Rewiring of peatland plant-microbe networks outpaces species turnover

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Abstract Enviro-climatological changes are thought to be causing alterations in ecosystem processes through shifts in plant and microbial communities. However, how links between plant and microbial communities change with enviro-climatological change is likely to be less straightforward, but may be fundamental for many ecological processes. To address this, we assessed the composition of the plant community and the prokaryotic community –using amplicon-based sequencing– of three European peatlands that were distinct in enviro-climatological conditions. Bipartite networks were used to construct site-specific plant-prokaryote co-occurrence networks. Our data show that between sites, plant and prokaryotic communities differ and that turnover in interactions between the communities was complex. Essentially, turnover in plant-microbial interactions is much faster than turnover in the respective communities. Our findings suggest that network rewiring does largely result from novel associations between species that are common and shared across the networks. Turnover in network composition is largely driven by novel interactions between a core community of plants and microorganisms. Taken together our results indicate that the functional role of species is context dependent, and that changes in enviro-climatological conditions will likely lead to network rewiring. Integrating turnover in plant-microbe interactions into studies that assess the impact of enviro-climatological change on peatland ecosystems is essential to understand ecosystem dynamics and must be combined with studies on the impact of these changes on ecosystem processes.

Keywords microbial and plant diversity, plant-microbe interactions, bipartite networks, 16S rRNA, 16S amplicon sequencing, peatlands.

Introduction

Throughout the present interglacial period, northern peatlands have acted as carbon (C) sinks, resulting in over 500 Gt of C (Yu et al. 2010) to be locked away in these ecosystems. Recently it has even been suggested that northern peatlands may contain twice as much C (Nichols & Peteet 2019), i.e. > 1000 Gt, yet these numbers are debated (Ratcliffe et al. 2020). Nevertheless, maintaining a positive carbon balance (C uptake > C release) in peatlands is of great relevance to mitigate carbon-climate feedbacks. The carbon sink function of peatlands largely depends on the imbalance between gross primary production and decomposition, which in undisturbed peatlands is largely in favour of the former. Indeed, peatland plants, and *Sphagnum* mosses in particular, produce recalcitrant litter (Clymo 1965, Dorrepaal et al. 2005) and release anti-microbial compounds (Fudyma et al. 2019; Hamard et al. 2019), which together with acidic and anoxic environmental conditions impede microbial breakdown of organic matter and thus facilitate the accumulation of plant remains. Hence, the C sink function of peatlands is controlled by an interplay between abiotic and biotic factors.

Climate and environmental change is often linked to alterations in the biotic composition of ecosystems (Walther et al. 2002, Pimm et al. 2014), which more often than not has consequences for ecosystem processes (Bardgett et al. 2013, Rillig et al. 2019). The effects of climate change on peatland ecosystem processes can either be direct or indirect. Examples of direct effects abound. Increased nitrogen deposition (Aldous 2002, Bragazza et al. 2006), drought (Fenner & Freeman 2011; Estop-Aragonés et al. 2016), and warming (Dorrepaal et al. 2009; Wilson et al. 2016), amongst others, have been linked to carbon loss. Nevertheless, responses to enviro-climatological change (ECC) can be inconsistent (Zhang et al. 2018) likely due to indirect effects of climate change on peatland processes, or to non-linear responses in plant and soil communities (Jassey et al. 2018, Lamentowicz et al. 2019). Indeed, the effects of warming and drought on carbon cycling in peatlands have previously shown to largely depend on the vegetation (Ward et al. 2013, Dieleman et al. 2015, Rupp et al. 2019). Moreover, Wang et al. (2015) and Fenner & Freeman (2020) respectively propose a plant-controlled metabolomic and a biogeochemical mechanism that protects C loss during drought. Following the train of thought that progressive changes in enviro-climatological conditions cause shifts in the composition of plant communities (Robroek et al. 2017), convergent shifts in the microbial community can be expected.

Indeed, peatland microbial community structure and activity are strongly connected to plant community assemblage (Bragina et al. 2014, Chronakova et al. 2019, Martí et al. 2019b, Ivanova et al. 2020). Hence, interactions between plant and microorganisms are the cornerstone that shape carbon-related processes in peatlands (Lindo et al. 2013) and it is critical that we understand how ECC affects these interactions to forecast the consequences for ecosystem processes (Kostka et al. 2016).

Most species do not exist in isolation but are embedded in trophic, non-trophic, mutualistic or antagonist interaction networks (Tylianakis et al. 2008, Bascompte 2009, Kéfi et al. 2016). In these networks, the number of species and interactions vary depending on species identity, functional traits present and enviro-climatologic niche (Schleuning et al. 2016). Network assemblage, in turn, can affect the resilience and stability of the network, which ultimately underlies the robustness of ecosystem functions to enviro-climatic change (Allesina et al. 2009, Morriën et al. 2017, de Vries et al. 2018). The effects of changes in plant community composition on soil microbial communities, however, are thought to be independent of the environmental context (Fanin et al. 2019), leading to complex responses of plant-microbe interactions to ECC. Hence, how environmental conditions regulate plant-microbe associations (i.e. networks) and how this relates to within-community responses (i.e. plant and microbial communities) is essential knowledge leading to improved understanding on the effects of ECC on peatland processes.

To assess how ECC affects plant-microbe interactions, we characterized plant and microbial communities in three European *Sphagnum*-dominated peatlands. We assessed how enviro-climatological context relates to compositional differences in plant and 16S rRNA-derived microbial communities and how this plays out on plant-microbial bipartite networks. We postulate that enviro-climatic change will trigger both communities to turnover, leading to changes in the network structure. The influence of enviro-climatologic conditions on plant-microbe associations can manifest in various ways: (i) no difference in biotic communities, nor in network associations, (ii) no difference in biotic communities but a rewiring in network association with altered enviro-climatological conditions, (iii) differences in the composition in one or both communities with congruent differences in network associations, and (iv) differences in biotic community compositions with a disproportionate rewiring of interaction networks. Congruent with the effects of environmental conditions on peatland plant

communities (Robroek et al. 2017), we expect that differences in plant-microbe network structure are largely driven by turnover in one or both communities.

Material and Methods

Site description and enviro-climatic data. Three geographically distinct European *Sphagnum*-dominated peatlands were selected for this study. Degerö Stormyr (64°88′N, 19°56′E, 277 m above sea level (asl)), Sweden, is a minerogenic peatland with ombrotrophic elements. Cena Mire (56°25'N, 23°81'E, 12 m asl), Latvia, is an ombrotrophic raised bog. Dosenmoor, (54°95'N, 10°02'E, 28 m asl), Germany, is a restored ombrotrophic raised bog. These sites vary in degree of conservation status, but all sampling locations were located in an ombrotrophic part of each peatland with vegetation characteristic for natural peatlands. Full vegetation characteristics of the sites can be found on https://doi.org/10.5061/dryad.g1pk3.

