

RESEARCH ARTICLE

Patterns and drivers of fungal community depth stratification in *Sphagnum* peat

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One sentence summary: Fungi living in peat are influenced by depth and vegetation.

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ABSTRACT

Peatlands store an immense pool of soil carbon vulnerable to microbial oxidation due to drought and intentional draining. We used amplicon sequencing and quantitative PCR to (i) examine how fungi are influenced by depth in the peat profile, water table and plant functional group at the onset of a multiyear mesocosm experiment, and (ii) test if fungi are correlated with abiotic variables of peat and pore water. We hypothesized that each factor influenced fungi, but that depth would have the strongest effect early in the experiment. We found that (i) communities were strongly depth stratified; fungi were four times more abundant in the upper (10–20 cm) than the lower (30–40 cm) depth, and dominance shifted from ericoid mycorrhizal fungi to saprotrophs and endophytes with increasing depth; (ii) the influence of plant functional group was depth dependent, with Ericaceae structuring the community in the upper peat only; (iii) water table had minor influences; and (iv) communities strongly covaried with abiotic variables, including indices of peat and pore water carbon quality. Our results highlight the importance of vertical stratification to peatland fungi, and the depth dependency of plant functional group effects, which must be considered when elucidating the role of fungi in peatland carbon dynamics.

Keywords: Ericaceae; ericoid mycorrhiza; peat; soil depth; sedge; water table

INTRODUCTION

Northern peatlands are characterized by extremely high soil carbon density, sequestering almost one third of the world's soil organic carbon stocks in ~3% of land area (Page, Rieley and Banks 2011). Carbon accumulates in peatlands because anoxic, phenol-

rich, water-saturated conditions depress rates of decomposition relative to primary production (Rydin and Jeglum 2013). In many locations around the world, peatlands are experiencing water table (WT) declines due to climate change-related droughts, and drainage for forestry and agriculture (Rydin and Jeglum 2013). Such declines in WT expose formerly anoxic peat to oxic

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conditions favorable to aerobic microbial metabolism and decomposition, and are likely to have important influences on microbial community structure (Freeman, Ostle and Kang 2001; Jaatinen, Laiho and Vuorenmaa 2008; Trinder, Johnson and Artz 2008). The switching of large areas of peatlands from net sinks to net sources of carbon may act as a positive feedback to climate change (Bardgett, Freeman and Ostle 2008; Bridgman et al. 2008). However, our understanding of the responses of peatlands to drainage and climate change stresses are incomplete without understanding how altered WT interacts with other factors, including plant functional groups (PFGs) and peat depth, to influence the structure and function of microbial communities involved in decomposition (Andersen, Chapman and Artz 2013).

Hydrologically driven shifts in the relative dominance of PFGs may influence communities of microorganisms, such as fungi. Of particular importance, fungal community structure and function could be regulated by differences in root traits among PFGs. At one end of the spectrum, peatland sedge (*Carex* and *Eriophorum* spp.) roots typically lack coevolved mycorrhizal symbionts (e.g. Thormann, Currah and Bayley 1999) but locally oxic conditions created by their aerenchyma (spongy tissues with air channels that permit gas exchange into otherwise anoxic peat) likely have strong influences on free-living fungi. In contrast, ericaceous shrubs (Ericaceae) are sensitive to anoxic conditions due to their lack of aerenchyma, but host ericoid mycorrhizal fungi (ErMF) with extracellular enzymes that enable depolymerization of complex organic molecules to gain access to limiting nutrients (e.g. nitrogen, phosphorous; Cairney and Burke 1998; Cairney and Meharg 2003; Read, Leake and Perez-Moreno 2004). Experimental work indicates that drier peat conditions promote dominance by ericaceous shrubs, whereas sedges can sometimes be favored by more moist conditions (Weltzin et al. 2003; Breeuwer et al. 2009; Potvin et al. 2015). The link between plants and fungi suggests that shifts in the dominance of PFGs due to WT alteration could change the structure and function of peatland fungal communities.

Fungal communities can also exhibit vertical stratification within peat profiles (Artz et al. 2007; Lin et al. 2014), and the causes of vertical stratification are likely intertwined with the effects of WT and PFG on fungi. A suite of abiotic variables change between surface and deep peat, including water content, oxygen availability, redox potential, temperature, dissolved organic carbon (DOC), bulk density and peat humification (i.e. the level of decomposition) (Hribljan et al. 2014; Lin et al. 2014; Tfaily et al. 2014; Potvin et al. 2015). Depth gradients of many of these chemical and physical characteristics are largely a consequence of water saturation and age, creating a contrast between the more frequently oxic, lower bulk density, fibric peat (acrotelm) and the typically water saturated, anoxic, denser, more sapric deeper peat (catotelm). PFGs also have divergent influences on abiotic properties of peat (Andersen, Chapman and Artz 2013), and different PFGs can thus be expected to modulate the effect of depth in the peat profile on fungal communities. For example, sedge aerenchyma allows living sedge roots to penetrate deeper into peat than roots of Ericaceae, potentially moderating the depth gradient in oxygen and root-derived labile resources. In contrast, shallowly rooted Ericaceae, with enzymatically active ErMF symbionts, may be expected to sharpen the distinction between upper and lower depths in a peat profile. These interactive effects of PFG with peat depth should be further modified by WT, because the WT level defines the major environmental context within which plant roots interact with peat and fungi. When a WT is low, oxygen is available to a greater fraction of the peat profile, which should reduce the importance of oxygenation by

sedge aerenchyma on the rhizosphere and be less limiting to the growth of ericaceous roots, associated symbionts and aerobic free-living fungi. Because they are intertwined, the individual and interactive effects of depth in the peat profile, WT and PFG are difficult to understand without direct experimental manipulation.

Here, we characterize the fungal community during the first year of a peatland mesocosm experiment, PEATcosm (Potvin et al. 2015). The experiment is aimed at understanding how peatland community and ecosystem processes are influenced by PFG and WT level, and how depth in the peat profile modulates the effects of these factors. Our primary objective with this sampling was to characterize the change in fungal community structure with depth in the peat profile. We hypothesized that (H1) the steep physical, chemical and biological gradients associated with depth in the peat profile cause fungal community structure to be vertically stratified. Specifically, we predicted that surface peat has the greatest overall fungal abundance and is dominated by ErMF fungi, whereas deeper in the peat profile fungal abundance declines and saprotrophic fungi become increasingly important. Our next objective was to test for a short-term response of fungi to PFG removal and WT decline. Relative to the effect of peat depth, we expected the effects of these factors to be small during the first season of the experiment. Nevertheless, when they do occur, we hypothesized that (H2) divergent traits between PFGs have differential effects on fungal community structure. Experimental removal of different PFGs should therefore alter fungal community structure in different ways. In particular, the distinction in community structure between upper and lower peat depths should be greatest in the presence of Ericaceae and the absence of sedges; this is likely, because of both the influence of Ericaceae roots and ErMF symbionts in the upper peat, and the potential ability of sedges to homogenize fungal communities along depth gradients by bringing oxygen to deep peat. Furthermore, we hypothesized that (H3) WT level influences fungal community structure, due to WT effects on abiotic characteristics of peat and the plant community. We specifically predicted that the relative abundance of ErMF and overall fungal abundance should increase as WT declines with experimentally simulated drought conditions. Our final objective was to test the relationship between fungal community structure and abiotic characteristics of peat and pore water (e.g. humification, carbon quality, temperature). We hypothesized that (H4) fungal community variation is coupled with variation in abiotic characteristics of peat and pore water, because these abiotic characteristics are influenced by the activities of fungi (e.g. decomposition), and some represent important resources for, or constraints on, fungi. In particular, variation in abiotic peat and pore water characteristics should mirror changes in fungal community structure between depths in the peat profile, and exhibit corresponding shifts with experimental manipulations of WT and PFG.

MATERIALS AND METHODS

Experimental study system

PEATcosm is a multifactorial peatland mesocosm experiment located at the Houghton Mesocosm Facility, USDA Forest Service, Northern Research Station, Forestry Sciences Laboratory in Houghton, Michigan (N47.11469°, W88.54787°). The experiment includes 24 mesocosms, each composed of a single ~1 m³ intact peat monolith excavated from an oligotrophic peatland in Meadowlands, MN, USA (N47.07278°, W92.73167°) in May 2010, and

installed in the Houghton Mesocosm Facility. Monoliths were obtained from lawn habitat, with existing vegetation dominated by the ericaceous shrubs *Chamaedaphne calyculata* (L.) Moench., *Kalmia polifolia* Wangenh. and *Vaccinium oxycoccus* L., and the sedge *Carex oligosperma* Michx., above a moss layer of *Sphagnum* species and *Polytrichum strictum* Brid. (Potvin et al. 2015). No experimental treatments were imposed during the 2010 growing season. The experiment included a two-level WT treatment and a three-level PFG treatment, with four replicate spatial blocks representing each of the six unique factor-level combinations. In June 2011, PFG manipulation was initiated with clipping of ericaceous shrubs (Sedge treatment), sedges (Ericaceae treatment) or unclipped as a PFG control (unmanipulated treatment; $n = 8$ for each treatment). Ericaceae and Sedge treatments were subsequently maintained by clipping new growth of excluded species as needed on a weekly basis. WT manipulations were also initiated in June 2011 (12 mesocosm bins with high and 12 with low WT; hereafter referred to as high and low, respectively). WT manipulation was designed to match typical seasonal WT dynamics for average (high) and summer drought (low) years, and was carried out using rain-out shelters, artificial rainwater addition and drainage in the spring at the acrotelm-catotelm boundary (~25 cm depth). In 2011, to avoid stress to mosses after initiation of the PFG treatment, the WT manipulation was minimal but distinct between treatments; high and low WT treatments differed by ~5 cm through the season, with the high averaging ~7 cm and the low ~12 cm below the peat surface during the peat sampling period (Fig. S1, Supporting Information). See Potvin et al. (2015) for additional details on design and treatments.

