BNF	GPP	R	NPP	% Contribution
Water column	0.03 (0.02 - 0.06)	5 (3 - 7)	46 (30 - 55)	-	-
Reef flat	0.62 (0.22 - 1.18)	205 (178 - 240)	109 (86 - 145)	96 (88 - 112)	11.7 (4.4 - 22.9)
Reef crest	0.61 (0.15 - 1.07)	246 (222 - 266)	137 (121 - 165)	109 (88 - 139)	10.0 (2.6 - 19.2)
Sand belt	0.49 (0.14 - 0.83)	149 (117 - 173)	85 (75 - 97)	65 (42 - 95)	13.7 (4.0 - 23.8)
Transition zone	0.41 (0.14 - 0.80)	268 (234 - 303)	154 (134 - 186)	114 (95 - 156)	6.7 (2.6 - 14.2)
Forereef	0.50 (0.10 - 0.78)	327 (284 - 359)	190 (167 - 223)	137 (116 - 187)	6.6 (1.6 - 12.0)

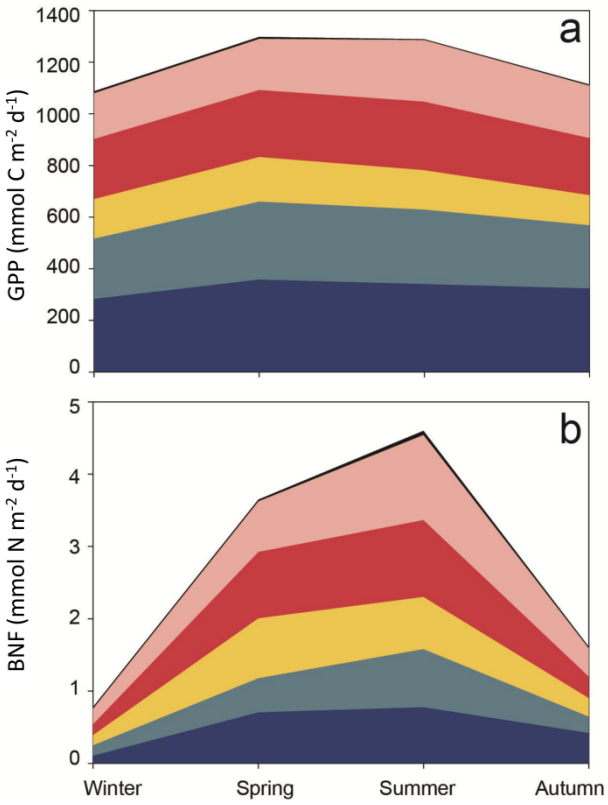

Fig. 6-4. GPP (a) and BNF (b) in the different reef habitats in the different seasons. Different colours represent different habitats as in the habitat map in Fig. 6-1, and black represent the contribution of the water column.

Discussion

This study represents one of the very few field investigations succeeding early fundamental works (Wiebe et al. 1975; Larkum et al. 1988) that generates a dinitrogen fixation (BNF) budget for a coral reef community based on individual rates of all dominant benthic substrates. To our knowledge, it is the first to calculate a seasonal BNF budget by combining substrate-specific rates extrapolated to their respective benthic 3D surface areas with a GIS analysis of the reef area under study. Finally, this study is the first effort to constrain previous global estimates with an assessment of the associated uncertainty (Table 6-3), propagated according to the propagation of errors for linear combinations (Miller and Miller 2005). BNF rates for the individual substrates investigated (Appendix 4 - Table 4) fall well within those reported by previous literature, when these values exist (see Cardini et al. (2014) for a review). At the global scale, no such detailed budget has been attempted previously, and values provided in Table 6-3 show that the estimates obtained here are reasonably robust against the associated uncertainties. However, there is considerable uncertainty in any such budget, and the increased complexity of the analyses introduces new uncertainties in our budget that include: (i) deviations from the theoretical C_2H_4:N_2 ratio in different communities due to, for example, the ratio of N incorporated into biomass versus the N released; (ii) spatial variations in activity associated with the same substrates caused by the effect of depth (i.e., light availability) on the measured processes; (iii) changes of ecosystem stoichiometry over space and time; (iv) changes in estimates of global coral reef areas. Nonetheless, some results appear robust:

A clear decoupling exists between the substrates contributing most to GPP and the ones that contribute most to BNF (Fig. 6-3). This indicates that generally neglected benthic substrates such as turf algae, 'bare' hard substrates and sediments contribute indirectly to the overall benthic production by providing bioavailable N to the reef ecosystem (Wiebe et al. 1975; Shashar et al. 1994b; den Haan et al. 2014). Importantly, other reef organisms provide both ecosystem functions thanks to a symbiosis (e.g. hard corals) or an inherent capability (e.g. microbial mats). These components are fundamental to both processes, with their contribution strongly depending on their benthic coverage.

In coral reef ecosystems, BNF and GPP are strictly benthos-related processes. The entire water column above the reef contributed with rates of BNF and GPP which were ca. 20-fold and 50-fold lower, respectively, than those contributed by the benthos (Table 6-2, Fig. 6-4).

Regionally important reef-contiguous habitats (e.g., sandy lagoon areas) may be major contributors of new N into the reef ecosystem as previously indicated (Capone et al. 1992;

Shashar et al. 1994b; O'Neil and Capone 2008). However, overlooked high-coral cover areas contribute new N at similar rates when accounting for their three-dimensional surfaces (Fig. 6-4). Moreover, these habitats are unrivalled in terms of GPP, shaping coral reef ecosystems productivity and growth.

BNF is highly susceptible to changes in environmental conditions, with 4-fold higher areal rates in spring and summer compared to winter and autumn in all reef habitats (Fig. 6-4). This suggests that BNF will likely respond to a greater extent than GPP to future climate change. Because of the tight coupling of BNF with the reef C cycle, with BNF sustaining ca. 10 % of NPP of the entire reef system on an annual basis, and up to ca. 20 % of NPP in summer (Table 6-2), BNF dynamics need to be accounted for when projecting the future of coral reef ecosystems productivity in response to climate change.

Concluding remarks

In an attempt to scale our results and provide estimates of global N inputs from benthic N_2 fixation in coral reef ecosystems, we used the global reef area provided by Burke et al. (2011) to quantify the contribution by the reef-framework (i.e., reef flat, reef crest, transition zone and fore reef), while the difference between the estimates by Smith (1978) and Burke et al. (2011) was used to quantify the contribution by the reef-contiguous soft bottom communities (i.e. sand belt). In addition, the fringing reef under study is exposed to seasonally changing environmental conditions. The stratified summer resembles typical conditions of tropical reef communities worldwide, which are characterized by warm and highly oligotrophic waters, while the mixed season has similar conditions to the ones found in marginal reef communities where nutrient concentrations are higher and temperature and light intensity are lower. Consequently, we present here different estimates based on annual averages and on summer measurements only, as the latter ones may more closely approximate the scale and significance of N_2 fixation in tropical reef systems worldwide (Table 6-3). These estimates, particularly those based on summer measurements, are similar to those presented by Capone and Carpenter (1982). However, they based their calculations on early measurements of N_2 fixation in algal mats (Webb et al. 1975; Wiebe et al. 1975) which were one order of magnitude higher than those measured in subsequent studies in different locations for similar substrates (Wilkinson et al. 1984; Charpy-Roubaud et al. 2001; Charpy-Roubaud and Larkum 2005). In any case, our estimates approach that of Capone and Carpenter (1982) due to the meanwhile increase of global reef area estimates. Ranging from 1.60 to 2.58 Tg N y^{-1}, our extrapolation define coral reef ecosystems among the benthic communities contributing most to the inputs of fixed N on a global scale.

Areal GPP and BNF rates were highest in spring and summer: it follows that the environmental factor controlling most these two processes at the reef scale is light availability, which also increased in spring and summer (Table 6-1). Secondarily, temperature is also exerting an important control on BNF, as summer individual rates were the highest. This is consistent with previous studies that found BNF to be light and temperature dependent on coral reef substrates (Wilkinson et al. 1985; Larkum et al. 1988). Conversely, the high concentrations of dissolved inorganic nutrients did not determine a decrease of overall reef BNF in spring. Thus, it is possible that future anthropogenic global warming will more strongly affect BNF in coral reef ecosystems, rather than human induced eutrophication of the coastal zones. As yet, large uncertainties remain in the potential responses, requiring further assessments. It is clear, however, that the tight coupling between BNF and reef primary productivity will determine feedback loops where perturbations in BNF will have repercussions in the C cycle, and vice versa.

Table 6-3. Estimate of the total contribution of combined nitrogen to the global nitrogen cycle by nitrogen fixation in benthic coral reef environments, revised after Capone and Carpenter (1982) (with data from Wiebe et al. (1975)) and (Larkum et al. 1988). Values are means with SE or range of values in parentheses.

Study location	Community	Time of year	Temperature (°C)	Areal rate (μmol N m^{-2} d^{-1})	Area (10^3 km^2)	N fixed (Tg N yr^{-1})	Reference
Aqaba, Jordan	Reef framework community	Annual average	(22.0 - 27.5)	536 (69)	249.7[a]	0.68 (0.09)	This study
Aqaba, Jordan	Soft bottom community	Annual average	(22.0 - 27.5)	488 (62)	367.3[a]	0.92 (0.12)	This study
Aqaba, Jordan	Total reef areas	Annual average	(22.0 - 27.5)	-	617.0[a]	1.60 (0.15)	This study
Aqaba, Jordan	Reef framework community	Summer	27.0 (0.2)	956 (151)	249.7[a]	1.22 (0.19)	This study
Aqaba, Jordan	Soft bottom community	Summer	27.0 (0.2)	723 (110)	367.3[a]	1.36 (0.21)	This study
Aqaba, Jordan	Total reef areas	Summer	27.0 (0.2)	-	617.0[a]	2.58 (0.28)	This study
Enewetak Atoll	Algal mats	January - February	(27 - 36)	4890 (1643)	110.0[b]	2.8	Capone and Carpenter (1982)
One Tree Reef	Various substrates	Annual range	(20 - 30)	(156 - 1330)	-	-	Larkum et al. (1988)

[a] The estimate from Burke et al. (2011) was used for reef framework areas and the estimate by Smith (1978) was used to calculate the areal extent of reef-associated soft bottom communities. [b] The old estimate from De Vooys (1979) was used as in the original publication.

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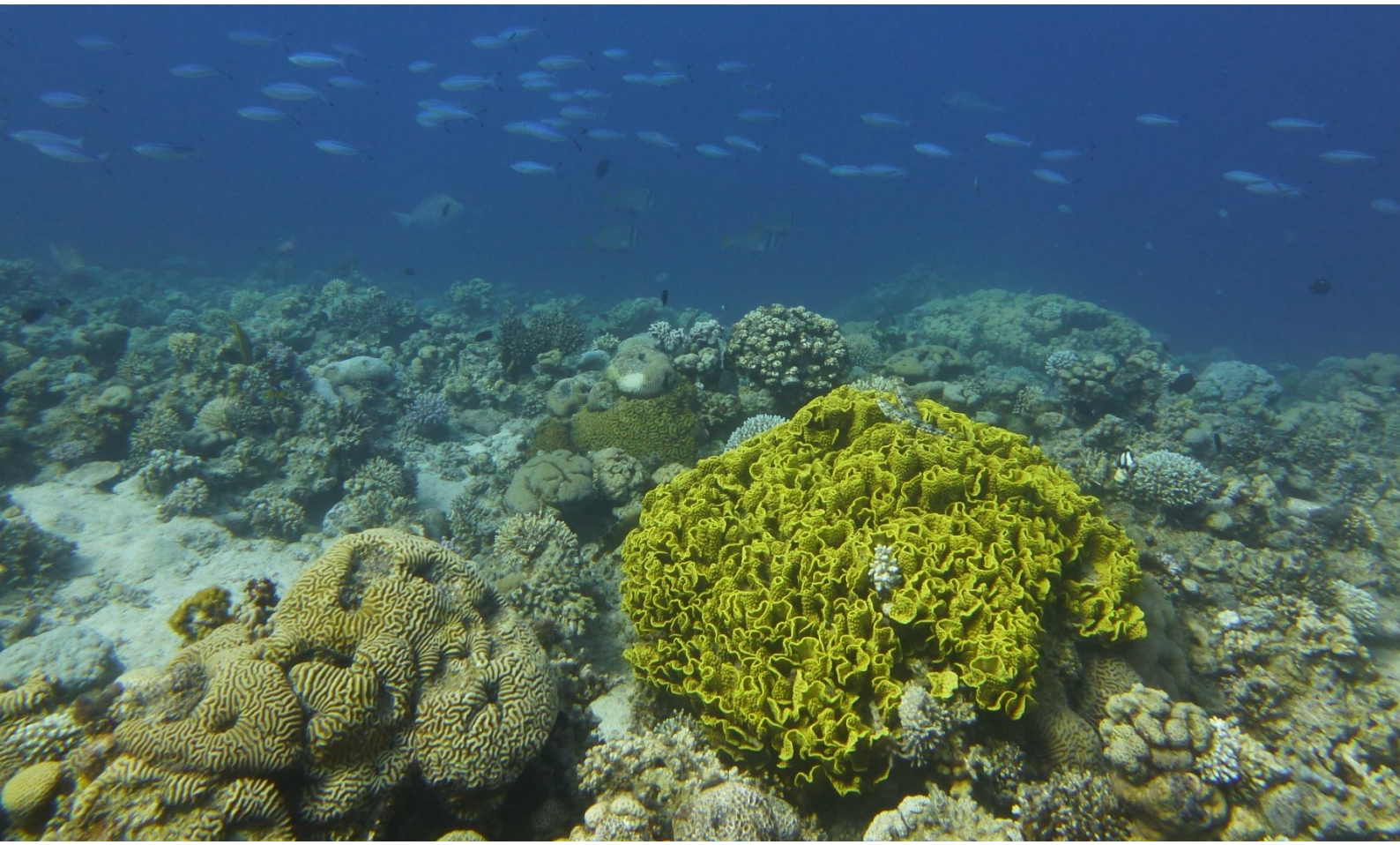
Chapter 7
Seasonal photosynthetic primary production by dominant benthic organisms in a Red Sea coral reef



Abstract

Coral reefs in the Northern Red Sea experience pronounced seasonal variations in environmental factors such as water temperature, light, and nutrient availability. This allows study of related effects on primary production by different functional groups. The present study therefore quantified primary production of all dominant benthic primary producers (corals, macroalgae, microphytobenthos, and cyanobacterial mats) from a Jordanian fringing reef (29° 27' 31" N, 34° 58' 26" E) by measuring net photosynthesis (P_n) and respiration (R) using stirred respirometry chamber incubations conducted during all four seasons of 2013. Annual mean P_n was highest for the macroalga *Caulerpa* (901 nmol O_2 cm^{-2} h^{-1}) and lowest for the soft coral *Sarcophyton* and sedimentary microphytobenthos (212 and 223 nmol O_2 cm^{-2} h^{-1} respectively). Primary production of sedimentary microphytobenthos exhibited the strongest response to seasonality: 5.7 times higher P_n in spring than in winter. Hard coral R rates were highest of all groups in every season, likely due to calcification and heterotrophy. Gross photosynthesis to respiration ratios were highest for algae and cyanobacterial mats, indicating these groups as most efficient at primary production. In the majority of groups where P_n was significantly different between seasons, P_n increased with increasing light availability from winter to spring. Xeniidae was the only group which P_n was significantly lower in summer than spring, indicating potential nutrient limitation that other groups may have alleviated through heterotrophy, or symbiotic dinitrogen fixation. The relatively high P_n and benthic cover hint to hard corals as the dominant primary producers in the investigated reef.

This chapter is under review at *Marine Biology*: van Hoytema N, Bednarz VN, Cardini U, Naumann MS, Al-Horani FA, Wild C. The influence of seasonality on primary production by dominant benthic organisms in a Red Sea coral reef.

Chapter 7 Benthic functional group primary production

Introduction

Coral reefs are known as marine biodiversity hot-spots characterized by high productivity in nutrient poor environments (Odum and Odum 1955; Hatcher 1988). The basis for this high productivity is photosynthesis, performed in coral reefs by a diverse community of benthic organisms. Coral reef primary production is primarily characterized by hard and soft corals with their endosymbiotic photosynthetic dinoflagellates of the genus *Symbiodinium*; i.e. zooxanthellae (Fabricius and Klumpp 1995; Wooldridge 2010; Stambler 2011). Other photosynthetic organisms occur in a wide range of habitats on (e.g. epilithic algae) and within (e.g. endolithic algae, sedimentary microphytobenthos) reef substrates (Fong and Paul 2011; Titlyanov and Titlyanova 2012). The two groups of primary producers which, in general, contribute most to total reef primary production are corals and turf algae (Adey 1998). These groups can display high rates of photosynthesis (Hatcher 1988; Smith et al. 2013), and comprise a large fraction of the benthic cover in Indo-Pacific and Caribbean reefs: benthic cover of both corals and turf algae ranges between 20 and 40% (Adey and Steneck 1985; Klumpp and McKinnon 1992; Jantzen et al. 2013).

The response of photosynthetic organisms (e.g. corals and macroalgae) to different light levels is species-specific, and they can adapt to changes in light by altering their photosynthetic apparatus (Porter et al. 1984; Carpenter 1985; Lesser and Shick 1989; Talarico and Maranzana 2000). Photosynthetic organisms exhibit a linear relationship between photosynthesis and light availability at low light levels, while photosynthesis asymptotically approaches a maximum rate as light levels increase (Falkowski & Raven 1997). Supra-optimal levels will cause photo-inhibition and –damage, reducing the photosynthetic efficiency (Long et al. 1994; Franklin et al. 1996). Temperature affects all metabolic processes (Gillooly et al. 2001), and photosynthesis increases with temperature up to a maximum above which temperature starts to have a detrimental effect on photosynthesis: e.g. 30 °C for macroalgae and zooxanthellae (Iglesias-Prieto et al. 1992; Kubler and Davison 1993). Inorganic nutrient concentrations in coral reefs are low compared to other marine coastal habitats (Kleypas et al. 1999), and both nitrogen and phosphorus can limit primary production in coral reefs (Larned 1998). The zooxanthellae-coral symbiosis shows the unique capability, compared to other reef primary producers, to alleviate nutrient limitation by heterotrophic feeding (Goreau et al. 1971). Further, certain microbial primary producers (e.g. cyanobacteria) are capable of atmospheric dinitrogen fixation in times of inorganic nitrogen shortage and may live in symbiosis with reef organisms such as corals (Lesser et al. 2004; Cardini et al. 2014).

The Gulf of Aqaba at the northern tip of the Red Sea contains some of the most northern warm-water coral reefs in the world (ca. 30 °N). Because of their relatively high latitude location,

these reefs are exposed to pronounced seasonal variations in key environmental factors. Light availability in the Gulf of Aqaba follows the seasonal cycle of the northern hemisphere with highest light levels from March to September (Winters et al. 2009). Surface water temperatures follow an annual cycle of 21 - 29 °C with lowest temperatures usually in March and highest temperatures in August/September (Carlson et al. 2014). This, together with exceptionally high temperatures throughout the water column (minimum 21 °C), leads to a cycle of surface water cooling and deep water mixing with inorganic nutrient upwelling in winter, and stratification down to 200 m water depth in summer. This stratification causes strongly oligotrophic conditions in surface waters of the Gulf and on the fringing coral reefs (Silverman et al. 2007; Carlson et al. 2014). These local circumstances offer the rare opportunity to study the effects of seasonal variation in key environmental factors on coral reef biogeochemical functions, particularly benthic primary production.

Many previous studies of benthic coral reef primary production have investigated the coral reef as a whole through flow respirometric in-situ approaches; i.e. not differentiating primary production by different groups of primary producers (Odum and Odum 1955; Barnes 1983; Atkinson and Grigg 1984; Silverman et al. 2007). Other studies investigated primary production of individual functional groups, but focused on particular groups: e.g. soft corals (Fabricius and Klumpp 1995), or macroalgae (Ateweberhan et al. 2006). In addition, coral reef research has until now mainly focused on the Pacific and Caribbean, while the Red Sea with its strong seasonal pattern has received much less attention (Berumen et al. 2013; Loya et al. 2014) Coral reef environmental conditions are predicted to become more variable in the near future due to global and local stressors such as ocean warming and eutrophication (D'Angelo and Wiedenmann 2014; Hoegh-Guldberg 2014). Knowledge on the responses of the diverse groups of coral reef primary producers to changing environmental parameters is therefore important to predict what impact those stressors may have on the functioning of coral reef ecosystems as a whole.

The objectives of this study were thus to quantify photosynthesis and respiration rates of all dominant benthic primary producers in a seasonal resolution and to evaluate the effect of seasonally changing environmental parameters (light, temperature, dissolved inorganic nutrient concentrations) on those key metabolic processes.

Materials and methods

Research area

This study was conducted at the Marine Science Station (MSS) of the University of Jordan and Yarmouk University, situated at the northern tip of the Gulf of Aqaba. The MSS is located approximately 10 km south of the city of Aqaba and is adjacent to a marine reserve containing a 1 km long fringing coral reef which borders the coastline in a crescent shape (location: 29° 27' 31" N, 34° 58' 26" E). Four experimental campaigns, each encompassing four weeks, were performed in February, April, September, and November 2013, respectively. The timing of these periods (hereinafter called winter, spring, summer, and fall respectively) was determined beforehand from literature to best cover the annual cycle of environmental conditions in this area of the Red Sea (Silverman et al. 2007; Carlson et al. 2014). Environmental monitoring and sample collection took place at the coordinates given above on the fore-reef slope at 10 m water depth.

Environmental monitoring

Monitoring was performed during all seasons to quantify the annual range of environmental key parameters in the research area. Water temperature and irradiance (lux) were measured in-situ at 1 min frequency by HOBO data loggers (Onset HOBO Pendant UA-002-64; temperature accuracy: ± 0.53 °C, spectral detection range: 150 – 1200 nm). The irradiance measurements (lux) were converted into photosynthetically active radiation (PAR) by a minute-by-minute measurement in a flow through aquarium under in-situ like light conditions (see below) between 08:00 and 14:00 (n = 353) with a HOBO pendant data logger and a PAR sensor (LI-COR LI-192SA underwater quantum sensor) in parallel. The lux and PAR values showed strong correlation ($R^2 = 0.83$), and a linear relationship was estimated (lux = PAR * 52.0) that was very similar to the factor of 51.2 given by Valiela (1984).

Inorganic nutrient concentrations were measured from four replicate water samples collected weekly between 08:00 and 10:00 within a 10 min time frame using clean 5 L high-density polyethylene canisters. Samples were immediately transported to the laboratory where subsamples for analyses of ammonium, phosphate, nitrite, and nitrate concentrations were filtered through sample pre-rinsed cellulose acetate filters (nominal pore size 0.45 µm). Inorganic nutrient concentrations were measured following standard methods (Murphy and Riley 1962; Strickland and Parsons 1968; Holmes et al. 1999). Ammonium was measured fluorometrically using a Trilogy Fluorometer (Turner Designs), while phosphate, nitrite and nitrate were measured

photometrically with a JASCO-V630 photometer. Detection limits for ammonium, phosphate, and the nitrogen oxides were 0.09, 0.01, and 0.02 µmol L^{-1}, respectively.

Benthic cover

The benthic cover at 10 m water depth in the study area was measured at the beginning of each sampling period by triplicate benthic line-point intercept transects (Nadon and Stirling 2006). Each transect length was 50 m with point intervals of 0.5 m resulting in a total of 300 data points per benthic survey. Percent cover data revealed that the reef was dominated by hard and soft corals (combined 57 - 63%; Fig. 7-1). Macroalgae and turf algal communities were far less abundant, but revealed seasonality in their benthic cover. Turf algae cover was highest in winter, while macroalgae were most abundant in spring (Fig. 7-1). Carbonate sediments and coral rock with only a thin biofilm both covered between 10 and 20% of the seafloor. Cyanobacterial mats comprised less than 4% of the benthos in all seasons, but were substantially more abundant in summer than other seasons (Fig. 7-1). These groups amount to an annual average of 98.4% of the total benthic cover. The dense turf algal communities (termed turf algae here-after) consisted of a variety of crustose coralline algae (CCA), cyanobacteria, and filamentous algae (Larkum et al. 2003). The studied reef was characterized by large areas of "bare" coral rock which is only covered by a thin biofilm of CCA and other primary producers (Bahartan et al. 2010). In these, the dense filaments, typical of the turf algae group, were lacking and the carbonate substrate was clearly visible (this group will here-after be termed coral rock).

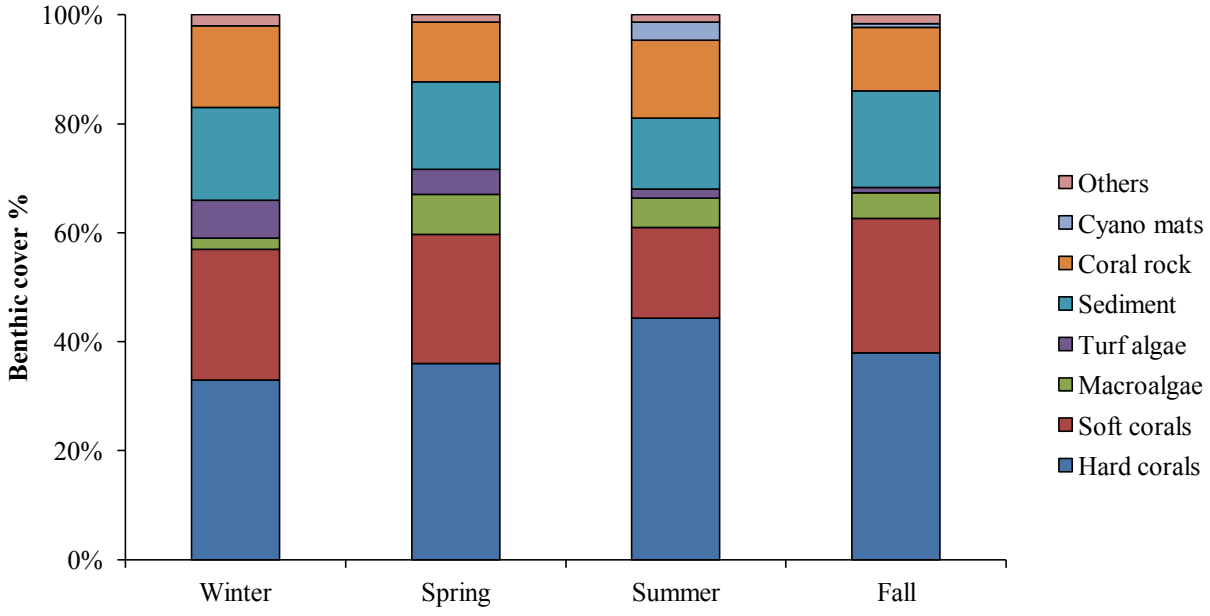


Fig. 7-1. Seasonal comparison of mean percentage benthic coverage by dominant substrate types in the research area.

Specimen collection

Specimens of the above mentioned functional groups of benthic primary producers were collected in each experimental campaign for measurement of net photosynthesis (P_n) and dark respiration (R) via oxygen (O_2) fluxes. Hard corals were represented by four genera: *Acropora*, *Pocillopora*, *Stylophora, and Goniastrea*, together comprising 37.8 – 57.2 % of total hard coral coverage in the study area. The family Xeniidae and genus *Sarcophyton* were the dominant soft corals; 93.0 – 98.7 % of the total seasonal soft coral coverage consisted of these two groups. The genera *Lobophora* and *Caulerpa* represented 91.7 – 100 % of macroalgal benthic cover. Carbonate sediment with its microbial community (here-after called sediment) was treated as a bulk group of which the microorganisms were not identified. Previous studies at the same location revealed that the sediment biota in the study area consists of a diverse community of phototrophic and heterotrophic microbes (Wild et al. 2006; Schottner et al. 2011). Samples of cyanobacterial mats growing on the sediment in the research area were also treated as a bulk group. Cyanobacterial mats as those sampled are several mm thick and consist of a community of cyanobacteria, photosynthetic bacteria, sulfur bacteria, and other microbes (Charpy et al. 2012).

Hard corals, soft corals, turf algae-covered coral skeleton branches, and coral rock were collected by carefully chiseling fragments or whole organisms off the reef. Specimen replication for all groups was n = 8 per group and season. Soft corals of the family Xeniidae were difficult to identify to genus level in-situ. They were therefore sampled based on family level identification, but all collected colonies exhibited comparable morphology, coloration and pulsating polyps. Macroalgae were collected by carefully prying them off their substrate with forceps. Specimens were transported back to the MSS laboratory in transparent plastic containers within 60 min of sampling. Hard and soft corals, as well as turf algae-covered branches, were fixed to ceramic tiles with epoxy glue (Reef Construct, Aqua Medic). Coral rock fragments were stable without fixation. Macroalgae were fixed to ceramic tiles or Petri dishes with rubber bands while protecting the algal tissue from getting damaged. Sediment and cyanobacterial mat samples were collected with round PVC sediment corers (corer mouth surface area 14.52 cm^2). Open corers were inserted into the sediment to 10 cm depth, after which the corer were dug out and their top and bottom were sealed with styrofoam plugs. The corers were then transported in a vertical position back to the lab where the top 1 cm of the core was extruded and transferred into a Petri dish with the same internal diameter as the sediment corer. Transporting the mat and sediment back to the laboratory in the corer was observed to cause the least disturbance and preserved the stratification of the samples. Mean soft coral surface area was 85.93 ± 38.37 cm^2 (mean ± SD), while all other groups had a mean surface area of 40.31 ± 19.67 cm^2 (see below for surface area determination).

