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Interactive comment on "Preferential protein depolymerization as a preservation mechanism for vascular litter decomposing in *Sphagnum* peat" *by* Hendrik Reuter et al.

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Reviewer comment: The main aim of the study was to evaluate the fate of plant litter nitrogen in a decomposition experiment involving litters and peaty soils with contrasting N status. It was necessary to distinguish between two fractions of protein nitrogen in the litter: (1) remaining original N that has not been depolymerized by decomposers' enzymes and (2) newly synthesized microbial N. The authors proposed a novel approach how to distinguish the two fractions; they measured precise FTIR spectra to evaluate peaks of total protein nitrogen and microbial DNA phosphorus (assuming that the DNA P is associated only

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with the microbes). Assuming constant microbial N:P stoichiometry they could express the microbial N fraction. I am not a microbiologist, so I am not able to review critically the assumptions leading up to the evaluation of preferential protein depolymerization. However, I appreciate the careful explanation of all the evaluation steps supported by references. The manuscript is well and clearly written but I would like to discuss following issues.

Authors reply: We would like to thank you for taking the time to review our manuscript. We furthermore thank you for the concise summary of our study and for your helpful and constructive comments which will improve the manuscript. Below we will respond to all issues raised and indicate how it was assisting for the revision of the manuscript accordingly.

[1] How is the microbial N invested in extracellular enzymes accounted for? [2] How relevant is this fraction in the evaluation of the N fate in the decomposing litter? [3] How can it differ in N-poor/rich soils and litters? [4] How this fraction can affect the proposed method leading to the evaluation of preferential protein depolymerization?

[1] Our methodological approach taken is only capable of quantifying microbial N from the FTIR peak heights of DNA bands. Plant bound N is determined as total litter N minus microbial N. The extracellular enzyme N-pool, located outside the microbial cells, yet part of the microbial N-fraction, is not considered as a standalone paramter.
[2] Unfortunately, we were not able to find quantitative data on the importance of this N-pool in the scientific literature. Enzymatic studies commonly do not quantify this pool directly but measure enzyme activities. This can partly be rationalized by a lack of efficient exoenzyme extraction methods that avoid cell lysis and a co-extraction of cell-bound enzymes.
Yet, we assume that the exoenzyme N-pool will be small and negligible compared to plant and microbial N. This can be illustrated indirectly as a fraction of DON in soil porewater should be composed of microbial exoenzymes. The detritus mud in this

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study can be considered as an environment with high microbial activity in which we measured about 10 mg/L DON. If we assume that 1 g leaf litter is soaked with 2 mL water which contains 10 mg/L DON (a fraction of which is the exoenzymes), the DON would only sum up to 20 μg water-soluble N per gram leaf litter, a rather small amount compared to the 10-30 mg total (insoluble) N per gram litter.

[3] In soils, especially mineral soils, the "exoenzyme N-pool" can account for a high fraction of the total N because exoenzymes can be immobilized and stabilized on mineral surfaces. Within leaf litter, this immobilization process should be less relevant and the exoenzymes themself will in all systems account for a very small fraction of the total organic N.

The interpretation of C/N-changes in decomposing litter using the stoichiometric decomposition theory acknowledges the effects of C-limitation or N-limitation as a function of soil-N or litter-N. But this is mostly discussed in terms of microbial biomass growth and decomposition activity, without explicitly mentioning variations in the extracellular enzyme release rate.

[4] As discussed in the manuscript, there were no indicators for N-limitation in any decomposed leaf sample. The CUEs were very similar for all litters what indicated a similar microbial biomass growth over the decomposition path within all samples. This uniformity should also apply to the exoenzyme production and release rate.

It can be supposed that the preferential depolymerization of proteins over other plant biopolymers, as observed in the poor *Sphagnum* peat soil, is induced by a higher formation and release of protease, the protein depolymerizing exoenzyme. Yet, this will only very minorly affect the overall exoenzyme N-pool because protease is only one enzyme out of many different enzymes required to depolymerize the complex plant tissue.

