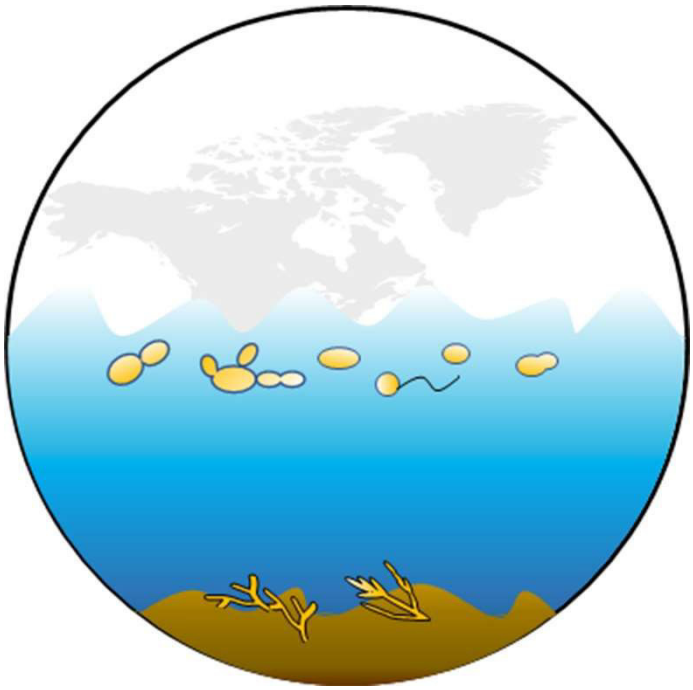
Spatial resolution of aquatic fungal communities driven by the interplay of environmental factors and ecological processes



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Spatial resolution of aquatic fungal communities driven by the interplay of environmental factors and ecological processes

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Summary

Aquatic fungi are a widespread group of organisms and occupy key roles in important ecological processes such as nutrient cycling. Due to their evolutionary development in the aquatic realm and secondary colonization steps of marine systems, they possess diverse ecologically relevant properties. However, little is known about the interplay between diversity and structure of aquatic fungal communities and the determinants. In particular, the spatial resolution of aquatic fungal communities is poorly understood. As a consequence, little attention has been paid to fungi, inhibiting the development of a holistic picture of aquatic ecological processes. Therefore, the aim of my dissertation was to understand how aquatic fungal communities in (i) rivers and adjacent coastal zone and (ii) benthic systems are spatially resolved by the interaction of ecological processes or single to multiple environmental factors.

In my PhD thesis, I resolved the mycopelagic and mycobenthic community structures with tag sequencing (18S rDNA gene sequence or Internal Transcribed Spacer 2 (ITS2)). This allowed me to show in my first project that the assembly processes of mycopelagic river communities differ drastically even over the shortest distances and that there is an interplay between environmental factors and ecological processes of selection and distribution. In my second project, in addition to tag sequencing, I conducted an extensive literature and database search for the purpose of a global biogeographic resolution of marine benthic fungi. This allowed me to create a conceptual picture of distribution patterns of benthic fungi for the first time. Surprisingly, the 100 km mark was a distance above which fungal distribution was strongly limited. Through a trait analysis, I could further show that fungal distribution is significantly influenced by individual traits such as morphology or trophic mode. In my third project, I conducted the first incubation experiments on the influence of anoxia on mycobenthic communities from coastal regions. I additionally corroborated the collected data with meta-society data from published datasets. Thus, I was able to investigate the adaptability and diverse strategies of mycobenthos to cope with anoxic conditions and to detect individual key players for the different oxic conditions in general.

Overall, this work highlights the importance of understanding the interaction of spatial distribution patterns, ecological processes and diverse environmental factors to predict and model the assembly of individual fungal communities. This is the only way to make reliable predictions of changing fluxes in

aquatic ecosystems, including all relevant groups of organisms. My work is an important part of this, because it opens up new perspectives on fungal assembly processes on which further research can be built.

Zusammenfassung

Aquatische Pilze sind eine weit verbreitete Organismengruppe und sie nehmen Schlüsselrollen in wichtigen ökologischen Prozessen wie zum Beispiel dem Nährstoffkreislauf ein. Aufgrund ihrer evolutiven Entwicklung im aquatischen Bereich sowie sekundäre Besiedlungsschritte mariner Systeme verfügen sie über diverse ökologisch interessante Eigenschaften. Allerdings ist nur wenig über das Zusammenspiel von Diversität und Struktur aquatischer Pilzgemeinschaften und den beeinflussenden Faktoren bekannt. Vor allem ist die räumliche Auflösung aquatischer Pilzgemeinschaften kaum erforscht. Als Konsequenz wurden bisher Pilze kaum beachtet, wodurch die Entwicklung eines holistischen Bildes aquatischer ökologischer Prozesse gehemmt ist. Ziel meiner Dissertation war es daher, zu verstehen, wie aquatische Pilzgemeinschaften in (i) Flüssen und angrenzender Küstenzone und (ii) benthischen Systemen durch die Interaktion ökologischer Prozesse oder einzelner bis mehrerer Umweltfaktoren räumlich aufgelöst werden.

In meiner Doktorarbeit habe ich die mykopelagischen und mykobenthischen Gesellschaftsstrukturen mit tag-Sequenzierung aufgelöst (18S rDNA Gensequenz oder Internal Transcribed Spacer 2 (ITS2)). Dadurch konnte ich in meinem ersten Projekt zeigen, dass die Assemblierungs-Prozesse mykopelagischer Flussgemeinschaften sich bereits über kürzeste Entfernungen drastisch unterscheiden und es zu einem Zusammenspiel von Umweltfaktoren sowie ökologischen Prozessen von Selektion und Verteilung kommt. In meinem zweiten Projekt habe ich neben der tag-Sequenzierung eine umfassende Literatur- und Datenbankrecherche zwecks einer globaler biogeographischer Auflösung mariner benthischer Pilze durchgeführt. Die erlaubte mir, erstmals ein konzeptionelles Bild von Verbreitungsmustern benthischer Pilze zu entwerfen. Überraschenderweise war die 100 km-Marke eine Distanz, ab der die pilzliche Verbreitung stark limitiert war. Durch eine Merkmals-Analyse konnte ich weiterhin zeigen, dass die pilzliche Verbreitung durch individuelle Merkmale wie Morphologie oder trophischer Modus signifikant beeinflusst wird. In meinem dritten Projekt habe ich erstmals den Einfluss von Anoxie auf mykobenthischen Gemeinschaften aus Küstenregionen in Inkubationsexperimenten durchgeführt. Die erhobenen Daten habe ich zusätzlich mit Meta-Gesellschaftsdaten aus publizierten Datensätzen unterfüttert. Somit konnte ich die Anpassungsfähigkeit und diverse Strategien des Mykobenthos mit anoxischen Bedingungen umzugehen, untersuchen und einzelne Keyplayer für die unterschiedlichen oxischen Bedingungen im Allgemeinen detektieren.

Insgesamt unterstreicht diese Arbeit, wie wichtig das Verständnis des Zusammenwirkens räumlicher Verteilungsmuster, ökologischer Prozesse sowie diverser Umweltfaktoren ist, um die Assemblierung

einzelner Pilzgemeinschaften vorherzusagen und zu modellieren. Nur dies erlaubt, verläßliche Voraussagen von sich änderenden Stoffflüssen zu machen unter der Einbeziehung aller relevanter Organismengruppen. Meine Arbeit ist ein wichtiger Bestandteil davon, denn sie eröffnen neue Sichtweisen auf pilzliche Assemblierungsprozesse, auf denen weiterführende Forschungsarbeiten aufbauen können

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Yanyan Yang

List of abbreviations

18S rRNA	18S of ribosomal ribonucleic acid
28S rRNA	28S of ribosomal ribonucleic acid
BCP	Biological carbon pump
CARD-FISH	Catalyzed reporter deposition-fluorescence in situ hybridization
CAZys	Carbohydrate-active enzymes
COI	Cytochrome c oxidase I
DDR	Distance decay relationship
DMF	Dark matter fungi
DNA	Deoxyribonucleic acid
DOIs	Digital object identifiers
DOM	Dissolved organic matter
eDNA	Environmental deoxyribonucleic acid
eRNA	Environmental ribonucleic acid
FPOM	Fine particulate organic matter
HTS	High-throughput sequencing
ITS	Internal transcribed spacer
LCA	Lowest common ancestor
LSU	Large subunit of ribonucleic acid
NGS	Next-Generation Sequencing
OMZs	Oxygen minimum zones
ONT	Oxford Nanopore Technologies
OTUs	Operational taxonomic units
PacBio	Pacific Bioscience
POM	Particulate organic matter
PSU	Practical salinity units
QPEs	Quantitative Process Estimates
RPB1	RNA polymerase II subunit 1
RPB2	RNA polymerase II subunit 2
SHs	Species hypotheses
SIP	Stable isotope probing
SMRT	Single-molecule real-time
SSU	Small subunit of ribonucleic acid
WoRMS	World Register of Marine Species

Contents

1 Introduction

1.1 Diversity and composition structure of the aquatic fungal communities

The first fungal taxon evolved around one billion years ago (Loron *et al.*, 2019) from freshwater habitats (Berbee *et al.*, 2017). Two notable evolutionary events coincided in the fungal community: the transition from aquatic to terrestrial environments (Spatafora *et al.*, 2017; Voigt *et al.*, 2021), and the retransition from terrestrial to aquatic environments (Nagy *et al.*, 2014). Retransitions from a terrestrial to an aquatic lifestyle has been clarified by Schoch *et al.* (2009): such transitions likely occurred at least three times in freshwater and up to six times in marine environments. Over the long history of evolution, fungi have evolved a large taxonomic and metabolic diversity (Baker, 2020; Hassett *et al.*, 2020; Wijayawardene, 2020). With their unique capacities and physiology, aquatic fungi have become the first choice for natural product mining, to aid in the production of food and medicine (El-Elimat *et al.*, 2021). The diverse ecological functions of aquatic fungi have drawn increasing attention in recent years, as they participate in the regulation of nutrient fluxes in many freshwater and marine habitats (Grossart *et al.*, 2019; Naranjo-Ortiz and Gabaldon, 2019b). Fungi are very diverse regarding their composition and physiology in the environment, and their strong adaptive capability enables them to occupy most niches on the Earth (Naranjo-Ortiz and Gabaldon, 2019b; 2020). Current estimations suggest up to 5 million fungal species in natural environments (Blackwell, 2011; Hawksworth and Lucking, 2017; Amend *et al.*, 2019), of which 151,435 species are identified (*http://www.speciesfungorum.org*, accessed 2022.09.04), spanning 20 phyla (Wijayawardene, 2020; Galindo *et al.*, 2021). It is also noted that all these phyla have been found in aquatic ecosystems (Riess *et al.*, 2018; Grossart *et al.*, 2019; Queiroz *et al.*, 2020).

Marine environment represents the largest ecosystem on Earth. Marine fungi, however, have been largely neglected for a long time (Gonçalves *et al.*, 2022). The first definition of “marine fungi” was proposed by Johnson and Sparrow (1961) based on their physiological characteristics, such as the requirement of high salinity for growth (>30‰). Their definition was further developed by Kohlmeyer and Kohlmeyer (1979) restricting “marine fungi” to two ecological groups: obligate and facultative marine fungi. Obligate marine fungi grow and sporulate only in marine or estuarine environments, while facultative marine fungi are mainly freshwater or terrestrial taxa but are halotolerant or halophilic. As

fungi isolated from marine samples cannot be easily classified as marine obligate or facultative fungi, the terms "marine-derived fungi" (Christophersen *et al.*, 1998; Bonugli-Santos *et al.*, 2015) and "marine (*sensu strictu*)" (Overy *et al.*, 2014) are widely used. Marine-derived fungi, refer to fungi isolated from marine environments, whereby marine "*sensu strictu*" refers to fungi whose complete life cycle occurs only in marine environments. These classifications, however, only convey about the fungi-obtained source, and fail to indicate their ecological significance (Pang *et al.*, 2016). Subsequently, a broader definition proposed by Pang *et al.* (2016) suggested that marine fungi should include all repeatedly recovered fungi that can grow, sporulate, develop symbiotic relationships with other organisms, adapt, evolve, or be metabolically active in the marine environment. Recently, Grossart *et al.* (2019) proposed that aquatic fungi rely on aquatic habitats for the whole or part of their life cycle. Based on their degree of adaption, activity, and dependence on aquatic environments, aquatic fungi are classified into three categories: indwellers, periodic immigrants, and versatile immigrants. Indwellers are well adapted and constantly active in aquatic habitats; periodic immigrants are less adapted to and only periodically active in aquatic habitats; versatile immigrants are the least adapted to and only sporadically active in aquatic habitats. In this thesis, the term "marine fungi" refers to fungi which inhabit parts or whole of their life cycle in marine environments.

Currently, there are only 1380 classified marine fungal species (7 phyla) at WoRMS (World Register of Marine Species, an online resource with a comprehensive list of names of marine organisms, accessed 2022.09.21) (Jones *et al.*, 2019). This is not surprising given the very limited research on marine fungi compared to their terrestrial counterparts (Bärlocher and Boddy, 2016).

1.1.1 Morphological diversity and structure of the fungal communities

Prior to the molecular era, the classification of all aquatic fungi taxa was based on morphological characterizations (Gautam *et al.*, 2022)), such as mycelium structure, spore pigmentation, size and shape, cape, stipe and lamellae (Agerer, 2001; Chethana *et al.*, 2021). They comprise a hyper-diverse composition ranging from completely unicellular monopolar organisms to highly complex syncytial filaments that may form macroscopic structures (Spatafora *et al.*, 2017). These structures vary under different environmental conditions, indicating the remarkably adaptive capabilities of the fungal kingdom (Naranjo-Ortiz and Gabaldon, 2020). Although the entire fungal kingdom possesses complex morphological characteristics, it can be broadly classified into three categories: unicellular fungi,

multicellular fungi, and dimorphic fungi (Fig. 1).

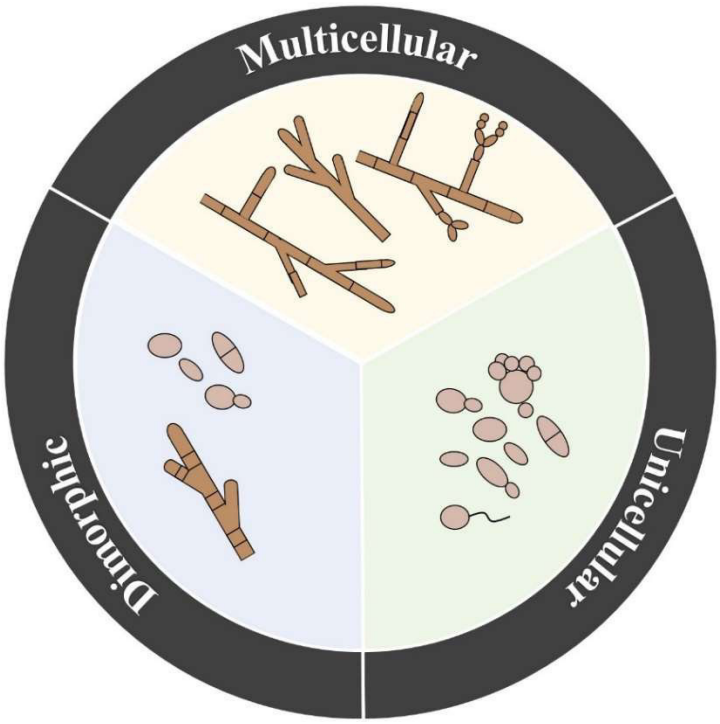

Figure 1. Cellular morphologies of the fungal community. This figure is made according to the growth morphologies of dimorphic fungi by Boyce and Andrianopoulos (2015) with modification.

1.1.1.1 Unicellular fungi

Unicellular fungi comprise two different types: zoosporic fungi and yeasts, neither of which is a taxonomic unit, but rather a fungal morphologic form shared by many unrelated lineages (Boekhout *et al.*, 2021). Compared to multicellular form, unicellular fungi are characterized by small size with a high surface/volume ratio, which lowers their energetic expenditure for osmotic balance. These characteristics provide advantages in high-osmotic stress and oligotrophic environments, where unicellular fungi are among the main microbial components (Naranjo-Ortiz and Gabaldon, 2019b; El Baidouri *et al.*, 2021).

Zoosporic fungi were identified based on producing motile spores using a single posterior flagellum for locomotion in at least one phase of their life cycle (Galindo *et al.*, 2021; Voigt *et al.*, 2021). Active motility of zoospores is one of the most important mechanisms of fungi in liquid phase dispersal (Gleason and Lilje, 2009) and migrating to find a new resource (Heaton *et al.*, 2020). This feature leads to the fact that zoosporic fungi can inhabit diverse aquatic ecosystems, from marine ecosystems to

freshwater systems and from tropical to arctic regions (Gleason *et al.*, 2011; Kilias *et al.*, 2020).

Yeasts are thought to be a reverse transition from multicellular back to a unicellular lifestyle through convergent evolution (Nagy *et al.*, 2014), and they form various lineages in the tree of the whole fungal kingdom (Boekhout *et al.*, 2021). It has been shown that yeasts occupy a wide variety of niches in many natural and anthropogenic aquatic ecosystems (Kutty and Philip, 2008; Boekhout *et al.*, 2021; El Baidouri *et al.*, 2021). Currently, 441 genera in *Ascomycota* and *Basidiomycota* are classified as yeast/dimorphic yeast form (Põlme *et al.*, 2021). They have compact genomes (Nagy *et al.*, 2020) that contain fewer intergenic contents and introns, suggesting that their regulatory networks are typically streamlined (Naranjo-Ortiz and Gabaldon, 2019b). This leads to a reduced ability to secrete hydrolytic enzymes, utilize mycelial growth to break into the substrate and produce complex secondary metabolites to regulate their surrounding environment (Nagy *et al.*, 2020). Thus, yeasts require close and sustained interactions with other microorganisms in environments (El Baidouri *et al.*, 2021). Concurrently, it also confers them considerable advantages to adapt to aquatic environments, where the supply of nutrients was considered mobile (El Baidouri *et al.*, 2021).

1.1.1.2 Multicellular fungi

The development of multicellularity in fungi is considered to have started with the loss of the flagellum in the ancestral unicellular fungi (Berbee *et al.*, 2017; Spatafora *et al.*, 2017; Heaton *et al.*, 2020). This multicellularity is a crucial step for their terrestrialization, i.e., adaptation to land environments (Naranjo-Ortiz and Gabaldon, 2019b).

Over evolution, multicellular fungi produced slender filaments called hyphae (singular hypha) that may or may not be septate during their development (Verrecchia, 2000), which can bring a bunch of advantages, e.g., increased size, complexity, and metabolic functioning (Berbee *et al.*, 2017). This enabled them to; (i) forage for much larger organisms by secreting hydrolytic enzymes (Rodriguez-Jasso *et al.*, 2010; Aydin *et al.*, 2017) and utilize mycelial growth to physically break into the substrate (Dollhofer *et al.*, 2015); (ii) start division of labors, or functional specialization, like germ-line and soma (Simpson, 2012); (iii) enhance stress resistance, like secreting extracellular matrix to control their surroundings (Lyons and Kolter, 2015); and (iv) escape from predators, like form stable colonies by self-replicating (Boraas *et al.*, 1998; Lyons and Kolter, 2015). Additionally, fungi are osmotrophic microbes, therefore whenever the resource in a living environment is immobile and difficult to digest, the

multicellular structure (hyphae) outperforms the unicellular structure (Heaton *et al.*, 2020). This outperformance is the result of continuous cytoplasmic transport of nutrients, which enables hydrolytic enzymes benefits for a longer period (Nagy *et al.*, 2020). Furthermore, the efficient space-searching strategy (Hanson *et al.*, 2006; Asenova *et al.*, 2016) formed by polarized growth and fractal branches of mycelium enables fungi to traverse soil intervals and nutritional plaques (Fricker *et al.*, 2017; Heaton *et al.*, 2020).

It is likely that with these considerable benefits of multicellularity, fungi can grow indefinitely and spread over enormous regions while remaining connected through the hyphal network; they can transport nutrients and metabolites through their mycelia and withstand severe environmental conditions (Groß-Schmölders *et al.*, 2020). Therefore, filamentous fungi have an advantage over other growth forms to become the dominant growth mode in sediment environments.

1.1.1.3 Dimorphic fungi

Dimorphic fungi are defined by the ability to switch between multicellular hyphal and unicellular yeast growth forms (Boyce and Andrianopoulos, 2015; Bahram and Netherway, 2021). It is a particularly powerful trait that enables fungi to respond to changes in environments and is essential for pathogenicity (Boyce and Andrianopoulos, 2015; Bahram and Netherway, 2021).

However, the current reports of strategies for dimorphism transition are based only on terrestrial fungal taxa. For example, thermophilic dimorphic fungal pathogens, such as *Histoplasma capsulatum*, *Blastomyces* species, *Sporothrix* species, etc., whose morphological transformation is temperature-dependent, acquire the capacity to convert from a hyphal form to a single-celled yeast form when infecting animal hosts (Li and Nielsen, 2017). Moreover, it is shown that *Mucor circinelloides*, whose morphological transformation is oxygen-dependent, usually grows as a mycelium under oxic conditions and as a budding yeast anaerobically (Orlowski, 1991). Apart from oxygen, the morphological transition between budding yeast and hyphal growth of *Candida albicans* is triggered by multiple environmental factors, such as temperature, pH, serum, and carbon dioxide (Li and Nielsen, 2017).

Although there are no studies applied to the dimorphism strategy of aquatic fungal taxa, dimorphic fungi can survive in many aquatic environments (Sampaio *et al.*, 2004). For dimorphic taxa, it was suggested that yeasts are saprotrophic forms that contribute to dispersion, whereas the filamentous stage has the

capacity to infect and explore the host's resources (Sampaio *et al.*, 2004). The morphologically diverse life cycles of dimorphic fungi likely enable them to better adapt to environmental stresses or changes in aquatic environments.

1.1.2 Phylogenetic diversity and structure of the fungal communities

Considering very limited differences among fungal taxa regrading morphological characteristics, as well as their morphological hysteresis and plasticity, it is problematic to classify fungi solely based on physiological indicators (morphology and internal structure). DNA sequencing (molecular method) with multiple maker genes, either individually or in combination, provides an important cutting-edge technology in exploring and classifying fungal communities (Nilsson *et al.*, 2019a). In addition, a complementary approach termed the "polyphasic taxonomic approach" was proposed by Das *et al.* (2014), suggesting that the combined morphological and molecular-based identification should be applied to get an accurate phylogenetic relationship.

The main molecular approaches for fungal identification are either based on sequence similarity or phylogenetic trees (Reich and Labes, 2017). Initially, the internal transcribed spacer (ITS) region was announced as the official fungal classification barcode based on the comparison of sequence similarity among sequence reads (Schoch *et al.*, 2012). However, given their higher divergence rates and untrustworthy alignments, it is tricky to obtain a high taxonomic resolution based on ITS sequences from aquatic fungi (Reich and Labes, 2017). With such shortcomings, many environmental sequences could only be classified at the fungal kingdom or phylum level (Nilsson *et al.*, 2016). Consequently, UNITE *(https://unite.ut.ee/)*, as the best-known ITS database, assigned digital object identifiers (DOIs) to identify the undescribed fungal taxa (Kõljalg *et al.*, 2016). On the other hand, phylogenetic placement allows the classification of unknown/novel fungal lineages too (Yarza *et al.*, 2017), such as basal fungi, which are dominant in aquatic environments (Panzer *et al.*, 2015). Therefore, the 18S rRNA (small subunits of ribosomal RNA) gene is commonly used in studies addressing aquatic fungi with environmental samples (Panzer *et al.*, 2015; Banos *et al.*, 2018). Phylogenetic exploitation of high-throughput sequencing (HTS) data has offered a lot of insights into the diversity and relationships of various fungal lineages (Gautam *et al.*, 2022).

The whole fungal kingdom possesses a high diversity of phylogeny and can be broadly divided into three categories: *Dikarya*, *Zygomycetous* fungi and *Zoosporic* fungi (Fig. 2).

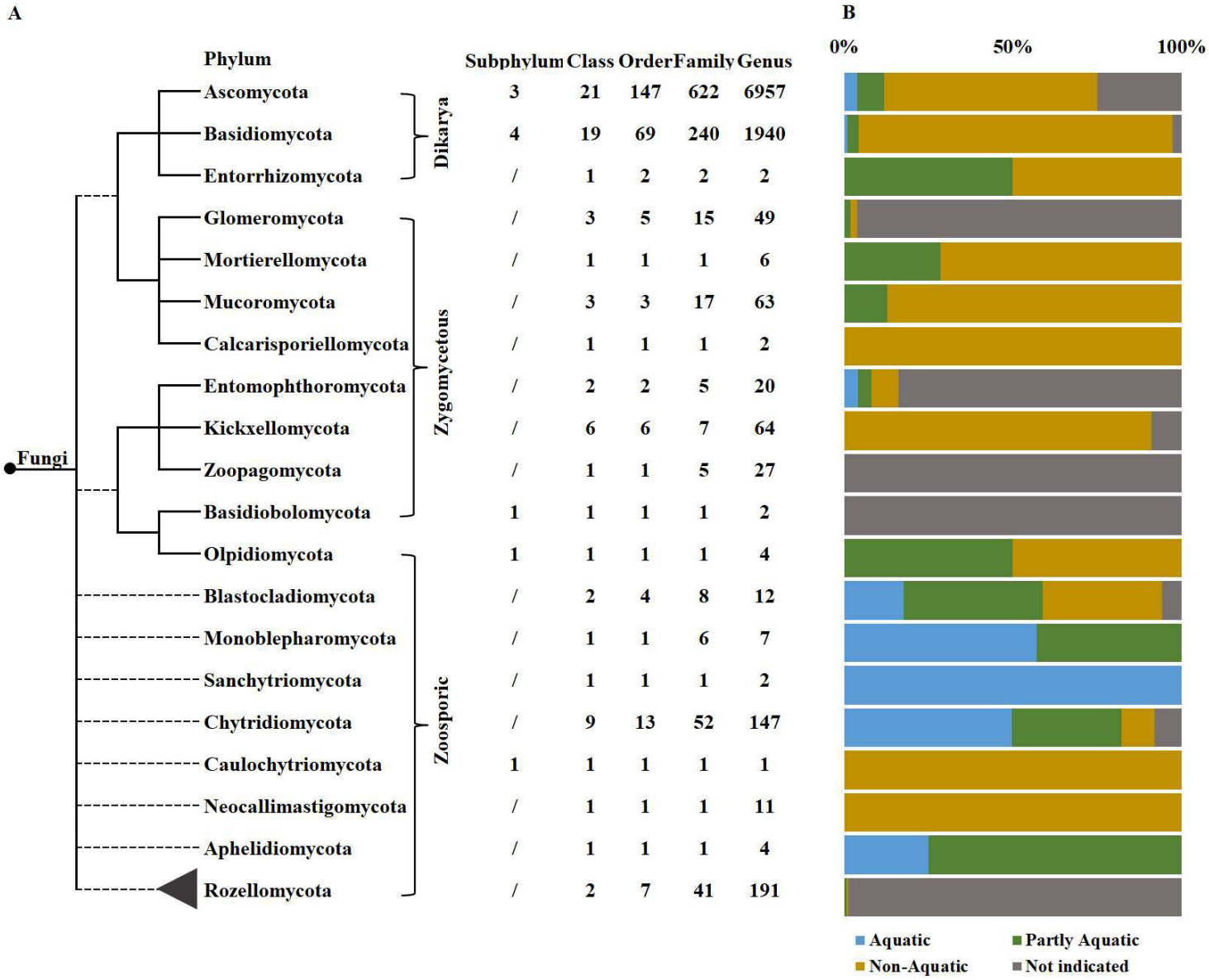

Figure 2. A. Sketch of a reference phylogenetic tree with all identified fungal groups based on four genes: SSU, LSU, RPB1 and RPB2, Solid line: bootstrap > 85, dotted line: bootstrap < 85. The number of subphyla, classes, orders, families, genera are collected from previous studies (Wijayawardene, 2020; Galindo et al., 2021);

B. Proportions of distribution for aquatic fungal at each phylum. Habitat distribution data were collected for identified genera from Põlme et al. (2021).

1.1.2.1 Dikarya

In the identified fungal Kingdom (Fig. 2), *Dikarya* is by far the most diverse and well-studied group (Wijayawardene, 2020). *Dikarya* is characterized by a sexual cycle that includes the fusion of hyaline bodies with meiotic decoupling, resulting in mycelium with two nuclear populations (dikaryotic mycelium). Most of them have septate hyphae and use ergosterol as a membrane sterol. Some lineages may even develop multicellular reproductive or vegetative structures (Naranjo-Ortiz and Gabaldon, 2019a). Notably, aquatic *Dikarya* do not produce fruiting bodies, and they usually have few macro characteristics to discern (Van den Wyngaert and Kagami, 2022). In total, *Dikarya* includes three phyla: *Ascomycota*, *Basidiomycota* and *Entorrhizomycota* (a tiny group of root endophytes). *Ascomycota* is known for its high diversity (Naranjo-Ortiz and Gabaldon, 2019a). They range from simple yeasts to very complex macroscopic fruiting bodies. Spores of *Ascomycota*, ascospores, are produced by an ascus, a sac-like structure formed by dikaryon hypha (Pöggeler *et al.*, 2006). *Basidiomycota* is distinguished by the formation of specialized club-like cells known as basidia, which typically yield four sexual spores. It is the most sophisticated fungi, in terms of cell cycle and multicellularity (Schmidt-Dannert, 2016). *Entorrhizomycota*, which was traditionally placed within *Ustilaginomycotina*, was proposed to be elevated to the phylum level by Bauer *et al.* (2015).

Currently, among the identified/accepted groups, only 154 genera of *Ascomycota* (filamentous) and 6 genera of *Basidiomycota* (filamentous and dimorphic) were defined from marine environments (Põlme *et al.*, 2021). However, it has been shown that *Dikarya* are the dominant fungal groups both in pelagic open oceans (Hassett *et al.*, 2020) and deep-sea oceans (Bass *et al.*, 2007; Xu *et al.*, 2018), and mainly in yeast form (Panzer *et al.*, 2015). Additionally, some *Dikarya* groups, such as *Pestalotiopsis* and *Cladosporium*, are frequently found in mangrove leaves (Raghukumar, 2004) or wood-associated environments (Fryar *et al.*, 2004; El-Sharouny *et al.*, 2009).

1.1.2.2 Zygomycetous fungi

Zygomycetous fungi can form true mycelia (coenocytic hyphae). It is generally assumed that the emergence of zygomycetous fungi is associated with the destruction of the fungal flagellum and the rise of filamentous terrestrial fungi (Kiss *et al.*, 2019; Chang *et al.*, 2022). According to some models, zygomycetous fungi are believed to be the most ancient terrestrial phyla that evolved from flagellated aquatic ancestors (Voigt *et al.*, 2021). Zygomycetous fungi are classed together based on evolutionary

affinity, yet there are virtually no morphological similarities between them. Most zygomycetous fungi are saprotophs or parasites of metazoans, amoebae, or other fungi, including highly specialized forms (Naranjo-Ortiz and Gabaldon, 2019b). Among them, *Mucoromycota* is the most studied and largest category of zygomycetous fungi, with most species existing as saprobes and parasites.

Although *Calcarisporiellomycota*, *Entomophthoromycota*, *Glomeromycota*, *Mortierellomycota*, and *Mucoromycota* are previously considered terrestrial lineages, aquatic lineages of these clades have been gradually uncovered (Fig. 2) (Riess *et al.*, 2018; Grossart *et al.*, 2019; Queiroz *et al.*, 2020; Põlme *et al.*, 2021). Some lineages of zygomycetous fungi are thought to be a diverse ecological group that lives parasitically or mutualistically in the digestive tract of aquatic arthropods (Lichtwardt, 2012), or as a parasite of amoebae (Corsaro *et al.*, 2018) and algae (Jones *et al.*, 2014). A weekly sampling study of pelagic marine mycoplankton communities by Banos *et al.* (2020), showed that *Mucoromycota* was the dominant group at one summer-point in the year. In the same year, Queiroz *et al.* (2020) indicated the presence of highly diverse *Glomeromycota* in global aquatic ecosystems. Additionally, the presence of zygomycetous fungi with relatively less abundance was revealed from seawater(Richards *et al.*, 2015) and deep-sea sediments (Yang *et al.*, 2020).