We collected variables that describe climatic and environmental conditions in the selected peatlands. Four bioclimatic variables (mean annual temperature, temperature seasonality, mean annual precipitation, precipitation seasonality) were extracted from the WorldClim database (Hijmans et al. 2005), and averaged over a 5-year (2005-2009) period that preceded our sampling campaign. Atmospheric deposition data were produced using the EMEP (European Monitoring and Evaluation Programme)-based IDEM (Integrated Deposition Model) model (Pieterse et al. 2007) and consisted of grid cell (50 × 50 km) averages of total nitrogen and sulfur deposition.

Field sampling of plant and microbial communities. Abundance data for all vascular plant and bryophyte species from randomly selected lawn (n =3) and hummock (n = 3) microhabitats (0.25 m^2 quadrats; six in total) were collected in the summer of 2010. Microhabitats were sampled in pairs, with each lawn-hummock pair separated < 2 m. This sampling strategy was chosen as to represent the nature of the whole peatland as much as possible. Vascular plants and *Sphagnum* mosses were identified to the species level. Non-*Sphagnum* bryophytes were identified to the family level. Rarefaction analysis indicated that our sampling adequately captured species richness in our peatlands.

In all plots (3 sites, 2 microhabitats, 3 replicates; 18 in total), we collected a 5 cm^3 peat sample from the acrotelm – defined as the peat layer between the peatland surface and the lowest water table level, which largely contains living plants – and the catotelm – the peat layer that underlies the acrotelm, is largely anoxic, and contains mostly dead plant remains (Clymo 1984). Hence, we collected a total of 36 samples from 18 plots. Sampling depth for the acrotelm samples was 5 cm below the surface, while sampling depth of the catotelm samples was 12.5 cm and 37.5 cm below the surface for the lawns and hummocks, respectively (Martí et al. 2015); Suppl. Fig. 1). Sampling was conducted using a 10-cm wide Holmen auger (Holmen 1964), which was carefully cleaned with 70% alcohol between sampling plots. Samples were refrigerated at 4°C, transported to the laboratory, and stored at -20°C prior to RNA extraction.

RNA isolation, amplicon sequencing and data handling. Total RNA was extracted from 0.75 g of wet peat (7.8% ± 2.5 dry peat) using the FastRNA®Pro Soil-Direct Kit and FastPrep® Instrument (MP Biomedicals, Santa Ana, CA) as per the manufacturer's instructions. Quality and concentration were determined on a Qubit fluorometer (Invitrogen, Eugene, OR, USA). Extracted RNA was reverse transcribed to complementary DNA (cDNA) using the lllustraTM Ready-to-Go RT-PCR Bead (GE-Healthcare, Uppsala, Sweden). Briefly, 2 µl of the extracted RNA and 2.5 µl of the random primer pd(N)6 (1.0 µg/µl) (GE-Healthcare, Uppsala, Sweden) were mixed, denatured at 97°C for 5 min, and then chilled on ice. One IllustraTM Ready-to-Go RT-PCR Bead and RNAse-free water were added to a final volume of 50 µl. Next, reverse transcription, consisting of a chain of 30 mins at 42°C followed by 5 mins at 95°C was used to create cDNA. RNA and cDNA were stored at -80°C and -20°C, respectively.

A 485 base pair (bp) fragment flanking the V3-V4 region of prokaryotic 16S rRNA was amplified using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') modified from (Yu et al. 2005). Each 25-µl PCR mixture contained one Illustra PuReTaq Ready-To-Go PCR Bead (GE Healthcare, Uppsala, Sweden), 0.5 µM of each primer, 5 µl of cDNA and RNAse-free water. The 35 PCR cycles, performed on a MyCycler™ Thermal Cycler (Bio-Rad Laboratories), consisted of 90 s at 95°C, 30 s at 95°C, 30 s at 56°C, 30 s at

72°C and a final 5 mins at 72°C. PCR products were verified on 1% agarose gel and purified with a GeneJET PCR Purification Kit (Fermentas, Vilnius, Lithuania). Purified PCR product concentrations were determined using the Quant-iT dsDNA HS Assay Kit and the Qubit fluorometer (Invitrogen) and adjusted to 5 ng μl^{-1}. To link sequence reads to samples, a second PCR on the initial product was performed using the primer pair 341F/806R with adapters and a unique barcode. The number of cycles was reduced to 10. Bands of ca. 530 bp were cut out and purified by High Pure PCR Cleanup Micro Kit (Roche Diagnostics GmbH, Mannheim, Germany) as per the manufacturer's instructions. These fragments were quantified using a Qubit™ dsDNA HS Assay Kit and the Qubit™ fluorometer (Invitrogen) and qPCR (Mx-3000, Stratagene). The sample amplicons were mixed to equal concentrations (4×10^5 copies μl^{-1}) and subjected to two-region 454-pyrosequencing on a 70 × 75 GS PicoTiterPlate (PTP) using a GS FLX pyrosequencing system.

Sequence data were sorted, trimmed, filtered and quality checked (QS ≥ 25 for trimming, and lengths ≥ 150 bp) using the Qiime 1.6.0 data analysis pipeline (Caporaso et al. 2010). Chimeras were detected and removed using UCHIME in reference mode with the MicrobesOnline gold set (Edgar et al. 2011). Briefly, operational taxonomical units (OTUs) were selected at 97% identity with a confidence threshold of 50%. Representative sequences were selected randomly and classified against the SILVA database version 132 (https://www.arb-silva.de/). Unifrac distances for beta diversity were calculated from trees generated with Fasttree. The input multiple sequence alignments were generated using PyNAST against gg2011 template alignments. From the 36 libraries, we obtained a total of 772,742 sequences with an average of 21,465 sequences per library, except for one sample from Cena Mire –lawn, acrotelm– which had 135 reads and was therefore removed from the analysis. OTUs that were present in fewer than 20% of the samples were removed from the analyses as well as OTUs identified as chloroplast. A total of 464,327 sequences were left, corresponding to 4707 OTUs. From those, 74% (3690) of the OTUs corresponded to bacterial taxa and 20% (141) to archaeal taxa.6% of the OTUs were unclassified.

Plant-prokaryote bipartite networks. Bipartite networks were used to detect co-occurrence between plants and prokaryotes as a proxy for site-specific plant-prokaryote associations (Faust & Raes 2012).

