Discussion of comments by Tim Moore on bg-2019-176

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Reviewer comment: This is an interesting manuscript which explores the fate of N during the early stages of decomposition of plant litter under anaerobic conditions. In essence, the authors examine the depolymerisation of N in litters and substrates of varying N content and FTIR is adapted to provide identification of the chemical changes in the decomposing litter, with an estimate of the microbial uptake and depolymerisation components and stoichiometric relationships. The manuscript is well structured and written and provides some insight into the depolymerisation process, which has been proposed for several years, but which has been difficult to analytically identify. I hasten to add that I have a very limited knowledge of the dark art of FTIR spectra analysis, so look to other reviewers to evaluate the veracity of the FTIR section of the manuscript. I have noted some grammatical/typographical and stylistic errors and suggestions, and place them in the pdfs, which are hopefully attached.

Authors reply: We thank you for your careful reading of the manuscript. We appreciate your comments on improvements of the writing, which are highly valuable for us as non-native speakers.
We also thank you for your detailed comments and suggestions, which we would like to discuss in detail. Please do not hesitate to post additional comments if you feel that some of our answers require further explanation or discussion.

As a preliminary outline: There are five main aspects which might be discussed in more detail in the manuscript. These are
(1) the missing correlation between nutrient status of the wetland and N content of the corresponding undecomposed plant litter,
(2) the variations in litter quality/decomposability, i.e. why does the medium-N leaf litter decomposes more slowly than the other two leaf litters,
(3) the role of dissolved organic nitrogen,
(4) the time-dependency of the reported process (preferential N decomposition) over the decomposition path, and
(5) the repeatability of the results in an oxic decomposition experiment.

A reply to point (5) must include both, a discussion of the applicability of the FTIR method in an oxic decomposition study and a discussion whether preferential N decomposition can be expected to be a relevant process in aerobic ecosystems. We suggest to change the "Conclusions" to "Conclusions and perspectives" in order to cover these aspects.
Point (3), the role of DON, will be adressed at selected points throughout the manuscript.
Point (1), (2), and (4) are interesting aspects, some of which had already been discussed by us during data analysis and manuscript writing. We believe that a discussion of these points might overload the main document and suggest to discuss these points solely here in the interactive discussion. As documents herein are citable, we suggest to add citations in the manuscript at relevant positions that lead the interested reader to this file.

I provide the following more detailed comments and suggestions for consideration by page and line number:

1, 0 While I think that the preservation of vascular litter in Sphagnum peat is a useful product of the work, I think it has a broader impact, and most litter entering Sphagnum (and other) peats decays initially under aerobic conditions, rather than the anaerobic burial used in this experiment. Thus I would suggest a more generic title, emphasizing the more original approaches taken.

We agree that the current title does not cover some aspects of the manuscript. We will change the title to:

- Unraveling preferential protein depolymerization from litter in response to external nitrogen availability in three anoxic wetland soils through a novel FTIR routine

1, 2 What does 'relative' mean here? It could be N accumulation relative to N (implying a lowering of the C:N ratio) or it could be a larger N mass, relative to the initial litter. Please clarify.

We thank you for this comment and will change the beginning of the abstract:

Phragmites australis litters were incubated in three saturated anoxic wetland soils of different nutrient status for 75 days and litter nitrogen (N) dynamics were analyzed by elemental analyses and infrared spectroscopy (FTIR). At the end of the incubation time, the N content in the remaining litter tissue had increased in most samples. Yet, the increase of N content was less pronounced when litters had been decomposed in a more N-poor wetland soil. FTIR was used to quantify the relative content of proteins...

4, 25 The experiment was conducted under anaerobic conditions, or at least litter placed in containers into which substrates had been added and presumably under saturated or waterlogged conditions. I think this is important, partly because of the conditions created (anoxic) and, as I note above, most peatland vascular litter does not decompose initially under anaerobic conditions. Thus, I think the experimental details of these containers and substrates/litter need to be better described. Also, were they incubated at 'room temperature'? Furthermore, are the results of this study likely to be repeated, quantitatively or qualitatively, if the experiment was to be repeated under aerobic conditions, which is probably the situation in many wetlands. Of course, one could argue that the initial aerobic decomposition is followed by anaerobic, as the litter becomes buried and goes beneath the water table.

In order to visualize the details of the experiment more precisely we would like to present two photos. Figure 1a shows the container filled with detritus mud (high-N substrate) after sampling and during the transport to the lab. Figure 1b shows the prepared litterbags with rhizome and leaf litter before incubation. The containers with sampled substrates were transported to the basement of our institute in June 2013, and three days later the litterbags were placed in each container, close to the bottom. We will include Figure 1a to the "Materials and Methods" section in the revised manuscript and furthermore describe the experimental design in more detail.

