# Stable carbon isotopic composition of peat columns, subsoil and vegetation on natural and forestry-drained boreal peatlands

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# Stable carbon isotopic composition of peat columns, subsoil and vegetation on natural and forestry-drained boreal peatlands

We studied natural and forestry drained peatlands to examine the effect of over 34 years lowered water table on the $\delta^{13}C$ values of vegetation, bulk peat and subsoil. In the seven studied sites, $\delta^{13}C$ in the basal peat layer was 1.1‰ and 1.2‰ lower than that of the middle-layer and surface layer, respectively. Furthermore, there was a positive correlation between the $\delta^{13}C$ values of the basal and surface peat layers, possibly due to C recycling within the peat column. In the same mire complex, natural fen peat $\delta^{13}C$ values were lower than those of the nearby bog, possibly due to the dominance of vascular plants on fen and the generally larger share of recycled C in the fens than in the bogs. Furthermore, natural and 51-years previously drained fen and bog, on the opposite sides of a ditch on the same mire complex showed no significant differences in $\delta^{13}C$ values. Plant $\delta^{13}C$ values were lower, while $\delta^{13}C$ values of subsoil were higher in the drained than in the natural side of the fen.

Keywords: carbon dioxide, *Sphagnum,* fen, bog, diagenesis, drainage, carbon cycle, Suess effect

## Introduction

The factors affecting bulk peat $\delta^{13}C$ are often related to water-table height in relation to peat surface. Fens have a naturally higher water table than bogs and vascular plants growing on fens are depleted in $^{13}C$ compared to *Sphagnum* peat in bogs [1]. Higher external diffusion resistance in *Sphagnum* photosynthesis in moist conditions increases $\delta^{13}C$ values of plant tissue, while in dry conditions $\delta^{13}C$ decreases [2]. Wetness also increases carbon dioxide ($CO_2$) recycling [2] and methanogenesis, and $^{13}C$-depleted $CO_2$ derived from methane ($CH_4$) oxidation by methanotrophic symbionts in *Sphagnum* species leads to formation of $^{13}C$ depleted peat [3,4].

Photosynthesis and aerobic respiration are relatively fast processes connected to daily

variability in light intensity and temperature moving carbon between peat and the atmosphere in the aerated peat surface layer, where water table fluctuates (acrotelm) [5]. Below the acrotelm, much slower and mostly anoxic degradation continues in the anaerobic, cold and acidic peat column (catotelm) [5]. This is the location where carbon is stored as peat. In general, aerobic microbial decomposition increases $\delta^{13}C$ of the remaining peat while anaerobic decay preserves or slightly decreases $\delta^{13}C$ of the peat columns [6,7].

Based on $^{14}C$ dating, a considerable amount of respired $CO_2$ is recycled through photosynthesis back into the peat profiles [8]. Use of $^{13}C$ depleted $CO_2$ respired from peat in addition to the atmospheric $CO_2$ may affect $^{13}C/^{12}C$ in peat columns. In addition to this, the concentration of the primary source of peat carbon, atmospheric $CO_2$, has increased from preindustrial (1000 –1600 AD) levels of 278 – 284 ppm [9] to present levels of ~400 ppm [10]. Whereas $\delta^{13}C$ of $CO_2$ has decreased from the preindustrial $\delta^{13}C$ values of – 6.5‰ [9] to the clearly lower present $\delta^{13}C$ values of ~–9 – –8 ‰ [10]. These changes are due to fossil fuel burning and land clearing [11].

Increased temperatures and drier conditions due to climate change will affect northern peatlands [12]. Drying will decrease peatland water tables, enabling increased aeration of peat surface layers. This leads to aerobic processes in the previously anaerobic layers; leading to an increase in C flow to the atmosphere. Furthermore, persistent water-level drawdown gradually leads to changes in the vegetation type. Since water-table height is important in determining the $\delta^{13}C$ of growing vegetation on peatlands [2,4], drying should affect not only the surface peat $\delta^{13}C$, but also the deeper peat layers exposed to air as the water table decreases. Increased forest growth has been connected to climatically warm periods by stratigraphical evidence, showing that forests have commonly grown on peatlands in drier climates [13,14].

Drainage for forestry involves permanent lowering of the water table by ditching to initiate forest growth. Long lasting water table decrease, increased shading by tree stands and concomitant increases in forest species (*Vaccinium mytillus, Vaccinum vitis-idaea*) and declines of Sphagna and mire dwarf shrubs [15], modifies nutrient status and light penetration in the peat surface. Prerequisites for changes in the $\delta^{13}C$ values of deep peat and subsoil carbon are changes in C-cycling processes and C flows; thus, detected changes in $\delta^{13}C$ could indicate that drying affects the peat column and subsoils. The mineral subsoil under mires is a continuous C sink and store [16], but it has rarely been studied.

The extent of the ecological change to drained peatlands is positively dependent on the nutrient-level and wetness of the original site [15,17]. Drainage for forestry leads to increased carbon loss from peat especially on drained fens [18–21]. Drainage of bogs can also lead to carbon loss [18,20], while drained bogs with a small decrease in the water table and poor forest growth can still function as carbon sinks [19,21]. Leaching of dissolved organic carbon (DOC) from peatlands increases to some extent in drained sites [22]. In addition, $CH_4$ fluxes decrease in drained bogs and can end in drained fens [23]. Drainage also alters the microbial community composition and its location [24,25]. Secondary succession initiated by drainage also affects vegetation pattern and thus increases aboveground litter input to the peat surface [26]. All these changes may also affect the $\delta^{13}C$ of peat.

Changes due to human impact on peatlands have been studied using the $\delta^{13}C$ of bulk peat [6,7,19,27]. To our knowledge only Krüger et al. (2015, 2016) [7,19] have compared the $\delta^{13}C$ values of paired natural and drained sites, which were similar before the artificial drainage. The effects of long-term drainage on the bottom peat and on the mineral subsoil $\delta^{13}C$ below the peat interface have not been studied. Respiratory $CO_2$ from decaying

vegetation (and peat) is ^{13}C-depleted compared to atmospheric $CO_2$ [28,29], and it is readily available to vegetation on the peatlands. However, the effect of drying-induced increases in the release and possible increased use of ^{13}C-depleted $CO_2$ from peat on the $\delta^{13}C$ of vegetation on peatlands has not been studied.

We studied peatlands drained for forestry 34 - 80 years earlier. Sites at Lakkasuo have both a bog and a fen ecosystem, each with adjacent drained and natural parts divided by a border ditch. According to a previous $\delta^{13}C$ study of the same sites [19], the natural sides of the bog and fen are not showing signs of degradation, thus comparison of natural sides to drained sides was possible. The first objective of this study was to study if growing peat $\delta^{13}C$ is influenced by use of $CO_2$ and $CH_4$ carbon from aerobic and anaerobic degradation processes (internal carbon cycling) besides $\delta^{13}C$ of atmospheric $CO_2$, the primary carbon source. In addition to this, we studied partially drained peatland pairs, to assess if changes in the whole ecosystem affect the $\delta^{13}C$ of understorey vegetation and the whole peat profile. Furthermore, we wanted to know if drainage of a fertile fen also affects subsoil $\delta^{13}C$.

**Materials and methods**

*Sites and their land use*

The study sites were in the coniferous forest area in the southern boreal zone of Finland (Fig.1, Table 1). Lakkasuo, an eccentric peatland complex (61º47´N, 24º18´E) in Orivesi, has natural fen (LakFN) and bog (LakBN) sites with adjacent drained fen (LakFD) and bog (LakBD) sites along a border ditch. Both sites were ditch-drained in 1961, 51 years before the peat profile sampling and 53 years before the bottom peat and subsoil

sampling. The original ditch depth was 0.7 m and spacing of ditches was 40–60 m; the upstream side of the peatland is pristine. The distance between LakF and LakB is 0.5 km. Subsoil in Lakkasuo is sand. The bottom of the bog is 4.5 m higher than that of the fen. Due to this, northern parts of Lakkasuo are fed by groundwater from the nearby esker springs and as subsurface runoff, however, in the ombrotrophic part, the surface of the mire is higher than the groundwater level in the esker. Due to this difference in water and nutrient source, change from minerotrophy to ombrotrophy is clear [30]. The area of the natural fen is 8 ha and the area of the bog is 20 ha [30].

Based on an investigation made in 2011 (Sup. Tab. 1) (Peatland database of the Geological Survey of Finland 2017)[31], *Sphagnum and Carex* dominate the upper part of the peat profiles of LakFN and LakFD, while *Lignum* (remains of lignin containing plants), *Sphagnum* and *Carex* dominate the deepest peat layers. Thus, based on vegetation, natural and drained sides of LakF were quite similar before drainage. There were differences in peat profiles of LakB. The peat profile of LakBN consisted of *Eriophorum* and *Sphagnum* to a depth of 150 cm and *Phragmites* appears in the depth layers from 150 to 250 cm. LakBD is dominated by *Sphagnum* to a depth of 50 cm and different to LakBN, *Eriophorum* is abundant in LakBD from a depth of 120 cm to the bottom of the peat. The basal peat layer of LakBN consists of *Lignum, Carex* and *Sphagnum,* but in LakBD *Eriophorum* also exists.

There were three sites in the Lammi area (61º4´N, 25º0´E, ca 150 m a.s.l.). They included the natural Villikkalansuo fen lagg (VilFN; area 4 ha) and the drained Laaviosuo bog (LaaBD) (area 67 ha) sites on the same peatland complex but at a distance of 1.2 km from each other. The third site was the Lovonsuo drained mire (LovFD). Based on the stump annual rings, it was estimated that the Lovonsuo mire (LovFD) (area 1.5 ha) was drained in the 1920's, thus the tree stand is now mature (Table 1).

Weather data is from weather stations located nearest to the sites for the period of 1981-2010 [32]. The annual average temperatures for the Lakkasuo and Lammi areas were 3.5 and 4.2 °C and precipitation 711 and 645 mm year$^{-1}$, respectively (Table 1). All the sites are usually covered by snow from November to the end of April [32]. The precipitation pattern during the snow-free season is quite similar for both locations: the driest month is May (42 - 45 mm) while the highest precipitation is in July (86 - 92 mm) [32].

