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2 DR. LOUIS J LAMIT (Orcid ID : 0000-0002-0385-6010)

3 DR. JAY T. LENNON (Orcid ID : 0000-0003-3126-6111)

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

10 **dependent**

11

12 **Running title**: Peat microbial communities and global change

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14 Louis J. Lamit[1,*], Karl J. Romanowicz[1,*], Lynette R. Potvin[2,*], Jay T. Lennon[3], Susannah G.

15 Tringe[4], Rodney A. Chimner[1], Randall K. Kolka[5], Evan S. Kane[1,2], Erik A. Lilleskov[2]

16

17 [1]College of Forest Resources and Environmental Science, Michigan Technological University,

18 Houghton, MI 49931, USA.

19 [2]USDA Forest Service Northern Research Station, Houghton, MI 49931, USA.

20 [3]Department of Biology, Indiana University, Bloomington, IN 47405, USA.

21 [4]DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720,

22 USA.

23 [5]USDA Forest Service Northern Research Station, Grand Rapids, MN 55744, USA

24 [*] Current Addresses. Louis J. Lamit: Department of Biology, Syracuse University, Syracuse, NY

25 13244, USA, and Department of Environmental and Forest Biology, State University of New

26 York College of Environmental Science and Forestry, Syracuse, New York 13210, USA.

27 Karl J. Romanowicz: Department of Ecology and Evolutionary Biology, University of

28 Michigan, Ann Arbor, MI 48109, USA. Lynette R. Potvin: Isle Royale National Park,

29 Houghton, MI 49931, USA.

30 Corresponding author: LJ Lamit. 315-446-4368, ljlamit@syr.edu, ORCID Identifier 0000-0002-

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32 **Abstract -** Peatlands store one-third of Earth's soil carbon, the stability of which is uncertain due
33 to climate change-driven shifts in hydrology and vegetation, and consequent impacts on
34 microbial communities that mediate decomposition. Peatland carbon cycling varies over steep
35 physicochemical gradients characterizing vertical peat profiles. However, it is unclear how
36 drought-mediated changes in plant functional groups (PFGs) and water table (WT) levels affect
37 microbial communities at different depths. We combined a multi-year mesocosm experiment
38 with community sequencing across a 70 cm depth gradient, to test the hypotheses that vascular
39 PFGs (Ericaceae vs. sedges) and WT (high vs. low) structure peatland microbial communities in
40 depth-dependent ways. Several key results emerged. 1) Both fungal and prokaryote (bacteria and
41 archaea) community structure shifted with WT and PFG manipulation, but fungi were much
42 more sensitive to PFG whereas prokaryotes were much more sensitive to WT. 2) PFG effects
43 were largely driven by Ericaceae, although sedge effects were evident in specific cases (e.g.,
44 methanotrophs). 3) Treatment effects varied with depth: the influence of PFG was strongest in
45 shallow peat (0-10, 10-20 cm), whereas WT effects were strongest at the surface and middle
46 depths (0-10, 30-40 cm), and all treatment effects waned in the deepest peat (60-70 cm). Our
47 results underscore the depth-dependent and taxon-specific ways that plant communities and
48 hydrologic variability shape peatland microbial communities, pointing to the importance of
49 understanding how these factors integrate across soil profiles when examining peatland
50 responses to climate change.
51

52 **Keywords -** Ericaceae, microbial community, peatlands, plant functional group, soil depth, water
53 table
54

55 **Introduction**

56 Hydrology is the main driver of wetland ecosystem structure and function (Mitsch &
57 Gosselink, 2015), and climate change-driven alterations to hydrology are having extensive
58 impacts on earth's wetlands (Junk et al., 2013; Moomaw et al., 2018). Because wetlands are
59 globally important carbon storage reservoirs and methane sources, their responses to climate
60 change will likely feed back to further modulate climate (Bardgett, Freeman, & Ostle, 2008;
61 Davidson & Janssens, 2005; Zhang et al., 2017). This is a particularly important issue for

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62 carbon-accumulating wetlands (peatlands) which contain approximately one-third of Earth's soil
63 carbon, more than twice the carbon stored aboveground in Earth's tropical rain forests (Joosten
64 & Couwenberg, 2008). Drier conditions in peatlands can alter carbon cycles and expose carbon
65 formerly sequestered below the water table (WT) to aerobic microbial oxidation (Bragazza,
66 Parisod, Buttler, & Bardgett, 2013; Bridgham, Pastor, Dewey, Weltzin, & Updegraff, 2008;
67 Davidson & Janssens, 2005; Freeman, Ostle, & Kang, 2001; Kane et al., 2019).

68 In addition to direct effects on hydrology, climate change can also alter peatland plant
69 communities, which has extended consequences for carbon cycling (Bragazza et al., 2013;
70 Dieleman, Branfireun, McLaughlin, & Lindo, 2016; Jassey et al., 2018; Potvin, Kane, Chimner,
71 Kolka, & Lileskov, 2015). Bogs and poor fens of the northern hemisphere are dominated by
72 *Sphagnum* mosses whose highly recalcitrant tissues form the bulk of peat-building organic
73 matter (van Breeman, 1995; Rydin & Jeglum, 2013). Growing in the *Sphagnum* matrix are dwarf
74 shrubs in the Ericaceae and graminoids in the Cyperaceae (hereafter Sedges), two vascular plant
75 functional groups (PFGs) with distinctive chemical, morphological and carbon allocation traits
76 that influence ecosystem processes (Crow & Wieder, 2005; Dorrepaal, Cornelissen, Aerts,
77 Wallén, & van Logtestijn, 2005; Rydin & Jeglum, 2013; Ward et al. 2015). Ericaceae lack
78 aerenchyma but form adventitious roots as their stems are buried in accumulating peat (Rydin &
79 Jeglum, 2013). This allows Ericaceae to proliferate in the acrotelm, the frequently oxic, upper
80 part of peat soil profiles (Moore, Bubier, Frolking, Lafleur, & Roulet, 2002; Wallén, 1987). In
81 contrast, aerenchyma allows sedges to dominate wetter sites, and to extend active roots into the
82 catotelm, the deeper anoxic peat below the WT (Moore et al., 2002; Rydin & Jeglum, 2013).
83 Strong evidence supports a shift towards dominance by Ericaceae when peatlands become drier
84 (Bragazza et al., 2013; Breeuwer et al., 2009; Chimner, Pypker, Hribljan, Moore, & Waddington,
85 2017; Malhotra et al., 2020; Potvin et al., 2015; Weltzin, Bridgham, Pastor, Chen, & Harth,
86 2003), a pattern driven in part by the divergent root traits of Ericaceae and sedges.

87 The roots of PFGs interact with peatland microbial communities to further modulate
88 ecosystem processes. Ericaceae roots form mutualistic symbioses with ericoid mycorrhizal fungi
89 (ErMF) which have the enzymatic capacity to degrade some forms of complex organic matter to
90 access immobilized nutrients (Cairney & Burke, 1998; Martino et al., 2018; Read, Leake, &
91 Perez-Moreno, 2004). Ready access to host photosynthate should make ErMF taxa strong
92 competitors with free-living saprotrophs (Verbruggen, Pena, Fernandez, & Soong, 2017). The

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93 differential abilities of ErMF vs saprotrophic fungi to degrade organic matter, coupled with the
94 high phenolic content of ericaceous litter (Bragazza et al., 2013; Dorrepaal et al., 2005), have
95 consequences for decomposition rates and ecosystem carbon storage (Bragazza et al., 2013;
96 Orwin, Kirschbaum, St John, & Dickie, 2011; Ward et al., 2015; Verbruggen et al., 2017) that
97 potentially carryover to affect microbial taxa that utilize byproducts of decomposition. Although
98 both PFGs are colonized by endophytic fungi with poorly known functions, peatland sedges do
99 not typically host mycorrhizal fungi (Thormann, Currah, & Bayley, 1999; Weishampel &
100 Bedford, 2006). Therefore, some of the most important effects of sedges on microbial
101 communities are likely mediated through their influence on free-living taxa. Sedge roots can be
102 sources of labile carbon that fuel processes such as fermentation and methanogenesis, while
103 aerenchyma-enabled rhizosphere oxygenation may allow aerobic microorganisms to be active
104 around roots in water saturated conditions and promote unique biogeochemical processes at the
105 sharp oxygen concentration gradients associated with rhizospheres (Chanton et al., 2008; Lamers
106 et al., 2012; Rupp, Kane, Dieleman, Keller, & Turetsky, 2019). A number of studies provide
107 important experimental evidence that PFGs uniquely influence aspects of peatland microbial
108 communities or associated carbon cycling processes (e.g., Robroek et al., 2015; Rupp et al.,
109 2019; Ward et al., 2015). However, there remains a large gap in our understanding of who in the
110 microbial communities is affected, because tools that provide the resolution necessary to fully
111 characterize the composition of diverse fungal, bacterial and archaeal communities (e.g., high
112 throughput amplicon sequencing) have not been extensively applied.