The term "room temperature" will be changed to 21°C.

We agree that the placement of freshly fallen leaf litter into an anoxic environment does not mimic the common pattern of litter decomposition in wetlands. Naturally, leaf litter is initially decomposed in the actrotelm and only becomes buried after extensive aerobic decomposition. Only for the incubated rhizomes direct anaerobic decomposition can be expected. A rapid burial of leaf litter and its anaerobic decomposition might also happen in the rewetted fen where sedimentation rates are high. Our study specifically aimed to compare the process of anaerobic decomposition in peatlands and to search for differences in the enzymatic breakdown of organic matter which might explain a part of the rapid conversion of organic matter to DOM,

Figure 1. (a) The container with the detritus mud (high-N substrate) from the rewetted fen Stangenhagen. Litterbags were placed in the containers in the anoxic zone of the substrate close to the bottom. **(b)** Litterbags with leaf and rhizome litter before incubation.

CO_2, or CH_4 in some anoxic soils or its stabilization in other soils. The use of rather labile fresh litter was needed to study these processes over a short experimental time. Thus, our experiment can possibly be compared to reciprocal peat core or litter transplantation studies which similarly try to investigate the basic mechanisms of C and N cycling in specific ecosystems through modifications like placing high-N litter in a low-N environment.

Yet, it is true that most studies on N cycling focus on aerobic environments, e.g. leaf litter in mineral soils. So the question whether the analytical method can be applied to aerobic environments and whether similar effects can be expected will be of interest for many readers. We tend to believe that this is the case, but additional experiments are needed to affirm this. We would like to note that even for past decomposition studies, our analytical approach might be applicable if litter samples are still available, stored under cold and dry conditions.

We will change the "Conclusions" of the manuscript to "Conclusion and perspectives":

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4 Conclusion and perspectives

In wetland ecosystems, the disentangling of gross N transformations is central for the assessment of C cycling, N cycling, and biogeochemical responses to increased external nutrient inputs, especially of N (Van Groenigen et al., 2015). Here, we have presented a new methodical approach which enables the disentangling of plant and microbial N in decomposed litter by using DNA signals in FTIR spectra as a marker for microbial N. This approach allows to quantify how much microbial N has formed and how much plant N is remaining in litter at a certain stage of decomposition.We have demonstrated that substrate-dependent, i.e. decomposition site dependent, variations in litter N accumulation were not caused by variations in the amount of existing microbial N in litter at a certain stage of decomposition, but instead by variations in the remaining amount of still unprocessed plant-N. This indicates a decrease of gross protein depolymerization when litter decomposes in a nutrient-rich environment, and suggests that microorganims preferentially use inorganic N from porewater instead of organic N from litter when those two pools compete as consumable N sources. The influence of the decomposition site and its nutrient status on gross protein depolymerization in decomposing vascular litter has not been detectable in former studies. Instead, net N mineralization/immobilization patterns in decomposing litter are often perceived as being predetermined solely by the initial litter C/N ratio and the rate of microbial N accumulation.

~~For litter decomposition in *Sphagnum* peat, we found high bulk N losses from litter due to preferential protein depolymerization. Although not directly adressed in this study, we assume that sphagnan, a compound released from *Sphagnum* mosses, is responsible for this effect. Sphagnan is known to bind free amino groups, so that a fraction of the depolymerized amino~~

~~acids might become inaccessible for microbial decomposers. We suggest that preferential protein depolymerization might be an adaptive mechanism to compensate amino acid losses to sphagnan. Preferential protein depolymerization leads to a gradual N depletion of the unprocessed organic matter in litter, what can be considered as a gradual decrease of litter decomposability, a potential mechanism for long-term preservation of vascular litter in *Sphagnum* peat.~~

Litter decomposition has here been studied in anoxic and waterlogged wetland soils. While initial litter C/N ratios are usually successful predictors of N mineralization patterns in aerobic environments, e.g. mineral soils, we here found bulk litter N dynamics that did not fit into that established concept. The strength of the site-dependency of C/N-patterns in litter is propably caused by the waterlogged conditions. Nutrients can more easily be exchanged between litter and porewater compared to less humid mineral soils. Additionally, microbial nutrient demand is lower in anoxic environments why we were able to test a large gradient in initial litter N content without causing N limitation.

Yet, the here shown ability of microbial decomposers to preferentially release N from decomposing plant tissue is probably not limited to anoxic systems. In particular for the aerobic decomposition of low N litter, which in some cases has been shown to proceed without net N immobilization, preferential protein depolymerization is an alternative mechanism to the concurrently assumed lowered microbial CUE through overflow respiration. Microbial N in litter, determined through the here reported FTIR method, might be an easily available parameter to investigate trends in preferential protein depolymerization and CUE in future aerobic litterbag studies.

