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Rewetting and Drainage of Nutrient-Poor Peatlands Indicated by Specific Bacterial Membrane Fatty Acids and a Repeated Sampling of Stable Isotopes (δ^{15}N, δ^{13}C)

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Peatland degradation impairs soil functions such as carbon storage and the existence of biodiversity hotspots. Therefore, and in view of the ongoing climate change, an efficient method of evaluating peatland hydrology and the success of restoration efforts is needed. To understand the role of microbial groups in biogeochemical cycling, gaseous loss and isotopic fractionation that lead to specific isotopic depth patterns (δ^{13}C, δ^{15}N), we integrated previously published stable isotope data with a membrane fatty acid (mFA) analysis related to various microbial groups that are known to be common in peatlands. We performed two sampling campaigns to verify the observed stable isotope depth trends in nutrient-poor peatlands in Northern Europe. Cores were taken from adjacent drained (or rewetted) and undrained sites. Fungal-derived mFA abundance was highest in the uppermost part of the drained layer. We found increasing bacterial-derived mFA concentrations with depth peaking in the middle of the drained layers, which correlates with a δ^{15}N peak of bulk material. The results support our hypothesis that changing peatland hydrology induce a shift in microbial community and metabolism processes and is therefore also imprinted in stable isotope values. Under waterlogged conditions overall levels of microbial-derived mFAs were generally low. Drained layers showed simultaneous changes in microbial abundance and composition and depth trends in stable isotope bulk values. Bacteria, particularly acidobacteria, can be expected to dominate increased denitrification with low oxygen saturation accompanied by increased δ^{15}N bulk values in the remaining substrate. Interestingly, cores from recent rewetted peatlands show no depth trend of δ^{15}N in the layers grown under rewetting conditions; this is congruent with relatively low concentrations of microbial-derived mFAs. Hence, we conclude that stable isotopes, especially δ^{15}N values, reflect changing microbial metabolic processes, which differ between drained and undrained - and especially also for recent rewetted–peatlands. As today stable isotope measurements are routine measurements, these findings enable us to get cost- and time efficient reliable information of drainage and restoration success.

Keywords: peatland degradation, stable isotopes, membrane fatty acids, soil microbiology, biochemistry, element cycling

INTRODUCTION

A unique biodiversity, slow rates of decomposition and the storage of significant quantities of carbon characterize wetland soils; this is especially true for nutrient-poor peatlands (Moore and Basiliko, 2006). The protection of biodiversity and successful peatland restoration could save 1.91 (0.31–3.38) gigatons (Gt) of CO_2-equivalent greenhouse gas emissions (Leifeld and Menichetti, 2018). Furthermore, 6% of the greenhouse effect is contributed by N_2O Schulze et al. (2009), which is also released by degraded peatlands due to impaired soil functioning (Palmer and Horn, 2015). For other ecosystems, microbial communities and their major role in biochemical cycling of carbon and nitrogen in soil are well documented, but little is known of the microbial community and its function in peatlands (Elliott et al., 2015). In particular, information about microbial community structures in different layers and their influence on biochemical processes under rewetting conditions is widely unknown (Elliott et al., 2015). Thus, more reliable information about peatland degradation and restoration success is needed.

Peat soil can be divided into three layers. In the uppermost part, the acrotelm, most biological metabolism and nutrient cycling takes place (Asada et al., 2005; Artz, 2013). In the lower layer, the anaerobic, water-saturated catotelm, microbial metabolism is suppressed due to the lack of oxygen (Asada et al., 2005; Artz, 2013; Lin et al., 2014). In between, the mesotelm is characterized by a fluctuating water table and facultative anaerobic conditions, which therefore leads to shifting levels of microbial abundance and activity (Asada et al., 2005; Artz, 2013; Lin et al., 2014). Drainage of peatlands expands the mesotelm, wherein formerly preserved organic substrates can be decomposed (Zedler and Kercher, 2005). If rewetting occurs, the former mesotelm will become anaerobic again, and aerobic microbial activity will be inhibited (Asada et al., 2005; Andersen et al., 2010).

Whereas microbial metabolism of carbon is discussed in several papers (see review of Blodau (2002)), nitrogen cycling in peatlands is less well studied. Nitrogen fixation has been reported to only occur in surface layers of peat, (Lin et al., 2014). However, the N_2O producing microbial mediated nitrification and denitrification of organic matter as well as other chemical processes occur also in deeper layers (Bremner, 1997; Palmer and Horn, 2015). For peatlands, Palmer et al. (2010) report denitrification of organic matter as the main N_2O source. Denitrification causes a reduction of nitrate and nitrite by converting them to nitric oxide (NO) and N_2O and, ultimately, to dinitrogen (N_2; Novák et al., 1999). Especially for the deep, anaerobic layer, Lin et al. (2014) reported extremely low values for denitrification and other N-cycling processes and, therefore, a conservation of the substrate.

Stable isotopes of carbon and nitrogen are known indicators of peatland hydrology (Alewell et al., 2011; Krüger et al., 2016; Groß-Schmölders et al., 2020; Kohl et al., 2015). For $\delta^{13}C$, Alewell et al. (2011), Krüger et al. (2016), Novak et al. (1999), Hobbie et al. (2017) and Biester et al. (2014) report an enrichment of ^{13}C with depth due to an increasing degree of organic matter decomposition. Substrates have a natural and specific range of

^{13}C values (Lerch et al., 2011). As lignin, cellulose and lipids are known to be depleted in ^{13}C, glucose, amino acids, pectin and hemicellulose are enriched in ^{13}C (Lerch et al., 2011). In undrained wetlands, the combination of these substrates is mostly preserved due to the waterlogged conditions. If drainage takes place, the original bulk soil $\delta^{13}C$ value is changed by degradation and microbial metabolism processes. Kohl et al. (2015) state that an increasing $\delta^{13}C$ depth trend is a consequence of a switch in dominant microbial decomposition, which has stronger effects than the residual enrichment of recalcitrant compounds such as lignin. Kohl et al. (2015) stated out, that fungi are main decomposer in the uppermost soil horizons and bacteria are more prominent in deeper horizons. With this switch of dominant microbial groups, also the decomposed material switches and therefore the ^{13}C bulk values change.

Additionally, a positive correlation between increased microbial metabolism and $\delta^{15}N$ was previously presented (Gro-Schmölders et al., 2020). Fractionation of stable isotopes during microbial metabolism of nitrate and ammonium occurs, since most organisms prefer the lighter and more frequently occurring ^{14}N (Kohzu et al., 2003; Robinson et al., 1998). As a result, plants in particular incorporate and translocate the lighter ^{14}N upwards to stem and foliar, which leads to an enrichment of heavier ^{15}N in the remaining bulk material (Högberg et al., 1996). Additionally, the mycorrhizal uptake of lighter ^{14}N into plants increases the $\delta^{15}N$ values of bulk material (Hobbie and Högberg, 2012). Furthermore, with ongoing microbial metabolic processes in peat, the $\delta^{15}N$ values increase as long as microbial metabolism occurs, and lighter ^{14}N will be leached, translocated or lost via outgassing (Novák et al., 1999; Damman, 1988; Niemen, 1998). In 2010, Goldberg et al. (2010) showed, that increasing oxygen concentrations in drained fens leads to higher N_2O release by nitrification, which is followed by increasing $\delta^{15}N$ values in the remaining substrate. Thus, microbial abundance and stable isotope ratios are closely linked, especially for some microbial groups that are more active in nitrogen cycling than others and therefore play a greater role (Tfaily et al., 2014). Fungi have a low demand for nitrogen, making them less likely to be a main driver of increasing $\delta^{15}N$ values (Thormann, 2005). In contrast, acidobacteria are one of the main bacterial groups in peat and are highly active in nitrogen cycling; in particular, they are involved in denitrification and N fixation (Ward et al., 2009). Accordingly, their abundance can be expected to have a close link to carbon and especially nitrogen stable isotope depth trends (Weijer et al., 2010).