Fungal sampling and molecular methods

One core per mesocosm was collected between 31 August and 13 September 2011, ~3 months after initiation of experimental manipulations. Peat cores were extracted using a 2.54 cm diameter aluminum corer sharpened at the leading edge and fitted to an electric drill. The 10–20 cm (acrotelm) and 30–40 cm (catotelm) depth increments from each core were split lengthwise and one half (for DNA analysis) was immediately flash frozen in liquid nitrogen, then stored at -80°C . Each sample was pulverized in a mortar and pestle under liquid nitrogen, and then ground to a fine powder with liquid N in an electric coffee grinder. Total soil DNA was isolated from 0.5 g of ground, wet peat using a PowerSoil DNA Isolation kit followed by purification with a PowerClean DNA Clean-Up kit (MoBio Laboratories Inc., Carlsbad, CA, USA). To enable wet to dry-mass conversion, a subsample of ground peat from each core was weighed wet and again after oven drying for 36 h at 60°C .

Fungal abundance was estimated in each sample using quantitative PCR (qPCR) following Lau and Lennon (2011). Briefly, the first internal transcribed spacer region (ITS1) was amplified with the primers ITS1f and 5.8S (Fierer, Vilgalys and Jackson 2005). Each 30 μL reaction included 1 μL of DNA template, 0.5 μL of each primer (10 μmol), 14.5 μL of DNase-free water, and 13.5 μL of 5 PRIME 2.5x Real-MasterMix SYBR ROX (5 Prime, Inc. Gaithersburg, MD, USA). PCR assays were performed with an Eppendorf Mastercycler realplex² system using the thermal cycle conditions of Fierer, Vilgalys and Jackson (2005). Standards were generated from a *Trichosporon* sp. isolate using the TOPO TA Cloning Kit (Invitrogen; Carlsbad, CA, USA). Plasmids were extracted from transformed cells (Sambrook and Russell, 2001), and the M13 forward and reverse primers from the cloning kit were used to generate PCR products for a standard curve. The

standard curve ranged from 10^2 to 10^7 copies per μL , with coefficients of determination (R^2) of 0.96–0.99 and amplification efficiencies of 0.93–0.99. Melting curve analyses provided no evidence for primer dimers. Three analytical replicates of each sample were run through the preceding qPCR process, data were averaged per sample and values were expressed as ITS1 gene copies per gram dry peat.

To further characterize fungal communities in each sample, community amplicon sequencing was conducted at the US Department of Energy Joint Genome Institute (JGI, Walnut Creek, CA, USA). Sample prep followed Caporaso et al. (2012), and utilized a PerkinElmer SciClone NGS G3 Liquid Handling Workstation (Waltham, MA, USA) and 5 PRIME's HotMasterMix amplification kit. The fungal ITS2 region was targeted with the forward primer sequence fITS9 (Ihrmark et al. 2012) and the reverse primer ITS4 (White et al. 1990). The full-length primer contained an Illumina adapter sequence, an 11-bp index (on the reverse primer only) which was unique to each sample, a primer pad, a 0–3 bp spacer pad and the ITS2 primer sequence. Prepared amplicon libraries were normalized, pooled and quantified using KAPA Biosystem's (Wilmington, MD, USA) next-generation sequencing library qPCR kit using a Roche LightCycler 480 real-time PCR instrument. The quantified amplicon pool was sequenced with an Illumina MiSeq (San Diego, CA, USA) using 2×250 bp paired-end chemistry. Data are available through the JGI genome portal (project ID 1 021 300, <http://genome.jgi.doe.gov/>).

Bioinformatics

The Itagger pipeline, version 1.1 (https://bitbucket.org/berkeleylab/jgi_itagger), was used for initial data processing. Duk (<http://duk.sourceforge.net/>) was used to filter PhiX 174, human and Illumina adapter sequences from demultiplexed reads. Primers were removed with Cutadapt (Martin 2011). Reads were quality trimmed based on the expected error rate over a 5 base window at their 3' ends and merged with Pandaseq (minimum overlap = 15 bp, quality threshold = 0.25; Masella et al. 2012) if their combined length was ± 3 standard deviations of the mean ITS2 length. The 5' and 3' ends of merged reads were trimmed by 94 and 35 bases, respectively, to remove the conserved 5.8S and 28S rRNA gene-flanking regions. Reads were then discarded when their expected number of errors (calculated as the product of error probabilities from Phred scores) exceeded 3. Sequences were dereplicated at 100% identity and operational taxonomic units (OTUs) were clustered iteratively at 99%, 98%, 97%, 96% and 95% identity with USEARCH (Edgar 2010). Reference-based chimera detection was run with UCHIME (Edgar, Haas and Clemente 2011) using UNITE (2011–07–22 release; <https://unite.ut.ee>). Clusters formed at 95% sequence similarity were used in subsequent analyses. Using 95% sequence similarity is slightly more conservative than the frequently used 97% cut-off; however, there is no single similarity cut-off that is perfect for delineating species in sequence datasets. We felt that it was most important to guard against superfluous OTU propagation, which may be common in environmental sequence datasets. A recent mock community study using the ITS2 region suggested that similarity cut-offs lower than the typically used 97% may yield a more accurate number of OTU clusters (Taylor et al. 2016).

Further processing, using OTUs generated from the Itagger pipeline, proceeded as follows. Taxonomy was assigned using the Ribosomal Database Project (RDP) Classifier with confidence set at 0.5 (Porras-Alfaro et al. 2014), implemented in

QIIME 1.9 (Caporaso et al. 2010). The RDP Classifier was trained with the UNITE 7 species hypothesis dynamic clustering dataset (released 2 March 2015; <https://unite.ut.ee/repository.php>; Kõljalg, Nilsson and Abarenkov 2013), supplemented with additional ITS sequences from non-fungal eukaryotic lineages obtained from the NCBI nucleotide database (Accession numbers: JF444765.1, JN853795.1, KF977223.1, AY398500.1, AY455777.1, GU097876.1, AY070244.1, HQ156450.1, JF742525.1, KC594036.1, AF317109.1, AY368576.1, AF401150.1, AY346506.1, AY570231.1, AY836783.1, FJ572393.1, AY396437.1, JF801558.1, KPU48597.1; <http://ncbi.nlm.nih.gov>). OTUs from non-fungal lineages and those that the RDP Classifier could not assign to a lineage were then filtered from the dataset. OTUs whose taxonomy was resolved only to fungal class, or higher, were subjected to BLASTn searches in the NCBI nucleotide database. These OTUs were retained only if BLASTn hits were of clear fungal origin and had an E-value $\leq 1 \times 10^{-20}$. OTUs represented by less than 10 sequences were removed to limit sources of sequencing error. OTUs were tentatively assigned to functional groups using FUNGuild (Nguyen et al. 2015), complemented with our own literature searches. Functional assignments are based on the best available knowledge; however, we stress that these are putative. The final OTU matrix was rarefied to 20 000 sequences per sample.

