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**The impact of long-term water level draw-down on microbial biomass: a comparative study from two peatland sites with different nutrient status**

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## ABSTRACT

We examined the effects of long-term (51 years) drainage on peat microbial communities using phospholipid fatty acid (PLFA) analysis. We analysed the peat profiles of natural and adjacent drained fen and bog sites. Viable microbes (i.e. microbial PLFA) were present in relatively large amounts even in the deepest peat layers of both peatland sites, a finding that warrants further investigation. Microbial biomass was generally higher in the fen than in the bog. Microbial community structure (indexed from PLFA) differed between the fen and bog sites and among depths. Although we did not exclude other factors, the effect of drainage on the total microbial biomass and community structure was not limited to the surface layers, but extended to the deepest layers of the fen and bog. Long-term drainage increased the total microbial PLFA biomass in the surface, subsurface and bottom layers of the fen, but decreased it in the surface and bottom layers of the bog site. Drainage also increased the characteristic FAs of Gram-positive and Gram-negative bacteria in the surface and subsurface layers of the fen, and decreased them in the bottom layers of the bog site. The characteristic fungal FA was only reduced in the surface layers of the bog site by drainage. Thus, by affecting the microbial community beyond the surface layers, long-term peatland water-level draw-down can alter the microbial contribution to deeper peat organic matter stabilization. This suggests that long-term drainage may have a more significant climate change effect than revealed by the surface layer analyses alone.



**Key words:** Fen; Bog; PLFA; long-term drainage; microbial biomass; Microbial community structure.


## 1. INTRODUCTION

Peatlands are crucial global carbon (C) stores [1,2], containing about 15 – 30% of all terrestrial organic C (OC); equivalent to 455 Gt ($10^{15}$ g) C [1]. Microbes are key actors (as catalysts) in all peat

biogeochemical processes, controlling the peat OC accumulation and decomposition [3]. They also contribute to the peat C exchange via respiration and, upon cell death, necromass addition to the peat soil organic matter (SOM), via the microbial carbon pump (MCP; [4]). Different microbial groups, with complementary enzymatic activities and different responses to environmental variables, interact in the peat C-cycling processes [5]. For example, the Gram–negative and Gram–postive bacteria are mainly associated with the mineralization steps involving labile and more recalcitrant C materials, respectively [6], and the exo-enzymatic capabilities of fungi make them important in the decomposition of macromolecules and recalcitrant C materials [7,8]. Changes in climate factors, such as hydrology, affect microbial community biomass and activity, both spatially and temporarily [9,10].

Models predict a warmer global climate (average temperature increases of about 4$^{o}$C) up to the year 2100 [11] and, under these scenarios, increased evapotranspiration due to increased temperatures would lead to a lower water table (WT) in peatlands [12]. Persistent draw-down of the peatland WT affects the niches of peatland microbes by increasing the thickness of the aerated surface layer [10]. The impacts of changed hydrology on the microbial community depends on the peatland type, intensity of change and the extent of change in space and time [9,13-15]. While changes in microbial niches may lead to increased diversity in the short-term, repeated replacement of specialist by generalist microbes may lead to loss of diversity in the long-run [14]. Changes in plant species cover following water level draw-down also modifies the influence of temperature and water content on peat microbial activities [16]. Studies have suggested that drainage could increase or decrease total microbial biomass or the biomass of some microbial groups, depending on the peatland type and depth [9,13]. Jaatinen et al [13] showed that fungi and actinobacteria suffer from drainage in a nutrient-rich fen, but that in a drained bog, while fungi either suffer or benefit, actinobacteria abundance remains the same or increases. Fungi and bacteria generally benefit (undergo biomass increase) from persistent drainage of wet mesotrophic fen sites, though actinobacteria suffer or show only minor responses [9,15]. Changes in peat C accumulation and decomposition activities following drainage have also been related to changes in the structure of below-ground microbial communities

[5,16]. Altered microbial diversity, due to drainage-induced changes in the quality and quantity of OC inputs, coupled with better oxygen availability, could increase the rate of soil OC (SOC) cycling; this leads to changes in the balance of peat-atmosphere C exchange [5,10,17].

Phospholipid-derived fatty acids (PLFA) are reliable quantitative biomarkers of viable microbes, since they are short-lived and readily metabolized upon cell death. Microbial biomasses, community structure and community responses to changing peat hydrology at different sites, have been studied with PLFAs [9,13,18]. Differences in microbial community structures between peatlands and the effects of treatments (e.g drainage) have also been analysed in several studies based on PLFAs alone [13,18-21], and their results are similar to those obtained using other molecular methods [22].

To our knowledge, previous studies on the effects of drainage on microbial communities (like those mentioned above), focused on the upper layers of peatlands (e.g. [13], [22]). However, drainage-induced increases in oxygenation coupled with temperature changes in the surface layers could prompt dissolved OC (DOC) release to deeper depths via the "enzymatic latch" process [23]. This increase in the flow and lability of DOC [24], coupled with deeper deposition of labile root exudates by roots of vascular plants [25-27], could modify the biomass of microbial communities and their composition in deeper peatland layers. Recent molecular evidence [17] and higher bulk peat stable C isotope ($\delta^{13}C$) values, indicating peat degradation in the bottom of drained peat, supports this view [17,28].

This study examined the effect of long-term drainage on microbial communities in depth profiles from surface to bottom layers of two peatland sites differing in nutrient status. We compared the biomasses and structures of the microbial communities (indexed by total and relative abundance of PLFA, respectively) between the natural and drained sides of fen and bog, representing boreal peatlands of different fertility after 51 years of water level draw-down. We also specifically investigated the effects of drainage on some selected microbial groups. We hypothesised that long-term WT decrease will (1) increase microbial biomass and (2) influence the microbial community

structure in the deep anoxic layers. We also hypothesized that (3) there will be higher microbial biomass increase in the fen than in the bog site, due to long-term drainage.

## 2. MATERIAL AND METHODS

### 2.1. Study sites

The study was conducted at two peatland sites (one fen and one bog) within the Lakkasuo boreal mire complex (61°47´N, 24°18´E, ca.150 m a.s.l.), in the Orivesi area in central Finland. At the nearest weather station to the sites in Juupajoki Hyytiälä (61°85´N, 24°29´E) the mean annual temperature was 3.5 °C and precipitation 711 mm for the period of 1981–2010 [29]. The sampling year was wetter than this long-term mean, with whole year precipitation of 907 mm and an average temperature of 3.2 °C. The mire complex comprises a large variety of typical Finnish mire site types [30]. Part of the Lakkasuo peatland was ditch-drained in 1961 (51 years before sampling) so that there are adjacent natural and drained sides of different fertility along a border ditch (Fig. S1). There were differences in the original fertility, WT and vegetation composition between the natural ombrotrophic cotton grass pine bog with *Sphagnum fuscum* hummocks (bog) and the natural minerotrophic tall sedge fen (fen) sites sampled. Drainage caused marked changes to the hydrology, peat and vegetation properties, carbon dioxide ($CO_2$) and $CH_4$ fluxes, especially at the drained fen ([28,30-33]; summarized in Table 1). For example, the six-month average WTs before the sampling date were -8.0 and -34.9 cm for the natural and drained fens, respectively, whereas it was -12.0 and -16.4 cm for the natural and drained bogs, respectively (Fig. 1). $CO_2$ fluxes increased in both sites whereas $CH_4$ fluxes ceased in the fen and were reduced by half in the bog after 30–32 years of drainage. Thus, there is strong evidence for significant, long-term changes in peat characteristics and greenhouse gas fluxes. The pH increased from the surface downwards in the natural and drained sides of both sites (Fig. S2). In general, the bog site is more acidic than the fen site and this was confirmed by previously

reported pH values (Fig. S2 & S3). Although temperatures vary seasonally, the temperature in deep peat is rather constant (~ 6 - 8 °C). The bulk densities (BD) at different depths of the drained and natural sides are the same in the bog site, but different in the fen site (Fig. S2).

