Abschließender Sachbericht

Titel des Vorhabens:

Linking aquatic mycodiversity to ecosystem function (“MycoLink”)

Leibniz-Einrichtung: IGB, Leibniz-Institut für Gewässerökologie und Binnenfischerei
Aktenzeichen: SAW-2014-IGB-1
Projektlaufzeit: 01.06.2014-28.02.2018
Ansprechpartner:
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Stand: 04. Dezember 2015

Final report

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Executive summary

The kingdom Fungi comprises an enormous amount of evolutionary diversity. Current estimates range from 1.5 – 6 million species within 12 phyla. The large majority of species are not described, despite Fungi being a quantitatively important and functionally important of the aquatic microbiota in freshwater systems. Aquatic fungi play a largely unrecognized key role in the degradation of polymeric, refractory OM, which render this enormous terrestrial organic matter pool accessible for bacterial degradation. Fungal diversity (community structure and function) is thought to greatly depend on quantity and quality of OM, with serious implications for freshwater OM and energy cycling as well as greenhouse gas emissions, both potentially affecting global C-cycles.

MycoLink aimed to develop and apply genetic (DNA metabarcoding) and chemotaxonomic (PFLA) markers to estimate fungal species composition and functional diversity in laboratory cultures and natural ecosystems to develop and test novel approaches for the high throughput cultivation, preservation, and metabolic characterization of aquatic fungi using strains representing major taxonomic groups.

We developed an automated pipeline for the combined analysis of sequence data from 5.8S and ITS2 genes and used both regions to classify fungi. ~90% of OTUs tested were classified to Class or better with 5.8S compared to ~50% with ITS2. We also developed long-read (~4500 bp) metabarcoding by adapting 3rd-generation sequencing technology (PacBio). Longer markers contain SSU, ITS, and LSU regions and provide greater taxonomic resolution by making use of 3 databases. Three isolation methods applied to four substrates (water, sediment, CPOM and FPOM) collected from three lakes yielded 262 cultures, among which 112 species were identified. The study also yielded about 20 potentially new species. Estimates from our data predicted 40 (water) - 150 (CPOM) species from a single substrate, likely higher in sediments. 85% (183 species) of the isolates were able to grow on humic acid medium and many could produce laccases (40%) and peroxidases (30 %). We documented the presence in the genome of peroxidases, laccases and cytochrome P450s, as well as multiple proteins for cellulose and hemicellulose degradation in *Clavariopsis aquatica*. Experimental study of gene expression indicates these were actively involved in carbon decomposition. Cultures were dominated by ascomycete species, most previously known as plant pathogens or saprobes. There was very high species turnover with < 5% of species shared between lakes or habitats. We examined environmental samples apanning a large gradient in humic matter concentration were screened for fungal ecosystem functions and diversity. Applying a Bayesian mixed model to PLFA data from sediments of the littoral zones of twenty lakes revealed that lakes with high quantities of aged and preprocessed organic matter had significantly higher abundances of aquatic fungi, whereas fresh organic matter was correlated with bacterial abundance.

Important outputs from the project include reference and type material of prominent aquatic fungi that was collected, authenticated and safeguarded in permanent voucher collections. Sequences obtained during this project will improve identification of aquatic fungi with public databases in the future, allowing for comparison of habitats and regions to determine if species are cosmopolitan or specialized. By allowing for the simultaneous use of three global databases (Unite, SILVA, RDP LSU), long-read DNA metabarcoding provided better taxonomic resolution than any single marker. The universal nature of the rRNA operon and our recovery of >100 non-fungal OTUs indicate that long-read DNA metabarcoding holds promise for studies of eukaryotic diversity more broadly. Our observations of OM processing in many aquatic isolates and in our genome-sequenced species *C. aquatica* indicate clearly that some aquatic fungi are able to modify lignin; most likely in order to facilitate the utilization of lignocellulose as a carbon and energy source.

1. Ausgangsfragen und Zielsetzung des Vorhabens; *initial questions / objectives*

The central hypotheses of MycoLink were:

(1) Fungi are a quantitatively important and highly diverse component of the aquatic microbiota in freshwater systems, and possess a multitude of novel, so far unknown physiological capabilities. New molecular approaches that target aquatic fungi will unravel an enormous, thus far undiscovered taxonomic and functional diversity.

(2) The composition and ecological role of natural communities of aquatic freshwater fungi is governed by their high functional diversity. Aquatic fungi are adapted to their environment by specific metabolic pathways that confer these microorganism a decisive role in the natural carbon cycle.

(3) Aquatic fungi play an unrecognized key role in the degradation of polymeric, refractory OM, which render this enormous terrestrial organic matter pool accessible for bacterial degradation. A better definition of different functional types of aquatic fungi and knowledge on their environmental dynamics are required to reliably quantify biogeochemical processes related to the fungal presence.

(4) Fungal diversity (community structure and function) greatly depends on quantity and quality of OM, as well as on the stage of OM decomposition. This relationship greatly varies with ecological features of the studied ecosystems, in particular, pH and temperature. It has serious implications for freshwater OM and energy cycling as well as greenhouse gas emissions, both potentially affecting global C-cycles.

The aims and objective of MycoLink were:

WP1 aimed to develop and apply genetic markers to estimate fungal species composition and functional diversity in laboratory cultures (WP2) and natural ecosystems (WP3). Necessary development steps include (1) identification of suitable marker genes, (2) development of PCR- and hybrid-capture- based analysis of these genes in a variety of sample types, and (3) adaptation of bioinformatics pipelines.