Small method modifications should be considered for the applicability of the method in aerobic decomposition studies. These modifications include an optimization of the calibration curve, either through the addition of very low-N litters as calibration samples to a decomposition study or through the artificial mixing of undecomposed litter with microbial biomass. Furthermore, the contribution of fungi must be considered, which we assumed to be absent in anoxic soils. Differences in the amont of DNA per biomass units and in the C/N ratio should be considered for the decomposer biomass in aerobic systems. Finally, the applicability of the same calibration curve for decomposed litters of different plant species still has to be investigated.

Table 1. I was a bit confused by * decomposition in home soil. I would have thought the 'home soil' would be high-N leaf with high-N substrate, medium with medium etc., but this is not the pattern observed. I wondered why.

To answer your question we would like to present some data of a study we did earlier which includes the same leaf litter used in the decomposition experiment. In that preliminary study we compared the chemical composition of *P. austrails* leaf litters of wetlands with different nutrient status. The data are presented in Table 1 and Table 2. The decision on the sampling sites in the decomposition study was actually based on data of Table 2.

The expected correlation between soil N and leaf litter N is partly observed. Leaf litter from the mesotrophic wetlands of group 3 have nitrogen contents below 1.2%. The eutrophic wetlands of group 1 and 2 have leaf litter N contents ranging from 1.5 to 2.1%. In our study, leaf litter from the medium-N site has a lower N content than the litter from the high-N site, as expected. The acidic kettle mires, which all have peat moss floating mats and small populations of *P. australis*, fall out of this pattern. *P. australis* litter from these nutrient poor sites of group 4 have comparably high litter N contents. In our experiment, the most nutrient rich litter belongs to the most nurtient poor site, which is the kettle mire Kablow Ziegelei.

In the textbook by Reddy and DeLaune (2008, p. 315) it is written that "nitrogen content of plant tissue is inversely related to biomass. It is expected for plants with high biomass production that concentration of nitrogen may be lower as a result of dilution and distribution within the tissue." Even though Reddy and DeLaune's statement is based on data from McJannet et al. (1995), who compare different plant species, we believe that a similar mechanism is responsible for the high N content in *P. australis* litter of group 4 sites. At these sites the *P. australis* plants were much smaller (shoot size and leaf area) compared to litter from more nutrient rich sites.

Another aspect which we would like to mention is the effect of silica. The ash content of the high-N leaf litter is only about 3%, while it is 7 and 10% for the other two leaf litters. The infrared spectrum of the high-N litter, shown in red in Figure 2a, shows a very low absorption intensity in the carbohydrate region. The black line is a difference spectrum which shows that a missing peak at 1095 cm^{-1} causes this difference. In combination with bands at 800 and 470 cm^{-1} this peak is identified as biogenic silica (Figure 2b). The lack of silica in the leaves from the poor *Sphagnum* fen is also reflected by the C content, which is about 48% vs. 44% in leaves from the nutrient-richer sites. In line with a higher C content, the N content of the high-N leaf tissue could be expected to be about 10% higher at a similar organic matter composition, simply because the tissue is less diluted with inorganic constituents.

Yet, the C contents remained relatively stable throughout the decomposition experiment for all litters (Table S3 in the SI), so changing concentrations of inorganic constituents did not cause changes in N content in our study. Furthermore, conclusions in the manuscript are mostly based on C/N ratios and percentual C and N losses, which are invariable to the ash content.

Table 1. The twelve analysed Brandenburgian peatlands. Hydrogenetic mire type classification was performed according to Succow and Joosten (2001). The peatlands are divided into four groups with respect to nutrient levels. The three peatlands in the first group have been drained intensively in the past and have been rewetted in recent years resulting in the formation of flooded mires and new ecosystems.

	Sampling Site	Hydrogenetic mire type	Ecological mire type	pH	Latitude, Longitude
	Moor bei Stangenhagen	Flood mire	euthrophic	6.2	52.204294°, 13.091846°
1	Moor bei Menzlin	percolation mire	euthrophic	6.6	53.88333°, 13.63333°,
	Niedermoor Hasenfelde	Terrestrialisation mire	euthrophic	7.2	52.68333°, 14.38333°
	Moor bei Anklam	Percolation Mire	euthrophic	6.9	53.79599°, 13.83202°
2	Glieningmoor	Terrestrialisation mire	euthrophic	6.2	52.349555°, 14.199986°
	Maxsee Niederung	Percolation mire	euthrophic	7.0	52.46308°, 13.97963°
	Töpchin Süd	Terrestrialisation mire	mesotrophic calcareous	6.6	52.161700°, 13.577400°
3	Triebschmoor	Terrestrialisation mire	mesotrophic calcareous	7.0	52.3454°, 13.80413°
	Pätzer Hintersee	Terrestrialisation mire	mesotrophic subneutral	6.3	52.20639°, 13.632832°
	Dollgengrund	Terrestrialisation mire	mesotrophic subneutral	5.7	52.00000°, 14.03333°
4	**Moor bei Kablow Ziegelei**	Kettle mire	oligotrophic acid	4.4	52.32571°, 13.72182°
	Kleiner Milasee	Kettle mire	oligotrophic acid	4.2	52.153220°, 13.957115°