Changes in manuscript: Exoenzymes are along with the readily depolymerized amino acids a small and rapidly cycling water-soluble N-pool in decomposing litter which is commonly not quantitatively discussed in studies on C/N dynamics in litter. We, therefore, decided not to discuss this pool explicitly, but only to mention the two

N-pools in litter which are plant N and microbial N. Yet, we will mention exoenzymes as part of DON which will receive more attention in the revised manuscript.

The concept also does not mention that the extracellular enzymes may mediate N acquisition from dissolved organic N, which was not analyzed in the soil water. How relevant is this N pool in the tested soils?

DON was quantified as 0.82 ± 0.19 mg/L in the low-N substrate, 0.73 ± 0.16 mg/L in the medium-N substrate, and 8.12 ± 0.77 mg/L in the high-N substrate. N acquisition from DON might thus be a relevant process in the high-N environment, a process that we overlooked in the present manuscript. We thank reviewer 2 for this comment and will discuss this process in the revised manuscript.
In our study, the potential effect of DON parallels that of porewater ammonium. DON and ammonium are external N sources available to microbes. Furthermore, both external N sources are only available in the high-N substrate, for which effects of ammonium are discussed. A consideration of DON along with DIN can thus easily be implemented.

Changes in manuscript: We will include DON along with ammonium as an additional external N source.

Why anoxic conditions were chosen for the experiment? Most plant litters, also in peatland habitats, are first exposed to oxic conditions. Do you think the conclusions are fully applicable also in oxic decomposition where fungal decomposition often prevails?

It is true that above-ground leaf litter in peatland habitats initially decomposes under oxic conditions. However, if peatlands are inundated as it is the case after rewetting of degraded peatlands, the plant litter might reach the low-oxygen or even anaerobic zone of the detritus layer within few days only and decompose anaerobically at an early stage of decomposition. Anaerobiosis in this newly formed mud layer might cause carbon sequestration which is well documented (Cabezas et al. 2014). On the

other hand, anoxic decomposition can be very efficient in anoxic environments like rewetted fens, indicated by the high CO_2 and CH_4 release from these ecosystems. The elucidation of carbon preservation mechanisms in anoxic environments is of high importance. We likewise chose anoxic decomposition as we had noticed, that anoxic litter decomposition in peatlands had only limitedly been studied using a litterbag experimental design.
We tend to believe that the capability of microbes to adjust their protein depolymerization activity in response to external N availability is not limited to anoxic soils, at least when litter decomposes subaqueously or under very humid conditions where diffusion of porewater N is high so that microorganisms can easily access external N sources. Yet, further experiments are needed to test these assumptions. The described analytical approach should generally apply to litterbag studies in oxic environments, but a different microbial biomass C/N ratio and a different DNA:N ratio must be expected when fungal decomposition prevails.

Changes in manuscript: We will change the "Conclusions" of the manuscript to "Conclusions and perspectives" in order to discuss the potential outcome of the reported method in oxic decomposition studies. The revised version of the conclusions can be found in the answer to Reviewer 1.

Other comments: Chapter 2.3 Infrared Spectroscopy: I think that more details about the target compounds and their absorption bands can be provided here in the method description than only later in the Results and Discussion.

We agree and will add information on how quantitative data for amide I and amide II bands as indicators for litter protein content and semiquantitative data for DNA bands were extracted from the FTIR spectra.

P4, L21: How effective was the 17-h period in leaching the litter? Is it possible that a significant proportion of the mass loss can be still attributed to the leaching and not entirely to microbial activity?

