The impact of long-term water level draw-down on general 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 analyzed the peat profiles of natural and adjacent drained fen (nutrient-rich) and bog (nutrient-poor) sites at the Lakkasuo mire complex in central Finland. Interestingly, viable microbes (i.e. microbial PLFA) were present at relatively large amount even in the deepest (over 3000 years old) peat layers of both peatland sites, a finding which warrants further investigations. 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 affected also the deepest layers of the fen and the bog. We could not analyze specific microbial species (PLFA limitation), but long-term drainage increased the Gram-positive and Gram-negative bacteria characteristic FAs in the surface and subsurface layers of the fen, and decreased them in the bottom layers of the bog site. The fungal characteristic FA was reduced only in the surface layers of the bog site. Our conclusion is that, by affecting the microbial community beyond the surface layers, long-term peatland water-level draw-down can enhance microbial contribution to deeper peat OM stabilization via the microbial C pump (MCP) or increased microbial peat degradation and carbon release from the bottom peat layers. Thus, suggesting a more significant climate change effect of drainage than revealed by 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]. Microbial biomass (MB) is a key actor (as

catalyst) in all peat biogeochemical processes, controlling the OC sequestration and decomposition [3]. They also contribute to the peat C exchange via respiration and necromass addition to the peat soil organic matter (SOM; upon cell death) via the microbial carbon pump (MCP; [4]). Although there are still some gaps in the understanding of the relative contribution of all the microbial groups, the consensus is that different microbial groups, with complementary enzymatic activities and different responses to environmental variables, interact in the peat C-cycling processes [5]. For example, while the Gram–negative and Gram–postive bacteria are mainly associated with the mineralization steps involving labile and more recalcitrant C materials respectively [6], 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, affects microbial community and biomass, both spatially and temporarily [9,10].

Models predict warmer global climate (average temperatures increases of about 4^{o}C) up to 2100 [11] and, under these scenarios, increased evapotranspiration due to increased temperatures would lead to lower water levels in peatlands [12]. Persistent draw-down of the peatland water table 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 on a short-term, repeated replacement of specialist by generalist microbes may lead to loss of diversity in the long-run [14]. Also, changes in plant species cover, following water level draw-down, modifies the influence of temperature and water content on peat microbial activities [16]. Different studies have suggested that, drainage could increase or decrease microbial biomass (total or some groups), depending on the peatland type and depth [9,13]. Jaatinen et al [13] showed that, fungi and actinobacteria suffer from drainage in nutrient-rich fen, but in the drained bog, while fungi either suffer or benefit, actinobacteria remain the same or become more abundant. In general, fungi and bacteria are said to benefit (biomass increase) from persistent drainage of wet mesotrophic fen sites while actinobacteria suffer or show only minor response [9,15]. More

studies have also related the changes in peat C sequestration and decomposition activities, following drainage, to changes in the structure of below-ground microbial communities [5,16]. Changed microbial diversity, due to drainage-induced higher quality and quantity of OC inputs, coupled with more oxygen availability, could increase the rate of SOC cycling; leading to changes in the balance of peat-atmosphere C exchange [5,10,17].

As reliable quantitative biomarkers of viable microbes (since they are short-lived and readily metabolized upon cell death), PLFA analysis have been used to characterise microbial communities' responses to changing peat hydrology in different sites [9,13,18]. Differences in microbial community structures between peatlands and treatments (e.g drainage) have also been analysed in several studies based on PLFAs (indexed by PLFAs) alone [13,18-21], and their results compared very well with those obtained, even recently, using other molecular methods [22].

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

This study was therefore aimed at examining the effect of long-term drainage on the microbial communities in the depth profiles (top–bottom) beyond the surface, especially the bottom layers, of two peatland sites differing in nutrient status. We compared the biomass and structure of the microbial communities (indexed by total and relative abundance of PLFA, respectively) between the natural and drained sites of fen and bog, representing boreal peatlands of different fertility after 51 years of

water level draw-down. Specifically, we also investigated the effects of drainage on some selected microbial groups. We hypothesised that (1) long-term water table decrease will increase microbial biomass in the deep anoxic layers and (2) influence the microbial community structure in the anoxic layers. We also hypothesized that long-term drainage will increase microbial biomass more in the fen than in the bog site.