The water table level was highest in LakFN and VilFN and deeper in the LakBN and the drained sites, LakFD, LakBD, LaaBD and LovFD (Table 1). Previous studies from Lakkasuo with corresponding natural and drained sides showed that long-term drainage has decreased water tables and also subsided the peat surface [17,23]. At LakF, peat subsided 23 cm due to drainage and the average water table was 24 cm deeper on the drained side compared to the natural side in 1991 and 1992 (Table 1). At LakB, subsidence was 10 cm, while water table was on average 11 cm deeper on the drained side (Table 1). The minimum detected water table was as low as 59 cm in LakFD and 43 cm in LakBD [23]. This pattern of water table difference between natural and drained sites at Lakkasuo has remained [24,25]. Peat temperature at a depth of 30 cm was on average 0.6 °C lower at LakFD and 1.7 °C lower at LakBD than it was in their natural counterparts (Table 1).

Based on surface peat N content, VilFN was the most fertile (3.4 ± 0.2 %) of the studied sites (Fig. 2c,h and 3c,h,m). At LakFD N% was greater than it was in its natural counterpart (Fig. 2c). The surfaces of LaaBD and LovFD were also nitrogen poor (Fig. 3h,m). LovFD represented the earliest drainage in this study, its tree stand is mature and the original peatland type can no longer be reconstructed. However, even though the N% (Fig. 3m) is now as low as usually found in the bogs, LovFD is considered to originally have been a fen, based on the vigorous forest growth and location nearby an esker. Carbon

balance estimation based on comparison of carbon amounts in the natural and drained sides has earlier been made for LakFN and LakFD pairs, and LakBN and LakBD pairs at Lakkasuo [17,19].

### *Sampling and analysis of peat profiles*

The samples from peatlands in the Lammi area, LaaBD, VilFN and LovFD were collected in September 2011. Three replicate sample profiles were taken with a 15 m distance between sampling points using a Russian pattern side-cutting sampler (half cylinder diameter 50 mm, length 500 mm).

Drying temperatures for peat dry weight determination are often in the range of 60 – 90 °C to prevent possible charring, oxidation, and vaporization of substances other than pore water [33], however, effects of sample preparation on $\delta^{13}C$ of bulk peat with a dead microbial biomass (necromass) are not well known. Therefore, the effect of drying method on $\delta^{13}C$ values was tested for peat samples from VilFN, LaaBD and LovF using three drying methods prior to $\delta^{13}C$ analyses. The basic method was oven drying at 70 °C. The existence of carbonates was tested by HCl acid fumigation of already dried and ground subsamples [34]. A ~ 5 mm layer of peat was kept in open glass vials (height 20 mm, diam. 20 mm) under an inverted glass dish with 12 M HCl in a glass vial overnight to remove inorganic carbon by exposure to HCl fumes. After acid fumigation, samples were kept overnight in an oven at 70 °C and weighed into tin capsules. Freeze-drying of the frozen samples was considered to keep possible volatile components of necromass intact in the peat. Frozen samples were freeze dried (Labonco Freeze Dryer, Model 77560) and mixed after drying. A portion of the dried sample mix was put into 2 mL Eppendorf tubes and homogenized using a Retsch MM301 vibrating ball mill. The homogenised samples were then weighed into tin capsules.

At Lakkasuo, peat profiles were sampled in November 2012. The sampling at Lakkasuo was done a few meters away from former boardwalks, where gas fluxes and environmental variables were measured in 1991 and 1992 [23]. Three replicate sample profiles were collected at a distance of at least 3 m apart starting at the surface below the green vegetation (hereafter "surface peat") to the deepest layer above the mineral sub soil (hereafter "basal peat"), using the Russian pattern side-cutting sampler. The samples were cut on site to the desired length, put into polyethylene bags, mixed and cooled immediately in a cooling box containing crushed ice.

Samples were later stored in a freezer at -20 °C until preparation for analysis. Samples were dried at 70 °C for 24 hours, grinded with a Retsch MM301 vibrating ball mill, stored in Eppendorf vials and weighed into tin capsules (Elementar Microanalysis Limited, UK). Dry bulk density (BD) of peat was determined by dividing dry weight (g) of a sample by its volume ($dm^{-3}$), thus BD is in g $dm^{-3}$.

Sampling depths for peat profiles and sample lengths are shown in Table 1. Individual samples are coded in text, so that for a surface sample, e.g. from LakFN from 0 - 25 cm, the depth is in bold superscript ($LakFN^{0-25}$). Mid-layer sample depths are marked as subscript ($LaaFD_{80-130}$), while in the deepest peat samples depth is in subscript underlined, so a LakFN basal peat sample from depth of 135 – 160 cm is marked as $LakFN_{\underline{135-160}}$.

### Sampling and analyses of the bottom peat and subsoil samples

The effects of drainage on subsoil were studied at LakF in November 2014. Based on previous studies, the ecosystem change was clearest in LakF. Profiles of the bottom peat and subsoil from the Lakkasuo natural fen (LakFNbot) (n = 3) and from the Lakkasuo drained fen (LakFDbot) (n = 3) were sampled using a Russian pattern side-cutting sampler (half cylinder diameter 27 mm, length 500 mm). The 50 cm long profiles

containing peat and subsoil were wrapped on site in plastic sheet and stored in a cool box for preparation later the same day. The profiles were cut into 5 cm long pieces, dried at 70 °C without acid fumigation and weighed to get their water content and dry bulk density. Subsamples were ground using an inverted spatula in glass vials and weighed into tin capsules for analysis of $\delta^{13}C$, C% and N%. Hereafter, "bottom peat" is used for the peat samples from peat profiles connected to subsoil, while the term "basal peat" is used when the whole peat columns are considered. The interface between the bottom peat and subsoil was defined for each of the 50 cm long profiles by determining where a rapid decrease in N% occurred between the 5 cm slices. This interface was set as the zero level between the bottom peat and subsoil in figures and in statistical analysis. In two natural and two drained side bottom profiles, this interface was between subsamples 20 - 25 cm and 25 - 30 cm, whereas in the remaining two profiles it was between subsamples 25 - 30 cm and 30 – 35 cm, calculated from the top of the 50 cm long profile.

### *Sampling and analysis of plants*

Plants were collected from LakFN, LakFD, LakBN and LakBD, in November 2014 and analyzed for $\delta^{13}C$, C% and N%. Plants were selected from the field layer vegetation and limited to trees less than 1.5 m in height, in order to sample only plants grown after drainage. Plants were dried at 70 °C for 24 hours. Needles, leaves or stem pieces of individual plants were cut to get a ~3 mg sample of each plant, which was then folded in a tin capsule and analyzed with a Thermo Finningan Advantage IRMS (Germany) coupled with the elemental analyzer Flash EA 1112 (Italy) (below). The plant species studied from each site are listed in Sup. Tab. 2.

### Stable carbon isotope, C% and N% analyses

Bulk peat samples from Lakkasuo fen and bog sites were analyzed with a Vario Pyro Cube coupled with an Isoprime 100 (Elementar, Germany). Other peat and plant samples were analyzed with a Thermo Finningan Advantage IRMS (Germany) coupled with an elemental analyzer Flash EA 1112 (Italy). Stable isotope composition was expressed in the delta notation as a ‰ deviation of the heavy-to-light isotope abundance ratio in the sample from that of a standard, Vienna PD pelemnite. The results are reported relative to the standard scale.

$$\delta\ ^{13}C = (\frac{\left(\frac{^{13}C}{^{12}C}\right)sample}{\left(\frac{^{13}C}{^{12}C}\right)standard} - 1) * 1000 \qquad (1)$$

For C% and N%, a certified birch leaf standard (Elementar Microanalysis, UK) was used as a reference. This same in-house standard was used as an internal $\delta^{13}C$ isotopic standard on the Lsvec - LSB-19 scale [35]. In each run, the same amount of the house standard was analyzed for $\delta^{13}C$ after every 5 samples to correct for drift, while four standards corresponding to a range of sample C amounts were weighed at the beginning of each run for linearity correction. Repeated analysis of the birch leaf standard (n = 8 − 10) in each run had S.D < 0.2 for $\delta^{13}C$, < 0.5 for C% and < 0.2 for N%.

### Statistical analyses

The effect of the drying method on VilFN, LaaBD and LovFD samples from different depth zones was tested separately for surface, middle and basal peat using 1-ANOVA. Furthermore, differences in $\delta^{13}C$, C%, N%, C/N ratio, bulk density and water content between bottom peat and subsoil and between natural and drained sides of LakFNbot

were tested using independent sample *t*-tests. Individual sample values above and below the interface between bottom peat and subsoil were included in the analysis. The effect of peatland type (fen vs. bog) and drainage on $\delta^{13}C$, C% and N% of plant tissue collected from LakF and LakB was also tested using a *t*-test.

We tested the effect of drainage on peat $\delta^{13}C$, C%, N% and C/N ratio of Lakkasuo natural and drained side pairs from each depth by independent sample *t*-test. We acknowledge that when comparing natural and drained pairs from the same depth using current peat surface as a reference level, we are not comparing the same original peat depth layers except in the basal peat. In the peat surface layer, subsidence, shrinkage and respiratory carbon loss deepens the surface from the original level and new material from altered vegetation accumulates as litter on the surface [23].

In analyses of all the studied peatlands, data was grouped into three groups: surface peat representing the interface with the atmosphere; middle layer peat under the surface and above the basal peat layer, and the basal peat layer (Table 1). Differences in the $\delta^{13}C$, C%, N% and C/N ratio of surface, middle layer and basal peat samples between natural and drained peatlands were tested using site- and depth-specific average values as replicates in an independent sample *t*-test. In addition, differences in $\delta^{13}C$, C%, N% and C/N ratio between surface (n = 7), mid layer (n = 18) and basal peat (n = 7) were tested using site- and depth-specific average values as replicates in 1-ANOVA. Furthermore, the correlations between $\delta^{13}C$, C%, N% and C/N ratio of surface, middle and basal peat layers were tested by linear regression analysis. Pair-wise post-hoc tests for 1-ANOVAs were conducted using the least significant difference (LSD) technique with Hochberg-Bonferroni corrected α-values in each partial test. Analyses were conducted using IBM SPSS Statistics version 25.

**Results**

*Effect of drying method on $\delta^{13}C$ of bulk peat*

There were no significant differences in $\delta^{13}C$ values between freeze-dried and oven dried or acid fumigated samples irrespective of the site and depth zone.