Chemical and physical characteristics of pore water and peat

We measured a suite of abiotic characteristics to investigate potential correlations with fungal community structure. Pore water was collected on 22 September 2011 from piezometers covered on their ends with 37 μm nylon mesh and installed at 20 and 40 cm depths. Samples were filtered (0.45 μm) and acidified with hydrochloric acid. DOC and total dissolved nitrogen (TDN) concentrations were measured using a Shimadzu TOC-V Combustion Analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Three optical properties indicative of DOC composition were also calculated. First, specific ultraviolet absorbance (SUVA_{254}) was calculated by dividing UV absorbance at $\lambda = 254$ nm by total DOC concentration. The SUVA_{254} index should increase linearly with DOC aromaticity (Weishaar et al. 2003). The second property, E2:E3 (UV absorbance ratio of $\lambda = 254$ nm to $\lambda = 365$ nm), decreases as molecular size of dissolved organic matter increases (De Haan and De Boer 1987). The third optical property, E4:E6 (UV absorbance ratio at $\lambda = 465$ nm to $\lambda = 665$ nm) increases with DOC aromaticity and is inversely related to DOC humification (lower values = more decomposed; Zhang and He 2015). Total phenolics were quantified using Hach (Loveland, CO, USA) reagents scaled to a microplate (Sinsabaugh, Reynolds and Long 2000), at 700 nm absorbance on a SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, CA). Ammonium was determined spectrophotometrically using Hach salicylate and cyanurate reagents, also scaled to a microplate. Temperature was continuously recorded in each mesocosm (see Potvin et al. 2015). We used the average temperature over 1 month (August 15 to September 15), from probes at 20 and 40 cm depths. pH was measured (all but three samples) on fresh peat collected during microbial coring using a peat slurry (1 g peat: 30 mL deionized water), with a Denver Instrument Model 220 pH meter (Bohemia, New York). The von Post score, an ordinal index of peat decomposition (see Rydin and Jeglum 2013), was measured on peat from both sampling depths collected in May 2011 (prior to initiation of experimental treatments).

Statistical analyses

A suite of analyses were used to address hypotheses 1 to 3, focused on understanding how depth in the peat profile, PFG and WT influence fungi. First, linear mixed models were run with the following response variables: ITS1 gene abundance, OTU richness (S), Pielou's OTU evenness (J') and the relative abundance and richness of the three most abundant functional groups (saprotrophs, ErMF, root endophytes). Additionally, we examined the relative abundance of the three most common putative ErMF lineages: *Rhizoscyphus ericae* (= *Pezoloma ericae*), *Sebacinales* group B (= *Serindipitaceae* spp.) and *Oidiodendron maius*. Relative abundances were calculated as the total number of sequences representing a specific taxon or functional group divided by the total number of sequences in a sample (20 000). Linear mixed models included PFG (Sedge, Ericaceae, Unmanipulated), WT (High, Low), sampling depth (10–20 cm, 30–40 cm), all two and three-way interactions, and block as fixed factors. Individual mesocosm bin was included as a random effect. Variables were log or square root transformed when necessary. Models were fit in R 3.0.2 (R Core Team, 2013) with the package *lme4* (Bates et al. 2014), fixed effects were tested with the *lmerTest* package using the Kenward-Roger approximation and post hoc tests, when appropriate, were run with the *lsmeans* and *multcompView* packages (Graves et al. 2012; Lenth and Hervé 2015).

To test responses of fungal composition, relative abundance matrices of fungal OTUs and orders were analyzed using distance-based permutation MANOVA (PERMANOVA) and non-metric multidimensional scaling (NMDS), with Bray-Curtis dissimilarity. PERMANOVA models included the same factors as described above for linear mixed models, including individual mesocosm bin as a random effect. Type III sums of squares were used for PERMANOVA, with null distributions created by permuting residuals from partial models lacking the factor being tested (Anderson, Gorley and Clarke 2008). Prior to PERMANOVA and NMDS, matrices were fourth root transformed to down-weight the influence of the most abundant taxa (Clarke and Gorley 2006). The variance in community composition explained by each NMDS axis was estimated by calculating the coefficient of determination (R^2) between the original Bray-Curtis matrix and the Euclidean distances between communities on an ordination axis (McCune and Grace 2002). Indicator 'species' analysis was run to understand which OTUs and orders were driving the strongest patterns in the dataset, and a chi-squared test was used to test whether the functional groups of indicator OTUs shifted between sampling depths. PERMANOVA was also conducted on the OTU matrix after transformation to presence-absence, and the square root of the variance component for the depth effect was used to estimate the average percentage change in OTU membership between communities from one sampling depth to the other (i.e. OTU turnover between depths; Anderson, Gorley and Clarke 2008). PERMANOVA was conducted in Primer 6.1.15 with PERMANOVA+ 1.0.5 (PRIMER-E, Plymouth, UK). NMDS and indicator species analysis were run in R 3.0.2 with the packages *vegan* (Oksanen et al. 2013) and *indicspecies* (De Cáceres and Legendre 2009), respectively.

To further understand the effects of PFG, WT and depth in the peat profile, we examined whether shifts in relative abundances were mirrored by similar shifts in qPCR-adjusted abundances for the dominant functional groups (ErMF, root endophytes, saprotrophs). This adjustment was accomplished by multiplying a functional group's relative abundance (the proportion of sequences out of 20 000) by a sample's total fungal ITS1 gene abundance (ITS1 gene copies per gram dry peat). This conversion

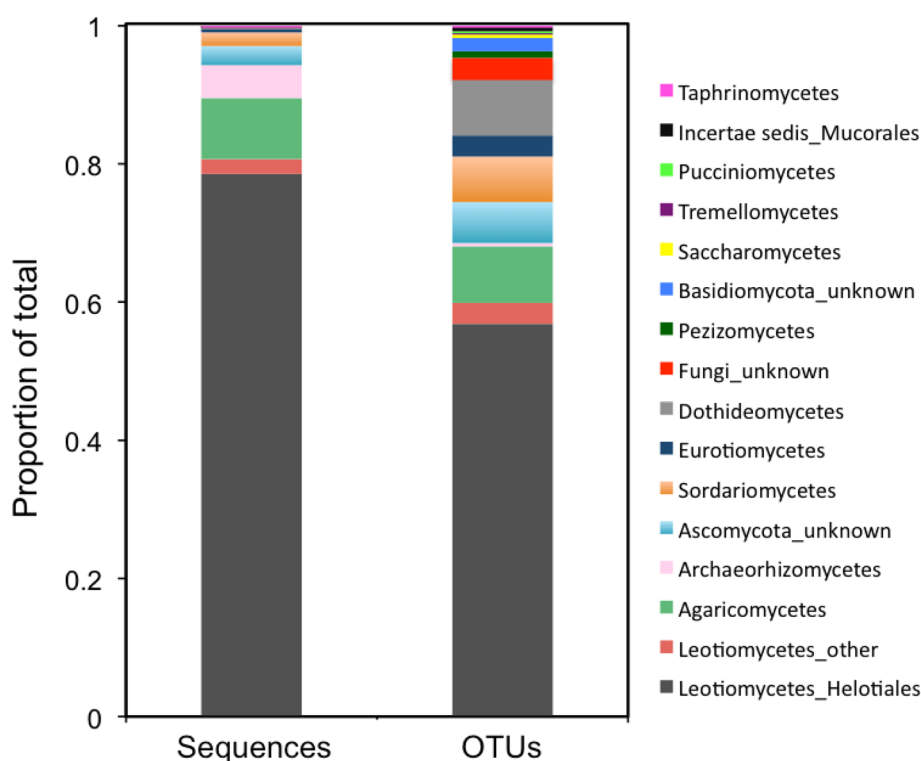


Figure 1. Composition by relative abundance and number of OTUs, of the rarefied sequence matrix. OTUs are grouped by class, unless otherwise noted. Graphs are ordered from bottom to top by decreasing number of total sequences per class.

generated a qPCR-adjusted abundance that should semiquantitatively reflect variation in a functional group's total abundance among samples. We recognize that artifacts may arise from biases associated with sequencing, and the use of ITS2 sequence data in conjunction with qPCR data generated using ITS1; however, we believe that this metric is informative because it adjusts for the huge decline in fungal abundance with depth. qPCR-adjusted data were tested with the linear mixed model approach described above.

The final set of analyses tested hypothesis 4, that fungal communities covary with pore water and peat characteristics. To understand the sources of variation in abiotic variables, their responses to sampling depth, PFG and WT were examined using the linear mixed model approach as described above for fungal community variables. However, the effect of depth on von Post humification was tested with a paired t-test (paired within mesocosm), with *P*-values obtained through permutation using the *broman* package (Broman and Broman 2014) in R. Vectors for each abiotic variable were then fit to NMDS ordinations using the 'envfit' function in the R package *vegan*. Because pore water variables primarily responded to peat depth (see Results section), we focused these analyses on understanding covariation between abiotic variables and the fungal community across the peat depth gradient.

RESULTS

The fungal community

A diverse community was recovered through sequencing. The dataset contained a total of 5 205 263 sequences (22 697–190 244 per sample) and 1489 OTUs, after clustering and chimera filtering but prior to further OTU filtering. The RDP classifier

categorized the majority of these remaining OTUs as fungal; however, upon manual checking some OTUs were unclassifiable or matched non-fungal lineages. Furthermore, the RDP classifier identified some OTUs as fungal, but did not provide taxonomy below the kingdom or phylum; nearly all of these OTUs were unclassifiable through BLASTn or strongly matched non-fungal lineages. After removing OTUs with uncertain identities and those represented by less than 10 sequences, the dataset included 4977 065 fungal sequences (20 970–182 143 sequences per sample; Fig. S2, Supporting Information). The final dataset contained 630 OTUs (56–325 OTUs per sample; Fig. S2, Supporting Information), with OTU reference sequences being 160 bp on average (range = 100–214 bp). Rarefaction to 20 000 sequences per sample reduced the number of OTUs to 623 (50–226 OTUs per sample). The OTUs represented three phyla, at least 31 orders from 12 classes, and were dominated by the Ascomycota order Helotiales (Table S1, Supporting Information; Fig. 1).