Stangenhagen, Menzlin and Hasenfelde (group 1) have been drained intensively in the past decades. In recent years, these sites have been rewetted and can now be classified as flooded mires in an early regeneration state. Anklam, Glieningmoor and Maxsee Niederung (group 2) are ground water fed, nutrient-rich mires in natural state. Töpchin Süd, Triebschmoor and Pätzer Hintersee (group 3) are nutrient-poor mires in near pristine condition, while Dollgen, Kablow-Ziegelei and Kleiner Milasee (group 4) are nutrient poor, acidic pristine mires.
Moor bei Stangenhagen: high-N substrate, Töpchin Süd: medium-N substrate, Moor bei Kablow Ziegelei: low-N substrate.

Table 2. Elemental composition and polyphenol contents of P. australis leaf litter from 12 fens of different nutrient status.

	Sampling Site	C	N	C:N	P	C:P	Polyphenols	Ash
		(mg/g)	(mg/g)	(mass)	(mg/g)	(mass)	(mg/g)	(mg/g)
	Moor bei Stangenhagen	437.5 ± 1.8	14.6 ± 0.1	29.9 ± 0.1	0.81 ± 0.01	54.0 ± 0.9	18.4 ± 2.0	103.3± 1.7
1	Moor bei Menzlin	454.1 ± 0.2	20.5 ± 0.1	22.1 ± 0.1	1.40 ± 0.03	32.4 ± 0.9	17.4 ± 3.1	58.4± 1.4
	Niedermoor Hasenfelde	464.1 ± 2.1	18.1 ± 0.4	25.6 ± 0.4	1.58 ± 0.01	29.4 ± 0.3	27.1 ± 1.1	46.8± 0.9
	Moor bei Anklam	464.3 ± 0.2	18.7 ± 0.3	24.9 ± 0.5	1.29 ± 0.01	25.9 ± 0.1	18.4 ± 2.6	50.6± 1.2
2	Glieningmoor	471.2 ± 0.9	13.7 ± 0.1	34.5 ± 0.3	0.59 ± 0.01	79.3 ± 0.1	20.2 ± 4.0	43.7± 3.4
	Maxsee Niederung	432.3 ± 1.1	14.7 ± 0.2	29.4 ± 0.3	0.82 ± 0.01	53.0 ± 0.9	17.4 ± 3.0	93.3± 1.4
	Töpchin Süd	447.9 ± 0.8	9.2 ± 0.1	48.6 ± 0.1	0.65 ± 0.02	69.1 ± 2.0	11.9± 1.4	73.6± 1.8
3	Triebschmoor	410.2 ± 0.1	11.7 ± 0.1	35.0 ± 0.5	0.50 ± 0.01	81.6 ± 2.1	19.0± 2.1	138.2± 1.5
	Pätzer Hintersee	442.7 ± 3.5	11.8 ± 0.1	37.4 ± 0.2	0.65 ± 0.02	68.2 ± 1.2	10.9± 1.2	77.1± 2.3
	Dollgengrund	461.8 ± 0.4	17,2 ± 0.1	26.9 ± 0.1	0.89 ± 0.01	51.6 ± 0.3	21.6± 3.7	60.4± 0.4
4	**Moor bei Kabow Ziegelei**	480.0 ± 1.7	20.3 ± 0.4	23.7 ± 0.4	0.90 ± 0.01	59.6 ± 0.4	32.4± 4.4	31.6± 2.4
	Kleiner Milasee	486.2 ± 0.3	20.6 ± 0.1	23.6 ± 0.1	0.70 ± 0.01	69.5 ± 0.8	26.9± 2.6	34.1± 1.8

Leaf litters were collected from north-east German peatlands during December 2012. Polyphenol content was determined following the Folin-Ciocalteu Assay. Ash content is the remaining mass after 3 h combustion at 450°C. Leaf litter was not preleached before analysis, so its elemental composition differs slightly compared to data in the main manuscript.