WP2 aimed to develop and test novel approaches for the high throughput cultivation, preservation, and metabolic characterization of aquatic fungi using strains representing major taxonomic groups of aquatic fungi (selected within WP1) as well as phylogenetically related reference strains available at DSMZ, CBS (The Netherlands), MUCL (Belgium), MUM (Portugal), VKM (Russia) and other collections. High-throughput cultivation methods were tested including selective media and dilution-to-extinction. Reliability of cultivation approaches such as species richness, required sampling effort, and new species discovery rate needed to be estimated at this step. Isolation efforts targeted representative species that were identified as being dominant and active components of ecosystems by WP1 and WP3. Contribution of fungi to OM decomposition in aquatic habitat as well as their specific adaptations to the aquatic environment was studied on a collection of isolates. Degradation experiments utilized natural DOC and POC in WP2. Preservation methods were developed

for a dedicated and diverse collection of novel types of aquatic fungi that will be made available to the scientific community.

WP3 aimed to elucidate the ecological role of aquatic fungi in experimental and natural aquatic habitats using a range of biochemical analysis including compound-specific stable isotope probing (13C-PLFA) and LC-OCD/OND. The impact of OM quality on fungi biodiversity and function has been investigated by combining these analyses with fluorescent OM characterization and subsequent PARAFAC analysis. A major focus of WP3 has been set on the degradation of recalcitrant OM by fungi. Environmental samples spanning a large gradient in humic matter concentration have been screened for fungal ecosystem functions (WP3), diversity (WP1) and the presence of selected functional genes (based on the metabolic screening of WP2). Additionally, humic matter degradation by fungi has been studied in experimental ecosystems at IGB in Lake Grosse Fuchskuhle and LakeLab mesocosms in Lake Stechlin (www.seelabor.de). Field experiments and samplings have been complimented by controlled laboratory studies using different humic matter concentrations to characterize aquatic fungi isolated from the environment. Additionally, diversity and host-parasite-interactions between algae and parasitic fungi (chytrids) have been investigated.

2. Entwicklung der durchgeführten Arbeiten einschließlich Abweichungen vom ursprünglichen Konzept, wissenschaftliche Fehlschläge, Probleme in der Vorhabenorganisation oder technischen Durchführung;

WP1 and WP2 were modified to address the higher than expected salary costs, slow grawth rate in cultivation and limited reliability of identification of fungi from environmental sequence libraries. The whole-genome sequencing aspect of the project was reduced because of higher salary costs associated with more highly qualified post-doctoral scientists. O make up for this, we applied for and obtained funding from the Joint Genome Instiute of the US Department of Energy as part of their Community Sequencing Program. This funding was used to cover costs of whole-genome DNA/RNA sequencing. The slow growth of aquatic fungi in culture meant that not all target species could be whole-genome sequenced, and those that are were behind schedule. To date, all sequence identification pipelines use short ITS1 or ITS2 sequences compared against a reference database, often either UNITE, MycoBank or NCBI GenBank. Because identification of fungi in lakes is a cornerstone of the project, two essential modifications were made. A collection of cultures identified using a multi-locus sequence approach (MLSA) served as an additional reference dataset to improve identification of species with no ITS1/ITS2 sequences available from public databases. Accordingly, present identification pipelines were modified to implement a training dataset and manual curation of taxonomic assignment results. Short ITS1 and ITS2 sequences often do not provide a reliable identification due to the lack of variability, resolution or reference data for this region. This results in a high proportion of unclassified environmental sequences. In order to overcome this problem, almost the entire ribosomal DNA polycistron (~4.5kb) was amplified and sequenced using the PacBio sequencing platform.

WP3: To understand the fungal diversity in aquatic systems, and their functional influence, it is necessary to obtain detailed information about the genetic pool of fungi using high-throughput sequencing methods such as Illumina Amplicon sequencing. As the sequencing length is restricted to about 500 bp the choice of the marker gene is important. Internal

transcribed spacer (ITS) regions have the advantage that they can successfully resolve differences on species and even strain level which can be a problem for SSU and LSU rRNA genes. On the other hand, reference databases are biased mainly towards sequences related to Ascomaycota and Basidiomycota, rendering it difficult to obtain reliable taxonomic information for environmental sequences. Therefore, we decided to use two different genetic markers, the 5.8S-ITS2 region as well as part of the LSU rRNA gene region.

3. Darstellung der erreichten Ergebnisse und Diskussion im Hinblick auf den relevanten Forschungsstand, mögliche Anwendungsperspektiven und denkbare Folgevorhaben;

WP1: The kingdom Fungi comprises an enormous amount of evolutionary diversity. Current estimates range from 1.5 – 6 million species within 12 phyla. The large majority of species are not described and those that are often require specialist identification. The internal transcribed spacer (ITS) region of the rRNA operon is widely used as a DNA barcode for fungi in metabarcoding studies. However in the absence of a sufficiently similar reference sequence, query sequences may be classified simply as fungi. We developed two different approaches to advance metabarcoding methods for studying fungi in fresh water environments, that contain many novel species. Both of them aim to use multiple marker regions from the rRNA operon that are amplified and sequenced together to combine advantages of different regions.

