JYU DISSERTATIONS 39

Promise Mpamah

Response of Microbial Biomass and Carbon Dynamics to Changing Hydrological Conditions in Old Peat Deposits

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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Editors
Timo Marjomäki
Department of Biological and Environmental Science, University of Jyväskylä
Ville Korkiakangas
Open Science Centre, University of Jyväskylä

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ABSTRACT

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Peatlands play key roles in the global ecosystem carbon (C) balance, as important C-stores. Their efficiency as long-term C-stores or atmospheric C sink is affected by the rate of microbial mineralization. Most studies on the climate change effects on the microbial communities and C-cycling in peatlands have focused on the surface layers. This thesis compared microbial biomass, community composition and internal C cycling processes in drained vs. natural peat profiles with focus on deep old peat. Using the phospholipid fatty acid (PLFA) and rRNA gene analyses, the study particularly looked at the impact of long-term drainage and the short-term effects of temperature and water content on the microbial communities as well as measured how these affected peat decomposition process e.g. by the bulk peat $\delta^{13}C$ analysis and CO_2/CH_4 gas release. The results showed that low microbial activity and biomass are the major factors responsible for the low C losses and activities from old peat. Although the MBC measured by chloroform fumigation extraction (CFE) did not show drainage effects, drainage increased the total microbial PLFA biomass (m^{-3}) up to the deepest layers of the fen but decreased it in the bog site. In the combined site data, drainage also decreased the average (± SE) proportion of archaeal to prokaryotic DNA from 44.9 ± 2.4 % to 20.1 ± 2.6 %. The differences found in PLFAs over the peat column because of drying were likely due to concomitant changes in vegetation cover. Although the living MBC appears to be a small fraction of peat total C (TC; highest range from 0.8 % to 2.0 % in the fen peat site), the study indicated that the MBC might contribute substantially more (both as living MBC and necromass) to the C storage of peatlands at regional or global scales than originally thought. The study identified microbial biomass as a primary constraint in peat decomposition and C cycling.

Keywords: Carbon loss; long-term peatland drainage; microbial biomass carbon; old peat; 16S rRNA gene; PLFA; stable isotopes; CFE.

Promise Anyalewachi Mpamah, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address

Promise Anyalewachi Mpamah
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
promise.a.mpamah@jyu.fi

Supervisors

Docent Hannu Nykänen
University of Eastern Finland
Department of Environmental Science
P.O. Box 1627
FI-70211 Kuopio
Finland

Assistant Professor Christina Biasi
University of Eastern Finland
Department of Environmental Science
P.O. Box 1627
FI-70211 Kuopio

Docent Sami Taipale
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Dr. Antti J. Rissanen
Tampere University of Technology
Laboratory of Chemistry and Bioengineering
P.O. Box 541
FI-33101 Tampere
Finland

Professor Marja Tiirola
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Reviewers

Docent Kari Minkkinen
University of Helsinki
Department of Forest Science
Latokartanonkaari 7
FI-00014 University of Helsinki
Finland

Professor Marcus Andreas Horn
Leibniz University of Hannover
Herrenhäuser Straße 2, 30419
Hannover
Germany

Opponent

Professor Ülo Mander
University of Tartu
Department of Geography
Ülikooli 18
50090 Tartu
Estonia

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LIST OF ORIGINAL PUBLICATIONS

The thesis was based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

I was responsible for the laboratory work for paper I and II and partly involved in the laboratory work of III and IV. I was involved in the planning of papers I and II together with Hannu Nykänen and Christina Biasi, while Sami Taipale and Antti J. Rissanen joined the planning at the later stages. I was equally involved in the planning of III, together with Hannu Nykänen and Antti J. Rissanen. I was involved in the sampling and fieldwork for papers I, II and III, but only partly analyzed the samples (PLFA) for IV. I was responsible for writing papers I, and II with contributions from all the co-authors. I participated in the writing of papers III and IV. All papers were finished together will all co-authors. Additionally, samples for the cell biomass and DNA works (fall 2017) outside the papers were collected by me and Hannu Nykänen. I made the chloroform-fumigation extraction and Dr. Katharina Kujala performed the DNA extractions and provided the data of qPCR analyses.

I Mpamah P.A., Taipale S., Rissanen A.J., Biasi C. & Nykänen H. 2017. The impact of long-term water level draw-down on microbial biomass: a comparative study from two peatland sites with different nutrient status. *European Journal of Soil Biology* 80: 59–68.

II Mpamah P.A., Rissanen A.J., Taipale S., Biasi C. & Nykänen H. 2018. Estimate of microbial biomass carbon stock in northern peatland. Manuscript.

III Nykänen H., Mpamah P.A. & Rissanen A.J. 2018. Stable carbon isotopic composition of peat columns, subsoil and vegetation on natural and forestry-drained boreal peatlands. *Isotopes in Environmental and Health Studies*, doi: 10.1080/10256016.2018.1523158.

IV Diáková K., Čapek P., Kohoutová I., Mpamah P.A., Bárta J., Biasi C., Martikainen P.J. & Šantrůčková H. 2016. Heterogeneity of carbon loss and its temperature sensitivity in East-European subarctic tundra soils. *FEMS Microbiology Ecology* 92(9), doi: 10.1093/femsec/fiw140.

ABBREVIATIONS

ANOVA	analysis of variance
Cex	extractable carbon
CFE	chloroform fumigation-extraction
CFI	chloroform fumigation-incubation
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DW	dry weight
MBC	microbial biomass carbon
Nex	extractable nitrogen
NMDS	non-metric multidimensional scaling
OC	organic carbon
OM	organic matter
PERMANOVA	permutational multivariate analysis of variance
Pex	extractable phosphorous
PLFA	phospholipid fatty acid
rRNA	ribosomal RNA
SIR	substrate induced respiration
SOC	soil organic carbon
SOM	soil organic matter
TC	total carbon
WT	water table

1 INTRODUCTION

1.1 Background

Peatlands cover about 3 % of the global land surface area (Joosten and Clarke 2002, Yu *et al*. 2011). Despite this relatively small areal extent, they play key roles as important carbon (C) stores in the global C budget (Immirzi *et al*. 1992, Turunen *et al*. 2002). Peat is the most efficient terrestrial C reservoir (Roulet 2000, Anon. 2010) and about one third of the terrestrial C pool, amounting to ca. 450–550 Pg C, are globally stored in peatlands (Immirzi *et al*. 1992, Sabine *et al*. 2004, Parish *et al*. 2008, Yu *et al*. 2011, Jungkunst *et al*. 2012). Also, as a long-term sink of atmospheric C [32.3 g C m^{-2} a^{-1}; (Yu 2012)], peatlands are considered to be a cost-effective measure in the adaptation and mitigation of climate change (Gorham 1991, Reed 2009, Lunt *et al*. 2010). The majority of peatland occur in the north, boreal and subarctic regions. In Finland for instance peat is reported to be the most important long-term C store, that exceeds the C stored in vegetation or mineral soil. The average C storage capacity in Finnish peatlands is about 72 kg C m^{-2} (Kauppi *et al*. 1997) which is more than the global average.

Formation of contemporary peat deposits has been a dynamic process with periods of alternating accumulation and degradation throughout their history (Page *et al*. 2004, Hirano *et al*. 2007). Peatlands, especially if drained, have the capacity for a huge positive feedback to the climate via increased C emissions (Cao *et al*. 1996, Turetsky *et al*. 2014). Hence, the amount of C stored or released by peatlands, naturally or via human influence, are very significant in the global C cycle (Gorham 1991).

1.2 Carbon storage and dynamics in peatlands

Incompletely decayed plant and animal materials that accumulated over a very long period of time (thousands of years) in usually waterlogged and acidic conditions are referred to as peat (Charman 2002, Rydin and Jeglum 2006).

Peatlands are ecosystems, where more than 0.3–0.4 m of peat has accumulated (Charman 2002, Harris and Bryant 2009). This definition and especially thickness of peat accumulation in a peatland may vary from one region to another (Joosten and Clarke 2002). Although there are shallower depths in the permafrost peatlands, recent estimate of peat depth in the boreal and Arctic peatlands is put at an average of 2.3 m (Gorham 1991). About 10 % of natural peat deposits are plant materials at different stages of decomposition, while up to 90 % of the wet weight is water.

Over centuries or millennia, about 2–16 % of the net primary production of peatland ecosystems is deposited as peat (Päivänen and Vasander 1994). Depending on the peatland type, the organic matter (OM) content of the partly decomposed plant residues in peat ranges from 20 % up to 90 % and more [mean > 65 %; (Charman 2002)]. For example, in the nutrient poor bog peatlands, which exclusively depend on atmospheric deposition for nutrients, the OM content of the dominant (living and dead biomass) *Sphagnum* moss litter can be well over 90 % (Gorham 1995). In the more fertile minerotrophic peatlands (fen) with higher water levels, the OM content of the more labile dominant sedges (*Carex*) is lower (Minkkinen *et al*. 1999, Laiho 2006, Jaatinen *et al*. 2007). On the average, about half of peat OM is C (Klingenfuß *et al*. 2014).

The rate of C accumulation on the surface of peatlands depends on the site type, vegetation and natural state of the site (Mäkilä 2011). Very high interannual and between-site variation in C accumulation rates in different peatland types and sites have been reported (Yu *et al*. 2012). However, Tolonen and Turunen (1996) showed that the average long-term C accumulation rates in g C m^{-2} a^{-1} was much higher in the bogs (24.0 ± 0.5, range 6.6–85.8, n = 548) than in the fens (15.1 ± 0.1, range 2.8–49.1, n = 373). They also showed that in the Finnish peatlands younger than approximately 9000 years, the long-term C accumulation rate was much higher and almost double in the southern compared to the northern mires. These differences in the C accumulation rates are probably due to variation in the internal processes that limits decomposition in peatlands (Clymo 1984, Belyea and Baird 2006). Variable depths for bogs and fens have also been reported. For example, mean depths of 1.88–2.55 m for bogs and 1.37–2.15 m for fens were reported for peatlands in the southern to northern parts of Ontario Hudson Bay Lowlands (Riley 2011).

The efficiency of peatlands as a long-term C store or atmospheric C sink is usually affected by net primary production on the one hand and microbial mineralization on the other hand (Yu 2012). The average net primary production is estimated at 2510.4 kJ m^{-2} a^{-1} and 1464.4 kJ m^{-2} a^{-1} for polar tundra and boreal forest, respectively. In natural peatlands, complete microbial breakdown of the deposited plant materials is impeded by factors such as prevalent anoxic condition, low soil temperatures and low pH, caused by continuous water logging and flooding of peatlands (Moore and Basiliko 2006, Artz 2009). Additionally, nutrient availability and quality of peat forming plant litters are low (Van Breemen 1995). Thus, natural peatlands are usually a net CO_2 sink with varying CO_2 sink capacity dependent on weather conditions, such as, temperature and hydrological regimes (Yu, 2011). Peat hydrology also

serves as one of the main factors controlling the diversity and composition of peat microbial communities and the associated rate of C cycling (Moore and Basiliko 2006, Andersen *et al*. 2013, Mishra *et al*. 2014). While natural peatlands are usually net CO_2 sinks, they are strong net sources of CH_4 due to the degradation of OM in anoxic soil conditions (Turetsky *et al*. 2014). In the deeper anoxic peat layers without O_2, final decomposition by methanogens produces CH_4, part of which is oxidized in the oxic layers by methanotrophs while the rest escapes to the atmosphere. Boreal and arctic peatlands are globally significant CH_4 sources (Moore and Knowles 1987, Crill *et al.* 1992, Whalen and Reeburgh 2000, Christensen *et al.* 2003). Nevertheless, because CO_2 uptake usually exceeds CH_4 release, natural peatlands generally represent a net C sink (Whalen and Reeburgh 2000). However, especially drying of peatlands may turn peatlands from C sinks to C sources particularly by increasing the release of CO_2 (Silvola *et al.* 1996, Minkkinen *et al.* 1999, Maljanen *et al.* 2010, Simola *et al.* 2012). The huge peat C-store is labile and sensitive to changes in climate and land use management, e.g. changes in hydrology as well as temperature regime, and is thus highly sensitive to global change (Shurpali *et al*. 1995, Trumbore *et al*. 1996, Lohila *et al*. 2004, Vanhala *et al*. 2007, Maljanen *et al*. 2009, Shurpali *et al*. 2009).

1.2.1 Effects of climate change and drainage on peatland carbon cycles

A complex interplay between the quality and quantity of OM, microbial communities and prevailing environmental conditions such as hydrological status and temperature governs the C cycling processes in peatlands (Jungkunst *et al*. 2012, Andersen *et al*. 2013). Ultimately, peat OM transformation is regulated by temperature and water saturation (anaerobic condition) status due to their influence on microbial activities (Yavitt *et al*. 1997, Deslippe *et al*. 2012, Jungkunst *et al*. 2012, Andersen *et al*. 2013, Männistö *et al*. 2013, Tveit *et al*. 2013, Peltoniemi *et al*. 2015). Climate change as well as natural and anthropogenic disturbances affect peatlands in different ways (Turetsky *et al*. 2002, Davidson and Janssens 2006, Moore and Basiliko 2006, Dorepaal *et al*. 2009, Andersen *et al*. 2013). Climatic changes can induce positive or negative feedbacks from peatlands by altering their C storage capacity and greenhouse gas balance (Turetsky *et al*. 2002, Andersen *et al*. 2006, Maljanen *et al*. 2010). For example, climate warming of about 1° C for few decades can trigger a global increase in peatland heterotrophic respiration, estimated now at 38–100 Tg C a^{-1} (Dorrepaal *et al*. 2009). Hence, the recent and predicted global climate warming may create a large and positive feedback to the atmosphere by increasing peat decomposition rates (Dorrepaal *et al*. 2009, Vicca *et al*. 2009). However, the temperature sensitivity of soil C-respiration is usually soil specific and dependent on other factors such as substrate quantity and quality, oxygen availability, nutrient limitation and extracellular enzyme activities (Allison *et al*. 2010). Based on kinetic theory, there is an indirect relationship between OC quality and temperature sensitivity: the lower the OC quality (more recalcitrant OM), the higher the temperature sensitivity (Mikan *et al*. 2002, Knorr *et al*. 2005,

Davidson and Janssens 2006, Conant *et al*. 2008, Wetterstedt *et al*. 2010). On the contrary, experimental data often show a lower temperature sensitivity of more recalcitrant OC compared to that of more labile OC (Biasi *et al*. 2005, Gershenson *et al*. 2009). Alteration of peat water table or saturation status by global warming may lead to enhanced aerobic respiration and reduction in CH_4 emissions (Nykänen *et al*. 1998, Maljanen *et al*. 2010, Yrjälä *et al*. 2011). The balance between the increased CO_2 emissions and the concomitant reduction in CH_4 emissions determines the shift in peatlands climatic influence due to lowered water table. Change in vegetation structure (plant functional type) is also very important factor in peat C balance. Ombrotrophic peatlands have faster peat C-accumulation rates and lower CH_4 emissions than the minerotrophic ones. Change towards drier conditions may trigger ombrotrophication.

An extreme case of drying can be observed when peatlands are drained for agricultural, forestry and/or peat extraction (horticultural and energy production uses) purposes, which is done by lowering the water level. This usually leads to increased peat decomposition and degradation. Global estimates showed that about 15 % (about 50 Mha) of the world total peatland area [about 398.5 Mha; (Lappalainen and Zurek 1996)] has been drained and degraded for different purposes such as agriculture, forestry and peat harvesting (Joosten 2009). In Europe for example, the water level in about 60 % of the original peatland area (about 96 Mha) (Lappalainen and Zurek 1996) have been altered for different purposes such as 50 % for forestry (about 28.8 Mha), 30 % for agriculture (about 17 Mha) and 10 % for peat extraction (about 5.8 Mha) (Joosten 1997, Vasander *et al*. 2003). In Fennoscandia and Russia, a total peatland area of more than 10 Mha has been drained for forestry (Minkkinen *et al*. 2008). More than half of the Finnish peatland has also been drained for forestry use, during the 20th century (Laine *et al*. 2006). Persistent draw-down of the peatland water table affects peatland microbes by increasing the thickness of the aerated surface layer thus stimulating aerobic respiration (Maljanen *et al*. 2010). In the longer-term, plant species cover and OM quality change following water level draw-down, modifying net primary production (which is usually increased), and the influence of temperature and water content on peat microbial activities (Laiho 2006). Depending on the peatland type (nutrient status) and other climate conditions, drainage-induced changes in microbial functions lead to changes in the rate of surface layer OM decomposition (Minkkinen *et al*. 1999). It also leads to changes in general peat–greenhouse gas dynamics (e.g. CO_2 and CH_4) and C balance (Nykänen *et al*. 1995, 1998, Minkkinen and Laine 1998, Maljanen *et al*. 2010, Yrjälä *et al*. 2011, Andersen *et al*. 2013). Global CO_2 emissions of about 2000 Tg a^{-1} (Parish *et al*. 2008, Joosten 2009) from drained peatlands (including fires), accounts for almost 6 % of total global CO_2 emissions or 25 % of CO_2 emissions from whole land use, land use change and forest sector (Canadell 2011, Ritchie and Roser 2018). For example, between 1990 and 2008, the global CO_2 emission from drained peatlands increased from 1058 Tg a^{-1} to 1298 Tg a^{-1}, with the EU being the second largest emitter (174 Tg CO_2-eq^{-1} a^{-1}) behind Indonesia (Joosten 2009). As long as

peatlands remains drained and continually oxidized, C emissions (e.g. CO_2) can continue for a very long time, ranging from decades to even centuries, thereby releasing the huge amount of C stored in peatlands. Although CH_4 is a stronger GHG than CO_2 and CH_4 emissions lower the radiative forcing created by CO_2, simultaneously reduced CH_4 emissions cannot offset the warming impact of high CO_2 released in drained peatlands (Foster *et al.* 2007).

1.3 Peat microbial communities involved in carbon cycling

Microbes are the most active components of soil biogeochemical processes in both terrestrial and aquatic ecosystems including peatlands (Paul and Voroney 1980, Marinari *et al*. 2006). As the ultimate controller of C mineralization, microbes take part in all the biochemical processes of soil OC (Paul and Voroney 1980), playing key roles in the biomass decomposition and C cycling processes in peat (Xu *et al*. 2010). Soil microbes, irrespective of climatic conditions, contribute to soil–atmosphere C exchange either via respiration or through fermentation. In general, different microbial groups play different roles in soil C cycling. The abundances, diversity and activities of key microbial groups like bacteria, fungi and archaea are in parallel with the specific site environmental variables and vary between peatland types (Lin *et al.* 2012). While Gram-negative bacteria are associated with the initial degradation steps, involving mainly plant-derived labile C, the Gram-positive bacteria are able to degrade more recalcitrant C materials such as those from root or dead fungal biomass (Waldrop and Firestone 2004, Kramer and Gleixner 2006, Denef *et al*. 2007). Fungi are the principal organic matter decomposing microbes in the soil, especially in acidic ecosystems like peatlands (Williams and Crawford 1983, Thormann 2006). They accomplish this via their extensive hyphal growth and synthesis of diverse suites of extracellular enzymes (Deacon 1997, Thormann 2006). While many fungi such as the saprobes and mycorrhizal fungi readily degrade fresh and labile OC, others such as basidiomycetes and ascomycetes can also degrade complex and recalcitrant macromolecules due to their exo-enzymatic capabilities (Thormann 2006, Rabinovich *et al*. 2004, Treonis *et al*. 2004, Denef *et al*. 2007). Functional and taxonomic succession of fungi during decomposition varies with litter quality (Thormann 2003, 2006, Talbot *et al*. 2013, Gittel *et al*. 2014a, b). The archaea group of organisms carries out the final stage of peat OM degradation that occurs in the anoxic peat layers (Juottonen *et al.* 2006). Methanogenic archaea are important actors in the peat biogeochemical C cycling, with the unique ability to produce methane (CH_4). There is distinct spatial niche separation between the microbial groups (bacteria, fungi and archaea) in peatland (Lin *et al.* 2012). While earlier studies reported that fungi (Thormann 2006) usually dominate peatland surface with extensive hyphal growth, recent studies suggest generally a less important role for fungi in the C cycling of peatland (Winsborough and Basiliko 2010, Lin *et al.* 2012). In deep peat layers (catotelm) where fungi metabolism is limited by anoxia, the

prokaryotes (bacteria and archaea) are the major players in the peat OM mineralization (Chanton *et al*. 1995, Winsborough and Basiliko 2010). All the microbes in peatlands grow on the deposited plant residues or other OM, building their biomass and maintaining their metabolisms with the plant-derived C. This involves simultaneous microbial production and oxidation of C compounds and the consequent feedback to the atmosphere, via the emission of gases such as CH_4 and CO_2. While the general microbial C pathways are known, there is currently lack of evidence linking microbial community structure and physiology to the emission of C compounds such as CH_4 and CO_2, from peatlands under changing climatic conditions and water regimes (Carney *et al*. 2007, Allison *et al*. 2010, Joergensen *et al*. 2011).

In general, soil microbes are dynamic with high spatial and temporal heterogeneity in their characteristics (Xu *et al*. 2010). They vary with depth in their community structure and activities due to stratified resource inputs and changing habitats (Artz 2009, Putkinen *et al*. 2009, Preston *et al*. 2012, Lin *et al*. 2014). Microbial biomass is more sensitive to disturbance or environmental change than the bulk soil organic C, SOC: (Bergstrom *et al*. 1998, Marinari *et al*. 2006). The built-up or decay of SOC is directly and indirectly controlled by microbial activities. Therefore, changes in microbial community composition and structure can be good indicators of disturbances or changes in environments like peatlands (Sparling 1992, Rice *et al*. 1996, Franzluebbers *et al*. 1999, Allison *et al*. 2010). Additionally, upon cell death, part of the soil (peat) living MB is added to the non-living fraction of soil organic matter (SOM) as "necromass" (Kindler *et al*. 2006, 2009, Miltner *et al*. 2009, 2012, Liang and Balser 2011, Liang *et al*. 2011). Unlike the living MB, the amount of the necromass in the SOM increases with depth (Miltner *et al*. 2009, 2012). There are currently no models or studies that estimate microbial necromass in peatlands.

1.3.1 Microbial and carbon dynamics in peat profiles with focus on deep peat

Microbial metabolism or activities are the main biotic drivers of peat C cycling, which are constrained by a combination of factors such as availability of O_2, other electron acceptors and nutrients, OM quality, low pH and temperature (Artz 2009, Singh *et al*. 2010, Xu *et al*. 2011, Graham *et al*. 2012). Differences in these constraints along peat profiles lead to vertical stratification of peat microbial communities (Sundh *et al*. 1997, Andersen *et al*. 2013). Fresh organic matter deposited in the surface layers becomes less labile and more recalcitrant with increasing depth. The low energy yield from the recalcitrant peat biomass in deep peat layers decreases microbial activities (Artz 2009). Other constraints such as low O_2 availability, pH and temperature hinders further decomposition of deep peat, leading to the accumulation or rather increase in the proportion of the most complex polymers (Turetsky *et al*. 2000). Not only in peat, but also in mineral soils, unfavourable environmental conditions are causing low microbial biomass and activities. A study with cryoturbated soil from the Arctic showed low microbial activities at depth in OM otherwise rich in C and N (the OM is sub-ducted from the surface down due to frost action; Gittel *et al*. 2014a,

Schnecker *et al*. 2014). In "normal" non-cryoturbated soils, to maintain metabolisms, decomposition processes in deeper peat depths requires specialized species adapted to the changes in the OM quality and redox conditions (Sundh *et al*. 1997, Artz 2006, 2009) among other factors. This leads on the one hand to spatial partitioning where communities with different growth pattern and enzymatic capabilities occupy different niches at various depths, (Peltoniemi *et al*. 2012, Gittel *et al*. 2014a) or where species replace other species with time (Morales *et al*. 2006, Artz 2009).

