

Biogeosciences Discuss., author comment AC1  
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## Reply on RC1

Lisa Noll et al.

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Author comment on "Climate and geology overwrite land use effects on soil organic nitrogen cycling on a continental scale" by Lisa Noll et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-41-AC1>, 2022

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### **This study studied the factors of land use and bedrock on protein depolymerization across**

a 4000-km transect in Europe, and highlighted the important role of climate and soil properties on N cycling at large scales. The sampling scheme is attractive, and many interesting biochemical indicators are measured. This study stated N cycling was controlled by substrate availability. This conclusion is not novel, actually which has been widely acknowledged for a long time. Generally, I think this paper fits the scope of this Journal, however, more should be added to highlight the novelty of this study. Some conclusions are confusing, the author had clarified the importance of substrate availability, which was related with OM input or vegetation, however, it also declared that the land use effect was insignificant. Those ideas are not consistent. It's better to clarify them. Besides, the writing should be improved. For instance, some paragraphs lack the key points, and some sentences are confusing or too long to understand. See the details as follows.

We thank the reviewer for the critical comments on our manuscript.

Minor comments to RC1 suggestions/criticisms: Our conclusion that N cycling is controlled by substrate availability is not our conclusion, we concluded that substrate (protein) availability drives gross rates (in situ rates) of protein depolymerization in soils across a continental transect. This is different from pouring some substrate on a soil and finding changes in net nitrification or net N mineralization, as we here measured gross process rates. Moreover, we here refer to organic N and not inorganic N cycling. We are aware that protein addition to soils increased proteolytic activities in arctic soils (Weintraub & Schimel, 2005), but this also does not refer to a gross process but rather to potential activities, which we here demonstrated not to be the driver of in situ depolymerization rates. Our MS here is only the second paper showing substrate limitation of the depolymerization processes in soils, after Noll et al. 2019 at the regional scale, but here at a large scale. This is also the major novelty of this study. In the revised MS we will improve this. We will also improve the text to highlight the key points in each paragraph (upfront or at the end). Too long or confusing sentences will be cut and rewritten.

Below are itemized replies to the referee comments. The line numbers refer to those of the original manuscript.

**Line 40: The key point of this paragraph is not clear, clarify it. And since land use is your**

main sampling scheme, you can introduce more about it.

The aim of the study was to disentangle the large scale controls on gross protein depolymerization rates and organic nitrogen cycling. Since land use is one determinant of soil functions and soil microbial community structure and function, different land uses were included in the study design. However, land use certainly was not determining the study in terms of main sampling scheme. We here nested land use in large scale climatic and geological controls along this continental transect, but searching for sites distributed across whole Europe where we found at least two (at best: three) land uses in close vicinity (max. few 100s meter). Moreover, effects of land use are expected to be more prominent at a smaller regional to local scale and exact land use data were not accessible for this data set. On the continental scale climate and bedrock considered the main drivers of organic N cycling as demonstrated by our results, though this has been rarely studied in such a systematic way. Therefore, land use was an important factor in our sampling scheme, yet not the one and only, and likely being strongly overprinted by large scale changes in climate and geology. We will clarify this in the introduction and discussion section of the revised manuscript. We also will add 1-2 more sentences on land use effects on soil organic N dynamics.

**Line 50. This reference was in contrast with your contents, discuss it later.**

The reference is not in contrast with our results. In the study by Mooshammer et al. (2012) the effects of elemental stoichiometry (C:N:P) on gross protein depolymerization rates were studied in decomposing beech litter. In contrast, in more decomposed organic horizons, processes of organic matter stabilization are increasingly important and might mask the stoichiometric effects reported from litter studies. We will further clarify this in the introduction and discussion section of the revised manuscript.

