Dear Referee,

Thank you, for your helpful comments, which will help to improve our paper considerably.

1.The authors' central hypothesis ('maximum 15N enrichment at maximum microbial diversity) seems to come out of nowhere, and it is unclear to me how the authors came up with this hypothesis except as a post-hoc justifying their results. I do not see why greater microbial diversity should necessarily imply greater nutrient limitation as it matters little to the nutrient whether it is taken up by fungi or bacteria (fungi compete with themselves as much for nutrients as with bacteria and vice versa, abundance does not equal activity, etc.). It is also unclear to me how this conclusion is supported by the presented data, which shows that the 15N maximum occurs at the same depth of the change fungal to bacterial dominance, but does not provide evidence that one is related to the other. I don't see why these changes in microbial community composition would provide evidence for greater nitrogen limitation at the depth of the 15N maximum. However, I'm not sure if this rather speculative interpretation of 15N being driven by microbial community composition is actually needed in this paper – I think de- scribing the differences between drained and undrained peatlands provides valuable information by itself.

Answer:
Influence of microbial diversity: Regarding your comment on microbial diversity and increased $\delta^{15}N$ values, we are sorry for being so unclear; we will be more precise with the description of the assumed relationship between the two parameters microbial metabolism processes and $\delta^{15}N$ values. We hypothesize that the microbial abundance (see answer 3) as well as the microbial community composition has an influence on the $\delta^{15}N$ values. With an increasing diversity, $\delta^{15}N$ values of the reaming substrate should increase, because (1) different microbial communities prefer different sources (Dijkstra et al. 2006, Dröllinger et al. 2019) and (2) with increasing bacterial abundance, fungi have to use also recalcitrant sources, because bacterial metabolism will outcompete fungi for the easily degradable substances (Rousk and Bååth, 2007, Winsborough, C. and Basiliko, 2010). Hence, with increasing microbial diversity also the diversity of the mineralized organic fractions increases (Thormann 2006). With an increased diversity of nitrogen sources more release of lighter ^{14}N is possible and the ratio of ^{15}N:^{14}N in the remaining substrate should increase. We realize that these are hypotheses to explain the observed patterns, but to our understanding they are the most likely ones and in describing them, we hope for an eager discussion in the community.

We have implemented this to the manuscript in section 3.6 (L501-508).

2. I think the manuscript could be improved by presenting/discussing the results in a different way. In the present version, the author very much focus on changes in d15N and other parameters with depth. I would recommend to start by comparing drained and undrained soils – I think it would be helpful if the authors first identify how drainage has changed the parameters measured in the soil profiles (e.g., drainage increase 15N values in the mesotelm). The authors can then discuss which processes led to an increase in 15N in drained peatlands (relative to intact neighbor sites), and

why these processes were strongest in the center of the mesotelm and less pronounced towards the surface and the catotelm edge.

Answer:
Different way to present: We have followed your advice and structured our results accordingly. We will start, as you suggested, with an overview of drained and undrained peatlands and the influence of hydrology on the measured biogeochemical parameters. This will be followed by an introduction to the processes, which leads to increased $\delta^{15}N$ values, and might explain the observed pattern in the mesotelm.

We have implemented these changes and restructured chapter 3 accordingly.

3. The authors could also improve the manuscript by providing a more detailed view on the processes that cause the N isotope fractionation in these soils. In particular, they do not propose a fate for the 14N-depleted nitrogen fraction. How does this carbon get lost from the soil profile (in drained relative to intact peatlands)? It does not simply get transported downwards in the soil profile as no large difference in d15N was observed in the catotelm (Fig. 2). Mineralization is a likely mechanism, does that mean that more depleted 15N is leached out of the soil profile and exported from the peatland? Or are there stronger gaseous losses (N2O, denitrification) in drained peatlands? What is the role of plant and microbial uptake of 15N in this process?

Answer:
Nitrogen cycling: We for sure do not claim that we fully understand the observed patterns yet, but that we see consistent patterns and (in combination with the fatty acid analysis) develop ideas what the origin of these patterns might be. Sorry, if this was not clear from the manuscript, we will add a sentence referring to this. We will further insert a section about N isotope fractionation in peatland soils and the underlying processes, which might be leading to ^{14}N depletion in the remaining substrate during drainage. In general, the ^{15}N:^{14}N ratio of plant material (here mostly sphagnum mosses) is lower than the values of microbes and bulk material (Aldous, 2002, Lichtfouse et al. 1995). Microbes prefer to mineralize the lighter, more frequent ^{14}N (Dijkstra et al., 2006, Novák et al, 1999). Since plants incorporate the microbial mineralized nitrogen they have a low ^{15}N:^{14}N ratio (Lichtfouse et al. 1995). Contrary, microbial biomass is enriched in ^{15}N, probably as the result of processing the lighter ^{14}N during mineralization and hence incorporation of the remaining heavier ^{15}N. In addition, caused by the preferential mineralization of lighter nitrogen, the heavier ^{15}N might be enriched in the remaining humic substances (Novák et al, 1999). The effect of the latter to ^{15}N:^{14}N bulk values is probably enhanced due to the loss of ^{15}N-depleted material during leaching (Damman 1988, Niemen 1998), denitrification and the release of N_2O (Kohzu 2003, Niemen 1998).
In natural peatlands, microbial activity is low and mostly visible in the uppermost, aerobe part of the peat (acrotelm). With the onset of the waterlogging, anaerobe conditions in the catotelm microbial activity is inhibited. This leads to small or even negligible changes of the original (light) plant isotopic ratio below the acrotelm. (Dijkstra, 2008)
In contrast, in drained peatlands the aerobic mesotelm expands and simultaneously microbial activity increases (Moore & Basiliko 2006, Roswell 1976). In an extended mesotelm a higher amount of mineralization and the release of N_2O takes place. With

increased mineralization the ^{15}N:^{14}N ratio in the remaining substrate should increase, as long as ^{14}N will be mineralized preferentially (Dijkstra, 2008). However, because of the faster and more complete decomposition with increasing microbial activity (Damman, 1988) metabolism of ^{15}N increases as well and fractionation will be less. This pattern leads to only small increases in the ^{15}N:^{14}N ratio of the bulk material, as all isotopes are used and fractionation is lowered in the middle of the mesotelm, where microbial activity is the highest. Actually, the best way to test for the combined effects of all these different processes on the isotopic fingerprinting would be to set up a conceptual model. However, we feel this is beyond our possibilities at the moment, but we certainly look for opportunities (e.g., cooperation) in the future.

We have implemented this mainly in chapter 3.6 (L463 – 514).

4. PLFA analysis: The authors use a non-standard method to extract/purify/derivatize PLFAs for analysis. While this is not a problem in itself, this method looks like a total fatty acid extraction to me. At least, it extracts and recovers free fatty acids (as shown by the use of the internal standard nonadecanoic acid). Please provide information how phospholipids were separated from glycolipids and neutral lipids in this method.

Answer:
FA analysis: You are totally right. We have extracted all membrane fatty acids and did not separate phospholipid fatty acids. We are really sorry for this incorrect classification in the first version of our manuscript. We aimed to distinguish between fatty acids of microbes, fungi and plants and we were able to detect these changes by the extraction of total membrane fatty acid values, because the used markers (i-C15:0 and a-C-15:0 for Gram positive - bacteria and C18:2ω9c for fungi) are not restricted to phospholipid fatty acids (Bajerski, Wagner & Mangelsdorf 2017; Finotti et al. 1992; Piotrowska-Seget & Mrozik 2003).

We have implemented this in fatty acid sections 2.3, 3.5 and for the whole manuscript, especially for L 27-36 and L128-138.

Some language issues: - I would prefer the more descriptive term 'maximum' rather than 'turning point', which implies some change in direction in processes. I think this would also improves the clarity in a central point of the manuscript.

Answer:
Language issue: You are right, "maximum" would also be a very good term for our observed pattern, but we decided to use "turning point" because if we compare different sites and layers, a maximum in one site or depths layer might not be the absolute maximum, which leads to confusion. Furthermore, what we are really looking at are changes in depth trends. As such, we think, turning point is a better term. Furthermore, we already used the term already to describe the observed isotope patterns in some previous publications and would thus like to stay with it.

L16: 'stable isotope signatures': See this advice on 'Isotope terminology' from Z. Sharp's 'Isotope Geochemistry' book (https://digitalrepository.unm.edu/unm_oer/1/): Mistake: "The isotopic signature of the rock was d18O = 5.7‰"' Recommended Expression"Thed18Ovalueoftherockwas5.7‰T husthisrockhastheoxygenisotope signature of the mantle." Explanation: "The word signature should be used to

describe the isotopic composition of a significant reservoir like the mantle, the ocean, or a major part of the system being studied, not to the isotopic composition of ordinary sample" - L311: 'equilibrium' between fungi and bacteria – I don't think equilibrium is the correct concept here. Maybe change from fungal to bacterial dominance, but even that is not

Answer:
Thank you for the explanation for the word "signature", we have changed it to "composition". In addition, we deleted the term "equilibrium" and have decided to use "change towards higher bacterial decomposition".

Minor comments: L12-15: The first three sentences have very little to do with the content of this manuscript. L304-306: highly speculative and not well referenced. Figure 2: check axis ticks for BD and C/N, start these axis at 0. Tables 3-6 could be places in a supplement.

Answer:
L12-15: We rewrote the sentences.
L304-306: We added references to these sentences (Lerch et al., 2011; Rousk and Bååth, 2007).
Figure 2: The axes start now with 0 and ticks are checked.
Tables 3-6 will be placed in the supplement.

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Dear Referee,

Thank you, for your helpful comments, which will improve our paper considerably.

1. To me, the Introduction is the part of the manuscript that requires the most attention. The research methods and questions need to be prepared in more detail and especially the role of roots and mycorrhiza should be considered in more detail.

Answer: We have rewritten the introduction and have insert a more detailed part for research methods and questions (see also answer 7). You are right; we have not sufficiently discussed the expected role of roots and mycorrhiza. We are sorry for that and have now complemented a paragraph with it. Our study sites are open peatlands with a small amount of vascular plants (result of our vegetation analysis). Hence, mycorrhiza should also play a minor role. But of course, there might still be an effect of mycorrhizal activity and rooting in our study sites. Mycorrhiza mediate the uptake of nitrogen into plants. Rooting and the existence of mycorrhiza leads to enriched ^{15}N values in the remaining bulk material (Högberg et al., 1996), because (1) mycorrhiza preferentially process lighter ^{14}N and transfer them to plants (Adams and Grierson, 2001, Asada et al., 2005a, Högberg et al., 1996, Kohzu et al., 2003, Robinson et al., 1998) and (2), even without mycorrhizal activity, plants preferentially incorporate the lighter ^{14}N. But, because of the small amount of vascular plants and therefore also of mycorrhiza, these cannot not be the main drivers for our observed pattern.

We have implemented this in the introduction (L113-120).

2. For consideration in the journal SOIL, the soil type/classification need to be adequately described.

Answer: You are right, we have forgotten to describe the soil type adequately and have inserted this classification to the manuscript. All investigated sites are classified as Histosols (organic soils). Histosols are classified as soils with a cumulative organic layer and an organic matter amount of 35% or higher in at least half of the uppermost 80-100 cm (IUSS, 2015). In addition all investigated peatland soils are Sphagnum peat, because of their mean annual temperatures (between +1.2°C and +7 °C) and their annual precipitation between 523ppm and 1600ppm (Eurola et al., 1984, Vitt et al., 2006). Lakkasuo and Degerö Stormyr are classified as Northern eccentric bogs and the peatlands in the black forest are characterized as ombrotrophic bogs (Eurola et al., 1984).

We have implemented this in section 2.1 (L160-165; L169, L175).

3. In some figures, but also text the acrotelm-mesotelm designations are an issue: Is there an acrotelm or not? Figure 3 shows an acrotelm/upper mesotelm, but in the remainder of the manuscript, an acrotelm is not mentioned. On the other hand, an "upper" and "lower" mesotelm are introduced. Please find a consistent way to handle the issue.

Answer: We are sorry, for not being clear enough with our definition. Yes, there is an acrotelm and a mesotelm in all sites. We have forgotten to mention this in figure 3 and have changed it accordingly. The acrotelm is the uppermost part of the peatland with living sphagnum vegetation (Morris et al., 2011). The deeper part, with dead plant material and in permanent waterlogged, anaerobe conditions, is called catotelm (Morris et al., 2011). In between the acrotelm and the catotelm, with fluctuating conditions, the mesotelm is located (Clymo and Bryant, 2008, Lin et al., 2014). With drainage, the mesotelm is expanding and a supplementary separation is reasonable, because the condition within the mesotelm differ a lot from aerobe, light and warm conditions (upper mesotelm) to semi-oxic, dark and cold conditions in the lower mesotelm (Artz, 2014, Lin et al., 2014). These changed conditions are the reason for the changed microbial metabolic pathways and are therefore critical for the 14N:15N ratio we see in the data sets (Lin et al., 2014).

We have implemented this in Figure 3 and to the introduction (L50-63).

4. Please check the manuscript again for signs of sloppiness: Throughout the manuscript, the abbreviations for Tables and Figures are inconsistently spelled; sometimes capitalize and sometimes not. Somewhere in the text, I quit nothing this in "Specific comments". In the references section, journal titles are generally spelled out, but sometimes not. Please edit following journal guidelines

Answer: Thank you for pointing this out. We have checked and deleted mistakes in tables and figures as well as in the reference section.

5. Specific comments: L12; L14; L16; L28; L32; L74; L219; L221; L247; L250; L287; L246; L443; L444

Answer: Thank you for your careful and constructive review of the manuscript. We have changed and improved the mentioned sentences.

6. L38-39: There are quite a few approaches to describe "peatland condition". But what is "peatland condition"? And are the methods you are proposing more time and cost efficient than others? You are hypothesizing that 15N isotopes could be such a tool. Fine, but PLFA analysis isn't that cheap and you are also heavily relying on that method. Please explain in more detail.

Answer: With the wording "peatland conditions" we are referring to the hydrology status, whether it is natural, drained or rewetted. We have changed the wording to hydrology status. You are right; FA analysis is not a cheap and easy method. We have done this analysis to support our hypothesis based on stable isotopes, and only the latter we refer to as a time- and cost efficient method to indicate drainage and rewetting. We do not suggest establishing an approach as a routine analysis, which uses both methods. The three main methods today to measure the hydrology status are

(1) a macro analysis of peatland vegetation, (2) gas emission measurements and (3) measurement of growth heights of peatland vegetation. Method (1) was also done in this study. We wanted to prove, that our investigated stable isotope patterns are related to decomposition and that they are not primarily a consequence of the vegetation assemblages. But this method is time consuming and needs a high level of expert knowledge and is thus very costly. Method (2), the measurement of gas exchange in peatlands (Baldocchi et al., 1988) measures current gas emissions and therefore provides an indirect measurement of ongoing decomposition processes (Bubier et al., 2003). But it is not able to give information on drainage history and gas exchanges at another time of the year (Bubier et al., 2003). Furthermore, this method is also very intensive in analytical equipment and expert knowledge needed. A third available method (3) is the measurement of the growth of peatland vegetation. But there are several problems with this method: Firstly, not only the sole growth of mosses indicates peat growth. It is important how much vegetation material enters the catotelm and is therefore stored under aerobe conditions. Secondly, peat shrinks and swells with water supply. Hence measuring peat height at different times would lead to completely different assumptions for peatland growth (Clymo, 1970). And thirdly, peat growth is really slow and it would need decades to get a positive reply with this method to indicate successful restoration efforts (Clymo, 1970, Fenton, 1980). Summing up, there are methods available to get information of the success of restoration effort, but these methods are lacking some important information or/ and are expensive and time consuming. Hence, there is a need for a new and less expensive and time-consuming indicators, which could be done not only by specific experts. We believe that bulk isotopes can be such suitable indicators, but we need to prove that with the FA method.

We have implemented this in the introduction (L64-92).

7. This is a general phenomenon Introduction chapter in general: Biogeochemical transformations as a consequence of rewetting re not introduced, but in the last paragraph of the introduction, you are looking for changes of 13C and 15N with the onset of the rewetting process.

Answer: Thank you, for your comment. You are right; we have missed to introduce our hypothesis of the influence of rewetting to stable isotopes. Rewetting increases the water table height and therefore enlarges the anaerobe catotelm (Andersen et al., 2006). We hypothesize, that the observed stable isotope pattern for drained horizons will be conserved, when formerly aerobe parts will get rewetted (Andersen et al., 2006). With rewetting the conditions in the former mesotelm will get anaerobe and microbial activity will be inhibited (Andersen et al., 2006, Asada et al., 2005b, Thormann et al., 1999). Hence, no or only few metabolism processes take place and stable isotope patterns shouldn`t change anymore. For the upper part of the rewetted peat, we expect to find natural conditions and vegetation growth, like in natural peatlands. Hence, we expect to find the same stable isotope pattern, as we see in natural peat.

We have implemented this in the introduction (L60-64)

8. L70-80: This paragraph should be rewritten. It lists methods, but the aim/objective/hypothesis is not sufficiently clear. Many methods are listed without

having been introduced before. Please introduce these methods. When looking at d15N, not only decomposition must be considered, but also mycorrhizal activity. Are you expecting root effects on d15N?

Answer: We have improved the wording of the mentioned paragraph and inserted an introduction to the mentioned methods (bulk density and carbon:nitrogen ratio measurements). Our aim is to find an answer for the depth trends of carbon and nitrogen stable isotopes corresponding to the hydrology status, which were investigated in previous studies. Our main hypothesis is that microbial metabolic pathways are the drivers behind these stable isotope depth trends. The hydrology status determines the abundance of microbial communities. With changing hydrology microbial abundance changes significantly (Kohl et al., 2013) and therefore also stable isotope values must change (Tfaily et al., 2014). Vice versa this would mean that stable isotope values reflect the hydrology status, which we aim to test. We hypothesize, that drained conditions lead to expanded microbial abundance, because of the attendance of oxygen also in deeper horizons. We aim to find significant links between this pattern and the observed stable isotope pattern. For natural and rewetted conditions we hypothesize to find low values of stable isotopes in accordance to low microbial abundance.

We have restructured and revised the whole introduction.

For mycorrhizal- and rooting effects please see answer 1.

9. Chapter 2.2. Coding of the sites is inconsistent. Some codes appear to relate to minerotrophic or ombrotrophic hydrology or drained vs. natural status, but others don't. Please code in a consistent way. Chapter 2.3: What does LOD1, LON3, DDC3, DNM1 mean? Did you take replicate cores at these sites? From which depth were samples taken?

Answer: We have changed the coding, to be more consistent. We have now named them as follows: first letter of the site + hydrology status (plus with subscript, if needed, for additional information) (Tab.1). Yes, we had three replicates per site and analyzed samples of the upper 60 cm of the cores. The cores were sliced in 2 cm sections and every second layer was analyzed, giving a 4 cm depth resolution. We have mentioned it in section 2.2 (L134/135 and L148).

Table 1: Labeling of all drilling sites

Location	Labeling
Degerö	
natural mire	DN
drained	DD
Lakkasuo	
minerotrophic natural	LN_m
minerotrophic drained	LD_m
ombrotrophic natural	LN_o
ombrotrophic drained	LD_o
Breitlohmisse	
natural mire	BN

natural dry	BN_{dry}
drained	BD_1
near the mire edge	BD_2
Rotmeer	
natural mire	RN
drained, with Sphagnum	RD_1
drained, without Sphagnum	RD_2
Ursee	
natural mire	UN
drained	UD

10. L 283-284: This sentence is incomprehensible. Does fungal biomass decrease in peatlands? Where? When? Please explain.

Answer: Yes, our hypothesis is, that with increasing depth and changing hydrological conditions (darker, less oxygen) fungi will be outcompeted by bacteria, which means, that fungal biomass must decrease, whereas bacterial biomass increases with depth. In the uppermost part (acrotelm and upper mesotelm) fungal biomass is the highest, whereas in the deeper part of the mesotelm bacterial biomass will increase. In the catotelm all microbial biomass is strongly reduced because of the anaerobe conditions. In natural peatlands a small amount of fungal biomass is also visible in the acrotelm, but in a much lower scale than for drained sites. (Thormann, 1999)

We have implemented it in the chapters 3.5 (L440-457) and 3.6 (L479-491).

11. Supplementary data: This xls. file is not for publication. It requires formatting and translation.

Answer: We have re-formatted the supplementary data to make it more comprehensible for readers.