113 Several key points highlight the importance of accounting for WT and sampling depth
114 when understanding PFG effects on peatland microbial communities. First, there is clear
115 evidence that microbial communities shift with changes in WT (Emsens et al., 2020; Jassey et
116 al., 2018; Urbanová & Barta, 2016), however concomitant shifts in vegetation can sometimes
117 make it difficult to fully decouple WT from PFG effects. Second, microbial communities can
118 change dramatically with increasing depth in peat profiles, in part a direct result of WTs
119 excluding obligate aerobes from living in anoxic conditions deep in peat profiles (Artz et al.,
120 2007; Asemaninejad, Thorn, & Lindo, 2017; Asemaninejad et al., 2019; Andersen, Chapman, &
121 Artz, 2013; Emsens et al., 2020; Kotiaho et al., 2013; Lamit et al., 2017; Lin et al., 2014). This is
122 one reason why the abundance of fungi, most of which prefer oxic conditions (Kavanagh, 2011),
123 drops dramatically with depth in peatlands (Golovchenko, Dobrovol’skaya, & Inisheva, 2002;

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124 Lamit et al. 2017; Lin et al. 2014). Third, microbial community depth stratification should also
125 be a product of the unique effects of plant roots along peat profiles, with different PFGs having
126 distinct depth effects based on their differences in rooting depth. Taken together, these points
127 emphasize the need for experimental decoupling of WT and PFG effects on peatland microbial
128 communities, within the context of depth.

129 We conducted a multi-year mesocosm experiment to examine how microbial
130 communities are shaped by seasonal drought and contrasting PFGs. We hypothesized, **H1)**
131 manipulation of PFGs will shift microbial community structure, with these effects being unique
132 to each PFG and strongly depth-dependent. We specifically predicted that Ericaceae removal
133 will have its strongest effect in the upper peat profile, while sedge removal should influence
134 communities along a greater length of the peat profile. Next, we hypothesized, **H2)** WT
135 manipulation will shift microbial community structure, with these effects also being strongly
136 depth-dependent. Although WT impacts are likely broad, the most distinct effects of WT should
137 occur at depths where the WT is the most dynamic and distinct between the treatments, and is
138 known to manifest a strong influence on peat and porewater chemistry (Kane et al., 2019; Lin et
139 al., 2014). Given that the depth-dependent effects of each PFG are in part a consequence of the
140 different ways that sedges and Ericaceae interact with oxic vs anoxic conditions, we further
141 hypothesized, **H3)** the responses of microbial communities to manipulation of one factor (PFG or
142 WT) will be dependent on the level of the other factor and these interactive effects will in turn be
143 dependent on depth in the peat profile. Understanding how climate change mediated changes in
144 WT and PFGs impact microorganisms along peat depth gradients is important because these are
145 some of the earth's most taxonomically and functionally diverse groups of organisms and
146 because their activities influence the most carbon-rich ecosystems on earth, peatlands.
147

148 **Materials and Methods**

149 *Experimental study system*

150 PEATcosm was a mesocosm experiment designed to test the influence of seasonal
151 drought and PFG on peatland ecosystems. Detailed descriptions of the experimental design, peat
152 characteristics, porewater chemistry and vegetation can be found in Kane et al. (2019), Lamit et
153 al. (2017), and Potvin et al. (2015). The experiment contained twenty-four ~1 m^3 peat monoliths
154 excavated from an oligotrophic acidic (pH = ~ 4) *Sphagnum* peatland in Minnesota, USA

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155 (N47.07278°, W92.73167°), in May 2010. The monoliths were installed in the Houghton
156 Mesocosm Facility, USDA Forest Service, Northern Research Station, Forestry Sciences
157 Laboratory in Houghton, Michigan (N47.11469°, W88.54787°). Monoliths were naturally
158 vegetated by a continuous layer of *Sphagnum* mosses (primarily *Sphagnum rubellum*, but some
159 *S. magellanicum and S. fuscum*), with *Polytrichum strictum* also present, and a vascular
160 community of Ericaceae (primarily *Chamaedaphne calyculata*, *Kalmia polifolia*, *Vaccinium*
161 *oxycoccus*) and sedges (*Carex oligosperma*, *Eriophorum vaginatum*). PFG manipulation was
162 initiated in June 2011 and included Ericaceae removal, sedge removal and unmanipulated
163 vegetation treatments (n = 8 per PFG treatment). PFG treatments were carried out by a
164 combination of gentle removal of target species' stems plus roots when avoidance of moss
165 damage was possible, followed by clipping all remaining aboveground tissues. PFG treatments
166 were subsequently maintained by clipping growth of excluded PFGs on a weekly basis, as
167 needed. WT manipulations were imposed by maintaining 12 mesocosms at average (high WT)
168 and 12 at summer drought (low WT) conditions (n = 4 replicates per WT x PFG treatment). WT
169 manipulation was carried out with rain-out shelters, artificial rainwater addition and controlled
170 drainage in the spring and after heavy rains at the acrotelm-catotelm boundary (~25 cm depth).
171 The depth separation of WT treatments was small in year one (2011), intermediate in year two
172 (2012), and the greatest in years three and four (2013, 2014) to simulate strong summer drought
173 (Fig S1).
174
175 *Peat sampling and molecular methods*
176 Peat for the focal dataset presented here was collected from four depth increments below
177 the peat surface (0-10, 10-20, 30-40, 60-70 cm) in late August/early September in year three of
178 the experiment, using a 5.08 cm diameter circular corer fitted to an electric drill. Additional
179 samples for complementary datasets were collected in late August/early September of year one
180 and in late July of year four using a 2.54 cm (year one) or 7.62 cm (year four) diameter corer
181 from two depth increments (10-20, 30-40 cm). Different diameter corers and sampling dates
182 were necessary to accommodate a variety of intended uses for the peat, which varied depending
183 on the year, and coring multiple times a season with a smaller corer would have created
184 excessive disturbance in the mesocosms. However, in all years each 10 cm depth increment was
185 not homogenized but instead a vertical split representative of approximately 25 ml of peat was

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186 subsampled from each for DNA work, ensuring the volume represented by the sampled material
187 remained comparable among years. Upon collection, samples were flash frozen in liquid nitrogen
188 and stored at -80 ºC.

189 Samples were pulverized using a mortar and pestle under liquid nitrogen, followed by a
190 coffee grinder. DNA was extracted from 0.5 g of ground peat from each sample using a
191 PowerSoil DNA Isolation kit, cleaned with a PowerClean DNA Clean-Up kit (MoBio
192 Laboratories; now Qiagen, Germantown, MD, USA), and quantified with a Qubit Fluorometer
193 (Invitrogen, Life Technologies, Carlsbad, CA, USA). DNA amplicon sequencing was conducted
194 at the U.S. Department of Energy Joint Genome Institute (JGI, Walnut Creek, California, USA;
195 now Berkeley, California) following Caporaso et al. (2012), with small modifications (see
196 Coleman-Derr et al., 2016; Tremblay et al., 2015). PCR amplification utilized the primers 515F
197 and 806R (Caporaso et al., 2012) targeting the bacterial and archaeal 16S V4 region, and fITS9
198 (Ihrmark et al., 2012) and ITS4 (White, Bruns, Lee, & Taylor, 1990) targeting the fungal ITS2
199 region. Primers were fitted with Illumina adaptors and the reverse primer contained an 11bp
200 barcode. Samples were pooled into equimolar portions and sequenced on an Illumina MiSeq
201 platform (Illumina, Inc., San Diego, CA) using 2 x 250 bp (year one) or 2 x 300 bp (years three,
202 four) chemistry.
203
204 *Bioinformatics*

205 Processing of DNA sequence reads proceeded as follows. Illumina adapters and PhiX
206 174 were removed with BBDuk (sourceforge.net/projects/bbmap/), and 3' and 5' PCR primers
207 were trimmed with Cutadapt 1.18 (Martin, 2011). Paired reads were merged with BBmerg
208 (Bushnell, Rood, & Singer, 2017), and those with expected error rate >1 and/or ambiguous bases
209 were removed with VSEARCH 2.5.1 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). The
210 5.8S (94 bases) and 28S (35 bases) flanks were trimmed from ITS2 reads with Cutadapt, and
211 resulting amplicons < 95 bases long were filtered. 16S V4 reads were not trimmed, but those
212 exceeding ± 8 bases from the median length (253 bases) were excluded. Chimera detection and
213 removal was implemented with the VSEARCH plug-in for QIIME 2 (Bolyen et al., 2019) using
214 the UNITE UCHIME ITS2 reference dataset (v. 7.2; Nilsson et al., 2015), and the SILVA 16S
215 dataset (128 QIIME release; Quast et al., 2013). De novo operational taxonomic units (OTUs)
216 were created with the QIIME 2 VSEARCH plug-in by first clustering at 98.5% similarity, then

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217 clustering the resulting OTU reference sequences at 97% similarity. 97% OTUs were curated
218 with LULU (min match = 90, min relative cooccurrence = 0.95; Frøslev et al., 2017), followed
219 by the removal of OTUs with < 10 reads in the dataset (Lamit et al., 2017).