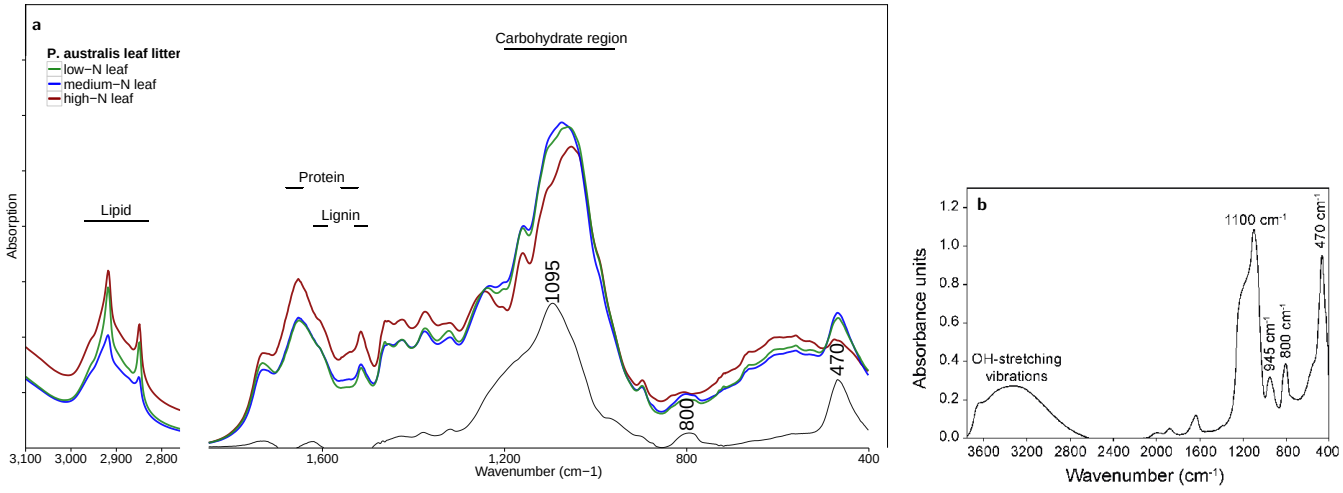

Figure 2. (a) Infrared spectra of undecomposed *P. australis* leaf litter. In black the difference spectrum "medium-N litter minus high-N litter" that extracts the peak which causes the drop in absorption intensity of the high-N litter in the carbohydrate region. **(b)** The spectrum of biogenic silica (from Meyer-Jacob et al., 2014).

7, 4 Litter bag experiments usually entail the early stages of decomposition, in this case 21 to 45% over 75 days. One wonders what the patterns may have been if the study allowed sampling earlier and later: in other words, are the processes identified here time-dependent in the decomposition path?

The main process which we identified is the preferential depolymerization of proteins in leaf litter as a parameter which seems to be characteristic for a specific decomposition site. This is a so far mostly neglected process. For example, it is written in Wikipedia, that "whether nitrogen mineralizes or immobilizes depends on the carbon-to-nitrogen ratio (C:N ratio) of the decomposing organic matter" (Wikipedia, n.d.). The basis for the discovery of this relationship is stoichiometric considerations which revealed a "global stoichiometry of litter nitrogen mineralization" (Manzoni et al., 2008) and the initial litter C/N ratio is identified as a predictor wether N mineralization or immobilization dominates.

We only have one sampling time (after 75 days), but large differences in overall C losses, ranging from 21 to 45%. Yet, many stoichiometric models which are the basis for some conceptual views on N cycling in decomposing litter do not use decompositon time as a parameter (Manzoni, 2017). N dynamics in litter are seen as a function of C loss. According the stoichiometric models, this could be seen as a gradient in the decomposition path and within these concepts we think that there might be no specific time-dependeny of these processes.

Still it would be worth to discuss a potential outcome if different sampling times were allowed. We believe that the first few decomposition days will probably be governed by leaching processes. Even when preleached leaf litter is used like in our study, an initial (probably abiotic) increase of the C/N ratio is often observed in this phase. Stoichiometric models often exclude this phase and start which pre-decomposed litter. This effect of abiotic leaching is however not observable in our study, because abiotic N losses should be litter specific and independent of the porewater chemistry of a specific site.

For very long decomposition times, other conceptual assumptions might need a re-evaluation. Our study is somehow caught between the chairs. We started with the approach by Tremblay and Benner (2006) who quantified the amount of microbial biomass and microbial N in decomposed litter using different markers. While Tremblay and Benner (2006) stopped at this point, we went one step further and use plant N and microbial N data in decomposed litter to caclulate CUEs and nNUEs. For this we use equations from isotope tracer experiments, which directly relate consumption and excretion. But tracer experiments

cover very short experiment times, so active elemental fluxes are compared. We consequently used a simplified model which assumes that plant organic matter breakdown and microbial biomass growth are the only two processes involved, what is not realistic over the full decomposition path. In our picture the final outcome of litter decomposition would be no remaining plant OM and newly formed microbial biomass, whose amount corresponds to about 10% of the initial plant C. In nature, close to no organic matter remains at the end of decomposition.