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Relevant changes:

We have revised the whole chapters 1 (introduction) and 3 (discussion), with major changes and implementations of the comments as followed:

1. Revised Introduction and implementation of the reviewer comments
    a. L27-36
    b. L50-63
    c. L64-92
    d. L113-120
    e. L128-138
2. Implementation of reviewer comments in chapter 2
    a. L160-165
    b. L169
    c. L175
    d. L233-259
3. Revised discussion chapter (3) and implementation of the reviewer comments:
    a. L440-457
    b. L479-491
    c. L463-514
4. Figures 1-3 were revised
5. Table 1 was revised
6. Tables 4-7 were moved to the supplement

Switch of fungal to bacterial degradation in natural, drained and rewetted oligotrophic peatlands reflected in $\delta^{15}N$ and fatty acid composition

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Abstract. ~~During the last centuries major parts~~Since the last centuries European peatlands are degrading along with drainage, land use and climate changes. Increasing ~~Peatland biodiversity and essential ecosystem functions (e.g. flood prevention, groundwater purification and CO_2 sink) were dramatically impaired. Moreover, climate change threatens peatlands in the near future. Increasing~~ pressure to peatland ecosystems calls for a more cost-efficient method to indicate the current state of peatlands and the success of restoration effort. ~~Metabolism processes~~Metabolic pathways in peatland soils are imprinted in stable isotope compositions due to differences in microorganism communities and their metabolic pathways. Therefore, we hypothesize that depth profiles of nitrogen stable isotope values provide a promising opportunity to detect peatland decomposition or restoration. We studied five peatlands: Degerö Stormyr (Northern Sweden), Lakkasuo (Central Finland) and three mires in the Black Forest (Southern Germany). At all locations, cores were taken from adjacent drained (or rewetted) and natural sites to identify $\delta^{15}N$ trends that could indicate changes due to drainage and restoration. At all drained (and rewetted) sites we found a distinct peak ("turning point") of the $\delta^{15}N$ values in the center of the drained horizon. ~~. To verify our interpretation C, the C/N ratio and the bulk density were measured and a microscopic analysis of the macro residuals in the peat cores was made.~~We did a fatty acid (FAs) analysis to link our results to microbial community composition. As marker we distinguished between

one fungal-derived FA (C18:2ω9c) and four bacterial-derived FAs. For bacteria, we looked for one general bacterial-derived FA (C14:0), two FAs for gram-positive bacteria (i-C15:0; a-C15:0) and one FA for gram-negative bacteria (C16:1ω9c) ~~and bacterial-derived PLFAs~~. In accordance with other studies, our results suggest, that fungi dominate the microbial metabolism in the upper, aerobic peat horizon. This is reflected by depleted δ^{15}N values. Downwards the drained horizon conditions slowly switch to oxygen limitation. In consequence fungal-derived FAs decrease whereas bacterial-derived FAs rise. The highest diversity of microbial-derived FAs is indicated by the δ^{15}N turning point. Below the δ^{15}N turning point, oxygen is increasingly limited and concentrations of all microbial-derived FAs are decreasing down to the onset of the permanently waterlogged, anaerobic horizon. Peatland cores with restoration success show, above the formerly drained horizon, again no depth trend of the isotopic values. Hence, we conclude that δ^{15}N stable isotope values reflect microbial community composition, which differs between drained and natural peatlands.

1 Introduction

In Europe 70% of the peatlands are degraded (Joosten and Couwenberg, 2001). Leifeld and Menichetti (2018) reported that degraded peatlands account for five percent of the anthropogenic CO_2 emission. Despite this dramatic peat decline, we lack reliable and transferable tools providing time- and cost-efficient information of the peatland hydrology status.

~~Even though peatlands cover only 3-4 % of the Earth's land surface (Leifeld and Menichetti, 2018), they act as an enormous sink for greenhouse gases in natural conditions (Yu et al., 2011; Joosten, 2008). In Europe 70% of the peatlands are currently degraded (Joosten and Couwenberg, 2001). These degrading peatlands account for five percent of the anthropogenic CO emission (Leifeld and Menichetti, 2018; Zedler and Kercher 2005). Despite this dramatic peat decline, we lack reliable and transferable tools for providing time- and cost-efficient information of peatland condition.~~Peatland soils consist of three different horizons. Most biological metabolism and nutrient cycling takes place in the acrotelm (uppermost aerobic peat horizon with living vegetation) (Asada et al., 2005a; Artz, 2013; Morris et al., 2011). In the water-saturated catotelm (deeper, anaerobic horizon) organic substrates are decomposed at much smaller rates owing to anoxic conditions (Asada et al., 2005a; Artz, 2013; Lin et

al., 2014). In the mesotelm, the peat horizon situated between acrotelm and catotelm, water table levels and oxygen content fluctuate, resulting in shifting aerobic and anaerobic conditions and shifting metabolism processes (Asada et al., 2005a; Artz, 2013; Lin et al., 2014). Clymo and Bryant (2008) therefore defined the mesotelm as a "transition horizon". In degraded peatlands the mesotelm is expanded and former preserved organic substrate is decomposed (Zedler and Kercher, 2005). In an expanded mesotelm conditions differ from aerobic, light and warm conditions in the upper mesotelm to semi-oxic, dark and cold conditions in the lower mesotelm (Artz, 2014; Lin et al., 2014). The conditions in the former mesotelm will get anaerobic and microbial activity will be inhibited with rewetting (Andersen et al., 2006; Asada et al., 2005b; Thormann et al., 1999).

Derived by the thickness of these horizons, we distinguish between three different hydrological statuses of peatlands (natural, drained and rewetted). We determined the hydrological status by a vegetation analysis, the humification index (HI) after von Post (Silc and Stanek, 1977) and the measurement of the water table height. Natural and rewetted sites have a high water table near the surface and are mainly formed by Sphagnum mosses with low humification indices. Drained sites are characterised by low water tables, higher grades of humification, less Sphagnum and more other moss species. However, determination of macro residuals in more or less degraded peat is time-consuming, needs highly specialised expert knowledge and is thus limited to a small number of samples.

Other common methods to measure peatland hydrology currently are gas emission measurements and measurement of growth heights of peatland vegetation. Gas measurements (e.g. CO_2, N_2O, CH_4) provide an indirect measurement of ongoing decomposition processes (Baldocchi et al., 1988). The method is expensive and labour intensive and does not to give information on drainage history and process dynamics beyond the specific measurement time (Bubier et al., 2003). Measuring vegetation growth is connected to several problems: (i) not only the sole growth of mosses indicates peat growth, but rather the balance of growth and degradation. It is important how much vegetation material enters the catotelm and is therefore stored under anaerobic conditions. (ii) Peat shrinks and swells with water supply. Hence, measuring peat height at different water table heights would lead to different assumptions for peatland growth (Clymo, 1970). And thirdly, peat growth is slow and an unambiguous

result on the success of restoration efforts might need decades of measurements (Clymo, 1970, Fenton, 1980).

As such and in search for practical indicators, we measured bulk density (BD), carbon/nitrogen ratio (C/N) and bulk stable isotope values. BD acts as an indicator for decomposition, because decomposition processes lead to higher compaction of the peat soil and therefore increasing BD values (Novak et al., 2008). The C/N ratio indicates the degree of decomposition (Malmer and Holm, 1984; Kuhry and Vitt, 1996). With increasing decomposition a preferential loss of C over N takes place and the C/N ratio decreases. Stable isotopes depth patterns of $\delta^{13}C$ and $\delta^{15}N$ in peat have been ~~We~~ found in previous studies (e.g. Krüger et al., 2016, Alewell et al., 2011) to be specific ~~depth patterns of $\delta^{13}C$ and $\delta^{15}N$ depending on~~for peatland hydrology (drained, rewetted or natural), but studies were unable to find an explanation of these depth patterns. As degradation is mostly connected to drainage, we hypothesized that an increase of microbial activity is responsible for the change in isotope patterns.

~~We hypothesize that nitrogen (N) stable isotopes could serve as such a tool.~~

~~In natural peatlands most biological metabolism and nutrient cycling takes place in the acrotelm (uppermost aerobic peat horizon with living vegetation) (Asada et al., 2005; Artz, 2013). In the water-saturated catotelm (deeper, anaerobic horizon) organic substrates are decomposed at much smaller rates owing to anoxic conditions and low pH values (Asada et al., 2005; Artz, 2013; Lin et al., 2014). In the mesotelm, a peat horizon situated between acrotelm and catotelm, water table levels and oxygen content constantly fluctuate, resulting in shifting oxic and anoxic conditions and shifting metabolism processes (Asada et al., 2005; Artz, 2013; Lin et al., 2014). Clymo and Bryant (2008) therefore defined the mesotelm as a "transition horizon".~~

Stable C and N isotopes are correlated with vegetation composition and microbial decomposition processes. As decomposition induces an enrichment of heavy isotopes (^{15}N, ^{13}C), vegetation is mostly more depleted in ^{15}N and ^{13}C than microbial and recycled substrate. Alewell et al. (2011) and Krüger et al. (2014) reported distinct changes in $\delta^{13}C$ values for palsa peat with the onset of decomposition of hummocks. Various authors observed the same trend with decomposition in peatlands of other climate conditions (Krüger et al., 2016; Novak et al, 1999; Hobbie et al., 2017; Biester et al., 2014). The distinct $\delta^{13}C$ depth pattern is a consequence of the use of different sources by fungi and bacteria as investigated

by Kohl et al. (2015) for peat profiles. They conclude that an increasing $\delta^{13}C$ signal is caused by differences in biomass synthesis and carbon sources used by fungi and bacteria, which was also reported by Lichtfouse et al. (1995) and Baumann et al. (2013). We found also distinct changes in $\delta^{15}N$ with drainage. It is known that plants preferentially incorporate the lighter ^{14}N (Högberg, 1997), an effect that is strongly enhanced by mycorrhizal uptake of nitrogen into plants (Hobbie and Högberg, 2012). Plant rooting and the existence of mycorrhiza leads to enriched $\delta^{15}N$ values in the remaining bulk material (Högberg et al., 1996), because plants and mycorrhiza preferentially process lighter ^{14}N (Adams and Grierson, 2001; Asada et al., 2005a; Högberg et al., 1996; Kohzu et al., 2003; Robinson et al., 1998). However, our study sites are open peatlands with a low occurrence of vascular plants and mycorrhiza. Hence, these mechanisms cannot be the main drivers of our observed $\delta^{15}N$ depth patterns. Tfaily et al. (2014) reported changing microbial abundance and metabolic pathways are correlated with $\delta^{15}N$ values. Vice versa this would mean that $\delta^{15}N$ values could reflect the hydrology status. Therefore, we assume $\delta^{15}N$ values allows us conclusions whether the observed peatland have a natural, drained or rewetted hydrology status. ~~The hydrology status determines the abundance of microbial communitie~~

Following previous studies, we use specific terms for the points of change in the stable isotope depth pattern. The points where the stable isotope signals undergo a sudden directional shift with depth are called “turning points” according to Alewell et al. (2011). Furthermore, the bottom of the mesotelm and the onset of the underlying catotelm are marked by the $\delta^{13}C$ turning point.

To test the idea of changing dominant microbial communities as drivers for isotope depth patterns, we did a fatty acid (FA) analysis of four investigated sites – two drained and two natural sites in Degerö Stromyr (Mid Sweden, 70 km form Umea) and Lakkasuo (Southern Finland, 14 from north from Orivesi). FAs are valid markers to indicate the abundance of specific microbial communities in the peat, because they are specific and persistent compounds of cell membranes of different species (Bajerski, Wagner and Mangelsdorf, 2017; Finotti et al. 1992; Piotrowska-Seget and Mrozik 2003; Reiffarth et al., 2016). Therefore FAs enable us to make qualitative and quantitative statements about the relative abundance of different microbial communities. We will test the existence of four bacterial markers (C14:0 as general marker, i-C15:0 and a-C15:0 indicative for gram positive, C16:1ω9c indicative for

gram negative) (Vestal and White 1989; Willers et al., 2015; Zelles, 1997) and one fungal marker (C18:2ω9c) (Sundh, Nilsson and Borga, 1997; Elvert et al., 2003; Willers et al., 2015).

We hypothesize microbial abundance and diversity are the drivers for the distinct observed ~~different~~ $\delta^{15}N$ depth pattern in natural, drained or rewetted ~~hydrology statuses~~peats. We assume $\delta^{15}N$ depth pattern can therefore be used as an inexpensive and less time-consuming tool to get reliable information of peatland hydrology. ~~Our aim is to evaluate ∂15N depth trends as indicators of specific peatland conditions and to study whether ∂15N depth trends of natural and drainage-affected sites indicate, in parallel to ∂13C, a shift in dominant microbial communities, reflected by specific PLFAs. We are also interested to see if there are distinct changes of ∂13C and ∂15N isotope signatures with the onset of rewetting processes. We match isotope depth (C, N) with bulk density (BD) and carbon/nitrogen ratio (C/N). BD acts as an indicator for decomposition, because decomposition processes are leadin to higher compaction of the peat soil and therefore increasing BD values. The C/N ratio gives us also information about the degree of decomposition. With increasing decomposition the C/N ratio decreasess.With an additional microscope analysis of the macro-residuals in the peat horizon, we will get information of the humification indices (HI and the vegetation assemblages.~~

~~relatively relatively~~ ~~To test our hypothesis of changing dominant microbial communities as drivers for isotope patterns, we do a PLFA analysis of four investigated sites – two drainage-affected and two natural sites in Degerö Stromyr and Lakkasuo. We will test the existence of two Gram positive – bacterial (i-C15:0; a-C15:0) markers and one fungal (C18:2ω9c) marker (Sundh, Nilsson and Borga, 1997; Elvert et al., 2003). In the Swedish site Degerö Stormyr we add information of tree ring development as an indicator of peatland dynamics.~~

2 Material and methods

2.1 Site description

We studied five oligotrophic peatlands (Tab. 1, Tab. 2). All investigated sites are classified as Histosols (organic soils). Histosols are classified as soils with a cumulative organic layer and an organic matter amount of 35% or higher in at least half of the uppermost 80-100 cm (IUSS, 2015). In addition all

investigated peatland soils are Sphagnum peats, because of their mean annual temperatures (between +1.2°C and +7 °C) and their annual precipitation between 523ppm and 1600ppm (Eurola et al., 1984; Vitt et al., 2006).

Degerö Stormyr (200 m above see level (a.s.l.)) is situated in Northern Sweden, at the Kulbäcksliden Experimental Forest near Vindeln, between the rivers Umeälven and Vindelälven (Eurola, Hicks & Kaakinen, 1984). It is an acidic mire with minerotrophic conditions and consists of interconnected small mire patches divided by ridges of glacial till. Degerö Stormyr is classified as Northern eccentric peatland (Eurola et al., 1984). The climate is characterized as cold with no dry seasons and cold summers (Dfc-zone after Köppen-Geiger classification; Peel et al., 2007). In Degerö ditches were installed at the beginning of the 20th century, were closed in 2017 and a naturally reestablishment of sphagnum took place afterwards. The water table is at the surface in the natural part (DN) (Nielsson et al., 2008) and in around 10-15 cm depths at the drained location (DD).

Lakkasuo (150 m a.s.l.), Central Finland, is a~~n~~ Northern, eccentric peatland complex (Eurola et al., 1984) with two parts. In the southern part the conditions are ombrotrophic, whereas the northern part is minerotrophic (Minkkinen et al. 1999). Lakkasuo is also located in the cold climate zone, with no dry seasons and cold summers (Dfc-zone after Köppen-Geiger classification; Peel et al. 2007). The 1961 installed ditches (70 cm depth, spacing of 40 m – 60 m) affect approximately 50 % of the peatland (Minkkinen et al., 1999). In the ombrotrophic natural site (LN_o) the water table was around 13 cm below ground surface. The ombrotrophic drained site (LD_o) had a water table of 26 cm depth (average), whereas the water table is near the surface at the minerotrophic natural site (LN_m) and in an average depth of 36 cm in the minerotrophic, drained site (LD_m) (Minkkinen et al., 1999) (Tab. 1, Tab. 2).

In the Black Forest three mires were investigated: Breitlohmisse, Ursee and Rotmeer. They are located in the temperate climate zone with no dry seasons and warm summers (Cfb-zone after Köppen-Geiger; Peel et al., 2007). In the mires of the Black Forest ditches were installed in the middle of the 20th century. Breitlohmisse (810 m a.s.l., 50 km southeast of Baden-Baden) is minerotrophic and is located in the Northern part of the Black Forest. The mire is mostly lanced with ditches for huntsman ships (BN_d). The ditches are naturally refilled with Sphagnum. The water table is at an average depth of 15 cm in the natural center (BN, BN_d), and is found at lower depths near the degraded edges of the mire

(BD_1, BD_2). Rotmeer (960 m a.s.l., 40 km southeast of Freiburg i.B.) and Ursee (850 m a.s.l., 45 km southeast of Freiburg i.B.) are both in the Southern Black Forest. Rotmeer consists of an ombrotrophic center (RN) (water table at the surface), surrounded by a minerotrophic part with signs of decomposition (RD_1, water table around 12 cm depth) and without mosses at the edges (RD_2, water table below 12 cm depth). Urmeer is minerotrophic. A quaking bog forms the center with the water table at the surface (UN), whereas the edges had a lower water table (UD) (Tab. 1, Tab. 2).

2.2 Soil sampling and bulk analyses

In May 2012 (Breitlohmisse), June 2012 (Rotmeer), July 2012 (Ursee) and September 2013 (Degerö and Lakkasuo) three volumetric peat cores were drilled per site with a Russian peat corer (Eijkelkamp, The Netherland) at a medium stage of small-scale topography. In Degerö cores were sampled in the assumed natural center of the mire (DN) and in one-meter distance to a drainage ditch (one meter depth) (DD). In Lakkasuo we took cores at the natural sites (ombrotrophic natural (LN_o), minerotrophic natural (LN_m)) and the drained locations (ombrotrophic drained (LD_o), minerotrophic drained (LD_m)). For Ursee two cores were taken, one in the natural center (UN) and one at the drained edge of the mire (UD). In Breitlohmisse and Rotmeer we took cores in a transect from natural (BN, RN) to strong drained (BD_2, RD_2) sites. Each core has a composite length of one meter. Here, we focus on the uppermost 60 cm because this part included the drained horizon and no major changes in isotopic composition were observed at the natural sites below the mesotelm. In all investigated peatlands, the catotelm starts in the natural sites below 10 cm depth and varied in drained sites, but was always visible below 40 cm depth.

Directly after drilling HI were determined for each horizon with the von Post scale. The von Post scale has a range form 1 to 10. HI 1 indicates natural condition with undecomposed, completely visible vegetation residuals. HI 10 represents a strongly decomposed horizon without visible vegetation residuals. (Silc and Stanek, 1977)

The cores were encased in plastic shells and covered with plastic wrap, stored in coolers, and transported to the laboratory. The cores were sliced in 2 cm sections and every second layer was analysed, giving a 4 cm depth resolution. Samples were oven-dried at 40 °C for 72 h, and homogenized

with a vibrating ball mill (MM400, Retsch, Germany). Stable C and N isotopic compositions were measured with an elemental analyser combined with an isotope ratio mass spectrometer (EA-IRMS) (Inegra2, Sercon, Crewe, UK). Carbon isotopic composition ($^{13}C/^{12}C$) was expressed relative to Vienna Pee-Dee Belemnite (VPDB) standard and reported in delta notation (‰), stable nitrogen isotopes were expressed relative to the atmospheric nitrogen standard and reported in delta notation (‰). C/N was determined with the mass relationship of the measured bulk content of C and N. Bulk density was measured with volumetric samples, which were weighted before and after drying.

In Degerö tree rings of seven individual trees were analysed (Pinus sylvestris) to obtain information of growth conditions and to enhance therefore our knowledge of drainage history.

2.3 Fatty acid analysis

Four cores (per site one drained and one natural core) were selected to do a fatty acid analysis: two sites in Lakkasuo, LD_o 1 and LN_o 3 and two sites for Degerö Stromyr, DD 3 and DN 1. We took subsamples from all cores in the acrotelm (respectively at the end of the mesotelm in DD) and in the catotelm. At the drained sites DD 3 and LD_o 1 we took also samples in the middle and at the end of the mesotelm.

We processed 0.2 – 1.1 g of sample for the lipid extraction with a mixture of CH2Cl2 : MeOH (9:1 v/v) in an Accelerated Solvent Extractor (Dionex ASE 350). 50 µl of an internal standard (0.4 mg/ml, nonadecanoic acid) was added before processing each sample.

The total lipid extracts (TLE) were saponified by adding 2 ml of KOH dissolved in MeOH (12%) and putting it in the oven for 3 hours at 80°C.

Following the method of Elvert et al. (2003) TLE was afterwards pooled with 1 ml KCl (0.1 mol) and the neutral fraction was extracted by agitating three times with hexane. Neutral fraction in the supernatant was separated, dried under a stream of N_2, and stored in the fridge for later analysis. We acidified the rest of the TLE with fuming hydrochloric acid to a pH of 1. The acid fraction was extracted by agitating again three times with hexane. The acid fraction in the supernatant was separated and hexane was reduced to almost dryness under a stream of N_2. Then the acid fraction was methylated by adding 1 ml Boron-Trifluoride (BF_3) in MeOH (12-14%) and putting it in the oven for 1 hour at 60°C. Afterwards the resulting fatty acid methyl esters (FAMEs) fraction was pooled with KCl (0.1

mol) extracted by agitating again three times with hexane and transferred in 2 ml vials. The FAMEs were quantified with a Trace Ultra gas chromatograph (GC) equipped with a flame ionization detector (FID) (Thermo Scientific, Waltham, MA, USA). The carrier gas (helium) had a constant flow of 1.2 ml per minute and the GC-FID was set to splitless mode. Detector temperature was 320°C and the samples (dissolved in hexane) were injected by 300°C. The starting temperature of the oven was 50°C. The temperature was increased by 10°C per minute to 140°C. The temperature was held for 1 minute before it was increased up to 300°C. This temperature was held for 63 minutes.

To identify the fungal and bacterial markers, we used the Bacterial Acid Methyl Esters standard (BAME, Supelco Mix). The standard includes the following FAs as marker for bacteria: C14:0 (general bacterial marker; Willers et al., 2015, Zelles, 1997), i-C15:0 and a-C-15:0 (for Gram positive – bacteria; Zelles, 1997;, O`Leary and Wilkinson, 1988; Tunlid and White, 1992) and C16:1ω9c (for Gram negative – bacteria; Willers et al., 2015; Zelles, 1997). For fungi, the standard includes C18:2ω9c (Andersen et al., 2010; Sundh, Nilsson and Borga, 1997; Zelles, 1997;, O`Leary and Wilkinson, 1988; Vestal and White, 1989). Quantification of the FAs was done using the internal standard, C19:0 FA, after correcting for the methyl group, added during methylation reaction.

2.4 Data evaluation and statistical analysis

As we were interested in comparing the depth trends of all single profiles with each other, we first normalized the depths of the cores. This was done using the depth of the $\delta^{15}N$ turning point (see chapter 3.1) in each drained profile as the anchor point serving as normalized depth (normD). The normalized depth of this anchor point was set to 20 cm depth (normD = 20 cm, Fig. 1) in each single core. In the corresponding natural cores, we have transferred the values from the same depth related to the drained core into the same norm depth. For example the values of the natural site (DN) in depth of 13 cm (depth of the turning point of $\delta^{15}N$ in the corresponding DD core) were set to 20 cm normD.