220 The OTU matrices were further filtered and annotated. The QIIME 2 naive Bayes
221 feature-classifier plug-in (Bokulich et al., 2018; Pedregosa et al., 2011) was used to assign
222 taxonomy with the UNITE all eukaryote dynamic species hypothesis dataset for fungi (v. 8.0,
223 released 02.02.2019; Kõljalg et al., 2013) and the SILVA 16S +18S dataset for prokaryotes (128
224 QIIME release; Quast et al., 2013). OTUs not assigned the taxonomy of target lineages were
225 excluded. Next, ITS2 OTU representative sequences were aligned to the UNITE species
226 hypothesis dynamic fungal dataset (v. 8.0, released 02.02.2019) using BLAST in QIIME 2, and
227 reads that did not match at least ≥ 70% of their length to fungi with a similarity of ≥ 75% were
228 filtered (Tedersoo et al., 2015). To further remove potential non-target sequences, the above 16S
229 V4 pipeline was run with 28 marine samples originally sequenced on the same plate as the year
230 one peat samples. Although rare in the dataset, OTUs that occurred in year one peat samples and
231 the marine samples, and had BLAST matches to salt-tolerant taxa reported from marine/saline
232 systems in NCBI DNA sequence database (http://blast.ncbi.nlm.nih.gov) were removed as
233 potential contaminants. The datasets were then rarified to 5000 reads per sample prior to all
234 statistical analyses. Tentative functional groups were assigned with FAPROTAX (Louca,
235 Parfrey, & Doebeli, 2016) and FUNGuild (Nguyen et al., 2016), with further refinement based
236 on literature searches.

237

238 *Statistical analyses*

239 Our first set of analyses examined overall patterns of OTU composition over the course
240 of the experiment, with the aim of identifying when treatment effects began to manifest most
241 distinctly and if the focal dataset (year three) was representative of the overall treatment effects
242 of the experiment. PerMANOVA (Anderson, 2001) was used to separately examine the two peat
243 depths sampled in all years (10-20 cm, 30-40 cm), with each analysis including the fixed effects
244 of WT (high, low), presence/absence of Ericaceae, presence/absence of sedges, year, and all
245 possible interactions among these effects. Each model also included block as a fixed effect, and
246 individual mesocosm as a random effect to account for non-independence of samples from the
247 same mesocosm. Modeling treatments in this way allowed for assessment of the presence of each

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248 vascular PFG (as opposed to the removal of the other PFG) because each PFG was present in
249 mesocosms with and without the other PFG, but a direct Ericaceae by sedge interaction could not
250 be tested. These analyses were complemented with canonical analysis of principal coordinates
251 (CAP; Andersen & Willis 2003) to visualize communities. PerMANOVA and CAP were run
252 using Bray-Curtis dissimilarity. For all PerMANOVA models, we also report the square root of
253 the estimated component of variation for each factor; these are in Bray-Curtis units (scaled
254 between 0 and 100), and can be used for comparing the relative importance of terms in a model
255 for explaining the overall variation in community composition (Andersen, Gorley & Clarke,
256 2008). Removal or pooling of model terms with negative estimates for components of variation
257 (Andersen, Gorley & Clarke, 2008) had little effect on the significance tests or estimates of
258 components of variation for the remaining terms in any of the PerMANOVAs we ran, therefore
259 all terms were always retained for simplicity. Prior to analyses, OTU matrices were relativized as
260 proportions of sample read totals followed by 4th root transformation to down-weight dominant
261 OTUs. PerMANOVAs were conducted with Type III sums of squares, using permutation of
262 residuals from partial models. PerMANOVA was run in Primer 6.1.15 with PERMANOVA+
263 1.0.5 (PRIMER-E, Plymouth, UK), and other analyses utilized *Vegan* (Oksanen et al., 2019) in R
264 3.6.3 (R core team, 2020).

265 To gain deeper insight into the depth-specific microbial community responses to PFG and
266 WT manipulations, we focused our more detailed analyses on microbial communities along the
267 70 cm depth gradient sampled in year three. First, PerMANOVAs were run using the equivalent
268 model structure as those for the multi-year analysis described above, substituting a depth effect
269 for the year effect. Second, PerMANOVAs were also run individually for each depth using the
270 fixed effects of WT, presence/absence of Ericaceae, presence/absence of sedges, block and two-
271 way interactions between WT and the presence/absence of each PFG. Third, CAP ordinations
272 were used to visualize OTU composition. Fourth, indicator species analysis was used to identify
273 the 25 strongest indicator OTUs associated with each depth and treatment using the R package
274 *indicspecies* (De Cáceres & Legendre, 2009). Fifth, linear mixed models were used to examine
275 the responses of OTU richness and total archaea, and the relative abundances of a limited set of
276 select functional groups with known relevance to carbon cycling (ErMF, lignocellulose-
277 degrading fungi, methanotrophic bacteria, methanogenic archaea). These models included the
278 fixed effects of depth, WT, presence/absence of sedges and Ericaceae, and all possible

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279 interactions among these fixed effects, plus the fixed effect of block and the random effect of
280 mesocosm. Linear mixed models were fit in R with *lmerTest* (Kuznetsova, Brockhoff, &
281 Christensen, 2017) using the Kenward-Roger approximation for *F*-tests. The *emmeans* package
282 (Russell, 2020) was used to generate marginal means from linear mixed models, and the
283 *effectsize* package (Ben-Shachar, Makowski & Lüdecke, 2020) was used to obtain partial-ω^2
284 values to use as effects sizes for comparison between model terms.

285 The final set of analyses utilized structural equation modeling with the depth gradient
286 data from year three (see *a priori* model in Fig. S2). Our specific goals were to: 1) test if the
287 effect of PFG manipulation on prokaryote and fungal communities was primarily due to the
288 alteration of plant species composition as opposed to unmeasured variables affected by PFG
289 manipulation, 2) examine the potential for WT to modulate the effects of PFG on prokaryote and
290 fungal communities through modification of the plant community, and 3) measure how the
291 strength of PFG and WT effects on prokaryote and fungal communities changed with depth.
292 Separate models were created for each microbial community at each depth. Fungal and
293 prokaryote communities were represented using Bray-Curtis dissimilarities calculated from the
294 same 4th root transformed OTU matrices described above. The treatment factors of WT (high vs
295 low) and PFG treatments (unmanipulated, Ericaceae removal, sedge removal) were each
296 represented in the model using pairwise distances denoting zero for pairs of samples from the
297 same treatment and one for pairs of samples from different treatments. Sedge and Ericaceae
298 communities were each represented using Bray-Curtis dissimilarity calculated from point-
299 intercept data from Potvin et al., (2015) measured in year three. Prior to calculating pairwise
300 dissimilarities, values in the Ericaceae and sedge data matrices were expressed as the % of
301 intercepts represented by each species to emphasize shifts in absolute abundances, and a small
302 constant was added as a dummy species to each matrix to account for some mesocosms lacking
303 members of sedges or Ericaceae. Model sub-components were tested with multiple regression on
304 distance matrices (Lichstein, 2007) with ranked dissimilarities/distances using *ecodist* (Goslee &
305 Urban, 2007) in R, and model fit was assessed with directional separation tests (Shipley, 2000)
306 calculated manually. However, unfolded pairwise matrices were examined with *piecewiseSEM*
307 (Lefcheck, 2016) to corroborate model parameters and verify the basis sets for directional
308 separation tests.
309

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310 **Results**

311 Diverse communities were recovered through amplicon sequencing. The rarefied fungal
312 ITS2 dataset contained 189 samples (sample size: year one = 48, year three =96, year four = 45;
313 945,000 sequences), and in all years was dominated by Helotiales (Ascomycota), followed by
314 Agaricales, Sebacinales and Polyporales (Basidiomycota) (Fig. S3A). In total, there were 1,193
315 fungal OTUs, with an average of 64.1 OTUs per sample (stdev = 17.8, range = 25-117 OTUs).
316 The rarefied prokaryote dataset contained 191 samples (sample size: year one = 48, year three =
317 96, year four = 47; 955,000 sequences). In all years, bacteria were dominated by Acidobacteria
318 and Proteobacteria, while Archaea were dominated by Euryarchaeota, followed by
319 Thaumarchaeota and Bathyarchaeota (Fig. S3B). There was a total of 7,353 prokaryote OTUs,
320 with an average of 606.0 OTUs per sample (stdev = 158.1, range = 235-897 OTUs).
321

322 ***Response of community composition across four years of WT and PFG manipulation***