Distinct microbial community compositions with different enzymatic and degradation potentials exist in different depths of peatlands (Gittel *et al*. 2014a). Though the general importance of fungi in C cycling of peatlands is still discussed (Winsborough and Basiliko 2010, Lin *et al*. 2012), vertical stratification of fungi is characteristically seen to be more pronounced in the reduction of the biomass (spores number, biomass and hyphal length) with increasing depth than alteration in the community members (Artz 2009, Andersen *et al*. 2013, Gittel *et al*. 2014a). The large numbers of polyphenol degrading fungi found in the lower segment of peatlands (Jassey *et al*. 2011) and the stable abundance of yeasts throughout the peat profile (Golovchenko *et al*. 2002, Artz *et al*. 2007) however, points at some exceptions. Vertical stratification of the bacteria community involves changes in both composition and size of the community (Andersen *et al*. 2103, Gittel *et al*. 2014a). The consensus is a decrease in diversity and biomass with depth (Golovchenko *et al*. 2007, Jaatinen *et al*. 2007) after a maximum at the interface between the oxic and anoxic (intermediate) layers (Sundh *et al*. 1997, Dedysh *et al*. 2006). The archaeal group of microbes carries out the final anaerobic decomposition of peat organic matter, producing CH_4 from formate, acetate or CO_2 + H_2 (Zinder 1993). Archaea are dominant in permanently waterlogged and anoxic deep layers, shifting from hydrogenotrophic to acetoclastic communities with increasing depth (Galand *et al*. 2002, Cadillo-Quiroz *et al*. 2006). The archaea and/or some consortium of organisms in deep peat layers are also involved in the anaerobic oxidation of CH_4, though the entire processes is not understood now (Smemo and Yavitt 2007, 2011). There is also evidence that other chemotrophic bacteria such as sulphate reducing bacteria (SRB) play key roles in the anaerobic degradation of organic matter in deep peat layers, but their complete identity is still lacking (Pester *et al*. 2012, Andersen *et al*. 2013). However, similarity in the potential oxidative enzyme activities (per gram dry soil) between arctic soil horizons suggests a decoupling of microbial community composition and SOM properties (Gittel *et al*. 2014b). According to Schnecker *et al*. (2014) C and N availability (chemical composition of SOM) is mainly regulated by enzyme activities. The enzyme activities are in turn related to microbial diversity or abundance of a particular microbial group. Low microbial diversity/abundance may not only be a possible explanation for the persistent accumulation of peat due to low decomposition rate of OC in deep peat layer, but also implies that altering the microbial composition and activities would alter decomposition dynamics in deep peat layers.

1.4 Molecular approaches to investigate soil microbial processes

Culture-dependent methods used in microbial studies have been replaced by an array of modern culture-independent techniques (Andersen *et al*. 2013). This was due to challenges such as time consumption and the large number of uncultivable microbial population (Amann *et al*. 1995, Morris *et al*. 2002), and rapid progress made in the development of molecular methods in recent times. Currently, there are several culture-independent biochemical and molecular methods used in the study of microbial community composition, abundance, diversity, phylogeny and functioning in different ecosystems, including peatlands, especially those undergoing environmental changes (Krieg 1994, Gevers and Coenye 2007, Liu and Stahl 2007, Andersen *et al*. 2013). Phospholipid fatty acids (PLFA) and nucleic acid (DNA and RNA)-based methods are common molecular approaches. Chloroform fumigation–extraction/incubation (CFE/CFI), on the other hand, are common and basic biochemical methods to measure microbial biomass C and N content. The nucleic acid-based methods include quantitative polymerase chain reaction (qPCR), molecular fingerprinting methods [e.g. denaturing gradient gel electrophoresis (DGGE)] and next generation sequencing (e.g. 16S or 18S rRNA gene amplicon sequencing, metagenomics and metatranscriptomics). The use of these methods depends on the focus of the research and the design of the experiment. The research hypothesis and available resources also determine the preference of one method over the other. Although different studies have demonstrated the sensitivity of these different methods (Krieg 1994, Gevers and Coenye 2007, Liu and Stahl 2007), they all have their limitations, as outlined in the next paragraph.

The CFE/CFI method is a suitable method for direct measurement of microbial biomass or biomass C and N from fresh or old soil samples (Philippot *et al*. 2012, Setia *et al*. 2012). However, correction for added chloroform C may be required in order to assess the concentration of biomass C in soils such as clay (Ocio and Brookes 1990a, Alessi *et al*. 2011). Also, the correction factor (K_{EC}) applied to calculate microbial biomass C from the C additionally made extractable by the fumigation is still controversial (Martens 1995). Use of DNA-based methods include quantifying microbial groups or specific functional genes (e.g. qPCR), assessment of biodiversity and community structure (e.g. sequencing of 16S rRNA gene amplicons). RNA-based methods give insight to the active members of the community. e.g., quantify functions by quantifying actively expressed genes [reverse transcriptase-qPCR (RT-qPCR)]. RNA-based methods can also be used to identify active members of the community by sequencing mRNA (e.g. reverse-transcriptase-PCR followed by sequencing) (Li and Liu 2003, Everett *et al*. 2010, Rincon-Florez *et al*. 2013). However, nucleic acid based methods often require trained personnel, complex procedures and specialized equipment. Most of them are also expensive and have methodological/extraction biases.

The PLFA-analysis that was mostly used in this study is very sensitive and considered reliable quantitative measure of living microbes, since they are short-lived and readily metabolized upon cell death (White *et al*. 1979, Mancuso *et al*. 1990). It is a rapid and inexpensive way of analysing the biomass and primarily the composition of microbial communities in the soil (Ramsey *et al*. 2006). PLFA-method is sometimes used (alone or together with other techniques like RNA and DNA sequencing) to estimate the biomass, as well as to study the diversity and community structure of mixed microbial communities in varying environments (Le Chevalier 1977, White *et al*. 1979, Mancuso *et al*. 1990, Kaur et al 2005). Based on PLFA analysis alone, several studies have analysed the changes in peat biogeochemical cycles, such as responses of microbial communities to changing conditions in peatlands (Borga *et al*. 1994, Frostegård and Bååth 1996, Sundh *et al*. 1997, Jaatinen *et al*. 2007, Mäkiranta *et al*. 2009, Andersen *et al*. 2010). It generally shows the overall fingerprint of microbial communities in an ecosystem and helps to understand the various disturbances or alterations in natural ecosystems, such as peatlands (Borga *et al*. 1994, Ramsey *et al*. 2006). In combination with isotopically (e.g.^{13}C) labelled substrates, PLFA analysis (PLFA-SIP) can reveal the metabolically active part of a microbial community and provide valuable information on substrate utilization patterns of microbes (Boschker and Middelburg 2002, Ruess and Chamberlain 2010). Ramsey *et al*. (2006) suggested that the PLFA analysis method might be more sensitive in detecting treatment effects on microbial community composition than the nucleic acid based method such as PCR (length heterogeneity PCR and DGGE)-based profiling method. For instance, the relative abundance of fungi and bacteria (fungal/bacterial ratio) used in comparing soils and treatments were better differentiated by the PLFA method than by the PCR method (Frostegård and Bååth 1996, Klamer and Bååth 2004). In addition, ^{13}C-DNA methods are inferior to ^{13}C-PLFA methods, since DNA contains little C and thus huge ^{13}C incorporation is required for sensitive analysis. However, the PLFA analysis method is very weak in phylogenetic resolution of microbial community, compared to the nucleic acid (rRNA)-based methods (Frostegård *et al*. 2011).

1.5 Isotope approaches to study soil carbon cycle

There are two naturally occurring stable isotopes of C: ^{12}C (98.93%) and ^{13}C (1.07%). The differences in the natural ^{13}C/^{12}C isotopic ratios are small but useful for C turnover studies. To make the differences easily recognizable, the C isotopic data is generally reported using the delta (δ) notation. The δ^{13}C values are calculated as a deviation (‰) of the heavy-to-light isotope abundance ratio in the sample from that of a standard (Vienna Pee Dee Belemnite, VPDB). This is calculated according to equation (i) bellow:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{VPDB}} - 1\right) \text{x } 1000 \qquad \text{(i)}$$

where R_{sample} is the abundance ratio ($^{13}C/^{12}C$) of a sample and R_{VPDB} is the abundance ratio of VPDB (0.0111802).

Most natural processes fractionate C isotopes, using a larger proportion of the lighter isotope (^{12}C), resulting in the enrichment of the source with the heavier isotope (^{13}C) (Ågren *et al*. 1996). The rate and degree of fractionation varies between processes and systems. The largest fractionation is found in photosynthesis, leading to labile materials depleted in ^{13}C in plants as compared to the atmosphere. The $\delta^{13}C$ of deposited biomass at different soil environments, such as minerotrophic and ombrotrophic peatlands, may differ due to differences in their vegetation (Ménot and Burns 2001, Skrzypek *et al*. 2008, Krüger *et al*. 2014). For example, the biomass from plants with C3 and C4 photosynthetic pathways have $\delta^{13}C$ values of around –27 ‰ and –13 ‰, respectively, but there are also differences in individual C3 species growing on variable peatlands (Rajagopalan *et al.* 1999). Soil-depth profile $\delta^{13}C$ values are a reflection of the combined effects of fractionation processes during photosynthesis in plants and microbial decomposition processes (Hornibrook *et al*. 2000, Krull and Retallack 2000, Krull *et al*. 2000, Werth and Kuzyakov 2010). Microbial respiration is known to not or only slightly fractionate against the heavy isotope ^{13}C isotope enrichment in the remaining soil OM with depth, at least in upland soils (Ågren *et al*. 1996, Alewell *et al*. 2011). Despite this trend, recalcitrant or slowly decomposing materials that accumulate at depth, such as lignin, are relatively low in ^{13}C (Ågren *et al*. 1996, Benner *et al*. 1987). There is apparent contradiction in these two facts, but most likely the balance between these two mechanisms shapes the isotopic depth profiles in the soils and in most cases the respiratory discrimination against ^{13}C, overlays the accumulation of ^{13}C depleted material, causing a progressive enrichment of ^{13}C in residual SOM. The $\delta^{13}C$ values in soil–depth profiles also vary with physiological conditions, degree of isotopic fractionation among depths and presence of inorganic C compounds (Krüger *et al*. 2014). Changes from aerobic to anaerobic metabolic pathways or decomposition rates, alters the isotopic signature of soils. According to Krüger *et al*. (2014), while aerobic decomposition leads to increase of $\delta^{13}C$ values, it remains stable or decreases with depth during anaerobic decomposition. Thus, in peatlands, the general decrease in $\delta^{13}C$ of SOC with depth is not always seen and follows more complicated trends.

Biogeochemical processes such as biomass degradation in peat and other soils have been analyzed using the isotopic signatures of elements such as C and N (Lynch *et al*. 2006, Schaub and Alewell 2009, Alewell *et al*. 2011, Andersson *et al*. 2012, Krüger *et al*. 2014). In general, they showed that the bulk $\delta^{13}C$ of peat or soil dominated by aerobic decomposition increases with depth while it decreases with depth in anaerobic decomposition dominated peat or soil (Alewell *et al*. 2011, Andersson *et al*. 2012, Krüger *et al*. 2014). Peatland drainage in Sourthern Finland (Krüger *et al*. 2016), land use intensification in Northern Germany (Krüger *et al*. 2015) and peat degradation due to permafrost

thawing in northern Sweden (Alewell *et al*. 2011, Krüger *et al*. 2014) have been monitored using the stable C and N isotopes depth profiles. Change in degradation mechanism due to differences in hydrology reflected in the $\delta^{13}C$ profile of hollow site (Alewell *et al.* 2011). The $\delta^{13}C$ analyses have also shown evidence of environmental changes and C turnover in different soil ecosystems (Arrouays *et al*. 1995, Freitas *et al*. 2001, Lynch *et al*. 2006, Pries *et al*. 2013), changes in permafrost peat organic matter accumulation (Andersson *et al*. 2012) and microbial CH_4 production in wetlands (Krull *et al*. 2000, Conrad 2005). Decomposition during composting lead to $\delta^{13}C$ depletion from –12.8 ± 0.6 ‰ to –14.1 ± 0.0 ‰ (Lynch *et al.* 2006). Hence, $\delta^{13}C$ analysis may provide detailed insights into the C dynamics of peatlands (Amelung *et al*. 2008).

1.6 Aims of the study

The main aim of this work was to study the drivers of C cycling and recycling processes, especially in the deep old peat layers, and the sensitivity of these processes to changes in environmental conditions. In addition, the study investigated the role of microbial biomass as a source of C addition to SOM, besides mediating mineralization, focusing on deep old peatland deposits. More specifically, the aims were:

- To examine the effect of changed hydrology on the internal C cycling and storage pattern in the whole peat column (top to bottom) via the effect on the abundance and structure of peat microbial communities, and bulk peat $\delta^{13}C$ (I, III).

- To determine the factors (biotic and abiotic) controlling decomposition and C loss from old peat compared to the "recent", young peat (IV).

- To quantitatively estimate the contribution of MBC to the total peat C, besides the role of microbes as the drivers of mineralization process (II).

Long-term effects of water-level drawdown on the internal C cycling and storage pattern of especially deep old peatland layers were examined using PLFA, CFE, qPCR and bulk peat $\delta^{13}C$ values analysis in two mire complexes comprising natural and drained fen and bogs (I, II, III). Factors driving decomposition and hence C losses from old and young peat soils were examined via a laboratory incubation study, using peat soil samples from East-European subarctic tundra (Seida) (IV). MBC contribution to SOM was measured using PLFA, CFE and bulk peat C analysis in two mire complexes comprising natural and drained fen and bogs (II, III).

2 MATERIAL AND METHODS

2.1 Study sites

The study combined direct analysis of field samples of 7 peatland sites (Fig. 1) from two different mire complexes (one in central and one in southern Finland) and a laboratory incubation study of peat samples from East-European subarctic tundra (Seida). For I, II and III, 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 were sampled. There was clear difference in the original fertility, water table and vegetation composition of the fen and the bog. Part of both peatland sites were ditch-drained in 1961 (51 years before sampling) so that there are adjacent natural and drained sites of different fertility along a border ditch. These sites were chosen because of the length of time their water table level has been lowered. There were also marked changes in their hydrology, peat and vegetation properties and greenhouse gas emissions which were clearly evident in the results of previous studies on drainage effects, for example, on CO_2 and CH_4 emissions (Silvola *et al*. 1996, Nykänen *et al*. 1998, Minkkinen *et al*. 1999, Laine *et al*. 2004). Additionally, for II and III, two more peatland sites in the southern part of Finland (Lovonsuo and Laaviosuo bog, both near the Lammi area; 61°4´N, 25°0´E, ca 150 m a.s.l.), which also have a long-time span of water-table draw down (about 33 years before sampling for Laaviosuo) were studied. Hereafter and in the attached publications, the natural Laaviosuo site is referred to as Villikkalansuo. The Lovonsuo site was drained ~90 years before sampling. There was no corresponding natural site left in Lovonsuo. Hence, the original peatland type cannot be reconstructed, and the forest is mature. Article IV centered on the incubation of very old peat which had been uplifted to the surface and modern, about 50 years old peat (considered to be "recent peat" or young peat, Biasi *et al*. 2014) from the Eastern-European discontinuous permafrost zone in the subartic, southern tundra (Seida; Fig. 1 in IV). This laboratory study was good fit for the field-based studies because old peat had been exposed to the surface

already for a considerable length of time, which allowed studying both long-term and short-term effects of climate change (changes in temperature and oxygen conditions) on microbial and C dynamics, particularly in old deep peat.

Additional reference sites REF 1 and REF 2 studied using the qPCR analysis were located at the North edge of Rimminvuoma peatland complex in the Lapland area of Finland 67° 929′ N, 25° 380′ E and 67° 933′ N, 25° 380′ E, respectively. Both sites are nutrient poor natural fens. The average surface pH, mean annual temperature and precipitation for the site are 6.4 ± 0.1, –0.5 °C and 500–600 mm, respectively. The maximum peat depth is 180 cm (average around 150 cm) and the water depth was at the surface at the time of sampling. The peatland is generally snow-covered from October to May, with the main run-off event occurring in May after the snow melts. The vegetation cover consists mainly of mosses (e.g. *Sphagnum* spp.), *Eriophorum angustifolium*, *Carex* sp. and *Trichophorum cespitosum*. The three most dominant plants are *Warnstorfia* sp., *Carex livida* and *Drosera rotundifolia*. The vegetation in REF 2 was essentially similar as in REF 1, but due to scarcity of research work, site information is limited. The maximum peat depth is 210 cm and the groundwater level extended to the surface by the time of sampling. The entire sites covered in this study are listed in Table 1.

TABLE 1 List of the entire sites covered in the study and their abbreviations. Detailed descriptions of each site are provided in the articles.

Site	Location	Peatland type	Hydrological status	Sampled after drainage	Abbreviation	Study/Article
Lakkasuo	Central Finland	Fen	Natural	–	LakFN	I, II, III, qPCR
			Drained	51 yrs	LakFD	
	Central Finland	Bog	Natural	–	LakBN	
			Drained	51 yrs	LakBD	
Villikkalansuo	Southern Finland	Fen	Natural	–	VilFN	II, III, qPCR
Laaviosuo		Bog	Drained	~30 yrs	LaaBD	
Lovonsuo		Fen	Drained	~80 yrs	LovFD	
Seida	Subarctic tundra, Russia	Peat Plateau	Natural	–	–	IV
Rimminvuoma	Lapland, Finland	Poor fen	Natural	–	REF 1	Thesis summary (qPCR)
		Poor fen	Natural	–	REF 2	

FIGURE 1 Peatland sites covered in this study, Lakkasuo fen and bog a1) fen natural side, a2) fen drained side, a3) bog natural side, a4) bog drained side, b) Villikkalansuo natural, c) Laaviosuo drained and d) Lovonsuo drained.

2.2 Methods

2.2.1 Data collection methods

The main methods used in this study are listed in Table 2. Microbial biomass, community structure and the impact of long-term drainage were determined using PLFA and rRNA gene analysis. Short-term effect of temperature and water content on microbial communities as well as their effect on peat decomposition processes were studied by laboratory incubation, including CO_2/CH_4 gas release. The bulk peat $\delta^{13}C$ analysis was also used to analyses the long-term effect of water content on peat decomposition up to the mineral sub-soil while the MBC and its contribution to total peat C was analysed using CFE or indirectly via PLFA using a conversion factor.

TABLE 2 Summary of the main methods used in this thesis. Detailed descriptions of each method are provided in the articles.

Method	Article	Reference
PLFA analysis	I, II, IV	Tavi *et al.* 2013
CO_2 and CH_4 production & consumption rate analysis	IV	Čapek *et al.* 2015
Plant and soil $\delta^{13}C$, N % & C % analysis	III	Ménot and Burns 2001, Kruger *et al.* 2014
Peat $\delta^{13}C$ isotope analysis	III	Coplen *et al.* 2006
Peat C and N % analysis	I, III, IV	Kruger *et al.* 2014
DOC analysis	IV	Weishaar *et al.* 2003
Chloroform fumigation extraction	II, IV	Vance *et al*. 1987a
Microplate fluorometric and photometric assays of extracellular enzymes potential activity	IV	Marx *et al.* 2001
Gross ammonification and nitrification rates	IV	Kirkham and Bartholomew 1954, 1955
Extractable nutrient analysis	IV	Čapek *et al.* 2015
16S rRNA gene analysis	Summary	Philippot *et al.* 2012,

2.2.2 Sample collection

Peatland samples for I, II and III were collected in segments, from 4–5 depths starting from the surface to the deepest layers above mineral soils, using the Russian pattern side-cutting sampler (5 x 50 cm; diameter x length) (Belokopytov and Beresnevich 1955). The samples were collected from both the natural and adjacent drained sites except in Lovonsuo, where there was no corresponding natural site left. The basal peat and sub-soil samples from Lakkasuo fen (drained and natural) used in III were collected using the same

instrument but with different dimensions (half cylinder diameter 27 mm, lenght 500 mm). Plant needles, leaves or stem pieces for $\delta^{13}C$ analysis (III) were collected from the natural and drained Lakkasuo fen and bog.

For the incubation study (IV), 3 replicates of soil samples consisting of peat were collected from the surface layers of three surface types characteristic of the Seida region: First, "recent peat" was sampled from the surface of a peat plateau, or tundra bog, a dry peatland which had been elevated after permafrost aggradation. "Old peat" was sampled from the surface of bare peat areas (so-called peat circles) which are randomly distributed on this peat plateau. Samples were also taken from the surface of the dominant upland tundra site for comparison. Cryogenic processes such as deep-ice formation in the arctic landscape, lead to the uplifting of old peat deposits from the permafrost layer to the well-drained surface of the peat plateau (Seppälä 2003, Payette *et al*. 2004, Kuhry 2008, Marushchak *et al*. 2011, Kaverin *et al*. 2016). There, wind abrasion and erosion likely removed the young upper parts of the peat layers, leaving the old peat at the surface of the peat circles and exposed to ambient climatic conditions (Seppälä 2003, Payette *et al*. 2004, Kuhry 2008, Marushchak *et al*. 2011, Kaverin *et al*. 2016). After removing visible roots from the recent peat and upland soil (there are no plants growing on the bare peat), the soils were stored at 4 °C for 4 months to allow for the depletion of labile substrate (e.g. fine root residues, root exudates) in the soil samples. The chemical and biological characteristics of the homogenized soil was analysed before the start of incubation. The incubation experiment was aimed at analysing effects of oxygen status and temperature on CO_2 and CH_4 production and underlying microbial controls.

In October 2017, additional peat samples used for CFE and qPCR analysis were collected from the same sites and depths as in I, II and III using the same instrument. Additional samples from REF sites 1 and 2 were also collected from the same depths using the same instrument, the Russian pattern side-cutting soil corer.

2.2.3 Microbial biomass measurements

In this study, three methods, PLFA, qPCR and CFE, were used to characterize the microbial biomass in different peat layers (Table 3). While microbial communities in peat may consist mainly of bacteria, archaea and possibly some fungi, none of these tools can fully describe the communities alone, but have different benefits and disadvantages. Note that molecular tools starting from DNA may include biomarkers of non-living organisms and external DNA (relic DNA, see Carini et al. 2016).

TABLE 3 Summary of our research focus when studying microbial communities in peat layers by different methodologies (PLFA = phospholipid fatty acids, qPCR = quantitative polymerase chain reaction, CFE = chloroform fumigation extraction).

Method	Main focus	Comments	References
PLFA	PLFAs with C chain length between 10–20 used to study the biomass and community structure of microbes excluding archaea.	Characteristic PLFAs for tracking Gram-negative bacteria, Gram-positive bacteria and fungi were used.	White *et al*. 1979, Ramsey *et al*. 2006
qPCR	Quantification of specific microbial groups or functional genes and assessment of biodiversity and community structure of living or non-living microbes, even relic DNA.	qPCR focused only on the prokaryotes, i.e. bacteria and archaea.	Philippot *et al*. 2012, Rincon-Florez *et al*. 2013, Carini *et al*. 2016
CFE	Direct measurement of all microbial biomass carbon (MBC), including living and non-living microbes.	Bulk analysis for MBC, the efficiency of this method in peat samples with over 90% water content is still a concern.	Vance *et al*. 1987a, Setia *et al*. 2012

2.2.3.1 PLFA analysis and conversion to MBC (I, II and IV)

PLFA analysis was done for all the peatland sites studied in Finland and for the peat sampled from the Russian arctic site, to examine the variation of microbial biomass and community structure with depth and age. PLFA analysis was also used to study the effect of drainage on the microbial community biomass and structure (I, II). Total lipid extraction from freeze-dried samples (> 3 g dry weight) and separation were done according to Tavi *et al*. (2013). After evaporation to dryness under nitrogen flow, the phospholipids fraction was methylated using the protocol in Virtue *et al*. (1996), but at 60–80 °C for 2 h. Methylated fatty acids were analysed using an Agilent 6890 GC connected to an Agilent 5973 mass selective detector while separation of methylated fatty acids was done, using a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm; J & W Scientific) and He as a carrier gas. PLFA peaks were identified by (a)

comparison of peaks with the spectra and retention times of the fatty acid methyl ester (FAME) standard (Supelco 37 component FAME mix), (b) using the mass ions (m/z ions) and mass spectra measured in SCAN mode. The library for microbial identification system (Wiley 275.L MS library and NIST 02.L MS library) was also used in the PLFA peaks identification.

In this study, only PLFAs between C10 and C20, which represents the main range for prokaryotic PLFAs and a few other microbial groups, such as fungi were analysed. Saturated FAs, 12:0, 14:0, 16:0, 17:0, 18:0 & 20:0 were grouped as general microbial PLFA. Iso- and anteiso-branched PLFAs, i14:0, i15:0, a15:0, i16:0, a17:0 & i17:0 were grouped as Gram-positive bacteria FAs (O'leary and Wilkinson 1988, Sundh *et al*. 1997, Taipale *et al*. 2009). Monounsaturated fatty acids (MUFAs) 16:1ω9, 16:1ω7, 17:1ω9 and 17:1ω7 and cyclopropyl fatty acids (Cy-FAs) cy17:0 and cy19:0 were grouped as Gram-negative bacteria FAs (Taipale *et al*. 2009, Frostegård *et al*. 2011). The 16:1ω5, 18:1ω9c and 18:2ω6c FAs were grouped as fungi FAs (Olsson 1999, Kaiser *et al*. 2010, Frostegård *et al*. 2011).

The contribution of the MBC_{PLFA} to the C storage of peat, as well as the impact of long term water level draw-down on peat MBC_{PLFA}, were estimated in II. Total microbial PLFA (nmol g^{-1} DW) were converted to MBC in mg g^{-1} dry weight (DW) of peat using the Joergensen and Emmerling (2006) F_{PLFA}-factor of 5.8 (see equation ii below), assuming that the possible over- or underestimation of some microbial group MBC by a single conversion factor (Blagodatskaya and Kuzyakov 2013) does not affect the result significantly.

$$MBC\ [\mu g\ (g\ DW)^{-1}] = total\ PLFA\ [nmol\ (g\ DW)^{-1}] \times F_{PLFA} \quad \text{(ii)}$$

where *MBC* = microbial biomass C and F_{PLFA} = F_{PLFA}–factor of 5.8 (Joergensen and Emmerling 2006).