Many DNA metabarcoding studies sequence a part of the 5.8S region located between ITS1 and ITS2, when sequencing the ITS2. We performed an in silico analysis of 5.8S and ITS sequences from the UNITE database and found that while the 5.8S region was too conserved for species-level identification, it outperformed ITS for producing higher level classifications, even in the absence of closely related reference data. We then developed an automated pipeline for the combined analysis of 5.8S and ITS2, whereby data from both regions, derived from a single DNA metabarcode sequence that is widely used in fungal diversity studies, were used to classify fungi. To evaluate the pipeline, we amplified part of the 5.8S gene together with ITS2 from sediment and water samples from 20 freshwater lakes in North-East Germany. 86% of the OTUs from these samples could be classified at least to the class level with the 5.8S while with the ITS2 only 46% could be classified to this level. In many studies the part of the 5.8S is sequenced to provide a conserved primer binding site, but it is discarded before the analysis. We show that it can be used to complement ITS2 data and help with high level taxonomic classification for sequences where ITS2 is failing to give any classification. This is especially helpful in understudied environments like freshwater lakes, where database coverage is poor.

The use of the 5.8S as complementary marker already improved higher level classification of novel fungal species, but its very high conservation make classification at more specific taxonomic ranks than class difficult. The SSU and LSU regions might be more suitable, but their sequencing together with the ITS is prevented by the short read lengths (<600 bp) of Illumunia sequecing. We explored the use of longer sequencing reads of several thousand bp, that are now possible with third-generation sequencing. Increased marker lengths

provide greater taxonomic resolution and allow for phylogenetic methods of classification, but longer reads may be subject to higher rates of sequencing error and chimera formation. In addition, most bioinformatics tools for DNA metabarcoding were designed for short reads and are therefore unsuitable. We used Pacific Biosciences circular consensus sequencing (CCS) to DNA-metabarcode environmental samples using a ca. 4,500 bp marker that included most of the eukaryote SSU and LSU rRNA genes and the complete ITS spacer region. We developed an analysis pipeline that reduced error rates to levels comparable to short-read platforms. Validation using a mock community indicated that our pipeline detected 98% of chimeras de novo. We recovered 947 OTUs from water and sediment samples in a natural lake, 848 of which could be classified to phylum, 397 to genus, and 330 to species. By allowing for the simultaneous use of three global databases (Unite, SILVA, RDP LSU), long-read DNA metabarcoding provided better taxonomic resolution than any single marker. We foresee the use of long reads enabling the cross-validation of reference sequences and the synthesis of ribosomal rRNA gene databases. The universal nature of the rRNA operon and our recovery of >100 non-fungal OTUs indicate that long-read DNA metabarcoding holds promise for studies of eukaryotic diversity more broadly.

Fungi are ecologically very important decomposers of lignocellulose. Besides “white rot” and “brown rot” Basideomycota which use different peroxidases, laccases and proteins of the cytochorme P450 super-family to degrade lignin to access cellulose and hemicellulose, limited lignin modification capabilities have also been reported for terrestrial Ascomycota. We investigated the presence of proteins for the modification of lignin and its constituents in the genome of the exclusively aquatic Ascomycota (hymphomycete) *Clavariopsis aquatica*. In addition we measured differential gene expression of *C. aquatica* when grown on lignocellulosic substrates compared to growth on a sugar rich substrate. We found differential expression of potential peroxidases, laccases and cytochrome P450s, as well as significant over representation of proteins for cellulose and hemicellulose degradation among the differential expressed genes. This observation strongly suggests that *C. aquatica* is able to modify lignin to some extent; perhaps in order to facilitate the utilization of lignocellulose as a carbon and energy source.

WP2: High-throughput dilution-to-extinction plating that is frequently applied to recover microorganisms from terrestrial habitats showed to be least efficient for aquatic habitats irrespectively of the studied substrate. Traditional cultivation on a set of media with a gradient of nutrient availability was reliable in terms of species diversity and work load. Most efficient but also time consuming was the method of single-colony and conidia picking. Three isolation methods applied to four substrates (water, sediment, CPOM and FPOM) collected from three lakes yielded 262 cultures, among which 112 species were identified. The study also yielded about 20 potentially new species.

CPOM yielded the highest diversity among analyzed substrates. Species richness curves of the isolates obtained were close to saturation in CPOM, FPOM and water samples from lakes Kettle Hole and Fuchskuhle SW indicating that the sampling effort was sufficient for the cultivation experiments. On the contrary, the number of species did not reach saturation for in sediments from lakes Kettle Hole and Fuchskuhle SW and in water from the lake Stechlin, suggesting that higher diversity of fungi could be isolated through an increased effort. The estimators predicted between 40 (water) and 150 (CPOM) species from a single substrate. In

agreement with previous observations, a high number of rare species was detected and lead to higher species richness estimates.

The retrieved cultures were dominated by ascomycete species, most of which previously known as plant pathogens or saprobes. There was low number of species shared between lakes and between different substrates. Lakes showed between 0 % (Fuchskuhle & Kettle Hole) and 4.5 % (Kettle Hole & Stechlin) overlap in fungal species. Similarly, up to 4.5 % of species were shared were between substrates. The following fungi were most common in the studied lakes or substrates: *Arthrinium sacchari*, *Aureobasidium pullulans*, *Cladosporium cladosporioides* (species complex), *Cladosporium herbarum*, *Epicoccum nigrum*, *Microsphaeropsis olivacea*, *P. brevicompactum*, *Penicillium piscarium*, *P. simplicissimum*, and *Sarocladium strictum*.

