

**Thank you for the opportunity to revise our manuscript addressing the reviewers' constructive comments. We have taken into account all of their comments and feel this has led to a far clearer and useful contribution that should be of interest to the Biogeosciences readership.**

**Please find our responses given in italics below after each individual comment or section provided by the reviewer.**

[line numbers refer to those found in the edited version with tracked changes]

### **Response to Reviewer 1**

Soil organic matter diagenetic state informs boreal forest ecosystem feedbacks to climate change”

The manuscript deals with the fate of the soil organic carbon in a climatic gradient from a cold to a warm region. The authors used several metrics to assess the influence of climate change on the cycling of C and N in soils. They applied the lignin diagenetic index, and evaluated the variation of this index in different soils strata. In general, the manuscript is well organized and easy to follow. I recommend the manuscript for publication after some minor revision.

***Response:*** *Thanks for your positive feedback.*

Minor comments:

Line 230 I did not find the AADI data in the original paper (Philben et al., 2016).

***Response:*** *The AADI is not referred to exactly as such in Philben et al. (2016). Rather it is simply referred to as ‘the degradation index’ in that paper as that work only contains amino acids datasets (e.g. see figure 3 in Philben et al. 2016). This has been clarified to avoid confusion. [see lines 913-917]*

Figure 1, This figure is very interesting. Why do the authors think that in some parameters (e.g. V ad/A<l, S/V, %side chain alteration, diOHBAVV, S Ad/Al) there is a great variation in the deepest soil horizon? Does the H horizon reflect older and more variable long-term effect of climate?

***Response:*** *Good question. We suspect that the increase in variation observed for some LP parameters within the deepest horizon reflects a combination of variation in how degraded the lignin is, the impact of hydrology (e.g. Hernes et al., 2007) and root inputs or processes (Otto and Simpson, 2006) on these signatures. The deepest layer (H) exhibits the greatest degradative state. However, with spatial heterogeneity particularly in hydrology and root inputs (mainly concentrated at the interface between the organic layer and surface of the mineral soil and thus right around the H layer) we might expect greater variation in resulting degradative state as timing and magnitude of new inputs of root or dissolved organic matter, which both carry lignin phenol signatures, varies. We have added a phrase in the revised manuscript to refer to this possibility. [see lines 1045-1049]*

Figure 2 The linear relationship is very strong, it may help the reader if the authors include a description for example “increase lignin degradation” to the right of the plot.

**Response:** *This is a great suggestion! An arrow and phrase to help link the ratio to the degree of degradation has now been added to this figure.*

Figure 3, In previous figure, the y axes was “layer”, and the factor analyzed was the “depth”, please homogenized the figures.

**Response:** *Thank you for pointing out the various nomenclature used for the same variable. We noticed that the figures also refer to horizon interchangeably with depth and layer. We have reviewed all figures, captions and text to be sure that we are consistent with the soil classification terms. The revised manuscript now correctly refers to the soil layer for the organic layer as a whole and the L, F and H as the individual soil horizons within that layer and used to understand the impact of soil depth on factors presented or assessed throughout.*

Figure 4, the deepest soils layer (H) has a broad variation compare with surface, any possible explanation?

**Response:** *See comment above. Importantly for this figure, the amino acid-based degradation index contributes to variation in this ratio, cumulatively increasing variation with depth with the spatial variation in lignin signatures, with those of amino acid signatures, such as lower amino acid content at depth due to the increased degradative state. We have added information conveying the probable causes as described above in addition to the reduced amino acid content at depth in the revised manuscript. [see lines 1045-1049]*

Line 436, Add “The” before SOC and SON.

**Response:** *Thank you for noticing this typo and we fixed that error in the revised manuscript.*

## **Responses to Reviewer 2**

### General comments

The manuscript still lacks in my opinion clarity and structure, and it is not easy to follow. In particular, the hypotheses being tested are described in just 4 lines, and it is very hard to see the connections of those hypotheses with the whole (rather long) introduction or their relevance. The whole manuscript seems written before the hypotheses, to be honest, and the hypotheses just added at the end as a patch to make some former referee happy and not as a logical tool. They are not even directly considered in the conclusions.

