# Temporal and spatial dynamics of peat microbiomes in drained and rewetted soils of three temperate peatlands

**Haitao Wang[1]*, Micha Weil[1], Dominik Zak[2, 3], Diana Mu□nch[1], Anke Günther[4], Gerald Jurasinski[4], Tim Urich[1]***

[1] Institute of Microbiology, University of Greifswald, Germany

[2] Department of Bioscience, Aarhus University, Denmark

[3] Department of Biogeochemistry and Chemical Analytics, Leibniz-Institute of Freshwater Ecology and Inland Fisheries Berlin, Germany

[4] Chair of Landscape Ecology and Site Evaluation, University of Rostock, Germany

**Contacting details:**

H. Wang: haitao.wang@uni-greifswald.de

M. Weil: micha.weil@uni-greifswald.de

D. Zak: doz@bios.au.dk

D. Mu□nch: diana.muench1@gmx.de

A. Günther: anke.guenther@uni-rostock.de

G Jurasinski: gerald.jurasinski@uni-rostock.de

Tim Urich: tim.urich@uni-greifswald.de

* Corresponding to: haitao.wang@uni-greifswald.de or tim.urich@uni-greifswald.de

## Abstract

**Background:** Drainage of high-organic peatlands for agricultural purposes has led to increased greenhouse gas emissions and loss of biodiversity. In the last decades, rewetting of peatlands is on the rise worldwide, to mitigate these negative impacts. However, it remains still questionable how rewetting would influence peat microbiota as important drivers of nutrient cycles and ecosystem restoration. Here, we investigate the spatial and temporal dynamics of the diversity, community composition and network interactions of prokaryotes and eukaryotes, and the influence of rewetting on these microbial features in formerly long-term drained and agriculturally used fens. Peat-soils were sampled seasonally from three drained and three rewetted sites representing the dominating fen peatland types of glacial landscapes in Northern Germany, namely alder forest, costal fen and percolation fen.

**Results:** Costal fens as salt-water impacted systems showed a lower microbial diversity and their microbial community composition showed the strongest distinction from the other two peatland types. Prokaryotic and eukaryotic community compositions showed a congruent pattern which was mostly driven by peatland type and rewetting. Rewetting decreased the abundances of fungi and prokaryotic decomposers, while the abundance of potential methanogens was significantly higher in the rewetted sites. Rewetting also influenced the abundance of ecological clusters in the microbial communities identified from the co-occurrence network. The microbial communities changed only slightly with depth and over time. According to structural equation models rewetted conditions affected the microbial communities through different mechanisms across the three studied peatland types.

**Conclusions:** Our results suggest that rewetting strongly impacts the structure of microbial communities and, thus, important biogeochemical processes, which may explain the high variation in greenhouse gas emissions upon rewetting of peatlands. The improved understanding of functional mechanisms of rewetting in different peatland types lays the foundation for securing best practices to fulfil multiple restoration goals including those targeting on climate, water, and species protection.

**Keywords:** Prokaryotes, Eukaryotes, Rewetting, Peatland soils, Alder forest, Coastal fen, Percolation fen

## Background

Peatlands store over 30% of the earth's soil carbon although they only cover nearly 3% of the global land area [1]. Waterlogging contributes to the high level of soil organic carbon since peat, consisting of

incompletely decomposed organic matters derived mostly from plant residues, accumulates when the oxygen is deficient [2]. However, drainage of wetlands including peatlands for agricultural use contributed to half of the loss of the global wetlands [1, 3]. Drainage of peatlands results in decreased emissions of methane ($CH_4$) but significantly increased emissions of carbon dioxide ($CO_2$) and potentially increased emissions of nitrous oxide ($N_2O$), ultimately contributing to increased warming potential effect [2, 4-6]. The conversion of natural fens to agricultural lands also leads to biodiversity loss due to intensive dehydration, tillage and fertilization [1]. Rewetting of drained peatlands aims at mitigating these negative impacts by restoring peatlands. By increasing the water level, the existing peat carbon pool is conserved as a result of the quickly reestablished anoxia. Under anoxic conditions phenol oxidase becomes inactivated promoting the accumulation of recalcitrant phenolic compounds released by plants which in turn limits the activity of hydrolase enzymes and eventually leads to low decomposition rates [7-9]. This benefit, however, might be offset since deficiency of oxygen can also promote carbon loss through elevated production and emission of $CH_4$ in particular if sites become inundated and colonized by dense stands of hydrophytes and/or helophytes [10]. Which effect prevails in a given rewetted peatland is strongly driven by the prevailing microbial decomposition processes. Therefore, a better understanding of the microbial communities and their processing of organic matter is paramount to an informed and optimized management of rewetted peatlands.

Fungi are considered to be the major agents of plant litter decomposition, while bacteria as well as some invertebrates can, to lesser extent, also be significant contributors [11]. The decomposition of litter and deadwood eventually leads to the release of $CO_2$ to the atmosphere. This process is called soil respiration, and is a crucial element of all carbon cycling on Earth [12]. The fate of organic carbon and nitrogen is also influenced by microbial communities associated with methane cycling and nitrogen cycling in which $CH_4$ and $N_2O$ are produced [13, 14]. While decent efforts were invested to investigate the influence of rewetting on the emissions of greenhouse gases (GHGs) [15-18], extracellular enzyme activities [19], dissolve organic carbon [20, 21], and water chemistry [15, 19], little is known about the changes of microbial communities driving these processes.

Since microbes are both the most diverse organism group on the planet and the major agents of biogeochemical cycling [22], their diversity and community structures are of great importance to ecosystem functioning. It is especially yet unclear how rewetting impacts the peat communities regarding all domains of life (archaea, bacteria and eukaryotes). Furthermore, unraveling the dynamics of

community composition together with relevant environmental factors helps to carve out the predictable spatial and temporal patterns [23], thus better perceiving the ecological consequences resulting from these patterns. It has been shown that decomposition rates and GHG emissions follow seasonal patterns [24-27], but the temporal dynamics of prokaryotic and eukaryotic communities in peat-soils are still poorly understood. A better understanding of microbial community dynamics in rewetted peatlands could lay the foundation for maintaining peat-soil quality and health, thus guiding future management of peatland restoration.

About two decades ago a peatland restoration program was initiated in the state of Mecklenburg-Vorpommern (M-V) in Northern Germany, and in this course over 20,000 ha of peat-soils in this state were rewetted [28]. The relatively high number of rewetted sites in different peatland types provides an excellent opportunity to study the influence of rewetting on peat microbial community dynamics in the three most relevant peatland types of the region, namely alder forest, coastal fen and percolation fen. The temporal dynamics of prokaryotic (archaeal and bacterial) and eukaryotic communities were assessed through high-throughput sequencing with 16S and 18S rRNA gene amplicons, respectively. Additional edaphic variables, including water content, pH, organic carbon and nutrients, were monitored. The diversities and community compositions of prokaryotes and eukaryotes were compared between the rewetted and drained sites within each peatland type. Our objectives are 1) to investigate the temporal changes of microbial diversities and community compositions in different seasons, 2) to reveal the influence of rewetting on the diversities, community compositions, network interactions and the potential functions of relevant biogeochemical processes, and 3) to reveal the functional mechanisms of rewetting on shaping the microbial communities and functions in the three different peatland types. We hypothesize that rewetting drained fens will influence diversity and community composition of both prokaryotes and eukaryotes, and changes in these communities will follow a seasonally temporal pattern. Moreover, the functional mechanisms of rewetting will be different in these three peatland types *inter alia* due to differences in salinity, vegetation and soil organic matter composition. This study finally intends to provide a theoretical guide for future planning and land management of drained peatlands.

## Results

### Study sites and soil edaphic properties

The six study sites are located in M-V in Northeastern Germany (Additional file 1: Fig. S1) and cover the three major peatland types (alder forest, coastal fen and percolation fen) in M-V. For each peatland type, two sites were selected. Both of them were drained in the past but one of the two has been rewetted. The alder forest presumably formed under water-logged conditions. Both sites were drained in the past and used as managed forest for many decades. The drained alder forest site (AD) is still drained, and the rewetted site (AW) was getting rewetted when the nearby Bauernmoor was rewetted in 2003. The coastal fen sites are located near Greifswald at the Karrendorf peninsula. The whole area was drained already in 1850 and has been used for pasture since. From the 1960s drainage was enforced and the areas were used as intensive grasslands. The drained site (CD) is still behind a dike but is now in an area that is used for extensive cattle grazing, the rewetted site (CW) is located in a part of the area that has been rewetted in 1993 by removing the old dikes. These parts are now flooded regularly, mostly during autumn and winter. The two percolation fens are located in one percolation fen complex at the rivers Recknitz and Trebel, respectively. Both sites also have a long drainage history with early drainage in the 18th century and much more intense drainage in the 1960s. Both sites were used as intensive grassland. While the drained site (PD) is still under medium intensive grassland use, the wet site (PW) has been rewetted in 1997 and was not actively managed since then.