Just 10% (22 out of 215) isolates belong to the ecological group of aquatic hyphomycetes (e.g. members of Helotiales, Hypocreales) that are typical for this habitat. Other species included common plant pathogens (e.g. members of Agaricales , Capnodiales, Exobasidiales, Pleosporales, Xylariales), wood-rotting species (Agaricales), mycoparasites (Cystobasidiales, Kriegeriales) and saprobes (Cystofilobasidiales, Pleosporales, Saccharomycetales, Tremellales). All isolates were screened for their ability to grow on humic acid medium (HAA) and to produce laccases (by means of ABTS) and peroxidases (using Azure B). A total of 85% (183 species) of the isolates were able to grow on HAA. The production of laccases and peroxidases was observed in 39.5% and 29.7 % of isolates, respectively. Most peroxidase producers were isolated from the dystrophic lake Fuchskuhle SW (17 species) followed by lakes Kettle Hole (8) and Stechlin (8). All species were able to grow submerged in water and sporulation was observed in 56 species, of which 14 were previously known as aquatic saprobes.

The culture-independent metabarcoding approach yielded 90,772 ITS2 sequences from 12 samples of the four substrates of the three lakes. The water samples yielded the highest numbers of reads. The taxonomic assignment was made with several common pipelines and, additionally, manually curated using MycoBank, NCBI GenBank and the collection of isolates. As a result, a total of 1,779 classified OTUs were curated and merged into 572 OTUs of which 74.5% were classified on species level, and ca. 90 % of OTUs were assigned to the genus level. The efficiency of taxonomic assignment of environmental sequences achieved with the selected approach is substantially higher than in any other study of a species-rich sequence library from either aquatic or terrestrial habitats. It also shows that previous metabarcoding approaches tend to overestimate fungal diversity.

Species richness in lakes estimated from the results of the barcoding approach ranged from 168 in the lake Kettle hole to 228 in lake Fuchskuhle SW and to 352 species in lake Stechlin. Similarly to cultivation experiments, only 3.3-8.6 % species were shared among lakes and 0.9-5.6 % of species were common among substrates. Almost 90% of the isolated species were also detected by metabarcoding. However, in addition to our culture-dependent methods, metabarcoding detected members of phyla Cryptomycota, Chytridiomycota, Zoopagomycota and Neocallimastigomycota (all together 33 species). Most species of the orders Chytridiales and Rhizophydiales (Chytridiomycota) occurred in sediments of lakes Stechlin and Fuchskuhle SW, whereas members of the phylum Cryptomycota were detected

in water from the lake Fuchskuhle SW and in sediment from the lake Kettle Hole. Metabarcoding also detected seven lichenized species. Roughly 50 % of the species identified in metabarcoding experiments belonged to the eco-category of either saprobes or plant pathogens. Both life strategies were equally present in all lakes. Aquatic saprophytes (e. g. aquatic hyphomycetes) occurred in all three lakes.

The majority of fungi were detected in a single substrate, only 30% of fungi were associated with two or three substrates. Metabarcoding showed fungal communities to vary among lakes and substrates. Substrates, but not lakes, predicted the occurrence of cultivated fungi, while metabarcoding suggests the distribution of differing fungal communities according to the type of substrate and lake more clearly. Community structure correlated with the quality of carbon, the temperature and available nitrogen.

The fungal collection of DSMZ will provide specimens isolated during this project. The project showed that aquatic fungi, their phylogenetic relationships, and molecular barcodes are often missing. This hampers successful research in aquatic habitats. Using the experience obtained during the project, reference and type material of prominent aquatic fungi could be collected, authenticated and safeguarded in DSMZ. Sequences obtained during this project will improve identification of aquatic fungi with public databases in the future.

WP3: Using a gradient of up to twenty lentic ecosystem differing largely in nutrients and dissolved organic matter (DOM) composition and quantity we were able to demonstrate that aquatic fungi are diverse and that they differ in their community structure and abundance, which are linked to the physico-chemical lake characteristics and organic matter characteristics. The occurrence and diversity of host organisms (e.g. algae and cyanobacteria) structures the occurrence and diversity for parasitic fungi. Novel species that parasitize on different algal hosts could be described which have implications on the lake phytoplankton communities and their potential blooms (van der Wyngaert et al., 2018a, b). This information could be of relevance for a potential future use of parasitic zoosporic fungi (chytrids) as a natural biocide against harmful blooming cyanobacteria. We could also demonstrate that aquatic fungi can degrade complex organic molecules using isolates that were cultured from a variety of habitats such as groundwater of two contrasting lakes (Perkins et al., in prep.; Table 1, Figure 1). On the other hand fungi can be also involved in the transformation and formation of humic substance (Rojas-Jimenez et al., 2017).

Table 1: Results of the degradation assays for different polymeric substrates. Degradation positive activities are shown in a semi-quantitative scale: -, + and ++ represent no, low and high activities, respectively. Capacity of degrading ABTS was detected as a color change, demonstrating laccase activity. Decolorization of Remazol Brilliant Blue (RBBR) is related to lignin peroxidase activity, while decolorization of PolyR-478 (PolyR), Bromocresol Green (Bromo), Toluidine (Tol), and Congo Red (Congo) is related to enzymatic degradation of polymeric, triarylmethane, and heterocyclic substrates, respectively.