323 Changes in OTU composition over time supported the hypotheses that PFG and WT
324 manipulation alter microbial communities (H1, H2) but provided no evidence for their
325 interaction (H3). At the 10-20 cm depth, fungal and prokaryote composition were influenced by
326 the presence of Ericaceae and WT manipulation (Table 1; Fig. 1A, 1B). The components of
327 variation from the PerMANOVA models indicate that the main effect of Ericaceae on fungal
328 composition was ~50% greater than that of WT at 10-20 cm, while the main effect of Ericaceae
329 on prokaryotes was 25% less than for WT (Table 1). At the 30-40 cm depth, WT was a slightly
330 stronger influence on fungi than was the presence of Ericaceae, while the influence of WT on
331 prokaryote composition was more than twice the strength of the marginally significant influence
332 of Ericaceae (Table 1; Fig. 1C, 1D). At both depths, many of the treatment effects manifested
333 most strongly in years three and four (significant treatment by year interactions; Table 1), which
334 is visually apparent in CAP ordinations (Fig. 1). When integrating over the course of the
335 experiment there was no evidence for a sedge effect on OTU composition at either depth (Table
336 1). Importantly, patterns in OTU composition across years confirmed that our focal depth
337 gradient dataset (year three) was representative of the broader PFG and WT effects over the
338 course of the experiment (Fig. 1).
339

340 ***Community responses over the year three 70 cm peat depth gradient***

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341 *Community depth stratification* — Depth had the largest influence on OTU composition
342 of any factor included in the full depth gradient PerMANOVA models (Table 1), and all depths
343 showed uniqueness in fungal and prokaryote communities relative to other depths (Fig 2, S3).
344 All depths had indicator OTUs that were members of the Helotiales, while 0-10 and 60-70 cm
345 depths also had many indicator OTUs representing a broader set of additional fungal lineages
346 (Table S1). Fungal indicators of the 0-10 and 10-20 cm depths included ErMF, plant pathogens,
347 general saprotrophs and lignocellulose degraders, while indicators of the 30-40 and 60-70 cm
348 depths included non-mycorrhizal root associates, general saprotrophs and lignocellulose
349 degraders (Table S1). Many prokaryote indicators, especially in the 0-10, 10-20 and 30-40 cm
350 depths, were from acid tolerant groups (e.g., Acidobacteriaceae, Acetobacteraceae). In the
351 deepest depth (60-70 cm) there was an increase in Deltaproteobacteria and archaeal indicator
352 OTUs, many of which are adapted to reduced conditions (e.g., methanogens, sulfate reducers;
353 Table S1). Prokaryote and fungal OTU richness decreased with depth, while total archaea
354 relative abundance increased (Table 2; Fig. S4).
355 *Community composition responses to PFG and WT across the 70 cm depth gradient* — In
356 support of H1, fungal and prokaryote OTU composition exhibited depth-specific responses to
357 PFG manipulation, although responses were only driven by Ericaceae (Table 1, 3). The influence
358 of Ericaceae on fungi and prokaryotes was distinctive in the upper depths (0-10, 10-20 cm) and
359 disappeared in the deeper depths (30-40, 60-70 cm; Table 1, 3; Fig. 2). Microbial communities in
360 mesocosms with and without Ericaceae exhibited depth-specific differences in their top indicator
361 OTUs, but there were also general patterns. ErMF OTUs were some of the top fungal indicators
362 of mesocosms with Ericaceae, while root endophytes and lignocellulose degraders were top
363 indicators of mesocosms lacking Ericaceae (Table S1). Of particular note is the lignocellulose-
364 degrading genus *Galerina* whose members are top indicators of the absence of Ericaceae at 0-10
365 and 10-20 cm (Table S1), and have high relative abundances in these depths (Fig. S5).
366 Mesocosms lacking Ericaceae included indicators from 10 different bacteria phyla and indicators
367 of mesocosms containing Ericaceae were primarily Proteobacteria, but both treatments included
368 some Acidobacteria indicators (Table S1).
369 Fungal and prokaryote OTU composition also exhibited depth-dependent responses to
370 WT manipulation, in support of H2, but there was no evidence for WT x PFG interactions (H3).
371 The WT effect in the upper depths was stronger in the surface (0-10 cm) than subsurface (10-20

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372 cm) peat for both communities. Despite this, at the upper two depths the strength of the WT
373 effect on fungal OTU composition was subordinate to the Ericaceae effect, whereas this pattern
374 was reversed for prokaryotes (Table 3, Fig. 2). Prokaryote composition was the most divergent
375 between WT groups at the 30-40 cm peat depth, while the influence of WT on fungi at this depth
376 was clearly far weaker than for prokaryotes (Table 3; Fig. 2). Neither fungal nor prokaryote
377 composition responded to WT at 60-70 cm (Table 3). The responses of composition were driven
378 by large depth-specific shifts in indicator OTUs for both fungi and prokaryotes (Table S1). For
379 example, fungal indicators of the high WT treatment in the surface peat (0-10 cm) were primarily
380 non-ErMF taxa likely associated with living or recently dead plant tissues, and indicators of the
381 low WT treatment included a higher proportion of ErMF (Table S1). In contrast, indicators of the
382 high WT treatment at 30-40 cm represented a very broad range of functions whereas the 30-40
383 cm low WT indicators were primarily non-mycorrhizal root-associates (Table S1). Interestingly,
384 an OTU assigned to the Methanomicrobia (hydrogenotrophic methanogens) was an indicator of
385 the high WT treatment in the three upper depths (Table S1), and three known methanotrophs
386 were top indicators of the 10-20 cm depth high WT treatment.

387 *Microbial functional group and OTU richness responses to PFG and WT across the 70*
388 *cm depth gradient* —Microbial functional groups and OTU richness exhibited complex
389 responses to PFG, WT and/or PFG x WT interactions, lending support to all hypotheses (Table
390 2; Fig. 3). Total ErMF relative abundance was strongly suppressed by Ericaceae removal and the
391 high WT treatment in the upper two depths (0-10, 10-20 cm; Table 2, Fig. 3A). Total
392 lignocellulose degrading fungi increased in relative abundance in the absence of Ericaceae,
393 especially in the upper depths (Table 2; Fig. 3B). The response of lignocellulose degraders
394 remained significant after including total ErMF relative abundance as a covariate in the mixed
395 model, indicating that the response was not solely driven by the removal of ErMF from the DNA
396 pool (Ericaceae effect: $F_{(1,21.3)} = 4.87$, $P = 0.038$; marginal means ± 1 SE : Ericaceae present =
397 0.08 ±0.01, Ericaceae absent = 0.17 ± 0.05). Methanogens and total archaea had elevated relative
398 abundances in high WT, except at 60-70 cm, with methanogen relative abundance peaking in the
399 30-40 cm high WT treatment (Table 2; Fig. 3C, S4C). Ericaceae removal tended to increase
400 methanotroph relative abundance with high WT and decrease it in low WT at most depths
401 (especially prominent at 10-20 and 30-40 cm) whereas sedge removal generally decreased
402 methanotroph relative abundance (Table 2; Fig. 3D). The major fungal and prokaryote taxa

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

405 OTU richness results partially supported H1, H2 and H3. High WTs clearly depressed
406 fungal richness in the surface peat (0-10 cm), although WT was not a significant overall effect in
407 the model (Table 2; Fig S4A). Prokaryote richness was influenced by interactions among
408 Ericaceae, WT, depth and sedges, with results only marginally significant in some cases (Table
409 2; Fig. S4B). For example, in the 0-10 cm depth the highest richness occurred in mesocosms
410 lacking Ericaceae, and at the 30-40 cm depth prokaryote OTU richness was elevated in low WT
411 treatments but this pattern reversed at 60-70 cm (Fig. S4B). Sedge removal tended to elevate
412 prokaryote OTU richness in the high WT treatment at the 0-10 cm depth, whereas a similar
413 effect was evident in the low WT treatment at the 10-20 cm depth (Fig. S4B).

414 *Structural equation modeling* — SEM supported the hypotheses concerning depth-
415 dependent effects of PFG and WT (H1, H2), with several key results. First, the variation in
416 fungal composition explained by the models was greatest in the 0-10 and 10-20 depths, the
417 greatest variation in prokaryote composition was explained by models for the 0-10 and 30-40 cm
418 depths, and the models for the 60-70 cm depth explained almost no variation in either
419 community (Fig. 4A, 4B). Second, PFG treatment effects were stronger on fungi than
420 prokaryotes and were most pronounced at 0-10 and 10-20 cm, while WT effects were stronger on
421 prokaryotes than fungi and were more pronounced in the 0-10 and 30-40 cm depths (Fig. 4).
422 Third, WT influenced Ericaceae composition, but the effect of WT on microbial communities
423 through Ericaceae was small (Table S2).