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) and for the period of 1981–2010, the mean annual temperature was 3.5 °C and precipitation 711 mm [29]. The sampling year was wetter than this long term mean, with a 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 sites of different fertility along a border ditch (Fig. S1). There were differences in the original fertility, water table 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. Especially at the drained fen, but less so at the drained bog, drainage caused marked changes to the hydrology, peat and vegetation properties, carbon dioxide (CO_2) and CH_4 fluxes as shown in previous studies ([28,30-33]; summarized in Table 1). For example, the average of six-month water table before the sampling date were -8.0 and -34.9 cm for the natural and drained fen whereas it was -12.00 and -16.38 cm for the natural and drained bog, respectively (Fig. 1). Also, CO_2 fluxes increased in both sites and 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 also confirmed by the previously reported pH values (Fig. S2 & S3). Although temperature measurements vary seasonally, the temperature in deep peat is rather constant (~ 6 - 8 °C). The bulk densities (BD) at different depths between the drained and natural are the same in the bog site but different in the fen site (Fig. S2).

2.2. Soil sampling and water table level measurement

In 2012 (November 22nd), three replicates sets of peat samples, from points located at least three meters apart, were collected from 4–5 depths (0 – 25 cm, 25 – 50 cm, 5 – 100 cm and deepest 25 cm) starting at the surface and extending to the deepest layer above 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 sites. The samples were collected into polyethylene bags, mixed and cooled immediately (in a box with crushed ice) after collection and later stored at -20 °C until analysis. Part of the samples were oven dried for analysis of their C and N content (Flash EA 1112 elemental analyser, Thermo Finnigan) after grinding to fine powder, using certified birch leaf standard (Elementar Microanalysis, UK) as reference. Continuous (3-hourly) water table (WT) level measurements were recorded by automatic WT-HR 64K logger (Fig. 1). The logger values were calibrated by manual measurements.

2.2.1. Additional peat properties measurements

Volumetric samples (from the same depths as initial samples) were used also 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 as described above (5.2 * 50 cm; half cylinder diameter * length) on 14 October, 2015. Sampling and depth measurement were started under living *Sphagnum* carpet. Samples were transferred from sampler into plastic bags (Aromata, Lidl Stiftung & Co, Neckarsulm, Germany), and mixed in the bag before pH probe electrodes coupled with temperature sensor (WTW P3 pH/conductivity with electrode SenTix 41; Weilheim, Germany) were inserted to peat. pH and temperature values were recorded after one minute. For 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

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 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. Then the top organic layer was transferred, dried (under nitrogen stream) and re-dissolved in 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} until 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 content [µg g^{-1} dry weight of soil (dw) and % of PLFAs] were calculated. In order to account for the variation in the peat compaction between the drained and natural sides, between the fen and bog sites and among 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).

2.3.1. Gram-negative, Gram-positive and Fungal characteristics FAs

PLFA biomarkers common among general microbial groups like Gram-negative bacteria, Gram-positive bacteria and fungi were selected and grouped. Iso- and anteiso branched PLFAs, i14:0, i15:0,

a15:0, a17:0 and i17:0, typical of Gram-positive bacteria [18,41,42] were grouped as Branched FA (BrFA). 16 monounsaturated fatty acids (MUFAs), 16:1ω5, 16:1ω6c, 16:1ω7c and 16:1ω8c, as well 18 MUFAs, 18:1ω5, 18:1ω6c, 18:1ω7c, 18:1ω7t and 18:1ω8c, both of which are typical of Gram-negative bacteria [42] were grouped separately. The 18:2ω6 FA typical of fungi [21] was studied as fungi FA.