Organisms were maintained in an outside flow-through aquarium of 800 L with an exchange rate of 4000 L h^{-1} of water pumped straight from the coral reef at 10 m water depth, thus resembling ambient in-situ conditions of temperature and inorganic nutrients. Light conditions were adjusted to in-situ conditions at 10 m water depth using layers of plastic mesh and monitored with data loggers (Onset HOBO Pendant UA-002-64). Mean differences between in-situ PAR and flow-through aquarium PAR levels measured during the incubations were low on average, 21.8, 56.4, 26.4, and 19.6 μmol photons m^{-2} s^{-1} for winter, spring, summer, and fall respectively. Hard and soft corals were acclimatized for at least 7 d, and macroalgae for at least 24 h, before incubation measurements to recover from extraction. Sediment, turf algae, coral rock, and cyanobacterial mats were incubated on the day of collection (R measurement 4-10 h after collection) to minimize changes in their biotic communities before incubation. The same specimens used for the R measurement, were measured for P_n the following day.

Quantification of primary production

Closed cell respirometric incubations under in-situ-like conditions took place in the flow-through aquarium for measurements of P_n and R using seawater which was pumped straight from the coral reef at 10 m water depth. Incubations were performed separately in glass chambers of 1 L for hard corals, soft corals, macroalgae, turf algae, coral rock, and cyanobacterial mats (n = 6-8). Sediment was incubated in 0.5 L chambers (n = 8). In addition, seawater controls (n = 8) with and without fixation tiles were run in parallel to correct for metabolic activity in the incubation seawater and potentially due to microbiota on the tiles. Magnetic stirrer plates (Cimarec™ i Telesystem Multipoint Stirrers, Thermo Scientific™) and Teflon-coated stir bars in the chambers provided water movement during the incubations at 600 rpm.

R measurements took place in the dark at least 1 h after sunset to ensure full darkness, while P_n measurements were performed the following day between 12:00 and 14:00 (10:00 and 16:00 for sediment incubations), which represents the most stable and maximum light conditions of the day (according to PAR sensor measurements every sampling period; Appendix 5 - Fig. 1). All specimens, except for sediments, were incubated for 60 - 90 min for P_n, and 90 - 120 min for R. Sediments were incubated for 120 - 330 min for P_n and 180 - 360 min for R to ensure that the difference between O_2 measurements at the start and end of the incubations was significantly larger for chambers with sediments than for seawater control chambers. All equipment used to mount organisms in the chambers was thoroughly cleaned before each incubation with a fine brush to remove epi-biota. Organisms were placed, without air exposure, into the chambers onto a mesh which separated them from the stir bar. Salinity and temperature corrected dissolved O_2 concentrations (mg L^{-1}) were measured in each chamber with an O_2 optode and a conductivity

probe (MultiLine® IDS 3430, WTW GmbH, Weilheim, Germany, accuracy: ± 0.5% of measured value) at the start and end of each incubation. All air was removed from the chambers as they were closed after the start measurement. Temperature and light availability were monitored during the incubations by a data logger in an additional incubation chamber.

Surface area quantification and data treatment

The surface area of incubated specimens was quantified after the incubations in the flow-through tank while organisms were under in-situ conditions showing no signs of stress (e.g. polyp contraction in soft corals). The surface areas of hard and soft corals, turf algae, and coral rock were measured using advanced geometry (Naumann et al. 2009; Bednarz et al. 2012). Macroalgae surface areas were measured by flattening the fragments between two panes of glass, taking a planar photograph, and quantifying the surface area by digital image analysis (Image J, version 1.48; Schneider et al. 2012). The macroalgae total surface area was then calculated by doubling the planar surface area (Haas et al. 2010). Sediment and cyanobacterial mat surface areas were assumed to be the circular surface area sampled by the sediment corer: 14.52 cm^2.

P_n and R rates were calculated by subtracting start from end O_2 concentrations and relating to incubation duration (mg O_2 production/consumption L^{-1} $hour^{-1}$). These O_2 rates were then corrected for the seawater control O_2 fluxes before being normalized to chamber volume and organism surface area. Fluxes in ng O_2 cm^{-2} h^{-1} were then divided by the molar mass of O_2 to obtain fluxes in nmol O_2 cm^{-2} h^{-1}. Gross photosynthesis (P_g) was estimated by adding positive R rates to their corresponding P_n rates: ($P_g = P_n + |R|$), and the P_g:R ratio, an estimation of photosynthetic efficiency, was derived by dividing P_g by R.

Statistical analyses

Fully crossed general linear models (LM) in R (version 3.0.2, R Development Core Team, 2013) were used to estimate the influence of 'group', 'season', and their interaction on the physiological measurements (P_n, R, P_g:R). In total, three models were run, one with each of the three respective response parameters (P_n, R, P_g:R) as a function of season and group.

Prior to running the models, the distributions of the respective response parameters were checked. Where necessary, data were square root- or log-transformed, after making all values positive by a standard addition, to reduce skewness. To confirm the assumptions of normally distributed and homogenous residuals, qqplots and scatterplots of the residuals plotted against the fitted values were visually inspected in R (Quinn and Keough 2002). Model stability (i.e. dffits

and dfbetas; Cohen and Cohen 2008) was checked for each of the models, as well as leverage and Cook's distance. All values fell within acceptable ranges.

All models were fitted in R using the function 'lm'. To establish the significances of the full models, likelihood ratio tests (R function 'ANOVA' with the argument test set to 'F') were used to compare their deviances with those of the null models comprising only the intercept. To test significances of individual terms (interaction terms and main factors), the deviances of the respective full models were compared with those of the corresponding reduced models not comprising the respective factor and/or term of interest. Post hoc t-tests were then performed to compare seasons for each group and groups in each season. Differences between groups or seasons were deemed significant at $\alpha < 0.05$.

Results

Environmental monitoring

The environmental parameters exhibited seasonal trends as expected from literature (e.g. Carlson et al. 2014) (Table 7-1). Temperature was stable from winter to spring, increased to 27 °C in summer, and decreased again towards fall. Light peaked in spring and summer, while inorganic nutrient concentrations showed the opposite trend to temperature with lowest concentrations in summer.

Table 7-1. Seasonal measurements of environmental parameters in the research area. Temperature and irradiance values based on 1 min frequency measurements between 12:00 and 14:00 on all days encompassing each respective sampling period (n = 18-27 days). Inorganic nutrient concentrations are based on weekly measurements in the four weeks encompassing each sampling period. Values are given as mean ± SD. Feb = February, Apr = April, Sep = September, Nov = November. PAR = photosynthetically active radiation. Nitrogen oxides = nitrate + nitrite concentrations.

	Winter (Feb)	Spring (Apr)	Summer (Sep)	Fall (Nov)
Temperature (°C)	22.7 ± 0.2	23.0 ± 0.2	27.2 ± 0.3	25.3 ± 0.2
PAR (µmol photons m^{-2} s^{-1})	147 ± 60	281 ± 61	320 ± 63	162 ± 63
Ammonium (µmol L^{-1})	0.32 ± 0.09	0.46 ± 0.07	0.11 ± 0.01	0.28 ± 0.12
Phosphate (µmol L^{-1})	0.11 ± 0.01	0.10 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Nitrogen oxides (µmol L^{-1})	0.71 ± 0.15	0.56 ± 0.12	0.06 ± 0.02	0.22 ± 0.23

Group-specific primary production

As all parameters showed a significant effect of the interaction between group and season (Table 7-2), comparisons were analyzed between groups in each season, and seasons for each group. *Caulerpa* P_n (696 – 1005 nmol O_2 cm^{-2} h^{-1}) (lowest – highest seasonal mean) was significantly higher than all groups in all seasons except for *Goniastrea* (spring and fall), turf algae (summer), and cyanobacterial mats (fall) (Fig. 7-2, Appendix 5 - Tables 1-4). The other macroalga, *Lobophora*, exhibited far lower P_n rates than *Caulerpa* (234 – 353 nmol O_2 cm^{-2} h^{-1}), it always ranked among the four groups with lowest P_n. *Sarcophyton* average P_n rates were significantly lower than those of the hard corals in all seasons (Appendix 5 - Tables 1-4). Xeniidae, however, showed no significant difference in P_n compared to the majority of the four hard corals over all seasons (10 out of 16 comparisons). Sediment exhibited the lowest P_n rates of all groups in winter, summer, and fall (81 – 232 nmol O_2 cm^{-2} h^{-1}). However, sediment spring P_n (459 ± 202 nmol O_2 cm^{-2} h^{-1}) was significantly higher than that of *Sarcophyton*, *Lobophora*, and coral rock (209 – 292 nmol O_2 cm^{-2} h^{-1}). Average R rates for the four hard coral genera (177– 324 nmol O_2 cm^{-2} h^{-1}) were significantly higher than other groups in all seasons except for *Sarcophyton* in spring, and coral rock and cyanobacterial mats in fall. Sediment and *Lobophora* R were among the lowest three groups in all seasons (45 – 92 nmol O_2 cm^{-2} h^{-1}). *Caulerpa* revealed the significantly highest P_g:R in both winter and fall (14.08, 20.42 respectively). *Caulerpa* P_g:R was also highest in spring and summer, but was not significantly different from sediment, cyanobacterial mats, and Xeniidae in spring and turf algae in summer. Xeniidae P_g:R was significantly higher than *Sarcophyton* and all hard corals, except for *Goniastrea* in spring.

Table 7-2. Results of the three fully crossed general linear models for net photosynthesis, respiration, and gross photosynthesis (P_g) to dark respiration (R) ratio with factors 'group' and 'season' as well as their interaction.

Parameters	Effect	df	$df_{residuals}$	F	p-value
Net photosynthesis (nmol O_2 cm^{-2} h^{-1})	Group (Gr)	11	354	43.41	**<0.001**
	Season (Se)	3	354	12.61	**<0.001**
	Gr x Se	32	322	3.27	**<0.001**
Respiration (nmol O_2 cm^{-2} h^{-1})	Group (Gr)	11	360	68.10	**<0.001**
	Season (Se)	3	360	17.22	**<0.001**
	Gr x Se	33	327	4.21	**<0.001**
P_g:R ratio	Group (Gr)	11	352	49.99	**<0.001**
	Season (Se)	3	352	1.69	0.167
	Gr x Se	32	320	6.37	**<0.001**

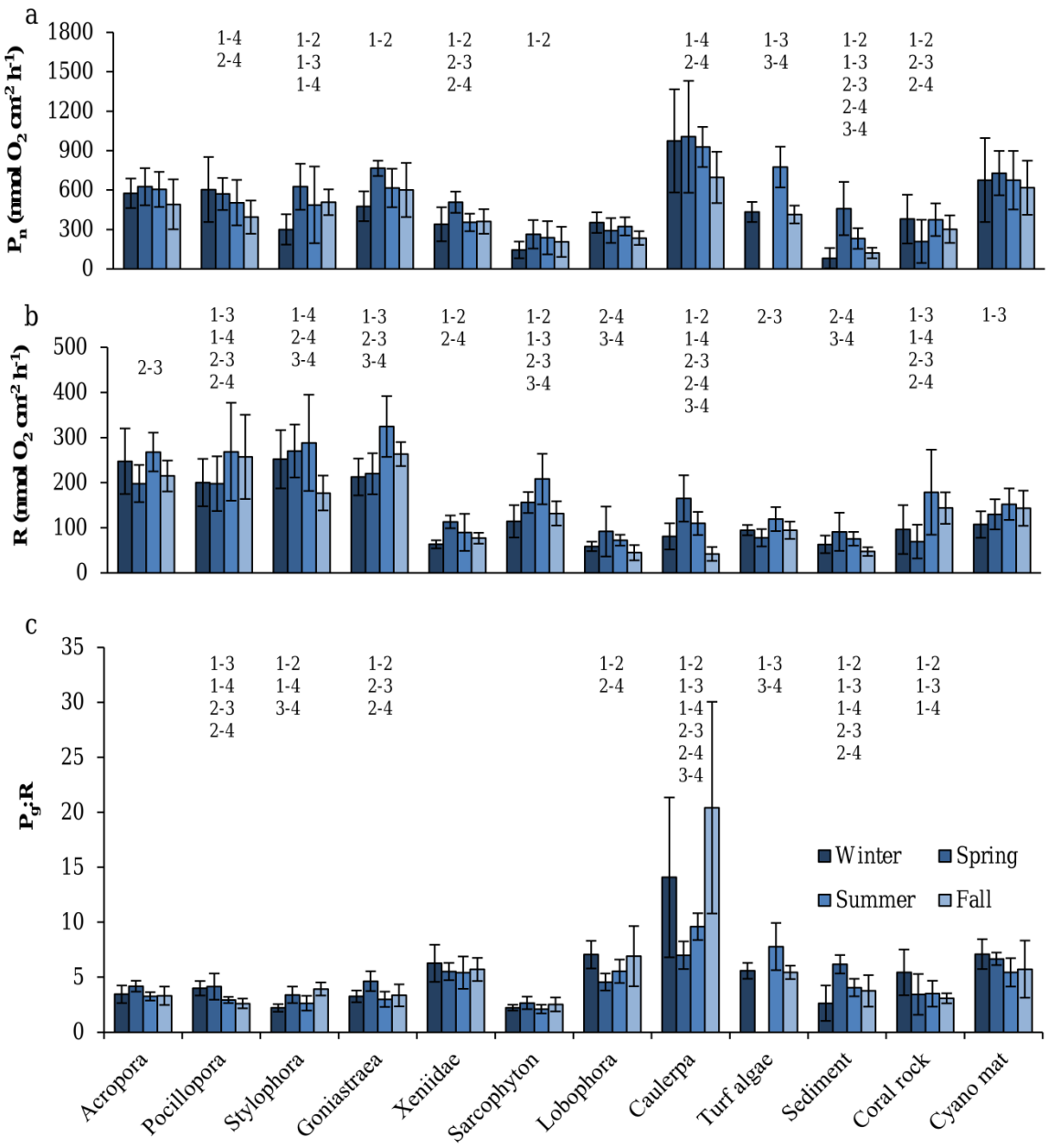


Fig. 7-2. Seasonal comparison of mean primary production parameters (n = 6-8 per group per season), a) net photosynthesis (P_n), b) dark respiration (R), c) gross photosynthesis to dark respiration ratio (P_g:R). Error bars indicate ± SD. 1 = winter, 2 = spring, 3 = summer, 4 = fall; linked numbers indicate a significant difference between the named seasons. Light availability during the P_n incubation of turf algae in spring was uncharacteristically low and non-representative for spring (73.8 ± 26.3 µmol photons m^{-2} s^{-1}). The spring P_n rate and P_g:R ratio of turf algae were therefore omitted from the presented data set.

Seasonal effects

Acropora, *Lobophora*, and cyanobacterial mats were the only groups exhibiting no significant difference in P_n between the seasons, while all others showed group-specific primary production rates in response to the effect of seasonality in environmental factors (Fig. 7-2). *Stylophora*,

Goniastrea, Xeniidae, *Sarcophyton*, and sediment exhibited significant increases in P_n from winter to spring (Fig. 7-2); coinciding with an increase in light levels. Only *Stylophora* P_n was still significantly higher in summer than in winter. R rates of *Acropora*, *Pocillopora*, *Goniastrea*, *Sarcophyton*, turf algae, coral rock, and cyanobacterial mats were significantly higher in summer, during highest water temperatures, than in other seasons. Group-specific P_g:R ratios showed various responses to the seasonality. *Acropora*, Xeniidae, *Sarcophyton*, and cyanobacterial mats all showed no significant difference in P_g:R between seasons. *Pocillopora* and *Goniastrea* both exhibited highest P_g:R in spring, while macroalgae revealed the opposite trend with spring P_g:R being significantly lower than in winter and fall.

To quantitatively compare the effect of seasonality on the different group-specific metabolic rates, we divided the highest seasonal average for each group by its lowest seasonal average (Table 7-3). Sediment P_n clearly displayed the strongest response to seasonality. The highest average rate (spring) was 5.67 times as high as the lowest average rate (winter). All other groups ranged from 1.18 to 2.08. *Caulerpa* showed the strongest reaction to seasonality in R, followed by coral rock (3.94 and 2.58 respectively). As P_g:R is based on both P_n and R, *Caulerpa* and sediment also revealed strongest seasonality in that parameter (2.92 and 2.34 respectively). Overall, the lowest response to seasonality was exhibited by *Acropora* and cyanobacterial mats (Table 7-3).

Table 7-3. Ratios of highest seasonal average value to lowest seasonal average for the metabolic parameters. P_n = net photosynthesis, R = dark respiration, P_g:R = gross photosynthesis to dark respiration ratio.

Group	P_n	R	P_g:R
Acropora	1.30	1.35	1.28
Pocillopora	1.53	1.36	1.59
Stylophora	2.08	1.63	1.78
Goniastrea	1.61	1.53	1.55
Xeniidae	1.50	1.79	1.16
Sarcophyton	1.82	1.82	1.26
Lobophora	1.51	2.05	1.55
Caulerpa	1.39	3.94	2.92
Turf algae	1.87	1.53	1.43
Sediment	5.67	1.92	2.34
Coral rock	1.82	2.58	1.76
Cyano mat	1.18	1.42	1.32

Discussion

This study provides a first comparative overview of primary production by diverse benthic coral reef organisms under the influence of seasonally changing environmental conditions. The seasonality measured in the reef under study has two main components: a) the increase in light availability from winter to spring and subsequent decrease from summer to fall, and b) the increase in water temperature from spring to summer and the consequent stratification in the water column leading to reduced inorganic nutrient concentrations in summer and fall. The annual cycle of temperature and inorganic nutrient concentrations was as expected from literature (Naumann et al. 2010; Carlson et al. 2014).

Group-specific primary production

Only few studies compared metabolic rates of such diverse groups of coral reef primary producers (Jantzen et al. 2013; Naumann et al. 2013; Smith et al. 2013). Hard corals, and turf algae are well-known for their high contribution to coral reef primary production (Adey 1998). The Jordanian reefs are characterized by a low cover of turf algae compared to reefs in other parts of the world (Bahartan et al. 2010). Turf algae P_n is relatively low in winter, and fall compared to other benthic primary producers, while turf algae in the Caribbean and Pacific regions can have P_n higher than other dominant groups there (Smith et al. 2013). The low turf algae P_n in low light winter and fall, combined with significantly increased P_n in nutrient-poor and high light summer are an indication that turf algae in this study may have been primarily limited by light availability and may overcome nutrient limitation in summer by the presence of cyanobacteria capable of nitrogen fixation (Larkum et al. 2003). Turf algae can be light saturated at low irradiance levels (Larkum et al. 2003; Copertino et al. 2006), or require high light conditions (Carpenter 1985; Klumpp et al. 1987). Several previous studies found that turf algae may quickly adapt to changes in light availability by alteration of their community composition (Copertino et al. 2006; Littler et al. 2010; Fricke et al. 2014). Thus a different turf algae community during summer may have caused the increased P_n rates. In addition, the oligotrophic conditions of the Gulf of Aqaba may also limit turf algae primary production, limiting their benthic cover. The reefs on the opposite side of the Gulf, near Eilat, Israel, have shifted to turf algae dominance. A possible cause of this may be eutrophication due to urban development and aquaculture (Bahartan et al. 2010). A better definition of seasonal changes in turf-algal communities will be required to further understand the ecology and function of this diverse group on coral reefs (Connell et al. 2014).

P_n rates of the soft coral Xeniidae were comparable to P_n rates of the hard corals, while *Sarcophyton* exhibited P_n rates which were substantially lower. Generally, soft corals rely more

on heterotrophy than hard corals (Fabricius and Klumpp 1995), but soft corals vary in this regard. Xeniidae is generally fully autotrophic (Schlichter et al. 1983), although evidence of some heterotrophy has been found (Lewis and Post 1982). *Sarcophyton* on the other hand is able to meet its respiratory demand with heterotrophy alone (Sorokin 1991). Certain Xeniidae (the presently studied included) perform polyp pulsation which increases O_2 exchange with the surrounding water, thereby reducing hyperoxic tissue conditions, and increasing photosynthetic efficiency (Kremien et al. 2013; Wild and Naumann 2013). This process may explain increased P_n of Xeniidae compared to *Sarcophyton*, as well as the relatively high P_g:R of Xeniidae compared to all other corals. Although hard coral P_n was higher than that of Xeniidae in the majority of cases, their concurrent high R resulted in lower P_g:R ratios. The high R rates of the hard corals, as well as *Sarcophyton*, were found in other coral reef metabolism studies (e.g. Eidens et al. 2012; Naumann et al. 2013; Smith et al. 2013). This high R may be due to night time calcification and/or heterotrophic feeding (Wijgerde et al. 2012).

The effect of seasonal environmental variation

Light availability represents the primary factor controlling photosynthesis (Falkowski and Raven 1997). P_n of two genera of hard corals, both soft corals, and sediment increased significantly from winter to spring, as light levels increased, while temperature and inorganic nutrient concentrations remained more comparably stable. Surprisingly, *Acropora*, *Pocillopora*, macroalgae, coral rock and cyanobacterial mat P_n did not increase with increasing irradiance levels. Photosynthetic organisms have various ways of adapting to changes in ambient light levels. Symbiotic corals have different densities and chlorophyll a contents of their zooxanthellae in the Red Sea in winter and summer (Mass et al. 2007), and are capable of varying their photo-adaptive capacity (Brown et al. 1999; Titlyanov et al. 2002). Sedimentary microbial community composition and abundance in the studied reef is highly dynamic and can change over time, selecting for organisms most capable of exploiting current environmental conditions (Schottner et al. 2011). The lack of a significant increase in sediment R between winter and spring may indicate that the change in P_n was more likely due to a compositional change towards a more phototrophic community than overall microbial abundance. The dynamic nature of the microbial community inhabiting the top layer of coral reef sediments and its capacity to respond to changing environmental conditions is underlined by the 5.7-fold higher P_n in spring compared to winter. The strong seasonality in primary production by the sediment community was also found in the Great Barrier Reef (Uthicke and Klumpp 1998). The change in water temperature there, 22.5 – 30.0 °C, explained most of the variation in sediment primary production and the sediment community was capable of fast photo-adaptation, causing productivity to differ minimally between days with high and low light

availability. Our findings indicate that on a seasonal scale, the increased light availability caused the strongest change in sediment primary productivity.

Metabolic rates of organisms generally increase with temperature (Gillooly et al. 2001). Photosynthesis by marine primary producers, therefore, also increases with temperature (Coles and Jokiel 1977; Terrados and Ros 1992; Reynaud et al. 2003). High temperatures (> 30 °C) can cause detrimental effects to photosynthesis (Iglesias-Prieto et al. 1992; Kubler and Davison 1993). This was, however, not the case in the present study since the maximum temperatures measured (27.2 ± 0.3 °C) were below that limit, and were comparable to those measured in previous years (Haas et al. 2010; Rasheed et al. 2012). In addition, northern Red Sea primary producers may be very tolerant to high temperatures because extremely high temperatures (> 32 °C in summer) in the southern Red Sea form a selective thermal barrier, which allows only thermal tolerant strains to migrate north to the Gulf of Aqaba (Fine et al. 2013).

Inorganic nutrient concentrations measured in the reef were similar to other oligotrophic coral reef systems (Sorokin 1995; Brown et al. 1999; Kleypas et al. 1999), but seasonally variable. Increased inorganic nutrient concentrations can facilitate photosynthesis in primary producers from oligotrophic habitats (Littler and Littler 1992; Bednarz et al. 2012), while photosynthesis by coral reef organisms may be nutrient limited under highly oligotrophic conditions, if this limitation is not alleviated through processes such as heterotrophic feeding (Grottoli et al. 2006), or dinitrogen fixation by symbiotic microorganisms (Lesser et al. 2004). The importance of this nitrogen fixing symbiosis for corals in general, remains to be determined (Grover et al. 2014). In the hard and soft corals which exhibited significant differences between the seasons in P_n, only Xeniidae P_n was significantly lower in summer than in spring. P_n consists of simultaneous P_g and R and increased R due to increased temperature may therefore decrease P_n. Xeniidae R rates were, however, not significantly higher in summer than spring. As mentioned above, Xeniidae performs little to no heterotrophic feeding (Schlichter et al. 1983). The Xeniidae under study also exhibited the lowest dinitrogen fixation rates of a wide range of corals (chapter 3; Bednarz et al. 2015). These factors indicate that Xeniidae summer P_n may well have been nutrient limited.

Macroalgae P_n remained stable from spring to summer. One explanation for this may be that macroalgae can rely on nutrients derived from their substrate at low water-flow (< 5 cm s^{-1}) in nutrient-depleted waters (Larned 1998). Water flow speed 1m above the substrate averaged 4.8 ± 2.9 cm s^{-1} (mean ± SD) between March and November 2013 (N. van Hoytema unpublished data), suggesting that *Caulerpa* and *Lobophora* may have relied on nutrients derived from their substrate to maintain P_n during summer. Reef framework and sediments of the studied reef have been found to act as a source of inorganic nutrients to the surrounding environment (Rasheed et

al. 2002). Macroalgae P_g:R ratios were remarkable compared to the other groups because their spring values were significantly lower than the other seasons. This appears to be primarily due to a significantly higher R while P_n remained more constant. Increased macroalgae R in spring may have been due to increased growth as indicated by the measured increase in macroalgae benthic cover from winter to spring (Ateweberhan et al. 2006), while macroalgae R may be increased during their growth season (e.g. Gomez and Wiencke 1996). *Lobophora* mainly inhabits shaded areas on the reef in between coral branches and under overhangs (N. van Hoytema personal observation), and may therefore be adapted to lower light levels than provided during the incubations. This could have led to light inhibition and lower P_n than the other macroalga *Caulerpa*.

Sediment P_n dropped significantly from spring to summer, potentially due to the reduced nutrient availability. The lack of a co-occurring change in R indicates that the decrease in P_n was not due to increased temperature raising the general metabolism. Coral reef sediments are inhabited by dinitrogen fixing microbes (i.e. diazotrophs) which can alleviate nitrogen limitation (Charpy et al. 2012; Cardini et al. 2014). However, carbonate sediments are very low in bio-available phosphate due to phosphorus adsorption to the calcium carbonate, especially under oligotrophic conditions, resulting in phosphate limitation (McGlathery et al. 1994). Cyanobacterial mats show the highest rates of benthic nitrogen fixation per unit surface area in coral reefs (chapter 5; Cardini et al. 2014). Further, cyanobacterial mats may also obtain required phosphate directly from the water column, thereby alleviating phosphate limitation. Indeed, cyanobacterial mats can assimilate phosphate from the water column more efficiently than eukaryotic primary producers under low phosphate conditions (Donald et al. 1997). These two processes may explain why cyanobacterial mats were capable of sustaining P_n rates in times of low nutrient availability.

Ecological implications

In this Jordanian fringing reef, hard corals belong to the top primary producers in the reef throughout the year with at least three out of four genera in the top six P_n rates in each season. Taking into account their high benthic cover throughout the year, this finding underlines their importance for the production of new organic matter in the oligotrophic environment of the northern Red Sea. Turf algae in this high latitude reef show lower P_n during the low light seasons winter and fall and have far lower benthic cover than the corals, indicating a relatively low contribution to whole reef primary production in this fringing reef. In addition to primary production, hard corals also distribute bio-available carbon further throughout the reef (Wild et al. 2004; Naumann et al. 2010; Naumann et al. 2012), and they are the primary reef builders;

constructing the coral reef habitat through calcification (Gattuso et al. 1999). These factors combined indicate that corals in this reef play a pivotal role in the maintenance of the high productivity and biodiversity that characterize coral reefs in nutrient poor environments such as the Gulf of Aqaba.

The Jordanian coast line is currently undergoing fast urban development with potentially detrimental effects to the reefs. First indications have been found that more developed areas along the Jordanian coast are showing an increase in turf algal cover (Al-Zibdah and Colgan 2013). As with the reefs on the Israeli side of the Gulf, further eutrophication of Jordanian waters may lead to a coral reef phase shift from coral to turf algae dominance. While the turf algae under eutrophied conditions may be able to reach primary production levels of corals, as found in other areas (Smith et al. 2013), they do not have the same ecosystem engineering capabilities as corals (Gattuso et al. 1999; Wild et al. 2004). A shift from corals to algae would therefore have severely detrimental effects on the coral reef community as a whole (Done 1992; Hughes et al. 2010).

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Chapter 8
Ecosystem-engineered seasonal carbon cycling in a Red Sea coral reef

Abstract

Coral reefs in the northern Red Sea experience oligotrophic conditions, particularly in summer due to water column stratification. These conditions may limit reef carbon (C) fixation via gross primary production (GPP). Recent research revealed that reef cavity sponges in the Pacific and Caribbean potentially take up dissolved organic carbon (DOC) at rates comparable to GPP. This sponge mediated C uptake may exceed pelagic microbial DOC uptake via the microbial loop. Coral reefs in the northern Red Sea harbor high densities of cavity sponges that could play a major role in local reef C cycling, but related knowledge is lacking. Therefore, this study investigated the contributions of GPP and sponge DOC uptake to the coral reef C cycle over all seasons of the year 2013 in a fringing reef close to Aqaba, Jordan. C fluxes of the benthic, pelagic, and cavity communities were empirically quantified using a series of incubation experiments conducted in seasonal resolution. These data were then combined into inverse trophic models to quantify the contributions by the different processes to overall reef C uptake. Findings revealed that DOC uptake by cavity sponges and primary production by hard and soft corals dominated the C flows through the reef in all seasons. Primary production was highest during spring with its relatively high light and nutrient availability (spring GPP: 308 mmol C m^{-2} d^{-1}). Sponge DOC uptake was more than 3-fold higher during summer and fall compared to winter and spring (summer DOC uptake: 535 mmol C m^{-2} d^{-1}), and between 3-fold (winter) and 10-fold (summer) higher than pelagic microbial DOC uptake (seasonal means: 43 – 48 mmol C m^{-2} d^{-1}). Annual DOC production within the coral reef was only 3-14 % of the DOC uptake by the sponge community, indicating that sponges in this hydrodynamically open fringing reef not only recycle C, but also efficiently capture allochthonous C for the coral reef community in its oligotrophic environment.