424 Fourth, at the 0-10 cm depth the effect of PFG treatment primarily acted through changes
425 in Ericaceae composition, which was not the case for the 10-20 cm depth. Initial model fit tests
426 at 10-20 cm suggested a need for a direct path from PFG treatment to fungal composition (model
427 fit $P = 0.012$). This direct path from PFG treatment to fungal OTU composition (partial *rho* =
428 0.254, Fig. 4A) was nearly equivalent to the compound effect of PFG treatment acting through
429 Ericaceae composition at 10-20 cm (compound effect partial *rho* = 0.240; Table S2). Similarly,
430 but to a lesser degree, initial model fit tests at 10-20 cm also suggested a need for a direct path
431 from PFG treatment to prokaryote composition (model fit $P = 0.086$). Although the direct path
432 from PFG treatment to prokaryote OTU composition was only marginally significant (partial *rho*
433 = 0.179, $P = 0.086$, Fig. 4B), we decided to retain it in the model because its strength was much

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434 greater than the compound effect of PFGs acting through Ericaceae composition at 10-20 cm
435 (compound effect partial *rho* = 0.04; Table S2). The direct path from Ericaceae composition to
436 prokaryote composition was modest but significant (partial *rho* = 0.136, $P = 0.026$), when the
437 direct path of PFGs on prokaryote composition was not included the model.
438
439 **Discussion**
440 Our results reveal the strength and depth-dependence of WT and PFG effects on
441 microbial communities. The strikingly greater impact of PFG on fungi near the surface
442 contrasted with the stronger impact of WT on prokaryotes across a broader range of depths.
443 These patterns can be explained by abiotic and biotic factors: the intolerance of most fungi to
444 anoxic conditions (Kavanagh, 2011) constraining most taxa to shallow peat, and the colocation
445 of the dominant ErMF with their shallowly-rooted Ericaceae hosts (Moore et al., 2002; Wallén,
446 1987). In contrast, the broad range of moisture niches, metabolic pathways, and redox tolerance
447 among soil prokaryotes (e.g., Bodelier & Dedysh, 2013; Lennon, Aanderud, Lehmkuhl, &
448 Schoolmaster, 2012) and the strong sensitivity of prokaryote communities to changes in soil
449 moisture (e.g., Bapiri, Bååth, & Rousk, 2010; Barnard, Osborne, & Firestone, 2013), explain
450 their shift with WT treatments in both drier surface peat as well as at acrotelm/catotelm boundary
451 where redox conditions are most dynamic (Kane et al., 2019; Tfaily et al., 2018). These depth-
452 dependent effects indicate that WT and PFG are among the key shapers of the vertical
453 physicochemical gradients that structure peatland microbial communities (Andersen, Chapman
454 & Artz 2013; Artz et al., 2007; Lin et al., 2014), the activities of which then feed back to
455 modulate carbon cycling along the peat profile (Chanton et al., 2009; Kane et al., 2019; Lin et
456 al., 2014; Tfaily et al., 2018). Although discussions on wetland carbon cycling usually emphasize
457 the role of anoxic reducing conditions (e.g., Schlesenger & Bernhart, 2013), most carbon inputs
458 from primary production in bogs and poor fens derive from senesced *Sphagnum* in the largely
459 oxic acrotelm (van Breeman, 1995; Rydin & Jeglum, 2013), making aerobic organisms
460 instrumental as the initial transformers of peatland organic matter. As this partially degraded
461 organic matter transitions into the catotelm, anaerobic metabolism becomes paramount, which is
462 reflected in the OTU composition in the deeper depths of PEATcosm and other studies (e.g., Lin
463 et al., 2014; Wang et al., 2019). Hence, the microbial community present at a peat depth sets
464 bounds on how the community can change with drought and changes in dominant PFGs, and the

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465 integrated responses of fungi and prokaryotes along the profile may influence the magnitude of
466 CO_2 and CH_4 released from peatlands under different climate change scenarios.
467
468 ***Responses to PFG manipulation***
469 As predicted, the influence of Ericaceae was greatest in the upper peat, which suggests a
470 restructuring of the community involved in aerobic carbon cycling with changes in dominant
471 PFGs. The presence of Ericaceae can have a strong impact on fungal communities in surface peat
472 (Ward et al., 2015; Kennedy, Mielke, & Nguyen, 2018), and observational studies indicate that
473 links between microbial communities and vegetation composition decline with depth in the peat
474 profile (e.g., Artz et al., 2007; Lin et al., 2014). ErMF showed a marked decrease in abundance
475 when Ericaceae were removed, indicating a preference for host photosynthate despite the free-
476 living saprotrophic capabilities of some ErMF (Martino et al., 2018). Of particular interest is that
477 relative abundance of the dominant ErMF Ascomycota, *Hyaloscypha ericae* (deprecated
478 synonyms are *Pezoloma ericae* and *Rhizoscyphus ericae*), was depressed less by Ericaceae
479 removal than the dominant ErMF Basidiomycota, *Serindipita* spp., possibly indicating a greater
480 degree of host dependency in the latter or more dormant propagules in the former. Ericaceae also
481 influenced a variety of non-ErMF fungi and prokaryotes, which may be driven by several
482 mechanisms. ErMF may competitively suppress saprotrophs (Verbruggen et al., 2017). In
483 particular, one of the most abundant saprotroph genera in surface depths, *Galerina*, responded
484 very positively to removal of Ericaceae. These *Galerina* species are *Sphagnum* peatland
485 specialists (Gulden, Stensrud, Shalchian-Tabrizi, & Kauserud. 2005, Castellano 2003), and the
486 lignocellulose degrading capability of fungi in the genus (Nagendran, Hallen-Adams, Paper,
487 Aslam, & Walton. 2009; Riley et al., 2014) suggest that these species are adapted to *Sphagnum*
488 as a substrate. If their activity is suppressed by ErMF it could also lead to greater accumulation
489 of partially-degraded *Sphagnum* litter, because ErMF do not possess the complete suite of lignin-
490 degrading enzymes, most notably class II peroxidases. Although *Sphagnum* does not technically
491 produce lignin, it does have analogous chemical components that resist hydrolytic decomposition
492 (Bengtsson, Rydin, & Hájek. 2018). Some taxa may also utilize the byproducts from ErMF
493 decomposition of organic matter. Ericaceae tissues also represent a direct input of carbon into
494 surface peat through exudates, senescence, and leaching phenolics that may act as unique
495 microbial substrates and/or inhibitors (Weigang, Artz, & Johnson, 2008).

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496 Two unexpected findings about the PFG effects are worth noting. First, SEM suggested
497 the effect of Ericaceae in the 10-20 cm peat was partially due to factors influenced by PFG
498 manipulation aside from direct changes in Ericaceae composition. These may include
499 subsidence, peat accumulation rates and other physicochemical parameters influenced by PFG
500 (Kane et al., 2019; Potvin et al., 2015). Additionally, aboveground plant community data may
501 not fully reflect the density of some or all Ericaceae species roots (even non-relativized, as we
502 used in the SEM), especially in the 10-20 cm depth where root density is high; this may reduce
503 the explanatory power of the Ericaceae community matrix thus elevating the strength of the
504 direct path from PFG manipulation to the microbial community. Second, inconsistent with our
505 hypothesis, sedges had a limited effect on microbial communities. Oxygenation and substrates
506 from sedge roots are important shapers of microbial-driven processes in sedge dominated
507 minerotrophic fens (Chanton et al., 2008; Rupp et al., 2019), and of diverse microbial
508 communities associate with sedges rhizospheres (Hough et al., 2020). The modest sedge effect in
509 PEATcosm might be due to their relatively low biomass in the ombrotrophic habitat we focused
510 on, which became more variable through the course of the experiment (Potvin et al., 2015). Our
511 findings contrast with a study, also in an ombrotrophic peatland, where microbial phospholipid
512 fatty acid (PLFA) composition responded more to sedge removal than Ericaceae removal
513 (Robroek et al., 2015). However, it is difficult to directly compare PLFA and amplicon
514 sequencing results, and results from Ward et al. (2015) suggest no sedge influence on fungal
515 composition. It is also possible that the *Eriophorum*-dominated sedge communities in Robroek et
516 al. (2015) have a greater effect on microbial communities than our *Carex*-dominated sedge
517 communities.
518
519 ***Responses to WT manipulation***
520 As predicted, the microbial response to WT manipulation was depth-dependent. In both
521 WT treatments, the upper 20 cm of peat was above the WT for a considerable time period prior
522 to sampling, with the 0-10 cm depth being above the WT surface for most of the growing season.
523 Distance above the WT interacts with peat density, porosity, and capillarity to drive differences
524 in moisture availability that may have directly affected the response of microbial communities in
525 the unsaturated upper peat to WT manipulation. Importantly, changes in taxa associated with
526 methane cycling (methanogens, methanotrophs) and the processing of complex organic matter