## 2.2. Soil sampling and water table level measurement

### 2.2.1. Initial soil sampling and water table measurement

In 2012 (November 22$^{nd}$), three replicate sets of peat samples were collected from random points within each site, located several meters apart along a 50 m boardwalk. Soil was sampled from 4–5 depths (0 – 25 cm, 25 – 50 cm, 50 – 100 cm and deepest 25 cm) starting at the surface and extending to the deepest layer above the mineral soil. Using a Russian pattern side-cutting sampler (5 x 50 cm; [34,35]), samples were collected in segments along the profile from both the drained and adjacent natural sides. The samples were put into polyethylene bags, mixed and cooled immediately after collection in a box with crushed ice, and later stored at -20 °C until analysis. Part of the samples were oven dried and ground into a fine powder for analysis of their C and N content (Flash EA 1112 elemental analyser, Thermo Finnigan), with a certified birch leaf standard (Elementar Microanalysis, UK) used as a reference. Continuous (3-hourly) WT level measurements were recorded with an automatic WT-HR 64K logger (Fig. 1). The logger values were calibrated by manual measurements.

### 2.2.2. Additional peat properties measurements

Volumetric samples (from the same depths as the initial samples) were used for pH and temperature measurements, as well as for bulk density determination (Fig. S2). Sampling was done with a similar, but smaller, Russian pattern side-cutting sampler to that described above (5.2 * 50 cm; half cylinder diameter * length) on 14 October, 2015. Sampling and depth measurements were started

under a living *Sphagnum* carpet. Samples were transferred from the sampler into plastic bags (Aromata, Lidl Stiftung & Co, Neckarsulm, Germany) and were mixed in the bag before insertion of a pH electrode coupled with a temperature sensor (WTW P3 pH/conductivity with electrode SenTix 41; Weilheim, Germany). Values of pH and temperature were recorded after one minute. To determine bulk density, the samples in the bags were dried in the oven (Memmert, UM 500, Schwabach, Germany) at 80°C until there was no change in the dry weight.

## 2.3. PLFA analysis

### 2.3.1. PLFA extraction and quantification

PLFA analysis was done following the protocol used by Tavi et al. [36] with some modifications. Freeze-dried and mixed peat samples from each depth profile were weighed into 50 ml extraction tubes (> 3 g dry weight of peat) using tools cleaned with methanol. Total lipids were extracted from the samples using a 1:2:0.8 (vol:vol:vol) ratio of chloroform–methanol–50 mM phosphate buffer [37]. Tubes were closed under a nitrogen flow, mixed and shaken at 200 rpm overnight. Dipentadecanoylphostatidylcholine ($C_{38}H_{76}NO_8P$) (Larodan Fine Chemicals) was added as an internal standard for quantification of PLFAs. After shaking for another five minutes, the samples were centrifuged at 2500 rpm for 15 minutes. The volume of the supernatant was measured and adjusted with chloroform and phosphate buffer to a ratio of 1:1:0.9 (vol:vol:vol) of chloroform-methanol-phosphate buffer. Samples were centrifuged again (2500 rpm, five min.) and the lower organic phase (total lipids) was evaporated to dryness. The total lipids were fractionated on a silicic acid column (Agilent silica-based HF Bond Elut LRC-SI, 500 mg, Varian), into neutral, glyco-, and phospholipids using 10, 20 and 10 ml of chloroform, acetone and methanol, respectively. The phospholipids fraction was evaporated to dryness under nitrogen flow and methylated using the protocol in Virtue et al. [38], but at 60–80 °C for two hours. Methylation standard nonadecanoic acid

($C_{20}H_{40}O_2$) (Sigma-Aldrich) was added just before methylation and was used to quantify the methylation efficiency. To collect methylated fatty acids (FAs), two ml of hexane/chloroform (4:1, vol:vol) were added to the samples, after which the samples were vortexed and centrifuged at 2000 rpm for five minutes. The top organic layer was then transferred, dried (under a nitrogen stream) and re-dissolved in a known volume of n-hexane.

The methylated FAs were analysed using an Agilent 6890 GC connected to an Agilent 5973 mass selective detector. The methylated FAs were separated with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm), using helium as a carrier gas. The samples were injected by splitless injection using the constant flow mode and using similar settings as Kaneko et al. [39]. The initial oven temperature was 50 °C, and subsequently it was increased by 30 °C $min^{-1}$ to 140 °C and then by 5 °C $min^{-1}$ to 320 °C. This final temperature was held for 20 min leading to a total run time of 60 min. Peaks were identified based on their relative retention times and mass spectra measured in SCAN mode. The retention times of the peaks were also compared with the retention times of the fatty acid methyl esters (FAME) in the standard mix (Supelco 37 component FAME mix). The internal standard PC (Dipentadecanoylphostatidylcholine) 15:0 was used for quantitative analysis. Dimethyl disulphide (DMDS) adducts were prepared, analysed and used in the determination of the position of double bonds in the monounsaturated FAMEs [40]. The FA contents [µg $g^{-1}$ dry weight of soil (dw) and % of PLFAs] were calculated. In order to account for variation in the peat compaction between the drained and natural sides, between the fen and bog sites and among the different depth layers, the amount of microbial FA in µg $g^{-1}$ dw was converted to g $m^{-3}$ using the dry bulk densities (BD; Fig. S2).

2.3.2. Characteristic FAs of Gram-negative and Gram-positive bacteria and fungi

PLFA biomarkers common among general microbial groups, such as Gram-negative bacteria, Gram-positive bacteria and fungi, were selected and grouped. Iso- and anteiso branched PLFAs,

182 i14:0, i15:0, a15:0, a17:0 and i17:0, typical of Gram-positive bacteria [18,41,42] were grouped as
183 Branched FA (BrFA). 16 monounsaturated fatty acids (MUFAs), 16:1ω5, 16:1ω6c, 16:1ω7c and
184 16:1ω8c, as well as 18 MUFAs, 18:1ω5, 18:1ω6c, 18:1ω7c, 18:1ω7t and 18:1ω8c, both of which are
185 typical of Gram-negative bacteria [42], were grouped separately. The 18:2ω6 FA, typical of fungi,
186 [21] was studied as fungi FA.
187
188 2.4. Data analysis
189
190 The effect of drainage (at each site and depth layer) and depth (at each natural and drained side)
191 on the total microbial PLFA biomass, and the absolute amounts and relative abundances of the
192 selected microbial group FAs (16 MUFAs, 18 MUFAs, BrFA and fungi FA), were tested using
193 independent sample t-test and one-way ANOVA, respectively. Correlations of the total microbial
194 PLFA biomass with pH and BD were tested using Spearmann correlation analysis. Multivariate
195 analyses of the PLFA profiles were based on Bray-Curtis dissimilarities calculated among samples
196 using $\log_{10}(x+1)$ transformed data of the relative abundances (% composition) of the PLFA. The data
197 were assessed graphically using non-metric multidimensional scaling (NMS) constrained to 2
198 ordination axes. NMS was done for the whole (natural and drained) fen and bog site data to depict
199 the overall patterns among sides, drainage and depth zones. Furthermore, the effect of depth and
200 drainage on PLFA profiles in both fen and bog were tested using 2-way permutational multivariate
201 analysis of variance (PERMANOVA) [43,44] with both factors as fixed factors. Mantel´s test was
202 used to analyse correlations of the PLFA profiles with pH and BD. The Mantel correlation test result
203 is only meaningful when the result is positive, which shows correlation. The positive sign in our
204 correlation results means that, the larger the change in the pH or BD, the larger the change in microbial
205 community structure (PLFA structure). ANOVA was done using IBM SPSS Statistics version 23.
206 NMS and Mantel´s tests were performed using PC-ORD version 6.0 ([45]; PC-ORD. Multivariate

analysis of ecological data. MjM Software, Gleneden Beach, Oregon, USA). PerMANOVA was done using FORTRAN program by Anderson [46].

