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. Since the last centuries European peatlands are degrading along with drainage, land use and climate changes. 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. 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. 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). 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 $\delta^{15}N$ 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 $\delta^{15}N$ turning point. Below the $\delta^{15}N$ 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 $\delta^{15}N$ 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.

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 found in previous studies (e.g. Krüger et al., 2016; Alewell et al., 2011) to be specific 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.

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.

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 $\delta^{15}N$ depth pattern in natural, drained or rewetted 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.

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 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).

The trends of the single eight 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 $p < 0.05$ between $\delta^{15}N$ in the center of the mesotelm compared to the $\delta^{15}N$ values in 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).

3.5 Changing 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), fungi will be outcompeted by bacteria with increasing depth and changing hydrological conditions (darker, less oxygen). 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 is generally faster than fungal metabolism and needs higher amounts of nitrogen (Brunner et al., 2013). We assume that bacteria and fungi compete most over decomposable substrates (not only nitrogen) at the $\delta^{15}N$ turning point, resulting in the highest turnover rates with an enrichment of $\delta^{15}N$ in the remaining peat, similar to reports from mineral soils with aerobic decomposition (Alewell, et al. 2011; Nadelhofer, et al 1996). As such, we assume that besides the highest microbial activity, also the diversity of microbial metabolism peak at the $\delta^{15}N$ turning point (Fig. 4). This would also be related to the highest $\delta^{15}N$ 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 $\delta^{15}N$ 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 decrease in microbial metabolism and 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 the nitrogen isotopic depth trends of peatlands are suitable indicators of the natural, drained or rewetted 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 rewetting of the peatland. Summing up, $\delta^{15}N$ depth profiles in peat might give more insights into a switch of microbial metabolism, 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 status, restoration success and drainage history.

Author contribution

Miriam Groß-Schmölders: sampling, measurements, evaluation and analysis of data, manuscript writing
Jan Paul Krüger: sampling and measuring
Axel Birkholz: measurements, help in analytics
Kristy Woodard: discussion
Pascal von Sengbusch: peat microscopy and vegetation analysis.
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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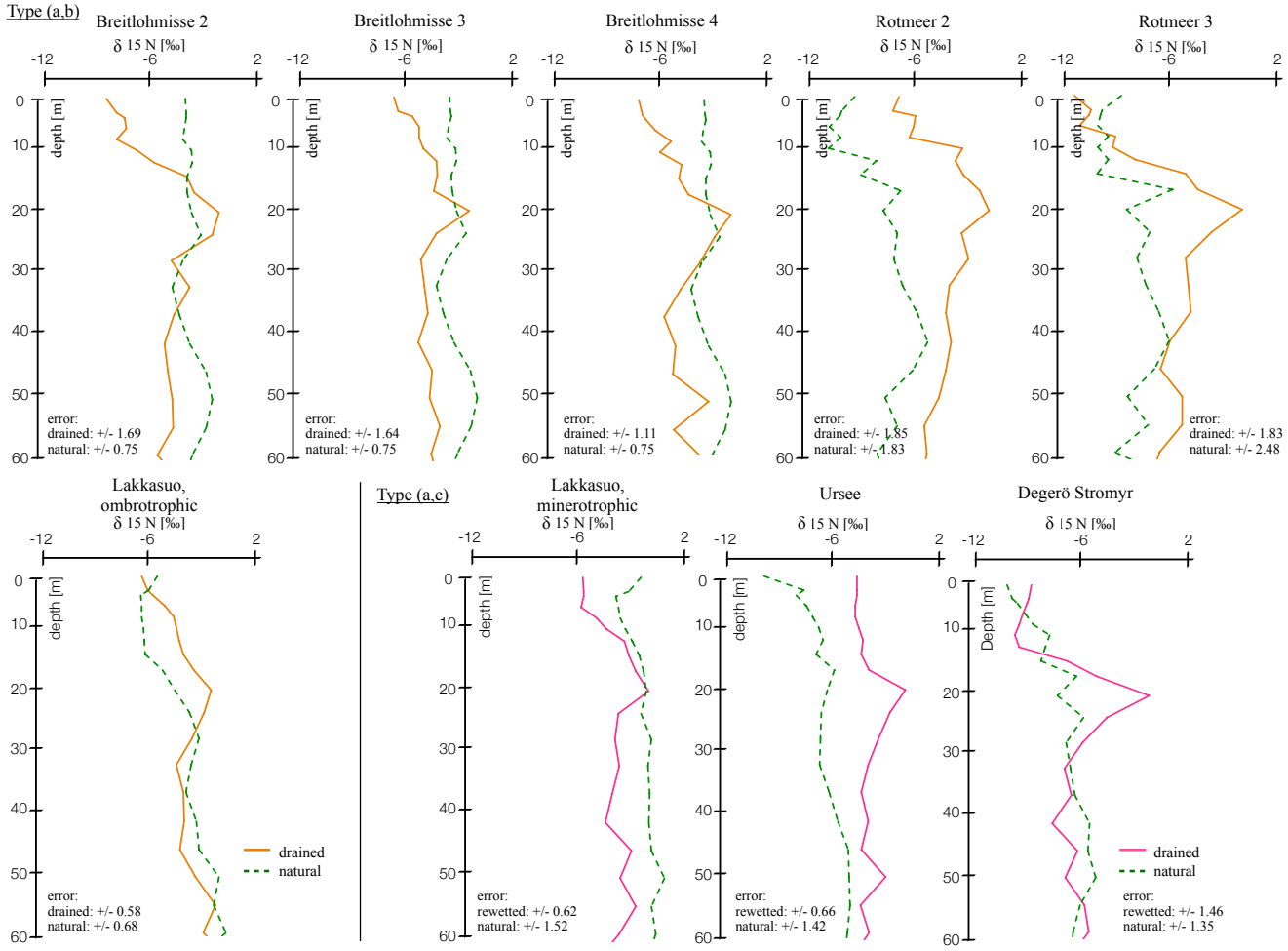