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527 (lignocellulose degraders, ErMF) highlight the potential extended effects of WT on carbon
528 cycling even in non-saturated peat. Our results contrast a recent mesocosm study by
529 Asemaninejad et al. (2018) who did not detect an effect of WT manipulation on fungal
530 communities at any depth in the profile. However, the discrepancy in results may be due to the
531 more limited WT depth differential between treatment levels in Asemaninejad et al. (2018),
532 which were maintained at a stable level for the course of the experiment. Moss species
533 composition and productivity were also influenced by WT treatment in PEATcosm (Potvin et al.
534 2015), and we suspect that the stronger WT effect on fungi and prokaryotes in the 0-10 cm depth
535 than the 10-20 cm depth is in part due to the role of *Sphagnum* mosses in structuring their
536 microbiomes (Kostka et al., 2016). The potential foundational influence of moss in acid
537 *Sphagnum* peatlands is one factor making these systems distinct from minerotrophic fens, where
538 moss has a less clear influence on microbial communities in surface peat (Emsens et al., 2020).
539 Microbial community responses in the deeper peat were likely driven by WT inundation
540 and oscillation. WT manipulation elicited some of its strongest responses (especially on
541 prokaryotes) at the 30-40 cm depth, where peat was perennially underwater in the high WT
542 treatment but seasonally above the WT in the low WT treatment. This should promote
543 communities capable of aerobic decomposition during the driest part of the season and anaerobic
544 decomposition on the shoulders of the growing season in the low WT treatment, and
545 communities associated with slower carbon transformations typical of anoxic reduced conditions
546 in the high WT treatment, a contention supported by our indicator species analyses. Interestingly,
547 methanogens reached their greatest relative abundance in the high WT treatment at the 30-40 cm
548 depth. This might reflect a preference for anoxic conditions combined with inputs of fresh, labile
549 substrates for fermenters and syntrophs to generate H_2 and CO_2 used in hydrogenotrophic
550 methanogenesis (Conrad, 1999). Oscillation between oxic and anoxic conditions at the interface
551 between the acrotelm and catotelm (i.e., the mesotelm) is associated with rapid organic matter
552 transformations (Kane et al., 2019; Lin et al., 2014; Tfaily et al., 2018), and shifts in microbial
553 composition in the low WT treatment at 30-40 cm indicate a downward extension of this
554 biogeochemical "hotspot" during drought. In contrast, the community at the 60-70 cm depth was
555 continuously submerged far below the WT surface, buffered from changes in the WT level and
556 less affected by roots, suggesting that deep peat microbial communities and their influences on
557 carbon cycling may not be strongly affected by seasonal drought, at least in the short term.

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558

559 ***Interactions between PFG and WT***

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

571 Climate change-driven shifts in peatland soil moisture can influence microbial
572 communities indirectly by shifting the composition of plant communities (e.g., Bragazza et al.,
573 2013; Jassey et al., 2018). Evidence for this in PEATcosm was not strong; although the low WT
574 treatment promoted Ericaceae cover and productivity, with some species responding more than
575 others (Potvin et al., 2015), the compound path effects from WT through Ericaceae composition
576 to the microbial communities were very small. Our SEMs may have underestimated the path
577 coefficient between WT and Ericaceae composition because mesocosms lacking Ericaceae did
578 not have a community to respond to WT treatment. However, we suspect that the strong effect of
579 simply having or not having Ericaceae overshadowed the impact of WT on microbial
580 communities acting through modification of the plant community. Over a longer time scale the
581 influence of WT on microbial communities acting through vegetation change should become
582 stronger, which would represent an indirect pathway in natural systems for climate change-
583 related droughts to influence microbial communities.

584

585 ***Conclusions***

586 Our results demonstrate the importance of WT and PFG in structuring peatland microbial
587 communities along peat depth profiles. Peatlands in many regions are experiencing increased
588 temperatures, changes in long-term precipitation patterns, and other anthropogenic disturbances

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589 that influence WT dynamics and cause shifts in plant communities; the influence of these factors
590 on carbon cycling will be contingent on how they influence microbial communities. Importantly,
591 microbial lineages and functional groups do not all respond equivalently to WT and PFG
592 manipulations, and their responses are depth-dependent. The strong mutualistic interactions of
593 fungi and Ericaceae appear to be the driver of the greater PFG effect on fungal than prokaryote
594 communities. The dominance of ErMF in shallow oxic peat indicates a large potential for
595 Ericaceae to shape microbial community structure and function near the surface, where most new
596 organic matter enters the peat profile. The fact that when Ericaceae are removed lignocellulose
597 degraders respond very positively in relative abundance begs the question of whether this
598 represents a functional release of those taxa in the absence of mycorrhizal competition.
599 Similarly, the significant prokaryote response to PFG manipulations indicates changing
600 resources and conditions driven by plant traits can also structure these communities, especially
601 methanotrophs; but the weaker prokaryote (vs. fungal) response to PFG vs. WT manipulation
602 suggests redox conditions predominate in structuring these communities. The vertical complexity
603 in responses, likely driven by declining PFG influence and increasing influence of declines in
604 both redox potential and organic matter quality with depth, highlights the necessity of accounting
605 for depth stratification when understanding the responses of peatland microbial communities to
606 global change.
607
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616
617 **Data Accessibility**
618 - These sequence data have been submitted to the National Center for Biotechnology Information
619 (NCBI) Sequence Read Archive under accession number PRJNA650129.

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620 -Vegetation data is available through Pangea: doi.pangaea.de/10.1594/PANGAEA.902313.

621

622 **Author Contributions**

623 E.A. Lilleskov, E.S. Kane, R.K. Kolka, J.T. Lennon, R.A. Chimner and S.G. Tringe conceived
624 the study and obtained funding. L.R. Potvin, E.A. Lilleskov, E.S. Kane, K.J. Romanowicz, S.G.
625 Tringe and L.J. Lamit performed the research. L.J. Lamit analyzed the data. L.J. Lamit and E.A.
626 Lilleskov wrote the paper, with all coauthors contributing to revisions.

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Table 1. PerMANOVA full model results of OTU composition responses treatment factors across years or depths.[†‡ §]

Multi-year models	Ericaceae	Sedge	WT	Year	Ericaceae x WT	Sedge x WT	Ericaceae x Year	Sedge x Year	WT x Year	Ericacae x WT x Year	Sedge x WT x Year
	F (df) *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*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

70cm depth gradient models	Ericaceae	Sedge	WT	Depth	Ericaceae x WT	Sedge x WT	Ericaceae x Depth	Sedge x Depth	WT x Depth	Ericacae x WT x Depth	Sedge x WT x Depth
	F (df) *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*F (df)* *P*, √Var	*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

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

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

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

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917 **Table 2.** Linear mixed model results for the relative abundances of functional groups, total archaea and OTU richness in the 70 cm
918 depth gradient sampled in year three.[†‡ §]
919

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

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921 [†]All response variables were log10 transformed prior to analysis, except fungal and prokaryote OTU richness.

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922 ‡ Models also included individual *mesocosm* bin as a random effect and *Block* as a fixed effect. No hypothesis test was applied to these
923 factors.
924 § ω_p^2= partial-ω^2 values. Negative values are reported as zero for simplicity.

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Table 3. PerMANOVA results for community composition responses to treatments within individual depths of the 70 cm peat depth gradient sampled in year three.[†‡]

Taxa and depth of model	**Ericaceae** *F (df)* *P,* √Var	**Sedge** *F (df)* *P,* √Var	**WT** *F (df)* *P,* √Var	**Ericaceae x WT** *F (df)* *P,* √Var	**Sedge x WT** *F (df)* *P,* √Var
<u>Fungi</u>					
00-10cm	3.25 (1, 15)	1.27 (1, 15)	2.31 (1, 15)	1.08 (1, 15)	1.04 (1, 15)
	<0.001, 20.03	0.163, 6.89	**0.001**, 15.27	0.367, 5.45	0.433, 3.74
10-20cm	3.20 (1, 15)	1.32 (1, 15)	1.55 (1, 15)	0.73 (1, 15)	0.87 (1, 15)
	0.002, 20.67	0.141, 7.86	**0.051**, 10.36	0.822, 0.00	0.647, 0.00
30-40cm	1.45 (1, 15)	0.84 (1, 15)	1.67 (1, 15)	0.78 (1, 15)	0.687 (1, 15)
	0.105, 9.81	0.675, 0.00	**0.051**, 12.13	0.740, 0.00	0.848, 0.00
60-70cm	0.92 (1, 15)	0.85 (1, 15)	1.69 (1, 15)	0.56 (1, 15)	0.78 (1, 15)
	0.5.2, 0.00	0.580, 0.00	0.094, 12.30	0.898, 0.00	0.672, 0.00
<u>Prokaryotes</u>					
00-10cm	1.79 (1, 15)	1.00 (1, 15)	2.73 (1, 15)	0.96 (1, 15)	0.99 (1, 15)
	0.005, 10.19	0.513, 0.00	**<0.001**, 15.09	0.579, 0.00	0.536, 0.00
10-20cm	2.01 (1, 15)	1.01 (1, 15)	2.11 (1, 15)	0.96 (1, 15)	1.02 (1, 15)
	0.002, 11.72	0.474, 1.05	**0.001**, 12.27	0.554, 0.00	0.462, 2.25
30-40cm	1.11 (1, 15)	0.88 (1, 15)	4.16 (1, 15)	0.92 (1, 15)	0.86 (1, 15)
	0.292, 4.00	0.598, 0.00	**<0.001**, 21.28	0.529, 0.00	0.631, 0.00
60-70cm	0.96 (1, 15)	0.78 (1, 15)	1.47 (1, 15)	0.67 (1, 15)	0.58 (1, 15)
	0.429, 0.00	0.687, 0.00	0.135, 7.52	0.832, 0.00	0.913, 0.00

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

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

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

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940 ordination points. Dashed lines are provided to show distinction between the strongest effects

941 observed in the latter two years (years three + four).