In a second step, because we were mainly interested in trends and not the absolute values, we normalized the isotopic values themselves, because the range of $\delta^{15}N$ varied considerably between the

sites, whereas the trends show consistent patterns (Fig. 1). Therefore, to be able to do a meaningful comparison we set therefore the value of $\delta^{15}N$ at the turning point to zero in each profile:

$$normalized\ \delta^{15}N\ [‰] = \delta^{15}N\ [‰] - \delta^{15}N\ [‰]\ at\ turning\ point$$

Using the same procedure, all other parameters ($\delta^{13}C$, C/N, BD) were normalized using the same anchor point (e.g., $\delta^{15}N$ turning point):

$$normalized\ value\ (\delta^{13}C\ [‰],\ BD,\ C/N) = value\ (\delta^{13}C\ [‰],\ BD,\ C/N) - value\ (\delta^{13}C\ [‰],\ C/N,\ BD)\ at\ \delta^{15}N\ turning\ point$$

Using the above procedures means to decide on the depth of the δ15N turning points, which we backed up statistically with a t-test ($p \leq 0.05$) and an integrated change point analysis with the software package "changepoint" in R (version 1.0.153). These analyses were done for each of the drained sites separately and also in addition with an average of all locations. For the t-test, we analysed for each depth if $\delta^{15}N$ values in the drained horizon are of the same population as the values of the natural sites (H0: drained and natural values are of the same population). For the changepoint analysis, the variance of $\delta^{15}N$ was evaluated with a linear gradient over the whole drained peat profile against the variance of three/ four separated linear gradients (rewetted part (if present), upper mesotelm, lower mesotelm, catotelm). Here, we define the starting point of the drained horizon with the onset of a shift in the δ15N values upward and the end of this horizon with the stabilization of the $\delta^{15}N$ values towards the surface.

We also determined the slopes of each single core to get information on the strength of differences of the isotopic values with depth. First, the whole peat profile of each drained core was analysed as one trend (called "overall profile"). Second, profiles were separated into different horizons: (i) rewetted horizon (if present), (ii) upper mesotelm, (iii) lower mesotelm and (iv) catotelm. If values were clearly changing with depth slopes were closer to zero. In horizons with stabilized values slopes were distinct higher or lower zero.

In the following we present only the normalized data. Raw data without normalization are available in the supplementary information.

2.5 Tree ring width and microscope analysis of peat

The investigation of the tree ring width of seven surrounding trees (Pinus sylvestris) in Degerö Stormyr was done with a hand-operated wood driller (Djos/ Sweden, 5 mm diameter). Samples were fixed on wooden carriers. The tracheids (elongated cells of the xylem of vascular plants) were cut with a sharp carbon blade and analysed with an impinging light binocular (60x – 160x amplification).

Peat samples of four study sites were analysed using an impinging light binocular (60x – 160x amplification) to get an overview of the vegetation assemblages and to differentiate horizons. For detailed information (distinction of Sphagnum species) the samples were elutriated with water, pigmented with methyl-blue and analysed under a transmitted light microscope (100x – 640x amplification).

3 Results and discussion

3.1 Depth profile of vegetation assemblage and water table defining the hydrological statuses

Following our indicators (HI, vegetation assemblages), we defined three types of hydrological statuses: (a) natural, (b) drained up to the surface, and (c) profiles with a rewetted horizon above the drained horizon (Fig. 1).

All sites, which we attributed as “natural” (type (a)), had a water table near the surface (<10 cm, section 2.1), and macro-residuals were highly visible throughout the profile, HIs were low and the main living vegetation was Sphagnum spp. (Tab.3, Tab. S4).

All drained sites had higher HIs even if no direct modifications in the vegetation assemblage could be documented. For type (b), there was little or no Sphagnum visible at the surface and the water table was found at lower depths (Section 2.1). Macro-residuals were more strongly affected by decomposition and HIs were high up to the surface. Especially the ombrotrophic-drained site (LDM) was influenced by

drainage. Here, mosses of drier environments replaced Sphagnum species or mosses were completely absent (Tab. 3, Tab.S4).

For type (c), vegetation assemblages were mainly composed of Sphagnum spp. and the water table was near the surface. HIs were low in the rewetted horizon and macro-residuals were preserved well (Section 2.1, Tab. 3, Tab. S4). With the onset of the upper mesotelm, HIs and decomposition of macro-residuals was high. In the lower mesotelm, the HIs were decreasing and more macro-residuals were visible. In the catotelm, the quality of macro residuals was higher than in the mesotelm and the HIs were even lower (Tab. 3, Tab.S4).

3.2 Tree ring width are verifying the rewetted hydrological status of Degerö

Tree ring width is a marker for the wellbeing and/or growth rate of trees. Young trees have a small circumference coupled with high growth rates, which leads to thicker tree rings. Tree rings get smaller with increasing age of the tree. If there are no environmental stressors like heat, increasing wetness or drought, tree rings are bigger and the cell lumen is higher compared to trees at sites with environmental stress. With increasing environmental stress tree ring width decreases (Stoffel et al., 2010). Before 1992, tree rings at the drained site (DD) site showed only a slightly decreasing trend, which could be due to aging (average of 1.3 mm width in the 1930s to an average width of 0.9 mm in the late 1980s). The draining ditches in Degerö Stormyr were established in the beginning of the 20th century, which supports these results, with dryer and therefore better growth conditions for trees. From 1992 onwards, tree ring widths decreased, reaching 0.2 mm in 1998 and thereafter. These results suggest a restoration to a wetter, e.g. more natural hydrological status. Rewetted hydrological conditions are not favourable for tree growth and thus lead to smaller tree ring width.

3.3 Biogeochemical parameters and hydrological status

Biogeochemical composition of peatlands strongly reflects the related hydrological status.

As typical for natural peatlands, our investigated natural sites have an average C/N ratio of 57 (Tab. S7). This is in line with results from Malmer and Holm (1984) and Kuhry and Vitt (1996) which found the C/N ratio in the acrotelm of oligotrophic peatlands to be higher than 35, mostly between 50-90. The

values in the mesotelm were lower compared to both, acrotelm as well as catotelm, most likely due to higher decomposition rates and the release of CO_2 (Tab. S7). As typical for peatlands, BD in our peatlands low due to the high amount of plant residuals in the soil and low values of mineralization (Novak et al., 2008), with 0.02 kg m^{-3} at the surface and increased with increasing decomposition and compaction of plant material downwards to 0.04 kg m^{-3} in the mesotelm (Tab. S8). BD was also increasing in the catotelm (average of 0.05 kg m^{-3}, Tab. S8), following the increased gravimetric pressure.

In contrast, the biogeochemical parameters of drained sites have a very different pattern. The lower C/N ratio in the acrotelm (average of 41, Tab. S7) and the mesotelm (average of 35, Tab. S7) indicates higher mineralization rates with gaseous release of carbon and nitrogen. In the catotelm with natural, anaerobic conditions, the C/N ratio were in the same range, as in the natural sites (average of 49, Tab. S7). BD of the acrotelm and mesotelm (average of 0.07 kg m^{-3}, Tab. S8) also increased as a consequence of the enhanced decomposition processes.

These results are in line with for the hydrology statuses indicated by the vegetation analysis (Section 3.1).

3.4 Stable nitrogen isotope depth trends as indicators for the hydrology status

While mineral soils have been shown to have continuous increasing values of $\delta^{15}N$ (Nadelhoffer et al, 1996~~;~~ Högberg et al, 1997), we found increasing $\delta^{15}N$ and $\delta^{13}C$ values with depth down to particular, isotopic specific turning points in drained peatland soils (Fig. 1). ~~We defined three types of peatland conditions from the observed depth trends of ∂15N: (a) natural, (b) drainage-affected up to the surface, (c) profiles with a rewetted horizon above the drainage-affected horizon. (fig. 1)~~

The trends of the single e~~E~~ight out of nine studied drained peatlands as well as the average trend confirm the existence of a $\delta^{15}N$ turning point. We determined a significant difference with $p < 0.05$ ~~for the difference in~~between $\delta^{15}N$ in the center of the mesotelm ~~in contrast~~compared to the $\delta^{15}N$ values in ~~undisturbed~~ undrained horizons, (Tab. S1) with a non-significant difference for one drained site: Breitlohmisse (BD_1). The latter was most likely related to generally higher $\delta^{15}N$ values of the natural site in Breitlohmisse (BN) compared to a smaller increase of $\delta^{15}N$ to the related drained site (BD_1). The

depth of $\delta^{15}N$ turning point (center of the mesotelm) differs from $\delta^{13}C$ turning point (end of the mesotelm) for all investigated sites (Fig. 2).

Changed slope values of the separated horizons indicate significant trend changes (Tab. S3). In anaerobic conditions (natural, catotelm) with stabilized isotopic values with depth, slopes were distinctly different to 0 [cm/‰]. $\delta^{15}N$ values seem to change within the mesotelm rapidly and slope values were closer to zero. Most interesting was a switch to negative trend values at the $\delta^{15}N$ turning point in all investigated drained sites, which marks the beginning of the lower mesotelm. (Tab. S3)

In a natural hydrological status (type (a)), all investigated parameters had a low variability and indicated a natural, wet mire hydrology status (Fig. 1). There were two exceptions: Breitlohmisse natural (BN) (40 - 60 cm normD) and Rotmeer natural (RN) (30 – 50 cm nromD), with trend instabilities of $\delta^{15}N$. This might indicate some minor drainage or disturbance in the wetland sites we classified as "natural" (Fig. 1).

In contrast, the values of the drained sites showed significant trends. We found two different trend types in the drained sites: Type (b) and (c) (Fig. 1). For type (b) we distinguished six sites: Lakkasuo ombrotrophic drained (LD_o), Breitlohmisse natural dry (BNd_d, Breitlohmisse drained 1 (BD1), Breitlohmisse 4 (BD_2), Rotmeer drained 1 (RD_1) and Rotmeer drained 2 (RD_2) with clear signs of decomposition up to the surface. Type (c) was visible in three sites: drained site Degerö Stromyr (DD), minerotrophic drained site Lakkasuo (LD_m) and Ursee 1 (UD). At type (c) sites the isotopic values, C/N and BD were stabilized again above the mesotelm. Therefore, they are assumed to be in a "new" natural status (Fig. 1, Fig. 2).

Below 8 cm (normD, average profile) all drained profiles showed the typical signs of the upper mesotelm with increasing values of $\delta^{15}N$, $\delta^{13}C$ and BD, down to the $\delta^{15}N$ turning point, and decreasing C/N. Below the $\delta^{15}N$ turning point, in the lower mesotelm, $\delta^{15}N$ values were decreasing. In this horizon $\delta^{13}C$ values, C/N and BD were increasing. The end of the lower mesotelm was mostly linked to a clear shift in $\delta^{13}C$ trend to either stable values or a slow decreasing trend; hence, we called this point $\delta^{13}C$ turning point (28 cm normD, average profile) (e.g. Krüger et al. 2014). Constant C/N, BD and $\delta^{15}N$ values below the $\delta^{13}C$ turning point served also as indicators for reduced compaction and decomposition. Most likely the $\delta^{13}C$ turning point marked the onset of permanent waterlogged

anaerobic conditions (e.g. Krüger et al. 2016). The similarity in trends in these deeper parts of the drained sites to those of the catotelm in the natural sites supported the assumption of an intact catotelm below the $\delta^{13}C$ turning point (Fig. 1, Fig. 2.). (For the single $\delta^{13}C$, C/N and BD values of all peat cores see supplementary information).

3.2 Depth profile of vegetation assemblage and water table in connection with isotope depth pattern

All sites, which we attributed as "natural" (type (a)), had a water table near the surface (<10 cm), and macro-residuals were highly visible throughout the profile, HIs were low and the main living vegetation was Sphagnum spp. (tab. 6, tab.7).

All drainage-affected sites had higher HIs even if no direct modifications in the vegetation assemblage could be documented. For type (b), there was little or no Sphagnum visible at the surface and the water table was found at lower depths. Macro-residuals were more affected by decomposition and HIs were high up to the surface. These results were in line with our interpretation of isotope signatures of a drainage-affected horizon up to the surface. Especially the ombrotrophic-drained site (LOD) was influenced by drainage. Here Sphagnum species seem to have disappeared and were replaced by mosses of drier environments or mosses were completely absent (tab. 7).

For type (c), vegetation assemblages were mainly composed of Sphagnum spp. and the water table was near the surface. HIs were low in the rewetted horizon and macro-residuals were preserved well (tab. 6, tab. 7). With the onset of the upper mesotelm, HIs and decomposition of macro-residuals was high. In the lower mesotelm, the HIs were decreasing and more macro-residuals were visible. In the catotelm, the quality of macro residuals was higher than in the mesotelm and the HIs were even lower.

3. Tree ring width are verifying isotope signals of changing

Tree ring width is a marker for the wellbeing and/or growth rate of trees. Young trees have a small scope coupled with high growth rates, which leads to thicker tree rings. Tree rings get smaller with increasing age of the tree. If there are no environmental stressors like heat, increasing wetness or drought, tree rings are bigger and the cell lumen is higher compared to trees at sites with environmental stress. With increasing environmental stress tree ring width decreases (Stoffel et al., 2010). Before

~~1992, tree rings at the site () site showed only a slightly decreasing trend, which could be due to aging (average of 1.3 mm width in the 1930s to an average width of 0.9 mm in the late 1980s). The draining ditches in Degerö Stormyr were established in the beginning of the 20th century, which supports these results, with dryer and therefore better growth conditions for trees. From 1992 onwards tree ring widths decreased, reaching 0.2 mm in 1998 and thereafter. These results were concurrent with the isotope analysis, because both suggest a restoration of natural wet . ewetted no longer suitable for trees and lead to smaller tree ring width according to adverse environmental conditions for tree growth. These findings underpin our suggestion of rewetted at this site in Degerö.~~

3.5 Changing ~~Linkage of microbial abundance and isotopic signature~~microbial FAs and nitrogen stable isotope depth pattern

Fungal-derived FAs (80% of all microbial-derived FAs) were the dominant fraction near the surface. In the catotelm the microbial-derived FA values were decreased down to 30% compared to the acrotelm and the mesotelm with a clear dominance of bacterial derived FAs (98%), as a consequence of the anaerobic conditions (Fig. 3).

The latter is congruent with the results of Thormann (1999), f~~F~~ungi will be outcompeted by bacteria with increasing depth and changing hydrological conditions (darker, less oxygen~~)~~). ~~Hence, fungal biomass decreases, whereas bacterial biomass increases (Thormann, 1999)~~. In the acrotelm of the natural sites, 70% less microbial-derived FA compared to the acrotelm of the drained sites confirmed the clear link between microbial abundance and the hydrological status. In contrast, we found similar values of microbial FAs in the catotelm for drained and natural sites. This suggests, that drainage did not affect the catotelm.

In the drained sites the enhanced microbial-derived FA abundance could be caused by the improved conditions for metabolism processes by drainage: enhanced oxygen abundance and relatively high nutrient availability of the prior conserved plant material (Peltoniemi et al., 2009). In the acrotelm and the upper mesotelm fungal-derived FAs were dominating (77%). At the $\delta^{15}N$ turning point lower values of fungal markers (23%) and increased bacterial-derived FAs (67%) could be found. In the lower mesotelm the abundance of microbial-derived FAs was generally decreased and 69% of the detected FAs were bacterial-derived. (Fig. 3)

3.6 Microbial metabolism mirrored by stable isotope patterns

Our findings suggest that nitrogen stable isotope values are linked to microbial abundance and diversity. We found a clear correlation for stable isotope depth pattern and microbial derived FAs in all sites (r^2=0.4), with high values of nitrogen stable isotopes being linked to high amounts of microbial derived FAs.

Generally, plants are depleted in ^{15}N compared to atmospheric nitrogen (which is, per definition, 0 ‰, because air is used as the nitrogen isotopic standard) due to the general preference of plants for the lighter isotope ^{14}N. As such, the average signal of the relatively undecomposed peat (e.g., the acrotelm of the natural/rewetted sites, the catotelm) is -10 to -4 ‰. These plant signals are imprinted in the acrotelm (average of -6.09 ‰; Tab. S5). Furthermore, $\delta^{15}N$ values of plants (here mostly sphagnum mosses) are lower than the values of microbes and bulk material (Aldous, 2002; Lichtfouse et al. 1995). Microbes prefer to mineralize the lighter ^{14}N and plants incorporate (and therefore extract) the microbial mineralized lighter nitrogen (Dijkstra et al., 2006; Novák et al, 1999). Contrary to plants, microbial biomass is enriched in ^{15}N, probably as the result of processing and releasing the lighter ^{14}N during mineralization and hence sequestering the remaining heavier ^{15}N. In addition, caused by the preferential mineralization of lighter nitrogen, the heavier ^{15}N might be also enriched in the remaining humic substances (Novák et al, 1999). The effect of the latter to $\delta^{15}N$ bulk values is probably also enhanced due to the loss of ^{15}N-depleted material during leaching (Damman, 1988; Niemen, 1998), denitrification and the release of gaseous nitrogen (Kohzu, 2003; Niemen, 1998). Our values confirm these reported patterns with highest $\delta^{15}N$ values in the mesotelm (average of -3.63 ‰; Tab. S5) and the correspondence of high microbial activity (reflected by the highest values of microbial-derived FAs) to the $\delta^{15}N$ turning point (Fig. 3, Fig. 4). In acid bogs under aerobic conditions, fungi will dominate the general metabolism in upper peat soils (Thormann et al., 2003). This is pictured by the highest amount of fungal-derived FAs in the acrotelm and the upper mesotelm (Fig. 3). Fungi are preferred decomposers of primary plant material (Wallander et al., 2009; Thormann et al., 2004) hence the depleted plant isotopic signal is relatively preserved in the upper most aerobic horizons. Furthermore, fungi have a relatively low nitrogen demand compared to bacteria (Myers et al. 2012). With increasing depth and increasing oxygen limitation fungal metabolism decreases (Thormann, 2011). In parallel, the

amount of bacterial-derived FAs increases (Fig. 3) as Lin et al. (2014), Hu et al. (2011) and Bauersachs et al. (2009) also reported. They found evidence for bacterial-dominated decomposition in hypoxic conditions. This is in line with the findings of Kohl et al. (2015) and Schmidt and Bölter (2002), who also reported a switch from fungal to bacterial dominance in the mesotelm. Also Andersen et al (2013), Wallander et al. (2009), Winsborough and Basiliko (2010) and Myers et al. (2012) stated out, that fungal biomass is decreasing in peatland soils with depth. In addition, bacterial metabolism ~~needs higher amounts of nitrogen~~is generally faster than fungal metabolism and needs higher amounts of nitrogen (Brunner et al., 2013). ~~In summary, with increasing depth and increasing bacterial metabolism most of the available nitrogen will be immobilized, which results in no or low fractionation of the bulk material (e.g., no preferential loss of the lighter N). Hence, the N turning point could be caused by the N limitation of peatland ecosystems with low oxygen availability.~~ We assume that ~~the~~ ~~δ^{15}N turning point~~ bacteria and fungi compete most over decomposable substrates (not ~~necessarily~~ only nitrogen) at the δ^{15}N turning point~~)~~, resulting in the highest turnover rates with an enrichment of δ^{15}N in the remaining peat, similar to reports ~~as we know~~ from mineral soils with aerobic decomposition (Alewell, et al. 2011~~;~~, Nadelhofer, et al 1996). ~~Tfaily et al. (2014) also reported the highest N values within the mesotelm. This pattern is reflected of derived s at the N turning point (. 3).~~As such, we assume that besides the highest microbial activity, also the diversity of microbial metabolism peak at the δ^{15}N turning point (Fig. 4). This would also be related to the highest δ^{15}N values, because (1) different microbial communities prefer different sources (Dijkstra et al. 2006; Dröllinger et al. 2019) and (2) with increasing bacterial abundance, fungi have to also use recalcitrant (isotopically lighter) sources, because bacterial metabolism will outcompete fungi for the easily degradable substances (Rousk and Bååth, 2007; Winsborough, C. and Basiliko, 2010). Hence, with increasing microbial diversity also the diversity of the mineralized organic fractions increases (Thormann 2006).

To summarize, with an increased diversity of utilized nitrogen sources, more release of lighter ^{14}N is possible and the δ^{15}N values in the remaining substrate should increase (Fig. 3, Fig.4). However, because of the faster and more complete decomposition with increasing microbial activity (Damman, 1988), metabolism of ^{15}N increases as well and fractionation will be less (Lerch et al., 2011). These

contrasting patterns must lead to only small increases in the $\delta^{15}N$ values of the bulk material, if all nitrogen are used, fractionation will be lower at the $\delta^{15}N$ turning point.

In the lower mesotelm, oxygen limitation increases, leading to a general decreas~~eing~~ in microbial metabolism and ~~decreasing~~ related concentrations of microbial-derived FAs (Fig. 3). The decreasing microbial metabolism leads to simultaneously decreasing $\delta^{15}N$ values because an increasing amount of intact vegetation (with low $\delta^{15}N$ values) will be conserved (Fig. 3, Fig. 4).

Finally, with the establishment of permanently waterlogged anaerobic conditions in the catotelm (also indicated by the $\delta^{13}C$ turning point), FA concentration decreases sharply to near zero values. Here, decomposition processes are largely inhibited, which leads to stable $\delta^{15}N$ and $\delta^{13}C$ values, close to the original vegetation signals (Alewell et al., 2011; Krüger et al, 2015) (Fig. 1, Fig3).