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Chapter 8 Seasonal carbon cycling model

Introduction

Coral reefs thrive under oligotrophic conditions (Kleypas et al. 1999). They maintain high gross primary production (GPP) due to efficient utilization and recycling of scarcely available dissolved and particulate nutritious organic material (Wild et al. 2004; de Goeij et al. 2013). Coral reef primary production is characterized by the symbiosis of coral hosts with photosynthetic dinoflagellates of the genus *Symbiodinium* (i.e. zooxanthellae; Wooldridge 2010; Stambler 2011). This symbiosis thrives in oligotrophic surroundings because the coral host supplements the zooxanthellae with nutrients from heterotrophic feeding or nitrogen fixation by symbiotic microbes, while the zooxanthellae provide carbohydrates to the host (Yellowlees et al. 2008; Cardini et al. 2014). Part of the zooxanthellate photosynthates is released as mucus by the coral and enters the pelagic particulate and dissolved organic carbon (POC and DOC respectively) pools in the coral reef (Naumann et al. 2010). POC released as mucus may function as a pelagic particle trap, forming highly enriched aggregates with suspended materials that then sink rapidly to the coral reef where they are remineralized, thereby providing vital nutrients and organic matter to the reef system (Wild et al. 2004; Mayer and Wild 2010). Besides corals, algae also release substantial DOC (Haas et al. 2010). Up to 80 % of carbon (C) released by reef primary producers dissolves immediately in seawater (Wild et al. 2004). The primary pathway in which this DOC is made re-available to higher trophic levels in oceanic waters is through the microbial loop (Azam and Malfatti 2007). However, bacterioplankton uptake rates were insufficient to explain DOC removal from coral reef waters (de Goeij and van Duyl 2007). Another pathway of DOC recycling to higher trophic levels is mediated by sponges (Yahel et al. 2003; de Goeij et al. 2008a). They take up vast quantities of DOC and simultaneously expel large amounts of choanocytes and mesohyl cells which are available for consumption by higher trophic levels (de Goeij et al. 2013; Alexander et al. 2014; Maldonado 2015). Sponges can be very abundant in cavities riddling coral reefs (Richter et al. 2001). Their total C uptake and release may therefore have a substantial impact on total reef C cycling. To gain a holistic understanding of coral reef C cycling, it is important to assess all C cycling processes described above together.

The coral reefs of the northern Red Sea experience relatively strong variation in light availability and water temperature due to their high latitude location. The annual fluctuation in sea surface temperature (21 – 29 °C) combined with relatively warm deeper water layers (year round ~ 21 °C, water depth > 200 m) result in an annual cycle of deep water mixing from December until May and stratification down to 200 m water depth from June until November (Carlson et al. 2014). Inorganic nutrients are brought up to surface water layers during deep water mixing, while they are trapped in deeper waters during stratification, creating extremely oligotrophic conditions

in coral reef surrounding surface waters (Rasheed et al. 2002; Silverman et al. 2007; Rasheed et al. 2012). These local conditions offer the rare opportunity to study the effects of seasonal variation in key environmental factors on C cycling within the coral reefs.

Several studies have investigated coral reef primary production and organic C cycling (e.g. Charpy and Charpy-Roubaud 1991; Hata et al. 2002; Alldredge et al. 2013). However none have done so, while also taking the sponge C cycling loop into account. The vast majority of previous studies have been performed on platform reefs, or reef flats of fringing reefs, leaving the deeper reef slopes of fringing reefs relatively unexamined (e.g. Gattuso et al. 1993; Chisholm and Barnes 1998; Silverman et al. 2007), while reef slopes can comprise up to 85% of total coral reef area (Smith 1978). In addition, many coral reef primary production studies have investigated the reef community as a whole through methods such as flow respirometry, or eddy correlation, making it impossible to determine the relative contributions by functional groups (Long et al. 2013; Koweek et al. 2015). Finally, the Red Sea has received less research effort than the Caribbean and Pacific regions (Berumen et al. 2013; Loya et al. 2014), while its seasonal variation in environmental conditions makes it an ideal natural laboratory to investigate the effect of that variation on coral reef functioning.

This study combines measurements of benthic and pelagic primary production and C cycling in a seasonal resolution to study the effect of changing environmental conditions on these processes in a northern Red Sea reef. Thereby, it differentiates dominant groups of benthic primary producers, enabling the assessment of their individual contributions to C cycling. To combine the various data, four seasonal trophic food web models were developed. Such models have been developed for coral reefs (e.g. Johnson et al. 1995; Opitz 1996; Niquil et al. 1998; Varkey et al. 2012; Heymans et al. 2014), but to our knowledge only one model exists for the Red Sea with a focus on the Eritrean coast (Tsehaye and Nagelkerke 2008). It, like most other coral reef food web models, focuses on fisheries aspects and therefore the top of the food web. The models presented here incorporate the seasonal aspect of the northern Red Sea and studies the foundation of the food web for the less researched reef fore-slope and its overlying water column.

Materials and methods

Study site

The field work for this study was conducted in the Gulf of Aqaba, at the Marine Science Station (MSS) of the University of Jordan and Yarmouk University, 10 km south of Aqaba. Just off the MSS is a ca. 1 km long fringing reef in which all monitoring data and samples were collected at

29° 27' 31" N, 34° 58' 26" E and 10 m water depth. This location on the fore-reef slope, known as "U7", has been studied over the last 40 years (e.g. Mergner and Schuhmacher 1974; Bednarz et al. 2015). All sampling and monitoring was performed at 10 m water depth within 100 m of "U7" during four 4-week periods in 2013 in February, April, September, and November. The timing of these periods (hereafter called winter, spring, summer, and fall) was previously determined from literature to best cover the annual cycle of environmental conditions in the Gulf of Aqaba (Silverman et al. 2007; Carlson et al. 2014).

Benthic cover

The "U7" area contains a 5 x 5 m^2 rope grid. Vertical pictures were taken of the 25 grid cells on one day in each season and the benthic composition of the research area was quantified using digital image analysis (CPCe image analysis software; Kohler and Gill 2006). 100 random points were placed on every photo and the benthic group underneath was determined down to the lowest possible taxa or substrate type. These data were then grouped into functional groups (Table 8-1). Sediment and coral rock were defined as "bare" reef sand and solid reef structure which were not overgrown by any of the other categories. The functional group data were recalculated to percentage benthic cover in each season. Surface sponge benthic cover was determined by in-situ measurements of sponge surface area within a 0.25 m^2 planar quadrat which was placed every 10 m along three serial 50 m transect lines through the research area at 10 m water depth (n = 15). This was done to improve the estimate of sponge cover as many surface sponges are relatively small and found within cracks and crevices, making them hard to quantify from planar photographs. The benthic cover of sponges in reef cavities within the reef structure was taken from literature at 0.82 m^2 sponge cover per m^2 planar reef surface (Richter et al. 2001). The water column above the reef was assumed to include 8 m^3. Current measurements throughout the water column indicated that the top 2 m were more wind-driven than the 8 m below, reaching substantially higher flow rates than the bottom 8 m (annual mean 25 ± 13 cm s^{-1} and 5 ± 1 cm s^{-1} respectively (mean ± SD); N. van Hoytema unpublished data). It was therefore assumed that there was little exchange between the top layer and the deeper water column.

Environmental monitoring and metabolic incubations

The following is a brief overview of methods used for monitoring of environmental conditions in-situ, measurements of heterotrophic bacterial abundance in-situ, metabolic incubations of benthic functional groups and plankton, and sampling and maintenance of specimens for those incubations. These methods have been fully described in materials and methods sections of previous chapters: environmental monitoring, chapter 1; heterotrophic bacteria abundance,

chapter 1; specimen sampling and maintenance, chapters 1 and 7; photosynthesis and respiration incubations, chapters 1 and 7; POC and DOC uptake and release incubations for benthic functional groups, chapter 2. Any addition to methods described in those chapters is given below.

Light (lux) and water temperature at 10 m water depth were measured during all seasons. The lux measurements were converted to photosynthetically active radiation (PAR) by a conversion factor calculated from a simultaneous minute-by-minute measurement of lux and PAR (08:00 to 14:00 on one day n = 353) using a HOBO pendant logger and a LI-COR LI192SA underwater quantum sensor: lux = PAR x 52.0, $R^2 = 0.83$ (Long et al. 2012). Water samples were collected on a weekly basis in the four weeks encompassing each study period for measurements of water column status parameters and planktonic net photosynthesis (Pn) and respiration (R), as briefly described below, (n $week^{-1}$ = 4 - 6). Samples were collected 1 m above the seafloor in clean high density poly-ethylene (HDPE) containers (volume: 5 L) using SCUBA. Subsamples were taken from the containers within 20 min of collection to measure concentrations of inorganic nutrients (NH_4^+, PO_4^{3-}, and NO_x; $NO_x = NO_2^- + NO_3^-$), particulate organic carbon (POC), dissolved organic carbon (DOC), and chlorophyll a (Chl a). Nutrient concentrations were measured according to Murphy and Riley (1962, Strickland and Parsons (1968), and Holmes et al. (1999) using a Trilogy Fluorometer (Turner Designs) for NH_4^+, and a JASCO-V630 photometer for PO_4^{3-} and NO_x. Subsamples for POC (1L), filtered onto pre-combusted (450 °C, 5 h) GF/F filters (nominal pore size 0.7 µm), were dried (40 °C for 48 h) pending analysis. Filters were acidified (0.1 N HCl) prior to measurement to remove any inorganic C. Measurements were performed on a EuroVector elemental analyzer (EURO EA 3000; analytical precision ≤ 0.1 %). Subsamples for DOC (50 mL) were vacuum filtered (max. pressure 20kPa) through pre-combusted GF/F filters straight into pre-acid-washed (0.4 mol L^{-1} HCl) 30 mL HDPE sample bottles, directly acidified with 80 µL of 18.5 % HCl, and stored at 4 °C in the dark until analysis by high-temperature catalytic oxidation on a Shimadzu TOC-V_{CPH} total organic C analyser using reference water samples (CRM program, Hansell Research Lab, USA, DA Hansell and W Chan; Batch 13, Lot #08-13, 41-45 µmol C L^{-1}) as a positive control after every 10 samples. Chl a subsamples (1L) were filtered onto pre-combusted GF/F filters and stored in the dark at -80 °C. Chl a was later extracted in 90 % acetone for 12 h at 4 °C in the dark and measured on a Trilogy fluorometer using the non-acidification module (CHL NA #046, Turner Designs).

Specimens of benthic functional groups hard and soft corals, macroalgae, turf algae, coral rock, and sediment as well as surface and cavity sponges were collected for quantification of metabolic fluxes (n = 8). The hard corals were represented by the four genera *Acropora*, *Stylophora*, *Goniastrea*, and *Pocillopora*, the two main groups of soft corals were the family Xeniidae, and genus *Sarcophyton*, and macroalgae benthic cover was dominated by the genera

Lobophora and *Caulerpa*. Turf algae, sediment, and coral rock were treated as bulk groups containing communities of various biotas (Larkum et al. 2003; Schottner et al. 2011). Surface sponges were represented by the genera *Mycale*, *Hemimycale*, *Amphimedon*, *Callyspongia*, and *Negombata*, and cavity sponges by *Chondrilla*. Fragments of hard corals, soft corals, macroalgae, turf algae covered dead coral branches, coral rock, and sponges were collected with hammer and chisel or tweezers. Sediment samples were collected with corers. Sediment cores were extruded from their corers and the top 1 cm was sliced off and placed in a Petri dish with the same surface area as the core (14.52 cm^2). All specimens were maintained in an outside flow through aquarium (800 L, 4000 L h^{-1} flow rate) with water coming from the coral reef at 10 m water depth, thus resembling ambient in-situ conditions of temperature and inorganic nutrients. Light conditions were adjusted to in-situ conditions at 10 m water depth using layers of plastic mesh and monitored with data loggers (Onset HOBO Pendant UA-002-64). The same specimens (or a subset thereof) were used for all metabolic incubations.

Closed cell respirometric incubations under in-situ-like conditions for measurements of net photosynthesis (Pn) and respiration (R) took place in the flow-through aquarium. Planktonic metabolic rates were measured with 0.5L glass chambers filled with water from the weekly environmental monitoring. Start O_2 measurements were taken with an O_2 optode and a conductivity probe (MultiLine® IDS 3430, WTW GmbH, Weilheim, Germany, accuracy: ± 0.5% of measured value). The chambers were then closed while removing all air. Chambers for R were placed in dense plastic bags for incubation in the dark and all chambers were moved to the flow through aquarium. Pn incubations under in-situ like conditions lasted from 10:00 until sunset. R incubations lasted for 24 h. After the incubations, end O_2 concentrations were immediately measured. Difference between start and end measurements were normalized for time and volume resulting in rates of nmol O_2 L^{-1} h^{-1}. Benthic specimen incubations were performed using water from the flow through aquarium in 1 L glass chambers for hard corals, soft corals, macroalgae, turf algae, and coral rock, 2 L chambers for sponges (see below), and 0.5 L chambers for sediment (n = 6 - 8). In addition, seawater controls (n = 8) were run in parallel to correct for metabolic activity in the incubation seawater. Chambers were stirred with magnetic stirrer plates. R measurements took place in the dark at least 1 h after sunset to ensure full darkness, while Pn measurements were performed the following day between 12:00 and 14:00 (10:00 and 16:00 for sediment incubations), which represents the most stable and maximum light conditions of the day as determined by light measurements (Appendix 5 - Fig. 1). All specimens, except for sediments and sponges, were incubated for 60 - 90 min for Pn, and 90 - 120 min for R. Sediments were incubated for 120 - 330 min for Pn and 180 - 360 min for R due to lower metabolic activity, and sponges were incubated for 180 min for R due to the larger chamber volume. The measurement

procedure was identical to that of the water column incubations. Pn and R rates were calculated by subtracting start from end O_2 concentrations and relating to incubation duration (mg O_2 production/consumption L^{-1} $hour^{-1}$). These O_2 rates were then corrected for the seawater control O_2 fluxes before being normalized to chamber volume and organism surface area (see below). Fluxes were recalculated to nmol O_2 cm^{-2} specimen surface area h^{-1}. Photosynthetic and respiratory quotients were assumed to be 1 for all measured specimens, leading to a direct conversion to nmol C cm^{-2} h^{-1} (Gattuso et al. 1996; Carpenter and Williams 2007).

Organic matter uptake and release by corals, macroalgae, turf algae, coral rock, and sponges were quantified using beaker incubations (Herndl and Velimirov 1986; Naumann et al. 2010). A selection of specimens used for the O_2 flux incubations (n = 6) were incubated from 10:00 until 16:00 h in open 1L glass chambers in the flow through aquarium with simultaneous seawater control incubations. Sponge methodology was different, see below. The setup was covered with transparent plastic foil to prevent contamination with airborne particles while leaving small openings on the sides for air exchange. Chambers were not stirred to prevent water currents from modifying the structural composition through e.g. POC dissolution to DOC. DOC and POC samples were collected at the start and end of the incubation and measured as described in the environmental monitoring section. Differences in DOC and POC between start and end samples were corrected for the control incubations and then normalized to time, volume, and specimen surface area. Incubations of cavity and surface sponges were conducted in chambers containing 2 L of seawater and over 3 h to take into account the fast filtration activity of sponges. These incubations were stirred to keep POC suspended and thereby available to the sponges for filtration. All organic matter fluxes were recalculated to nmol C cm^{-2} h^{-1}.

The surface area of all specimens used in all metabolic incubations was quantified to normalize metabolic fluxes. Specimen surface areas were measured as described in chapter 7 using advanced geometry and digital image analysis. In addition, sponge surface areas were also measured by advanced geometry (Naumann et al. 2009).

Heterotrophic bacterial abundance in the water column was measured to constrain flows pertaining to the microbial loop. Samples (2 mL) were collected in March (n = 4; used for winter and spring) and November (n = 53; used for summer and fall) and fixed with 0.1 % paraformaldehyde (final concentration), frozen with liquid nitrogen, and then stored at -80 °C until analysis. The analysis was performed with a flow cytometer (FACSCalibur, Becton Dickinson, 488 nm excitation laser). Samples were stained with SYBR Green 1 (conc. 1 per 1000) for 30 min prior to analysis, sorted at a flowrate of ca. 0.06 µL min^{-1} for 1 min and subsequently gated on a side scatter versus green fluorescence density plot.

Food web model

To comparatively study C flows through the coral community, linear inverse food web models (LIMs) were developed for the four seasons. A LIM consists of matrix equations with equalities and inequalities (Soetaert and van Oevelen 2009). Model constructions and solutions were run in R (version 3.0.2, R Development Core Team, 2013) using the R-package LIM (http://lib.stat.cmu.edu/R/CRAN/web/packages/LIM/index.html). The equalities contain the topology of the food web and single value real world data from measurements on the flows such as respiration and primary production. The inequalities place constraints on the food web flows such as a minimal fraction of assimilated C must go to respiration or respiration must fall between two values. The LIM is the same for all four seasons and consists of three major areas: the water column, the coral reef benthos, and the cavity sponge community within the coral reef. The water column food web is based on a LIM for the water column in a Pacific Atoll (Niquil et al. 1998). It consists of phytoplankton, zooplankton, bacterioplankton, protozoa, POC and DOC. These C compartments are termed pelagic particulate organic carbon (PPC) and pelagic dissolved organic carbon (PDC) from hereon to differentiate them from organic C in sediment and cavities. It is assumed that all biotic compartments respire. In addition to this, phytoplankton performs photosynthesis and is grazed by protozoa and zooplankton. It also releases PPC and PDC to the water column. Zooplankton excretes PPC and PDC and grazes on phytoplankton, protozoa and PPC. Protozoa excrete PPC and PDC, and feed on PPC, PDC, bacterioplankton, and phytoplankton. Bacterioplankton feeds on PDC and releases PDC. Finally, PPC dissolves into PDC, and there is an export flow from zooplankton to higher trophic levels not described in the model. As the modeled fringing reef is relatively hydrodynamically open, the water residence time is measured in hours, compared to years for a Pacific atoll lagoon (Niquil et al. 1998; Naumann et al. 2012), inflows of C are allowed for all water column compartments. The consistency of the imported C has to reflect the relative biomasses of C for the water column compartments as calculated from the environmental monitoring and from literature on the research area (see below). The coral reef benthos consists of hard corals, soft corals, macroalgae, turf algae, coral rock, sediment, and surface sponges. All these compartments respire and all photosynthesize except for surface sponges (see discussion). Hard and soft corals feed heterotrophically on zooplankton, protozoa, and bacteria. Coral rock with its filter feeding community does the same, but also feeds on phytoplankton (Yahel et al. 2006). In addition, all these compartments can take up, and release, PDC and PPC. Sediment consists of the following sub-compartments: sediment biota, sediment dissolved organic carbon (SDC) and sediment particulate organic carbon (SPC). The sediment biota both takes up and releases SPC and SDC. PPC settles out of the water column to the SPC, and SDC diffuses out to PDC (Chipman et al.

2012). There is also a direct flow from hard corals to SPC based on the release of mucus strings which quickly settle to the sediment (Mayer and Wild 2010; Naumann et al. 2012). All benthic compartments have an export flow which simulates removal of C by processes such as feeding by organisms not included in the model. The surface and cavity sponges respire and it was assumed for the sponges that any C fluxes into and out of the organisms are through assimilation. C which flows through the sponge without being assimilated was ignored. Both sponge compartments assimilate PPC, PDC, phytoplankton, protozoa, and bacteria. The release of sponge cells as part of the sponge loop was simulated as release to PPC for surface sponges. Cavity sponges release cells to cavity particulate organic carbon (CPC). It was assumed that CPC was separate from PPC and only available to detritivores not included in the model. Additionally, both surface and cavity sponges have an export flow to spongivores not included in the model.

The LIM in each season consists of 104 flows, 16 compartments, and 133 equalities, or inequalities. All data introduced to the model was added as ranges (inequalities) to incorporate variability in measurements, or uncertainty of literature values, except for when a flow was 0. This occurred only for certain PPC or PDC uptake rates for the benthos when functional groups in certain seasons only showed release of either PPC or PDC, the uptake rate was then set to 0 (max. 4 flows per model). The models were solved for the flow values by a Monte-Carlo sampling method (Soetaert and van Oevelen 2009). Briefly, 10.000 food web structures were sequentially sampled resulting in 10.000 estimates for each C flow. All estimates were different, but consistent with the matrix equations constructed from the food web topology and entered values. The mean and standard deviation of the sample collection for each flow as presented in the results section represent the best estimate and a measure of its uncertainty respectively. The model unit for all flows is mmol C m^{-2} reef surface d^{-1}; see below for respective conversions of rates to this unit.

Data treatment for model input

Benthic cover

The two dimensional (2D) benthic cover percentages for the benthic compartments measured using the photo-quadrats were recalculated to three dimensional (3D) surface areas per planar m^2 of reef surface. This was done by 3D/2D conversion factors which were calculated from two measurements of specimens used for the primary production incubations (Table 8-1). First, the 3D surface area of specimens was determined using the same methodologies as used in the metabolic incubations. Second, vertical photographs were taken from all specimens under natural conditions. The vertical pictures were then digitally analyzed with Image J (Schneider et al. 2012) to quantify specimen 2D planar surface area. The 3D surface areas were divided by corresponding

2D planar surface areas to obtain conversion factors. The soft coral conversion was based on measurements of Xeniidae since this group dominated soft coral benthic cover. Branching hard coral conversion was based on the average of measurements on *Acropora* and *Stylophora* by Naumann et al. (2009). Their measurements, which were done on larger fragments and coral colonies than the fragments used here for the primary production incubations, better resembled the size of colonies in-situ, therefore resulting in more accurate conversion factors. Other hard coral and coral rock conversion factors were based on incubated specimens of the genus *Goniastrea* and coral rock respectively. The benthic cover analysis from the "U7" photographs included more categories than incubated. Conversion factors for these categories were derived from conversion factors that were available from direct measurements. Dead coral (no live tissue, but skeletal structure more clear than in coral rock) was assumed to be the average of branching and other corals. The macroalgae *Caulerpa*, growing in loose bundles, was directly measured for its 3D/2D conversion, while *Lobophora* and other macroalgae were assumed to adhere to their substrate. Their conversion was therefore based on an average of coral rock and dead coral. Rubble (small fragments of reef structure lying on sediment areas) was assumed to have the same conversion factor as coral rock. Carbonate sediment was given a conversion factor of 1. The turf algae conversion factor was calculated as the average of its underlying substrates (coral rock, rubble, dead coral, and sediment) weighed by the relative cover of turf algae on each of these substrates. All these conversions resulted in cm^2 3D functional group surface per 2D planar m^2 reef area (Table 8-1) which were then combined with the measured C fluxes from the incubations to produce values per group per m^2 reef area (Appendix 6 - Tables 2-3). The 8 m^3 water column was integrated to the planar reef surface. Surface sponges benthic cover was already measured three dimensionally in the planar quadrat measurements and could be used directly while the 3D cavity sponge surface area was already available per 2D m^2 reef area from Richter et al. (2001).

Biomasses

To specify the consistency of the C entering the reef from offshore, estimates were made in each season for biomasses of the water column compartments (Appendix 6 - Table 1). PDC and total PPC (including all plankton) concentrations were measured in the environmental monitoring (Table 8-2). Phytoplankton biomass was based on Chl a concentration assuming µg C = 60 x µg Chl a (Yahel et al. 1998). Bacterioplankton biomass was based on measured heterotrophic bacteria abundances; assuming 20 femtogram C bacterial $cell^{-1}$ (Lee and Fuhrman 1987). The protozoa compartment was assumed to consist of ciliates and heterotrophic flagellates. Ciliate biomass was taken as minimum and maximum values for Gulf of Aqaba surface waters from Claessens et al. (2008). Heterotrophic flagellate biomass was calculated from cell abundances ranging from 5 x 10^5 to 1 x 10^6 L^{-1} (Berninger and Wickham 2005), a flagellate cell volume of

15.09 μm^3 (Borsheim and Bratbak 1987), and a mass of 200 femtogram C μm^3 flagellate $cell^{-1}$ (Van Duyl et al. 1990). Zooplankton biomass in Jordanian fringing reefs ranges between 10 and 20 mg dry weight m^{-3} (Al-Najjar and El-Sherbiny 2008). C content was assumed to be 40% of dry weight (Parsons et al. 1984). The detrital component of total PPC (described in other sections as PPC) was determined by subtracting plankton biomasses from total PPC. Ranges were used for the biomasses based on seasonal mean ± SD of the weekly environmental monitoring to incorporate variation within each season. The fractions that pelagic compartments comprised of total water column C were used to constrain the consistency of C entering the reef (Appendix 6 - Table 1). In addition, a range of 2 - 12 mmol C m^{-2} d^{-1} PPC sedimentation to SPC was entered (Naumann et al. 2012), as well as a direct hard coral to SPC mucus string flow of 1.0 - 2.6 mmol C m^{-2} d^{-1} (Mayer and Wild 2010; Naumann et al. 2012).

Table 8-1. Surface areas of dominant benthic functional groups. Benthic cover quantified from 25 1 m^2 photoquadrats per season (100 random points per photoquadrat). 3D/2D conversion factors used to recalculate benthic cover % to 3D cm^2 for each functional group per planar 2D m^2 reef area. Surface sponge surface area determined by in-situ 3D measurements with a quadrat placed every 10 m along 150 m line transect. Cavity sponge surface area based on Richter et al. (2001). NA = not applicable.

	Benthic cover (%)				3D/2D	Surface area (cm^2 m^2 planar $reef^{-1}$)			
	Winter	Spring	Summer	Fall		Winter	Spring	Summer	Fall
Branching corals	2.71	2.99	2.72	3.97	7.58	2051	2265	2060	3007
Other corals	12.17	12.32	12.94	13.96	2.89	3516	3558	3739	4035
Xeniidae	32.04	28.61	17.96	19.21	6.45	20676	18461	11588	12395
Other soft coral	0.98	2.19	1.85	2.30	6.45	635	1415	1195	1487
Lobophora	0.49	0.86	0.86	0.86	3.88	191	332	335	335
Caulerpa	0.50	2.72	0.24	0.12	1.84	92	500	45	23
Other macroalgae	0.00	0.45	0.12	0.17	3.88	0	175	48	64
Turf algae	3.33	3.28	3.38	3.30	2.77	921	910	936	914
Coral Rock	13.96	13.16	18.84	17.56	2.53	3539	3337	4776	4451
Sediment	28.21	25.22	31.10	30.16	1.00	2821	2522	3110	3016
Surface sponges					NA	400	400	400	400
Total	94.39	91.80	90.02	91.62		34842	33875	28231	28326
Cavity sponges					NA	8200	8200	8200	8200

Metabolic rates

The hourly benthic and water column Pn and R rates were extrapolated to daily fluxes by the following calculations. Pn and corresponding R were summed and then multiplied by average seasonal hours of daylight (Table 8-2) to calculate daily gross primary production (GPP). R was

multiplied by 24 h to obtain daily community respiration (Rday). Net community production (NCP) was calculated by subtracting Rday from GPP. The PR ratio was finally calculated by dividing GPP by Rday. PPC and PDC uptake/release rates of all benthos compartments were extrapolated to d^{-1} by multiplying by 24 h while taking into account relative differences between day and night. Night PPC fluxes are 0.67 and 0.55 times those during the day for corals and algae respectively (Haas et al. 2010; Naumann et al. 2010). The algae factor was applied to all non-coral groups. It was assumed that PDC fluxes behaved similarly to PPC fluxes. All these extrapolations were performed on the original rates measured for all replicates incubated. Flows to and from compartments were constrained by the minimum and maximum measured rates from the incubations to incorporate variation between replicate specimens or samples (Appendix 6 - Tables 2-3). For the PPC and PDC fluxes, this meant that groups might both take up and release C as some replicates showed net uptake while others showed release. Planktonic GPP resulted in negative values for some replicates in each season due to higher O_2 consumption in Pn incubations than in R incubations. Because of this, minimum Planktonic GPP in all seasons was set to 0. Planktonic Rday was used as the sum of respiration by all planktonic compartments. Seasonal sponge Rday and PPC or CPC release rates were based on values measured during October. These values were assumed to be applicable to summer and fall, but were adjusted for winter and spring. The sponge genus *Mycale* was incubated in all seasons (chapter 4). Relative differences in summer-fall vs. winter and spring in Rday and PPC or CPC flows in those incubations were used to adjust the overall surface and cavity sponge flows to winter and spring conditions.