2.4. Data analysis

The effect of drainage (at each site and depth layer) and depth (at each natural and drained site) on total microbial PLFA biomass, the absolute amount and the relative abundance of the selected microbial group FAs (16 MUFAs, 18 MUFAs, BrFA and fungi FA) were tested using independent sample t-test and one-way ANOVA, respectively. Correlations of the total microbial PLFA biomass with pH and BD were tested using Spearmann correlation analysis. Multivariate analyses of the PLFA profiles were based on Bray-Curtis dissimilarities calculated among samples using the $\log_{10}(x+1)$ transformed data of the relative abundances (% composition) of the PLFA. The data were assessed graphically using non-metric multidimensional scaling (NMS) constrained to 2 ordination axes. NMS was done for the whole (natural and drained) fen and bog site data to depict the overall patterns among sites, drainage and depth zones. Furthermore, the effect of depth and drainage on PLFA profiles in both fen and bog was tested using 2-way permutational multivariate analysis of variance (PERMANOVA) [43,44] with both factors as fixed factors. Mantel´s test was used to analyse correlations of the PLFA profiles with pH and BD. ANOVA was done using IBM SPSS Statistics version 23. NMS and Mantel´s test 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 analysed only PLFAs between C10 and C20 representing the range for mainly prokaryotic PLFAs and a few other microbial groups like 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 natural bog site for all the depths, except in the 25 – 50 cm depth, where the amount was not different between the fen and bog (Fig. 2A, Table S1). There was no correlation between pH and total microbial PLFA biomass either in the combined dataset of the fen and bog, or the fen and bog separately. In all but the 50 – 100 cm depth of the fen site, total microbial PLFA biomass was higher in the drained compared to the natural site. In the bog site, the total microbial PLFA biomass was only different between the drained and natural sites at the top and bottom layers, where the amount was smaller in the drained site (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 site 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 the amount in the 25–50 cm layer, but similar to the amount 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, which was not the case in the natural fen (Table S2). In the natural bog, the amount microbial PLFA biomass was higher in the surface layer than in all the other depth layers, which were similar to one another (Table S2).

As visualised with NMS ordination, there were differences in the microbial community PLFAs structure between the fen and bog sites (Fig. 3). NMS (R^2-values of axes) 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. Gram-negative, Gram-positive and Fungal characteristics FAs

The overall concentration of all the major microbial group PLFA followed the same trend as the total microbial PLFA, being higher in the fen than the bog site especially in the drained site (Fig. 2B). They were also mostly highest in the surface than deeper layers of both sites. The amount (g m^{-3}) of 16 C monounsaturated fatty acids (16 MUFAs) was higher in the top two layers (0 – 25 and 25 – 50 cm) of the drained compared to the natural fen site, but lower in the bottom layer (only) of the drained compared to the natural bog site (Fig. 2B). The relative contribution of 16 MUFAs to the total microbial PLFA (% contribution) did not differ between the drained and the natural sites in both fen and bog, and in all the sampled depths, except in the 50 – 100 cm depth of the bog site (Fig. 4A). Depth affected the amount of 16 MUFAs (g m^{-3}) only in the drained fen and natural bog sites. 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 bog natural, where it was higher in the surface layer (0–25 cm) than in 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) was only higher in the sub-surface layer (25 – 50 cm) of the drained compared to the natural fen site, and lower only in the bottom layer of the drained compared to the natural bog site (Fig. 2C). The relative contribution of 18 MUFAs to the total microbial PLFA (% contribution) was not different between the drained and the natural sites in both fen and bog (Fig. 4B). There were depth differences, in the amount of 18 MUFAs (g m^{-3}), in all the sites except in the drained bog. In the sites 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 depth effect on the relative contribution of 18 MUFAs to the total PLFAs in all the sites, except in the bog drained (Fig. 4B, Table S3).

The amount (g m^{-3}) of terminally branched fatty acids (BrFAs) was higher in the top two layers (0 – 25 and 25 – 50 cm) of the drained compared to the natural fen site, but lower in the sub-surface (25 – 50 cm) and bottom layers of the drained compared to the natural bog site (Fig. 2D). The relative contribution of BrFAs to the total microbial PLFA (% contribution) was not different between the drained and the natural site in both fen and bog (Fig. 4C). There were depth differences, in the amount (g m^{-3}) of BrFAs, only 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 site are higher than in the other depth layers, which were similar to one another (Fig. 2D, Table S2). There was no depth effect on the relative contribution of BrFAs to the total PLFAs in all the sites, except in the bog natural site (Fig. 4C, Table S3).

The amount (g m^{-3}) of fatty acids, characteristic of fungi (fungi FA), were not different between the drained and natural fen site but was lower only in the surface layer (0 – 25 cm) of the drained compared to natural bog site (Fig. 2E). Neither drainage nor depth affected the relative contribution of fungal FA to the total microbial PLFA (% contribution) in both fen and bog sites (Fig. 4D, Table S3). There were also no depth differences, in the amount (g m^{-3}) of fungal FAs in all the sites (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 than in the bog (Table 1 and Fig. S2) [5,13,14,19,47]. Concomitant differences in their vegetation cover affect microbes due to soil structure and C substrate availability differences. Biomass from fen vegetation is easier to decompose than biomass of bog vegetation consisting largely of recalcitrant *Sphagnum* mosses [5,13,48]. The roots of sedges in fens, provide better soil stability, and macro pore structure than that 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 sites; Table 1) [28,49,50]. Differences in microbial biomasses were also accompanied by differences in the microbial community structures between the fen and bog, due to similar reasons (above) and the natural difference in the wetness of the sites [13,47].