**Response:** *We appreciate the reviewer’s input on the structure of the introduction and linkages with the hypotheses within the manuscript as a whole, and particularly in the conclusion. In the revised manuscript, we have shortened and restructured the introduction and completely revised the final paragraph. Some of the key context has been moved out of that paragraph and restructured to support the motive of the hypotheses. The final paragraph has been completely rewritten to clarify the hypotheses (see next comments specific to the hypotheses). We also revised the discussion and conclusion section to better tie back into the two hypotheses. These*

revisions also prompted related edits in the abstract. We feel that these revisions help clarify the study, its findings and their implications for readers.

Concerning Hypotheses 1: the diagenetic state of SOC seems akin to "quality" or "recalcitrance" of SOC (this relationship needs to be clarified in the intro, or at least explained better why diagenetic state of SOC is relevant). This is the main determinant of SOC kinetics, but the stocks are not just determined by the kinetics.

**Response:** *The reviewer's comment here importantly suggests edits to the introduction were needed to clarify the term diagenetic state, why we used it, and how it may be useful in conjunction with ecosystem fluxes in understanding the trajectories of SOC stocks. This is a very important point that underlies the implications of this manuscript and therefore, revisions to the introduction have also focused on making this point much clearer to readers. [see lines 215-219]*

*The diagenetic state of SOC (or SON) measured at any given time or site is the net result of the rate of organic matter inputs and the rate of the combined action of degradation or physiochemical alteration, so not just a result of SOC kinetics in isolation. This concept is a pillar to organic geochemistry as a discipline (e.g., see text books such as those by Killops and Killops or Engel and Macko), and it is important that readers are fully on board with this definition. Thus, the revised introduction provides an explanation of diagenetic state, how it relates to SOC composition (quality and recalcitrance), and how it may be useful in understanding the trajectories of SOC stocks when determined using an approach that accurately accounts for any changes in the composition of SOC inputs. This is a great example of cross domain terminology and thinking (à la Hedges and Oades, 1997) that is essential to define the terminology for a broader readership such as EGU Biogeosciences.*

*The terms quality or recalcitrance are more generalized terms often used in describing SOC (or SON) composition as it relates to microbial degradation and can be associated with the diagenetic state. For example, SOC that has been more diagenetically altered would be expected to exhibit a lower rate of degradation (e.g. rate of respiration at a standard temperature and moisture condition) and thus less bioreactive (or more recalcitrant to biological degradation). However, the diagenetic state is more specific as it refers to the degree to which SOC has been altered (biologically and physiochemically), and thus must be assessed independent of variation in the composition of inputs. This can be quite difficult when investigating the impact of climate even within a single biome given the fact that the composition of plant inputs can change in response to climate. For example, in boreal forests warming is often attributed to reduction in moss inputs and thus an increase in the proportion of vascular plant inputs. This is observed as a shift toward lower carbohydrate and increases in more aromatic SOC. Such a shift itself regardless of the degree of degradation or diagenesis can change the "quality" of the SOC in ways that can reduce its bioreactivity or increase its recalcitrance. Consequently, we observed a decrease in bioreactivity (or increase in recalcitrance) in the warmer forests relative to the colder forests where the elevated moss inputs are observed (Kohl et al. 2018). This common scenario in boreal forests represents a challenge for detecting and comparing the diagenetic state of SOC across these forests, stimulating our use of the lignin phenol index in this research.*

I also have concerns about how you tested such hypothesis, read below.

**Response:** Please see our response to this comment below.

Concerning hypothesis 2: I do not understand well how you tested it, You state at line 369: "we expect soil C and N cycling are coupled across these forests in association with climate warming" (and then proceed telling you could not really link them). That doesn't seem testing a hypothesis to me, and I really do not get the experimental approach you followed to test Hypothesis 2. Plus: C and N are not necessarily coupled, you can definitely have variations in the C:N ratios of ecosystems (for sure of plant organs). For example: <https://www.sciencedirect.com/science/article/pii/S0378112713004155>, if tree species composition changes due to climate change (or, as in your case, just different biomes) also the C:N ratio will change.