Figure 1: $\delta^{15}N$ depth profiles in all natural and drained (or rewetted) sites; with normalized depth and normalized $\delta^{15}N$ values (see chapter 2.4); trend types: (a) natural (green), (b) drained up to the surface (orange) and (c) rewetted above drainage (pink) (For single, non-normalized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

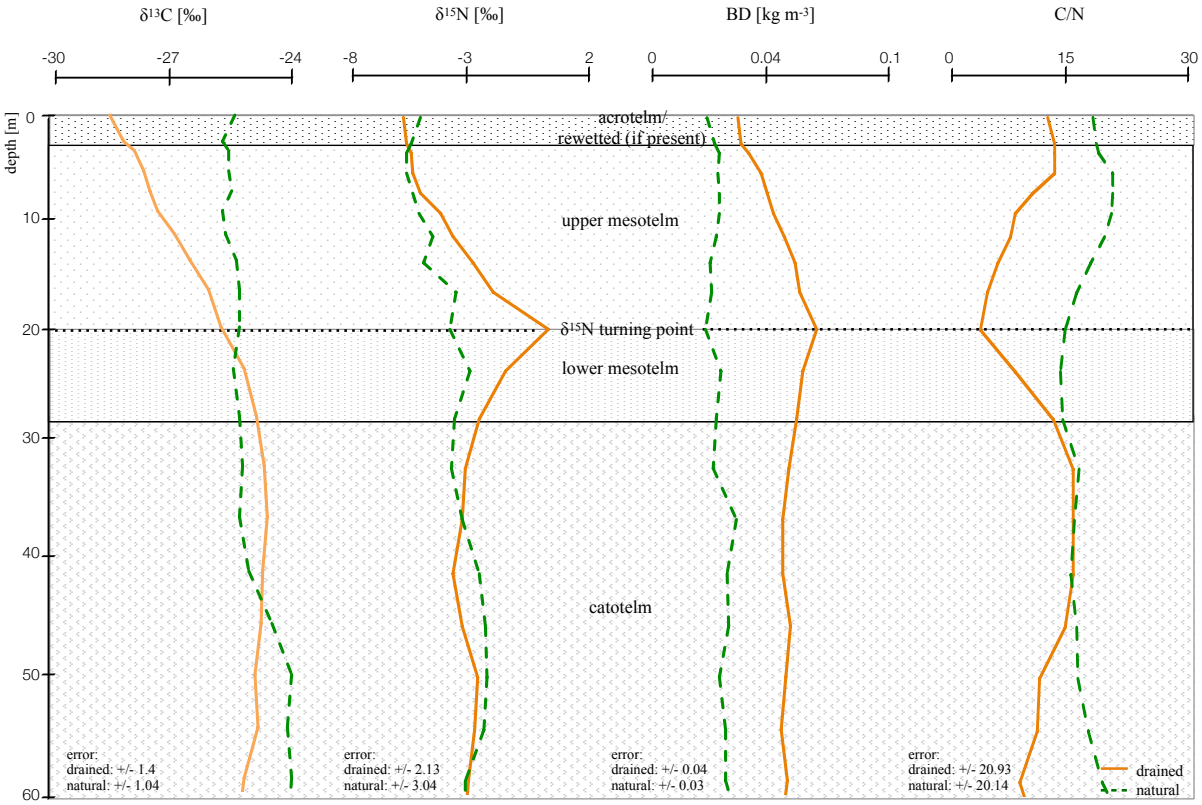
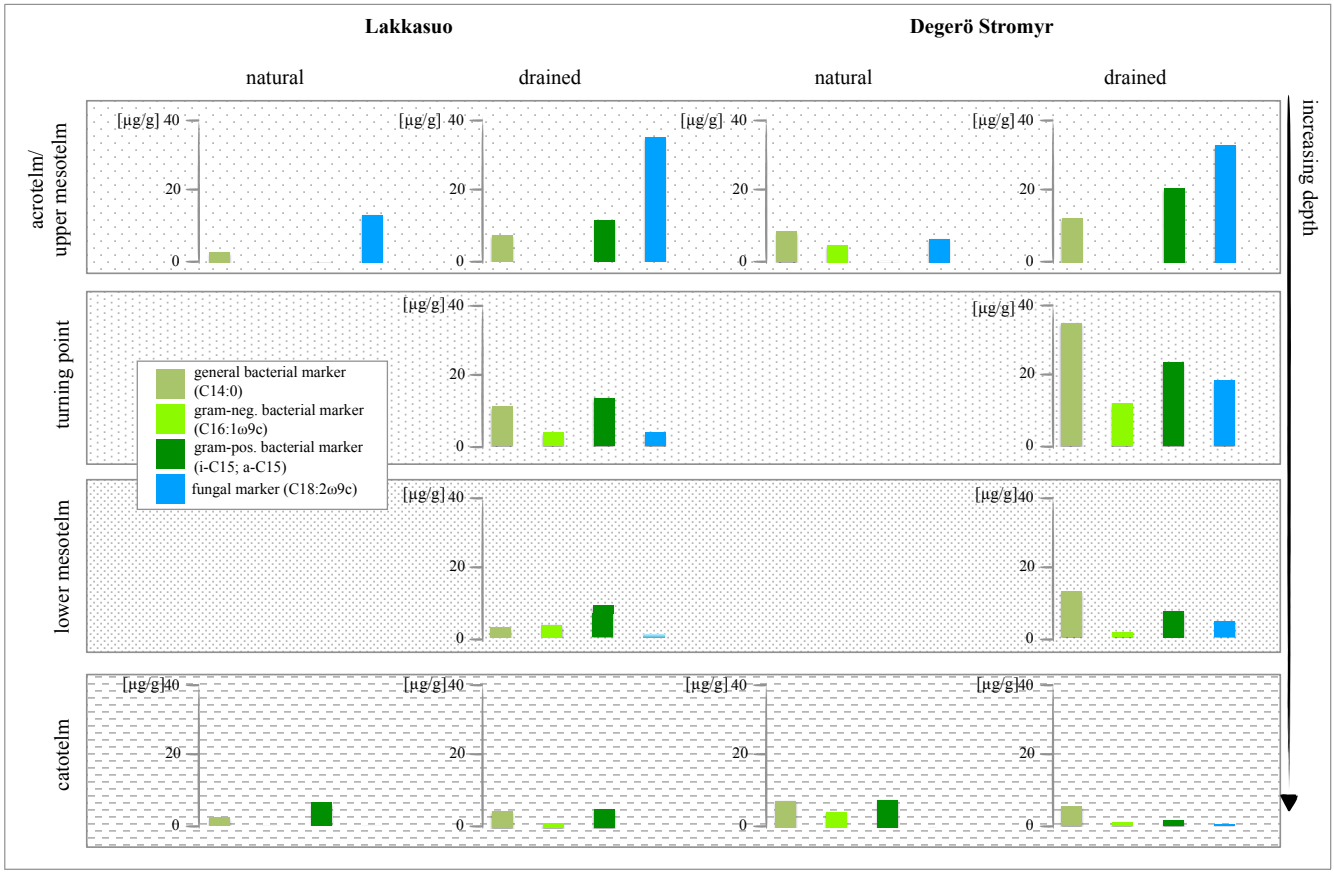


Figure 2: Mean depth trends ($\delta^{15}N$, $\delta^{13}C$, C/N and BD) of natural and drained sites of all nine investigated peatlands with normalized depth and normalization based on $\delta^{15}N$ compositions (see chapter 2.4; For single $\delta^{13}C$, C/N and BD values of all peat cores see supplementary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



Figur[1]e 3: Fatty acid concentrations of bacterial and fungal marker in natural and drained wetlands Lakkasuo and Degrö Stromyr [2]in different horizons.