There was viable microbial biomass in all the peat layers of both the fen and 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 in the bog, is likely due to differences in fertility, litter quality [47], with depth, between the bog and the fen (Table S4). Generally, the microbial biomass PLFA (g m^{-3}) increased with increasing nitrogen content (N %), C % and decreasing C/N ratios in the fen, but not 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 indicate changes in the potentially active microbial biomass, it cannot separate the active and non-active cells [51]. We also acknowledge that the turnover rate of PLFAs, in the deep anoxic peat layers, is unknown and could be considerably slower than in the oxic and

warmer surface peat layers. This means that FAs detection in deeper peat layers may not be indicative of similar cell activities as in the surface peat layers. However, the presence of potential (enzymatic) prokaryotic microbial activities [52] and active microbial populations [53] have been previously reported from deep peat layers (100 cm to 300 cm); thus supporting our finding of living microbes in bottom peat layers. Our community structure analyses (% PLFA profiles, Table 3, Fig. 3; see also 4.3 below), also indicated differences in the microbial groups occupying different depths, possibly due to community adaptations to depth-related changes in several factors, e.g. pH as shown here, oxygen availability, alternative electron acceptors and substrates [5,54]. There is high temperature variability at the at the surface, but low and stable temperature at the bottom (Fig. S2 and [31]). This also modifies community structure. Since there are large amounts of C stored in deeper peat layers, the significant amount of microbial biomass in these layers may have implications for the global C cycles, such as microbial-enhanced C flows from peat to mineral subsoil. There is an estimated average 13.6 g m^{-2} yr^{-1} C input from peat into mineral subsoils (Turunen et al. 1999) which may increase with changes in deep peat layers.

4.2. Effects of drainage on the biomass and community structure of microbes

Drainage affected the total microbial biomass in both the fen and bog peat sites due to factors including changes in the quality of available substrates, the availability of nutrients and increased thickness of the aerated layer [13,55]. In general, while drainage led to succession towards different ecosystem from the original in the fen, it led to smaller changes in the bog site. Typical fen species like tall sedges were succeeded by spruce swamp and forest species, like *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* appears. (see also Table 1). Tree stand volume increased from 0 in the natural to 111 m^{-3} ha^{-1} in the drained fen site, compared to the bog site where tree stand volume changed from 5 to 16 m^{-3} ha^{-1} after 38 years after drainage

[31]. The higher tree growth led to increased evapotranspiration and further decrease in the water table. 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 long-term effects of drainage were active. The means of six months WT depth 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 natural and drained bog, respectively. Also, the previously reported annual mean WT depths, were always much lower in the drained fen, than the drained bog for most part of the year (Fig. 1 and Fig. S4). The much lower WT in the drained fen therefore explains why there was drainage-induced changes on 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. Although the total microbial PLFA biomass in some subsurface layers (25 – 100 cm in bog and 50 -100 cm in fen) were not different between the natural and drained sites, the total microbial PLFA biomass in the deepest (bottom) layers of both the fen and bog sites were different between the natural and drained sites. Inconsistent pattern in the long-term drainage induced changes, especially in the subsurface layers, have been reported previously [13]. Our finding is possible because, the different microbial communities at the different depths in the pristine peatlands [5,13,54] have different sensitivity to changed hydrology; reacting differently to drainage-induced changes [5,13,15,19]. Also, since drainage effect on microbial communities in deeper anoxic layers depends on the movement of materials (e.g. DOC) from the surface to deeper layers [24,26,27], accumulation of materials to concentration able to cause significant changes in microbial biomass is more probable in the deepest layers than in the intermediate layers, many years after drainage [24].