**Response:** *The reviewer's comment points out further need to clarify the hypotheses and also the manuscript by restructuring the introduction and discussion. The revision now includes more explicit hypotheses within the final paragraph of the introduction. Restructuring of the introduction and revisions to the discussion have been completed to better clarify; (1) what diagenetic state is and how it can be used to understand the response of SOC stocks; (2) the challenges in obtaining the diagenetic state of SOC in boreal forest ecosystems across different climates where composition of SOC inputs can vary; and (3) how coupling measures of the diagenetic state of SOC and SON enable a comparisons of soil C and N cycling across ecosystems to determine if they are coupled and if the degree to which they are coupled is maintained across climates.*

*The comment here regarding the lines around 369 (first paragraph of the discussion) indicates that the reviewer was not aware that this section was meant to be context for the approach and findings of the study, and was not meant to indicate that we did not link soil C and N cycling. Rather, this section was meant to remind the reader of the challenges in making this linkage and how we over came those challenges in this study by combining the amino acid and lignin diagenetic state measures. Thus, in the revised manuscript we have removed this paragraph and improved the context within the introduction and discussion to avoid such confusion. Thus the revised introduction and discussion sections better clarifies:*

- 1. The use of diagenetic state to account for changing inputs and their influence on interpreting SOM composition. For example, we outline how this is particularly problematic for SOC in the boreal forest context. We reorganized and revised lines 63-80 where we discuss how varied plant inputs can alter the composition, and thus interpretation, of SOM including the C:N ratio which can vary as a function of different plant inputs which can even occur within a given biome such as studied here (shifts toward more vascular plants and less moss within warmer forests).*
- 2. How an approach combining the diagenetic state of SOC and SON, through the use of biomarker indices and proxies to assess the diagenetic state of SOC and SON, can be used to evaluate how the degree to which soil C and N cycling are coupled may vary over time or space (in particular see revised section 4.3 including final paragraph)*
- 3. How evaluating the degree of coupling between soil C and N can contribute to understanding the role (and limits) increased N cycling may play in supporting maintenance of SOC stocks. This includes added explanation within the discussion of how the cycling of C and N are not necessarily linked. For example, if maintenance of SOC stocks in the warmer forests were attributed to greater availability of external N sources (e.g. significant atmospheric N input) relative to the colder forest sites then we would not expect the ratio of the lignin phenol diagenesis to amino acid diagenesis ratio*

*to lower in the warmer region forests and not be similar to those observed in the colder forest sites. [see section 4.3]*

*These edits help to explain our hypotheses and how they were tested within this study.*

Materials and methods are sometimes explained in paragraphs scattered across the MS.

**Response:** *We have revised the materials and methods section by pulling out methodological explanations provided within the results and integrating them within the Methods section. For example, we omitted first statement in the Results section (original lines 249-251) and left that information in the methods section. The entire manuscript has been reviewed carefully to detect such inconsistencies and we found multiple instances within the results section and each of these were removed and integrated as needed into the revised Materials and Methods section [see revised sections 2.4, 2.5, 3.1, 3.2, 3.3 and 3.4 and new section 2.6].*

The authors need to work on the structure of the MS and on the logical consistency of what is being tested and how. The relevance of the results are also unclear (probably because of the above-mentioned lack of structure. Once you have an hypothesis to test you can also define its relevance in the introduction, before proceeding with the rest of the manuscript).

**Response:** *The logic of what is being tested and how it is addressed has been clarified through the revisions to the introduction, materials and methods, results and discussion. These include clarification on what the diagenetic state of SOC and SON is, how those can be assessed, what combining those measures can reveal in terms of soil C and N cycling, and the approach taken in this study. Additionally, the direct statement of the hypotheses within the introduction and also how they are linked to data analyses completed. See more details on how we will address this above in addition to new section 2.6.*

I also have some concerns about the study itself, which I will address here, while less specific comments.

In particular, my main concerns are:

Methodological issue: about the LPDI index construction and its validation. It is stated that it was an iterative process, but the iteration steps are not described properly in M&M or they were not clear to me.