1.1.2.3 Zoosporic fungi

Phylogenetic studies of zoosporic fungi showed that they diverged for a long term before the diversification of terrestrial fungi (Voigt *et al.*, 2021). However, the community composition of zoosporic fungi remains poorly known and their phylogenetic position is often uncertain (Galindo *et al.*, 2021; Voigt *et al.*, 2021). The definition of zoosporic fungi is mainly based on the ultrastructure analysis of their zoospores, which can use a single posterior flagellum for locomotion in at least one phase of their life cycle (Galindo *et al.*, 2021; Voigt *et al.*, 2021). It has been shown that by lacking chitinous cell walls (Gleason and Lilje, 2009; James and Berbee, 2012), zoospores mat be burst by unregulated osmosis (Carlile *et al.*, 2001) in marine ecosystems. Therefore, under higher osmotic pressure, zoospores must regulate water influx, encyst by retracting or shedding their flagella, and construct a chitinous cell wall, which usually occurs when adhered to a host (Carlile *et al.*, 2001; Letcher and Powell, 2018).

Based on multiple barcoding and meta-omic surveys, it was suggested that diverse zoosporic fungal groups can predominate over other fungi in freshwater (Panzer *et al.*, 2015). Many studies prove that zoosporic fungal parasites are associated with phytoplankton and zooplankton (Lepelletier *et al.*, 2014;

Frenken *et al.*, 2017). Additionally, numerous zooporic fungi have been found on microplastic debris too. They interact with attached bacteria, and render nutrients available to other organisms (Kettner *et al.*, 2019). Besides using large amounts of organic matter derived by phytoplankton (Schöl *et al.*, 2014), zoospores can also use endogenous food reserves (Carlile *et al.*, 2001; Picard *et al.*, 2009). Therefore, zoosporic fungi are dominant in environments with low concentrations of phytoplankton.

Chytridiomycota, known as parasites and saprotrophs of phytoplankton (Frenken *et al.*, 2017), are associated with extremely dense algae blooms in aquatic environments (Hassett and Gradinger, 2016). It is worth mentioning that *Chytridiomycota* sustains a high abundance even two months after the algae bloom studies, and changes from attached to a free-living lifestyle at the end of the algae bloom (Gutierrez *et al.*, 2016). Regarding another important zoosporic fungal taxon, *Rozellomycota* is the main parasite of *Chytridiomycota* (Gleason *et al.*, 2012) and phytoplankton (the green alga *Coleochaete* (*Charophyta*)) (Letcher and Powell, 2018). This newly classified phylum is believed to be as large and diverse as the rest of the whole fungal kingdom (Jones *et al.*, 2011a; Jones *et al.*, 2011b; Corsaro *et al.*, 2014). Previously, it is reported that 0.02-4.5% of the total 18S rDNA sequences were identified as *Rozellomycota* in the aquatic ecosystem (Livermore and Mattes, 2013). Recent studies state that *Rozellomycota* is dominant and abundant in lakes with low temperatures (Rojas-Jimenez *et al.*, 2017; Lepère *et al.*, 2019). Lepère *et al*. (2019) have discussed the role of *Rozellomycota* in freshwater environments and shown that it is estimated as the most diverse group of fungi. For example, over 17% of the *Rozellomycota* operational taxonomic units (OTUs) are found in freshwater lakes and accounted for a large proportion of the total rRNA reads.

1.1.2.4 Fungi *incertae sedis*

Using cultivation approaches, multiple species of filamentous fungi and yeasts have been isolated. However, recent advances in molecular tools reveal that there is still a large fraction of uncultivated fungal taxa inhabiting aquatic ecosystems. The shift from culture-based studies to environmental DNA (eDNA)-based investigations has greatly advanced our understanding of the diversity and distribution of aquatic fungi but also created unexpected challenges. To define this great diversity of undescribed fungi revealed by molecular analysis of eDNA samples, the term “Dark matter fungi” (DMF) was proposed by Grossart *et al.* (2016). DMF are likely to be abundant across the entire fungal kingdom. At high taxonomic rank, marine and aquatic fungi have been shown to contain numerous novel and

undescribed taxa (Manohar and Raghukumar, 2013). However, considering this high proportion of unannotated sequences in environmental samples (Reich and Labes, 2017), conducting target statistics without classification is particularly problematic. As described in section 1.1.2, there are currently two approaches for classifying the unknown/novel fungal sequences. Compared to DOIs system in UNITE, the phylogenetic approach has additional advantages in terms of detection, grouping and classification (Yarza *et al.*, 2017). Therefore, reliable phylogenetic reference trees are needed for aquatic/marine mycology.

The documentation of DMF in the early divergent branches of the fungal tree expands our knowledge of the phylogeny and ecology of this group. *Rozellomycota*, a highly phylogenetic diverse group, can be found in a large range of ecosystems and is almost exclusively known through environmental sequences (Livermore and Mattes, 2013; Tedersoo *et al.*, 2017). However, enhanced cultivation of fungal strains for phylogenetic and physiological characterization is critical, as it allows for reliable linkage of phylogenetic, morphological, and physiological characteristics (Grossart *et al.*, 2019).

1.1.3 Diversity of fungal trophic modes

Fungi exist in a variety of lifestyles, including as saprotrophs, pathotrophs and symbiotrophs (Fig. 3) (Nguyen *et al.*, 2016). They metabolize organic material derived from animals, plants, algae, bacteria and other fungi. However, in many species, the trophic modes can be highly variable, including shifts between symbiotrophic, pathogenic and saprotrophic strategies (Fig. 3) (Põlme *et al.*, 2021). The study of fungal characteristic connections can reveal potential evolutionary or physiological tradeoffs that determine functional guild responses to environmental stressors (Zanne *et al.*, 2020; Põlme *et al.*, 2021).

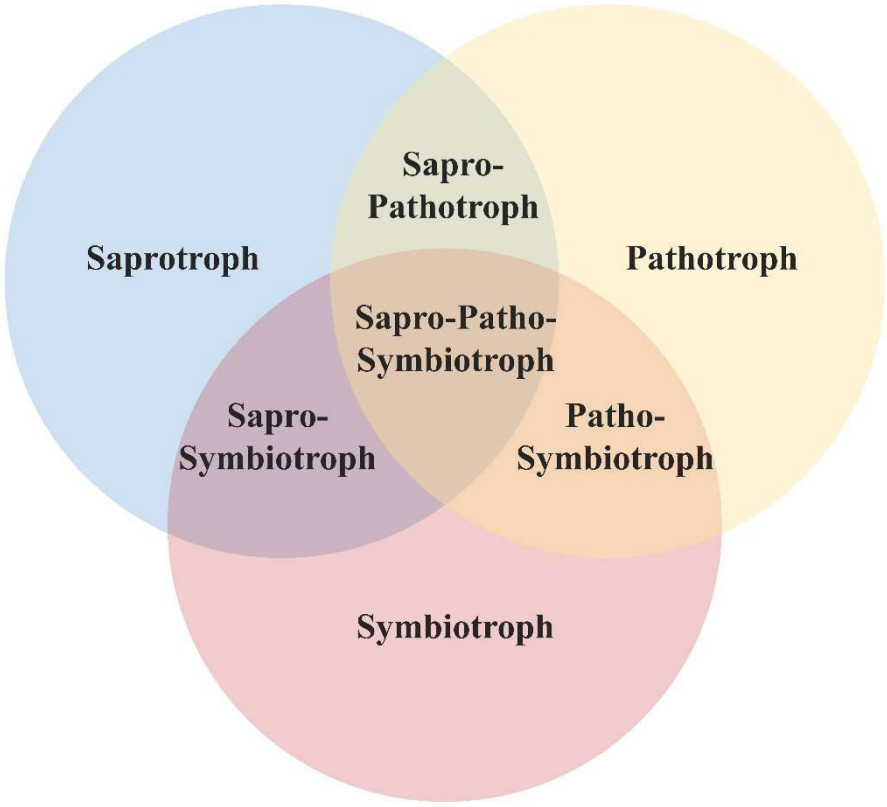

Figure 3. The trophic modes of fungal communities from FUNGuild (Nguyen et al., 2016).

1.1.3.1 Saprotroph

Fungi are known as decomposers of organic matter, which makes them essential for the cycling of carbon and other nutrients. The evolution of fungal decomposers involves not only the improvement of colonization and penetration capacities, but also the creation of novel routes to disrupt resistant structural components of intractable organic matter (Naranjo-Ortiz and Gabaldon, 2019b). In terrestrial ecosystems, saprotrophic fungi are well known as the primary degraders of organic matter and as key regulators of nutrient cycles (van der Wal *et al.*, 2013), which is also beginning to be evidenced in aquatic environments (Amend *et al.*, 2019; Gladfelter *et al.*, 2019; Grossart *et al.*, 2019). This conclusion is supported by multiple meta-transcriptome analyses of the marine fungal community (Chrismas and Cunliffe, 2020; Baltar *et al.*, 2021; Orsi *et al.*, 2022). Similar to their terrestrial counterparts, aquatic fungi have also been shown to harbor diverse carbohydrate-active enzymes (CAZys) repertoires, which are also actively expressed (Chrismas and Cunliffe, 2020; Baltar *et al.*, 2021; Orsi *et al.*, 2022). Therefore, these fungi are biochemical engineers, breaking down considerable amounts of organic carbon in aquatic environments into simpler biomolecules that are then assimilated into cells (Raghukumar, 2017).

Recently, Orsi *et al.* (2022) suggestted that marine fungi play relevant roles in the cycling of marine organic matter in the water column and sediments similar to those of prokaryotes. In heterogeneous mangrove sediments, saprotrophic fungi act as keystone species and control the overall microbial community structure through a synergistic interaction with other biological factors, such as bacteria and archaea (Marie Booth *et al.*, 2019). They are in charge of converting polymeric compounds into dissolved or particulate organic matter that may be used by other consumers in the food web (Richards *et al.*, 2012), such as lysine and methionine, and sterols (an crucial precursor for the production of cholesterol in marine animals) as well as numerous vitamins (Phillips, 1984). Furthermore, saprotrophic fungi would likely attach to organic and synthetic particles, such as plastic, since they can readily assimilate the carbon from these particles (Bochdansky *et al.*, 2017; Lacerda *et al.*, 2020). It was reported that in marine plastics, primarily saprotrophic fungal groups have accumulated in highly variable phylogenetic assemblages (Lacerda *et al.*, 2020).

1.1.3.2 Pathotroph

Pathotrophic fungi are those that can cause diseases when obtaining nutrients from their host (Zeilinger *et al.*, 2016). They follow a necrotrophic rather than biotrophic strategy (Zanne *et al.*, 2020). Their genomes include high copy numbers of genes coding for chitinases, phosphate transporters, polyketide synthases, and effector proteins that induce virulence, as well as CAZys (such as the hemicellulose-degrading β-xylosidase enzyme) (Ohm *et al.*, 2012; Lange *et al.*, 2019). However, from an evolutionary perspective, some pathotrophic fungi have evolved to become obligate parasites that do not produce toxins but often secrete effectors to suppress the host immune system (Zeilinger *et al.*, 2016). Parasitism is one of nature's most common ecological interactions, exisiting in practically every aquatic ecosystem (Ilicic and Grossart, 2022). Parasitic fungi play a significant role in shaping pelagic food web structure, facilitating energy transfer, and controlling disease (Kagami *et al.*, 2007).

As a consequence, multiple organisms could be attacked by fungi in marine ecosystems, such as animals, zooplankton, phytoplankton, other fungi, and even large bacteria. Numerous *Ascomycota* and *Basidiomycota* are known to be pathogens of multiple organisms in marine systems (Zanne *et al.*, 2020; Pang *et al.*, 2021; Põlme *et al.*, 2021). Among them, the most representative ones are *Cladosporium* (*Ascomycota*) and *Malassezia* (*Basidiomycota*). *Cladosporium*, known as the pathogen of phytoplankton (Cooley *et al.*, 2019), was shown to utilize polysaccharides with extracellular β-glucosidase (Cunliffe *et*

al., 2017). *Malassezia*, as a widespread ocean generalist, includes a range of trophic strategies from saprophytic to biotrophic, and could be opportunistic pathogens of coral and sponge (Amend, 2014). Some zoosporic fungi are known as pathogens too. They breakdown various substrates, including chitin, cellulose, and keratin, as well as some of the most resistant compounds, including lignin and sporopollenin, by releasing large numbers of extracellular enzymes (Lange *et al.*, 2019). It was reported that *Aphelidiomycota*, *Chytridiomycota* and *Rozellomycota* may be the most common parasites of microfauna, protozoans and algae in aquatic ecosystems (Grossart *et al.*, 2019). For example, chytrids can utilize phytoplankton organic matter efficiently, which thereby can influence the quality and quantity of phytoplakton, and feeds back to higher trophic levels, such as zooplankton (Kagami *et al.*, 2014). In general fungi and bacteria appear to be more antagonistic. However, it was shown that chytrid fungi can parasitize *Achromatium oxaliferum*, the largest freshwater heterotrophic bacterium (Schorn and Cypionka, 2018). Another well-known fungal pathogenic group, *Rozellomycota*, represents the intracellular parasites of fungi (Gleason *et al.*, 2012), phytoplankton (Letcher and Powell, 2018) and amoebae (Corsaro *et al.*, 2014). However, it remains poorly characterized.

Recently, the ecological potential of parasitic fungi in marine ecosystems has been discussed, suggesting that their ecological role is more important than previously considered (Ilicic and Grossart, 2022). However, the diversity of parasitic fungi, their ecological activities, and their interactions with other microbes are yet unexplored.

1.1.3.3 Symbiotroph

Symbiotrophic fungi obtain nutrients by exchanging resources with hosts (Nguyen *et al.*, 2016; Wutkowska *et al.*, 2021). Some fungi have evolved into successful symbionts in natural environments (Zeilinger *et al.*, 2016). They live in symbiotic associations with plants, algae, animals and other organisms (Naranjo-Ortiz and Gabaldon, 2019b). However, most of the current reports on synthetic interactions between fungi and other organisms have been conducted for terrestrial fungi. Varieties of host–fungal symbiotic interactions exist, such as mycorrhizal, endophytic and lichenized fungi (Bahram and Netherway, 2021). Endophytic fungi have been found to provide several benefits for the physiology and functioning of their hosts (Peay *et al.*, 2016), such as protection against pathogens (Arnold *et al.*, 2003). Mycorrhizal fungi are responsible for facilitating plant nutrient and water uptake in exchange for photosynthetic carbon from the host (Zeilinger *et al.*, 2016), as well as protecting plants against

pathogens, herbivores, and several abiotic stressors (Smith and Read, 2010). Along the co-evolution between fungi and their host, most mycorrhizal fungi have lost the capability to produce certain types of CAZys associated with lignin degradation (Kohler *et al.*, 2015). Additionally, fossilized microbial consortia of fungi and prokaryotes are found in the submarine igneous crust, suggesting that fungi can establish symbiotic relationships with chemoautotrophic prokaryotes (Bengtson *et al.*, 2014; Ivarsson *et al.*, 2016).

However, there are few reports of symbiotic interactions between fungi and other organisms in aquatic ecosystems. Recently, a co-culture experiment of *Mortierella elongata* (a terrestrial fungal taxa, *Mucoromycota*) and *Nannochloropsis oceanica* (marine algae) revealed their mutualistic interactions (Du *et al.*, 2019). This suggests the possibility of mutualistic interactions between phytoplankton and fungi in marine environments. Thereby, the presence of symbiotrophic fungi and their potential ecological roles are there to-be-explored.

Box 1: Obstacles for targeting the fungal diversity

- The lack of well-established reference databases, inaccurate fungal morphological identification methods, and non-specific molecular markers are hindering the resolution of fungal community analysis.
- Regarding a phylogeny-based approach, a challenge to resolve relationships within the fungal phylogenetic tree arises due to missing data on the main branch of the tree.
- Metagenomic approaches combining taxonomic, phylogenetic, and functional diversity in coordinated surveys are needed to better decipher the diversity patterns of these fungi and their functional roles at a global scale.

1.2 Why & what do we find …

A central topic in microbial ecology is to understand the processes that govern the assembly of communities and the dynamics of populations over space and time (Suárez-Castro *et al.*, 2022). It was already shown that fungal communities can be affected by multiple factors, e.g., geographic distance, abiotic/biotic factors, habitat specialization or fungal traits. However, we still lack a comprehensive understanding of the forces that shape the aquatic fungal communities in both spatial and temporal scales (Fig. 4); and how do they affect the structure and distribution of the fungal community?

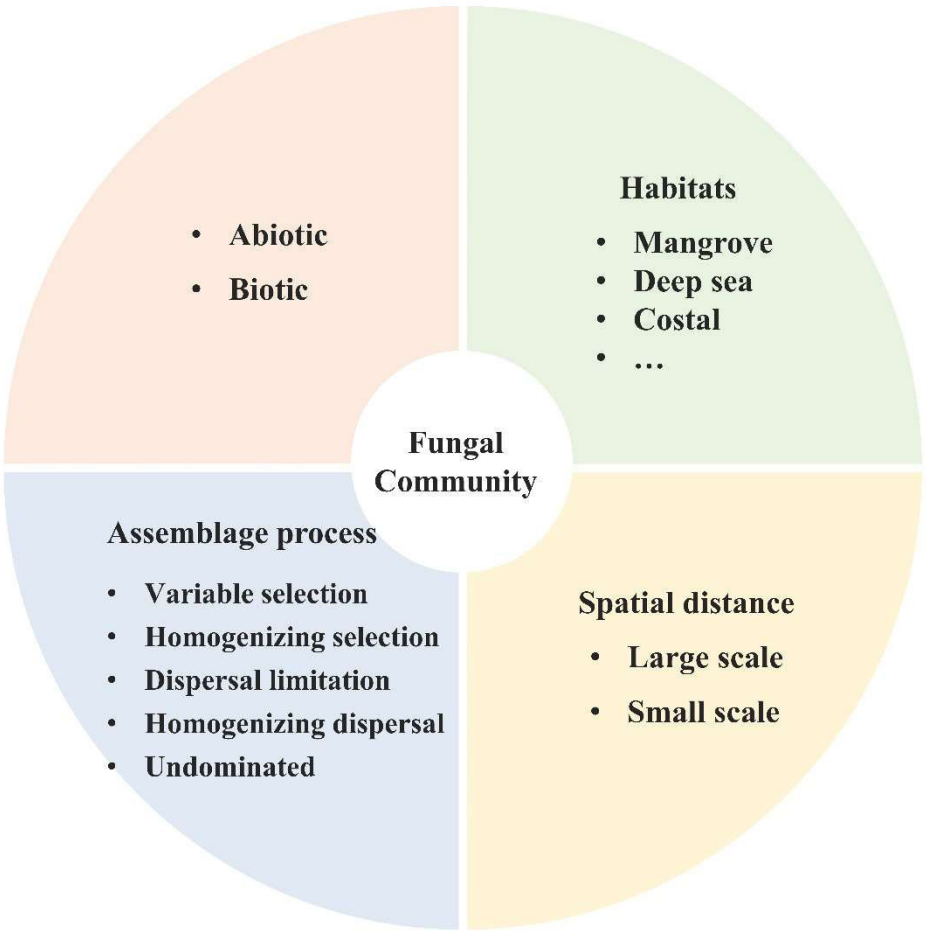

Figure 4. Driving factors of fungal community

1.2.1 Metadata analysis

It has been shown that numerous biotic and abiotic factors, such as variable biological, chemical, and physical elements, including the availability of substrata for colonization, the type of the substratum, and interference competition, affect the composition and distribution of the aquatic fungal community (Jones, 2011).

1.2.1.1 Abiotic factors

Abiotic factors refer to all non-living chemical or physical factors in the environment. Given the high diversity of fungal taxa within a community, it is likely that fungal taxa differ in their ability to take up nutritional resources (e.g., carbon, nitrogen, and phosphorus) and in their tolerance to chemical or physical pressures. Therefore, oscillations in abiotic factors can affect the structure and function of aquatic fungal communities at multiple organizational scales (Boyd *et al.*, 2014).

The availability of nutrients is known to play a major role in shaping fungal communities (Shearer *et al.*, 2007). Fungal biomass is mainly relevant to total nutrient content (Liu *et al.*, 2015). Multiple studies have shown that nutrients such as dissolved organic carbon, total nitrogen, nitrate, phosphate, silicate, and sulfide, are important environmental drivers of fungal communities (Taylor and Cunliffe, 2016; Tisthammer *et al.*, 2016; Rojas-Jimenez *et al.*, 2019). In the aquatic area, a number of chemical and physical factors have been shown to influence fungal communities, such as pH, salinity, temperature, depth and oxygen (Taylor and Cunliffe, 2016; Tisthammer *et al.*, 2016; Reich *et al.*, 2017; Cheung *et al.*, 2018; Tian *et al.*, 2018; Morales *et al.*, 2019; Rojas-Jimenez *et al.*, 2019; Banos *et al.*, 2020; Carter *et al.*, 2020). Additionally, abiotic factors contribute to the variation of fungal morphology and their trophic guilds (Zhao *et al.*, 2019). For example, temperature and oxygen can cause the morphological transformation of dimorphic fungi ,which in turn triggers their transformation between saprophytic and pathogenic forms (Li and Nielsen, 2017).

Therefore, to summarize, the diversity, composition and structure of aquatic fungal communities are significantly associated with abiotic factors.

1.2.1.2 Biotic factors

Biotic factors incorporate all the actions or activities of living organisms within an environment. There are multiple biotic interactions between aquatic fungi and other microbes, such as zooplankton and phytoplankton. These interactions can be positive or negative, and direct or indirect, and play an important role in shaping the fungal community structure (Banos *et al.*, 2020).

More specifically, different species of corals and sponges have significant effects on different fungal communities (Amend *et al.*, 2012; Nguyen and Thomas, 2018). Host algae will regulate the composition of the fungal populations associated with them by producing antifungal compounds (Kubanek *et al.*,

2003). Community investigation of root-associated fungi shows that it depends on host associations rather than other biotic and abiotic factors (Wutkowska *et al.*, 2021). Fungi and bacteria are found living together in a wide variety of environments, i.e., endofungal bacteria, which can play a key role in modulating the basic biology of their host fungi (Deveau *et al.*, 2018). A study in costal sediments has shown that interkingdom biotic fators are also vital in shaping the fungal community structure (Cheung *et al.*, 2018). These competitive and promotional interactions have also been proven using co-cultivation experiments with fungi and bacteria on leaves, to assess the functional performances and compositions of the fungal community. The results highlight the potential impact of fungal–fungal and fungal–bacterial interactions on the overall fungal community structure (Baudy *et al.*, 2021). Moreover, *Rozellomycota*, as the main parasite, manipulates the diversity of *Chytridiomycota* (Gleason *et al.*, 2012). Furthermore, fluctuations in the biotic interactions of aquatic fungi also reflect on their different fungal trophic patterns (Nguyen *et al.*, 2016) , which in turn affect their community structure. Like zoosporic fungi, they are key players in the marine ecosystem (Gleason *et al.*, 2011), since their trophic mechanisms function as a "bridge" between larger phytoplankton and zooplankton (Kagami *et al.*, 2007).

1.2.2 Habitats

Due to the high diversity and variabilities of taxa, fungi can colonize and adapt to different aquatic habitats, from freshwater, and brackish water to marine water, including pelagic and benthic sediments (Panzer *et al.*, 2015; Grossart *et al.*, 2019). The transition of microbial communities between habitat types is thought to be infrequent (Paver *et al.*, 2018). Therefore, as the natural environment of fungal taxa, habitat type is an important factor driving the composition and structure of fungal communities. Despite the close associations between fungi and the habitats, their distribution and role in the host and environment, as well as the cross-habitat determinants of their community composition, remain poorly studied (Bahram *et al.*, 2021).

1.2.2.1 Pelagic zone

The pelagic zone is a region of oceanic waters that are found throughout the oceans and at all depths. The regional and vertical distribution of pelagic fungi is shaped by several factors, such as the abundance of nutrients and dissolved oxygen, sunlight, water temperature, pressure, and salinity, etc (Morales *et al.*, 2019). Pelagic fungi possess an extremely diverse community and may have different niches, like cell-free or particle-attached.

Freshwater pelagic systems are known to harbor high organic carbon loads, due to influences of terrestrial and aquatic processes (Brett *et al.*, 2017). It has been suggested that freshwater fungi have the same capabilities as terrestrial fungi in metabolizing organic matter through biotransformation (Krauss *et al.*, 2011). Therefore, freshwater fungi can fragment litter, generating fine particulate organic matter (FPOM) (Witzgall *et al.*, 2021). Fungal assemblages in freshwater showed the highest diversity on the phylum level (Panzer *et al.*, 2015), and host a high number of uncharacterized groups (Lepère *et al.*, 2019). It was shown that *Chytridiomycota* predominate over other zoosporic fungi in freshwater (Panzer *et al.*, 2015). However, it has recently been shown that Rozellomycota are among the most diverse group of active fungi in freshwater ecosystems (Lepère *et al.*, 2019).

In the pelagic marine realm, yeasts of *Basidiomycota* and *Ascomycota* from the marine realm form a distinct cluster within the fungal phylogenetic tree (Panzer *et al.*, 2015; Priest *et al.*, 2021). However, it is suggested that marine fungi, as an ecological assemblage, include all classes of zoosporic chytrids, basidiomycetes and ascomycetes (Pang *et al.*, 2016; Hassett *et al.*, 2020). Among the marine fungal communities, lignicolous fungi are the best-studied groups. They are estimated to comprise 10% of all known marine fungi (Bugni and Ireland, 2004), and inhabit driftwood, trapped wood and test blocks/panels submerged in marine ecosystems (Garzoli *et al.*, 2015). Notably, a recent study by Orsi *et al.* (2022) shows that the facultative yeast *Malassezia* is the main fungal forager of pelagic diatom extracellular polymeric substances (dEPS).

Brackish water is an intermediary between freshwater and marine (Paver *et al.*, 2018), and a large progressive salinity raise zone. It can occur in estuaries and other transitory water bodies with limited exchanges of oceanic water, such as the Baltic Sea (Rojas-Jimenez *et al.*, 2019). There is a lack of available information about fungal communities in brackish ecosystems and the differences in their composition at various salinity levels. 8PSU (practical salinity units) was suggested as a threshold for fungal communities along a salinity decline, and below this threshold, *Chytridiomycota* was the dominant group.

1.2.2.2 Benthic zone

The benthic zone is a region of oceanic bottoms. The marine benthic sedimentary zones are the largest habitats on the planet. In addition to storing carbon over geological time, it performs climate-relevant functions. Fungi have been described as one of the most significant groups of microorganisms

responsible for organic matter degradation in sediments and peat areas (Marie Booth *et al.*, 2019; Retter *et al.*, 2019). With global research, a clear distinction between marine fungal communities in pelagic and benthic region was observed (Tisthammer *et al.*, 2016). The significant difference between mycoplankton and mycobenthos communities was also verified in gene expression (Orsi *et al.*, 2022).

Among multiple driving factors, such as altitude, mean annual temperature, C/N ration, dissolved organic carbon and total nitrogen etc., depth has a significant effect on benthic fungal communities in the coastal region (Tian *et al.*, 2018; Marie Booth *et al.*, 2019; Nagano *et al.*, 2020). However, this conflicts with the results of Rojas-Jimenez *et al.* (2020), in which depth had no significant effect. This may be explained by the different drivers of benthic fungal communities in coastal and deep-sea regions. Nevertheless, this also highlights the need for further understanding of the ecological role of fungi and their main drivers in benthic ecosystems.

Costal sediments are known to be highly dynamic systems and can shift between oxic and anoxic conditions. The bioturbation of benthic animals can intermittently transport oxygen to the deep anaerobic zone, directly or indirectly affect the changes in the microbial flora and cause the particles to migrate vertically (Papaspyrou *et al.*, 2005; Kristensen *et al.*, 2011). Therefore, costal sediments appear to harbor an unusual community of fungi that can adapt to saline-submerged habitats and alter their composition in oxic/anoxic conditions (Redou *et al.*, 2015; Lee *et al.*, 2019). The importance of fungal degradation in anoxic sediments has just recently been discovered (Marie Booth *et al.*, 2019). Among the coastal regions, differences in the fungal community exist between mangroves sediment and mudflat areas, even though both are dominated by *Ascomycota* (Cheung *et al.*, 2018). Similar differences have also been found by Picard (2017) between wetland sediments, intertidal sand and sediment core, where *Chytridiomycota* is also a dominant group. Additionally, *Chytridiomycota* is found to be seasonally dominating the arctic sea floor sediments (Hassett and Gradinger, 2016).

1.2.3 Spatial distance

The continuous development of metagenomic approaches has allowed the investigation of the diversity patterns of fungi and their functional roles at a global scale. Such studies are quite informative by expanding our understanding of the taxonomy, phylogeny, metabolic potential and functional diversity of fungi at different spatial scales (Tedersoo *et al.*, 2014; Tedersoo *et al.*, 2020; Tedersoo *et al.*, 2021b). The first global-scale analysis of marine fungal community structure was applied by Tisthammer *et al.*

(2016), which revealed a significant distinction between the pelagic and benthic fungal communities. Morales *et al.* (2019) proposed that at global scale, marine fungi contribute to multiple biogeochemical cycles in the planktonic ocean and may play an important role in ecosystem function by providing key nutrients. By combining over 600 HST datasets and billions of shotgun and amplicon sequences, Hassett *et al.* (2020) revealed the dominant groups of marine planktonic fungi from various oceanographic regions. The evidence suggests that the geographic pattern of microorganisms falls into at least two categories: endemic taxa and cosmopolitan taxa (Hanson *et al.*, 2012; Tedersoo *et al.*, 2014; Tedersoo *et al.*, 2022). Apart from metagenomics, distance decay relationship (DDR; the decrease in community similarity with increasing geographic distance) is one of the most common biogeographic patterns, also applicable to aquatic fungi (Tian *et al.*, 2018; Zhao *et al.*, 2019). As a function of geographic distance, longitude and latitude are proven to be the primary factors determining the variation of fungal community structure over large spatial scales (Liu *et al.*, 2015; Duarte *et al.*, 2016). Currently, there are already some thresholds defined as distance barriers for aquatic fungal communities, such as 50 km and 100 km as regional scales (Tian *et al.*, 2018) and 1000 km as larger barriers for the fungal community from wetland sediments (Wu *et al.*, 2013). Above 1000km, similar studies on aquatic bacteria have defined 3000km as spatial scale of their biodiversity distribution (Martiny *et al.*, 2006). However, there is a lack of available studies on larger distance barriers for global distribution patterns of aquatic fungi. Therefore, this also highlights the need for further understanding of the spatial scales of fungal global distribution in aquatic ecosystems.

1.2.4 Assemblage process

Along with the extensive discovery of biogeographic patterns in fungal communities, environmental factors exhibit different intensities of the driving force at different spatial scales. At local and regional scales, environmental variations mainly drive the changes in microbial community composition (Wu *et al.*, 2013); while at large scales, community similarity decreases primarily as a function of the geographic distance (Duarte *et al.*, 2016; Duarte *et al.*, 2017). That is, selection pressure increases or decreases along environmental gradients, so that the assemblage process of aquatic fungal communities cannot be explained by environmental selection alone and multiple factors must be considered in combination. Currently, there are four processes considered to drive microbial biogeographic patterns: selection, drift, dispersal and mutation (Hanson *et al.*, 2012). Stegen *et al.* (2015) extend a statistical framework, which can distinguish between five different assemblage processes, namely, variable

selection, homogenizing selection, dispersal limitation, homogenizing dispersal, and undominated process like ecological drift. This model allows the quantification of microbial community interplay to assess different assembly processes.