*General trends in $\delta^{13}C$, C% and N% of bulk peat at the study sites*

In general, peat profiles in Lakkasuo (Fig. 2 a,f) had a larger range of $\delta^{13}C$ values (-29.8 - -26.6 ‰) than those in the Lammi area (-28.0 - -26.4 ‰) (Fig. 3 a,f,k). The lowest $\delta^{13}C$ value was in $LakBD_{255-280}$ (Fig. 2f), while the highest $\delta^{13}C$ was found from $LovFD_{30-80}$ (Fig. 3k). In general, the fen peat $\delta^{13}C$ values were lower than those of the bog peat at the Lakkasuo mire (Fig. 2a,f). The amount of nitrogen and C% were larger in fens than in bogs, leading to a higher C/N ratio in the bogs (Fig. 2b-d, g-i; 3b-d, g-i, l-n). LovFD was probably a fen before drainage, due to its location near the esker and current mature tree stand, however, the N% is low in the whole peat profile (Fig. 3m). The upper peat profile bulk density was clearly higher on LakFD compared to LakFN (Fig. 2e), while on LakB differences were smaller and the effect of drainage was not clear (Fig. 2j). In the Lammi area peatlands, VilFN had increasing BD towards the bottom (fig. 3e), while at LaaBD variation in BD was minor (Fig. 3j). On LovFD, BD is clearly higher at the surface compared to rest of the peat profile (Fig. 3o).

The overall average $\delta^{13}C$ value for all the studied peatlands had significant differences between depth layers (Table 2). Post hoc comparisons showed that $\delta^{13}C$ in basal peat was significantly lower (1.2‰) than the surface peat and (1.1‰) lower (p = 0.004) than the mid-layer peat (Table 2). The content of C, N%, and the C/N ratio did not differ between

peat layers (Table 3). There was also a positive correlation between surface and mid-layer peat $\delta^{13}C$ values (linear regression: $r^2 = 0.77$, $p = 0.009$, $n = 7$), but not between basal peat and mid-layer peat values. There was a clear positive correlation in the average $\delta^{13}C$ values between surface and basal peat of different sites (linear regression: $r^2 = 0.71$, $p = 0.009$, $n = 7$). Surface and basal peat of the sites at Lakkasuo were more depleted in $^{13}C$ than those of the Lammi area or of a raised bog in Scotland[39] (Fig. 4). The LakBD basal peat was more depleted in $^{13}C$ than others in the regression line and did not fit the pattern of the other sites (Fig. 4). Furthermore, a bog site from northern Minnesota, USA, had depleted $\delta^{13}C$ values in bottom layers and enriched $\delta^{13}C$ values in surface layers[36] (Fig. 4).

### *Effect of drainage on the $\delta^{13}C$, C% and N% of bulk peat of natural and drained pairs*

There were no differences in the averages of $\delta^{13}C$, C%, N% and C/N ratio between the natural ($n = 3$) and drained ($n = 4$) surface, middle or basal peat layers (t-test, $p > 0.05$). Yet, the N% of the basal peat layer tended to be smaller on the drained sites ($1.4 \pm 0.3\%$) than on the natural sites ($2.2 \pm 0.1\%$). However, N% in the mid layer was significantly smaller on the drained sites ($1.0 \pm 0.1\%$) compared to natural sites ($1.9 \pm 0.3\%$) [t-test: $t(16) = 2.92$, $p = 0.010$], thus the C/N ratio in the mid layer was significantly larger for the drained sites ($58.7 \pm 5.4\%$) compared to natural sites ($32.2 \pm 6.3\%$) [t-test: $t(16) = -2.98$, $p = 0.009$].

It was possible to study the effects of drainage in Lakkasuo natural and drained pairs. Drainage decreased $\delta^{13}C$ slightly in the whole peat profile of LakF, although not significantly at any depth (Fig. 2a). Drainage did not change LakBD $\delta^{13}C$ values of the three uppermost layers, but there was a significant decrease in LakBD$_{255-280}$ [t-test, $t(4) =$

5.996, p = 0.04) (Fig. 2f). Drainage increased bulk peat C% in LakFD$^{0-25}$ [t(4) = -3.797, p = 0.019], and LakFD$_{50-100}$ [t(4) = -5.447, p = 0.022] (Fig. 2b). In LakB, the C% was smaller in the whole profile of the drained side, but the difference was significant only in LakBD$_{50-100}$ [t(4) = 3.05, p = 0.038] and LakBD$_{255-280}$, [t(4) = -3.292, p = 0.000] (Fig. 2g). There was no change in N% due to drainage in LakF. There was significant decrease of N% in LakBD$_{50-100}$ [t(4) = 2.821, p = 0.048] and in LakBD$_{255-280}$ [t(4) = 3.621, p = 0.022] (Fig. 2h). C/N ratio remained similar in LakF after drainage, although there was a small increase in LakFD$_{25-50}$. On LakB, the C/N ratio increased in the drained side peat profile, but the increase was significant only in LakBD$_{50-100}$ [t(4) =-3.292, p = 0.030] (2i).

### Bottom peat and subsoil stratigraphy at Lakkasuo fen

Bottom peat was enriched in $^{13}$C towards the interface between the peat and subsoil on LaKFbotN and LaKFbotD. In contrast, in the subsoil below the interface, $^{13}$C-depletion took place, which was more pronounced in the natural side (Fig. 5a). The natural side of the subsoil was 1.0‰ depleted in $^{13}$C compared to the bottom peat [t(19.2) = 4.77, p = 0.000] (Table 3, Fig. 5a). Such a difference was not detected on the drained side. Furthermore, $\delta^{13}$C values of the subsoil of the natural side were 1.3‰ lower than in the drained side [t(26) = -4.17, p = 0.000], but not in the bottom peat (Table 3, Fig. 5a). The amount of C, N%, C/N ratio, BD and volumetric water content were significantly different between the bottom and the subsoil (t-test, p<0.05), both on natural and drained sides of LakF between the bottom peat and the subsoil.

There was a tendency for $\delta^{13}$C value, BD, C/N ratio and volumetric water content to be higher and C% and N% smaller in the bottom peat at the drained than at the natural side (Table 3, Fig. 5b).

### *δ¹³C, C% and N% of plants at LakF and LakB*

Plant species changed on the drained side of LakF and LakB (Sub. Tab. 2). There was a 2.9‰ decrease in $\delta^{13}C$ of the plants in LakFD compared to LakFN [$t(11) = 3.25$, $p = 0.008$] (Table 4). On the drained bog, the decrease of $\delta^{13}C$ was not significant (Table 4). In LakFD, plants were depleted in $^{13}C$ compared to LakBD plants [$t(15) = -2.85$, $p = 0.012$], but there was no significant difference between LakFB and LakBN. There was no difference in the C% or N% of the plants (t-test, $p > 0.05$) in natural and drained sides. There were also no differences in the C% or N% of the plants between natural fen and bog [$t(9)$, $p > 0.05$).

## Discussion

Microbial metabolites and microbial biomass after cell death are added to soil organic matter (SOM) as necromass. Contrary to living microbial biomass, the amount of necromass in SOM increases with depth [37]. We predicted that this can also happen in peat soils with slow degradation and that microbial necromass there can have different $\delta^{13}C$ values due to methanogenesis or methanotrophy compared to original peat C originating from plants. Thus, bulk peat consisting of both also includes signal of microbial necromass C. We further predicted that fraction of necromass C would oxidize or evaporate differently at 70 °C compared to plant derived biomass, but not during freeze drying. If necromass escapes, and $\delta^{13}C$ of necromass is different, drying would modify the $\delta^{13}C$ values of the bulk peat samples. However, our results showed no difference in $\delta^{13}C$ following the different drying methods for different depth zones, which indicates that the necromass C has similar $\delta^{13}C$ as plant remains. Similarly, fumigation with HCL did not change bulk peat $\delta^{13}C$ values, thus showing that there were no carbonates in the

peat.

In the studied Lakkasuo bog, the charcoal layer at a depth of 58 cm was dated to AD 1845 by dendrochronology [38]. Thus, our 0 - 25 cm and 25 – 50 cm samples from Lakkasuo bog, and at least the 0 - 25 cm layer of the fen, included $\delta^{13}C$ from the depleted atmospheric $CO_2$ of the industrial era in the natural and drained sides of the border ditch. Despite this, the studied surface peat layers were $^{13}C$ enriched compared to basal peat, as was the case in some other studies [4,39]. Thus, processes capturing and storing C in the surface peat column were overruling peat depletion due to the $^{13}C$ Suess effect, even though the $^{13}C$ Suess effect was decreasing $\delta^{13}C$ values in the studied surface peat layers similar to in 30 cm long *Sphagnum* peat cores in northern Finland [27].

Clymo and Bryant (2008) [40] found uniform $\delta^{13}C$ values in a depth profile of a 7-m deep raised Scottish peat bog, whereas a bog with a once extremely low water table (1.4 m) in northern Minnesota had the lowest $\delta^{13}C$ values in the surface and the highest in the middle peat layer [36]. In general, plausible explanations for low $\delta^{13}C$ values in basal peat are a high input of respired C back to aquatic vegetation and uptake of recycled C derived from methanogenesis during the wet initiation phase of peatlands [4]. Thus, peat growth in height in relation to minerogenic water sources leads to gradual change from fens to bogs, and to enrichment of the surface peat $^{13}C$. Furthermore, peat in fens is generally $^{13}C$-depleted compared to bogs [1,4,19], as it was in this study at Lakkasuo. Lignin in vascular plants typical to fens leads to $^{13}C$-depletion [41] compared to *Sphagnum* peat in bogs. Anaerobic microbial organic matter degradation of vascular plants further accelerates peat $^{13}C$ depletion, since microbes preferentially use isotopically heavier compounds instead of more recalcitrant lignin that has a lower $\delta^{13}C$ [42]. The beginning of all bogs in Finland is minerogenous [43], and in this study *Carex*, *Eriophorum* and *Phragmites* remains were found in the basal peat from the studied sites.