**Response:** *We thank the reviewer for noting that the structure of the iteration steps were not clear to a reader. Much of this information was in the supporting information, including figures used to inform the final LPDI and we will revise the manuscript to better reflect these steps. The revised manuscript now lays out the process in a stepwise fashion so it is more easily reproducible (see revised section 2.4 Lignin phenol diagenetic index (LPDI) development, application, and validation). This revision also included the inclusion of a flowchart figure (Supplemental Figure S2)*

Validation of the LPDI index extrapolated by the PCA model: I understood correctly, the only validation is the comparison between your LPDI index (the first PCA component) and an NMR ratio. I have no idea what you refer to with "measured LPDI" since your LPDI is a PCA component, but in any case, the agreement between your LPDI and the NMR ratio should be shown in detail. I could not even understand what the agreement was between the two, and this

is the only link with some sort of physical reality of your index. It is crucial. On top of that, a PCA model will likely be overfitted, and it would be best to have this validation on independent samples (you measure the NMR ratio on them, apply your PCA model coming from your study and different samples to derive the LPDI estimate, and then measure the  $R^2$ ).

**Response:** *Addressing this comment has served to significantly clarify our methodology including unpackaging the information synthesized within Figure 2.*

*Briefly, our revisions to this section of the manuscript (Lines 728-730; 748-765; 773-780; 887-906) include:*

- 1. A clearer explanation of the lignin phenol index, which was modeled after the amino acid index presented and utilized successfully in similar contexts in Dauwe et al. 1999; Menzel et al. 2015 and Philben et al. 2016. The lignin phenol concentrations were indeed measured on actual samples (see Figure 1 for those data). The PCA simply serves as a data reduction tool on the lignin phenols datasets only, in order to better track changes in the multiple indices and ratios presented in Figure 1 for lignin. This is now more clearly explained within this section.*
- 2. A clearer explanation of the validation steps used to predict the LPDI on samples we did not measure it on. Briefly, Figure 2 shows the relationship reviewer suggests where we measured the NMR ratio and the LPDI actually measured on individual samples. We note the figure also shows predictions of LPDI based on the NMR ratio and that may lead to some confusion thus we modified the figure and its caption to address this point of confusion. The NMR ratio itself is completely independent of the LPDI, does not use the same datasets to derive, and the relationship in Figure 2 shows an agreement between these two completely independent assessments of lignin phenol diagenesis. We have modified the manuscript text and figures to increase clarity around these points.*

Foundations of the experimental setup: the diachronic approach chosen in the MS might present a lot of issues, that are not discussed, while results are compared with synchronic approaches (warming on a single site). A climatic gradient IS NOT warming. Environments in different climates are likely already at equilibrium (more or less), while rapid warming resulting from an experimental manipulation brings the system far from its previous equilibrium state. I do not think the results are comparable, and I have doubts that a sequence over climates can offer information on climate warming in general.

Successful warming experiments that have used climatic gradients that I am aware of have taken a sample from one location and physically moved replicates of the same sample over the gradient.

**Response:** *We agree with the reviewer that results from across a climate transect such as this do not provide the same information as those observed from experimental warming (whether synchronic or as part of a soil transplanting experiment across climates), and we did not intend to imply that they were equivalent or comparable. References to experimental warming studies made within the manuscript were primarily made to discuss contrasts between the two approaches in order to explain what we learn from the two approaches through understanding what each is able to provide. For example (original lines 409-415):*

*“The lack of change in lignin diagenetic state across these boreal forest sites, despite the +5.2°C MAT of climate warming, contrasts with the increase in the diagenetic state of lignin observed over 14 months of experimental*

warming in a temperate forest (Feng et al., 2008). This may be due to a lack of additional ecosystem responses to warming (e.g., enhanced soil inputs; Melillo et al., 2011) that were not captured over the shorter time scale. Climate warming impacts on ecosystem properties, such as altered litter inputs (Pisani et al., 2016) and shifts in climate conditions such as MAP (Duboc et al., 2014; Pisani et al., 2014), can serve as drivers of lignin decomposition and its diagenetic state.“

Obviously, such instances were not clearly described. For example, in this case above we should have more clearly indicated that describing the differences in the two approaches was meant to convey the role of ecosystem processes altered by climate change attributed to warming and changes to water availability over decades to centuries not typically captured by experimental warming conducted within one or several years. We have identified all instances such as this throughout the introduction and discussion and revised them in order to indicate the contrasts observed and what they mean with regard to understanding SOC responses to climate change. This also includes editing the reference to the comparison of sites from that of warming to attributes of a warmer relative to colder climate along this transect.