Estimation of MBC-stock in northern peatlands was done using the peat volume approach (Yu 2012) as

$$C_{MB} = \sum_i (A_i \times \overline{D_i} \times \overline{BD_i} \times \overline{CC_i}) \quad \text{(iii)}$$

Where C_{MB} = microbial biomass C stocks in peat, i = peatland type (type means different depths of different peatland types like fen natural or fen drained), A_i = area of peatland type i, $\overline{D_i}$ = the mean peat depth for type i, $\overline{BD_i}$ = the mean bulk density for the peat type i and CC_i = the mean of MBC for peat type i.

2.2.3.2 qPCR analysis of bacterial and archaeal 16S rRNA genes

The analysis of 16S rRNA genes was done for all the peatland sites studied in Finland, including two additional (reference) sites in Lapland, to examine the variation of prokaryotic (bacteria and archaea) biomass and structure with depth and drainage. Extraction of DNA was done with the beat-beating technique using Powerlyzer Power Soil DNA Kit (MoBio/Qiagen). DNA-

extracts were diluted to 1–5 ng μl^{-1} and measurements were done in duplicate. The archaeal and bacterial 16S rRNA genes were quantified using the archaeal specific primer set S-D-Arch-0787-a-S-20 (ATTAGATACCCSBGTAGTCC)/S-D-Arch-1043-a-A-16 (GCCATGCACCWCCTCT) (Yu et al. 2005) and the bacterial specific primer set 27F (5'AGAGTTTGATCMTGGCTCAG 3')/338R (5' TGCTGCCTCCCGTAGGAGT-3') (Lane 1991), respectively. Standards for the archaeal and bacterial 16S rRNA gene quantitative analysis were PCR products that were cloned into cloneJet vector (Thermo Fisher) and amplified from the vector. Amplified PCR standards were purified using Ampure XP (Agilent), quantified using on Tape Station HS DNA analysis (Agilent), and diluted with water for the qPCR run (range 10^2 to 10^8 copies μl^{-1}). qPCR conditions quantification of bacterial 16S rRNA genes were as follows: Initial denaturation (95 °C, 5 min), 25 cycles of denaturation (95 °C, 30 s), annealing (52 °C, 30 s), and elongation (72 °C, 1 min), and a final elongation (72 °C, 2 min). Fluorescence was recorded during the elongation step. qPCR conditions for the quantification of archaeal 16S rRNA genes were as follows: Initial denaturation (95 °C, 5 min), 40 cycles of denaturation (95 °C, 30 s), annealing (58 °C, 30 s), elongation (72 °C, 1 min), fluorescence recording (78 °C, 15 s), and a final elongation (72 °C, 2 min). Fluorescence was recorded at 78 °C because primer dimers interfered with the fluorescence recording at 72 °C. Inhibition of the humic acids and other PCR inhibitors was checked with qPCR from 5 DNA extracts by spiking a given concentration of standard to the qPCR reaction mixture. When using the chosen template concentration, the test showed no sample inhibition. Based on the qPCR analysis, concentrations of the archaeal and bacterial 16S rRNA gene templates were calculated per gram dry weight and per volume of extracted peat samples.

2.2.3.3 Chloroform Fumigation Extraction (CFE) of microbial biomass carbon

CFE analysis was done for all the peatland sites studied in Finland and for the peat sampled from the Russian arctic site, to examine the variation of total MBC_{CFE} with depth, age and drainage of peatlands. Total MBC was determined using the fumigation-extraction procedure in Vance *et al.* (1987a), as modified and described by Preston *et al.* (2012). For each measurement, two peat samples (10 g fresh weight) were taken from the homogenized sample and the other of them was fumigated with ethanol-free chloroform ($CHCl_3$) in the dark for 24 h. Due to the high moisture content of the peat samples (average H_2O content of 91.4 % with a range of 79.2–95.4 % for Lakkasuo sites and average of 92.3 % with a range of 85.5–94.8 % for sites in Lammi), 500 µl of $CHCl_3$ was directly added to the surface before fumigation to aid cell lysis (Ocio and Brookes 1990b). Repeated evacuation was used to remove $CHCl_3$ residue and vapour after fumigation. Fumigated and non-fumigated samples were extracted with 40 ml of 0.5 M K_2SO_4. Dissolved OC (DOC) extracts were filtered through Filtropur S 0.45 µm (Germany) syringe filters and analyzed with Shimadzu TOC-LCPH total OC analyzer (Shimadzu, Japan). MBC was calculated by subtracting the non-fumigated extractable DOC from the fumigated extractable DOC after correcting for differences in initial peat moisture content. MBC_{CFE}

was corrected for non-extractable C using KEC (efficiency of C extraction) of 0.38 (Vance *et al.* 1987a). Estimation of MBC_{CFE}-stock in northern peatlands were done using the peat volume approach (Yu 2012) as shown in equation (iii) under 2.2.3.1.

2.2.4 Carbon isotope ($\delta^{13}C$) analysis as well as carbon and nitrogen content of peat and vegetation (III)

Dried peat samples from all the sites were homogenized using a Retsch MM301 vibrating ball mill. Plant needles, leaves and stem pieces from LakFN, LakFD, LakBN and LakBD were dried at 70 °C for 24 h. The $\delta^{13}C$, N % and C % of Lakkasuo (fen and bog) bulk peat were analysed with Vario Pyrio Cube coupled to Isoprime 100 (Elementar, Germany) while other peat and plant samples were analysed with Thermo Finningan Advantage IRMS coupled to elementar analyser Flash EA 1112 (Italy). Additionally, the effect of variable drying methods on $\delta^{13}C$ values were tested for peat samples from VilFN, LaaBD and LovF using three drying methods prior to $\delta^{13}C$ analyses. The methods were oven drying at 70 °C, oven drying at 70 °C after acid fumigation (to test the existence of carbonates) and freeze drying (using Labonco Freeze Dryer, Model 77560). There was no difference in the result of three method and hence the simplest oven drying method was used in the sample analysis. Stable isotope compositions were expressed in the delta notation as explained in section 1.5.

For the C % and N % analysis, a certified birch leaf standard (Elementar Microanalysis, UK) was used as reference. This same in-house standard was used as an internal $\delta^{13}C$ isotopic standard on the Lsvec–LSB-19 scale (Coplen *et al.* 2006). 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 4 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 %.

2.2.5 Incubation experiment (IV)

After the samples were exported from sub-arctic tundra to Europe, they were kept at 4 °C for about 4 months before initiation of the experiment. Prior to incubation, the water content of the peat samples was adjusted to 80 % or 100 % water holding capacity (WHC) for aerobic and anaerobic treatment, respectively. Aerobic and anaerobic peat samples were incubated for 23 weeks at temperature range (4 ˚C, 12 ˚C and 20 ˚C) to determine temperature and moisture/oxygen sensitivity of C losses from old and young peat as well as upland tundra soil. Incubation setup and experimental conditions followed Čapek *et al.* (2015) with modification of the oxygen-free artificial atmosphere in the anaerobic samples to 99 % He and 1 % CO_2.

Differences in microbial community structure between the recent and old peat were investigated using PLFA analysis before the incubation experiment.

PLFA extraction was done with similar procedure as in 2.2.3.1. Total lipids were extracted and dried from wet soil samples (equivalent to about 2 g dry weight) using a mixture of chloroform, methanol and 50 mM phosphate buffer in a volume ratio of 1:2:0.8 respectively (Bligh & Dyer 1959). After separation, the phospholipids fraction was methylated (base catalyzed) and extracted as methyl esters (Suutari *et al.* 1990). Separation and analysis of methylated fatty acids and PLFA peaks identification was done according to chapter 2.2.3.1, while dimethyl disulfide (DMDS) adducts were prepared, analysed and used in the determination of the position of double bonds in the monounsaturated FAME (Nichols *et al.* 1986). (Further details on PLFA profiles including bioindicators used for general microbial biomass, Gram-positive and Gram-negative bacteria as well as fungi are in Table 5).

Chloroform fumigation extraction method was used to assess C, N and P content in microbial biomass (CMB, NMB and PMB, respectively) before and after the incubation. Nutrient content in microbial biomass was calculated as a difference between fumigated and non-fumigated sample. The yield of the fumigation method was corrected to incomplete extraction of microbial C (Kec = 0.38; Vance *et al.* 1987a), microbial N (Ken = 0.4; Brookes *et al.* 1985) and microbial P (Kep = 0.4; Brookes *et al.* 1982). Microbial stoichiometry, i.e. the proportion of elements in microbial biomass, was expressed here as $C:N_{MB}$ and $C:P_{MB}$.

Fluxes of CO_2 and CH_4 were measured weekly during the first month and biweekly during the remaining incubation period. Gas samples were analyzed using a gas chromatograph (Agilent 7820A GC, Agilent Technologies, Santa Clara, USA) equipped with thermal conductivity and flame ionization detectors. DOC in filtered soil extracts (1:20 m/v) was described by specific UV absorbance which is defined as absorptivity at a wavelength of 254 nm normalized to concentration of DOC ($SUVA_{254}$; L mg C^{-1} m^{-1}) while the aromatic composition of the DOC was calculated according to Weishaar *et al.* (2003) using equation (v) below

$$\text{Aromaticity (\% DOC)} = 6.52 \,.\, SUVA_{254} + 3.63. \qquad \text{(v)}$$

where $SUVA_{254}$ = Specific UV absorbance of water sample measured at wavelength of 254 nm and normalized for DOC concentration.

Extractable nutrient (Cex, Nex, Pex) contents were determined before and after the incubation. The potential activity of extracellular enzymes was determined by microplate fluorometric and photometric assays under standard conditions. The loss of OC initially present in the soils (C_{LOSS}) was defined as CO_2 and CH_4 production. Specific CO_2 production was defined as actual CO_2 production rate relative to microbial biomass (C_{MB}). Further chemical analyses, such as total OC and N and their isotopic ratios, were done on soil samples dried at 60 ˚C and ground with a ball mill similar to the one described in 2.2.4 above.

At the end of the incubation period, gross ammonification and gross nitrification rates were determined using pool dilution technique (Kirkham and

Bartholomew 1954, 1955). An aliquot of 500 µL ^{15}N-enriched labeling solution (0.25 mM) was added to each sample aliquot in the form of $^{15}NH_4Cl$ (10 at%) for gross ammonification and $K^{15}NO_3$ (10 at%) for gross nitrification experiments. The labeled aliquots (2 for each sample) were kept at the respective temperature of the sample incubation. One was extracted after 4 h and the other after 24 h. They were immediately extracted with 15 mL of 0.5 M K_2SO4. Microdiffusion technique was used to prepare the extracts for the determination of $^{15}N/^{14}N$ ratio in NH_4+ or NO_3- (Brooks *et al.* 1989). The filter papers with the samples were dried in an H_2SO_4 atmosphere and then analyzed for $^{15}N/^{14}N$ ratio by a continuous-flow isotope ratio mass spectrometer (IRMS; Thermo Finnigan DELTA XPPlus, San Jose, CA, USA) coupled to an elemental analyzer (Thermo Finnigan Flash EA 1112 Series) and an open split interface (Thermo Finnigan Conflow III). Standards (USGS40) were analyzed in order to correct the results for drift during the sample runs and nonlinearity due to sample amount. Net N mineralization rates were calculated as the sum of accumulated $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ during the incubation period divided by the number of incubation days.

2.2.6 Statistical analyses (I–IV, Summary)

Statistical analyses (Table 4), were mainly done using the IBM SPSS Statistics and the R software, while basic calculations were done using Microsoft Excel version 2016. Data (e.g. on C_{LOSS}) were checked for normality by the Shapiro-Wilk test. Differences in the parameters measured, such as amount of PLFA, total C (TC), MBC, among sites, depths and between ecosystems were tested using independent *t*-test analysis of mean values (I, II) and one-way ANOVA (I). Correlation of total microbial PLFA, MBC, TC and MBC/TC with site parameters like C/N and C, were tested using the Spearman's correlation analysis (I, II). In I, 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. In III, effect of drainage, drying method and depth on the bulk peat $\delta^{13}C$ and C % were tested using two-way ANOVA. Student's *t*-test was used to test the effects of soil type (peat vs subsoil) and peatland type (fen vs bog) on the peat $\delta^{13}C$, C % and N % (of peat bottom), including how drainage impacted on these effects. Student's *t*-test was also used to test the effects of drainage and peatland type (fen vs bog) on the $\delta^{13}C$, C % and N % of the plant. In IV, absolute differences in C_{LOSS} among soils, temperatures and oxygen levels were evaluated by factorial ANOVA followed by post-hoc Tukey HSD test. A multiple linear regression model with stepwise selection was applied to find the best predictor of C_{LOSS} among all measured soil parameters, i.e. chemical and microbial parameters, and enzyme potential activities, using the R software (R Core Team 2014). Chemical, microbial and enzymatic parameters were evaluated for changes between initial and final measurements using *t*-test and differences among soils and treatments were tested using factorial ANOVA (IV).

TABLE 4 Statistical analyses used in this study. Detailed descriptions of each analysis are provided in the articles.

Analysis	Description
NMDS	I
t-test	I, II, III, IV
one-way ANOVA	I, III
two-way ANOVA	III
Spearman's correlation	I, II, III
PERMANOVA	I
Mantel's test	I
bivariate Spearman correlation test	III
post-hoc Tukey HSD test	IV
Shapiro-Wilk test of normality	IV
Ridge regression	IV
Factorial ANOVA	IV

3 RESULTS

3.1 General differences in microbial biomass between natural fen and bog and over the soil profile

There was higher total microbial PLFA biomass and MBC_{PLFA} in the natural fen compared to the natural bog site ($p < 0.001$). Contrary to that, there was no significant difference in the average amount of MBC_{CFE} (g^{-1} dw) and the number of total prokaryotic 16S rRNA gene copies (g^{-1} dw) between natural fen and natural bog sites. There is a possibility also that MBC_{PLFA} overestimated the MBC compared to MBC_{CFE} (II). There were consistent depth differences in microbial biomass and MBC in both the natural fen ($p < 0.001$) and natural bog ($p < 0.001$) sites as determined by several different techniques except the prokaryotic 16S rRNA gene copies in the fen site (I, Fig. 2). The total microbial PLFA biomass, MBC_{PLFA} and MBC_{CFE} were highest in the surface layer especially in the fen site, but also in the bog (Fig. 2). The total number of bacterial 16S rRNA copies per gram or volume peat (g^{-1} dw or m^{-3}) had no consistent pattern, though it tended to decrease with depth in the bog. On the other hand, the number of archaeal 16S rRNA gene copies (g^{-1} dw or m^{-3}) increased with depth in all the sites. As a result, there was no consistent depth trend in the total number of prokaryotic 16S rRNA gene copies per volume of peat (m^{-3}) in both natural fen and bog. The site in subarctic is special as it is a naturally drained site and the old peat is lifted up to the surface, but also here, total microbial (bacterial and fungal) PLFA and C_{MB} content was lower in the old previously deep peat as compared to surface peat (Table 5, Fig. 4A in IV). There was also consistent reduction in the amount of microbial PLFA, MBC and number of 16S rRNA gene copies from the surface to deeper layers in Villikkalansuo natural site. E.g., there were microbial PLFA (0.22 ± 0.05 and 0.12 ± 0.02 mg g^{-1} dw), MBC_{PLFA} (1.33 ± 0.13 and 0.67 ± 0.03 mg g^{-1} dw) and 16S rRNA ($5.32 \times 10^8 \pm 1.56 \times 10^7$ and $2.14 \times 10^8 \pm 1.01 \times 10^8$ copies g^{-1} dw) in the surface and bottom layers respectively.

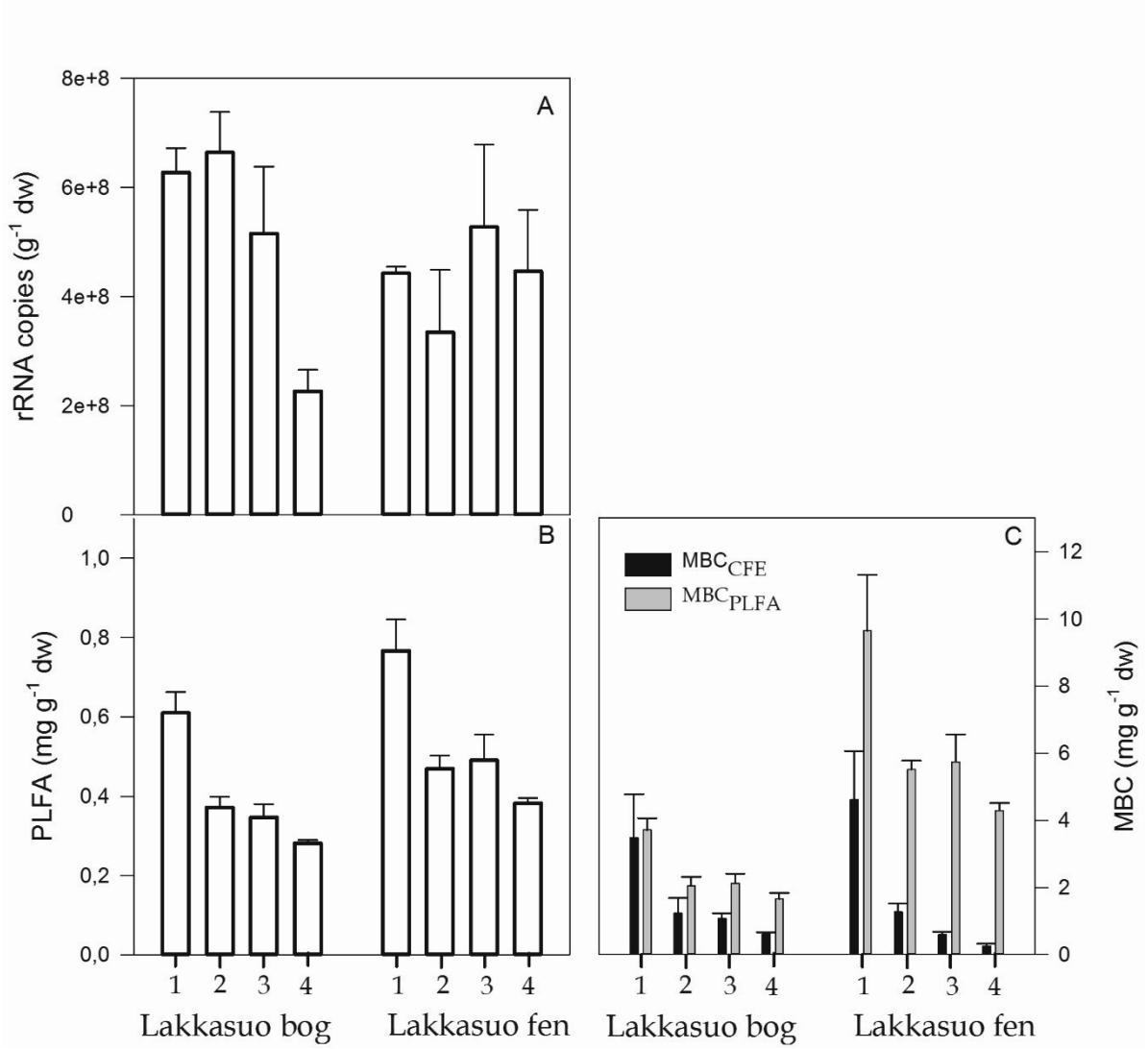

FIGURE 2 Mean (± SE, n =3) (A) number of 16S rRNA gene copies (g^{-1} dw), (B) microbial PLFA (mg g^{-1} dw) and (C) microbial biomass carbon estimated by CFE and via PLFA (mg g^{-1} dw) in different depths (1–4) of Lakkasuo bog and fen. Starting from the surface, depths 0–25 cm = 1, 25–50 cm = 2, 50–100 cm = 3 and bottom (deepest 25 cm) layer = 4. Data: I,II and additional sample.

3.2 General differences in microbial community composition between natural fen and bog and over the soil profile.

NMS ordination of relative PLFA composition showed that microbial community structure was different between the natural fen and bog sites (I). The NMS ordination further indicated that microbial community structure within each of the sites was explained by depth and hydrological status (Fig. 3 in I). The effect of depth and hydrological status on microbial community structure was also independent of each other in both the fen and bog sites (Table 2 & 3 in I). While microbial community structure also correlated with the

pH in both the fen and bog sites, it correlated with the bulk density in the bog site alone. The overall concentration of the entire major microbial group PLFAs followed the same trend as the total microbial PLFA, being higher in the fen than the bog site, and mostly highest in the surface layers (Fig. 4 in I). While there was depth effect on the amount and relative contribution of 16 monounsaturated fatty acids (16 MUFAs), characteristic of Gram-negative bacteria, only in the natural bog, there was depth effect on the amount and relative contribution of 18 MUFAs, also characteristic of Gram-negative bacteria, in the natural fen but not the bog site. There was no depth effect on the amount of terminally branched fatty acids (BrFAs), which are characteristic of Gram-positive bacteria, in both the natural fen and bog site. However, the relative contribution of BrFAs to total PLFA was affected by depth in the natural bog. There was no depth effect on the amount and relative contribution of Fungal FA in both the natural fen and bog sites. When assuming equal amplification efficiencies and abundance of rRNA operons in the archaeal and bacterial DNA, the percentage of archaeal DNA in the total prokaryotic (bacteria + archaea) DNA varied among peatland sites. While bacterial DNA decreased with depth from the surface, the archaeal DNA increased with depth from the surface. It ranged from as low as 0.36 ± 0.07 % in the 25–50 cm depth of the Lovonsuo drained site to as high as 69.61 ± 4.70 % in the bottom layer of reference site 1 (a natural site) (Fig. 3). In some of the natural peatlands, especially the reference sites 1 & 2 from northern Finland, archaea were the dominant prokaryotes. On average, the proportion of archaea increased with depth.

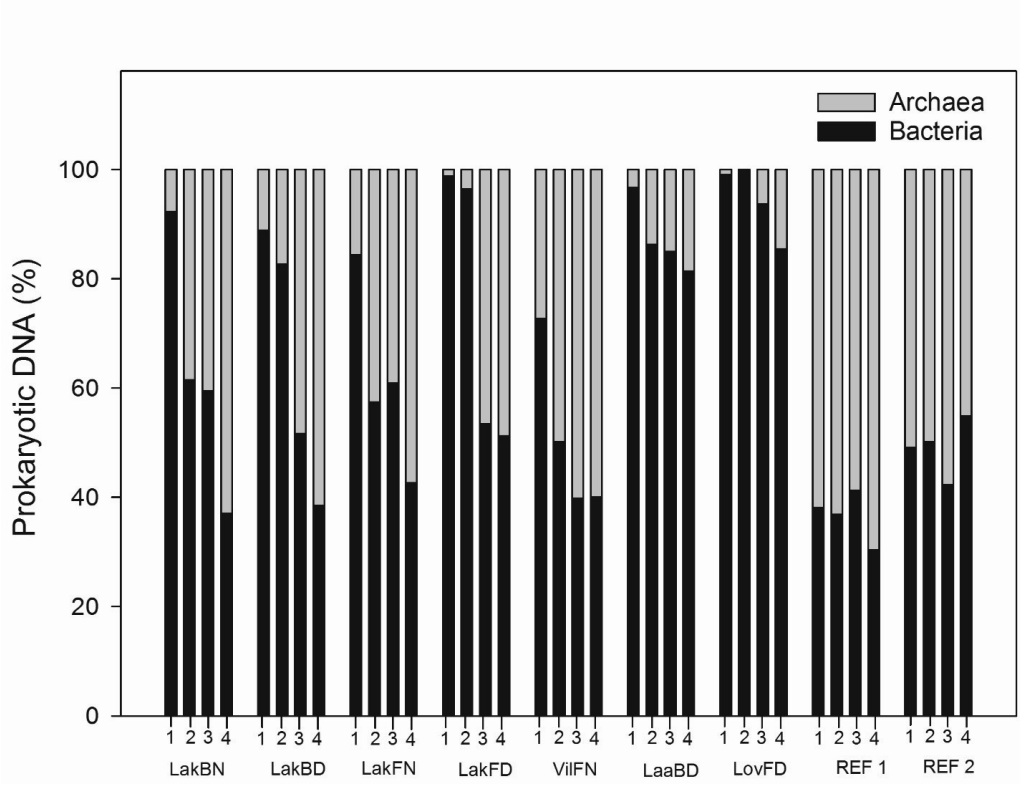

FIGURE 3 The proportion (percentage) of archaeal and bacterial DNA in the total prokaryotic DNA (extracted starting quantity) of the Lakkasuo bog natural and drained (LakBN & LakBD), Lakkasuo fen natural and drained (LakFN & LakFD), Villikkalansuo natural (VilFN), Laaviosuo drained (LaaBD), Lovonsuo drained (LovFD) and two reference natural peatland sites from the Northern part of Finland (REF 1 and REF 2). Starting from the surface, depths 0–25 cm =1, 25–50 cm = 2, 50–100 cm = 3 and bottom (deepest 25 cm for the site and deepest 50 cm for LaaBD) layer = 4. Data: additional sample.