Natural selection can affect the fungal speciation over time, but also is the main factor for fungal diversification. It is the driving force behind the evolution of adaptive characteristics and is crucial in the production of phenotypic and genetic variety in fungal populations (Chethana *et al.*, 2021). Potential selection factors include all physical, chemical and biological characteristics of an organism's environment. Organismal dispersal refers to the active or passive movement of organisms through space from its natal/asexual reproductive site to a new one where it reproduces sexually or asexually (Tesson *et al.*, 2016). As a consequence of dispersal, population and ecosystem dynamics can be affected, and this may also exert an impact on evolution via subsequent adaptation to novel environments. Although fungal communities in general show a strong pattern of regional endemism and dispersal limitation, long-distance dispersal can occur over short time scale through the aerial movement of spores, or over longer time scale as species comigrate (Peay *et al.*, 2016). Geographic isolation affects aquatic fungi dispersal (Chethana *et al.*, 2021). However, from an evolutionary perspective, aquatic fungi have evolved different dispersal strategies: e.g. zoospores or amoebic spores, spore appendages, resistant spores, fragments of hyphae, the whole thallus, or as passenger on/in particles or host tissue, and with small body size which benefit much more than larger, multicellular organisms (Dayarathne *et al.*, 2020). Some fungal taxa can take the advantage of abiotic factors, such as wind, waterflow or thermal fluxes, to passively disperse (Tesson *et al.*, 2016). Ecological drift (i.e., stochastic changes in population sizes), a process occurring when selection is relatively weak and organisms rarely move between communities, causing taxon abundances to vary, and lowering diversity within communities and increasing differences among otherwise similar communities (Stegen *et al.*, 2015). A study of the fungal community in saline agricultural soils has revealed that the assembly process is mainly through stochastic (Zhao *et al.*, 2019).

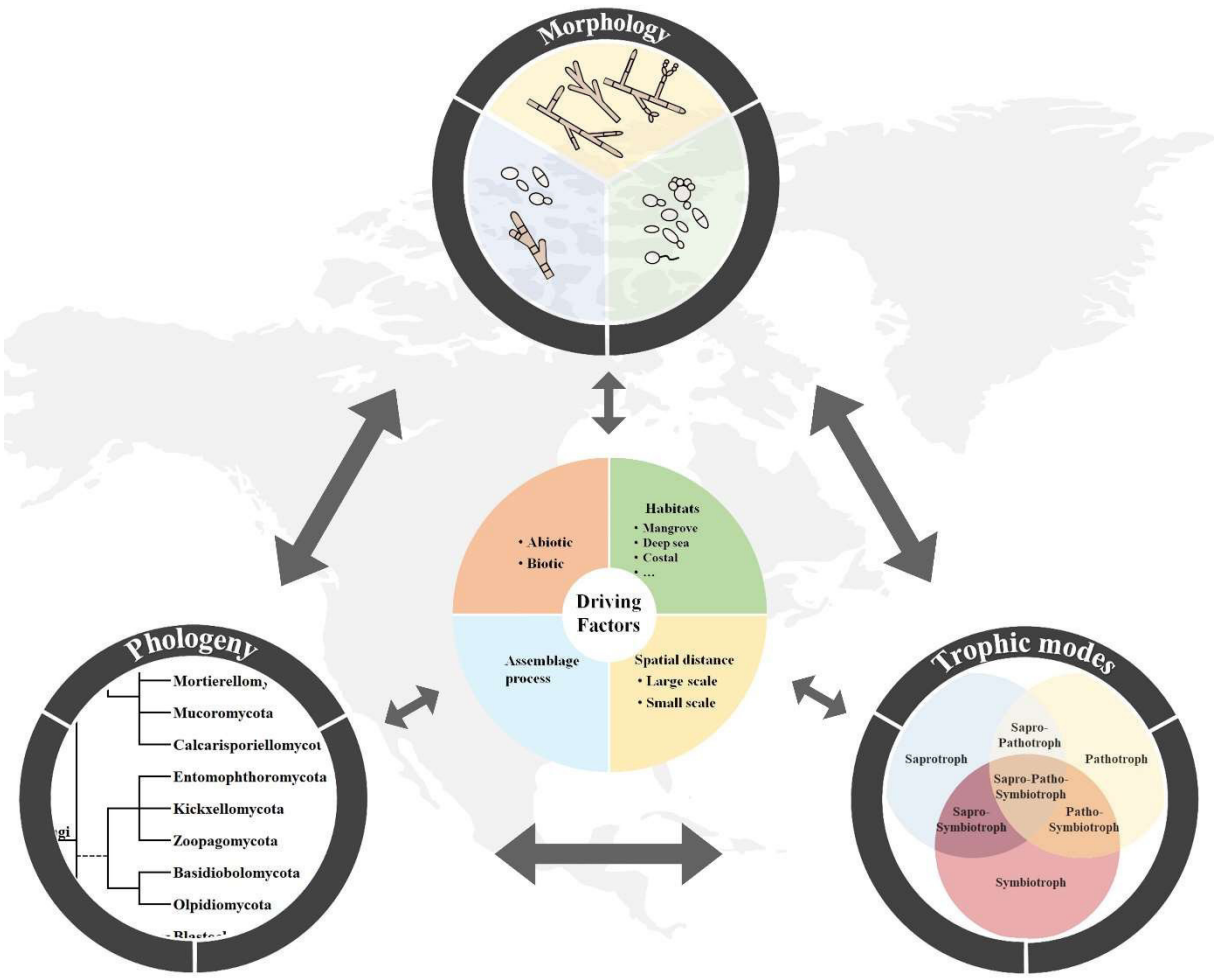

Figure 5. Proposed triangle of global fungal community diversity that is driven by multiple factors, and all links between them. This figure is made according to the triangle of fungal traits from Bahram and Netherway (2021) with modification.

Box 2: Obstacles for studying driving factors

- The complexity of fungal communities at the taxonomic and functional levels makes it difficult to find clear patterns linking community composition to ecosystem function and to understand the influence of biotic and abiotic factors on them (Fig. 5).
- A very important question that needs to be addressed is the global geographic distribution of marine benthic fungi, and their drivers.

1.3 Concept development of aquatic fungi

1.3.1 Why does concept development matter?

For understanding the integration of microbial communities in ecosystem functioning, concept development is essential. Through conceptual models, an explanation of what might happen in actual or predicted situations can be summarized, visualized and explained (Smith *et al.*, 2016), as well as contribute to developing our understanding of their biodiversity, abundance, distribution, and ecological importance (Amend *et al.*, 2019). There is growing evidence that fungi play a mediating role between different organisms and ecosystems, and that this role has the potential to influence the macroecology and evolution of these organisms. This suggests that fungal interactions are an ecological driver that interconnects different levels of biological and ecological organization of their hosts, competitors and antagonists with the environment and ecosystem function (Bahram and Netherway, 2021). Mycologists have been interested in deciphering the global distribution patterns of aquatic fungi to better understand global species richness, patterns of biodiversity, and the extent of cosmopolitan versus endemism. Therefore, in future studies, it is important to consider the contribution of biotic interactions to aquatic fungal communities as it helps to improve predictions of the fungal community composition and global distribution in aquatic ecosystems. Although awareness of the importance of fungi in a variety of aquatic ecosystems is gradually increasing, there is still a need for a conceptual framework to organize these studies, which are mainly focused on individual ecosystems (e.g., open seas, estuaries, rivers, and lakes).

1.3.2 Developed concepts

Azam *et al.* (1983) proposed the term “microbial loop”, referring to the pathway by which dissolved organic carbon, starting with bacteria, becomes available at higher trophic levels through incorporation into the marine food web. Subsequently, “ocean carbon pump”, also known as “biological carbon pump” (BCP), is defined as the biologically driven process by which the ocean sequesters carbon from atmospheric and terrestrial runoff into the ocean interior and seafloor sediments (Volk and Hoffert, 1985). Over a decade after the concept of microbial cycling has been introduced, viruses and their influence on carbon and nutrient cycling in photic waters are slowly being noticed. “Viral shunt” has been proposed by Wilhelm and Suttle (1999), referring to the reintroduction and recycling of the primary production of marine phytoplankton due to viral shunt.

It took almost another two decades before fungi were considered to be players in the microbial cycle (Fig. 6). “Mycoloop” was proposed by Kagami *et al.* (2007); it describes that nutrients from large inedible algae are transferred to zooplankton via the zoospores of parasitic chytrids. Grossart *et al.* (2019) integrated three conceptual models comprising the natural and artificial environments for aquatic fungi, and their ecological roles. Results show a diverse habitat for aquatic fungi, from high montane lentic habitats down to the deep open ocean, from human intervention in landscapes; and describe three major processes by which aquatic fungi transform and incorporate xenobiotic and autotrophic organic matter into the food web: mycoloop Kagami *et al.* (2007), mycoflux and benthic shunt. These conceptual frameworks have paved the way for a better understanding of the aquatic fungal community. “Mycoflux” was introduced by Grossart *et al.* (2019), and refers to currently unknown pelagic fungal interactions, their ecology and their effects on the aquatic carbon pump. “Benthic shunt” indicates a specific pathway, referring to the ability of fungi to colonize and degrade the benthic organic matter, and to transport it to higher trophic levels (Grossart *et al.*, 2019). Recently, the term “fungal shunt” is proposed by Klawonn *et al.* (2021), referring to the photosynthetic carbon transformant by fungal parasites to chytrid sporangia and their free-swimming zoospores. This process facilitates the carbon transfer to higher trophic levels, bypassing the microbial cycle and viral shunt. The ecological role of parasitic fungi in the biological carbon pump is well explained with “fungal shunt”, “mycoloop” and “mycoflux”(Ilicic and Grossart, 2022). However, in marine environments, “benthic shunt” has not been described, although a similar mechanism is expected. To uncover it, the global distribution analysis of benthic marine fungi was needed. The four major biochemical processes of fungal shunt, mycoloop, mycoflux, and benthic shunt draw a picture that aquatic fungi are an active, dynamic, and functional group in the whole aquatic ecosystem (Fig. 6).

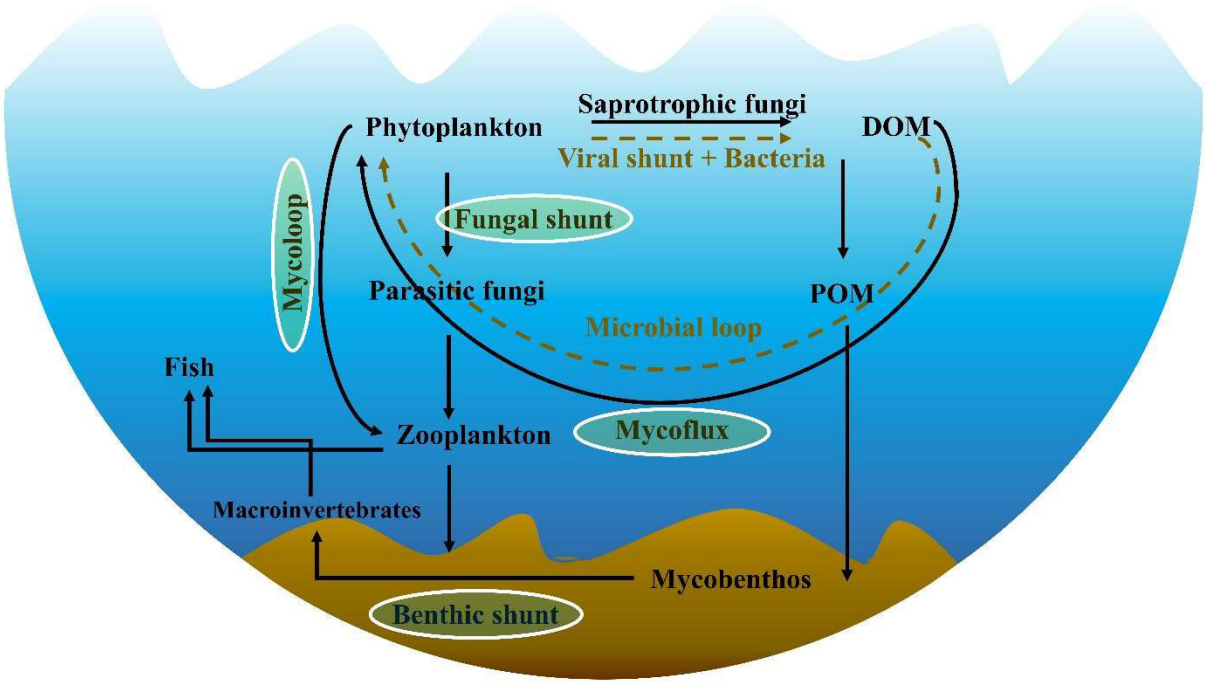

Figure 6. Fungi are key components of biological carbon pump. Four major biochemical processes in which aquatic fungi are involved, namely the fungal shunt, mycoloop, mycoflux, and benthic shunt. Combined information by Azam et al. (1983); Wilhelm and Suttle (1999); Kagami et al. (2007); Grossart et al. (2019); Ilicic and Grossart (2022).

Box 3: Obstacles to develop ecological concepts

- Conceptual framework on the global distribution analysis of benthic marine fungi is needed.
- Conceptual barriers which may arise due to concepts on terrestiral fungi must be overcome in the process of conducting research on marine fungi.

2 Aims and objectives

Aquatic fungal communities are highly complex assemblages on taxonomic, as well as on functional levels. They hold prominent positions in manifold ecological processes. Despite technical advances in sequencing methods and data integration, our understanding of the drivers and processes that lead to the place- and time-specific composition of a particular aquatic fungal community is still poor and lacks spatial resolution. Therefore, the overarching goal of my dissertation was to understand how aquatic fungal communities in (i) river and adjacent river plumes, and (ii) benthic systems are spatially resolved under the interaction of ecological processes or single to multiple environmental factors.

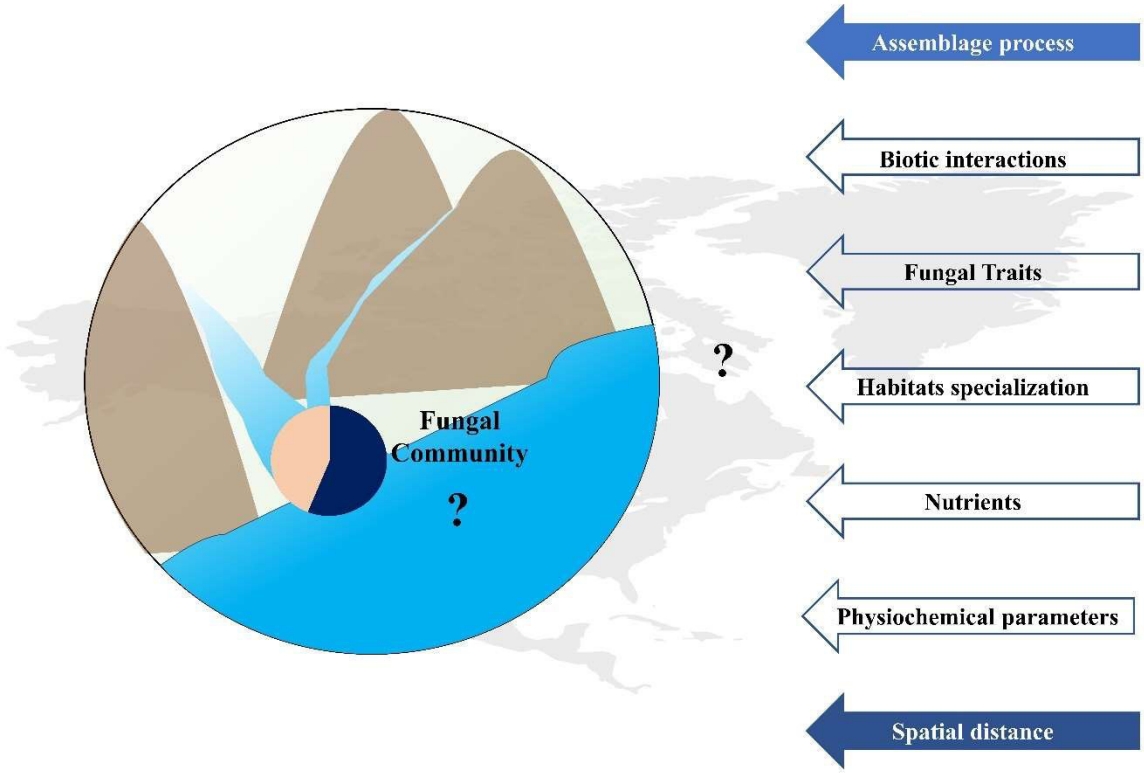

Figure 7. Aims and objectives of this thesis.

3 List of publications, manuscripts, and cooperation projects

3.1 Publications and manuscripts

Paper I

Mycoplankton biome structure and assemblage processes differ significantly along a transect from the shallow freshwater area of the Elbe River down to the river plume and the adjacent marine waters

Yanyan Yang, Stefanos Banos, Gunnar Gerdts, Antje Wichels, Marlis Reich

published in *Front. Microbiol*., (2021)

https://doi.org/10.3389/fmicb.2021.640469

Paper II

The biogeography of marine benthic fungi: limitations in distribution, spatial and deterministic factors, and impact of taxon-specific traits

Yanyan Yang, Rolf Nimzyk, Marlis Reich

(Currently revising the manuscript for resubmission to *Limnology and Oceanography Letters*)

Paper III

Effects of oxygen content on the mycobenthic community of coastal sediments

Yanyan Yang, Carmen Alicia Rivera Pérez, Tim Richter-Heitmann, Rolf Nimzyk, Michael Friedrich, Marlis Reich

(Manuscript prepared for submission to Scientific Reports)

3.2 Cooperation projects

As I have developed a high expertise in fungal community analysis and especially in taxonomic resolution of undescribed taxa, I contributed to several cooperation works related to fungal community analysis. All these projects are in the state of manuscript preparation.

Project I

The Plastisphere – Marine fungi communities in the plastics age

Inga V. Kirstein, Marlis Reich, Yanyan Yang, Maike Timmerman, Antje Wichels, Gunnar Gerdts

AWI Helgoland, Germany

Project II

Assessing the effect of salmon aquaculture on fungal diversity

Elmedina Husanovic, Kim Præbel, Marlis Reich, Marta Turon, Owen S Wangensteen, Teppo Rämä, Yanyan Yang etc. (alphabetical order)

UiT, Norway

Project III

Mycobiome in copepods

Ximena Dubinsky-Velasquez, Marlis Reich, Yanyan Yang, Tamar Guy-Haim etc.

IOLR, Israel

Declaration on the contribution of the candidate to a multi-author article/manuscript which is included as a chapter in the submitted doctoral thesis

Chapter: Paper I

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 15 %
Experimental work and/or acquisition of (experimental) data:	ca. 50 %
Data analysis and interpretation:	ca. 40 %
Preparation of Figures and Tables:	ca. 40 %
Drafting of the manuscript:	ca. 40 %

Chapter: Paper II

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 15 %
Experimental work and/or acquisition of (experimental) data:	ca. 80 %
Data analysis and interpretation:	ca. 60 %
Preparation of Figures and Tables:	ca. 90 %
Drafting of the manuscript:	ca. 50 %

Chapter: Paper III

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 15 %
Experimental work and/or acquisition of (experimental) data:	ca. 40 %
Data analysis and interpretation:	ca. 60 %
Preparation of Figures and Tables:	ca. 80 %
Drafting of the manuscript:	ca. 50 %

Date:

Signatures:

4 Chapters

4.1 Paper I

Mycoplankton biome structure and assemblage processes differ significantly along a transect from the shallow freshwater area of the Elbe River down to the river plume and the adjacent marine waters

Yanyan Yang, Stefanos Banos, Gunnar Gerdts, Antje Wichels, Marlis Reich

Front. Microbiol., (2021, Apr)

https://doi.org/10.3389/fmicb.2021.640469

4.2 Paper II

The biogeography of marine benthic fungi: limitations in distribution, spatial and deterministic factors, and effects of taxon-specific traits

Short title: Biogeographic patterns of marine benthic fungi

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Keywords: Biogeography, distance-decay, sediment, dispersal, traits

Abstract

Benthic fungi are involved in the marine carbon cycle through their decomposition activities. However, little is known about their distribution and drivers of local occurrence, limiting predictions of microbial functionality. Using a meta-analysis combining fungal-specific high-throughput sequencing (HTS) data from 213 samples, we aimed to determine fungal distribution patterns and factors influencing them such as habitat specialization and fungal traits.

Fungal taxon distribution was limited and geographic distance overlaid habitat specialization. Overall, four different distribution patterns were identified, of which the large-scale patterns (> 2,500 km) resembled those described for bacteria and microeukaryotes. Surprisingly, there was a significant reduction in fungal taxon distribution around the 100-km distance. Individual traits such as morphology and trophic mode had a small but significant effect on distribution, suggesting that the effects of individual species-specific traits may sum to a larger effect. Based on the results, we developed a first conceptual framework for the distribution of benthic fungi.

Scientific Significance Statement

Microbial community composition and its resulting activities have major consequences on ecological processes such as organic matter decomposition in the seafloor. Local community composition is a function of microbial distribution. Fungi, as prominent members of microbial communities, are involved in microbe-driven processes by taking on various roles, but little is known on their distribution patterns and their causes. We show that the distribution of benthic fungi is limited and probably controlled by a whole bouquet of factors in which geographic distance, habitat specialization but also traits related to life history have an influence on distribution. Based on the results we propose a new conceptual framework on the biogeography of benthic marine fungi.

1 Introduction

Biogeography is defined as the distribution of organisms over space and time. Because each species possesses an individual bouquet of functional traits, the composition of a local community of organisms has implications for functional ecosystem processes such as the marine carbon cycle. For example, the absence of a particular taxon may lead to partial degradation of a certain organic metabolite and/or its entry into long-term storage (Dittmar et al., 2021). The local occurrence of a taxon depends on various ecological processes including taxon-specific distribution, adaptability to local conditions, or organismic interactions (Stegen et al., 2012). Local distribution patterns add up to large scale distribution patterns with their own dynamics and influencing variables. Knowledge of these biogeographic patterns is the basis for understanding the relationship between local microbial composition and functional diversity and provides explanatory approaches to get to the bottom of functional differences in similar niches.

Benthic bacterial biogeography is well studied (Zinger et al., 2011). For example, their global distribution is strongly affected by ocean basins (Schauer et al., 2010). Furthermore, their distribution is characterized by taxon-specific habitat specialization (Wang et al., 2013). In contrast, benthic fungal biogeography is poorly understood, although they are actively involved in carbon turnover (Orsi et al., 2022). In an initial study by Tisthammer *et al.* (2016), it was shown that filamentous Ascomycota biogeographically dominate benthic fungal communities. This raises the question of the extent to which morphology influences distribution patterns. Fungi can take the form of unicellular organisms such as zoospores, amoeboid-like cells or yeasts as well as filamentous growth forms. The latter form in particular may be advantages for sediment life; for example, hyphae can grow three-dimensionally through the sediment in search of nutrients. Another factor affecting distribution patterns may be the trophic feeding mode of fungal taxa, especially since many fungi change this during their life cycle.

However, it is likely that both taxon-specific and environmental or spatial factors influence the occurrence of individual fungal taxa.

To study reliable distribution patterns at the molecular level, a marker gene must be chosen that resolves taxa down to the "species" level. For fungi, there are several marker genes that differ in their resolution across taxon groups (Reich and Labes, 2017). The one that allows species-level resolution for a wide variety of taxonomic groups is the internal transcribed spacer (ITS) (Schoch et al., 2012); however, it does not serve as a marker for other marine organisms. This limits the number of HTS datasets published to date, as eukaryotic datasets from global sampling projects are based on 18S rRNA gene sequences (Cordier et al., 2022).

Our goal was to provide an initial conceptual framework of benthic fungal distribution patterns and their influencing factors, considering also taxon-specific traits. To this end, we performed a meta-analysis of published ITS2-HTS datasets from 213 samples and asked the following questions: What are critical distances for the distribution of benthic fungi? What is the relationship between endemic and cosmopolitan species? Can spatial patterns dominate over deterministic factors such as habitat type? To what extent do characteristics such as morphology and trophic feeding mode influence distribution patterns?

2 Materials and Methods

2.1 Search query for suitable data sets for biogeographical analyses

We searched for HTS datasets from fungal surveys in marine sediments using the ITS2 published not later than January 2021. Therefore, we conducted a comprehensive literature research using the search engines like “Web of Science” (https://www.webofscience.com/wos/woscc/basic-search), “Google Scholar” (https://scholar.google.com/), or the database of European Nucleotide Archive (ENA). Finally, we assigned samples to different habitat types using the catalogue of Environmental Ontology (ENVO) (Buttigieg et al., 2016) (Supplementary Table 1).

2.2 Sequence analysis

We analyzed all sequences as one dataset using the PIPITS pipeline v2.5 (Gweon et al., 2015). This included quality filtering, processing and taxonomic classification based on the RDP Classifier v2.10 (Wang et al., 2007) using the UNITE (Nilsson et al., 2019) fungal ITS reference training data set V2 (https://sourceforge.net/projects/rdp-classifier/files/RDP_Classifier_TrainingData/). In a final step, we removed all OTUs from the final OTU table that were detected with less than 10 sequences and only in one sample.

Due to the high number of undescribed fungal OTUs in the final OTU table, we tried to improve the taxonomic classification of the most prominent OTUs by using additional classification options: BLASTn (Altschul et al., 1990) was performed on the UNITE webpage (accessed 04.04.2022) by enabling beside the UNITE-own database the INSD database. We evaluated results manually by including possible Species Hypothesis (SH) (Kõljalg et al., 2013) using a 3% distance between species threshold. We additionally used the secondary structure of the ITS2 via the web-based ITS2 Database (Schultz et al., 2006; Ankenbrand et al., 2015) (accessed 04.04.2022) applying the default settings.

We defined OTUs as cosmopolitan in the case that they were present in (i) at least 8 out of the 10 projects

and (ii) ≥ 55 % of samples of the whole dataset. Contrarily, we defined OTUs endemic in the case that they occurred only in < 5 % of all samples. The FUNGuild (Nguyen et al., 2016) tool was used to assign the trophic mode, growth morphology and ecological guild affiliation to single OTUs using the default parameters. In the case, where OTUs assigned to genus level could not be affiliated to guilds, we conducted another search on the datasheet of the FungalTrait database v1.2 (Polme et al., 2020).

2.3 Statistical analysis

We ran all statistical analyses within the R environment v4.1.2 (R Core Team, 2015) and if not differently stated with the *vegan* package (Oksanen et al., 2013). We tested significant dominance of a fungal morpho- and trophotype in sediments with Tukey´s post-hoc test (TukeyHSD) using the default settings in the core R package *stats*. Next, we normalized data with total standardization methods and computed Bray-Curtis dissimilarity matrix between pairs of samples. To test for a significant influence of geographic distance on community dissimilarity, we applied a Mantel test with Kendall´s rank correlation and 9,999 permutations. To identify potential patterns of fungal distribution, we calculated the proportion of shared OTUs between each possible sample pair and plotted against sample pair distance confirming a significant change with TukeyHSD. In addition, we calculated the proportion of different morpho- and trophic types among shared OTUs. Finally, we applied regression analysis to test for a spatial influence on the occurrence of specific morpho- and trophic types, by applying the function "lm".

To test the impact of habitat type and geographical distance on fungal dissimilarity, we ran variation partitioning analysis (VPA) with the “varpart” function. Prior to VPA, we calculated eigenfactors and – values to carry out a spatial eigenfunction using the “distance-based Moran’s eigenvector maps” (dbMEM) function of the package *adespatial* (Dray et al., 2017). Then, we tested eigenfunctions with a positive eigenvalue for significance ($P \leq .05$) by dbRDA-based forward selection function “ordistep”.

3 Results

3.1 Key figures of the composite data set

A total of 10 studies met our search criteria and included 213 independent samples (Fig. 1A; Supplementary Table 1). Sample locations ranged 108 latitudes and 174 longitudes. The distance between individual samples spanned from < 1 km to ~16,000 km. The combined dataset held 11,149 OTUs with 11.046,524 fungal ITS2-sequences. Fungal OTUs were divided across 14 phyla, of which Ascomycota dominated OTUs with 52% of relative sequence abundance. 31 % of OTUs could not be classified further than the kingdom level (Fig. 1B). Only six of all OTUs were classified as cosmopolitan represented by 8.9 % of all sequences and 0.05 % of all OTUs including two *Cladosporium* and one *Malassezia* OTU. 7,244 OTUs were defined as endemic OTUs (65% of OTUs and 25 % of all sequences) (Fig. 1C, E).

Using both the FUNGuilds and FungalTraits databases, classification of OTUs into ecological guilds was possible for 4,107 OTUs (37 % of all OTUs). Out of those, saprotrophic feeding mode was with 37 % of the OTUs significantly more represented than others (TukeyHSD, $P < .001$). Furthermore, 4,082 OTUs (37 %) could be grouped morphologically; the filamentous growth form dominated significantly with 3,471 of the classifiable OTUs (85 %) (TukeyHSD, $P < .001$), while yeast growth was reported only for 558 OTUs (14 %) (Fig. 1D, Supplementary Table 2).

3.2 Fungal distribution patterns on distant geographical scales

The Mantel test revealed a significant relation of increasing distance with increasing community dissimilarity ($P < .001$, $R^2 = 0.142$). Similarly, the average proportion of shared OTUs in two samples decreased significantly with increasing distance between samples from 45 % to 2 % at 10 km and 16,000 km, respectively (TukeyHSD, $P < .01$). Particularly striking decreases of the portion of shared OTUs was observed at the 100 km and 2,500 km marks with 20 % and 11 % loss, respectively, both being

significant. An exception to the observed trend was the percentage of shared OTUs at the local level < 1 km distance, which was 19 % lower than that from the 10 km mark and being also significant. Based on these results, fungal distribution patterns were classified into four scales with significant OTU turn-over: local (< 1 km), small (>1 km - 100 km), intermediate (>100 km - 2,500 km), and large (> 2,500 km) (Fig. 2A, Supplementary Table 3).

3.3 Factors impacting fungal distribution patterns

Distance-decay analyses identified a significant effect of morpho- and trophotype on taxon distribution (Fig. 2B; Supplementary Table 4). Thus, the percentage of shared OTUs with filamentous type increased significantly with distance ($R^2 = 0.15$, $P < .001$), whereas it decreased significantly with yeast form ($R^2 = 0.06$, $P < .001$) (Fig. 2B).

dbMEM identified 212 spatial eigenvectors, from which five were positive along Moran I. Forward selection attested all five positive eigenvectors having a significant impact on the fungal composition ($P \leq .05$) (Supplementary Table 5). VPA showed that spatial distance explained more of the observed variability than the habitat type with 11.3% under spatial control, 7.6% under habitat type and 5.2% shared between the two factors. 75.5% stayed unexplained (Fig. 2C).

4 Discussion

Here, we present a wide-scale study of the biogeography of marine benthic fungi and develop an initial conceptual framework by defining thresholds for distribution patterns and identifying scale-dependent factors. Although a limitation of biogeographic analyses is often non-uniform sampling, the number of samples in our study is greater than or equal to that for biogeographic studies of pelagic fungi conducted by Tisthammer *et al.* (2016) and Hassett *et al.* (2019), respectively. Furthermore, the patterns identified in our study are very consistent with those of bacteria and microeukaryotes (Schauer et al., 2010; Cordier et al., 2022). Therefore, we believe that our results provide a solid foundation on which new studies can be developed.

4.1 Large (> 2,500 km) and intermediate (< 2,500 km) scale distribution patterns

The separation of large and intermediate biogeographic scales of benthic fungi occurred at the 2,500 km mark. A similar value is given by Schauer *et al*. (2010) for benthic bacterial communities, who consider ocean basins as barriers to bacterial dispersal. Thus, a division of biogeographic scales into intermediate and large appears to have general validity for the dispersal of benthic microorganisms. Long-distance dispersal of microorganisms is always passive (Martiny et al., 2006). Passive dispersal of benthic fungi can take the form of spores, filaments, or attached via suspended sediment particles transported by currents and water movement (Li et al., 2018). The rate of their descent, and thus the traversal of greater distances, depends in part on the structure and weight of the spores/inoculum. For example, some spores of aquatic fungi exhibit specific appendages that either allow them to remain in the water longer or through which they attach to particles (Kohlmeyer and Kohlmeyer, 1979). Furthermore, vectors can positively influence fungal long-distance dispersal like micro- and macroorganisms (Singh et al., 2016) or drift wood (Rämä et al., 2014). However, our results indicate provincialism of benthic fungi,

suggesting that long-distance dispersal does not have a significant influence on benthic fungal community assemblage. Consequently, only 0.05 % of all fungal OTUs showed a cosmopolitan occurrence. Cosmopolitan distribution is likely achieved by taxa that are highly performant in dispersal, respond flexibly to diverse environmental factors, and thus can competitively colonize many different ecological niches, such as the two cosmopolitan *Cladosporium* species detected here. *Cladosporium* species produce numerous small conidia that form dense branching chains. Thus, they are well adapted to be spread easily in large numbers over long distances. Given that almost all (5/6) cosmopolitan OTUs are filamentous in their morphological trait, the question arises whether yeast cells may be distributed less effectively compared to the small conidia/spores produced by many filamentous taxa. Another explanation may be a better and more long-lasting establishment of filamentous fungi in the sediment compared to yeasts.