The use of the climate transect here was intended to understand the combined impact of all responses (microbial, plant and hydrologic change) to the warmer and wetter climate predicted for the region and over several decades to a century rather than immediate responses to warming alone where the soil system is brought far from its equilibrium state. However, we feel that investigations of ecosystems and their soils across climate sequences can and do offer information on how they are likely to respond to climate change over decadal and century time scales when studied within biomes or over millennia in the case of studies across biomes (see successful studies such as: Kane et al. 2005; Norris et al. 2010; Giardina et al. 2014; Ziegler et al. 2017; Gu et al. 2022). The clarification on what can be learned using such an approach versus an experimental warming approach has been integrated into the introduction and discussion sections of the manuscript to address this concern. This includes what we are not able to assess using the climate transect approach (e.g. not providing soil responses to the disequilibrium conditions imposed by short term soil warming). The Methods section now briefly reiterates these points that separate this approach from experimental (synchronic or diachronic) approaches that study warming (Lines 648-653). Finally, we now clarify what we mean here through the use of the term warming as it is meant to convey the changes in climate in the boreal region studied and associated with climate warming. This has now been done through edits described above where warming was replaced with attributes of a warmer relative to colder climate along the transect. Thus the differences are not simply due to an increase in soil or air temperature but increases in T and precipitation as well as ecosystem properties that occur in association with longer term (decades to century) increases in T and precipitation. For example, greater soil DOM losses (Bowering et al. 2022), decreases in moss inputs as well as the potential for plant responses to increased N cycling not captured in shorter term experimental warming studies.

Similarly, you should dig into the concept of equilibrium of SOC. An increase in inputs always results in an increase in outputs. Sure, it brings the equilibrium stocks up, but it's not a linear relationship. SOC decays universally as an exponential function (at least the vast majority of its variance is explained by it), so 10 tons more inputs are not going to result in 10 tons more stocks, but maybe 1 ton because the more the inputs, the higher the fluxes. You should probably try first to model the stocks you observed with such a relatively simple approach and then proceed to explain any eventual residual variance, if any.

**Response:** We agree that increases in soil inputs are often associated with increased losses. Indeed, in a system in SOC equilibrium, this must be the case, and thus greater inputs do not

*always lead to an increase in SOC stocks. Because inputs and losses are often quite well matched in magnitude in most ecosystems (i.e. NEP = the small difference between two very large fluxes that of gross primary production and respiration (and lateral losses)), it is difficult to assess whether SOC stocks are increasing or decreasing over yearly or even decadal timescales. This is why it is useful to couple ecosystem fluxes with measures of SOM diagenetic state in order to assess how soil C stocks may respond to climate change (Billings et al. 2008). We simply cannot resolve differences in the inputs and losses well enough to inform the trajectory of SOC stocks in response to the collective effects of differing climate in these forests.*

*Losses of SOC include losses of C via CO<sub>2</sub> through the process of decay as well as lateral losses as DOC. We have quantified these and other important C fluxes in these forests. To model SOC stocks within these forests in a meaningful way we would need to assess losses and inputs, both of which vary across the forest sites. When we compare these inputs and losses across the transect, we find a deficit of inputs across all sites relative to losses, with the data varying such that the average values overlap among the multiple latitudinal regions (Ziegler et al. 2017). This is likely due to the fact that we are unable to account for all inputs (mosses, roots), and suggests we cannot discern meaningful differences in latitudinal variation in these fluxes.*

*Though we appreciate that SOC often exhibits exponential decay patterns over time, we are unsure how such a model would provide more information to this emerging story developing along this transect. We interpret the reviewer's suggestion to mean that we need to do a better job of incorporating this broader, C-cycling context into the start of the paper, which would better clarify the motive for our approach. Therefore, in a revised manuscript, we have made significant edits to the introduction to clarify the challenges in detecting net changes in SOC stocks and how the diagenetic state of SOM provides evidence for inferring the temporal trajectory of SOC stocks (i.e., maintained, lost, or accruing) and its links to SON cycling. [see revised introduction as well as new first paragraph of discussion section ]*

Specific comments:

Paragraph 2.4: describe in detail the iteration steps (some details are later after line 275)

**Response:** *Per the reviewer's comments and our responses above we have described this approach in more detail within the Materials and Methods section, and also include a visualization of the steps (ie. a flowchart of the workflow; new Figure S2) provided in the supporting materials.*

Paragraph 3.3: Introduce a detailed explanation of the validation approach in M&M, and describe with measurements the results of the validation here.