The soil properties varied significantly among the six sites (Additional file 2: Table S1). Rewetting generally increased the soil moisture in all three peatland types (Additional file 1: Fig. S2 and Additional file 1: Fig. S3), with a significant increase in alder forest (Additional file 2: Table S1). Since September 2017, the water level was always higher in the rewetted sites compared with their drained counterparts with the exception that CW and CD showed similar water level fluctuations since May 2018 (Additional file 1: Fig. S4). The soil temperature followed a clear seasonal pattern from August 2017 to July 2018 (Additional file 1: Fig. S5). Moreover, dissolved organic matter (DOM) was higher in the wet sites, especially in the alder forest and coastal fen and the concentrations of the three considered DOM compounds were in most of the rewetted sites (Additional file 2: Table S1). The concentrations of DOM and single compounds also increased sharply from April to November in 2017 and then decreased to lower levels in February 2018 (Additional file 1: Fig. S2). All rewetted sites showed lower nitrate concentrations compared to their still-drained counterparts, while this was only the case in AW and CW for nitrite and ammonium (Additional file 2: Table S1). Nitrate showed a similar temporal pattern as

DOM in AD but a contrasting trend in the other sites, while ammonium concentrations were increasing since April in all sites except for CD (Additional file 1: Fig. S2). Water-extractable P content was higher in AW than in AD (Additional file 2: Table S1), and it significantly decreased since April in AW, CD and PD (Additional file 1: Fig. S2). pH and salinity were significantly higher in the rewetted sites compared with their drained counterparts (Additional file 2: Table S1). pH and salinity were not compared among seasons since they were measured with different methods in April and the other seasons. However, there were less variations of these soil properties with depths, with only a few changes observed in certain sites (Additional file 1: Fig. S3).

**Microbial diversities**

The diversities of both prokaryotes and eukaryotes were lower in the coastal sites compared with the other sites (Fig. 1a, Additional file 1: Fig. S6, and Additional file 2: Table S1). Prokaryotic diversities changed significantly across seasons in AD, CD, PD and PW, while eukaryotic diversities, including fungi, protists and metazoa, showed less significant changes across seasons (Fig. 1a). Under wet conditions prokaryotic diversity was higher in coastal fen and protist diversity was higher in alder forest and coastal fen (Additional file 2: Table S1). Metazoan diversity, however, was lower in all wet sites while diversities of fungi and protists were lower in the wet percolation fen (Additional file 2: Table S1). Prokaryotic diversity declined with depth in AW and CW (Additional file 1: Fig. S6) as well as the diversities of fungi and metazoa (Additional file 1: Fig. S6). Both prokaryotic and eukaryotic diversities showed positive correlation with nitrate and negative correlation with salinity and prokaryotic diversity was also positively correlated with other soil properties (Additional file 1: Fig. S7).

**Microbial community compositions**

The prokaryotic communities were dominated by Acidobacteria (19.4%), Betaproteobacteria (11.7%), Alphaproteobacteria (11.1%), Actinobacteria (7.69%) and Chloroflexi (6.41%) (Additional file 1: Fig. S8). The taxa distributions showed similar patterns during these four seasons, but obvious differences among sites were observed. For instance, the Acidobacteria were more abundant in the drained sites compared with the rewetted sites, while Betaproteobacteria showed a contrasting trend (Additional file 1: Fig. S8). Actinobacteria were more abundant in the coastal fens while Cloroflexi were more abundant in

the other two peatland types (Additional file 1: Fig. S8). These variations were reflected in the nonmetric multidimensional scaling (NMDS) plot where the study sites grouped very well (Fig. 1b). However, the samples from percolation fen and alder forest showed considerable overlap resulting from the numerous shared amplicon sequence variants (ASVs) between these two peatland types (Fig. 1b). In addition, these two peatland types shared much less ASVs with the coastal fen than they shared with each other (Fig. 1b). The changes in prokaryotic community composition were mostly driven by peatland types (PERMANOVA, $R^2$=0.255, $P$=0.001). The hydrological state (drained/rewetted) also showed a significant but less strong influence (PERMANOVA, $R^2$=0.080, $P$=0.001) on the prokaryotic community. However, season showed no significant impact on prokaryotic community composition (PERMANOVA, $R^2$=0.017, $P$=0.168, Additional file 1: Fig. S9a), while depth showed a significant but weak impact (PERMANOVA, $R^2$=0.026, $P$=0.001, Additional file 1: Fig. S9b). NMDS was also conducted separately for each site to exclude the site effect, and the results showed that the interaction of depth and season exhibited strong impact on community composition in all sites except AD (PERMANOVA, $P$<0.05, Additional file 1: Fig. S10).

The eukaryotic communities were dominated by fungi (Ascomycota, 25.6%; Basidiomycota, 12.5%; Glomeromycota, 3.09%), Nematoda (10.5%), Cercozoa (9.53%), Apicomplexa (5.01%) and Arthropoda (4.47%) (Additional file 1: Fig. S11). Specifically, Nematoda and Mortirellales were more abundant in the drained sites compared with the rewetted sites and Apicomplexa were more abundant in the percolation fens compared with the other two peatland types (Additional file 1: Fig. S11). The eukaryotic community composition exhibited a congruent pattern as prokaryotes but with less overlapping between percolation fen and alder forest (Fig. 1c). There were significant differences between peatland types (PERMANOVA, $R^2$=0.189, $P$=0.001), and between drained and rewetted sites (PERMANOVA, $R^2$=0.071, $P$=0.001), while there was no significant impact of season (PERMANOVA, $R^2$=0.018, $P$=0.069, Additional file 1: Fig. S9c) and only a weak impact of depth (PERMANOVA, $R^2$=0.018, $P$=0.002, Additional file 1: Fig. S9d). Similar to prokaryotes, we also found that the interaction between depth and season exhibited a strong impact on community composition in all sites except AD (PERMANOVA, $P$<0.05, Additional file 1: Fig. S12).

The prokaryotic and eukaryotic community compositions were driven by soil properties in a similar way. Salinity, which was higher in the coastal fen sites, differentiated the coastal from the other fens, while nitrate was more abundant and related with alder forest (Fig. 1b and c). Moisture, pH, DOM and its

components, P and ammonium content, were generally highest in PW, and they also differentiated the rewetted from the drained sites (Fig. 1b and c). The community composition of eukaryotes was significantly correlated with that of prokaryotes (Mantel test, r=0.852, *P*=0.001). By analyzing plant ASVs separately, we also found distinct clusters of the samples grouped by sites (Additional file 1: Fig. S9e). The Mantel tests showed that the plant community composition was strongly and significantly correlated with both prokaryotic (r=0.551, *P*=0.001) and eukaryotic (r=0.634, *P*=0.001) community compositions.

**Differentially abundant taxa and predicted functions**

The prokaryotic ASVs were collapsed into functional groups based on their taxonomies using FAPROTAX. 8 dominant and representative functions regarding carbon and nitrogen cycles, and decomposition were analyzed (Fig. 2). Rewetting significantly promoted functions including methanogenesis, denitrification, sulfate respiration and fermentation in certain peatland types, while the others including cellulolysis, nitrification and nitrogen fixation were over-represented in the drained sites (Fig. 2). Methanotrophy was over-represented in the rewetted sites of alder forest and percolation fen but in the drained site of coastal fen (Fig. 2). While the predicted functions showed few temporal patterns (Additional file 1: Fig. S13), there were more differences between depth sections (Additional file 1: Fig. S14). Some differentially abundant ASVs were dominant and could be assigned to certain functions according to FAPROTAX. Within Actinobacteria, several dominant ASVs of *Acidothermus* genus involved in cellulolysis were more abundant in AD and PD than in their rewetted counterparts (Fig. 3) supporting the finding that microbes conducting cellulolysis were more abundant in the drained sites (Fig. 2). Two *Flavobacterium* ASVs with high fold changes were more abundant in CD than in CW, and they are aerobic chemoheterotrophs (Fig. 3). One ASV of *Desulfobulbus* driving sulfate reduction was more abundant in CW than in CD (Fig. 3), which is in line with the result that sulfate respiration was stronger in the rewetted sites (Fig. 2). Some ASVs of *Sulfurimonas* were also more abundant in the rewetted site of coastal fen (Fig. 3). The ASVs belonging to phylum Nitrospirae were mostly more abundant in the rewetted sites, while several ASVs of *Ca*. Nitrosotalea were more important in the drained sites (Fig. 3).