Co-occurrence networks were computed at site level. Hence data for the hummock and lawn microhabitats were pooled; however, separate networks were constructed to link the plant community with the acrotelm and with the catotelm prokaryote communities. Hellinger-transformed matrices, comprising data of bacterial and archaeal OTUs, vascular plants and *Sphagnum* species, were used to calculate Spearman's correlation coefficients for plant-prokaryote associations (Berry & Widder 2014). Significance for each permutation was assessed using a permutation test with 10,000 repetitions. A null distribution of correlations was estimated based on correlations calculated for the permuted data. Only correlations > 0.6 (positive and negative) and significantly larger in magnitude than expected by chance for an overall false discovery rate of 0.05 (using the Benjamini-Hochberg FDR correction) were adopted in the co-occurrence matrix (Sander et al. 2017). Each co-occurrence matrix was transformed into an adjacency (binary) matrix based on the presence or absence of links. Networks were then produced using the *igraph* and *bipartite* R packages (references in Supplementary), where each network comprised positive and negative plant-prokaryote associations. Each network was further analysed in terms of topography, and network dissimilarity (Berry & Widder 2014, Williams et al. 2014).

First, we evaluated the topological attributes of the networks using a set of indices focussed on species interactions, which were calculated for interaction between the plant and prokaryotic communities. *Effective partners* was defined as the number average of links for individuals in a specific network. *Partner diversity* is the diversity (PD; Shannon-based) of interactions between species from the two levels of the network. *Partner specificity* (PS) was calculated as the coefficient in variation of interactions, normalised to values between 0 and 1 (Poisot et al. 2012b), where PS = 0 indicates low specificity and PS = 1 indicates high specificity. These three indices were calculated from a top-down and bottom up perspective. Top-down accounts for the number of interactions plant species have with prokaryotic 'species'. Bottom-up accounts for the number of interactions prokaryotes have with species in the plant community. Essentially, in a network top-down and bottom up networks do not necessarily have to match (Suppl. Fig. 2). Next, we identified network attributes using a set of plant-prokaryote interaction indices. *Linkage density* (LD) is the marginal total-weighted diversity of interactions between species. *Generality* of the plant community (G_p) is calculated as the mean effective number of prokaryotic 'species' per plant species weighted by the total number of interactions. It describes the

degree of specialization in the plant community. Low values indicate a high degree of specialization. *Generality* for the prokaryotic community (G_m) is calculated as the mean effective number of plant species per prokaryotic 'species' weighted by the total number of interactions, and represents the degree of specialization in the prokaryotic community. *Robustness* (R) is a measure of robustness of the network to species loss, calculated as the area under the extinction curve of the network. The curve, essentially, describes the dependency –for survival– of species from one level on species of another level. R = 1 corresponds to a situation where most prokaryotes survive while most plant species are eliminated, or vice versa. R = 0 corresponds to a situation where species loss form one level results in the loss of most to all network connections. *Partner diversity* in the networks (PDn) is the diversity (Shannon-based) of the number of interactions for the species in each level of the bipartite network. *Species richness* (SR) is the number of species in the bipartite network community. *Niche specialization* (N) represents the site scores from a NMDS analysis, and represent the niche characteristics of each community (Devictor et al. 2010). The indices G, R, PD SR and N were calculated separately for the two levels –plants and prokaryotes– in the bipartite networks.

Network turnover. To test if enviro-climatic conditions affected species turnover and turnover in bipartite networks, we calculated pairwise β-diversity in species composition and plant-prokaryote networks in each site using the methods proposed by Legendre & De Cáceres (2013) and Poisot et al. (2012a), respectively. For pairwise β-diversity in species composition, we used a site × species abundance matrix. Total β-diversity (BD) was partitioned into species contributions (BD_S; degree of variation of individual species in each site) and local contribution (BD_L; comparative indicators of the ecological uniqueness of the sites) to β-diversity (Legendre & De Cáceres 2013). For β-diversity in co-occurrence networks, we use a site × species pairs matrix (adjacency binary matrix). As differences in networks can arise either through changes in species composition and/or realized interactions between species, we specifically focused on the dissimilarities in interactions between networks (BDN) that originates from differences in species interactions due to species turnover (BDN_{NS}) or from novel

interactions between common network species (BDN_{CS}) (Koleff et al. 2003, Canard et al. 2012, Poisot et al. 2012a).

*Data handling and statistical analyses***.** Analysis of Variance tests were used to test for differences in pore water nutrients, plant species and prokaryote richness, diversity (Shannon H'), evenness (Pielou's J), and plant-prokaryote network indices between sites and sampling depth (for prokaryote data only). Prior to testing for turnover in prokaryotic community composition across the different habitats (β-diversity), relative abundance of each OTU was calculated within each sample. We used redundancy analysis (RDA) to relate enviro-climatologic factors to plant and prokaryotic communities (Hellinger-transformed matrices). To avoid apparent spatial trends in plant and prokaryotic communities, we detrended biotic communities using partial RDAs (*p*RDA) with latitude and longitude as co-variables. A subset of enviro-climatological variables was selected from the complete set of enviro-climatologic variables using stepwise selection based on Akaike Information Criterion (AIC) to avoid issues of multicollinearity and overparameterization in the RDA models. Then, based on this subset, we selected the variables that best described environmental differences between sites: seasonality in temperature and total nitrogen deposition. The robustness and significance of the RDAs was then tested using permutation tests. All statistical analyses were performed in *R* version 3.1.2 using the *vegan, diverse, phyloseq, nlme, dplyr and tidyr* packages (references in Supplementary).

Results

Mean annual temperature (MAT) was lowest in Degerö Stormyr and highest in Dosenmoor, while seasonality in temperature (TS) followed an opposite pattern (Table 1). Mean annual precipitation (MAP) varied from 710 mm in the Cena Mire to 760 mm in Dosenmoor. Seasonality in precipitation (PS) did not vary much between sites (Table 1). Total nitrogen and sulfur deposition were lowest in Degerö Stormyr. Total nitrogen deposition values in Dosenmoor were almost double as compared to those in Cena Mire. For sulfur, Cena Mire and Dosenmoor had similar deposition values (Table 1).

Plant and prokaryote community composition. Vascular plant and bryophyte species richness and evenness were highest in Cena Mire (Table 2). Detected archaeal richness did not differ among the three sites. Richness of the acrotelm archaeal communities was at least three times higher as compared to richness of the catotelm archaeal communities (Table 2). Bacterial richness, opposite to the patterns observed in the plant community, was lowest in Cena Mire, for both acrotelm and catotelm (Table 2).

Redundancy analysis (RDA) showed that all communities (plants, acrotelm prokaryotes, and catotelm prokaryotes) well separated in the ordination space (Suppl. Fig. 3). RDA analysis explained 27% (vascular plants), 8% (prokaryote, acrotelm) and 19% (prokaryote, catotelm) of the variance (adjusted R^2) of each community. Total nitrogen deposition and temperature seasonality both explained much of the differences in community structure for all three communities ($P \leq 0.05$). Notably, the three communities showed similar patterns in their species-environment relationships. Communities in Degerö Stormyr were related to high seasonality in temperature, and communities in Dosenmoor were related to high total nitrogen deposition (Suppl. Fig. 3).

