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Interactive comment on "Switch of fungal to bacterial degradation in natural, drained and rewetted oligotrophic peatlands reflected in δ^{15}N and fatty acid composition" *by* Miriam Groß-Schmölders et al.

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Dear Referee,

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

1.The authors' central hypothesis ('maximum 15N enrichment at maximum microbial diversity) seems to come out of nowhere, and it is unclear to me how the authors came up with this hypothesis except as a post-hoc justifying their results. I do not see why

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

Answer: Regarding your comment on microbial diversity and increased δ15N values, we are sorry for being so unclear; we will be more precise with the description of the assumed relationship between the two parameters microbial metabolism processes and δ15N values. We hypothesize that the microbial abundance (see answer 3) as well as the microbial community composition has an influence on the δ15N values. With an increasing diversity, δ15N values of the reaming substrate should increase, because (1) different microbial communities prefer different sources (Dijkstra et al. 2006, Drollinger et al. 2019) and (2) with increasing bacterial abundance, fungi have to use also recalcitrant sources, because bacterial metabolism will outcompete fungi for the easily degradable substances (Rousk and Bååth, 2007, Winsborough, C. and Basiliko, 2010). Hence, with increasing microbial diversity also the diversity of the mineralized organic fractions increases (Thormann 2006). With an increased diversity of nitrogen sources more release of lighter 14N is possible and the ratio of 15N:14N in the remaining substrate should increase. We realize that these are hypotheses to explain the observed patterns, but to our understanding they are the most likely ones and in describing them, we hope for an eager discussion in the community.

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

Answer: We have followed your advice and structured our results accordingly. We will start, as you suggested, with an overview of drained and undrained peatlands and the influence of hydrology on the measured biogeochemical parameters. This will be followed by an introduction to the processes, which leads to increased δ15N values, and might explain the observed pattern in the mesotelm.

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

Answer: We for sure do not claim that we fully understand the observed patterns yet, but that we see consistent patterns and (in combination with the fatty acid analysis) develop ideas what the origin of these patterns might be. Sorry, if this was not clear from the manuscript, we will add a sentence referring to this. We will further insert a section about N isotope fractionation in peatland soils and the underlying processes,

which might be leading to 14N depletion in the remaining substrate during drainage. In general, the 15N:14N ratio of plant material (here mostly sphagnum mosses) is lower than the values of microbes and bulk material (Aldous, 2002, Lichtfouse et al. 1995). Microbes prefer to mineralize the lighter, more frequent 14N (Dijkstra et al., 2006, Novák et al, 1999). Since plants incorporate the microbial mineralized nitrogen they have a low 15N:14N ratio (Lichtfouse et al. 1995). Contrary, microbial biomass is enriched in 15N, probably as the result of processing the lighter 14N during mineralization and hence incorporation of the remaining heavier 15N. In addition, caused by the preferential mineralization of lighter nitrogen, the heavier 15N might be enriched in the remaining humic substances (Novák et al, 1999). The effect of the latter to 15N:14N bulk values is probably enhanced due to the loss of 15N-depleted material during leaching (Damman 1988, Niemen 1998), denitrification and the release of N2O (Kohzu 2003, Niemen 1998). In natural peatlands, microbial activity is low and mostly visible in the uppermost, aerobe part of the peat (acrotelm). With the onset of the waterlogging, anaerobe conditions in the catotelm microbial activity is inhibited. This leads to small or even negligible changes of the original (light) plant isotopic ratio below the acrotelm. (Dijkstra, 2008) In contrast, in drained peatlands the aerobic mesotelm expands and simultaneously microbial activity increases (Moore & Basiliko 2006, Roswell 1976). In an extended mesotelm a higher amount of mineralization and the release of N2O takes place. With increased mineralization the 15N:14N ratio in the remaining substrate should increase, as long as 14N will be mineralized preferentially (Dijkstra, 2008). However, because of the faster and more complete decomposition with increasing microbial activity (Damman, 1988) metabolism of 15N increases as well and fractionation will be less. This pattern leads to only small increases in the 15N:14N ratio of the bulk material, as all isotopes are used and fractionation is lowered in the middle of the mesotelm, where microbial activity is the highest. Actually, the best way to test for the combined effects of all these different processes on the isotopic fingerprinting would be to set up a conceptual model. However, we feel this is beyond our possibilities at the moment, but we certainly look for opportunities (e.g., cooperation)

in the future.

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

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

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

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

L16: ‘stable isotope signatures’: See this advice on ‘Isotope terminology’ from Z. Sharp’s ‘Isotope Geochemistry’ book (https://digitalrepository.unm.edu/unm_oer/1/): Mistake: “The isotopic signature of the rock was d18O = 5.7‰ ÌĞ” Recommended Ex-

pression“Thed18Ovalueoftherockwas5.7‰ÌĞhusthisrockhastheoxygenisotope signature of the mantle.” Explanation: “The word signature should be used to describe the isotopic composition of a significant reservoir like the mantle, the ocean, or a major part of the system being studied, not to the isotopic composition of ordinary sample” - L311: ‘equilibrium’ between fungi and bacteria – I don’t think equilibrium is the correct concept here. Maybe change from fungal to bacterial dominance, but even that is not

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

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

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

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