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Peatland microbial community responses to plant functional group and drought are depth-dependent

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Abstract

Peatlands store one-third of Earth's soil carbon, the stability of which is uncertain due to climate change-driven shifts in hydrology and vegetation, and consequent impacts on microbial communities that mediate decomposition. Peatland carbon cycling varies over steep physicochemical gradients characterizing vertical peat profiles. However, it is unclear how drought-mediated changes in plant functional groups (PFGs) and water table (WT) levels affect microbial communities at different depths. We combined a multiyear mesocosm experiment with community sequencing across a 70-cm depth gradient, to test the hypotheses that vascular PFGs (Ericaceae vs. sedges) and WT (high vs. low) structure peatland microbial communities in depth-dependent ways. Several key results emerged. (i) Both fungal and prokaryote (bacteria and archaea) community structure shifted with WT and PFG manipulation, but fungi were much more sensitive to PFG whereas prokaryotes were much more sensitive to WT. (ii) PFG effects were largely driven by Ericaceae, although sedge effects were evident in specific cases (e.g., methanotrophs). (iii) Treatment effects varied with depth: the influence of PFG was strongest in shallow peat (0–10, 10–20 cm), whereas WT effects were strongest at the surface and middle depths (0–10, 30–40 cm), and all treatment effects waned in the deepest peat (60–70 cm). Our results underline the depth-dependent and taxon-specific ways that plant communities and hydrologic variability shape peatland microbial communities, pointing to the importance of understanding how these factors integrate across soil profiles when examining peatland responses to climate change.

KEYWORDS
Ericaceae, microbial community, peatlands, plant functional group, soil depth, water table

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1 | INTRODUCTION

Hydrology is the main driver of wetland ecosystem structure and function (Mitsch & Gosselink, 2015), and climate change-driven alterations to hydrology are having extensive impacts on Earth's wetlands (Junk et al., 2013; Moomaw et al., 2018). Because wetlands are globally important carbon storage reservoirs and methane sources, their responses to climate change will probably feed back to further modulate climate (Bardgett et al., 2008; Davidson & Janssens, 2005; Zhang et al., 2017). This is a particularly important issue for carbon-accumulating wetlands (peatlands) which contain approximately one-third of Earth's soil carbon, more than twice the carbon stored above ground in Earth's tropical rain forests (Joosten & Couwenberg, 2008). Drier conditions in peatlands can alter carbon cycles and expose carbon formerly sequestered below the water table (WT) to aerobic microbial oxidation (Bragazza et al., 2013; Bridgham et al., 2008; Davidson & Janssens, 2005; Freeman et al., 2001; Kane et al., 2019).

In addition to direct effects on hydrology, climate change can also alter peatland plant communities, which has extended consequences for carbon cycling (Bragazza et al., 2013; Dieleman et al., 2016; Jassey et al., 2018; Potvin et al., 2015). Bogs and poor fens of the northern hemisphere are dominated by *Sphagnum* mosses whose highly recalcitrant tissues form the bulk of peat-building organic matter (van Breeman, 1995; Rydin & Jeglum, 2013). Growing in the *Sphagnum* matrix are dwarf shrubs in the Ericaceae and graminoids in the Cyperaceae (hereafter sedges), two vascular plant functional groups (PFGs) with distinctive chemical, morphological and carbon allocation traits that influence ecosystem processes (Crow & Wieder, 2005; Dorrepaal et al., 2005; Rydin & Jeglum, 2013; Ward et al., 2015). Ericaceae lack aerenchyma but form adventitious roots as their stems are buried in accumulating peat (Rydin & Jeglum, 2013). This allows Ericaceae to proliferate in the acrotelm, the frequently oxic, upper part of peat soil profiles (Moore et al., 2002; Wallén, 1987). In contrast, aerenchyma allows sedges to dominate wetter sites, and to extend active roots into the catotelm, the deeper anoxic peat below the WT (Moore et al., 2002; Rydin & Jeglum, 2013). Strong evidence supports a shift towards dominance by Ericaceae when peatlands become drier (Bragazza et al., 2013; Breeuwer et al., 2009; Chimner et al., 2017; Malhotra et al., 2020; Potvin et al., 2015; Weltzin et al., 2003), a pattern driven in part by the divergent root traits of Ericaceae and sedges.

The roots of PFGs interact with peatland microbial communities to further modulate ecosystem processes. Ericaceae roots form mutualistic symbioses with ericoid mycorrhizal fungi (ErMF) which have the enzymatic capacity to degrade some forms of complex organic matter to access immobilized nutrients (Cairney & Burke, 1998; Martino et al., 2018; Read et al., 2004). Ready access to host photosynthate should make ErMF taxa strong competitors with free-living saprotrophs (Verbruggen et al., 2017). The differential abilities of ErMF vs. saprotrophic fungi to degrade organic matter, coupled with the high phenolic content of ericaceous litter (Bragazza et al., 2013; Dorrepaal et al., 2005), have consequences for decomposition rates and ecosystem carbon storage (Bragazza et al., 2013; Orwin et al., 2011; Verbruggen et al., 2017; Ward et al., 2015) that potentially carry over to affect microbial taxa that utilize byproducts of decomposition. Although both PFGs are colonized by endophytic fungi with poorly known functions, peatland sedges do not typically host mycorrhizal fungi (Thormann et al., 1999; Weishampel & Bedford, 2006). Therefore, some of the most important effects of sedges on microbial communities are probably mediated through their influence on free-living taxa. Sedge roots can be sources of labile carbon that fuel processes such as fermentation and methanogenesis, while aerenchyma-enabled rhizosphere oxygenation may allow aerobic microorganisms to be active around roots in water-saturated conditions and promote unique biogeochemical processes at the sharp oxygen concentration gradients associated with rhizospheres (Chanton et al., 2008; Lamers et al., 2012; Rupp et al., 2019). A number of studies provide important experimental evidence that PFGs uniquely influence aspects of peatland microbial communities or associated carbon cycling processes (e.g., Robroek et al., 2015; Rupp et al., 2019; Ward et al., 2015). However, there remains a large gap in our understanding of who in the microbial communities is affected, because tools that provide the resolution necessary to fully characterize the composition of diverse fungal, bacterial and archaeal communities (e.g., high-throughput amplicon sequencing) have not been extensively applied.

Several key points highlight the importance of accounting for WT and sampling depth when understanding PFG effects on peatland microbial communities. First, there is clear evidence that microbial communities shift with changes in WT (Emsens et al., 2020; Jassey et al., 2018; Urbanová & Barta, 2016), although concomitant shifts in vegetation can sometimes make it difficult to fully decouple WT from PFG effects. Second, microbial communities can change dramatically with increasing depth in peat profiles, in part a direct result of WTs excluding obligate aerobes from living in anoxic conditions deep in peat profiles (Andersen et al., 2013; Artz et al., 2007; Asemaninejad et al., 2017, 2019; Emsens et al., 2020; Kotiaho et al., 2013; Lamit et al., 2017; Lin et al., 2014). This is one reason why the abundance of fungi, most of which prefer oxic conditions (Kavanagh, 2011), drops dramatically with depth in peatlands (Golovchenko et al., 2002; Lamit et al., 2017; Lin et al., 2014). Third, microbial community depth stratification should also be a product of the unique effects of plant roots along peat profiles, with different PFGs having distinct depth effects based on their differences in rooting depth. Together, these points emphasize the need for experimental decoupling of WT and PFG effects on peatland microbial communities, within the context of depth.

We conducted a multiyear mesocosm experiment to examine how microbial communities are shaped by seasonal drought and contrasting PFGs. We hypothesized, (H1) manipulation of PFGs will shift microbial community structure, with these effects being unique to each PFG and strongly depth-dependent. We specifically predicted that Ericaceae removal will have its strongest effect in the upper peat profile, while sedge removal should influence communities along a greater length of the peat profile. Next, we hypothesized, (H2) WT

manipulation will shift microbial community structure, with these effects also being strongly depth-dependent. Although WT impacts are probably broad, the most distinct effects of WT should occur at depths where the WT is the most dynamic and distinct between the treatments, and is known to manifest a strong influence on peat and porewater chemistry (Kane et al., 2019; Lin et al., 2014). Given that the depth-dependent effects of each PFG are in part a consequence of the different ways that sedges and Ericaceae interact with oxic vs. anoxic conditions, we further hypothesized, (H3) the responses of microbial communities to manipulation of one factor (PFG or WT) will be dependent on the level of the other factor and these interactive effects will in turn be dependent on depth in the peat profile. Understanding how climate change-mediated changes in WT and PFGs impact microorganisms along peat depth gradients is important because these are some of the Earth's most taxonomically and functionally diverse groups of organisms and because their activities influence the most carbon-rich ecosystems on Earth, peatlands.