4.2 Small-scale distribution patterns (< 100 km)

At the small-scale level, the degree of proportional overlap in the number of the same OTUs in two different fungal communities was highest at 40%. This number speaks for factors that promote an even dispersal and successful establishment of fungal inoculum within this distance. Substrate input with an areal distribution of upper water productivity (Friedland et al., 2018; Renaut et al., 2018) may be one such factor. It promotes benthic microbial activity and diversity (Zinger et al., 2011; Ramírez et al., 2021), which leads to increased biomass and thus increased inoculum mass. Additionally, the sinking particles act as a transport vector of fungi from upper water layers to the benthos. For example, Ramirez et al. (2021) identified an areal sedimentation of particles populated with fungi from productive water columns as the cause of a ubiquitous fungal phylotype-specific biosignature in surface sediment. Similarly, Cordier et al. (2022) named particle export from the water surface as one of the main structuring factors for benthic eukaryotic communities. The protists contained therein possessed a much

more homogeneous community structure over long distances compared to larger organism groups. Another factor can be attributed to a homogenizing effect of dispersal; it may operate because the abiotic factors present do not counteract dispersal and colonization as shown, for example, for bacterial communities in permeable sediment (Stegen et al., 2015). In general, however, a distance of 100 km can be defined as a critical threshold for the dispersal of benthic fungi, although the amplitude of variation is relatively large probably due to species-specific dispersal strategies and local environmental conditions.

4.3 Local distribution patterns (< 1 km)

The high variability of fungal OTUs across locally neighbouring communities was notable. Sediment is very heterogeneous with respect to the availability of oxygen, nutrients, organic matter or electron donor and acceptor in a very small space. The strong partitioning of neighbouring microbial communities (Li et al., 2016; Barone et al., 2022) is thus, a function of a dramatic change of niche conditions over short distances. Filamentous fungi have an advantage over other growth forms in terms of substrate heterogeneity: they can optimally explore the three-dimensional space with their fine-structured mycelium and translocate biomass from nutrient-depleted to -rich regions. This may explain why taxa with filamentous growth significantly dominated the benthic fungal communities across all regions and habitat types studied here. This goes hand in hand with a significant dominance of the saprotrophic lifestyle. One possible strategy of saprotrophic fungi may be the encrustation of their hyphae with particulate matter and a subsequent aggregate formation from dissolved organic material as shown by Damare and Raghukumar (2008). Aggregate formation may be one of the reasons for high local taxon patchiness.

Bioturbation is another factor that can cause local differences of microbial communities already in the mm range. The activity of benthic animals leads to the formation of structurally and biologically distinct

environments that can be unevenly distributed (Kogure and Wada, 2005). Adaptation to microniches leads to a high number of endemic species explaining partly our results. Bioturbation can occur in all sediments, from coastal sediment (Taylor and Cunliffe, 2015; Booth et al., 2019) to deep-sea sediment (Soltwedel and Vopel, 2001). The strength of influence depends on the size of the benthic fauna, whether they are stationary or mobile, and their mode of movement and resulting structure in the sediment. Bioturbation acts in both vertical and horizontal directions. The high OTU turn-over at the local level due to possible taxon-specific ecology/morphology and biotic interactions are probably some reasons for the high percentage of unexplained variation in the VPA analysis, in addition to other unmeasured factors.

4.4 Conclusions

Based on the results, we conceptualized a first framework on the distribution of benthic marine fungi (Fig. 3). Surprisingly, we found a clearly limited distribution at small and intermediate distances. Building on our results in which all taxa were treated equally, we propose that next studies shall be conducted at the level of individual taxonomic units to clarify which bunch of traits is responsible for individual taxa being able to disperse and successfully establish at the target site, whereas others are not. Furthermore, based on our results, we would like to open the discussion on the extent to which the signal of adaptation to a particular niche can be overridden by geographic distance. In other words, can niche-specific fungal specialists in very similar but geographically distant niches differ from each other because the long distance between the two niches prevents colonization/infection of the other niche, rather than because they lack the necessary traits?

However, these questions also highlight the importance of a trait-based biogeography that includes primarily functional traits linked to fitness, dispersal and performance. New tools are on the way that allow more accurate species delineation. In addition, databases are developing that bring together

information on the individual traits of organisms. We believe this opens new avenues for the science of microbial biogeography, in which the organism's life history will be placed at the center

5 Acknowledgements

We acknowledge the University of Bremen for financial support.

6 Data availability statement

All used datasets are openly accessible (for accession numbers, see Supplementary Table 1).

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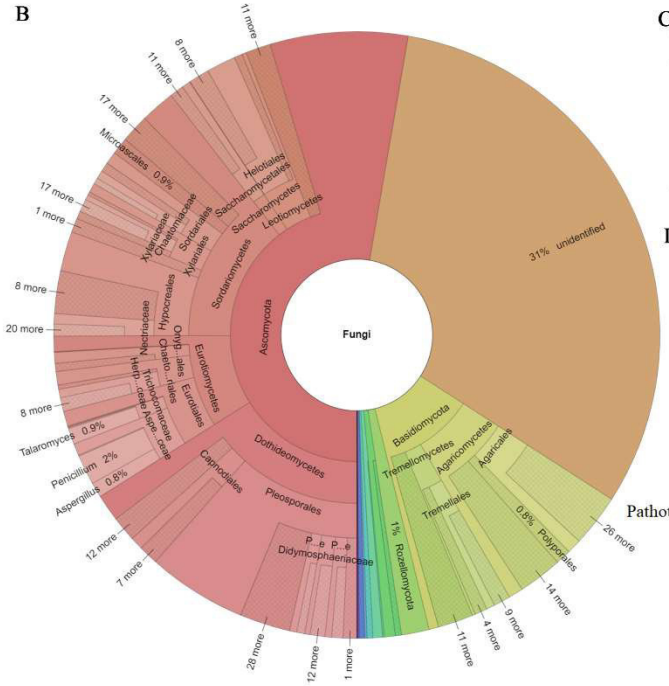
8 Figures

Figure 1: Key figures of the composite dataset (A) Global sampling map with circles indicating study sites (detailed information can be found in the Supplementary Table 1); (B) Krona chart showing taxonomic classification of fungal OTUs of the composite (interactive chart can be browsed over Supplementary File 2.html); (C) Numbers on endemic and cosmopolitan OTUs; (D) Proportion of fungal OTUs assigned to morphological and trophic traits, ***: significant difference to other types (TukeyHSD, P < .001); (E) Detailed information on taxonomy and ecology of cosmopolitan OTUs.

A

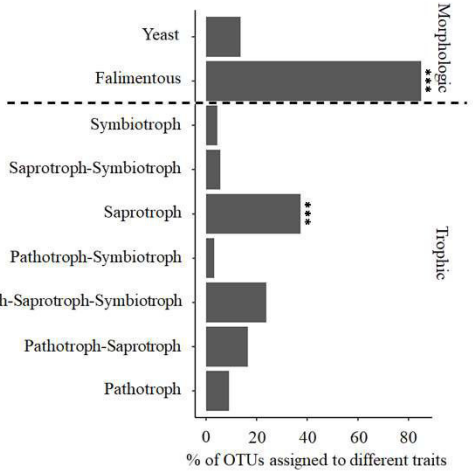
B



C

	OTU [No./%]	Sequence [No./%]
Endemic Species	7,244 / 65	2,745,918 / 25
Cosmopolitan species	6 / 0.05	983,519 / 8.9
Rest	3,899 / 35	7,317,087 / 66
Total	11,149 / 100	11,046,524 / 100

D



E

OTUs	Taxanomy	Trophic	Morphology	Sequence No.	Proportion of sample[%]
OTU9855	Cladosporium	Pathotroph-Saprotroph	Filamentous	558,838	97.2
OTU17447	Cladosporium	Pathotroph-Saprotroph	Filamentous	181,446	75.1
OTU9047	Didymellaceae	Pathotroph-Saprotroph	Filamentous	99,030	56.3
OTU8707	Pleosporaceae	Pathotroph-Saprotroph-Symbiotroph	Filamentous	79,050	78.4
OTU1290	Malassezia	Pathotroph-Saprotroph	Yeast	36,285	62.4
OTU17183	Cladosporiaceae	Unassigned	Unassigned	28,870	63.8

Figure 2: Impact of spatial factors on fungal distribution (A) Proportion of shared OTUs as a function of increasing distance between pairs of samples. Based on TukeyHSD, four distance thresholds were defined indicating a significant change of shared OTU number ($P < .001$). $: distance threshold imply significant change of shared OTUs with all other distances, #: distance threshold does only partly imply significant change of shared OTUs with other distances (for details see Supplementary Table 3); (B) Distance-decay analysis showing a significant relation between geographic distance and proportion of shared OTUs with a yeast (blue) or filamentous (red) morphotype (TukeyHSD, $P < .001$). (C) Variation partitioning analysis attested spatial distance a greater impact on observed differences in fungal community than habitat type. Significance was tested by dbRDA-based forward selection; ***, $P = .001$).

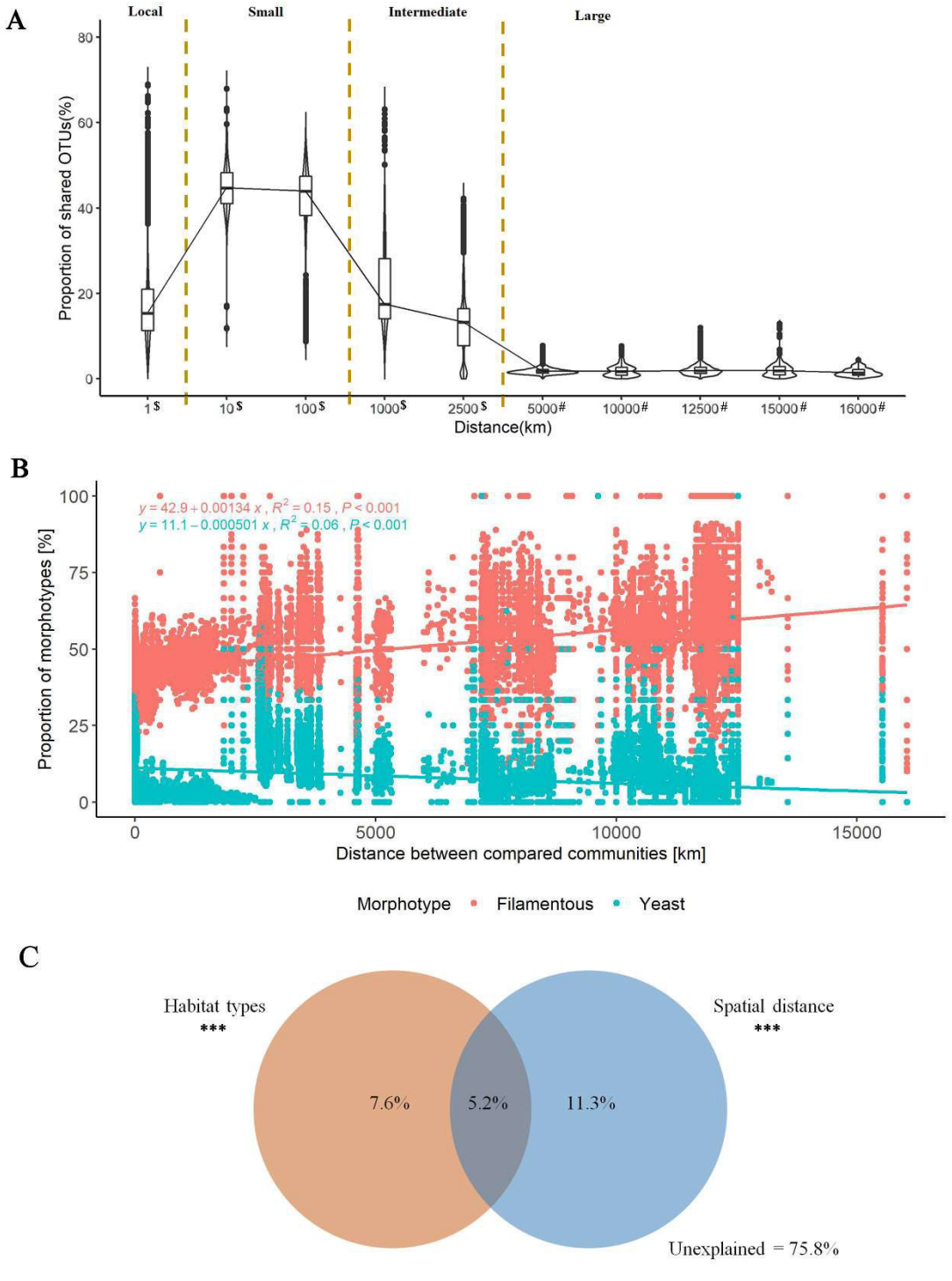
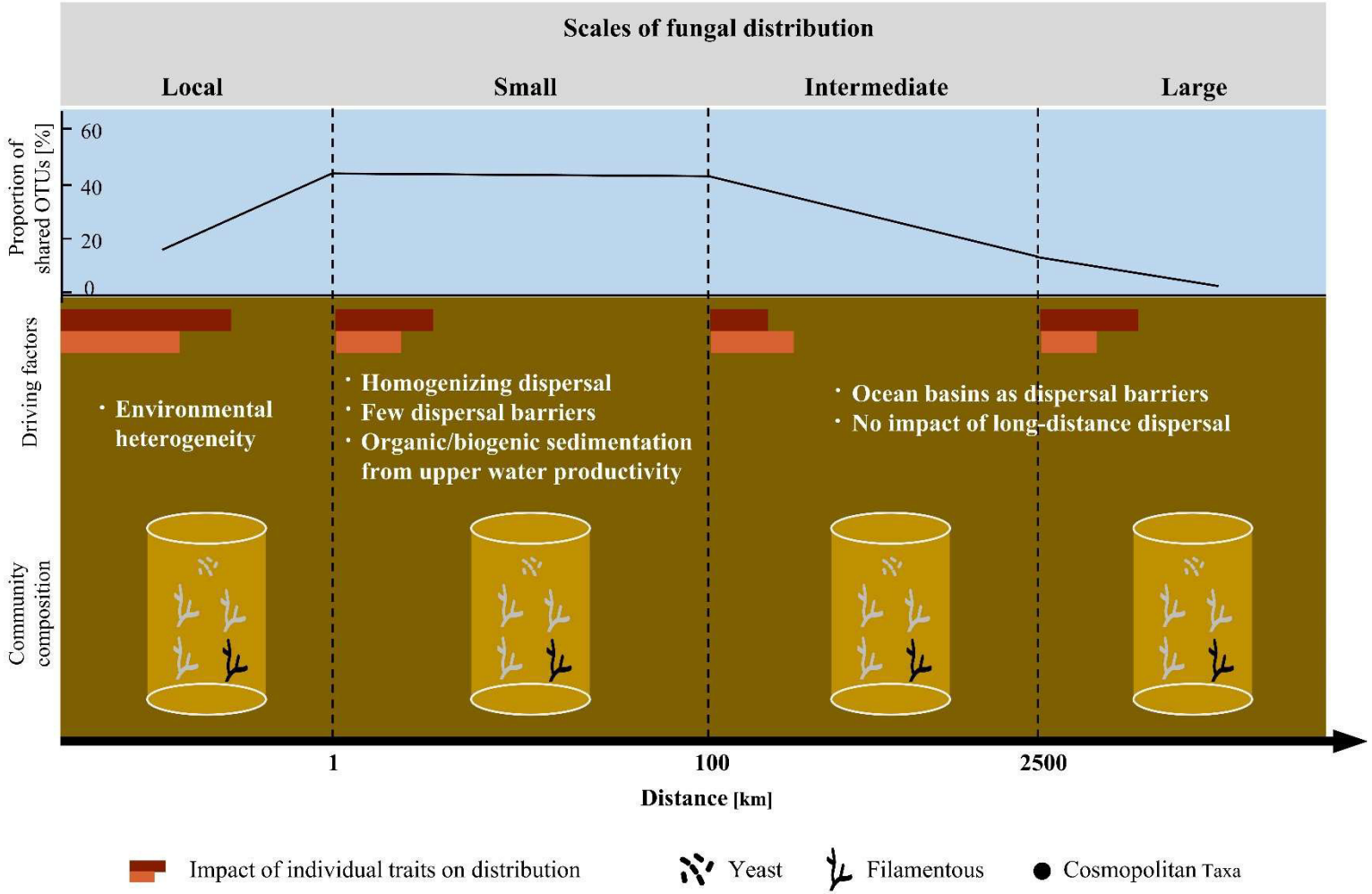


Figure 3: Conceptual framework for the distribution patterns of benthic fungi and possible influencing factors. Benthic fungi are limited in their distribution and four distribution patterns can be identified: local (< 1 km), small (1 - 100 km), medium (100 - 2,500 km), and large (> 2,500 km). The change from one distributional scale to the next is accompanied by a large increase/decrease in the number of shared taxa between the compared communities. Different factors may act within each scale. Taxon-specific traits have a joint effect on taxon distribution and may operate at all distributional scales. Filamentous fungi dominate benthic fungal communities, and cosmopolitan taxa may occur in two communities that are even geographically distant.



4.3 Paper III

Effects of oxygen content on the mycobenthic community of coastal sediments

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Keywords: Marine fungi, anoxic, community incubation, incubation time

Abstract

In coastal marine sediments, anoxia is a regular phenomenon that can develop rapidly and over low spatial resolution. Benthic fungi occupy a prominent position in the marine carbon cycle. However, anoxia can be challenging, as fungal respiration is most energy-efficient when using oxygen as an electron acceptor. Which part of the benthic fungal community (mycobenthos) can cope with onset of anoxia and are there strategies and traits being beneficial?

In this study, we incubated the first centimetres of mycobenthos inhabited coastal sediments under oxic and anoxic conditions. Dynamics of the mycobenthos were followed via ITS2-tag Illumina sequencing. Additionally, we conducted a comprehensive meta-analysis on published fungal sequence data from environmental studies of marine coastal sediments.

Only a quarter of all members were present under both, another quarter only under anoxic conditions. OTUs showed different strategies with short, rapid response to anoxia, while others increased in frequency only after prolonged anoxia. The strategy of dimorphism proved to be significantly beneficial, while taxonomy and other traits had little influence on dynamics. Based on our results, it can be assumed that different niches open up over the duration of prolonged anoxic conditions, in which individual taxa develop based on their strategies.

1 Introduction

Seabed microorganisms play an important role in the oceanic carbon cycle and food webs. Through their decomposition activities, they control the rate and yield of carbon turnover with consequences for the long-term carbon storage in the seabed (review on chemical, physical and biological properties). Fungi are part of the microbial benthos called mycobenthos and can metabolize similar amounts of carbon as bacteria (Orsi *et al.*, 2022).

Oxygen availability is an important factor for microbial activity, as the use of molecular oxygen as an electron acceptor in microbial respiration yields the highest energy output. Many microbes have evolved alternative energy yielding mechanisms to be able to thrive in niches with low or no oxygen content (Orsi, 2018). Fungi exhibit the broadest energetic metabolic and physiological diversity among eukaryotes (Martin *et al.*, 2003). This includes adaptation strategies at the organelle level such as reduced mitochondria or hydrogenosomes (Yarlett *et al.*, 1986; Marvin-Sikkema *et al.*, 1993; Abers *et al.*, 2002), diverse energy yielding pathways like fermentation, aerobic or anaerobic respiration (Panagiotou *et al.*, 2005; Morozkina and Kurakov, 2007) and the use of alternative electron acceptors to oxygen (Kobayashi *et al.*, 1996). These findings are based on physiological experiments mainly conducted on a few terrestrial fungal species. Even at the community level, there are only a limited number of mycobenthos focused studies that have investigated the influence of oxygen concentration on the fungal diversity and composition (Jebaraj and Raghukumar, 2009; Jebaraj *et al.*, 2010). As a consequence, no conclusions can be drawn on how oxygen concentration affects mycobenthic diversity and how this changes the functional role of mycobenthos, e.g., in the context of carbon turnover, in rate and quality.

The mycobenthos of coastline sediments faces special challenges with regard to oxygen availability: The oxygen penetration is limited to the first millimetres to centimetres (Rasmussen and Jørgensen, 1992), while the underlying sediment is anoxic. The organic input is high due to the proximity to the coast and increased phytoplankton activity. Thus, microbial respiration increases in the upper centimetres leading to local anoxic conditions (Precht *et al.*, 2004). On the other side, numerous factors promote oxygen penetration into deeper, anoxic sediment layers, such as water turbulences and

bioturbation, which further interplay with the sediment structure and high oxygen concentration in the water layers above the sediment (Bertics and Ziebis, 2010; Shang *et al.*, 2013). As a result, oxygen availability can quickly change over local and temporal scale. It can be assumed that a large proportion of the fungal taxa living here can respond flexibly to a change between oxic and anoxic conditions. The aim of this study was to investigate (i) which proportion and taxa of such a natural community from oxic sediment layers can thrive under anoxic conditions, and (ii) whether the adaptability is related to taxonomy, specific functional traits or temporal duration of conditions. For this purpose, mycobenthic communities were sampled from the oxic layers of the northern Wadden Sea and incubated under oxic and anoxic conditions. Diversity was analysed by ITS2-tag sequencing. We also conducted a meta-analysis on published mycobenthic sequence data from coastal marine sediments and investigated whether taxonomy, morphology or trophic modi influence the occurrence of taxa under the two conditions tested.

2 Material and Methods

2.1 Incubation Experiments

Three sediment cores were taken on the 11th of October in 2015 at low tide from the mudflats of the Wadden Sea of Dorum-Neufeld, Germany (E 8.3031, N 53.4435), using sterilised polycarbonate drills (25.5 cm length and 5.5 cm diameter) in a transect and with a distance of 10 m to each other. The cores were cooled and transported directly to the laboratory, where all further steps were carried out under sterile conditions. The first 3 cm of the aerobic layers were separated and used further. As a control for the naturally occurring mycobenthic community, three subsamples/drill core were taken and stored at -20°C until DNA extraction. The remaining sediments were mixed and used to prepare slurries for oxic and anoxic incubations in Erlenmeyer flasks and 120 ml serum bottles, respectively. Each incubation contained a slurry of 20 g sediment and 60 ml artificial seawater (ASW) (Horne, 1969). Three replicates were prepared per condition. After 24 h, 48 h, 72 h, 8 d, 15 d, and 22 d, 6 ml of slurry were retrieved from each microcosm with a sterile needle and stored at -20°C until further treatment resulting into 39 samples (seven time points, 3 replicates/condition, 2 conditions (oxic, anoxic)).

2.2 DNA extraction and Illumina sequencing on samples of the incubation experiment

DNA from the 36 incubation samples and three of the original sediment was extracted using the NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instruction. Fungal internal transcribed spacer (ITS) region 2 was amplified as described in Banos *et al.* (2018) but using the fungi-specific primers fITS7 (5′-GTGARTCATCGAATCTTTG-3′) (Ihrmark *et al.*, 2012)and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.*, 1990). PCR, library preparations and sequencing were performed at LGC Genomics GmbH (Berlin, Germany) using the IlluminaMiseq chemistry for 2 x 300 bp reads (Illumina, Berlin, Germany) following the manufacturer's instructions.

2.3 Meta-analysis on publically available fungal-specific ITS2 HTS data sets

Literatures were screened for High-Throughput Sequencing (HTS) datasets on mycobenthos from sediments of coastal shelfs targeting the ITS2 and published not later than October 2021. In short, a comprehensive literature research was conducted using the search engines like "Web of Science" (*https://www.webofscience.com/wos/woscc/basic-search*), "Google Scholar" (*https://scholar.google.-com/*), or the database of European Nucleotide Archive (ENA). Data was grouped into oxic or anoxic sediment according to contextual information in the papers. Additionally, papers and textbooks were screened for physiological proof of adaptation strategies of fungi towards hypoxia/anoxia. Key words like "anoxic and fungi", "anaerobic and fungi", and "oxygen minimum zone" were used. Finally, we assigned samples to oxic conditions according to the sampling depth (0-8cm) and anoxic conditions using the definition in the original studies (Table S1_ Information on composite dataset).

2.4 Sequence analysis

All sequences from both the published datasets and the experiment were analyzed as one dataset using the PIPITS pipeline v2.8 (Gweon *et al.*, 2015). Due to the high number of undescribed fungal OTUs in the final OTU table, attempts were made to improve the taxonomic classification of the most abundant species, generalists and OTUs that stood out in the statistical analyses by using additional classification options. BLASTn (Altschul *et al.*, 1990) was performed on the UNITE webpage (accessed on 22.08.2022) by enabling beside the UNITE-own database the INSD database and applying classification thresholds over different phylogenetic levels as suggested by Nilsson *et al.* (2019). Results including possible Species Hypothesis (SH) (Kõljalg *et al.*, 2013) were manually evaluated. As a last option, undescribed OTUs were attempted to be classified based on the secondary structure of the ITS2 via the web-based ITS2 Database (Schultz *et al.*, 2006; Ankenbrand *et al.*, 2015) (accessed on 25.04.2022) applying the default settings. To assign the trophic mode, and growth morphology to single OTUs, FUNGuild was used with the default parameters (Nguyen *et al.*, 2016). Whenever OTUs assigned to genus level could not be affiliated with guilds, a second search was conducted on the FungalTrait datasheet v1.2 (Põlme *et al.*, 2021).

2.5 Statistics

Krona charts were generated on the taxonomic composition using KronaTools-2.8.1 (Ondov *et al.*, 2011). If not differently stated, all statistical analyses were carried out within the *R environment* v4.1.3. (R-Core-Team, 2022). Rarefaction curves were generated with the R package *iNEXT* (Hsieh *et al.*, 2016). OTU richness (Chao1), diversity (Shannon) and evenness (Gini-Simpson) was calculated using the "estimate_richness" function in the package *phyloseq* (Mcmurdie and Holmes, 2013). To identify a possible influence of the time of incubations on the mycobenthic assemblage process, linear regression (n = 18; $P < 0.05$) was run using the "lm" function. Cosmopolitan genera were defined as if they were present under both oxic/anoxic conditions, and >=50% samples. Impact of oxygen availability on taxa abundance over all taxonomic levels and alpha-diversity was calculated using Tukey's post-hoc test (TukeyHSD). Similarly, significant occurrence of a given morphological trait or trophic modes under one condition was also calculated with the Tukey's post-hoc test. Taxonomic groups were based on total relative abundance, and trophic modes, morphologic groups were based on OTUs proportions. Next, the OTU-to-sample matrix was normalized via the total standardization using the "decostand" function in *vegan 2.5-7* (Oksanen *et al.*, 2020). Then, a Bray-Curtis dissimilarity matrix between pairs of samples was calculated. The dissimilarity matrix was used for principal component analysis (PCoA). Significance of dissimilarity between sample groups was tested by PERMANOVA (FDR adjusted $P < .05$) using the "adonis2" function of the package *vegan.* To identify genera/OTUs that contributed most to the observed dissimilarity between different conditions, the similarity percentage (SIMPER) analysis was done using the "simper" function in *vegan*.

3 Results

3.1 Facts on the whole dataset

Via the literature survey, seven fungi specific ITS2-HTS datasets were identified comprising 161 samples, 132 from oxic and 29 from anoxic sediments. The combined sequence analysis on meta-samples and incubation samples identified a total of 11,376 fungal OTUs (10,202,128 sequences). 614 OTUs (928,656 sequences) and 11,038 OTUs (9,273,472 sequences) were detected in the incubation and meta-analysis samples, respectively (Table S2_ Fully annotated OTU table). All rarefaction curves levelled off reaching a plateau indicating sufficient sequencing depth to capture most of the mycobenthic diversity (Fig. S1). OTUs were classified into 14 fungal phyla, 44 classes, 121 orders, 306 families and 826 genera. Half (51.4%) OTUs (46.4% of sequences) was belong to *Ascomycota*, followed by *Basidiomycota* of 11.9% OTUs (24.1% of sequences). Unidentified fungi occupied 33.0% of OTUs (28.7% of sequences). *Cladosporium* and *Alternaria* were identified as cosmopolitan genera under incubation experiment and meta-analysis, with higher abundance under anoxic conditions (*Cladosporium*-5.27%, 5.44%, Alternaria-1.63%, 4,41%) of incubation experiment and meta-analysis than oxic conditions (*Cladosporium*-1.73%, 1.55%, *Alternaria*-1.19%, 1,19%). Furthermore, additional 21 *Ascomycota* genera, *Vishniacozyma* and *Mortierella* were identified as cosmopolitan genera in meta-analysis (Table 1, Table S3).

In the incubation experiments, 27% (165) of all OTUs were detected in both oxic and anoxic conditions, while 23% (140) of OTUs were found only in anoxic incubations (Table1, Fig. 1A). In the meta-analysis, 15% (1,659) of all OTUs were found in both sediment types while 6% (699) of OTUs were detected only in anoxic sediments (Table 1). *Ascomycota* dominated all datasets with 23% (108) and 31% (96), and 53% (5,496) and 56% (1,318), of all OTUs in oxic and anoxic conditions of the incubation experiment and meta-analysis, respectively. *Basidiomycota* accounted for 11.2% (53), 18.0% (55), 11.1% (1146) and 16.8% (397) of all OTUs in oxic and anoxic conditions of the incubation experiment and meta-analysis, respectively. Unidentified fungi accounted for 64 % (304) of all OTUs in the oxic incubation approaches, while they represented 48 % (146), 32 % (3,287) and 26 % (607) of

all OTUs in anoxic incubations and oxic and anoxic sediments of the meta-analysis, respectively (Fig. S2, Fig. S3).

After trimming with FUNGuilds and FungalTraits, identification of OTUs into trophic modes was possible for 4089 OTUs including 4,365,582 sequences; out of the identification: saprotroph was the dominant group, with 35.3% (73 OTUs) for oxic incubation samples, 38.7% (74 OTUs) for anoxic incubation samples, 37.9% (1430 OTUs) for oxic environment samples, and 31.5% (328 OTUs) for anoxic environment samples (Fig. S4). Saprotroph-symbiotroph showed a significant difference (Tukey HSD, $P < 0.001$) between natural oxic (3.7%) and anoxic (5.8%) conditions.

Furthermore, 4064 OTUs including 4,363,876 sequences were assigned with morphological modes: filamentous species was the dominant group, with 71.6% (36 OTUs) for oxic incubation samples, 79.6% (36 OTUs) for anoxic incubation samples, 86.7% (3251 OTUs) for oxic environment samples, and 78.2% (813 OTUs) for anoxic environment samples (Fig. S5). For meta-analysis, filamentous fungi and facultative yeasts showed a significant difference (Tukey HSD, $P = 0.031$ and $P < 0.001$). For incubation samples, dimorphic yeasts showed a significant difference between the oxic and anoxic incubation (Tukey HSD, $P = 0.002$).

3.2 Alpha- and Beta-diversity

For the incubation experiments, no significant differences in alpha diversity were reported between oxic and anoxic conditions (TukeyHSD, $P > 0.45$). However, linear regression identified a time-dependent effect. Thus, OTU richness increased significantly in the oxic incubation approaches ($R^2 = 0.61$, $P < 0.001$) over the time of incubations. In contrast, OTU richness ($R^2 = 0.24$, $P = 0.038$), diversity ($R^2 = 0.30$, $P = 0.018$) and evenness ($R^2 = 0.27$, $P = 0.027$) decreased (Fig. 1B).