The observed positive correlations between basal and middle layer and basal and surface peat $\delta^{13}C$ may be common to peatlands, since $\delta^{13}C$ data from some other peatlands [39] also fit the same pattern, even though different peat profiles exist (Fig. 4). In general, methane oxidation producing $^{13}C$-depleted $CO_2$ mostly happens in aerobic peat and inside *Sphagnum* cells [44], thus the signal of $^{13}C$-depleted $CH_4$ ($\delta^{13}C$ –94 - -38 ‰) [45] can be returned to the same peat column as plant biomass or as microbial biomass. Besides methane oxidation, a plausible explanation for surface and basal peat $\delta^{13}C$ correlation may be the continuous return of $CO_2$ from peat respiration back to vegetation through photosynthesis, thus refixation of $CO_2$ released from the same vertical location. Based on $^{14}C$ dating, $20 \pm 5\%$ of peat carbon in the peat profile originates from peat $CO_2$ respiration [8]. Since isotopic fractionation of atmospheric $CO_2$ to moss tissue is 23.5‰ [46], $CO_2$ that is respired from peat is also similarly depleted in $^{13}C$ compared to atmospheric $CO_2$. The downward leaching DOC [47–49] of the same vertical location may also contribute to the similarity of $\delta^{13}C$ values of peat profiles from different locations. We can predict that internal C cycling in anaerobic and aerobic processes is also important in modifying peat $\delta^{13}C$ stratigraphy as well as atmospheric $CO_2$ $\delta^{13}C$.

LakF peat had a lower $\delta^{13}C$ than the LakB peat in almost the whole peat column in this study, as was the case in another study of the same sites [19]. The distance between the fen and bog sites at Lakkasuo is 0.5 km, and they are about the same age. Thus, they have developed in similar weather and atmospheric $CO_2$ concentration and $\delta^{13}C$ of atmospheric $CO_2$. The same factors explaining peat profile $\delta^{13}C$ stratigraphy (above) also affect the $\delta^{13}C$ differences between fen and bog: vascular plants are $^{13}C$-depleted [1] compared to *Sphagnum* and there is also a bigger share of recycled C in fens than in bogs. Recycling of $CH_4$ derived C contributes more to $^{13}C$-depletion of fen peat than of bog peat since fens both produce and oxidize more $CH_4$ than bogs.

In natural peatlands, uptake of $CO_2$ through photosynthesis is at least slightly larger than that lost during respiration, methanogenesis and leaching combined. The balance of C flows leading to their $\delta^{13}C$ values in pristine peat columns changes due to drainage. Drainage-induced changes were clearly detected as subsidence, clear water table decrease, increased respiration, decreased $CH_4$ emissions [17,23,50] and as significant change in C% and N% found at some depths in this study. However, low $\delta^{13}C$ of LakBD$_{255\text{-}280}$ could actually be due to it already being in the subsoil, as the C% was also extremely low. Plants growing on the drained side of LakF with an increased source of $^{13}C$-depleted $CO_2$ were also $^{13}C$-depleted. Aerobic respiration of $CO_2$ clearly increased due to drainage: respiration increased from 190 to 360 g C m$^{-2}$ a$^{-1}$ in LakF, and from 160 to 240 g C m$^{-2}$ a$^{-1}$ in LakB [23]. This increase in $CO_2$ release should have led to clear $^{13}C$-enrichment of the remaining peat, but it was not detected in this study. One explanation is counteracting processes depleting $^{13}C$ of the remaining peat. Decrease in $CH_4$ flow contributes to $\delta^{13}C$ balance, since $\delta^{13}C$ of $CH_4$ is lower (range -94 - 38 ‰) [45] than that of $CO_2$, even though C flows related to $CH_4$ are smaller than those of $CO_2$. In LakFN, C release as $CO_2$ was six times larger than as $CH_4$, while this ratio was 34 for LakBD [23]. After drainage, the originally large $CH_4$ flux ended in LakFD, and in LakB the initially smaller $CH_4$ fluxes on the natural side decreased to half that on the drained side. Decrease of $CH_4$ fluxes would keep the remaining peat C $^{13}C$ depleted, when the production of $^{13}C$-depleted $CH_4$ decreases. In addition, Fernandez et al. (2003) [41] showed that in 10 month aerobic incubations of vascular plants, the initially released $CO_2$ was depleted in relation to the original plant materials, but later enriched, and that the net effect in long lasting incubations was a $^{13}C$ depletion of the remaining plant material.

In drained peatlands better aeration increases respiration and in theory leads to depletion of the remaining peat. Lack of a drainage effect on the $^{13}C$ values in this study contrasts

with the results of Krüger et al. (2016). Our coarse sampling did not catch the small vertical variability and small (0.3 -1.1‰) but consistent ^{13}C-enrichment of peat columns from a depth of 5 cm to 93 cm of the same drained fen and ^{13}C-enrichment in drained bog from a depth of 13 cm to 41 cm and ^{13}C-depletion from 43 cm to 93 cm [19]. In their study, only bog and fen surface peat using same sampling depth interval average (0 - 25 cm) as was used in this study had same average $\delta^{13}C$ values on natural and drained sides [19], similarly as we found in this study. It is not clear why drainage effect was not visible in other depths in our data collected one year earlier from the same site. Since in both studies sampling, drying and analysis techniques were quite similar, the only possible explanation is slightly different location of sampling points in the same sites.

Carbon accumulates in subsoil under peat since DOC percolating through the peat column is absorbed in mineral soil in reducing conditions under anoxic peat, aided by slow mineralization in anaerobic conditions [51,52]. Carbon input from peat into the mineral subsoil was 13.6 g m$^{-2}$ year$^{-1}$ [16] and storage was 3.9 – 10.7 kg m$^{-2}$ of C [16,51,52]. Thus, subsoils form a globally important C reservoir, since the area of subsoil under peatlands corresponds to that of peatlands. Despite this, there are only a few studies of C storage and processes in subsoil [16,51,52]. Carbon in the subsoil of the LakFN was ^{13}C-depleted compared to the bottom peat above it, but was ^{13}C-enriched on the drained side. If we suppose that drained and natural side subsoil had similar $\delta^{13}C$ values before drainage, then the best explanation for subsoil C enrichment is that the aeration of subsoil is increased due to oxygenated water flow through the peat column to the subsoil. The aerobic layer deepened, so that the deepest measured WT was only 70 cm above the subsoil, and also temperature of the peat decreased on the drained side [17,23,53]. Although saturated vertical hydraulic conductivity of peat is low, 0.7 x 10$^{-5}$ – 1.3 x 10$^{-2}$ m s$^{-1}$ [54], it allows water to pass through the whole remaining peat column in a few

minutes or days. Levy et al. (2014) showed with water stable isotopes that meteoric water penetrated to basal peat of natural fen and bog [55], thus supporting earlier findings of DOC flow through peat columns. However, it is unclear, how water moves in saturated peat or subsoilt. Diffusion moves $CO_2$, $CH_4$ and $O_2$ to all directions, even diffusion is slower ($10^{-7}$- $10^{-9}$ $m^2$ $s^{-1}$) than advection [54]. Thus, aerobic respiration could enrich the remaining subsoil C in $^{13}C$. In addition to this, $CH_4$ oxidation may initially increase due to better aeration, but aeration decreases $CH_4$ production and thus the source of $^{13}C$ depleted $CH_4$ diminishes, which may also contribute to enrichment of subsoil. Increase in water flow on the drained side was supported by increased volumetric water content in the bottom peat and subsoil, as a decrease in peat N%, and also by an insignificant decrease in bottom peat and subsoil C% (tab. 3, Fig. 4). Furthermore, since the carbon in subsoil originates from DOC and is retained in the subsoil under anoxic peat due to reducing conditions [51,52], if redox then increases, bound C will also be released. Even though leaching is not fractionating between $^{12}C$ and $^{13}C$ of the remaining C, it may enhance C mineralization.

Although the vegetation pattern of the bottom peat was similar in both sides, and increase in C content of the mineral soil started at the same time as peatland started to develop, it is possible that the difference found here is an artefact of our limited data, and that the difference was due to spatial variability of peat and subsoil $\delta^{13}C$ before drainage.

**Conclusions**

In this study, surface peat $\delta^{13}C$ followed the $\delta^{13}C$ of the basal peat layer. This can be explained by recycling of respired $CO_2$ back to vegetation, downward leaching of DOC, and by methanogenesis and methanotrophy, which are all processes that move C in the

same location. The higher carbon release and uptake as $CO_2$ and $CH_4$ in the fen than in the bog, together with the dominance of lignin containing vascular plants, is a plausible explanation for the lower $\delta^{13}C$ values of the fens and basal peat.

We compared a pair of natural and drained sites of originally the same peatland, which were divided by ditching 51 years ago, as a possible method to study the effects of drying on peat by $\delta^{13}C$. There were no clear changes in $\delta^{13}C$, even when C%, N% and C/N ratio changed at some depths. It is also possible, that three replicates with low vertical resolution were not enough to capture variation of peat $\delta^{13}C$ in natural and drained pairs, or that processes depleting or enriching $^{13}C$ were in balance. Increase in $\delta^{13}C$ values of subsoil can be explained by increased water flow in subsoil leading to changes in microbial processes due to increased aeration. We are confident that drainage-driven $\delta^{13}C$-depletion of vegetation was reliable, because there were documented changes in surface vegetation and increase in $\delta^{13}C$ depleted $CO_2$ flows from decaying peat.

### References

[1]    Andersson RA, Meyers P, Hornibrook E, et al. Elemental and isotopic carbon

        and nitrogen records of organic matter accumulation in a holocene permafrost

peat sequence in the east European Russian arctic. J. Quat. Sci. 2012;27:545–552.

[2]  Price GD, McKenzie JE, Pilcher JR, et al. Carbon-isotope variation in Sphagnum from hummock-hollow complexes: implications for Holocene climate reconstruction. The Holocene. 1997;7:229–233.

[3]  Nichols JE, Walcott M, Bradley R, et al. Quantitative assessment of precipitation seasonality and summer surface wetness using ombrotrophic sediments from an Arctic Norwegian peatland. Quat. Res. 2009;72:443–451.

[4]  Jones MC, Peteet DM, Sambrotto R. Late-glacial and Holocene δ15N and δ13C variation from a Kenai Peninsula, Alaska peatland. Palaeogeogr. Palaeoclimatol. Palaeoecol. 2010;293:132–143.

[5]  Ingram HAP. Soil Layers in Mires: Function and Terminology. J. Soil Sci. 1978;29:224–227.

[6]  Krüger JP, Leifeld J, Alewell C. Degradation changes stable carbon isotope depth profiles in palsa peatlands. Biogeosciences. 2014;11:3369–3380.

[7]  Krüger JP, Leifeld J, Glatzel S, et al. Biogeochemical indicators of peatland degradation - A case study of a temperate bog in northern Germany. Biogeosciences. 2015;12:2861–2871.