While most of the fungal ASVs could not be matched to the database, 15 functional guilds were identified with FUNGuild, and only 8 of them showed significant differences between the drained and the rewetted sites (Additional file 1: Fig. S15). Rewetting promoted the fungi that might be plant pathogens, algal or

fungal parasites, and soil or plant saprotroph in the alder forest and the percolation fen (Additional file 1: Fig. S15). Soil saprotroph and arbuscular mycorrhizal fungi (AMF) were abundant and were over-represented in CW, while AMF or fungi that might be some certain saprotrophs were more important in AD and PD (Additional file 1: Fig. S15). The analysis of differentially abundant taxa supported these results. The ASVs of *Archaeorhizomyces* were identified as soil saprotrophs, and one of these abundant ASVs was more important in PW than in PD (Fig. 4). The ASVs belonging to Tremellales (Fungal Parasite-Undefined Saprotroph) were more abundant in the drained site (Fig. 4). The Glomeromycota phylum was identified as AMF. One Archaeosporales ASV was dominant in AD, and the two differentially abundant AMF ASVs were found in PD, while there were more abundant AMF ASVs in CW than in CD (Fig. 4). For protists, the Apicomplexa including ASVs of Eugregarinorida, Gregarina and *Monocystis agills* was more important in the drained sites compared to the rewetted sites (Fig. 4). For metazoa, the abundant ASVs of Acari belonging to Arthropoda were found in AD and in both CW and CD, while several nematoda ASVs belonging to Rhabditida, Tylenchida and Enplea were mostly found to be more abundant in the drained sites (Fig. 4).

Since microbes carrying out cellulolysis showed high abundance (Additional file 1: Fig. S13 and S14), we are considering them as the major prokaryotic decomposers. Both the prokaryotic (cellulolysis) and eukaryotic decomposers (fungi) were significantly and negatively correlated with the water content (Additional file 1: Fig. S16).

**Co-occurrence network**

In total, 6,290 significant correlations were captured among 1,769 prokaryotic and 708 eukaryotic ASVs, and all of them were positive (Fig. 5a). These correlations could indicate the potential interactions among different microbial taxa. We identified 8 major ecological clusters (modules) from this network which might potentially drive some specific functions in the ecosystem (Fig. 5a). These clusters were differently distributed across sites, and the abundances of prokaryotes and eukaryotes within each module showed consistent patterns (Fig. 5b). Module I and III were dominant in some rewetted sites, while module IV, V and VI showed higher abundances in the drained sites. Module II was more abundant in the alder forest while module VII was more abundant in the coastal fen. Elements of Module VIII were mainly distributed in AW and PD. However, there was no distinct change of these modules with depths or seasons (Fig. 5b). Since all prokaryotes and eukaryotes with a certain module responded similarly, we

averaged their abundances for each module. The correlation analysis exhibited that all these modules were significantly correlated with soil moisture, nitrate concentration and salinity (Additional file 1: Fig. S7). Some of them were also correlated with DOM and pH (Additional file 1: Fig. S7).

**Structural equation modelling (SEM)**

We designed a *priori* theoretical model based on the causal relationships among the variables (Additional file 1: Fig. S17). By conducting the group analysis and comparing the constrained and unconstrained models, we observed significant differences of the models for the three peatland types. In the alder forest, rewetting showed no direct effects on prokaryotic and eukaryotic diversities but had direct effects on the other response variables. Rewetting could also indirectly influence these microbial parameters through changing soil moisture and then DOM which showed significant impacts on these responses (Fig. 6). DOM also covaried with Nitrate, pH and plant community composition, indicating that DOM might also be controlled by these properties (Fig. 6). Moreover, the different plant community compositions between drained and rewetted sites also influenced the eukaryotic community composition, ecological clusters and predicted functions (Fig. 6). In the coastal fen, rewetting directly influenced all the responses. The indirect effects of rewetting were mainly mediated by nitrate, DOM and pH, but not by soil moisture (Fig. 6). As salinity strongly covaried with nitrate and differed significantly between CD and CW (Additional file 1: Fig. S7and Additional file 2: Table S1), salinity in addition to pH might be the key driver of rewetting effect in coastal fen. The plant community compositions only mediated the influence of rewetting on eukaryotic functions and ecological clusters (Fig. 6). In the percolation fen, rewetting showed direct effects on all the response variables excluding prokaryotic diversity. It also initially influenced the soil moisture which then influenced nitrate, DOM and pH. The pH was an important mediator which was influenced directly by rewetting and moisture, and influenced all the responses excluding eukaryotic communities (Fig. 6). However, DOM and plant community composition showed less impact on the response variables (Fig. 6). The $R^2$ values of eukaryotic diversity were low in all study sites, and the prokaryotic diversity was also weakly explained in alder forest and percolation fen (Fig. 6).

**Discussion**

Our results show that rewetting drained fens influences the diversity and community composition of both prokaryotes and eukaryotes, but different patterns were found for the three peatland types. In the following temporal and spatial aspects are considered in detail besides certain specific factors driving the

change of microbial communities. Finally, the overall impact of rewetting on microbial communities is discussed in the broader context of ecological restoration and GHG emissions.

**1. Temporal and vertical patterns of the prokaryotic and eukaryotic communities**

In our study, most of the detected soil parameters showed significant temporal changes in addition to soil temperature (Additional file 1: Fig. S2 and S5), the most important indicator of seasonality. These parameters probably drove the temporal patterns observed in the prokaryotic and some of the eukaryotic diversities (Fig. 1a), as well as some of the temporal changes of predicted functions (Additional file 1: Fig. S13). However, both prokaryotic and eukaryotic community compositions at sites showed no obvious temporal changes, indicating that peat microbial communities do not react strongly to weather driving temporal changes of environmental conditions. This resilience to external forces such as temperature and nutrient supply might result from the internal feedback mechanisms including competition, viral infection and predator-prey interlinkages, that maintain a relatively stable state of community in seasonally changing environments [23]. Moreover, the water level we monitored since September 2017 (Additional file 1: Fig. S4) and the water content (Additional file 1: Fig. S2) showed no obvious seasonal patterns. The stable water status among seasons might also have contributed to the slow changes in the microbial communities since soil moisture was one of the most important environmental factors shaping the community compositions (Fig. 1b and c). Also, if water-saturation or high-water levels occur, respectively it may create homogeneity and weak niche differentiation that leads to stronger interactions between microbes [29], resulting in high resilience to environmental stresses. These results support that peatlands can stand and be resilient to gradual, long-term changes in climate and water conditions [2].

The decrease of fungal and metazoan diversities with increasing depth (Additional file 1: Fig. S6) suggests the general pattern that these two eukaryotic groups prefer to live in upper soil layers where oxygen is sufficiently available. It has been demonstrated that environmental gradients drive changes in microbial community composition along soil depth [30]. Our study found that depth showed a weak yet significant effect on the microbial community compositions. While many soil parameters we recorded changed only slightly with depth, distinct moisture depth profiles were observed in sites with higher water content (Additional file 1: Fig. S3). This indicated that oxygen related to water content might contribute to depth profiles of microbial communities, which was also supported by significant changes in

oxygen-sensitive microbial processes with depths, including methanogenesis, nitrification, cellulolysis and fermentation (Additional file 1: Fig. S14). Despite of the weak impact of depth and season, their interaction showed strong and significant effect on both prokaryotic and eukaryotic community compositions in all sites except AD (Additional file 1: Fig. S10 and S12), suggesting that the influence of these two factors was covered by the influence of site. It also indicated that temporal shifts of compositions only existed at certain depths in a single site. However, one year of observation is a limited basis for understanding seasonal patterns as these patterns include an annual repeating pattern, not just changes over a short period [23]. In addition, we experienced quite unusual weather patterns over the course of our study period with very wet end of 2017 followed by an exceptionally dry year 2018. Therefore, a longer-term monitoring of microbial dynamics in peatlands at a smaller scale is needed in future studies.