Incubation time has a more important effect than oxygen [PERMANOVA, (R2, F-value, FDR adjusted P-value)] on fungal community composition, oxygen: 0.044, 1.726, 0.005; time: 0.195, 1.541, $P < 0.001$; oxygen*time: 0.155, 1.224, 0.025 (Fig. 1C). PCoA did not show a clear separation of oxic and anoxic incubations (both axes together explain 20.9% of the total variation). However, along Axe 1

(12.5%), the oxic incubation over day 15 and 22 separated, which was confirmed by PERMANOVA as a significant change in beta diversity (R2 = 0.1, F-value = 4.23, FDR adjusted $P < 0.001$) (Fig. 1D).

The SIMPER analyses (Table. S4) indicated that the fungal communities (23 genera) showing significant difference between oxic and anoxic incubations: *Lacrymaria* (*Basidiomycota*, saprotroph-filamentous) contributed most (COD = 6,25%) to the difference and was more abundant under anoxic (relative abundance = 9.6%) and oxic (relative abundance = 4.4%) incubation. Furthermore, for meta-analysis, 26 genera showed significant difference between oxic and anoxic conditions: *Psathyrella* (*Basidiomycota*, saprotroph-filamentous) contributed most (COD = 7.19%) to the difference and was more abundant under anoxic (relative abundance = 3.9%) than oxic (relative abundance = 2.0%) condition.

3.3 Response of different taxa to anoxic incubation

No phylum showed significant differences between oxic and anoxic incubations. However, one class (*Rhizophydiomycetes*) showed significantly (Tukey HSD, $P < 0.05$) higher abundance over anoxic incubation. Four orders (*Hymenochaetales*, *Microbotryales*, *Rhizophydiales*, and *Verrucariales*) showed significantly higher abundance over anoxic incubation. 10 families showed (*Cucurbitariaceae*, *Diatrypaceae*, *Gyroporaceae*, *Hygrophoraceae*, *Myxotrichaceae*, *Pucciniaceae*, *Pucciniastraceae*, *Schizoporaceae*, *Teratosphaeriaceae*, *Verrucariaceae*) significantly higher abundance over anoxic incubation. 22 genera (*Acericola*, *Blumeria*, *Bovista*, *Candida*, *Catenulostroma*, *Chalciporus*, *Golovinomyces*, *Gyroporus*, *Hebeloma*, *Hygrophorus*, *Itersonilia*, *Lycoperdon*, *Melampsoridium*, *Neocatenulostroma*, *Neocucurbitaria*, *Oidiodendron*, *Parasola*, *Phacidiella*, *Puccinia*, *Sarcopodium*, *Striaticonidium*, *Xylodon*) showed significantly higher abundance over anoxic incubation. Among them, *Candida* can be found from other community research (anoxic), and anoxic fermentation. *Oidiodendron* can be found from other community research (anoxic) (Fig. 2).

Among the OTUs (305 OTUs) which existed under anoxic incubation, 18% (55 OTUs) can quickly respond actively within the first 24 hours of anoxia with increasing frequency. 22.6% (69 OTUs) responded with a slower reaction to anoxic incubation with increasing frequency showing after 22 days. 2% (6 OTUs) reacted with a relatively equal frequency over 22 days anoxic incubation (Fig. 3).

4 Discussion

4.1 Duration of anoxic conditions is decisive factor in structuring the mycobenthic community

In this study, we investigated the adaptability of a mycobenthic community from coastal surface sediment to anoxia. We hypothesised that due to the heterogeneity of oxygen availability in the source habitat, a large proportion of taxa are adapted to at least transient anoxia. This priming effect is documented, for example, for freshwater hyphomycetes isolated from river soil with temporary developed anoxia, which survived anoxic incubations compared to other fungal taxa (Field and Webster, 1983). Similar observations were made for anoxic incubation experiments for fungal communities from peat soils, resulting in a drastic reduction of vital fungal biomass up to 40% in communities from upper layers, while no such reduction was observed for communities from deeper soil layers (Kurakov *et al.*, 2011). In our study, the oxygen content of the incubations had a significant effect on the structure of the communities, but the temporal factor was the determinant variable. Half of all OTUs were found under anoxic conditions with 18% of the OTUs reacting within the first 24 hours of anoxia with increasing frequency. In sediments, anoxia can develop quickly and develop over microscales (Hietanen and Lukkari, 2007). Ortega-Arbulu *et al.* (2019) reported in incubation experiments a response of mycobenthic communities to the establishment of anoxic conditions even within 7 hours. We were able to group fungal taxa according to their response patterns over the entire incubation period into three groups, likely reflecting different adaptive strategies of taxa within the mycobenthic community (Jebaraj and Raghukumar, 2009). These results suggest that new and different niches continuously evolve during the establishment of anoxia and even during the duration of anoxia. For a mycobenthic community in a very dynamic environment in terms of oxygen content, this means that there is a continuous change in composition along the temporal occurrence of the diverse niches similar to what has been reported for mycobenthic communities in continuously disturbed sediment (Galand *et al.*, 2016).

4.2 Adaptability to anoxia is not conserved at higher taxonomic levels

The high number of undescribed species in fungal communities (Grossart *et al.*, 2016) makes it difficult to correlate the observed community response and fungal taxonomy and characteristics (Yang *et al.*, 2021). In this study, we were able to improve the taxonomic resolution for some of the most abundant or statistically relevant OTUs by manual annotation, using diverse sequence comparison algorithms and the secondary structure of ITS2 (Schultz *et al.*, 2006; Kõljalg *et al.*, 2013; Ankenbrand *et al.*, 2015; Nilsson *et al.*, 2019) for taxonomic classification. The number of undescribed OTUs was significantly higher in oxic sediments. In the meta-analysis, OTU richness and diversity were additionally significantly higher. Hence, our data support the hypothesis of Orsi (2018) that oxic sediments harbour a much greater undescribed fungal diversity than anoxic ones. Among the taxonomically classifiable OTUs, significant occurrence under anoxic conditions increased at lower taxonomic ranks such as family or genus level. This suggests that fungal adaptability to anoxic conditions is not conserved at higher taxonomic levels. *Ascomycota* and *Basidiomycota* were equally represented among the significant taxa, along with other phyla, suggesting that adaptability may be widespread throughout the fungal kingdom with similar or different strategies. An example of the former is the convergent evolution of lactic acid-producing fermentative members of the *Blastocladiomyocta* and *Oomycota* (Kittelmann *et al.*, 2017). *Ascomycota* dominated all communities studied but showed no significance in terms of oxygen content. Recently, Yang et al. (Paper II) were able to provide evidence in a mycobenthic biogeographical study that Ascomycota are generally the dominant phylum of the mycobenthos. This breaks down the previous view of benthic community structures, as anoxic sediments in particular were previously considered to be Ascomycota-dominant; with the exception of deep-sea sediments (Orsi, 2018).

4.3 Impact of fungal traits effecting adaptability to anoxia

The biogeographic study by Yang et al. (Paper II), in press mentioned above further confirms our results that filamentous growth form dominated all communities even being significant in oxic sediments of the meta-analyses. Filamentous fungi have the advantage of penetrating the sediment three-dimensionally with their hyphae. This allows them to grow through sediment areas with

unfavourable conditions, such as nutrient deficiency, by supplying hyphae in nutrient-poor regions via nutrient transport from hyphal areas with good nutrient supply (Groß-Schmölders *et al.*, 2020). Similarly, cell components that need to be synthesised in an oxygen-dependent manner may be transported to oxygen-poor regions of the mycelium. It can be hypothesized that two different energy production processes can also take place in hyphae of different sediment areas similar to cable bacteria (Pfeffer *et al.*, 2012). At least from *Fusarium oxysporum*, it is known that both denitrification and oxygenic respiration are carried out simultaneously when oxygen availability is limited (Morozkina and Kurakov, 2007). In general, however, it is probably difficult to correlate the filamentous growth form in sediments with oxygen concentration changing over the shortest distances, since already one mycelium with its three-dimensional spatial spread (Fricker *et al.*, 2017; Heaton *et al.*, 2020) can be in several niches at the same time.

Different morphotypes were significantly related to anoxic conditions, such as dimorphic yeasts in our incubation experiments. Dimorphic yeasts can reversibly change from a filamentous to a yeast growth form during environmental perturbations. In addition to anoxia, various factors such as temperature, pH or metabolites (Szaniszlo, 1985; Bossche *et al.*, 1993) are listed as triggers for a morphological switch, often it is even an interaction of diverse factors (Li and Nielsen, 2017). Which growth form is formed in anoxia depends on the fungal species (Ruiz-Herrera and Sentandreu, 2002), but the morphological change is connected to physiological changes in which very different gene patterns are activated (Doiphode *et al.*, 2009). Dimorphism, thus, gives fungal taxa an advantage in responding to environmental changes and to be able to occupy different ecological niches.

In the anoxic sediment of the meta-analysis, facultative yeasts were significantly more abundant. This is consistent with observations that most aquatic yeasts tested were described as weakly fermentative (Kutty and Philip, 2008). According to Fell and van Uden (1963), benthic yeasts are mainly found in the upper sediment centimetres, as oxygen availability is a limiting factor for most of them. Thus, they cannot independently synthesise unsaturated fatty acids and sterols under anaerobiosis. However, due to fermentation, they can quickly respond actively to temporarily occurring anaerobic phases and thus may occupy this specific niche. However, first step to a fermentative lifestyle was probably the

exploration of anaerobic niches and several yeast lines have laterally evolved anaerobic growth (Dashko *et al.*, 2014). Among them are some *Saccharomycetaceae* why it is not surprising that they were detected in sulfidic anoxic sediments as part of the active fungal community (Orsi *et al.*, 2022).

Saprotrophy was the predominant trophic mode in both oxic and anoxic sediments. Little is known about the source and yield of benthic saprotrophic fungal decomposition in the ocean. The question of how mycobenthic carbon turnover is influenced by a fermentative or respiratory (oxic or anoxic) mode is also poorly understood. First insights into this topic were recently provided by Orsi *et al.* (2022) who showed that members of the *Mucoromycota* and *Chytridiomycota* can actively metabolise the necromass of bacteria.

Furthermore, in our project, fungi with mutlitrophic forms were abundant; the saprotrophic-symbiotic mode was even significant in the oxic sediment in the meta-analysis. The high diversity of mixed trophic forms is not surprising, as the marine benthos holds a high eukaryotic diversity within and across different kingdoms (Alongi and Sasekumar, 1993). Interactions of benthic fungi range from diatoms (Scholz *et al.*, 2016; Ilicic and Grossart, 2022), invertebrates (Marchese *et al.*, 2021), bacteria (Baudy *et al.*, 2021) to foraminifera (Vohník, 2021). However, experimental evidence of fungal symbioses is limited to work on seagrass (Borovec and Vohnik, 2018) and mangrove roots (Gladfelter *et al.*, 2019). Rojas-Jimenez *et al.* (2020) recently pointed to positive correlations between fungi in oxic deep-sea sediments. Despite the qualitative compilations of the two fungal trait databases FUNGUILDS (Nguyen *et al.*, 2016) and FunTraits (Põlme *et al.*, 2021), conclusions about marine fungal interactions based on sequence datasets have to be handled with care, as the majority of information in the databases was derived from terrestrial environments. The marine realm poses significantly different challenges for fungi (Amend *et al.*, 2019; Gonçalves *et al.*, 2022), and thus the weighting of the individual trophic modes may shift significantly due to differences in marine interaction partners and environmental conditions.

4.4 Further considerations

This discussion demonstrates that the adaptability of individual fungal taxa to anoxia is controlled by various traits and factors. The regulation of whether, at what time and how strongly a fungal taxon

proliferates in anoxic sediments presumably takes place via precise fine-tuning. For example, germination from inoculum/spore and proliferation in a given niche depends on intrinsic activation patterns and assertiveness over already growing taxa (Hesham *et al.*, 2020). Both factors may play an important role for taxa that proliferated with a time lag after the setup of anoxic incubations. In anoxic conditions, the presence of alternative electron acceptors to oxygen is a factor that has not yet been mentioned. Their presence and concentration also control the composition of aquatic mycobenthos (Song *et al.*, 2019). So far, only the *Neocallimastigomycota* have been identified as a truly anoxic group of fungi (Gruninger *et al.*, 2014). For the rest of the kingdom, a wide range of physiological mechanisms have been described by which they respond to low to zero oxygen concentration (Dumitru *et al.*, 2004; Morozkina and Kurakov, 2007; Jebaraj and Raghukumar, 2009; Jebaraj *et al.*, 2010). Some taxa possess only one mechanism while others have evolved a complex regulatory system to continue their vital activity under hypoxia and anoxia (Gleason *et al.*, 2019). Of the few fungal taxa for which physiological adaptation strategies to hypoxia/anoxia have been experimentally shown, almost all were detected at least at the genus level in our study. They were always represented with higher/equal frequency in oxic versus anoxic sediment. Based on our results, we hypothesise that benthic fungi that are host-independent (including facultative mutualists) have largely adapted secondarily to anoxic conditions or are temporarily adaptive due to intrinsic traits. Taxa from the basal fungal groups are excluded from this hypothesis, as their true diversity is as yet obscure (Voigt *et al.*, 2021) and therefore other unknown physiological mechanisms may have evolved as part of an evolution in anoxia and/or host-dependence (Berg *et al.*, 2022).

5 Conclusion

The paucity of studies on mycobenthos in general, and on its physiological adaptation to oxygen concentrations, in particular, hinders the development of a conceptual framework for its life in anoxia. Furthermore, there is a lack of understanding of how filamentous fungi occupy the three-dimensional space of the sediment. Should this be analogous to the terrestrial realm, where fungi significantly structure the soil, actively shift and release nutrients via their hyphae, and even create new niches for other organisms (Deveau *et al.*, 2018), there must be a paradigm shift in our understanding of the

benthic system. However, until now, fungi have been largely overlooked in conceptual studies of the seafloor (Grossart *et al.*, 2019). Intensive studies are needed to fill this gap. Thus, the benthic universe is open for mycologists to explore!

Data availability statement

The generated sequence datasets can be obtained from the European Nucleotide Archive (ENA) with the accession number PRJEB54572. The fully annotated OTU table can be accessed over the Supplementary Table S2, representative sequences for each OTU over the Supplementary File S1 (.fasta-format).

Author contributions

MR and MF designed the study and acquired the necessary finances; RN, TRH, CRP, YY and MR analysed the data; YY and MR wrote the manuscript. All authors read and approved the final version of the manuscript.

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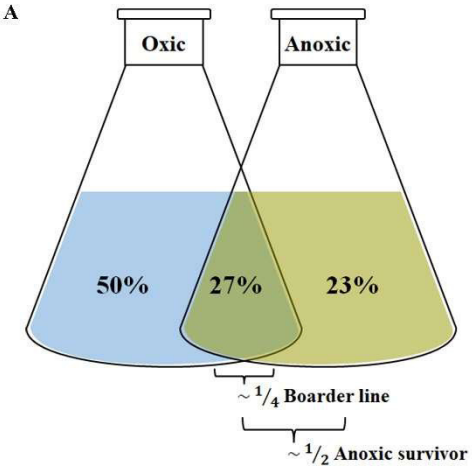
Table 1. Summary of difference between incubated conditions and meta-analysis, showing the dominant group, proportion of anoxic OTUs, cosmopolitan genus, most contributed genus by SIMPER, groups which showing significant difference and alpha-diversity.

		Incubation	Meta-analysis
Dominant group	Phylum	Ascomycota	
	Morphology	Filamentous growth	
	Trophic Modes	Saprotrophic	
% of anoxic OTUs (only)		50% (23%)	21.4% (6%)
Cosmopolitan Genus		Cladosporium, Alternaria	Ascomycota (23), Vishniacozyma, Mortierella
Most contributed genus (Dominant condition)		Lacrymaria (Anoxic)	Psathyrella (Anoxic)
Group with significant difference (Dominant condition)		/	Basidiomycota (Anoxic) Mucoromycota (Oxic)
		Dimorphic yeast (Anoxic)	Yeast (Anoxic), Facutative yeast (Anoxic), Filamentous (Oxic)
Alpha-Diversity comparison		Time-dependent decreasing of diversity (Anoxic) Time-dependent increasing of diversity (Oxic)	Higher in Oxic conditions

Figure 1: Comparison between samples under oxic and anoxic incubation. (A) shared OTUs under anoxic and oxic incubations; (B) trends of alpha-diversity (Chao1 and Shannon) under anoxic and oxic incubations; (C) Significance of effecting factors (oxygen and time) between oxic and anoxic incubations was tested with the PERMANOVA (adonis); (D) Principal component analysis (PCoA) ordinating fungal communities based on their Bray-Curtis dissimilarity. Samples under long oxic incubation clustered as one significant different group (PERMANOVA, $P < 0.001$).



B

α - diversity	Chao1	Shannon
Oxic	↗*	↗
Anoxic	↘*	↘*

C

	R2	F-value	P^{FDR}
Time	0.195	1.541	0.000*
Oxygen	0.044	1.726	0.005*
Oxygen:Time	0.155	1.224	0.025*

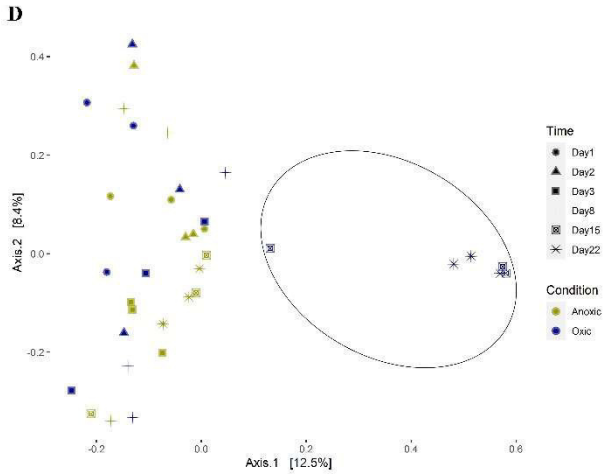
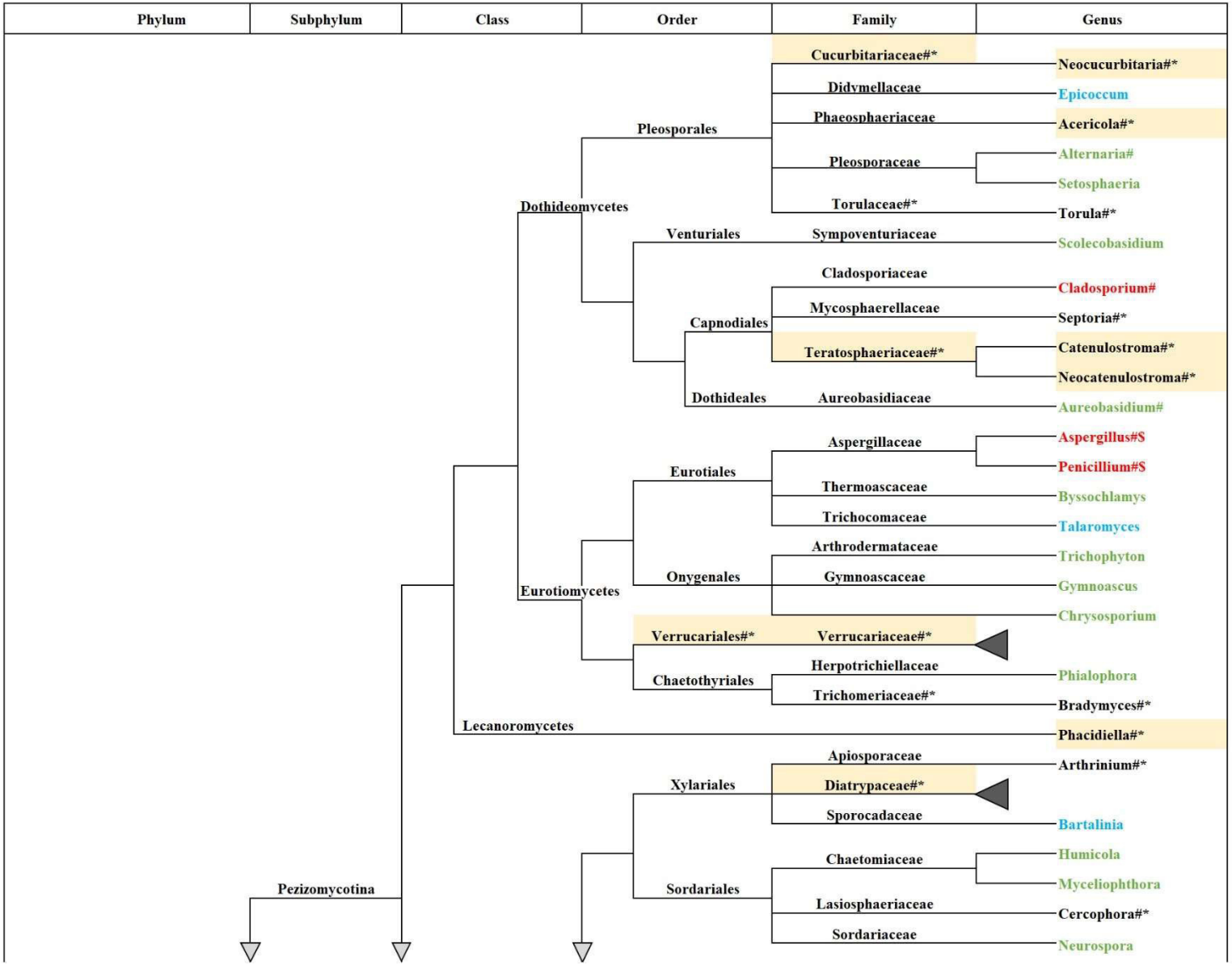
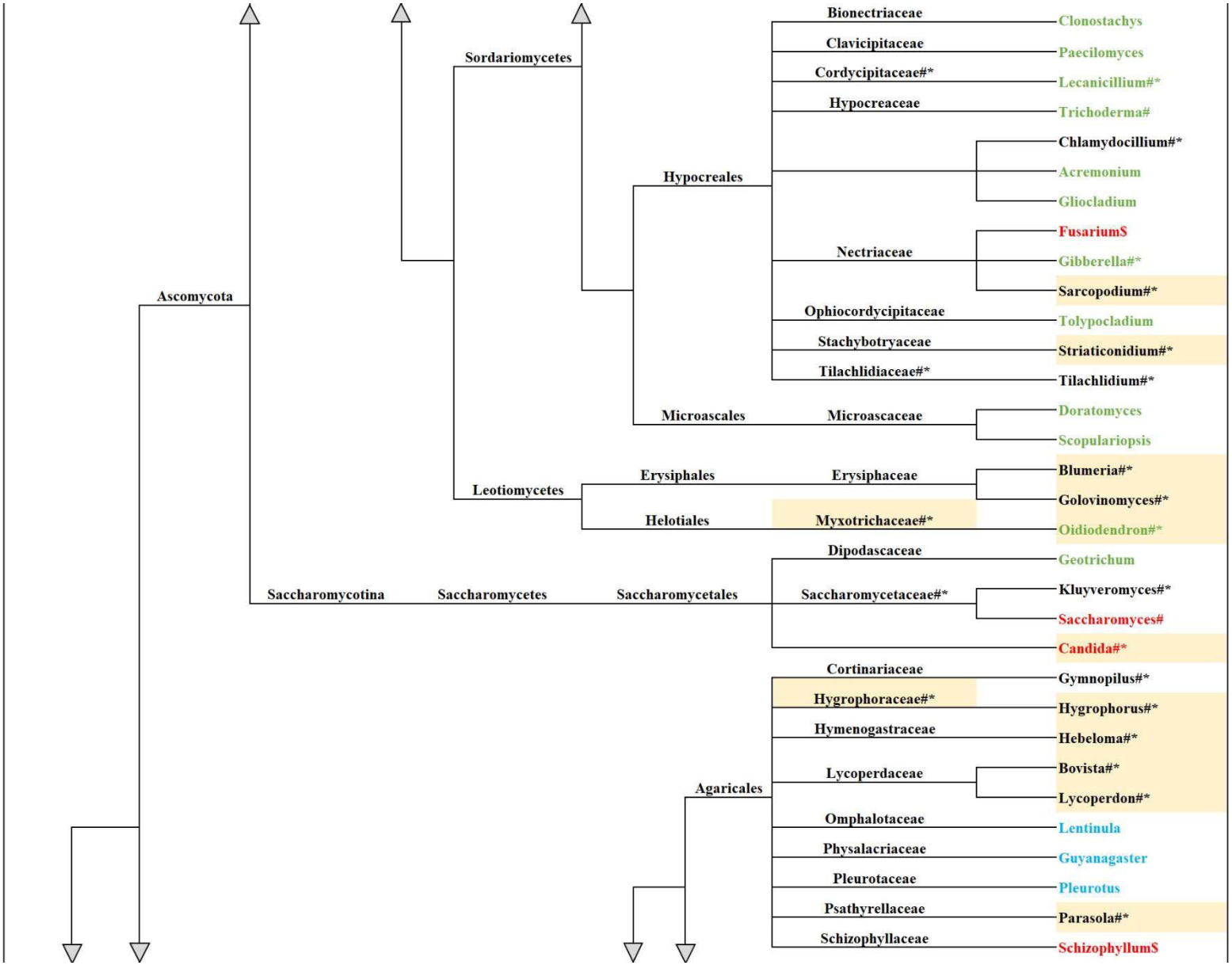


Figure 2: Sketch of the fungal phylogenetic tree to explain the groups which respond to anoxic incubations. #: present in incubation samples; *: showing significance between oxic and anoxic incubation samples; Highlighted with yellow: higher abundance at anoxic incubation; blue marked genera have physiological proof with anoxic fermentation; green marked genera were present in anoxic samples with communities' analysis; red marked genera were present in anoxic samples with fermentation and communities' analysis; $: genera have physiological proof with anaerobic respiration.


Phylum
Subphylum
Class
Order
Family
Genus
Pezizomycotina
Lecanoromycetes
Dothideomycetes
Eurotiomycetes
Pleosporales
Venturiales
Capnodiales
Dothideales
Eurotiales
Onygenales
Verrucariales#*
Chaetothyriales
Xylariales
Sordariales
Cucurbitariaceae#*
Didymellaceae
Phaeosphaeriaceae
Pleosporaceae
Torulaceae#*
Sympoventuriaceae
Cladosporiaceae
Mycosphaerellaceae
Teratosphaeriaceae#*
Aureobasidiaceae
Aspergillaceae
Thermoascaceae
Trichocomaceae
Arthrodermataceae
Gymnoascaceae
Verrucariaceae#*
Herpotrichiellaceae
Trichomeriaceae#*
Apiosporaceae
Diatrypaceae#*
Sporocadaceae
Chaetomiaceae
Lasiosphaeriaceae
Sordariaceae
Neocucurbitaria#*
Epicoccum
Acericola#*
Alternaria#
Setosphaeria
Torula#*
Scolecobasidium
Cladosporium#
Septoria#*
Catenulostroma#*
Neocatenulostroma#*
Aureobasidium#
Aspergillus#S
Penicillium#S
Byssochlamys
Talaromyces
Trichophyton
Gymnoascus
Chrysosporium
Phialophora
Bradymyces#*
Phacidiella#*
Arthrinium#*
Bartalinia
Humicola
Myceliophthora
Cercophora#*
Neurospora


Ascomycota
Saccharomycotina
Saccharomycetes
Saccharomycetales
Saccharomycetaceae#*
Saccharomyces#
Candida#*
Kluyveromyces#*
Dipodascaceae
Geotrichum
Leotiomycetes
Erysiphales
Erysiphaceae
Blumeria#*
Golovinomyces#*
Helotiales
Myxotrichaceae#*
Oidiodendron#*
Sordariomycetes
Hypocreales
Bionectriaceae
Clonostachys
Clavicipitaceae
Paecilomyces
Cordycipitaceae#*
Lecanicillium#*
Hypocreaceae
Trichoderma#
Chlamydocillium#*
Acremonium
Gliocladium
Nectriaceae
FusariumS
Gibberella#*
Sarcopodium#*
Ophiocordycipitaceae
Tolypocladium
Stachybotryaceae
Striaticonidium#*
Tilachlidiaceae#*
Tilachlidium#*
Microascales
Microascaceae
Doratomyces
Scopulariopsis
Agaricales
Cortinariaceae
Gymnopilus#*
Hygrophoraceae#*
Hygrophorus#*
Hymenogastraceae
Hebeloma#*
Lycoperdaceae
Bovista#*
Lycoperdon#*
Omphalotaceae
Lentinula
Physalacriaceae
Guyanagaster
Pleurotaceae
Pleurotus
Psathyrellaceae
Parasola#*
Schizophyllaceae
SchizophyllumS

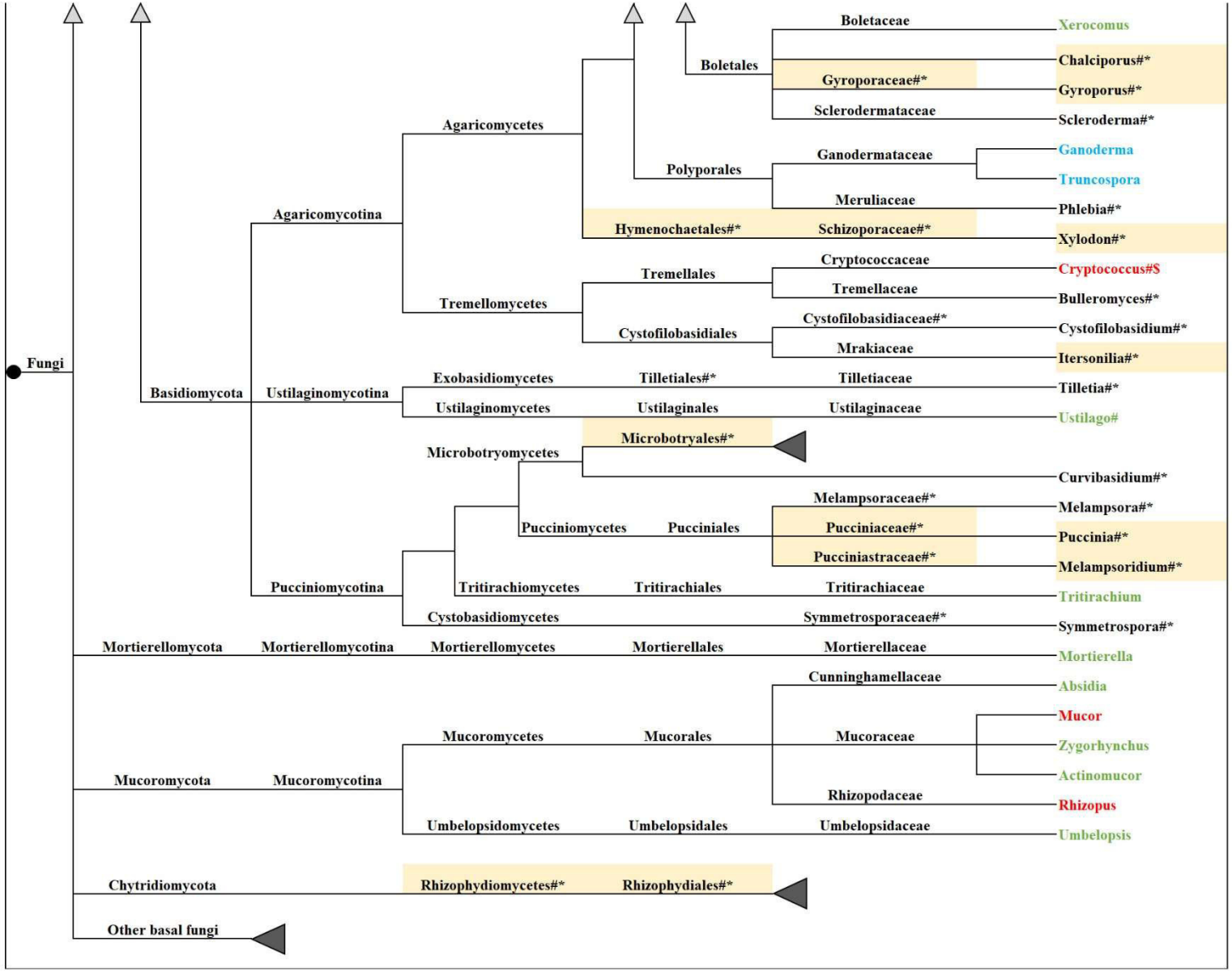
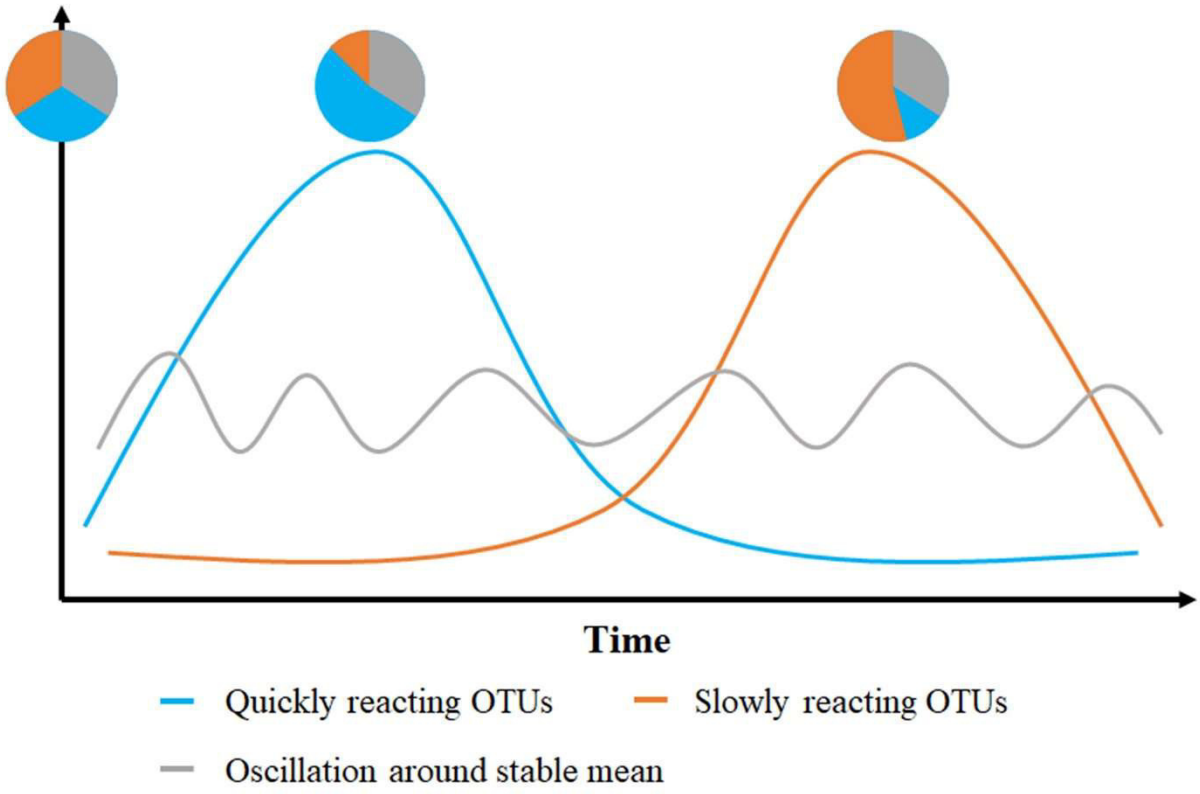