Across sites, and pooled for the acro- and catotelm communities, bacterial to archaeal ratios exceeded 3:1 (Fig. 1 a). Acidobacteria (35%), Proteobacteria (29%), Verrucomicrobia (13%), and Actinobacteria (9%) were the most abundant bacterial phyla across sites (Fig. 1 b). Relative abundances in bacterial phyla were similar across sites. Composition of the archaeal community, on the other hand, seemed site-specific (Fig. 1 c). In Degerö Stormyr, methanogens of the order Methanomicrobiales (50%) and Methanobacteriales (37%) were the dominant archaeal orders. Archaeal communities in Cena Mire were dominated by one single order, Methanomicrobiales (83%). In Dosenmoor, Thermoplasmata (39%) and an uncultured archaeon (28%) likely associated to the phyla Thaumarchaeota and Crenarchaeota were most dominant. Of the classified orders in this site, Nitrosotaleales (30%), Methanomicrobiales (22%) and Methanosarcinales (19%) had highest prevalence.

Network structure The number of plant-prokaryote pairs in bipartite networks differed between the three sites (site effect, $F_{1,2} = 65$, $P = 0.01$; site x depth, $F_{1,2} = 2.2$, $P = 0.27$), with > 70% fewer

interactions in Cena Mire and Dosenmoor as compared to Degerö Stormyr (Fig. 2). A lower number of bipartite interactions results from lower numbers of both positive and negative associations (Fig. 2).

A couple of vascular plants, *Andromeda polifolia* and *Rhynchospora alba*, and one moss species, *Sphagnum rubellum,* were important species in plant-prokaryote networks in all three sites (Fig. 2). OTUs classified as belonging to the genera *Occallatibacter*, *Acidothermus*, *Verrucomicrobia*, and Phycisphaera-related group WD2101 and OTUs assigned to the family Methylacidiphilaceae were present in all three networks of the acrotelm prokaryotic communities (Fig. 2). The bacterium "*Candidatus* Koribacter", a bacterium in the family Acidobacteriaceae, and a bacterium in the family Pedosphaeraceae were important network species of the catotelm prokaryotic communities (Fig. 2).

For all sites, β-diversity for bipartite (plant-prokaryote) networks (BDN) exceeded β-diversity for species composition (BD), indicating that network turnover is higher than species turnover between sites ($P \leq 0.001$; Fig. 3). Indeed, network compositions (i.e. pairwise comparison between networks) between sites were more dissimilar (acrotelm, BDN = 0.91; catotelm, BDN = 0.89) than species compositions (i.e. pairwise comparison between communities) between sites (acrotelm, BD = 0.32; catotelm, BD= 0.31). Turnover in species composition between sites, β-diversity (BD), was equally driven by species and local contributions (BD_S and BD_L). Network β-diversity (BDN) was mostly driven by turnover in interactions (both direction and number) between species that are common in the three sites (BDN_{CS}), rather than by novel interactions driven by changes in the community composition (BDN_{NS}; Fig. 3).

Differences in the number of bi-partite interactions between sites seem to relate to differences in the numbers of effective partners between the respective communities, which were always highest in Degerö Stormyr (acrotelm: $P < 0.001$; catotelm: $P < 0.001$; Fig. 4 a, b). This is further supported by differences in diversity in plant-prokaryotic partners, which were higher in Degerö Stormyr as compared to Cena Mire and Dosenmoor (acrotelm: $P < 0.001$; catotelm: $P < 0.001$, Fig 4 c, d). Moreover, the number, but also diversity, of prokaryotic partners with the plant community (top-down links), were higher than the number of plant partners with the prokaryote community (bottom up links).

This means that plant species link to a higher number of prokaryotes, than prokaryotes to plants. Partner specificity was lowest in Degerö Stormyr for both levels of organisation in the bi-partite networks (acrotelm: $P < 0.001$; catotelm: $P < 0.05$, Fig. 4 e, f). Specificity of the links of prokaryotes with plants were larger than the specificity of plants links to prokaryotes (acrotelm: $P < 0.001$; catotelm: $P < 0.001$).

At the network level, the overall linkage density (LD), generality (G), partner diversity (PD) and species richness (SR) in the bipartite networks was higher in Degerö Stormyr as compared to Cena Mire and Dosenmoor; a pattern found in both acrotelm and catotelm bipartite networks (Suppl. Fig. 4). The bipartite networks, acro- and catotelm, in Degerö Stormyr were strongly sensitive to the loss of prokaryotes (low robustness) but not to the loss of plants. Oppositely, bipartite networks in Cena Mire and Dosenmoor were more sensitive to the loss of plants as compared to prokaryote loss (Suppl. Fig. 4). As compared to Degerö Stormyr, networks in Cena Mire and Dosenmoor show a high degree in niche specialization, especially in the plant communities (Suppl. Fig. 4). Such differences in network indices between sites were driven by both total nitrogen deposition and temperature seasonality (Fig. 5). While compositional network indices (linkage density, partner diversity, number of effective partners) and generality indices decreased with increasing nitrogen deposition, stability network indices (robustness, niche extinctions, modularity) increased. Opposite patterns were found with increasing temperature seasonality (Fig. 5). These results indicate that plant-microbial interactions change in complexity, specificity and evenness in the distribution of species interactions with changes in environmental conditions, and that these patterns are likely driven by the turnover of species between peatland sites.

Discussion

Using data on plant and prokaryotic community composition in three *Sphagnum*-dominated peatlands, we evidence that plant-prokaryotic networks are site-specific. As these peatlands are situated in three distinct environmental zones (Metzger et al. 2005) our data suggest that these networks are vulnerable to the effects of enviro-climatological change, and turnover at a faster rate than the individual biotic levels that comprise these networks. Hence, a turnover in the plant community as shown in earlier

research (Gunnarsson et al. 2002, Pinceloup et al. 2020) will lead to complex changes in plant-microbe interactions that could affect ecosystem processes in the longer term (Morriën et al. 2017).