The total microbial PLFA biomass at the bottom layers of the drained and natural sites were different in both sites. This was due to changes in the flow and constituent of the leachate water [24], deeper deposition of labile root exudates by vascular plants and differences in the amount and quality of DOC reaching the bottom layers in the drained sites [26,27]. Although the roots of vascular plants may not reach the bottom layers, their root exudates will get deeper and influence microbes in the bottom layers, via water movement, faster than those deposited by the shallow roots of mosses. The

result of DOC isotopic analysis and tritium analysis in pore water, by Charman et al [24], supported the downward movement of younger C via water movement in ombrotrophic boreal peatland. The study also showed that the ^{14}C age of CO_2 and CH_4 from the deeper peat layers, were much younger than the surrounding peats. They attributed this to the transfer of DOC and gaseous C compounds to deeper peats, via water movement, and the microbial usage of younger C. Furthermore, they concluded that low hydraulic conductivity in peats may not be a real limitation to water movement, on a long time scale. Although mainly in a fen peat site, Krüger et al. [28] also reported the effect of drainage in the deeper peat profiles, which showed higher $\delta^{13}C$ values (a qualitative indicator of peat degradation) in the drained compared to the natural site. They also reported much older peat ages in the drained sites of both fen and bog. This also suggests the effect of drainage on deeper peat microbial activities, as microbes use younger C more enriched with ^{14}C (leaving ^{14}C depleted older peat behind). The overall effect of drainage on microbial biomass was reflected considerably in the increased CO_2 and decreased CH_4 efflux, especially in the drained fen (Table 1). By affecting the deep peat microbial biomass, long-term drainage may have additional significant C balance effect than the enhanced carbon losses from surface peat (Kruger et al 2016). Increased microbial biomass in in bottom peat layers, due to drainage, can either contribute to the peat OM stabilization via the microbial C pump (MCP) process ([4]; not studied in peatland yet) or enhance deep peat microbial degradation (Kruger et al 2016) and carbon flow to sub-soil (Turunen et al 1999). Further studies are needed to elaborate on this.

Drainage increased total microbial PLFA biomass in the surface, subsurface and bottom layers of the fen, supporting our first hypothesis, but decreased it in the surface and bottom layers of the bog site, contrary to our first hypothesis. This is possibly due to differences in their nutrient status, substrate and vegetation changes[13,22,47], as already discussed above. Other reasons could be the usually slight decrease in the WT at the drained bog compared to fen over time (Fig. 1 and Fig. S4), the originally lower quality and availability of the substrate in the bog, which have become poorer after 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 sites, especially at the bottom layer. High C/N ratio usually indicates low substrate quality, which also could explain the low microbial biomass in drained bog. We cannot conclusively explain the differences in C/N ratios between the bog sites; 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, according to our third hypothesis, drainage increased the total microbial biomass more in the fen more than the bog site.

Similar to several previous studies, peat microbial community structure in this study was indexed by the relative composition of microbial PLFAs [13,18-21,56]. According to our second hypothesis, our result showed that long-term drainage affected the microbial community structure in all the depth layers including the bottom (deepest) layers. Jaatinen et al. [13] and Urbanová and Bárta [22], studied the effect of long-term drainage on microbial communities only in the surface layers (0 -30 cm) of peatlands. They also showed that long-term drainage, and the resulting change 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; [31] 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 sites of both the fen and bog sites (by Mantel´s test), explains in part, the changes in the microbial community structures in the different depth layers [13]. Other reasons for the change in microbial community structure include changes in the anoxic/oxic condition of the surface layer and the available litter quality in different depth, occasioned by the drainage-induced changes in the prevailing plant communities and structures (see table 1 and paragraph 1 of 4.2) [16,19,55].

4.3. Gram-negative, Gram-positive and Fungal characteristics FAs

Similar to previous studies [13-15], drainage and depth-induced effects on the biomass of the microbial group characteristic FAs, varied among the microbial groups and between the fen and bog sites. The higher concentration of all the major microbial group characteristic FAs, except fungal characteristic FA, in the natural and drained fen than the bog site was due to the same reasons given for total PLFA biomass (in 4.1). The biomass of both the Gram-negative and Gram-positive bacteria characteristic FAs were increased in the surface and subsurface layers of the fen site, probably due to drainage-induced increase in aeration and substrate availability, but decreased in the bottom layer of the bog site, possibly due to low substrate quality. The fungal characteristic FA was only reduced in the surface layer of bog, likely due to low biomass quality and differences in the sensitivity of fungal species, to drainage [15]. For example, Peltoniemi et al [15] showed that basidiomycetes are more sensitive than ascomycetes, though they are within the same fungal phyla represented by the same characteristic FA.