Site	Strain	ABTS	RBBR	Bromo	PolyR	Tol	Congo
Fuku FX3	*Cadophora sp. KR27*	++	+	+	+	++	+
	Penicillium buchwaldii KR28	-	++	-	-	-	-
	Penicillium chrysogenum KR31	-	-	-	-	-	++
	Parengyodontium album KR32	-	-	-	-	+	+
	Trichoderma viride KR33	-	+	-	-	+	-
	Fusarium sporotrichioides KR34	-	+	-	-	-	-
	Gibberella avenacea KR35	++	++	+	++	+	+
	Isaria farinosa KR36	++	++	+	++	+	++
	Cladosporium cladosporioides KR38	++	++	-	++	++	++
	Cladosporium herbarum KR39	++	++	-	++	++	+
Stechlin NW1	*Umbelopsis sp. KR40*	-	-	-	-	-	-
	Mortierella alpina KR41	-	-	-	-	-	+
	Neoascochyta paspali KR42	+	+	-	+	+	+
	Mortierella gamsii KR43	-	-	-	-	++	++
	Neonectria sp. KR45	+	-	-	-	-	+
	Cladosporium ramotenellum KR46	++	++	-	++	++	+
	Neonectria lugdunensis KR47	++	+	-	+	-	+
Stechlin NW250	*Dictyochaeta sp. KR48*	++	+	-	++	-	+
	Coniothyrium palmicola KR49	++	-	-	-	-	+
	Parengyodontium album KR50	-	-	-	-	-	+
	Cladosporium cladosporioides KR51	++	-	-	-	-	+
	Cladosporium herbarum KR52	++	-	-	-	-	+
	Truncatella spartii KR53	-	-	-	-	+	+
	Ciboria sp. KR54	++	-	-	+	+	+
	Penicillium chrysogenum KR55	++	-	-	-	-	+
	Ramularia vizellae KR56	-	-	-	-	-	-
	Penicillium nothofagi KR57	+	-	-	-	-	-

Figure 1: Degradation assay for fungal isolates using different complex substrates. The capability for degradation was made visible by a color change of the substrate.

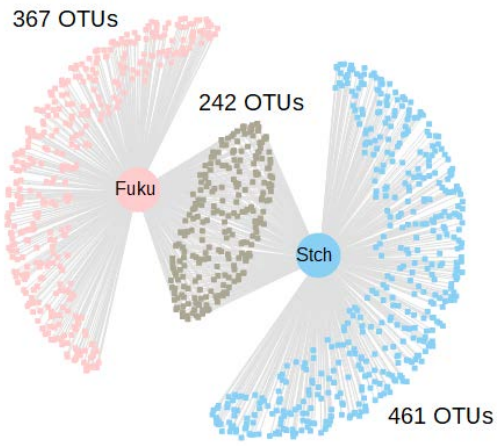

Figure 2: A network illustrating the shared and unique fungal OTUs of the two main sampling sites. Red colour nodes represent OTUs unique to groundwater boreholes around Lake Grosse Fuchskuhle (Fuku), while blue nodes represent OTUs unique to boreholes around Lake Stechlin (Stch). Grey nodes indicate OTUs that are shared by both groundwater bodies.

Furthermore, using high-throughput amplicon information from the LSU rRNA gene clear taxonomic and phylogenetic differences in the fungal community were visible (Figure 2).

Fungal sequences from aquatic environments are still limited in the general sequence databases such as GenBank or UNITE. The generated sequence data in the Mycolink project using different genetic markers, e.g. 5.8S-ITS2, LSU or almost full length rRNA operon, helps to increase the database information for finding possible environmental reference sequences. This is for example important for the comparison of habitats in different regions of the world to see if species are cosmopolitan or if they occur only in certain places with very specific conditions for their survival and reproduction. This has been also shown for prokaryotes where the continuous sequencing effort from samples from various habitats enabled the setup of a detailed database that helped to restructure the prokaryotic taxonomy. To expand the view from aquatic fungal diversity to fungal abundance we applied phospholipid fatty acids (PLFA) as chemotaxonomic markers. We refined a method applying a Bayesian mixing model on PLFA and verified its applicability and limits. In the context of these experiments we also extracted PLFA from various species of higher fungi and chytriodomycota, isolated in the course of this project. PLFA patterns of aquatic fungi were only scarcely reported before. These findings open up the opportunity to use this well established and low cost method for aquatic environmental studies. Comparison of aquatic isolates and with literature data from terrestrial species revealed that differences in morphology and taxonomy are more important than the origin of the isolate to explain differences in PLFA composition. Extracting the PLFA of Zoospores of saprophytic and parasitic chytrids revealed that these are distinct from the PLFA patterns of sessile life stage, showing higher amounts of monounsaturated fatty acids and saturated fatty acids.
Applying the Bayesian mixed model method to sediments of the littoral zones of twenty lakes we could show, that lakes with high quantities organic matter, with higher quantities of aged and preprocessed organic matter show significantly higher abundances of aquatic fungi (Figure 3). On the contrary we could identify fresh organic matter to be correlated with bacterial abundance (Figure 4).