In line with earlier work where it was suggested that changes in enviro-climatological conditions in peatland ecosystems leads to a significant turnover in the composition of the plant community (Robroek et al. 2017), plant community composition was highly distinctive between sites. Moreover, our study confirms previous suggestions that enviro-climatological conditions are important drivers that underlie the composition of the vegetation, and that changes in these drivers result in alterations of the plant community (Gunnarsson et al. 2002, Pinceloup et al. 2020) and associated biotic interactions (Wiedermann et al. 2007). Indeed, we note that site-specificity of plant communities is paralleled in the prokaryotic community, a pattern that is likely explained by the strong links between plant and microbial communities (Chronakova et al. 2019; Ivanova et al. 2020). Despite the variation in prokaryotic community composition between sites, the archaea to bacteria ratio remains strikingly stable. These results would imply that both microbial groups are equally affected by alterations in enviro-climatological conditions. Yet, our data also highlights that bacterial community composition was remarkably similar between sites. These results mirror earlier findings where bacterial community composition was reported to be rather stable under experimental warming (Weedon et al. 2017). Archaeal communities, on the other hand, were highly dissimilar between the three peatland sites. In Degerö Stormyr and Cena mire, methanogens dominated the archaeal community. In these sites, biogeochemical drivers likely promoted a classical anaerobic degradation of organic matter to methane in the absence of other terminal electron acceptors. The dominating methanogens, Methanobacteriales and Methanomicrobiales, characteristic for acid peatlands, are hydrogenotrophic and form methane from hydrogen/CO_2 and/or formate (Kotsyurbenko et al. 2007, Martí et al. 2015). Interestingly, the Methanomicrobiales family *Methanoregulaceae* identified in the present study dominated the methanogen population in a comparable set of peatlands along a nitrogen deposition gradient (Martí et al. 2015). Reductions in the relative abundance of methanogens in Dosenmoor may be the result of higher deposition levels of nitrogen and sulfur. The presence of oxidized forms of these elements, may favour nitrogen oxide and sulphate reduction, resulting in increased competition for methanogens. This is supported by the higher relative abundance of the phyla Nitrosotaleales and Thaumarchaeota (Suppl.

Table 1), which are known ammonia-oxidizing archaea in acid environments (Lehtovirta-Morley et al. 2011, Lin et al. 2015), in the Dosenmoor. Furthermore, Thermoplasmata –highly abundant in the Dosenmoor– are suggested to be involved in the transformation of sulphite and possibly organosulphonates (Lin et al. 2015). Given the high sulfur deposition in the Dosenmoor, these compounds prospectively are abundant. Here, it should be stressed that relative abundance does not necessarily correspond to the actual abundance and/or the physiological activity by the microorganisms displayed. To exemplify, increased methanogen activity has been shown in peatlands with high nitrogen deposition (Martí et al. 2019a). Hence, the main part of the organic matter degradation likely follows the methanogenic route, also at the Dosenmoor.

Beyond the differences in community composition, our work shows that plant-microbe network structure differs between sites. As the turnover of plant-microbe interactions outpaces the turnover of plant and prokaryote communities, these results suggest that changes in enviro-climatological conditions are key in driving the observed rewiring of plant-prokaryote interactions. Interestingly, the highest numbers of effective partners and partner diversity were found in the plant community. Prokaryotes have significantly lower numbers of plant partners, but their associations with plant species are much more specific. This pattern is consistent with observations of plant-specific microbial community compositions (Bragina et al. 2012, Hamard et al. 2019), yet our results also suggest that compared to the rather specific nature of microbial associations to plant species the plant-microbiome is relatively non-specific. An explanation for this observation would be that one plant species can provide resources or niche space for a wide array of microbes, which in turn are rather specific (Suppl. Fig. 2). These findings would then ultimately indicate that plants are important drivers for plant-prokaryote interactions and is in line with the general idea that the plant community has a direct effect on the prokaryotic species occurrences and community composition and activity (Fisk et al. 2003, Wardle et al. 2004, Robroek et al. 2015, Yavitt & Williams 2015), but the opposite is not necessarily true.

Network size and complexity differ between sites and seem to erode from the most northern (Degerö Stormyr) to the most southern (Dosenmoor) site. These findings echo earlier findings where, as a response to warming, networks were observed to become less complex (Galiana et al. 2014). A

faster loss of network interactions as compared to species loss has earlier been observed and has been suggested to have major implications upon ecosystem processes and services (Valiente-Banuet et al. 2014). Indeed, decreased network complexity has been connected to efficiency in soil carbon uptake in ex-arable land, even without major changes in the composition of plant communities (Morriën et al. 2017). Our data suggest that seasonality in temperature and nutrient deposition modulates network indices that are related to composition, stability and generality of plant-microbe networks. As the effects of both variables are opposing, clear consequences of changing are hard to distil from our data. Nevertheless, our findings strongly parallel previous findings on specialization in ecological networks (Olesen & Jordano 2002), and suggest that lower seasonality and greater nutrient availability lead to narrower and more specialized niches. Hence, more optimal enviro-climatological conditions allow for potential species co-evolution, which increases local adaptations and favours network specialization (Dalsgaard et al. 2011, 2013). Such increase in specialization indicates an increased level of nestedness and would in the long term weaken network stability. Network properties were strongly modified across sites because of the vulnerability of prokaryotes to enviro-climatological changes. Bacteria in the genera *Occallatibacter*, *Acidothermus*, *Verrucomicrobium,* bacteria in the Phycisphaera-related group WD2101 (yet uncultured Planctomycetes of the order Tedisphaerales) and bacteria in the family Methylacidiphilaceae (phylum Verrucomicrobia) occurred in all three acrotelm networks. These genera have been previously found in soils, and have been characterised as chemoorganotrophic, strictly aerobic, moderately acidophilic, and chemoorganotrophic mesophiles. The bacterium "*Candidatus* Koribacter", a bacterium in the family Acidobacteriaceae, and a bacterium in the family Pedosphaeraceae were important network species in catotelm prokaryotic communities.

With our research we show that while differences in environmental properties, such as temperature and nutrient deposition, between sites markedly relate to differences in the composition of plant and prokaryote, acro- and catotelm, communities, turnover in plant-prokaryote networks exceeds species turnover in either one of both communities. Specifically, we show that species turnover, likely caused by enviro-climatological changes resulted in a reshuffling of plant and microbial communities, which then led to a significant rewiring of plant-microbial interactions. Our results suggest that increasing

network specialization may have been driven by a shift in physiological and metabolic activity across one organisational level (Blazewicz et al. 2020; Williams & de Vries 2020), which then cascaded to the other level. Our findings highlight that while spatial patterns of diversity across trophic levels are indeed key to understand network specialization as forwarded by Galiana et al. (2019), and that the contribution of novel associations between species that are common and shared across the networks is most prevalent. Hence, turnover in network composition is largely driven by novel interactions between plants and microbes that are common across networks and to a lower extent by species new to a metacommunity due to species turnover, suggesting that differences in enviro-climatological conditions result in alterations in the functional role of species. The latter will likely lead to network rewiring as observed in our study, which in the long term has unforeseen consequences for important ecosystems functions such as carbon uptake.

Data and materials availability All data that support this manuscript are available through DANS, the Netherlands institute for permanent access to digital research resources (https://dans.knaw.nl/en). Nucleotide sequence accession numbers have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB36623.

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initiated the data collection. BJMR and MM led the field sampling. VEJ, MM and BJMR analysed all data, with help of MGD, AJV and BHS for the microbial data, and led the writing of the manuscript to which all co-authors contributed. The first and second author contributed equally to this paper.