3.3 Correlation analysis

Although the focus of the different techniques used in different analyses in this study are not the same (Table 3), the data generated from them were correlated since they are analyzing the same community. In the combined site (Lakkasuo fen and bog, Laaviosuo and Lovonsuo) data, there was correlation between the total PLFA biomass (µg g^{-1} dw) and number of prokaryotic (bacteria + archaea) (ρ = 0.307, p = 0.010) as well as bacterial (ρ = 0.303, p = 0.010) 16S rRNA gene copies (g^{-1} dw). When the sites were separated, there was correlation between the total PLFA biomass (µg g^{-1} dw) and number of prokaryotic (bacteria + archaea) 16S rRNA copies (g^{-1} dw) in Laaviosuo (ρ = 0.606, p = 0.002) and Lakkasuo bog (ρ = 0.563, p = 0.004) but not in the Lakkasuo fen (ρ = -0.224, p = 0.304). There was also correlation between the total PLFA biomass (µg g^{-1} dw) and number of bacterial 16S rRNA copies (g^{-1} dw) in Laaviosuo (ρ = 0.623, p = 0.002) and Lakkasuo bog (ρ = 0.578, p = 0.003) but not in the Lakkasuo fen (ρ = -0.236, p = 0.268. There was no correlation between the total microbial PLFA

biomass (m^{-3}) and the number of 16S rRNA copies per volume (m^{-3}) of bacteria and archaea combined or separated. In the combined data set for all the peatland sites, the MBC_{PLFA} (g^{-1} dw) correlated ($\rho = 0.464$, $p < 0.001$) with the MBC_{CFE}. There was also correlation between the MBC_{CFE} (g^{-1} dw) and the number of prokaryotic ($\rho = 0.409$, $p = 0.001$) as well as bacterial ($\rho = 0.439$, $p < 0.001$) rRNA copies (g^{-1} dw). There was a negative correlation between the MBC_{CFE} (g^{-1} dw) and the number of archaeal ($\rho = -0.250$, p-value = 0.041) rRNA copies (g^{-1} dw).

3.4 Effects of climate change on microbial biomass in peat

3.4.1 Effects of drying/ drainage

The whole-peat profile (top to bottom) microbial PLFA (Fig. 2A in I) analysis (I, II) and qPCR analysis of prokaryotic 16S rRNA gene (Fig. 3) showed that long-term water table draw-down affected the microbial biomass and community structure up to the deepest depth layers. While drainage increased the microbial community PLFA volumetric biomass (m^{-3}) in the surface, sub-surface and deepest layers of the nutrient-rich fen peatland, it decreased it in the surface and deepest layers of the nutrient-poor bog (I) (Fig. 2A in I). In all but the 50–100 cm depth of the fen site, total microbial PLFA biomass was higher in the drained than the natural side. In the bog site, the total microbial PLFA biomass was only different between the drained and natural side at the top and bottom layers, where the amount was smaller in the drained side (Fig. 2A in I). NMS ordination of relative PLFA composition, followed by a two-way factorial (drainage and depth) analysis (PERMANOVA), showed that, independent of depth effect, drainage affected microbial community structure in both the fen and bog sites (Table 2 and 3 in I).

The drainage-induced effects on specific major microbial groups, inferred from the absolute and relative concentrations of PLFA (I), varied among the microbial groups and between the fen and bog sites. The amount (g m^{-3}) of 16 MUFAs (FAs characteristic of Gram-negative bacteria) was higher in the top two layers (0–25 and 25–50 cm) of the drained side of the fen than the natural side, but lower in the bottom layer (only) of the drained side of the bog than the natural side. The relative contribution of 16 MUFAs to total PLFA (% contribution) did not differ between the drained and the natural sides in either fen or bog except at the 50–100 cm depth of the bog site (Fig. 4A in I). The amount (g m^{-3}) of 18 MUFAs (another FAs, characteristic of Gram-negative bacteria) was higher in the sub-surface layer (25–50 cm) of the drained fen site compared to the natural fen side, and lower in the bottom layer of the drained bog side compared to the natural bog side (Fig. 2C in I). The relative contribution of 18 MUFAs to the total microbial PLFA (% contribution) did not differ between the drained and the natural sides of either fen or bog (Fig. 4B in I). The amount (g m^{-3}) of terminally branched fatty acids (BrFAs), which are

characteristic of Gram-positive bacteria, was higher in the top two layers (0–25 and 25–50 cm) of the drained fen than the natural fen side, but lower in the sub-surface (25–50 cm) and bottom layers of the drained bog than the natural bog side (Fig. 2D in I). The relative contribution of BrFAs to the total microbial PLFA (% contribution) did not differ between the drained and natural sides of either fen or bog (Fig. 4C in I). The amount (g m^{-3}) of fatty acids characteristic of fungi (fungi FA) did not differ between the drained and natural fen sides, but was lower in the surface layer (0–25 cm) of the drained bog side than the natural bog side (Fig. 2E in I). Neither drainage nor depth affected the relative contribution of fungal FA to the total microbial PLFA (% contribution) in either fen or bog sites (Fig. 4D in I). The ratios of bacteria:archaea DNA in the total prokaryotic DNA was clearly higher in the drained sites especially in the surface layers, while some of the natural peatlands (especially the Ref sites 1 & 2 from northern Finland) were dominated by archaea DNA (Fig. 3). Water table draw-down affected the archaea microbial group up to the deepest depth layers. In the sites without corresponding adjacent natural and drained sites (VilFN, LaaBD, LovFD) the number of archaeal 16S rRNA copies (m^{-3}) were generally lower in the drained sites compared to the natural sites (Fig. 4). In the combined site data, the average (± SE) proportion of archaeal to total prokaryotic DNA decreased from 44.9 ± 2.4 % in the natural site to 20.1 ± 2.6 % in the drained sites. Overall, effects of drainage were site specific, variable between different microbial groups and dependent on layers. In general, however, effects of drainage on microbial biomass and community persisted down to the bottom layers of the peatlands.

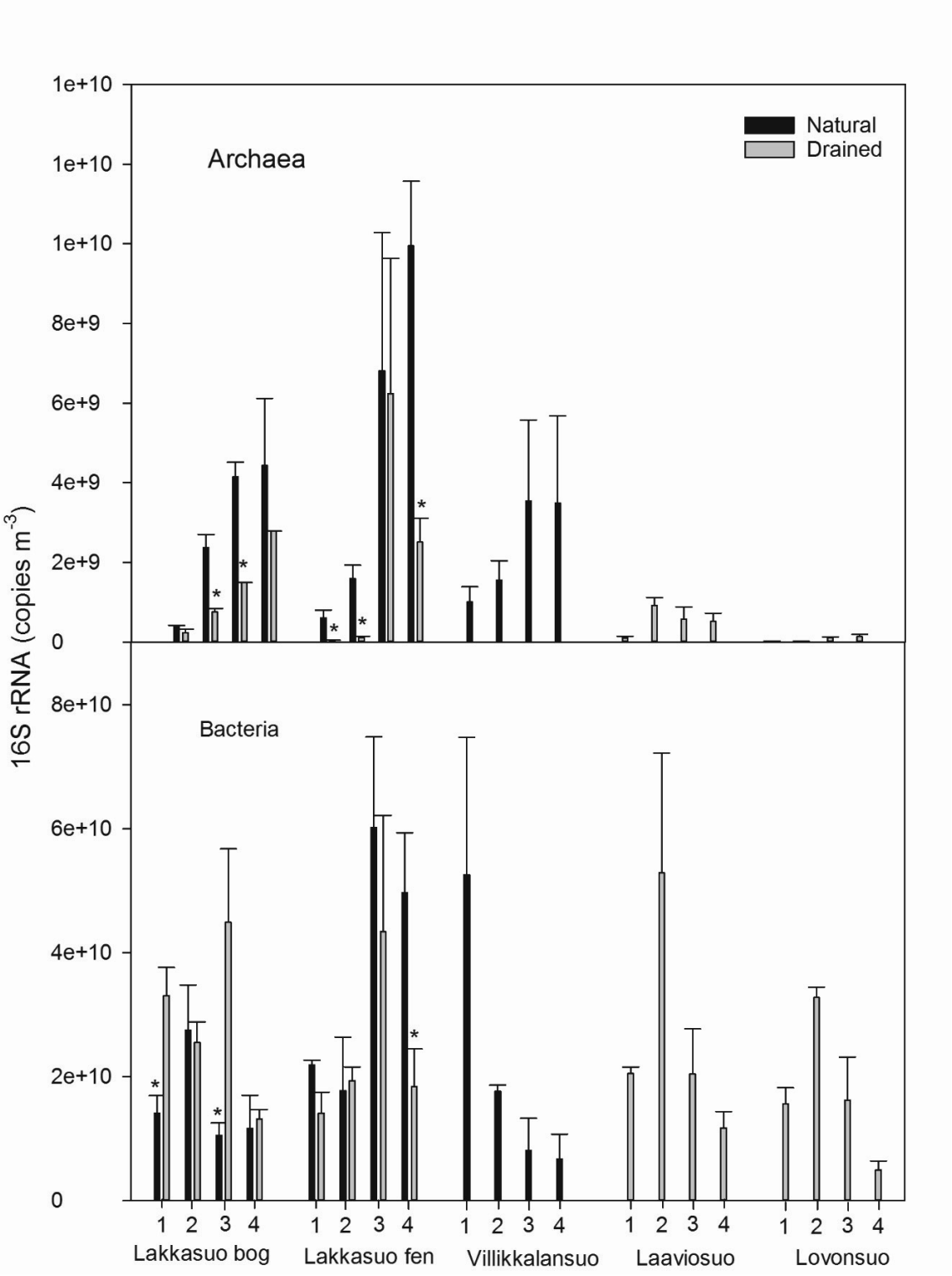

FIGURE 4 Estimated mean (± SE, n = 3) numbers of archaeal and bacterial 16S rRNA copies m^{-3} in different depths (1–4) of Lakkasuo bog, Lakkasuo fen, Villikkalansuo natural, Laaviosuo drained and Lovonsuo drained. Significant differences ($p < 0.05$) between the drained and natural sides at the Lakkasuo bog and fen, and in each depth based on independent sample *t*-tests, are denoted by asterisk (*). Starting from the surface, depths 0–25 cm = 1, 25–50 cm = 2, 50–100 cm = 3 and bottom (deepest 25 cm for other sites and deepest 50 cm for Laaviosuo drained) layer = 4.

In the Lakkasuo fen and bog sites with distinct adjacent natural and drained sites, the number of archaeal 16S rRNA copies were also mostly lower in the drained site of all the sampled depths in both the bog and fen sites. The number of archaeal 16S rRNA copies in all the depths of the drained sides were significantly lower ($p < 0.05$) than those in the natural sides except for the 0–25 cm and bottom depths in the bog site and 50–100 cm depth in the fen site. For the bacterial 16S rRNA copies (m^{-3}), there was no consistent trend with depth in either the natural or the drained sites. In the few depths where there is significant difference between the natural and the drained sites, the number of bacterial 16S rRNA copies (m^{-3}) was lower in the drained site. Unlike the PLFA biomass and 16S rRNA copies, the MBC_{CFE} varied mainly with depth but not between the natural and the drained sites in all the depth layers, except in the bottom layer of the bog site (Fig. 5).

Historical water table measurements in the studied sites [Lakkasuo mire complex (I)] showed that the water table depth was always lower in the drained fen than the drained bog for most part of the year [Fig. S4, Supplementary materials (I)]. Long-term drainage decreased the bulk peat stable C isotope ratio ($\delta^{13}C$ values) down to the deepest depth layers (III). However, the effect was statistically significant only in the bottom of Lakkasuo bog site. There was enrichment of the bulk $\delta^{13}C$ values of the mineral sub-soil under the drained compared to the natural Lakkasuo fen site (III) (Fig. 6).

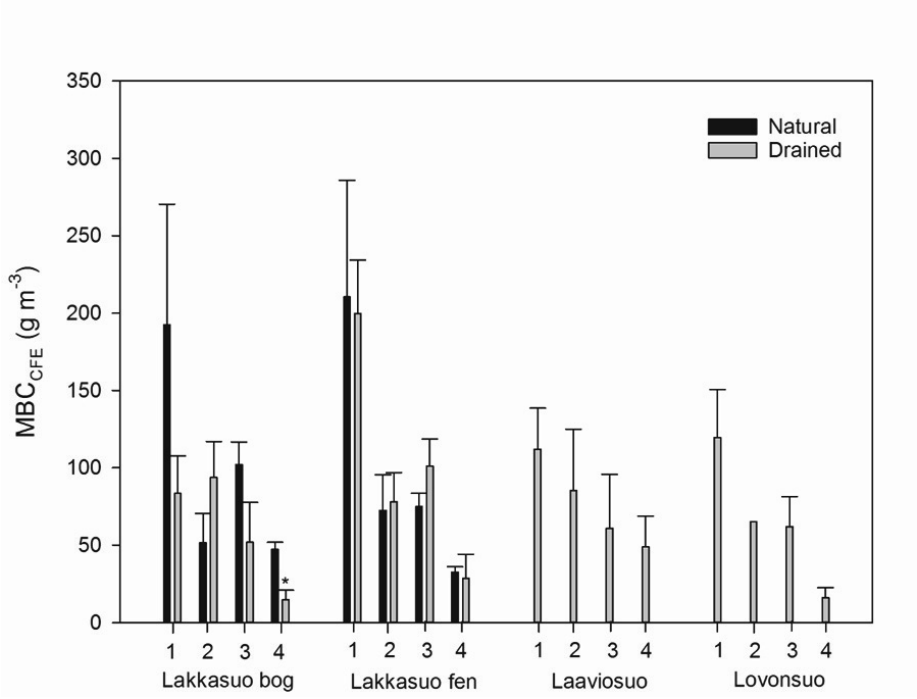

FIGURE 5 Mean (± SE, n =3) amount of microbial biomass carbon (g m^{-3}) in different depths (1–4) of Lakkasuo bog, Lakkasuo fen, Laaviosuo drained and Lovonsuo drained sites. There were no statistically significant differences ($p < 0.05$) between the drained and natural sides in all the depths at the Lakkasuo bog and fen, based on independent sample t-tests. Starting from the surface, depths 0–25 cm = 1, 25–50 cm = 2, 50–100 cm = 3 and bottom (deepest 25 cm for other sites and deepest 50 cm for Laaviosuo drained) layer = 4.

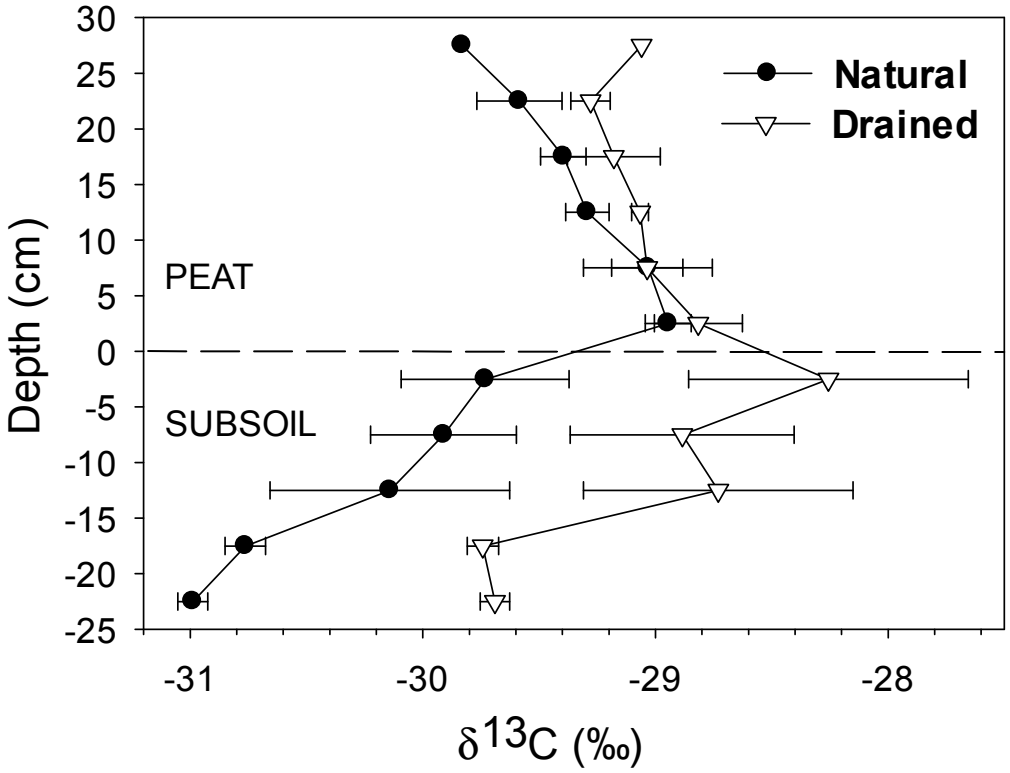

FIGURE 6 Estimated Average $\delta^{13}C$ (± S.E, n = 3) of peat bottom and subsoil profile from Lakkasuo fen site. Zero level indicates transition zone between peat and subsoil; above zero is peat and below zero is subsoil.

3.4.2 Carbon decomposition rates and effects of temperature on C losses from old and young peat as well as underlying factors

The incubation study (IV) showed extremely low microbial biomass and correspondingly very low C-losses from the old peat compared to the recent peat (Table 5, Fig. 2 in IV). Under both aerobic and anaerobic conditions, microbial biomass was the strongest and the most stable predictor of C-losses compared to all other determined soil properties. The effect of temperature on microbial biomass varied with factors such as soil quality, nutrient limitations and pre-adaptation of the microbial community to existing conditions such as O_2 availability. Under aerobic condition, microbial biomass (C_{MB}) was lower ($p < 0.001$) in the old compared to recent peat, representing 0.22 % and 1.01 of OC, respectively. Microbial biomass remained unchanged throughout the incubation period in both the old and recent peat (Fig 4A in IV). Soil type (soil quality) accounted for 37 % of the variability in C-losses, but again the main controlling factor was microbial biomass, while temperature and oxygen status had a secondary effect on C-losses. The cumulative C-loss relative to OC integrated over the incubation period was lower in the old peat compared to the recent peat (Fig. 2 in IV). While 0.7 % of OC was lost from the old peat over the 23 weeks at 20 °C, 3.1 % of OC was lost from the recent peat. While there was no CH_4 consumption detected in the aerobically incubated old peat, there

was steady consumption of CH_4 at ambient concentrations from the headspace of the recent peat aerobic incubation vessels.

Under anaerobic conditions, where microbial biomass in the old peat with poorer soil quality increased during incubation, it decreased in the recent peat with higher quality, under the same conditions in the whole temperature range (Fig. 4A in IV). Anaerobic C-losses were lower, and more variable compared to aerobic treatment, but like the aerobic conditions, cumulative C-losses were lower in the old compared to the recent peat (Fig. 2 in IV). Cumulative C-losses under anaerobic conditions represented the sum of CO_2 and CH_4 produced, however, production of CH_4 was not detected in the old and the recent peat soils for the whole incubation period. The C-loss from old peat was lower by 24.5 %–40.1 % as compared to aerobic conditions, regardless of the temperature. In the recent peat, C-loss were lower by 21.6 %–69.1 % compared to aerobic conditions and this was positively affected by temperature. Generally, the difference between anaerobic and aerobic conditions were thus smaller for old vs recent peat, indicating better adaptation of soil microbes to anaerobic conditions in old peat even thousands of years after aeration (uplifting).

Like the peatlands from the boreal region and relative to the OC, microbial biomass (CMB) was lower ($p < 0.001$) in the old (deep peat) compared to recent peat (surface peat). In addition, the amount of characteristic fatty acids for all the microbial groups (general microbial biomass, Gram-positive and Gram-negative bacteria and fungi) were lower in the old peat than in the young peat. The characteristic FAs for the microbial groups mirrored the depth and drainage effects on microbial groups measured in the peatlands from the boreal region (Table 5). E.g., the relative abundance of fungi characteristic FA was the least and mostly affected by depth (old vs. recent peat) than the other microbial groups. The microbial biomass remained unchanged throughout the aerobic incubation period in both the old and recent peat but increased in the old peat and decreased in the recent peat during anaerobic incubation, just like the effect of drainage on microbial PLFA from boreal peatlands.

TABLE 5 PLFA profiles including characteristic fatty acids for general microbial biomass, Gram-positive and Gram-negative bacteria and fungi. Values are given as mean molar concentrations (nmol g^{-1} dw) ± standard deviations (n = 3), and the relative abundance of each group is expressed as mean molar % ± S.D of the total PLFA.

	PLFA (nmol g^{-1} dw)	**Recent peat**	**Old peat**
General biomass	12:0	2.8 ± 2.1	6.0 ± 2.3
	14:0	28.4 ± 9.2	23.8 ± 7.2
	16:0	273.8 ± 85.1	114.7 ± 23.9
	17:0	13.4 ± 1.6	24.9 ± 6.0
	18:0	55.4 ± 11.2	35.6 ± 1.1
	20:0	22.9 ± 4.1	13.5 ± 3.6
	sum	**396.8 ± 101.8**	**218.5 ± 41.4**
	rel. abundance (%)	**29.2**	**34.0**
Gram-positive bacteria	i14:0	7.6 ± 1.9	5.1 ± 1.2
	i15:0	129.5 ± 57.9	80.8 ± 15.8
	a15:0	40.8 ± 22.8	40.8 ± 10.2
	i16:0	53.7 ± 31.1	20.2 ± 6.5
	i17:0	12.9 ± 4.8	10.0 ± 3.1
	br17:0	28.1 ± 21.3	15.1 ± 8.9
	sum	**272.6 ± 132.3**	**172.1 ± 45.4**
	rel. abundance (%)	**20.0**	**26.8**
Gram-negative bacteria	16:1ω9	79.7 ± 41.3	25.8 ±10.8
	16:1ω7	95.1 ± 69.9	18.8 ± 6.3
	cy17:0	27.9 ± 16.1	28.7 ± 7.4
	17:1ω9	14.9 ± 11.0	14.3 ± 2.0
	18:1ω7	82.8 ± 40.8	24.6 ± 13.6
	cy19:0	69.9 ± 23.8	72.2 ± 13.9
	sum	**370.3 ± 192.1**	**184.4 ± 33.9**
	rel. abundance (%)	**27.2**	**28.7**
Fungi	16:1ω5	10.9 ± 2.3	9.4 ± 3.3
	18:1ω9c	147.5 ± 76.2	31.7 ± 11.4
	18:2ω6c	161.8 ± 110.4	25.8 ± 9.7
	sum	**309.3 ± 183.8**	**57.5 ± 20.9**
	rel. abundance (%)	**23.5**	**10.4**
Total PLFAs	**total conc. (nmol g^{-1} dw)**	**1360 ± 586**	**642 ± 140**
	(nmol g^{-1} OC)	**2955 ± 1273**	**1503 ± 328**

3.5 Chemical, biological and physical characteristics of SOC in deep peat

From the incubation study (IV), under different temperature and oxygen regimes, a multiple regression analysis showed that microbial biomass variation was the most stable and strongest predictor of C losses from old peat as compared to the "recent" peat (IV). The deep old peat had lower decomposition rate, microbial biomass (Table 5) and biomass-specific CO_2 production compared to the "recent" vegetated peat (IV). The study also showed that soil quality (the soil type), which accounted for 37 % of the reported C-loss variability during the incubation period, was the second strongest predictor of C-losses while the temperature and O_2 had secondary effects on C-losses. There was higher DOC aromaticity (IV) and C:N ratios (I) in the old peat compared to the recent (young) peat. One exception to this, was the low C:N ratio in the permafrost-affected old uplifted peat (IV) because it is originally fen peat which has been uplifted as fen often underlies bogs. The $\delta^{13}C$ values of bottom old peat were generally more negative than the intermediate and surface layers (III), indicating more recalcitrant biomass like lignin or more anaerobic degradation of biomass in the bottom old peat. The peat soil in the more fertile fen site also had lower C:N ratios than the bog site (I). The bulk peat C per unit volume increased with depth in all the peatlands covered in this study (II). The old peat sample used in the incubation study, as well as the deep peat in other studies, also had higher bulk density than the recent peat (IV). The extractable C_{CFE} expressed on a gram of OC bases (μmol g^{-1} OC) was also higher in the old peat than the recent peat (IV).