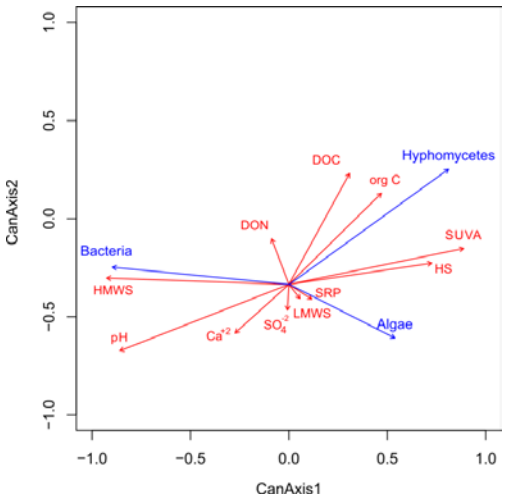

Figure 3: Influence of different of various parameters on bacterial, fungal (Hyphomycetes) and algal abundance in littoral sediments. (HMWS- high molecular weight substances, DON – dissolved organic nitrogens, DOC – dissolved organic carbon, org C – organic carbon in sediment, SUVA - specific absorption units (254nm), HS - humic substances, SRP - soluble reactive phosphorus, LWS - low molecular weight substances).

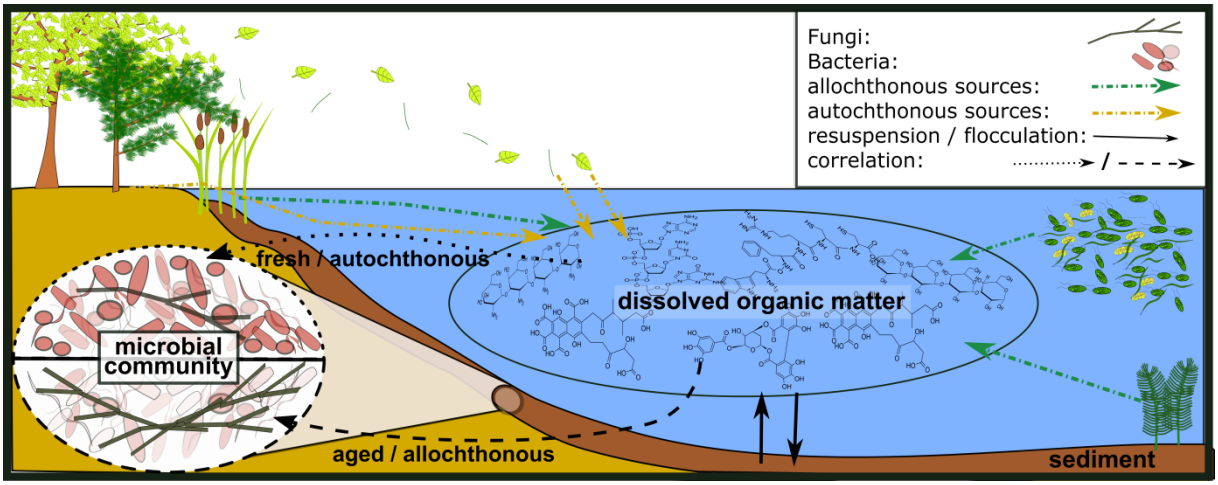

Figure 4: Summary of dependencies of fungal bacteria ratios on organic matter quality in lake ecosystems.

4. Stellungnahme, ob Ergebnisse des Vorhabens wirtschaftlich verwertbar sind und ob eine solche Verwertung erfolgt oder zu erwarten ist; Angaben zu möglichen Patenten oder Industriekooperationen;

1. The processes of lignin modification and cellulose / hemicellulose degradation by *Clavariopsis aquatica* are of potential use in biofuel production, with the research here potentially contributing to a better biological understanding.

2. Long-read DNA metabarcoding as developed within the project is highly likely to be adapted in ecological monitoring by both government agencies and private companies. This is a major outcome of the MycoLink project with economical potential for the many SMEs offering genetic methods for biomonitoring of eukarotes.

3. The parasitic zoosporic fungi (chytrids) could be further investigated as a natural biocide against harmful blooming cyanobacteria

5. Angabe der Beiträge von möglichen Kooperationspartnern im In- und Ausland, die zu den Ergebnissen des Vorhabens beigetragen haben;

5.1 Whole-genome sequencing collaborators (WP1)

Our successful proposal for whole-genome and transcriptome sequencing of aquatic fungi to the US Department of Energy's JGI-Community Sequencing Program (JGI proposal ID 1663, funded for 5 yr) included the following partners.
https://genome.jgi.doe.gov/portal/WhoGenvironments/WhoGenvironments.info.html

1. Agricultural Research Organization Newe Ya'ar, Israel
2. Helmholtz Centre for Environmental Research - UFZ, Germany
3. Mount Allison University, New Brunswick, Canada
4. Jena Microbial Resource Collection, Germany
5. University of Vienna, Austria
6. Monash University, Malaysia
7. University of Maine, USA
8. Aalborg University, Denmark
9. University of Sydney, Australia
10. GEOMAR Helmholtz Centre for Ocean Research, Germany
11. Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, France
12. University of Lorraine, France

5.2 Other collaborations

1. Helmholtz Centre for Environmental Research – UFZ (Germany) provided facilities and support for carrying out the *Clavariopsis aquatica* experiments (see publication 17)
2. Reinhold Hanel Thünen Institute (Germany) provided fungal samples from Sargasso