2 | MATERIALS AND METHODS

2.1 | Experimental study system

PEATcosm was a mesocosm experiment designed to test the influence of seasonal drought and PFG on peatland ecosystems. Detailed descriptions of the experimental design, peat characteristics, porewater chemistry and vegetation can be found in Kane et al. (2019), Lamit et al. (2017) and Potvin et al. (2015). The experiment contained 24 ~1-m^3 peat monoliths excavated from an oligotrophic acidic (pH = ~4) *Sphagnum* peatland in Minnesota, USA (47.07278°N, 92.73167°W), in May 2010. The monoliths were installed in the Houghton Mesocosm Facility, USDA Forest Service, Northern Research Station, Forestry Sciences Laboratory in Houghton, Michigan (47.11469°N, 88.54787°W). Monoliths were naturally vegetated by a continuous layer of *Sphagnum* mosses (primarily *Sphagnum rubellum*, but some *S. magellanicum* and *S. fuscum*), with *Polytrichum strictum* also present, and a vascular community of Ericaceae (primarily *Chamaedaphne calyculata*, *Kalmia polifolia*, *Vaccinium oxycoccus*) and sedges (*Carex oligosperma*, *Eriophorum vaginatum*). PFG manipulation was initiated in June 2011 and included Ericaceae removal, sedge removal and unmanipulated vegetation treatments (n = 8 per PFG treatment). PFG treatments were carried out by a combination of gentle removal of target species' stems plus roots when avoidance of moss damage was possible, followed by clipping all remaining above-ground tissues. PFG treatments were subsequently maintained by clipping growth of excluded PFGs on a weekly basis, as needed. WT manipulations were imposed by maintaining 12 mesocosms at average (high WT) and 12 at summer drought (low WT) conditions (n = 4 replicates per WT × PFG treatment). WT manipulation was carried out with rain-out shelters, artificial rainwater addition and controlled drainage in the spring and after heavy rains at the acrotelm–catotelm boundary (~25 cm depth). The depth separation of WT treatments was small in year one (2011), intermediate in year two (2012) and the greatest in years three and four (2013, 2014) to simulate strong summer drought (Figure S1).

2.2 | Peat sampling and molecular methods

Peat for the focal data set presented here was collected from four depth increments below the peat surface (0–10, 10–20, 30–40, 60–70 cm) in late August/early September in year three of the experiment, using a 5.08-cm-diameter circular corer fitted to an electric drill. Additional samples for complementary data sets were collected in late August/early September of year one and in late July of year four using a 2.54-cm (year one) or 7.62-cm (year four) diameter corer from two depth increments (10–20, 30–40 cm). Different diameter corers and sampling dates were necessary to accommodate a variety of intended uses for the peat, which varied depending on the year, and coring multiple times a season with a smaller corer would have created excessive disturbance in the mesocosms. However, in all years each 10-cm depth increment was not homogenized but instead a vertical split representative of ~25 ml of peat was subsampled from each for DNA work, ensuring the volume represented by the sampled material remained comparable among years. Upon collection, samples were flash frozen in liquid nitrogen and stored at −80°C.

Samples were pulverized using a mortar and pestle under liquid nitrogen, followed by a coffee grinder. DNA was extracted from 0.5 g of ground peat from each sample using a PowerSoil DNA Isolation kit, cleaned with a PowerClean DNA Clean-Up kit (MoBio Laboratories; now Qiagen), and quantified with a Qubit Fluorometer (Invitrogen, Life Technologies). DNA amplicon sequencing was conducted at the U.S. Department of Energy Joint Genome Institute (JGI) following Caporaso et al. (2012), with small modifications (see Coleman-Derr et al., 2016; Tremblay et al., 2015). Polymerase chain reaction (PCR) amplification utilized the primers 515F and 806R (Caporaso et al., 2011) targeting the bacterial and archaeal 16S V4 region, and fITS9 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) targeting the fungal ITS2 region. Primers were fitted with Illumina adaptors and the reverse primer contained an 11-bp barcode. Samples were pooled into equimolar portions and sequenced on an Illumina MiSeq platform (Illumina) using 2 × 250 bp (year one) or 2 × 300 bp (years three, four) chemistry.

2.3 | Bioinformatics

Processing of DNA sequence reads proceeded as follows. Illumina adapters and PhiX 174 were removed with BBDuk (sourceforge.net/projects/bbmap/), and 3′ and 5′ PCR primers were trimmed with CUTADAPT 1.18 (Martin, 2011). Paired reads were merged with BBMERG (Bushnell et al., 2017), and those with expected error rate > 1 and/or ambiguous bases were removed with VSEARCH 2.5.1 (Rognes et al., 2016). The 5.8S (94 bases) and 28S (35 bases) flanks were trimmed from ITS2 reads with CUTADAPT, and resulting amplicons < 95 bases

long were filtered. 16S V4 reads were not trimmed, but those exceeding ±8 bases from the median length (253 bases) were excluded. Chimera detection and removal was implemented with the VSEARCH plug-in for QIIME 2 (Bolyen et al., 2019) using the UNITE UCHIME ITS2 reference data set (v7.2; Nilsson et al., 2015), and the SILVA 16S data set (128 QIIME release; Quast et al., 2013). *De novo* operational taxonomic units (OTUs) were created with the QIIME 2 VSEARCH plug-in by first clustering at 98.5% similarity, then clustering the resulting OTU reference sequences at 97% similarity. Then, 97% OTUs were curated with LULU (min match = 90, min relative cooccurrence = 0.95; Frøslev et al., 2017), followed by the removal of OTUs with < 10 reads in the data set (Lamit et al., 2017).

The OTU matrices were further filtered and annotated. The QIIME 2 naive Bayes feature-classifier plug-in (Bokulich et al., 2018; Pedregosa et al., 2011) was used to assign taxonomy with the UNITE all eukaryote dynamic species hypothesis data set for fungi (v8.0, released February 2, 2019; Kõljalg et al., 2013) and the SILVA 16S +18S data set for prokaryotes (128 QIIME release; Quast et al., 2013). OTUs not assigned the taxonomy of target lineages were excluded. Next, ITS2 OTU representative sequences were aligned to the UNITE species hypothesis dynamic fungal data set (v8.0, released February 2, 2019) using BLAST in QIIME 2, and reads that did not match at least ≥ 70% of their length to fungi with a similarity of ≥ 75% were filtered (Tedersoo et al., 2015). To further remove potential nontarget sequences, the above 16S V4 pipeline was run with 28 marine samples originally sequenced on the same plate as the year one peat samples. Although rare in the data set, OTUs that occurred in year one peat samples and the marine samples, and had BLAST matches to salt-tolerant taxa reported from marine/saline systems in the NCBI DNA sequence database (http://blast.ncbi.nlm.nih.gov) were removed as potential contaminants. The data sets were then rarified to 5,000 reads per sample prior to all statistical analyses. Tentative functional groups were assigned with FAPROTAX (Louca et al., 2016) and FUNGUILD (Nguyen et al., 2016), with further refinement based on literature searches.

2.4 | Statistical analyses

Our first set of analyses examined overall patterns of OTU composition over the course of the experiment, with the aim of identifying when treatment effects began to manifest most distinctly and if the focal data set (year three) was representative of the overall treatment effects of the experiment. Permutational analysis of variance (PerMANOVA) (Anderson, 2001) was used to separately examine the two peat depths sampled in all years (10–20, 30–40 cm), with each analysis including the fixed effects of WT (high, low), presence/absence of Ericaceae, presence/absence of sedges, year, and all possible interactions among these effects. Each model also included block as a fixed effect, and individual mesocosm as a random effect to account for nonindependence of samples from the same mesocosm. Modelling treatments in this way allowed for assessment of the presence of each vascular PFG (as opposed to the removal of the other PFG) because each PFG was present in mesocosms with and without the other PFG, but a direct Ericaceae by sedge interaction could not be tested. These analyses were complemented with canonical analysis of principal coordinates (CAP; Anderson & Willis 2003) to visualize communities. PerMANOVA and CAP were run using Bray–Curtis dissimilarity. For all PerMANOVA models, we also report the square root of the estimated component of variation for each factor; these are in Bray–Curtis units (scaled between 0 and 100), and can be used for comparing the relative importance of terms in a model for explaining the overall variation in community composition (Anderson et al., 2008). Removal or pooling of model terms with negative estimates for components of variation (Anderson et al., 2008) had little effect on the significance tests or estimates of components of variation for the remaining terms in any of the PerMANOVAs we ran, and therefore all terms were always retained for simplicity. Prior to analyses, OTU matrices were relativized as proportions of sample read totals followed by 4^{th} root transformation to down-weight dominant OTUs. PerMANOVAs were conducted with Type III sums of squares, using permutation of residuals from partial models. PerMANOVA was run in PRIMER 6.1.15 with PERMANOVA+1.0.5 (PRIMER-E), and other analyses utilized vegan (Oksanen et al., 2019) in R 3.6.3 (R Core Team, 2020).