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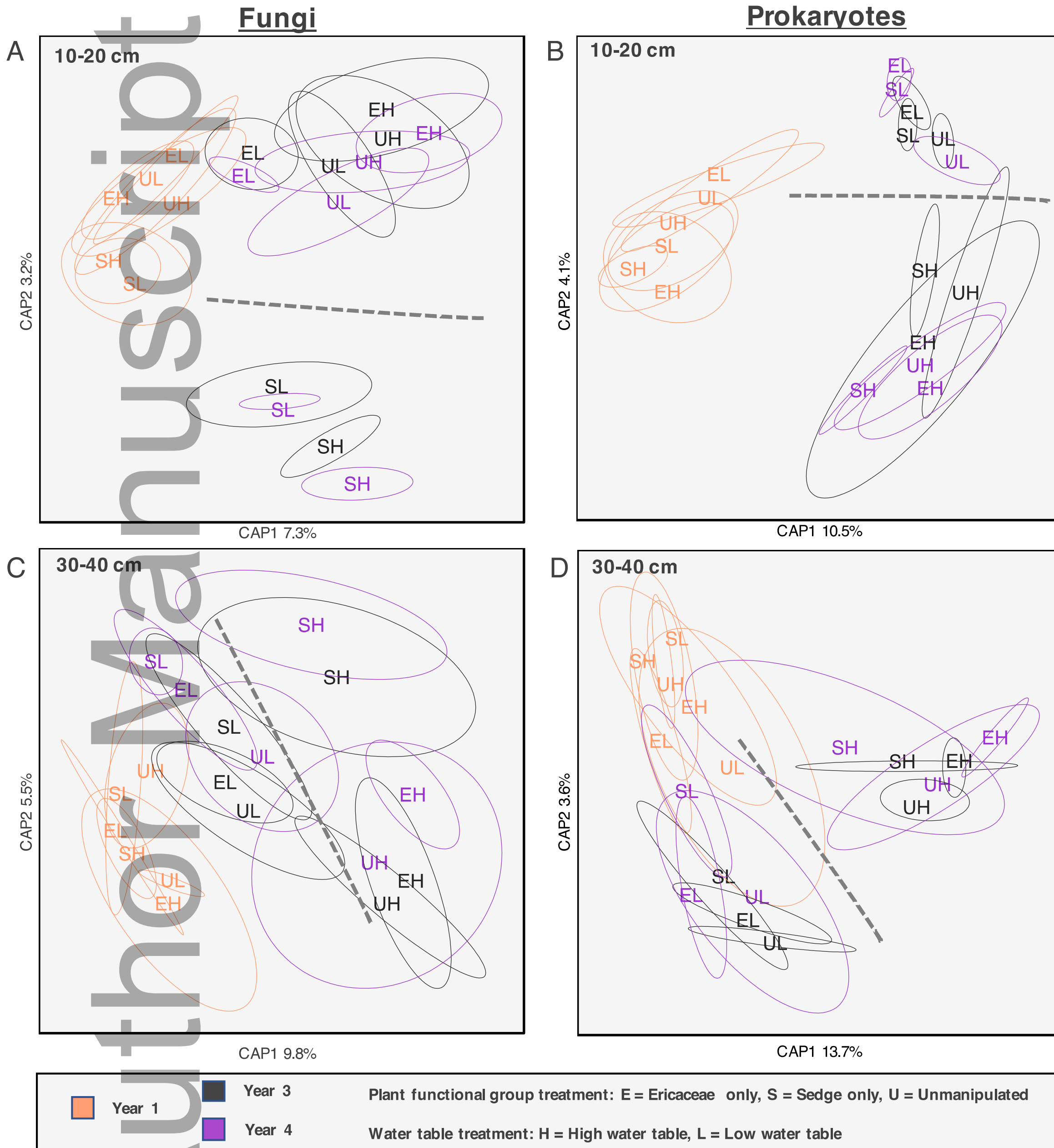

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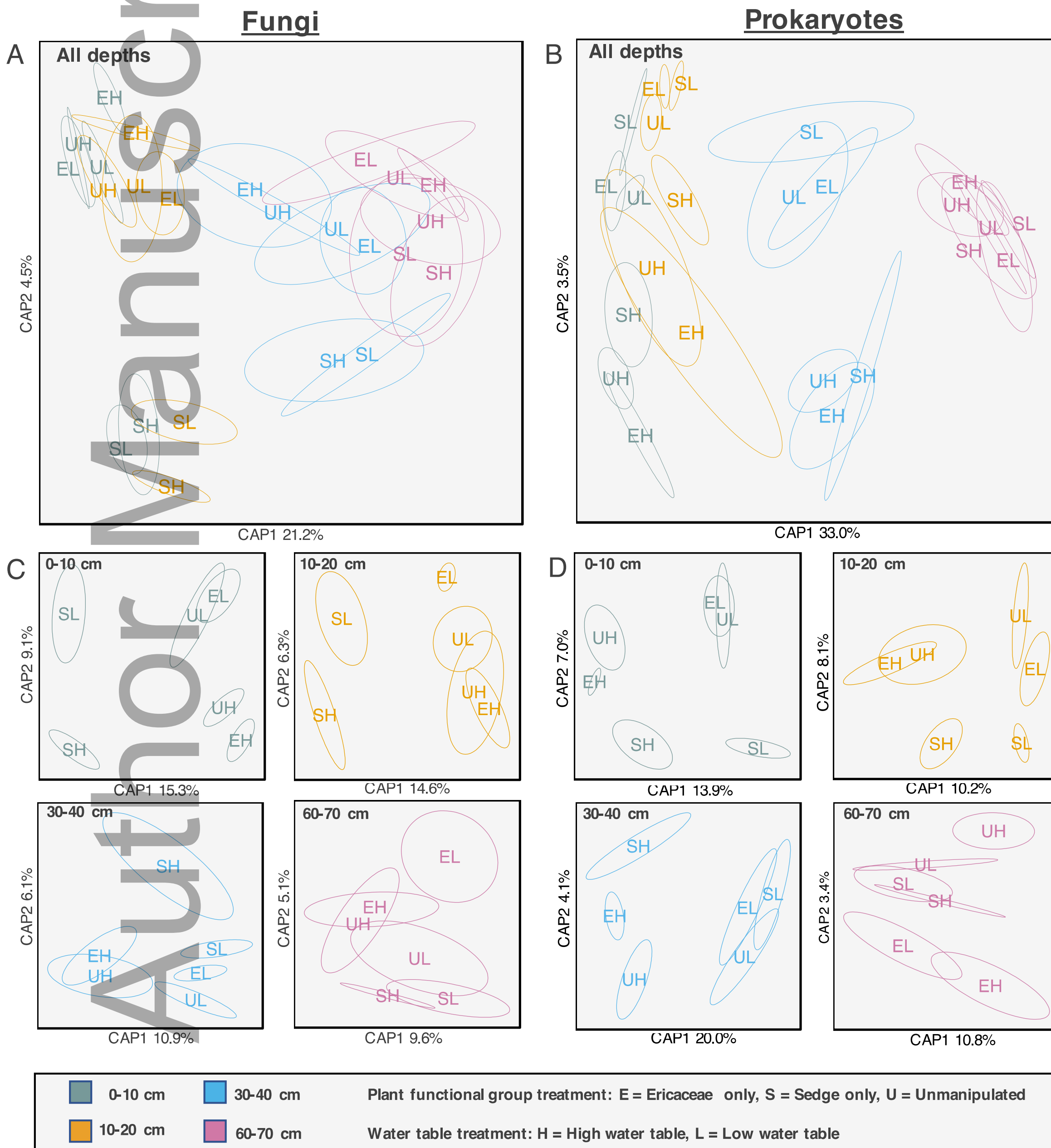
944 **Figure 2.** Canonical analysis of principal coordinates (CAP) ordinations with fungal (A, C) and
945 prokaryote (B, D) operational taxonomic unit composition (OTU) across the 70 cm depth
946 gradient sampled in year three. Ordinations were first (A, C) conducted by constraining by
947 sampling depth, plant functional group treatment (E = Ericaceae only, S = Sedge only, U =
948 Unmanipulated) and water table treatment (H = high, L = low), and only by water table and plant
949 functional group treatments for datasets within each depth (B, D). Ellipses represent 95%
950 confidence intervals of the ordination points.