6. Qualifikationsarbeiten, die im Zusammenhang mit dem Vorhaben entstanden sind;

6.1 Heeger F. **Genomics Approaches to the Study of Diversity and Function of Aquatic Fungi**, Inaugural-Dissertation to obtain the academic degree *Doctor rerum naturalium* (Dr. rer. nat.), submitted to the Department of Biology, Chemistry and Pharmacy of Freie Universität Berlin. Submitted 31.05.2018

6.2 Taube R. **The role of aquatic fungi in lake ecosystems – the influence of organic matter and the opportunities of fatty acids as quantitative markers.** Inaugural-Dissertation to obtain the academic degree *Doctor rerum naturalium* (Dr. rer. nat.),
To be submitted to the Institute of Biology and Biochemistry of Universität Potsdam. Planned submission 30.11.2018

6.3. Perkins A. **Role of carbon on the diversity and physiology of groundwater fungi**. Master thesis, submitted to the Department of Biology, Macquarie University Sydney, Australia. BSc completion on 25.10.2016

Liste der Publikationen aus dem Vorhaben

MycoLink PIs, students and post-docs indicated with **bold text**

Published or accepted for publication

1. **Bourne EC**, PR Johnston, E Funke, **MT Monaghan** (In press). Gene expression analysis of litter-associated fungi using RNAseq. Ch. 39 in *Methods to Study Litter Decomposition: a Practical Guide* (eds. Graça MAS, Bärlocher F, Gessner, MO). Springer, Dordrecht

2. Frenken T, E Alacid, SA Berger, **EC Bourne**, M Gerphagnon, **H-P Grossart**, AS Gsell, BW Ibelings, M Kagami, FC Küpper, PM Letcher, A Loyau, T Miki, JC Nejstgaard, S Rasconi, A Reñé, T Rohrlack, K Rojas-Jimenez, DS Schmeller, B Scholz, K Seto, T Sime-Ngando, A Sukenik, DB Van de Waal, S Van den Wyngaert, E Van Donk, J Wolinska, **C Wurzbacher**, R Agha. 2017.Integrating chytrid fungal parasites into plankton ecology. Research gaps and needs. *Environmental Microbiology* 19:3802–3822.

3. **Heeger F**, **EC Bourne**, **C Baschien**, **A Yurkov**, B Bunk, C Spröer, **J Overmann**, **CJ Mazzoni**, **MT Monaghan**. 2018. Long-read DNA metabarcoding of ribosomal rRNA in the analysis of fungi from aquatic environments. *Molecular Ecology Resources* https://onlinelibrary.wiley.com/doi/abs/10.1111/1755-0998.12937.

4. Hölker F, **C Wurzbacher**, C Weißenborn, **MT Monaghan**, SIJ Holzhauer, **K Premke**. 2015. Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. *Philisophical Transactions of the Royal Society B* 10.1098/rstb.2014.0130.

5. Nilsson RH, AFS Taylor, RI Adams, **C Baschien**, Bengtsson-Palme J, ... **A Yurkov**, ... K Abarenkov. 2018. Taxonomic annotation of public fungal ITS sequences from the built environment – a report from an April 10–11, 2017 workshop (Aberdeen, UK). *MycoKeys* 28: 65.

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7. Rojas-Jimenez K, JA Fonvielle, H Ma, **H-P Grossart**. 2017. Transformation of humic substances by the freshwater Ascomycete *Cladosporium* sp. *Limnology and Oceanography* 62:1955-1962.

8. Rojas-Jimenez K, **C Wurzbacher**, **EC Bourne**, A Chiuchiolo, JC Priscu, **H-P Grossart**. 2017. Early diverging lineages within *Cryptomycota* and *Chytridiomycota*

dominate the fungal communities in ice-covered lakes of the McMurdo Dry Valleys, Antarctica. *Scientific Reports* 7:15348.

9. Schulze-Makuch D, D Wagner, SP Kounaves, K Mangelsdorf, KG Devine, J-P de Vera, P Schmitt-Kopplin, **H-P Grossart**, V Parro, M Kaupenjohann, A Galy, B Schneider, A Airo, J Frösler, AF Davila, FL Arens, L Cáceres, F Solis Cornejo, D Carrizon, L Dartnell, J DiRuggiero, M Flury, **L Ganzert**, MO Gessner, P Grathwohl, L Guan, J Heinz, M Hess, F Keppler, D Maus, CP McKay, RU Meckenstock, W Montgomery, EA Oberlin, AJ Probst, JS Saenz, T Sattler, J Schirmack, MA Sephton, M Schloter, J Uhl, B Valenzuela, G Vestergaard, L Wörmer, P Zamorano. 2018. Transitory microbial habitat in the hyperarid Atacama Desert. *Proceedings of the National Academy of Sciences* 115:2670-2675.

10. **Taube R**, **L Ganzert**, **H-P Grossart**, G Gleixner, **K Premke**. 2018. Organic matter quality structures benthic fatty acid patterns and the abundance of fungi and bacteria in temperate lakes. *Science of the Total Environment* 610–611:469-481.

11. van den Wyngaert S, Seto K, Rojas-Jimenez K, Kagami M, **H-P Grossart**. 2018. A new parasitic chytrid, *Staurastromyces oculus* (*Rhizophydiales*, *Staurastromycetaceae* fam. nov.), infecting the freshwater desmid *Staurastrum* sp. *Protist* 168:392-407.