To gain deeper insight into the depth-specific microbial community responses to PFG and WT manipulations, we focused our more detailed analyses on microbial communities along the 70-cm depth gradient sampled in year three. (i) PerMANOVAs were run using the equivalent model structure as those for the multiyear analysis described above, substituting a depth effect for the year effect. (ii) PerMANOVAs were also run individually for each depth using the fixed effects of WT, presence/absence of Ericaceae, presence/absence of sedges, block, and two-way interactions between WT and the presence/absence of each PFG. (iii) CAP ordinations were used to visualize OTU composition. (iv) Indicator species analysis was used to identify the 25 strongest indicator OTUs associated with each depth and treatment using the R package indicspecies (De Cáceres & Legendre, 2009). (v) Linear mixed models were used to examine the responses of OTU richness and total archaea, and the relative abundances of a limited set of select functional groups with known relevance to carbon cycling (ErMF, lignocellulose-degrading fungi, methanotrophic bacteria, methanogenic archaea). These models included the fixed effects of depth, WT, presence/absence of sedges and Ericaceae, and all possible interactions among these fixed effects, plus the fixed effect of block and the random effect of mesocosm. Linear mixed models were fit in R with lmertest (Kuznetsova et al., 2017) using the Kenward-Roger approximation for *F*-tests. The EMMEANS package (Russell, 2020) was used to generate marginal means from linear mixed models, and the effectsize package (Ben-Shachar et al., 2020) was used to obtain partial-ω^2 values to use as effects sizes for comparison between model terms.

The final set of analyses utilized structural equation modelling with the depth gradient data from year three (see a priori model in Figure S2). Our specific goals were to: (i) test if the effect of PFG manipulation on prokaryote and fungal communities was primarily due

to the alteration of plant species composition as opposed to unmeasured variables affected by PFG manipulation, (ii) examine the potential for WT to modulate the effects of PFG on prokaryote and fungal communities through modification of the plant community, and (iii) measure how the strength of PFG and WT effects on prokaryote and fungal communities changed with depth. Separate models were created for each microbial community at each depth. Fungal and prokaryote communities were represented using Bray–Curtis dissimilarities calculated from the same 4^{th} root-transformed OTU matrices described above. The treatment factors of WT (high vs. low) and PFG treatments (unmanipulated, Ericaceae removal, sedge removal) were each represented in the model using pairwise distances denoting zero for pairs of samples from the same treatment and one for pairs of samples from different treatments. Sedge and Ericaceae communities were each represented using Bray–Curtis dissimilarity calculated from point-intercept data from Potvin et al. (2015) measured in year three. Prior to calculating pairwise dissimilarities, values in the Ericaceae and sedge data matrices were expressed as the percentage of intercepts represented by each species to emphasize shifts in absolute abundances, and a small constant was added as a dummy species to each matrix to account for some mesocosms lacking members of sedges or Ericaceae. Model subcomponents were tested with multiple regression on distance matrices (Lichstein, 2007) with ranked dissimilarities/distances using ecodist (Goslee & Urban, 2007) in R, and model fit was assessed with directional separation tests (Shipley, 2000) calculated manually. However, unfolded pairwise matrices were examined with piecewisesem (Lefcheck, 2016) to corroborate model parameters and verify the basis sets for directional separation tests.

3 | RESULTS

Diverse communities were recovered through amplicon sequencing. The rarefied fungal ITS2 data set contained 189 samples (sample size: year one = 48, year three = 96, year four = 45; 945,000 sequences), and in all years was dominated by Helotiales (Ascomycota), followed by Agaricales, Sebacinales and Polyporales (Basidiomycota) (Figure S3A). In total, there were 1,193 fungal OTUs, with an average of 64.1 OTUs per sample (*SD* = 17.8, range = 25–117 OTUs). The rarefied prokaryote data set contained 191 samples (sample size: year one = 48, year three = 96, year four = 47; 955,000 sequences). In all years, bacteria were dominated by Acidobacteria and Proteobacteria, while Archaea were dominated by Euryarchaeota, followed by Thaumarchaeota and Bathyarchaeota (Figure S3B). There was a total of 7,353 prokaryote OTUs, with an average of 606.0 OTUs per sample (*SD* = 158.1, range = 235–897 OTUs).

3.1 | Response of community composition across four years of WT and PFG manipulation

Changes in OTU composition over time supported the hypotheses that PFG and WT manipulation alter microbial communities (H1, H2) but provided no evidence for their interaction (H3). At 10–20 cm depth, fungal and prokaryote composition were influenced by the presence of Ericaceae and WT manipulation (Table 1; Figure 1a,b). The components of variation from the PerMANOVA models indicate that the main effect of Ericaceae on fungal composition was ~50% greater than that of WT at 10–20 cm, while the main effect of Ericaceae on prokaryotes was 25% less than for WT (Table 1). At 30–40 cm depth, WT was a slightly stronger influence on fungi than was the presence of Ericaceae, while the influence of WT on prokaryote composition was more than twice the strength of the marginally significant influence of Ericaceae (Table 1; Figure 1c,d). At both depths, some of the treatment effects manifested most strongly in years three and four (significant treatment by year interactions; Table 1), which is visually apparent in CAP ordinations (Figure 1). When integrating over the course of the experiment there was no evidence for a sedge effect on OTU composition at either depth (Table 1). Importantly, patterns in OTU composition across years confirmed that our focal depth gradient data set (year three) was representative of the broader PFG and WT effects over the course of the experiment (Figure 1).

3.2 | Community responses over the year three 70-cm peat depth gradient

3.2.1 | Community depth stratification

Depth had the largest influence on OTU composition of any factor included in the full-depth gradient PerMANOVA models (Table 1), and all depths showed uniqueness in fungal and prokaryote communities relative to other depths (Figure 2; Figure S3). All depths had indicator OTUs that were members of the Helotiales, while 0–10 and 60–70 cm depths also had many indicator OTUs representing a broader set of additional fungal lineages (Table S1). Fungal indicators of the 0–10 and 10–20 cm depths included ErMF, plant pathogens, general saprotrophs and lignocellulose degraders, while indicators of the 30–40 and 60–70 cm depths included nonmycorrhizal root associates, general saprotrophs and lignocellulose degraders (Table S1). Many prokaryote indicators, especially in the 0–10, 10–20 and 30–40 cm depths, were from acid-tolerant groups (e.g., Acidobacteriaceae, Acetobacteraceae). In the deepest depth (60–70 cm) there was an increase in Deltaproteobacteria and archaeal indicator OTUs, many of which are adapted to reducing conditions (e.g., methanogens, sulphate reducers; Table S1). Prokaryote and fungal OTU richness decreased with depth, while total archaea relative abundance increased (Table 2; Figure S4).

3.2.2 | Community composition responses to PFG and WT across the 70-cm depth gradient

In support of H1, fungal and prokaryote OTU composition exhibited depth-specific responses to PFG manipulation, although responses

TABLE 1 PerMANOVA full model results of OTU composition responses treatment factors across years or depths[a,b,c,d]

Multiyear models	Ericaceae *F* (*df*) *p*, √Var	Sedge *F* (*df*) *p*, √Var	WT *F* (*df*) *p*, √Var	Year *F* (*df*) *p*, √Var	Ericaceae × WT *F* (*df*) *p*, √Var	Sedge × WT *F* (*df*) *p*, √Var	Ericaceae × Year *F* (*df*) *p*, √Var	Sedge × Year *F* (*df*) *p*, √Var	WT × Year *F* (*df*) *p*, √Var	Ericacae × WT × Year *F* (*df*) *p*, √Var	Sedge × WT × Year *F* (*df*) *p*, √Var
Fungi 10–20 cm	4.05 (1, 15) **<0.001**, 15.0	1.06 (1, 15) 0.382, 2.09	2.20 (1, 15) **<0.001**, 9.4	4.62 (2, 54) **<0.001**, 17.1	0.96 (1, 15) 0.545, 0.0	1.08 (1, 15) 0.355, 3.4	1.39 (2, 36) **0.045**, 8.0	0.99 (2, 36) 0.485, 0.0	1.65 (2, 36) **0.007**, 10.2	0.98 (2, 36) 0.498, 0.0	0.93 (2, 36) 0.616, 0.0
Fungi 30–40 cm	1.8 (1, 15) **0.016**, 8.3	0.76 (1, 16.1) 0.805, 0.0	2.47 (1, 15.4) **0.002**, 11.3	4.49 (2, 33) **<0.001**, 17.0	0.78 (1, 15) 0.780, 0.0	0.77 (1, 16.1) 0.791, 0.0	1.06 (2, 33) 0.344, 3.2	0.96 (2, 33) 0.526, 0.0	1.19 (2, 33) 0.200, 5.5	0.93 (2, 33) 0.605, 0.0	0.89 (2, 33) 0.666, 0.0
Prokaryotes 10–20 cm	2.07 (1, 15.6) **0.002**, 7.4	0.96 (1, 15) 0.575, 0.0	3.28 (1, 15.6) **<0.001**, 10.8	4.2 (2, 35) **<0.001**, 15.1	0.99 (1, 15.6) 0.693, 0.0	1.05 (1, 15) 0.379, 2.2	1.13 (2, 35) 0.188, 4.3	0.89 (2, 35) 0.729, 0.0	1.39 (2, 35) **0.019**, 7.5	0.93 (2, 35) 0.646, 0.0	0.89 (2, 35) 0.736, 0.0
Prokaryotes 30–40 cm	1.74 (2, 15) 0.059, 6.5	0.75 (1, 15) 0.781, 0.0	4.79 (1, 15) **<0.001**, 14.7	4.49 (2, 36) **<0.001**, 14.8	0.81 (1, 15) 0.674, 0.0	1.23 (1, 15) 0.200, 5.2	0.87 (6, 36) 0.763, 0.0	0.83 (2, 36) 0.851, 0.0	2.06 (2, 36) **<0.001**, 11.5	0.99 (2, 36) 0.471, 0.0	0.93 (2, 36) 0.6245, 0.0
70-cm depth gradient models	**Ericaceae *F* (*df*)*p*, √Var**	**Sedge *F* (*df*)*p*, √Var**	**WT *F* (*df*)*p*, √Var**	**Depth *F* (*df*)*p*, √Var**	**Ericaceae × WT *F* (*df*)*p*, √Var**	**Sedge × WT *F* (*df*)*p*, √Var**	**Ericaceae × Depth *F* (*df*)*p*, √Var**	**Sedge × Depth *F* (*df*)*p*, √Var**	**WT × Depth *F* (*df*)*p*, √Var**	**Ericacae × WT × Depth *F* (*df*)*p*, √Var**	**Sedge × WT × Depth *F* (*df*)*p*, √Var**
Fungi	3.02 (1, 15) **<0.001**, 13.2	1.22 (1, 15) 0.173, 4.4	1.15 (1, 15) 0.080, 5.5	12.89 (3, 54) **<0.001**, 30.2	0.74 (1, 15) 0.876, 0.0	0.76 (1, 15) 0.840, 0.0	1.44 (3, 54) **0.009**, 8.2	0.916 (3, 54) 0.684, 0.0	2.12 (3, 54) **<0.001**, 13.1	0.80 (3, 54) 0.910, 0.0	0.88 (3, 54) 0.764, 0.0
Prokaryotes	1.89 (1, 15) **0.002**, 5.8	0.96 (1, 15) 0.570, 0.0	3.37 (1, 15) **<0.001**, 9.5	18.39 (3, 54) **<0.001**, 33.4	0.93 (1, 15) 0.636, 0.0	0.80 (1, 15) 0.856, 0.0	1.30 (3, 54) **0.015**, 6.2	0.89 (3, 54) 0.831, 0.0	2.37 (3, 54) **<0.001**, 13.2	0.86 (3, 54) 0.913, 0.0	0.89 (3, 54) 0.852, 0.0