Metabolic constraints

Metabolic constraints were placed on compartments which flows were less defined by measurement data to regulate the balance between uptake (i.e. ingestion), assimilation, and production of biomass. Sediment, coral rock, zooplankton, and protozoa all were constrained by assimilation efficiencies: assimilation is 40 - 80% of uptake (Banse 1979; Hendriks 1999), and production efficiencies: production is 30 - 60% of assimilation (Calow 1977; Banse 1979; Hendriks 1999). In addition, coral rock grazing on phytoplankton was constrained specifically (Yahel et al. 2006). Rates in ng Chl a cm^{-2} rock surface h^{-1} for March and September - November were converted for winter-spring and summer-fall respectively to daily fluxes in the reef assuming the Chl a to C conversion mentioned above and the benthic cover of coral rock in the reef in each season. Total zooplankton uptake was constrained by an upper limit using the maximum biomass estimate and water temperature with the following formula: uptake = 0.49 x biomass x $e^{0.0693T}$ (Vezina and Platt 1988). Zooplankton and protozoa grazing on other biota were constrained by grazing rates: zooplankton: 0.007 to 0.013 d^{-1}, protozoa: 0.15 to 1.1 d^{-1} for bacteria and 0.15 to 1.3

d^{-1} for phytoplankton (Grossart and Simon 2002; Sommer et al. 2002). Protozoan production was also constrained by a growth rate of 0.15 d^{-1}: range implemented in the models: 0.05 d^{-1}* min biomass to 0.25 d^{-1}* max biomass (Sakka et al. 2000). In addition, zooplankton and protozoa excretion (excretion = uptake - assimilation) was constrained as 0.33 to 1 x Rday (Niquil et al. 1998). Bacterioplankton flows were constrained by production = 0.1 to 0.6 x uptake (del Giorgio and Cole 1998) and growth = 0.15 to 1.3 d^{-1} x biomass (Grossart and Simon 2002; Sommer et al. 2002). Phytoplankton flows were constrained by Rday = 0.05 to 0.3 x GPP, and excretion = 0.05 to 0.5 x GPP (Niquil et al. 1998). Heterotrophic feeding by hard corals was constrained as 0.2 to 0.6 x Rday (Grottoli et al. 2006). The same constraint was placed on the soft corals, but heterotrophic feeding was only assumed for the fraction not consisting of Xeniidae since this family has an almost completely autotrophic lifestyle (Schlichter et al. 1983). Sponge flows not directly measured were constrained by Rday and POC release data from incubations, as well as a C assimilation (PPC + PDC) to POC release efficiency of 11 to 24 % (de Goeij et al. 2013).

Statistical analyses

The first 1000 solutions sets of every season were analyzed using Primer-E v6 (Clarke and Gorley 2006) with PERMANOVA extension (Anderson 2001) for univariate distance-based permutational nonparametric analyses of variance (PERMANOVA). Solution sets were square root transformed, after which a Bray-Curtis similarity matrix was constructed. A PERMANOVA with Type III sums of squares was used with 999 permutations and residuals under a reduced model due to the size of the dataset. A main test and subsequent pair-wise tests were performed for the factor season assuming a significant difference at $p < 0.05$. The comparison between reduced solution sets was visualized with a multi-dimensional scaling plot (MDS).

Results

The environmental monitoring revealed a clear separation between seasons in light, temperature, and inorganic nutrient concentrations (Table 8-2). Chl a concentrations were lower during low nutrient summer, while PDC concentrations were higher in that season.

All seasonal models were solvable when allochthonous C was allowed to flow into the reef. However, when this inflow was turned off, the summer and fall models were no longer solvable. The models in those seasons required inflow of external organic C to balance their loss terms, while the winter and spring models could rely solely on internal GPP. Significant differences were found between all seasons overall, and between each two seasons separately for the reduced solution sets used in the PERMANOVA (Appendix 6 - Table 4). The MDS visualized

a strong difference between the flow estimate collections of winter and spring vs. summer and fall. Additionally, summer and fall were more comparable to each other than winter and spring (Fig. 8-1).

Table 8-2. Seasonal measurements of environmental parameters in the research area. Temperature and irradiance values based on 1 min frequency measurements between 12:00 and 14:00 on all days encompassing each respective sampling period (n = 18-27 days). Inorganic nutrient, Chl a, PPC, and PDC concentrations based on weekly measurements in the four weeks encompassing each sampling period. Values are given as mean ± SD. Feb = February, Apr = April, Sep = September, Nov = November. PAR = photosynthetically active radiation. Nitrogen oxides = nitrate + nitrite concentrations. Chl a = chlorophyll a, PPC = pelagic particulate organic carbon, PDC = pelagic dissolved organic carbon.

	Winter (Feb)	Spring (Apr)	Summer (Sep)	Fall (Nov)
Temperature (°C)	22.7 ± 0.2	23.0 ± 0.2	27.2 ± 0.3	25.3 ± 0.2
PAR (µmol photons m^{-2} $s^{-1)}$	147 ± 60	281 ± 61	320 ± 63	162 ± 63
Hours of daylight	11.22	12.82	12.38	10.65
Ammonium (µmol L^{-1})	0.32 ± 0.09	0.46 ± 0.07	0.11 ± 0.01	0.28 ± 0.12
Phosphate (µmol L^{-1})	0.11 ± 0.01	0.10 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Nitrogen oxides (µmol L^{-1})	0.71 ± 0.15	0.56 ± 0.12	0.06 ± 0.02	0.22 ± 0.23
Chl a (µg L^{-1})	0.21 ± 0.03	0.21 ± 0.01	0.10 ± 0.01	0.19 ± 0.05
PPC (µmol L^{-1})	6.16 ± 1.29	10.25 ± 2.95	7.96 ± 2.46	8.81 ± 0.98
PDC (µmol L^{-1})	72.92 ± 5.21	71.95 ± 7.54	90.49 ± 1.03	86.24 ± 2.50

Benthic cover

The combination of planar benthic cover estimates and 3D/2D conversion factors resulted in a benthic cover dominated by soft corals (Table 8-1). Soft coral benthic cover declined from spring to summer, coinciding with an overall reduction in total 3D surface area per planar m^2. The decline of soft coral cover led to an increase in coral rock as this substrate was uncovered. However, the lower 3D/2D conversion factor of coral rock compared to soft corals resulted in the overall decline in 3D surface area. Macroalgae benthic cover peaked in spring, while turf algae cover was stable over the seasons.

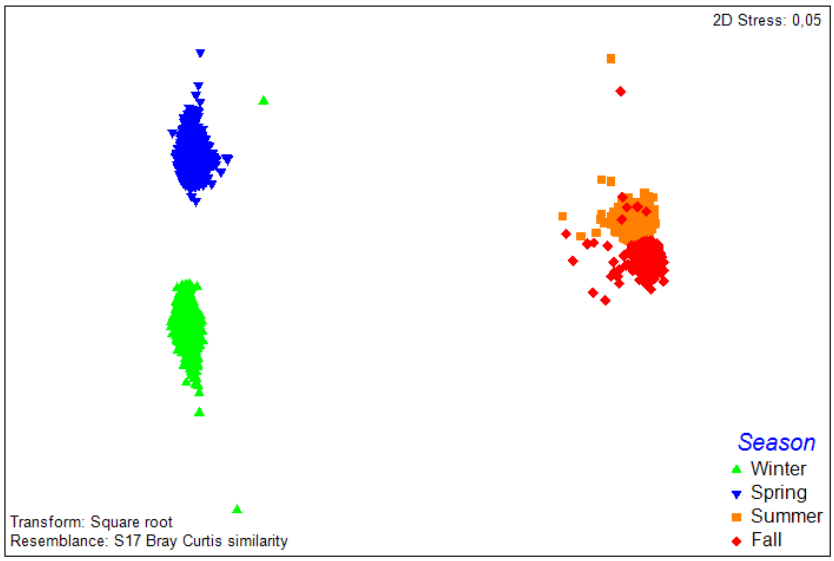

Fig. 8-1. Multi-Dimensional Scaling plot of first 1000 solution sets of every season. Based on Bray-Curtis similarity matrix after solution sets were square root transformed.

Model output

The mean flow values returned by the four seasonal models cover five orders of magnitude (Fig. 8-2), the one exception being DOC uptake by macroalgae in spring (mean: 6.2 x 10^{-4} mmol C m^{-2} d^{-1}, not depicted in Fig. 8-2 and Appendix 6 - Fig 2). The largest flows in all seasons were cavity sponge DOC uptake, hard and soft coral GPP, and bacterioplankton DOC uptake, ranging from ca. 40 to 540 mmol C m^{-2} d^{-1} (Fig. 8-2, Appendix 6 - Fig. 1-4). The smallest flows in all seasons were grazing by zooplankton and soft corals, as well as organic C uptake and release by macroalgae: all flows < 1 mmol C m^{-2} d^{-1}. The standard deviations of the flows are a measure of how well they are constrained. Overall, the flows in each season appear well constrained, especially so for the larger and therefore more important flows (Appendix 6 - Fig. 1-4). The coefficient of variation (CoV, i.e. standard deviation normalized to its corresponding mean) for the flows in each season also reveals good constraint. CoV < 0.5 for 51 to 56 % of the flows and < 0.7 for 73 to 75 % of the flows over the seasons. Total reef GPP was highest in spring, while Rday was highest in summer and fall (Fig. 8-3). Mean NCP was positive in winter and spring, close to 0 in summer, and negative in fall. Correspondingly, mean PR ratio was 1.3 and 1.4 for winter and spring respectively. Summer mean PR was 1.0 and fall mean PR was 0.83, indicating that the reef C balance was more heterotrophic in summer and fall than winter and spring. This is also visible in the relative balance between GPP and the required inflow of external organic C (Fig. 8-3; Table 8-3). GPP/inflow was 1.3 and 1.5 in winter and spring, but declined strongly to 0.4 and 0.3 in summer and fall. Organic C inflow was dominated by DOC; it constituted 88 - 92 % of total inflow (Table 8-3).

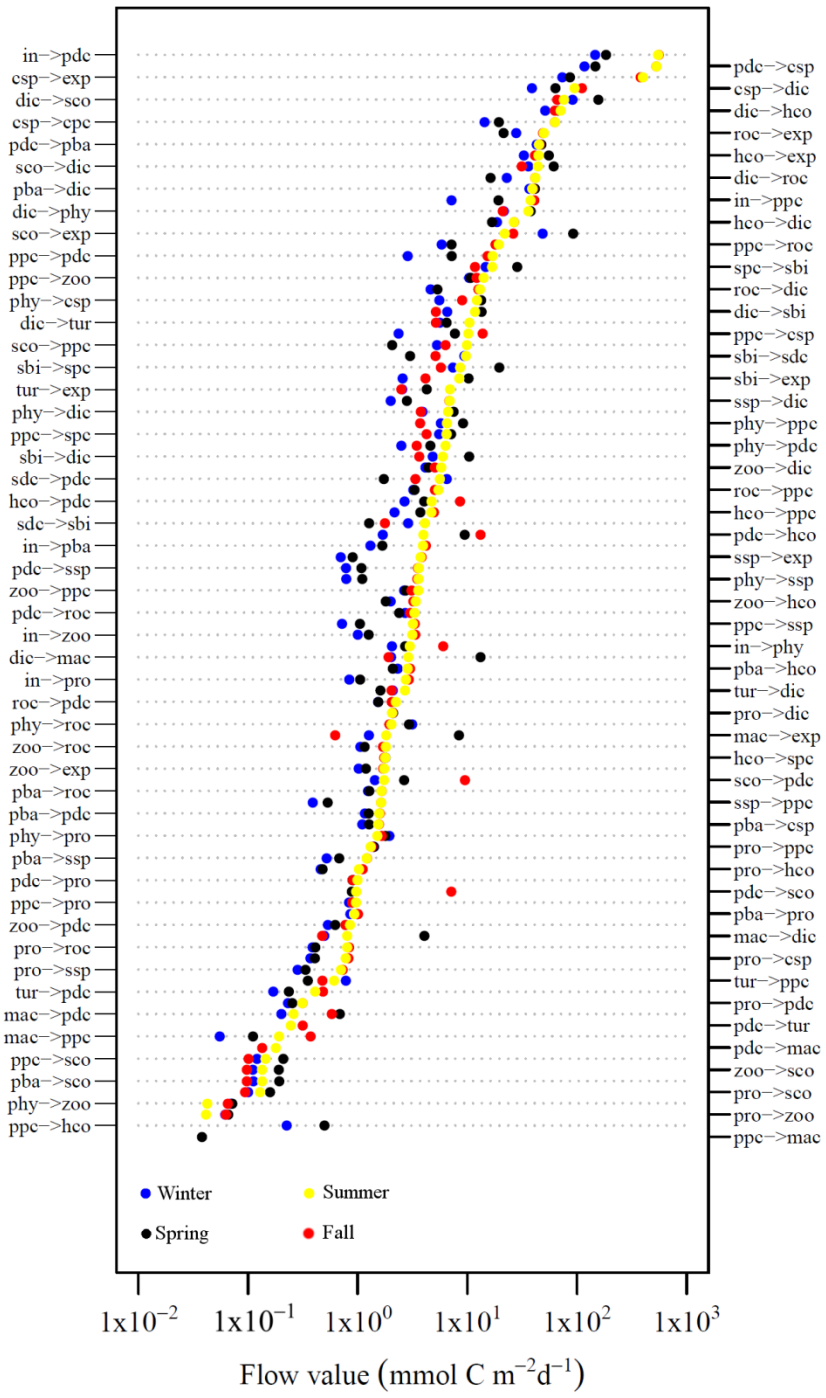

Fig. 8-2. Means of all flows in the seasonal food web models. Ordered for flows in the summer model (high to low). cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.

Hard and soft corals were the dominant contributors to GPP (Fig 8-4). Their combined contribution ranged from 59 % in summer to 71 % in spring. Planktonic GPP was relatively stable and contributed between 11 and 14 % to total GPP. The benthos was most important for Rday during winter (26, 30, 43 % contribution to Rday for sponges, water column, and benthos respectively). However, sponge respiration increased strongly from spring to summer and fall, causing higher overall Rday, and a relative contribution of sponges up to 48%, while benthos contribution declined to 31 % (Fig. 8-5). Combined surface and cavity sponge DOC uptake is one of the largest processes in the reef, outranking system GPP in summer and fall (Fig. 8-6). Sponge DOC uptake was also up to more than 10-fold higher than either benthic or pelagic DOC uptake in summer and fall (Fig. 8-7). Total system DOC uptake was 10 to 20-fold higher than system DOC release; indicating heavy reliance on the inflow of allochthonous DOC (Fig. 8-8). Export out of the model e.g. due to unmodeled predation was dominated by flows from cavity sponges, hard and soft corals, and coral rock (Table 8-4).

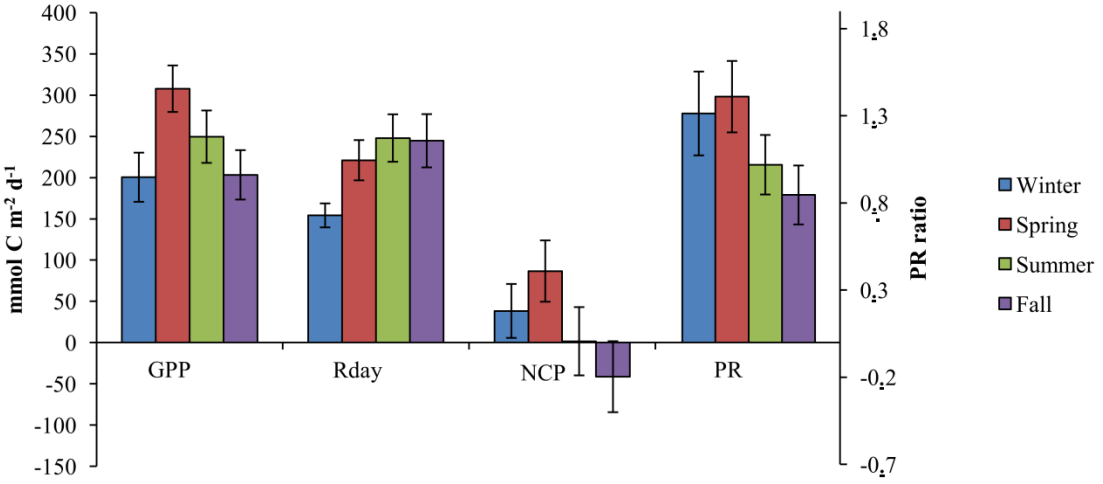

Fig. 8-3. Metabolic parameters of the coral reef community over the seasons. Error bars indicate ± SD. GPP = gross primary production, Rday = community respiration, NCP = net community production, PR = gross primary production to community respiration ratio. GPP, Rday, and NCP are related to the left y-axis, PR ratio is related to the right y-axis.

Table 8-3. Inflow of C into the model over the seasons. Composition of inflow was constrained by water column biomasses from environmental monitoring and literature (Appendix 6 - Table 1). Water column integrated to reef surface: all values in mmol C m^{-2} d^{-1}, values given as mean ± SD.

	Winter	Spring	Summer	Fall
Protozoa	0.84 ± 0.18	1.05 ± 0.20	2.74 ± 0.53	2.92 ± 0.57
Zooplankton	1.00 ± 0.15	1.26 ± 0.17	3.13 ± 0.52	3.34 ± 0.54
Bacteria	1.30 ± 0.06	1.68 ± 0.13	3.93 ± 0.09	4.17 ± 0.10
Phytoplankton	2.06 ± 0.10	2.71 ± 0.17	2.98 ± 0.16	6.02 ± 0.70
PPC	7.17 ± 0.64	19.23 ± 2.34	37.55 ± 6.18	40.82 ± 1.14
PDC	146.08 ± 5.23	183.73 ± 8.29	548.50 ± 12.74	556.41 ± 12.87
Total	158.44 ± 5.70	209.66 ± 9.63	598.85 ± 14.01	613.67 ± 14.24

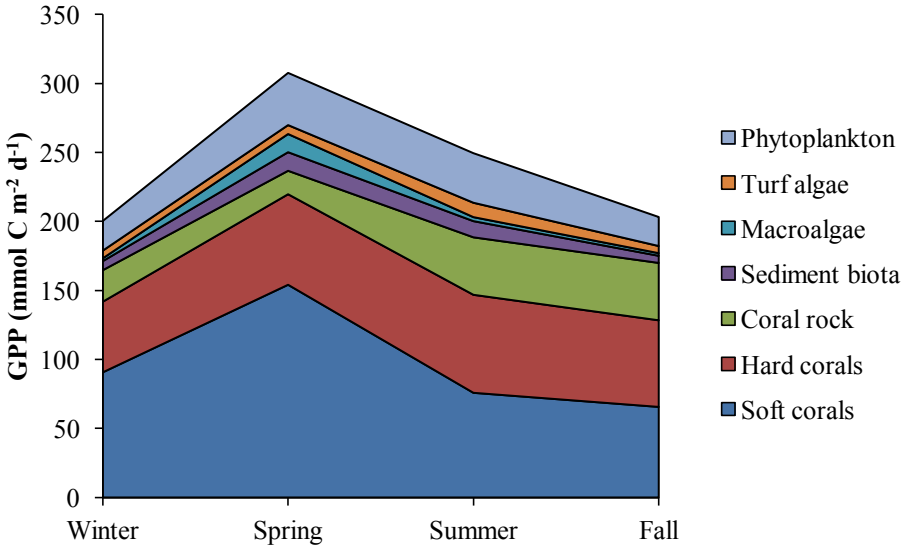

Fig. 8-4. Gross primary production (GPP) over the seasons for the photosynthetic functional groups.

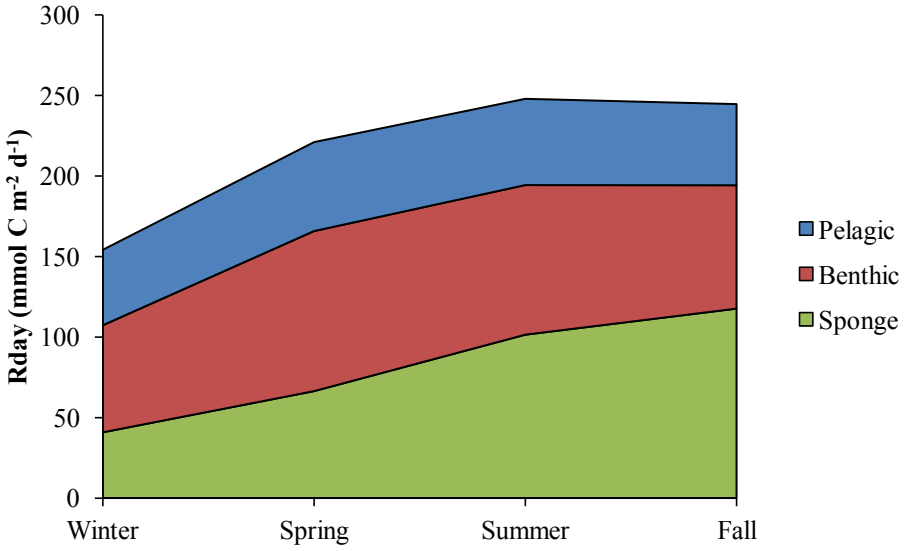

Fig. 8-5. Community respiration (Rday) for the main coral reef compartments over the seasons. Sponge = surface sponges and cavity sponges.

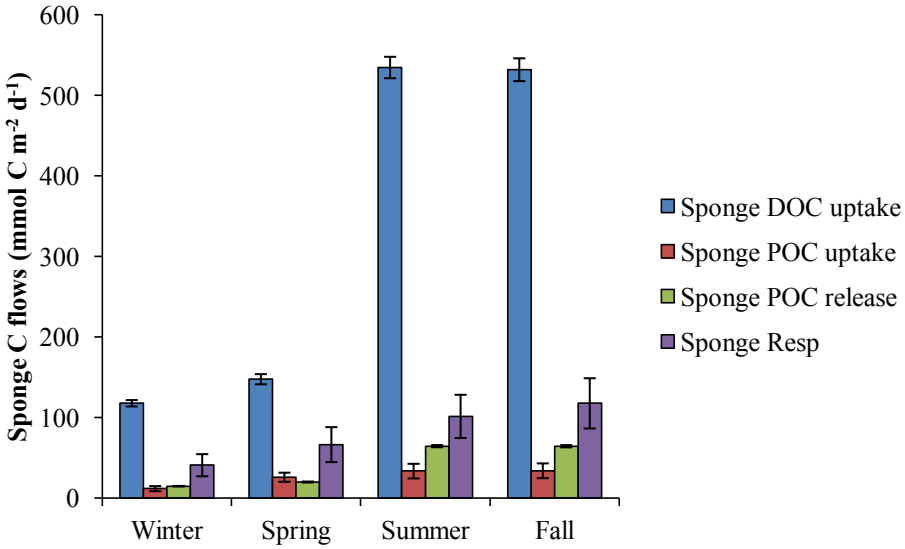

Fig. 8-6. Summed carbon flows of the cavity and surface sponges over the seasons, values given as mean ± SD. DOC = dissolved organic carbon, POC = particulate organic carbon, Resp = respiration.

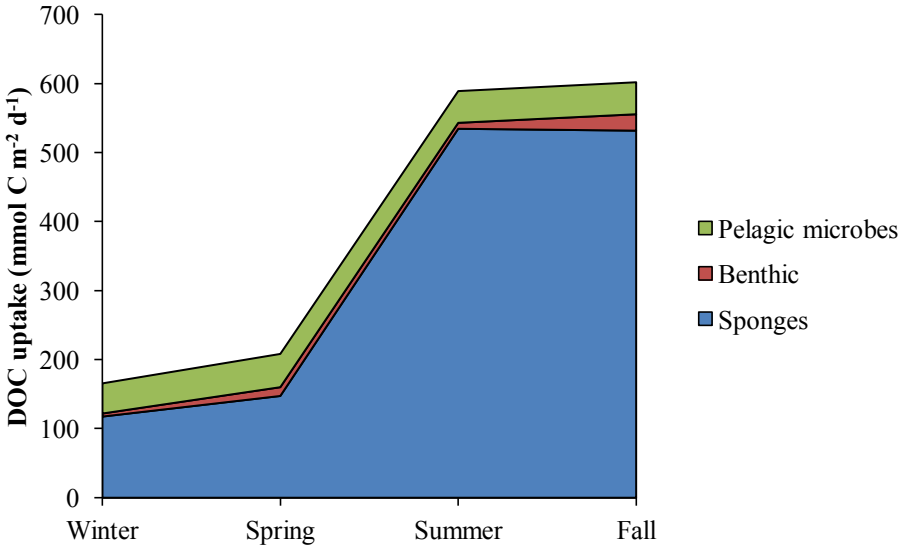

Fig. 8-7. Net dissolved organic carbon (DOC) uptake by the main coral reef compartments over the seasons. Sponges = surface sponges + cavity sponges.

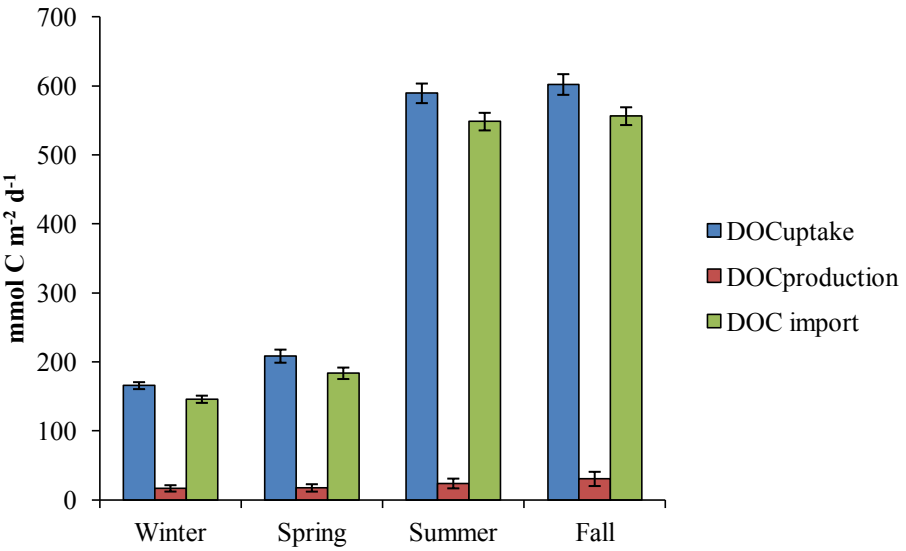

Fig. 8-8. Comparison of dissolved organic carbon (DOC) uptake by the model biota, DOC production within the model, and DOC import from outside the model over the seasons. All values given as mean ± SD.

Table 8-4. Export of C from model compartments over the seasons. Export flows simulate processes not included in the model. All values in mmol C m^{-2} d^{-1}, values given as mean ± SD.

	Winter	Spring	Summer	Fall
Zooplankton	1.02 ± 0.87	1.19 ± 0.96	1.75 ± 1.46	1.70 ± 1.39
Macroalgae	1.26 ± 0.59	8.38 ± 3.90	1.82 ± 0.59	0.62 ± 0.42
Turf algae	2.55 ± 0.72	4.25 ± 1.14	6.95 ± 1.09	2.49 ± 0.51
Sediment	2.57 ± 1.83	3.00 ± 1.68	8.42 ± 3.12	4.14 ± 2.07
Coral rock	27.84 ± 8.41	21.42 ± 9.97	49.88 ± 11.83	49.09 ± 15.02
Hard corals	32.66 ± 17.71	55.26 ± 21.94	44.58 ± 27.67	41.40 ± 25.37
Soft corals	48.59 ± 25.65	92.12 ± 19.26	21.87 ± 13.79	26.10 ± 14.93
Surface sponges	0.70 ± 0.49	0.90 ± 0.62	3.74 ± 2.33	3.81 ± 2.36
Cavity sponges	73.23 ± 13.82	86.27 ± 21.50	398.63 ± 28.26	380.23 ± 32.18

Discussion

Modeled community C cycling

The annual ranges of mean GPP, Rday, NCP, and PR are comparable to GPP found for other reefs (Table 8-5). In general, metabolic rates were more comparable to other reef slopes than reef crests and flats. Those shallower reef areas receive substantially more light, and may have increased rugosity covered by primary producers, leading to increased levels of GPP and corresponding Rday (Long et al. 2013). NCP and PR show that the reef community is net autotrophic in winter

and spring, relatively balanced in summer, and net heterotrophic in fall. This is similar to measurements from a reef flat across the Gulf of Aqaba in Eilat, Israel (Silverman et al. 2007). GPP was highest in spring when light levels and inorganic nutrients were relatively high. The reduced nutrient concentrations in summer may have limited GPP while light was readily available (Larned 1998). Rday was highest in summer and fall, coinciding with highest temperatures. Metabolism of organisms in general increases with increasing temperature (Gillooly et al. 2001). These parameters together with the autotrophic and heterotrophic character of the community during the winter-spring and summer-fall seasons respectively indicate that community metabolism in this reef is strongly affected by seasonal changes in environmental conditions.

Table 8-5. Comparison of metabolic parameters with literature. All values in mmol C m^{-2} d^{-1} and given as means, or ranges. GPP = gross primary production, Rday = community respiration, NCP = net community production, PR = gross primary production to community respiration ratio. An O_2:C factor of 1 was assumed when O_2 fluxes were transformed to C fluxes (Gattuso et al. 1996; Carpenter and Williams 2007).