In stoichiometric models, the underlying equation for C loss is

$$\frac{dC(t)}{dt} = -D + D \cdot CUE \cdot \frac{C}{C_0} \tag{1}$$

where D is the decomposition rate in C units (Manzoni, 2017). We ignored the term C/C_0. Stoichiometric models use the litter bulk C and N, and a decomposed litter sample that reached a high N content due to microbial biomass accumulation will not be different to a litter sample which comes with a high initial plant N content.

While we believe that for 75 days our methods are suitable, these might need to be adapted for long decomposition times. We think that the DNA band, which we quantified through FTIR, is a marker for the living microbial biomass in litter. At the end of decomposition, we would expect that non-microbial C and N fractions in litter are not only plant OM, but a mixture of plant OM and remains of dead microbial biomass. A decrease of the (what we termed) plant OM C/N ratio could thus be expected over time. But such hypothesis would need more experiments. For our experiment, which lasted 75 days, we believe that such secondary processes like the cycling of microbial necromass can still be neglected.

We actually have performed another decomposition experiment with different sampling times. The aim of the study was to investigate how the breakdown of plant organic matter and the formation of DOM differs under different redox conditions. The experimental setup included leaf litter (from the rewetted fen Stangenhagen) with an inoculum in carbonate-buffered pure water (Figure 3). As N cycling was not the primary aim of that experiment, we would like to present here how our method performs over time (Table 3).

Without any natural environment and with the leaf litter as the sole C and nutrient source, the conditions for microbial growth were probably rather harsh, a possible reason why we measured comparably low litter decomposition rates. For the first two

Figure 3. Mesocosms prepared for the anoxic decomposition experiment.

sampling dates our method does not detect any microbial biomass in the litters, but also the C/N-ratio of the litter did not change substantially in this time. For the last two sampling dates we found microbial N. The overall picture of these data suggest that preferential N depolymerization was not a relevant process in this study, the C/N ratio of the plant OM remained rather constant. The emerging pattern thus corresponds to the established C/N ratio concept in that an increase in litter N content over time occurs due to the growth of microbial biomass within the litter.

Table 3. Selected C and N data of leaf litter, of its microbial N content, microbial CUEs and nNUEs, and parameters of the plant biomass fraction of litter. The litter encompasses the remaining plant biomass and the newly formed microbial biomass.