4. Conclusion

Our results confirmed that ~~show significant differences in~~the nitrogen isotopic depth trends of peatlands are suitable indicators of the natural, drained ~~and~~ or rewetted ~~peat profiles~~hydrological status. We validated our isotopic hypothesis with microscope analysis of the vegetation remains in the cores as well as the investigation of tree rings as indicators for changed hydrological status in the past. An analysis of gram-positive and gram-negative bacterial-derived FAs versus fungal- derived FAs underpinned our hypothesis with the expected changes in microbial abundance with depth. The aerobic acrotelm was characterized by a high fungal abundance with low nitrogen demand and turnover. The upper mesotelm was the transition to a mixture of decreasing fungal and increasing bacterial abundance, competing on organic substrates and resulting in an enrichment of $\delta^{15}N$ values. In the lower mesotelm microbial decomposition generally decreased, but was dominated by bacterial abundance and finally microbial metabolism was strongly impeded and $\delta^{15}N$ values stabilized in the anaerobic catotelm.

Carbon isotope compositions are also changing with drainage, but they are neither a suitable indicator for a switch in microbial abundance within the drained horizon, nor for the trend induced by ~~with~~ rewetting of the peatland~~, as it is visible for the nitrogen compositions due nitrogen limitation and recycling processes~~. Summing up, $\delta^{15}N$ depth profiles in peat might give more insights into ~~the degree of~~a switch of microbial metabolism~~transformation~~, because they reflect more precisely different

microbial abundance than carbon isotope compositions. Therefore, we conclude that $\delta^{15}N$ depth profiles could act as a reliable and efficient tool to get fast and easy information about the hydrological~~y~~ status, restoration success and drainage history.

Author contribution

~~Christine Alewell and Jens Leifeld are the supervisors of the project.~~ Miriam Groß-Schmölders: sampling, measurements, evaluation and analysis of data, manuscript writing

~~,~~ Jan Paul Krüger: sampling and measuring

~~,~~ Axel Birkholz: measurements, help in analytics

~~and~~ Kristy Woodard: discussion ~~were doing the measurements~~

Pascal von Sengbusch: peat ~~was doing the~~ microscopy and vegetation analysis. ~~Miriam Groß-Schmölders prepared the manuscript with contribution of all co-authors.~~

Jens Leifeld: project idea, supervision and discussion

Christine Alewell: project idea, supervision, discussing and writing

Competing interests

The authors declare that they have no conflict of interest.

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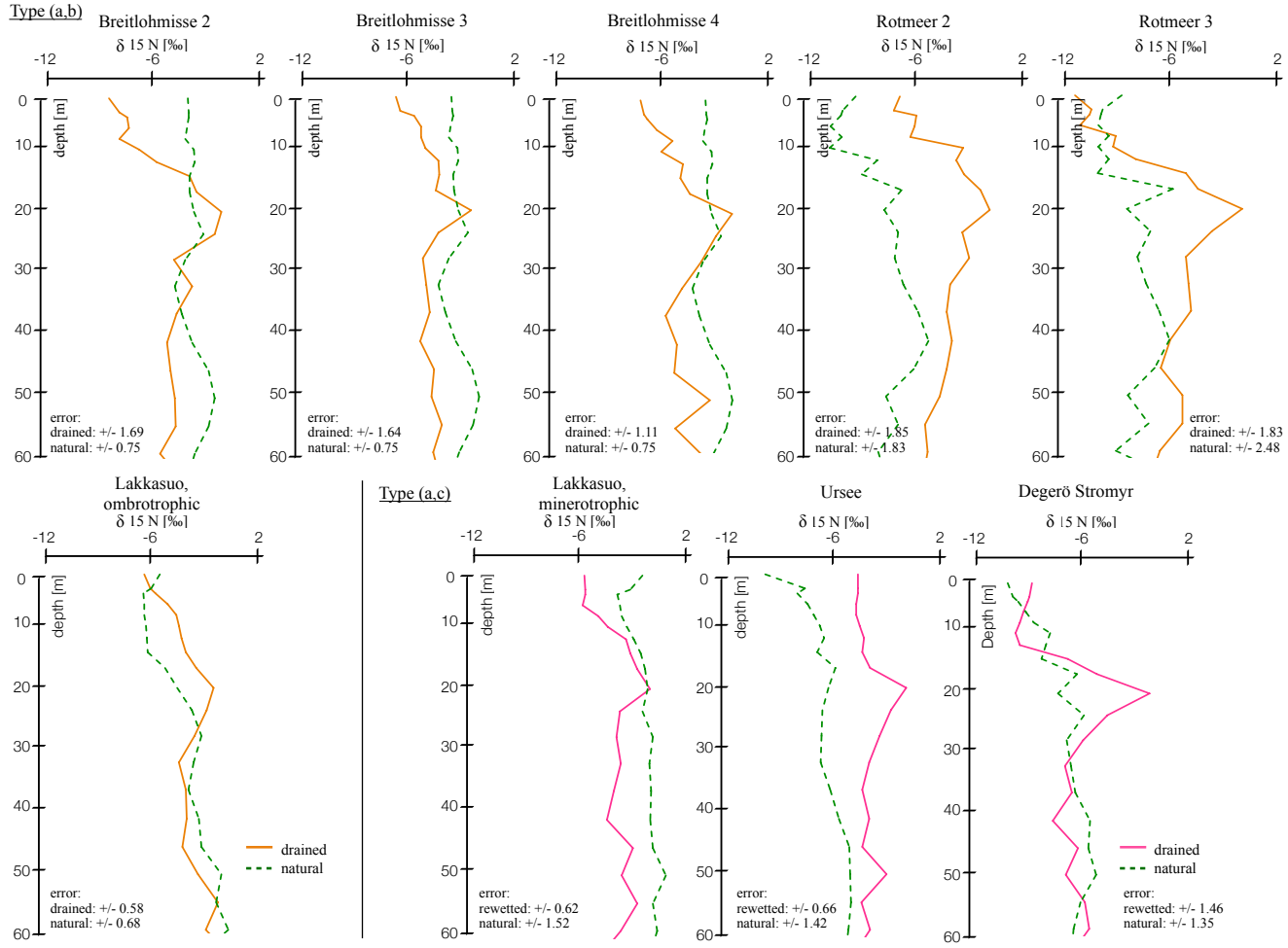

Figure 1: $\delta^{15}N$ depth profiles in all natural and drained (or rewetted) sites; with normalized depth and normalized $\delta^{15}N$ values (see chapter 2.4); trend types: (a) natural (green), (b) drained up to the surface (orange) and (c) rewetted above drainage (pink) (For single, non-normalized values see supplementary information).

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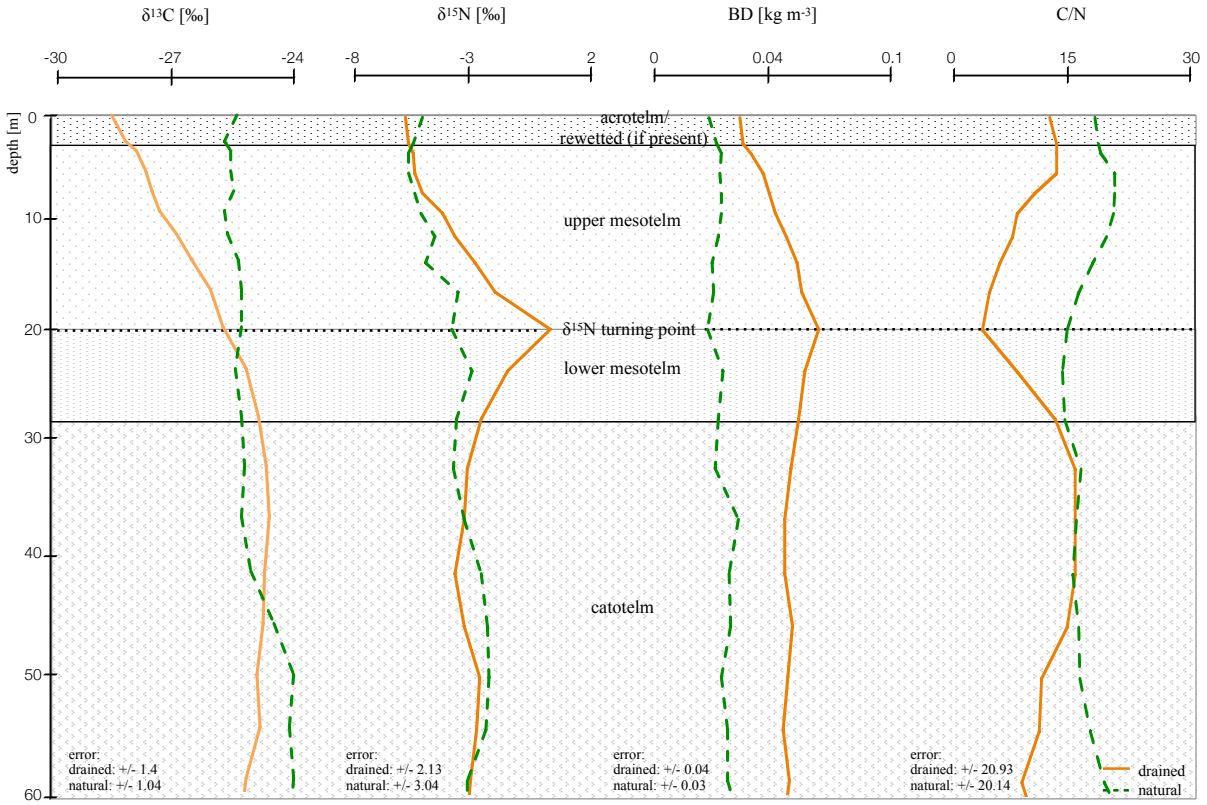

Figure 2: Mean depth trends ($\delta^{15}N$, $\delta^{13}C$, C/N and BD) of natural and drained sites of all nine investigated peatlands with normalized depth and normalization based on $\delta^{15}N$ compositions (see chapter 2.4; For single $\delta^{13}C$, C/N and BD values of all peat cores see supplementary information).

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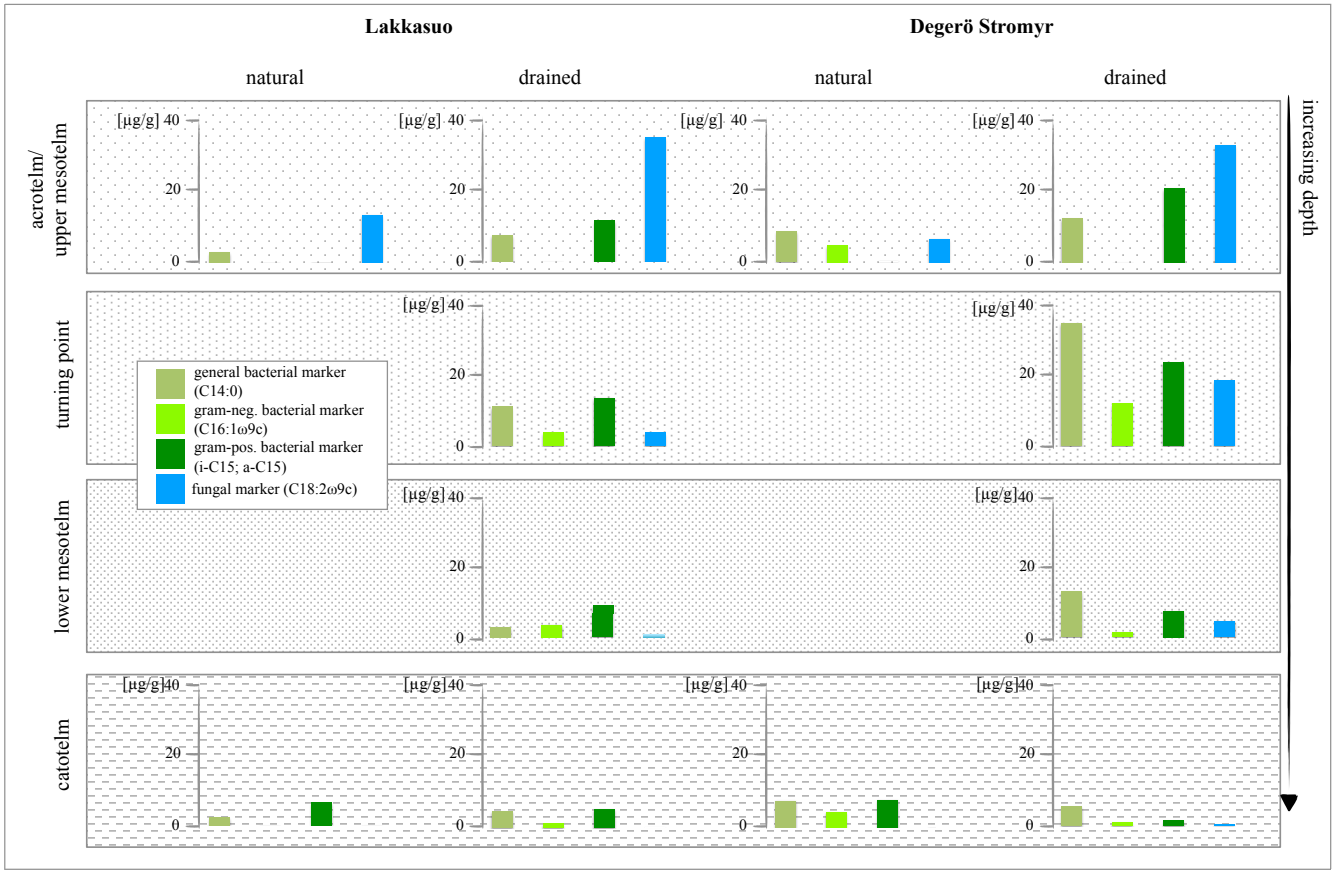

Figur[1]e 3: Fatty acid ~~A amount~~concentrations ~~-~~of bacterial and fungal marker in natural and drained wetlands Lakkasuo and Degrö Stromyr~~LD$_0$, LN$_0$, DD and DN~~ [2]in different horizons.

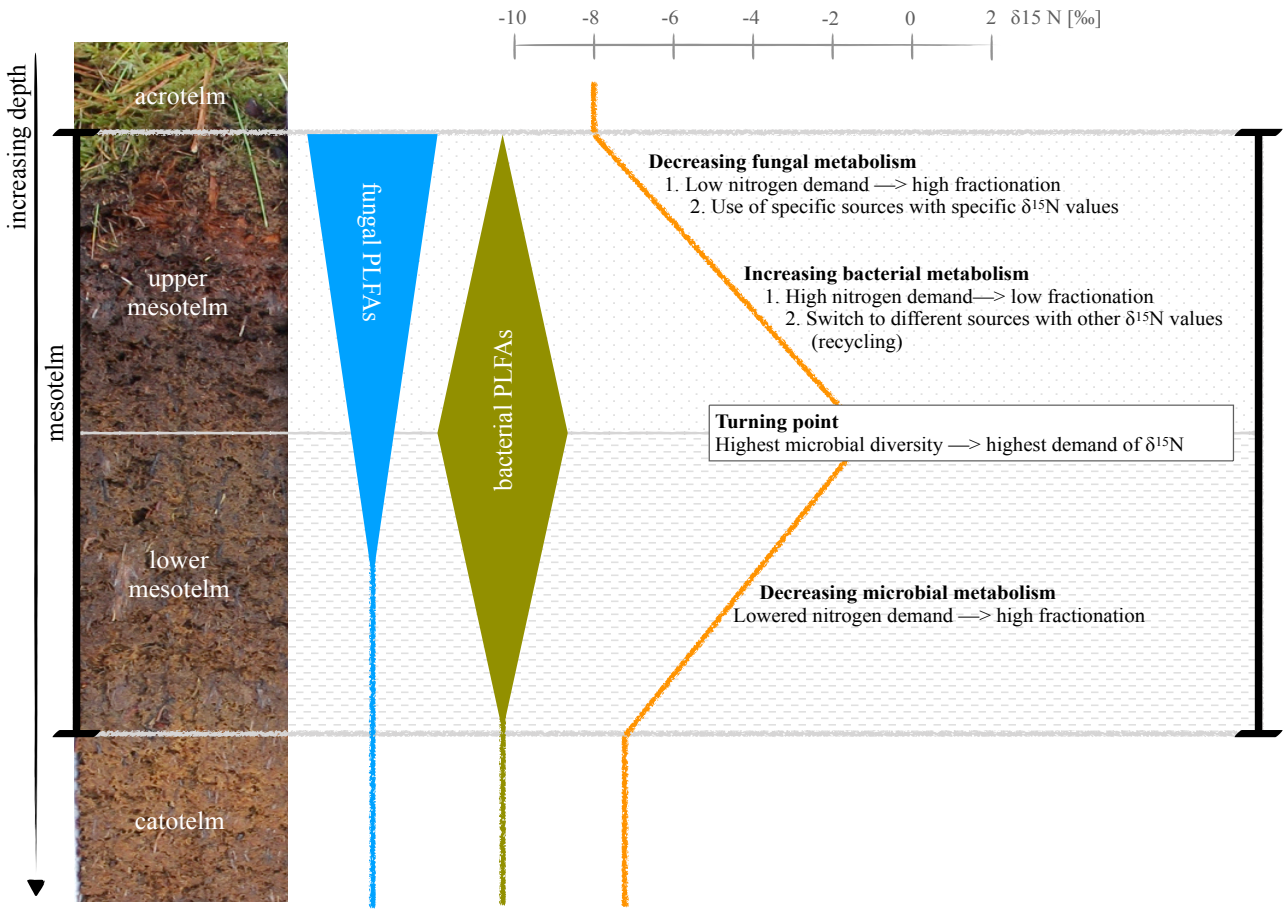

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Figure 4: Hypothesis of a microbial switch (fungi to bacteria) with depth, reflected by specific FAs, and its influence of the $\delta^{15}N$ depth trend; example photo and $\delta^{15}N$ values of the ombrotrophic, drained site in Lakkasuo (LD_0) (note all isotope values are normalized to zero at turning point).

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Table 1: Labeling of all drilling sites

Location	Labeling
Degerö	
natural mire	DN
drained	DD
Lakkasuo	
minerotrophic natural	LN_m
minerotrophic drained	LD_m
ombrotrophic natural	LN_o
ombrotrophic drained	LD_o
Breitlohmisse	
natural mire	BN
natural dry	BN_d
drained	BD_1
near the mire edge	BD_2
Rotmeer	
natural mire	RN
drained, with Sphagnum	RD_1
drained, without Sphagnum	RD_2
Ursee	
natural mire	UN
drained	UD

Table 2: Overview of studied mires; coordinates (lat./long.); mean annual temperature (MAT); annual precipitation (P); Sphagnum mosses (Sph.) (Laine et al., 2004; Nielsson et al., 2008; DWD 2018, Alexandersson et al., 1991; Armbruster et al., 2003)

Country	Mire	lat/long..	MAT	P	Main vegetation on top	
			[°C]	[mm]	natural	drained
Sweden	*Degerö Stromyr*	64°11'lat., 19°33'long.	+1.2	523	Sph. majus	Sph. balticum
Finland	*Lakkasuo*	61°48'lat., 24°19'long.	+3	700	Sph. angustifolia	Sph. angustifolia
Germany (Black Forest)	*Breitlohmisse*	48°41'lat., 8°25'long.	+7	835	Sph. capillifolium	Sph. capillifolium
	Ursee	47°51'lat., 8°25'long.	+7	1600	-	-
	Rotmeer	47°52'lat., 8°6'long.	+7	1600	Sph. rubellum	Sph. rubellum patches

Table 3: Description of vegetation of four of the study sites; Sphagnum mosses (Sph.)

Site	Horizon	Main species	Description
Degerö (DD)	rewetted horizon	*Sph. balticum*	Yellow, good preserved Sph.-turf, detached Sph. cymbifolia, *Vaccinium oxycoccos Eriophorum vaginatum, Andromeda polifolia* & *Cladopodiella fluitans*
	mesotelm	*Sph. balticum*	Darker, grayish; Sph.-turf, some *Eriophorum vaginatum,* detached Sph. cymbifolia, *Vaccinium oxycoccos, Andromeda polifolia*
	catotelm	*Sph. balticum*	Yellow; Sph.-turf, more Sph. cymbifolia, some *Eriophorum vaginatum,* detached, *Vaccinium oxycoccos, Andromeda polifolia*
Lakkasuo (LD_o)	upper mesotelm	*Sph. rubellum*	Dark brown; Sph.-turf, mostly *Sph. rubellum* with *Pleutrozium schreberi* in the uppermost part
	lower mesotelm	*Sph. rubellum*	Dark brown, grayish; Sph.-turf, mostly *Sph. rubellum* and *Sph. balticum*
	catotelm	*Sph. rubellum*	Light brown, yellow; Sph.-turf, mostly *Sph. rubellum*
Breitlohmisse (BN_d)	upper meostelm	*Sph. capillifolium*	Brown; Sph.-turf mostly *Sph. capillifolium* and *Sph. cymbifolia*, much *Ericaceous roots* and some *Eriophorum vaginatum* stems
	mesotelm	*Sph. cymbifolia*	Dark brown; Sph.-turf mostly *Sph. capillifolium* and *Sph. cymbifolia*, some *Ericaceous roots* and *Eriophorum vaginatum*
	catotelm	*Sph. cymbifolia*	Lighter, reddish; Sph.-turf mostly *Sph. capillifolium* and *Sph. cymbifolia*, some *Sph. acutifolia*
Rotmeer (RD_1)	upper mesotlem	*Sph. acutifolia*	Brown-reddish, yellow; Sph.-turf, mostly *Sph. acutifolia*, some *Sph. rubellum,* detached *Eriophorum vaginatum*
	mesotelm	*Sph. cymbifolia*	Dark brown, grayish; Sph.-turf, mostly *Sph. cymbifolia*, and *Sph. acutifolia*, some *Sph. rubellum,* detached *Eriophorum vaginatum*
	catotelm	*Sph. cymbifolia*	Reddish, yellow; Sph.-turf, mostly *Sph. cymbifolia*, some *Sph. acutifolia,* detached *Eriophorum vaginatum*

Dear Referee,

Thank you, for your helpful comments, which will help to improve our paper considerably.