[a]*Ericaceae* = presence/absence Ericaceae, *Sedge* = presence/absence sedges, *WT* = water table manipulation, *Year* = year sampled, *Depth* = peat sampling depth.

[b]Models also included individual *mesocosm* as a random effect and *Block* as a fixed effect. No hypothesis test was applied to these factors.

[c]√Var = the square root of the estimated component of variation for each factor. Negative estimates are reported as zero for simplicity.

[d]Bold indicates P-values equal or less than 0.05

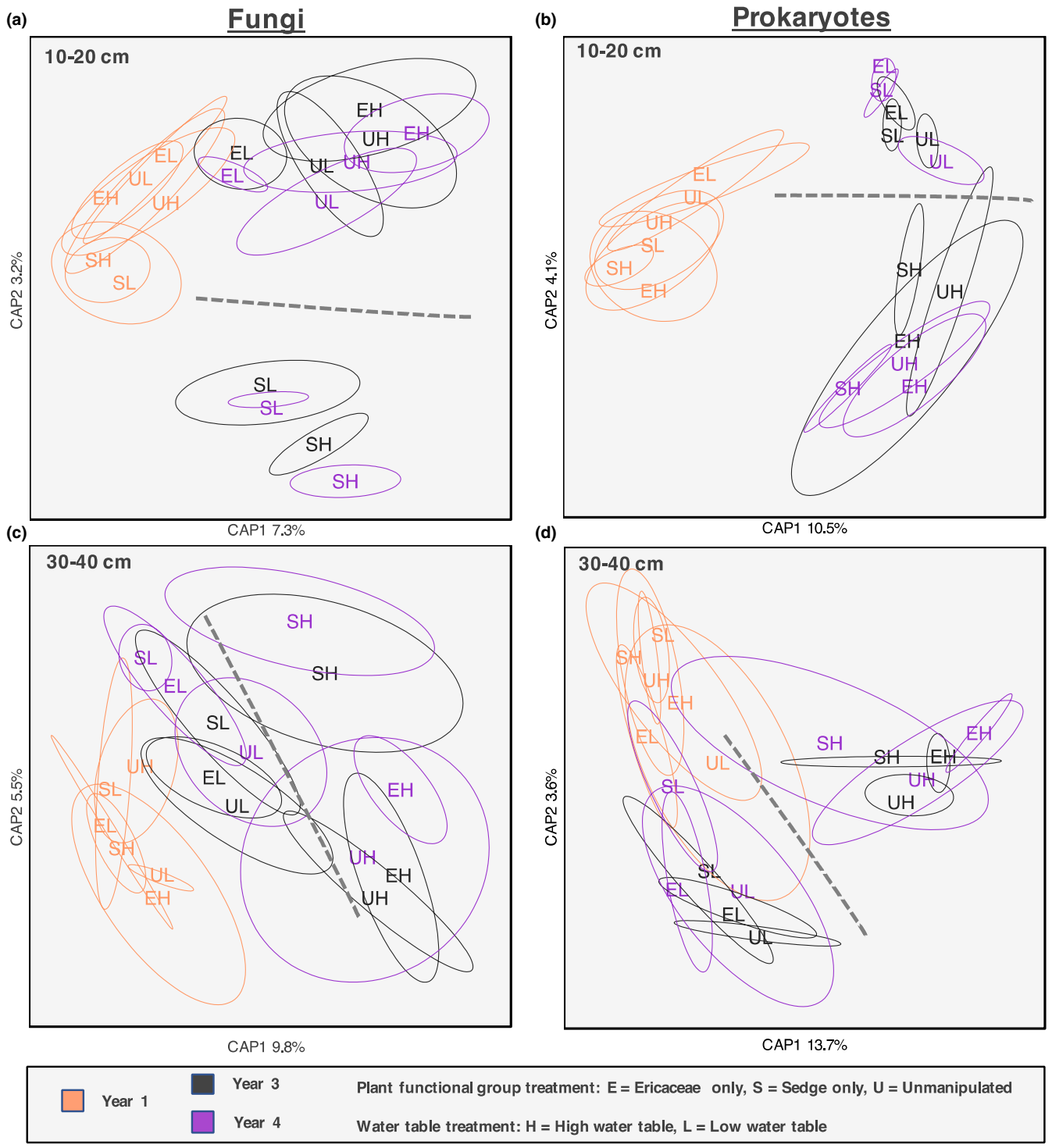


FIGURE 1 Canonical analysis of principal coordinates (CAP) ordinations with fungal (a, c) and prokaryote (b, d) operational taxonomic unit (OTU) composition at the 10–20 cm (a, b), and 30–40 cm (c, d) depths sampled in years one, three and four. Ordinations were constrained by year, plant functional group treatment (E = Ericaceae only, S = Sedge only, U = Unmanipulated), and water table treatment (H = high, L = low). Ellipses represent 95% confidence intervals of the ordination points. Dashed lines are provided to show distinction between the strongest effects observed in the latter two years (years three + four) [Colour figure can be viewed at wileyonlinelibrary.com]

were only driven by Ericaceae (Tables 1 and 3). The influence of Ericaceae on fungi and prokaryotes was distinctive in the upper depths (0–10, 10–20 cm) and disappeared in the deeper depths (30–40, 60–70 cm; Tables 1 and 3; Figure 2). Microbial communities in mesocosms with and without Ericaceae exhibited depth-specific differences in their top indicator OTUs, but there were also general patterns. ErMF OTUs were some of the top fungal indicators of mesocosms with Ericaceae, while root endophytes and lignocellulose