Conflict of interests – The authors declare no conflicts of interest.

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Table 1. Bioclimatic data for Degerö Stormyr, Cena Mire, and Dosenmoor. MAT = Mean annual temperature (°C), TS = Seasonality in temperature, MAP = Mean annual precipitation (mm), PS = Seasonality in precipitation; N_{tot} = Total nitrogen deposition (mg m^{-2} yr^{-1}), S_{tot} = Total sulfur deposition (mg m^{-2} yr^{-1}).

	MAT	TS	MAP	PS	N_{tot}	S_{tot}
Degerö Stormyr	1.2	9.3	730	26	130	96
Cena Mire	6.4	7.9	710	31	760	344
Dosenmoor	8.0	5.9	760	19	1260	6144

Table 2. Plant species and prokaryote richness (OTUs) and evenness (Pielou's J). Values are means ± standard errors. Prokaryotic richness and evenness have been described for the acrotelm and catotelm communities separately. Different letters represent significant difference ($P \leq 0.05$) in richness or evenness data for the sites as tested by analysis of variance within four sub-communities (vascular plants, bryophytes, archaea and bacteria).

Plant community		Richness	Evenness
Vascular plants	Degerö Stormyr	6.5 ± 1.23^{b}	0.8 ± 0.05^{a}
	Cena Mire	12.0 ± 1.94^{a}	0.2 ± 0.05^{b}
	Dosenmoor	7.2 ± 0.40^{b}	0.8 ± 0.06^{a}
Bryophytes	Degerö Stormyr	1.8 ± 0.17^{b}	0.5 ± 0.15
	Cena Mire	3.3 ± 0.56^{a}	0.5 ± 0.12
	Dosenmoor	1.7 ± 0.21^{b}	0.7 ± 0.12

Prokaryotic community		Acrotelm (oxic)		Catotelm (anoxic)	
		Richness	Evenness	Richness	Evenness
Archaea	Degerö Stormyr	14 ± 2.0	0.8 ± 0.05	51 ± 4.5	0.4 ± 0.06
	Cena Mire	30 ± 13.3	0.6 ± 0.08	74 ± 7.0	0.4 ± 0.05
	Dosenmoor	38 ± 14.2	0.7 ± 0.05	84 ± 11.9	0.5 ± 0.06
Bacteria	Degerö Stormyr	1367 ± 95.0^{a}	0.8 ± 0.00	1511 ± 99.4^{a}	0.8 ± 0.01^{a}
	Cena Mire	1231 ± 109.0^{b}	0.8 ± 0.02	838 ± 105.9^{b}	0.7 ± 0.04^{b}
	Dosenmoor	1615 ± 88.3^{a}	0.8 ± 0.01	1246 ± 139.2^{a}	0.7 ± 0.01^{a}

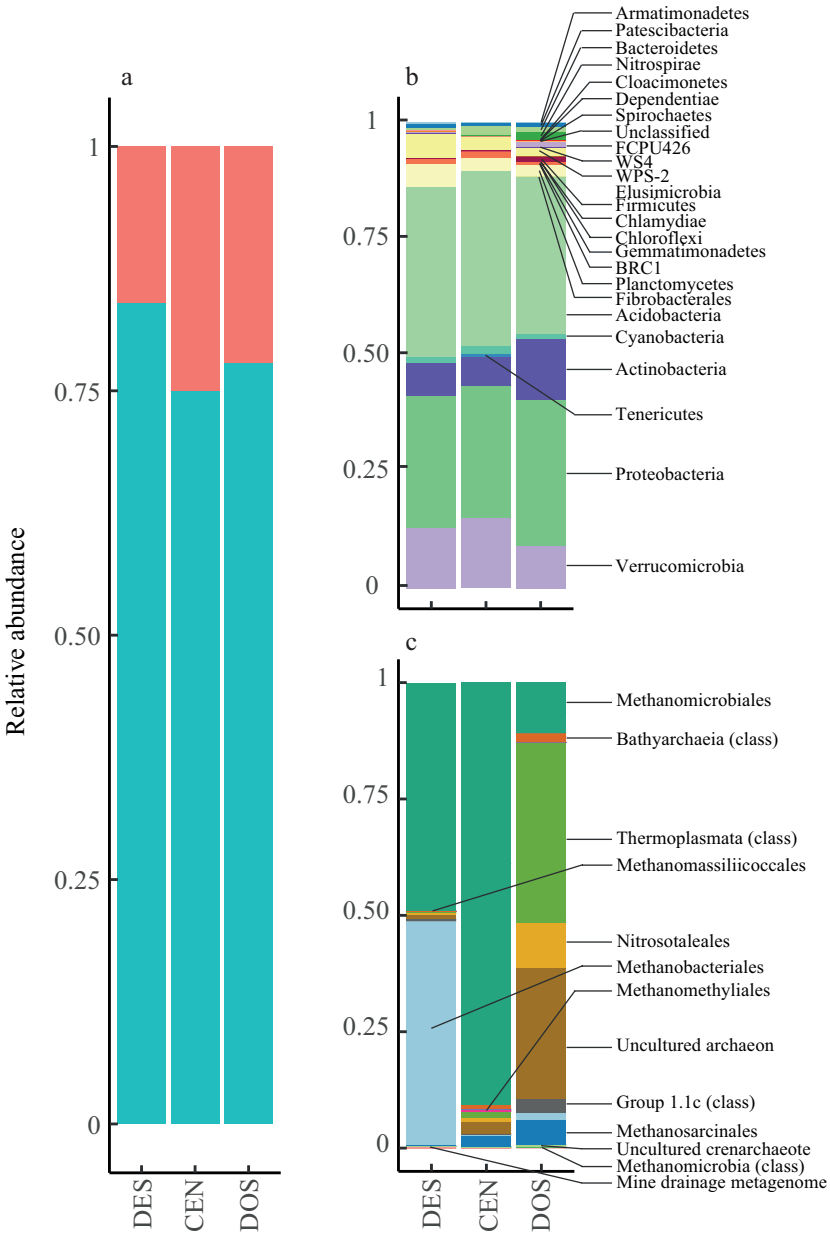

Figure 1. Comprehensive view of the sequence content of peat soil prokaryotic libraries per site: DES = Degerö Stormyr, CEN = Cena Mire, DOS = Dosenmoor. Segments that compose each bar represent the total number of sequences normalised to the total number of sequences in the libraries per site. a) View of the relative abundance of bacteria (blue) and archaea (red) in the site-specific communities. b) View of the relative abundance of bacterial 'species' in the respective site-specific communities. Each segment represents identified bacterial phyla. c) View of the relative abundance of archaeal 'species' in the respective site-specific communities. Each bar segment represents identified archaeal orders.