3.6 Microbes as dynamic storage of C in peat

The study (I, II) demonstrated the presence of viable microbes (i.e. microbial PLFA) at a relatively large amount even in the deepest (over 3000 years old) peat (Fig. 2A in I) layers. Mean (± S.E) peat MBC_{PLFA} estimated from total PLFA biomass (using a conversion factor) in all the sites and depths studied (excluding Seida from the arctic) was 2.7 ± 0.3 (mg g^{-1} dw) with the maximum amount of 9.6 ± 1.7 (mg g^{-1} dw) in the surface of the Lakkasuo fen natural and minimum amount of 0.6 ± 0.1 (mg g^{-1} dw) in the bottom layer of the Lovonsuo drained site. Mean (± S.E) peat MBC_{CFE} estimated by the CFE method, in the same sites (Lakkasuo fen and bog, Laaviosuo drained and Lovonsuo drained) and depths was 1.2 ± 0.3 (mg g^{-1} dw) with the maximum amount of 4.6 ± 1.5 in the surface of Lakkasuo fen natural and minimum amount of 0.2 ± 0.1 (mg g^{-1} dw) in the bottom layer of Lovonsuo drained site. While MBC_{PLFA} varied between natural and drained sites as well as between depths, MBC_{CFE} only varied between depths. In both cases, MBC was highest in the surface layers. In

general, the average (± S.E) contribution of MBC_{PLFA} to total C was about 0.43 ± 0.04 % with the highest range of 0.81 ± 0.05 % to 1.95 ± 0.34 % in the fen site. Based on CFE, average MBC_{CFE} contribution to total peat C was about 0.26 ± 0.03 % with the highest range of 0.04 ± 0.01 % to 0.94 ± 0.30 % in the fen site. Unlike MBC_{PLFA}, there was no difference in the average MBC_{CFE} contribution to total C between the fen and bog site. The MBC-stock in Northern peatland could be up to 1508 Tg C (based on MBC_{PLFA}) or up to 725 Tg C (based on MBC_{CFE}) using average peat depth of 2.3 m according to Gorham (1991) (II).

4 DISCUSSION

PLFA analysis showed higher microbial biomass in the fen than in the bog, but this difference was not observed when looking at microbial biomass based on the rRNA gene copy numbers. While the genetic analysis included only bacterial and archaeal rRNA genes, the PLFA analysis included the fungi but not the archaea. The assumption of equal recovery ("lost factor") for all the samples in the DNA extraction protocol, which is probably not the case, is a source of error in the quantitative analysis, as well as the DNA outside the cells (relic DNA), and assumption of equal numbers of rRNA copies per biomass in different samples. Actually, the PLFA-analysis, which was mostly used in this study, is considered a more reliable quantitative measure of living microbes, since fatty acids are short-lived and readily metabolized upon cell death (White *et al.* 1979, Mancuso *et al.* 1990). In overall, PLFA analysis is considered more sensitive in detecting treatment effects on microbial community composition than the nucleic acid based methods (Ramsey *et al.* 2006). For instance, the relative abundance of fungi and bacteria (fungal/bacterial ratio) used in comparing soils and treatments were better differentiated by the PLFA method than by the PCR method (Frostegård and Bååth 1996, Klamer and Bååth 2004).

Higher nutrient content and pH in the fen compared to bog site, coupled with differences in their vegetation cover (I), mostly explains the higher microbial PLFA biomass in the fen site (Jaatinen *et al.* 2007, Andersen *et al.* 2013). Biomass of fen vegetation is more easily decomposed than that of bog vegetation, which consists largely of recalcitrant *Sphagnum* mosses (Jaatinen *et al.* 2007, Andersen *et al.* 2013, Mandic-Mulec *et al.* 2014). The roots of sedges in fens provide better soil stability and macro pore structure than those of *Sphagnum* mosses on the bogs. Depth variation in the total microbial PLFA biomass, MBC_{PLFA}, MBC_{CFE} and 16S rRNA, especially for archaea is most likely due to depth related differences in the fertility, redox potential or oxygen availability and litter quality in each of the sites (Preston *et al.* 2012, Lin *et al.* 2014). For example, the C/N ratios increased with depth, indicating poorer biomass quality in both the fen and bog sites (I). Also, in the Lakkasuo fen but not in the Lakkasuo bog site, microbial biomass decreased with increasing C/N

ratios as well as decreasing N % and C % (I). Soil organic matter is also more decomposed, naturally older and thus more recalcitrant at depth. Thus, PLFA content was e.g. lower in the old peat sampled from subarctic tundra as compared to the young peat though C/N ratios were actually higher in the young peat. In addition to the differences in the location of the sites, variation in the composition of prokaryotic DNA, especially the proportion of archaeal vs. bacterial 16S rRNA gene copies (Fig. 3 & 4) among the sites, is best explained by similar reasons as those stated above for differences in the amount of microbial PLFA between the sites (Borga *et al.* 1994, Jaatinen *et al.* 2007, Kim *et al.* 2008, Andersen *et al.* 2013). Increasing relative abundance of archaea rRNA gene copies from about 1 % at the surface to up to 60 % of the total rRNA gene copies below 75 cm depth in peatland, have been previously reported (Lin *et al.* 2014). This therefore suggests that the archaeal microbial groups play key roles in the C cycle (e.g. methanogens) of deep layers.

Although the main focus of the three different methods used in the study to analyze the microbial communities differs slightly (Table 3), the correlation (though weak) between the data generated by them suggests they can be used together or alternatively. Preference for the use of all or one of the method over the other depends on the focus of the research, design of the experiment, research hypothesis and available resources.

4.1 Effects of climate change on microbial biomass in peat

In addition to the low nutrient availability and low quality of peat forming plant litters (Van Breemen 1995), complete microbial breakdown of deposited plant materials is impeded by factors such as prevalent anoxic condition, low soil temperatures and pH, caused by continuous water logging and flooding of peatlands (Moore and Basiliko 2006, Artz 2009). The diversity and composition of peat microbial communities and the associated rates of C cycling (Moore and Basiliko 2006, Andersen *et al.* 2013, Mishra *et al.* 2014) are sensitive to changes in these factors (Shurpali *et al.* 1995, Trumbore *et al.* 1996, Lohila *et al.* 2004, Vanhala *et al.* 2007, Maljanen *et al.* 2009, Shurpali *et al.* 2009).

Drainage of peatlands leads to subsidence and increased in the bulk density of peat surface layers (Minkkinen and Laine 1998). The degree of subsidence depends on the thickness and wetness of the peat as well the depth of the drainage ditches (Lukkala 1949). This implies that though the layers compared in this study are from the same depths relative to the present peat surface, subsidence of peat surface after drainage means that samples from the drained sites (except the bottom layer samples) may be from different original depths (Minkkinen and Laine 1998). Most of the results are therefore reported per volume (m^{-3}) to take care of the bulk density variation while subsidence is believed to be mostly limited to the topmost peat layers.

4.1.1 Long-term effects of drying

The rate of peat litter decomposition varies with the structure and composition of peat microbial communities (Laiho 2006, Straková *et al.* 2010) among other factors. Water table depth (hydrology) affects the location where major biogeochemical processes occur in the peat profiles (Jaatinen *et al.* 2007, Yrjälä *et al.* 2011, Andersen *et al.* 2013, Urbanová and Bárta 2016). Hence, similar to the results from several previous studies, water level drawdown affected the biomass and community structure (I, II) of peat microbial communities in this study (Yrjälä *et al.* 2011, Urbanová and Bárta 2016). Most studies on the effects of drainage on peat microbial communities and C cycling focused on the surface layers (Jaatinen *et al.* 2005, 2007, Urbanová and Bárta 2016). The whole-peat profile (top to bottom) MBC_{CFE} analysis did not show drainage effect, except in the bottom layer of the bog site (Fig. 5), possibly because CFE was not so efficient in highly water saturated (averagely ≥ 90 %) and low pH (3.33–5.33) samples as used in the analysis (Vance *et al.* 1987b, Haney *et al.* 1999, 2001, Alessi *et al.* 2011). However, the whole-peat profile microbial PLFA (Fig. 2A in I) and 16S rRNA gene (Fig 4) analyses (I, II) showed that, long-term water table draw-down affected the microbial community (biomass and structure) up to the deepest depth layers. Although the water table was still clearly above the deepest layers, while drainage increased the microbial community PLFA biomass (m^{-3}) in the surface, sub-surface and deepest layers of the nutrient-rich fen peatland, it decreased it in the surface and deepest layers of the nutrient-poor bog (I) (Fig. 2A in I). In addition, the number of archaeal 16S rRNA copies (g^{-1} dw or m^{-3}) were also generally lower in the drained compared to the natural sites up to the deepest depths. This was possibly due to changes in microbial activities because of deeper deposition of labile root exudates by roots of vascular plant (Walker *et al.* 2016) coupled with differences in the amount and quality of DOC transferred from the surface layers of the drained site (Charman *et al.* 1999, Jokinen *et al.* 2006, Laine *et al.* 2014). Earlier studies reported that C flow (via water) from the surface to the deeper anaerobic peat layers were negligible (Siegel et al. 1995). Recent findings have shown that DOC derived in part from surface processes, rather than deeper peat decomposition, fuels anaerobic respiration in deep peat column (Aravena *et al.* 1993, Charman *et al.* 1994, 1999, Chasar *et al.* 2000, Chanton *et al.* 1995, 2008, Clymo & Bryant 2008, Corbett *et al.* 2013), up to 7 m depth in one of the studies (Clymo & Bryant 2008). Coupled with reported differences in the radiocarbon enrichment of DOC, CO_2 and CH_4 gases in deep peat relative to deep peat soil (Charman *et al.* 1999, Chasar *et al.* 2000, Chanton *et al.* 2008) this supports the finding that surface drainage affected microbial communities in the deepest peat layers. Also, as a qualitative indicator of peatland microbial degradation (Alewell *et al.* 2011, Krüger *et al.* 2014, 2015, 2016), the drainage-induced variation in the bulk-peat $\delta^{13}C$ values up to the mineral sub-soil (III) (Fig. 6), supports the results on the effect of drainage on peat microbial biomass beyond the surface layers.

The specific impact of drainage on peat microbial communities as inferred from the PLFA varied between the studied peatland types (I, II). While drainage

increased the microbial community PLFA biomass in the nutrient-rich fen peatland, it decreased it in the nutrient-poor bog (I). The fen and bog sites studied here for drainage effects were drained at the same time, located in the same place (e.g. in the Lakkasuo mire complex) and therefore exposed to similar climatic conditions, such as temperature and precipitation. Hence, differences in the drainage-induced effects, between the bog and fen (I, II, III) sites, resulted from difference in other factors. These factors include the original nutrient status, extent of vegetation change and the quality of available litter or substrate, between the peatland types (Laine and Vanha-Majamaa 1992, Minkkinen *et al.* 1999, Laiho 2006, Jaatinen *et al.* 2007, Preston *et al.* 2012, Urbanová and Bárta 2016). In general, while drainage in the studied fen site led to a succession towards a different ecosystem from the original, in the bog site it led to smaller changes. Thirty-eight years after drainage, tree stand volume increased from nothing in the natural fen to 111 m^{-3} ha^{-1} in the drained fen side and from 5 in the natural bog to 16 m^{-3} ha^{-1} in the drained bog side (Minkkinen *et al.* 1999). The increased tree growth in the drained fen led to greater evapotranspiration and further decrease in the WT. Historical water table measurements in the studied sites [Lakkasuo mire complex (I)] showed that the water table depth was always lower in the drained fen than the drained bog for most part of the year (e.g Nykänen *et al.* 1998, Minkkinen *et al.* 1999, Laiho 2006, Jaatinen *et al.* 2007, Pearson *et al.* 2015, Peltoniemi *et al.* 2016). Unlike the PLFA biomass, the number of archaeal 16S rRNA copies were lower in the drained side of both the fen and bog sites (Fig. 4). Since PLFA analysis did not account for the archaea, this therefore suggest that different microbial species respond differently to drainage effect (Jaatinen *et al.* 2007, 2008, Kim *et al.* 2008, Peltoniemi *et al.* 2009). The reduction in archeae as a result of drainage is likely due to the reduction of anaerobic, methane-producing organisms which otherwise dominate the anaerobic peat community (Ma and Lu 2011, Ma *et al.* 2012).

As in previous studies, this study (I) also showed that the drainage-induced effects on specific major microbial groups, inferred from the absolute (volumetric) and relative (%) concentrations of PLFA (I) and number of 16S rRNA copies, varied among the microbial groups and between the fen and bog sites (Jaatinen *et al.* 2007, Kim *et al.* 2008, Peltoniemi *et al.* 2009). There was higher concentrations of all the FAs characteristic of the major microbial groups, except the fungal characteristic FA, in the drained fen than the bog site. The biomass of both the Gram-negative and Gram-positive bacteria characteristic FAs were increased in the surface and subsurface layers of the drained fen site, but decreased in the bottom layer of the bog site. The fungal characteristic FA was only reduced in the surface layer of the bog site. 16S rRNA gene analysis showed that while the prokaryotic DNA in natural peatlands are dominated by the archaea DNA, drainage increases the ratio of bacteria:archaea DNA in the total prokaryotic DNA especially in the surface layers. Although the same dominant microbial taxa are present in these differing peatland types (Preston *et al.* 2012), the drainage-induced effects on specific microbial groups differ between peatland types and depths (Jaatinen *et*

al. 2007, 2008, Peltoniemi *et al.* 2009). This was probably because drainange-induced effects depends on other factors such the sensitivity of the specific microbial group to hydrological changes, the extent of the oxic condition and the vegetation characteristics of the site (Borga *et al.* 1994, Peltoniemi *et al.* 2009, Andersen *et al.* 2013). For example, Peltoniemi *et al.* (2009) showed that fungi were more sensitive to drainage than actinobacteria in the same site, even though their responses to drainage depended on the peatland type. They also reported that, between the fungal phyla, basidiomycetes might be more sensitive to drainage than ascomycetes. However, since PLFA analysis method is very weak in phylogenetic resolution of microbial communities (Frostegård *et al.* 2011), the 16S rRNA analysis gives further clarification on this at least for bacteria and archaea. Using techniques with higher taxonomic resolution than PLFA-analysis, e.g. next-generation sequencing of rRNA gene amplicons, could give better insight to the drainage-induced effects than observed with the total microbial community PLFA (I).

4.1.2 Short-term and long-term effects of water/oxygen content and short-term effects of temperature in subarctic peat soils

Like the peatlands from the boreal region and relative to the OC, microbial biomass (CMB) was lower ($p < 0.001$) in the old (deep peat) compared to recent peat (surface peat). In addition, the amount of characteristic fatty acids for all the microbial groups (general microbial biomass, Gram-positive and Gram-negative bacteria and fungi) were lower in the old peat than in the young peat. This was possibly due to the depth related differences in the fertility and litter quality between the surface and deep peat layers (Mandic-Mulec *et al.* 2014). The microbial biomass remained unchanged throughout the incubation period in both the old and recent peat. However, like the depth and drainage effects on microbial groups measured in the peatlands from the boreal region, the relative abundance the microbial groups were differently affected with fungi characteristic FA being more affected by depth and drainage (old and aerobic peat incubation) than the other microbial groups. This is because fungi are more sensitive to drainage than other microbial groups like actinobacteria (Peltoniemi *et al.* 2009). Another reason being the contrasting responses to drainage by the different species in these groups (Jaatinen *et al.* 2007, 2008, Peltoniemi *et al.* 2009).

Together with hydrology, temperature is one of the ultimate controllers of peat OM transformation due to its effect on peat microbial communities (Yavitt *et al.* 1997, Deslippe *et al.* 2012, Jungkunst *et al.* 2012, Andersen *et al.* 2013, Männistö *et al.* 2013, Tveit *et al.* 2013, Peltoniemi *et al.* 2015). Temperature influences on microbial communities and particularly their activities (Laiho 2006). The incubation study (IV) showed that the effect of temperature increase on microbial biomass does not only vary with factors such as the peat biomass quality, but also with the pre-adaptation of the microbial community to existing conditions such as O_2 availability. Whereas microbial biomass in the old peat with poorer SOM quality increased at higher temperature under anaerobic

condition, it decreased at higher temperature in the recent peat with richer SOM quality under the same conditions (Fig. 2A in I). This suggests that at least part of the microbial community in the old peat thrives in the anaerobic conditions with their metabolism adjusted to the absence of O_2. Again, microbes with a slow anaerobic metabolism could increase in biomass under anaerobic conditions, whereas under aerobic conditions aerobes that are more efficient would suppress their growth.

Microbial mediated C-losses were positively affected by temperature in both the young and old peat soils (IV). Previous studies considered the physiological temperature response of basal microbial metabolism as the main controller of the temperature sensitivity of C-losses (Brown *et al.* 2004, Allen *et al.* 2005, Allen and Gillooly 2009, Yvon-Durocher *et al.* 2012). Contrary to this, results of the incubation study showed that microbial communities in both the recent and old peat samples reacted to higher temperatures with an increased production of hydrolytic enzymes. This possibly raised the temperature sensitivity of C-losses beyond the basic microbial physiological response in two ways. First, enzyme synthesis requires energy in the form of ATP, which is formed during oxidative phosphorylation while producing CO_2. Therefore, increased enzyme production at higher temperatures enhances CO_2 production above the basal metabolic rate. Secondly, when the hydrolytic enzyme pool is higher at higher temperatures, microbial community is supplied with the increased substrate level, again reinforcing CO_2 production above the basal metabolic rate. In agreement with previous studies, the result further showed that the degree of temperature sensitivity of peat C-losses varies among sites (soil types), depending on factors such as substrate quantity and quality, oxygen availability, nutrient limitation and extracellular enzyme activities (Allison *et al.* 2010). For example, higher temperature induced a larger difference between the aerobic and anaerobic cumulative C-losses in recent peat compared to the old peat (no difference). This also indicates tight adaptation of the peat microbial community to aerobic conditions in the surface, as microbial biomass in recent peat was negatively affected by anaerobic condition at higher temperatures.

4.1.3 Effects of plants

The vegetation cover on a particular site determines the type of deposited biomass available to the microbial communities (Ménot and Burns 2001, Skrzypek *et al.* 2008, Krüger *et al.* 2014). Microbial communities rely on the energy derived from the decomposition of available biomass for growth. The influence of other factors, such as temperature, pH and O_2 availability, on microbial metabolism or biomass decomposition depends on the quality and quantity of available plant biomass (Laiho 2006, Artz 2009, Singh *et al.* 2010, Xu *et al.* 2011, Graham *et al.* 2012). This study (IV) showed extremely low microbial biomass and correspondingly very low C-losses from the old peat without vegetation compared to the recent peat with vegetation (Table 5). The lack of fresh input of organic materials from plants is considered one of the main

reasons for this (Gittel *et al.* 2014b, Schnecker *et al.* 2014, Wild *et al.* 2014, Čapek *et al.* 2015, Gentsch *et al*. 2015). The OC in deep old peats represent a complex substrate for microbial decomposition. Continuous input of fresh OC from plant litter and root exudates can stimulate the development of microbial community and facilitate microbial mineralization of complex compounds (Wild *et al.* 2014). Although recent findings have shown that DOC derived from surface processes partly fuels microbial respiration in deep anaerobic peat layers (Aravena *et al.* 1993, Charman *et al.* 1994, 1999, Chasar *et al.* 2000, Chanton *et al.* 1995, 2008, Clymo and Bryant 2008, Corbett *et al.* 2013), the quantity and quality of DOC is not exactly the same as in the surface layers. The OC in deep old peats is further protected from microbial decay by the low abundance of fungi as the main primary decomposers as well high compression of the old peat (Gittel *et al.* 2014b, Lin *et al.* 2014). Compression-induced limited dispersion and spatial separation of microbial cells in deep old peat can limit the development of microbial community (Kuzyakov *et al.* 2000, Wild *et al.* 2014). Deep rooting plants can counter or at least reduce the effect of compaction in deep peats. Increased tree growth can also lead to greater evapotranspiration and decrease in the WT of peat site, resulting in increased microbial growth (Jokinen *et al.* 2006, Laine *et al.* 2014, Walker *et al.* 2016). This was evident in the drainage-induced increases in the total microbial PLFA biomass in the surface and sub-surface layers of the fen, but a decrease was evident only in the surface layer of the bog site (I). This means that the constrained microbial community development in old deep peat layers can be released by succession towards a different ecosystem, due to drying caused by global warming. This could trigger increased decomposition of old peat currently stored in deeper layers of peatlands.

4.2 Chemical, biological and physical controls of SOC in deep peat

OC cycling in peatland is governed by a complex interplay between chemical (e.g. quality of OM), physical (e.g. prevailing environmental conditions) and biological (e.g. microbial communities and vegetation) factors (Jungkunst *et al.* 2012, Andersen *et al.* 2013). The dynamic and continuous peat accumulation processes involve the addition of new biomass to the peat surface, while the age of peat generally increases with depth (Page *et al.* 2004, Hirano *et al.* 2007). After very intense biochemical processes in the active surface layers, the bulk of peat C, which are usually stored in the anaerobic deeper layers are more recalcitrant, decomposed and homogenized. Results from this study showed higher DOC aromaticity (IV) and C:N ratios (I) in the old peat compared to the recent (young) peat, indicating poorer substrate quality in the old peat. The low C:N ratio in the permafrost-affected old uplifted peat (IV) was due to the high amount of nitrogen in the sample and the fen-type origin, and not the quality of

the peat SOM. The $\delta^{13}C$ values of bottom peat were also generally more negative than the intermediate and surface layers, indicating possibly more recalcitrant compounds with lower $\delta^{13}C$ (III). The poorer OC quality in deep peat means less sensitivity to other decomposition controlling factors, such as temperature and hydrology (Biasi *et al.* 2005, Gershenson *et al.* 2009, Allison *et al.* 2010, Jungkunst *et al.* 2012, Andersen *et al.* 2013) as well as being resilient to microbial decomposition. For example, higher temperatures induced a larger difference between the aerobic and anaerobic cumulative C-losses in recent peat compared to the old peat where there was no difference (IV). This line of view is supported by the overall peat C loss in the fen and gain (with 2 out of 4 methods used to estimate biomass) in the bog site, reported by Krüger *et al.* (2016) after long-term drainage. As confirmed by the C:N ratio analysis (I), the biomass of fen vegetation is more easily decomposed than that of bog vegetation, which consists largely of recalcitrant *Sphagnum* mosses (Jaatinen *et al.* 2007, Andersen *et al.* 2013, Mandic-Mulec *et al.* 2014).

The old deep peat layers are continuously anaerobic due to high water saturation. Analysis of the bulk stable C isotope ratio of the peat profile (top to bottom) (III) showed that long-term water table draw-down affected peat $\delta^{13}C$ values even in deeper depth layers (Fig. 6), though statistically significant only in the bottom of bog and the mineral subsoil (Fig. 2 and Table 4 in III). Since all the peat forming plants on the studied peatlands are using C3 pathway of photosynthesis, the differences in the $\delta^{13}C$ profiles resulted from different plant species and processes (including microbial) modifying or moving C-containing compounds first between the atmosphere and vegetation, and then within the peat column. Krüger *et al.* (2016) reported higher $\delta^{13}C$ (possibly indicating higher peat decomposition rate) up to 93 cm depth in the drained compared to the natural fen site in Lakkasuo. Specifically, drainage depleted the $\delta^{13}C$ values in the peat columns (III) (though not statistically significant), but clearly enriched it in the subsoil of the drained compared to the natural fen. In the drained compared to the natural bog, drainage had no effect on the $\delta^{13}C$ in the surface, enriched it in middle layer and depleted it in the bottom layer though only statistically significant in the bottom layer (III). These peat column results agree with the overall peat C gain (with 2 out of 4 methods) in the bog site, but not the loss in the fen, due to drainage (Ojanen *et al.* 2013, Krüger *et al.* 2016). In addition to this, the bulk peat C per unit volume increased with depth in all the peatlands covered in this study (II). The old peat sample used in the incubation study also had higher bulk density than the recent peat (IV). The extractable C expressed on a gram of OC bases (μmol g^{-1} OC) was also higher in the old peat than the recent peat (IV), this is because the deeper old peat is more decomposed and compressed than the surface younger peat. Compaction in the old non-vegetated peat leads to higher bulk densities (Repo *et al.* 2009, Marushchak *et al.* 2011, Biasi *et al.* 2014) and is the reason for the relatively high CO_2 emissions from the uplifted old bare peat (Biasi *et al.* 2014), despite the low decomposition rate as confirmed in IV. This compressed nature of the deep old peat constrains microbial cell dispersion, limits the pore spaces, water fluxes and gaseous exchanges (Ekschmitt *et al.* 2008, Schmidt *et al.* 2011, Biasi *et al.*

2014). Therefore, as confirmed in I, II & IV, this leads to high spatial separation and limited dispersion of microbial cells (Ekschmitt *et al.* 2008, Xu *et al.* 2010, Schmidt *et al.* 2011) and possibly limits the development of the microbial community in deep peat (Kuzyakov *et al.* 2000, Wild *et al.* 2014). The old deep peat layers are also continuously exposed to low pH and temperature. A combination of these factors limits microbial development and C losses in deep peat layers (Jaatinen *et al.* 2007, Peltoniemi *et al.* 2009, Allison *et al.* 2010, Preston *et al*. 2012, Preston and Basiliko 2016).