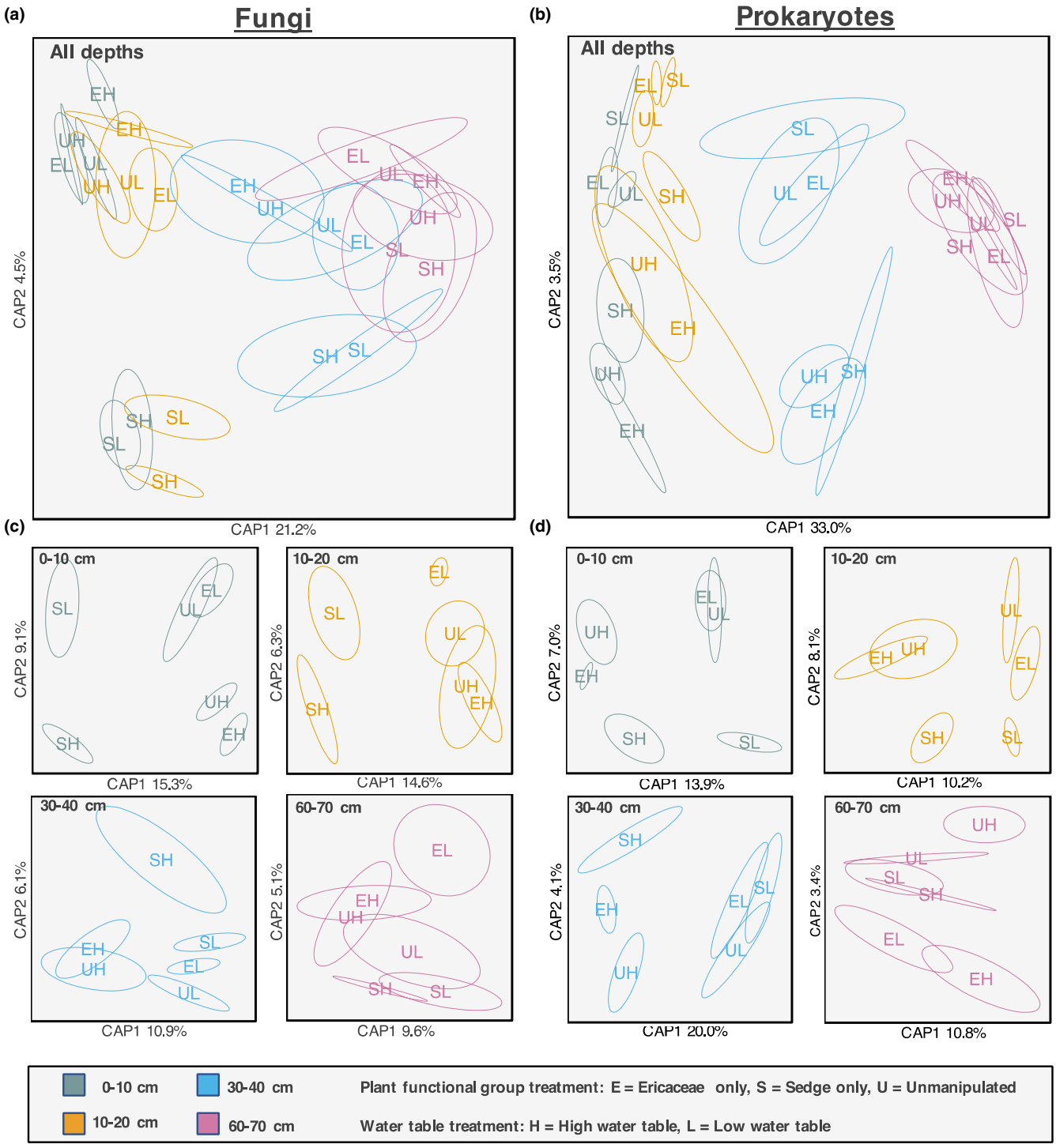


FIGURE 2 Canonical analysis of principal coordinates (CAP) ordinations with fungal (a, c) and prokaryote (b, d) operational taxonomic unit composition (OTU) across the 70-cm depth gradient sampled in year three. Ordinations were first (a, c) conducted by constraining by sampling depth, plant functional group treatment (E = Ericaceae only, S = Sedge only, U = Unmanipulated) and water table treatment (H = high, L = low), and only by water table and plant functional group treatments for data sets within each depth (b, d). Ellipses represent 95% confidence intervals of the ordination points [Colour figure can be viewed at wileyonlinelibrary.com]

degraders were top indicators of mesocosms lacking Ericaceae (Table S1). Of particular note is the lignocellulose-degrading genus *Galerina* whose members are top indicators of the absence of Ericaceae at 0–10 and 10–20 cm (Table S1), and have high relative abundances in these depths (Figure S5). Mesocosms lacking Ericaceae included indicators from 10 different bacteria phyla, and indicators of mesocosms containing Ericaceae were primarily Proteobacteria, but both treatments included some Acidobacteria indicators (Table S1).

Fungal and prokaryote OTU composition also exhibited depth-dependent responses to WT manipulation, in support of H2, but

TABLE 2 Linear mixed model results for the relative abundances of functional groups, total archaea and OTU richness in the 70-cm depth gradient sampled in year three[a,b,c,d]

Ericaceae $F_{(1,15)}$ p ω_p^2	Sedge $F_{(1,15)}$ p ω_p^2	WT $F_{(1,15)}$ p ω_p^2	Depth $F_{(3,54)}$ p ω_p^2	Ericaceae × WT $F_{(1,15)}$ p ω_p^2	Sedge × WT $F_{(1,15)}$ p ω_p^2	Ericaceae × Depth $F_{(3,54)}$ p ω_p^2	Sedge × Depth $F_{(3,54)}$ p ω_p^2	WT × Depth $F_{(3,54)}$ p ω_p^2	Ericaceae × WT × Depth $F_{(3,54)}$ p ω_p^2	Sedge × WT × Depth $F_{(3,54)}$ p ω_p^2
Fungi										
OTU richness										
0.02, 0.890 0.00	0.22, 0.647 0.05	2.71, 0.120 0.09	7.42, **<0.001** 0.25	0.01, 0.910 0.06	0.00, 0.975 0.00	0.19, 0.901 0.00	0.39, 0.763 0.00	1.94, 0.134 0.05	0.22, 0.879 0.00	0.30, 0.822 0.00
Ericoid mycorrhizal fungi										
18.60, **<0.001** 0.51	2.24, 0.155 0.07	4.72, **0.046** 0.18	47.90, **<0.001** 0.71	3.95, 0.065 0.15	0.22, 0.646 0.00	2.00, 0.124 0.05	0.04, 0.989 0.00	3.80, **0.015** 0.13	0.64, 0.592 0.00	0.37, 0.778 0.00
Lignin degraders										
11.68, **0.004** 0.39	0.15, 0.708 0.00	0.06, 0.813 0.00	4.11, **0.011** 0.14	0.07, 0.798 0.00	0.11, 0.743 0.00	1.13, 0.347 0.01	1.25, 0.301 0.01	1.70, 0.177 0.04	0.34, 0.794 0.00	0.23, 0.873 0.00
Prokaryotes										
OTU richness										
4.13, 0.060 0.16	0.04, 0.851 0.00	0.16, 0.693 0.00	98.88, **<0.001** 0.84	0.85, 0.372 0.00	0.04, 0.853 0.00	4.2, **0.009** 0.14	0.49, 0.694 0.00	2.73, **0.053** 0.08	0.56, 0.647 0.00	2.50, 0.069 0.07
Methanotrophs										
0.01, 0.942 0.00	4.98, **0.041** 0.19	0.10, 0.756 0.00	7.74, **<0.001** 0.26	9.79, **0.007** 0.34	0.89, 0.361 0.00	0.80, 0.502 0.00	1.30, 0.284 0.02	4.86, **0.005** 0.17	2.63, 0.059 0.08	1.12, 0.348 0.01
Methanogens										
0.41, 0.534 0.00	1.88, 0.191 0.05	25.26, **<0.001** 0.59	39.20, **<0.001** 0.66	1.58, 0.228 0.03	0.94, 0.347 0.00	1.28, 0.290 0.01	0.62, 0.604 0.00	8.17 **<0.001** 0.27	0.60, 0.615 0.00	1.17, 0.329 0.01
Total Archaea										
2.35, 0.146 0.07	1.86, 0.193 0.05	1.95, 0.183 0.05	73.32, **<0.001** 0.79	0.19, 0.669 0.00	0.83, 0.378 0.00	1.75, 0.168 0.04	0.22, 0.881 0.00	5.04, **0.004** 0.17	0.76, 0.521 0.00	2.08, 0.113 0.05

[a]All response variables were $\log_{10}$-transformed prior to analysis, except fungal and prokaryote OTU richness.

[b]Models also included individual *mesocosm* bin as a random effect and *Block* as a fixed effect. No hypothesis test was applied to these factors.

[c]ω_p^2 = partial-ω^2 values. Negative values are reported as zero for simplicity.

[d]Bold indicates P-values equal or less than 0.05.

TABLE 3 PerMANOVA results for community composition responses to treatments within individual depths of the 70 cm peat depth gradient sampled in year three.[a,b,c]

Taxa and depth of model	Ericaceae *F* (*df*) *p*, √Var	Sedge *F* (*df*) *p*, √Var	WT *F* (*df*) *p*, √Var	Ericaceae × WT *F* (*df*) *p*, √Var	Sedge × WT *F* (*df*) *p*, √Var
Fungi					
0–10 cm	3.25 (1, 15) **<0.001**, 20.03	1.27 (1, 15) 0.163, 6.89	2.31 (1, 15) **0.001**, 15.27	1.08 (1, 15) 0.367, 5.45	1.04 (1, 15) 0.433, 3.74
10–20 cm	3.20 (1, 15) **0.002**, 20.67	1.32 (1, 15) 0.141, 7.86	1.55 (1, 15) **0.051**, 10.36	0.73 (1, 15) 0.822, 0.00	0.87 (1, 15) 0.647, 0.00
30–40 cm	1.45 (1, 15) 0.105, 9.81	0.84 (1, 15) 0.675, 0.00	1.69 (1, 15) **0.050**, 12.13	0.78 (1, 15) 0.740, 0.00	0.69 (1, 15) 0.848, 0.00
60–70 cm	0.92 (1, 15) 0.5.2, 0.00	0.85 (1, 15) 0.580, 0.00	1.69 (1, 15) 0.094, 12.30	0.56 (1, 15) 0.898, 0.00	0.78 (1, 15) 0.672, 0.00
Prokaryotes					
0–10 cm	1.79 (1, 15) **0.005**, 10.19	1.00 (1, 15) 0.513, 0.00	2.73 (1, 15) **<0.001**, 15.09	0.96 (1, 15) 0.579, 0.00	0.99 (1, 15) 0.536, 0.00
10–20 cm	2.01 (1, 15) **0.002**, 11.72	1.01 (1, 15) 0.474, 1.05	2.11 (1, 15) **0.001**, 12.27	0.96 (1, 15) 0.554, 0.00	1.02 (1, 15) 0.462, 2.25
30–40 cm	1.11 (1, 15) 0.292, 4.00	0.88 (1, 15) 0.598, 0.00	4.16 (1, 15) **<0.001**, 21.28	0.92 (1, 15) 0.529, 0.00	0.86 (1, 15) 0.631, 0.00
60–70 cm	0.96 (1, 15) 0.429, 0.00	0.78 (1, 15) 0.687, 0.00	1.47 (1, 15) 0.135, 7.52	0.67 (1, 15) 0.832, 0.00	0.58 (1, 15) 0.913, 0.00

