

Replies to reviewer#1

I reviewed this short, but very interesting manuscript by Spohn et al., who used thermal oxidation and radiocarbon dating to show that old organic matter in Swedish forest soils is strongly enriched in nitrogen. The approach taken is generally interesting, but the authors would need to prove, that the N-enrichment is not an artefact of the thermal oxidation. This is most likely very difficult. The so-called Maillard reaction, which is well known in the pyrogenic carbon community and also food chemistry, is the condensation of sugars with amino acids under high temperatures (see e.g.: Nitrogen Enrichment during Soil Organic Matter Burning and Molecular Evidence of Maillard Reactions | Environmental Science & Technology). Although thermal oxidation might be a way to isolate biogeochemically meaningful organic matter fractions, they do not remain unchanged. The KCL extraction done by the authors to quantify the amount of mineral nitrogen in the samples after thermal fractionation is not enough, because here it's about the formation of aromatic nitrogen. Unfortunately, the methodological choice here is most likely not suitable to test the hypothesis of the authors. As a consequence, the manuscript needs to be rejected at that stage. A simple physical fractionation might have been the better choice to separate younger and older OM.

We thank the reviewer for the thoughtful comments.

The reviewer wrote that our finding that old soil organic matter fractions are rich in nitrogen might be an artefact because of the Maillard reaction. The Maillard reaction is a reaction of an amino acid and a sugar that together form a melanoidin at elevated temperatures (with highest reaction rates between 140 to 165 °C). During this reaction an organic nitrogen compound (an amino acid) reacts with another organic compound (a sugar) to form a new organic nitrogen compound (a melanoidin). Importantly, no inorganic nitrogen is involved in this reaction, and the organic nitrogen remains organic nitrogen.

We have no evidence showing that the Maillard reaction did not occur in the soils during the thermal treatment. However, if it occurred, it should not affect the conclusions of the study, as we will explain in the following.

We analyzed the $\Delta^{14}\text{C}$ of the soil fractions obtained by ramped thermal fractionation and related them to the carbon (C) and nitrogen (N) contents as well as to the C:N ratio. We found that fractions with low $\Delta^{14}\text{C}$ content have a low C:N ratio.

The reviewer seems concerned about the fact that the product of the Maillard Reaction is more thermostable than the educts (i.e., reactants) of the reaction. If this were the case, this would not affect the conclusion of the study. This is because our conclusion is not based on the thermostability of the compounds and neither on the assumption that the thermostability of the organic matter does not change during the fractionation process. Our main conclusion is solely based on the observation that fractions with low $\Delta^{14}\text{C}$ content have a low C:N ratio. We will address this aspect in the revised version of the Discussion.

If we assume for a moment that all organic matter in the fractions obtained by thermal fractionation had been created by the Maillard Reaction, the observation that $\Delta^{14}\text{C}$ content have a low C:N ratio would still be correct, and it would show that the C in these fractions is on average old (compared to the untreated soil). If the organic matter of a compound formed by the Maillard reaction has a low $\Delta^{14}\text{C}$, this is due to the $\Delta^{14}\text{C}$ of the reactants. Importantly, the Maillard reaction does not create new organic nitrogen from inorganic nitrogen.

In addition, it seems rather unlikely that the Maillard reaction caused (or contributed to) the formation of an organic matter fraction with a very low C:N ratio, which is in the range of the C:N ratio of amino acids, peptides, and proteins. The product of the Maillard reaction has a C:N ratio that is higher than the C:N ratio of the amino acid that reacts in this reaction due to the incorporation of the C from the sugar. Thus, the Maillard reaction does not lead to formation of organic matter with a very low C:N ratio (as

found in our study), but to the formation of organic matter that has a C:N ratio which is higher than the C:N ratio of amino acids, peptides, and proteins. Hence, it seems unlikely that the Maillard reaction has a large contribution to the formation of organic matter in the thermostable fractions. We will mention this in the Discussion in the revised version of the manuscript.

The reviewer also commented on the inorganic N quantification. The purpose of this measurement was to confirm that the inorganic N that is formed by the combustion of organic N during the ramped thermal fractionation does not remain in the sample. If we had detected inorganic nitrogen in the samples, we would have to subtract the inorganic N from the total N to obtain the organic N content. We will add a sentence in the Methods section to explain this more clearly.

Apart from this fundamental comment, I'd like to mention that although short papers are nice to read, I found this one a bit too superficial in some parts. Surely, the authors mentioned a couple of times, that N-rich organic compounds might have a higher affinity to be sorped and stabilized, but readers would probably like to get some more background and deeper understanding on that. Also, the step of organic matter transformation (e.g. by microbes) is somewhat missing. We know that a substantial part of mineral associated OM consists of microbial necromass, which is per se quite enriched in N. Although the authors have no data on this, there could be more in-depth discussion on the different stabilization pathways. I also miss a bit of an implications section in the discussion (what does that mean for the ecosystem, for the different biogeochemical cycles, how far can you generalize these results, and who should care about them). Also, a proper conclusions section is missing. We agree that the Discussion can be extended. Particularly, we will discuss with more detail the contribution of microbial biomass to nitrogen-rich, mineral-associated soil organic matter in the revised version of the manuscript. In addition, we agree that also the implications of the results can be discussed more explicitly. We will add implications of our findings for both the carbon and the nitrogen cycle at the end of the Discussion, and we will extend the conclusions.

Specific comments:

Line 12: It could also indicate, that organic matter becomes enriched in its transformation processes and that more processed organic matter persists longer. We agree with this comment, and we will revise this paragraph of the Introduction accordingly.

Introduction: Maybe at least mention the 'microbial carbon pump' and how this relates to N We agree. We will write about the microbial carbon pump and the contribution of microbial biomass to nitrogen-rich, mineral-associated soil organic matter in the Introduction and Discussion of the revised manuscript.

Line 53: Could you please elaborate a bit more on your exclusion criteria of Podzols? To avoid strong eluviation, you could have selected via OM content. Yes, we could potentially also have selected the soils based on OM content. However, since we were interested in studying the effect of forest type (coniferous vs. broadleaf), we chose all soils to have the same soil order to exclude soil order as a potential confounding factor. We will explain this more clearly in the revised version of the manuscript.

Line 58: Why larger than 55 years? We selected stands that have a stand age larger than 55 years to base the study on soils that have a long continuity in organic matter inputs. The mean stand age is 88 years. A higher stand age would have been even better. However, if we had selected the sites based on a high stand age, we would have less sites to include in this study. Still, given that the stand ages are relatively high it is likely that these forests were never clear cut and that there is a continuity in forest type beyond the current stand age. We will explain this better in the Methods of the revised version of the manuscript.

Line 74: Why so restricted to the 0-10 cm layer? We chose the uppermost 10 cm of the soils because this is where the soil organic matter contents are highest. Thus, the findings are relevant for a large proportion of the organic matter of the soils.

Line 75: So you took the soils from the archive? Could be mentioned. We thought that this is clear based on the sentence in lines 49 to 51, but we will add this explicitly in line 75.

Line 78: Why 35°C? This seems rather low. This has been the standard drying temperature for the Swedish National Forest Inventory for the last 40 years. Forest soils are usually dried at a rather low temperature.

Line 1745ff: This whole section seems to belong to the section above (on thermal fractionation). Maybe move upwards? We agree that this section fits better behind the first section of the Discussion. We will change the order of the sections.

Figure 2: I don't really like the x-axis names. Soil, 325°C, 400°C. Please consider changing. We will change the names to Soil, Fraction 1 and Fraction 2.