**2. Major factors driving changes in prokaryotic and eukaryotic communities**

By investigating the dynamics of prokaryotic and eukaryotic communities in peat soils, we observed a congruent pattern between these two communities as well as their responses to the environmental factors, which is supported by a previous study showing a similar pattern [31]. In the coastal sites we found a significant lower prokaryotic and eukaryotic diversity than in the two freshwater peatland types, which was mainly due to the varying salinity that was negatively correlated to both diversities (Additional file 1: Fig. S7). Salinity also differentiated the coastal sites from the other two types in terms of community composition. Since salinity is a key factor in influencing biogeochemical cycles, increased salinity can shape specific microbial guilds [32-34]. Given that CW is frequently flooded by the brackish seawater from the Baltic Sea, the high saline condition in CW strongly increased the abundance of microbes carrying out sulfate respiration while decreasing the abundance of those carrying out methanogenesis (Additional file 1: Fig. S13). Since sulfate is the main electron acceptor in saline habitats, sulfate reducers with a higher affinity with substrates and a higher energy gain outcompete methanogens under these conditions [35]. Moisture was another important factor that shaped the communities, and it was significantly and positively correlated with diversities of prokaryotes and protists, as well as with other properties including pH and concentrations of nutrients including organic compound, nitrogen and P (Additional file 1: Fig. S7). This is in line with a previous study showing that increased water level by permanent inundation released large amounts of nutrients into pore water, especially phosphorus and ammonium [15]. Therefore, higher moisture might initially increase the availability of nutrients, which

then increases the microbial diversities and shapes the community structures in years of rewetting. Interestingly, nitrate concentrations were much higher in AD and thus differentiated AD from the other sites (Fig. 1b and 2c). This might be due to either the high nitrification activity generating nitrate and/or due to higher external nitrate import via groundwater or precipitation. Moreover, vegetation was also an important factor that cannot be neglected. According to our data, the plant community composition was strongly correlated with both prokaryotic and eukaryotic community compositions, suggesting that plants might be the driving factor of the congruent patterns observed between prokaryotes and eukaryotes.

**3. Influence of rewetting on microbial communities regarding ecological consequences**

Rewetting potentially promoted methanogenesis in AW and PW, but not in CW (Fig. 2), due to the high amount of sulfate reducers in CW which is regularly flooded with sea water. The higher abundance of methanogens in rewetted sites seems to be the main reason for the increase of $CH_4$ production and thus potentially emission after rewetting [36]. It has been shown that $CH_4$ dominated the balances of annual greenhouse gases in decades after rewetting a bog in Lower Saxony [16]. The dominance of $CH_4$ was also observed in riparian wetlands after 12-year rewetting [37]. Since the fens in our study all have been rewetted for over 12 years before our measurements commenced, the rewetting could have contributed to high $CH_4$ emissions, which should to be tested with measurements of GHG exchange in the future. However, it is also noteworthy that potential methanotrophs consuming $CH_4$ were also more abundant in AW and PW (Fig. 2). ANME groups were only found in AW in our study, with a relative abundance of 0.15%, resulting in the highest methanotrophy abundance in AW, and this indicated that rewetting in this site potentially accumulated the anaerobic methane oxidation. Hence, the alder forest and the coastal fen seem to be the better choice to be rewetted regarding consumption and production of $CH_4$, respectively, but this is of course a rather narrow perspective since the whole GHG budgets have to be considered.

One purpose of rewetting drained peatlands is to restore the carbon storage by cutting off oxygen supply with inundation, thus decreasing the emitted $CO_2$ from soil respiration. One major process is the decomposing of litter and deadwood by decomposers comprising some major bacteria and fungi [11, 12]. Microbes that carry out cellulolysis seemed to be the major prokaryotic decomposers in our study due to their high abundance (Additional file 1: Fig. S13 and S14). We found that abundances of both cellulolysis and fungi were strongly and negatively correlated with water content (Additional file 1: Fig. S16), suggesting an underlying mechanism of the long-known fact that water content is a key factor controlling

the microbial decomposition activity in peat-soils. Considering that rewetting generally elevated the water level, our results also illustrate the known fact that rewetting of drained peat-soils could inhibit the microbial decomposition, thus conserving the carbon. This is further supported by some studies showing that rewetting can reduce the carbon loss from peatlands by investigating net ecosystem changes of $CO_2$ and $CH_4$ [15, 38, 39].

Nitrification and denitrification are sources of $N_2O$ and the function prediction supported the changes in these processes under wet conditions (Fig. 2). Since the relative abundances of potential denitrifiers were much lower compared with those of nitrifiers (Additional file 1: Fig. S13 and S14), the production of $N_2O$ in these fens seems to stem primarily from nitrification. Potential nitrifiers were over-represented in drained sites of all peatland types (Fig. 2), supporting the finding that rewetting generally reduces the emission of $N_2O$ [17, 39].

Rewetting also showed influences on the eukaryotic communities. The lower diversities of fungi and metazoa in rewetted sites might have been driven by the deficiency of oxygen under wet conditions since both groups prefer aerobic living conditions. Some protists including Eugregarinorida, Gregarina and *Monocystis* are known parasites of invertebrates, and they were over-represented in drained sites (Fig. 4). Some metazoa known as parasites of plants and animals, including Tylenchida, Rhabditida and Enpleas, were also over-represented in drained sites (Fig. 4). These results illustrate that rewetting might help survivals of certain animal or plant species living in that area.

## Conclusions

Our study showed the significant influence of rewetting on both prokaryotic and eukaryotic communities in peat soils. To our knowledge, this is the first study that comprehensively described the changes of peat communities regarding all domains of life across seasons after long-term rewetting, which bridges the gap between rewetting practice and the induced ecological consequences. Congruent responses of prokaryotic and eukaryotic communities to rewetting were observed and were driven by important abiotic and biotic factors. Significant changes in potential microbial functions, fungi and animals indicate important environmental consequences of rewetting relevant to carbon balance, GHG productions and parasitism. Rewetting also induced different mechanistic relationships between microbial parameters and environmental factors between different types of peatlands, suggesting that habitat-specific responses of soil and water properties to rewetting should be aware, given that these are important factors driving

biogeochemical processes. Nevertheless, lack of temporal dynamics of the peat communities needs to be verified with future studies in a longer term of investigation.

## Methods

### Soil sampling

The peat soils were sampled from these six sites in April 2017 (spring), August 2017 (summer), November 2017 (autumn) and February 2018 (winter). At each site, samples were taken at three spots as replicates at depths of 5-10 cm, 15-20 cm and 25-30 cm from the top soils. Samples were taken using a soil core and then stored in sterilized Nasco Whirl-PAK baggies. The collected soils were immediately blended and transported to the laboratory with an ice box. Soils were kept at 4 $^{o}$C before being processed on the next day of sampling. In total, 216 samples (3 depths × 6 sites × 4 seasons × 3 replicates) were collected.

### Soil edaphic properties

Soils moisture was gravimetrically measured by drying the soil over night at 90 $^{o}$C until mass constancy. Dissolved organic carbon (DOC) was extracted from 3 g fresh soil in 30-mL 0.1 M NaCl solution on a shaker for 30 min at 200 rpm. Then the extracts were filtered using 0.45 µm sodium acetate filters. To minimize possible artifacts acetate filters were carefully pre-rinsed with ca. 100 ml deionized water and filtered samples were stored not longer than 1 week at 4°C before conducting DOM analysis [40]. A size-exclusion chromatography (SEC) with organic carbon and organic nitrogen detection (LC-OCD-OND analyzer, DOC-Labor Huber, Karlsruhe, Germany) was used to detect the concentrations and composition of DOC based on size categories [41]. The detected organic compounds were categorized into three groups: (i) biopolymers (BP-S), (ii) humic or humic-like substances including building blocks (HL-S), and (iii) low molecular-weight substances (LM-S). The total amount of these three groups was calculated as DOM. The ammonium molybdate spectrometric method (DIN EN 1189 D11) was used to determine soluble reactive phosphorus with a Cary 1E Spectrophotometer (Varian). The photometry CFA method (Skalar SAN, Skalar Analytical B.V., The Netherlands) was used to calorimetrically determine N-$NH_4^+$ and N-$NO_x^-$ concentrations according to the guidelines in EN ISO 11732 (DEV-E 23) and EN ISO 13395 (DEV, D 28), respectively. The pH and salinity of water samples taken from wells installed at each study site were measured manually by digital meters in April 2017. The

data of the other three seasons were manually measured at groundwater wells with the water sensors (Aquaread AP-2000 / AP-2000-D). Water levels and soil temperatures were also continuously monitored at each site since then using Campbell Scientific CR300 Dataloggers (Logan, USA) and HOBO Dataloggers (Bourne, USA), respectively.