## 3. RESULTS

### 3.1. Total microbial PLFA biomass and structure

We only analysed PLFAs between C10 and C20, which represents the main range for prokaryotic PLFAs and a few other microbial groups, such as fungi. The most common FA in the samples was C16:0 (a universal FA), which contributed (mean ± SE) 10.0 ± 0.3 % to the whole depth PLFA profile of the fen site (natural + drained) and 10.1 ± 0.4 % to the whole depth PLFA profile of the bog site (natural + drained). The total microbial PLFA biomass (g $m^{-3}$) was higher in the natural fen than the natural bog site at all the depths, except for the 25 – 50 cm depth, where it did not differ between the fen and bog (Fig. 2A, Table S1). There was no correlation between pH and the total microbial PLFA biomass either in the combined dataset of the fen and the bog, or the fen and the bog separately. In all but the 50 – 100 cm depth of the fen site, total microbial PLFA biomass was higher in the drained than the natural side. In the bog site, the total microbial PLFA biomass was only different between the drained and natural side at the top and bottom layers, where the amount was smaller in the drained side (Fig. 2A, Table S1).

There were also depth differences in the total microbial PLFA biomass in both the natural and drained fen, but only in the natural side of the bog (Fig. 2A, Table S2). In both the natural and drained fen, the amount of microbial PLFA biomass in the surface layer (0–25 cm) was higher than in the 25–50 cm layer, but similar to that in the bottom layer. In the drained fen, the total microbial PLFA biomass was also higher in the surface layer than in the 50–100 cm layer (Table S2). In the natural bog, the amount of microbial PLFA biomass was higher in the surface layer than in the other depth layers, which did not differ from one another (Table S2).

As visualised by NMS ordination, there were differences in the microbial community PLFA compositions between the fen and bog sites (Fig. 3). NMS further suggests that the variation in the microbial community structure was explained by both depth and drainage in both the fen and bog sites (Fig. 3). This was confirmed by a two-way factorial (drainage and depth) analysis (PERMANOVA), which showed that drainage and depth independently affected the microbial community structure in both the fen and bog sites (Tables 2 & 3). There was correlation between pH and the community structure in the combined dataset of the fen and bog (Mantel´s test, $r = 0.42$, $p < 0.001$, $n = 16$) as well as in the fen (Mantel´s test, $r = 0.48$, $p < 0.05$, $n = 8$) and bog (Mantel´s test, $r = 0.52$, $p < 0.05$, $n = 8$) alone. Correlations between BD and community structure were also detected in the combined dataset (Mantel´s test, $r = 0.30$, $p < 0.01$, $n = 16$) as well as in the bog (Mantel´s test, $r = 0.57$, $p < 0.01$, $n = 8$), but not in the fen.

### 3.2. Characteristic FAs of Gram-negative and Gram-positive bacteria and fungi

The overall concentration of all the major microbial group PLFAs followed the same trend as the total microbial PLFA, being higher in the fen than the bog site, especially in the drained side (Fig. 2B). They were also mostly highest in the surface layer than the deeper layers of both sites. The amount (g $m^{-3}$) of 16 C monounsaturated fatty acids (16 MUFAs), which are characteristic of Gram-negative bacteria, was higher in the top two layers (0 – 25 and 25 – 50 cm) of the drained side of the fen than the natural side, but lower in the bottom layer (only) of the drained side of the bog than the natural side (Fig. 2B). The relative contribution of 16 MUFAs to the total microbial PLFA (% contribution) did not differ between the drained and the natural sides in either fen or bog except at the 50 – 100 cm depth of the bog site (Fig. 4A). Depth only affected the amount of 16 MUFAs (g $m^{-3}$) in the drained fen and natural bog sides. In both cases, the amount (g $m^{-3}$) of 16 MUFAs was higher in the surface (0 – 25 cm) layer than the other depth layers, which were similar to one another (Fig. 2B, Table S2). There was also no depth effect on the relative contribution of 16 MUFAs to the total

PLFAs except in the natural bog, where it was higher in the surface layer (0–25 cm) than at the other depths, which were similar to one another (Fig. 4A, Table S3).

The amount (g $m^{-3}$) of 18 C monounsaturated fatty acids (18 MUFAs), which are also characteristic of Gram-negative bacteria, was higher in the sub-surface layer (25 – 50 cm) of the drained fen side compared to the natural fen side, and lower in the bottom layer of the drained bog side compared to the natural bog side (Fig. 2C). The relative contribution of 18 MUFAs to the total microbial PLFA (% contribution) did not differ between the drained and the natural sides of either fen or bog (Fig. 4B). There were depth differences in the amount of 18 MUFAs (g $m^{-3}$) in all sides except in the drained bog. In the sides with depth differences, the amount (g $m^{-3}$) of 18 MUFAs was higher in the surface (0 – 25 cm) layer than the other depth layers, which were similar to one another (Fig. 2C, Table S2). There was also a depth effect on the relative contribution of 18 MUFAs to the total PLFAs in all the sides, except the drained bog side (Fig. 4B, Table S3).

The amount (g $m^{-3}$) of terminally branched fatty acids (BrFAs), which are characteristic of Gram-positive bacteria, was higher in the top two layers (0 – 25 and 25 – 50 cm) of the drained fen than the natural fen side, but lower in the sub-surface (25 – 50 cm) and bottom layers of the drained bog than the natural bog side (Fig. 2D). The relative contribution of BrFAs to the total microbial PLFA (% contribution) did not differ between the drained and natural sides of either fen or bog (Fig. 4C). There were only depth differences in the amount (g $m^{-3}$) of BrFAs in the drained sides of both the fen and bog. The amount of BrFAs in the surface layer (0 – 25 cm) of the drained fen and the top two layers (0 – 25 and 20 – 50 cm) of the drained bog sides were higher than in the other depth layers, which were similar to each other (Fig. 2D, Table S2). There was no depth effect on the relative contribution of BrFAs to the total PLFAs in any of the sides, except the natural bog side (Fig. 4C, Table S3).

The amount (g $m^{-3}$) of fatty acids characteristic of fungi (fungi FA) did not differ between the drained and natural fen sides, but was lower in the surface layer (0 – 25 cm) of the drained bog side than the natural bog side (Fig. 2E). Neither drainage nor depth affected the relative contribution of fungal FA to the total microbial PLFA (% contribution) in either fen or bog sites (Fig. 4D, Table S3).

There were also no depth differences in the amount (g $m^{-3}$) of fungal FAs in any of the sides (Fig. 2E).