The fungal community and depth in the peat profile

In support of hypothesis 1, there was a large shift in the fungal community with increasing depth in the peat profile. Fungal ITS1 gene abundance was 4-fold greater at the 10–20 cm than the 30–40 cm depth, and OTU evenness increased slightly with depth (Table 1; Fig. 2a and c). However, there was no evidence of an OTU richness response to depth (Table 1; Fig. 2b).

Composition changed with sampling depth, at both the order and OTU level (Table 1; Fig. 3). Furthermore, the identity of OTUs occurring in the community changed by an average of ~21% between sampling depths (i.e. there was a turnover in ~21% of the community's OTUs from one depth to the other, derived from the square root of the variance component for the depth effect from the presence-absence matrix). Indicator species analysis

Table 1. Mixed model results for the effect of PFG, depth to WT and depth in 1 the peat profile (depth) on fungal community variables.^{a,b,c,d}

Response variable ^b	PFG ($F_{2,15}$ P)		WT ($F_{1,15}$ P)		Depth ($F_{1,18}$ P)		PFG × WT ($F_{2,15}$ P)		PFG × depth ($F_{2,18}$ P)		WT × depth ($F_{1,18}$ P)		PFG × WT × depth ($F_{2,18}$ P)	
ITS1 gene abundance	2.13	0.154	0.74	0.404	41.14	<0.001	0.21	0.815	0.93	0.413	2.54	0.129	0.79	0.468
Rarefied OTU richness	2.44	0.121	1.48	0.243	0.03	0.855	0.28	0.760	0.43	0.658	0.05	0.823	0.23	0.797
Pielou's OTU evenness	1.21	0.325	3.34	0.087	14.26	0.001	0.46	0.641	0.33	0.724	0.00	0.901	0.78	0.475
OTU composition	0.96	0.554	0.79	0.782	13.25	<0.001	0.86	0.731	0.76	0.811	0.89	0.552	0.91	0.585
OTU composition (presence/absence)	1.01	0.452	0.79	0.744	11.33	<0.001	0.89	0.686	0.85	0.689	0.95	0.502	0.92	0.570
Order composition	1.70	0.035	0.83	0.608	10.56	<0.001	0.57	0.931	0.69	0.773	1.15	0.326	0.84	0.619
Ericoid mycorrhizal fungi														
Relative abundance	1.80	0.199	0.03	0.867	14.11	0.001	0.00	0.998	1.09	0.356	0.04	0.843	2.68	0.096
qPCR-adjusted abundance	2.09	0.158	0.43	0.524	40.22	<0.001	0.03	0.969	1.46	0.258	0.70	0.413	1.23	0.316
OTU richness	0.26	0.773	0.47	0.502	9.05	0.008	0.22	0.803	0.73	0.494	0.58	0.456	0.75	0.485
Root endophytes														
Relative abundance	1.42	0.271	0.30	0.594	102.58	<0.001	0.53	0.598	1.49	0.251	4.72	0.043	2.90	0.081
qPCR-adjusted abundance	0.48	0.625	0.01	0.917	2.94	0.104	2.05	0.163	0.14	0.871	0.03	0.861	0.23	0.796
OTU richness	4.22	0.035	1.34	0.265	39.11	<0.001	0.137	0.873	1.00	0.387	0.01	0.906	2.29	0.130
Total saprotrophs														
Relative abundance	1.08	0.366	1.21	0.289	35.12	<0.001	0.75	0.488	0.12	0.901	0.34	0.565	0.80	0.465
qPCR-adjusted abundance	0.01	0.986	4.71	0.046	3.77	0.068	2.00	0.170	0.48	0.629	3.79	0.067	2.52	0.109
OTU richness	2.94	0.083	2.40	0.142	15.37	0.001	2.12	0.154	0.31	0.739	0.05	0.820	0.22	0.808
<i>Rhizoscyphus ericae</i>														
Relative abundance	0.22	0.804	0.00	0.959	40.77	<0.001	0.51	0.608	1.32	0.291	0.50	0.487	1.49	0.251
qPCR-adjusted abundance	1.01	0.389	0.11	0.749	65.96	<0.001	0.15	0.862	1.73	0.205	0.06	0.815	1.46	0.259
Sebacinales Group B														
Relative abundance	3.10	0.075	0.01	0.926	4.50	0.050	0.24	0.788	0.23	0.789	0.24	0.630	0.36	0.702
qPCR-adjusted abundance	3.85	0.045	0.10	0.753	20.95	<0.001	0.13	0.881	0.69	0.515	1.22	0.284	0.21	0.816
<i>Oidiodendron maius</i>														
Relative abundance	1.72	0.212	0.69	0.419	14.75	0.001	0.19	0.825	1.50	0.249	2.68	0.119	0.70	0.510
qPCR-adjusted abundance	4.33	0.033	0.06	0.805	23.91	<0.001	0.83	0.453	1.64	0.222	2.14	0.161	0.26	0.771

^aModels included individual mesocosm (random effect) and block (fixed effect); no hypothesis test was applied to these factors.^bF for univariate variables are F-ratios for mixed linear models, and F for composition are pseudo-F-ratios from PERMANOVA.^cOTU = operational taxonomic unit.^dBold indicates $0.1 > P > 0.05$, and bold italics indicate $P \leq 0.05$. Greater than 16% of the tests are significant at $P \leq 0.05$, which is more than are expected by chance. Interpretation of $0.1 > P > 0.05$ should be treated with caution.

identified a suite of indicators for each peat depth (Table S2, Supporting Information), and the functional group to which indicator OTUs tended to belong differed between depths ($X^2 = 31.21$, $P < 0.001$). Indicator OTUs of the 10–20 cm depth were typically ErMF, whereas indicators of the 30–40 cm depth were primarily saprotrophs and root endophytes (Table S2; Fig. 3a). At the order level, the Rhytismatales, Archaeorhizomycetales, Sebaciales and Xylariales were identified as indicators of the 10–20 cm depth, while the Polyporales and Coniochaetales were indicators of the 30–40 cm depth (Table S2; Fig. 3b).

The dominant fungal functional groups were also influenced by depth in the peat profile. ErMF relative abundance decreased more than one third, and OTU richness decreased by approximately one fourth, from the 10–20 to 30–40 cm depths (Table 1; Fig. 4a and c). In contrast, saprotroph relative abundance was more than 5-fold greater at the 30–40 cm than 10–20 cm depth, and OTU richness increased by one-third from the upper to lower depth (Table 1; Fig. 4d and f). The relative abundance of

root endophytes increased 6-fold, and OTU richness nearly doubled, from the 10–20 to 30–40 cm depth (Table 1; Fig. 4g and i). However, root endophyte relative abundance exhibited a complex three-way interaction with other factors (see details below).

qPCR-adjusted abundances provided a different view of functional group responses to depth in the peat profile. After qPCR adjustment, ErMF still decreased with increasing depth, and at the 30–40 cm depth were only one sixth of their value at the 10–20 cm depth (Table 1; Fig. 4b). However, the depth effect on root endophytes lost statistical significance after qPCR adjustment (Table 1; Fig. 4e). Although depth remained a marginally significant effect on saprotrophs after qPCR adjustment, its effect was largely obscured by its interaction with WT (Table 1; Fig. 4h).

Each of the three putative ErMF lineages examined individually (*Rhizoscyphus ericae*, *Oidiodendron maius*, Sebaciales Group B) decreased sharply with increasing depth (Table 1; Fig. 5). This decrease was observed in relative and qPCR-adjusted abundances (Fig. 5).