Fungi
Basidiomycota
Agaricomycotina
Agaricomycetes
Boletales
Boletaceae
Xerocomus
Gyroporaceae#*
Chalciporus#*
Gyroporus#*
Sclerodermataceae
Scleroderma#*
Polyporales
Ganodermataceae
Ganoderma
Truncospora
Meruliaceae
Phlebia#*
Hymenochaetales#*
Schizoporaceae#*
Xylodon#*
Tremellomycetes
Tremellales
Cryptococcaceae
Cryptococcus#S
Tremellaceae
Bulleromyces#*
Cystofilobasidiales
Cystofilobasidiaceae#*
Cystofilobasidium#*
Mrakiaceae
Itersonilia#*
Ustilaginomycotina
Exobasidiomycetes
Tilletiales#*
Tilletiaceae
Tilletia#*
Ustilaginomycetes
Ustilaginales
Ustilaginaceae
Ustilago#
Pucciniomycotina
Microbotryomycetes
Microbotryales#*
Curvibasidium#*
Pucciniomycetes
Pucciniales
Melampsoraceae#*
Melampsora#*
Pucciniaceae#*
Puccinia#*
Pucciniastraceae#*
Melampsoridium#*
Tritirachiomycetes
Tritirachiales
Tritirachiaceae
Tritirachium
Cystobasidiomycetes
Symmetrosporaceae#*
Symmetrospora#*
Mortierellomycota
Mortierellomycotina
Mortierellomycetes
Mortierellales
Mortierellaceae
Mortierella
Mucoromycota
Mucoromycotina
Mucoromycetes
Mucorales
Cunninghamellaceae
Absidia
Mucoraceae
Mucor
Zygorhynchus
Actinomucor
Rhizopodaceae
Rhizopus
Umbelopsidomycetes
Umbelopsidales
Umbelopsidaceae
Umbelopsis
Chytridiomycota
Rhizophydiomycetes#*
Rhizophydiales#*
Other basal fungi

Figure 3: Relative frequencies changing of OTUs under anoxic incubation along incubated time, showing three different distribution patterns: blue marked quickly responding OTUs, orange marked slowly responding OTUs, and grey marked OTUs which oscillated around the stable mean.



5 Discussion

Throughout history, aquatic fungi have proven themselves as ubiquitous actors across the Earth's ecosystem and as key components in nutrient cycles. However, there are still many obstacles and bottlenecks in identifying aquatic fungi and exploring their ecological functions. The aim of this study is to understand how aquatic fungal communities are spatially resolved under the interaction of ecological processes or single to multiple environmental factors in (i) river and adjacent river plumes, and (ii) benthic systems.

5.1 Why conducted fungal community analysis?

Our knowledge on the diversity and distribution of aquatic fungi is fragmentary in many aspects, and thus there is an urgent need to conduct community analysis. Community analysis is the first step to understanding the role of fungi in ecosystems, followed by testing of the hypothesis developed based on the community structure. This process helps to understand "*What exists in a given aquatic ecosystem?*" and "*How does the community change over the environment, time, and space?*" (Hajibabaei *et al.*, 2011). However, even if it is possible to get a consistent sample set to test environmental or spatial factor related hypotheses, several obstacles still occur due to technical issues. In the following section, some of the most common obstacles in the analysis of aquatic fungi communities, which also include the ones encountered in this project, are discussed, such as a lack of well-established reference databases, inaccurate taxonomic assignments, and a large number of undescribed taxa, etc.

5.2 Exploration of novel niches

An obstacle to aquatic fungal community analysis is that even though assessments of fungi biodiversity have been conducted worldwide (from polar to tropic regions), many sites and ecosystems remain unknown (Grossart *et al.*, 2019). This project has dealt with a large diversity of the fungal community in aquatic ecosystems, ranging from pelagic water to benthic sediments. Diversity and community analysis provide insights into the ecological role of fungi and more effective maintenance of sustainable ecosystems in the future. In addition, it contributes to further understanding of the productivity, disturbance resistance and recovery capacity of the ecosystems. At the same time it suggests a high opportunity for species to develop specific niches (Logares *et al.*, 2017). In turn, this means there are

more aquatic fungal niches waiting to be uncovered, such as the "plastisphere" (Zettler *et al.*, 2013), and "marine snow"(Bochdansky *et al.*, 2017).

5.3 Resolution strategies for unclassified fungi

Although up to 5 million fungal species are estimated worldwide(Blackwell, 2011), only a small fraction of them have been named and represented by a GenBank sequence (Porter and Golding, 2011). Moreover, the proportion of unidentified fungal clusters has been reported to exceed the number of identified fungal clusters in GenBank (Hibbett D and D., 2011). Increasing evidence suggests that the map of mycobiome diversity was redrawn by HTS studies (Nilsson *et al.*, 2019a). They uncovered an enormous unclassified taxonomic and functional diversity, which can only be assigned at the kingdom level (Hassett *et al.*, 2020). This hinders targeted statistics, comparability, ecological function prediction and identification on aquatic fungi.

There are possible solutions to better classify fungal sequences and describe fungal diversity, such as through phylogenetic placement in a reference tree. This involves the integration of sequences into an existing phylogenetic reference tree based on phylogenetic criterion without changing the overall topology of the entire tree, providing the possibility to classify unknown/novel fungal lineages (Reich and Labes, 2017). A reliable phylogenetic tree of fungal 18S rRNA gene sequences based on curated alignment has been constructed by Yarza *et al.* (2017). However, this fungal phylogenetic tree still needs to be enriched with novel fungal sequences. In paper I, the selected fungal phylogenetic reference tree originally contains 9329 representative 18S rRNA gene sequences, covering the whole fungal kingdom (Yarza *et al.*, 2017), and was further enriched with (i) 254 fungal 18S rRNA gene sequences of the SILVA dataset SSURef_128 not yet present in the tree; (ii) 210 reference sequences of so-far unrecognized soil-inhabiting order-level clades described by Tedersoo *et al.* (2017), namely clade GS02, GS07-11, GS13, GS15, and GS19, and (iii) 79 sequences of newly identified basal fungal taxa ((Seto *et al.*, 2020; Simmons *et al.*, 2020) , and Lee *et al.*, unpublished data (INSDC accession numbers: KJ668047- KJ668085)), namely *Zygorhizidiales*, and *Chytridiales*. In the end, there are only 50 OTUs that are assigned as fungi, not lower taxonomic levels. With phylogenetic placement, they are assigned as "basal fungi", forming 3 different clusters/branches.

Another good strategy is the assignment of DOIs to fungal sequences of unknown taxonomy. This

approach is employed by the UNITE database and involves the realignment of sequences to the database and subsequent clustering of unknown sequences into based on similarity thresholds to form sequence hypotheses (SHs) (Nilsson *et al.*, 2019b).

However, since there is no pipeline/program covering all databases and classification principles, it is recommended to manually check the taxonomic assignment and evaluate the affinity for different taxonomic levels (Tedersoo *et al.*, 2015). In paper II and paper III, I attempted to improve the taxonomic classification of the most prominent OTUs by using additional classification options: (i) the results of the RDP classifier were checked manually; (ii) BLASTn (Altschul *et al.*, 1990) was performed on the UNITE webpage; (iii) the secondary structure of the ITS2 was used via the web-based ITS2 Database (Schultz *et al.*, 2006; Ankenbrand *et al.*, 2015). In the end, we found that approximately 25% OTUs still remain unidentified in marine sediments, which calls for expanded sequencing of new specimens and cultures. Nevertheless, this also highlights the utilization of 18S rRNA to address the analysis of marine fungal communities.

Additionally, even if the taxonomy cannot be resolved completely, the sequences are a basis from which more targeted studies on a specific taxon can be conducted. It allows the designing of 18S rRNA-targeted oligonucleotide probes of unidentified fungal lineages used for catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH), such as the visualization of *Rozellomycota* (Priest *et al.*, 2021).

5.4 Emerging Technologies – long read sequencing

Currently, multiple marker genes are used for taxa identification based on molecular approaches, such as 18S rRNA (~1800bp) (Karst *et al.*, 2018), ITS (~800bp) (Porter and Golding, 2011), 28S rRNA(~2900bp) (Khot *et al.*, 2009), etc. However, due to technical limitations, full-length marker genes cannot be directly sequenced by cost-effective high-throughput sequencing methods (Next-Generation Sequencing Technology, NGS). Therefore, most of the environmental biodiversity screening is dependent on short DNA reference sequences, covering only a single or two variable regions of the marker gene. In this project, paper I amplified V7-V8 region of SSU, and paper II and paper III amplified ITS2 region. As discussed in the above section, compared to ITS, 18S RNA has additional advantages in terms of classification, detection, and grouping for marine fungal communities. However, ITS2

allowed the identification of more OTUs at the species level. Additionally, to get accurate taxonomy, the lowest common ancestor (LCA) rule was applied in this project. As it was recommended to get a reliable classification at higher taxonomic level rather than wrong classification down to the genus or species level (Reich and Labes, 2017).

A major limitation with short read sequencing is that the length of the reads does not provide enough phylogenetic power for accurate taxonomic classification at below genus-level. This also often results in a single species being classified in different taxa when different marker genes are used (Heeger *et al.*, 2018). Furthermore, the large diversity of undescribed fungi, (DMF), is likely to be abundant across the entire tree (Grossart *et al.*, 2016), and it has often been documented that sequences without any homologies in several databases exist (Page, 2016). This low taxonomic resolution power has been ascribed to the large number of non-cultivable and undescribed fungal taxa but also the "short-reads" of the rRNA barcodes (Tedersoo *et al.*, 2014). However, it has been proposed that third-generation sequencing combined with eDNA-metabarcoding technology to sequence the entire rRNA operon could overcome this (including SSU, ITS and LSU) (Tedersoo *et al.*, 2017; Jamy *et al.*, 2020; Latz *et al.*, 2022).

The advantage of long-, over short read sequencing over short reads sequencing is increasing the accurate identification of microbial community diversity. Third-generation sequencing systems, such as, Pacific Bioscience (PacBio), Oxford Nanopore Technologies (ONT), Illumina and 10x Genomics, provide much longer reads than previous generations (Goodwin *et al.*, 2016). The Single-molecule real-time (SMRT) technology is the most widely used long-read platform from PacBio. The Synthetic long reads technology is used by Illumina and 10x Genomics (Goodwin *et al.*, 2016). Additionally, the mock community demonstrated that the SMRT-Sequel (PacBio) is more efficient for metabarcoding of complex samples, while MinION (Nanopore) with high error rates can be useful for identifying major pathogens quickly and accurately (Loit *et al.*, 2019; Mafune *et al.*, 2019; Castano *et al.*, 2020).

Although they have been around for several years, the read quality has only been improved enough recently to enable them to be relevant in metabarcoding and community analysis (Tedersoo *et al.*, 2021a). By taking advantage of this long reads sequencing technology, full-length marker genes sequencing has become obtainable to achieve accurate "species" classification and identification (Aragona *et al.*, 2022), which can be used in many fields, such as species evolution, species abundance, environment and ecology, etc. However, long reads sequencing has a critical drawback, as the comparable datasets and

bioinformatic pipelines are still missing. (Nilsson *et al.*, 2019a).

A study tested the feasibility of applying single-molecule real-time (SMRT) technology to barcode database construction, with the most complex samples tested having mitochondrial cytochrome c oxidase I (COI) amplicons in DNA from up to 10,000 different samples (Hebert *et al.*, 2018). The results suggest that as its sequencing cost decreases further, SMRT technology will be a powerful method for constructing DNA barcode databases. Moreover, metabarcoding analysis of tree roots (Marciulyniene *et al.*, 2021), leaves and tubers (Loit *et al.*, 2019), soil (Tedersoo *et al.*, 2018; Jamy *et al.*, 2020; Tedersoo *et al.*, 2020; Furneaux *et al.*, 2021), mangrove sediments (Zhang *et al.*, 2021), and lake water/sediment(Heeger *et al.*, 2018) has demonstrated that long-read sequencing targeting SSU, LSU rRNA genes and the full length of ITS regions can be used for broad ecological studies that require the accurate characterization of fungal biomes from complex environmental samples (Hoang *et al.*, 2022).

5.5 Driving factors – Where are we now?

Understanding the occurrence and maintenance of biodiversity is always the key topic in the study of ecology. However, the interlinkages between aquatic fungal community composition and structure with their potential drivers are still in the mist.

Currently, microbial biogeography has started to shift from the description of distribution patterns to the study of microbial community structuring processes. Therefore, besides the multiple environmental factors, it is also important to understand which assemblage processes affect the aquatic fungal communities and whether they differ between distributed patterns. However, there is still a lack of studies on the structuring processes of aquatic fungal communities at a global scale. Particularly, for benthic fungi, there are no studies on their structuring processes. In paper I, an ecological framework of quantitative process estimates (QPEs) method (Stegen *et al.*, 2015) was applied, which revealed that the assembly processes underlying the mycobiomes differed significantly.

Although the patterns can be discerned at smaller spatial and taxonomic scales, as well as between certain functional groups, the distribution patterns and abundance of fungal communities are not determined by one single factor (Nilsson *et al.*, 2019a). In paper I, it is revealed that multiple environmental factors working with habitat types and spatial distance affect the distributions of fungal communities and can drastically change over very short distances. At a smaller scale, environmental

factors play a bigger role in shaping the composition of fungal communities, compared to geographic distance. These findings are in agreement with previous studies on fungal distribution (Tisthammer *et al.*, 2016; Duarte *et al.*, 2017; Tian *et al.*, 2018; Nilsson *et al.*, 2019a). In paper II, it is shown that the distribution of benthic fungi is limited and probably controlled by a whole bouquet of factors in which geographic distance, habitat specialization but also traits related to life history influence the distribution. Together with paper I, it is shown that at a large scale, community similarity decreases as a function of the geographic distance (Duarte *et al.*, 2016; Duarte *et al.*, 2017).

In paper III, the effect of oxic/anoxic incubation was further discussed. Oxygen seems to act as a significant factor in shaping the structure of fungal communities. Furthermore, fungal communities have developed different adaptive strategies at different time scales, including multiple taxa, morphological types, and trophic forms, to respond to oxic and anoxic environments. Based on thorough screening of current literature (paper III), apart from the known anaerobic phylum *Neocallimastigomycota*, there are 87 genera (the majority are *Ascomycota* and *Basidiomycota*) showing potentially adaptive strategies to anoxic conditions. Among them, 18 genera were verified to harbor anaerobic growth capacities through fermentation or anaerobic respiration. However, with the development of new oxygen-sensing technologies, oxygen detection limits are being pushed lower and lower (Berg *et al.*, 2022). This brings up further thoughts about "what is a truly anoxic environment?". According to Berg *et al.* (2022): an environment is defined as "apparently anoxic", when oxygen content is <3 nmol L^{-1}. Anoxic refers to the condition when oxygen content is zero. This suggests the need to re-evaluate the fungal taxa that can handle anoxic environments. However, perhaps the question is not really whether a fungus is strictly an anaerobic species, but rather physiological mechanisms by which aquatic fungi thrive in oxygen-limited habitats, such as marine oxygen minimum zones (OMZs), sediments, stratified lakes, and anoxic microniches within marine snow particles. Though no specific biotic factors were studied in this project, they do play a significant role in shaping marine fungal communities. Perhaps they are responsible for the unexplained variations (paper I and paper II).

5.6 Concept developing of aquatic fungi at a global scale

HTS studies often recover tens of thousands OTUs, which help to describe the global fungal biodiversity in targeted habitats. Multiple global research studies have been applied to the terrestrial fungal community (Tedersoo *et al.*, 2014; Tedersoo *et al.*, 2021b). To boost further research in marine fungal

diversity, biogeography and macroecology, global surveys are needed. Present global research has revealed an omnipresence of fungi in the world's oceans, and they may play an important role in ecosystem function by providing key nutrients (Tisthammer *et al.*, 2016; Morales *et al.*, 2019; Hassett *et al.*, 2020). Based on this, it is possible to develop concepts for understanding the integration of microbial communities in ecosystem functioning. The developed concepts “mycoloop” and “mycoflux” have well explained that pelagic fungi play a mediating role between different organisms and ecosystems (Kagami *et al.*, 2007; Grossart *et al.*, 2019). Although, “benthic shunt” was proposed to refer to the ecological role of benthic fungi, it has not been explicitly described for marine ecosystems (Grossart *et al.*, 2019). To readjust and fill this gap, the first step is to understand the global composition and distribution patterns of marine benthic fungi. Paper II provides an initial conceptual framework of distribution patterns of marine benthic fungi. It showed that the distance of 100 km was identified as the critical threshold for the benthic fungal dispersal, which agreed with the lake sediments (Tian *et al.*, 2018). In addition, similar to 3000 km as distance barriers for marine bacteria (Martiny *et al.*, 2006), 2500 km appears to have the general validity for the dispersal of benthic marine fungi (paper II). Although through continuous research, a corner of the marine fungal community at a global scale has been gradually uncovered, the global distribution patterns of the whole marine fungal community remain largely enigmatic.

6 Challenges and prospects

As shown by the three papers in this thesis, marine fungal communities are diverse in pelagic and benthic freshwater and marine environments. Considering that the ocean is the largest continuous habitat on Earth while only 1901 species of marine fungi have been described, the fungal diversity in global aquatic systems is greatly underestimated. Therefore, marine mycology is currently facing many serious challenges and gaps (Amend *et al.*, 2019).

The most pressing issue to be addressed is how to handle the growing need for new species description from marine with the current reference databases. This is mainly caused by the scarcity of taxonomic and evolutionary studies on marine strains, resulting from which many gaps still exist in phylogenetic trees and reference databases. A problem that followed from this, is the overrepresented terrestrial sequence data in databases that bias the process of primer design for marine fungal taxa (Amend *et al.*, 2019). This has greatly limited the development of the molecular biology of marine mycology. Furthermore, along with the development of third-generation sequencing systems, the need for optimal fungi-specific primer pairs targeting the full-length marker genes keeps growing. Therefore, there is an intense need for more research on marine fungal communities, which will enrich the public sequence library. Thereby, it will also improve the potential primer biases which are caused by the overrepresented terrestrial sequence databases. Improved primer pairs would allow for discovering more specific marine phyla and in turn for covering the entire fungal kingdom.

Culturability is accepted as one of the major barriers and limitations to marine mycology (Overy *et al.*, 2019). Although multiple studies have isolated some obligate marine fungal cultures, this is grossly insufficient, which is primarily a result of under-sampling. Many niches, substrates, and geographic sites are currently understudied concerning bioprospecting. Additionally, marine fungi are valuable resources for subsequent research and utilization. Such as, setting up and maintaining a laboratory model to explore the interactions between fungi and other coexisting organisms. Applying genomics and transcriptomics on specific isolates allows for exploring their molecular strategies as a response to different changes or growth pressure. Therefore, there is an urgent need to strengthen the research on the culture of marine fungal taxa. When new research projects are planned, environmental RNA (eRNA) with long read sequencing, stable isotope probing (SIP) with labelled phytoplankton polysaccharides and additional methods for isolation should be attempted. Combined with the meta-genomics and meta-

transcriptomics, will greatly facilitate a deeper understanding of the marine fungal enzymatic abilities in the microbial loop and carbon cycling. This would allow for the discovery of metabolically active fungal communities. Thereby, more targeted isolation could be screened.

Other challenges/gaps are the lack of genome and genetic data on marine fungi, available deep RNAseq or proteomic data sets, and difficulties to maintain a laboratory model setting to explore the interactions between fungi as well as the biotic and abiotic factors. These data are of crucial relevance to ecological, taxonomic, and biotechnological research of marine mycology. Although there are some first insights, the overall scope/state of research is still far from sufficient.

Additionally, future studies require researchers to innovate research models with new technologies, ideas, and mechanisms from an integrated multidisciplinary and comprehensive perspective. There is a growing need for reproducibility and public data availability of fungal communities. Therefore, another challenge is to commence the ecological research of marine mycology at a global scale, with a standardized set of sampling and processing protocols, which, however, has not yet been established, like Ocean Sampling Day etc.

Despite these drawbacks and limitations, as a mycologist, I believe that with targeted efforts, these challenges can be overcome.

7 Conclusions

This thesis aimed to understand how aquatic fungal communities in (i) river and adjacent river plumes, and (ii) benthic systems are spatially resolved under the interaction of ecological processes or single to multiple environmental factors.

My work resulted into: (i) a first explanatory approach of different assemblage processes and their influence on mycopelagics; (ii) a first conceptual framework for distribution patterns, distance threshold and influencing traits on the biogeography of mycobenthos at a global scale; and (iii) a first incubation experiment on community level to assess the ability of different fungal taxa to survive under anoxic conditions. During my analysis, the need to understand the structuring processes between multiple environmental factors at different spatial scales was highlighted. Furthermore, for technical contributions, this thesis enriched a reliable phylogenetic tree of fungal 18S rRNA gene with novel fungal sequences, especially for zoosporic fungal sequences. This facilitates the assignment of unidentified fungi, especially to DMF, thus aiding in future recognition of different environmental clades.

However, the work also points to persistent deficiencies in techniques and databases that hinder good resolution of aquatic mycobiomes. There is still (i) extremely limited number of available datasets on aquatic fungi precluding the development of meaningful frameworks, especially for the benthic realm; (ii) extremely limited number of obligate marine fungal cultures hindering the hypothesis verification for the functionalism of marine fungi; and (iii) limitations in accessibility and numbers of driving factors restricting the comprehensive understanding of the structuring forces of microbial community.

Therefore, to get a clear picture of aquatic fungal community, next steps could be applied in (i) summarizing publicly available databases that targeted multiple marker genes; (ii) including more specific fungal niches and influencing factors; and (iii) isolating more taxa with targeted functions.

Taken together, this is the basis for future work.

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9 Appendix

9.1 Supplementary material of Paper I

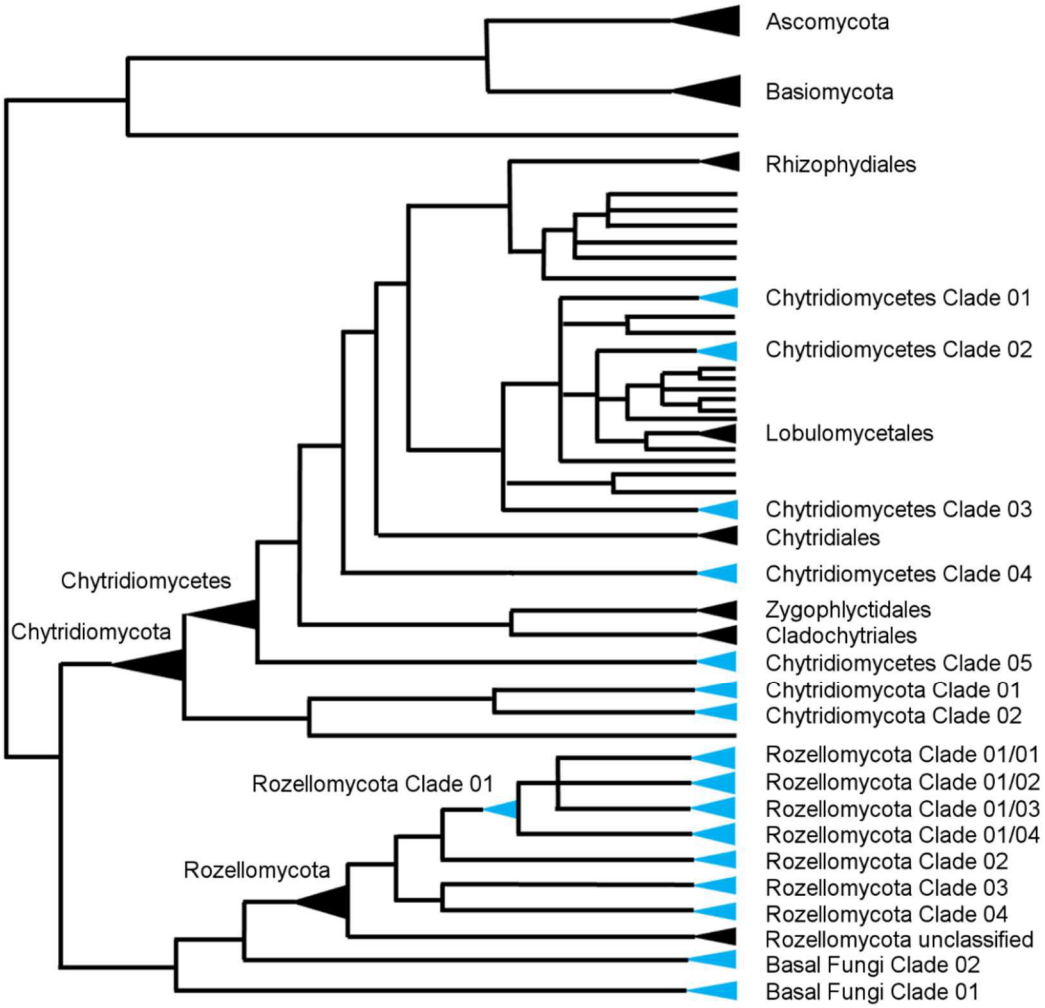
Supplementary Material

Myoplankton biome structure and assemblage processes differ significantly along a transect from the shallow freshwater area of the Elbe River down to the river plume and the adjacent marine waters

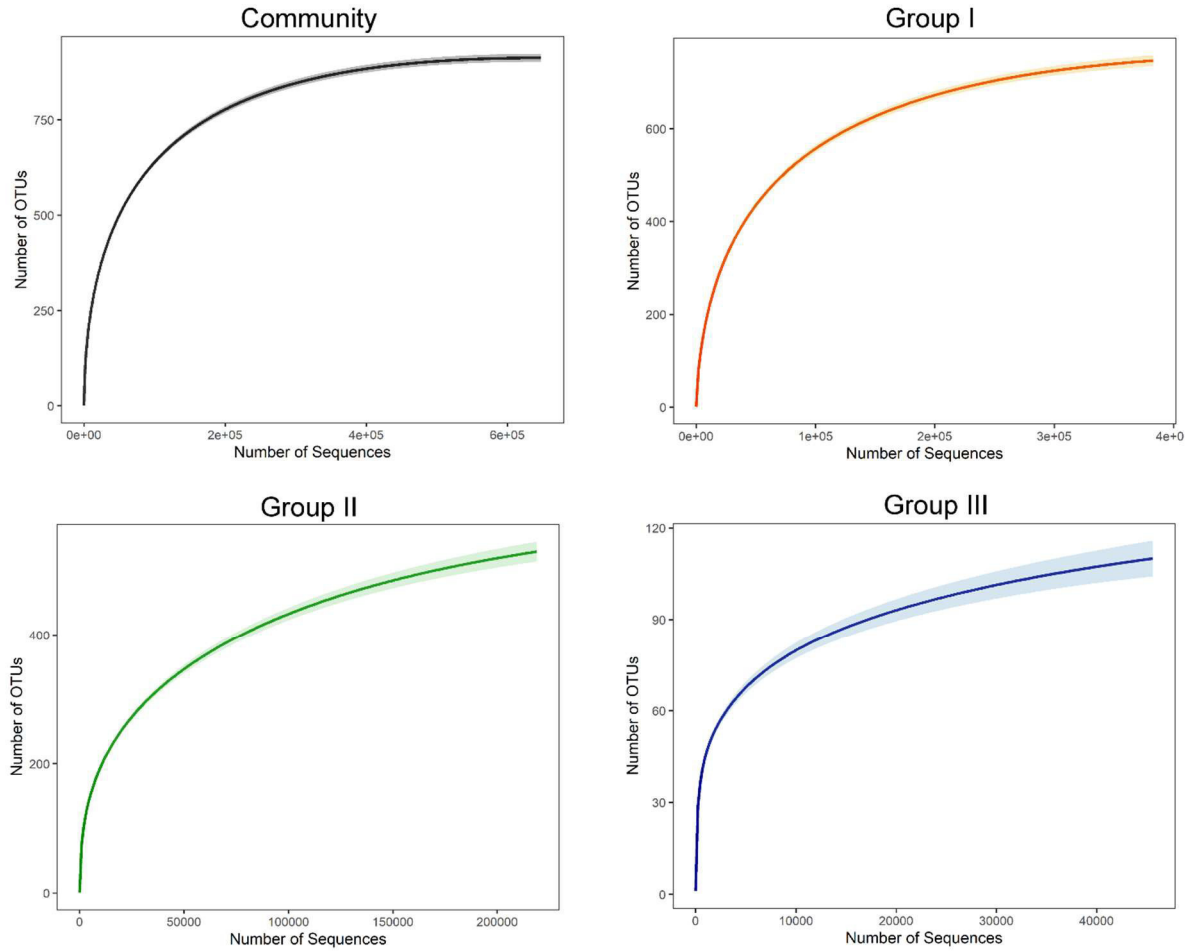
Yanyan Yang, Stefanos Banos, Gunnar Gerdts, Antje Wichels, Marlis Reich

Supplementary Figures

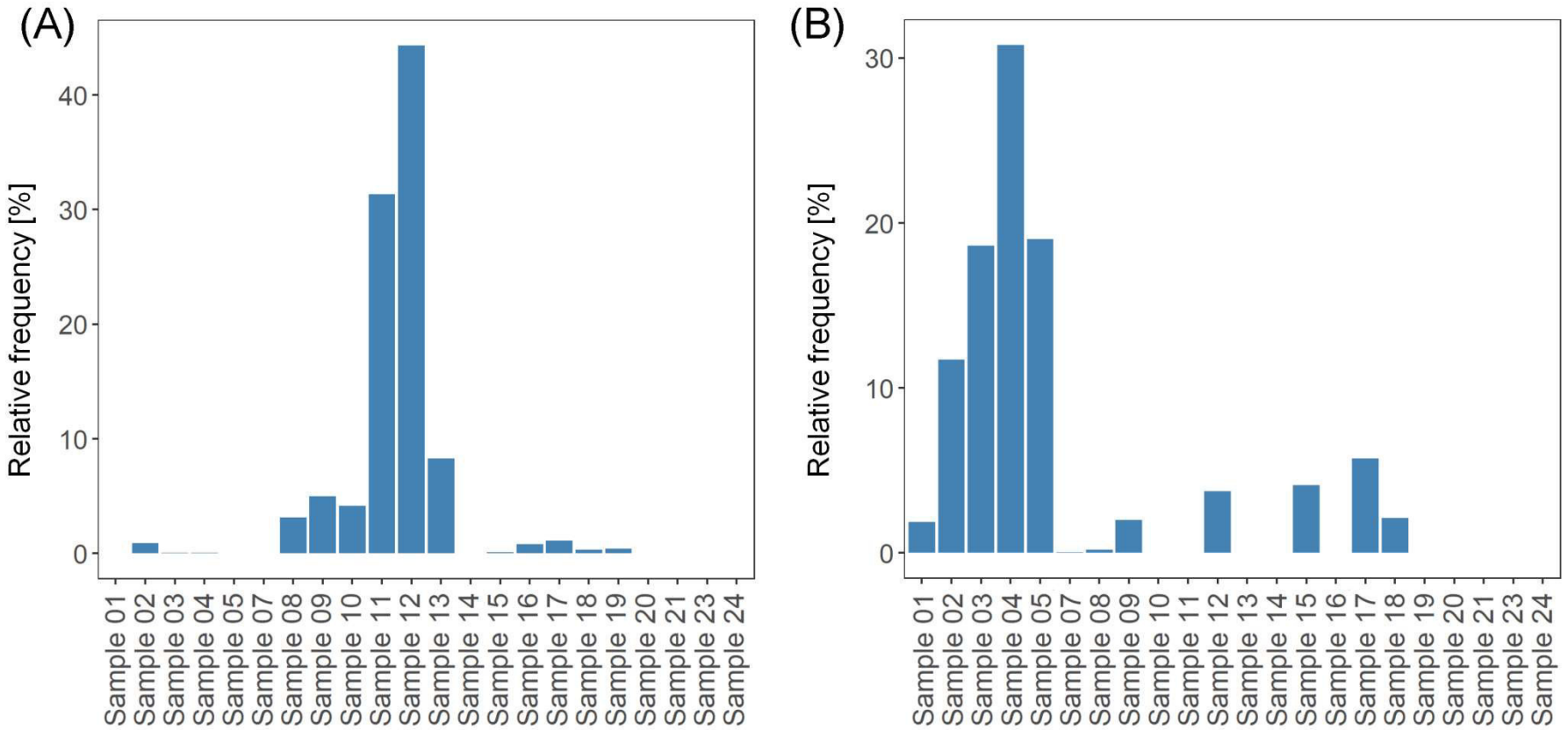
Supplementary Figure S1. Sketch of the fungal phylogenetic tree to explain why environmental clades were classified at different taxonomic levels. Novel diversity clades are colored in blue. Their taxonomic position depends on the branch on which the clade is located and if this branch has a reliable taxonomic assignment.