We did not collect data on mass loss or DOC during the leaching procedure, so we cannot precisely answer your first question. However, it can be assumed that first leaching is the dominant process and that with increasing time the microbial decomposition becomes the dominant process indeed coupled partly with ongoing leaching processes (Asaeda et al. 2002). It must be noted that leaching was already taking place under in-situ conditions. The weeks preceding the leaf litter sampling were rather rainy, so leaching by rain-water will already have occurred in the field. The leaching in the lab was done to remove water-soluble organic matter, but also to reduce effects of the inhomogeneous nature of the natural leaching by rain between leaf parts (leaf top vs. leaf bottom, etc.) as well as between sampling sites.
We have data of a leaching experiment for which the leaf litter from the kettle-mire Kablow-Ziegelei was used. 4.5 g leaf litter was leached in 1 L water for 24 h. DOC (0.2 μm filtered) reached 92 mg/L what corresponds to 4.4% of the initial leaf C.
Even though all leaves were pre-leached, significant amounts of organic matter were likely lost from the litter by abiotic leaching during the 75 days. Yet, such a leaching effect should be litter specific, unaffected by differences in water chemistry of the three decomposition substrates. Theoretically, the low C-losses of the medium-N leaf litter in all substrates could be caused by a lower extent of leaching compared to the other two leaf litters. Yet, the low amounts of microbial N in these samples indicate a lower microbial decomposability of this sample as a cause of the low C-loss. The most important findings in this study, which are the site-specific differences in the decomposition process for each litter type (preferential protein depolymerization), cannot be explained by litter-specific leaching effects.

Changes in manuscript: As we did not collect data on the leaching process we decided not to mention the leaching in more detail in the manuscript.

P4, L23: How was the rhizome litter defined? (I expect that a continuum between living and highly decomposed rhizomes can be found in the soil).

The rhizome litter was cut from living *P. australis plants*, rinsed with distilled water and

freeze-dried. We thank you for this comment, as we forgot to mention, that the rhizome tissue was indeed living plant tissue. We became aware that the term "litter" does not strictly apply to belowground living plant tissue and will change the term "rhizome litter" to "rhizomes" or "rhizome tissue" in the manuscript.

P12, L12 and Figure 4b: The linear model has the intercept very close to zero (as indicated by the trendline in the graph), obviously statistically not different from zero. What is the relevance of the zero intercept? Does it support the assumption of the entirely microbial origin of the rhizome litter N and P?

The trend line in Figure 4b indeed has an intercept very close to zero. The exact formula of the trendline presented in Figure 4b in the manuscript is: y= 0.05996 + 0.29411x.
Indeed, the trendline shown in the original Figure 4b was not statistically different from zero. To account for the DNA:N homeostasis, we used a trendline forced through zero. The formula of that trendline was y=0.31452x, as denoted in the manuscript. We will replace Figure 4b with the used trendline intercepting zero. The novel Figure 4b with the changed trendline has been added to the end of this document.
The finding, that N values and DNA signals lead a trendline very close to zero for the rhizomes was indeed an important finding during the method development. At that time, we tried to find an overall pattern in the complex and incomprehensible dataset of litter C/N changes. We were aware that only very little plant N could remain within the decomposed rhizomes while leaf litter N had to be a mixture of plant and microbial N. Assuming microbial homeostasis, we were searching for a potential marker of microbial N in the litter using infrared spectroscopy. The DNA bands were a promising start, but in the scientific literature, these had only been reported in the spectra of microbial cell tissue. The attempt to extract these bands by using the second derivative spectra of FTIR difference spectra was an experimental approach that could easily have reached the limits of infrared spectroscopy in terms of resolving power and signal-to-noise ratio. Yet, we found a positive signal for all decomposed

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litter samples at the position where DNA-band should appear. The N-to-DNA trend line of the rhizomes, closely passing the origin, was an important confirmation that we did extract a DNA signal as this pattern was expected from stoichiometric considerations. Furthermore, it allowed quantifying microbial N in leaf litter samples. This quantification of microbial N ultimately allowed the calculation of CUE-values which were very constant for all leaves, what again was a pattern consistent with the stoichiometric decomposition theory. Only then we were rather confident that we positively extracted DNA signals and that the site-dependent deviations in N dynamics were due to variations in remaining plant N what indicated site-dependent preferential protein depolymerization.