[8]  Jungner H, Sonninen E, Possnert G, et al. Use of bomb-produced 14C to evaluate the amount of CO2 emanating from two peat bogs in Finland. Radiocarbon. 1995;37:567–573.

[9]  Rubino M, Etheridge DM, Trudinger CM, et al. A revised 1000 year atmospheric $\delta^{13}$ C-CO$_2$ record from Law Dome and South Pole, Antarctica. J. Geophys. Res. Atmos. 2013;118:8482–8499.

[10]  Oceanography SI of. Scripps CO2 program [Internet]. 2017. Available from:

http://scrippsco2.ucsd.edu/#.

[11]   Keeling CD. The Suess effect: 13 Carbon- 14 Carbon interrelations. Environ. Int.
       1979;2:229–300.

[12]   Gorham E. Role in the Carbon Cycle and Probable Responses to Climatic
       Warming. Ecol. Appl. 1991;1:182–195.

[13]   Blytt A. Forsøg til en Theori om Invandringen af Norges Flora under vexlende
       regnfulde og tørre Tider. Nyt Mag. Naturvidensk. 1867;21:279–362.

[14]   Kesitalo J. Reserves of peat and ligneous material in the mires of the Siikajoki
       valley and the Oulujärvi area. Nordia. 1982;16:1–109.

[15]   Laine J, Vasander H, Laiho R. Long-term effects of water level drawdown on the
       vegetation of drained pine mires in southern Finland. J Appl ecol. 1995;32:785–
       802.

[16]   Turunen J, Tolonen K, Tolvanen S, et al. Carbon accumulation in the mineral
       subsoil of boreal mires. Global Biogeochem. Cycles. 1999;13:71–79.

[17]   Minkkinen K, Vasander H, Jauhiainen S, et al. Post-drainage changes in
       vegetation composition and carbon balance in Lakkasuo mire, Central Finland.
       Plant Soil. 1999;207:107–120.

[18]   Pitkänen A, Turunen J, Tahvanainen T, et al. Carbon storage change in a partially
       forestry-drained boreal mire determined through peat column inventories. Boreal
       Environ. Res. 2013;18:223–234.

[19]   Krüger JP, Alewell C, Minkkinen K, et al. Calculating carbon changes in peat
       soils drained for forestry with four different profile-based methods. For. Ecol.
       Manage. 2016;381:29–36.

[20]   Simola H, Pitkänen A, Turunen J. Carbon loss in drained forestry peatlands in
       Finland, estimated by re-sampling peatlands surveyed in the 1980s. Eur. J. Soil

Sci. 2012;63:798–807.

[21] Ojanen P, Lehtonen A, Heikkinen J, et al. Soil CO2 balance and its uncertainty in forestry-drained peatlands in Finland. For. Ecol. Manage. 2014;325:60–73.

[22] Sallantaus T. Response of leaching from mire ecosystems to changing climate. In: Kanninen M, Heikinheimo P, editors. Finnish Res. Program. Clim. Chang. Second Prog. Report. Acad. Finland,. Helsinki: Academy of Finland; 1993. p. 291–296.

[23] Nykänen H, Alm J, Silvola J, et al. Methane fluxes on boreal peatlands of different fertility and the effect of long-term experimental lowering of the water table on flux rates. Global Biogeochem. Cycles. 1998;12:53–69.

[24] Jaatinen K, Fritze H, Laine J, et al. Effects of short- and long-term water-level drawdown on the populations and activity of aerobic decomposers in a boreal peatland. Glob. Chang. Biol. 2007;13:491–510.

[25] Mpamah PA, Taipale S, Rissanen AJ, et al. The impact of long-term water level draw-down on microbial biomass: A comparative study from two peatland sites with different nutrient status. Eur. J. Soil Biol. 2017;80:59–68.

[26] Straková P, Penttilä T, Laine J, et al. Disentangling direct and indirect effects of water table drawdown on above- and belowground plant litter decomposition: Consequences for accumulation of organic matter in boreal peatlands. Glob. Chang. Biol. 2012;18:322–335.

[27] Esmeijer-Liu AJ, Kürschner WM, Lotter AF, et al. Stable Carbon and Nitrogen Isotopes in a Peat Profile Are Influenced by Early Stage Diagenesis and Changes in Atmospheric CO(2) and N Deposition. Water. Air. Soil Pollut. 2012;223:2007–2022.

[28] Ghashghaie J, Badeck F-W, Lanigan G, et al. Carbon isotope fractionation during

dark respiration and photorespiration in C3 plants. Phytochem. Rev. 2003;2:145–161.

[29] Brüggemann N, Gessler A, Kayler Z, et al. Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: A review. Biogeosciences. 2011;8:3457–3489.

[30] Laine J, Komulainen VM, Laiho R, et al. Lakkasuo - a guide to mire ecosystem. Metsäekologian Laitos, Helsingin Yliopisto; 2004.

[31] Geological Survey of Finland. Peatland database of Geological Survey of Finland [Internet]. 2017. Available from: upa.gtk.fi/paikkatieto/meta/tutkitut_turvealueet.html.

[32] Pirinen P, Simola H, Aalto J, et al. Tilastoja Suomen ilmastosta 1981 - 2010. Raportteja. Ilmatieteen laitos; 2012.

[33] O'Kelly BC, Sivakumar V. Water Content Determinations for Peat and Other Organic Soils Using the Oven-Drying Method. Dry. Technol. 2014;32:631–643.

[34] Harris D, Horwath W, van Kessel C. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J. 65:1853–1856.

[35] Coplen TB, Brand WA, Gehre M, et al. New guidelines for $\delta^{13}C$ measurements. Anal. Chem. 2006;78:2439–2441.

[36] Hobbie EA, Chen J, Hanson PJ, et al. Long-term carbon and nitrogen dynamics at SPRUCE revealed through stable isotopes in peat profiles. Biogeosciences. 2017;14:2481–2494.

[37] Miltner A, Bombach P, Schmidt-Brücken B, et al. SOM genesis: Microbial biomass as a significant source. Biogeochemistry. 2012;111:41–55.

[38] Alm J, Tolonen K, Vasander H. Determination of recent apparent carbon

accumulation in peat using dated fire horizons. Suo 1992; 43191-194. 1992;43:191–194.

[39]  Waldron S, Hall AJ, Fallick AE. Enigmatic stable isotope dynamics of deep peat methane. Global Biogeochem. Cycles. 1999;13:93–100.

[40]  Clymo RS, Bryant CL. Diffusion and mass flow of dissolved carbon dioxide, methane, and dissolved organic carbon in a 7-m deep raised peat bog. Geochim. Cosmochim. Acta. 2008;72:2048–2066.

[41]  Fernandez I, Mahieu N, Cadisch G. Carbon isotopic fractionation during decomposition of plant materials of different quality. Global Biogeochem. Cycles. 2003;17:n/a-n/a.

[42]  Benner R, Fogel ML, Sprague EK, et al. Depletion of 13C in lignin and its implications for stable carbon isotope studies. Nature. 1987;329:708–710.

[43]  Laine J, Vasander H. No Title. In: Vasander H, editor. Peatlands Finl. helsinki: Finnish Peatland society; p. 10–20.

[44]  Nichols JE, Isles PDF, Peteet DM. A novel framework for quantifying past methane recycling by Sphagnum-methanotroph symbiosis using carbon and hydrogen isotope ratios of leaf wax biomarkers. Geochemistry, Geophys. Geosystems. 2014;15.

[45]  Holmes ME, Chanton JP, Tfaily M., et al. CO2 and CH4 isotope compositions and production pathways in a tropical peatland. Glob. Biogeochem. Cycles, 29,1–18,. 2015;29:1–18.

[46]  Brooks JR, Flanagan LB, Buchmann N, et al. Carbon isotope composition of boreal plants: Functional grouping of life forms. Oecologia. 1997;110:301–311.

[47]  Charman DJ, Aravena R, Bryant CL, et al. Carbon isotopes in peat, DOC, CO2, and CH4 in a holocene peatland on Dartmoor, southwest England. Geology.

1999;27:539–542.

[48]    Chasar L, Chanton J. Radiocarbon and stable carbon isotopic evidence for
transport and transformation of dissolved organic carbon, dissolved inorganic
carbon, and CH4 in a. Global Biogeochem. Cycles. 2000;14:1095–1108.

[49]    Tfaily MM, Cooper WT, Kostka JE, et al. Organic matter transformation in the
peat column at Marcell Experimental Forest: Humification and vertical
stratification,No Title. J. Geophys. Res. Biogeosci.,. 2014;119:661–675.

[50]    Silvola J, Alm J, Ahlholm U, et al. CO2 fluxes from peat in boreal mires under
varying temperature and moisture conditions. J. Ecol. 84:219–228.

[51]    Turunen J, Moore TR. Controls on carbon accumulation and storage in the
mineral subsoil beneath peat in Lakkasuo mire, central Finland. Eur. J. Soil Sci.
2003;54:279–286.

[52]    Moore TR, Turunen J. Carbon Accumulation and Storage in Mineral Subsoil
beneath Peat. Soil Sci. Soc. Am. J. 2004;68:690.

[53]    Straková P, Anttila J, Spetz P, et al. Litter quality and its response to water level
drawdown in boreal peatlands at plant species and community level. Plant Soil.
2010;335.

[54]    Rezanezhad F, Price JS, Quinton WL, et al. Structure of peat soils and
implications for water storage, flow and solute transport: A review update for
geochemists. Chem. Geol. 2016;429:75–84.