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952 **Figure 3.** Marginal means (± 1SE) for relative abundances (Rel. Abund.) of ericoid mycorrhizal
953 fungi (A), lignocellulose degrading fungi (B), methanogenic archaea (C) and methanotrophic
954 bacteria (D) for the presence/absence (solid lines = presence, dashed lines = absence) of
955 Ericaceae (left panel) or Sedges (right panel) by water table treatment (green = low water table,
956 blue = high water table) along the 70 cm peat depth gradient. Marginal means are estimated from
957 linear mixed models (see Table 2).
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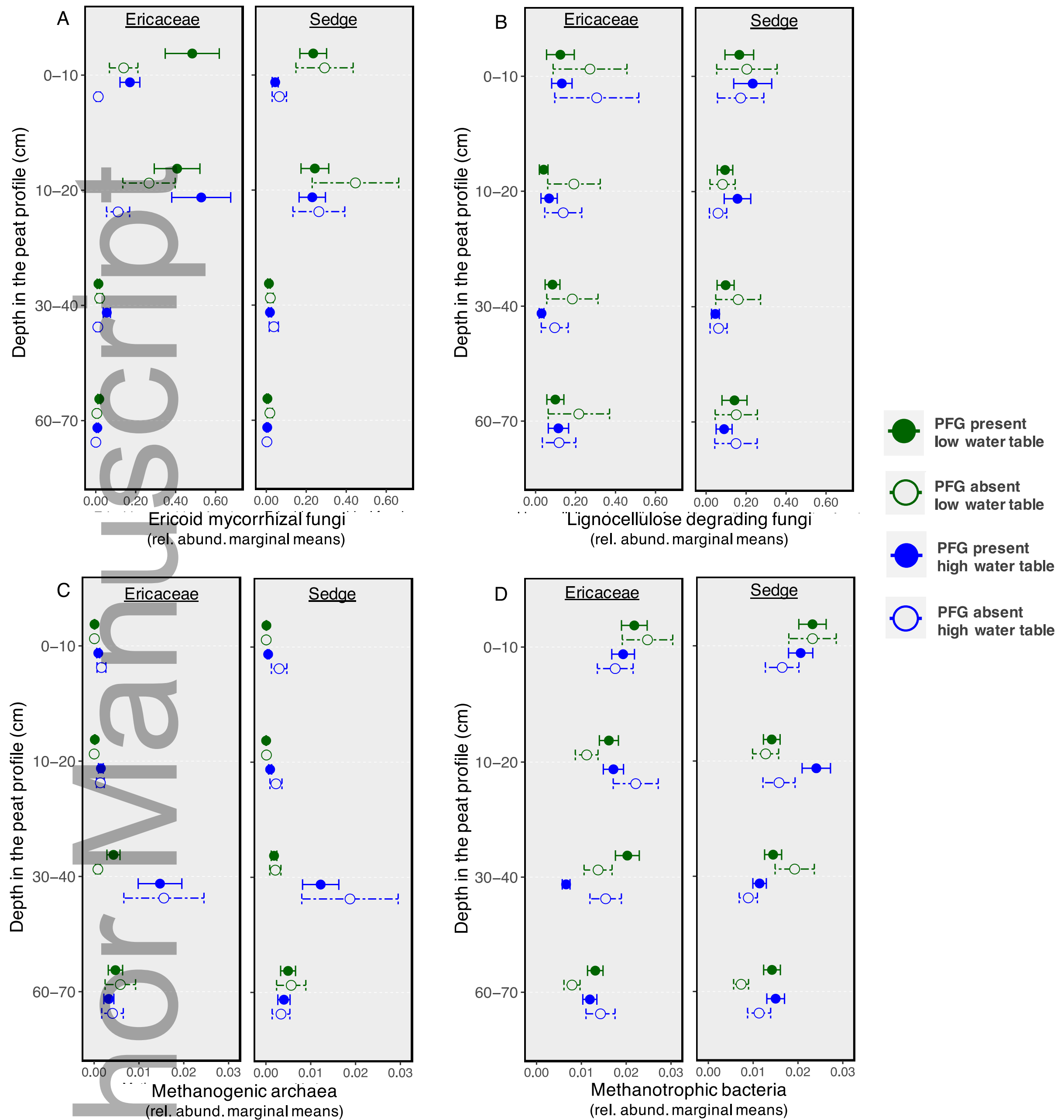

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964 **Figure 4.** Structural equation model results for fungi (A) and prokaryotes (B), and associated

965 model fit statistics and total effects (C), linking microbial responses to water table (WT

966 treatment), plant functional group manipulation (PFG treatment) and the vegetation community

967 (Ericaceae composition, Sedge composition). Path widths are scaled proportional to their path

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968 coefficients (*Rho* or partial-*Rho* values) and are dashed when not significant at an alpha-level of
969 0.05. Each variable is represented by a dissimilarity/distance matrix. Separate models were run
970 for each depth and each taxonomic group, using samples from year three of the experiment, and
971 A and B both represent the combined results of four different models. The core of the models
972 (black arrows) were equivalent for all models because the same plant community data was used
973 in each; paths and estimates specific to each depth's model are color coded. Models for the 10-20
974 cm depth required the addition of a direct path from PFG treatment to fungal or prokaryote
975 composition to obtain reasonable model fit, but models at other depths did not require this
976 additional path.

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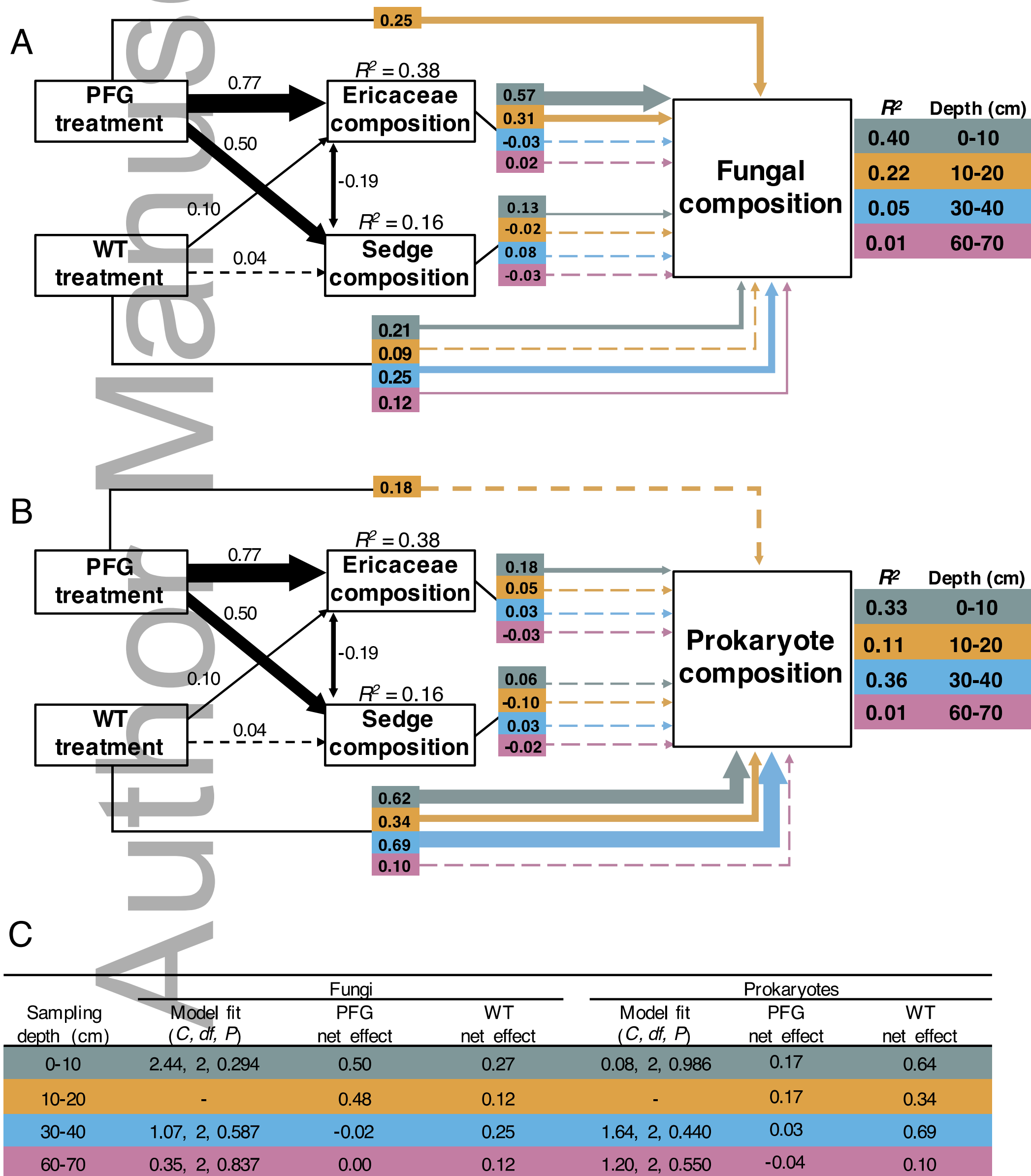

Sampling depth (cm)	Fungi Model fit (*C*, *df*, *P*)	Fungi PFG net effect	Fungi WT net effect	Prokaryotes Model fit (*C*, *df*, *P*)	Prokaryotes PFG net effect	Prokaryotes WT net effect
0-10	2.44, 2, 0.294	0.50	0.27	0.08, 2, 0.986	0.17	0.64
10-20	-	0.48	0.12	-	0.17	0.34
30-40	1.07, 2, 0.587	-0.02	0.25	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

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