12. van den Wyngaert S, Rojas-Jimenez K, Seto K, Kagami M, **H-P Grossart**. 2018. Diversity and hidden host specificity of chytrids infecting colonial volvocacean algae. *Journal of Eukaryotic Microbiology*. 10.1111/jeu.12632

13. **Wurzbacher C**, N Warthmann, **EC Bourne**, K Attermeyer, M Allgaier, JR Powell, H Detering, S Mbedi, **H-P Grossart**, **MT Monaghan**. 2016. High habitat-specificity in fungal communities in oligo-mesotrophic, temperate Lake Stechlin. *MycoKeys* 16:17-44.

14. **Wurzbacher C**, K Attermeyer, MT Kettner, C Flintrop, N Warthmann, S Hilt, **H-P Grossart**, **MT Monaghan**. 2017. DNA metabarcoding of unfractionated water samples relates phyto-, zoo- and bacterioplankton dynamics and reveals a single-taxon bacterial bloom. *Environmental Microbiology Reports* 9:383–388.

15. **Wurzbacher C**, A Fuchs, K Attermeyer, K Frindte, **H-P Grossart**, M Hupfer, P Casper, **MT Monaghan**. 2017. Shifts among Eukarya, Bacteria, and Archaea define the vertical organization of a lake sediment. *Microbiome* 5:41.

In Preparation

1. **Baschien C, J Barkowski, S Huang, B Bunk, J Overmann**. Isolation and metabarcoding of aquatic mycodiversity of lakes along a humic substance gradient.

2. **Bourne EC, F Heeger, C Wurzbacher, R Taube, L Ganzert, K Premke, H-P Grossart, CJ Mazzoni, C Baschien, MT Monaghan**. Fungal community composition of 20 north-temperate lakes along a dissolved organic matter gradient.

3. **Ganzert L**, T Hornick, JN Woodhouse, S Berger, J Nejstegaard, MO Gessner, **H-P Grossart**. Response in aquatic fungal community structure after a simulated terrestrial run-off of refractory organic matter in a lake ecosystem.

4. **Ganzert L**, M Freese, R Hanel, **H-P Grossart**. Spatial fungal diversity and community structure in the Sargasso Sea.

5. **Ganzert L**, **R Taube**, **EC Bourne**, **F Heeger**, **K Premke**, **C Baschien**, **C Wurzbacher**, **CJ Mazzoni**, **MT Monaghan**, **H-P Grossart**. Influence of lake characteristics on the diversity and assembly of lower aquatic fungi in 20 different temperate lakes.

6. **Heeger F**, **C Wurzbacher**, **EC Bourne**, **CJ Mazzoni**, **MT Monaghan**. 5.8S as a low variability complementary marker to ITS2 improves high-level classifications of aquatic fungi.

7. **Heeger F**, **EC Bourne**, C Wurzbacher, E Funke, A Lipzen, I Grigoriev, V Ng, G He, D Schlosser, **MT Monaghan**. Identification of enzymes for lignocellulose degradation in *Clavariopsis aquatica*.

8. Perkins A, **L Ganzert**, K Rojas-Jiménez, J Fonvielle, GC Hose, **H-P Grossart**. Highly diverse fungal communities in carbon-rich aquifers of two contrasting lakes in Northeast Germany.

9. **Taube R,** J Fabian, R Agha, S van den Wyngaert, M Gerphagnon, M Kagami, **K Premke**. Phospholipid derived fatty acids as a tool to detect and quantify saprotrophic and parasitc aquatic fungi.

Darstellung der Maßnahmen zur Sicherung und Verfügbarmachung der im Vorhaben produzierten Forschungsdaten;

1. 1. Bioinformatics pipeline for the analysis of long DNA reads (in this case PacBio) for fungal metabarcoding using SSU, ITS and LSU as marker region: https://github.com/f-heeger/long_read_metabarcoding

2. Bioinformatics pipeline for the analysis of combined ITS2 and 5.8S amplicons: https://github.com/f-heeger/two_marker_metabarcoding

3. Python and R code used for the analysis of *Clavariopsis aquatica* RNA-Seq data: https://github.com/f-heeger/caquatica_expression

4. Circular Consensus PacBio Reads from isolate and environmental samples were submitted to the NCBI Short Reads Archive under the accession numbers SRR6825182 - SRR6825222 , consensus sequences of the isolate samples were submitted to GenBank under the accession numbers MH047187 - MH047202

5. Raw RNA-Seq read data of *Clavariopsis aquatica* was submitted to the NCBI Short Read Archive under the accession numbers SRR6976392 - SRR6976405

6. Reference UNITE datasets are being constantly improved, and the latest release includes, among others, annotation results obtained during the project, see UNITE Community (2017): UNITE release. Version 01.12.2017. UNITE Community. (https://doi.org/10.15156/BIO/587481 and https://doi.org/10.15156/BIO/587475) and Nilsson et al. (2018) in above list of publications.

7. GenBank Accession Numbers: KY350145-KY350147, KY555729-KY555735, MG605050-MG605055

8. SRA data Fungal diversity and community structure in groundwater of two contrasting temperate lakes: PRJNA485560

9. SRA data Fungal diversity and community structure in 20 different temperate lakes in Northeast Germany: PRJNA485773

Liste möglicher Pressemitteilungen und Medienberichte.