To examine microbial abundance, we measured the concentrations of specific membrane fatty acids (mFAs). Membrane fatty acids are valid markers to indicate the abundance of specific microbial communities. Sundh et al. (1997) and Torres and Pancost (2016) demonstrated that mFAs are persistent and, to a high degree, insoluble compounds in peat soil. Membrane fatty acids vary based on their origin (plants, specific microbial groups, etc.; Bajerski et al., 2017; Finotti et al., 1993; Piotrowska-Seget and Mrozik, 2003; Reiffarth et al., 2016; Willers et al., 2015). Therefore, based on an analysis of the quantity of mFAs present, the relative abundance

of certain microbial communities might be assessed (Piotrowska-Seget and Mrozik, 2003; Torres and Pancost, 2016). We tested the existence of four bacterial markers and one fungal marker:

- i-C15:0 and C16:1ω7c, which, in combination, are indicative of acidobacteria (Damast et al., 2011; Dedysh and Damsté, 2018; Myers and King, 2016);
- C14:0 and C17:0, which are generally indicative of bacteria (Willers et al., 2015; Zelles, 1997); and
- C18:2ω6c, which is indicative of saprotrophic fungi (Sundh et al., 1997; Elvert et al., 2003; Willers et al., 2015).

To differentiate between wetland soil functioning as carbon storage and biodiversity hotspots in undrained, rewetted and drained sites, we investigate the influence of drainage and rewetting on microbial-derived mFA abundance and stable isotopic values. We studied two peatlands with different drainage histories, using a high spatial resolution of 4 cm in the uppermost 50 cm of the peat columns. In both investigated peatlands' ditches were installed to drain the sites for agricultural use (Mikkenen et al.m, 1999; Nielsson et al., 2008). In Lakkasuo, Southern Finland, we located a site that had experienced continuous drainage since 1961 (Minkkinen et al., 1999). In the Swedish Degerö Stormyr, ditches were installed at the beginning of the 20th century (Nielsson et al., 2008). They have filled up naturally with sphagnum over the last 20 years and sites are thus rewetting. We compared undrained with drained sites in Lakkasuo and undrained with rewetted sites in Degerö Stormyr. Furthermore, to verify our previous results regarding stable isotopes as markers for peatland hydrology and drainage history (Minkkinen et al., 1999), we investigated depth trends at two points in time (2013 and 2017) to verify pattern stability over time.

We define a sudden directional change in the stable isotope depth patterns as "turning points," according to Alewell et al. (2011) and Groß-Schmölders et al. (2020). The $\delta^{15}N$ turning point is located in the middle of the mesotelm, where $\delta^{15}N$ values are highest. In contrast, the $\delta^{13}C$ turning point marks the bottom of the mesotelm and the onset of the underlying catotelm, above which the $\delta^{13}C$ values start to decrease continuously up to the surface.

The contribution of this paper is to examine the microbial composition of peat soil with respect to stable isotope fractionation and test the persistence of stable isotope depth trends with a repeated sampling approach.

We hypothesize the following:

- Bacterial abundance, especially that of acidobacteria, is highest in the mesotelm.
- Bacterial abundance is the main driver of the nitrogen stable isotope depth trend in nutrient-poor peatlands.
- Stable isotope depth patterns are persistent over a time span of four years (2013–2017) and are therefore reliable indicators of drainage and rewetting.

MATERIALS AND METHODS

Site Description

We investigated two nutrient-poor peatlands in northern Europe, both classified as fibric Histosol (HSf; IUSS, 2015; **Table 1**).

Degerö Stormyr (64°11′lat. 19°33′long.; 200 m above sea level (a.s.l.)) is situated in Northern Sweden, at the Kulbäcksliden Experimental Forest near Vindeln, between the rivers Umeälven and Vindelälven (Eurola et al., 1984). It is an acidic bog and consists of interconnected small mire patches divided by ridges of glacial till. The climate is characterized as cold with no dry seasons and cold summers (Dfc-zone after Köppen-Geiger classification; Peel et al., 2007). Mean annual temperature is +1.2°C and the annual precipitation has an average of 523 mm (Alexandersson et al., 1991). Ditches were installed in Degerö in the beginning of the 20th century but a natural reestablishment of sphagnum took place over the last few decades (>20 years). Therefore, we define this site as rewetted. In the undrained part the main moss species is *Sphagnum majus* Nielsson et al. (2008) and the water table is near the surface (**Table 1**). The humification index (HI) after von Post is low (H1-H2) and macro residuals are highly visible (**Table 1**; Groß-Schmölders et al., 2020). Also, biochemical parameters indicate undrained conditions. The carbon:nitrogen ratio (CN) in the acrotelm is 89, and the bulk density (BD) is low (0.02 kg m^{-3}), both is typical for undrained nutrient poor peatlands (**Table 1**; Groß-Schmölders et al., 2020). For the rewetted site the main moss species is *Sphagnum balticum* (Nielsson et al., 2008). The water table is near the surface, HI is low (H2) and macro-residuals are preserved well in the upper horizon (**Table 1**; Groß-Schmölders et al., 2020). In contrast the values of the former mesotelm indicate degradation: HI is higher (H3), less macro-residuals are visible, CN decreased to 41 and the BD increased (0.06 kg m^{-3}; **Table 1**; Groß-Schmölders et al., 2020).

Lakkasuo (150 m a.s.l.) in Central Finland is an eccentric peatland complex with two parts. The southern part is a bog with ombrotrophic conditions; whereas the northern part is a fen (Minkkinen et al., 1999). Only samples of the ombrotrophic bog are included for this study. 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). Mean annual temperature is +3°C and the average precipitation is 700 mm (Laine et al., 2004). Lakkasuo is still drained. Ditches installed in 1961 (70 cm depth, spacing of 40–60 m) affected approximately 50% of the peatland (Minkkinen et al., 1999). The main current moss species in undrained sites is *Sphagnum angustifolia* (Laine et al., 2004). HI is low (H2), a high number of macro-residuals is visible, and the water table is near the surface (<5 cm) (**Table 1**; Groß-Schmölders et al., 2020). Also, the biochemical parameters indicate undrained conditions: high CN (66) and low BD (0.02 kg m^{-3}; **Table 1**; Groß-Schmölders et al., 2020). In the drained site *Pleurozium* spp.*, a moss species of drier environments, is the main moss species and a high number of pine trees is abundant. Macro-residuals are strongly affected by decomposition, HI (H3-H4) and BD (0.06 kg m^{-3}) are high and CN (44) is low (**Table 1**; Groß-Schmölders et al., 2020).

TABLE 1 | Detailed information for the acrotelm/former mesotelm (only for Degerö rewetted) of the drained, rewetted and undrained sites of Degerö Stormyr and Lakkasuo at the surface (Nielsson et al., 2008; Mikkenen et al., 1999; Groß-Schmölders et al., 2020); av.: average, WT: water table below surface, C: carbon, N: nitrogen, CN: carbon: nitrogen ratio, BD bulk density [k m^{-3}], von Post Indices (vP).