**Line 64, add reference.**

We will add appropriate references such as Lauber et al. for global drivers of microbial community structure, and global meta-analyses on net and gross N mineralization and nitrification (published by Christoph Müller's group), and on soil and microbial C:N:P stoichiometries on the global level. We rephrased the sentence as follows:

*In conclusion, land use, bedrock and biogeographic region are likely key controls on soil nutrient status and edaphic properties and affect microbial community structure, substrate availability and microbial N and C demands as shown in Figure 1.*

**Line 96, what's the depth of the 'organic layers'?**

Organic layers were sampled down to the mineral soil. The depth of the individual organic horizons varied strongly from 2 cm to > 15 cm (max. 30 cm). This will be added to the revised MS.

**Line 180: This sentence is confusing, please rephrase it.**

We rephrased the sentence as follows:

*For statistical analyses of single variables, mineral soils were grouped by bedrock (limestone, sediments, silicates) or by land use (cropland, grassland, woodlands).*

**Line 340: Simplify the sentences**

We rephrased the sentence as follows:

*However, though vegetation N limitation increases with latitude (Kang et al, 2010) we showed here that depolymerization rates increased with latitude, indicating increasing labile organic N provisioning to microbes and plants at higher latitudes under lab conditions. This highlights differential element viz. nutrient limitation of plants and soil microbes across large spatial scales as proposed by Capek et al. (2018). This is also supported by the missing effects of resource C:N ratios and microbial C:N imbalances on depolymerization rates.*

**Lin 320-335: It's confusing in 4.1. 'land use had no effect on the response of depolymerization rates'**, however, above discussion was talking about the differences in different land use, and even attributed the difference to soil pH.

The described land use effects were significant for individual sampling sites. However, in the overall statistical analyses the effect of land use explained only 5% of the variability. We will clarify this in the discussion section of the revised manuscript.

**Line 343: pH was the main predictor in results, but the contribution of texture, mineral assemblage, how are they related to N cycling?**

In line 343 we stated that "Across all land use types NaOH-extractable protein and soil pH were the main predictors for gross protein depolymerization in mineral soils, indicating that soil properties that determine protein availability such as texture, mineral assemblage or soil pH need to be considered when addressing controls of soil organic N cycling.". This does not imply that as RC1 mentions "pH was the main predictor", and then asks how texture and mineral assemblage are related to N cycling". Soil pH mirrors the strength of Ca-bridging of negatively charged ligands (as protein-carboxylates) to negatively charged soil particles (clays), but also the weathering status of soils, which comes with the formation of secondary clays and Fe/Al oxyhydroxides. On the other hand, soil texture and mineral assemblage are also affected by geology (aside of weathering) and directly affect the amount of organic N bound and at the same time the binding strength of this interaction. Gross protein depolymerization rates were negatively correlated to clay content, indicating that the availability of proteins decreases with increasing clay content. Sorption experiments in artificial soils showed that at neutral soil pH (>7) clay minerals are the main sorption sites for organic N (Pronk et al., 2013). Aside from the stabilization on mineral surfaces, high clay contents, as found in limestone soils, promote soil aggregation and thereby the occlusion of organic matter and proteins rendering them inaccessible for enzymatic attack (Lützow et al., 2006). We will clarify this in section 4.1.

**Line 360: I don't think this indicate that 'stabilized compounds are available for microbial utilization'**

We rephrased the sentence as follows:

*In acidic soils, column experiments with embedded goethite revealed that sufficiently large amounts of stabilized C were re-dissolved by progressing percolation of dissolved OM and consequent subsequent exchange of adsorbed compounds. The re-dissolved compounds are thus available for microbial utilization (Leinemann et al., 2018).*

**Line 365: Add reference**

"Fe- and Al oxyhydroxides remained as a significant parameter in linear models and path analyses and should therefore be considered as important predictor for the potential of a soil to retain and accumulate SOM (Moni et al, 2007; Fang et al., 2019), high SOM promoting microbial biomass and activity (Xu et al., 2013; Hartman & Richardson, 2013)."

We will add appropriate references here, such as:

Moni, C., Chabbi, A., Nunan, N., Rumpel, C., & Chenu, C. (2007, December). Do iron and aluminium oxides stabilise organic matter in soil? A multi-scale statistical analysis, from field to horizon. In AGU Fall Meeting Abstracts (Vol. 2007, pp. B11G-04).