## 4. DISCUSSION

### 4.1. Biomass and community structure of microbes

The higher total microbial PLFA biomass in the natural fen compared to the bog is best explained by the higher nutrient content and pH in the fen (Table 1 and Fig. S2) [5,13,14,19,47]. Concomitant differences in vegetation cover affect microbes due to differences in soil structure and C substrate availability. Biomass of fen vegetation is more easily decomposed than that of bog vegetation, which consists largely of recalcitrant *Sphagnum* mosses [5,13,48]. The roots of sedges in fens provide better soil stability and macro pore structure than those of *Sphagnum* mosses on the bogs. The higher microbial PLFA biomass in the fen is also reflected in the higher $CO_2$ and $CH_4$ emissions from the fen (natural side; Table 1) [28,49,50]. Differences in microbial biomasses were also accompanied by differences in the microbial community structures of the fen and bog, which are due to similar reasons as those stated above in addition to differences in the natural wetness of the sites [13,47].

There was viable microbial biomass in all the peat layers of both the fen and the bog sites (Fig. 2). The drastic reduction in total microbial PLFA biomass from the surface layer to the 25–50 cm layer in the fen but not the bog is likely due to depth related differences in the fertility and litter quality [47] (Table S4). In general, the microbial biomass PLFA (g $m^{-3}$) in the fen increased with increasing nitrogen (N %) and carbon (C %) content and decreasing C/N ratios, but this was not the case in the bog site. There was no decrease in microbial biomass with depth (depth effect) in the bog site, probably due to poor substrate quality (higher C/N ratio). Quite surprisingly, the total microbial PLFA biomass did not differ between surface layers and bottom layers except in the natural bog. Although PLFA-analysis detects viable cells and indicates changes in the potentially active microbial biomass,

311 it cannot separate the active and non-active cells [51]. We also acknowledge that the turnover rate of
312 PLFAs in the deep anoxic peat layers is unknown and could be considerably slower than in the oxic
313 and warmer surface peat layers. This means that detection of FAs in deeper peat layers may not be
314 indicative of similar cell activities as in the surface peat layers. However, the presence of potential
315 (enzymatic) prokaryotic microbial activity [52] and active microbial populations [53] have previously
316 been reported for deep peat layers (100 cm to 300 cm), which supports our finding of living microbes
317 in bottom peat layers. Our community structure analyses (% PLFA profiles, Table 3, Fig. 3; see also
318 4.3 below) also indicated differences in the microbial groups occupying different depths, possibly
319 due to community adaptations to depth-related changes in several factors, e.g. pH as shown here,
320 oxygen availability, alternative electron acceptors and substrates [5,54]. There is high temperature
321 variability at the surface, but low and stable temperature at the bottom ( Fig. S2 and [31]), which
322 could also modify the community structure. Since there are large amounts of C stored in deeper peat
323 layers, the significant amount of microbial biomass in these layers may have implications for the
324 global C cycles, such as microbial-enhanced C flows from peat to mineral subsoil. There is an
325 estimated average 13.6 g $m^{-2}$ $yr^{-1}$ C input from peat into mineral subsoils [55], which may increase
326 with changes in deep peat microbial biomass and composition.
327
328 4.2. Effects of drainage on the biomass and community structure of microbes
329
330 Drainage increased total microbial PLFA biomass in the surface, subsurface and bottom layers of
331 the fen, supporting our first hypothesis, but decreased it in the surface and bottom layers of the bog
332 site, which is contrary to our first hypothesis. This is possibly due to differences in their nutrient
333 status, thickness of the aerated layer, available substrate and vegetation changes [13,22,47,56]. In
334 general, while drainage in the fen led to a succession towards a different ecosystem from the original,
335 in the bog site it led to smaller changes. Typical fen species, such as tall sedges, were succeeded by
336 spruce swamp and forest species, such as *Pinus sylvestris, Betula pubescens and Polytrichum*

*commune*. At the bog site, mosses and dwarf shrubs decreased while the forest species, mainly *Pinus sylvestris,* increased *and Pleurozium schreberi* appeared (see also Table 1). Thirty-eight years after drainage, tree stand volume increased from nothing in the natural fen to 111 $m^{-3}$ $ha^{-1}$ in the drained fen side and from 5 in the natural bog to 16 $m^{-3}$ $ha^{-1}$ in the drained bog side [31]. The increased tree growth in the drained fen led to greater evapotranspiration and further decrease in the WT. Although the WT depth at the time of sampling was about the same in both sites due to heavy rainfall in the previous weeks, the six-month mean WT depths before our sampling date were -8.0 and -34.9 cm at the natural and drained fen, respectively and, -12.0 and -16.4 cm for the natural and drained bog, respectively. The previously reported annual mean WT depths were also much lower in the drained fen than in the drained bog for most parts of the year (Fig. 1 and Fig. S4). The much lower WT in the drained fen therefore explains why there were drainage-induced changes in the total microbial PLFA biomass in the surface and sub-surface layers of the fen, but only in the surface layer of the bog site. Other reasons could be the originally lower substrate quality and availability in the bog, which has become poorer after a few decades of drainage (Table S4) [5,13,48]. Our C/N ratio results showed similar ratios along the profiles of both the natural and drained fen, suggesting that the increase in microbial PLFA biomass was not due to differences in peat quality. However, the C/N ratios in the bog profiles were significantly higher in the drained side, especially in the bottom layer. High C/N ratio usually indicates low substrate quality, which could also explain the low microbial biomass in the drained bog. We cannot conclusively explain the differences in C/N ratios between the natural and drained bog sides, which are possibly caused by loss of N after drainage. Since drainage increases N losses by leaching and plant uptake, it means that we are discussing both the direct and indirect effects of drainage. We also note that there could have been natural differences in the peat qualities of the bog site before drainage. However, we believe that our basic assumption that the original peat quality was similar holds true, and that the differences we found can be associated to drainage. Hence, in accordance with our third hypothesis, drainage increased the total microbial biomass more in the

362 fen than in the bog site. The overall effect of drainage on microbial biomass was reflected by the
363 increased $CO_2$ and decreased $CH_4$ efflux, especially in the drained fen (Table 1).

364 The total microbial PLFA biomass in the deepest (bottom) layers of both the fen and bog sites
365 were different between the natural and drained sides. This was due to changes in the flow and
366 constituents of the leachate water [24], deeper deposition of labile root exudates by vascular plants
367 and differences in the amount and quality of DOC reaching the bottom layers in the drained sides
368 [26,27]. Although the roots of vascular plants may not reach the bottom layers, their exudates will
369 get to microbes in the bottom layers via water movement at a faster rate than those deposited by the
370 shallow roots of mosses. The results of DOC isotopic analysis and tritium analysis of pore water, by
371 Charman et al [24], supported the downward movement of younger C via water movement in
372 ombrotrophic boreal peatland. The study also showed that $CO_2$ and $CH_4$ (based on $^{14}C$ dating) from
373 the deeper peat layers was younger than that from the surrounding peat. They attributed this to the
374 transfer of DOC and gaseous C compounds to deeper peat via water movement, and the microbial
375 usage of younger C. Furthermore, they concluded that low hydraulic conductivity in peat may not be
376 a real limitation to water movement over a long time scale. Although mainly studied in a fen peat
377 site, Krüger et al. [28] also reported the effect of drainage in the deeper peat profiles, which showed
378 higher $\delta^{13}C$ values (a qualitative indicator of peat degradation) in the drained than the natural side.
379 They also reported much older peat in the drained sides of both fen and bog. This also suggests an
380 effect of drainage on deeper peat microbial activity, as microbes use younger C that is more enriched
381 with $^{14}C$ and DOC is a potential source of relatively younger C (leaving $^{14}C$ depleted older peat
382 behind) [24]. By affecting the deep peat microbial processes [28] and biomass, long-term drainage
383 may have additional significant effects on the peat C balance, besides the enhanced C losses from
384 surface peat. Increased microbial biomass in the bottom peat layers due to drainage can either
385 contribute to the peat organic matter (OM) stabilization via the microbial C pump (MCP) process
386 ([4]; not studied in peatland yet), or enhance deep peat microbial degradation [28] and C flow to sub-
387 soil [55]. Further studies are needed to elaborate on this.