Location	av. WT [cm]	av. pH	av. C [kg m^{-2}]	av. N [kg m^{-2}]	CN	BD	vP
Lakkasuo	—	—	—	—	—	—	—
undrained	5	4.1	44.8	0.7	65.6	0.02	H2
Drained	26	3.8	48.1	1.0	44.2	0.06	H3-H4
Degerö Stormyr	—	—	—	—	—	—	—
undrained	0	4.8	42.9	0.4	88.8	0.02	H1-H2
Rewetted	10	4.8	45.2	0.7	58.8/41.1	0.02/0.06	H1-H2

Soil Sampling and Bulk Analyses

We drilled three volumetric peat cores per site in September 2013 and one in June 2017. Cores were drilled with a Russian peat corer (Eijkelkamp, Netherlands). Per site three Cores were taken (1–3). Cores were taken in the undrained parts (Degerö Stormyr (DU13$_{1-3}$, DU17); Lakkasuo (LU13$_{1-3}$, LU17)), and in 1-m distance to a drainage ditch (to 1-m depth) (Degerö Stormyr rewetted (DR13$_{1-3}$, DR17); Lakkasuo drained (LD13$_{1-3}$, LD17)). We drilled the cores in 2017 close to the location of 2013, with a GPS accuracy of 1.3 m, to monitor possible hydrology changes, which could be mirrored by stable isotope depth patterns and microbial FA abundance.

The cores were encased in hard plastic shells, stored in coolers, and transported to the laboratory. The cores were sliced in 2 cm sections for the isotope analyses. Every second section was analyzed, 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 signatures were measured an elemental analyzer combined with an isotope ratio mass spectrometer (EA-IRMS) (Inegra2, Sercon, Crewe, United Kingdom). Carbon isotopic composition (^{13}C/^{12}C) was expressed relative to Vienna Pee-Dee Belemnite (VPDB) standard and reported in delta notation (‰):

$$\delta^{13}C_{sample} = \left(\frac{R_s}{R_{std}} - 1\right)x100$$

R_s and R_{std} are the ratios of ^{13}C/^{12}C in the sample and VPDB standard ($R_{std} = 0.011182$).

Nitrogen stable isotopes were expressed relative to atmospheric nitrogen and reported in delta notation (‰):

$$\delta^{15}N_{sample} = \left(\frac{R_s}{R_{std}} - 1\right)x100$$

R_s and R_{std} are the ratios of ^{15}N/^{14}N in the sample and atmospheric nitrogen ($R_{air} = 0.0$).

Fatty Acid Analyses

We aimed to extract total membrane FAs to distinguish between FAs of different bacterial groups, fungi, and plants. We processed 0.2–1.1 g of sample for the lipid extraction with a mixture of CH$_2$Cl$_2$: MeOH (9:1 v/v) in an Accelerated Solvent Extractor (Dionex ASE 350). 0.1 µg/ml of an internal standard with 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 h at 80°C.

Following the method of Elvert et al. (2003), the TLE were polarized with 1 ml KCl (0.1 mol), and the neutral fraction was extracted by rinsing three times with hexane, dried under a stream of N2, and stored in the refrigerator 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 rinsing again three times with hexane dried under a stream of N$_2$. They were methylated by adding 1 ml Boron-Trifluoride (BF$_3$) in MeOH (12–14%) and putting it in the oven for 1 h at 60°C. Afterwards the FA fraction was polarized with KCl (0.1 mol) and transferred in 4 ml vials by rinsing three times with hexane. After one day, in which residues could settle, we transferred the upper part with hexane in 2 ml vials to measure the FAs. The FAs were quantified with a Trace Ultra gas chromatograph (GC) equipped with a flame ionization detector (FID) (Thermo Scientific, Waltham, MA, United States). 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 set to 320°C and the samples (dissolved in hexane) were injected at 300°C injector temperature. The starting temperature of the oven was 50°C and hold for 2 min. Then temperature was increased by 10°C per minute to 140°C. The temperature was held for 1 min before it was increased up to 300°C. This temperature was held for 63 min.

To identify the fungal and bacterial markers, we used the Bacterial Acid Methyl Esters standard (BAME, Supelco Mix). For bacteria, it includes the FAs C14:0 and C17:0 as general bacterial markers Zelles (1997), Willers et al. (2015)), i-C15:0 and C16:1ω7c for acidobacteria (Damasté et al., 2011; Dedysh and Damsté, 2018; Myers and King, 2016). The fatty acid C18:2ω9c was used as a marker for fungi (Andersen et al., 2010; Sundh et al., 1997; Zelles, 1997; O`Leary and Wilkinson, 1988; Vestal and White, 1989). All these markers are valid for overall membrane fatty acids and can be used to detect different microbial groups in soil (Finotti et al., 1993; Piotrowska-Seget and Mrozik, 2003; Bajerski et al., 2017). Quantification of the FAs was done using the injected internal standard C19:0 FA, after correcting for the methyl group, added during methylation reaction, normalized to dry weight of bulk material.
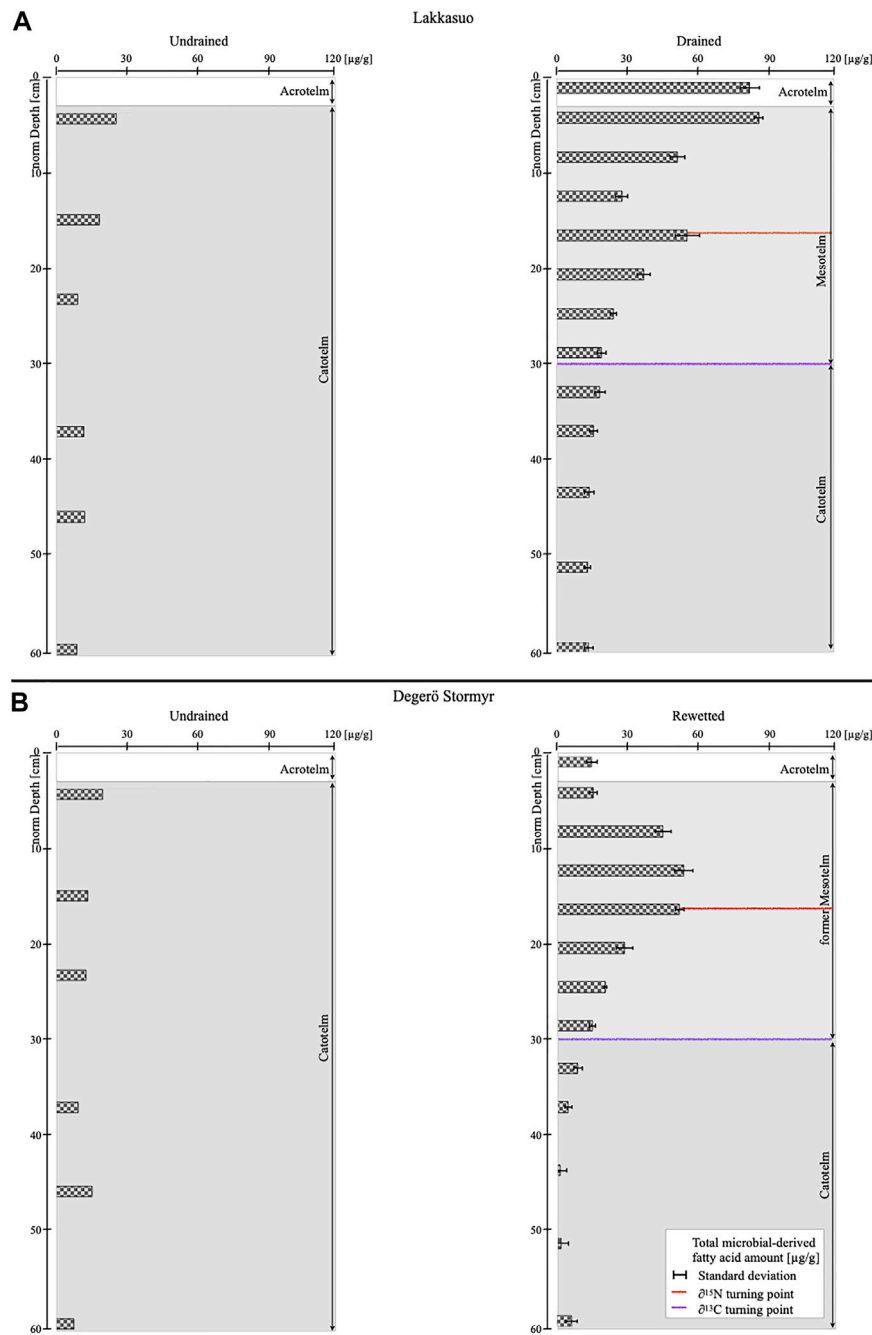
FIGURE 1 | Average values of total microbial-derived membrane fatty acid concentrations [µg/g] **(A)** Lakkasuo: undrained (LU_{1-3}) and drained ($LD13_{1-3}$) **(B)** Degerö Stormyr: undrained (DU_{1-3}) and rewetted ($DR13_{1-3}$); Red reference line gives the $\delta^{15}N$ turning point, violet reference line gives the $\delta^{13}C$ turning point.