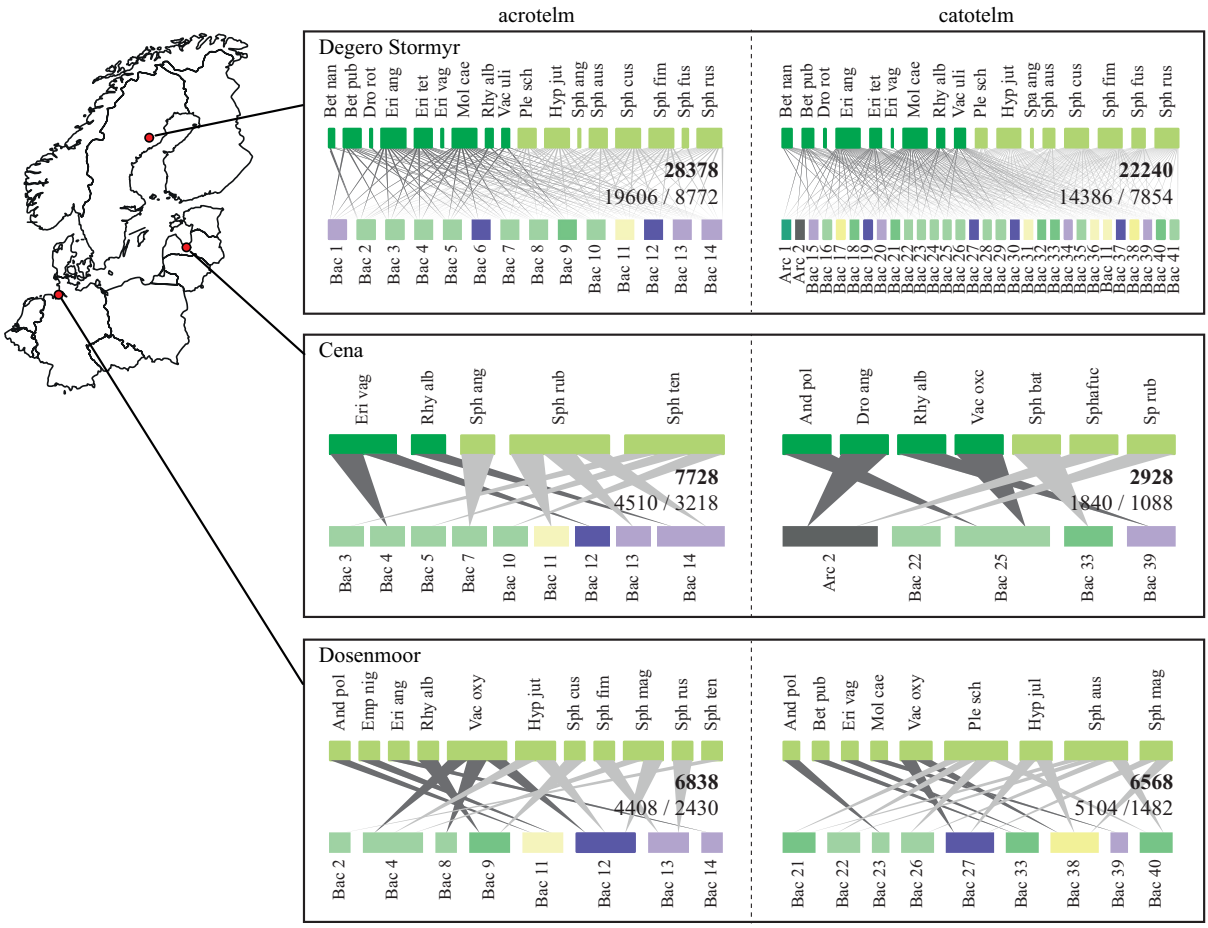

Figure 2. Bipartite network visualisation for the three European peatlands. Indicated are interactions between the plant community and acrotelm prokaryotic community (left) and between the plant community and catotelm prokaryotic community below (right). Plants are visualised at the top of the networks and coloured dark and light green for vascular plants and bryophytes, respectively. Prokaryotes are illustrated on the bottom of the networks and follow the colour scheme as in Fig.1. A complementary list of prokaryotes and their classification can be found in Suppl. Table 1. Size of the bars scales with relative abundance in the respective communities. For all sites, networks are filtered to only visualise interactions with 'keystone' prokaryotes, defined as those that had > 5 interactions in Degerö Stormyr. Interaction lines represent Spearman's rank correlations > 0.6. Dark grey interactions indicate vascular plant-prokaryote interactions, light grey indicates bryophyte-prokaryote interactions. Inset numbers indicate the full number of bi-partite (bold) interactions, further broken down in the number of positive / negative interactions.

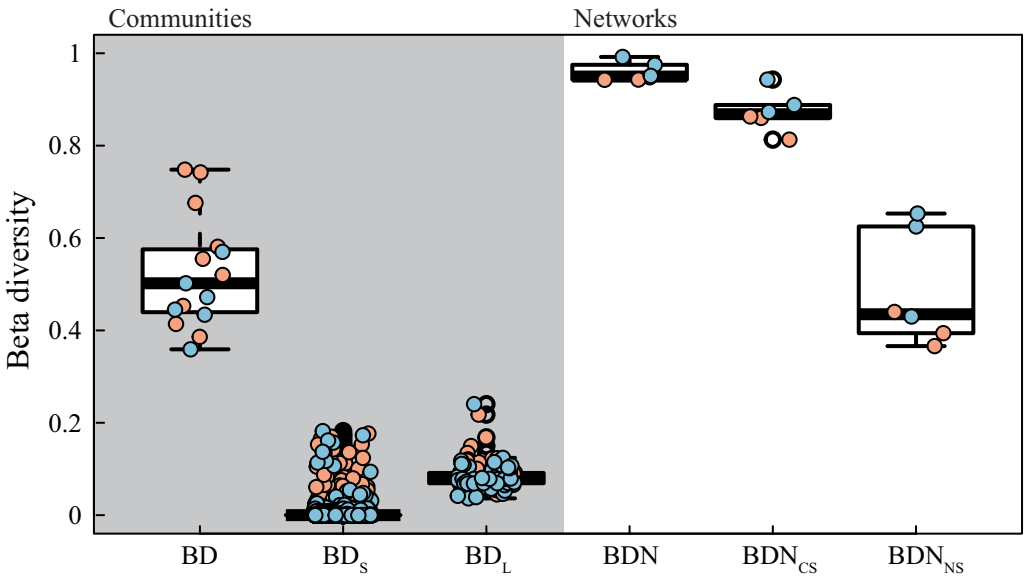

Figure 3. Turnover in species composition (species β-diversity) and association (network β-diversity) and their respective components across three peatlands (see methods). Red circles indicate plant and microbial communities in the acrotelm. Blue circles indicate plant and microbial communities in the catotelm.