**DNA extraction and sequencing**

DNA was extracted from 0.25 g soil using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions with some modification. Vortex in the bead beating step was replaced with a FastPrep®-24 5G instrument (MP Biomedicals, Santa Ana, USA), with an intensity of 5.0 m/s for 45 s. The extracted DNA samples were sent to LGC Genomics GmbH (Berlin, Germany) for 16S rRNA and 18S rRNA gene amplicon sequencing. Primers pairs of 515YF (5'-GTGYCAGCMGCCGCGGTAA-3')/B806R (5'-GGACTACNVGGGTWTCTAAT-3') [42] and 1183F (5'-AATTTGACTCAACRCGGG-3')/1443R (5'-AATTTGACTCAACRCGGG-3') [43] were used to amplify 16S rRNA (prokaryotes) and 18S rRNA (eukaryotes) genes, respectively. Amplicons were sequenced with Illumina Miseq 300 bp paired-end platform.

**Sequencing data analysis**

16S rRNA and 18S rRNA gene amplicon sequences were processed separately. For each processing, the raw sequence reads were demultiplexed with barcodes, adapters and primers removed using the Illumina *bcl2fastq* software. The data were then processed with *dada2* (v1.8.0) pipeline [44] in R v3.5. The qualities of the sequences were checked, and sequences failing to meet the filter scores (maxEE=2, truncQ=2, maxN=0) were discarded. The filtered sequences were de-replicated and clustered into ASVs, and paired-end sequences were merged. The chimeric sequences were then de-novo checked and removed. The final representative sequence of each ASV was assigned to taxonomy against a modified version of the SILVA SSUref_NR_128 database [45]. ASVs with only one sequence were removed. ASVs of 16S rRNA amplicons that were assigned to Chloroplast or mitochondria were also removed. Several samples were discarded due to their low sequence numbers (<1,000), resulting in a total of 209 samples for further analysis. Finally, 16S rRNA and 18S rRNA sequences were clustered into 25,864 and 15,937 ASVs, respectively.

Plant sequences accounted for ~33% of the 18S rRNA amplicons, since peat-soil is mainly formed with dead plants. We split plant sequences from eukaryotic ASVs to increase the resolution of the other eukaryotes, but the plant sequences were also analyzed accordingly and separately. The diversity (Shannon index) of both prokaryotes and eukaryotes (fungi, protists and metazoa) were calculated. The tables of ASV counts were normalized using metagenomeSeq's CSS [46], and the community compositions were analyzed using nonmetric NMDS based on Bray-Curtis dissimilarity distances. The 16S rRNA taxonomic profiles were converted to putative functional profiles using FAPROTAX which maps prokaryotic clades to established metabolic or other ecologically relevant functions [47]. However, some modifications were made. The ASVs involved in nitrification were further verified by blasting against NCBI database according to a previous study [48]. The anaerobic methanotrophic groups ANME were excluded from methanogenesis. Similarly, the fungal taxonomic profiles were also converted to ecological guilds using FUNGuild [49].

A co-occurrence network was constructed to explore the potential interactions between species, including prokaryotes, plants and other eukaryotes. ASVs with relative abundances lower than 0.01% were filtered. The pairwise Spearman's rank correlations were performed with *Hmisc* package [50] and all *P*-values were adjusted by the Benjamini and Hochberg false discovery rate (FDR) method. The cutoffs of correlation coefficient and adjusted *P*-value were 0.8 and 0.01, respectively. The significant correlations were visualized using *igraph* package [51]. Network consists of modules which are intensively connected themselves but sparsely connected to other modules. These ecological clusters (modules) were identified from this network using *igraph*. The relative abundances of prokaryotes and eukaryotes in each module were calculated as sum of the relative abundances of the prokaryotic and eukaryotic ASVs belonging to this module, respectively.

**Statistical analysis**

The statistical analyses were done with R v3.5. The significance of the difference among different seasons or depths was identified with non-parametric Kruskal-Wallis test using *vegan* package [52]. Kruskal-Wallis posthoc tests were conducted to compare the means of alpha diversity, soil properties, predicted functions and ecological clusters between each two groups of a factor using *PMCMR* package [53]. The ASVs (relative abundance > 0.01%) that were differently abundant in drained and rewetted sites were identified with differential expression analysis based on the Binomial distribution using *DESeq2*

package [54]. The same differential expression analysis was also performed on predicated prokaryotic and fungal functions. Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to test the significance of impacts of rewetting, peatland type, depth, season and their interactions on the prokaryotic and eukaryotic community compositions using *vegan* package. Mantel test was used to test the correlations between prokaryotic, eukaryotic and plant community compositions with *vegan* package. The pairwise Spearman's rank correlations were conducted to examine the relationships between diversities, soil properties, community compositions, predicted functions and ecological clusters using *Hmisc* package, and were plotted with *corrplot* package [55]. The soil properties were fitted into NMDS ordination, and the significant ones were kept using the envfit function in the *vegan* package. All the *P*-values for multiple comparisons were adjusted by FDR method and the null hypothesis was rejected while *P*-values were less than 0.05.

SEM was performed to further find out the mechanism of rewetting effect on the microbial features. We built a *priori* theoretical model based on the causal relationships among the variables (Additional file 1: Fig. S17). We assume that rewetting could influence the microbial communities either directly or indirectly through changing the water content and then the other soil parameters and the plant community composition. To reduce the complexity of the model, 4 soil parameters were chosen according to the correlation analysis (Additional file 1: Fig. S7). pH possibly drove DOM as well as other properties, while nitrate showed less relevance with other properties but was driven by salinity (Additional file 1: Fig. S7). We also reduced the dimensions of predicted functions and ecological clusters using NMDS based on Bray-Curtis dissimilarities. The axes of NMDS were utilized in this model. SEM was constructed using covariance-based method with *lavaan* package [56]. Since we found that peatland type mostly drove the microbial communities (Fig. 1b and c), it is interesting to find out the different functional mechanisms of rewetting across these 3 peatlands. We therefore implemented the group analysis using the *sem* function with peatland type as the group in *lavaan*. Before that, all variables were checked for normality, and the non-normally distributed ones were transformed using a two-step method [57]. Since the multivariate normality of the final dataset still showed significant multivariate skew and kurtosis, we used the Satorra-Bentler (S-B) procedure to correct the fitting statistics. The initial model showed a weak yet adequate fit to the data (S-B $\chi^2$=4.752, *P*=0.191). To improve this model, we then constrained the coefficients of two paths that were not significant in all 3 peatland types to zero (Additional file 1: Fig. S17). The constrained model and unconstrained model showed no significant

difference with scaled chi-square difference test ($P$=0.786). Using the same method, we also constrained that regressions among different fens were equal, and the significant difference ($P$<0.001) between constrained and unconstrained models indicated that our models were different between these 3 peatlands.

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**Availability of data and material**

All the sequencing files were deposited to the European Nucleotide Archive of EMBL (European Molecular Biology Laboratory). The study accession number is PRJEB36764.

**Authors' contributions**

HW, MW and TU conceived and designed the research; HW, MW and DM collected the samples and performed the lab work; DZ measured the soil edaphic properties, and AG provided the environmental data; GJ helped to generate the structure of this manuscript; HW analyzed the data and wrote the first draft of the manuscript, and all authors contributed to revisions. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

[1]Institute of Microbiology, University of Greifswald, Germany. [2]Department of Bioscience, Aarhus University, Denmark. [3]Department of Biogeochemistry and Chemical Analytics, Leibniz-Institute of

Freshwater Ecology and Inland Fisheries Berlin, Germany. [4]Chair of Landscape Ecology and Site Evaluation, University of Rostock, Germany.

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## Figure legends

**Fig. 1** (**a**) Changes in Shannon index of prokaryotes and eukaryotes including fungi, protists and metazoa in different seasons. Data are shown as mean values and the error bars represent standard errors. Asterisks indicate the significant changes in different seasons in a corresponding site (Kruskal-Wallis test, *$P$<0.05, **$P$<0.01). (**b**) Prokaryotic and (**c**) eukaryotic community compositions based on the Bray-Cutis dissimilarities shown as NMDS plots. Venn diagrams show the number of shared prokaryotic and eukaryotic ASVs associated with different peatland types. The main ordinations in NMDS plots show similarity between samples and the arrows show correlations between environmental variables and ordination axes. DOM, dissolved organic matter; BP-S, biopolymer substances; HL-S, humic-like substances; LM-S, low-molecular substances; P, phosphorus.