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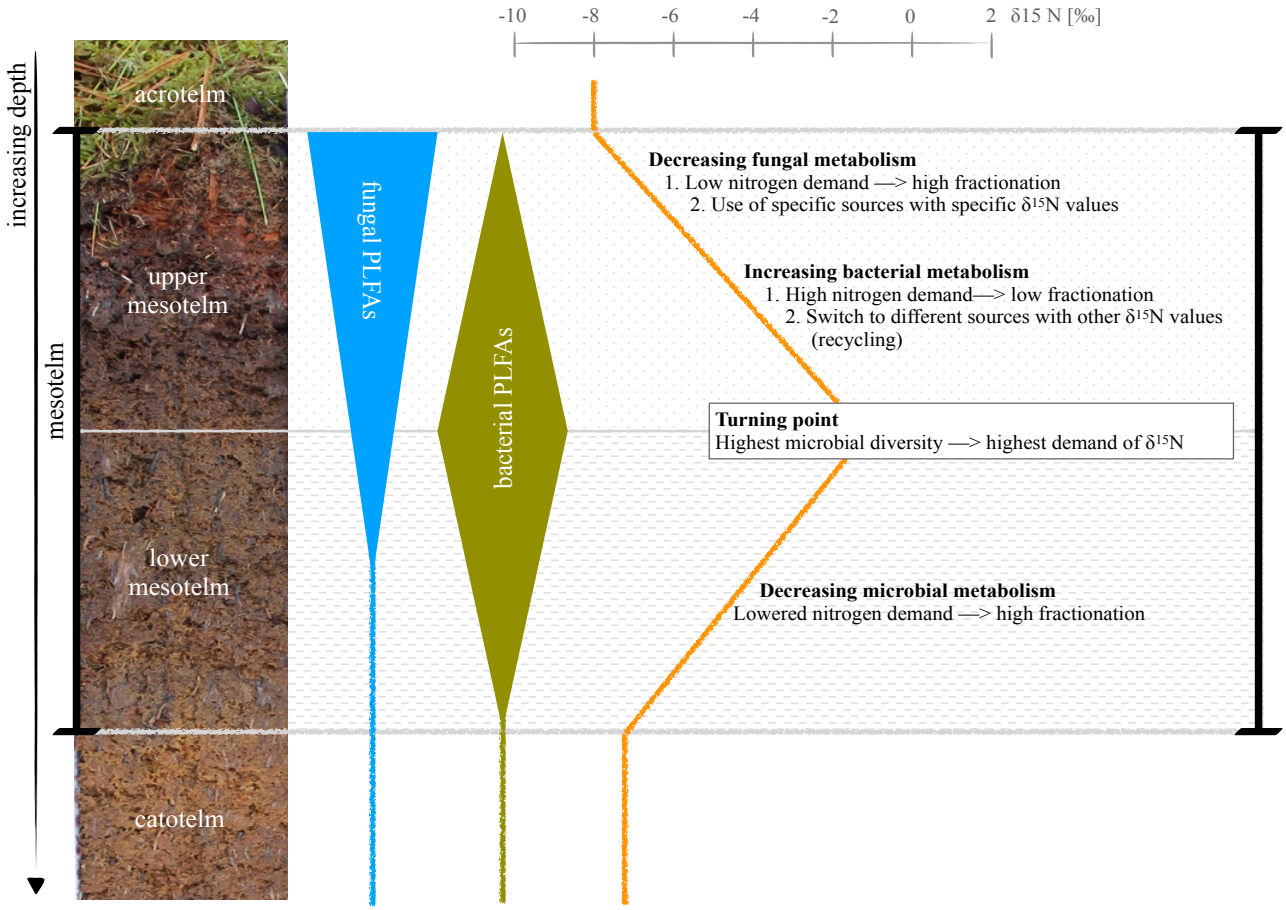

Figure 4: Hypothesis of a microbial switch (fungi to bacteria) with depth, reflected by specific FAs, and its influence of the $\delta^{15}N$ depth trend; example photo and $\delta^{15}N$ values of the ombrotrophic, drained site in Lakkasuo (LD_o) (note all isotope values are normalized to zero at turning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

730 **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

735 **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*