There were, however, no differences in the total microbial biomass in some subsurface layers (25 – 100 cm in bog and 50 -100 cm in fen) of the natural and drained sides. Inconsistent patterns in the long-term drainage-induced changes, especially in the subsurface layers, have been previously reported [13]. This is possibly because the different microbial communities at the different depths in the pristine peatlands [5,13,54] have different sensitivities to changed hydrology and react differently to drainage-induced changes [5,13,15,19]. Since the effect of drainage on microbial communities of the deeper anoxic layers also depends on the movement of materials (e.g. DOC) from the surface to deeper layers [24,26,27], accumulation of materials to concentrations that can cause significant changes in microbial biomass is less probable in the intermediate layers than the deepest layers many years after drainage [24].

Similar to in several previous studies, peat microbial community structure in this study was indexed by the relative composition of microbial PLFAs [13,18-21,57]. According to our second hypothesis, our results showed that long-term drainage affected the microbial community structures in all the depth layers including the bottom (deepest) layers. Jaatinen et al. [13] and Urbanová and Bárta [22] studied the effects of long-term drainage on microbial communities in the surface layers (0 -30 cm) of peatlands. They also showed that long-term drainage and the resulting changes in vegetation pattern altered the microbial community structures in different peatland types. Our own measurements and those from previous studies showed that the pH in both the fen and bog sites were mostly reduced by drainage in all the depth layers (Fig. S2 and S3). Therefore, the correlation between the microbial community structure and pH in the natural and drained sides of both the fen and bog sites (by Mantel´s test) partly explains the changes in the microbial community structures at the different depth layers [13]. Other reasons for the changes in microbial community structure include changes in the anoxic/oxic condition of the surface layer and the available litter quality at different depths, occasioned by the drainage-induced changes in the prevailing plant communities and structures (see table 1 and paragraph 1 of 4.2) [16,19,56].

414

415 4.3. Characteristic FAs of Gram-negative and Gram-positive bacteria, and fungi

416

417 Similar to in previous studies [13-15], drainage and depth-induced effects on the biomass of the
418 FAs characteristic of our microbial groups, varied among the microbial groups and between the fen
419 and bog sites. The higher concentrations of all the FAs characteristic of the major microbial groups,
420 except the fungal-characteristic FA, in the natural and drained fen than the bog site was due to the
421 same reasons given for total PLFA biomass (in 4.1). The biomass of both the Gram-negative and
422 Gram-positive bacteria characteristic FAs were increased in the surface and subsurface layers of the
423 drained fen side, probably due to drainage-induced increases in aeration and substrate availability.
424 However, they were decreased in the bottom layer of the bog site, possibly due to low substrate
425 quality, which was revealed by the C/N ratio in the drained side. The fungal characteristic FA was
426 only reduced in the surface layer of the bog, likely due to low peat biomass quality and differences in
427 the sensitivities of different fungal species to drainage [15]. For example, Peltoniemi et al [15] showed
428 that basidiomycetes are more sensitive to drainage than ascomycetes, though they are within the same
429 fungal phyla represented by the same characteristic FA.
430 Drainage-induced effects on the relative abundance of our selected microbial group characteristic
431 FAs were not observed, except for 16 MUFAs in the 50 – 100 cm depth of the bog site. This was
432 probably due to the contrasting drainage responses of the different species in these groups [9,13, 15].
433 Kim et al. [14] also reported no differences in the diversity and composition of denitrifiers and
434 methanogens in any of their sites following a short-term drought. The depth-induced effects were
435 also inconsistent among the groups and between the fen and bog sites (Fig.4, Table S3). This was
436 probably because these microbial groups contained different species with contrasting depth
437 stratification patterns. Our 25 cm sample depth-range may also be too long to reveal the inconsistent
438 changes observed in the shorter sampling depth-ranges for specific microbial groups (e.g. [9,13,22]).
439 For example, Lin et al. [52] reported an increasing proportion of yeast (*Saccharomyces*) and a

reduction of the white-rot fungi (*Agaricomycotina*) with depth, although both of them are fungi. The effect of drainage on the relative abundances of the mainly aerobic bacteria FAs (16 MUFAs) in the 50 – 100 cm layer of bog may be due to change from a permanently anoxic to at least episodic oxic conditions in the subsurface layers (see Fig. 1; [13]). Using techniques with higher taxonomic resolution than PLFA - analysis, e.g. next-generation sequencing of 16S rRNA gene amplicons, could give better insight to the drainage- and depth-induced effects observed with the total microbial community. Nevertheless, it is clear from our results that the depth-induced effects on the microbial community structure vary between the two sites and between the treatments. This suggests that the factors regulating depth-induced effects on specific microbial species (within the groups) differ between sites.

## 5. CONCLUSIONS

Our study shows that the biomass and the structure of *in-situ* microbial communities differ between the studied fen and bog sites. While long-term water level draw-down significantly increased the total microbial biomass in the affected layers of the fen, it decreased the total microbial biomass in the affected layers of the bog, which is likely due to the differences in their original nutrient statuses, the level of WT depth reduction and substrate quality. There was a considerable amount of living microbial biomass even in the deepest and oldest (3000-3400 years) layers of the studied peatlands. Although drainage mostly affects microbial community in the surface layers, the effect of long-term WT draw-down on deeper peat microbes is also important, due to the long-term storage of large amounts of C in deeper peat layers. Drainage-induced increase in microbial biomass, measured in this study for the fen site, can enhance microbial contribution to deeper peat OM stabilization via the microbial C pump (MCP) or increase microbial peat degradation and C release in the bottom peat layers. This suggests a more significant effect of drainage on climate change than revealed by surface layer analyses alone. Further studies are needed to elaborate on this. Since peatland drainage has been

used to model climate change effects on peatlands [11], our results might be revealing climate change effects on deep peat microbial biomasses.

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626

627 **Captions for figures**

628 **Fig. 1.** Average daily water table (WT) depth in the sampled peatland sites from May to December
629 of the sampling year

630

631 **Fig. 2.** Mean (± SE, n = 3) amount (g $m^{-3}$) of (A) microbial total PLFA, (B) 16 MUFAs, (C) 18
632 MUFAs, (D) terminally branched FAs and (E) FA characteristic of fungi in all the depths and sites
633 sampled. Significant differences ($p < 0.05$) between the drained and natural sides at each site in each
634 depth following independent sample t-tests are denoted by an asterisk (*). For depth effects Fn = fen
635 natural, Fd = fen drained, Bn = bog natural and Bd = bog drained, while sites with significant depth
636 effects (1-ANOVA, $p < 0.05$) are highlighted in bold. Bottom = 135 – 160 and 105 – 130 for fen
637 natural and drained, 217 – 242 and 245 – 270 for bog natural and drained respectively.

638

639 **Fig. 3.** Non-metric multidimensional scaling (NMS) ordination of PLFA [log10(x+1) of the relative
640 abundance of individual PLFAs]. Average (± SE, n = 3) NMS axes scores of the sites. Axes are
641 arbitrary; the closer the sample points are on the plot, the more similar they are in PLFA composition.
642 Depth 0–25 cm (○), 25–50 cm (Δ), 50–100 cm (□) and bottom layers (◇). The drained and natural
643 sides of both the fen and bog sites differ significantly ($p < 0.05$) in a two-way factorial (drainage and
644 depth) analysis (PERMANOVA, see Table 2).