**Fig. 2** The differentially abundant predicted functions of prokaryotes between rewetted and drained sites in three different mires. Positive log2-fold change values indicate significantly higher abundances in rewetted site (adjusted $P$<0.05), while negative values indicate significantly higher abundances in drained site (adjusted $P$<0.05). The size of the points indicates the relative abundance of the functional groups.

**Fig. 3** The differentially abundant ASVs of prokaryotes between rewetted and drained sites in three different mires. Positive log2-fold change values indicate significantly higher abundances in rewetted site

(adjusted $P<0.01$), while negative values indicate significantly higher abundances in drained site (adjusted $P<0.01$). The size of the points indicates the relative abundance of the ASVs.

**Fig. 4** The differentially abundant ASVs of eukaryotes between rewetted and drained sites in three different mires. Positive log2-fold change values indicate significantly higher abundances in rewetted site (adjusted $P<0.01$), while negative values indicate significantly higher abundances in drained site (adjusted $P<0.01$). The size of the points indicates the relative abundance of the ASVs.

**Fig. 5** (**a**) Co-occurrence network and identified modules. Prokaryotic nodes are shown as cycles while eukaryotic nodes are shown as triangles. (**b**) The distribution of relative abundances of prokaryotic and eukaryotic nodes within each module in different sites, depths or seasons. M, module; pro, prokaryote; euk, eukaryote.

**Fig. 6** Structural equation model showing the direct and indirect effect of rewetting on the diversities, community compositions, network ecological clusters and the predicted microbial functions. Paths that showed significant relationships ($P<0.05$) are shown. The numbers along with arrows are standardized path coefficients, and the numbers in the brackets indicate the number of axis (nMDS-axis 1 or axis 2). Direct effects of rewetting and moisture are shown as red and blue arrows, respectively. The gray double-sided arrows indicate the covariant relationships between the variables. Amount of variance explained by the model ($R^2$) is listed for all response variables. DOM, dissolved organic matter; Pro, prokaryote; Euk, eukaryote; Eco-clusters, ecological clusters derived from network analysis; Micro-functions, microbial functions derived from FAPROTAX.

## Additional files

**Additional file 1: Fig. S1.** Map showing locations of sampling sites. **Fig. S2.** Changes in soil edaphic properties in different seasons. **Fig. S3.** Changes in soil edaphic properties in different depths. **Fig. S4.** Groundwater level monitored every 15 min, shown as mean value of each day from 2017-9-22 to 2018-7-31. **Fig. S5.** Soil temperature monitored every 15 min at two depths (5 cm and 15 cm), shown as mean value of two depths and of each day from 2017-7-24 to 2018-7-31. **Fig. S6.** Changes in Shannon

index of prokaryotes and eukaryotes including fungi, protists and metazoa in different depths. **Fig. S7.** Pairwise Spearman's rank correlations between diversities, soil properties, community compositions, predicted functions and ecological clusters. **Fig. S8.** The distributions of prokaryotic phyla or classes in different sites, depths and seasons. **Fig. S9.** NMDS plots based on the Bray-Cutis dissimilarities showing prokaryotic community composition among seasons (**a**) and depths (**b**), eukaryotic community composition among seasons (**c**) and depths (**d**), and plant community composition (**e**) and fungal community composition (**f**) associated with peatland types and water status. **Fig. S10.** NMDS plots based on the Bray-Cutis dissimilarities showing prokaryotic community composition among seasons and depths in AD (**a**), AW (**b**), CD (**c**), CW (**d**), PD (**e**) and PW (**f**). **Fig. S11.** The distributions of eukaryotic phyla in different sites, depths and seasons. **Fig. S12.** NMDS plots based on the Bray-Cutis dissimilarities showing eukaryotic community composition among seasons and depths in AD (**a**), AW (**b**), CD (**c**), CW (**d**), PD (**e**) and PW (**f**). **Fig. S13.** Changes in predicted prokaryotic functions in different seasons. **Fig. S14.** Changes in predicted prokaryotic functions in different depths. **Fig. S15.** The differentially abundant predicted functions of fungi between rewetted and drained sites in three different peatlands. **Fig. S16.** Linear regression between gravimetric water content and relative abundance of prokaryotes carrying cellulolysis (**a**) or fungi (**b**). **Fig. S17.** A *priori* structural equation model. (PDF 16.6M)

**Additional file 2: Table S1.** Soil edaphic properties and Shannon indices of prokaryotes, fungi, protists and metazoa in different sites, in different depths, and in different seasons. (XLXS 12KB)

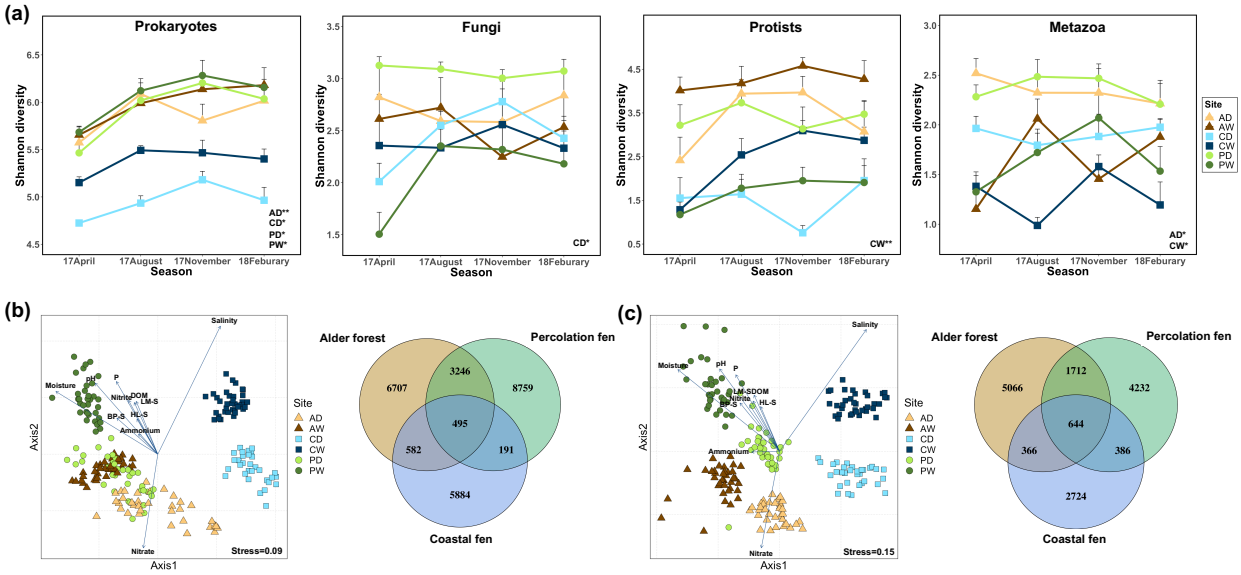


**Fig. 1** (**a**) Changes in Shannon index of prokaryotes and eukaryotes including fungi, protists and metazoa in different seasons. Data are shown as mean values and the error bars represent standard errors. Asterisks indicate the significant changes in different seasons in a corresponding site (Kruskal-Wallis test, **P*<0.05, ***P*<0.01). (**b**) Prokaryotic and (**c**) eukaryotic community compositions based on the Bray-Cutis dissimilarities shown as NMDS plots. Venn diagrams show the number of shared prokaryotic and eukaryotic ASVs associated with different peatland types. The main ordinations in NMDS plots show similarity between samples and the arrows show correlations between environmental variables and ordination axes. DOM, dissolved organic matter; BP-S, biopolymer substances; HL-S, humic-like substances; LM-S, low-molecular substances; P, phosphorus.