The incubation study (IV) (though for a permafrost-affected old peat), further showed that the lower sensitivity of the old peat OC to the C mineralization controlling factors was mainly due to both low microbial biomass (Table 5, Fig. 2 in IV) and biomass-specific CO_2 production. Under different temperature and oxygen regimes, a multiple regression analysis showed that microbial biomass variation was the most stable and strongest predictor of C losses from old peat as compared to the 'recent' vegetated peat (IV), meaning that low microbial biomass constrained C losses in the old peat even long time after aeration ("natural drainage"). This is likely because fresh C input from plants is lacking in these soils, as confirmed from the other studies here. Unlike the overall results of 16S rRNA and MBC_{CFE} analysis, I & II showed long-term drainage-induced microbial biomass PLFA increase in the fen and reduction in the bog site, when the same soil age classes are compared (Fig. 2A in I). Comparing this with the overall peat C losses in the fen and gain in the bog site, reported by Krüger *et al.* (2016) after long term drainage, emphasizes the importance of microbial biomass in peat C losses. Depth-dependent alteration of microbial biomass and composition has been reported (Artz 2009). As confirmed in I & II (as well as rRNA and MBC analysis), total microbial biomass is generally highest in the most active surface layers and decreases with depth. In accordance with the general metabolic theory (Brown *et al.* 2004, Allen and Gillooly 2009, Yvon-Durocher *et al.* 2012), the lower microbial biomass observed in the deeper old peat layers also means lower C losses (mineralization) and reduced sensitivity of other mineralization controlling factors, as recorded in the incubation study (IV). Lack of fresh OC input and root exudates from plants also constrains microbial community development and microbial mineralization of complex compounds in deep peat layers (Wild *et al.* 2014). Again, input of fresh C can override these primary constraints, as e.g. triggered by drainage and increase in deep-rooting plants.

4.3 Microbes as dynamic storage of C in peat

Most models and studies of C stabilization in the soil (including peat soil) have always focused on the chemical characterization of biomass and abiotic factors, paying little or no attention to the contribution of MBC (Balser 2005). This was because active microbial biomass is generally reported to constitute less than 5 % of soil organic matter (Wardle 1992, Dalal 1998). The total MBC is also often

less than 4 % of the soil organic C (Sparling 1992, Anderson and Joergensen 1997) or an average of about 1.2 % of the global soil C (Xu *et al.* 2013). This study (II and CFE analysis of MBC) did not only confirm the low percentage of living MBC contribution to the peat soil organic C, but also showed that on average, living MBC in the peat ecosystems constituted an even smaller fraction of C than the global soil average. The global soil average included mineral soils with higher microbial C percentages compared to peats. The average (± S.E) MBC_{PLFA} to total C fraction of about 0.43 ± 0.04 % for peat with the highest range of 0.81 ± 0.05 % to 1.95 ± 0.34 % in the fen site are within the range reported previously (range from 0.03 to 2.08 % in the surface layers) using different estimation methods in peatlands (Hall and Hopkins 2015, Preston and Basiliko 2016, Urbanova and Barta 2016). However, considering the substantial amount of OC stored in peatlands, as well as their very significant role in the global C cycle (Gorham 1991), looking at the fractional contribution of living MBC alone, may be misleading. This study (II) showed that, at the regional or global scale, the living MBC-stock in the peat ecosystems is substantial. Estimate by II showed that up to 1508 Tg C (MBC_{PLFA}) or 725 Tg C (MBC_{CFE}), out of the 455 000 Tg C in northern peatlands (Gorham 1991), exist as MBC. These figures do not include anaerobic microbes like the archaea that are not detectable by the PLFA method used. Although the average MBC values (g^{-1} dw) derived from total PLFA and used in this estimate were slightly higher than the MBC values (g^{-1} dw) measured via CFE, the efficiency of CFE method in very wet (averagely ≥ 90% H_2O content) and low pH (3.33–4.63) peat samples like the ones in this study is a serious concern (Vance *et al.* 1987b, Haney *et al.* 1999, 2001, Alessi *et al.* 2011). This is evident in the fact that the result did not show any difference between the natural and drained sides even in the highly studies surface layers. This is partly because chloroform (the microbial cell lysing reagent) is only partially or negligibly soluble in water. There is also high variability in the MBC_{CFE} amounts among replicates. According to Setia *et al.* (2012), the amount microbial biomass C extracted by direct extraction method (same method used in PLFA) is higher than the amount extracted by CFE, from the same samples. It therefore means that this result needs to be interpreted with certain caution.

In general, microbial biomass has a quicker turnover rate than plant residues and respond more rapidly to changes in soil condition than the rest of the soil OC (Bergstrom *et al.* 1998). This means that the contribution of microbial-derived C to the formation of soil OC (or C storage) is much higher than represented by the estimate in II from living MBC alone (Potthoff *et al.* 2008). The size of living microbial biomass or living MBC-stock alone does not indicate the actual long-term microbial contribution to soil (peat) C pool or storage. Upon the death and turnover of living soil microbial biomass, part of the biomass (including MBC) remains as necromass, which contributes significantly to the non-living part of soil organic matter (SOM) (Kindler *et al.* 2006, 2009, Miltner *et al.* 2009, 2012). Some microbial cellular components are preserved and added to the soil C pool due to their chemical structure and organo-mineral complexities (Sollins *et al.* 1996, Baldock and Skjemstad 2000, Lützow *et al.* 2006). The accumulation of the necromass and other recalcitrant

compounds of microbial origin, driven by the rapid turnover time of microorganisms, iteratively adds continuously to soil C-stores. A conceptual model has demonstrated that the residue of microbial cell envelope materials (containing part of the MBC) is significant to SOM formation (Miltner *et al.* 2012). Another microbial biomass turnover model (Absorbing Markov Chain approach) by Liang *et al.* (2011), also showed that bacterial necromass was for example about 40-fold the living microbial biomass. However, none of these studies or models specifically involved peatlands where OM mineralization and thus microbial growth and turnover is inhibited. This study (I, II) demonstrated the presence of viable microbes (i.e. microbial PLFA and rRNA) at a relatively large amount even in the deepest (over 3000 years old) peat layers. It is unlikely that these microbes are inactive and survived this long, considering the age of these deposits. Assuming they are active with high biomass turnover as in other soil types, the iterative turnover of the estimated living MBC-stock in II would clearly mean that the microbial biomass-derived C might play a more significant role in the organic matter stabilization and C storage in these ecosystems than were here acknowledged.

5 CONCLUSIONS

The fate of the large C stocks stored in deep peats is uncertain under the changing climatic conditions, since all the biotic and abiotic factors controlling the C storage are not yet understood. While part of this study demonstrated that viable microbes (i.e microbial PLFA) were present at a relatively large amount in the deepest (over 3 000 years old) peat layers, another part of the study showed that low microbial activity is the major factor responsible for the low C losses from old peat. These findings warrant further investigation. Reduced temperature sensitivity and insignificant effect of oxygen exposure on the C losses were detected in old peat deposits compared to the recent vegetated peat (from the arctic). This was explained by the adaptation of the old peat microbial community to anaerobic conditions, which also explains the decrease in the total microbial biomass (measured as PLFA and MBC) in the deeper depths of the old peat. The low microbial biomass and activities in the deep old peat mirrored the low OC quality, unfavourable nutrient stoichiometry and other factors, such as increased soil compaction in deeper layers. Primarily, it mirrored the low microbial biomass and likely constraints like lack of plants in the subarctic sites where old peat is uplifted to the surface. In much more abundant peatlands, like those from the boreal region, drainage may also stimulate plant growth and input of fresh C compounds. Then, projected climate-induced changes might release the constraints of microbial growth in aged peat layers by introducing fresh plant materials and root exudates to deeper peat layers, as succession would favor vascular plants and lower water table levels under warmer climatic conditions. This was shown by PLFA biomass increase in the surface and sub-surface layers of the fen where vegetation succession towards forest vegetation was seen and decrease in the surface layer of the bog site, where vegetation was not so much affected by the drainage. The study indicates that although old peat deposits deep in the soil horizon are less vulnerable to direct climatic changes when compared to the surface deposits, environmental changes can alter microbial community composition, increase microbial activities and trigger decomposition of the organic matter in the deeper layers by indirectly affecting substrate availability.

The study also showed that although the living MBC appears to be a small fraction of the TC, it might contribute more substantially (as both living MBC and necromass) to the C storage of peatlands at regional scale than originally thought. However, peatland drainage and the projected climate change-induced lowering of water table may have drastic changes in the microbial communities, which may reduce the MBC contribution to peatland total C and affect the bulk peat $\delta^{13}C$ values beyond the surface layers. The overall long-term effect of peatland drainage, and the projected global climate change induced lowering of the water level in peatlands, might not be the same for all sites. While long-term drainage significantly increased the total microbial biomass (PLFA) in the affected layers of the fen, it decreased the total microbial biomass (PLFA) in the affected layers of the bog. Lowering water table depth leads to increased thickness of aerated peat layers which affects the location where major biogeochemical processes and nutrient regeneration happens in the peat profiles.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Vanhojen turvekerrostumien mikrobeihin sitoutunut hiili muuttuvissa hydrologisissa olosuhteissa

Suot ovat tärkeitä hiilivarastoja globaalisti. Soiden turpeeseen on sitoutunut kolmannes maa-alueiden hiilestä. Suomessa turpeeseen sitoutunut hiili on tärkeä pitkän aikavälin hiilivarasto ja soihin ja sedimentteihin sitoutunut hiilimäärä on suurempi kuin kasvillisuuden ja kivennäismaan hiilivarastot yhteensä. Suot ovat dynaamisia ekosysteemejä, joiden hiilivarasto saattaa purkautua ilmastomuutoksen seurauksena. Muutokset soiden vesitaseessa muuttavat hapetus–pelkistysolosuhteita ja vaikuttavat mikrobiologiseen mineralisaatioon yhdessä ravinteiden ja lämpötilan kanssa. Turpeen hiilivarasto on siten herkkä maankäytön ja ilmaston muutoksille.

Yleensä oletetaan, että soiden syvempiin kerroksiin varastoitunut turve on vähemmän herkkä ympäristön muutoksille kuin pinta-turve, mutta myös ikääntynyt turve ja sen hiilikatoa säätelevät tekijät ovat tärkeitä soiden hiilivaraston säilymisen kannalta. Useimmat tutkimukset ilmastonmuutoksen vaikutuksista soiden mikrobiyhteisöihin ja hiilenkiertoon ovat kuitenkin keskittyneet soiden pintakerroksiin.

Tässä väitöskirjassa keskityin tutkimaan mineralisaatiota ja hiilen vähenemistä eri-ikäisissä turvekerroksissa vertaamalla syvempien kerrosten ikääntynyttä ja pintasuon nuorta kasvipeitteistä turvetta. Pitkäaikaisen vedenpinnan laskun (ojituksen) vaikutusta mikrobiyhteisöihin tutkittiin fosfolipidirasvahappo-analyysin (PLFA), kloroformi-fumigaation ja kvantitatiivisen polymeraasiketjureaktion (qPCR) avulla. Kuivumisen vaikutusta turveprofiilissa ja alapuolisessa mineraalimassa tutkittiin myös vakaiden hiili-isotooppien avulla ($^{12}C/^{13}C$-suhde). Lämpötilan ja kosteuden vaikutusta turpeen mineralisaatioon tutkittiin kokeellisesti nuoressa ja vanhassa kryoturbaation kohottamassa turpeessa.

Ojitetuilla ja luonnontilaisilla soilla tehty PLFA-analyysi osoitti, että eläviä mikrobeja oli suhteellisen suuria määriä jopa yli 3000 vuotta vanhoissa turvekerroksissa. Toisaalta kryoturbaation vuoksi pintaan kohonneen turpeen pieni mikrobiaktiivisuus oli tärkein syy siihen, että turve hajosi hitaasti muuttuneissa olosuhteissa. Nämä havainnot edellyttävät lisätutkimuksia. Kryoturbaatiossa pintaan nousseen turpeen pieni mikrobibiomassa ja matala mikrobiologinen aktiivisuus johtuvat turpeen orgaanisen aineen laadusta, epäsuotuisista ravinnesuhteista ja turpeen rakenteellisesta tiivistymisestä syvemmissä kerroksissa. Vaikka lämpötilan kokeellinen nosto lisäsi mikrobiologista aktiivisuutta ja hiilen katoa pinta- ja pohjaturpeessa, lämpötilan nousun vaikutus johtuu osittain mikrobiyhteisön nopeammasta sopeutumisesta vallitsevaan happitilanteeseen korkeammassa lämpötilassa.

Ojitettujen ja luonnontilaisten suoparien vertailu osoitti, että rasvahapoilla määritetty, lähinnä bakteereista ja sienistä koostuva mikrobibiomassa kasvoi ojituksen vuoksi rehevän suon pohjakerroksissa, mutta pieneni karun suon

pohjakerroksissa. Sen sijaan kaikkien mikrobiryhmien biomassaa kuvaavassa kloroformi-fumigaatiossa ei havaittu merkittävää kasvua ojituksen seurauksena. Tulosten ero johtunee mittaustavoista, sillä eri suotyypeistä ja kerroksista yhdistetyssä aineistossa ojitus vähensi arkeonien DNA:n määrää suhteessa kaikkien prokaryoottien sisältämään DNA:han (ojittamattomilla 44,9 % ja ojitetuilla 20,1 %), mikä johtuu muutoksista hapen saatavuudessa, pH:ssa ja hiilen laadussa.

Tulosten mukaan lisääntyvä bakteeribiomassa saattaa lisätä orgaanisen aineen hajoamista myös pohjaturpeissa muuttuneessa ilmastossa. Elävä mikrobibiomassa on pieni osa turpeen hiilen kokonaismäärästä (enimmillään 0,8–2,0 % ravinteisella suolla). Tutkimus osoitti kuitenkin, että mikrobibiomassalla ja siitä syntyvällä mikrobiperäisellä kuolleella biomassalla on sekä paikallista että maailmanlaajuista merkitystä. Havaittu keinotekoisen kuivatuksen vaikutus bakteerien ja arkeonien väliseen suhteeseen myös syvemmissä turvekerroksissa osoittaa, että ennustettu ilmastomuutoksen aiheuttama vedenpinnan tason lasku soilla voi vaikuttaa koko turveprofiilin mikrobiyhteisöihin ja siten soiden hiilivarastoihin.

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ORIGINAL PAPERS

I

THE IMPACT OF LONG-TERM WATER LEVEL DRAW-DOWN ON MICROBIAL BIOMASS: A COMPARATIVE STUDY FROM TWO PEATLAND SITES WITH DIFFERENT NUTRIENT STATUS

by

Promise A. Mpamah, Sami Taipale, Antti J. Rissanen, Christina Biasi &
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The impact of long-term water level draw-down on microbial biomass: A comparative study from two peatland sites with different nutrient status

Promise Anyalewachi Mpamah [a, *], Sami Taipale [a, b], Antti Juhani Rissanen [c], Christina Biasi [c], Hannu Kalevi Nykänen [a, b]

[a] University of Jyväskylä, Department of Biological and Environmental Science, Jyväskylä, Finland
[b] University of Eastern Finland, Department of Environmental Science, Finland
[c] Tampere University of Technology, Laboratory of Chemistry and Bioengineering, Tampere, Finland

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ABSTRACT

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

1. Introduction

Peatlands are crucial global carbon (C) stores [1,2], containing about 15–30% of all terrestrial organic C (OC); equivalent to 455 Gt (10^{15} g) C [1]. Microbes are key actors (as catalysts) in all peat biogeochemical processes, controlling the peat OC accumulation and decomposition [3]. They also contribute to the peat C exchange via respiration and, upon cell death, necromass addition to the peat soil organic matter (SOM), via the microbial carbon pump (MCP [4]). Different microbial groups, with complementary enzymatic activities and different responses to environmental variables, interact in the peat *C*-cycling processes [5]. For example, the Gram−negative and Gram−positive bacteria are mainly associated with the mineralization steps involving labile and more recalcitrant C materials, respectively [6], and the exo-enzymatic capabilities of fungi make them important in the decomposition of macromolecules and recalcitrant C materials [7,8]. Changes in climate factors, such as hydrology, affect microbial community biomass and activity, both spatially and temporarily [9,10].

Models predict a warmer global climate (average temperature increases of about 4 °C) up to the year 2100 [11] and, under these scenarios, increased evapotranspiration due to increased temperatures would lead to a lower water table (WT) in peatlands [12]. Persistent draw-down of the peatland WT affects the niches of peatland microbes by increasing the thickness of the aerated surface layer [10]. The impacts of changed hydrology on the microbial community depends on the peatland type, intensity of change and

* Corresponding author.
E-mail addresses: promise.a.mpamah@jyu.fi (P.A. Mpamah), sami.taipale@jyu.fi (S. Taipale), antti.rissanen@tut.fi (A.J. Rissanen), christina.biasi@uef.fi (C. Biasi), hannu.nykanen@uef.fi (H.K. Nykänen).

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the extent of change in space and time [9,13–15]. While changes in microbial niches may lead to increased diversity in the short-term, repeated replacement of specialist by generalist microbes may lead to loss of diversity in the long-run [14]. Changes in plant species cover following water level draw-down also modifies the influence of temperature and water content on peat microbial activities [16]. Studies have suggested that drainage could increase or decrease total microbial biomass or the biomass of some microbial groups, depending on the peatland type and depth [9,13]. Jaatinen et al. [13] showed that fungi and actinobacteria suffer from drainage in a nutrient-rich fen, but that in a drained bog, while fungi either suffer or benefit, actinobacteria abundance remains the same or increases. Fungi and bacteria generally benefit (undergo biomass increase) from persistent drainage of wet mesotrophic fen sites, though actinobacteria suffer or show only minor responses [9,15]. Changes in peat C accumulation and decomposition activities following drainage have also been related to changes in the structure of below-ground microbial communities [5,16]. Altered microbial diversity, due to drainage-induced changes in the quality and quantity of OC inputs, coupled with better oxygen availability, could increase the rate of soil OC (SOC) cycling; this leads to changes in the balance of peat-atmosphere C exchange [5,10,17].

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

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

This study examined the effect of long-term drainage on microbial communities in depth profiles from surface to bottom layers of two peatland sites differing in nutrient status. We compared the biomasses and structures of the microbial communities (indexed by total and relative abundance of PLFA, respectively) between the natural and drained sides of fen and bog, representing boreal peatlands of different fertility after 51 years of water level draw-down. We also specifically investigated the effects of drainage on some selected microbial groups. We hypothesised that long-term WT decrease will (1) increase microbial biomass and (2) influence the microbial community structure in the deep anoxic layers. We also hypothesized that (3) there will be higher microbial biomass increase in the fen than in the bog site, due to long-term drainage.

2. Material and methods

2.1. Study sites

The study was conducted at two peatland sites (one fen and one bog) within the Lakkasuo boreal mire complex (61°47′N, 24°18′E, ca.150 m a.s.l.), in the Orivesi area in central Finland. At the nearest weather station to the sites in Juupajoki Hyytiälä (61°85′N, 24°29′E) the mean annual temperature was 3.5 °C and precipitation 711 mm for the period of 1981–2010 [29]. The sampling year was wetter than this long-term mean, with whole year precipitation of 907 mm and an average temperature of 3.2 °C. The mire complex comprises a large variety of typical Finnish mire site types [30]. Part of the Lakkasuo peatland was ditch-drained in 1961 (51 years before sampling) so that there are adjacent natural and drained sides of different fertility along a border ditch (Fig. S1). There were differences in the original fertility, WT and vegetation composition between the natural ombrotrophic cotton grass pine bog with *Sphagnum fuscum* hummocks (bog) and the natural minerotrophic tall sedge fen (fen) sites sampled. Drainage caused marked changes to the hydrology, peat and vegetation properties, carbon dioxide (CO_2) and CH_4 fluxes, especially at the drained fen ([28,30–33]; summarized in Table 1). For example, the six-month average WTs before the sampling date were −8.0 and −34.9 cm for the natural and drained fens, respectively, whereas it was −12.0 and −16.4 cm for the natural and drained bogs, respectively (Fig. 1). CO_2 fluxes increased in both sites whereas CH_4 fluxes ceased in the fen and were reduced by half in the bog after 30–32 years of drainage. Thus, there is strong evidence for significant, long-term changes in peat characteristics and greenhouse gas fluxes. The pH increased from the surface downwards in the natural and drained sides of both sites (Fig. S2). In general, the bog site is more acidic than the fen site and this was confirmed by previously reported pH values (Figs. S2 and S3). Although temperatures vary seasonally, the temperature in deep peat is rather constant (~6–8 °C). The bulk densities (BD) at different depths of the drained and natural sides are the same in the bog site, but different in the fen site (Fig. S2).

2.2. Soil sampling and water table level measurement

2.2.1. Initial soil sampling and water table measurement

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

2.2.2. Additional peat properties measurements

Volumetric samples (from the same depths as the initial samples) were used for pH and temperature measurements, as well as for bulk density determination (Fig. S2). Sampling was done with a similar, but smaller, Russian pattern side-cutting sampler to that described above (5.2 * 50 cm; half cylinder diameter * length) on 14 October, 2015. Sampling and depth measurements were started under a living *Sphagnum* carpet. Samples were transferred from the sampler into plastic bags (Aromata, Lidl Stiftung & Co, Neckarsulm, Germany) and were mixed in the bag before insertion of a pH electrode coupled with a temperature sensor (WTW P3 pH/conductivity with electrode SenTix 41; Weilheim, Germany). Values of

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) [31], [2]Nykänen et al. (1998) [32], [3]Silvola et al. (1996) [33] or [4]Laine et al. (2004) [30]. Peat constituents: 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*. Dominant tree species marked with bold.

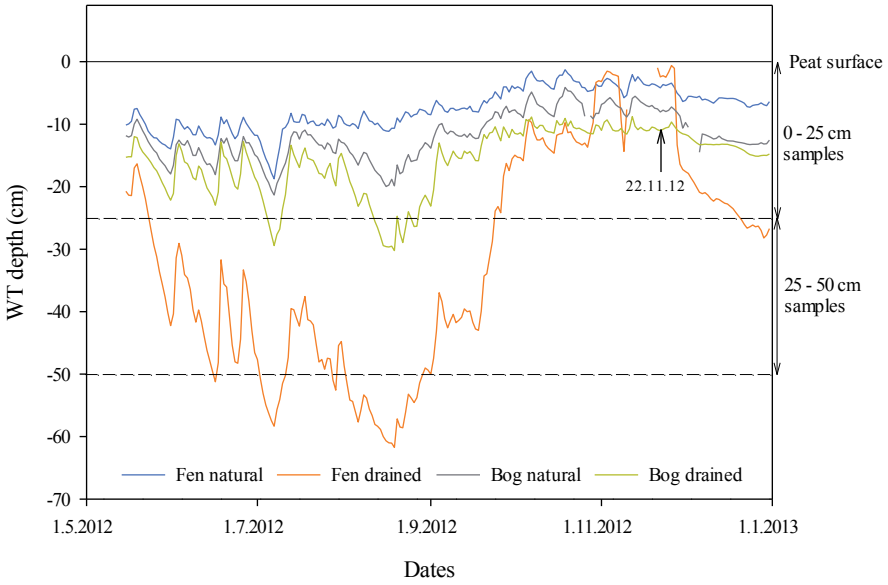

Fig. 1. Average daily water table (WT) depth in the sampled peatland sites from May to December of the sampling year.

pH and temperature were recorded after 1 min. To determine bulk density, the samples in the bags were dried in the oven (Memmert, UM 500, Schwabach, Germany) at 80 °C until there was no change in the dry weight.

2.3. PLFA analysis

2.3.1. PLFA extraction and quantification

PLFA analysis was done following the protocol used by Tavi et al. [36] with some modifications. Freeze-dried and mixed peat samples from each depth profile were weighed into 50 ml extraction tubes (>3 g dry weight of peat) using tools cleaned with methanol. Total lipids were extracted from the samples using a 1:2:0.8 (vol:vol:vol) ratio of chloroform–methanol–50 mM phosphate buffer [37]. Tubes were closed under a nitrogen flow, mixed and shaken at 200 rpm overnight. Dipentadecanoylphostatidylcholine ($C_{38}H_{76}NO_8P$) (Larodan Fine Chemicals) was added as an internal standard for quantification of PLFAs. After shaking for another 5 min, the samples were centrifuged at 2500 rpm for 15 min. 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, 5 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 2 h. 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 5 min. The top organic layer was then transferred, dried (under a nitrogen stream) and re-dissolved in a known volume of n-hexane.

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

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

PLFA biomarkers common among general microbial groups, such as Gram-negative bacteria, Gram-positive bacteria and fungi, were selected and grouped. Iso- and anteiso branched PLFAs, 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 as 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 side) on the total microbial PLFA biomass, and the absolute amounts and relative abundances 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 $\log_{10}(x+1)$ 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 sides, drainage and depth zones. Furthermore, the effect of depth and drainage on PLFA profiles in both fen and bog were 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. The Mantel correlation test result is only meaningful when the result is positive, which shows correlation. The positive sign in our correlation results means that, the larger the change in the pH or BD, the larger the change in microbial community structure (PLFA structure). ANOVA was done using IBM SPSS Statistics version 23. NMS and Mantel's tests were performed using PC-ORD version 6.0 ([45]; PC-ORD. Multivariate analysis of ecological data. MjM Software, Gleneden Beach, Oregon, USA). PerMANOVA was done using FORTRAN program by Anderson [46].