[a]Ericaceae = presence/absence Ericaceae, Sedge = presence/absence sedges, WT = water table manipulation, Depth = peat sampling depth.
[b]√Var = the square root of the estimated component of variation for each factor. Negative estimates are reported as zero for simplicity.
[c]Bold indicates P-values equal or less than 0.05.

there was no evidence for WT × PFG interactions (H3; Tables 1 and 3). The WT effect in the upper depths was stronger in the surface (0–10 cm) than subsurface (10–20 cm) peat for both communities. Despite this, at the upper two depths the strength of the WT effect on fungal OTU composition was subordinate to the Ericaceae effect, whereas this pattern was reversed for prokaryotes (Table 3; Figure 2). Prokaryote composition was the most divergent between WT groups at the 30–40 cm peat depth, while the influence of WT on fungi at this depth was clearly far weaker than for prokaryotes (Table 3; Figure 2). Neither fungal nor prokaryote composition exhibited a clear response to WT at 60–70 cm, although results for fungi were trending towards significance (Table 3). The responses of composition were driven by large depth-specific shifts in indicator OTUs for both fungi and prokaryotes (Table S1). For example, fungal indicators of the high WT treatment in the surface peat (0–10 cm) were primarily non-ErMF taxa probably associated with living or recently dead plant tissues, and indicators of the low WT treatment included a higher proportion of ErMF (Table S1). In contrast, indicators of the high WT treatment at 30–40 cm represented a very broad range of functions whereas the 30–40 cm low WT indicators were primarily nonmycorrhizal root-associates (Table S1). Interestingly, an OTU assigned to the Methanomicrobia (hydrogenotrophic methanogens) was an indicator of the high WT treatment in the three upper depths (Table S1), and three known methanotrophs were top indicators of the 10–20 cm depth high WT treatment.

3.2.3 | Microbial functional group and OTU richness responses to PFG and WT across the 70-cm depth gradient

Microbial functional groups and OTU richness exhibited complex responses to PFG, WT and/or PFG × WT interactions, lending support to all hypotheses (Table 2; Figure 3). Total ErMF relative abundance was strongly suppressed by Ericaceae removal and the high WT treatment in the upper two depths (0–10, 10–20 cm; Table 2, Figure 3a). Total lignocellulose-degrading fungi increased in relative abundance in the absence of Ericaceae, especially in the upper depths (Table 2; Figure 3b). The response of lignocellulose degraders remained significant after including total ErMF relative abundance as a covariate in the mixed model, indicating that the response was not solely driven by the removal of ErMF from the DNA pool (Ericaceae effect: $F_{(1,21.3)} = 4.87$, $p = 0.038$; marginal means ±1 *SE*: Ericaceae present = 0.08 ± 0.01, Ericaceae absent = 0.17 ± 0.05). Methanogens and total archaea had elevated relative abundances in high WT, except at 60–70 cm, with methanogen relative abundance peaking in the 30–40 cm high WT treatment (Table 2; Figure 3c; Figure S4C). Ericaceae removal tended to increase methanotroph relative abundance with high WT and decrease it with low WT at most depths (especially prominent at 10–20 and 30–40 cm) whereas sedge removal generally decreased methanotroph relative abundance (Table 2; Figure 3d). The major fungal and prokaryote taxa

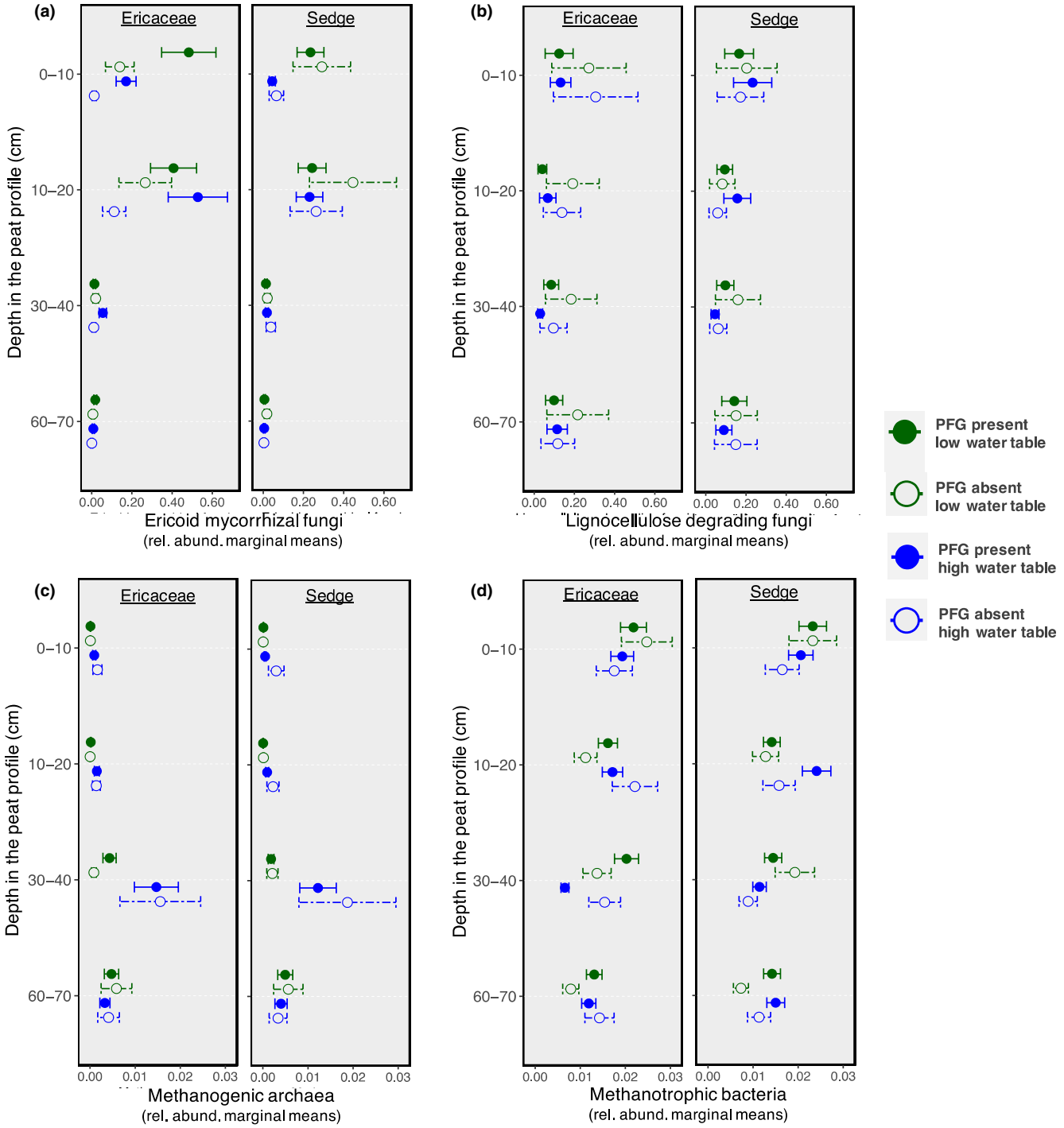


FIGURE 3 Marginal means (± 1*SE*) for relative abundances (Rel. Abund.) of ericoid mycorrhizal fungi (a), lignocellulose-degrading fungi (b), methanogenic archaea (c) and methanotrophic bacteria (d) for the presence/absence (solid lines = presence, dashed lines = absence) of Ericaceae (left panel) or sedges (right panel) by water table treatment (green = low water table, blue = high water table) along the 70-cm peat depth gradient. Marginal means are estimated from linear mixed models (see Table 2) [Colour figure can be viewed at wileyonlinelibrary.com]

within these functional groups often but not always followed the overall patterns of the group as a whole (Figure S5).

OTU richness results partially supported H1, H2 and H3. High WTs clearly depressed fungal richness in the surface peat (0–10 cm), although WT was not a significant overall effect in the model (Table 2; Figure S4A). Prokaryote richness was influenced by interactions among Ericaceae, WT, depth and sedges, with results only marginally significant in some cases (Table 2; Figure S4B). For example, at the 0–10 cm depth the highest richness occurred in mesocosms lacking Ericaceae, and at the 30–40 cm depth prokaryote OTU richness was elevated in low WT treatments but this pattern reversed at 60–70 cm (Figure S4B). Sedge removal tended to elevate

prokaryote OTU richness in the high WT treatment at the 0–10 cm depth, whereas a similar effect was evident in the low WT treatment at the 10–20 cm depth (Figure S4B).