Litter Type and	Litter[a]				Microbial biomass			Plant biomass				
Decomp. Environment	N_{litter}[a]	C_{loss}	N_{loss}	$(C/N)_{litter}$	$N_{microbial}$[b]	CUE[c]	nNUE[d]	N_{plant}[e]	$C_{depoly.}$[f]	$N_{depoly.}$[g]	α[h]	$(C/N)_{plant}$[i]
	(wt%)	(%)	(%)	(mass)	(wt%)	(%)	(%)	(wt%)	(%)	(%)	(ratio)	(mass)
Initial Litter	1.34 ± 0.05	-	-	32.1 ± 1.1	–	–	–	–	–	–	–	–
— Methanogenic conditions —												
19 days	1.25 ± 0.05	8.9 ± 1.8	15.2 ± 3.1	35.1 ± 1.0	-0.09 ± 0.06	-	–	–	–	–	–	–
54 days	1.29 ± 0.06	16.6 ± 0.7	21.4 ± 4.1	34.8 ± 1.5	-0.04 ± 0.02	-	–	–	–	–	–	–
153 days	1.57 ± 0.12	33.9 ± 1.1	23.1 ± 7.1	28.2 ± 2.1	0.21 ± 0.05	4.53 ± 1.15	32.7 ± 12.7	1.36 ± 0.09	37.3 ± 0.7	33.5 ± 5.3	0.90 ± 0.12	31.8 ± 1.2
383 days	1.68 ± 0.10	50.3 ± 2.0	36.3 ± 1.5	25.5 ± 1.5	0.52 ± 0.13	5.64 ± 0.96	34.8 ± 5.7	1.16 ± 0.04	53.1 ± 2.3	56.0 ± 2.8	1.05 ± 0.01	34.6 ± 0.6
— Sulfate reducing conditions —												
26 days	1.15 ± 0.19	15.9 ± 3.0	27.0 ± 12.6	38.5 ± 5.2	-0.11 ± 0.07	-	–	–	–	–	–	–
61 days	1.29 ± 0.15	24.4 ± 0.7	25.6 ± 8.0	33.5 ± 3.4	-0.01 ± 0.03	-	–	–	–	–	–	–
160 days	1.37 ± 0.09	33.4 ± 1.1	28.4 ± 2.7	30.4 ± 1.4	0.28 ± 0.09	6.45 ± 2.47	33.3 ± 7.0	1.09 ± 0.18	33.6 ± 1.8	43.2 ± 7.3	1.30 ± 0.30	37.7 ± 5.3
392 days	1.38 ± 0.09	46.0 ± 7.1	41.9 ± 2.4	30.3 ± 3.3	0.32 ± 0.14	4.03 ± 1.03	23.0 ± 7.0	1.06 ± 0.06	46.7 ± 5.7	55.0 ± 6.6	1.18 ± 0.03	37.7 ± 1.3
— Nitrate reducing conditions —												
19 days	1.19 ± 0.06	10.7 ± 1.3	21.0 ± 2.8	37.0 ± 1.6	-0.08 ± 0.03	-	–	–	–	–	–	–
54 days	1.16 ± 0.07	21.9 ± 2.3	33.1 ± 1.6	38.1 ± 1.8	0.04 ± 0.07	1.81 ± 2.73	5.6 ± 11.1	1.11 ± 0.12	24.3 ± 3.1	35.7 ± 5.4	1.50 ± 0.36	39.7 ± 3.4
145 days	1.25 ± 0.13	30.6 ± 3.3	36.2 ± 4.1	35.8 ± 4.0	0.21 ± 0.04	5.13 ± 0.56	22.9 ± 3.5	1.04 ± 0.11	34.4 ± 3.1	46.9 ± 4.2	1.38 ± 0.23	42.0 ± 5.0
375 days	1.46 ± 0.12	47.8 ± 2.2	42.4 ± 2.9	29.6 ± 2.6	0.25 ± 0.08	3.00 ± 0.73	18.3 ± 4.6	1.22 ± 0.11	50.0 ± 2.6	52.1 ± 3.3	1.04 ± 0.10	34.6 ± 2.4

[a] Decomposed litter contains plant and microbial N.

[b] $N_{microbial}$: N content in decomposed litter belonging to microorganisms (in wt% litter dry mass).

[c] CUE: Carbon use-efficiency.

[d] nNUE: Net nitrogen use-efficiency.

[e] N_{plant}: N content in decomposed litter belonging to plant OM (in wt% litter dry mass).

[f] $C_{depoly.}$: Fraction of initially present plant C which has been depolymerized.

[g] $N_{depoly.}$: Analogously to C_{depoly}.

[h] (α): Coefficient of preferential protein decomposition (N_{depoly}/C_{depoly}).

[i] $(C/N)_{plant}$: Carbon-to-nitrogen ratio of the plant OM fraction within the litter.

7, 8 Litter quality involves several attributes of the initial litter influencing decomposition rate, of which the C:N ratio is frequently cited. It was not borne out here, possibly because decomposition was under anaerobic conditions. Were there any other attributes of the litter which might explain this deviation, such as P content, lignin content etc.?

The medium-N leaf litter, originating from the eutrophic rewetted fen Stangenhagen, had the lowest decomposability of all samples. Initial phosphorus contents were 0.65, 0.81, and 0.90 mg/g for the low-N, medium-N and high-N leaf litters (Table 2, unleached). We did not measure P during the decomposition experiment, but these data suggest that variations in decomposability were unlikely due to differences in P content.

CuO lignin contents (Λ_6) accounted for 5.7, 4.7 and 5.9%. Decomposed leaf samples had lignin contents between 7.0 and 8.8%. So the medium-N leaf litter had the lowest initial lignin content, what would contrarily suggest a high decomposability. The medium-N litter had the highest ash content of all leaf litters (10.3%) (Table 2). Ash should mostly be silica in *P. australis* litter. The high-N and low-N leaves had ash contents of 3.2 and 7.4% and showed a very similar litter quality and we are not aware of an effect of mineral content on litter quality.

So I guess we can not say what caused the difference in decomposability. The rhizome litters were probably very decomposable due to the lability and high surface area of the aerenchym tissue, which in the living plant is protected from the environment through a stable outer rhizome surface. Yet, this simple explanation for the high C loss of the rhizomes is not easily predictable from their elemental composition or through FTIR spectroscopy, for which leaves and rhizomes look very similar. So possibly the explanation lies in the physical buildup of the biopolymers in the leaf litter, which leads to a more resistent litter tissue in leaves from the rewetted fen?