Statistical Analysis

For the FA analysis and the comparison of spatial variations for drained vs rewetted sites, we were interested in comparing the depth trends of all single profiles with each other. This was done by using the depth of the $\delta^{15}N$ turning point in each drained profile as the anchor point serving as normalized depth (normD) and set them to 17 cm depth (normD = 17 cm) in each single core.

Using the same procedure, $\delta^{13}C$ trends were normalized using the same anchor point (e.g., δ15N turning point) (for more detailed information, please see Groß-Schmölders et al., 2020).

For the cores of 2013 (3 replicates), variance, standard deviations, and spearman correlation coefficient (R) were calculated with the software R (version 1.0.153). We define an R-value above 0.4 as a strong correlation following McGrew and
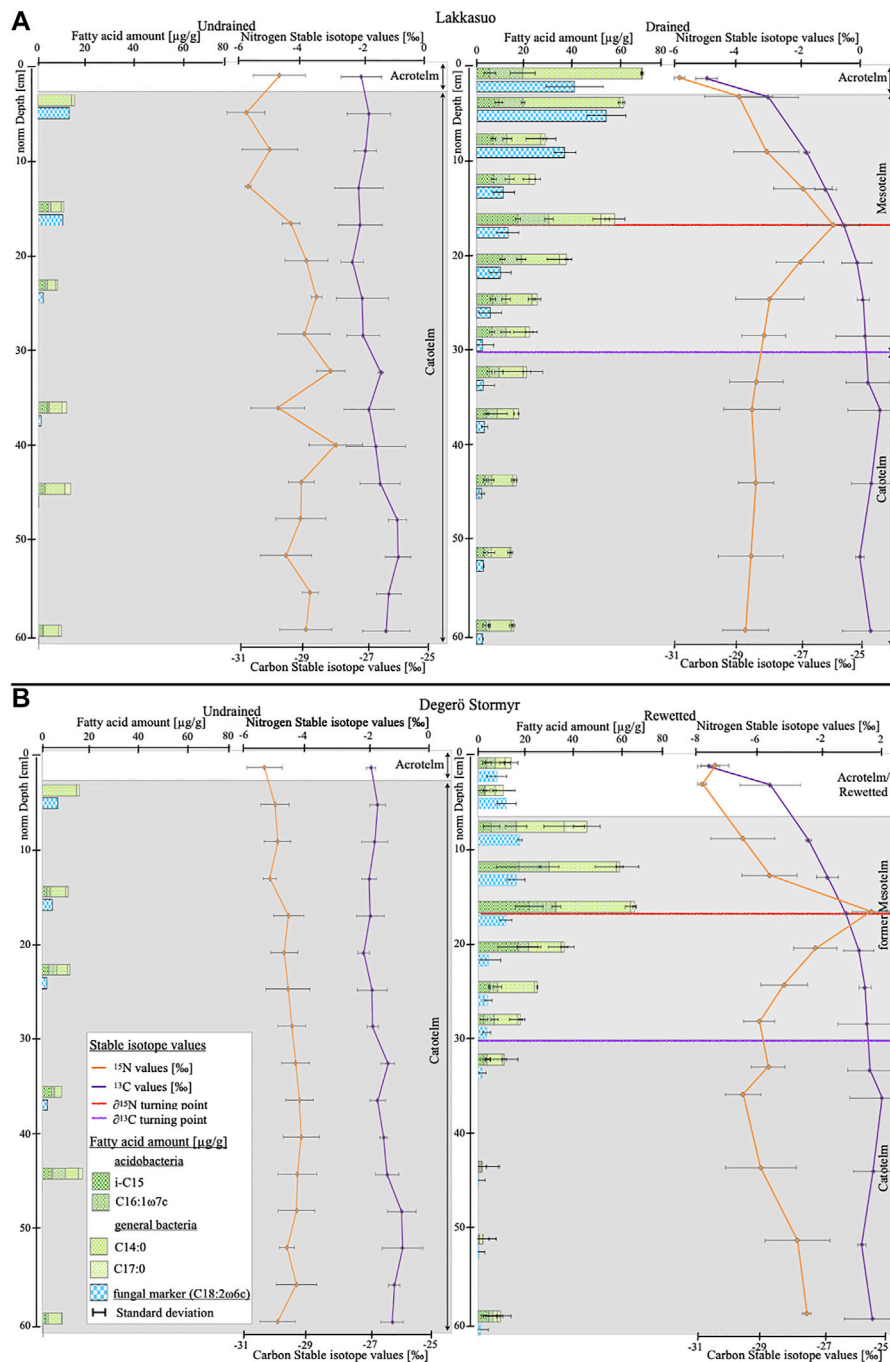
FIGURE 2 | Stable isotope depth trends [‰] (orange: $\delta^{15}N$, violet: $\delta^{13}C$) and fatty acids marker concentrations [µg/g], separated by different microbial groups **(A)** Lakkasuo: undrained (LU_{1-3}) and drained ($LD13_{1-3}$) **(B)** Degerö Stormyr: undrained (DU_{1-3}) and rewetted ($DR13_{1-3}$); Red reference line gives the $\delta^{15}N$ turning point, violet reference line gives the $\delta^{13}C$ turning point.
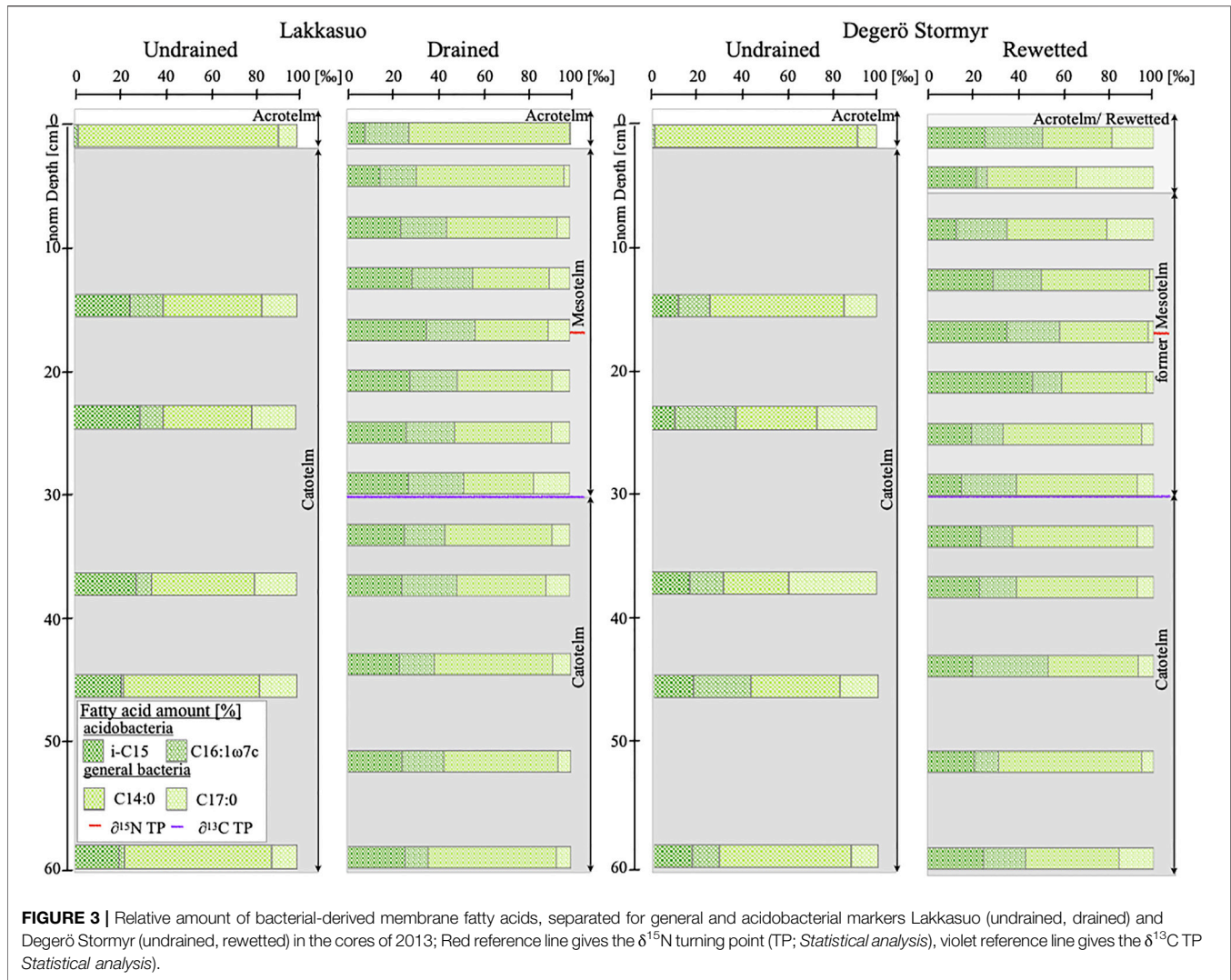
Monroe (2000) and define significance with $p < 0.05$ (McCune and Grace, 2002).