1.The authors' central hypothesis ('maximum 15N enrichment at maximum microbial diversity) seems to come out of nowhere, and it is unclear to me how the authors came up with this hypothesis except as a post-hoc justifying their results. I do not see why greater microbial diversity should necessarily imply greater nutrient limitation as it matters little to the nutrient whether it is taken up by fungi or bacteria (fungi compete with themselves as much for nutrients as with bacteria and vice versa, abundance does not equal activity, etc.). It is also unclear to me how this conclusion is supported by the presented data, which shows that the 15N maximum occurs at the same depth of the change fungal to bacterial dominance, but does not provide evidence that one is related to the other. I don't see why these changes in microbial community composition would provide evidence for greater nitrogen limitation at the depth of the 15N maximum. However, I'm not sure if this rather speculative interpretation of 15N being driven by microbial community composition is actually needed in this paper – I think de- scribing the differences between drained and undrained peatlands provides valuable information by itself.

Answer:
Influence of microbial diversity: Regarding your comment on microbial diversity and increased $\delta^{15}N$ values, we are sorry for being so unclear; we will be more precise with the description of the assumed relationship between the two parameters microbial metabolism processes and $\delta^{15}N$ values. We hypothesize that the microbial abundance (see answer 3) as well as the microbial community composition has an influence on the $\delta^{15}N$ values. With an increasing diversity, $\delta^{15}N$ values of the reaming substrate should increase, because (1) different microbial communities prefer different sources (Dijkstra et al. 2006, Dröllinger et al. 2019) and (2) with increasing bacterial abundance, fungi have to use also recalcitrant sources, because bacterial metabolism will outcompete fungi for the easily degradable substances (Rousk and Bååth, 2007, Winsborough, C. and Basiliko, 2010). Hence, with increasing microbial diversity also the diversity of the mineralized organic fractions increases (Thormann 2006). With an increased diversity of nitrogen sources more release of lighter ^{14}N is possible and the ratio of ^{15}N:^{14}N in the remaining substrate should increase. We realize that these are hypotheses to explain the observed patterns, but to our understanding they are the most likely ones and in describing them, we hope for an eager discussion in the community.

We have implemented this to the manuscript in section 3.6 (L501-508).

2. I think the manuscript could be improved by presenting/discussing the results in a different way. In the present version, the author very much focus on changes in d15N and other parameters with depth. I would recommend to start by comparing drained and undrained soils – I think it would be helpful if the authors first identify how drainage has changed the parameters measured in the soil profiles (e.g., drainage increase 15N values in the mesotelm). The authors can then discuss which processes led to an increase in 15N in drained peatlands (relative to intact neighbor sites), and

why these processes were strongest in the center of the mesotelm and less pronounced towards the surface and the catotelm edge.

Answer:
Different way to present: We have followed your advice and structured our results accordingly. We will start, as you suggested, with an overview of drained and undrained peatlands and the influence of hydrology on the measured biogeochemical parameters. This will be followed by an introduction to the processes, which leads to increased $\delta^{15}N$ values, and might explain the observed pattern in the mesotelm.

We have implemented these changes and restructured chapter 3 accordingly.

3. The authors could also improve the manuscript by providing a more detailed view on the processes that cause the N isotope fractionation in these soils. In particular, they do not propose a fate for the 14N-depleted nitrogen fraction. How does this carbon get lost from the soil profile (in drained relative to intact peatlands)? It does not simply get transported downwards in the soil profile as no large difference in d15N was observed in the catotelm (Fig. 2). Mineralization is a likely mechanism, does that mean that more depleted 15N is leached out of the soil profile and exported from the peatland? Or are there stronger gaseous losses (N2O, denitrification) in drained peatlands? What is the role of plant and microbial uptake of 15N in this process?

Answer:
Nitrogen cycling: We for sure do not claim that we fully understand the observed patterns yet, but that we see consistent patterns and (in combination with the fatty acid analysis) develop ideas what the origin of these patterns might be. Sorry, if this was not clear from the manuscript, we will add a sentence referring to this. We will further insert a section about N isotope fractionation in peatland soils and the underlying processes, which might be leading to ^{14}N depletion in the remaining substrate during drainage. In general, the ^{15}N:^{14}N ratio of plant material (here mostly sphagnum mosses) is lower than the values of microbes and bulk material (Aldous, 2002, Lichtfouse et al. 1995). Microbes prefer to mineralize the lighter, more frequent ^{14}N (Dijkstra et al., 2006, Novák et al, 1999). Since plants incorporate the microbial mineralized nitrogen they have a low ^{15}N:^{14}N ratio (Lichtfouse et al. 1995). Contrary, microbial biomass is enriched in ^{15}N, probably as the result of processing the lighter ^{14}N during mineralization and hence incorporation of the remaining heavier ^{15}N. In addition, caused by the preferential mineralization of lighter nitrogen, the heavier ^{15}N might be enriched in the remaining humic substances (Novák et al, 1999). The effect of the latter to ^{15}N:^{14}N bulk values is probably enhanced due to the loss of ^{15}N-depleted material during leaching (Damman 1988, Niemen 1998), denitrification and the release of N_2O (Kohzu 2003, Niemen 1998).
In natural peatlands, microbial activity is low and mostly visible in the uppermost, aerobe part of the peat (acrotelm). With the onset of the waterlogging, anaerobe conditions in the catotelm microbial activity is inhibited. This leads to small or even negligible changes of the original (light) plant isotopic ratio below the acrotelm. (Dijkstra, 2008)
In contrast, in drained peatlands the aerobic mesotelm expands and simultaneously microbial activity increases (Moore & Basiliko 2006, Roswell 1976). In an extended mesotelm a higher amount of mineralization and the release of N_2O takes place. With

increased mineralization the ^{15}N:^{14}N ratio in the remaining substrate should increase, as long as ^{14}N will be mineralized preferentially (Dijkstra, 2008). However, because of the faster and more complete decomposition with increasing microbial activity (Damman, 1988) metabolism of ^{15}N increases as well and fractionation will be less. This pattern leads to only small increases in the ^{15}N:^{14}N ratio of the bulk material, as all isotopes are used and fractionation is lowered in the middle of the mesotelm, where microbial activity is the highest. Actually, the best way to test for the combined effects of all these different processes on the isotopic fingerprinting would be to set up a conceptual model. However, we feel this is beyond our possibilities at the moment, but we certainly look for opportunities (e.g., cooperation) in the future.

We have implemented this mainly in chapter 3.6 (L463 – 514).

4. PLFA analysis: The authors use a non-standard method to extract/purify/derivatize PLFAs for analysis. While this is not a problem in itself, this method looks like a total fatty acid extraction to me. At least, it extracts and recovers free fatty acids (as shown by the use of the internal standard nonadecanoic acid). Please provide information how phospholipids were separated from glycolipids and neutral lipids in this method.

Answer:
FA analysis: You are totally right. We have extracted all membrane fatty acids and did not separate phospholipid fatty acids. We are really sorry for this incorrect classification in the first version of our manuscript. We aimed to distinguish between fatty acids of microbes, fungi and plants and we were able to detect these changes by the extraction of total membrane fatty acid values, because the used markers (i-C15:0 and a-C-15:0 for Gram positive - bacteria and C18:2ω9c for fungi) are not restricted to phospholipid fatty acids (Bajerski, Wagner & Mangelsdorf 2017; Finotti et al. 1992; Piotrowska-Seget & Mrozik 2003).

We have implemented this in fatty acid sections 2.3, 3.5 and for the whole manuscript, especially for L 27-36 and L128-138.

Some language issues: - I would prefer the more descriptive term 'maximum' rather than 'turning point', which implies some change in direction in processes. I think this would also improves the clarity in a central point of the manuscript.

Answer:
Language issue: You are right, "maximum" would also be a very good term for our observed pattern, but we decided to use "turning point" because if we compare different sites and layers, a maximum in one site or depths layer might not be the absolute maximum, which leads to confusion. Furthermore, what we are really looking at are changes in depth trends. As such, we think, turning point is a better term. Furthermore, we already used the term already to describe the observed isotope patterns in some previous publications and would thus like to stay with it.

L16: 'stable isotope signatures': See this advice on 'Isotope terminology' from Z. Sharp's 'Isotope Geochemistry' book (https://digitalrepository.unm.edu/unm_oer/1/): Mistake: "The isotopic signature of the rock was d18O = 5.7‰"' Recommended Expression"Thed18Ovalueoftherockwas5.7‰T husthisrockhastheoxygenisotope signature of the mantle." Explanation: "The word signature should be used to

describe the isotopic composition of a significant reservoir like the mantle, the ocean, or a major part of the system being studied, not to the isotopic composition of ordinary sample" - L311: 'equilibrium' between fungi and bacteria – I don't think equilibrium is the correct concept here. Maybe change from fungal to bacterial dominance, but even that is not

Answer:
Thank you for the explanation for the word "signature", we have changed it to "composition". In addition, we deleted the term "equilibrium" and have decided to use "change towards higher bacterial decomposition".

Minor comments: L12-15: The first three sentences have very little to do with the content of this manuscript. L304-306: highly speculative and not well referenced. Figure 2: check axis ticks for BD and C/N, start these axis at 0. Tables 3-6 could be places in a supplement.

Answer:
L12-15: We rewrote the sentences.
L304-306: We added references to these sentences (Lerch et al., 2011; Rousk and Bååth, 2007).
Figure 2: The axes start now with 0 and ticks are checked.
Tables 3-6 will be placed in the supplement.

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Dear Referee,

Thank you, for your helpful comments, which will improve our paper considerably.

1. To me, the Introduction is the part of the manuscript that requires the most attention. The research methods and questions need to be prepared in more detail and especially the role of roots and mycorrhiza should be considered in more detail.

Answer: We have rewritten the introduction and have insert a more detailed part for research methods and questions (see also answer 7). You are right; we have not sufficiently discussed the expected role of roots and mycorrhiza. We are sorry for that and have now complemented a paragraph with it. Our study sites are open peatlands with a small amount of vascular plants (result of our vegetation analysis). Hence, mycorrhiza should also play a minor role. But of course, there might still be an effect of mycorrhizal activity and rooting in our study sites. Mycorrhiza mediate the uptake of nitrogen into plants. Rooting and the existence of mycorrhiza leads to enriched ^{15}N values in the remaining bulk material (Högberg et al., 1996), because (1) mycorrhiza preferentially process lighter ^{14}N and transfer them to plants (Adams and Grierson, 2001, Asada et al., 2005a, Högberg et al., 1996, Kohzu et al., 2003, Robinson et al., 1998) and (2), even without mycorrhizal activity, plants preferentially incorporate the lighter ^{14}N. But, because of the small amount of vascular plants and therefore also of mycorrhiza, these cannot not be the main drivers for our observed pattern.

We have implemented this in the introduction (L113-120).

2. For consideration in the journal SOIL, the soil type/classification need to be adequately described.

Answer: You are right, we have forgotten to describe the soil type adequately and have inserted this classification to the manuscript. All investigated sites are classified as Histosols (organic soils). Histosols are classified as soils with a cumulative organic layer and an organic matter amount of 35% or higher in at least half of the uppermost 80-100 cm (IUSS, 2015). In addition all investigated peatland soils are Sphagnum peat, because of their mean annual temperatures (between +1.2°C and +7 °C) and their annual precipitation between 523ppm and 1600ppm (Eurola et al., 1984, Vitt et al., 2006). Lakkasuo and Degerö Stormyr are classified as Northern eccentric bogs and the peatlands in the black forest are characterized as ombrotrophic bogs (Eurola et al., 1984).

We have implemented this in section 2.1 (L160-165; L169, L175).

3. In some figures, but also text the acrotelm-mesotelm designations are an issue: Is there an acrotelm or not? Figure 3 shows an acrotelm/upper mesotelm, but in the remainder of the manuscript, an acrotelm is not mentioned. On the other hand, an "upper" and "lower" mesotelm are introduced. Please find a consistent way to handle the issue.

Answer: We are sorry, for not being clear enough with our definition. Yes, there is an acrotelm and a mesotelm in all sites. We have forgotten to mention this in figure 3 and have changed it accordingly. The acrotelm is the uppermost part of the peatland with living sphagnum vegetation (Morris et al., 2011). The deeper part, with dead plant material and in permanent waterlogged, anaerobe conditions, is called catotelm (Morris et al., 2011). In between the acrotelm and the catotelm, with fluctuating conditions, the mesotelm is located (Clymo and Bryant, 2008, Lin et al., 2014). With drainage, the mesotelm is expanding and a supplementary separation is reasonable, because the condition within the mesotelm differ a lot from aerobe, light and warm conditions (upper mesotelm) to semi-oxic, dark and cold conditions in the lower mesotelm (Artz, 2014, Lin et al., 2014). These changed conditions are the reason for the changed microbial metabolic pathways and are therefore critical for the 14N:15N ratio we see in the data sets (Lin et al., 2014).

We have implemented this in Figure 3 and to the introduction (L50-63).

4. Please check the manuscript again for signs of sloppiness: Throughout the manuscript, the abbreviations for Tables and Figures are inconsistently spelled; sometimes capitalize and sometimes not. Somewhere in the text, I quit nothing this in "Specific comments". In the references section, journal titles are generally spelled out, but sometimes not. Please edit following journal guidelines

Answer: Thank you for pointing this out. We have checked and deleted mistakes in tables and figures as well as in the reference section.

5. Specific comments: L12; L14; L16; L28; L32; L74; L219; L221; L247; L250; L287; L246; L443; L444

Answer: Thank you for your careful and constructive review of the manuscript. We have changed and improved the mentioned sentences.

6. L38-39: There are quite a few approaches to describe "peatland condition". But what is "peatland condition"? And are the methods you are proposing more time and cost efficient than others? You are hypothesizing that 15N isotopes could be such a tool. Fine, but PLFA analysis isn't that cheap and you are also heavily relying on that method. Please explain in more detail.

Answer: With the wording "peatland conditions" we are referring to the hydrology status, whether it is natural, drained or rewetted. We have changed the wording to hydrology status. You are right; FA analysis is not a cheap and easy method. We have done this analysis to support our hypothesis based on stable isotopes, and only the latter we refer to as a time- and cost efficient method to indicate drainage and rewetting. We do not suggest establishing an approach as a routine analysis, which uses both methods. The three main methods today to measure the hydrology status are

(1) a macro analysis of peatland vegetation, (2) gas emission measurements and (3) measurement of growth heights of peatland vegetation. Method (1) was also done in this study. We wanted to prove, that our investigated stable isotope patterns are related to decomposition and that they are not primarily a consequence of the vegetation assemblages. But this method is time consuming and needs a high level of expert knowledge and is thus very costly. Method (2), the measurement of gas exchange in peatlands (Baldocchi et al., 1988) measures current gas emissions and therefore provides an indirect measurement of ongoing decomposition processes (Bubier et al., 2003). But it is not able to give information on drainage history and gas exchanges at another time of the year (Bubier et al., 2003). Furthermore, this method is also very intensive in analytical equipment and expert knowledge needed. A third available method (3) is the measurement of the growth of peatland vegetation. But there are several problems with this method: Firstly, not only the sole growth of mosses indicates peat growth. It is important how much vegetation material enters the catotelm and is therefore stored under aerobe conditions. Secondly, peat shrinks and swells with water supply. Hence measuring peat height at different times would lead to completely different assumptions for peatland growth (Clymo, 1970). And thirdly, peat growth is really slow and it would need decades to get a positive reply with this method to indicate successful restoration efforts (Clymo, 1970, Fenton, 1980). Summing up, there are methods available to get information of the success of restoration effort, but these methods are lacking some important information or/ and are expensive and time consuming. Hence, there is a need for a new and less expensive and time-consuming indicators, which could be done not only by specific experts. We believe that bulk isotopes can be such suitable indicators, but we need to prove that with the FA method.

We have implemented this in the introduction (L64-92).

7. This is a general phenomenon Introduction chapter in general: Biogeochemical transformations as a consequence of rewetting re not introduced, but in the last paragraph of the introduction, you are looking for changes of 13C and 15N with the onset of the rewetting process.

Answer: Thank you, for your comment. You are right; we have missed to introduce our hypothesis of the influence of rewetting to stable isotopes. Rewetting increases the water table height and therefore enlarges the anaerobe catotelm (Andersen et al., 2006). We hypothesize, that the observed stable isotope pattern for drained horizons will be conserved, when formerly aerobe parts will get rewetted (Andersen et al., 2006). With rewetting the conditions in the former mesotelm will get anaerobe and microbial activity will be inhibited (Andersen et al., 2006, Asada et al., 2005b, Thormann et al., 1999). Hence, no or only few metabolism processes take place and stable isotope patterns shouldn`t change anymore. For the upper part of the rewetted peat, we expect to find natural conditions and vegetation growth, like in natural peatlands. Hence, we expect to find the same stable isotope pattern, as we see in natural peat.

We have implemented this in the introduction (L60-64)

8. L70-80: This paragraph should be rewritten. It lists methods, but the aim/objective/hypothesis is not sufficiently clear. Many methods are listed without

having been introduced before. Please introduce these methods. When looking at d15N, not only decomposition must be considered, but also mycorrhizal activity. Are you expecting root effects on d15N?

Answer: We have improved the wording of the mentioned paragraph and inserted an introduction to the mentioned methods (bulk density and carbon:nitrogen ratio measurements). Our aim is to find an answer for the depth trends of carbon and nitrogen stable isotopes corresponding to the hydrology status, which were investigated in previous studies. Our main hypothesis is that microbial metabolic pathways are the drivers behind these stable isotope depth trends. The hydrology status determines the abundance of microbial communities. With changing hydrology microbial abundance changes significantly (Kohl et al., 2013) and therefore also stable isotope values must change (Tfaily et al., 2014). Vice versa this would mean that stable isotope values reflect the hydrology status, which we aim to test. We hypothesize, that drained conditions lead to expanded microbial abundance, because of the attendance of oxygen also in deeper horizons. We aim to find significant links between this pattern and the observed stable isotope pattern. For natural and rewetted conditions we hypothesize to find low values of stable isotopes in accordance to low microbial abundance.

We have restructured and revised the whole introduction.

For mycorrhizal- and rooting effects please see answer 1.

9. Chapter 2.2. Coding of the sites is inconsistent. Some codes appear to relate to minerotrophic or ombrotrophic hydrology or drained vs. natural status, but others don't. Please code in a consistent way. Chapter 2.3: What does LOD1, LON3, DDC3, DNM1 mean? Did you take replicate cores at these sites? From which depth were samples taken?

Answer: We have changed the coding, to be more consistent. We have now named them as follows: first letter of the site + hydrology status (plus with subscript, if needed, for additional information) (Tab.1). Yes, we had three replicates per site and analyzed samples of the upper 60 cm of the cores. The cores were sliced in 2 cm sections and every second layer was analyzed, giving a 4 cm depth resolution. We have mentioned it in section 2.2 (L134/135 and L148).

Table 1: Labeling of all drilling sites

Location	Labeling
Degerö	
natural mire	DN
drained	DD
Lakkasuo	
minerotrophic natural	LN_m
minerotrophic drained	LD_m
ombrotrophic natural	LN_o
ombrotrophic drained	LD_o
Breitlohmisse	
natural mire	BN

natural dry	BN_{dry}
drained	BD_1
near the mire edge	BD_2
Rotmeer	
natural mire	RN
drained, with Sphagnum	RD_1
drained, without Sphagnum	RD_2
Ursee	
natural mire	UN
drained	UD

10. L 283-284: This sentence is incomprehensible. Does fungal biomass decrease in peatlands? Where? When? Please explain.

Answer: Yes, our hypothesis is, that with increasing depth and changing hydrological conditions (darker, less oxygen) fungi will be outcompeted by bacteria, which means, that fungal biomass must decrease, whereas bacterial biomass increases with depth. In the uppermost part (acrotelm and upper mesotelm) fungal biomass is the highest, whereas in the deeper part of the mesotelm bacterial biomass will increase. In the catotelm all microbial biomass is strongly reduced because of the anaerobe conditions. In natural peatlands a small amount of fungal biomass is also visible in the acrotelm, but in a much lower scale than for drained sites. (Thormann, 1999)

We have implemented it in the chapters 3.5 (L440-457) and 3.6 (L479-491).

11. Supplementary data: This xls. file is not for publication. It requires formatting and translation.

Answer: We have re-formatted the supplementary data to make it more comprehensible for readers.