Drainage-induced effects were not observed on the relative abundance of our selected microbial group characteristic FAs, except for 16 MUFAs in the 50 – 100 cm depth of the bog site. The depth-induced effects were also inconsistent among the groups and between the fen and bog sites (Fig.4, Table S3). This was probably because these microbial groups contained different species with contrasting depth stratification pattern, contrasting drainage responses and our 25 cm sample depth-range being too large, to reveal the inconsistent changes observed in the shorter sampling depth-ranges for specific microbial groups (e.g. [9,13,22]). 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. Kim et al. [14] also reported no differences in the diversity and composition of denitrifiers and methanogens in all their sites following a short-term drought. The effect of drainage on the mainly aerobic bacteria FAs (16

MUFAs), in the 50 – 100 cm layer of bog alone, may be due to change from a permanently anoxic to at least an episodic oxic condition 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-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 varies between the two sites and among treatments; suggesting that the factors regulating depth-induced effects on specific microbial species (within the groups) differs between sites.

5. CONCLUSIONS

Our study shows that the biomass and the structure of *in-situ* microbial communities differ between the two peatland types studied. While long-term water level draw-down significantly increased total microbial biomass in the affected layers of the fen, it decreased the total microbial biomass in the affected layers of the bog likely due to the differences in their original nutrient status, the level of water table depth reduction and quality of substrate. There is considerable amount of living microbial biomass in the deepest and oldest (3000-3400 years) layers of the studied peatlands. Even though drainage mostly affects microbial community in the surface layers, the effect of long-term peatland water-level draw-down on deeper layer microbes is also important because long-term storage of large amount of C in deep layers. Increased microbial biomass due to drainage measured in this study, for the fen site, can enhance microbial contribution to deeper peat OM stabilization via the microbial C pump (MCP) or increased microbial peat degradation and carbon release in the bottom peat layers. This suggests a more significant climate change effect of drainage than revealed by surface layer analyses alone. Further studies are needed to elaborate on this, and how well this artificial drainage corresponds to climate change-induced drying of peatlands.

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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

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

Table 2. Two-way factorial (drainage and depth) analysis (PERMANOVA) explaining the structural variation in the analyzed 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.

		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 simple-effect 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.

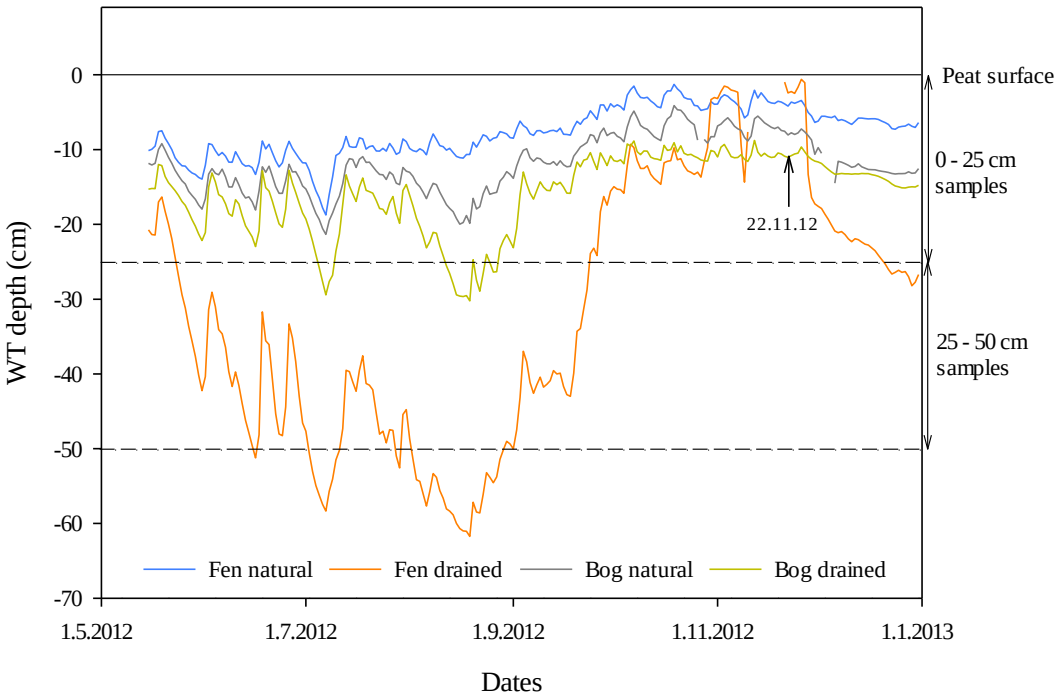

Fig. 1. Average daily water table (WT) depth in the sampled peatland sites from May to December of the sampling year.