Site	GPP	Rday	NCP	PR	Reference
Aqaba, Jordan, Reef slope, seasonal range	200 - 308	154 - 248	-41 - 87	0.8 - 1.4	This study
Eilat, Israel, Reef crest, winter	260	180	80	1.4	Silverman et al. (2007)
Eilat, Israel, Reef crest, summer	400	390	8	1.0	Silverman et al. (2007)
French Frigate Shoals, Reef flat, winter	356	213	142	1.67	Atkinson and Grigg (1984)
French Frigate shoals, Reef flat, summer	710	405	305	1.75	Atkinson and Grigg (1984)
Kaneohe bay, Hawaii, Reef flat	400	465	-65	0.86	Falter et al. (2008)
Florida Keys, Reef crest	944	566	378	1.7	Long et al. (2013)
Florida Keys, Reef slope	193	199	-6	0.97	Long et al. (2013)
Colombian Caribbean, Reef slope, seasonal range	250 - 305	136 - 147	103 - 169	1.7 - 2.2	Eidens et al. (2014)
Various Caribbean/Pacific, Reef slope	167 - 583	158 - 250	-83 - 425	0.5 - 5.5	Hatcher (1988)

Organic matter release by corals has been identified as an essential process in coral reef dynamics since this matter becomes available to other reef organisms and functions as a particle trap for planktonic matter which is then brought into the reef C and nutrient cycles (Wild et al. 2004; Mayer and Wild 2010). Total annual mean net organic C release by hard and soft corals (2.7 and 7.3 mmol C m^{-2} d^{-1} respectively) and their relation to total benthic GPP (1.1 and 3.1 % respectively) are comparable to values previously estimated for the studied reef (Naumann et al. 2012). The organic matter flows through the sponge compartments were based solely on POC release measured during incubations and a (POC+DOCin) / POCout turnover efficiency of 11-24% (de Goeij et al. 2013). Model estimations of sponge-related flows will therefore be compared to measurements from literature. The sponge POC release rates were lower, but comparable to

those found for three sponge genera from other locations when assuming cavity sponge biomass of 1.76 mol C m^{-2} planar reef (Table 8-6; Richter et al. 2001; Alexander et al. 2014). DOC uptake rates during summer and fall were comparable to measurements in the Caribbean (de Goeij et al. 2008b). Temperatures during those seasons were similar to those in the Caribbean research site on the island of Curacao (Alexander et al. 2014). The contribution of DOC to total TOC (TOC = POC + DOC) uptake was comparable to measurements of another sponge from the Gulf of Aqaba (Yahel et al. 2003). Summer and fall respiration rates measured for the sponges were also comparable to those measured by de Goeij et al. (2008b). Richter et al. (2001) estimated cavity community phytoplankton grazing in the studied reef. Seasonal mean phytoplankton grazing by cavity sponges as estimated by the model was lower, but did not include the remaining cavity community of filter feeders. All these data indicate that the flows estimated by the model fall within natural ranges measured in other sponges. Rates of DOC to POC transformation by the sponge loop were comparable to those estimated for Caribbean and Indo-Pacific reefs (de Goeij et al. 2013). Pelagic microbial DOC uptake and subsequent C availability to higher trophic levels through the microbial loop were also comparable to estimates from that study, and the sponge loop activity was up to tenfold higher than that of the microbial loop (Table 8-7).

Table 8-6. Comparison of sponge flows to literature. All values given as mmol C m^{-2} d^{-1} and ranges or mean ± SD. DOC = dissolved organic carbon, POC = particulate organic carbon, TOC = total organic carbon = DOC + POC.

	This study	Literature	Location	Reference
DOC uptake	118 - 535	645 ± 123	Curacao	de Goeij et al. (2008b)
DOC/TOC uptake	85 - 94 %	> 90 %	Eilat, Israel	Yahel et al. (2003)
POC production	15 - 65	44 - 316	Curacao	Alexander et al. (2014)
Phytoplankton uptake	6 - 13	74 ± 4	Aqaba, Jordan	Richter et al. (2001)
Respiration	41 - 118	137 ± 37	Curacao	de Goeij et al. (2008b)

Data quality

Food web flows estimated by a model are only as good as the data that were entered into it. Many of the data entered into the model were based on metabolic incubations performed ex-situ. Incubations such as these will always have an effect on measured rates since it is impossible to fully simulate in-situ conditions. Care was taken to create in-situ like conditions by using flow through water which was pumped straight from the coral reef at the correct water depth and screens to mimic in-situ light conditions. Photosynthesis and respiration incubations were kept to a minimum duration to prevent hyper- and hypo-oxic conditions. These incubations were stirred to minimize buildup of a boundary layer around specimens which may influence metabolic rates

(Dennison and Barnes 1988; Shashar et al. 1993). Organic matter incubations (except for sponges) were not stirred to prevent water currents from modifying the structural composition through e.g. POC dissolution to DOC. However, organic matter release is stimulated by water movement (Wild et al. 2012); organic matter flow rates given here should therefore be interpreted as conservative estimates. The midday incubations for Pn were performed under the highest and most stable light conditions of the day as measured during the environmental monitoring (Appendix 5 - Fig. 1). It is however possible that photosynthetic organisms experience photoinhibition during midday when peak light levels can damage their photosynthetic apparatus (Long et al. 1994; Franklin et al. 1996). However, coral reef primary producers in the Gulf of Aqaba were not found to show signs of photoinhibition, even at light levels far exceeding those found in this study (Levy et al. 2004; Schneider et al. 2009). The use of night time R as an estimate to calculate GPP is common practice (Falter et al. 2008; Long et al. 2013). However, R of organisms and substrates during daylight can be substantially higher than at night (Al-Horani et al. 2003; Glud 2008). Values given for GPP may therefore be underestimated. As incubations did not cover the entire daily cycle, rates were extrapolated to d^{-1}. Especially GPP may be overestimated by this since highest photosynthetic rates are reached during midday, assuming no photoinhibition (Levy et al. 2004; Schneider et al. 2009). The extrapolation of incubation rates to planar m^2 reef area may introduce substantial errors. However, metabolic rates for the reef community are within the range of those found in coral reefs using in-situ whole community methods as further discussed below, see table 8-5 (e.g. Hatcher 1988; Silverman et al. 2007). Sponges can have photosynthetic symbionts (Erwin and Thacker 2007). However, the dominant surface sponge in the study site displayed minimal levels of photosynthesis (chapter 4), and surface sponge 3D benthic surface area per planar m^2 reef area was 0.01 % of total 3D surface area. The potential surface sponge contribution to GPP was therefore assumed to be minimal and sponge photosynthesis was ignored. Uncertainty due to extrapolations and simulated in-situ conditions during measurements was introduced into the model by constraining flows where possible only to their minimum and maximum measured values.

The LIM as used in this study was solved under the assumption of steady state for each season. This assumption may be in error for a highly active community such as a coral reef. However, coral reefs generally display a balanced PR ratio, indicating that net growth of the system as a whole is minimal (Hatcher 1988). PR in the Aqaba reef varied over the seasons, but fluctuated around unity and was comparable to other reef communities. Additionally, Vezina and Pahlow (2003) investigated the effect of the steady state assumption in LIMs on simulated food webs which were in steady and transient state. They found that the steady state assumption did not significantly alter the accuracy of the ecosystem flow reconstructions.

The modeled reef community received allochthonous C in every season ranging from 158 to 614 mmol C m^{-2} d^{-1}. The mean water flow speed over the seasons was very stable at ca. 5 cm s^{-1} (van Hoytema unpublished data). This results in a water flow speed of 4.3 km d^{-1}. Total water moving through the model's 8 m^3 water column was therefore 34.6 x 10^3 m^3 d^{-1}. Total water column C ranged from 71.8 to 95 mmol C m^{-3}, resulting in a potential C flow of 2.4 x 10^6 to 3.3 x 10^6 mmol C m^{-2} d^{-1}. Offshore waters most likely have reduced total organic C due to a lack of benthic activity, but the massive amounts of water moving through the reef per day indicate that the inflows of C as modeled are possible, and the vast majority of C flowing through would not be used by the model.

Ecological implications

The community of this relatively high latitude reef revealed a clear response to the strong seasonality in environmental factors. The metabolic balance shifted from an autotrophic period during relatively high nutrient availability to a heterotrophic period during strongly oligotrophic conditions due to stratification, and increased temperatures. Concurrently, the amount of C made available to the reef community by the sponge loop increased more than 3-fold between these periods, reaching levels 10-fold higher than the pelagic microbial loop. This primarily cavity sponge-mediated pathway therefore may play an essential role in fulfilling this seasonally increased energy demand of the community. Indeed, sponge DOC uptake was more than 10-fold higher than DOC released by the whole reef community. The majority of sponge DOC uptake therefore consisted of allochthonous C which flowed into the system. The sponge loop may not just play a major part in internal reef cycling as posited by de Goeij et al. (2013). It may be a key component in capturing foreign C for the reef community in a hydrodynamically open reef system as the studied fringing reef. Trapping planktonic particles may increase the C concentration of coral released POM up to eightfold (Wild et al. 2004). This factor which was measured in the Great Barrier Reef and corrected for compaction of the aggregates as applied there, is unavailable for the studied reef. However, comparable gross enrichment of POM was found for the studied reef by Mayer and Wild (2010). A particle trapping capacity of seven times its own C content with subsequent introduction of that allochthonous C into the reef C cycle would place this pathway among the top processes bringing C into the reef community (Table 8-7). Potentially increased particle trapping rates in summer and fall as estimated in table 8-7 may additionally support the coral reef community during more heterotrophic seasons.

The cavities that riddle the coral reef framework have been described as the largest but least researched habitat in coral reefs (Richter et al. 2001). The high sponge DOC uptake values should be seen as conservative since the assumed cavity sponge benthic cover per planar reef m^2 was an average of several Gulf of Aqaba reefs; mean cavity surface area was higher for the

studied reef specifically (Richter et al. 2001). In addition, cavities in the reef structure extended deeper than could be measured by the experimental setup of Richter et al. (2001), indicating that rates of processes mediated by cavity-dwelling organisms may be higher per planar reef surface area than presently assumed. Further research into these cavities is warranted since the sponges and their microbial symbionts that inhabit them may additionally play a presently unquantified role in the nitrogen cycle which could provide essential nutrients to the wider reef community in its oligotrophic surroundings (Wilkinson and Fay 1979; Webster and Taylor 2012).

Table 8-7. Comparison of carbon cycling processes over the seasons as estimated by the models. All values in mmol C m^{-2} d^{-1} and given as mean ± SD. DOC = dissolved organic carbon, POC = particulate organic carbon, micr. = microbial. Pelagic micr. available = carbon from the microbial loop available to higher trophic levels = DOC uptake by bacteria and protozoa - excretion and respiration. The external POC trapping estimate is based on an 8-fold increase in carbon content by released coral mucus (Wild et al. 2004), it was calculated from coral POC release, not produced by the model.

	Winter	Spring	Summer	Fall
GPP	200 ± 30	308 ± 28	250 ± 32	203 ± 30
Rday	154 ± 15	221 ± 24	248 ± 29	245 ± 32
Sponge DOC uptake	166 ± 5	209 ± 9	590 ± 14	602 ± 15
Sponge net POC release	15 ± 0	20 ± 1	64 ± 1	64 ± 1
Pelagic micr. DOC uptake	44 ± 3	48 ± 10	46 ± 6	46 ± 6
Pelagic micr. available	3 ± 1	3 ± 1	3 ± 2	3 ± 2
Benthic heterotrophic feeding	17 ± 3	19 ± 3	33 ± 2	32 ± 2
Coral POC release	8 ± 3	5 ± 5	14 ± 5	15 ± 8
External POC trapping	61 ± 23	43 ± 27	116 ± 42	118 ± 60

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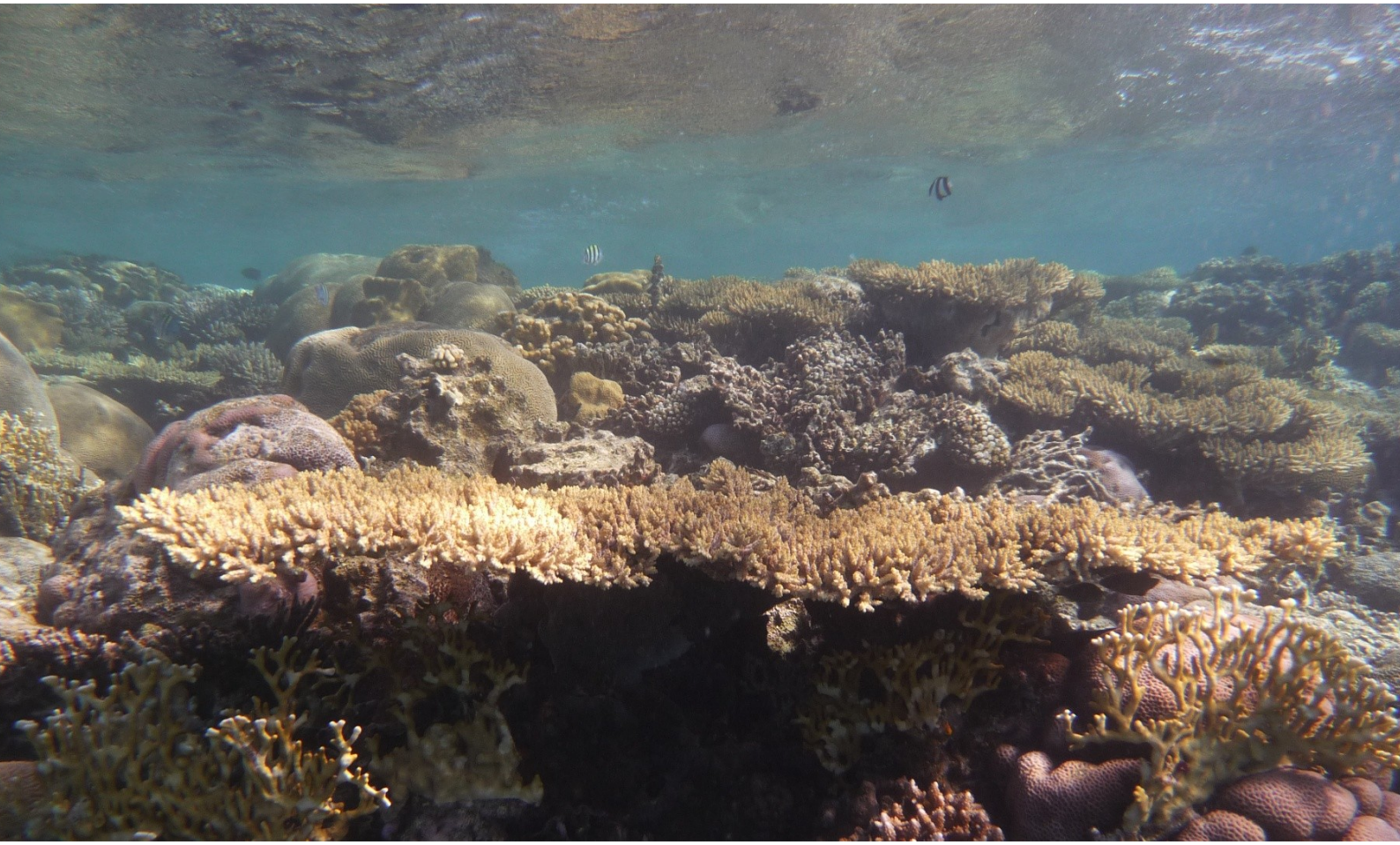
General Discussion



Key findings

The key findings of this thesis are summarized below in reference to the general objectives:

To quantify the seasonal variation in environmental conditions in the water column over a Red Sea fringing coral reef, and its effect on planktonic photosynthetic primary production and N_2 fixation.

Clear annual cycles were found for light availability, temperature, and inorganic nutrient concentrations. Light increased from winter to spring and decreased from summer to fall. Temperature and inorganic nutrient concentrations were closely linked, but negatively correlated. Water temperature increased while inorganic nutrient concentrations decreased from spring to summer. These annual cycles were as expected from literature (Silverman et al. 2007; Carlson et al. 2014). Planktonic primary production was reduced under the low nutrient conditions due to stratification. This decline coincided with a phytoplanktonic community shift from picoeukaryotes to *Prochlorococcus* sp.. The smaller *Prochlorococcus* sp. may have been less limited by the strongly oligotrophic conditions due to increased uptake efficiency (Finkel et al. 2010). However, this relative size-uptake efficiency relation is contested (Maranon et al. 2013). Mean dinitrogen (N_2) fixation rates were stable throughout the year. The potential contribution of N_2 fixation to nitrogen (N) demand for primary production increased more than 5-fold to ~ 21 % from the mixed to the stratified period. Dissolved organic carbon (DOC) concentrations increased from the mixed to the stratified period. This carbon (C) pool which is only accessible in the water column through the "microbial loop" (Azam and Malfatti 2007), may have additionally supported N_2 fixation during stratification as the microbial N_2 fixing community may have shifted from both autotrophic and heterotrophic to fully heterotrophic from the mixed to the stratified period (Rahav et al. 2013; Rahav et al. 2015). In addition, the increased DOC may have supported the planktonic food web during reduced primary production.

To comparatively quantify photosynthetic primary production by the dominant benthic functional groups under seasonal variation in environmental conditions.

Benthic primary production and its response to the seasonality were group-specific. While certain hard corals, macroalgae, and cyanobacterial mats revealed no significant photosynthetic response to the seasonality, net photosynthesis (Pn) of sedimentary microphytobenthos and the soft coral Xeniidae were substantially different between seasons. The relative response by the carbonate sedimentary microphytobenthos was strongest of all groups investigated in chapter 7, underlining the dynamic nature of sedimentary communities and their adaptive capacity to changing

environmental conditions (Schottner et al. 2011). The different response to seasonality by the community in neighboring silicate sediments (chapter 5) corroborates this dynamic nature as well as the difference in composition between these sediment communities (Wild et al. 2005; Schottner et al. 2011). Coral holobionts are mixo-trophic; they are able to obtain energy and nutrients from a variety of pathways (Fabricius and Klumpp 1995; Grottoli et al. 2006). There is however clear differentiation between groups in their reliance on various pathways. The soft coral Xeniidae showed highest gross photosynthesis to respiration (Pg:R) ratios throughout the year, indicating high photosynthetic efficiency. The polyp pulsation behavior of the Xeniidae investigated in this thesis has been proposed to be the source of this efficiency since moving the photosynthetic tissues through the water increases diffusion. High diffusion rates result in reduced hyperoxic conditions and consequently reduced limitation of primary production (Kremien et al. 2013). However, Xeniidae was the only coral group which displayed significantly reduced Pn rates in summer compared to spring, concurrent with a drop in inorganic nutrient concentrations due to stratification. The other groups were most likely able to maintain Pn due to processes such as heterotrophic feeding and symbiotic N_2 fixation. Xeniidae are exceptionally autotrophic among corals in their energy gain (Schlichter et al. 1983), and exhibited the lowest rates of N_2 fixation of all corals investigated (chapter 2-3). Xeniidae benthic cover changes rapidly over the seasons (chapter 8) and years (Tilot et al. 2008), indicating that this group may have more dynamic annual and multi-annual colonization and benthic cover loss cycles than the other corals investigated. The recently reported presence of invasive Xeniid soft corals in a Venezuelan Caribbean coral reef may well be cause for concern as these Xeniids are rapidly colonizing new substrate and overgrowing local hard corals, thereby reducing local biodiversity (Ruiz Allais et al. 2014). This rapid colonizing behavior may be amplified in Caribbean coral reefs where competitors for space are unaccustomed to it (Bax et al. 2003). Hard and soft corals combined high photosynthetic rates with high benthic cover over all seasons, indicating them as the dominant primary producers in the reef.

To quantify N_2 fixation by the dominant benthic functional groups to determine their relative contributions to total N_2 fixation and the potential contribution by N_2 fixation to N demand by primary production under seasonal variation in environmental conditions

N_2 fixation was ubiquitous throughout the coral reef as all investigated functional groups revealed diazotrophic activity (chapters 2-6). Over all seasons, microbial mats, turf algae, coral rock including dead coral skeletons, and carbonate sediment revealed highest rates per specimen surface area (chapters 4-6). These groups also performed the vast majority of total reef N_2 fixation (summed ~ 95 %; Table D-1) All these functional groups contain a variety of microbes capable of N_2 fixation (Larkum et al. 1988; Charpy et al. 2010; Cardini et al. 2014), and part of the N fixed

by this process becomes available to the wider reef community (Williams and Carpenter 1997; Mulholland et al. 2006). In general, N_2 fixation in the reef was highest in summer. N_2 fixation in that season may have been stimulated by the combination of highest temperatures, and lowest inorganic nutrient concentrations of the year, while high levels of GPP (in e.g. microbial mats and turf algae) would have provided the fuel needed for this energy-costly process (chapter 7).

To combine the data on all functional groups into an overarching estimate of primary production, further C cycling processes, and N_2 fixation in the coral reef to quantitatively compare the magnitudes of these processes.

Gross primary production (GPP) in the reef was dominated by corals. Hard and soft corals combined contributed ~ 60 - 80% of total GPP (chapter 8). Turf- and macroalgae represented only a small part of the benthic cover and while their group specific photosynthesis rates were among the highest measured of all functional groups (chapter 7), their combined contribution to GPP never exceeded 6.4%. The strongly oligotrophic conditions of the Gulf of Aqaba result in coral reefs with an exceptionally low algal cover compared to other areas (Adey and Steneck 1985; Klumpp and McKinnon 1992; Bahartan et al. 2010). However, indications have been found of anthropogenic disturbances leading to a shift from corals to algae (Bahartan et al. 2010; Al-Zibdah and Colgan 2013). Such a shift may have severe implications for coral reef functioning as corals are not only strong primary producers, but also ecosystem engineers of the habitat by building the reef structure (Gattuso et al. 1999). Furthermore, corals play a vital role by releasing organic matter as mucus which subsequently is involved in trapping planktonic particles, leading to an inflow of external nutrients and organic matter (chapter 8). GPP estimates produced by the model in chapter 8 indicate that GPP was highest in spring, when light availability and nutrients were high, primarily due to increased photosynthetic activity by the dominant soft coral Xeniidae. GPP of the other functional groups modelled in chapter 8 appears relatively stable over the seasons, indicating how successfully their primary production is attuned to the seasonally varying conditions in the Gulf of Aqaba through a range of adaptations described in chapter 7. The reef community acted net autotrophic in winter and spring, while it was metabolically balanced in summer and net heterotrophic in fall.

Functional group-specific contributions of N_2 fixation to net primary production (NPP = gross primary production - respiration) ranged several orders of magnitude (Table D-1). N_2 fixation by the sediment community in winter far exceeded estimated potential demand by its own NPP. Contrarily, soft corals did exhibit N_2 fixation in all seasons, but potential contribution of N_2 fixation to N demand of soft coral NPP was < 0.5% throughout the year. Highest total contribution of N_2 fixation to NPP was concurrent with highest N_2 fixation rates in summer when

N_2 fixation potentially provided 18% of N needed for NPP. The mean estimate of total N_2 fixation per m^2 reef surface ranged from 118 (winter) to 929 (summer) µmol N m^{-2} d^{-1} (Table D-1). These rates fall in between the two published estimates of total reef N_2 fixation; 145-328 µmol N m^{-2} d^{-1} at One Tree Island, Great Barrier Reef (Larkum et al. 1988), and 1200-1968 µmol N m^{-2} d^{-1} at Eilat, Israel (Shashar et al. 1994b).

Table 1. Mean net primary production (NPP; daily gross primary production (GPP) - daily respiration (Rday)) based on GPP and Rday measurements as entered into the model in chapter 8. Mean N_2 fixation (NF) calculated by combining N_2 fixation rates from chapters 2-6 with 3D surface area calculations from chapter 8; assuming a C_2H_4:N_2 conversion factor of 4:1 (Mulholland et al. 2004). Contr. % = % contribution of NF to nitrogen demand by NPP; assuming a C:N ratio of 550:30 (Atkinson and Smith 1983). values of NPP and NF given as mean (range). Micr. mats = microbial mats.

	NPP (mmol C m^{-2} d^{-1})	NF (µmol N m^{-2} d^{-1})	Contr. %	NPP (mmol C m^{-2} d^{-1})	NF (µmol N m^{-2} d^{-1})	Contr. %
	Winter			Spring		
Hard corals	14 (3 - 34)	3 (0 - 17)	0.39	33 (20 - 44)	6 (0 - 21)	0.31
Soft corals	61 (21 - 99)	3 (1 - 5)	0.08	99 (75 - 126)	2 (0 - 5)	0.03
Macroalgae	2 (1 - 2)	0.4 (0 - 2)	0.51	8 (3 - 15)	17 (2 - 54)	4.11
Turf algae	3 (3 - 5)	20 (11 - 28)	10.73	5 (4 - 6)	29 (11 - 50)	11.23
Coral rock	13 (3 - 22)	23 (5 - 50)	3.24	7 (-2 - 20)	146 (75 - 233)	35.84
Sediment	0.3 (-2 - 3)	40 (6 - 66)	259.50	12 (9 - 25)	159 (108 - 246)	23.71
Micr. mats	3 (2 - 6)	29 (12 - 51)	18.66	6 (4 - 7)	197 (128 - 226)	57.04
Total	97 (29 - 171)	118 (34 - 218)	2.23	170 (112 - 245)	556 (325 - 833)	5.98
	Summer			Fall		
Hard corals	20 (4 - 41)	26 (7 - 76)	2.35	15 (-3 - 20)	7 (0 - 46)	0.90
Soft corals	39 (33 - 45)	11 (6 - 16)	0.49	36 (15 - 55)	1 (1 - 3)	0.06
Macroalgae	2 (1 - 3)	11 (3 - 19)	10.67	1 (1 - 2)	3 (1 - 6)	5.12
Turf algae	8 (6 - 10)	126 (81 - 158)	29.94	3 (2 - 4)	30 (22 - 39)	18.94
Coral rock	14 (10 - 14)	471 (263 - 739)	59.94	7 (4 - 41)	171 (89 - 834)	44.97
Sediment	6 (3 - 10)	161 (72 - 268)	47.66	2 (0.1 - 3)	41 (17 - 60)	38.71
Micr. mats	8 (4 - 11)	124 (60 - 148)	29.66	4 (2 - 7)	109 (81 - 141)	54.00
Total	97 (60 - 134)	929 (492 - 1423)	17.52	67 (21 - 132)	363 (212 - 1128)	9.87

The seasonal model in chapter 8 revealed that the sponge community in the cavities riddling the reef structure may play a key role in both carbon (C) recycling and the capture of allochthonous C through the uptake of dissolved organic carbon (DOC) which dominated C flowing into the reef. In this, the sponges far outpaced the pelagic microbial loop as posited by de Goeij et al. (2013). Both the uptake of DOC and subsequent release of discarded cells (Alexander et al. 2014) increased 3-fold from spring to summer, potentially providing an energy source to the wider reef community during the more heterotrophic conditions of summer and fall (chapter 8).

The increased activity in both this process and whole reef N_2 fixation during summer oligotrophic conditions indicate that this Red Sea coral reef is finely attuned to its seasonally variable environment.

Outlook

Coral reefs around the world are facing global and local stressors, levels of which are unprecedented in recent history (Hoegh-Guldberg et al. 2007; Harnik et al. 2012). As they experience these anthropogenic disturbances such as sea surface warming, ocean acidification, and increased levels of eutrophication, the delicate equilibrium which allows them to thrive in their oligotrophic surroundings will be affected. Already, a large fraction of the world's reefs are deteriorating and shifting to other states, dominated by for example algae or soft corals (Hughes et al. 2003; Bellwood et al. 2004; Norstrom et al. 2009). This will have major consequences for their surrounding oceans and the coastal population making use of their ecosystem services (Moberg and Folke 1999; Burke et al. 2011). Scleractinian corals which are the key ecosystem engineers are projected to be strongly affected by their changing environment, and essential processes such as construction of the solid reef structure through calcification, primary production through symbiotic zooxanthellae and subsequent organic matter release, essential to whole reef C cycling, may well be compromised (Brown 1997; Hoegh-Guldberg et al. 2007; Wooldridge 2013). As shown in this thesis, a community shift away from scleractinian corals may not just impact reef construction due to reduced calcification. A shift to soft coral dominance, for example, may lead to a decline in de novo nutrient production through N_2 fixation, due to soft corals lacking a carbonate skeleton which houses an internal community of diazotrophic cyanobacteria (Shashar et al. 1994).

To optimize the conservation of these essential ecosystems, it is vital that we gain a thorough understanding of how nutrients and C cycle through them. An essential component that needs to be further investigated is the cryptic habitats that riddle the reef structure, and which have been described as the largest and yet the least researched habitat within coral reefs (Richter et al. 2001). Indeed, the N_2 fixation measurements presented in this thesis indicate that less researched functional groups such as communities in sediments, the reef coral rock structure, and microbial mats are vital to whole reef N_2 fixation. The seminal paper by Odum and Odum (1955) already described endolithic algae in coral skeletons as far outweighing the photosynthetic biomass represented by the zooxanthellae in coral tissues. Since these endolithic algae have also been shown to fix substantial amounts of N_2 (Shashar et al. 1994), they may be more significant contributors to the high levels of primary production than zooxanthellae on coral reefs . On a

larger scale, reef cavities harbor a vast community of sponges and other filter feeders (Richter et al. 2001; Scheffers et al. 2004). This cryptic biota is far removed from easy access for research purposes, but as shown in this thesis, it may be involved in C cycles which dominate coral reef metabolism and help support the reef community in periods of limited photosynthetic primary production. Current knowledge on such activity by the cryptic communities is scarce, but community composition appears to be highly localized, indicating specialization to local conditions (Scheffers et al. 2010). In addition, the abundant cavity communities may play a major role in maintaining their highly productive coral reefs in oligotrophic waters through roles in nutrient cycles (Webster and Taylor 2012). However, knowledge on the prevalence and quantity of these processes is currently lacking.