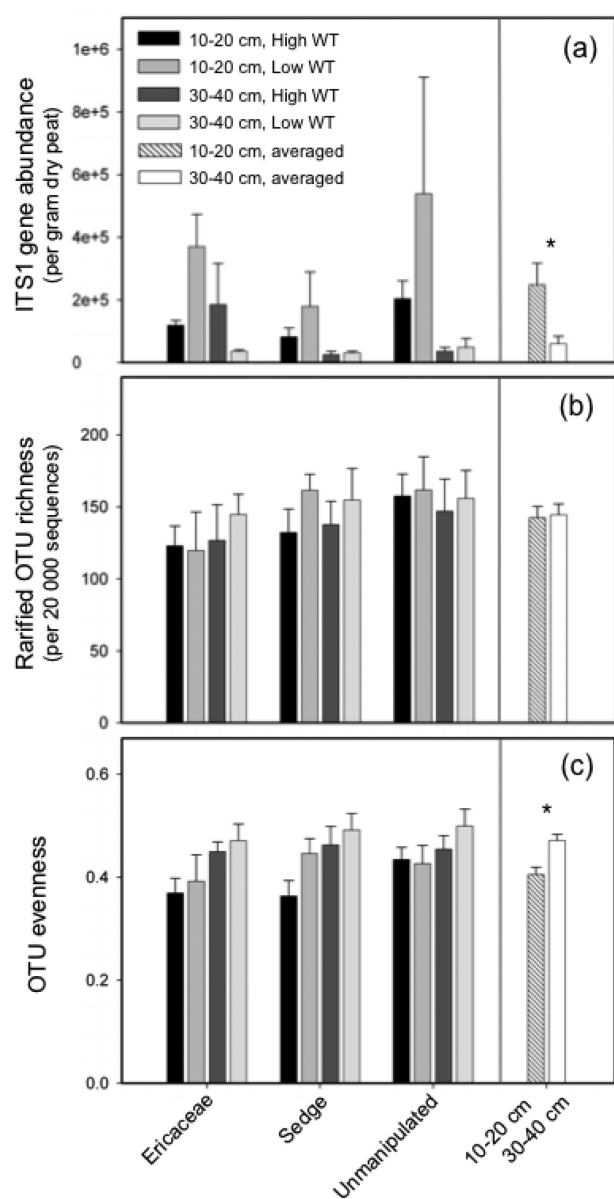


Figure 2. Total ITS1 gene abundance from qPCR (a), as well as OTU richness (b) and evenness (c), for each factor-level combination and averaged by depth. Bars are means ± 1 standard error of the raw data. Asterisk indicates a significant ($\alpha \leq 0.05$) main effect of sampling depth.

Fungal community responses to PFG and WT

Although PFG and WT effects were less pronounced than those of sampling depth, there was modest support for hypotheses 2 and 3. OTU evenness showed a marginal response to WT, being slightly greater in the low WT treatment within most PFG by depth factor levels (Table 1; Fig. 2c). However, neither ITS1 gene abundance nor OTU richness showed clear evidence of a response to PFG or WT (Table 1; Fig. 2a and b).

Fungal composition responded to PFG at the order level but not at the OTU level, and showed no evidence of a response to WT (Table 1; Fig. 3). At the 10–20 cm depth, ordination (Fig. 3b) coupled with post hoc PERMANOVA suggested that the composition of orders in mesocosms containing ericaceous shrubs was distinct from the Sedge treatment (Unmanipulated vs

Ericaceae: $P = 0.342$; Unmanipulated vs Sedge: $P = 0.039$; Ericaceae vs Sedge: $P = 0.062$). This PFG effect was not evident at 30–40 cm depth (Sedge vs Ericaceae: $P = 0.530$; Sedge vs Unmanipulated: $P = 0.945$; Ericaceae vs Unmanipulated: $P = 0.326$). Ordination also revealed that the Sedge treatment at the 10–20 cm depth was more similar to all treatment groups at the 30–40 cm depth than were the 10–20 cm Unmanipulated and Ericaceae treatments (Fig. 3b).

In some cases, responses to PFG and WT were exhibited by fungal functional groups. Root endophyte relative abundance exhibited a WT $\times$ depth interaction, although post hoc analyses revealed a complicated WT $\times$ depth response that was specific to each PFG treatment (Table 1 and Table S3, Supporting Information; Supplementary Material; Fig. 4d). Root endophyte OTU richness responded to PFG, where it was lowest in the Ericaceae relative to other treatments at both depths (Table 1 and Table S3, Supplementary Material; Fig. 4f). qPCR-adjusted saprotroph abundance responded significantly to WT (Table 1; Fig. 4h), being greater in the low compared to the high WT treatment at the 10–20 cm depth (Table S3, Supplementary Material). There were also several cases with marginally significant P -values that suggest incipient WT and PFG effects (e.g. three-way interactions for ErMF and root endophyte relative abundance; Table 1).

Some individual ErMF lineages also responded to PFG. While *Rhizoscyphus ericae* did not respond significantly to experimental manipulations, abundance of Sebaciniales Group B responded marginally to PFG (Table 1; Fig. 5a and c), and qPCR-adjusted abundances of both *Oidiodendron maius* and Sebaciniales Group B were affected by PFG. Specifically, the Unmanipulated and Ericaceae PFG treatments were generally higher than Sedge in these taxa, driven primarily by a PFG effect in the 10–20 cm depth only (Table 1 and Table S3, Supplementary Material; Fig. 5d and f).

Fungal community relationships with abiotic variables of peat and pore water

Consistent with hypothesis 4, some abiotic peat and pore water variables covaried with the fungal community (Table S4, Supplementary Material; Fig. 3). However, the abiotic variables were primarily influenced by depth in the peat profile; inconsistent with hypothesis 4, only one variable (pore water pH) exhibited responses to WT and PFG manipulation and these were very small in magnitude (Table 2 and S4, Supplementary Material). Compared to the 10–20 cm depth, the 30–40 cm depth had higher DOC, TDN, E4:E6 and Von Post values, but had lower temperature, E2:E3 and SUVA₂₅₄. The vectors with the strongest relationships in OTU- and order-level ordinations were von Post humification, temperature, and the E2:E3 and E4:E6 organic matter features (Table S5, Supplementary Material; Fig. 3). Von Post humification increased as composition shifted along NMDS axis 1 from the shallow to deeper depth, and this axis explained the majority of variation in the original Bray-Curtis distance matrices for both ordinations (Table S5, Supplementary Material; Fig. 3). In contrast, temperature and E2:E3 vectors increased from the deeper to shallower depth, although these variables were less collinear with NMDS axis 1 in the OTU-level ordination than was von Post humification. Many of the other pore water variables also exhibited significant relationships with fungal OTU composition, and there was a clear gradient in the community along which TDN, phenolics, NH_4^+ and DOC decreased, and SUVA₂₅₄ and E2:E3 increased.