In the following we present only the normalized data. Raw data without normalization are available in the **Supplementary Table**.

RESULTS

Microbial-Derived FA Quantities

Microbial-derived mFA abundance was found to be low over the whole profile in undrained sites, with an average of 16.05 µg/g

FIGURE 3 | Relative amount of bacterial-derived membrane fatty acids, separated for general and acidobacterial markers Lakkasuo (undrained, drained) and Degerö Stormyr (undrained, rewetted) in the cores of 2013; Red reference line gives the $\delta^{15}N$ turning point (TP; *Statistical analysis*), violet reference line gives the $\delta^{13}C$ TP *Statistical analysis*).

(±7.4) in Lakkasuo (undrained; LU) and 14.74 µg/g (±4.7) in Degerö Stormyr (undrained; DU). In the acrotelm, the quantity is higher (LU: 21.8 µg/g; DU: 27.7 µg/g) than in the catotelm (LU: 13.5 µg/g, ±4.4; DU: 13.28 µg/g, 3.4; **Figure 1**).

In the drained site at Lakkasuo (LD), we discovered a large quantity of microbial-derived mFAs in the acrotelm {LD13$_{1-3}$: 108.7 µg/g [mean, ±5.5 (standard deviation of n = 3]; **Figure 1**) and the mesotelm [62.27 µg/g (±3.7)]; all results from the 2013 sampling}. The highest microbial-derived mFA concentration was found at the $\delta^{15}N$ turning point (73.73 µg/g, ±6.74; **Figure 1**). In the catotelm, the values of mFAs were low in the LD sites (21.01 µg/g, ±2.4). The concentration of fungal-derived mFAs is decreasing from 40.33 µg/g (±11.7; **Figure 2**) in the acrotelm of LD13$_{1-3}$, to very low values at the end of the mesotelm and in the catotelm (2.88 µg/g, ±1.4 µg/g; **Figure 2**). Also, the percent of fungal-derived mFAs are decreasing from 49% of all microbial-derived mFAs in the acrotelm to 14% in the catotelm. Contrary to the continually decreasing trend in depth of the fungal-derived mFAs under drained conditions, the bacterial-derived mFA concentration is highest in the middle of the

mesotelm and peaks parallel to the $\delta^{15}N$ turning point (mean 60.17 µg/g, ±10.2; **Figure 2**). Bacterial-derived mFAs comprise up to 85% of total microbial-derived mFAs at this depth.

For the rewetted site in Degerö Stormyr (DR13$_{1-3}$), we detected low values of microbial-derived mFAs in the acrotelm and in the uppermost part of the former mesotelm [20.61 µg/g (±2.6)]. This is expected, because of the wet conditions which are not suitable for high microbial abundance. Below, in the deeper part of the former mesotelm microbial-derived mFA quantities increases [53.44 µg/g (±2.93)]. As in Lakkasuo, the microbial-derived mFA quantity also peaks at the $\delta^{15}N$ turning point in the middle of the formerly drained layer (71.55 µg/g, ±2.48; **Figure 1**). In the below catotelm, values were low for DR sites (8.80 µg/g, ±2.7; **Figure 1**).

In the rewetted cores DR13$_{1-3}$, we found that fungal-derived mFAs have the highest percentage of the mFAs detected in the acrotelm (35%) and in the upper part of the former mesotelm (32%, **Figure 3**). The highest total quantity of fungal-derived mFAs is in the upper mesotelm (16.75 µg/g, ±3.82; **Figure 2**). In the catotelm, the percentage (16%) and the total quantity of

fungal-derived mFAs (2.42 µg/g, ±2.17; **Figure 2**/3) is low. With decreasing fungal-derived mFA percentage, the bacterial-derived mFA percentage increases, from 65% in the acrotelm to 84% in the catotelm (**Figure 3**). Overall, bacterial-derived mFA abundance is highest at the $\delta^{15}N$ turning point (62.15 µg/g, ±5) and low in the catotelm (7.45 µg/g, ±3; **Figure 2**).

Stable Isotope Values

Carbon and nitrogen isotope values in undrained sites in Lakkasuo (LU13$_{1-3}$, LU17) and Degerö Stormyr (DU13$_{1-3}$, DU17) show no depth trend, whereas carbon isotope values vary less than nitrogen stable isotope values (**Figure 2**).

In LU13$_{1-3}$ and LU17, carbon isotope values range between −24.47‰ and −28.36‰ with a mean of −26.43‰ (±0.85). Nitrogen stable isotope values in these cores are between 0.51‰ and −6.56‰ (mean −3.15‰, ±1.78).

In DU13$_{1-3}$ and DU17, carbon stable isotope values are between −22.84‰ and −27.38‰ (mean −24.52‰, ±0.94). For nitrogen, the values range between −0.71‰ and −9.31‰ (mean −3.99, ±2.27).

In the drained site in Lakkasuo (LD13$_{1-3}$, LD17) and the rewetted site in Degerö Stormyr (DR13$_{1-3}$, DR17), values show a distinct depth pattern and vary more than in undrained sites (**Figure 2**). In both sites (LD and DR), carbon stable isotope values show a decreasing trend in the upper layers (above 30 cm normD, **Figure 2**). In LD13$_{1-3}$, carbon isotope values range between −24.53‰ and −31.04‰ (mean -26.67‰, ±1.23), and in DR13$_{1-3}$, between −23.30‰ and −29.74‰ (mean −26.33, ±1.33).