Additionally, to gain holistic understanding of coral reef C and nutrient balances, it is essential to study the various elements together. An imbalance in nutrient availability may potentially lead to breakdown of coral reef symbioses (Wiedenmann et al. 2013). Stoichiometry of elements in dissolved and particulate organic matter transported into the coral reef, and subsequent movements of those elements through the reef system, will have an impact on ecosystem functioning (Sitters et al. 2015). The ecosystem model presented in chapter 8 should therefore be seen as an initial step since it can be extended to contain flows of more than one element. The model can thereby incorporate stoichiometric relations between elements throughout the coral reef to better define flow magnitudes of those elements as they are cycled and transformed (van Oevelen et al. 2010). In conclusion, a multi-disciplinary approach using tools from ecophysiology, biogeochemistry, and ecological modeling, but also microbiology, molecular ecology, and population genetics will be essential to gain the level of understanding needed to preserve these tightly balanced ecosystems in the near future.

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Appendices

Appendix 1 - Table 1. Spearman rank correlation tests between status and process parameters for both seasons combined. Values are Spearman rank correlation coefficients; coefficients in italics are not significant. * = $p < 0.05$, ** = $p < 0.01$.

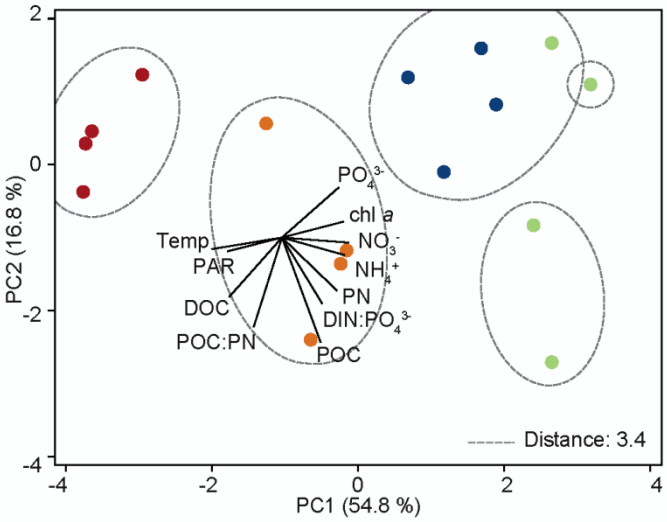
	Temp	Light	Chl.a	DOC	POC	PN	NH4	PO4	NOx	Pnet	BOD	Pgross	Nfix
Temp	X												
Light	0.48*	X											
Chl a	-0.75**	-0.67**	X										
DOC	0.73**	*0.20*	-0.55**	X									
POC	*-0.03*	*0.13*	*0.07*	*-0.14*	X								
PN	-0.42*	*-0.03*	*0.35*	*-0.34*	0.66**	X							
NH4	-0.71**	*-0.09*	*0.40*	-0.72**	*-0.06*	*0.10*	X						
PO4	-0.73**	*-0.03*	0.48*	-0.56**	*-0.06*	*0.30*	0.68**	X					
NOx	-0.80**	-0.63**	0.63**	-0.57**	*-0.26*	*0.18*	0.60**	0.69**	X				
Pnet	-0.59**	-0.56**	0.60**	-0.41*	*-0.36*	*0.15*	*0.34*	0.47*	0.85**	X			
BOD	*0.32*	0.59**	-0.48*	*0.06*	*0.36*	*-0.11*	*0.02*	*-0.19*	-0.62**	-0.60**	X		
Pgross	-0.44*	*-0.29*	*0.34*	-0.44*	*-0.17*	*-0.05*	0.51**	*0.40*	0.63**	0.72**	*0.06*	X	
Nfix	*0.11*	0.59*	*-0.41*	*0.37*	-0.57*	*-0.31*	*-0.21*	*0.22*	*-0.02*	*0.19*	*0.17*	*0.23*	X

Appendix 1 - Table 2. Spearman rank correlation tests between status and process parameters for the mixed season. Values are Spearman rank correlation coefficients; coefficients in italics are not significant. * = $p < 0.05$, ** = $p < 0.01$.

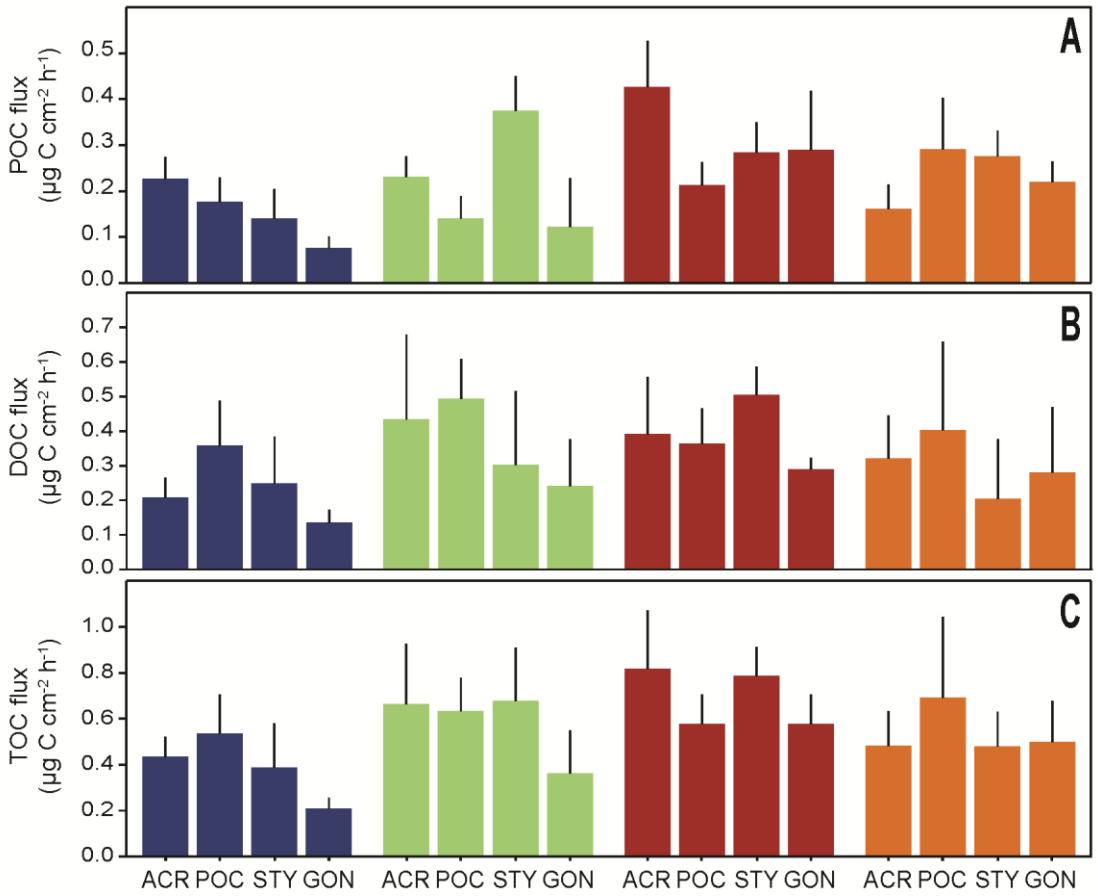
Temp	X												
Light	*0.15*	X											
Chl a	*0.02*	*-0.30*	X										
DOC	*-0.10*	*-0.01*	*-0.04*	X									
POC	*0.18*	*0.60*	*-0.14*	*-0.16*	X								
PN	*-0.07*	*0.34*	*-0.18*	*0.21*	0.76**	X							
NH4	*-0.21*	0.67*	*-0.26*	*-0.26*	*-0.01*	*-0.31*	X						
PO4	*-0.16*	*0.13*	*0.28*	*0.10*	*-0.22*	*-0.45*	*0.49*	X					
NOx	*-0.48*	-0.63*	*0.36*	*0.26*	-0.61*	*-0.53*	*-0.05*	*0.43*	X				
Pnet	*-0.15*	-0.79**	*0.38*	*0.06*	*-0.66**	*-0.56*	*-0.30*	*-0.04*	0.72**	X			
BOD	*0.15*	0.90**	*-0.35*	*-0.39*	*0.64**	*0.34*	*0.52*	*-0.18*	-0.71**	-0.63*	X		
Pgross	*-0.16*	*-0.07*	*0.05*	*-0.41*	*-0.27*	*-0.39*	*0.20*	*-0.22*	*0.18*	*0.55*	*0.20*	X	
Nfix	-0.79*	*<0.001*	*0.37*	*-0.14*	*-0.50*	*-0.54*	*0.61*	*0.36*	*0.54*	*0.39*	*0.18*	0.79*	
	Temp	Light	Chl.a	DOC	POC	PN	NH4	PO4	NOx	Pnet	BOD	Pgross	Nfix

Appendix 1 - Table 3. Spearman rank correlation tests between status and process parameters for the stratified season. Values are Spearman rank correlation coefficients; coefficients in italics are not significant. * = $p < 0.05$, ** = $p < 0.01$.

	Temp	Light	Chl.a	DOC	POC	PN	NH4	PO4	NOx	Pnet	BOD	Pgross	Nfix
Temp	X												
Light	0.92**	X											
Chl a	-0.86**	-0.90**	X										
DOC	*0.43*	*0.35*	*-0.32*	X									
POC	*-0.26*	*-0.29*	*0.29*	*-0.18*	X								
PN	*-0.08*	*-0.07*	*0.21*	*-0.06*	0.80**	X							
NH4	*-0.35*	*-0.41*	*0.12*	*-0.39*	*-0.14*	*-0.34*	X						
PO4	*0.24*	*0.20*	*-0.30*	*0.42*	*0.01*	*-0.27*	*-0.07*	X					
NOx	*-0.58*	*-0.60*	0.66*	*-0.17*	*0.27*	*0.13*	*0.16*	*-0.38*	X				
Pnet	*-0.32*	*-0.43*	*0.52*	*0.12*	*-0.06*	*0.18*	*0.15*	*-0.16*	0.67*	X			
BOD	*0.30*	*0.26*	*-0.50*	*-0.03*	*-0.10*	*-0.37*	*0.20*	0.58*	*-0.44*	*-0.32*	X		
Pgross	*-0.43*	*-0.55*	*0.41*	*-0.23*	*0.12*	*-0.02*	*0.51*	*0.19*	*0.52*	0.67*	*0.35*	X	
Nfix	0.89**	0.79*	*-0.75*	0.86*	*-0.75*	*-0.36*	*-0.71*	*0.57*	*-0.50*	*-0.11*	*0.46*	*-0.14*	X



Appendix 2 – Fig. 1. Principal Component Analysis (PCA) testing for multivariate change in environmental factors among the four seasons. Colors represent winter (blue), spring (green), summer (red), autumn (orange). Note that the percent variation explained by the PCs is indicated on the axes and refers to the fraction of the total variance explained by Irradiance (PAR), Temperature (Temp), Ammonium (NH_4^+), Nitrate (NO_3^-), Phosphate (PO_4^{3-}), Particulate organic C (POC), Particulate N (PN), Dissolved organic C (DOC), Chlorophyll *a* (chl *a*), POC:PN ratio (POC:PN), DIN:PO_4^{3-} ratio (DIN:PO_4^{3-}).



Appendix 2 – Fig. 2. Organic C fluxes of the four hard coral genera during the four seasons. Fluxes of particulate, dissolved and total organic C (POC, DOC and TOC, respectively) of *Acropora* (ACR), *Pocillopora* (POC), *Stylophora* (STY) and *Goniastrea* (GON) are presented here as means (n = 6) ± s.e.m. For statistical differences see Appendix 2 - Table 3 and 6. Colors represent winter (blue), spring (green), summer (red) and autumn (orange).

Appendix 2 - Table 1. Summary of the background water parameters monitored during the four different seasons at 10 m water depth. Values are means of four weeks of measurements (± s.e.m.).

Environmental variable	Winter (Feb)	Spring (Apr)	Summer (Sep)	Autumn (Nov)
Irradiance (PAR)	203 (6)	218 (24)	319 (9)	199 (5)
Temperature (°C)	22.5 (0.1)	22.8 (0.1)	27.5 (0.2)	25.2 (0.2)
Ammonium (µM)	0.32 (0.04)	0.46 (0.03)	0.11 (0.01)	0.28 (0.06)
Nitrate (µM)	0.34 (0.03)	0.44 (0.04)	0.04 (0.01)	0.13 (0.05)
Nitrite (µM)	0.37 (0.06)	0.12 (0.04)	0.02 (0.01)	0.02 (0.01)
Phosphate (µM)	0.11 (0.01)	0.10 (0.01)	0.04 (0.01)	0.04 (0.01)
POC (µM)	7.70 (0.21)	10.25 (1.48)	6.61 (0.61)	8.81 (0.49)
PN (µM)	0.93 (0.03)	1.27 (0.14)	0.74 (0.02)	0.87 (0.04)
DOC (µM)	76.62 (4.27)	71.95 (3.77)	84.71 (2.09)	80.81 (0.54)
Chlorophyll a (µg/L)	0.21 (0.01)	0.22 (0.02)	0.10 (0.01)	0.19 (0.02)
POC:PN ratio (mol/mol)	8.32 (0.19)	8.07 (0.59)	8.91 (0.63)	10.11 (0.48)
DIN:PO_4^{3-} ratio (mol/mol)	9.72 (0.96)	10.46 (1.01)	5.56 (1.64)	11.94 (2.15)

Appendix 2 - Table 2. Results of the PERMANOVA test for multivariate changes among the four seasons. Tested are differences in Irradiance (PAR), Temperature (Temp), Ammonium (NH_4^+), Nitrate (NO_3^-), Phosphate (PO_4^{3-}), Particulate organic C (POC), Particulate N (PN), Dissolved organic C (DOC), Chlorophyll *a* (chl *a*), POC:PN ratio (POC:PN), DIN:PO_4^{3-} ratio (DIN:PO_4^{3-}). ***, $p < 0.001$.

	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Season	3	113	37.695	8.713	***	9916
Residual	12	52	4.326			
Total	15	165				

Appendix 2 - Table 3. Results of the two-way analyses of variance. Tested are the effects of the factors Season, Genus and their interaction on N_2 fixation, Net photosynthesis (Pn), Respiration (R), Gross photosynthesis (Pg), POC, DOC and TOC fluxes, Zooxanthellae density (Zoox), Chlorophyll a × cm^{-2} (chl *a*), Chlorophyll *a* × zooxanthellae^{-1} (chl *a*/zoox). *, p < 0.05; **, p < 0.01; ***, p < 0.001; n.s., not significant.

Factor	N_2 fixation		Pn		R		Pg		POC fluxes		DOC fluxes		TOC fluxes		Zoox		chl *a*		chl *a*/zoox	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Season	49.6	***	5.2	***	5.5	**	3.2	*	2.8	*	0.8	n.s.	1.7	n.s.	47.3	***	18.6	***	51.8	***
Genus	8.5	***	4.3	**	1.4	n.s.	3.2	**	1.5	n.s.	0.8	n.s.	1.0	n.s.	6.1	**	2.7	n.s.	12.1	***
Season x Genus	2.9	**	2.5	**	3.0	**	1.9	*	1.3	n.s.	0.3	n.s.	0.3	n.s.	3.2	*	2.7	n.s.	12.7	***

Appendix 2 - Table 4. Results of a posteriori tests for the interaction term Season x Genus, if significant (cf. Appendix 2 - Table 3), looking at differences among seasons. Variables are N_2 fixation, Net photosynthesis (Pn), Respiration (R), Gross photosynthesis (Pg), Zooxanthellae density (Zoox), Chlorophyll *a* × zooxanthellae^{-1} (chl *a*/zoox). *, p < 0.05; **, p < 0.01; n.a., not available.

Variable	Factor level	ACR				POC				STY				GON			
		WIN	SPR	SUM	AUT	WIN	SPR	SUM	AUT	WIN	SPR	SUM	AUT	WIN	SPR	SUM	AUT
N_2 fixation	WIN																
	SPR					*											
	SUM	**	**		**	**	*		**	**	**		**	**	**		**
	AUT													**	**		
Pn	WIN																
	SPR									**				**			
	SUM																
	AUT									*							
R	WIN		*										*				
	SPR												**				
	SUM												**	*	*		
	AUT																
Pg	WIN																
	SPR									**				*			
	SUM													*			
	AUT																
Zoox	SPR	n.a.		*	n.a.	n.a.			n.a.	n.a.		**	n.a.	n.a.		**	n.a.
	SUM	n.a.			n.a.	n.a.			n.a.	n.a.			n.a.	n.a.			n.a.
chl *a*/zoox	SPR	n.a.			n.a.	n.a.			n.a.	n.a.			n.a.	n.a.			n.a.
	SUM	n.a.			n.a.	n.a.	**		n.a.	n.a.	**		n.a.	n.a.	*		n.a.

Appendix 2 - Table 5. Results of a posteriori tests for the interaction term Season x Genus, if significant (cf. Appendix 2 - Table 3), looking at differences among coral genera. Variables are N_2 fixation, Net photosynthesis (Pn), Respiration (R), Gross photosynthesis (Pg), Zooxanthellae density (Zoox), Chlorophyll *a* × zooxanthellae^{-1} (chl *a*/zoox). *, p < 0.05; **, p < 0.01; n.a., not available.

Variable	Factor level	WIN				SPR				SUM				AUT			
		ACR	POC	STY	GON	ACR	POC	STY	GON	ACR	POC	STY	GON	ACR	POC	STY	GON
N_2 fixation	ACR																
	POC					**											
	STY					*											
	GON										*			**	**	**	
Pn	ACR			**													
	POC			**													
	STY																
	GON			*											*		
R	ACR		*														
	POC															*	
	STY																
	GON															*	
Pg	ACR			**	*												
	POC			*													
	STY																
	GON																
Zoox	ACR		n.a.	n.a.	n.a.										n.a.	n.a.	n.a.
	POC	n.a.		n.a.	n.a.									n.a.		n.a.	n.a.
	STY	n.a.	n.a.		n.a.									n.a.	n.a.		n.a.
	GON	n.a.	n.a.	n.a.		*	**	*						n.a.	n.a.	n.a.	
chl *a*/zoox	ACR		n.a.	n.a.	n.a.										n.a.	n.a.	n.a.
	POC	n.a.		n.a.	n.a.					**			*	n.a.		n.a.	n.a.
	STY	n.a.	n.a.		n.a.					**	**		**	n.a.	n.a.		n.a.
	GON	n.a.	n.a.	n.a.										n.a.	n.a.	n.a.	

Appendix 2 - Table 6. Results of a posteriori tests for the factor Season, if the interaction term was not significant (cf. Appendix 2 - Table 3). Variables are POC fluxes and Chlorophyll *a* × cm^{-2} (chl *a*). *, p < 0.05; **, p < 0.01; n.a., not available.

Variable	Factor level	WIN	SPR	SUM	AUT
POC fluxes	WIN			*	
	SPR				
	SUM				
	AUT				
chl *a*	SPR	n.a.		**	n.a.
	SUM	n.a.			n.a.

Appendix 2 - Methods: Calculations applied for the coral C and N flux model

Calculations of carbon (C) and nitrogen (N) fluxes presented by the model were primarily based on data sets generated by the present study. In case this option was not available, conservative assumptions were made in choosing established literature data, obtained for similar tropical scleractinian species from comparable regions/environmental conditions, whenever possible.

1.1. Percent contribution of zooxanthellae-acquired N to zooxanthellae N demand (CZND)

Bioavailable N demand of zooxanthellae photosynthetic activity (ZND, µmol N cm^{-2} d^{-1}) was calculated from zooxanthellae daily gross photosynthesis (DPg) measured for the two respective seasons (spring and summer), assuming a conservative molar C:N ratio of 10 in the algal fraction (Muller-Parker et al. 1994), and a fraction of 0.1 of primary production fuelled by new N uptake (Rahav et al. 1989). For each particular uptake rate, the percent contribution of zooxanthellae-acquired N (calculated as explained in section 1.4 and 1.5) to ZND (CZND) was derived by the equation: CZND [%] = (N uptake rate to the zooxanthellae)/ZND*100.

1.2. Daily gross photosynthesis, respiration and CZAR

Measured gross photosynthesis rates (Pg, µg O_2 cm^{-2} h^{-1}) were converted to daily gross photosynthesis rates (DPg) (µmol C cm^{-2} d^{-1}) for spring and summer, assuming 12 h of daylight and 1.1 as photosynthetic quotient (Muscatine et al. 1981). Daily respiration (DR) was converted to µmol C cm^{-2} d^{-1} from the measured rates of dark respiration (R, µg O_2 cm^{-2} h^{-1}) for spring and summer, over a 24 h day cycle using 0.8 as respiratory quotient (Muscatine et al. 1981). The percent contribution of zooxanthellae-acquired C to daily animal respiration (CZAR) was calculated according to Muscatine et al. (1981), using T = 0.78 according to (Tremblay et al. 2012).

1.3. N uptake by N_2 fixation

N uptake rates derived from N_2 fixation (µmol N cm^{-2} d^{-1}) were calculated from our results for the two respective seasons using a theoretical molar ratio C_2H_4:N_2 of 3:1 without considering hydrogenase activity (Kayanne et al. 2005; Olson et al. 2009), but accounting for the 2 atoms of molecular N_2. For calculating CZND, the ratio of the N fixed by the N_2-fixing bacteria subsequently assimilated by the symbiotic zooxanthellae was assumed equal to 0.97, as described for the first time by Foster et al. (2011) for an association between a N_2 fixer and a eukaryotic unicellular alga.

1.4. C and N uptake by heterotrophy, and CHAR

Heterotrophic C uptake (HC, µmol C cm^{-2} d^{-1}) was calculated as HC = DR*25/100, assuming a conservative average 25% contribution of heterotrophically-acquired C to animal respiration

(CHAR) (Falkowski et al. 1993; Palardy et al. 2008; Tremblay et al. 2011) for spring and summer, resulting from relatively low and constant in-situ feeding rates on low year-round particulate organic C (POC) and N (PON) concentrations in reef-surrounding waters of the Gulf of Aqaba (cf. Appendix 2 - Table 1). The assumption of constant seasonal feeding was derived from non-variable seasonal observations of micro- and mesozooplankton depletion in local coral reef-overlying waters (Yahel et al. 2005), and from the locally constant, yet relatively insignificant, nanoplankton biomass (Lindell and Post 1995; Al-Najjar et al. 2007), which is accepted among pico- and nanoparticles as the most important contribution to C (84-94%) and N (52-85%) ingested by corals (Houlbrèque et al. 2004). Heterotrophic PON uptake (µmol N cm^{-2} d^{-1}) was derived from heterotrophic C uptake rates by applying local in-situ POC:PN ratios (cf. Appendix 2 - Table 1) and an average N assimilation efficiency of 90 % (Bythell 1988; Anthony 1999; Mills 2000; Piniak et al. 2003; Mills et al. 2004). A fraction of 0.2 of the calculated flux was assumed to be subsequently transferred from the host to the zooxanthellae (Cook 1972; Szmant-Froelich 1981; Piniak et al. 2003), and thus regarded relevant for zooxanthellae N demand and CZND calculations. Finally, total heterotrophic N uptake (µmol N cm^{-2} d^{-1}) was calculated for spring and summer as the sum of PON and dissolved organic N (DON) uptake, where DON uptake rate = dissolved free amino acids (DFAA) + urea uptake rates. Each respective DON uptake rate was calculated as described here below.

1.4.1. Dissolved free amino acids (DFAA)

Active carrier-mediated DFAA uptake at in-situ DFAA concentrations was calculated applying Michaelis-Menten kinetics ($V = (V_{max}*[DFAA])/(K+[DFAA])$) according to Grover et al. (2008), where V is DFAA uptake rate (nmol N cm^{-2} h^{-1}), V_{max} is maximum DFAA uptake rate (i.e. 7.52 nmol N cm^{-2} h^{-1}), [DFAA] is in-situ DFAA concentration (µmol l^{-1}) and K is DFAA concentration at half-maximal uptake rate (i.e. 1.23 µmol l^{-1}). [DFAA] was calculated by converting seasonal in-situ DOC concentrations (cf. Appendix 2 - Table 1) to DON concentrations applying DOC:DON = 14 (Tanaka et al. 2011) and assuming DFAA=0.1*DON (Bronk 2002). A fraction of 0.33 of the calculated flux was assumed to be taken up by the zooxanthellae rather than by the host (Grover et al. 2008), and thus regarded relevant for zooxanthellae N demand and CZND calculations.

1.4.2. Urea

Active carrier-mediated urea uptake at in-situ urea concentrations was calculated applying Michaelis-Menten kinetics ($V = (V_{max}*[Urea])/(K+[Urea])$) according to Grover et al. (2006), where V is urea uptake rate (nmol N cm^{-2} h^{-1}), V_{max} is maximum urea uptake rate (i.e. 0.20 nmol N cm^{-2} h^{-1}), [Urea] is in-situ urea concentration (µmol l^{-1}) and K is urea concentration at half-maximal uptake rate (i.e. 1.05 µmol l^{-1}), [Urea] = 0.3 µmol l^{-1} used as representative in-situ concentration (Bronk 2002). A fraction of 0.2 of the calculated flux was finally assumed to be

taken up by the zooxanthellae rather than by the host (Grover et al. 2006), and thus regarded relevant for zooxanthellae N demand and CZND calculations.

1.5. Dissolved inorganic N (DIN) uptake

Dissolved inorganic N (DIN) uptake (µmol N cm^{-2} d^{-1}) was calculated for spring and summer seasons as the sum of ammonium (NH_4^+) + nitrate (NO_3^-) uptake rates. Each respective uptake flux was calculated as described here below.

1.5.1. Ammonium (NH_4^+)

Active carrier-mediated NH_4^+ uptake at in-situ NH_4^+ concentrations was calculated applying Michaelis-Menten kinetics ($V = (V_{max}*[NH_4^+])/(K+[NH_4^+])$) according to Muscatine and D'elia (1978), where V is NH_4^+ uptake rate (nmol N cm^{-2} h^{-1}), V_{max} is maximum NH_4^+ uptake rate (i.e., 6.39 and 2.59 nmol N cm^{-2} h^{-1} for spring and summer season, respectively), $[NH_4^+]$ is in-situ NH_4^+ concentration (µmol l^{-1}) (cf. Fig. 2-1, Appendix 1 - Table 1) and K is NH_4^+ concentration at half-maximal uptake rate (Muscatine and D'elia 1978) (i.e. 0.58 µmol l^{-1}). V_{max} original units (µmol N mg chl *a* h^{-1}) were converted to nmol N cm^{-2} h^{-1} using present study seasonal chlorophyll a cm^{-2} data (cf. Fig. 2-2). A fraction of 0.9 of the calculated flux was assumed to be taken up by the zooxanthellae rather than by the host, and thus regarded as relevant for zooxanthellae N demand (Grover et al. 2002; Pernice et al. 2012) and CZND calculations.

1.5.2. Nitrate (NO_3^-)

Active carrier-mediated NO_3^- transport was calculated applying linear uptake kinetics ($V = 7.8273*[NO_3]$) at in-situ NO_3^- concentrations according to Bythell (1990), where V is NO_3^- uptake rate (nmol N cm^{-2} h^{-1}) and $[NO_3]$ is in-situ concentration (nmol l^{-1}) (cf. Fig. 2-1, Appendix 2 - Table 1). As NO_3^- assimilation is exclusive to zooxanthellae (Crossland and Barnes 1977; Leggat et al. 2007; Kopp et al. 2013), the calculated flux was assumed to be taken up at a ratio of 1 for CZND calculations.

1.6. Percent loss by organic C (or N) release of the total acquired C (or N) (LOC or LON)

Total organic C (TOC) release was calculated as the sum of the measured POC and DOC release rates (Appendix 2 - Fig. 2) and calculated on a per day basis (µmol C cm^{-2} d^{-1}). The percent loss by TOC release of the total acquired C (LOC) was calculated by the equation: LOC[%] = TOCrelease/(DPg+HC)*100. Total organic N (TON) release was calculated assuming POC:PON ratios of 11.0 in spring and of 13.4 in summer as measured by Naumann et al. (2010) at the sampling site, and an average DOC:DON ratio of 18.6 obtained from literature (Tanaka et al. 2008; Tanaka et al. 2009; Tanaka et al. 2010). The percent loss by TON release of the total

acquired N (LON) was calculated by the equation: LON [%] = TONrelease/∑(N uptake rates)*100.