Fang, K., Qin, S., Chen, L., Zhang, Q., & Yang, Y. (2019). Al/Fe mineral controls on soil organic carbon stock across Tibetan alpine grasslands. *Journal of Geophysical Research: Biogeosciences*, 124(2), 247-259.

Xu, X., Thornton, P. E., & Post, W. M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography*, 22(6), 737-749.

Hartman, W. H., & Richardson, C. J. (2013). Differential nutrient limitation of soil microbial biomass and metabolic quotients ( $q_{CO_2}$ ): is there a biological stoichiometry of soil microbes?. *PloS one*, 8(3), e57127.

**Line 376-378: this sentence is confusing, please simplify it**

We rephrased the sentence as follows:

*Sorption of proteins on clay and Fe-mineral surfaces is usually highest close to the isoelectric point of a specific protein.*

**Line 390: add support for this opinion**

In these paragraph we highlight one major outcome of this large scale study, which we consider robust based on multiple data lines presented in this MS, i.e. "The lack of correlation between gross depolymerization and peptidase activity implies that gross protein depolymerization rates are rather substrate limited than enzyme limited. Differences in protein depolymerization rates between alkaline, neutral and acidic soils are due to changes in substrate (protein) availability rather than due to changes in microbial community structure and in enzymatic activity.". The whole MS builds towards this "conclusion", we therefore consider it unnecessary to add further (?) support here.

**Line 405-420: Do you mean that the climate factors influence soil pH and then regulate the**

depolymerization rates? However, your data doesn't seem to support this, please explain it.

We concluded that climate factors drive chemical weathering of soil minerals. These changes are accompanied by the mobilization and hydrological losses of base cations such as K, Ca and Mg and consequently a drop of soil pH. However, our data show that depolymerization rates are not controlled by soil pH in the first place. Our data rather support that the observed effect of climate factors on depolymerization rates is explained by changes in soil chemical weathering and more specifically the formation of specific minerals. We will clarify this in the revised manuscript.

**Line 450: can you compared the contributions in the combined model including land use,**

soil properties, climate together?

As shown in Fig. 4 a-e land use did not affect the response of gross depolymerization rates to changes in soil properties (this would be seen in differences in the slopes of these relationships between croplands, grasslands and woody vegetation), but contributed to differences in soil pH and NaOH-extractable protein between land uses, as shown by

different intercepts of the linear regression models (Fig. 4f). The regression model on climate factors and land use (Fig. S5) also shows that land use is only a minor driver of depolymerization. Therefore, land use was not included in the final SEM and the SEM only explains the large scale soil physicochemical controls on protein depolymerization. Land use effects might become more dominant on regional scales, where effects of climate are smaller.

**Line 462: It's confusing, 'peptidase activity is a proxy of microbial N or N limitation'?**

This refers to the work by Allison, Sinsabaugh, Weintraub and others, and the theory of enzyme allocation. According to Allison et al. (Allison et al.,2010) "Extracellular enzymes allow microbes ... to acquire resources from complex molecules, .... We examine the hypothesis that extracellular enzyme producers are under evolutionary pressure to minimize the cost:benefit ratio of enzyme production. Consistent with this prediction, enzyme producers generally allocate more resources to enzymes that target limiting nutrients." According to this peptidase activity can be used as a proxy for microbial N limitation.

We rewrote this sentence as follows:

*The amount of NaOH-extractable protein was here identified as the most important direct predictor of protein depolymerization rates, while peptidase activity was a poor predictor of protein depolymerization, but rather reflects a proxy of microbial N limitation according to enzyme allocation theory (Allison et al., 2010).*

**Fig 5: 'black arrows' are missing in the figure. And how to identify the direct and indirect effects?**

The figure caption describes the black/white plot of Fig. 5. In the manuscript we showed the colored plot with red and blue arrows. We will correct the figure captions in the revised manuscript.

The indirect effects are effects of one parameter on depolymerization rates mediated by another parameter. For example mean annual precipitation has an indirect effect on depolymerization rates via NaOH-extractable protein.

**References**

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