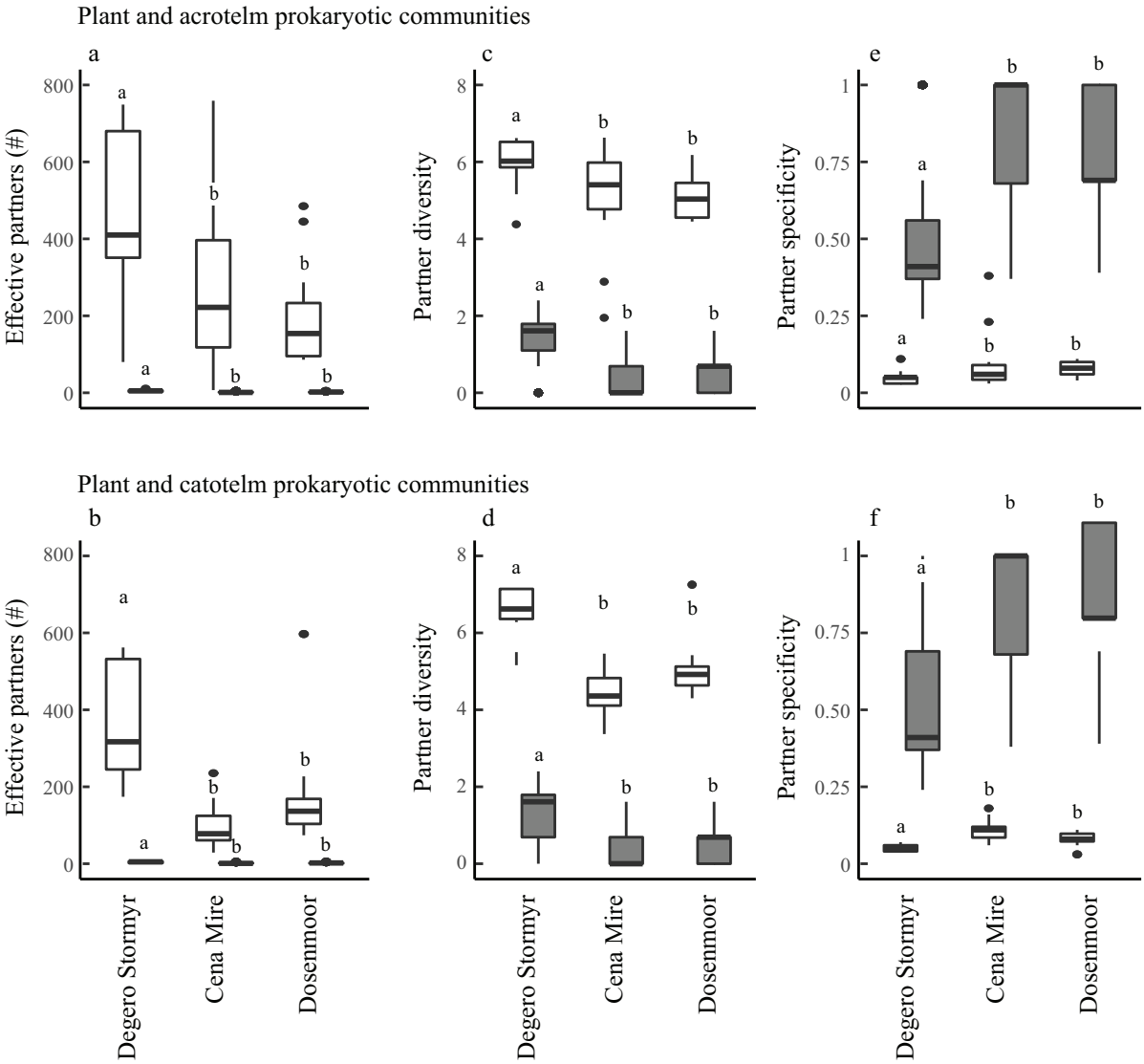

Figure 4. Boxplots of the species associations and network properties for the acrotelm (a, c, e) and catotelm (b, d, f) plant-prokaryote interactions. The number of specific partners (a, b), partner diversity (c, d) and partner specificity (e, f) have been calculated for plants (white boxplots: top down links; Suppl. Fig. 2) and prokaryotes (grey boxplots: bottom up links; Suppl. Fig. 2). Horizontal solid lines indicate median values. Different letters indicate differenced at the $P \leq 0.05$ level, based on analysis of variance using mean values.

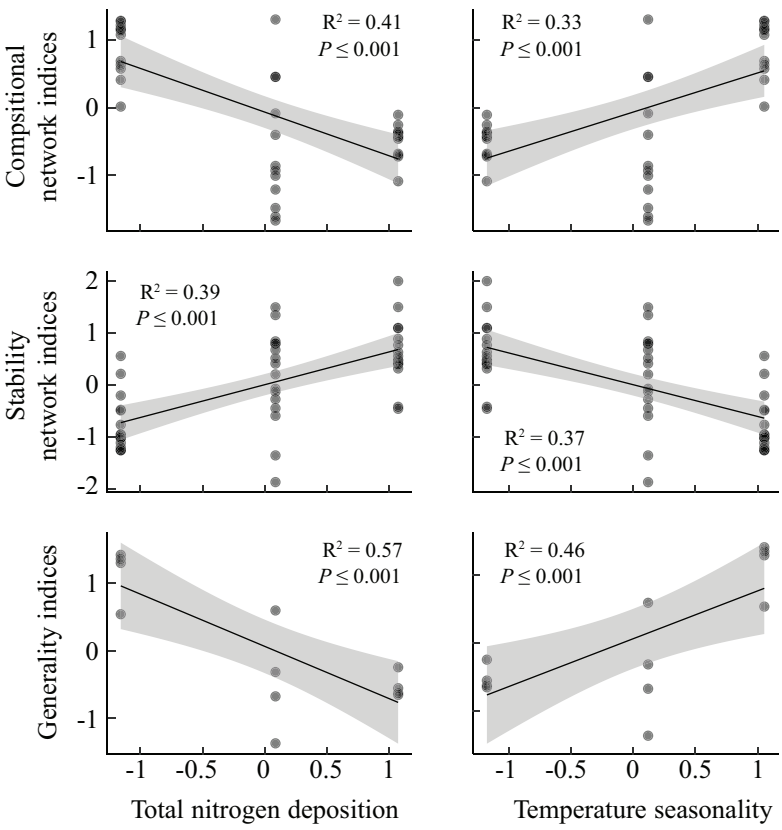

Figure 5 Linear regressions between the key enviro-climatological variables –total nitrogen deposition and temperature seasonality– and network indices. Network indices were grouped into three categories, network composition (linkage density, species richness, partner diversity), network stability (robustness, niche specialization, modularity) and network generality (generality indices). The grey area represents the 95% confidence interval of the mean. Data on specific network indices can be found in Suppl. Fig. 4.