[55]    Levy ZF, Siegel DI, Dasgupta SS, et al. Stable isotopes of water show deep
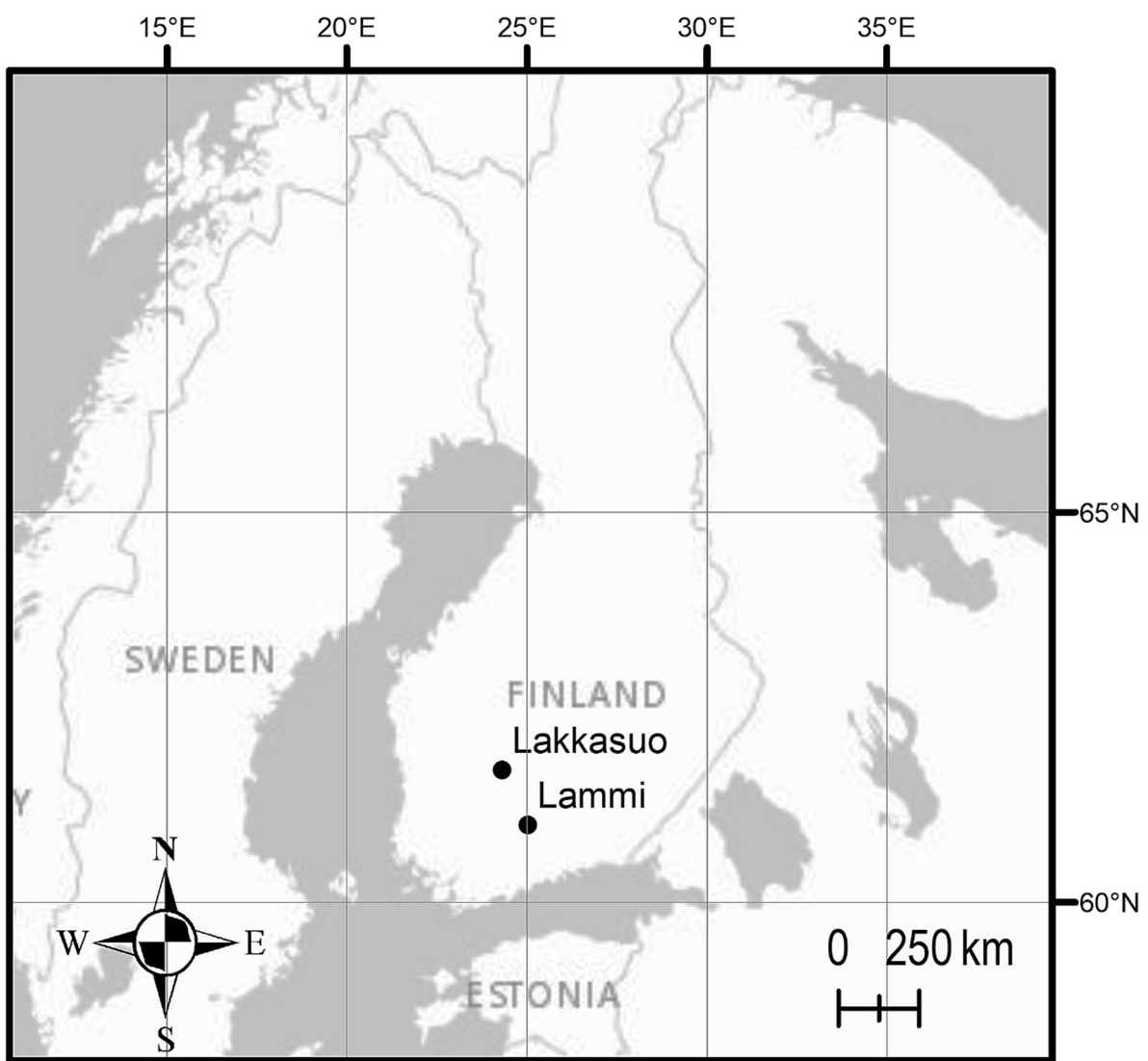seasonal recharge in northern bogs and fens. 2014;

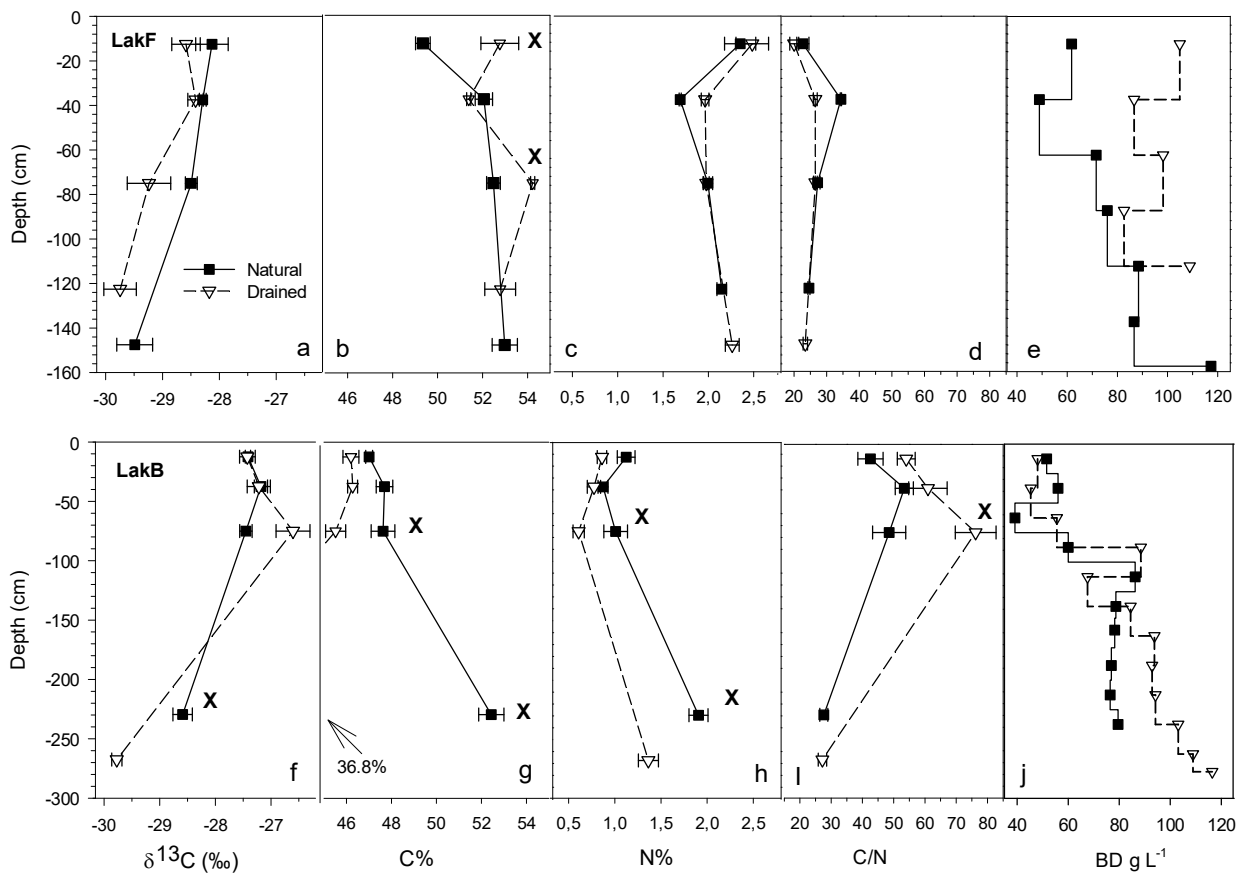Fig. 1. Location of the study areas, Lakkasuo and Lammi.

Fig. 2. Lakkasuo depth profiles of $\delta^{13}C$ (a,f), C% (b,g), N% (c,h), C/N-ratio (d,i), bulk density (BD) (e,j). (Average ± S.E., n = 3, except in BD, where n = 1). Statistically significant differences in $\delta^{13}C$, C%, N% and C/N ratio (t-test, $p < 0.05$) are marked with an X.