645

646 **Fig. 4.** Mean (± SE, n = 3) relative abundance (% contribution to total microbial PLFAs) of (A) 16
647 MUFAs, (B) 18 MUFAs, (C) terminally branched FAs and (D) FA characteristic of fungi at different
648 depths in the studied fen and bog sites. Significant differences ($p < 0.05$) between drained and natural
649 sides at each depth and for each microbial group following independent sample t-tests are denoted by
650 an asterisk (*). For depth effects Fn = fen natural, Fd = fen drained, Bn = bog natural and Bd = bog

651 drained, while sites with significant depth effects (1-ANOVA, $p < 0.05$) are highlighted in bold.
652 Bottom = 135 – 160 and 105 – 130 for fen natural and drained, 217 – 242 and 245 – 270 for bog
653 natural and drained respectively.

654

655 Table 1. General features of the study sites

| Site | **Fen** | | **Bog** | |
|---|---|---|---|---|
| Managements | Natural | Drained | Natural | Drained |
| Peatland type[1,2] | Tall sedge fen | Tall sedge fen planted with scots pines | Cottongrass pine bog with *Sphagnum fuscum* hummocks | Cottongrass pine bog with *Sphagnum fuscum* hummocks |
| Tree stand volume[1] ($m^{-3}$ $ha^{-1}$) | 0 | 111 | 5 | 16 |
| Peat thickness[1] (cm) | 168 | 140 | 267 | 244 |
| $CO_2$ flux[3] (g $CO_2$ - C $year^{-1}$) | 188 | 356 | 164 | 236 |
| $CH_4$ flux[2] (g $CH_4$ - C $year^{-1}$) | 31.0 | -0.0 | 4.8 | 2.7 |
| Peat bottom age[1] (years) | 3400 | | 3000 | |
| Peat constituent[1] | C (L, S, Er) | | S (Er, L) | |
| Vegetation[2,4] | Ap Cl Cr Ev Ps Sa Sp Sf Bn | Pc Ps Ac Bp Ce Dc Psc Sa **Psy Bp** | Sa Ap Ev Ps Rc Sf En **Psy** | Cs Dp Ev Psc Sr **Psy** |
| C (%) (surface) | 50.1±1.1 | 53.2±0.4 | 47.9±0.5 | 46.8±0.4 |
| N (%) (surface) | 2.5±0.3 | 2.3±0.4 | 1.2±0.2 | 0.86±0.1 |
| P (µg $g^{-1}$)[1] (surface) | 0.82 | 1.20 | 0.37 | 0.50 |

656 Lakkasuo mire complex features are adopted and modified from [1]Minkkinen *et al*. (1999) [31], [2]Nykänen *et al* (1998) [32], [3]Silvola *et al* (1996) [33] or [4]Laine et al.
657 (2004) [30]. Peat constituents: C = *Carex,* L = *Lignum,* S = *Sphagnum,* Er = *Eriophorum.* Vegetation cover: Ac, *Agrostitis capillaris*; Ap, *Andromeda polifolia*; Bp,
658 *Betula pubescent*; Bn, *Betula nana*; Ce, *Carex echinata*; Cl, *Carex lasiocarpa*; Cr, *Carex rostrate*; Cs, *Cladonia* sp.; Dc, *Dryopteris carthusiana*; Dp, *Dicranum*
659 *polysetum*; En, *Empetrum nigrum*; Ev, *Eriophorum vaginatum*; Pc, *Polytrichum commune*; Ps, *Polytrichum strictum*; Psc, *Pleurozium schreberi*; Psy, *Pinus*
660 *sylvestris*; Rc, *Rubus chamaeomorus*; Sa, *Sphagnum angustifolium*; Sf, *Sphagnum fuscum*; Sp, *Shagnum papillosum*; Sr, *Sphagnum russowii*. Dominant tree species
661 marked with bold.

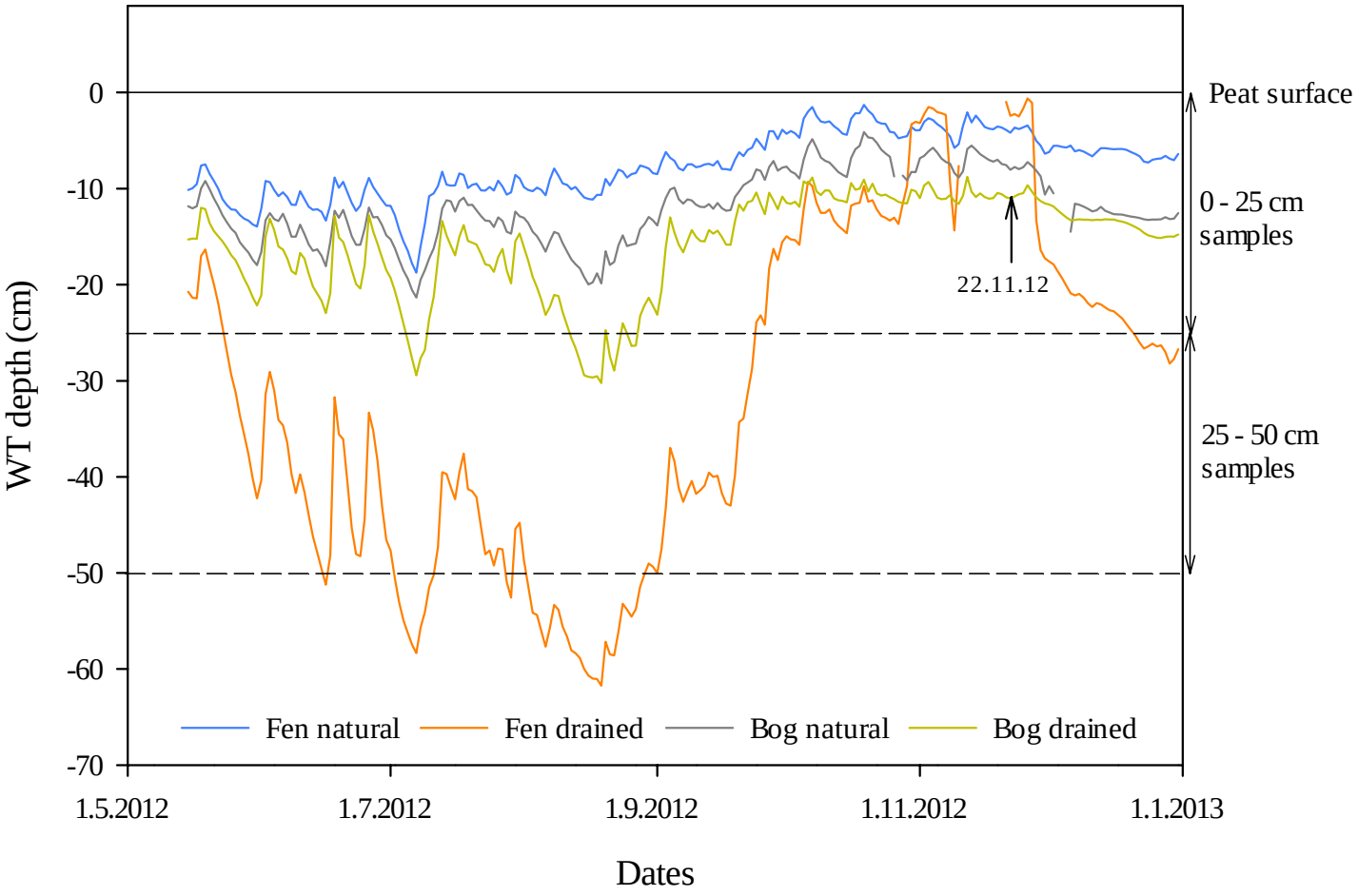
Table 2. Two-way factorial (drainage and depth) analysis (PERMANOVA) explaining the structural variation in the analysed microbial communities, between depths (0 –25 cm, 25 – 50 cm, 50 – 100 cm, bottom) and between drained and natural sides in fen and bog sites. Analysis was done with $\log_{10}$ (x+1) -transformed PLFA relative abundance (% composition) data. Significant depth and drainage effects ($p < 0.05$) are indicated with '*'.