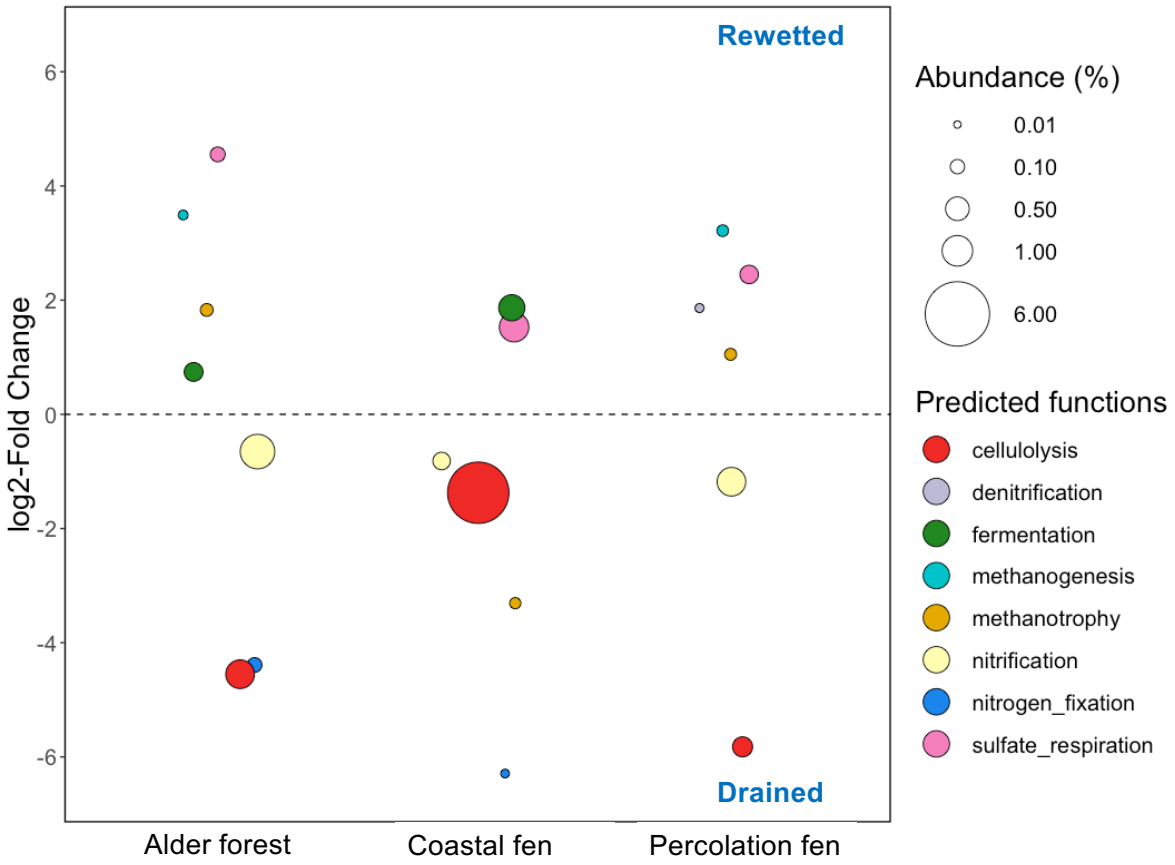


**Fig. 2** The differentially abundant predicted functions of prokaryotes between rewetted and drained sites in three different mires. Positive log2-fold change values indicate significantly higher abundances in rewetted site (adjusted $P<0.05$), while negative values indicate significantly higher abundances in drained site (adjusted $P<0.05$). The size of the points indicates the relative abundance of the functional groups.

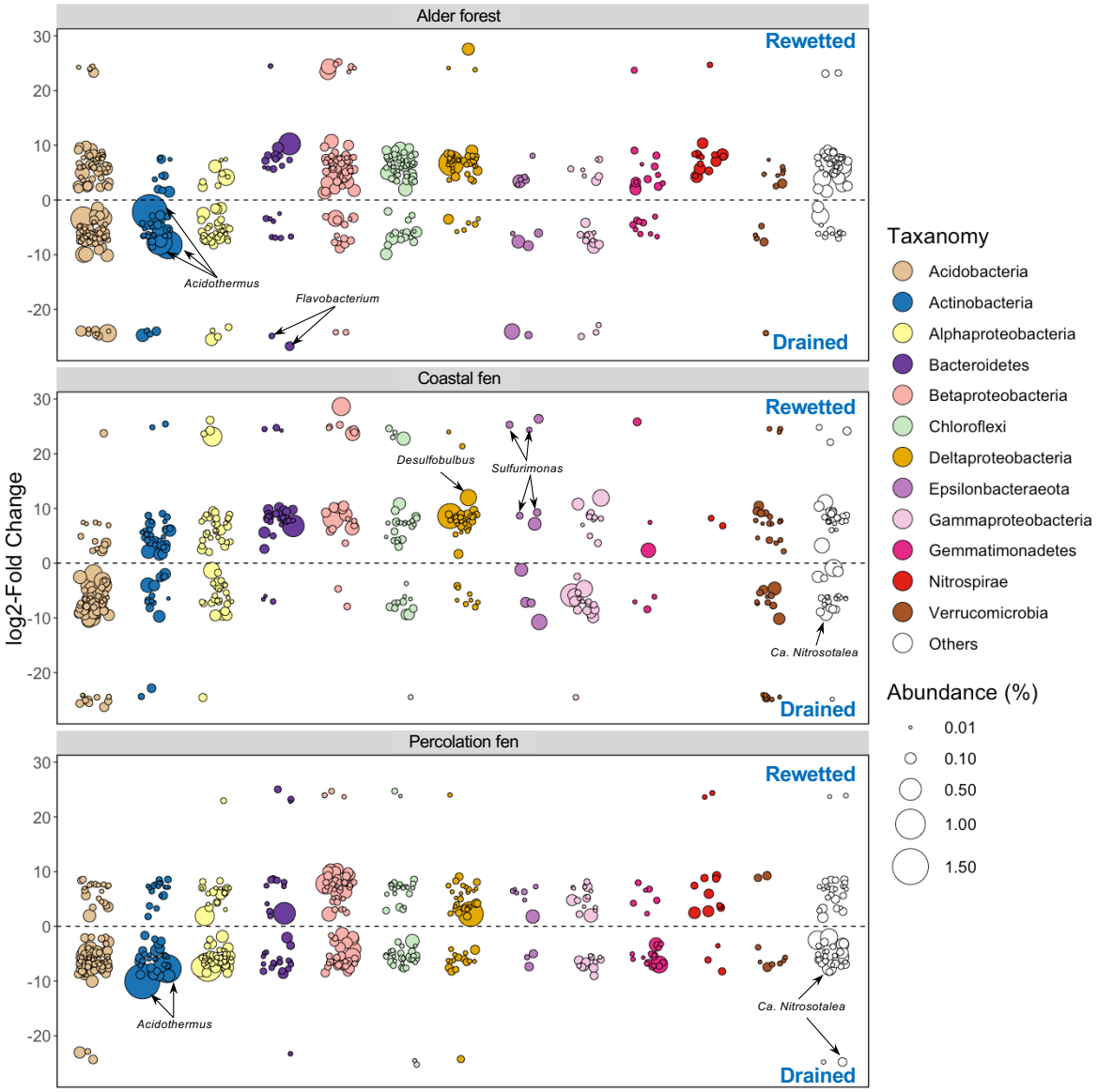


**Fig. 3** The differentially abundant ASVs of prokaryotes between rewetted and drained sites in three different mires. Positive log2-fold change values indicate significantly higher abundances in rewetted site (adjusted $P<0.01$), while negative values indicate significantly higher abundances in drained site (adjusted $P<0.01$). The size of the points indicates the relative abundance of the ASVs.