Nitrogen values in drained and rewetted sites show a peak in the mesotelm (17 cm normD, **Figure 2**). In LD13$_{1-3}$, $\delta^{15}N$ values are between 0.94‰ and −5.73‰ (mean 2.40, ±1.50). In DR131-3, δ15N values are between 1.83‰ and −10.64‰ (mean −3.42, ±2.38).

DISCUSSION

New Insights to Microbial Abundance in Undrained, Rewetted and Drained Sites, Identified by Membrane Fatty Acids

In the undrained sites LU and DU, microbial-derived mFA concentrations were highest in the acrotelm, which is in line with other studies (Asada et al., 2005; Artz, 2013). It is the result of environmental conditions in this layer (aerobic, rich of primary plant material). But the values of the acrotelm in the undrained sites are low compared to those of the drained sites, which is a sign of the intact carbon storage function of peat soils and could be the result of 1) the overall nutrient-poor conditions of the investigated sites Minick et al. (2019) with a reduced quantity of nutrients and 2) the incorporation of C in living vegetation (Artz et al., 2008; **Figure 1**).

The highest microbial-derived mFA concentration discovered in the drained site of Lakkasuo is in line with our previous study Groß-Schmölders et al. (2020) and Peltoniemi et al. (2009), as the facultative aerobic mesotelm, with its high content of available organic matter, provides optimal conditions for microbial metabolism and thus contains the highest microbial-derived mFA concentrations (**Figure 1**). This is also in line with the findings of Wang et al. (2019), who showed that the mesotelm is a hot spot for microbial communities, with the highest microbial diversity.

For the rewetted site in Degerö Stormyr, we assumed that the highest mFA concentration in the former mesotelm (the newly established catotelm after rewetting; **Figure 1**) could be the result of former microbial metabolism preserved from the past aerobic conditions that occurred due to drainage. This correlation was also reported by Torres and Pancost (2016). The low microbial-derived mFA quantities in the uppermost part of DR$_{1-3}$, similar to concentrations of DU (**Figure 1**) are likely a result of the recovery of undrained conditions.

Regarding microbial community assemblage, our results are congruent with previous studies (Groß-Schmölders et al. (2020), Thormann (2004)) demonstrating that fungal-derived mFA concentrations are dominant in the upper layers of drained sites (LD, **Figure 2**). This is congruent with the ecological niche for fungi being located near the surface, where there are large quantities of primary plant material and aerobic conditions (Thormann et al., 2004; Wallander et al., 2009; Strickland and Rousk, 2010). Here, fungal metabolism has a competitive advantage (De Boer et al., 2005; Thormann et al., 2003; Thormann, 2011).

In contrast to fungi, bacteria are better adapted to the facultative anaerobic conditions lower in the profile, (Winsborough and Basiliko, 2010). They are able to make use of a wider spectrum of substrates, which leads to an increase in ratios of bacterial-derived mFAs as depth increases within the mesotelm (**Figure 2**; Kohl et al., 2015).

The group of acidobacteria is of special interest here, as acidobacteria are highly abundant in soil, especially in peatlands (Hausmann et al., 2018; Damsté et al., 2015). They comprise 30% of all bacteria in nutrient-rich fens and up to 80% in pristine bogs (Serkebaeva et al., 2013). Acidobacteria are known to have a slow growth rate and are tolerant to depleted sites, which supports their abundance in nutrient-poor peatlands (Wang et al., 2019). Because of their capability to metabolize in facultative anaerobic to anaerobic conditions, acidobacteria are particularly visible in the mesotelm (Urbanova and Barta, 2014). Acidobacteria are always Gram-negative and exhibit a group of specific membrane compounds (Dedysh and Damsté, 2018). In particular acidobacteria mainly produce glycerol dialkyl glycerol tetraethers Weijers et al. (2010) and the membrane lipids i-C15:0, C16:1ω7c and 13,16-dimethyl octacosanedioic acid (Damsté et al., 2015; Damsté et al., 2018). The concentrations of these were linked to low pH values and decreasing oxygen availability (Weijers et al., 2010), which are typical conditions of the mesotelm. Our results are in line with these findings, as we found an increasing abundance of the mFAs i-C15:0 and C16: 1ω7c near the $\delta^{15}N$ turning point (**Figures 2**, **3**).

Microbial-Derived Membrane Fatty Acid Quantities and Isotopic Values

In undrained sites, we saw no depth trend in the isotopic values compared to the strong decrease of microbial-derived mFA

concentration in sublayers (**Figure 2**). We conclude that this is caused by the extreme environment of the catotelm, with low temperatures and anaerobic conditions. Hence, metabolism and decomposition processes are strongly inhibited, the mFA quantities and the isotopic fractionation rates are extremely low, and organic substrates are stored (Clymo, 1984; Frolking et al., 2001; Krüger et al., 2015).

Microbial-Derived Membrane Fatty Acid Quantities and Carbon Isotopic Values

Carbon stable isotope values increase with depth in all investigated drained sites by ~2.48‰ from the acrotelm to the catotelm (**Figure 2**). This increase was also found previously Alewell et al. (2011), Krüger et al. (2014) and is in the same range of what other studies have found for peatland soils (Kohl et al., 2015; Hobbie et al., 2017). Wynn et al. (2006) reported, that this trend is caused by the microbial communities involved and their preference for substrates. If more enriched substrates are degraded, the $\delta^{13}C$ values in the remaining substrate could decrease due to the enhanced gaseous loss of ^{13}C enriched CO_2 (Wynn et al., 2006). For example, glucose, pectin and hemicellulose, which are enriched in ^{13}C, are some of the preferred substrates for microbial metabolism and are processed in the uppermost peat soil layers. As a result, $\delta^{13}C$ values are low in the uppermost peat layers of degraded sites, as we see in LD (**Figure 2**). In contrast to the enriched $\delta^{13}C$ values of CO_2, methane (CH_4) is depleted in $\delta^{13}C$, which could balance the effect of enriched CO_2 in the remaining substrate. But as methane production mainly occurs in anerobic conditions, the effect of gaseous loss of depleted CH_4 is expected to play a minor role in drained peatlands (Yang et al., 2019). In addition, Hornibrook et al. (1997) reported that the $\delta^{13}C$ values of CH_4 are increasing with decreasing depth in peatlands, which could also minor the effect of depleted CH_4 in our sites. That the depleted $\delta^{13}C$ values in top layers of peat are caused by the preferred cycling and therefore gaseous loss of heavy ^{13}C isotopes can be verified by studies that reported that there is no preferred loss of lighter ^{12}C during microbial metabolism (Lerch et al., 2011). Increasing values of $\delta^{13}C$ in peat soil with depth are therefore related to a change in the processed substrates and their specific $\delta^{13}C$ values (Kohl et al., 2015). With increasing depth, recalcitrant, $\delta^{13}C$-depleted substrates such as lignin are also processed, which leads to increasing mobilization of lighter ^{12}C and further increasing $\delta^{13}C$ values in the remaining bulk soil with depth (Lerch et al., 2011). Further Boström et al. (2007) assume that the ^{13}C enrichment in drained soils with depth is a result of the increased contribution of microbial derived C with depth.