Appendix 2 - Table 7. Parameters used to build the C and N flux model

	Spring	Summer
*Daily gross photosynthesis (DPg, µmol C cm^{-2} d^{-1})	9.610 ± 1.695	9.235 ± 2.678
*Daily respiration (DR, µmol C cm^{-2} d^{-1})	6.643 ± 1.736	8.616 ± 2.547
Heterotrophic C uptake (HC, µmol C cm^{-2} d^{-1})	1.731 ± 0.404	2.148 ± 0.479
Total heterotrophic N uptake (µmol N cm^{-2} d^{-1})	0.247 ± 0.050	0.277 ± 0.053
Particulate organic N (PON) uptake (µmol N cm^{-2} d^{-1})	0.193 ± 0.046	0.217 ± 0.051
Dissolved free amino acids (DFAA) uptake (µmol N cm^{-2} d^{-1})	0.053 ± 0.004	0.059 ± 0.002
Urea uptake (µmol N cm^{-2} d^{-1})	0.001	0.001
*Total dissolved inorganic N (DIN) uptake (µmol N cm^{-2} d^{-1})	0.150 ± 0.022	0.018 ± 0.004
**Ammonium (NH_4^+) uptake* (µmol N cm^{-2} d^{-1})	0.068 ± 0.005	0.010 ± 0.001
**Nitrate (NO_3^-) uptake* (µmol N cm^{-2} d^{-1})	0.082 ± 0.016	0.008 ± 0.003
*Uptake from N_2 fixation (µmol N cm^{-2} d^{-1})	0.002 ± 0.001	0.010 ± 0.005
*Total organic C (TOC) release (µmol C cm^{-2} d^{-1})	1.168 ± 0.298	1.379 ± 0.262
Total organic N (TON) release (µmol N cm^{-2} d^{-1})	0.079 ± 0.023	0.087 ± 0.017
Zooxanthellae N demand (ZND) (µmol N cm^{-2} d^{-1})	0.096 ± 0.017	0.092 ± 0.027
Contribution of zooxanthellae-acquired C to animal respiration (CZAR, %)	110 ± 18	86 ± 14
Contribution of heterotrophically-acquired C to animal respiration (CHAR, %)	25	25
Contribution of zooxanthellae-acquired N (DIN) to ZND (CZND, %)	149 ± 22	18 ± 5
Contribution of zooxanthellae-acquired N (heterotrophic) to ZND (CZND, %)	59 ± 11	69 ± 12
Contribution of zooxanthellae-acquired N (N_2 fixation) to ZND (CZND, %)	2 ± 1	11 ± 5
Contribution of zooxanthellae-acquired N (total) to ZND (CZND, %)	209 ± 34	97 ± 21
Loss by organic C release of the total acquired C (LOC, %)	10 ± 3	12 ± 2
Loss by organic N release of the total acquired N (LON, %)	14 ± 4	28 ± 6

Parameters are either obtained from data sets generated by the present study (*) or calculated as described in the Appendix 2 - Methods section. Values are reported as means ± standard deviation.

Appendix 2 - References

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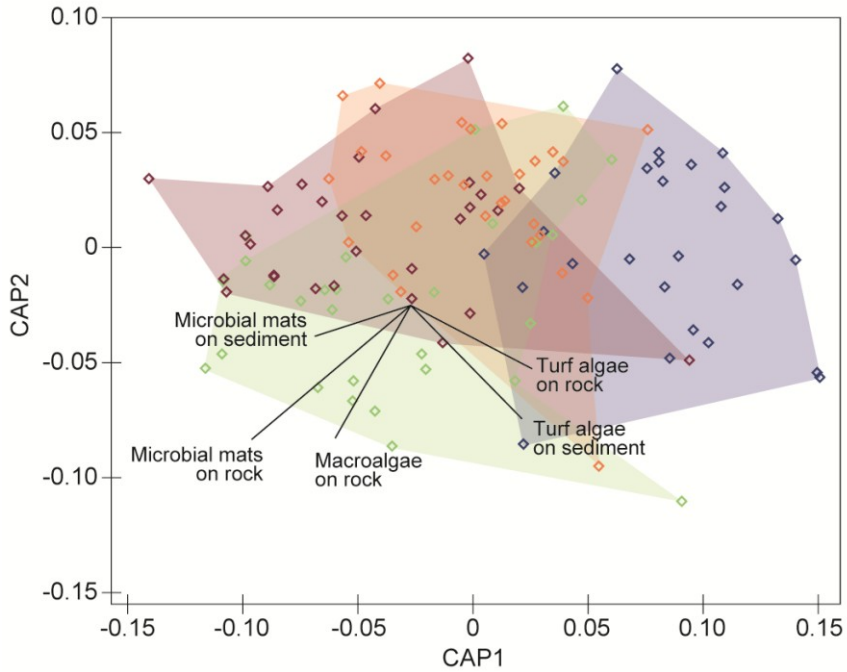
Appendix 3 - Table 1. Fully crossed two-factor general linear model with N_2 fixation, net photosynthesis (P_{net}), respiration (R), gross photosynthesis (P_{gross}), and the ratio of P_{gross}:R as a function of substrate and season.

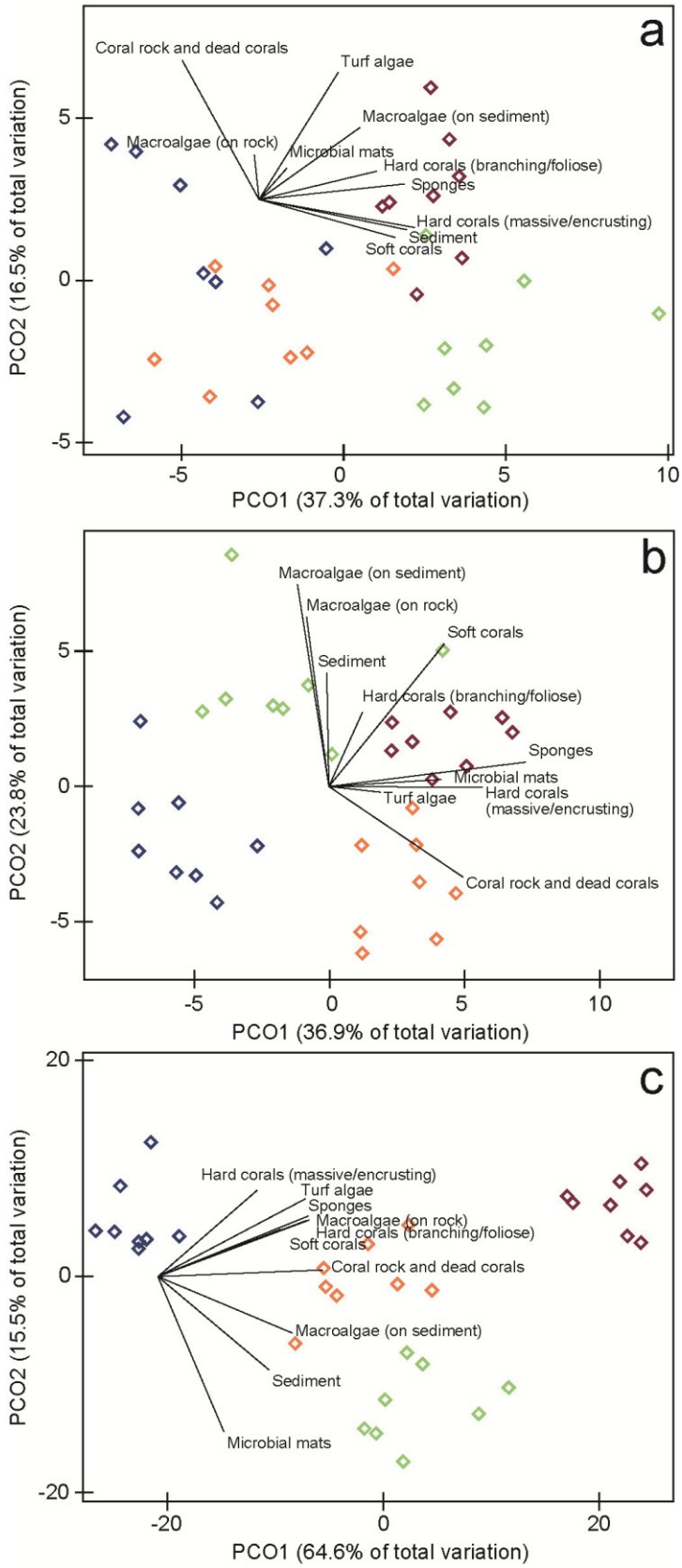
Parameter	Factor	df, $df_{residual}$	F	p
N_2 fixation	Season	6, 83	5.78	<0.001
	Substrate	3, 83	60.09	<0.001
	Season * Substrate	2, 77	299.37	<0.001
P_{gross}	Season	3, 79	12.68	<0.001
	Substrate	2, 79	63.64	<0.001
	Season * Substrate	5, 74	3.96	<0.001
R	Season	3, 89	18.00	<0.001
	Substrate	2, 89	156.27	<0.001
	Season * Substrate	6, 83	15.34	<0.001
P_{net}	Season	3, 80	6.65	<0.001
	Substrate	2, 80	210.64	<0.001
	Season * Substrate	5, 75	7.59	<0.001
P_{gross}:R	Season	3, 80	8.44	<0.001
	Substrate	2, 80	409.47	<0.001
	Season * Substrate	5, 75	6.52	<0.001

Appendix 3 - Table 2. Fully crossed three-factor general linear model with N_2 fixation as a function of substrate, season, and time of day (day vs. night).

	df, $df_{residual}$	F	p
Season	2, 122	106.12	<0.001
Substrate	2, 122	224.02	<0.001
Time of day	1, 122	6.86	<0.01
Season*Substrate	4, 114	6.22	<0.001
Season*Time of day	2, 114	9.28	<0.001
Substrate*Time of day	2, 114	10.39	<0.001
Season*Substrate*Time of day	4, 110	3.70	<0.01



Appendix 4 - Fig.1. Canonical Analysis of Principal Coordinates (CAP) plot showing differences in benthic community composition between seasons. Vectors are overlaid for benthic components that have a Spearman rank correlation greater than 0.4 with either of the resulting CAP axes. Colors represent winter (blue), spring (green), summer (red) and autumn (orange).



Appendix 4 - Fig.2. PCO plots showing differences in benthic community gross primary production (a) respiration (b) and N_2 fixation (c), between seasons. Benthic categories correlation vectors are overlaid on the plots to visualize relationships between variables and the ordination axes. Colors represent winter (blue), spring (green), summer (red) and autumn (orange).

Appendix 4 - Table 1. Cover (%) of all benthic categories at each reef habitat in all seasons. Values are means (n = 6) with SE in parentheses.

Benthic category	Season	Habitat				
		Forereef	Transition zone	Sand belt	Reef crest	Reef flat
Hard corals (branching/ foliose)	Winter	15.3 (1.7)	15.7 (2.0)	10.5 (1.9)	15.5 (2.4)	5.8 (3.0)
	Spring	17.3 (3.4)	14.8 (3.1)	6.8 (2.4)	13.3 (3.2)	6.0 (2.5)
	Summer	13.2 (3.9)	14.5 (2.9)	7.8 (1.7)	13.8 (3.0)	7.5 (3.1)
	Autumn	18.1 (1.9)	16.0 (1.9)	7.8 (2.0)	13.7 (1.8)	7.2 (1.7)
Hard corals (massive/ encrusting)	Winter	45.3 (3.4)	15.8 (7.2)	10.7 (6.8)	17.2 (2.1)	14.2 (1.8)
	Spring	37.0 (1.8)	20.5 (2.6)	8.3 (2.5)	13.5 (2.8)	13.2 (3.3)
	Summer	44.3 (4.5)	23.7 (4.6)	7.5 (4.1)	20.0 (8.6)	16.8 (8.4)
	Autumn	39.3 (3.8)	21.3 (3.3)	6.5 (4.0)	19.3 (2.6)	20.7 (2.1)
Soft corals	Winter	10.0 (1.5)	13.3 (2.0)	1.8 (3.0)	3.8 (3.9)	5.0 (4.6)
	Spring	7.0 (3.8)	14.6 (0.7)	3.2 (1.0)	5.5 (1.0)	5.3 (1.1)
	Summer	8.2 (1.0)	10.5 (1.2)	3.3 (1.1)	3.0 (0.8)	10.3 (2.2)
	Autumn	11.2 (1.6)	14.8 (2.6)	2.7 (4.1)	7.3 (4.2)	10.8 (4.3)
Coral rock ('bare')	Winter	8.2 (2.3)	8.8 (2.6)	9.7 (2.5)	22.1 (1.9)	17.5 (1.7)
	Spring	7.3 (2.0)	7.2 (2.5)	7.3 (4.1)	19.3 (4.4)	14.3 (2.8)
	Summer	8.5 (3.6)	7.8 (2.7)	7.7 (2.1)	21.3 (2.6)	20.7 (1.3)
	Autumn	9.2 (1.8)	5.2 (1.9)	8.0 (1.8)	20.7 (0.7)	15.7 (1.3)
Dead corals ('bare')	Winter	1.5 (0.9)	1.7 (1.1)	2.0 (0.8)	0.8 (0.7)	6.9 (0.6)
	Spring	1.8 (0.7)	1.3 (0.9)	1.0 (0.6)	0.2 (0.3)	2.3 (0.3)
	Summer	1.2 (0.4)	1.8 (2.0)	0.8 (2.3)	2.3 (2.0)	3.3 (0.6)
	Autumn	1.8 (0.5)	1.8 (0.5)	1.5 (0.4)	2.3 (0.4)	1.5 (0.5)
Microbial mats (on rock)	Winter	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.5 (0.0)
	Spring	4.5 (0.0)	1.3 (0.0)	3.3 (0.0)	5.8 (0.0)	2.0 (0.0)
	Summer	2.5 (0.6)	3.0 (1.0)	1.0 (1.1)	0.8 (1.2)	6.7 (1.4)
	Autumn	2.5 (1.3)	0.5 (1.4)	0.3 (1.0)	0.0 (0.6)	0.7 (0.8)
Turf algae (on rock)	Winter	6.5 (1.8)	8.0 (1.6)	3.7 (1.2)	12.0 (1.9)	10.8 (1.8)
	Spring	3.3 (2.0)	3.6 (1.1)	2.2 (2.2)	8.8 (2.6)	15.5 (1.2)
	Summer	3.0 (2.5)	2.2 (3.5)	1.5 (6.2)	5.7 (5.6)	3.8 (5.4)
	Autumn	8.0 (1.4)	2.7 (1.3)	1.7 (1.0)	3.0 (1.0)	12.7 (1.0)
Macroalgae (on rock)	Winter	2.7 (1.3)	4.2 (1.2)	3.5 (1.6)	6.7 (1.7)	4.0 (2.2)
	Spring	10.8 (2.0)	6.9 (1.8)	7.2 (2.3)	13.8 (2.8)	6.0 (3.0)
	Summer	7.8 (2.9)	5.0 (2.3)	3.7 (2.2)	7.7 (3.0)	1.8 (2.6)
	Autumn	1.7 (2.2)	3.2 (1.9)	3.0 (1.8)	9.5 (1.3)	3.0 (2.0)
Sponges	Winter	0.5 (0.3)	0.7 (0.3)	0.7 (0.3)	0.0 (0.3)	0.0 (0.2)
	Spring	0.5 (0.2)	0.5 (0.2)	0.7 (0.2)	0.0 (0.2)	0.0 (0.0)
	Summer	1.0 (0.0)	0.7 (0.0)	1.0 (0.0)	0.0 (0.2)	0.0 (0.2)
	Autumn	1.2 (0.3)	1.0 (0.3)	1.2 (0.3)	0.3 (0.3)	0.2 (0.4)
Sediment ('bare')	Winter	6.2 (3.2)	23.8 (5.7)	37.7 (6.0)	1.7 (3.6)	15.7 (3.6)
	Spring	7.5 (4.9)	25.0 (4.9)	50.3 (8.8)	0.7 (9.3)	16.8 (0.9)
	Summer	6.2 (3.2)	26.3 (3.7)	56.2 (1.0)	0.7 (3.5)	7.3 (4.5)
	Autumn	4.7 (3.5)	29.5 (4.9)	57.8 (5.4)	0.2 (4.2)	9.5 (6.8)
Microbial mats (on sediment)	Winter	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)
	Spring	0.3 (0.0)	0.3 (0.0)	1.3 (0.0)	0.0 (0.0)	0.2 (0.0)
	Summer	0.8 (0.1)	0.8 (0.2)	1.7 (0.2)	0.0 (0.2)	0.0 (0.2)
	Autumn	0.3 (0.2)	0.5 (0.2)	1.7 (0.1)	0.0 (0.2)	0.0 (0.5)
Turf algae (on sediment)	Winter	0.7 (0.5)	4.3 (2.1)	12.8 (2.2)	0.0 (2.2)	6.1 (1.3)
	Spring	0.2 (3.4)	0.0 (5.7)	0.5 (5.9)	0.0 (6.0)	1.0 (0.0)
	Summer	0.0 (0.6)	0.2 (1.7)	0.0 (1.7)	0.0 (1.8)	0.0 (1.0)
	Autumn	0.3 (0.2)	0.3 (0.2)	0.7 (0.0)	0.0 (0.0)	0.0 (0.0)

Appendix 4 - Table 1. continued

Benthic category	Season	Habitat				
		Forereef	Transition zone	Sand belt	Reef crest	Reef flat
Macroalgae (on sediment)	Winter	0.0 (0.0)	0.3 (0.0)	0.3 (0.3)	0.0 (0.3)	0.3 (0.3)
	Spring	0.0 (0.0)	1.3 (0.3)	0.7 (0.3)	0.0 (0.3)	0.2 (0.0)
	Summer	0.5 (0.1)	0.8 (0.1)	0.2 (0.2)	0.2 (0.2)	0.0 (0.2)
	Autumn	0.2 (0.0)	0.3 (0.1)	0.2 (0.2)	0.0 (0.8)	0.7 (0.8)
Millepora	Winter	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)	13.3 (0.0)	0.0 (0.0)
	Spring	0.0 (0.0)	0.0 (0.3)	0.3 (2.8)	9.0 (2.4)	0.2 (1.0)
	Summer	0.0 (2.9)	0.0 (3.8)	0.7 (0.0)	11.0 (0.0)	0.2 (0.0)
	Autumn	0.0 (0.0)	0.2 (0.0)	0.8 (0.0)	8.5 (0.0)	0.5 (0.0)
Crustose coralline algae (CCA)	Winter	1.2 (0.2)	0.2 (0.3)	1.7 (0.3)	3.5 (0.2)	3.3 (1.0)
	Spring	0.0 (1.0)	0.0 (0.9)	0.0 (1.2)	5.2 (1.6)	6.0 (1.6)
	Summer	0.0 (1.6)	0.0 (1.6)	0.0 (1.6)	7.8 (1.6)	10.8 (1.2)
	Autumn	0.2 (0.0)	0.0 (0.0)	0.0 (0.0)	7.7 (0.0)	9.0 (0.0)
Zoanthids	Winter	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.0 (0.0)	1.0 (0.0)
	Spring	0.2 (0.0)	0.2 (0.0)	0.0 (0.3)	2.0 (0.4)	3.7 (0.4)
	Summer	0.0 (0.5)	0.0 (0.6)	0.2 (0.6)	2.5 (0.6)	4.5 (0.2)
	Autumn	0.2 (0.2)	0.2 (0.2)	0.3 (0.2)	2.8 (0.2)	4.5 (0.0)
Coral rubble	Winter	1.5 (0.5)	3.0 (0.8)	4.5 (0.8)	0.7 (0.7)	6.1 (0.7)
	Spring	2.0 (0.6)	2.6 (0.8)	6.5 (1.2)	2.3 (1.3)	4.8 (0.7)
	Summer	2.8 (1.4)	2.3 (1.4)	6.7 (0.6)	3.0 (1.4)	4.3 (1.0)
	Autumn	1.3 (0.5)	2.5 (0.4)	5.8 (0.3)	3.2 (0.4)	1.3 (0.8)
Others	Winter	0.5 (0.3)	0.2 (0.2)	0.2 (0.2)	1.8 (0.2)	1.8 (0.0)
	Spring	0.2 (0.2)	0.3 (0.2)	0.3 (0.5)	0.5 (0.6)	2.5 (0.4)
	Summer	0.0 (0.4)	0.3 (0.3)	0.2 (0.4)	0.2 (0.5)	1.8 (0.6)
	Autumn	0.0 (0.2)	0.0 (0.1)	0.0 (0.2)	1.5 (0.2)	2.2 (0.2)

Appendix 4 - Table 2. Zonal statistics of each habitat, and percentage of area covered by the benthic categories investigated. Values are means with SD in parentheses.

Habitat	Mean depth ± SD (m)	Max depth (m)	Min depth (m)	Water volume (m^3)	Planar 2D area (m^2)	Slope 2D area (m^2)	2D area covered by benthic categories investigated (%)
Reef flat	0.7 (0.2)	2.2	0.5	38318	54214	54244	82.9 (3.9)
Reef crest	2.2 (1.0)	5.9	0.5	34541	15831	16071	78.1 (2.6)
Sand belt	5.2 (2.2)	15.6	1.0	135294	26186	26705	92.9 (0.4)
Transition zone	9.0 (2.9)	17.4	3.2	422929	46825	47997	97.0 (0.3)
Forereef	16.7 (2.6)	20.6	7.0	592619	35395	36616	97.5 (0.7)
Total reef area	6.8 (3.7)	20.6	0.5	1223700	178451	181633	89.7 (8.2)

Appendix 4 - Table 3. Description of the incubated categories, replication for each season and calculated 2D:3D conversion factors (where applicable).

Benthic category	Replication	2D:3D	Description / taxa incubated
Hard corals (branching/foliose)	n = 24	7.6	Acropora, Stylophora, Pocillopora
Hard corals (massive/encrusting)	n = 8	2.9	Goniastrea
Soft corals	n = 16	6.5	Xeniidae, Sarcophyton
Coral rock (‘bare’)	n = 8	2.5	Biogenic reef framework with the carbonate structure clearly visible
Dead corals (‘bare’)	-	5.2	Assumed equal to ‘bare’ coral rock
Microbial mats (on rock)	-	3.9	Assumed equal to microbial mats on sediment
Turf algae (on rock)	n = 8	3.9	Dense mats composed of a heterogeneous assemblage of filamentous algae
Macroalgae (on rock)	n = 8	3.9	Lobophora
Sponges	n = 8	3.9	Mycale
Sediment (‘bare’)	n = 8	1.0	Top 1 cm surface layer with its microphytobenthos
Microbial mats (on sediment)	n = 8	1.0	Top 1 cm surface layer covered by the mat
Turf algae (on sediment)	-	1.0	Assumed equal to turf algae on rock
Macroalgae (on sediment)	n = 8	1.8	Caulerpa
Seawater	n = 32	-	800 mL water volume per replicate

Appendix 4 - Table 4. Gross primary production (GPP), respiration (R) and N_2 fixation (BNF) of the benthic categories investigated in each season, after considering their respective three-dimensional structure (2D:3D), in mmol (C or N) m^{-2} benthos 3D area d^{-1}. Values are means (n = see Appendix 4 - Table 3) with SE in parentheses.

Benthic category	Season	GPP	R	BNF
Hard corals (branching/foliose)	Winter	648 (62)	424 (38)	0.035 (0.010)
	Spring	754 (26)	403 (19)	0.076 (0.014)
	Summer	733 (31)	500 (22)	0.303 (0.046)
	Autumn	618 (38)	393 (33)	0.050 (0.011)
Hard corals (massive/encrusting)	Winter	239 (16)	147 (9)	0.028 (0.010)
	Spring	342 (7)	153 (11)	0.017 (0.006)
	Summer	326 (17)	225 (17)	0.194 (0.040)
	Autumn	299 (23)	182 (7)	0.099 (0.033)
Soft corals	Winter	256 (23)	138 (8)	0.013 (0.004)
	Spring	403 (14)	208 (8)	0.021 (0.008)
	Summer	344 (15)	231 (14)	0.078 (0.012)
	Autumn	300 (21)	162 (9)	0.012 (0.003)
Coral rock ('bare')	Winter	145 (25)	58 (12)	0.136 (0.046)
	Spring	85 (22)	42 (8)	0.942 (0.121)
	Summer	168 (16)	109 (20)	2.113 (0.289)
	Autumn	136 (14)	88 (8)	0.804 (0.118)
Dead corals ('bare')	Winter	298 (53)	121 (24)	0.280 (0.094)
	Spring	175 (45)	87 (17)	1.944 (0.251)
	Summer	347 (34)	225 (42)	4.362 (0.596)
	Autumn	280 (30)	181 (15)	1.660 (0.243)
Microbial mats (on rock)	Winter	365 (66)	100 (11)	2.450 (0.439)
	Spring	400 (33)	121 (11)	9.535 (0.522)
	Summer	386 (40)	142 (12)	4.140 (0.328)
	Autumn	355 (32)	134 (13)	5.335 (0.410)
Turf algae (on rock)	Winter	246 (14)	88 (4)	0.828 (0.078)
	Spring	256 (18)	73 (6)	1.234 (0.206)
	Summer	417 (27)	111 (9)	5.207 (0.449)
	Autumn	237 (14)	88 (6)	1.263 (0.088)
Macroalgae (on rock)	Winter	192 (14)	55 (3)	0.082 (0.045)
	Spring	179 (24)	86 (18)	0.268 (0.110)
	Summer	184 (12)	68 (4)	1.045 (0.189)
	Autumn	130 (10)	42 (6)	0.268 (0.061)
Sponges	Winter	30 (8)	221 (13)	0.011 (0.001)
	Spring	139 (37)	407 (89)	0.037 (0.013)
	Summer	143 (18)	524 (18)	0.260 (0.042)
	Autumn	80 (18)	526 (35)	0.058 (0.014)
Sediment ('bare')	Winter	17 (3)	15 (2)	0.142 (0.030)
	Spring	66 (10)	22 (4)	0.630 (0.067)
	Summer	37 (4)	18 (1)	0.518 (0.079)
	Autumn	20 (2)	11 (1)	0.137 (0.022)

Appendix 4 - Table 4. continued

Benthic category	Season	GPP	R	BNF
Microbial mats (on sediment)	Winter	94 (17)	26 (3)	0.631 (0.113)
	Spring	103 (8)	31 (3)	2.455 (0.134)
	Summer	99 (10)	37 (3)	1.066 (0.084)
	Autumn	91 (8)	34 (3)	1.374 (0.105)
Turf algae (on sediment)	Winter	63 (4)	23 (1)	0.213 (0.020)
	Spring	66 (5)	19 (2)	0.318 (0.053)
	Summer	107 (7)	29 (2)	1.341 (0.116)
	Autumn	61 (4)	23 (2)	0.325 (0.023)
Macroalgae (on sediment)	Winter	233 (31)	36 (5)	0.003 (0.001)
	Spring	258 (37)	73 (8)	0.445 (0.161)
	Summer	229 (15)	49 (4)	0.358 (0.055)
	Autumn	169 (20)	19 (3)	0.157 (0.038)

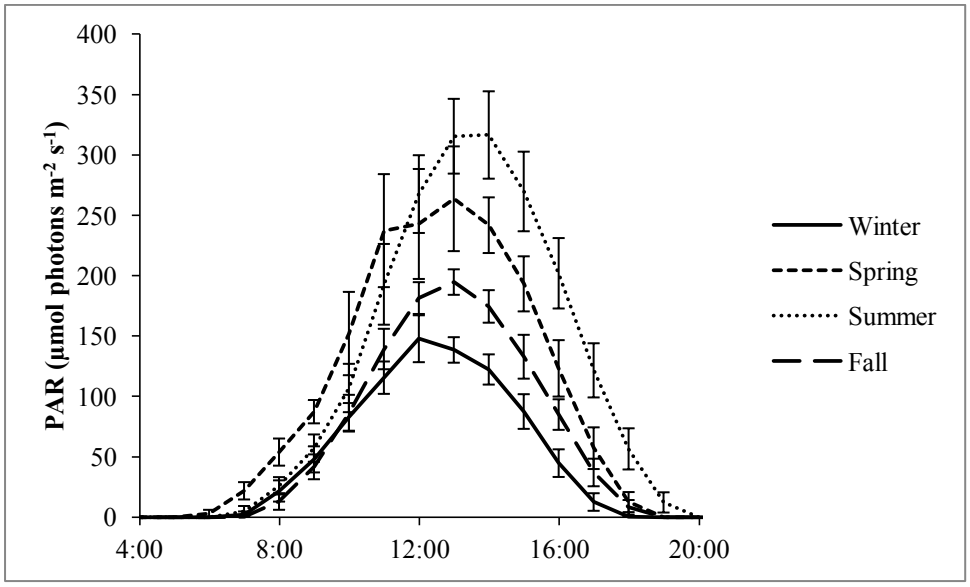
Appendix 4 - Table 5. Results of PERMANOVA looking at differences in benthic community cover among seasons and reef habitats. Shown are the main test (a) and the comparisons amongst seasons (b) and amongst reef habitats (c). Significant p-values have a grey background.

	Source	df	SS	MS	Pseudo F	P(perm)
(a)	Season (Se)	3	5035.9	1678.6	5.8197	0.0001
	Reef habitat (Rh)	4	42437	10609	36.782	0.0001
	Se x Rh	12	4048	337.34	1.1695	0.183
	Residuals	98	28267	288.44		
	Total	117	79788			
(b)	Winter	Winter				
	Spring	0.0001	Spring			
	Summer	0.0001	0.0213	Summer		
	Autumn	0.0005	0.0002	0.0107	Autumn	
(c)	Reef flat	Reef flat				
	Reef crest	0.0001	Reef crest			
	Sand belt	0.0001	0.0001	Sand belt	Transition	
	Transition zone	0.0001	0.0001	0.0001	zone	
	Forereef	0.0001	0.0001	0.0001	0.0001	Forereef

Appendix 4 - Table 6. Results of PERMANOVAs looking at differences in (a) gross primary production, (b) respiration and (c) N_2 fixation of the benthic community among the seasons, with the respective comparisons amongst seasons. Significant p-values have a grey background.