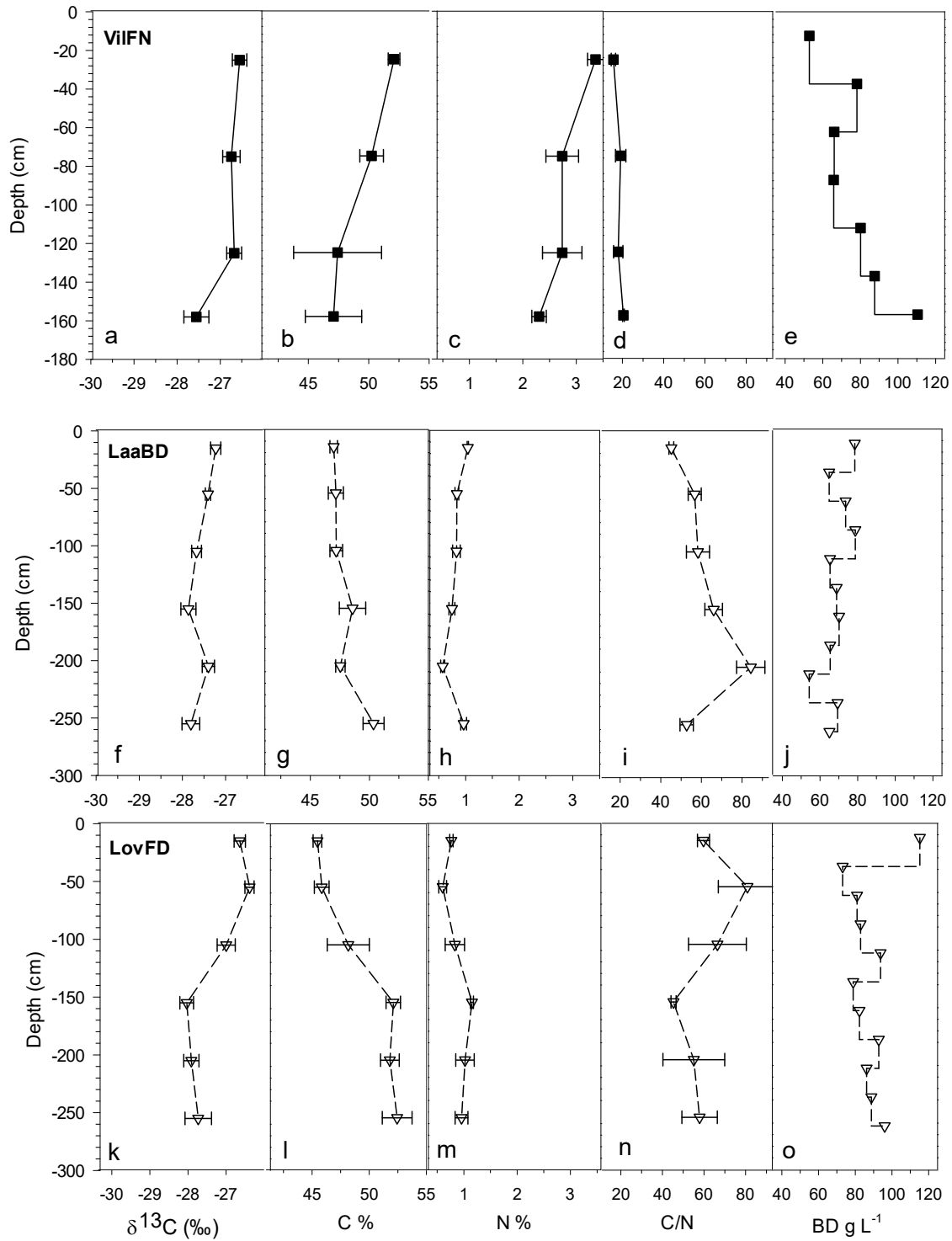Fig. 3. Profiles from the Lammi area: $\delta^{13}C$ (a,f,k), C% (b,g,l), N% (c,h,m), C/N-ratio (d,i,n) and BD (e,j,o). (Average ± S.E., n = 3, except in BD, where n = 1).
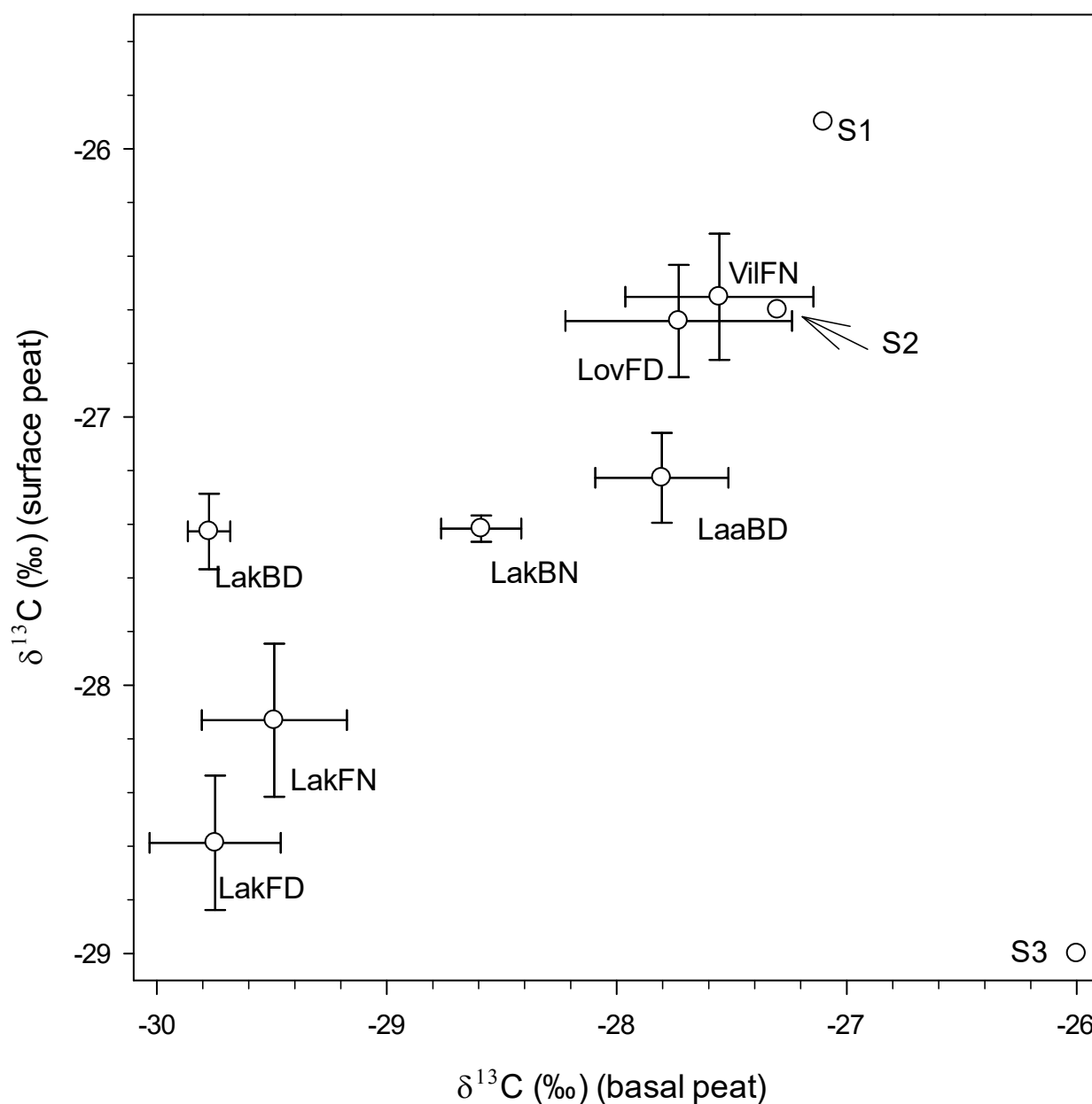
Fig. 4. Correlation between surface and basal $\delta^{13}C$ (average ± S.E., n = 3). Abbreviations: LakFN = Lakkasuo natural side, LakFD = Lakkasuo drained side, LovFD = drained Lovonsuo, LaaBD = drained Laaviosuo and VilFN = natural Villikkalansuo. Studied peatlands are supplemented with values from two fen sites in Waldron et al. 1999 [39] (S1 and S2) and data from one bog site Hobbie et al. 2017 [36]. Surface peat $\delta^{13}C$ = 0.63 x (bottom peat $\delta^{13}C$) - 9.47, ($r^2$= 0.71, n = 7). When S1 and S2 are included in the equation: surface peat $\delta^{13}C$ = 0.69 x (bottom peat $\delta^{13}C$) - 7.46, ($r^2$= 0.79, n = 9).
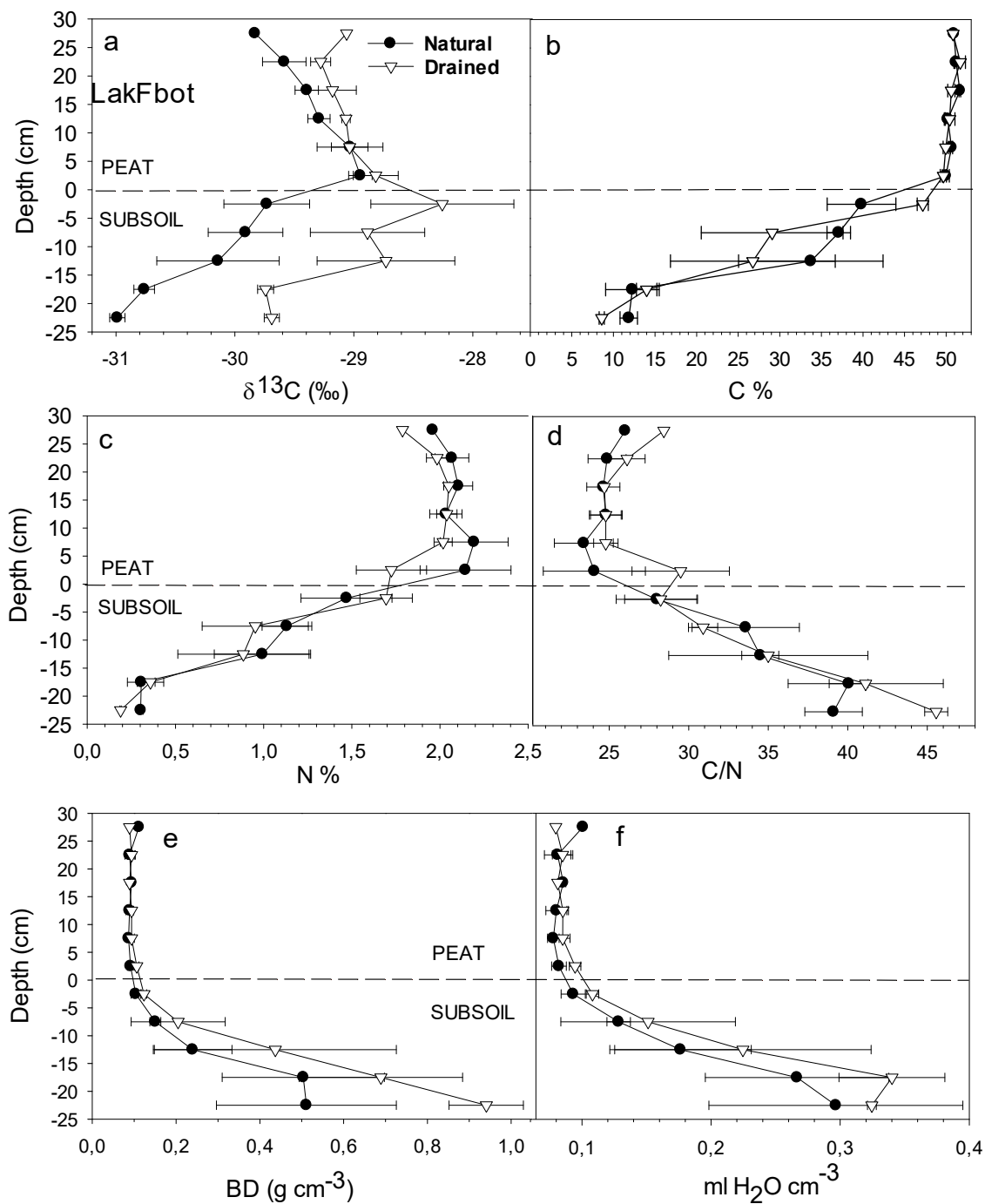
Fig. 5. Peat bottom profiles from natural and drained Lakkasuo Fen, i.e. LakFNbottom and LakFDbottom. A depth of zero indicates the transition zone between peat and subsoil; above zero is peat and below zero sub soil. $\delta^{13}C$ (a), C% (b), N% (c), C/N-ratio (d), bulk density (e) and volumetric water content (f).