With regard to specific microbial groups, Kohl et al. reported in 2015 that a high fungal-to-bacterial ratio in the microbial community is negatively correlated with $\delta^{13}C$ values in the remaining substrate; this is caused by changes in processed substrates and their carbon isotopic signals. Hence, fungal-to-bacterial ratio is decreasing, and $\delta^{13}C$ values are increasing due to a change in microbial metabolism processes and substrates used (Kohl et al., 2015). As Kohl et al. (2015) showed, the $\delta^{13}C$ values of bacteria (−40.1 to −30.6‰) and fungi (−31.1 to −24.6‰) stay the same in different depths of peat, but their ratios are changing,

and with them, the $\delta^{13}C$ values of the bulk material. This is in line with our data of a decreasing fungal-to-bacterial ratio and increasing $\delta^{13}C$ values with depth (**Figure 2**).

With regard to acidobacteria, Weijers et al. (2010) found that acidobacterial-derived mFAs have enriched $\delta^{13}C$ values compared to plants and in the same range then bulk. The reason for that could be the preferred cycling of glucose and pectin, which are enriched in $\delta^{13}C$ (Pankratov et al., 2008; Lerch et al., 2011). As the highest quantity of acidobacterial-derived mFAs is found in the mesotelm (**Figures 2, 3**), we found that the increasing metabolism rate of acidobacteria could be linked to the increasing $\delta^{13}C$ values (**Figure 2**). An increase in acidobacterial-derived mFAs is expected because acidobacteria are known to be autotrophs and are therefore able to assimilate CO_2 from the soil (Wiesenberg et al., 2008). As soil CO_2 is enriched in ^{13}C Wiesenberg et al. (2008), the increasing metabolism by acidobacteria increases the $\delta^{13}C$ values in the remaining substrate further.

Microbial-Derived Membrane Fatty Acid Quantities and Nitrogen Isotopic Values

Considering the parallel depth trend of the concentrations of bacterial-derived mFAs and $\delta^{15}N$ values (**Figure 4**), we conclude that nitrogen stable isotope values appear to be linked more closely to bacterial abundance than to overall microbial abundance. This interpretation is supported by a higher Spearman correlation index of $R = 0.54$ for bacteria compared to $R = 0.30$ for all microbes. Peaks in bacterial-derived mFA abundance and at $\delta^{15}N$ values occur in tandem and are visible for both sites investigated (**Figure 2**). The correlation is clear if we consider that the quantity of N in microbial biomass is a substantial part of N in bulk substrate of poor peatlands Lin et al. (2014), and thus, isotopic fractionation by microbes will influence the bulk isotopic value significantly.

We differentiated between acidobacterial and general bacterial markers. For both bacterial groups, the mFA concentrations increased towards the $\delta^{15}N$ turning point and were lowest in the catotelm (**Figure 2**).

For both peatland sites, we found the highest concentration of acidobacterial-derived mFAs reaching approximately 50% of all bacterial-derived mFAs in the mesotelm (**Figure 3**). In the upper mesotelm and the catotelm, the percentage is approximately 40% (**Figure 3**). Hence, our results are congruent with the results of Artz in 2013, which point to a characteristic depth trend with a peak in the mesotelm of acidobacterial populations in nutrient-poor peatlands (**Figures 2, 3**).

Acidobacteria are closely involved in nitrogen cycling (Urbanova and Barta, 2014; Eichhorst et al., 2018; Kalam et al., 2020). For example, they are involved in denitrification processes (Urbanova and Barta, 2014). They reduce nitrate, nitrite and possibly nitric oxide (Ward et al., 2009; Eichhorst et al., 2018; Kalam et al., 2020). As Eichhorst et al. (2018) reported, there are also evidence for the mobilization of ammonium by acidobacteria and the gaseous loss of N_2O. All these cycling processes are known to increase $\delta^{15}N$ values in bulk material Denk et al. (2017) and are observed predominantly in the mesotelm (Palmer and Horn, 2015; Oshiki et al., 2016). Thus, acidobacteria could be the key to forming the $\delta^{15}N$ depth trends

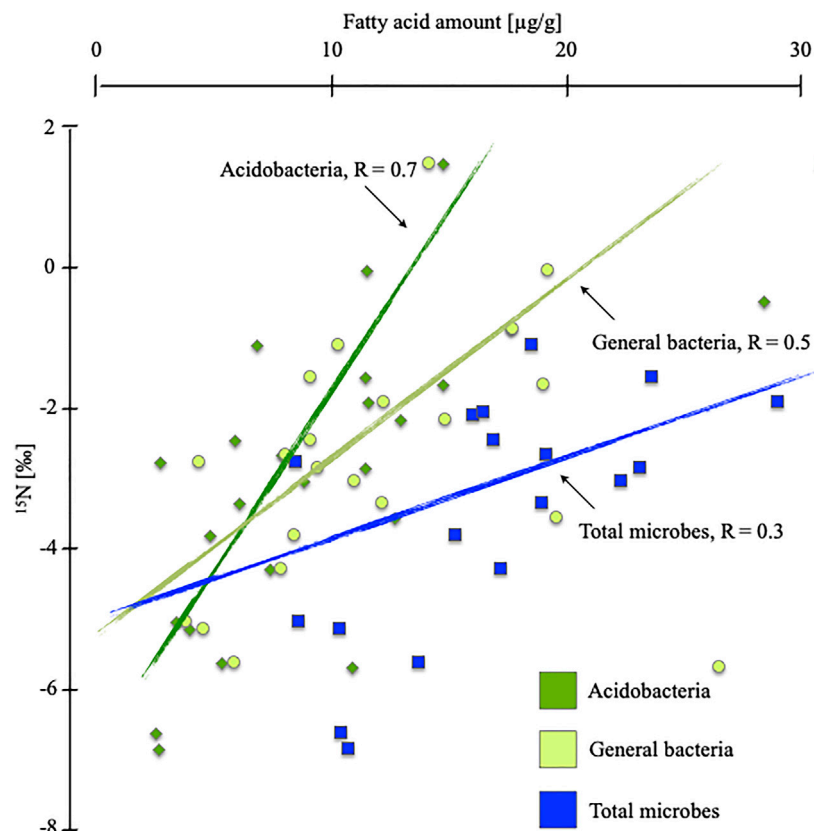**FIGURE 4** | Spearman correlation index (R) and correlation of $\delta^{15}N$ [‰] and microbial, general bacterial and acidobacterial membrane fatty acid marker amount [µg/g], for the rewetted site in Degerö Stormyr and the drained site in Lakkasuo.
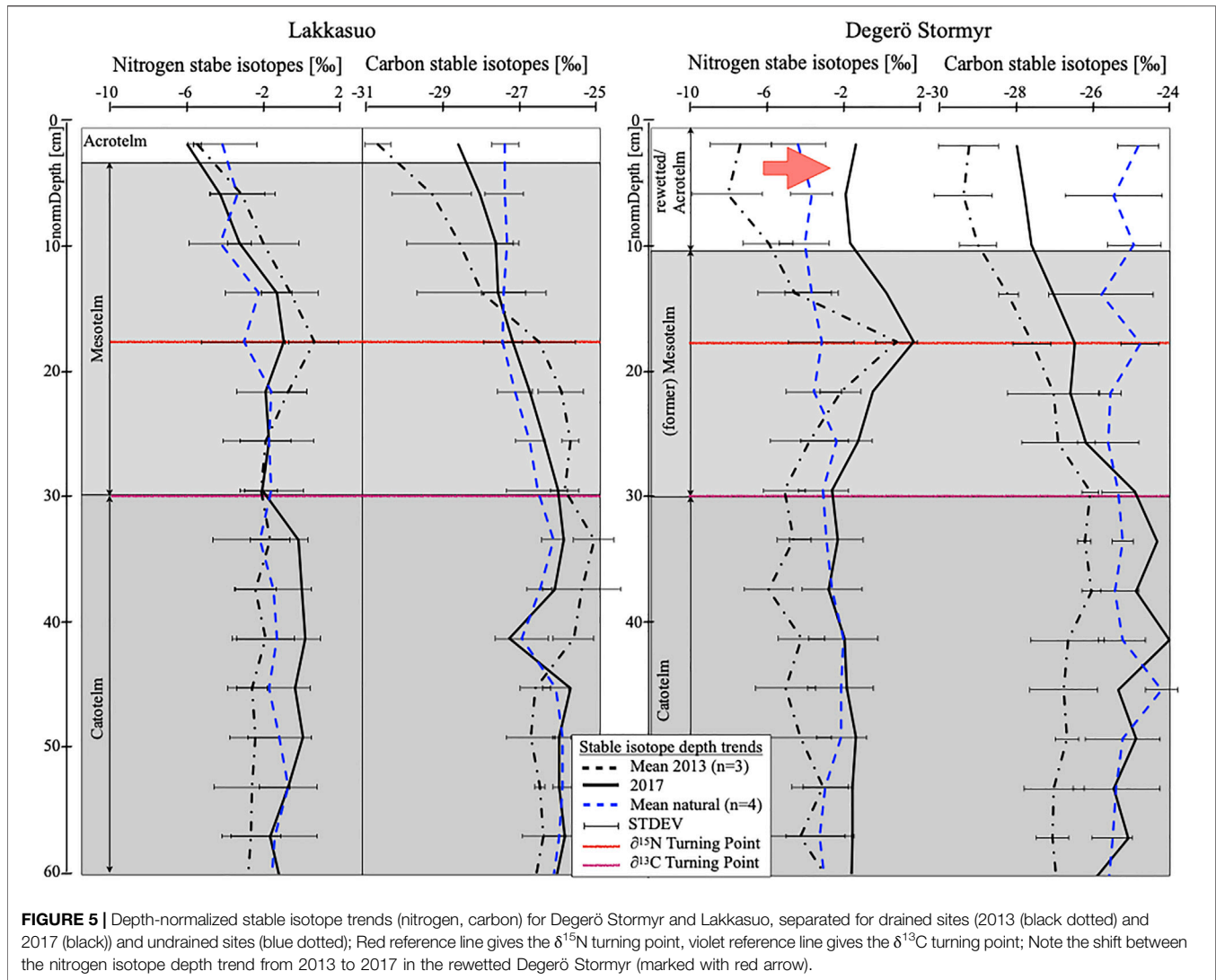
in nutrient-poor peatlands. We assume that as the occurrence of acidobacteria increases, more nitrogen will be processed. Lighter ^{14}N will be released in a gaseous state, leached or mineralized and then incorporated by plants and translocated upwards within the plants. These processes should lead to increasing $\delta^{15}N$ values in the remaining substrate (Hausmann et al., 2018). This correlation is illustrated by our data. Acidobacterial-derived mFA markers, shown in absolute concentrations (**Figure 2**) and in relation to other microbial markers, are highest at the $\delta^{15}N$ turning point (**Figures 2**, **3**). At the $\delta^{15}N$ turning point, the relative abundance of acidobacterial-derived mFAs in relation to all bacterial-derived mFAs is highest, with 49.92% for LD and 55.64% for DR (**Figure 3**). Overall investigated sites, $\delta^{15}N$ values and the acidobacterial-derived mFAs are highly significantly correlated (R = 0.66, $p < 0.05$; **Figure 4**).