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Relevant changes:

We have revised the whole chapters 1 (introduction) and 3 (discussion), with major changes and implementations of the comments as followed:

1. Revised Introduction and implementation of the reviewer comments
    a. L27-36
    b. L50-63
    c. L64-92
    d. L113-120
    e. L128-138
2. Implementation of reviewer comments in chapter 2
    a. L160-165
    b. L169
    c. L175
    d. L233-259
3. Revised discussion chapter (3) and implementation of the reviewer comments:
    a. L440-457
    b. L479-491
    c. L463-514
4. Figures 1-3 were revised
5. Table 1 was revised
6. Tables 4-7 were moved to the supplement

Switch of fungal to bacterial degradation in natural, drained and rewetted oligotrophic peatlands reflected in $\delta^{15}N$ and fatty acid composition

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Abstract. ~~During the last centuries major parts~~Since the last centuries European peatlands are degrading along with drainage, land use and climate changes. Increasing ~~Peatland biodiversity and essential ecosystem functions (e.g. flood prevention, groundwater purification and CO_2 sink) were dramatically impaired. Moreover, climate change threatens peatlands in the near future. Increasing~~ pressure to peatland ecosystems calls for a more cost-efficient method to indicate the current state of peatlands and the success of restoration effort. ~~Metabolism processes~~Metabolic pathways in peatland soils are imprinted in stable isotope compositions due to differences in microorganism communities and their metabolic pathways. Therefore, we hypothesize that depth profiles of nitrogen stable isotope values provide a promising opportunity to detect peatland decomposition or restoration. We studied five peatlands: Degerö Stormyr (Northern Sweden), Lakkasuo (Central Finland) and three mires in the Black Forest (Southern Germany). At all locations, cores were taken from adjacent drained (or rewetted) and natural sites to identify $\delta^{15}N$ trends that could indicate changes due to drainage and restoration. At all drained (and rewetted) sites we found a distinct peak ("turning point") of the $\delta^{15}N$ values in the center of the drained horizon. ~~. To verify our interpretation C, the C/N ratio and the bulk density were measured and a microscopic analysis of the macro residuals in the peat cores was made.~~We did a fatty acid (FAs) analysis to link our results to microbial community composition. As marker we distinguished between

one fungal-derived FA (C18:2ω9c) and four bacterial-derived FAs. For bacteria, we looked for one general bacterial-derived FA (C14:0), two FAs for gram-positive bacteria (i-C15:0; a-C15:0) and one FA for gram-negative bacteria (C16:1ω9c) ~~and bacterial-derived PLFAs~~. In accordance with other studies, our results suggest, that fungi dominate the microbial metabolism in the upper, aerobic peat horizon. This is reflected by depleted δ^{15}N values. Downwards the drained horizon conditions slowly switch to oxygen limitation. In consequence fungal-derived FAs decrease whereas bacterial-derived FAs rise. The highest diversity of microbial-derived FAs is indicated by the δ^{15}N turning point. Below the δ^{15}N turning point, oxygen is increasingly limited and concentrations of all microbial-derived FAs are decreasing down to the onset of the permanently waterlogged, anaerobic horizon. Peatland cores with restoration success show, above the formerly drained horizon, again no depth trend of the isotopic values. Hence, we conclude that δ^{15}N stable isotope values reflect microbial community composition, which differs between drained and natural peatlands.

1 Introduction

In Europe 70% of the peatlands are degraded (Joosten and Couwenberg, 2001). Leifeld and Menichetti (2018) reported that degraded peatlands account for five percent of the anthropogenic CO_2 emission. Despite this dramatic peat decline, we lack reliable and transferable tools providing time- and cost-efficient information of the peatland hydrology status.

~~Even though peatlands cover only 3-4 % of the Earth's land surface (Leifeld and Menichetti, 2018), they act as an enormous sink for greenhouse gases in natural conditions (Yu et al., 2011; Joosten, 2008). In Europe 70% of the peatlands are currently degraded (Joosten and Couwenberg, 2001). These degrading peatlands account for five percent of the anthropogenic CO emission (Leifeld and Menichetti, 2018; Zedler and Kercher 2005). Despite this dramatic peat decline, we lack reliable and transferable tools for providing time- and cost-efficient information of peatland condition.~~Peatland soils consist of three different horizons. Most biological metabolism and nutrient cycling takes place in the acrotelm (uppermost aerobic peat horizon with living vegetation) (Asada et al., 2005a; Artz, 2013; Morris et al., 2011). In the water-saturated catotelm (deeper, anaerobic horizon) organic substrates are decomposed at much smaller rates owing to anoxic conditions (Asada et al., 2005a; Artz, 2013; Lin et

al., 2014). In the mesotelm, the peat horizon situated between acrotelm and catotelm, water table levels and oxygen content fluctuate, resulting in shifting aerobic and anaerobic conditions and shifting metabolism processes (Asada et al., 2005a; Artz, 2013; Lin et al., 2014). Clymo and Bryant (2008) therefore defined the mesotelm as a "transition horizon". In degraded peatlands the mesotelm is expanded and former preserved organic substrate is decomposed (Zedler and Kercher, 2005). In an expanded mesotelm conditions differ from aerobic, light and warm conditions in the upper mesotelm to semi-oxic, dark and cold conditions in the lower mesotelm (Artz, 2014; Lin et al., 2014). The conditions in the former mesotelm will get anaerobic and microbial activity will be inhibited with rewetting (Andersen et al., 2006; Asada et al., 2005b; Thormann et al., 1999).

Derived by the thickness of these horizons, we distinguish between three different hydrological statuses of peatlands (natural, drained and rewetted). We determined the hydrological status by a vegetation analysis, the humification index (HI) after von Post (Silc and Stanek, 1977) and the measurement of the water table height. Natural and rewetted sites have a high water table near the surface and are mainly formed by Sphagnum mosses with low humification indices. Drained sites are characterised by low water tables, higher grades of humification, less Sphagnum and more other moss species. However, determination of macro residuals in more or less degraded peat is time-consuming, needs highly specialised expert knowledge and is thus limited to a small number of samples.

Other common methods to measure peatland hydrology currently are gas emission measurements and measurement of growth heights of peatland vegetation. Gas measurements (e.g. CO_2, N_2O, CH_4) provide an indirect measurement of ongoing decomposition processes (Baldocchi et al., 1988). The method is expensive and labour intensive and does not to give information on drainage history and process dynamics beyond the specific measurement time (Bubier et al., 2003). Measuring vegetation growth is connected to several problems: (i) not only the sole growth of mosses indicates peat growth, but rather the balance of growth and degradation. It is important how much vegetation material enters the catotelm and is therefore stored under anaerobic conditions. (ii) Peat shrinks and swells with water supply. Hence, measuring peat height at different water table heights would lead to different assumptions for peatland growth (Clymo, 1970). And thirdly, peat growth is slow and an unambiguous

result on the success of restoration efforts might need decades of measurements (Clymo, 1970, Fenton, 1980).

As such and in search for practical indicators, we measured bulk density (BD), carbon/nitrogen ratio (C/N) and bulk stable isotope values. BD acts as an indicator for decomposition, because decomposition processes lead to higher compaction of the peat soil and therefore increasing BD values (Novak et al., 2008). The C/N ratio indicates the degree of decomposition (Malmer and Holm, 1984; Kuhry and Vitt, 1996). With increasing decomposition a preferential loss of C over N takes place and the C/N ratio decreases. Stable isotopes depth patterns of $\delta^{13}C$ and $\delta^{15}N$ in peat have been ~~We~~ found in previous studies (e.g. Krüger et al., 2016, Alewell et al., 2011) to be specific ~~depth patterns of $\delta^{13}C$ and $\delta^{15}N$ depending on~~for peatland hydrology (drained, rewetted or natural), but studies were unable to find an explanation of these depth patterns. As degradation is mostly connected to drainage, we hypothesized that an increase of microbial activity is responsible for the change in isotope patterns.

~~We hypothesize that nitrogen (N) stable isotopes could serve as such a tool.~~

~~In natural peatlands most biological metabolism and nutrient cycling takes place in the acrotelm (uppermost aerobic peat horizon with living vegetation) (Asada et al., 2005; Artz, 2013). In the water-saturated catotelm (deeper, anaerobic horizon) organic substrates are decomposed at much smaller rates owing to anoxic conditions and low pH values (Asada et al., 2005; Artz, 2013; Lin et al., 2014). In the mesotelm, a peat horizon situated between acrotelm and catotelm, water table levels and oxygen content constantly fluctuate, resulting in shifting oxic and anoxic conditions and shifting metabolism processes (Asada et al., 2005; Artz, 2013; Lin et al., 2014). Clymo and Bryant (2008) therefore defined the mesotelm as a "transition horizon".~~

Stable C and N isotopes are correlated with vegetation composition and microbial decomposition processes. As decomposition induces an enrichment of heavy isotopes (^{15}N, ^{13}C), vegetation is mostly more depleted in ^{15}N and ^{13}C than microbial and recycled substrate. Alewell et al. (2011) and Krüger et al. (2014) reported distinct changes in $\delta^{13}C$ values for palsa peat with the onset of decomposition of hummocks. Various authors observed the same trend with decomposition in peatlands of other climate conditions (Krüger et al., 2016; Novak et al, 1999; Hobbie et al., 2017; Biester et al., 2014). The distinct $\delta^{13}C$ depth pattern is a consequence of the use of different sources by fungi and bacteria as investigated

by Kohl et al. (2015) for peat profiles. They conclude that an increasing $\delta^{13}C$ signal is caused by differences in biomass synthesis and carbon sources used by fungi and bacteria, which was also reported by Lichtfouse et al. (1995) and Baumann et al. (2013). We found also distinct changes in $\delta^{15}N$ with drainage. It is known that plants preferentially incorporate the lighter ^{14}N (Högberg, 1997), an effect that is strongly enhanced by mycorrhizal uptake of nitrogen into plants (Hobbie and Högberg, 2012). Plant rooting and the existence of mycorrhiza leads to enriched $\delta^{15}N$ values in the remaining bulk material (Högberg et al., 1996), because plants and mycorrhiza preferentially process lighter ^{14}N (Adams and Grierson, 2001; Asada et al., 2005a; Högberg et al., 1996; Kohzu et al., 2003; Robinson et al., 1998). However, our study sites are open peatlands with a low occurrence of vascular plants and mycorrhiza. Hence, these mechanisms cannot be the main drivers of our observed $\delta^{15}N$ depth patterns. Tfaily et al. (2014) reported changing microbial abundance and metabolic pathways are correlated with $\delta^{15}N$ values. Vice versa this would mean that $\delta^{15}N$ values could reflect the hydrology status. Therefore, we assume $\delta^{15}N$ values allows us conclusions whether the observed peatland have a natural, drained or rewetted hydrology status. ~~The hydrology status determines the abundance of microbial communitie~~

Following previous studies, we use specific terms for the points of change in the stable isotope depth pattern. The points where the stable isotope signals undergo a sudden directional shift with depth are called “turning points” according to Alewell et al. (2011). Furthermore, the bottom of the mesotelm and the onset of the underlying catotelm are marked by the $\delta^{13}C$ turning point.

To test the idea of changing dominant microbial communities as drivers for isotope depth patterns, we did a fatty acid (FA) analysis of four investigated sites – two drained and two natural sites in Degerö Stromyr (Mid Sweden, 70 km form Umea) and Lakkasuo (Southern Finland, 14 from north from Orivesi). FAs are valid markers to indicate the abundance of specific microbial communities in the peat, because they are specific and persistent compounds of cell membranes of different species (Bajerski, Wagner and Mangelsdorf, 2017; Finotti et al. 1992; Piotrowska-Seget and Mrozik 2003; Reiffarth et al., 2016). Therefore FAs enable us to make qualitative and quantitative statements about the relative abundance of different microbial communities. We will test the existence of four bacterial markers (C14:0 as general marker, i-C15:0 and a-C15:0 indicative for gram positive, C16:1ω9c indicative for

gram negative) (Vestal and White 1989; Willers et al., 2015; Zelles, 1997) and one fungal marker (C18:2ω9c) (Sundh, Nilsson and Borga, 1997; Elvert et al., 2003; Willers et al., 2015).

We hypothesize microbial abundance and diversity are the drivers for the distinct observed ~~different~~ $\delta^{15}N$ depth pattern in natural, drained or rewetted ~~hydrology statuses~~peats. We assume $\delta^{15}N$ depth pattern can therefore be used as an inexpensive and less time-consuming tool to get reliable information of peatland hydrology. ~~Our aim is to evaluate ∂15N depth trends as indicators of specific peatland conditions and to study whether ∂15N depth trends of natural and drainage-affected sites indicate, in parallel to ∂13C, a shift in dominant microbial communities, reflected by specific PLFAs. We are also interested to see if there are distinct changes of ∂13C and ∂15N isotope signatures with the onset of rewetting processes. We match isotope depth (C, N) with bulk density (BD) and carbon/nitrogen ratio (C/N). BD acts as an indicator for decomposition, because decomposition processes are leadin to higher compaction of the peat soil and therefore increasing BD values. The C/N ratio gives us also information about the degree of decomposition. With increasing decomposition the C/N ratio decreasess.With an additional microscope analysis of the macro-residuals in the peat horizon, we will get information of the humification indices (HI and the vegetation assemblages.~~

~~relatively relatively~~ ~~To test our hypothesis of changing dominant microbial communities as drivers for isotope patterns, we do a PLFA analysis of four investigated sites – two drainage-affected and two natural sites in Degerö Stromyr and Lakkasuo. We will test the existence of two Gram positive – bacterial (i-C15:0; a-C15:0) markers and one fungal (C18:2ω9c) marker (Sundh, Nilsson and Borga, 1997; Elvert et al., 2003). In the Swedish site Degerö Stormyr we add information of tree ring development as an indicator of peatland dynamics.~~

2 Material and methods

2.1 Site description

We studied five oligotrophic peatlands (Tab. 1, Tab. 2). All investigated sites are classified as Histosols (organic soils). Histosols are classified as soils with a cumulative organic layer and an organic matter amount of 35% or higher in at least half of the uppermost 80-100 cm (IUSS, 2015). In addition all

investigated peatland soils are Sphagnum peats, because of their mean annual temperatures (between +1.2°C and +7 °C) and their annual precipitation between 523ppm and 1600ppm (Eurola et al., 1984; Vitt et al., 2006).

Degerö Stormyr (200 m above see level (a.s.l.)) is situated in Northern Sweden, at the Kulbäcksliden Experimental Forest near Vindeln, between the rivers Umeälven and Vindelälven (Eurola, Hicks & Kaakinen, 1984). It is an acidic mire with minerotrophic conditions and consists of interconnected small mire patches divided by ridges of glacial till. Degerö Stormyr is classified as Northern eccentric peatland (Eurola et al., 1984). The climate is characterized as cold with no dry seasons and cold summers (Dfc-zone after Köppen-Geiger classification; Peel et al., 2007). In Degerö ditches were installed at the beginning of the 20th century, were closed in 2017 and a naturally reestablishment of sphagnum took place afterwards. The water table is at the surface in the natural part (DN) (Nielsson et al., 2008) and in around 10-15 cm depths at the drained location (DD).

Lakkasuo (150 m a.s.l.), Central Finland, is a~~n~~ Northern, eccentric peatland complex (Eurola et al., 1984) with two parts. In the southern part the conditions are ombrotrophic, whereas the northern part is minerotrophic (Minkkinen et al. 1999). Lakkasuo is also located in the cold climate zone, with no dry seasons and cold summers (Dfc-zone after Köppen-Geiger classification; Peel et al. 2007). The 1961 installed ditches (70 cm depth, spacing of 40 m – 60 m) affect approximately 50 % of the peatland (Minkkinen et al., 1999). In the ombrotrophic natural site (LN_o) the water table was around 13 cm below ground surface. The ombrotrophic drained site (LD_o) had a water table of 26 cm depth (average), whereas the water table is near the surface at the minerotrophic natural site (LN_m) and in an average depth of 36 cm in the minerotrophic, drained site (LD_m) (Minkkinen et al., 1999) (Tab. 1, Tab. 2).

In the Black Forest three mires were investigated: Breitlohmisse, Ursee and Rotmeer. They are located in the temperate climate zone with no dry seasons and warm summers (Cfb-zone after Köppen-Geiger; Peel et al., 2007). In the mires of the Black Forest ditches were installed in the middle of the 20th century. Breitlohmisse (810 m a.s.l., 50 km southeast of Baden-Baden) is minerotrophic and is located in the Northern part of the Black Forest. The mire is mostly lanced with ditches for huntsman ships (BN_d). The ditches are naturally refilled with Sphagnum. The water table is at an average depth of 15 cm in the natural center (BN, BN_d), and is found at lower depths near the degraded edges of the mire

(BD_1, BD_2). Rotmeer (960 m a.s.l., 40 km southeast of Freiburg i.B.) and Ursee (850 m a.s.l., 45 km southeast of Freiburg i.B.) are both in the Southern Black Forest. Rotmeer consists of an ombrotrophic center (RN) (water table at the surface), surrounded by a minerotrophic part with signs of decomposition (RD_1, water table around 12 cm depth) and without mosses at the edges (RD_2, water table below 12 cm depth). Urmeer is minerotrophic. A quaking bog forms the center with the water table at the surface (UN), whereas the edges had a lower water table (UD) (Tab. 1, Tab. 2).

2.2 Soil sampling and bulk analyses

In May 2012 (Breitlohmisse), June 2012 (Rotmeer), July 2012 (Ursee) and September 2013 (Degerö and Lakkasuo) three volumetric peat cores were drilled per site with a Russian peat corer (Eijkelkamp, The Netherland) at a medium stage of small-scale topography. In Degerö cores were sampled in the assumed natural center of the mire (DN) and in one-meter distance to a drainage ditch (one meter depth) (DD). In Lakkasuo we took cores at the natural sites (ombrotrophic natural (LN_o), minerotrophic natural (LN_m)) and the drained locations (ombrotrophic drained (LD_o), minerotrophic drained (LD_m)). For Ursee two cores were taken, one in the natural center (UN) and one at the drained edge of the mire (UD). In Breitlohmisse and Rotmeer we took cores in a transect from natural (BN, RN) to strong drained (BD_2, RD_2) sites. Each core has a composite length of one meter. Here, we focus on the uppermost 60 cm because this part included the drained horizon and no major changes in isotopic composition were observed at the natural sites below the mesotelm. In all investigated peatlands, the catotelm starts in the natural sites below 10 cm depth and varied in drained sites, but was always visible below 40 cm depth.

Directly after drilling HI were determined for each horizon with the von Post scale. The von Post scale has a range form 1 to 10. HI 1 indicates natural condition with undecomposed, completely visible vegetation residuals. HI 10 represents a strongly decomposed horizon without visible vegetation residuals. (Silc and Stanek, 1977)

The cores were encased in plastic shells and covered with plastic wrap, stored in coolers, and transported to the laboratory. The cores were sliced in 2 cm sections and every second layer was analysed, giving a 4 cm depth resolution. Samples were oven-dried at 40 °C for 72 h, and homogenized

with a vibrating ball mill (MM400, Retsch, Germany). Stable C and N isotopic compositions were measured with an elemental analyser combined with an isotope ratio mass spectrometer (EA-IRMS) (Inegra2, Sercon, Crewe, UK). Carbon isotopic composition ($^{13}C/^{12}C$) was expressed relative to Vienna Pee-Dee Belemnite (VPDB) standard and reported in delta notation (‰), stable nitrogen isotopes were expressed relative to the atmospheric nitrogen standard and reported in delta notation (‰). C/N was determined with the mass relationship of the measured bulk content of C and N. Bulk density was measured with volumetric samples, which were weighted before and after drying.

In Degerö tree rings of seven individual trees were analysed (Pinus sylvestris) to obtain information of growth conditions and to enhance therefore our knowledge of drainage history.

2.3 Fatty acid analysis

Four cores (per site one drained and one natural core) were selected to do a fatty acid analysis: two sites in Lakkasuo, LD_o 1 and LN_o 3 and two sites for Degerö Stromyr, DD 3 and DN 1. We took subsamples from all cores in the acrotelm (respectively at the end of the mesotelm in DD) and in the catotelm. At the drained sites DD 3 and LD_o 1 we took also samples in the middle and at the end of the mesotelm.

We processed 0.2 – 1.1 g of sample for the lipid extraction with a mixture of CH2Cl2 : MeOH (9:1 v/v) in an Accelerated Solvent Extractor (Dionex ASE 350). 50 µl of an internal standard (0.4 mg/ml, nonadecanoic acid) was added before processing each sample.

The total lipid extracts (TLE) were saponified by adding 2 ml of KOH dissolved in MeOH (12%) and putting it in the oven for 3 hours at 80°C.

Following the method of Elvert et al. (2003) TLE was afterwards pooled with 1 ml KCl (0.1 mol) and the neutral fraction was extracted by agitating three times with hexane. Neutral fraction in the supernatant was separated, dried under a stream of N_2, and stored in the fridge for later analysis. We acidified the rest of the TLE with fuming hydrochloric acid to a pH of 1. The acid fraction was extracted by agitating again three times with hexane. The acid fraction in the supernatant was separated and hexane was reduced to almost dryness under a stream of N_2. Then the acid fraction was methylated by adding 1 ml Boron-Trifluoride (BF_3) in MeOH (12-14%) and putting it in the oven for 1 hour at 60°C. Afterwards the resulting fatty acid methyl esters (FAMEs) fraction was pooled with KCl (0.1

mol) extracted by agitating again three times with hexane and transferred in 2 ml vials. The FAMEs were quantified with a Trace Ultra gas chromatograph (GC) equipped with a flame ionization detector (FID) (Thermo Scientific, Waltham, MA, USA). The carrier gas (helium) had a constant flow of 1.2 ml per minute and the GC-FID was set to splitless mode. Detector temperature was 320°C and the samples (dissolved in hexane) were injected by 300°C. The starting temperature of the oven was 50°C. The temperature was increased by 10°C per minute to 140°C. The temperature was held for 1 minute before it was increased up to 300°C. This temperature was held for 63 minutes.

To identify the fungal and bacterial markers, we used the Bacterial Acid Methyl Esters standard (BAME, Supelco Mix). The standard includes the following FAs as marker for bacteria: C14:0 (general bacterial marker; Willers et al., 2015, Zelles, 1997), i-C15:0 and a-C-15:0 (for Gram positive – bacteria; Zelles, 1997;, O`Leary and Wilkinson, 1988; Tunlid and White, 1992) and C16:1ω9c (for Gram negative – bacteria; Willers et al., 2015; Zelles, 1997). For fungi, the standard includes C18:2ω9c (Andersen et al., 2010; Sundh, Nilsson and Borga, 1997; Zelles, 1997;, O`Leary and Wilkinson, 1988; Vestal and White, 1989). Quantification of the FAs was done using the internal standard, C19:0 FA, after correcting for the methyl group, added during methylation reaction.

2.4 Data evaluation and statistical analysis

As we were interested in comparing the depth trends of all single profiles with each other, we first normalized the depths of the cores. This was done using the depth of the $\delta^{15}N$ turning point (see chapter 3.1) in each drained profile as the anchor point serving as normalized depth (normD). The normalized depth of this anchor point was set to 20 cm depth (normD = 20 cm, Fig. 1) in each single core. In the corresponding natural cores, we have transferred the values from the same depth related to the drained core into the same norm depth. For example the values of the natural site (DN) in depth of 13 cm (depth of the turning point of $\delta^{15}N$ in the corresponding DD core) were set to 20 cm normD.