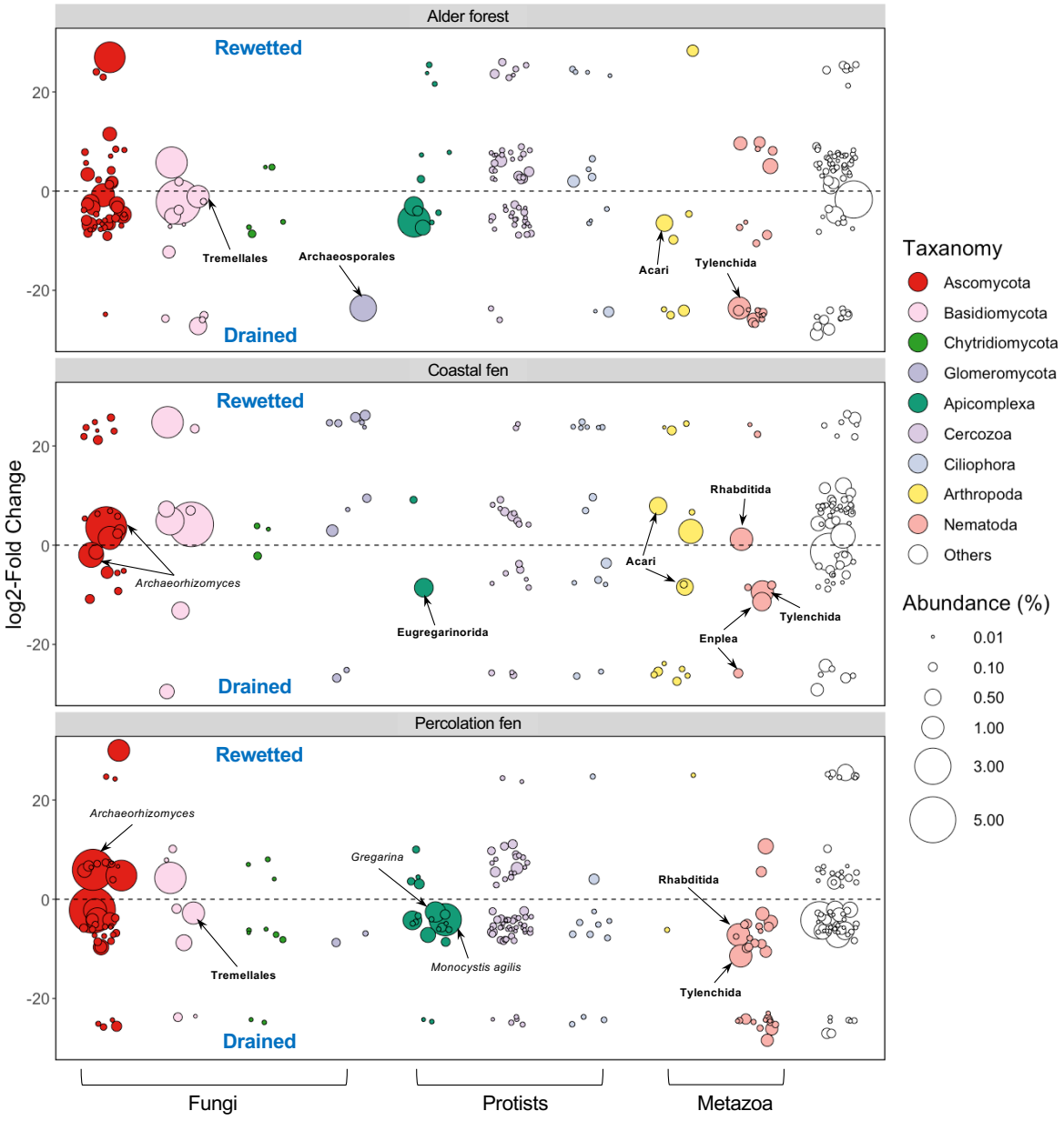


**Fig. 4** The differentially abundant ASVs of eukaryotes between rewetted and drained sites in three different mires. Positive log2-fold change values indicate significantly higher abundances in rewetted site (adjusted $P<0.01$), while negative values indicate significantly higher abundances in drained site (adjusted $P<0.01$). The size of the points indicates the relative abundance of the ASVs.

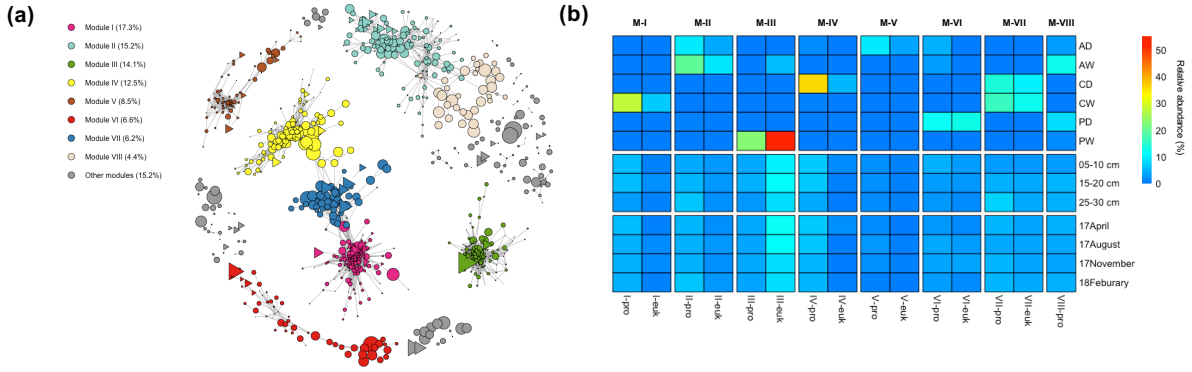


**Fig. 5** (**a**) Co-occurrence network and identified modules. Prokaryotic nodes are shown as cycles while eukaryotic nodes are shown as triangles. (**b**) The distribution of relative abundances of prokaryotic and eukaryotic nodes within each module in different sites, depths or seasons. M, module; pro, prokaryote; euk, eukaryote.

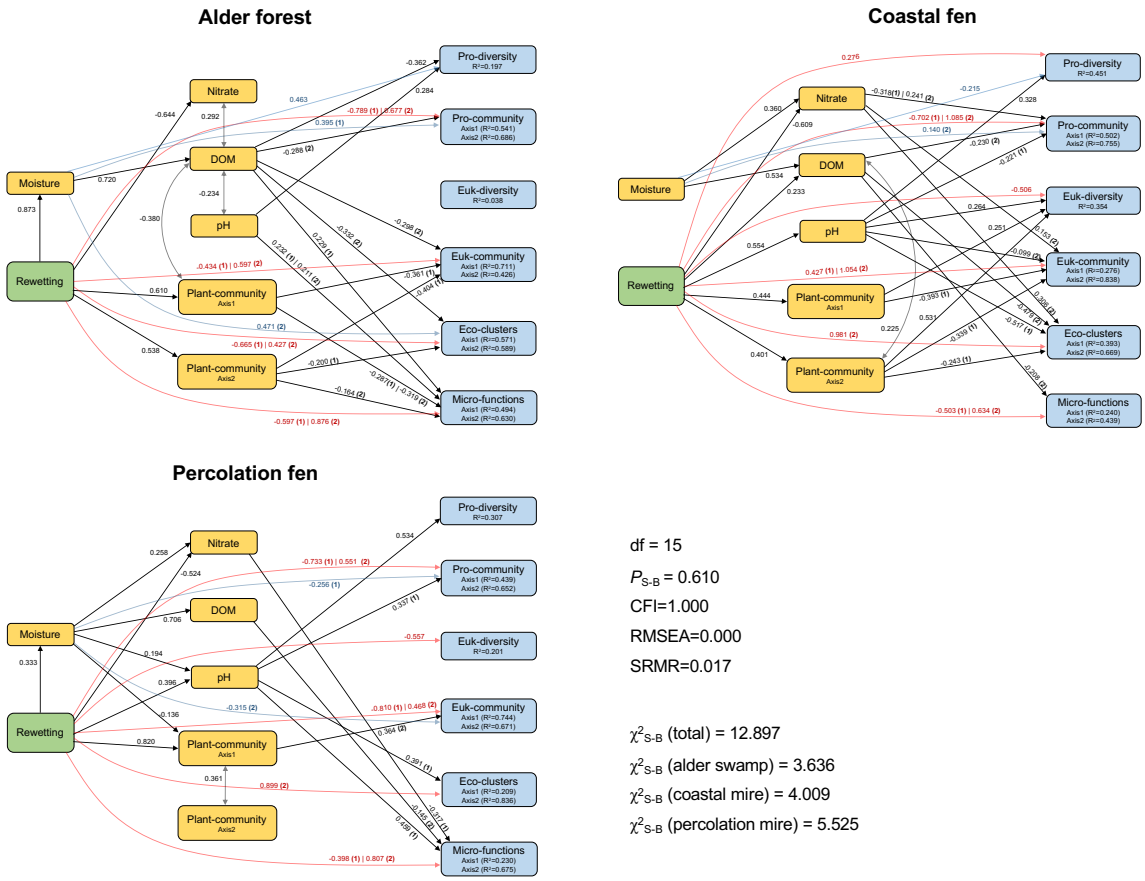


**Fig. 6** Structural equation model showing the direct and indirect effect of rewetting on the diversities, community compositions, network ecological clusters and the predicted microbial functions. Paths that showed significant relationships ($P$<0.05) are shown. The numbers along with arrows are standardized path coefficients, and the numbers in the brackets indicate the number of axis (nMDS-axis 1 or axis 2). Direct effects of rewetting and moisture are shown as red and blue arrows, respectively. The gray double-sided arrows indicate the covariant relationships between the variables. Amount of variance explained by the model ($R^2$) is listed for all response variables. DOM, dissolved organic matter; Pro, prokaryote; Euk, eukaryote; Eco-clusters, ecological clusters derived from network analysis; Micro-functions, microbial functions derived from FAPROTAX.