**Response:** *A clearer explanation of the validation steps used to predict the LPDI on samples we did not measure it on have been added to the M&M section. As described above, Figure 2 shows the relationship the reviewer suggests where we directly measured the NMR ratio and the LPDI on individual samples. We note the figure also shows predictions of LPDI based on the NMR ratio and that could have lead to some confusion. Thus we modified the figure to address this point of confusion. The NMR ratio itself is completely independent of the LPDI, does not use the same datasets to derive, and the relationship in Figure 2 shows an agreement between these two completely independent assessments of lignin phenol diagenesis. We appreciate the*



reviewer pointing out this issue and feel that the modification made in the revised text and figure clarify these points regarding the validation made. [see revised Fig 2 and revised section 2.4]

Discussion: here, you talk about some hypotheses. Describe all your hypotheses in the introduction, and then proceed to test them. Describe in M&M how you are going to test. Mention the result of the tests in the conclusions. This will add clarity.

**Response:** *This comment has been addressed through revisions made in the last part of the introduction (Lines 578-594) and the M&M section (Lines 919-920; 1016-1025) as previously described above and we agree with the reviewer that this should help clarify.*

Line 369-370: C and N cycles are not necessarily coupled one-to-one. C:N ratio can vary, and for example, a site can lose fertility as a consequence.

**Response:** *Indeed, we did not mean to imply that soil C and soil N would be coupled one-to-one but rather that the rates at which each is processed are linked. Given this and other related comments we have revised manuscript, through inclusion of examples such as sites of high or very low fertility. For example, sites with excessive atmospheric N inputs versus those in very remote high latitude ecosystems would be expected to be quite different in terms of the degree to which soil C and soil N cycles are linked. In the case where excess N is available, and thus low rates of SON mineralization with warming or within warmer climates (such as some high-latitude ecosystems; e.g. Meyer et al. 2006), SOC would become more diagenetically altered than SON. This would be detected as an increasing ratio of LPDI to AADI, and in turn would signify a reduction in SOC stocks, similar to what has been observed in response to artificial N fertilization in tundra soils, where losses of soil carbon were enhanced relative to plant productivity and soil inputs (Mack et al., 2004). Where N availability is lower (such as in the boreal forests studied here or temperate forests such as in Melillo et al. 2017) increases in soil C cycling – say with experimental warming or in warmer climates – would be expected to be coupled with increases in soil N cycling and thus the degradation states of the two would be coupled, and therefore, we would expect the LPDI to track well with the AADI. These explanations have now been incorporated into the revised introduction and discussion.*

Conclusions: your last statement should be motivated. How do you think this measurement could reduce uncertainty in models? And how do you think it could increase our understanding of such feedbacks? Other than that, conclusions should tell the reader if the hypotheses being tested were verified or not.

**Response:** *Good point, we agree we should be more specific here in order to clarify what these results provide. Specifically, the further application of the approach demonstrated here, which provides an assessment for how well the cycling of soil C is linked with the cycling of soil N, would allow us to understand where and when the maintenance of soil C stocks are controlled by the cycling of N within the ecosystem versus limited by other factors. Applying this to other systems or over time would provide an indication of ecosystem shifts in response to climate (or other environment change) that may limit forest productivity or affect forest nutrient allocation and thus impact the maintenance of soil C stocks. Development of such datasets would then inform the limits of this ecosystem-climate feedback (enhanced N cycling and availability supporting primary production and soil inputs) and thus inform land-atmosphere carbon exchange models by providing a means of establishing such limits. Past modeling studies have called for such improvements to the accuracy with which C-N cycles and their feedbacks are simulated (Thomas et al. 2013), and thus better observational constraints on C-N cycling and its*

response to climate change (Meyerholt et al. 2020). We also recognize that many models include assumptions about organic matter degradation states and that information is propagated into model kinetic equations, and representations. With more information on C compounds there would be more opportunity for representing true stoichiometry and the relationships between C quality and reaction rates in the model frameworks (T. O'Meara and B. Bond-Lamberty, personal communication). We suggest that this index may be useful for assessing C quality in ecosystems of interest. These ideas have been briefly incorporated into the new conclusion section as suggested.

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