In a second step, because we were mainly interested in trends and not the absolute values, we normalized the isotopic values themselves, because the range of $\delta^{15}N$ varied considerably between the

sites, whereas the trends show consistent patterns (Fig. 1). Therefore, to be able to do a meaningful comparison we set therefore the value of $\delta^{15}N$ at the turning point to zero in each profile:

$$normalized\ \delta^{15}N\ [‰] = \delta^{15}N\ [‰] - \delta^{15}N\ [‰]\ at\ turning\ point$$

Using the same procedure, all other parameters ($\delta^{13}C$, C/N, BD) were normalized using the same anchor point (e.g., $\delta^{15}N$ turning point):

$$normalized\ value\ (\delta^{13}C\ [‰],\ BD,\ C/N) = value\ (\delta^{13}C\ [‰],\ BD,\ C/N) - value\ (\delta^{13}C\ [‰],\ C/N,\ BD)\ at\ \delta^{15}N\ turning\ point$$

Using the above procedures means to decide on the depth of the δ15N turning points, which we backed up statistically with a t-test ($p \leq 0.05$) and an integrated change point analysis with the software package "changepoint" in R (version 1.0.153). These analyses were done for each of the drained sites separately and also in addition with an average of all locations. For the t-test, we analysed for each depth if $\delta^{15}N$ values in the drained horizon are of the same population as the values of the natural sites (H0: drained and natural values are of the same population). For the changepoint analysis, the variance of $\delta^{15}N$ was evaluated with a linear gradient over the whole drained peat profile against the variance of three/ four separated linear gradients (rewetted part (if present), upper mesotelm, lower mesotelm, catotelm). Here, we define the starting point of the drained horizon with the onset of a shift in the δ15N values upward and the end of this horizon with the stabilization of the $\delta^{15}N$ values towards the surface.

We also determined the slopes of each single core to get information on the strength of differences of the isotopic values with depth. First, the whole peat profile of each drained core was analysed as one trend (called "overall profile"). Second, profiles were separated into different horizons: (i) rewetted horizon (if present), (ii) upper mesotelm, (iii) lower mesotelm and (iv) catotelm. If values were clearly changing with depth slopes were closer to zero. In horizons with stabilized values slopes were distinct higher or lower zero.

In the following we present only the normalized data. Raw data without normalization are available in the supplementary information.

2.5 Tree ring width and microscope analysis of peat

The investigation of the tree ring width of seven surrounding trees (Pinus sylvestris) in Degerö Stormyr was done with a hand-operated wood driller (Djos/ Sweden, 5 mm diameter). Samples were fixed on wooden carriers. The tracheids (elongated cells of the xylem of vascular plants) were cut with a sharp carbon blade and analysed with an impinging light binocular (60x – 160x amplification).

Peat samples of four study sites were analysed using an impinging light binocular (60x – 160x amplification) to get an overview of the vegetation assemblages and to differentiate horizons. For detailed information (distinction of Sphagnum species) the samples were elutriated with water, pigmented with methyl-blue and analysed under a transmitted light microscope (100x – 640x amplification).

3 Results and discussion

3.1 Depth profile of vegetation assemblage and water table defining the hydrological statuses

Following our indicators (HI, vegetation assemblages), we defined three types of hydrological statuses: (a) natural, (b) drained up to the surface, and (c) profiles with a rewetted horizon above the drained horizon (Fig. 1).

All sites, which we attributed as “natural” (type (a)), had a water table near the surface (<10 cm, section 2.1), and macro-residuals were highly visible throughout the profile, HIs were low and the main living vegetation was Sphagnum spp. (Tab.3, Tab. S4).

All drained sites had higher HIs even if no direct modifications in the vegetation assemblage could be documented. For type (b), there was little or no Sphagnum visible at the surface and the water table was found at lower depths (Section 2.1). Macro-residuals were more strongly affected by decomposition and HIs were high up to the surface. Especially the ombrotrophic-drained site (LDM) was influenced by

drainage. Here, mosses of drier environments replaced Sphagnum species or mosses were completely absent (Tab. 3, Tab.S4).

For type (c), vegetation assemblages were mainly composed of Sphagnum spp. and the water table was near the surface. HIs were low in the rewetted horizon and macro-residuals were preserved well (Section 2.1, Tab. 3, Tab. S4). With the onset of the upper mesotelm, HIs and decomposition of macro-residuals was high. In the lower mesotelm, the HIs were decreasing and more macro-residuals were visible. In the catotelm, the quality of macro residuals was higher than in the mesotelm and the HIs were even lower (Tab. 3, Tab.S4).

3.2 Tree ring width are verifying the rewetted hydrological status of Degerö

Tree ring width is a marker for the wellbeing and/or growth rate of trees. Young trees have a small circumference coupled with high growth rates, which leads to thicker tree rings. Tree rings get smaller with increasing age of the tree. If there are no environmental stressors like heat, increasing wetness or drought, tree rings are bigger and the cell lumen is higher compared to trees at sites with environmental stress. With increasing environmental stress tree ring width decreases (Stoffel et al., 2010). Before 1992, tree rings at the drained site (DD) site showed only a slightly decreasing trend, which could be due to aging (average of 1.3 mm width in the 1930s to an average width of 0.9 mm in the late 1980s). The draining ditches in Degerö Stormyr were established in the beginning of the 20th century, which supports these results, with dryer and therefore better growth conditions for trees. From 1992 onwards, tree ring widths decreased, reaching 0.2 mm in 1998 and thereafter. These results suggest a restoration to a wetter, e.g. more natural hydrological status. Rewetted hydrological conditions are not favourable for tree growth and thus lead to smaller tree ring width.

3.3 Biogeochemical parameters and hydrological status

Biogeochemical composition of peatlands strongly reflects the related hydrological status.

As typical for natural peatlands, our investigated natural sites have an average C/N ratio of 57 (Tab. S7). This is in line with results from Malmer and Holm (1984) and Kuhry and Vitt (1996) which found the C/N ratio in the acrotelm of oligotrophic peatlands to be higher than 35, mostly between 50-90. The

values in the mesotelm were lower compared to both, acrotelm as well as catotelm, most likely due to higher decomposition rates and the release of CO_2 (Tab. S7). As typical for peatlands, BD in our peatlands low due to the high amount of plant residuals in the soil and low values of mineralization (Novak et al., 2008), with 0.02 kg m^{-3} at the surface and increased with increasing decomposition and compaction of plant material downwards to 0.04 kg m^{-3} in the mesotelm (Tab. S8). BD was also increasing in the catotelm (average of 0.05 kg m^{-3}, Tab. S8), following the increased gravimetric pressure.

In contrast, the biogeochemical parameters of drained sites have a very different pattern. The lower C/N ratio in the acrotelm (average of 41, Tab. S7) and the mesotelm (average of 35, Tab. S7) indicates higher mineralization rates with gaseous release of carbon and nitrogen. In the catotelm with natural, anaerobic conditions, the C/N ratio were in the same range, as in the natural sites (average of 49, Tab. S7). BD of the acrotelm and mesotelm (average of 0.07 kg m^{-3}, Tab. S8) also increased as a consequence of the enhanced decomposition processes.

These results are in line with for the hydrology statuses indicated by the vegetation analysis (Section 3.1).

3.4 Stable nitrogen isotope depth trends as indicators for the hydrology status

While mineral soils have been shown to have continuous increasing values of $\delta^{15}N$ (Nadelhoffer et al, 1996~~;~~ Högberg et al, 1997), we found increasing $\delta^{15}N$ and $\delta^{13}C$ values with depth down to particular, isotopic specific turning points in drained peatland soils (Fig. 1). ~~We defined three types of peatland conditions from the observed depth trends of ∂15N: (a) natural, (b) drainage-affected up to the surface, (c) profiles with a rewetted horizon above the drainage-affected horizon. (fig. 1)~~

The trends of the single e~~E~~ight out of nine studied drained peatlands as well as the average trend confirm the existence of a $\delta^{15}N$ turning point. We determined a significant difference with $p < 0.05$ ~~for the difference in~~between $\delta^{15}N$ in the center of the mesotelm ~~in contrast~~compared to the $\delta^{15}N$ values in ~~undisturbed~~ undrained horizons, (Tab. S1) with a non-significant difference for one drained site: Breitlohmisse (BD_1). The latter was most likely related to generally higher $\delta^{15}N$ values of the natural site in Breitlohmisse (BN) compared to a smaller increase of $\delta^{15}N$ to the related drained site (BD_1). The

depth of $\delta^{15}N$ turning point (center of the mesotelm) differs from $\delta^{13}C$ turning point (end of the mesotelm) for all investigated sites (Fig. 2).

Changed slope values of the separated horizons indicate significant trend changes (Tab. S3). In anaerobic conditions (natural, catotelm) with stabilized isotopic values with depth, slopes were distinctly different to 0 [cm/‰]. $\delta^{15}N$ values seem to change within the mesotelm rapidly and slope values were closer to zero. Most interesting was a switch to negative trend values at the $\delta^{15}N$ turning point in all investigated drained sites, which marks the beginning of the lower mesotelm. (Tab. S3)

In a natural hydrological status (type (a)), all investigated parameters had a low variability and indicated a natural, wet mire hydrology status (Fig. 1). There were two exceptions: Breitlohmisse natural (BN) (40 - 60 cm normD) and Rotmeer natural (RN) (30 – 50 cm nromD), with trend instabilities of $\delta^{15}N$. This might indicate some minor drainage or disturbance in the wetland sites we classified as "natural" (Fig. 1).

In contrast, the values of the drained sites showed significant trends. We found two different trend types in the drained sites: Type (b) and (c) (Fig. 1). For type (b) we distinguished six sites: Lakkasuo ombrotrophic drained (LD_o), Breitlohmisse natural dry (BNd_d, Breitlohmisse drained 1 (BD1), Breitlohmisse 4 (BD_2), Rotmeer drained 1 (RD_1) and Rotmeer drained 2 (RD_2) with clear signs of decomposition up to the surface. Type (c) was visible in three sites: drained site Degerö Stromyr (DD), minerotrophic drained site Lakkasuo (LD_m) and Ursee 1 (UD). At type (c) sites the isotopic values, C/N and BD were stabilized again above the mesotelm. Therefore, they are assumed to be in a "new" natural status (Fig. 1, Fig. 2).

Below 8 cm (normD, average profile) all drained profiles showed the typical signs of the upper mesotelm with increasing values of $\delta^{15}N$, $\delta^{13}C$ and BD, down to the $\delta^{15}N$ turning point, and decreasing C/N. Below the $\delta^{15}N$ turning point, in the lower mesotelm, $\delta^{15}N$ values were decreasing. In this horizon $\delta^{13}C$ values, C/N and BD were increasing. The end of the lower mesotelm was mostly linked to a clear shift in $\delta^{13}C$ trend to either stable values or a slow decreasing trend; hence, we called this point $\delta^{13}C$ turning point (28 cm normD, average profile) (e.g. Krüger et al. 2014). Constant C/N, BD and $\delta^{15}N$ values below the $\delta^{13}C$ turning point served also as indicators for reduced compaction and decomposition. Most likely the $\delta^{13}C$ turning point marked the onset of permanent waterlogged

anaerobic conditions (e.g. Krüger et al. 2016). The similarity in trends in these deeper parts of the drained sites to those of the catotelm in the natural sites supported the assumption of an intact catotelm below the $\delta^{13}C$ turning point (Fig. 1, Fig. 2.). (For the single $\delta^{13}C$, C/N and BD values of all peat cores see supplementary information).

3.2 Depth profile of vegetation assemblage and water table in connection with isotope depth pattern

All sites, which we attributed as "natural" (type (a)), had a water table near the surface (<10 cm), and macro-residuals were highly visible throughout the profile, HIs were low and the main living vegetation was Sphagnum spp. (tab. 6, tab.7).

All drainage-affected sites had higher HIs even if no direct modifications in the vegetation assemblage could be documented. For type (b), there was little or no Sphagnum visible at the surface and the water table was found at lower depths. Macro-residuals were more affected by decomposition and HIs were high up to the surface. These results were in line with our interpretation of isotope signatures of a drainage-affected horizon up to the surface. Especially the ombrotrophic-drained site (LOD) was influenced by drainage. Here Sphagnum species seem to have disappeared and were replaced by mosses of drier environments or mosses were completely absent (tab. 7).

For type (c), vegetation assemblages were mainly composed of Sphagnum spp. and the water table was near the surface. HIs were low in the rewetted horizon and macro-residuals were preserved well (tab. 6, tab. 7). With the onset of the upper mesotelm, HIs and decomposition of macro-residuals was high. In the lower mesotelm, the HIs were decreasing and more macro-residuals were visible. In the catotelm, the quality of macro residuals was higher than in the mesotelm and the HIs were even lower.

3. Tree ring width are verifying isotope signals of changing

Tree ring width is a marker for the wellbeing and/or growth rate of trees. Young trees have a small scope coupled with high growth rates, which leads to thicker tree rings. Tree rings get smaller with increasing age of the tree. If there are no environmental stressors like heat, increasing wetness or drought, tree rings are bigger and the cell lumen is higher compared to trees at sites with environmental stress. With increasing environmental stress tree ring width decreases (Stoffel et al., 2010). Before

~~1992, tree rings at the site () site showed only a slightly decreasing trend, which could be due to aging (average of 1.3 mm width in the 1930s to an average width of 0.9 mm in the late 1980s). The draining ditches in Degerö Stormyr were established in the beginning of the 20th century, which supports these results, with dryer and therefore better growth conditions for trees. From 1992 onwards tree ring widths decreased, reaching 0.2 mm in 1998 and thereafter. These results were concurrent with the isotope analysis, because both suggest a restoration of natural wet . ewetted no longer suitable for trees and lead to smaller tree ring width according to adverse environmental conditions for tree growth. These findings underpin our suggestion of rewetted at this site in Degerö.~~

3.5 Changing ~~Linkage of microbial abundance and isotopic signature~~microbial FAs and nitrogen stable isotope depth pattern

Fungal-derived FAs (80% of all microbial-derived FAs) were the dominant fraction near the surface. In the catotelm the microbial-derived FA values were decreased down to 30% compared to the acrotelm and the mesotelm with a clear dominance of bacterial derived FAs (98%), as a consequence of the anaerobic conditions (Fig. 3).

The latter is congruent with the results of Thormann (1999), f~~F~~ungi will be outcompeted by bacteria with increasing depth and changing hydrological conditions (darker, less oxygen~~)~~). ~~Hence, fungal biomass decreases, whereas bacterial biomass increases (Thormann, 1999)~~. In the acrotelm of the natural sites, 70% less microbial-derived FA compared to the acrotelm of the drained sites confirmed the clear link between microbial abundance and the hydrological status. In contrast, we found similar values of microbial FAs in the catotelm for drained and natural sites. This suggests, that drainage did not affect the catotelm.

In the drained sites the enhanced microbial-derived FA abundance could be caused by the improved conditions for metabolism processes by drainage: enhanced oxygen abundance and relatively high nutrient availability of the prior conserved plant material (Peltoniemi et al., 2009). In the acrotelm and the upper mesotelm fungal-derived FAs were dominating (77%). At the $\delta^{15}N$ turning point lower values of fungal markers (23%) and increased bacterial-derived FAs (67%) could be found. In the lower mesotelm the abundance of microbial-derived FAs was generally decreased and 69% of the detected FAs were bacterial-derived. (Fig. 3)

3.6 Microbial metabolism mirrored by stable isotope patterns

Our findings suggest that nitrogen stable isotope values are linked to microbial abundance and diversity. We found a clear correlation for stable isotope depth pattern and microbial derived FAs in all sites (r^2=0.4), with high values of nitrogen stable isotopes being linked to high amounts of microbial derived FAs.

Generally, plants are depleted in ^{15}N compared to atmospheric nitrogen (which is, per definition, 0 ‰, because air is used as the nitrogen isotopic standard) due to the general preference of plants for the lighter isotope ^{14}N. As such, the average signal of the relatively undecomposed peat (e.g., the acrotelm of the natural/rewetted sites, the catotelm) is -10 to -4 ‰. These plant signals are imprinted in the acrotelm (average of -6.09 ‰; Tab. S5). Furthermore, $\delta^{15}N$ values of plants (here mostly sphagnum mosses) are lower than the values of microbes and bulk material (Aldous, 2002; Lichtfouse et al. 1995). Microbes prefer to mineralize the lighter ^{14}N and plants incorporate (and therefore extract) the microbial mineralized lighter nitrogen (Dijkstra et al., 2006; Novák et al, 1999). Contrary to plants, microbial biomass is enriched in ^{15}N, probably as the result of processing and releasing the lighter ^{14}N during mineralization and hence sequestering the remaining heavier ^{15}N. In addition, caused by the preferential mineralization of lighter nitrogen, the heavier ^{15}N might be also enriched in the remaining humic substances (Novák et al, 1999). The effect of the latter to $\delta^{15}N$ bulk values is probably also enhanced due to the loss of ^{15}N-depleted material during leaching (Damman, 1988; Niemen, 1998), denitrification and the release of gaseous nitrogen (Kohzu, 2003; Niemen, 1998). Our values confirm these reported patterns with highest $\delta^{15}N$ values in the mesotelm (average of -3.63 ‰; Tab. S5) and the correspondence of high microbial activity (reflected by the highest values of microbial-derived FAs) to the $\delta^{15}N$ turning point (Fig. 3, Fig. 4). In acid bogs under aerobic conditions, fungi will dominate the general metabolism in upper peat soils (Thormann et al., 2003). This is pictured by the highest amount of fungal-derived FAs in the acrotelm and the upper mesotelm (Fig. 3). Fungi are preferred decomposers of primary plant material (Wallander et al., 2009; Thormann et al., 2004) hence the depleted plant isotopic signal is relatively preserved in the upper most aerobic horizons. Furthermore, fungi have a relatively low nitrogen demand compared to bacteria (Myers et al. 2012). With increasing depth and increasing oxygen limitation fungal metabolism decreases (Thormann, 2011). In parallel, the

amount of bacterial-derived FAs increases (Fig. 3) as Lin et al. (2014), Hu et al. (2011) and Bauersachs et al. (2009) also reported. They found evidence for bacterial-dominated decomposition in hypoxic conditions. This is in line with the findings of Kohl et al. (2015) and Schmidt and Bölter (2002), who also reported a switch from fungal to bacterial dominance in the mesotelm. Also Andersen et al (2013), Wallander et al. (2009), Winsborough and Basiliko (2010) and Myers et al. (2012) stated out, that fungal biomass is decreasing in peatland soils with depth. In addition, bacterial metabolism ~~needs higher amounts of nitrogen~~is generally faster than fungal metabolism and needs higher amounts of nitrogen (Brunner et al., 2013). ~~In summary, with increasing depth and increasing bacterial metabolism most of the available nitrogen will be immobilized, which results in no or low fractionation of the bulk material (e.g., no preferential loss of the lighter N). Hence, the N turning point could be caused by the N limitation of peatland ecosystems with low oxygen availability.~~ We assume that ~~the~~ ~~δ^{15}N turning point~~ bacteria and fungi compete most over decomposable substrates (not ~~necessarily~~ only nitrogen) at the δ^{15}N turning point~~)~~, resulting in the highest turnover rates with an enrichment of δ^{15}N in the remaining peat, similar to reports ~~as we know~~ from mineral soils with aerobic decomposition (Alewell, et al. 2011~~;~~, Nadelhofer, et al 1996). ~~Tfaily et al. (2014) also reported the highest N values within the mesotelm. This pattern is reflected of derived s at the N turning point (. 3).~~As such, we assume that besides the highest microbial activity, also the diversity of microbial metabolism peak at the δ^{15}N turning point (Fig. 4). This would also be related to the highest δ^{15}N values, because (1) different microbial communities prefer different sources (Dijkstra et al. 2006; Dröllinger et al. 2019) and (2) with increasing bacterial abundance, fungi have to also use recalcitrant (isotopically lighter) sources, because bacterial metabolism will outcompete fungi for the easily degradable substances (Rousk and Bååth, 2007; Winsborough, C. and Basiliko, 2010). Hence, with increasing microbial diversity also the diversity of the mineralized organic fractions increases (Thormann 2006).

To summarize, with an increased diversity of utilized nitrogen sources, more release of lighter ^{14}N is possible and the δ^{15}N values in the remaining substrate should increase (Fig. 3, Fig.4). However, because of the faster and more complete decomposition with increasing microbial activity (Damman, 1988), metabolism of ^{15}N increases as well and fractionation will be less (Lerch et al., 2011). These

contrasting patterns must lead to only small increases in the $\delta^{15}N$ values of the bulk material, if all nitrogen are used, fractionation will be lower at the $\delta^{15}N$ turning point.

In the lower mesotelm, oxygen limitation increases, leading to a general decreas~~eing~~ in microbial metabolism and ~~decreasing~~ related concentrations of microbial-derived FAs (Fig. 3). The decreasing microbial metabolism leads to simultaneously decreasing $\delta^{15}N$ values because an increasing amount of intact vegetation (with low $\delta^{15}N$ values) will be conserved (Fig. 3, Fig. 4).

Finally, with the establishment of permanently waterlogged anaerobic conditions in the catotelm (also indicated by the $\delta^{13}C$ turning point), FA concentration decreases sharply to near zero values. Here, decomposition processes are largely inhibited, which leads to stable $\delta^{15}N$ and $\delta^{13}C$ values, close to the original vegetation signals (Alewell et al., 2011; Krüger et al, 2015) (Fig. 1, Fig3).