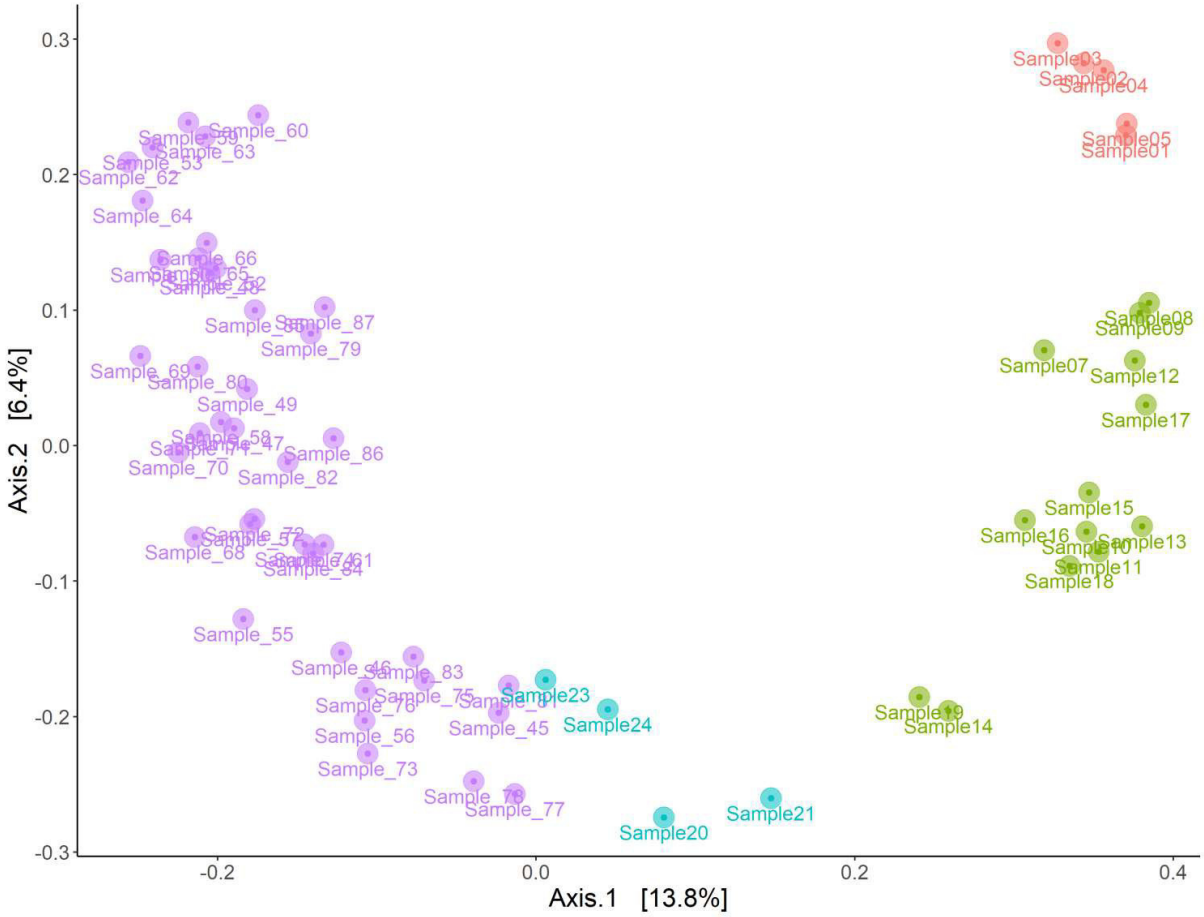
Supplementary Figure S2. Rarefaction curves calculated on all generated sequences (communities over the total transect) and sample group wise (see PCoA, Fig. 2). Shaded area indicates the 95%-confidence interval.



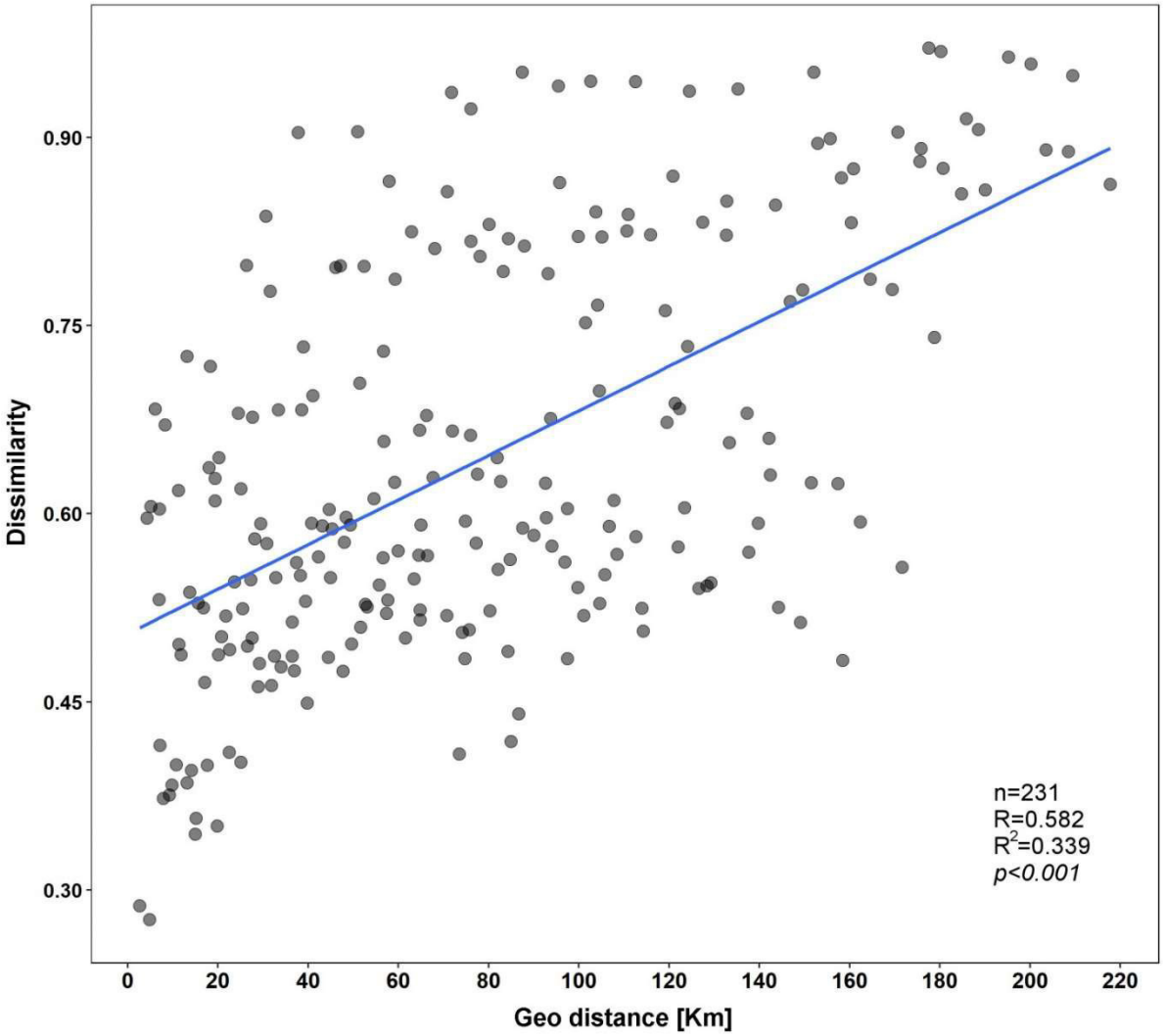
Supplementary Figure S3. Within the estuary of the Eble River, two different distribution patterns of the abundant OTUs were observed (samples 7-24). OTUs being present (A) over numerous adjacent sampling sites (>4; like OTU SMBZZZ14, Rhizophydiales, saprotroph), or (B) high frequency at single sampling sites if occurring in adjacent sites in a maximum of 3 adjacent sampling stations (like OTU SBMZZZ18, Chytridiomycota clade 01, saprotroph).



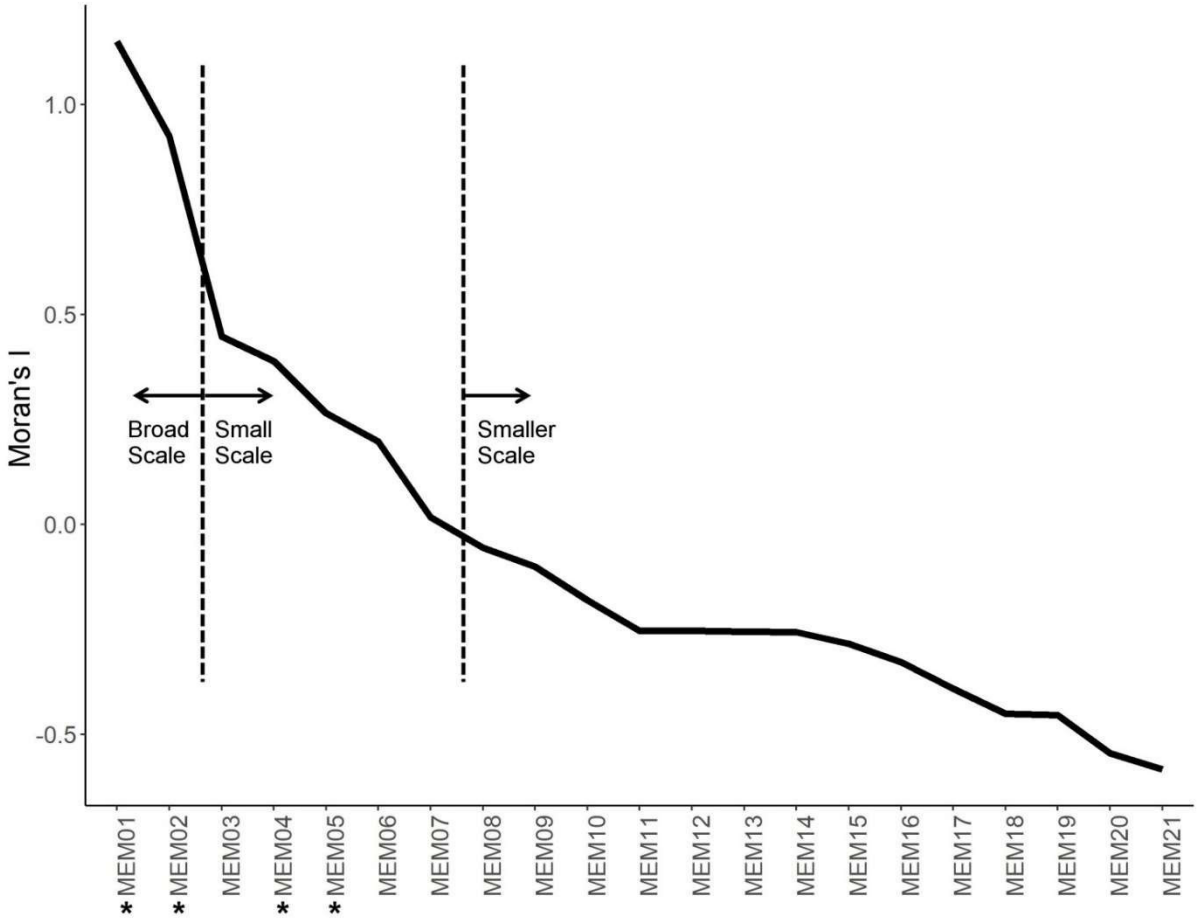
Supplementary Figure. S4: PCoA segregating the mycoplankton communities of the end members of the studied transect (marine environment, samples 20-24) with mycoplankton communities of Helgoland Roads described by Banos *et al*. (2020). PCoA is based on Generalized UniFrac values with the distance parameter of "0" accounting only for the phylogenetic structure and not abundances. Samples 1-24 are samples from the transect. Red, group I; green, group II; blue, group III. Group III are marine samples where the river plume faded off. Samples 45-87 in pink are from Helgoland Roads (Banos *et al.*, 2020, DOI: 10.3389/fmicb.2020.01305).



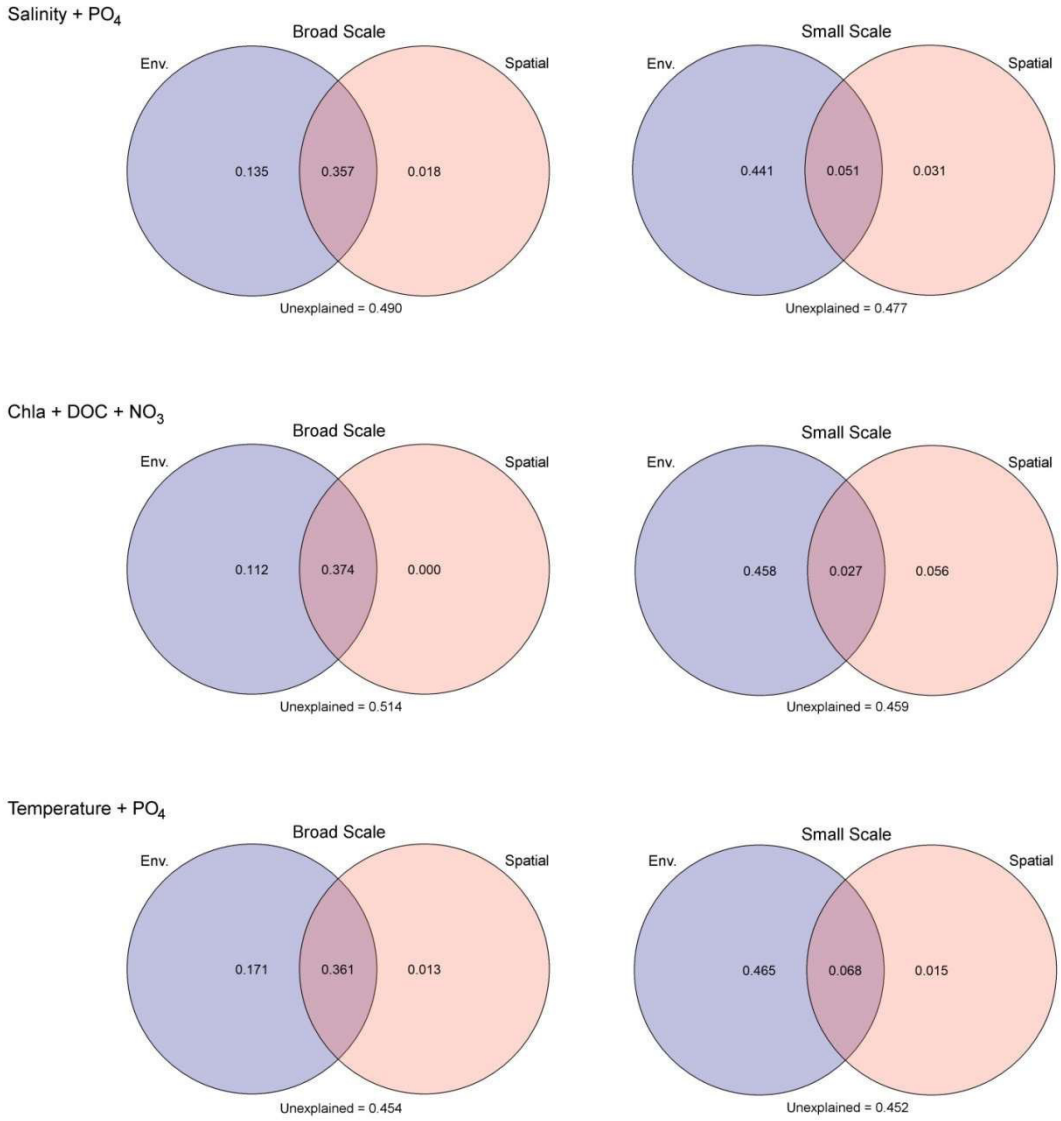
Supplementary Figure S5: Distance-decay analysis based on linear regression indicating a significant relationship of increasing phylogenetic dissimilarity among communities with increasing distance (km) of sampling sites. Phylogenetic dissimilarity calculated as Generalized UniFrac-value. Distance between sampling sites was calculated as cumulative water channel distance.



Supplementary Figure S6. Definitions of the two spatial submodules used for VPA analysis were based on the scalogram using the Moran's I coefficient as ordinate. *, significant eigenfunctions (forward selection, $P < .05$).



Supplementary Figure S6: VPA calculated with three different environmental models. Due to high collinearity among some of the environmental variables, three different models were calculated with db-RDA based forward selection. The best model was the one with salinity and PO_4. However, all models showed a similar trend indicating that observed variability among mycoplankton communities in the lower reaches of the Elbe River are mainly driven by environmental factors, which are partly under spatial control.



Supplementary Tables

Supplementary Table S1: Geographic location and grouping of samples. Sample grouping as given by PCoA and confirmed by PERMANOVA analysis ($P < 0.05$). Additionally, samples were manually grouped into samples of fresh, brackish, and marine water types based on their salinity value. Some samples were spared out from this analysis as they strongly vary in salinity over the course of a year and thus cannot be assigned into one of the groups

Sample	Ost	Nord	Temp. (°C)	DOC (µmol/l)	Chl a (µg/l)	SiO4 (µmol/l)	PO4 (µmol/l)	NO2 (µmol/l)	NO3 (µmol/l)	NH4 (µmol/l)	pH	Salinity (PSU)	Depth (m)
Sample1	10.552	53.370	24.2	432.22	91.58	60.42	0.34	1.03	43.22	1.84	8.94	<0.5[$]	2.50*
Sample2	10.427	53.401	24.7	417.63	68.06	48.05	0.09	0.87	33.74	1.18	9.07	<0.5[$]	2.50*
Sample3	10.367	53.428	24.1	422.77	72.04	49.90	0.19	1.23	51.72	1.60	8.92	0.41[#4]	2.28*
Sample4	10.173	53.395	22.2	365.34	55.69	88.40	0.24	1.16	56.80	2.74	8.34	0.43[#3]	3.50*
Sample5	10.143	53.415	22.3	413.65	56.74	62.71	0.36	0.96	46.37	2.85	8.54	<0.5[$]	3.50*
Sample6	9.984	53.474	20.9	409.12	50.37	46.63	0.31	0.93	41.05	3.61	8.31	NA	NA
Sample7	9.879	53.536	21.2	472.51	15.42	30.18	1.49	2.46	74.28	4.30	7.81	0.47[#2]	7.00*
Sample8	9.633	53.573	20.3	399.20	5.18	33.77	1.93	0.10	64.17	0.76	7.76	0.49[#1]	7.00*
Sample9	9.517	53.643	20	387.59	5.39	39.41	1.29	0.09	99.79	0.40	7.78	0.70	19.50*
Sample10	9.431	53.733	20	401.15	5.88	49.48	1.48	0.11	103.60	0.48	7.87	1.00	13.00*
Sample11	9.363	53.813	19	567.21	6.13	54.23	1.72	0.27	107.73	0.41	7.99	2.00	20.00*
Sample12	9.287	53.860	19	418.66	3.79	54.43	1.83	0.72	104.69	0.72	7.96	4.20	15.00*
Sample13	9.168	53.877	18	422.97	4.17	52.35	2.25	1.19	95.58	0.48	7.96	7.40	15.00*
Sample14	8.998	53.850	18	399.67	6.45	36.94	1.69	1.07	75.40	1.84	8.03	12.90	7.00*
Sample15	8.935	53.842	18	354.37	10.39	30.67	1.79	0.97	67.28	2.63	8.05	15.10	17.00*
Sample16	8.780	53.923	18	331.77	7.29	13.54	1.03	0.59	37.30	1.35	8.16	22.30	25.00*
Sample17	8.680	53.900	18.3	513.15	8.20	25.66	2.29	0.95	36.80	1.86	8.09	17.79	15.00
Sample18	8.500	53.950	18.1	315.26	6.62	9.64	1.31	0.59	18.79	1.44	8.17	27.03	13.00
Sample19	8.405	53.982	18	469.94	6.58	5.24	0.78	0.37	10.44	0.59	8.21	27.56	18.00
Sample20	8.312	53.990	18.1	280.26	4.62	4.00	0.46	0.20	4.07	0.25	8.18	29.88	18.00
Sample21	8.238	54.008	18	218.09	4.25	3.87	0.39	0.18	3.64	0.16	8.19	30.25	20.00
Sample22	8.083	54.050	17.8	177.19	3.48	4.39	1.38	0.18	3.00	0.83	8.20	30.22	20.00
Sample23	7.987	54.102	17.3	219.15	3.30	7.77	0.42	0.25	1.95	1.19	8.16	30.10	27.00
Sample24	7.892	54.152	17.6	179.61	2.73	5.04	0.17	0.17	1.26	0.12	8.22	31.35	53.00

#: values of the data portal "Fachinformationssystem (FIS)" of the FGG (FlussGebietsGemeinschaft, Magedeburg, Germany) Elbe; [1-4]distances from sample location of the FGG to sample location of this study:[1]0.6km, [2]0.5km, [3]0.2km, [4]1.5km

$: based on literature values: (Amann *et al*., 2014, DOI: 10.1007/s10533-013-9940-3; Carstens *et al*., 2004, DOI: 10.1002/aqc.652; Magath *et al*., 2013, DOI: 10.1111/jfb.12115)

*: using the digital relief model of the river from the Zentrales Datenmanagement (ZDM) of the Wasserstraßen- und Schifffahrtsverwaltung des Bundes

Supplementary Table S2: Collinearity of environmental parameters tested by Spearman rank order correlations. Only significant correlations (FDR adjusted $P < .05$) with high relevance ($R^2 \geq 0.5$) are shown. R, R^2-values are indicated in the table.

	Temperature	DOC	Chl *a*	SiO_4	PO_4	NO_2	NO_3	NH_4	pH	Salinity
DOC										
Chl *a*										
SiO_4										
PO_4										
NO_2										
NO_3										
NH_4			0.73,0.53			0.75,0.56				
pH					-0.82,0.68					
Salinity	-0.92,0.84			-0.83,0.69						
Depth	-0.81,0.65		-0.72,0.52							0.83,0.68

Supplementary Table S3: Taxonomic classification and trophic mode of the most abundant OTUs. Representative sequences of the OTUs were inserted into the phylogenetic reference tree using phylogenetic placement. Taxonomy of the branch where sequences were placed, were transferred on the OTU. Additionally, BLASTn was carried out and best BLAST hits are reported. Based on the classification over BLASTn, literature was screened for information on taxa specific trophic modes.

https://www.frontiersin.org/articles/file/downloadfile/640469_supplementary-materials_tables_3_xlsx/octet-stream/Table%203.xlsx/1/640469

Supplementary Table S4: Fully annotated OTU table. The table provides frequencies of OTUs and detailed taxonomic information from the phylogenetic tree. Representative sequence for each OTU can be found in the attached .fasta-file Supplementary File S1.

https://www.frontiersin.org/articles/file/downloadfile/640469_supplementarymaterials_tables_4_xlsx/octet-stream/Table%204.xlsx/1/640469

Supplementary Table S5: Correlation analyses of the abundant OTUs with environmental parameters using Pearson rank order correlations. Only significant correlations (FDR adjusted $P < .05$) are shown. R, R^2 values are shown in the table.

OTUs	Phylum	Temperature	DOC	Chl *a*	SiO_4	PO_4	NO_2
SMBZZZ15	Ascomycota		-0.43, 0.18		-0.45, 0.20	-0.50, 0.25	
SMBZZZ13	Ascomycota				-0.68, 0.46		-0.51, 0.26
SMBZZZ16	Ascomycota				-0.54, 0.29		-0.49, 0.24
SMBZZZ10	Chytridiomycota	0.71, 0.50		0.67, 0.45	0.67, 0.45		0.46, 0.21
SMBZZZ11	Chytridiomycota	0.63, 0.40		0.50, 0.25	0.76, 0.58		0.42, 0.18
SMBZZZ14	Chytridiomycota		0.47, 0.22			0.55, 0.30	
SMBZZZ23	Chytridiomycota	0.72, 0.52		0.70, 0.49	0.54, 0.29		0.44, 0.19
SMHZZZZZ	Chytridiomycota	0.77, 0.59	0.44, 0.19	0.43, 0.18	0.73, 0.53		
SMBZZZ17	Chytridiomycota		0.45, 0.20			0.74, 0.55	
SMBZZZ19	Chytridiomycota	0.58, 0.34		0.63, 0.40	0.46, 0.21		0.44, 0.19
SMBZZZ21	Chytridiomycota	0.69, 0.48		0.76, 0.58	0.43, 0.18		0.44, 0.19
SMBZZZ12	Chytridiomycota	0.89, 0.79	0.54, 0.29	0.79, 0.62	0.70, 0.49		
SMBZZZZ9	Chytridiomycota	0.57, 0.33	0.49, 0.24	0.43, 0.18	0.62, 0.38		
SMHZZZZ2	Chytridiomycota	0.61, 0.37		0.43, 0.18	0.65, 0.42		
SMHZZZZ3	Chytridiomycota	0.83, 0.69		0.46, 0.21	0.69, 0.48		
SMBZZZ18	Chytridiomycota	0.66, 0.44		0.62, 0.38	0.57, 0.33		0.48, 0.23
SMHZZZZ4	Basal Fungi clade 02	0.79, 0.62	0.58, 0.34	0.53, 0.28	0.67, 0.45		

Table is continued on the next page…

OTUs	Phylum	NO_3	NH_4	pH	Salinity	Depth
SMBZZZ15	Ascomycota	-0.79, 0.62		0.53, 0.28		
SMBZZZ13	Ascomycota	-0.58, 0.34	-0.47, 0.22		0.48, 0.23	
SMBZZZ16	Ascomycota	-0.57, 0.33			0.51, 0.26	
SMBZZZ10	Chytridiomycota		0.45, 0.20		-0.72, 0.52	-0.63, 0.40
SMBZZZ11	Chytridiomycota				-0.69, 0.48	-0.59, 0.35
SMBZZZ14	Chytridiomycota	0.63, 0.40		-0.47, 0.22		
SMBZZZ23	Chytridiomycota		0.54, 0.29		-0.75, 0.56	-0.64, 0.41
SMHZZZZZ	Chytridiomycota	0.47, 0.22			-0.80, 0.64	-0.53, 0.28
SMBZZZ17	Chytridiomycota	0.71, 0.50		-0.59, 0.35		
SMBZZZ19	Chytridiomycota		0.61, 0.37		-0.67, 0.45	-0.45, 0.20
SMBZZZ21	Chytridiomycota		0.62, 0.38		-0.70, 0.49	-0.61, 0.37
SMBZZZ12	Chytridiomycota		0.46, 0.21		-0.87, 0.76	-0.69, 0.48
SMBZZZZ9	Chytridiomycota	0.49, 0.24			-0.70, 0.49	-0.55, 0.30
SMHZZZZ2	Chytridiomycota	0.48, 0.23			-0.73, 0.53	-0.54, 0.29
SMHZZZZ3	Chytridiomycota	0.53, 0.28			-0.85, 0.72	-0.66, 0.44
SMBZZZ18	Chytridiomycota		0.59, 0.35		-0.66, 0.44	-0.67,0.45
SMHZZZZ4	Basal Fungi clade 02				-0.75, 0.56	-0.56, 0.31

Supplementary Table S5: Distance-based Moran's eigenvector (dbMEM) analysis identified seven eigenvectors with positive Moran I-values. Out of those, four were identified to have a significant effect on mycoplankton community structure as detected by dbRDA-based forward selection

	MEM1	**MEM2**	**MEM3**	**MEM4**	**MEM5**	**MEM6**	**MEM7**
Eigenvalues	0.204	0.164	0.079	0.069	0.047	0.035	0.003
Moran's I	1.15	0.924	0.448	0.389	0.265	0.197	0.017
Forward selection on positive MEM factors							
F-values	7.0365	4.0154		2.2569	1.9272		
P values	0.001	0.003		0.02	0.049		

Supplementary File S1: Representative sequences of all OTUs detected in this study. For detailed information on OTUs, see Supplementary Table S4.

https://www.frontiersin.org/articles/file/downloadfile/640469_supplementary-materials_datasheets_2_fasta/octet-stream/Data%20Sheet%202.FASTA/1/640469

Supplementary File S2: Phylogenetic tree as .tree-file. Phylogenetic reference tree (Yarza *et al.,* 2017), which was enriched with new fungal full length 18S rRNA gene sequences from the SILVA database. Next, generated sequences of this study were phylogenetically placed into the tree.

https://www.frontiersin.org/articles/file/downloadfile/640469_supplementary-materials_datasheets_3_zip/octet-stream/Data%20Sheet%203.zip/1/64046

9.2 Supplementary material of Paper II

Supplementary Material

The biogeography of marine benthic fungi: limitations in distribution, spatial and deterministic factors, and effects of taxon-specific traits

Yanyan Yang, Rolf Nimzyk, Marlis Reich

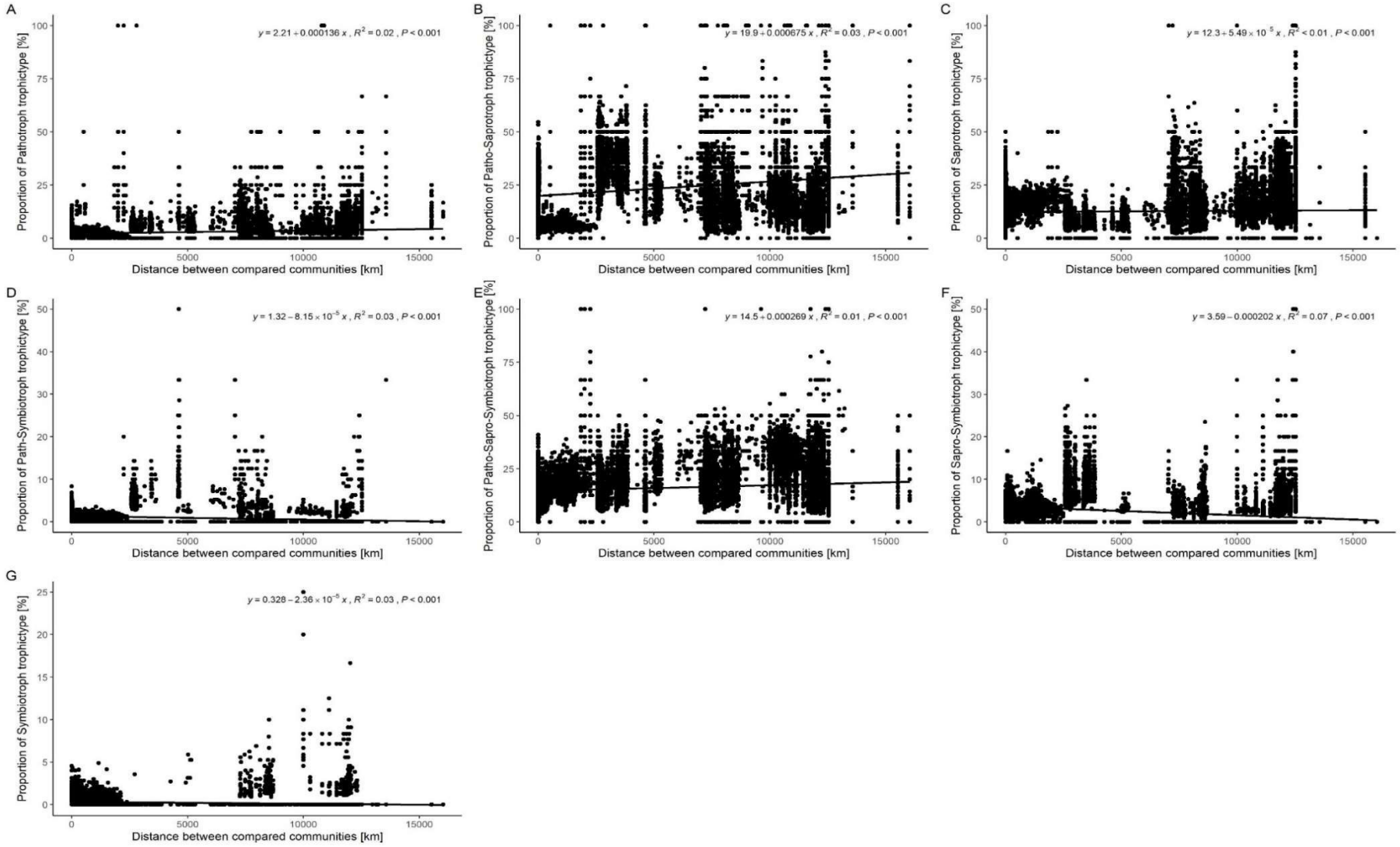
Supplementary Table 1: Information on composite dataset. The table provides information on samples locations and numbers, habitat types, accession numbers and related publications.