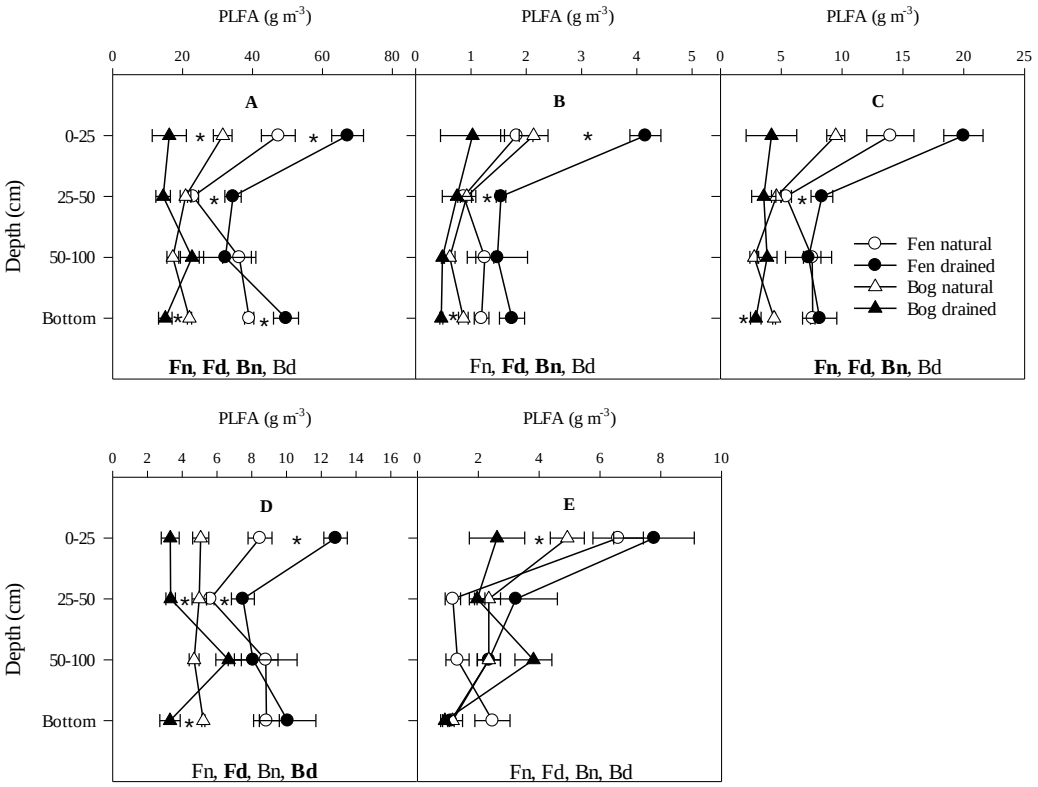

Fig. 2. Mean (± SE, n = 3) amount (g m^{-3}) of (A) microbial total PLFA, (B) 16 MUFAs, (C) 18 MUFAs, (D) terminally branched FAs and (E) FA characteristic of fungi in all the depths and sites sampled. Significant differences ($p < 0.05$) between the drained and natural sides at each site in each depth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