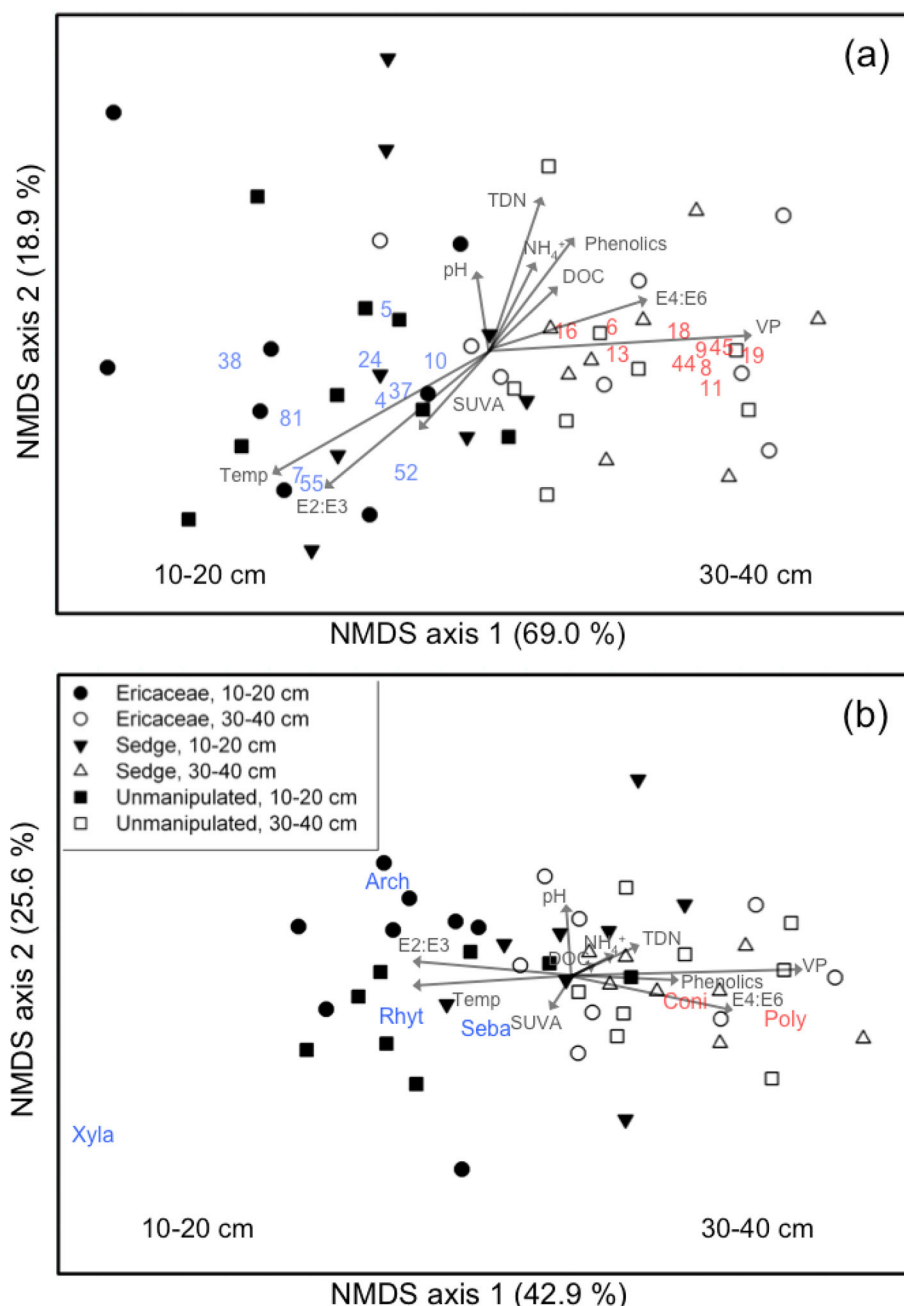


Figure 3. NMDS ordinations of fungal OTU (a) and order (b) composition. WT was not a significant factor and is omitted for clarity of presentation. Arrows represent vectors of variables with their lengths scaled to their relative magnitude, although vector lengths cannot be directly compared between the two ordinations (TDN = total dissolved nitrogen, DOC = dissolved organic carbon, Temp. = temperature, VP = von Post score). The locations of individual taxa with the highest indicator values for each depth (red = 10–20 cm indicators, blue = 30–40 cm indicators) are plotted by their OTU code numbers or the first four letters of their order (Arch = Archaeorhizomycetales, Coni = Coniochaetales, Polyporales = Poly, Rhyt = Rhytismatales, Seba = Sebaciniales, Xyla = Xylariales; only significant indicator orders that contained ≥ 3 OTUs are shown). See Tables S1 and S2 for OTU taxonomy and indicator species analysis results.

DISCUSSION

Fungal community stratification with depth in the peat profile

In support of hypothesis 1, depth in the peat profile had the strongest effect on fungi. Depth stratification of fungal communities has been documented by a number of studies in upland and peat soils (e.g. Artzt et al. 2007; Taylor et al. 2014). For example, in agreement with our findings, sharp decreases in total

fungal abundance within the upper 40 cm of peat were recently observed in a bog and poor fen (Lin et al. 2014). Such drops in fungal abundance likely reflect the intolerance of many fungi to anoxic conditions below the WT (Kavanagh 2011), combined with declining root subsidy to symbiotic fungi with depth.

As predicted, the fungal community shifted from ErMF to saprotroph dominance with increasing depth in the peat profile. This result was supported by relative abundance and OTU richness of functional groups, as well as indicator species analysis.

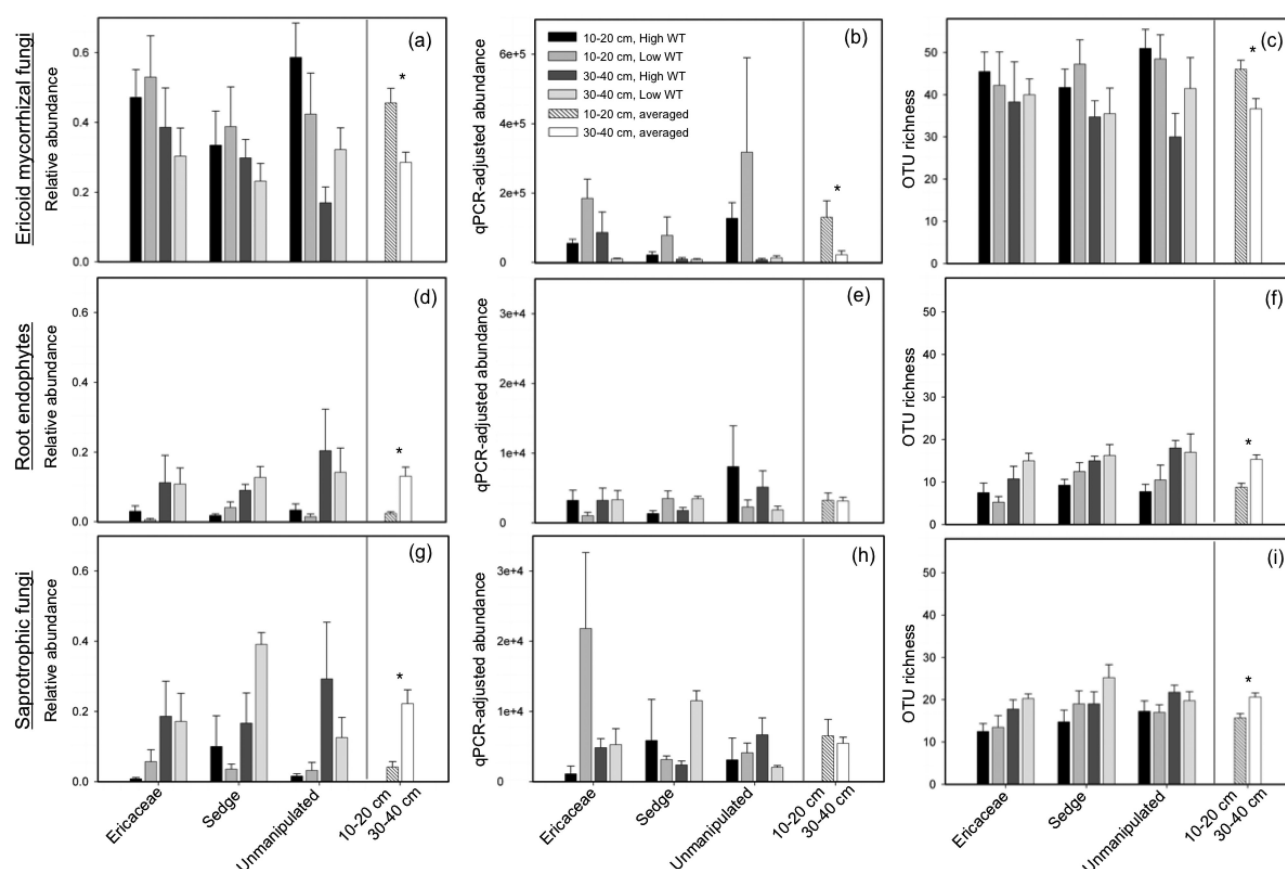


Figure 4. Relative abundance, qPCR-adjusted abundance and richness of ErMF (a–c), root endophytes (d–f) and saprotrophic fungi (g–i) for each factor-level combination and averaged by depth. Note the variation in y-axis scales. Bars are means ± 1 standard error. Asterisk indicates a significant ($\alpha \leq 0.05$) main effect of sampling depth; see Table S3 for pairwise post hoc tests between specific factor-level combinations.

Most indicators of the upper depth were putative ErMF OTUs, while those of the lower depth included many saprotrophs, as well as endophytes of unclear function. In fact, the only putative ErMF indicators of the deeper depth were classified as *Rhizoscyphus* sp. These OTUs are likely related to the confirmed ErMF *Rhizoscyphus ericae* as well as potentially non-ErMF fungi in the greater *R. ericae* aggregate; because their function has not been directly characterized, it is possible that they may not be ErMF. Roots at 30–40 cm depth are below the growing season typical WT minimum, the limit to active ericaceous roots (Wallén 1987; Moore et al. 2002). This suggests that the shift from ErMF to saprotroph dominance with increasing depth was also driven by aging and senescence of submerged ericaceous roots buried by accumulating peat. Importantly, ErMF (as a whole and the three lineages examined individually) still decreased with depth after qPCR adjustment while the depth effect on saprotrophs and endophytes diminished. This highlights the primary role of ErMF in driving the shift with depth, and indicates that saprotrophs and endophytes do not necessarily prefer the deeper depth.

Vertical stratification of communities may also be influenced by mycorrhizal fungi actively excluding saprotrophs (Gadgil and Gadgil 1971; Lindahl et al. 2007; Fernandez and Kennedy 2016). Extensive extracellular enzymatic capabilities and access to host-derived carbon likely make ErMF formidable competitors with saprotrophs for nutrients in recalcitrant organic matter. Most filamentous saprotrophic fungi likely prefer oxic conditions and should therefore have the greatest abundances in surface peat. A lack of saprotroph preference for the upper depth

may indicate that, despite favorable redox and litter quality, saprotrophs were inhibited by ErMF in the upper peat. In upland forests, it is hypothesized that mycorrhizal inhibition of saprotrophs creates depth stratification, where saprotrophs colonize litter at the soil surface and mycorrhizal fungi colonize more humified organic matter in subsurface horizons (Lindahl et al. 2007; Fernandez and Kennedy 2016). The vertical distribution of functional group dominance in our peat system was the inverse of this pattern, which likely reflects fundamental differences between the systems: deeper peat is water saturated and the entire soil profile is composed of organic matter. We did not sample the upper 0–10 cm of peat because most of it is represented by living moss, and so the 10–20 cm depth includes what may be considered new litter inputs; this is reflected by low von Post scores.