3.2.4 | Structural equation modelling (SEM)

SEM supported the hypotheses concerning depth-dependent effects of PFG and WT (H1, H2), with several key results. First, the variation in fungal composition explained by the models was greatest in the 0–10 and 10–20 cm depths, the greatest variation in prokaryote composition was explained by models for the 0–10 and 30–40 cm depths, and the models for the 60–70 cm depth explained almost no variation in either community (Figure 4a,b). Second, PFG treatment effects were stronger on fungi than prokaryotes and were most pronounced at 0–10 and 10–20 cm, while WT effects were stronger on prokaryotes than fungi and were more pronounced in the 0–10 and 30–40 cm depths (Figure 4). Third, WT influenced Ericaceae composition, but the effect of WT on microbial communities through Ericaceae was small (Table S2).

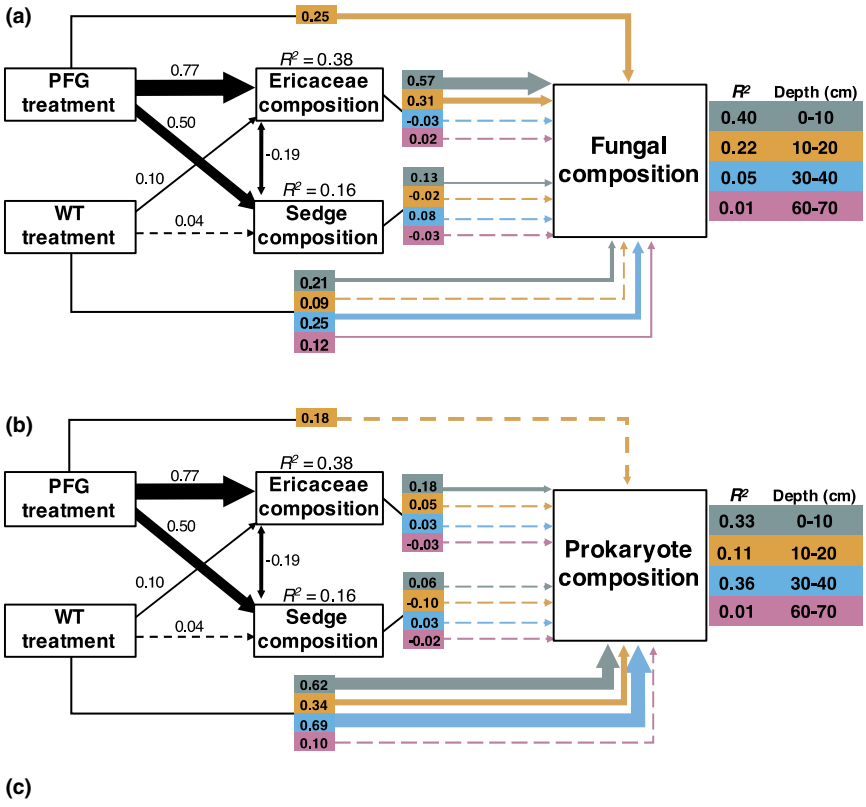
Fourth, at 0–10 cm the effect of PFG treatment primarily acted through changes in Ericaceae composition, which was not the case for the 10–20 cm depth. Initial model fit tests at 10–20 cm suggested a need for a direct path from PFG treatment to fungal composition (model fit $p = 0.012$). This direct path from PFG treatment to fungal OTU composition (partial *rho* = 0.25, Figure 4a) was nearly equivalent to the compound effect of PFG treatment acting through Ericaceae composition at 10–20 cm (compound effect partial *rho* = 0.24; Table S2). Similarly, but to a lesser degree, initial model fit tests at 10–20 cm also suggested a need for a direct path from PFG treatment to prokaryote composition (model fit $p = 0.086$). Although the direct path from PFG treatment to prokaryote OTU composition was only marginally significant (partial *rho* = 0.18, $p = 0.086$, Figure 4b), we decided to retain it in the model because its strength was much greater than the compound effect of PFGs acting through Ericaceae composition at 10–20 cm (compound effect partial *rho* = 0.04; Table S2). The direct path from Ericaceae composition to prokaryote composition was modest but significant (partial *rho* = 0.14, $p = 0.026$), when the direct path of PFGs on prokaryote composition was not included in the model.

4 | DISCUSSION

Our results reveal the strength and depth-dependence of WT and PFG effects on microbial communities. The strikingly greater impact of PFG on fungi near the surface contrasted with the stronger impact of WT on prokaryotes across a broader range of depths. These patterns can be explained by abiotic and biotic factors: the intolerance of most fungi to anoxic conditions (Kavanagh, 2011) constraining most taxa to shallow peat, and the colocation of the dominant ErMF with their shallowly rooted Ericaceae hosts (Moore et al., 2002; Wallén, 1987). In contrast, the broad range of moisture niches, metabolic pathways and redox tolerance among soil prokaryotes (e.g., Bodelier & Dedysh, 2013; Lennon et al., 2012) and the strong sensitivity of prokaryote communities to changes in soil moisture (e.g., Bapiri et al., 2010; Barnard et al., 2013) explain their shift with WT treatments in both drier surface peat as well as at the acrotelm–catotelm boundary where redox conditions are most dynamic (Kane et al., 2019; Tfaily et al., 2018). These depth-dependent effects indicate that WT and PFG are among the key shapers of the vertical physicochemical gradients that structure peatland microbial communities (Andersen et al., 2013; Artz et al., 2007; Lin et al., 2014), the activities of which then feed back to modulate carbon cycling along the peat profile (Chanton et al., 2009; Kane et al., 2019; Lin et al., 2014; Tfaily et al., 2018). Although discussions on wetland carbon cycling usually emphasize the role of anoxic reducing conditions (e.g., Schlesenger & Bernhart, 2013), most carbon inputs from primary production in bogs and poor fens derive from senesced *Sphagnum* in the largely oxic acrotelm (van Breeman, 1995; Rydin & Jeglum, 2013), making aerobic organisms instrumental as the initial transformers of peatland organic matter. As this partially degraded organic matter transitions into the catotelm, anaerobic metabolism becomes paramount, which is reflected in the OTU composition in the deeper depths of PEATcosm and other studies (e.g., Lin et al., 2014; Wang et al., 2019). Hence, the microbial community present at a peat depth sets bounds on how the community can change with drought and changes in dominant PFGs, and the integrated responses of fungi and prokaryotes along the profile may influence the magnitude of CO_2 and CH_4 released from peatlands under different climate change scenarios.

4.1 | Responses to PFG manipulation

As predicted, the influence of Ericaceae was greatest in the upper peat, which suggests a restructuring of the community involved in aerobic carbon cycling with changes in dominant PFGs. The presence of Ericaceae can have a strong impact on fungal communities in surface peat (Kennedy et al., 2018; Ward et al., 2015), and observational studies indicate that links between microbial communities and vegetation composition decline with depth in the peat profile (e.g., Artz et al., 2007; Lin et al., 2014). ErMF showed a marked decrease in abundance when Ericaceae were removed, indicating a preference for host photosynthate despite the free-living saprotrophic capabilities of some ErMF (Martino et al., 2018). Of particular interest is that the relative abundance of the dominant ErMF Ascomycota, *Hyaloscypha ericae* (deprecated synonyms are *Pezoloma ericae* and *Rhizoscyphus ericae*), was depressed less by Ericaceae removal than the dominant ErMF Basidiomycota, *Serindipita* spp., possibly indicating a greater degree of host dependency in the latter or more dormant propagules in the former. Ericaceae also influenced a variety of non-ErMF fungi and prokaryotes, which may be driven by several mechanisms. ErMF may competitively suppress saprotrophs (Verbruggen et al., 2017). In particular, one of the most abundant saprotroph genera in surface depths, *Galerina*, responded



(c)

Sampling depth (cm)	Fungi Model fit (C, df, P)	Fungi PFG net effect	Fungi WT net effect	Prokaryotes Model fit (C, df, P)	Prokaryotes PFG net effect	Prokaryotes WT net effect
0-10	2.44, 2, 0.294	0.50	0.27	0.08, 2, 0.986	0.17	0.64
10-20	-	0.48	0.12	-	0.17	0.34
30-40	1.07, 2, 0.587	-0.02	0.25	1.64, 2, 0.440	0.03	0.69
60-70	0.35, 2, 0.837	0.00	0.12	1.20, 2, 0.550	-0.04	0.10

FIGURE 4 Structural equation model results for fungi (a) and prokaryotes (b), and associated model fit statistics and total effects (c), linking microbial responses to water table (WT treatment), plant functional group manipulation (PFG treatment) and the vegetation community (Ericaceae composition, sedge composition). Path widths are scaled proportional to their path coefficients (*Rho* or partial-*Rho* values) and are dashed when not significant at an alpha-level of 0.05. Each variable is represented by a dissimilarity/distance matrix. Separate models were run for each depth and each taxonomic group, using samples from year three of the experiment, and (a) and (b) both represent the combined results of four different models. The core of the models (black arrows) was equivalent for all models because the same plant community data was used in each; paths and estimates specific to each depth's model are colour coded. Models for the 10–20 cm depth required the addition of a direct path from PFG treatment to fungal or prokaryote composition to obtain reasonable model fit, but models at other depths did not require this additional path [Colour figure can be viewed at wileyonlinelibrary.com]

very positively to removal of Ericaceae. These *Galerina* species are *Sphagnum* peatland specialists (Castellano, 2003; Gulden et al., 2005), and the lignocellulose-degrading capability of fungi in the genus (Nagendran et al., 2009; Riley et al., 2014) suggests that these species are adapted to *Sphagnum* as a substrate. If their activity is suppressed by ErMF it could also lead to greater accumulation of partially degraded *Sphagnum* litter, because ErMF do not possess the complete suite of lignin-degrading enzymes, most notably class II peroxidases. Although *Sphagnum* does not technically produce lignin, it does have analogous chemical components that resist hydrolytic decomposition (Bengtsson et al., 2018). Some taxa may also utilize the byproducts from ErMF decomposition of organic matter. Ericaceae tissues also represent a direct input of carbon into surface peat through exudates, senescence, and leaching phenolics that may act as unique microbial substrates and/or inhibitors (Weigang et al., 2008).