3. Results

3.1. Total microbial PLFA biomass and structure

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

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

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

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

The overall concentration of all the major microbial group PLFAs followed the same trend as the total microbial PLFA, being higher in the fen than the bog site, especially in the drained side (Fig. 2B). They were also mostly highest in the surface layer than the deeper layers of both sites. The amount (g m^{-3}) of 16 C monounsaturated fatty acids (16 MUFAs), which are characteristic of Gram-negative

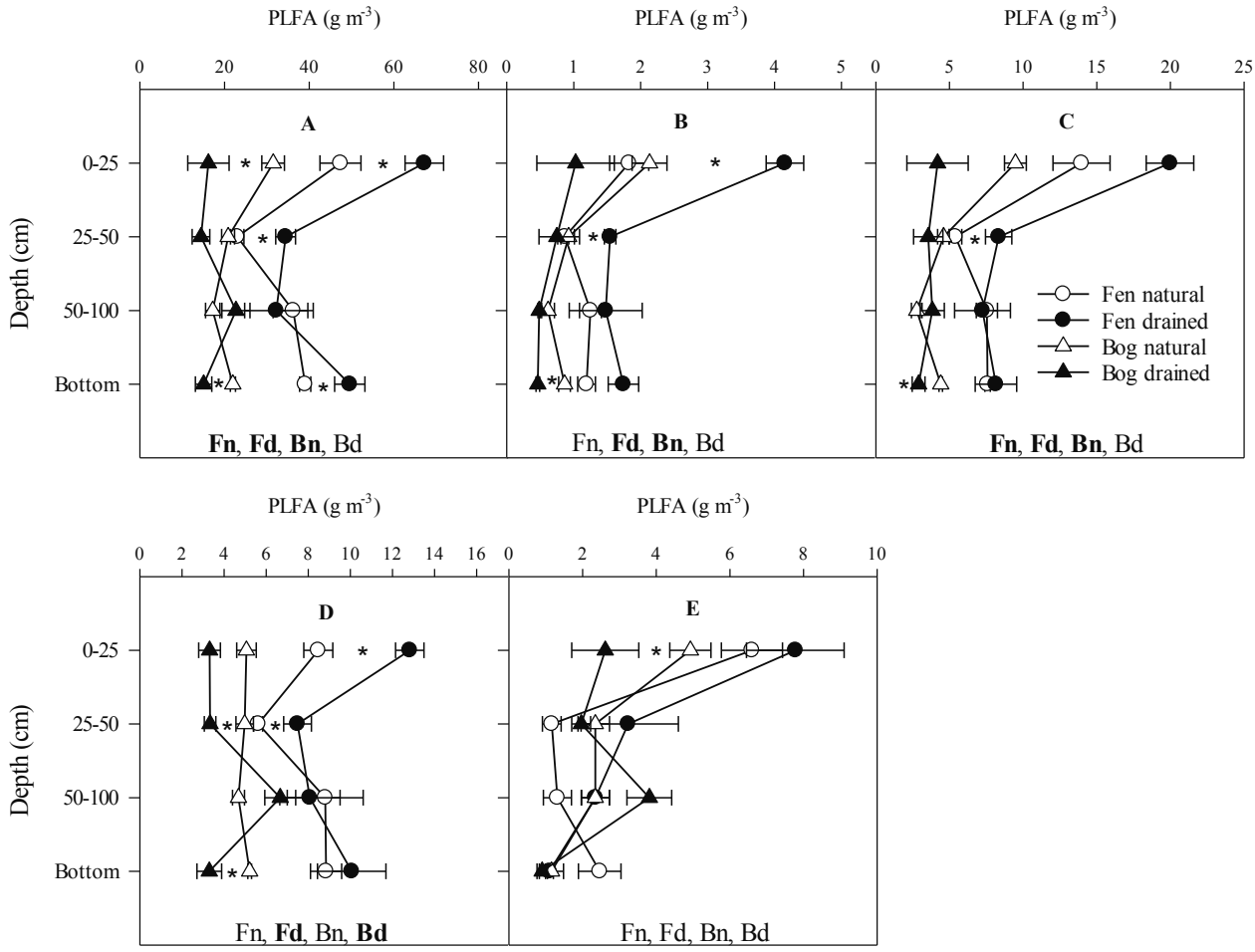

Fig. 2. Mean (±SE, n = 3) amount (g m^{-3}) of (A) microbial total PLFA, (B) 16 MUFAs, (C) 18 MUFAs, (D) terminally branched FAs and (E) FA characteristic of fungi in all the depths and sites sampled. Significant differences ($p < 0.05$) between the drained and natural sides at each site in each depth 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 (1-ANOVA, $p < 0.05$) 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.

bacteria, was higher in the top two layers (0–25 and 25–50 cm) of the drained side of the fen than the natural side, but lower in the bottom layer (only) of the drained side of the bog than the natural side (Fig. 2B). The relative contribution of 16 MUFAs to the total microbial PLFA (% contribution) did not differ between the drained and the natural sides in either fen or bog except at the 50–100 cm depth of the bog site (Fig. 4A). Depth only affected the amount of 16 MUFAs (g m^{-3}) in the drained fen and natural bog sides. In both cases, the amount (g m^{-3}) of 16 MUFAs was higher in the surface (0–25 cm) layer than the other depth layers, which were similar to one another (Fig. 2B, Table S2). There was also no depth effect on the relative contribution of 16 MUFAs to the total PLFAs except in the natural bog, where it was higher in the surface layer (0–25 cm) than at the other depths, which were similar to one another (Fig. 4A, Table S3).

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

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

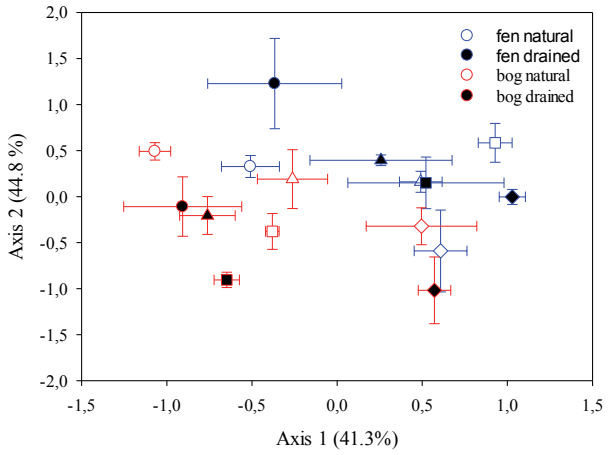

Fig. 3. Non-metric multidimensional scaling (NMS) ordination of PLFA [$\log_{10}(x+1)$ of the relative abundance of individual PLFAs]. Average (±SE, n = 3) NMS axes scores of the sites. Axes are arbitrary; the closer the sample points are on the plot, the more similar they are in PLFA composition. Depth 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 ($p \leq 0.05$) in a two-way factorial (drainage and depth) analysis (PERMANOVA, see Table 2).

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

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

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

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

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

of the sides, except the natural bog side (Fig. 4C, Table S3).

The amount (g m^{-3}) of fatty acids characteristic of fungi (fungi FA) did not differ between the drained and natural fen sides, but was lower in the surface layer (0–25 cm) of the drained bog side than the natural bog side (Fig. 2E). Neither drainage nor depth affected the relative contribution of fungal FA to the total microbial PLFA (% contribution) in either fen or bog sites (Fig. 4D, Table S3). There were also no depth differences in the amount (g m^{-3}) of fungal FAs in any of the sides (Fig. 2E).

4. Discussion

4.1. Biomass and community structure of microbes

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

There was viable microbial biomass in all the peat layers of both the fen and the bog sites (Fig. 2). The drastic reduction in total microbial PLFA biomass from the surface layer to the 25–50 cm layer in the fen but not the bog is likely due to depth related differences in the fertility and litter quality [47] (Table S4). In general, the microbial biomass PLFA (g m^{-3}) in the fen increased with increasing nitrogen (N %) and carbon (C %) content and decreasing C/N ratios, but this was not the case in the bog site. There was no decrease in microbial biomass with depth (depth effect) in the bog site, probably due to poor substrate quality (higher C/N ratio). Quite surprisingly, the total microbial PLFA biomass did not differ between surface layers and bottom layers except in the natural bog. Although PLFA-analysis detects viable cells and indicates changes in the potentially active microbial biomass, 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 detection of FAs 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 activity [52] and active microbial populations [53] have previously been reported for deep peat layers (100 cm–300 cm), which supports 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 surface, but low and stable temperature at the bottom (Fig. S2 and [31]), which could also modify the 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 [55], which may increase with changes in deep peat microbial biomass and composition.

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

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, which is contrary to our first hypothesis. This is possibly

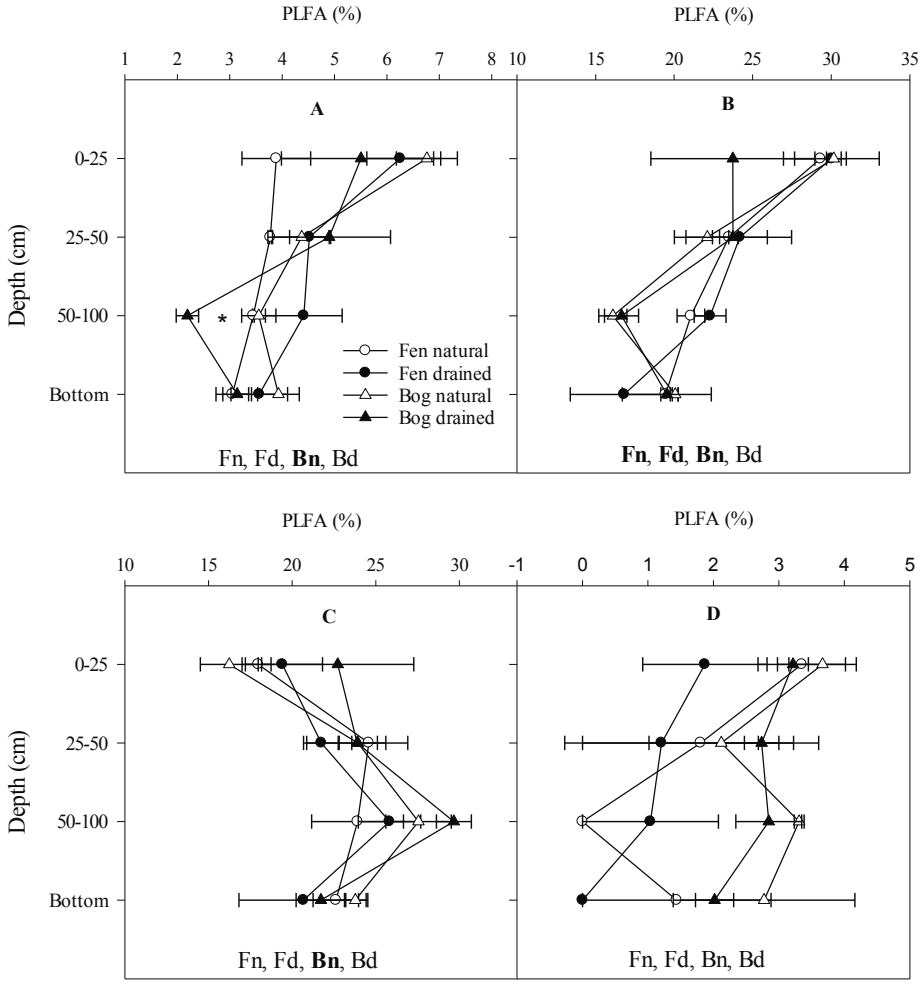

Fig. 4. Mean (±SE, n = 3) relative abundance (% contribution to total microbial PLFAs) of (A) 16 MUFAs, (B) 18 MUFAs, (C) terminally branched FAs and (D) FA characteristic of fungi at different depths in the studied fen and bog sites. Significant differences ($p < 0.05$) between drained and natural sides in each depth and for each microbial group following independent sample t-tests are denoted by an asterisk (*). For depth effects Fn = fen natural, Fd = fen drained, Bn = bog natural and Bd = bog drained, while sites with significant depth effects (1-ANOVA, $p < 0.05$) 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.

due to differences in their nutrient status, thickness of the aerated layer, available substrate and vegetation changes [13,22,47,56]. In general, while drainage in the fen led to a succession towards a different ecosystem from the original, in the bog site it led to smaller changes. Typical fen species, such as tall sedges, were succeeded by spruce swamp and forest species, such as *Pinus sylvestris*, *Betula pubescens* and *Polytrichum commune*. At the bog site, mosses and dwarf shrubs decreased while the forest species, mainly *Pinus sylvestris,* increased and *Pleurozium schreberi* appeared (see also Table 1). Thirty-eight years after drainage, tree stand volume increased from nothing in the natural fen to 111 m^{-3} ha^{-1} in the drained fen side and from 5 in the natural bog to 16 m^{-3} ha^{-1} in the drained bog side [31]. The increased tree growth in the drained fen led to greater evapotranspiration and further decrease in the WT. Although the WT depth at the time of sampling was about the same in both sites due to heavy rainfall in the previous weeks, the six-month mean WT depths before our sampling date were −8.0 and −34.9 cm at the natural and drained fen, respectively and, −12.0 and −16.4 cm for the natural and drained bog, respectively. The previously reported annual mean WT depths were also much lower in the drained fen than in the drained bog for most parts of the year (Fig. 1 and Fig. S4). The much lower WT in the drained fen therefore explains why there were drainage-induced changes in the total microbial PLFA biomass in the surface and sub-surface layers of the fen, but only in the surface layer of the bog site. Other reasons could be the originally lower substrate quality and availability in the bog, which has become poorer after a few decades of drainage (Table S4) [5,13,48]. Our C/N ratio results showed similar ratios along the profiles of both the natural and drained fen, suggesting that the increase in microbial PLFA biomass was not due to differences in peat quality. However, the C/N ratios in the bog profiles were significantly higher in the drained side, especially in the bottom layer. High C/N ratio usually indicates low substrate quality, which could also explain the low microbial biomass in the drained bog. We cannot conclusively explain the differences in C/N ratios between the natural and drained bog sides, which are possibly caused by loss of N after drainage. Since drainage increases N losses by leaching and plant uptake, it means that we are discussing both the direct and indirect effects of drainage. We also note that there could have been natural

differences in the peat qualities of the bog site before drainage. However, we believe that our basic assumption that the original peat quality was similar holds true, and that the differences we found can be associated to drainage. Hence, in accordance with our third hypothesis, drainage increased the total microbial biomass more in the fen than in the bog site. The overall effect of drainage on microbial biomass was reflected by the increased CO_2 and decreased CH_4 efflux, especially in the drained fen (Table 1).

The total microbial PLFA biomass in the deepest (bottom) layers of both the fen and bog sites were different between the natural and drained sides. This was due to changes in the flow and constituents 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 sides [26,27]. Although the roots of vascular plants may not reach the bottom layers, their exudates will get to microbes in the bottom layers via water movement at a faster rate than those deposited by the shallow roots of mosses. The results of DOC isotopic analysis and tritium analysis of 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 CO_2 and CH_4 (based on ^{14}C dating) from the deeper peat layers was younger than that from the surrounding peat. They attributed this to the transfer of DOC and gaseous C compounds to deeper peat via water movement, and the microbial usage of younger C. Furthermore, they concluded that low hydraulic conductivity in peat may not be a real limitation to water movement over a long time scale. Although mainly studied 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 than the natural side. They also reported much older peat in the drained sides of both fen and bog. This also suggests an effect of drainage on deeper peat microbial activity, as microbes use younger C that is more enriched with ^{14}C and DOC is a potential source of relatively younger C (leaving ^{14}C depleted older peat behind) [24]. By affecting the deep peat microbial processes [28] and biomass, long-term drainage may have additional significant effects on the peat C balance, besides the enhanced C losses from surface peat. Increased microbial biomass in the bottom peat layers due to drainage can either contribute to the peat organic matter (OM) stabilization via the microbial C pump (MCP) process ([4]; not studied in peatland yet), or enhance deep peat microbial degradation [28] and C flow to sub-soil [55]. Further studies are needed to elaborate on this.

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

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

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

Similar to in previous studies [13–15], drainage and depth-induced effects on the biomass of the FAs characteristic of our microbial groups, varied among the microbial groups and between the fen and bog sites. The higher concentrations of all the FAs characteristic of the major microbial groups, except the 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 drained fen side, probably due to drainage-induced increases in aeration and substrate availability. However, they were decreased in the bottom layer of the bog site, possibly due to low substrate quality, which was revealed by the C/N ratio in the drained side. The fungal characteristic FA was only reduced in the surface layer of the bog, likely due to low peat biomass quality and differences in the sensitivities of different fungal species to drainage [15]. For example, Peltoniemi et al. [15] showed that basidiomycetes are more sensitive to drainage than ascomycetes, though they are within the same fungal phyla represented by the same characteristic FA.

Drainage-induced effects on the relative abundance of our selected microbial group characteristic FAs were not observed, except for 16 MUFAs in the 50–100 cm depth of the bog site. This was probably due to the contrasting drainage responses of the different species in these groups [9,13,15]. Kim et al. [14] also reported no differences in the diversity and composition of denitrifiers and methanogens in any of their sites following a short-term drought. 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 patterns. Our 25 cm sample depth-range may also be too long to reveal the inconsistent changes observed in the shorter sampling depth-ranges for specific microbial groups (e.g. Refs. [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. The effect of drainage on the relative abundances of the mainly aerobic bacteria FAs (16 MUFAs) in the 50–100 cm layer of bog may be due to change from a permanently anoxic to at least episodic oxic conditions in the subsurface layers (see Fig. 1 [13]). Using techniques with higher taxonomic resolution than PLFA - analysis, e.g. next-generation sequencing of 16S rRNA gene amplicons, could give better insight to the drainage- and depth-induced effects

observed with the total microbial community. Nevertheless, it is clear from our results that the depth-induced effects on the microbial community structure vary between the two sites and between the treatments. This suggests that the factors regulating depth-induced effects on specific microbial species (within the groups) differ between sites.

5. Conclusions

Our study shows that the biomass and the structure of *in-situ* microbial communities differ between the studied fen and bog sites. While long-term water level draw-down significantly increased the total microbial biomass in the affected layers of the fen, it decreased the total microbial biomass in the affected layers of the bog, which is likely due to the differences in their original nutrient statuses, the level of WT depth reduction and substrate quality. There was a considerable amount of living microbial biomass even in the deepest and oldest (3000–3400 years) layers of the studied peatlands. Although drainage mostly affects microbial community in the surface layers, the effect of long-term WT draw-down on deeper peat microbes is also important, due to the long-term storage of large amounts of C in deeper peat layers. Drainage-induced increase in microbial biomass, measured in this study for the fen site, can enhance microbial contribution to deeper peat OM stabilization via the microbial C pump (MCP) or increase microbial peat degradation and C release in the bottom peat layers. This suggests a more significant effect of drainage on climate change than revealed by surface layer analyses alone. Further studies are needed to elaborate on this. Since peatland drainage has been used to model climate change effects on peatlands [11], our results might be revealing climate change effects on deep peat microbial biomasses.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2017.04.005.

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II

ESTIMATE OF MICROBIAL BIOMASS CARBON STOCK IN NORTHERN PEATLAND

by

Promise A. Mpamah, Antti J. Rissanen, Sami Taipale, Christina Biasi &
Hannu Nykänen 2018

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III

STABLE CARBON ISOTOPIC COMPOSITION OF PEAT COLUMNS, SUBSOIL AND VEGETATION ON NATURAL AND FORESTRY-DRAINED BOREAL PEATLANDS

by

Hannu Nykänen, Promise A. Mpamah & Antti J. Rissanen 2017

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

Hannu Nykänen[a], Promise A. Mpamah[b] and Antti J. Rissanen[c]

[a]Department of Environmental Science, University of Eastern Finland, Kuopio, Finland; [b]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland; [c]Laboratory of Chemistry and Bioengineering, Tampere University of Technology, Tampere, Finland

ABSTRACT

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 carbon (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 site of the fen.

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1. 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 the 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 the 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

CONTACT Hannu Nykänen hannu.nykanen@uef.fi
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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 to −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 myrtillus, Vaccinium vitis-idaea*) and declines of *Sphagnum* 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. [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 sites of the bog and fen are not showing signs of degradation, thus comparison of natural sites to drained sites was possible. The first objective of this study was to study if growing peat $\delta^{13}C$ is influenced by the 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 $\delta^{13}C$.

2. Materials and methods

2.1. Sites and their land use

The study sites were in the coniferous forest area in the southern boreal zone of Finland (Figure 1, Table 1). Lakkasuo, an eccentric peatland complex (61°47′N, 24°18′E) in Orivesi,

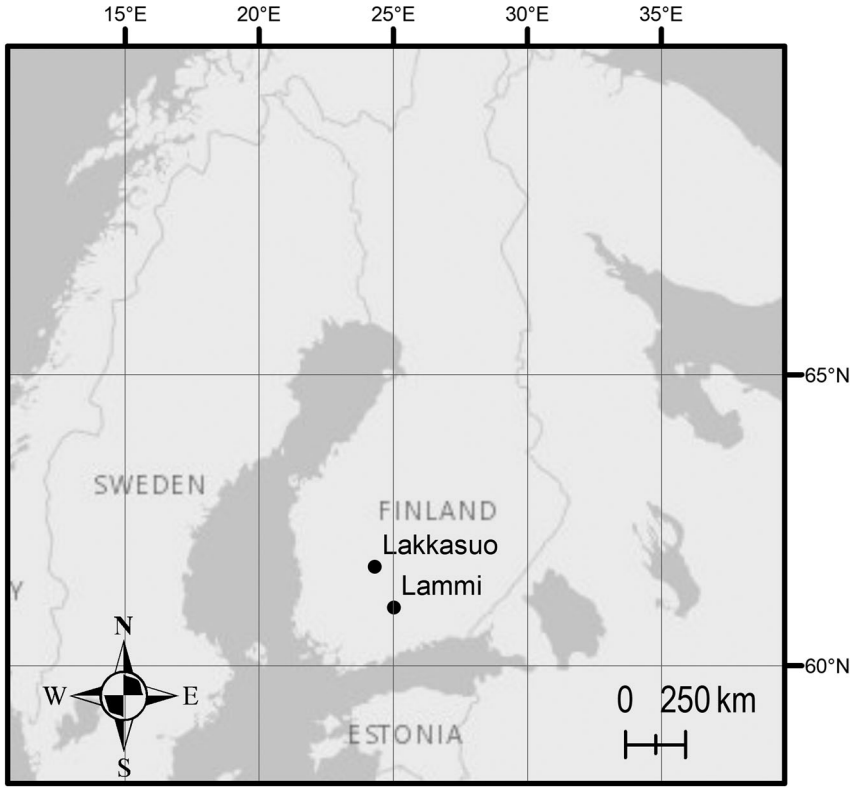

Figure 1. Location of the study areas, Lakkasuo and Lammi.

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

Location	Mean temp. (°C)[a]	Precipitation (mm a^{-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)[b]
Lakkasuo[c]	3.4	711	LakFN	0	−8	9.7		**0–25**, 25–50, 50–100, 135–160
			LakFD, 1961	111	−32	9.1	−23	**0–25**, 25–50, 50–100, 115–130
			LakBN	5	−10	11.2		**0–25**, 25–50, 50–100, 217–242
			LakBD, 1961	16	−21	9.5	−10	**0–25**, 25–50, 50–100, 255–280
Lammi area[d]	4.2	645	VilFN	0	0	n.d.	n.d	**0–50**, 50–100, 100–150, 150–165
			LaaBD, 1978	dense	−20 to −30	n.d.	n.d.	**0–30**, 30–80, 80–130, 130–180, 180–230, 230–280
			LovFD,∼1920	dense	< −30	n.d.	n.d.	**0–30**, 30–80, 80–130, 130–180, 180–230, 230–280

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

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 site 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 (Suppl. Table 1, [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 sites 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 (61°4′N, 25°0′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 1920s, 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 values were 711 and 645 mm a^{-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 lower in the LakBN and the drained sites, LakFD, LakBD, LaaBD and LovFD (Table 1). Previous studies from Lakkasuo with corresponding natural and drained sites 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 site compared to the natural site in 1991 and 1992 (Table 1). At LakB, subsidence was 10 cm, while the water table was on average 11 cm deeper on the drained site (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 nitrogen (N) content (in %), VilFN was the most fertile (3.4 ± 0.2 %) of the studied sites (Figures 2(c,h) and 3(c,h,m)). At LakFD N% was greater than it was in its natural counterpart (Figure 2(c)). The surfaces of LaaBD and LovFD were also nitrogen-poor (Figure 3(h,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% (Figure 3(m)) 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 the comparison of carbon amounts in the natural and drained sites has earlier been made for LakFN and LakFD pairs, and LakBN and LakBD pairs at Lakkasuo [17,19].