	Source	df	SS	MS	Pseudo F	P(perm)
(a)	Season	3	621.56	207.19	6.9098	0.0001
	Residuals	28	839.57	29.984		
	Total	31	1461.1			
	Winter	Winter				
	Spring	0.0003	Spring			
	Summer	0.0001	0.0001	Summer		
	Autumn	0.0132	0.0003	0.0005	Autumn	
(b)	Season	3	833.49	277.83	10.649	0.0001
	Residuals	28	730.5	26.089		
	Total	31	1564			
	Winter	Winter				
	Spring	0.0003	Spring			
	Summer	0.0002	0.0002	Summer		
	Autumn	0.0005	0.0003	0.0006	Autumn	
(c)	Season	3	10224	3407.9	33.877	0.0001
	Residuals	28	2816.6	100.59		
	Total	31	13040			
	Winter	Winter				
	Spring	0.0004	Spring			
	Summer	0.0002	0.0001	Summer		
	Autumn	0.0003	0.0002	0.0003	Autumn	

Appendix 5 - Fig. 1. Photosynthetically active radiation (PAR) (hourly mean ± SD of measurements min^{-1}) in the incubation tank between 04:00 and 20:00 hrs; averaged over 3 days during each sampling period. Selected days were comparable to all incubation days in weather conditions



Appendix 5 - Table 1. Comparison of benthic primary producers' metabolic rates in winter. Net photosynthesis and respiration rates are represented as nmol O_2 cm^{-2} h^{-1}. P_g:R = gross photosynthesis to respiration ratio. Values given as mean ± SD. Identical letters behind groups indicate no significant difference.

	Net photosynthesis		Respiration		P_g:R ratio	
Acropora	575 ± 113	a	247 ± 73	ab	3.45 ± 0.80	a
Pocillopora	604 ± 247	a	200 ± 52	b	4.00 ± 0.66	a
Stylophora	300 ± 116	b	252 ± 65	a	2.21 ± 0.34	b
Goniastrea	476 ± 114	ac	213 ± 41	ab	3.26 ± 0.53	a
Xeniidae	339 ± 130	bcd	63 ± 9	cf	6.27 ± 1.69	ce
Sarcophyton	144 ± 63	e	114 ± 36	d	2.23 ± 0.26	b
Lobophora	353 ± 79	bcf	59 ± 11	c	7.06 ± 1.25	c
Caulerpa	974 ± 393	g	81 ± 29	cef	14.08 ± 7.26	d
Turf algae	433 ± 77	adfh	95 ± 11	de	5.58 ± 0.71	ce
Sediment	81 ± 77	e	63 ± 19	cf	2.64 ± 1.60	b
Coral rock	379 ± 186	bch	96 ± 54	df	5.44 ± 2.08	e
Cyano mat	675 ± 319	a	107 ± 29	de	7.10 ± 1.35	c

Appendix 5 - Table 2. Comparison of benthic primary producers' metabolic rates in spring. Net photosynthesis and respiration rates are represented as nmol O_2 cm^{-2} h^{-1}. P_g:R = gross photosynthesis to respiration ratio. Values given as mean ± SD. Identical letters behind groups indicate no significant difference. Light availability during the net photosynthesis incubation of turf algae in spring was uncharacteristically low for that season (73.8 ± 26.3 µmol photons m^{-2} s^{-1}). The spring net photosynthesis rates and gross photosynthesis to respiration ratios of turf algae were therefore omitted from the data set.

	Net photosynthesis		Respiration		P_g:R ratio	
Acropora	625 ± 141	acd	198 ± 41	ac	4.18 ± 0.50	ac
Pocillopora	571 ± 122	abd	198 ± 61	ac	4.16 ± 1.19	abck
Stylophora	625 ± 175	acd	270 ± 59	b	3.40 ± 0.76	acd
Goniastrea	766 ± 59	cg	220 ± 45	bc	4.64 ± 0.90	bef
Xeniidae	508 ± 81	dh	113 ± 14	dfi	5.53 ± 0.78	egh
Sarcophyton	263 ± 109	ef	156 ± 23	ae	2.66 ± 0.57	d
Lobophora	292 ± 94	f	92 ± 55	fh	4.56 ± 0.77	afh
Caulerpa	1005 ± 426	g	165 ± 52	ag	7.00 ± 1.25	g
Turf algae			78 ± 19	h		
Sediment	459 ± 202	bh	91 ± 42	dfh	6.18 ± 0.83	g
Coral rock	209 ± 165	e	69 ± 38	h	3.44 ± 1.85	dk
Cyano mat	729 ± 168	ac	130 ± 34	egi	6.67 ± 0.57	g

Appendix 5 - Table 3. Comparison of benthic primary producers' metabolic rates in summer. Net photosynthesis and respiration rates are represented as nmol O_2 cm^{-2} h^{-1}. P_g:R = gross photosynthesis to respiration ratio. Values given as mean ± SD. Identical letters behind groups indicate no significant difference.

	Net photosynthesis		Respiration		P_g:R ratio	
Acropora	604 ± 133	adh	268 ± 43	ab	3.26 ± 0.38	ab
Pocillopora	504 ± 173	ab	268 ± 108	a	2.93 ± 0.28	a
Stylophora	487 ± 292	bcd	288 ± 106	ab	2.64 ± 0.68	ac
Goniastrea	615 ± 147	adh	324 ± 67	b	2.99 ± 0.69	a
Xeniidae	354 ± 66	be	90 ± 41	ce	5.42 ± 1.47	d
Sarcophyton	237 ± 126	e	208 ± 56	d	2.11 ± 0.40	c
Lobophora	323 ± 69	cef	73 ± 12	e	5.55 ± 1.07	d
Caulerpa	927 ± 153	g	110 ± 26	cf	9.61 ± 1.22	e
Turf algae	775 ± 155	gh	119 ± 27	fg	7.78 ± 2.14	e
Sediment	232 ± 79	e	75 ± 15	ce	4.06 ± 0.79	bf
Coral rock	374 ± 124	bf	179 ± 94	dh	3.50 ± 1.17	af
Cyano mat	675 ± 224	ah	152 ± 35	gh	5.45 ± 1.29	d

Appendix 5 - Table 4. Comparison of benthic primary producers' metabolic rates in fall. Net photosynthesis and respiration rates are represented as nmol O_2 cm^{-2} h^{-1}. P_g:R = gross photosynthesis to respiration ratio. Values given as mean ± SD. Identical letters behind groups indicate no significant difference.

	Net photosynthesis		Respiration		P_g:R ratio	
Acropora	491 ± 190	ab	215 ± 34	ab	3.31 ± 0.84	abc
Pocillopora	394 ± 127	be	257 ± 94	a	2.61 ± 0.45	a
Stylophora	508 ± 98	ab	177 ± 39	bf	3.93 ± 0.59	b
Goniastrea	600 ± 206	ac	263 ± 27	a	3.35 ± 1.00	ab
Xeniidae	361 ± 93	bde	77 ± 12	c	5.71 ± 1.05	d
Sarcophyton	206 ± 115	dfg	132 ± 27	d	2.52 ± 0.64	a
Lobophora	234 ± 51	f	45 ± 17	e	6.92 ± 2.75	d
Caulerpa	696 ± 195	ch	42 ± 15	e	20.42 ± 9.63	e
Turf algae	414 ± 68	bi	95 ± 19	c	5.44 ± 0.60	d
Sediment	120 ± 41	g	47 ± 10	e	3.76 ± 1.44	bc
Coral rock	302 ± 105	efi	144 ± 35	df	3.09 ± 0.45	ab
Cyano mat	618 ± 207	ah	143 ± 39	df	5.73 ± 2.59	d

Appendix 6 - Table 1. Pelagic biomass ranges used for constraining inflow of C and metabolic activity. Water column integrated to the reef surface area, all values in mmol C m^{-2}.

	Winter	Spring	Summer	Fall
Phytoplankton	7.10 - 9.38	6.91 - 10.25	3.48 - 4.39	5.80 - 9.21
Zooplankton	2.66 - 5.33	2.66 - 5.33	2.66 - 5.33	2.66 - 5.33
Protozoa	1.67 - 4.67	1.67 - 4.67	1.67 - 4.67	1.67 - 4.67
Bacteria	5.04 - 5.30	5.04 - 5.30	5.04 - 5.30	5.04 - 5.30
POC	22.47 - 34.93	42.08 - 80.08	31.07 - 63.68	47.47 - 53.76
DOC	541.64 - 625.03	515.25 - 635.94	715.75 - 732.16	669.94 - 709.91
Total	580.58 - 684.65	573.62 - 741.58	759.68 - 815.52	732.58 - 788.18

Appendix 6 - Table 2. Ranges of gross primary production (GPP) and community respiration (Rday) rates entered into the models. All values in mmol C m^{-2} d^{-1}.

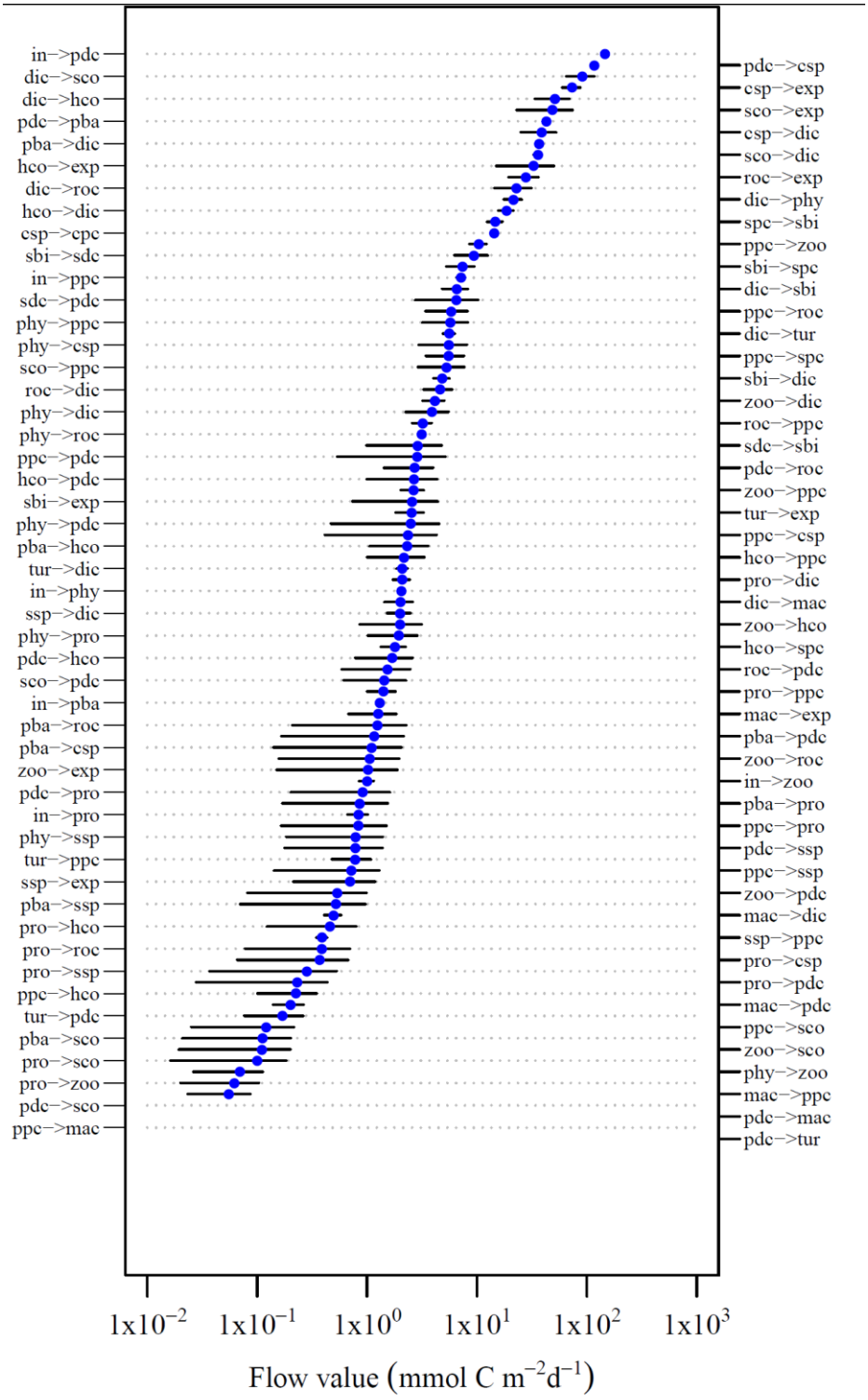
GPP	Winter	Spring	Summer	Fall
Water column	0 - 35.07	0 - 45.35	0 - 46.02	0 - 26.59
Hard corals	18.10 - 82.55	33.78 - 96.69	23.19 - 113.79	17.65 - 95.65
Soft corals	46.18 - 138.95	120.66 - 192.33	51.06 - 97.01	37.86 - 91.22
Macroalgae	0.98 - 3.02	5.24 - 21.34	1.95 - 3.82	1.06 - 2.47
Turf algae	4.18 - 7.01	4.60 - 8.27	7.67 - 13.20	3.93 - 6.41
Coral rock	5.12 - 38.65	0 - 33.45	21.55 - 61.60	15.47 - 67.08
Sediment	0.28 - 9.02	11.91 - 36.67	6.54 - 17.21	2.53 - 7.51
Rday				
Water column	22.36 - 50.68	33.65 - 110.62	35.72 - 101.07	33.28 - 99.98
Hard corals	15.30 - 48.79	13.47 - 52.56	19.66 - 72.40	21.08 - 75.32
Soft corals	25.58 - 39.84	45.67 - 66.12	18.14 - 52.39	22.60 - 36.01
Macroalgae	0.34 - 0.64	2.02 - 6.12	0.56 - 1.04	0.25 - 0.74
Turf algae	1.67 - 2.50	1.03 - 2.18	1.93 - 3.45	1.48 - 2.57
Coral rock	2.30 - 16.34	1.56 - 12.87	11.98 - 47.55	11.81 - 26.02
Sediment	2.12 - 5.94	3.16 - 11.39	3.95 - 7.23	2.45 - 4.24
Surface sponges	1.30 - 6.74	1.90 - 9.87	3.08 - 15.99	3.08 - 15.99
Cavity sponges	17.25 - 69.35	25.25 - 101.52	40.92 - 164.52	40.92 - 164.52

Appendix 6 - Table 3. Ranges of particulate and dissolved organic carbon flow rates (POC and DOC respectively) entered into the models. All values in mmol C m^{-2} d^{-1}. Negative values indicate net uptake, positive values indicate net release.

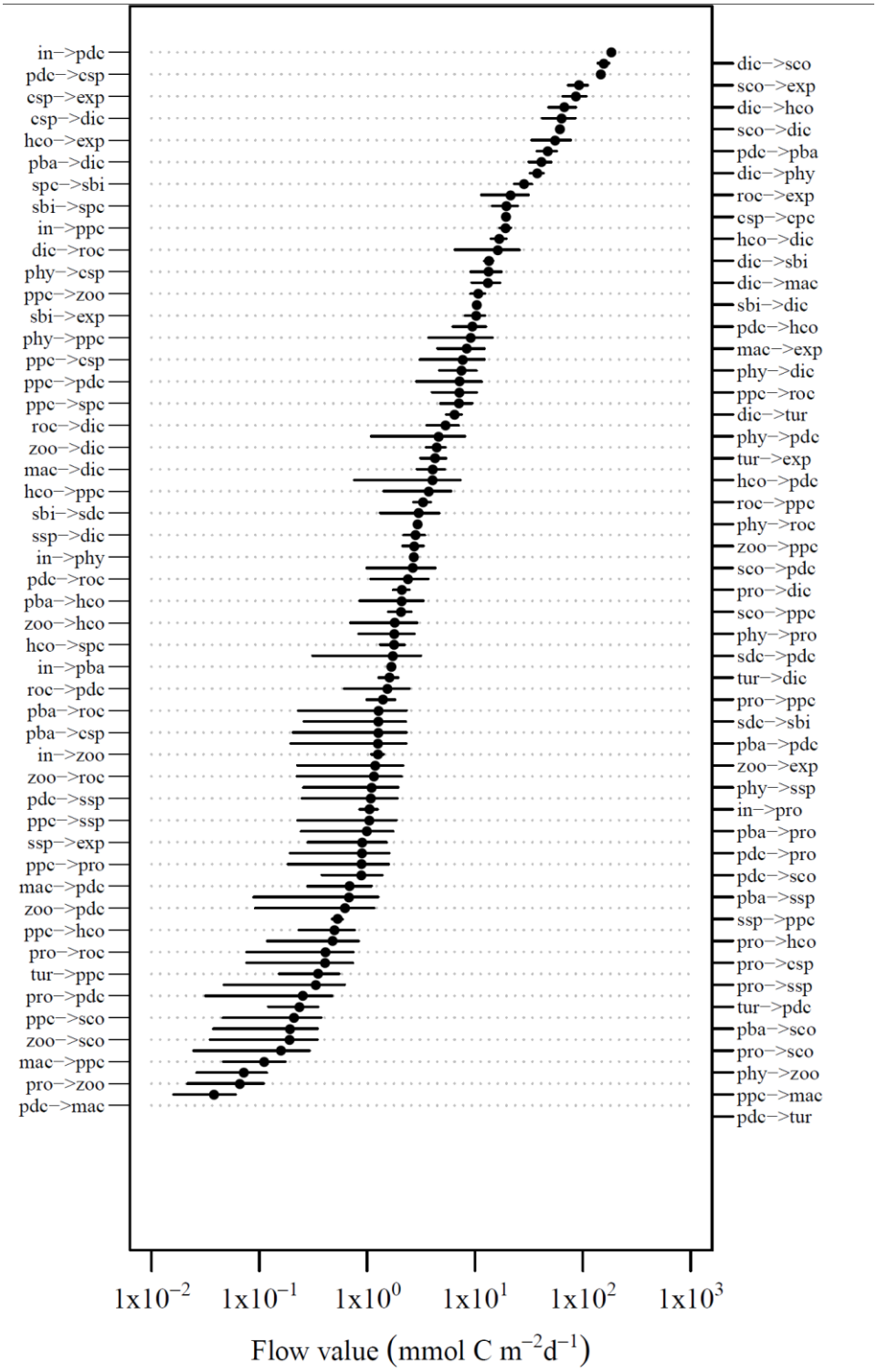
POC	Winter	Spring	Summer	Fall
Hard corals	-0.43 - 4.12	-0.93 - 8.45	0.57 - 8.88	0.92 - 9.46
Soft corals	0.42 - 9.09	1.21 - 2.92	2.31 - 17.82	3.11 - 9.59
Macroalgae	0.001 - 0.11	-0.08 - 0.22	0.16 - 0.22	0.19 - 0.58
Turf algae	0.24 - 1.30	0.004 - 0.69	0.38 - 0.83	0.32 - 0.64
Coral rock	0.57 - 4.23	0.56 - 4.12	0.79 - 5.84	0.72 - 5.34
Surface sponges	0.05 - 0.44	0.06 - 0.60	0.20 - 1.93	0.20 - 1.93
Cavity sponges	5.33 - 14.75	7.30 - 20.20	23.32 - 64.57	23.32 - 64.57
DOC				
Hard corals	-3.18 - 5.88	-13.67 - 14.76	-7.41 - 10.38	-20.13 - 46.16
Soft corals	0.04 - 2.90	-1.74 - 5.91	-1.92 - 3.53	-12.99 - 26.59
Macroalgae	0.09 - 0.31	-0.001 - 1.42	-0.36 - 0.52	-0.26 - 1.56
Turf algae	0.01 - 0.34	0.04 - 0.43	-0.49 - 0.82	-0.63 - 0.99
Coral rock	-4.68 - 3.31	-4.56 - 3.22	-6.46 - 4.56	-5.90 - 4.17

Appendix 6 - Table 4. Results of PERMANOVA main and pair-wise tests for the factor season on the reduced model solution set of the first 1000 solutions.

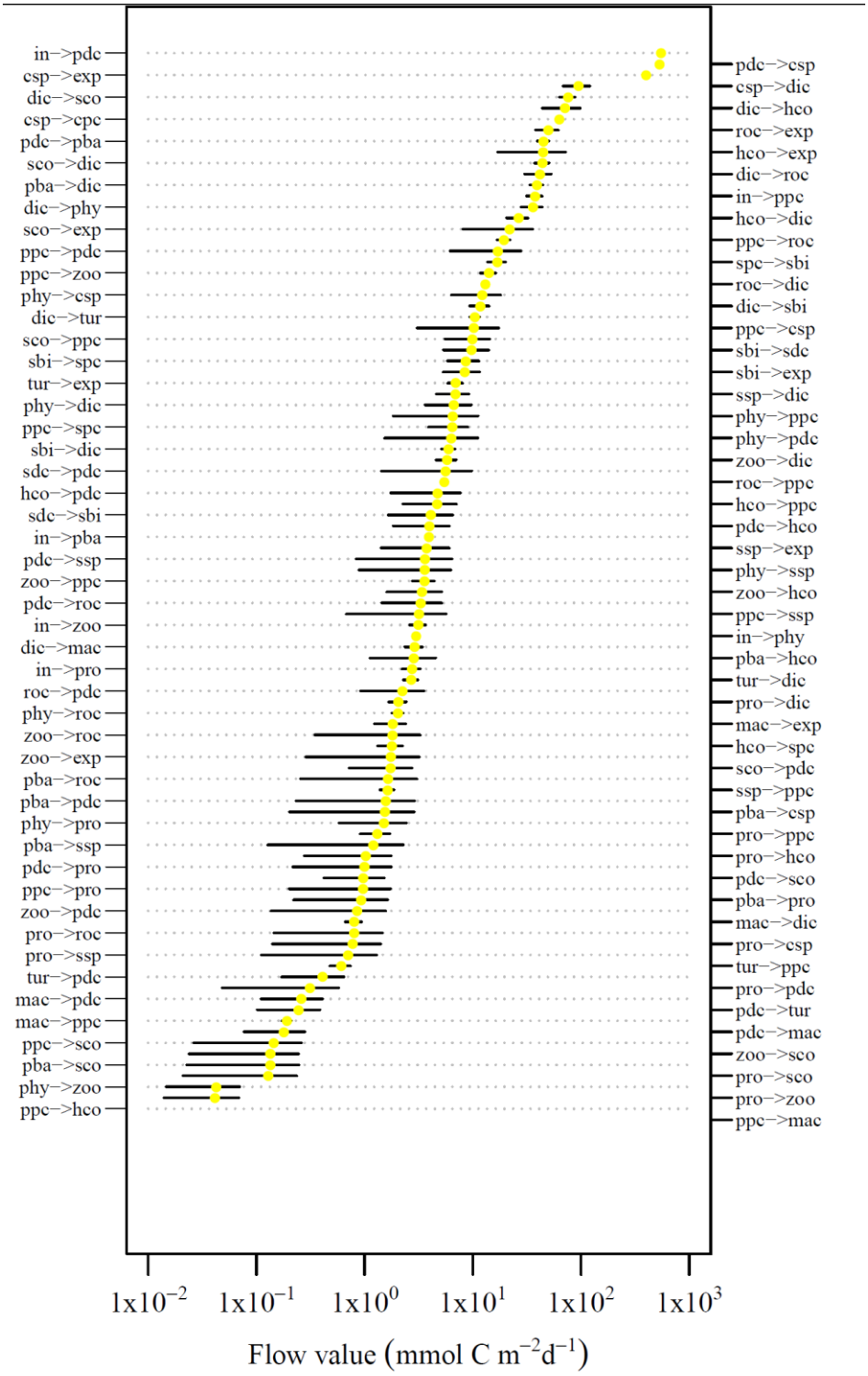
Comparison	Effect	df	SS	MS	Pseudo-F	p-value	Unique perms
Overall	Season	3	4.85×10^5	1.62×10^5	3938	0.001	997
	Residuals	3993	1.64×10^5	41.12			
	Total	3996	6.50×10^5				
					t	p-value	Unique perms
Pair-wise			Winter	Spring	43.61	0.001	999
			Winter	Summer	72.01	0.001	999
			Winter	Fall	74.78	0.001	998
			Spring	Summer	68.32	0.001	999
			Spring	Fall	75.27	0.001	999
			Summer	Fall	23.46	0.001	999



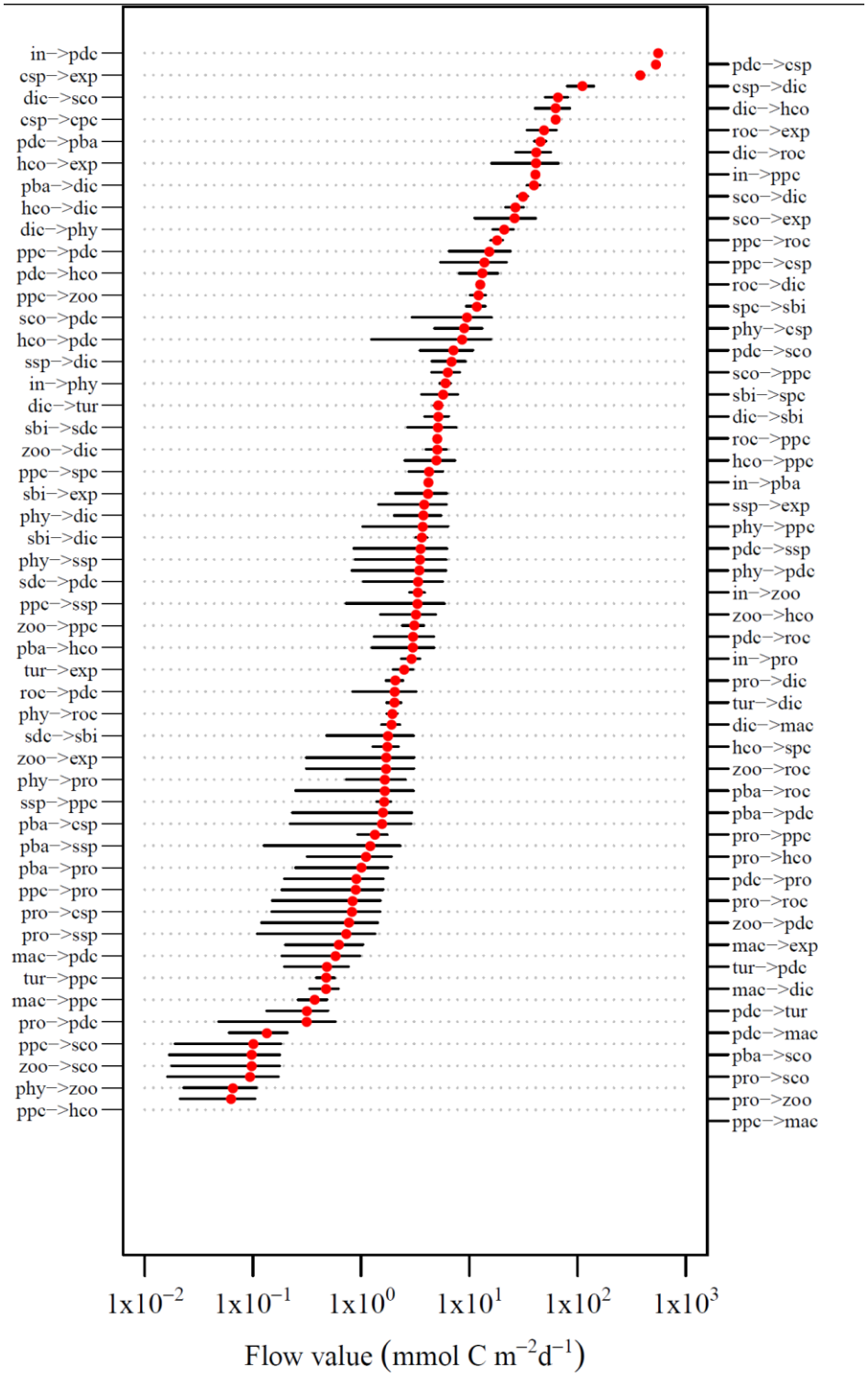
Appendix 6 - Fig. 1. Means and standard deviations of all flows of the winter model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.



Appendix 6 - Fig. 2. Means and standard deviations of all flows of the spring model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.



Appendix 6 - Fig. 3. Means and standard deviations of all flows of the summer model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.



Appendix 6 - Fig. 4. Means and standard deviations of all flows of the fall model. cpc = cavity particulate organic carbon. csp = cavity sponges, dic = dissolved organic carbon, exp = export, hco = hard corals, in = import, mac = macroalgae, pba = pelagic bacteria, pdc = pelagic dissolved organic carbon, phy = phytoplankton, ppc = pelagic particulate organic carbon, pro = pelagic protozoa, roc = coral rock, sbi = sediment biota, sco = soft corals, sdc = sediment dissolved organic carbon, spc = sediment particulate organic carbon, ssp = surface sponges, tur = turf algae, zoo = zooplankton.

Erklärung

Gemäß §6 der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007 versichere ich, dass die vorliegende Arbeit mit dem Titel,

„The importance of carbon dioxide and dinitrogen fixation for seasonal coral reef metabolism: from organism to ecosystem functioning"

1. ohne unerlaubte fremde Hilfe selbstständig verfasst und geschrieben wurde

2. keine anderen als die angegebenen Quellen und Hilfsmittel benutzt wurden

3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden

4. es sich bei den von mir abgegebenen Arbeiten um 3 identische Exemplare handelt.

Bremen, 24. Mai 2015

Nanne van Hoytema