Consistent with the hypothesis of suppression of saprotrophs in surface peat, our results indicate that taxa capable of decomposing recalcitrant plant material are relatively more important deeper in the peat. As an order and as individual OTUs, Polyporales were indicators of the deeper depth. Polyporales largely specialize in wood decomposition, and the individual Polyporales OTUs found as indicators are placed in genera (*Phanerochaete*, *Hypochnicium*) that have white rot capabilities (i.e. the enzymatic potential for complete mineralization of lignocellulose; Aust 1995). In a *Sphagnum* peat system, these fungi may perhaps utilize dead stems and larger roots of Ericaceae. Certain other non-polypore white rot fungi were also indicators of deeper peat, including *Hypholoma*, *Gymnopilus* and *Pleurotus*. In contrast, only one white rot fungus (*Ganoderma*) was an

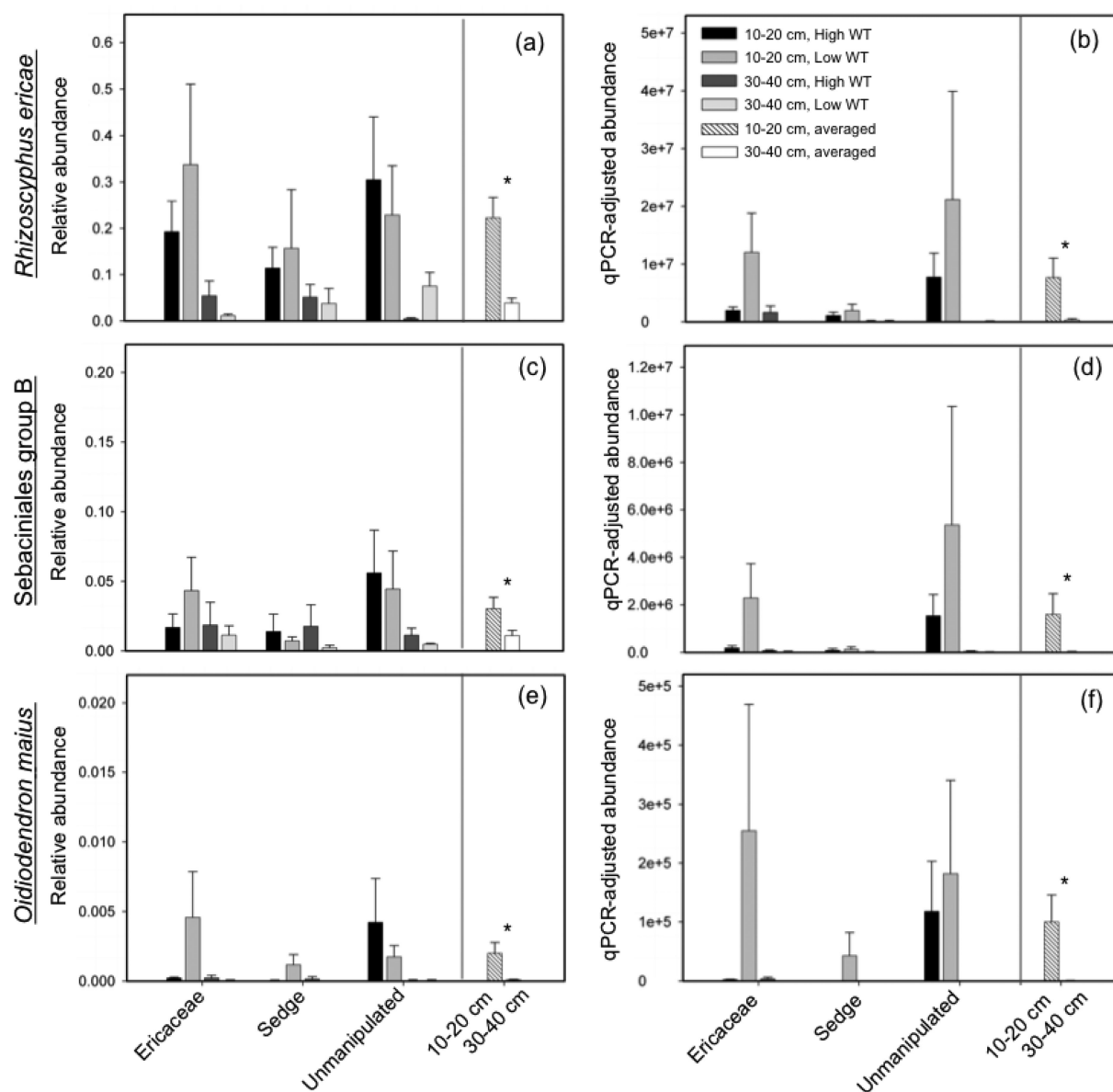


Figure 5. Relative and qPCR-adjusted abundances of the most common ericoid mycorrhizal fungal lineages in our study: *Rhizocyphus ericae* (a), Sebaciniales group B (b) and *Oidiodendron maius* (c), for each factor-level combination and pooled by depth. Note the variation in y-axis scales. Bars are means ± 1 standard error. Asterisk indicates a significant ($\alpha \leq 0.05$) main effect of sampling depth; see Table S3 for pairwise post hoc tests between specific factor-level combinations.

indicator of the shallower depth. The order Coniochaetales was also an indicator of the deeper depth. This order was primarily represented by the genus *Lecythophora*, which was assigned as a root endophyte but members of this genus can also function as soft rot fungi (e.g. Bugos, Sutherland and Adler 1988). Of the four orders that were indicators of surface peat, none is a white rot lineage: one contains fungi that are most likely ErMF in our system (Sebaciniales), one contains members with unknown functions (although some may be root associated; Archaeorhizomycetales) and two contain pathogens, endophytes and non-white rot saprotrophs (Xylariales and Rhytismatales). In fact, the 10–20 cm indicator OTUs found in these two orders are related to plant pathogens: *Physalospora vaccinii* (Xylariales) attacks cranberry fruit (Polashock et al. 2009) and *Colpoma* (Rhytismatales) can infect Ericaceae wood (Johnston 1991).

Although the results suggest that vertical stratification in the fungal community is due to environmental preferences, life histories and interactions among OTUs of active fungi, differential patterns of dormancy or preservation of DNA from dead fungal tissues may also influence vertical stratification. For example, extracellular relic DNA in soil can affect the picture of community structure revealed through environmental sequencing (Carini et al. 2016). However, the sharp decrease in fungal abundance with depth revealed through qPCR suggests that much of the DNA of fungi active in upper peat degrades as it becomes part of the deeper, more humified peat. Furthermore, results indicate that depth stratification in peat is strongly shaped by the presence of ErMF in the active rooting zone of host plants dependent on these fungi, lending additional support for the role of active fungi driving the patterns of depth stratification. The future

Table 2. Pore water and other peat variables (mean $\pm$ 1SD).