2.2. 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

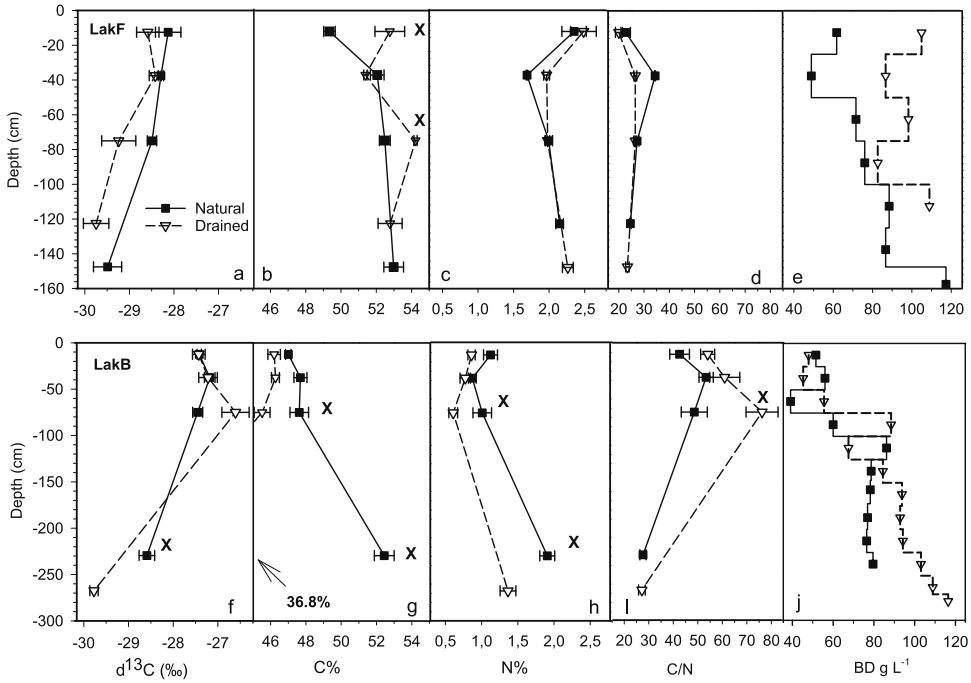

Figure 2. Lakkasuo depth profiles of $\delta^{13}C$ (a,f), C% (b,g), N% (c,h), C/N-ratio (d,i), bulk density (BD) (e,j). (Average ± S.E., $n = 3$, except in BD, where $n = 1$). Statistically significant differences in $\delta^{13}C$, C%, N% and C/N ratio (*t*-test, $p < .05$) are marked with an X.

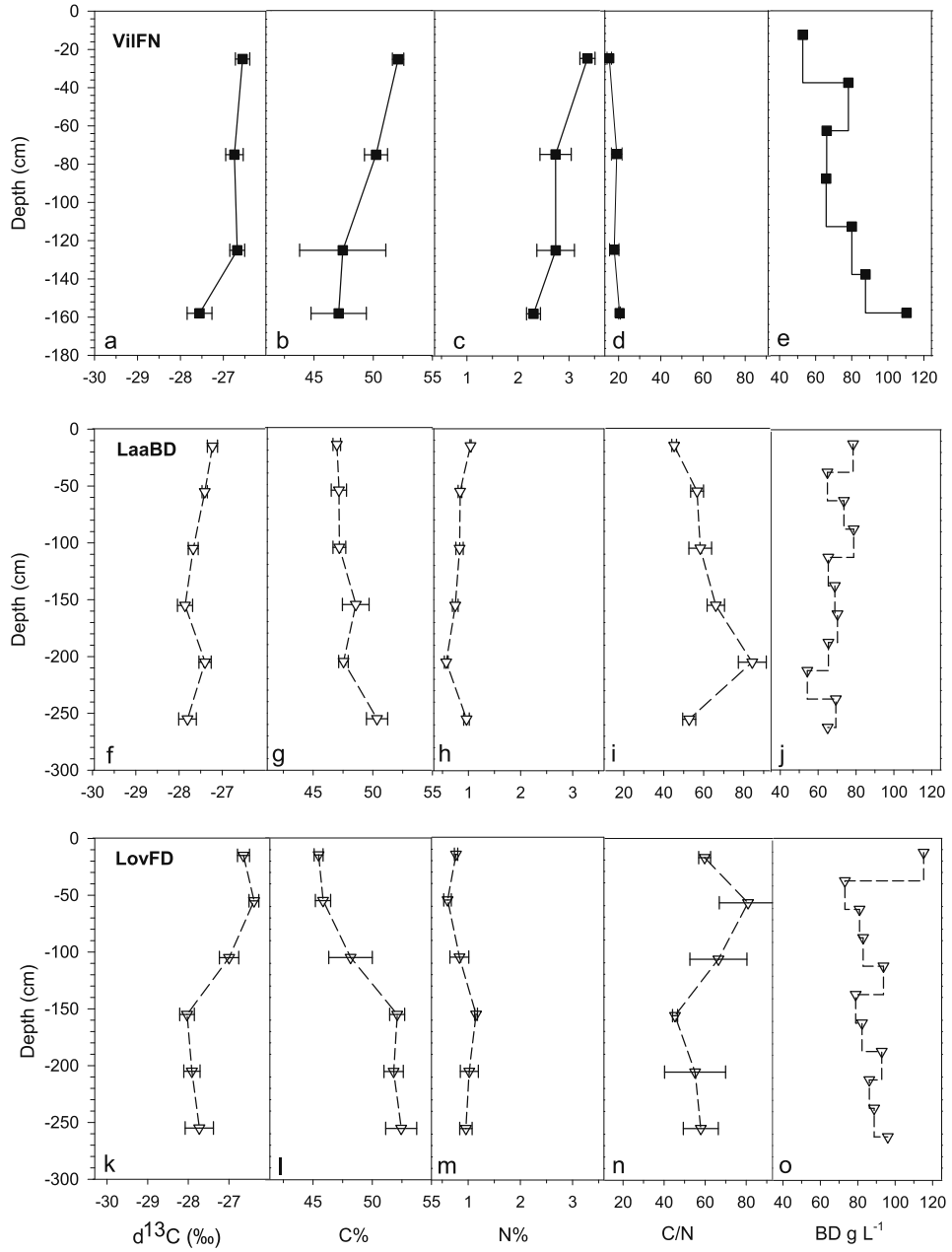

Figure 3. Profiles from the Lammi area: $\delta^{13}C$ (a,f,k), C% (b,g,l), N% (c,h,m), C/N-ratio (d,i,n) and BD (e,j,o). (Average ± S.E., $n = 3$, except in BD, where $n = 1$).

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, diameter 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 homogenized samples were then weighed into tin capsules.

At Lakkasuo, peat profiles were sampled in November 2012. The sampling at Lakkasuo was done a few metres 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 to 25 cm, the depth is in bold superscript (LakFN$^{\mathbf{0-25}}$). 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 $LakFN_{\underline{135-160}}$.

2.3. 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 site bottom profiles, this interface was between subsamples 20–25 and 25–30 cm, whereas in the remaining two profiles it was between subsamples 25–30 and 30–35 cm, calculated from the top of the 50 cm long profile.

2.4. Sampling and analysis of plants

Plants were collected from LakFN, LakFD, LakBN and LakBD, in November 2014 and analysed for δ^{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 analysed 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 Suppl. Table 2.

2.5. Stable carbon isotope, C% and N% analyses

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

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

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 δ^{13}C isotopic standard on the LSVEC – NBS-19 scale [35]. In each run, the same amount of the house standard was analysed for δ^{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 δ^{13}C, <0.5 for C% and <0.2 for N%.

2.6. 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 δ^{13}C, C%, N%, C/N ratio, bulk density and water content between bottom peat and subsoil and between natural and drained sites 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 δ^{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 δ^{13}C, C%, N% and C/N ratio of Lakkasuo natural and drained site 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 deepen 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 δ^{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 δ^{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 δ^{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.

3. Results

3.1. Effect of drying method on δ^{13}C of bulk peat

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

3.2. General trends in δ^{13}C, C% and N% of bulk peat at the study sites

In general, peat profiles in Lakkasuo (Figure 2(a,f)) had a larger range of δ^{13}C values (−29.8 to −26.6 ‰) than those in the Lammi area (−28.0 to −26.4 ‰) (Figure 3(a,f,k)). The lowest δ^{13}C value was in $\text{LakBD}_{255–280}$ (Figure 2(f)), while the highest δ^{13}C was found from $\text{LovFD}_{30–80}$ (Figure 3(k)). In general, the fen peat δ^{13}C values were lower than those of the bog peat at the Lakkasuo mire (Figure 2(a,f)). The amounts of N% and C% were larger in fens than in bogs, leading to a higher C/N ratio in the bogs (Figures 2(b–d, g–i); 3(b–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 (Figure 3(m)). The upper peat profile bulk density was clearly higher on LakFD compared to LakFN (Figure 2(e)), while on LakB differences were smaller and the effect of drainage was not clear (Figure 2(j)). In the Lammi area peatlands, VilFN had increasing BD towards the bottom (Figure 3(e)), while at LaaBD variation in BD was minor (Figure 3(j)). On LovFD, BD is clearly higher at the surface compared to rest of the peat profile (Figure 3(o)).

The overall average δ^{13}C value for all the studied peatlands had significant differences between depth layers (Table 2). Post hoc comparisons showed that δ^{13}C in basal peat was significantly lower (1.2 ‰) than in the surface peat and lower (1.1 ‰, $p = .004$) than in the mid-layer peat (Table 2). The content of C, N%, and

Table 2. Averages of δ^{13}C, C%, N% and C/N-ratio of natural and drained peat layers of all sites together.

Depth	*n*	δ^{13}C (‰)	C%	N%	C/N-ratio
Surface	7	−27.4 ± 0.3[a]	48.6 ± 1.1	1.7 ± 0.4	37.1 ± 6.6
Middle	18	−27.6 ± 0.2[a]	49.1 ± 0.7	1.3 ± 0.2	49.9 ± 5.0
Basal	7	−28.7 ± 0.4[b]	49.3 ± 2.2	1.7 ± 0.2	33.4 ± 5.8
F(2,29)		***5.58***	*0.083*	*1.13*	*2.27*
p		***.009***	*.921*	*.336*	*.122*

Note: Statistical differences between depth layers are expressed by different letters.
1-ANOVA, pairwise tests using LSD with Hochberg–Bonferroni corrected α-values.

the C/N ratio did not differ between peat layers (Table 2). There was also a positive correlation between surface and mid-layer peat δ^{13}C values (linear regression: $r^2 = 0.77$, $p = .009$, $n = 7$), but not between basal peat and mid-layer peat values. There was a clear positive correlation in the average δ^{13}C values between surface and basal peat of different sites (linear regression: $r^2 = 0.71$, $p = .009$, $n = 7$). 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 (Figure 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 (Figure 4). Furthermore, a bog site from northern Minnesota, USA, was depleted in ^{13}C in bottom layers and enriched in ^{13}C in surface layers [36] (Figure 4).

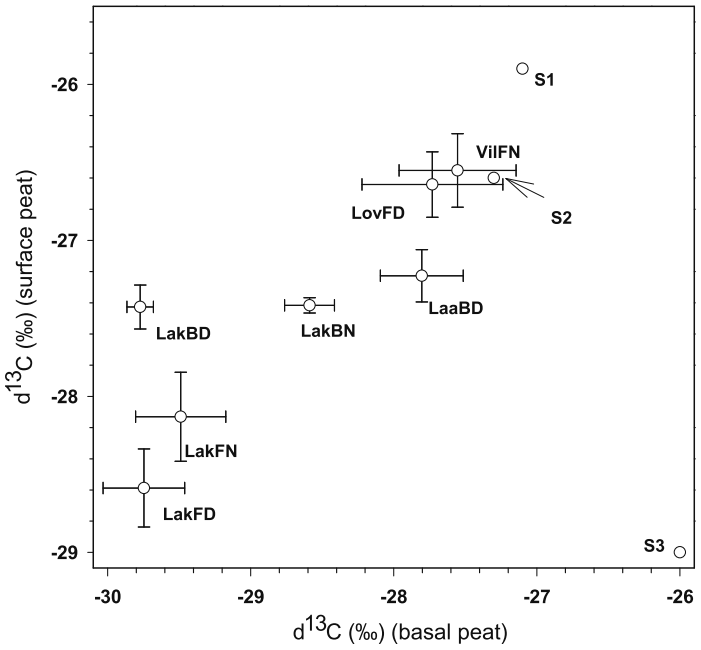

Figure 4. Correlation between surface and basal δ^{13}C (average ± S.E., $n = 3$). Abbreviations: LakFN = Lakkasuo natural site, LakFD = Lakkasuo drained site, LovFD = drained Lovonsuo, LaaBD = drained Laaviosuo and VilFN = natural Villikkalansuo. Studied peatlands are supplemented with values from two fen sites in Waldron et al. [39] (S1 and S2) and data from one bog site Hobbie et al. [36]. Surface peat $\delta^{13}C = 0.63 \times$ (bottom peat δ^{13}C) − 9.47, ($r^2 = 0.71$, $n = 7$); when S1 and S2 are included in the equation: surface peat $\delta^{13}C = 0.69 \times$ (bottom peat δ^{13}C) − 7.46, ($r^2 = 0.79$, $n = 9$).

3.3. 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, $p > .05$). Yet, the N% of the basal peat layer tended to be smaller on the drained sites (1.4 ± 0.3 %) than on the natural sites (2.2 ± 0.1 %). However, N% in the mid-layer was significantly smaller on the drained sites (1.0 ± 0.1 %) compared to natural sites (1.9 ± 0.3 %) [*t*-test: $t(16) = 2.92$, $p = .010$], thus the C/N ratio in the mid-layer was significantly larger for the drained sites (58.7 ± 5.4 %) compared to natural sites (32.2 ± 6.3 %) [*t*-test: $t(16) = -2.98$, $p = .009$].

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 (Figure 2(a)). Drainage did not change LakBD $\delta^{13}C$ values of the three uppermost layers, but there was a significant decrease in $LakBD_{\underline{255-280}}$ [*t*-test, $t(4) = 5.996$, $p = .04$) (Figure 2(f)). Drainage increased bulk peat C% in $LakFD^{0-25}$ [$t(4) = -3.797$, $p = .019$], and $LakFD_{50-100}$ [$t(4) = -5.447$, $p = .022$] (Figure 2(b)). In LakB, the C% was smaller in the whole profile of the drained site, but the difference was significant only in $LakBD_{50-100}$ [$t(4) = 3.05$, $p = .038$] and $LakBD_{\underline{255-280}}$, [$t(4) = -3.292$, $p = .000$] (Figure 2(g)). There was no change in N% due to drainage in LakF. There was a significant decrease of N% in $LakBD_{50-100}$ [$t(4) = 2.821$, $p = .048$] and in $LakBD_{255-280}$ [$t(4) = 3.621$, $p = .022$] (Figure 2(h)). 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 site peat profile, but the increase was significant only in $LakBD_{50-100}$ [$t(4) = -3.292$, $p = .030$] (Figure 2(i)).

3.4. 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 site (Figure 5(a)). The natural site of the subsoil was 1.0 ‰ depleted in ^{13}C compared to the bottom peat [$t(19.2) = 4.77$, $p = .000$] (Table 3, Figure 5(a)). Such a difference was not detected on the drained site. Furthermore, $\delta^{13}C$ values of the subsoil of the natural site were 1.3 ‰ lower than in the drained site [$t(26) = -4.17$, $p = .000$], but not in the bottom peat (Table 3, Figure 5(a)). The amount of C, N%, C/N ratio, BD and volumetric water content were significantly different between the bottom and the subsoil (*t*-test, $p < .05$), both on natural and drained sites of LakF.

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 site (Table 3, Figure 5(b)).

3.5. $\delta^{13}C$, C% and N% of plants at LakF and LakB

Plant species changed on the drained site of LakF and LakB (Suppl. Table 2). There was a 2.9 ‰ decrease in $\delta^{13}C$ of the plants in LakFD compared to LakFN [$t(11) = 3.25$, $p = .008$]

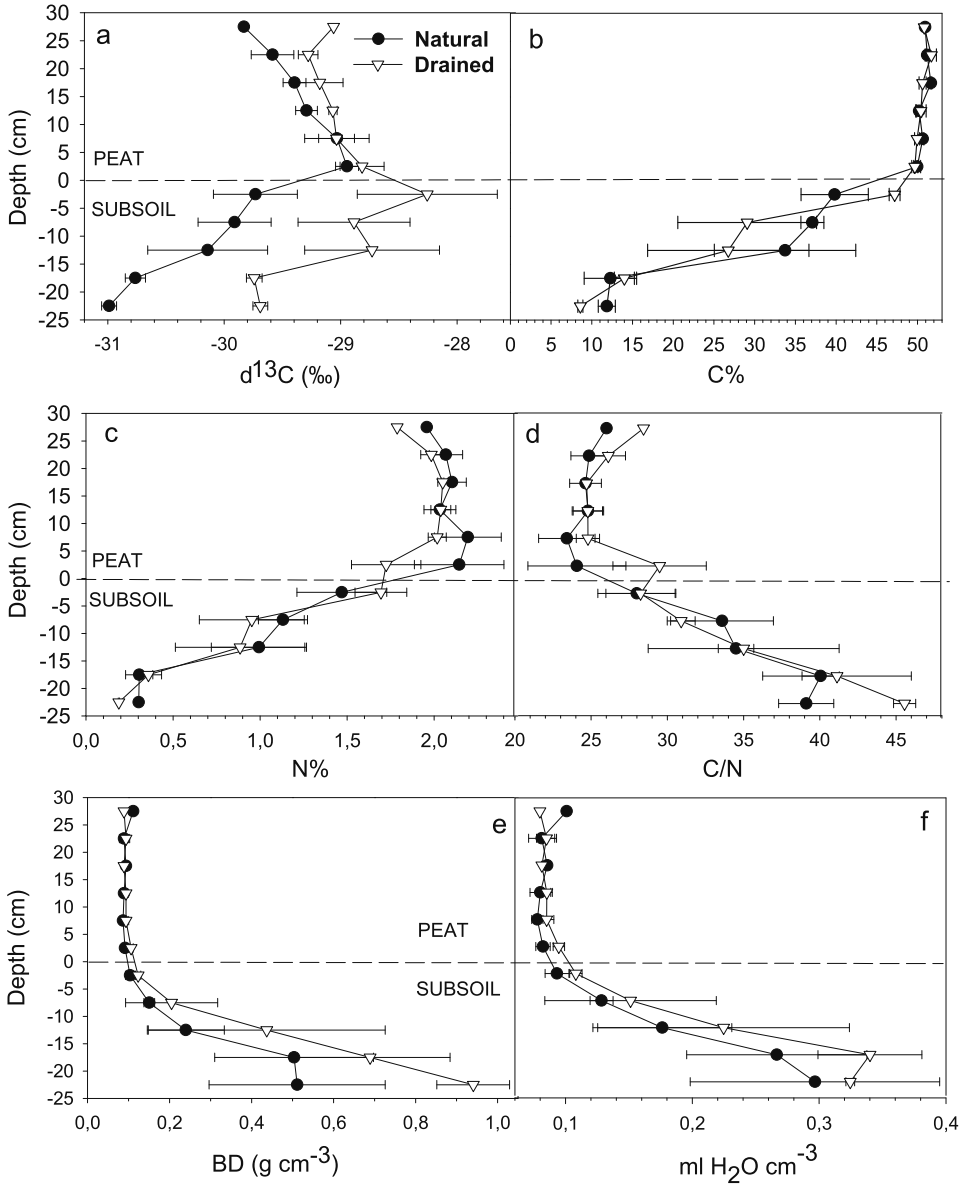

Figure 5. Peat bottom profiles from natural and drained Lakkasuo Fen, i.e. LakFNbottom and LakFD-bottom. A depth of zero indicates the transition zone between peat and subsoil; above zero is peat and below zero sub soil. $\delta^{13}C$ (a), C% (b), N% (c), C/N-ratio (d), bulk density (e) and volumetric water content (f).

(Table 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 [$t(15) = -2.85$, $p = .012$], but there was no significant difference between LakFB and LakBN. There was no difference in the C% or N% of the plants (*t*-test, $p > .05$) in natural and drained sites. There were also no differences in the C% or N% of the plants between natural fen and bog [t(9), $p > .05$).

Table 3. Lakkasuo fen bottom peat and subsoil δ^{13}C, C%, N%, C/N-ratio and volumetric water content (average ± S.E, $n = 16$ for peat and $n = 14$ for subsoil).

	LakFbotN	LakFbotD	LakFbotN vs. LakFbotD
δ^{13}C‰ peat	−29.3 ± 0.1	−29.1 ± 0.1	$t(30) = -1.95$, $p = .068$
δ^{13}C‰ subsoil	−30.3 ± 0.2	−29.0 ± 0.1	***t(26) = −4.17, p = .000***
	t(19.2) = 4.77	*t(15.0) = −0.239*	
	p = .000	*p = .815*	
C% peat	50.7 ± 0.2	50.5 ± 0.5	$t(30) = 0.81$, $p = .427$
C% subsoil	28.0 ± 3.8	26.3 ± 4.4	$t(26) = 0.52$, $p = .768$
	t(13) = 5.33	***t(13.1) = 5.55***	
	p = .000	***p = .000***	
N% peat	2.1 ± 0.1	2.0 ± 0.1	$t(30) = 0.938$, $p = .062$
N% subsoil	0.9 ± 0.1	0.9 ± 0.2	$t(26) = 0.082$, $p = .936$
	t(17.1) = 7.70	***t(15) = 6.15***	
	p = .000	***p = .000***	
C/N peat	24.5 ± 0.7	26.1 ± 0.7	$t(30) = 0.038$, $p = .104$
C/N subsoil	34.8 ± 1.4	35.5 ± 2.7	$t(26) = 0.295$, $p = .207$
	t(18.4) = −6.35	***t(15.7) = −3.93***	
	p = .000	***p = .001***	
BD (g dm^{-3}) peat	91.2 ± 3.0	94.9 ± 2.5	$t(30) = 0.94$, $p = .355$
BD (g dm^{-3}) subsoil	286.7 ± 65.0	440.0 ± 104.1	$t(21.9) = -1.30$, $p = .209$
	t(13.1) = −2.99	***t(13) = −3.37***	
	p = .000	***p = .005***	
H_2O cm^3 cm^{-3} peat	0.08 ± 0.002	0.09 ± 0.002	$t(30) = 0.341$, $p = .362$
H_2O cm^3 cm^{-3} subsoil	0.18 ± 0.034	0.22 ± 0.034	$t(26) = -0.855$, $p = .400$
	t(13.2) = −3.56	***t(13.1) = 3.99***	
	p = .003	***p = .002***	

Note: 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 the Independent sample *t*-test ($p < .05$) are expressed with bold text.

Table 4. Comparison of δ^{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 presented column-wise.

	δ^{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 = .008	$p = .157$	$p = .174$
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	$t(13) = 0.65$	$t(13) = 0.03$	$t(13) = 0.01$
	$p = .529$	$p = .973$	$p = .994$
LakFN vs. LakBN	$t(9) = 0.51$	$t(9) = 0.64$	$t(9) = -0.30$
	$p = .622$	$p = .536$	$p = .769$
LakFD vs. LakBD	***t(15) = −2.85***	$t(15) = -1.00$	$t(15) = 1.46$
	p = .012	$p = .335$	$p = .164$

Note: Significantly different values in the Independent sample *t*-test are expressed with bold text.

4. 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 δ^{13}C values due to methanogenesis or methanotrophy compared to original peat C originating

from plants. Thus, bulk peat consisting of both also includes signals 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 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 sites 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 [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 a 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 $\delta^{13}C$ value [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 (Figure 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 to −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 ± 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 $\delta^{13}C$ value 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 a significant change in C% and N% found at some depths in this study. However, low $\delta^{13}C$ of $LakBD_{\underline{255–280}}$ could actually be due to it already being in the subsoil, as the C% was also extremely low. Plants growing on the drained site 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 to −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 site decreased to half that on the drained site. 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. [41] showed that after 10 months of 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 $\delta^{13}C$ values in this study contrasts with the results of Krüger et al. [19]. 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 to 93 cm of the same drained fen and ^{13}C enrichment in drained bog from a depth of 13 to 41 cm and ^{13}C depletion from 43 to 93 cm [19]. In their study, only bog and fen surface peat using the same sampling depth interval average (0–25 cm) as was used in this study had the same average $\delta^{13}C$ values on natural and drained sites [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 the 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} a^{-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 LakFN was ^{13}C-depleted compared to the bottom peat above it, but was ^{13}C-enriched on the drained site. If we suppose that drained and natural site subsoils had similar δ^{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 site [17,23,53]. Although saturated vertical hydraulic conductivity of peat is low, $0.7 \times 10^{-5} - 1.3 \times 10^{-2}$ m s^{-1} [54], it allows water to pass through the whole remaining peat column in a few minutes or days. Levy et al. 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 subsoil. Diffusion moves CO_2, CH_4 and O_2 to all directions; diffusion is even 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 site 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% (Table 3, Figure 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 sites, and an 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 δ^{13}C before drainage.

5. Conclusions

In this study, surface peat δ^{13}C followed the δ^{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 δ^{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 ^{13}C depletion of vegetation was reliable because there were documented changes in surface vegetation and increase in ^{13}C-depleted CO_2 flows from decaying peat.

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Disclosure statement

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IV

HETEROGENEITY OF CARBON LOSS AND ITS TEMPERATURE SENSITIVITY IN EAST-EUROPEAN SUBARCTIC TUNDRA SOILS.

by

Kateřina Diáková, Petr Čapek, Iva Kohoutová, Promise A. Mpamah, Jiří Bárta, Christina Biasi, Pertti J. Martikainen & Hana Šantrůčková 2016

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