Supplementary Table 2: Fully annotated OTU table. The table provides information on taxonomy, morphology, trophic mode, classification to cosmopolitan/endemic distribution and sequence count of all fungal OTUs of the composite dataset. Representative sequence for each OTU can be found in Supplementary File 1.fasta.

Comparison		Change of proportions [%]	p
<1km	10km	25.7	**0.000**
	100km	21.6	**0.000**
	1000km	2.0	**0.000**
	2500km	-6.3	**0.000**
	5000km	-17.2	**0.000**
	10000km	-17.1	**0.000**
	12500km	-16.8	**0.000**
	15000km	-17.0	**0.000**
	16000km	-17.5	**0.000**
<10km	100km	-4.0	**0.000**
	1000km	-23.7	**0.000**
	2500km	-32.0	**0.000**
	5000km	-42.9	**0.000**
	10000km	-42.8	**0.000**
	12500km	-42.5	**0.000**
	15000km	-42.7	**0.000**
	16000km	-43.2	**0.000**
<100km	1000km	-19.6	**0.000**
	2500km	-27.9	**0.000**
	5000km	-38.8	**0.000**
	10000km	-38.7	**0.000**
	12500km	-38.4	**0.000**
	15000km	-38.6	**0.000**
	16000km	-39.1	**0.000**
1000km	2500km	-8.3	**0.000**
	5000km	-19.2	**0.000**
	10000km	-19.1	**0.000**
	12500km	-18.8	**0.000**
	15000km	-19.0	**0.000**
	16000km	-19.5	**0.000**
2500km	5000km	-10.9	**0.000**
	10000km	-10.8	**0.000**
	12500km	-10.5	**0.000**
	15000km	-10.7	**0.000**
	16000km	-11.2	**0.000**
5000km	10000km	0.1	0.976
	12500km	0.4	**0.000**
	15000km	0.2	0.853
	16000km	-0.3	0.997
10000km	12500km	0.3	0.010
	15000km	0.1	0.999
	16000km	-0.4	0.970
12500km	15000km	-0.2	0.895
	16000km	-0.7	0.453
15000km	16000km	-0.5	0.897

Supplementary Table 3: Identification of different distribution patterns: Significant change of proportions of shared OTUs among two communities separated by different geographic distances (see Fig. 2). Significance was tested with TukeyHSD ($P < .001$).

Supplementary Table 4: Distance-decay analysis indicates significant impact of geographic distance on proportion of trophic mode of shared OTUs of two compared communities**.** Significance test based on F-test ($P < .001$) in "lm" function of R.



Supplementary Table 5: Distance-based Moran's eigenvector (dbMEM) analysis identified five eigenvectors with a positive Moran I-value. All five showed a significant effect on mycoplankton community structure as detected by dbRDA-based forward selection ($P < .001$).

	MEM1	MEM2	MEM3	MEM4	MEM5
Eigenvalues	0.353	0.181	0.051	0.015	0.004
Moran's I	0.723	0.370	0.104	0.030	0.008
Forward selection on positive factors					
F-values	21.013	14.024	5.651	7.156	3.545
P values	0.001	0.001	0.001	0.001	0.001

Supplementary File 1.fasta: Representative sequences of fungal OTUs.

Supplementary File 2.html: Interactive Krona chart of taxonomic classification.

9.3 Supplementary material of Paper III

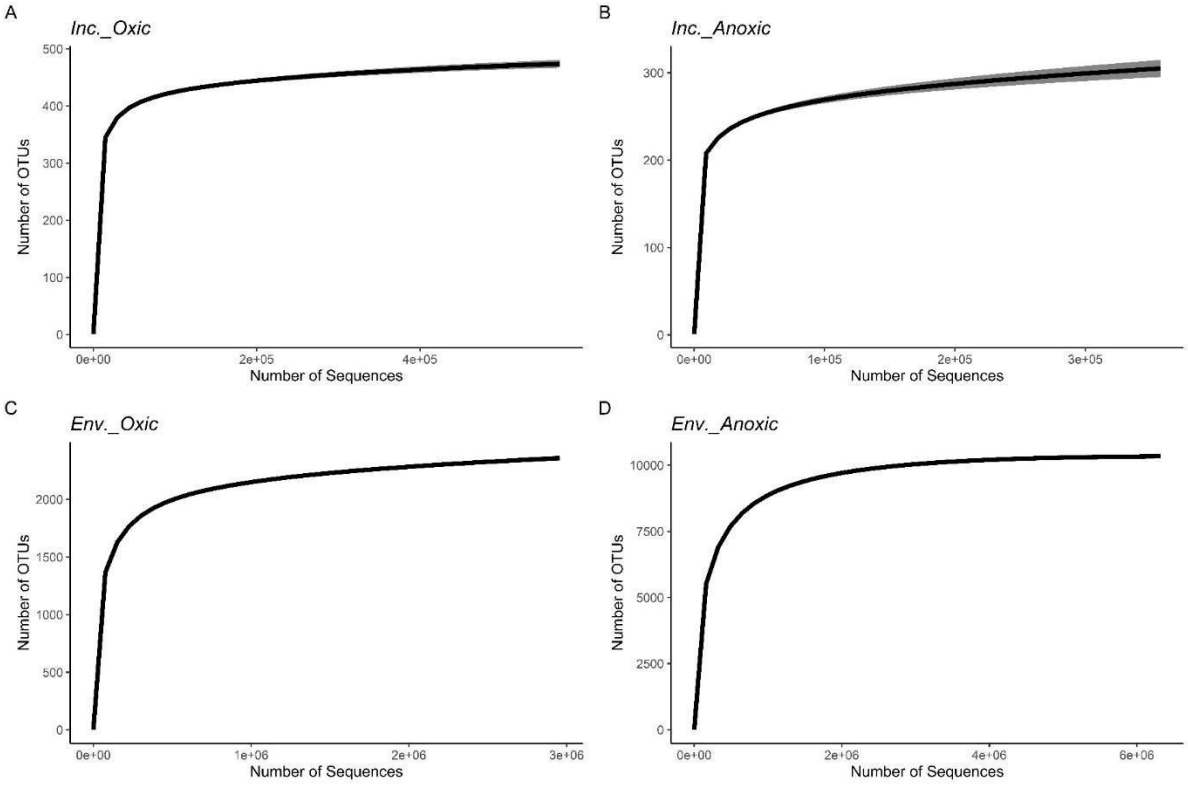
Supplementary Material

Effects of oxygen content on the mycobenthic community of coastal sediments

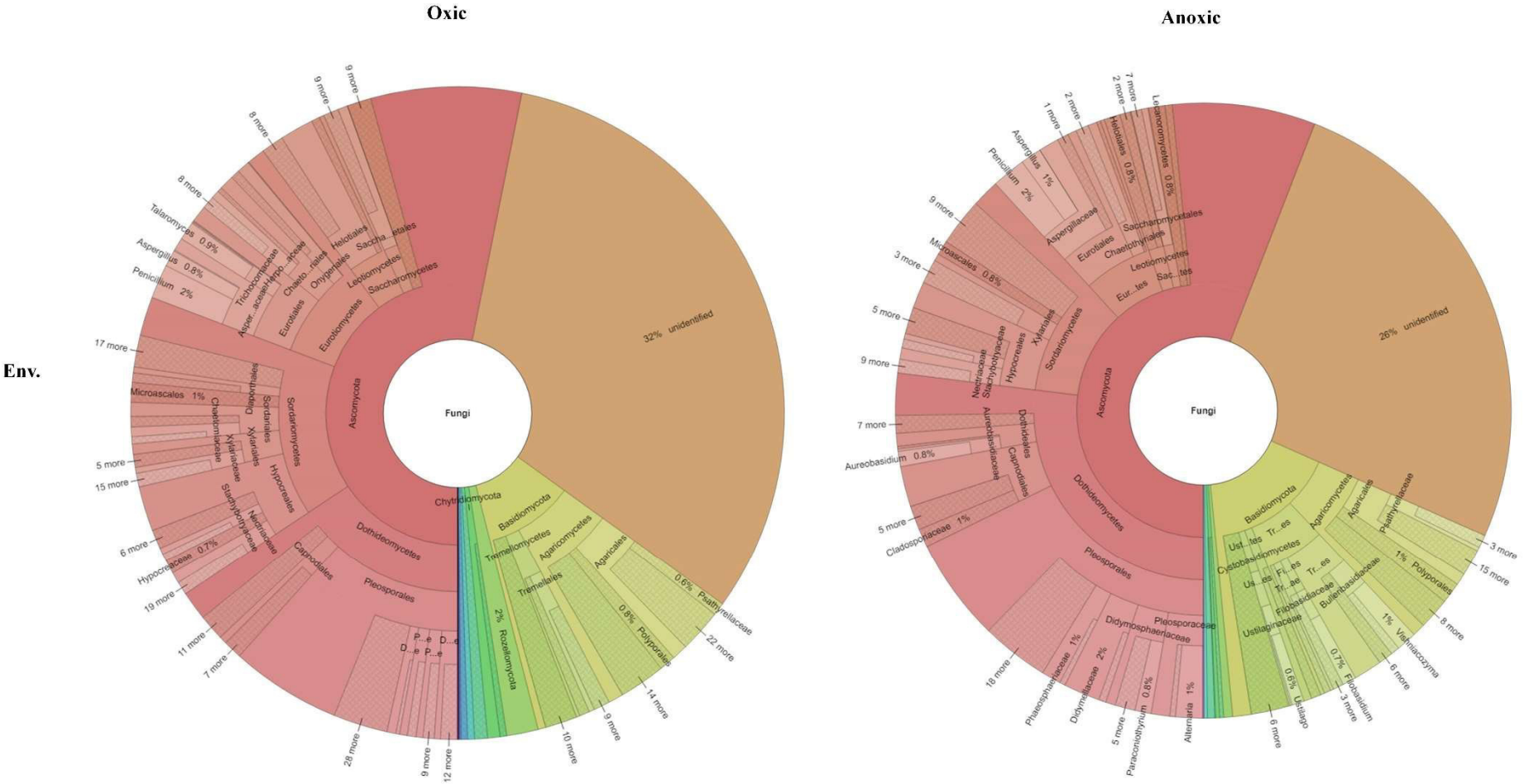
Yanyan Yang, Carmen Alicia Rivera Pérez, Tim Richter-Heitmann, Rolf Nimzyk, Michael Friedrich Marlis Reich

Supplementary Figures

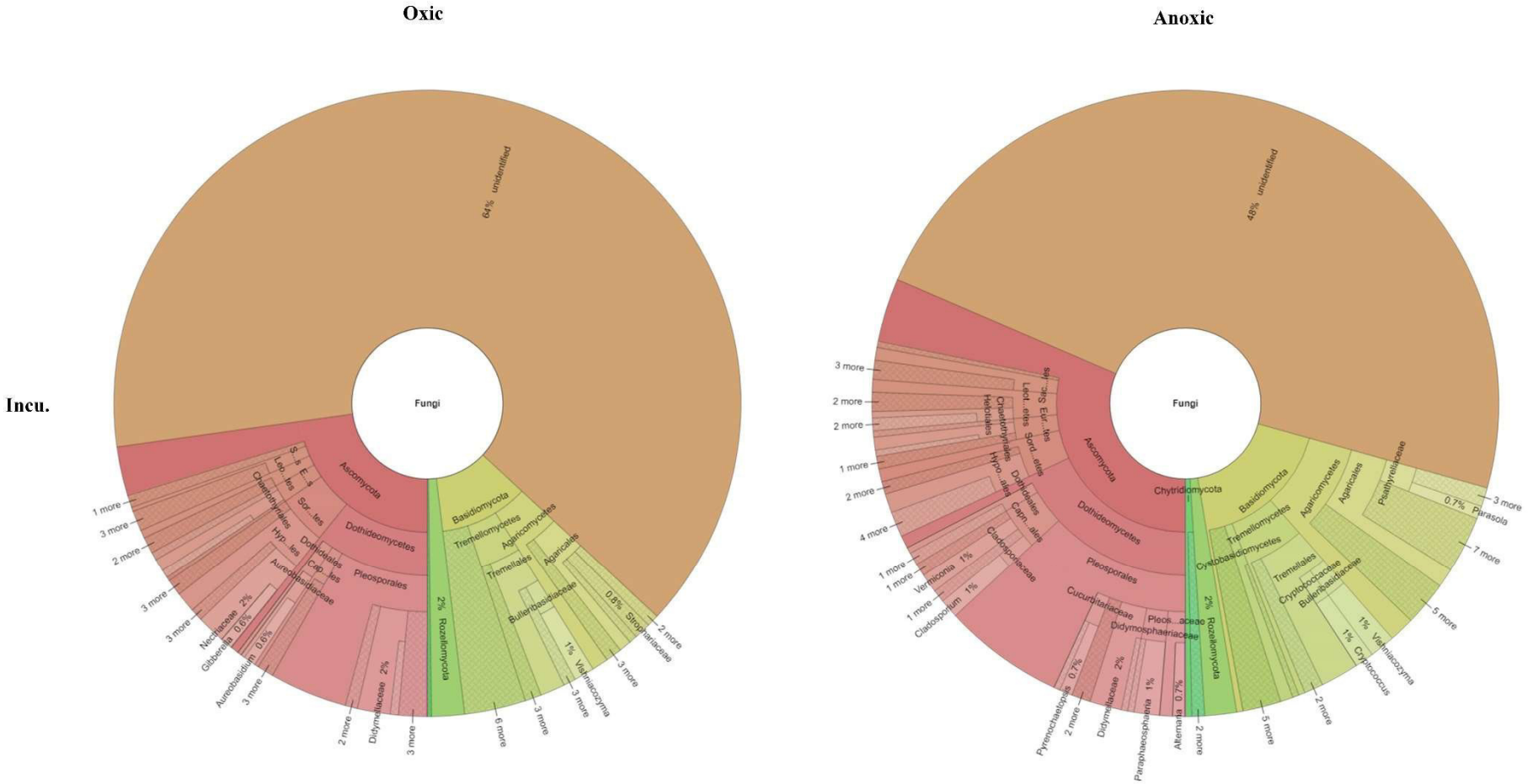
Supplementary Figure S1: Rarefaction curves calculated on all generated sequences (meta-samples and incubation samples**) with four conditions** (oxic and anoxic conditions of the incubation experiment and meta-analysis)**.** Shaded area indicates the 95%-confidence interval.



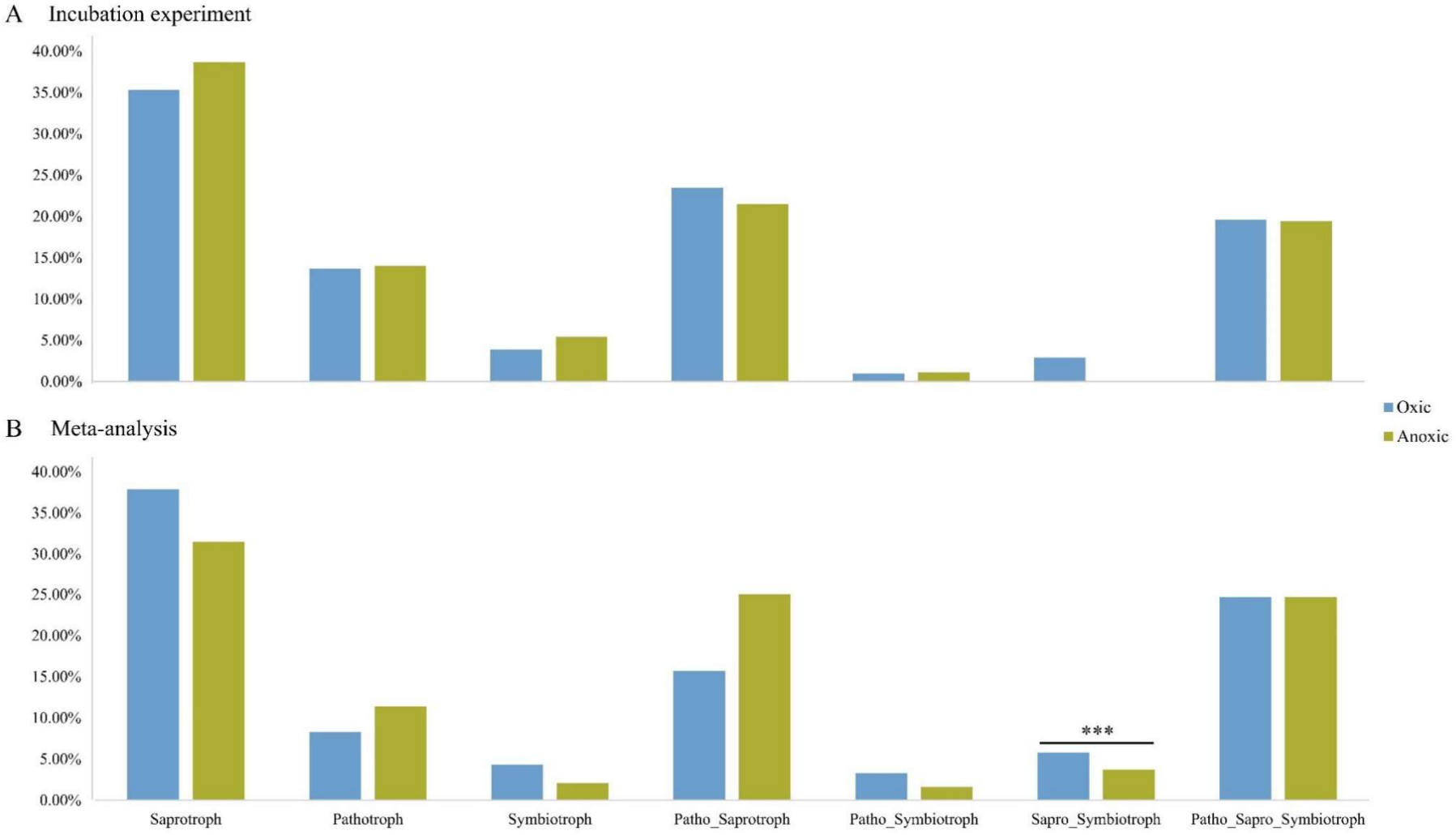
Supplementary Figure S2: Krona chart showing taxonomic classification of fungal OTUs of the environmental samples under anoxic and oxic conditions **(**interactive chart can be browsed over Supplementary File 2.html).



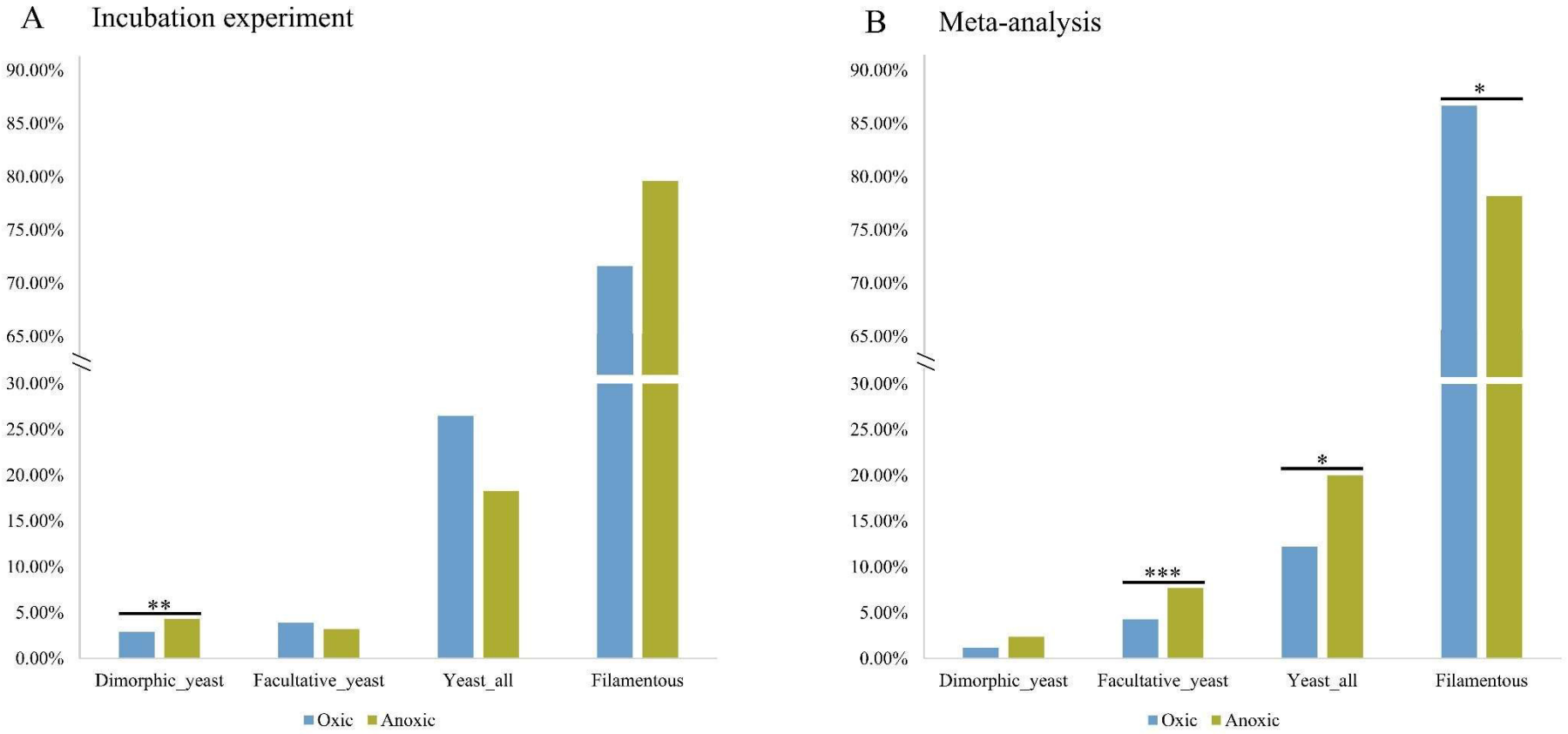
Supplementary Figure S3: Krona chart showing taxonomic classification of fungal OTUs of the incubated samples under anoxic and oxic conditions **(**interactive chart can be browsed over Supplementary File 3.html).



Supplementary Figure S4: Proportion of fungal OTUs assigned to trophic modes under anoxic and oxic conditions of the incubation experiment and meta-analysis, significance between oxic and anoxic conditions was tested by TukeyHSD: *** $P < .001$.



Supplementary Figure S5: Proportion of fungal OTUs assigned to morphological types under anoxic and oxic conditions of the incubation experiment and meta-analysis, significance between oxic and anoxic conditions was tested by TukeyHSD: *** $P < .001$, ** $P < .01$, * $P < .05$.



Supplementary Tables

Supplementary Table 1: Information on composite dataset. The table provides information on samples locations and numbers, habitat types, accession numbers and related publications.

Supplementary Table 2: Fully annotated OTU table. The table provides information on taxonomy, morphology, trophic mode, and sequence count of all fungal OTUs of the composite dataset. Representative sequence for each OTU can be found in Supplementary File 1.fasta.

Supplementary Table 3: Cosmopolitan genus for environmental samples and incubated samples, defined by existence under both oxic/anoxic conditions, and >=50% samples.

	Phylum	Class	Order	Family	Genus	Anoxic	Oxic
Environment	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	5.44%	1.55%
	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	0.18%	0.20%
	Ascomycota	Dothideomycetes	Dothideales	Dothideales_unidentified	Hortaea	1.45%	1.89%
	Ascomycota	Dothideomycetes	Pleosporales	Biatriosporaceae	Biatriospora	0.01%	0.12%
	Ascomycota	Dothideomycetes	Pleosporales	Cucurbitariaceae	Pyrenochaetopsis	0.04%	0.09%
	Ascomycota	Dothideomycetes	Pleosporales	Dictyosporiaceae	Dictyosporium	0.02%	0.34%
	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Paraconiothyrium	0.30%	1.40%
	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Paraphaeosphaeria	0.26%	0.78%
	Ascomycota	Dothideomycetes	Pleosporales	Periconiaceae	Periconia	0.05%	0.21%
	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	4.41%	1.19%
	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	0.00%	0.05%
	Ascomycota	Dothideomycetes	Pleosporales	Thyridariaceae	Roussoella	0.03%	0.51%
	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	0.00%	0.07%
	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Aspergillus	2.43%	2.20%
	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	1.00%	2.07%
	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Talaromyces	0.01%	0.80%
	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Pseudeurotium	0.01%	0.20%
	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetales_unidentified	Candida	0.03%	0.13%
	Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	Chordomyces	0.00%	0.09%
	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	0.01%	0.45%
	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_unidentified	Acremonium	0.17%	0.06%
	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales_unidentified	Emericellopsis	0.00%	0.21%
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	0.00%	0.13%
	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	0.25%	0.36%
	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	0.00%	0.10%
Incubation	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	5.27%	1.73%
	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	1.63%	1.19%

Supplementary Table 4: SIMPER analysis of the identified fungal genera, showing the abundance of each genus under anoxic and oxic conditions, avarage dissimilarities between the 2 conditions, and the contributions of each genus to the observed community dissimilarity (COD). Only genus with more than 0.5% contribution to the observed community dissimilarities are listed. Bold marked the abundance which is higher in anoxic conditions than oxic conditions.

Environmental samples

Genus	Abundance (Anoxic)	Abundance (Oxic)	Dissimilarity	P-value	COD	Trophic.Mode	Morphology	Phylum	Class	Order	Family
Psathyrella	**3.90%**	2%	0.048	0.017	7.19%	Saprotroph	Filamentous	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae
Alternaria	**4.40%**	1%	0.026	0.009	3.88%	Patho_Sapro_Symbiotroph	Filamentous	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae
Cladosporium	**5.40%**	1.50%	0.013	0.011	1.95%	Patho_Saprotroph	Filamentous	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae
Claviceps	0.20%	0%	0.005	0.03	0.72%	Pathotroph	Filamentous	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae

Incubation samples

Genus	Abundance (Anoxic)	Abundance (Oxic)	Dissimilarity	P-value	COD	Trophic.Mode	Morphology	Phylum	Class	Order	Family
Lacrymaria	**9.60%**	4.40%	0.043	0	6.25%	Saprotroph	Filamentous	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae
Wallemia	0.20%	**2.20%**	0.02	0.001	2.87%	Saprotroph	Filamentous	Basidiomycota	Wallemiomycetes	Wallemiales	Wallemiaceae
Cryptococcus	**1.80%**	0.40%	0.013	0	1.95%	Patho_Sapro_Symbiotroph	Dimorphic_yeast	Basidiomycota	Tremellomycetes	Tremellales	Cryptococcaceae
Parasola	0.70%	0%	0.012	0.001	1.82%	Saprotroph	Filamentous	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae
Agaricus	2.70%	0.40%	0.011	0.006	1.67%	Saprotroph	Filamentous	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae
Vishniacozyma	1.20%	1.20%	0.011	0.034	1.66%	Saprotroph	Yeast	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae
Phacidiella	0.10%	0%	0.01	0.011	1.54%	Pathotroph	Filamentous	Ascomycota	Lecanoromycetes	unidentified	unidentified
Yarrowia	0.30%	0.10%	0.008	0.006	1.23%	Saprotroph	Yeast	Ascomycota	Saccharomycetes	Saccharomycetales	Dipodascaceae
Pyrenochaetopsis	1.20%	0.70%	0.006	0.006	0.93%	Patho_Sapro_Symbiotroph	Filamentous	Ascomycota	Dothideomycetes	Pleosporales	Cucurbitariaceae
Buckleyzyma	0.70%	0.80%	0.005	0	0.71%	Saprotroph	Yeast	Basidiomycota	Cystobasidiomycetes	unidentified	Buckleyzymaceae
Hypholoma	0.50%	0.20%	0.005	0	0.71%	Saprotroph	Filamentous	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae
Torula	0%	0.20%	0.005	0.012	0.66%	Patho_Saprotroph	Filamentous	Ascomycota	Dothideomycetes	Pleosporales	Torulaceae
Acericola	0.60%	0%	0.004	0.02	0.55%	Patho_Saprotroph	Filamentous	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae

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