| | | **Fen** | | **Bog** | |
|---|---|---|---|---|---|
| | df | Rel. abundance | | Rel. abundance | |
| | | *F* | *p* | *F* | *p* |
| Drainage | 1 | 2.89 | 0.0194* | 6.76 | 0.0001* |
| Depth | 3 | 3.16 | 0.0005* | 7.52 | 0.0001* |
| Interaction | 3 | 1.08 | 0.3704 | 1.09 | 0.3854 |

**Table 3.** Difference in the structure of microbial community among depths in fen and bog sites, using the $\log_{10}$ (x+1) of PLFA relative abundance (% composition). Significant differences ($p < 0.05$) among depths from pair-wise analyses following 2-way (PERMANOVA; Table 2) analysis are shown with letters (different letter denotes significant differences among depths).

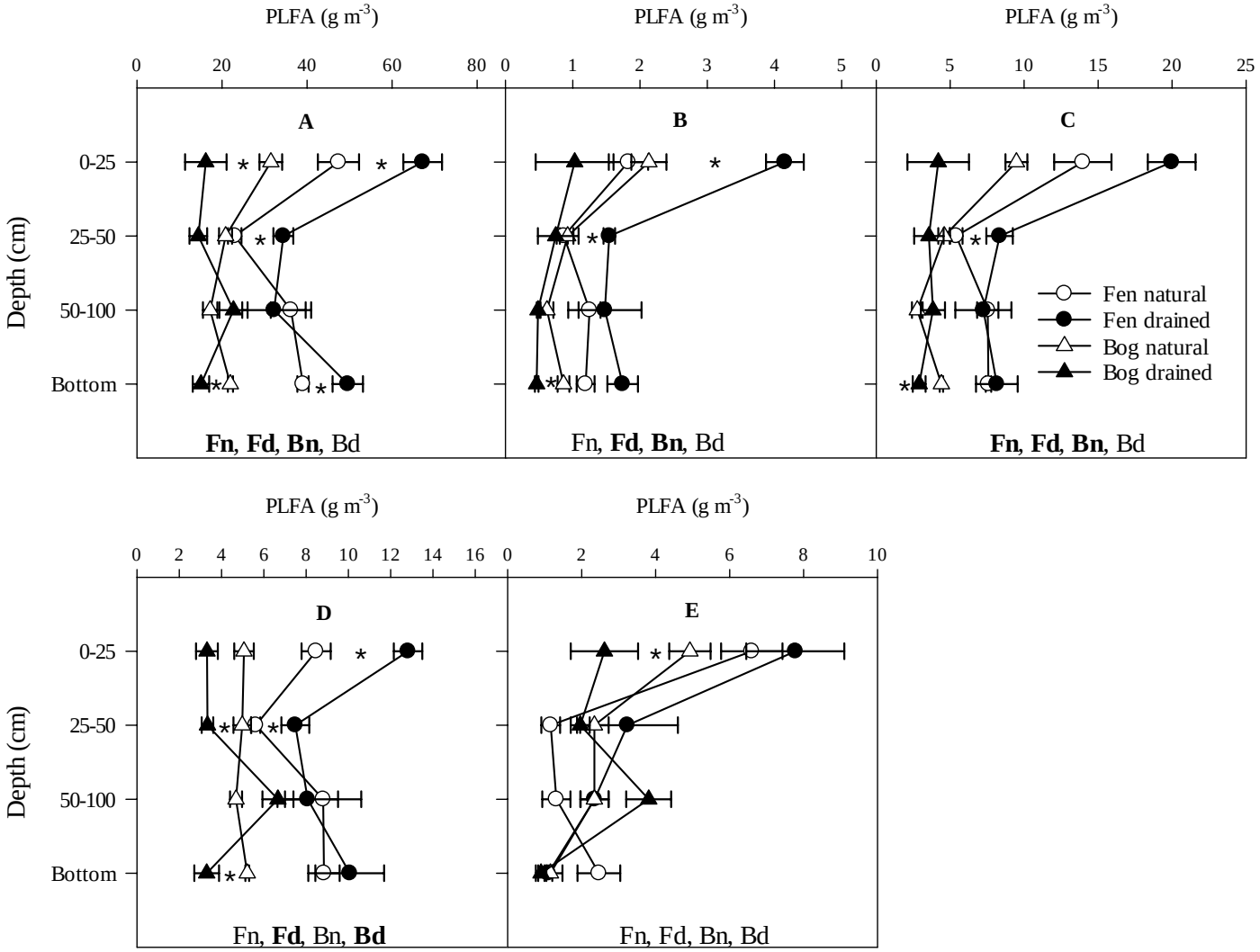
| **Depth (cm)** | Fen | Bog |
|---|---|---|
| 0 –25 | a | a |
| 25 – 50 | b | a |
| 50 – 100 | bc | b |
| bottom | c | c |

Bottom = 135 – 160 and 105 – 130 for fen natural and drained, 217 – 242 and 245 – 270 for bog natural and drained respectively.