Changes in the manuscript: We will replace Figure 4b (new version shown at the end of this document) and put some more emphasis on the importance of the trend line closely passing zero.

Technical comments: Table 1, first column: “soil substrate” can be clearer (as it is used also in the text)

We agree and will use the term "organic soil" instead of "substrate".

P4, L4: “N mineralization/immobilization”: does the slash sign denote a ratio or some- thing like “and/or”?

We will change the term "net N mineralization/immobilization" to "net N mineralization and/or immobilization". We thank you for bringing this imprecision to our attention.

P4, L12: Replace “sedge-brown moss peat” by “sedge–brown-moss peat”

It will be done.

P7, L1: The C/N in the senescent leaves was measured after the leaching? If so, “leached leaves” can be used.

We thank you for this comment and will use "leached leaves".

P19, L12: Although the data on CuO-oxidation lignin monomer products were not used in the paper, the supplement should contain a description of the method (or a reference).

We agree and will add a short paragraph on lignin analysis in chapter 2.2, mentioning that CuO lignin data of the organic soils can be found in the supplement information and lignin data of leaf litters in the supplementary datasets.

Thank you again for your review,
Hendrik Reuter, on behalf of all coauthors

References:

Asaeda, T., Nam, L. H., Hietz, P., Tanaka, N., Karunaratne, S.: Seasonal fluctuations in live and dead biomass of *Phragmites australis* as described by a growth and decomposition model: implications of duration of aerobic conditions for litter mineralization and sedimentation, Aquatic Botany, 73, 223-239, 2002.

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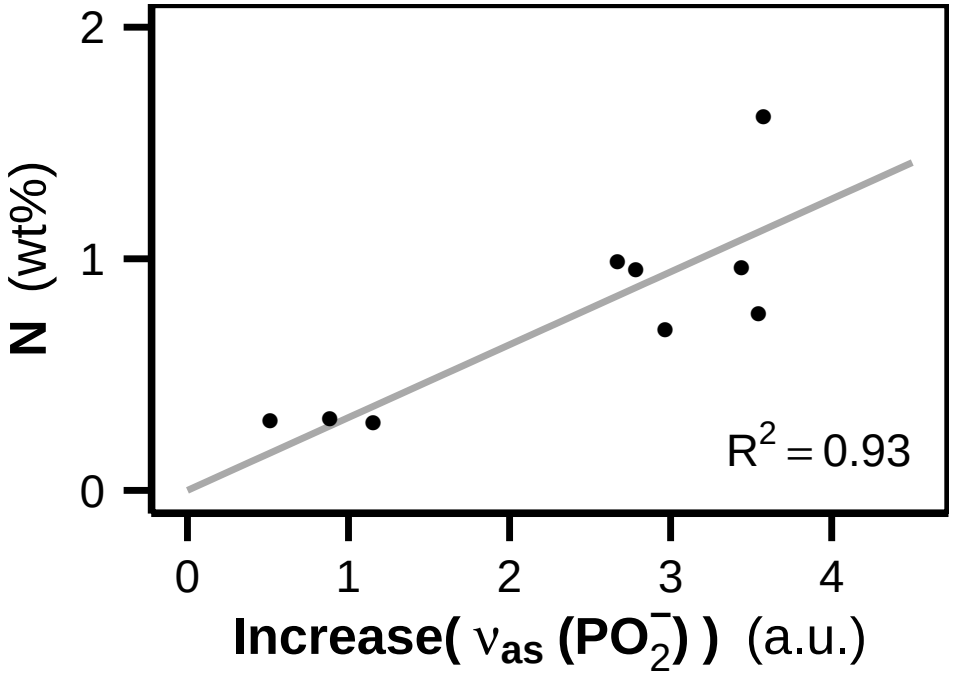

Fig. 1. Novel Figure 4b with the trendline forced through the origin.

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