Two unexpected findings of the PFG effects are of note. First, SEM suggested the effect of Ericaceae in the 10–20 cm peat was partially due to factors influenced by PFG manipulation aside from direct changes in Ericaceae composition. These may include subsidence, peat accumulation rates and other physicochemical parameters influenced by PFG (Kane et al., 2019; Potvin et al., 2015). Additionally, above-ground plant community data may not fully reflect the density of some or all Ericaceae species roots (even non-relativized, as we used in the SEM), especially at 10–20 cm depth where root density is high; this may reduce the explanatory power of the Ericaceae community matrix, thus elevating the strength of the direct path from PFG manipulation to the microbial community.

Second, inconsistent with our hypothesis, sedges had a limited effect on microbial communities. Oxygenation and substrates from sedge roots are important shapers of microbially driven processes in sedge-dominated minerotrophic fens (Chanton et al., 2008; Rupp et al., 2019), and of diverse microbial communities associated with sedge rhizospheres (Hough et al., 2020). The modest sedge effect in PEATcosm might be due to their relatively low biomass in the ombrotrophic habitat we focused on, which became more variable through the course of the experiment (Potvin et al., 2015). Our findings contrast with a study, also in an ombrotrophic peatland, where microbial phospholipid fatty acid (PLFA) composition responded more to sedge removal than Ericaceae removal (Robroek et al., 2015). However, it is difficult to directly compare PLFA and amplicon sequencing results, and results from Ward et al. (2015) suggest no sedge influence on fungal composition. It is also possible that the *Eriophorum*-dominated sedge communities in Robroek et al. (2015) have a greater effect on microbial communities than our *Carex*-dominated sedge communities.

4.2 | Responses to WT manipulation

As predicted, the microbial response to WT manipulation was depth-dependent. In both WT treatments, the upper 20 cm of peat was above the WT for a considerable time period prior to sampling, with the 0–10 cm depth being above the WT surface for most of the growing season. Distance above the WT interacts with peat density, porosity and capillarity to drive differences in moisture availability that may have directly affected the response of microbial communities in the unsaturated upper peat to WT manipulation. Importantly, changes in taxa associated with methane cycling (methanogens, methanotrophs) and the processing of complex organic matter (lignocellulose degraders, ErMF) highlight the potential extended effects of WT on carbon cycling even in nonsaturated peat. Our results contrast with a recent mesocosm study by Asemaninejad et al. (2018) who did not detect an effect of WT manipulation on fungal communities at any depth in the profile. However, the discrepancy in results may be due to the more limited WT depth differential between treatment levels in Asemaninejad et al. (2018), which were maintained at a stable level for the course of the experiment. Moss species composition and productivity were also influenced by WT treatment in PEATcosm (Potvin et al., 2015), and we suspect that the stronger WT effect on fungi and prokaryotes at 0–10 cm than at 10–20 cm depth is in part due to the role of *Sphagnum* mosses in structuring their microbiomes (Kostka et al., 2016). The potential foundational influence of moss in acid *Sphagnum* peatlands is one factor making these systems distinct from minerotrophic fens, where moss has a less clear influence on microbial communities in surface peat (Emsens et al., 2020).

Microbial community responses in the deeper peat were probably driven by WT inundation and oscillation. WT manipulation elicited some of its strongest responses (especially on prokaryotes) at 30–40 cm depth, where peat was perennially underwater in the high WT treatment but seasonally above the WT in the low WT treatment. This should promote communities capable of aerobic decomposition during the driest part of the season and anaerobic decomposition on the shoulders of the growing season in the low WT treatment, and communities associated with slower carbon transformations typical of anoxic reduced conditions in the high WT treatment, a contention supported by our indicator species analyses. Interestingly, methanogens reached their greatest relative abundance in the high WT treatment at 30–40 cm depth. This might reflect a preference for anoxic conditions combined with inputs of fresh, labile substrates for fermenters and syntrophs to generate H_2 and CO_2 used in hydrogenotrophic methanogenesis (Conrad, 1999). Oscillation between oxic and anoxic conditions at the interface between the acrotelm and catotelm (i.e., the mesotelm) is associated with rapid organic matter transformations (Kane et al., 2019; Lin et al., 2014; Tfaily et al., 2018), and shifts in microbial composition in the low WT treatment at 30–40 cm indicate a downward extension of this biogeochemical "hotspot" during drought. In contrast, the community at 60–70 cm depth was continuously submerged far below the WT surface, buffered from changes in the WT level and less affected by roots, suggesting that deep peat microbial communities and their influences on carbon cycling may not be strongly affected by seasonal drought, at least in the short term.

4.3 | Interactions between PFG and WT

The response of some components of the microbial community highlighted the potential for WT and PFG interactions. The negative effect that Ericaceae tended to have on aerobic methanotrophs in the high WT treatment might be explained by dense Ericaceae roots depleting rhizosphere O_2 through respiration, although other mechanisms of direct interference are possible (e.g., via antibiosis; Adeoyo et al., 2019). Additionally, at 10–20 cm depth, the more complete decline in ErMF after Ericaceae removal in high WT mesocosms suggests that the negative effects of host removal are compounded by anoxia when this depth is flooded during the shoulders of the growing season. Given the importance of ErMF and methanotrophs to peatland carbon cycling, these results indicate that the responses of subcomponents of the community to PFG × WT interactions may cause shifts in the functioning of microbial communities even when overall OTU composition responds more slowly.

Climate change-driven shifts in peatland soil moisture can influence microbial communities indirectly by shifting the composition of plant communities (e.g., Bragazza et al., 2013; Jassey et al., 2018). Evidence for this in PEATcosm was not strong; although the low WT treatment promoted Ericaceae cover and productivity, with some species responding more than others (Potvin et al., 2015), the compound path effects from WT through Ericaceae composition to the microbial communities were very small. Our SEMs may have underestimated the path coefficient between WT and Ericaceae composition because mesocosms lacking Ericaceae did not have a

community to respond to WT treatment. However, we suspect that the strong effect of simply having or not having Ericaceae overshadowed the impact of WT on microbial communities acting through modification of the plant community. Over a longer time scale the influence of WT on microbial communities acting through vegetation change should become stronger, which would represent an indirect pathway in natural systems for climate change-related droughts to influence microbial communities.

5 | CONCLUSIONS

Our results demonstrate the importance of WT and PFG in structuring peatland microbial communities along peat depth profiles. Peatlands in many regions are experiencing increased temperatures, changes in long-term precipitation patterns, and other anthropogenic disturbances that influence WT dynamics and cause shifts in plant communities; the influence of these factors on carbon cycling will be contingent on how they influence microbial communities. Importantly, microbial lineages and functional groups do not all respond equivalently to WT and PFG manipulations, and their responses are depth-dependent. The strong mutualistic interactions of fungi and Ericaceae appear to be the driver of the greater PFG effect on fungal than prokaryote communities. The dominance of ErMF in shallow oxic peat indicates a large potential for Ericaceae to shape microbial community structure and function near the surface, where most new organic matter enters the peat profile. The fact that when Ericaceae are removed lignocellulose degraders respond very positively in relative abundance begs the question of whether this represents a functional release of those taxa in the absence of mycorrhizal competition. Similarly, the significant prokaryote response to PFG manipulations indicates that changing resources and conditions driven by plant traits can also structure these communities, especially methanotrophs; however, the weaker prokaryote (vs. fungal) response to PFG vs. WT manipulation suggests redox conditions predominate in structuring these communities. The vertical complexity in responses, probably driven by declining PFG influence and increasing influence of declines in both redox potential and organic matter quality with depth, highlights the necessity of accounting for depth stratification when understanding the responses of peatland microbial communities to global change.

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AUTHOR CONTRIBUTIONS

E.A.L., E.S.K., R.K.K., J.T.L., R.A.C. and S.G.T. conceived the study and obtained funding. L.R.P., E.A.L., E.S.K., K.J.R., S.G.T. and L.J.L. performed the research. L.J.L. analysed the data. L.J.L. and E.A.L. wrote the paper, with all coauthors contributing to revisions.

DATA AVAILABILITY STATEMENT

The sequence data have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession no. PRJNA650129. Vegetation data are available through Pangea: doi.pangaea.de/10.1594/PANGAEA.902313.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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