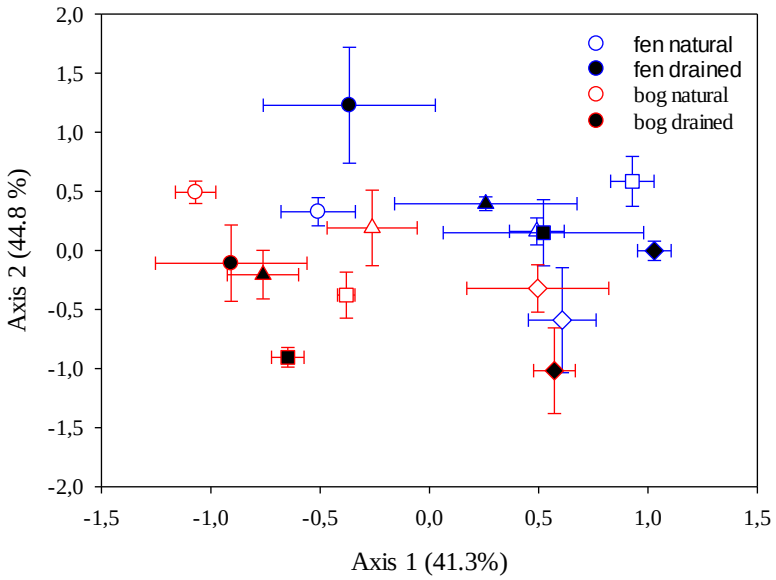

Fig. 3. Non-metric multidimensional scaling (NMS) ordination of PLFA [$\log_{10}(x+1)$ of the relative abundance of individual PLFAs]. Average (± SE, n = 3) NMS axes scores of the sites. Axes are arbitrary; the closer the sample points are on the plot, the more similar they are in PLFA composition. Depth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

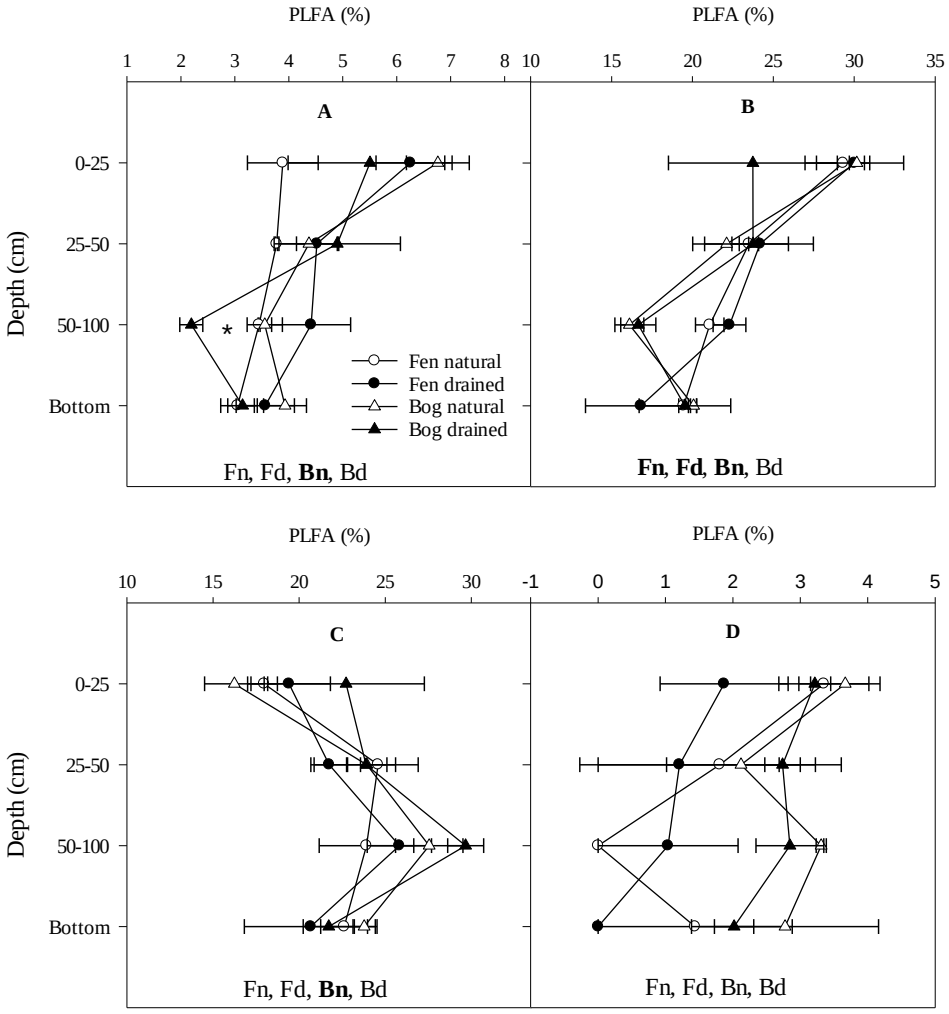

Fig. 4. Mean (± SE, n = 3) relative abundance (% contribution to total microbial PLFAs) of (A) 16 MUFAs, (B) 18 MUFAs, (C) terminally branched FAs and (D) FA characteristic of fungi at different depths in the studied fen and bog sites. Significant differences ($p < 0.05$) between drained and natural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