Table S1 While nitrate was essentially non-existent in the porewater from the three substrates, there was a major difference in NH4 and also DOC, the latter implying a large variation in dissolved organic nitrogen (DON), referred to p 18, l6. In Sphagnum peatlands, DON dominates the pore water, often forming 60-90% of the total dissolved nitrogen (TDN). It appears that TDN was not measured (allowing an estimate of DON) but could there be more consideration of DON in the understanding of the processes involved?

We will present a dataset of another sampling campaign in which TDN was quantified. For Stangenhagen these are data of a June 2015 sampling, so the values differ from those presented in the manuscript. DON in the *Sphagnum* site accounts for 0.82 mg/L, what is in the same range as 1 mg/L NH_4^+. We were not aware that DON makes up a substantial fraction of TDN in these sites and will mention this in the manuscript text as this might support the argumentation on the role of sphagnan.

High amounts of DON are measured in the high-N site porewater. We agree that we have overlooked DON as an additional external N source and, as suggested, will discuss DON at selected points in the revised manuscript.

Table 4. Selected Data on Peatland Type, Pore Water and Peat Chemistry of the Sampling Sites.

	low-N substrate	medium-N substrate	high-N substrate
Name	Kablow-Ziegelei	Töpchin Süd	Stangenhagen
Coordinates	52°3273'N, 13°7274'E	52°1617'N, 13°5774'E	52°2081'N, 13°0941'E
Fen type	kettle hole mire	terrestrialisation mire	former percolation mire
*Pore water**			
DOC (mg/L)	79.9 ± 15.8	28.4 ± 4.8	103.3 ± 1.3
DON (mg/L)	0.82 ± 0.19	0.73 ± 0.16	8.12 ± 0.77
pH	4.3 ± 0.1	6.6 ± 0.1	6.8 ± 0.1
Electrical conductivity (μS/cm)	60 ± 5	645 ± 18	1089 ± 100
SRP (mg/L)	0.02 ± 0.01	0.56 ± 0.10	4.22 ± 0.51
$NO_3{}^-$-N (mg/L)	0.02 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
$NH_4{}^+$-N (mg/L)	1.0 ± 0.6	2.1 ± 0.4	23.4 ± 4.3

I found it a little bit confusing that C and N ratio was expressed atomically, whereas everything appears to be on a mass basis; while atomic units are common in stoichiometric studies, most decomposition studies use mass.

We agree and will change C/N values to mass ratios.

Sequence of reference citations seems to vary between alphabetical and chronological and the format used in the References is variable.

Thank your for this comment, we will correct citation order and reference style.

In case the Supplement does not load, oxycoccos is mis-spelt and it is Electrical conductivity.

Thank you, we will correct these misspellings.

Thank you again for your review,

Hendrik Reuter, on behalf of all coauthors

References

Manzoni, S.: Flexible Carbon-Use Efficiency across Litter Types and during Decomposition Partly Compensates Nutrient Imbalances—Results from Analytical Stoichiometric Models, Frontiers in Microbiology, 8, 661, 2017.

Manzoni, S., Jackson, R. B., Trofymow, J. A., and Porporato, A.: The Global Stoichiometry of Litter Nitrogen Mineralization, Science, 321, 684–686, 2008.

McJannet, C., Keddy, P., and Pick, F.: Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups, Functional Ecology, pp. 231–238, 1995.

Meyer-Jacob, C., Vogel, H., Gebhardt, A. C., Wennrich, V., Melles, M., and Rosén, P.: Biogeochemical variability during the past 3.6 million years recorded by FTIR spectroscopy in the sediment record of Lake El'gygytgyn, Far East Russian Arctic, Climate of the Past, 10, 209–220, https://doi.org/10.5194/cp-10-209-2014, https://www.clim-past.net/10/209/2014/, 2014.

Reddy, K. R. and DeLaune, R. D.: Biogeochemistry of wetlands: science and applications, CRC press, 2008.

Succow, M. and Joosten, H., eds.: Landschaftsökologische Moorkunde, Schweizerbart Science Publishers, Stuttgart, Germany, 2001.

Tremblay, L. and Benner, R.: Microbial contributions to N-immobilization and organic matter preservation in decaying plant detritus, Geochimica et Cosmochimica Acta, 70, 133–146, 2006.

Van Groenigen, J., Huygens, D., Boeckx, P., Kuyper, T. W., Lubbers, I., Rütting, T., and Groffman, P.: The soil N cycle: new insights and key challenges, Soil, 1, 235–256, 2015.

Wikipedia: Mineralization(soil science), https://en.wikipedia.org/wiki/Mineralization_(soil_science)#cite_note-3, accessed: 2019-06-09, n.d.