4. Conclusion

Our results confirmed that ~~show significant differences in~~the nitrogen isotopic depth trends of peatlands are suitable indicators of the natural, drained ~~and~~ or rewetted ~~peat profiles~~hydrological status. We validated our isotopic hypothesis with microscope analysis of the vegetation remains in the cores as well as the investigation of tree rings as indicators for changed hydrological status in the past. An analysis of gram-positive and gram-negative bacterial-derived FAs versus fungal- derived FAs underpinned our hypothesis with the expected changes in microbial abundance with depth. The aerobic acrotelm was characterized by a high fungal abundance with low nitrogen demand and turnover. The upper mesotelm was the transition to a mixture of decreasing fungal and increasing bacterial abundance, competing on organic substrates and resulting in an enrichment of $\delta^{15}N$ values. In the lower mesotelm microbial decomposition generally decreased, but was dominated by bacterial abundance and finally microbial metabolism was strongly impeded and $\delta^{15}N$ values stabilized in the anaerobic catotelm.

Carbon isotope compositions are also changing with drainage, but they are neither a suitable indicator for a switch in microbial abundance within the drained horizon, nor for the trend induced by ~~with~~ rewetting of the peatland~~, as it is visible for the nitrogen compositions due nitrogen limitation and recycling processes~~. Summing up, $\delta^{15}N$ depth profiles in peat might give more insights into ~~the degree of~~a switch of microbial metabolism~~transformation~~, because they reflect more precisely different

microbial abundance than carbon isotope compositions. Therefore, we conclude that $\delta^{15}N$ depth profiles could act as a reliable and efficient tool to get fast and easy information about the hydrological~~y~~ status, restoration success and drainage history.

Author contribution

~~Christine Alewell and Jens Leifeld are the supervisors of the project.~~ Miriam Groß-Schmölders: sampling, measurements, evaluation and analysis of data, manuscript writing

~~,~~ Jan Paul Krüger: sampling and measuring

~~,~~ Axel Birkholz: measurements, help in analytics

~~and~~ Kristy Woodard: discussion ~~were doing the measurements~~

Pascal von Sengbusch: peat ~~was doing the~~ microscopy and vegetation analysis. ~~Miriam Groß-Schmölders prepared the manuscript with contribution of all co-authors.~~

Jens Leifeld: project idea, supervision and discussion

Christine Alewell: project idea, supervision, discussing and writing

Competing interests

The authors declare that they have no conflict of interest.

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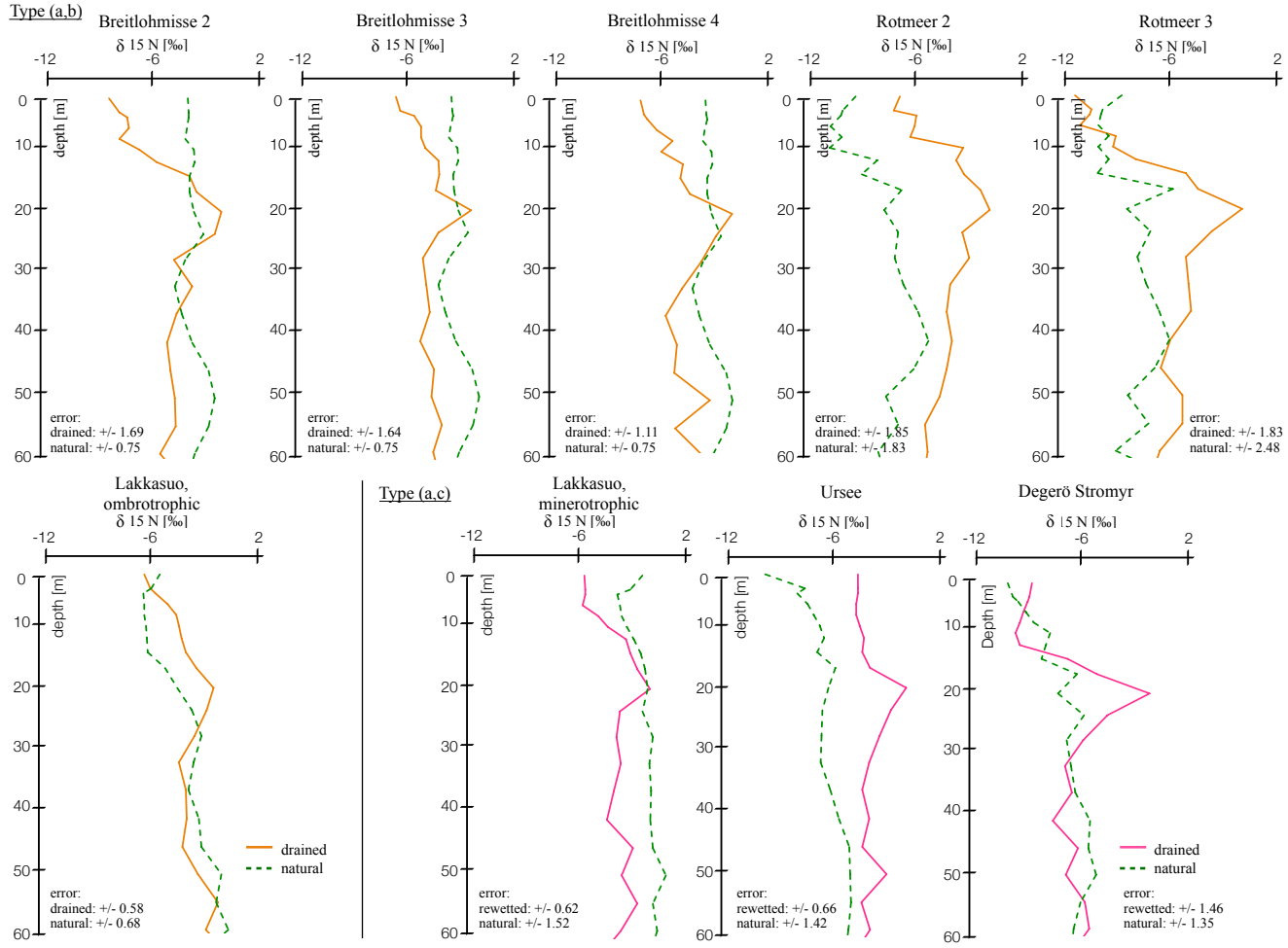

Figure 1: $\delta^{15}N$ depth profiles in all natural and drained (or rewetted) sites; with normalized depth and normalized $\delta^{15}N$ values (see chapter 2.4); trend types: (a) natural (green), (b) drained up to the surface (orange) and (c) rewetted above drainage (pink) (For single, non-normalized values see supplementary information).

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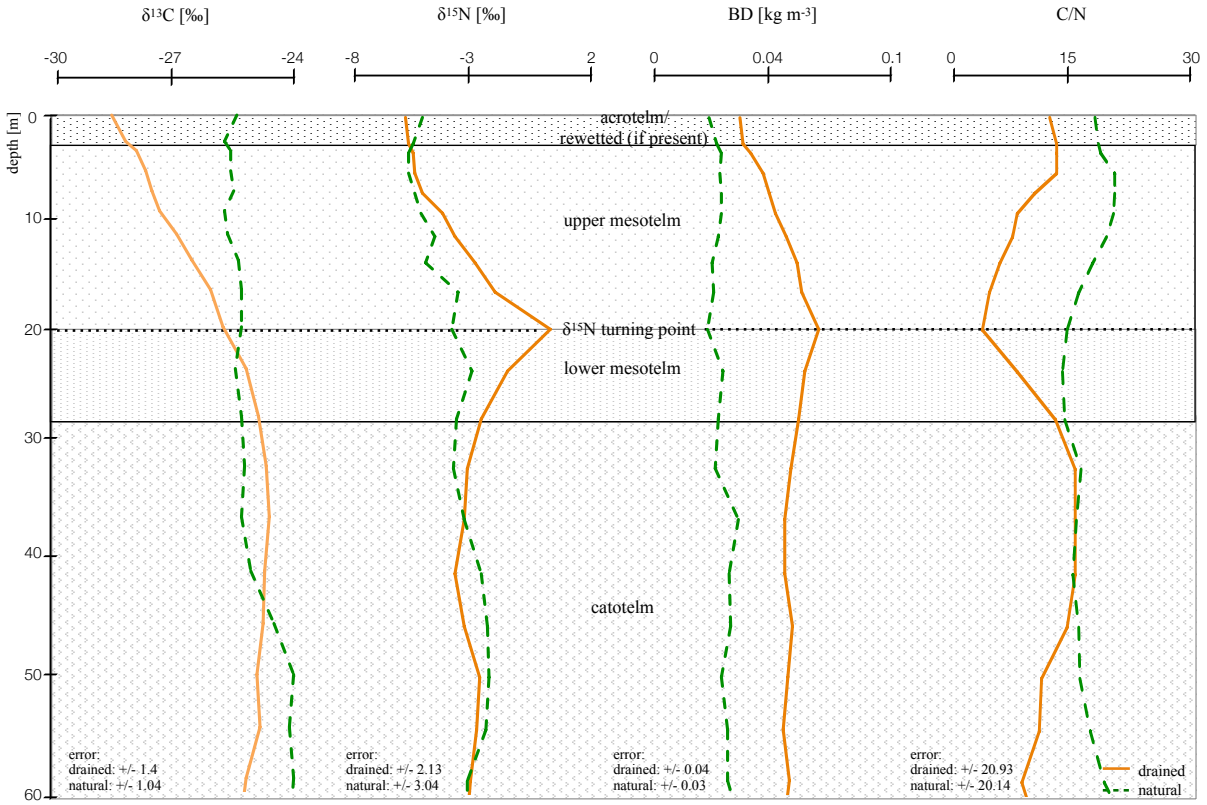

Figure 2: Mean depth trends ($\delta^{15}N$, $\delta^{13}C$, C/N and BD) of natural and drained sites of all nine investigated peatlands with normalized depth and normalization based on $\delta^{15}N$ compositions (see chapter 2.4; For single $\delta^{13}C$, C/N and BD values of all peat cores see supplementary information).

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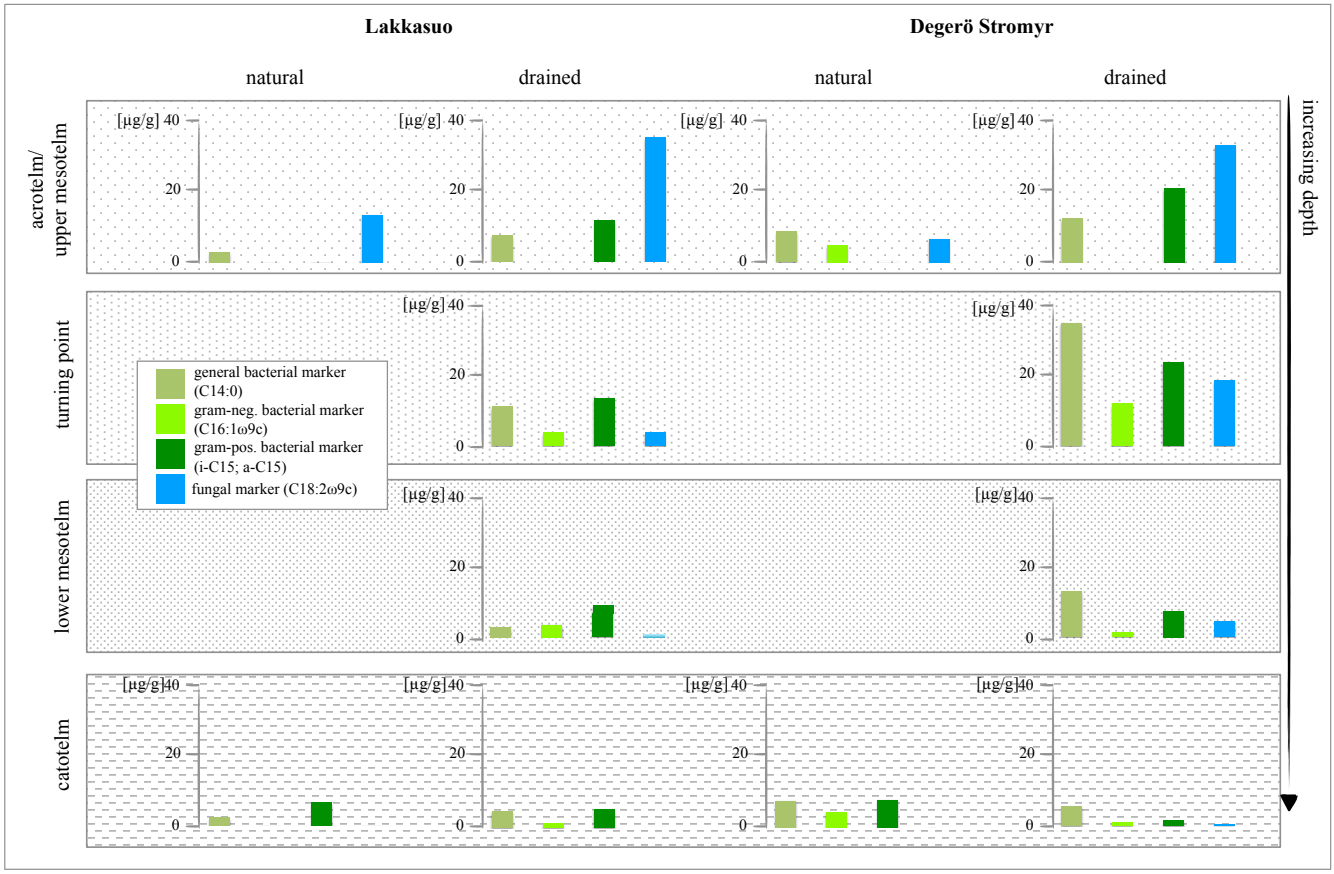
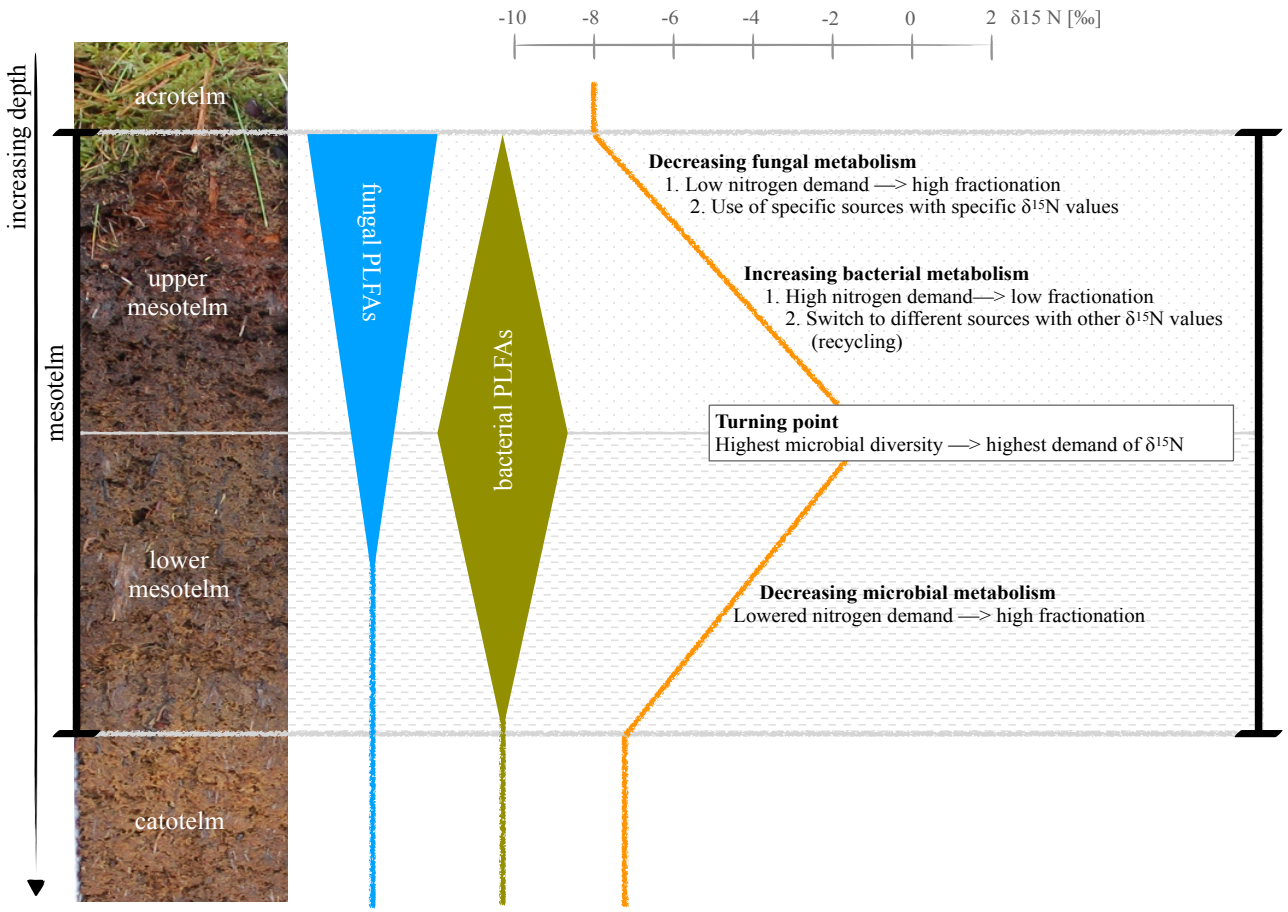


Figur[1]e 3: Fatty acid ~~A amount~~concentrations ~~-~~of bacterial and fungal marker in natural and drained wetlands Lakkasuo and Degrö Stromyr~~LD$_0$, LN$_0$, DD and DN~~ [2]in different horizons.



800

Figure 4: Hypothesis of a microbial switch (fungi to bacteria) with depth, reflected by specific FAs, and its influence of the $\delta^{15}N$ depth trend; example photo and $\delta^{15}N$ values of the ombrotrophic, drained site in Lakkasuo (LD_0) (note all isotope values are normalized to zero at turning point).

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Table 1: Labeling of all drilling sites

Location	Labeling
Degerö	
natural mire	DN
drained	DD
Lakkasuo	
minerotrophic natural	LN_m
minerotrophic drained	LD_m
ombrotrophic natural	LN_o
ombrotrophic drained	LD_o
Breitlohmisse	
natural mire	BN
natural dry	BN_d
drained	BD_1
near the mire edge	BD_2
Rotmeer	
natural mire	RN
drained, with Sphagnum	RD_1
drained, without Sphagnum	RD_2
Ursee	
natural mire	UN
drained	UD

Table 2: Overview of studied mires; coordinates (lat./long.); mean annual temperature (MAT); annual precipitation (P); Sphagnum mosses (Sph.) (Laine et al., 2004; Nielsson et al., 2008; DWD 2018, Alexandersson et al., 1991; Armbruster et al., 2003)

Country	Mire	lat/long..	MAT	P	Main vegetation on top	
			[°C]	[mm]	natural	drained
Sweden	*Degerö Stromyr*	64°11'lat., 19°33'long.	+1.2	523	Sph. majus	Sph. balticum
Finland	*Lakkasuo*	61°48'lat., 24°19'long.	+3	700	Sph. angustifolia	Sph. angustifolia
Germany (Black Forest)	*Breitlohmisse*	48°41'lat., 8°25'long.	+7	835	Sph. capillifolium	Sph. capillifolium
	Ursee	47°51'lat., 8°25'long.	+7	1600	-	-
	Rotmeer	47°52'lat., 8°6'long.	+7	1600	Sph. rubellum	Sph. rubellum patches

Table 3: Description of vegetation of four of the study sites; Sphagnum mosses (Sph.)

Site	Horizon	Main species	Description
Degerö (DD)	rewetted horizon	*Sph. balticum*	Yellow, good preserved Sph.-turf, detached Sph. cymbifolia, *Vaccinium oxycoccos Eriophorum vaginatum, Andromeda polifolia* & *Cladopodiella fluitans*
	mesotelm	*Sph. balticum*	Darker, grayish; Sph.-turf, some *Eriophorum vaginatum,* detached Sph. cymbifolia, *Vaccinium oxycoccos, Andromeda polifolia*
	catotelm	*Sph. balticum*	Yellow; Sph.-turf, more Sph. cymbifolia, some *Eriophorum vaginatum,* detached, *Vaccinium oxycoccos, Andromeda polifolia*
Lakkasuo (LD_o)	upper mesotelm	*Sph. rubellum*	Dark brown; Sph.-turf, mostly *Sph. rubellum* with *Pleutrozium schreberi* in the uppermost part
	lower mesotelm	*Sph. rubellum*	Dark brown, grayish; Sph.-turf, mostly *Sph. rubellum* and *Sph. balticum*
	catotelm	*Sph. rubellum*	Light brown, yellow; Sph.-turf, mostly *Sph. rubellum*
Breitlohmisse (BN_d)	upper meostelm	*Sph. capillifolium*	Brown; Sph.-turf mostly *Sph. capillifolium* and *Sph. cymbifolia*, much *Ericaceous roots* and some *Eriophorum vaginatum* stems
	mesotelm	*Sph. cymbifolia*	Dark brown; Sph.-turf mostly *Sph. capillifolium* and *Sph. cymbifolia*, some *Ericaceous roots* and *Eriophorum vaginatum*
	catotelm	*Sph. cymbifolia*	Lighter, reddish; Sph.-turf mostly *Sph. capillifolium* and *Sph. cymbifolia*, some *Sph. acutifolia*
Rotmeer (RD_1)	upper mesotlem	*Sph. acutifolia*	Brown-reddish, yellow; Sph.-turf, mostly *Sph. acutifolia*, some *Sph. rubellum,* detached *Eriophorum vaginatum*
	mesotelm	*Sph. cymbifolia*	Dark brown, grayish; Sph.-turf, mostly *Sph. cymbifolia*, and *Sph. acutifolia*, some *Sph. rubellum,* detached *Eriophorum vaginatum*
	catotelm	*Sph. cymbifolia*	Reddish, yellow; Sph.-turf, mostly *Sph. cymbifolia*, some *Sph. acutifolia,* detached *Eriophorum vaginatum*