Depth Trend of Stable Isotopes in Drained and Rewetted Sites

The comparison of stable isotope depth trends ($\delta^{15}N$, $\delta^{13}C$) revealed specific differences between undrained, drained, and rewetted sites, which remained stable over both years. Regardless of the sampling year, none of the cores of undrained sites ($DN13_{1-3}$, DN17, $LN13_{1-3}$, LN17) showed any depth trend of stable isotopes (**Figure 5**). The cores of the drained site Lakkasuo

($LD13_{1-3}$, LD17) showed trends within the drained layer (acrotelm and mesotelm). $\delta^{13}C$ values increased throughout the drained layer, and a peak of ^{15}N values (the $\delta^{15}N$ turning point) was visible in the mesotelm, which both is indicative for the ongoing loss of typical peatland soil functioning (e.g. carbon storage). In the rewetted site Degerö Stormyr ($DR13_{1-3}$, DR17), no depth trends in $\delta^{15}N$ values were observed in the layer likely formed during rewetting conditions above the layer formed during former drainage (**Figure 5**). Below the rewetted layer, the $\delta^{15}N$ depth trend of the former mesotelm was preserved in the newly established (>20 years of rewetting) catotelm. The decreasing trend of $\delta^{13}C$ values in DR also seems to have slowed down in the rewetted core in 2017 (**Figure 5**).

The time-dependent sampling was devised to test the robustness of the stable isotope depth pattern as an indicator for peatland hydrology in relation to the onset, duration and ending of a drainage event. With one exception, nitrogen stable isotope depth trends in both years - 2013 and 2017 - are very similar, confirming our hypothesis (**Figure 5**). The exception is the $\delta^{15}N$ depth trends in Degerö Stormyr, the site that was rewetted in recent decades, where a shift in the rewetted layer in 2017 towards more undrained depth trends compared to 2013 most likely indicates a successful restoration.

FIGURE 5 | Depth-normalized stable isotope trends (nitrogen, carbon) for Degerö Stormyr and Lakkasuo, separated for drained sites (2013 (black dotted) and 2017 (black)) and undrained sites (blue dotted); Red reference line gives the δ^{15}N turning point, violet reference line gives the δ^{13}C turning point; Note the shift between the nitrogen isotope depth trend from 2013 to 2017 in the rewetted Degerö Stormyr (marked with red arrow).

The changes in stable isotope values with rewetting occur simultaneously with changes in microbial-derived mFA quantities. This indicates that the changed environmental conditions lead to changing microbial community compositions and therefore changed metabolism processes (Asada et al., 2005). This is in line with the findings of Elliott et al. (2015), who also found distinct changes in microbial communities with rewetting. In drained peat sites, they also found increased values of acidobacteria, which we also found in our sites (**Figures 2**, **3**). Elliott et al. (2015) also indicated that changes in microbial communities are rapid after rewetting and could therefore also indicate relatively short rewetting times and the recovery of typical peatland soil functioning.

CONCLUSION

Our results confirm that the existence of specific microbial groups is correlated to stable isotope depth trends (δ^{15}N, δ^{13}C) of nutrient-poor peatlands, particularly that of nitrogen isotopes. An analysis of mFA markers for general bacteria as well as specific mFA markers for acidobacteria, and fungi revealed a high abundance of fungal-derived mFAs in the aerobic acrotelm. The upper mesotelm showed a transition to decreasing fungal-derived and increasing bacterial-derived mFA abundance (especially that of acidobacteria). As such, the δ^{15}N turning point seems to be driven in particular by the nitrogen cycling of bacterial metabolism, most prominently by acidobacteria. Downwards along the (former) mesotelm, δ^{15}N values decreased, likely due to low microbial metabolic rates. Finally, in the permanent anaerobic catotelm, where microbial metabolism is strongly impeded, δ^{15}N values show no further depth trend. Stable isotope depth trends (δ^{15}N, δ^{13}C) from two different years were able to confirm the persistence of these trends as indicators for ongoing drainage and therefore impaired soil functioning, e.g. as storage of carbon. Furthermore, δ^{15}N seems to indicate former drainage followed by rewetting processes. In summary, we conclude that microbial abundance as indicated

by group specific biomarkers can be confirmed as key for stable isotope depth trends and that differences in $\delta^{15}N$ depth profiles may be indicative for drainage and rewetting.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MG-S conducted the sampling, measurements, evaluation and analysis of the data and cowrote the paper with CA. AB assisted with the measurements and helped with the analytics. KK added to the discussion. JL and CA had the project idea, supervised and added to the discussion.

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