Table 1: General features of the study sites and sampling depths

| Location | Mean temp. (°C)[1] | Precipi-tation (mm yr$^{-1}$) | Site code and year of drainage | Tree stand volume (m$^{-3}$ ha$^{-1}$) | Average water table or during sampling (cm) | Temp. at -30 cm (°C) | Subsidence of drained side (cm) | Sampling depths from surface (cm)[2] |
|---|---|---|---|---|---|---|---|---|
| Lakkasuo[3] | 3.4 | 711 | LakFN | 0 | -8 | 9.7 | | **0 - 25**, 25 - 50, 50 - 100, <u>135 – 160</u> |
| | | | LakFD, 1961 | 111 | -32 | 9.1 | -23 | **0 - 25**, 25 - 50, 50 - 100, <u>115 – 130</u> |
| | | | LakBN | 5 | -10 | 11.2 | | **0 - 25**, 25 - 50, 50 - 100, <u>217 – 242</u> |
| | | | LakBD, 1961 | 16 | -21 | 9.5 | -10 | **0 - 25**, 25 - 50, 50 - 100, <u>255 – 280</u> |
| Lammi area[4] | 4.2 | 645 | VilFN | 0 | 0 | n.d. | n.d | **0 - 50**, 50 - 100, 100 - 150, <u>150 - 165</u> |
| | | | LaaFD,1978 | dense | -20 - -30 | n.d. | n.d. | **0 - 30**, 30 - 80, 80 - 130, 130 - 180, 180 - 230, <u>230 - 280</u> |
| | | | LovFD,~1920 | dense | < -30 | n.d. | n.d. | **0 - 30**, 30 - 80, 80 - 130, 130 - 180, 180 - 230, <u>230 - 280</u> |

[1]Weather data from [32]. [2]Surface samples are marked by bold, middle layer samples by normal text and bottom layer samples are underlined.
[3]Lakkasuo mire complex features are adopted and modified from [17,23]. [4]Description and measurements from VilFN, LaaFD and LovFD at Lammi area are our own.

Table 2. Averages of $\delta^{13}C$, C%, N% and C/N-ratio of natural and drained peat layers of all sites together. Statistical differences between depth layers are expressed by different letters.

| Depth | n | $\delta^{13}C$ (‰) | C % | N% | C/N-ratio |
|---|---|---|---|---|---|
| Surface | 7 | $-27.4 \pm 0.3^a$ | $48.6 \pm 1.1$ | $1.7 \pm 0.4$ | $37.1 \pm 6.6$ |
| Middle | 18 | $-27.6 \pm 0.2^a$ | $49.1 \pm 0.7$ | $1.3 \pm 0.2$ | $49.9 \pm 5.0$ |
| Basal | 7 | $-28.7 \pm 0.4^b$ | $49.3 \pm 2.2$ | $1.7 \pm 0.2$ | $33.4 \pm 5.8$ |
| F(2,29) | | *5.58* | *0.083* | *1.13* | *2.27* |
| p | | *0.009* | *0.921* | *0.336* | *0.122* |

1-ANOVA, pairwise tests using LSD with Hochberg-Bonferroni corrected α - values.

Table 3. Lakkasuo fen bottom peat and subsoil $\delta^{13}C$, C%, N%, C/N-ratio and volumetric water content (average ± S.E, n = 16 for peat and n = 14 for subsoil). Averages are from 4 sample layers above and below the interface between peat and subsoil. Results of t-tests between peat and subsoil are shown column-wise, and between natural (LakFbotN) and drained side (LakFbotD) of peat and subsoil are shown in the right column. Significantly different values in Independent sample *t*-test (p<0.05) are expressed with bold text.

| | LakFbotN | LakFbotD | LakFbotN vs. LakFbotD |
|---|---|---|---|
| $\delta^{13}C$‰ peat | -29.3 ± 0.1 | -29.1 ± 0.1 | *t(30) = -1.95, p = 0.068* |
| $\delta^{13}C$‰ subsoil | -30.3 ± 0.2 | -29.0 ± 0.1 | ***t(26) = -4.17, p = 0.000*** |
| | ***t(19.2) = 4.77 p = 0.000*** | *t(15.0) = -0.239 p = 0.815* | |
| C% peat | 50.7 ± 0.2 | 50.5 ± 0.5 | *t(30) = 0.81, p = 0.427* |
| C% subsoil | 28.0 ± 3.8 | 26.3 ± 4.4 | *t(26) = 0.52, p = 0.768* |
| | ***t(13) = 5.33 p = 0.000*** | ***t(13.1) = 5.55 p = 0.000*** | |
| N% peat | 2.1 ± 0.1 | 2.0 ± 0.1 | *t(30) = 0.938, p = 0.062* |
| N% subsoil | 0.9 ± 0.1 | 0.9 ± 0.2 | *t(26) = 0.082, p = 0.936* |
| | ***t(17.1) = 7.70 p = 0.000*** | ***t(15) = 6.15 p = 0.000*** | |
| C/N peat | 24.5 ± 0.7 | 26.1 ± 0.7 | *t(30) = 0.038, p = 0.104* |
| C/N subsoil | 34.8 ± 1.4 | 35.5 ± 2.7 | *t(26) = 0.295, p = 0.207* |
| | ***t(18.4) = -6.35 p = 0.000*** | ***t(15.7) = -3.93 p = 0.001*** | |
| BD (g $dm^{-3}$) peat | 91.2 ± 3.0 | 94.9 ± 2.5 | *t(30) = 0.94, p = 0.355* |
| BD (g $dm^{-3}$) subsoil | 286.7 ± 65.0 | 440.0 ± 104.1 | *t(21.9) = -1.30, p = 0.209* |
| | ***t(13.1) = -2.99 p = 0.000*** | ***t(13) = -3.37 p = 0.005*** | |
| $H_2O$ $cm^3$ $cm^{-3}$ peat | 0.08 ± 0.002 | 0.09 ± 0.002 | *t(30) = 0.341, p = 0.362* |
| $H_2O$ $cm^3$ $cm^{-3}$ subsoil | 0.18 ± 0.034 | 0.22±0.034 | *t(26) = -0.855, p = 0.400* |
| | ***t(13.2) = -3.56 p = 0.003*** | ***t(13.1) = 3.99 p = 0.002*** | |

Table 4. Comparison of $\delta^{13}C$, C% and N% (average ± S.E.) of plants between natural and drained sides of LakF (i.e. LakFN vs. LakFD) and LakB (i.e. LakBN vs. LakBD) and between fen and bog in the natural (LakFN vs. LakBN) and in drained (LakFD vs. LakBD) is column-wise. Significantly different values in Independent sample t-test are expressed with bold text.

|  | $\delta^{13}C‰$ | C% | N% |
|---|---|---|---|
| LakFN (n =5) | -29.2 ± 0.9 | 48.9 ± 1.3 | 0.82 ± 0.15 |
| LakFD (n = 8) | -32.1 ± 0.4 | 46.9 ± 0.6 | 1.17 ± 0.17 |
| LakFN vs. LakFD | **t(11) = 3.25** | *t(11) = 1.52* | *t(11) = -1.45* |
|  | **p = 0.008** | *p = 0.157* | *p = 0.174* |
|  |  |  |  |
| LakBN (n = 6) | -29.7 ± 0.5 | 47.9 ± 0.9 | 0.88 ± 0.14 |
| LakBD (n = 9) | -30.2 ± 0.5 | 47.9 ± 0.7 | 0.88 ± 0.12 |
| LakBN vs. LakBD | t(13) = 0.65 | t(13) = 0.03 | t(13) = 0.01 |
|  | p = 0.529 | p = 0.973 | p = 0.994 |
|  |  |  |  |
| LakFN vs. LakBN | t(9) = 0.51 | t(9) = 0.64 | t (9) = -0.30 |
|  | p = 0.622 | p = 0.536 | p = 0.769 |
| LakFD vs. LakBD | **t(15) = -2.85** | *t(15) = -1.00* | *t(15) = 1.46* |
|  | **p = 0.012** | *p = 0.335* | *p = 0.164* |

**Supplementary Tables**

Sup. Tab. 1. Main peat constituents and their relative proportions in six grade scale [1]. Peat plant remains constituents: C = *Carex*, L= *Lignum*, S= *Sphagnum*, ER = *Eriophorum* and PR = *Phragmites.*

| Depth (cm) | Peat constituents | Depth (cm) | Peat constituents |
|---|---|---|---|
| LakFN | | LakFD | |
| 0 – 30 | C2S4 | 0 - 30 | S2C4 |
| 30 - 50 | S2C4 | 30 - 50 | S2C4 |
| 50 - 70 | S2C4 | 50 - 80 | L1S2C3 |
| 70 - 100 | S2C4 | 80 - 100 | L1S2C3 |
| 100 - 150 | L1S2C3 | 100 - 150 | L2S2C2 |
| 150 - 190 | L1S2C3 | | |
| LakBN | | LakBD | |
| Depth (cm) | Peat constituents | Depth (cm) | Peat constituents |
| 0 – 50 | ER2S4 | 0 - 100 | S6 |
| 50 - 70 | ER1S5 | 100 - 120 | L1S5 |
| 70 - 100 | ER1S5 | 120 - 150 | ER1S5 |
| 100 - 150 | ER1S5 | 150 - 180 | ER1S5 |
| 150 - 200 | L1PR1S4 | 180 - 200 | ER1C2S3 |
| 200 - 250 | L1PR1C1S3 | 200 - 290 | ER1L1C2S2 |
| 250 - 260 | L2C2S2 | | |

**References**

[1] Lappalainen, E, Stén, C-G and Häikiö, J. Turvetutkimusten maasto-opas. Geologian tutkimuskeskus, Opas no. 12. Espoo 1984. ISBN 951-690-192-1.

Sup. Tab. 2. Analysed plant species, and type of sample. Mosses are marked with bold text.

| Site | Plants and number of parts analyzed. |
|------|--------------------------------------|
| LakFN | *Pinus sylvestris* [needles (n =1), trunk (n =1)], *Empetrum nigrum* [needles (n =1), trunk (n = 1)], *Carex sp.*(n =1) |
| LakFD | *Betula pubenscens* [leaves (n = 1), trunk (n = 1)], **Dicranum polysetum** [leaves (n = 1), trunk (n = 1)] , *Picea abies* [needles (n = 1), trunk (n = 1)], *Empetrum nigrum* [needles (n = 1) and trunk (n = 1)], *Agrostis capillaris* (n = 1) |
| LakBN | *Pinus sylvestris* [needles (n = 1), trunk (n = 1)], **Polytrichum commune (n = 1)**, *Vaccinium vitis-idaea* [needles (n = 1) and trunk (n = 1)], *Vaccinium myrtillus* (n = 1) |
| LakBD | *Calluna vulgaris* [needles (n = 1), trunk (n = 1)], *Empetrum nigrum* [needles (n = 1), trunk (n = 1)], *Vaccinium vitis-idaea* [needles (n = 1), trunk (n = 1)], *Pinus sylvestris* [(needles (n = 1), trunk (n = 1)], *Vaccinium uliginosum* (n = 1) |