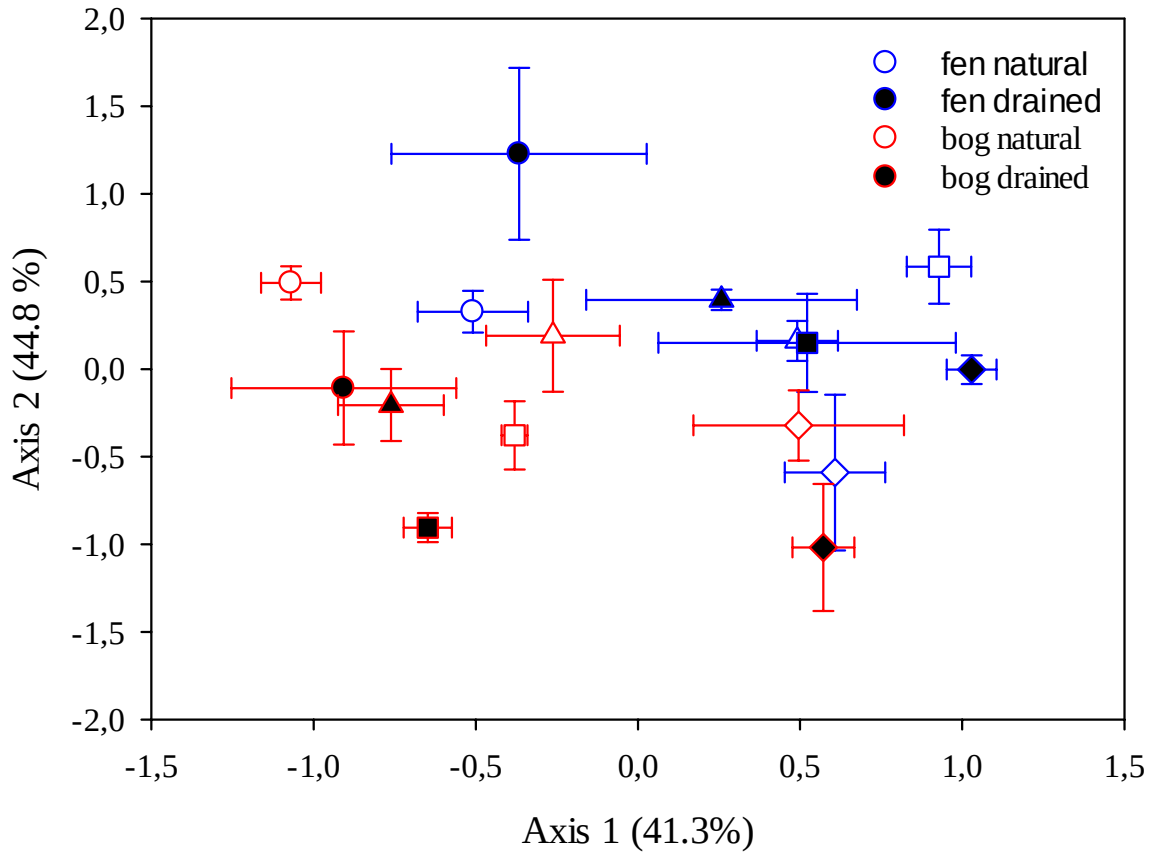

675 **Fig. 1.** Average daily water table (WT) depth in the sampled peatland sites from May to December
676 of the sampling year.

677



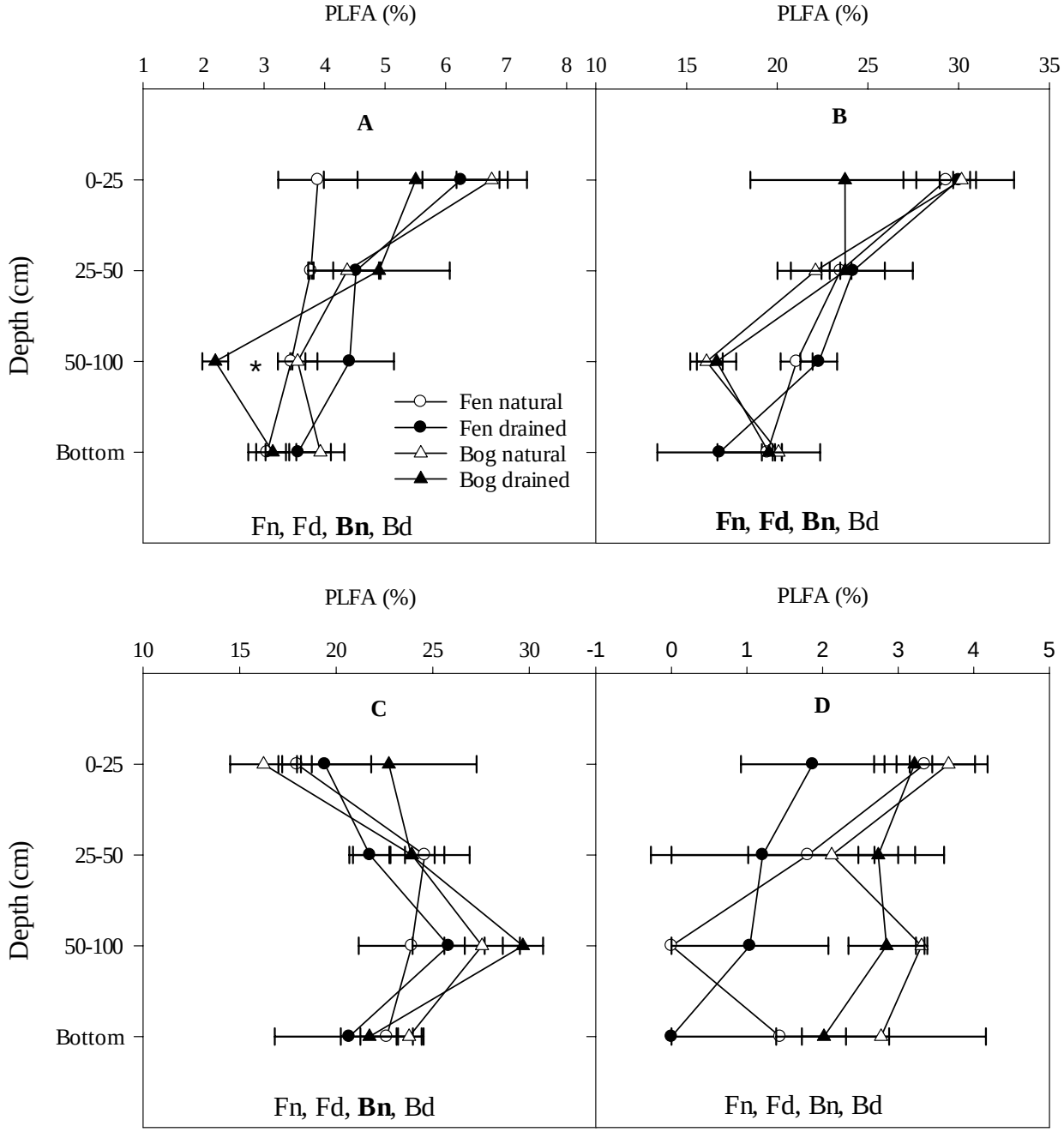

678 **Fig. 2.** Mean (± SE, n = 3) amount (g $m^{-3}$) of (A) microbial total PLFA, (B) 16 MUFAs, (C) 18
679 MUFAs, (D) terminally branched FAs and (E) FA characteristic of fungi in all the depths and sites
680 sampled. Significant differences ($p < 0.05$) between the drained and natural sides at each site in each
681 depth following independent sample t-tests, are denoted by asterisk (*). For depth effects Fn = fen
682 natural, Fd = fen drained, Bn = bog natural and Bd = bog drained, while sites with significant depth
683 effects (1-ANOVA, $p < 0.05$) are highlighted in bold. Bottom = 135 – 160 and 105 – 130 for fen
684 natural and drained, 217 – 242 and 245 – 270 for bog natural and drained respectively.

685




686 **Fig. 3.** Non-metric multidimensional scaling (NMS) ordination of PLFA [$\log_{10}(x+1)$ of the relative
687 abundance of individual PLFAs]. Average (± SE, n = 3) NMS axes scores of the sites. Axes are
688 arbitrary; the closer the sample points are on the plot, the more similar they are in PLFA composition.
689 Depth 0–25 cm (○), 25–50 cm (Δ), 50–100 cm (□) and bottom layers (◇). The drained and natural
690 sides of both the fen and bog sites differ significantly ($p < 0.05$) in a two-way factorial (drainage and
691 depth) analysis (PERMANOVA, see Table 2).

692




693 **Fig. 4.** Mean (± SE, n = 3) relative abundance (% contribution to total microbial PLFAs) of (A) 16
694 MUFAs, (B) 18 MUFAs, (C) terminally branched FAs and (D) FA characteristic of fungi at different
695 depths in the studied fen and bog sites. Significant differences ($p < 0.05$) between drained and natural
696 sides in each depth and for each microbial group following independent sample t-tests are denoted by
697 an asterisk (*). For depth effects Fn = fen natural, Fd = fen drained, Bn = bog natural and Bd = bog
698 drained, while sites with significant depth effects (1-ANOVA, $p < 0.05$) are highlighted in bold.
699 Bottom = 135 – 160 and 105 – 130 for fen natural and drained, 217 – 242 and 245 – 270 for bog
700 natural and drained respectively.