Response variable ^a	Depth (cm)	Ericaceae high WT	Ericaceae low WT	Sedge high WT	Sedge low WT	Unmanipulated high WT	Unmanipulated low WT	Overall mean	Statistically significant factors ^b
DOC (mg L ⁻¹)	20	118.2 $\pm$ 29.4	112.0 $\pm$ 25.1	86.4 $\pm$ 11.8	98.7 $\pm$ 25.9	112.7 $\pm$ 28.3	110.6 $\pm$ 16.3	106.4 $\pm$ 23.7	D
	40	123.3 $\pm$ 24.9	119.8 $\pm$ 25.7	91.4 $\pm$ 11.5	107.9 $\pm$ 27.4	119.9 $\pm$ 31.3	115.2 $\pm$ 14.2	112.9 $\pm$ 23.6	
Total phenolics (mg L ⁻¹)	20	18.22 $\pm$ 2.77	20.39 $\pm$ 4.57	18.11 $\pm$ 2.69	19.44 $\pm$ 4.25	20.68 $\pm$ 3.45	19.74 $\pm$ 3.18	19.43 $\pm$ 3.30	
	40	19.32 $\pm$ 2.10	21.47 $\pm$ 3.59	17.72 $\pm$ 3.01	20.78 $\pm$ 5.15	21.06 $\pm$ 3.51	19.40 $\pm$ 1.21	19.96 $\pm$ 3.23	
E2:E3	20	6.82 $\pm$ 0.68	6.47 $\pm$ 0.92	7.11 $\pm$ 0.57	6.91 $\pm$ 0.95	6.59 $\pm$ 0.88	6.33 $\pm$ 0.81	6.71 $\pm$ 0.77	D
	40	6.08 $\pm$ 0.75	5.75 $\pm$ 0.86	6.58 $\pm$ 0.42	6.20 $\pm$ 0.85	5.92 $\pm$ 0.93	5.89 $\pm$ 0.52	6.07 $\pm$ 0.71	
E4:E6	20	4.34 $\pm$ 0.50	4.05 $\pm$ 0.93	4.55 $\pm$ 0.43	4.54 $\pm$ 0.94	4.37 $\pm$ 0.94	4.20 $\pm$ 0.95	4.34 $\pm$ 0.74	D
	40	4.86 $\pm$ 0.66	5.57 $\pm$ 0.51	5.08 $\pm$ 0.69	5.40 $\pm$ 0.87	5.17 $\pm$ 0.78	5.16 $\pm$ 0.69	5.21 $\pm$ 0.67	
SUVA ₂₅₄	20	3.69 $\pm$ 0.87	4.27 $\pm$ 0.30	4.57 $\pm$ 0.47	4.62 $\pm$ 0.81	4.17 $\pm$ 0.70	4.18 $\pm$ 0.58	4.25 $\pm$ 0.66	D
	40	3.64 $\pm$ 0.71	3.95 $\pm$ 0.42	4.37 $\pm$ 0.29	4.20 $\pm$ 0.79	3.99 $\pm$ 0.66	3.97 $\pm$ 0.22	4.02 $\pm$ 0.54	
TDN (mg L ⁻¹)	20	3.37 $\pm$ 0.94	2.79 $\pm$ 0.68	2.57 $\pm$ 0.57	3.15 $\pm$ 1.28	2.67 $\pm$ 0.69	3.05 $\pm$ 0.57	2.93 $\pm$ 0.79	D
	40	3.53 $\pm$ 0.84	3.04 $\pm$ 0.78	2.58 $\pm$ 0.61	3.26 $\pm$ 1.19	3.01 $\pm$ 0.94	3.19 $\pm$ 0.62	3.10 $\pm$ 0.81	
NH ₄ ⁺ (mg L ⁻¹)	20	0.47 $\pm$ 0.35	0.25 $\pm$ 0.09	0.24 $\pm$ 0.18	0.41 $\pm$ 0.30	0.26 $\pm$ 0.19	0.40 $\pm$ 0.22	0.34 $\pm$ 0.23	
	40	0.53 $\pm$ 0.42	0.36 $\pm$ 0.16	0.20 $\pm$ 0.22	0.46 $\pm$ 0.43	0.25 $\pm$ 0.26	0.37 $\pm$ 0.17	0.36 $\pm$ 0.29	
pH	20	3.99 $\pm$ 0.09	3.79 $\pm$ 0.07	3.99 $\pm$ 0.16	3.99 $\pm$ 0.21	3.74 $\pm$ 0.09	3.89 $\pm$ 0.26	3.88 $\pm$ 0.17	D, PFG×D, PFG×WT×D
	40	3.81 $\pm$ 0.04	3.74 $\pm$ 0.11	3.87 $\pm$ 0.10	3.85 $\pm$ 0.13	3.81 $\pm$ 0.10	3.84 $\pm$ 0.17	3.82 $\pm$ 0.11	
Temperature °C	20	18.33 $\pm$ 2.25	17.86 $\pm$ 0.51	18.19 $\pm$ 1.06	18.13 $\pm$ 0.16	18.64 $\pm$ 0.66	17.96 $\pm$ 0.43	18.19 $\pm$ 0.99	D
	40	17.41 $\pm$ 1.46	17.41 $\pm$ 0.45	17.32 $\pm$ 0.34	17.35 $\pm$ 0.26	17.85 $\pm$ 0.82	17.40 $\pm$ 0.4	17.45 $\pm$ 0.69	
Von Post ^c	10–20							2.88 $\pm$ 0.95	D
	30–40							5.04 $\pm$ 1.00	

^aDOC = dissolved organic carbon, E2:E3 = ratio of absorption spectra at λ = 254 nm to λ = 365 nm, E4:E6 = ratio of absorption spectra at λ = 465 nm to λ = 665 nm, SUVA₂₅₄ = specific ultraviolet absorbance calculated as absorption spectra at λ = 254 nm divided by the DOC, TDN = total dissolved nitrogen.

^bResults of mixed model analyses or a permutation-based paired t-test (Von Post only), see Table S2, Supplementary Information. Bold indicates $0.1 > P > 0.05$, and bold italics indicate $P \leq 0.05$. D = depth in peat profile, PFG = plant functional group, WT = water table manipulation. An 'x' indicates interactions between factors.

^cThe Von Post humification index was quantified on samples taken prior to experimental manipulation, so its values are averages by depth only.

application of RNA sequencing (e.g. Lin et al. 2014) will shed further light on the active fungal lineages driving depth stratification in fungal community structure.

Rapid responses to PFG and WT manipulation

PFG and WT manipulation should provide evidence for the mechanisms causing depth stratification of fungal communities. If sedges homogenize the community, as we hypothesize, their presence should drive both depths of treatments in which they are present (Unmanipulated and Sedge) to be similar to each other and intermediate between the 10–20 cm and 30–40 cm depths in the treatment from which they were removed (Ericaceae). However, results (for *Oidiodendron maius*, Sebaciniales group B and order-level composition) show that PFG primarily influenced the upper depth, and communities in Sedge mesocosms at the 10–20 cm depth were intermediate between mesocosms with ericaceous shrubs at 10–20 cm depth (Ericaceae and Unmanipulated) and all communities at the 30–40 cm depth. This indicates that ericaceous roots and ErMF, which dominate the 10–20 cm depth, are stronger structuring agents for fungal communities than sedge roots present at both depths. This should facilitate depth stratification of fungal communities.

WT manipulation had the least effect on fungi, which is not surprising given the small depth difference of the initial WT treatment. Contrary to our hypotheses, the responses of ErMF and total fungal abundance to WT were too variable to be statistically significant. Instead, saprotrophs and root endophytes both responded to WT, where WT level tended to modulate the

effects of PFG or depth in the peat profile. Concerning root endophytes, their co-dominance in the deeper depth suggests they may not be entirely dependent on active host roots, perhaps acting saprotrophically on senescent roots and moss (Day and Currah 2011; Mandyam and Jumpponen 2015). Perhaps consistent with this interpretation, root endophyte relative abundance was primarily affected by WT at the 10–20 cm depth, where endophytes decreased with lower WT in treatments containing ericaceous shrubs (Ericaceae and Unmanipulated) and increased in the Sedge treatment. This could arise if reduced flooding stress on ericaceous roots favors ErMF over root endophytes. In Sedge mesocosms, lowered WTs might have favored endophyte colonization of living roots and/or saprotrophic utilization of dying residual shrub roots.

Many of the detectable rapid fungal responses to experimental manipulations were modest. Community inertia may slow the response of fungi to PFG manipulation due to survival of hyphae, dormant propagules and/or DNA in the absence of hosts, perhaps explaining why the Sedge treatment supported many ErMF OTUs. Facultative saprotrophy, as has been reported for some ErMF (e.g. *Oidiodendron maius*; Rice and Currah, 2006), may also mute the effects of PFG manipulation. Finally, misassignment of taxa to functional guilds, as discussed earlier for *Rhizoscyphus* sp., could blur the signal of community responses to PFG manipulation. The possibility of misassignment points to the tentative nature of functional group designation in amplicon sequencing datasets, highlighting the importance of efforts to characterize the natural history of a greater range of fungal species (Peay 2014).

Relationship of fungi with abiotic peat and pore water variables

Fungal community composition covaried with several properties of peat and pore water. This could have arisen from a causal link, with fungi affecting peat characteristics or vice versa, or correlation with another variable (e.g. presence of host roots or redox conditions associated with depth). Fungi associated with the 10–20 cm depth (e.g. ErMF) were living in less decomposed peat (lower von Post), with less degraded DOC that was of relatively lower molecular size (lower E4:E6, higher E2:E3), and had lower overall DOC and TDN concentrations, relative to fungi associated with the 30–40 cm depth (e.g. Polyporales). While the higher SUVA₂₅₄ observed in the shallower depth is at odds with the observed E2:E3 data, it is consistent with less-processed inputs from the breakdown of litter (lignin-like), which is supported by lower E4:E6 (Zhang and He 2015). Many of the differences between depths can be attributed to the 30–40 cm depth being composed of older peat. However, WT and PFG should have direct and indirect (via microbial community alteration) influences on the vertical stratification of abiotic peat and pore water variables; over time, experimental PFG and WT manipulation should outline how these factors promote such vertical stratification.

CONCLUSIONS

This study highlights the strong depth stratification of peatland fungal communities. The precipitous drop in total fungal abundance with increasing depth indicates that fungi thrive best in the oxic conditions near the surface. However, the shift in fungal composition with depth in the peat profile was driven by a strong decrease in ErMF that dominate the shallow oxic peat in the sphere of active host roots. The preference of ErMF for the upper peat may constrain saprotrophs and root endophytes to dominating communities in deeper peat, in low oxygen conditions that they may not prefer. Such patterns support the hypothesis that ErMF competitively suppress other fungi in surface peat. Furthermore, the rapid responses to PFG and WT manipulation highlight the importance of these factors in stratifying fungi by depth. Given the abundance of ErMF in surface peat, the likelihood that ErMF effectively compete with saprotrophs, and the potential for a lowered WT to increase ericaceous shrub abundance over time, climate-change mediated declines in WT should cause ErMF to become increasingly important players in peatland carbon cycling in the coming decades.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://femsec.org/) online.

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Conflict of interest. None declared.

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