Dear Anonymous Referee #1:

We appreciate your thoughtful review of our paper. Each of your comments is apt and useful for improving our paper. Below we respond to each comment and indicate how we plan to revise the manuscript accordingly. For clarity, we have copied your comments in their entirety in blue italics, and followed each with our response in normal font directly below.

1. *Upon initial reading of the paper I got a little confused trying to sort out exactly what "pheo-bulk meant. I think now I understand it depends on the material being discussed, for example it could be either: the difference between _15Npheo of leaves and bulk _15N of leaves or the difference between _15Npheo of soil and bulk _15N of soil organic matter. But this is different from comparing the _15Npheo in soil/litter with _15Npheo and _15Nbulk of leaves. I'm not sure if anything really needs to be changed, but maybe a sentence of clarification somewhere might help?*

We appreciate having this confusion pointed out and will expand the isotopic notation subsection of the methods section to provide clarifying explanation. We only use "pheo-bulk" in the paper as a subscript for $^{15}\varepsilon_{pheo\text{-}bulk,}$ which we use to describe biosynthetic isotopic fractionation within whole leaf (bulk) tissue. It would therefore be more descriptive and perhaps reduce confusion to change this terms to $^{15}\varepsilon_{pheo\text{-}leaf}$ and $^{15}\varepsilon_{chl\text{-}bulk}$ to $^{15}\varepsilon_{chl\text{-}leaf.}$ We use the symbol ε to provide a direct comparison with previously reported values of isotopic fractionation between chlorophyll and whole leaves (Chikaraishi et al., 2005). We do discuss isotope differences between isolated compounds and bulk samples for other materials, but not using the epsilon notation. In those cases, we describe a difference in isotope values by simple subtraction, which is abbreviated with delta notation in Table 3.

2. *The methods for HPLC should be more specific. For example what does "variable ratio" mean? (p. 6 line 24). What is the advantage of using two HPLC steps? Was the sample divided in half, then each part passed through each HPLC method/column? Or was it successive, i.e. sample goes through column A and into B?*

We will expand the methods section for HPLC to provide clarity on these points. Variable ratio means that over the course of the run, the ratio of solvents flowing through the HPLC column changes. We will provide the details of how the percentages of solvents were varied throughout the run. We will clarify that sample fractions corresponding to peaks collected from the first HPLC column run were subsequently run through a second column.

3. *I strongly suggest the authors consider depositing their data into a data repository where it can be easily accessed by anyone, in keeping with global scientific trends of making more data open access.*

We agree to deposit our data into a data repository for open access by anyone. We will make our database publicly available by uploading to PANGAEA (https://www.pangaea.de/).

4. *Do the authors have any theories for why trees exhibit more positive "pheo-bulk of leaves compared to herbaceous plants? Cellulose accumulation is mentioned in the paper but I'm unclear how it's connected to nitrogen isotope values?*

We agree that our findings invite further discussion of why trees in our study exhibit greater intra-leaf isotopic fractionation between pheophytin and whole leaf tissue ($^{15}\varepsilon_{pheo\text{-}bulk}$) than plants. Differences in rates of growth or pathways of N compound synthesis, breakdown, and redistribution are processes that would have potential for N isotope fractionation. Due to their size and longevity, trees have different N storage and redistribution requirements than herbaceous plants. Although bulk leaf $\delta^{15}N$ is not observed to change on abscission (Kolb and Evans, 2002), seasonal or within-season breakdown and redistribution of foliar N compounds could involve N isotope fractionation that results in partitioning of N isotopes within leaf compounds During the growing season, chlorophyll is continually broken down and replaced. As we mention in the paper, because the energy needed to break the Mg-N bond is substantially smaller than the covalent bond, Mg loss from Chl would theoretically be expected to have little or no N isotope fractionation. However, in perennial plants with long lives such as the trees in our study, it is possible that this process happens many times, compounding expression of isotopic fractionation from this process. It is worth noting that others have also observed intra-leaf patterns in N isotope fractionation among different plant types that are not well understood. Chikaraishi et al. (2005) noted that $^{15}\varepsilon_{chl\text{-}bulk}$ of chlorophylls from C4 plants show much greater discrimination against 15N than do C3 plants, despite biosynthesis via the same pathway. We will remove mention of cellulose accumulation; while a distinguishing character of trees, cellulose contains no nitrogen and would not directly affect $\delta^{15}N$ values.

5. *I read another paper recently (Wang et al 2019, GRL) that used bulk N isotope values of black carbon deposited in lake sediments as a proxy for regional N availability over the last 10,000 years. I wonder if the _15Npheo proxy in soil could be coupled or compared with that method, both to further validate both proxies but also to study changes in N availability in more detail.*

We appreciate the encouragement to expand our discussion of how the $\delta^{15}N_{pheo}$ proxy could be applied to investigate N dynamics. We will add this to our discussion section. We see two key opportunities for application of a $\delta^{15}N_{pheo}$ to advance understanding of N dynamics here, both alone and in combination with other proxies such as the black carbon proxy used in Wang et al. 2019, GRL.

First, the ability to track changes in foliar ^{15}N over time gives insight into factors affecting $\delta^{15}N$ of plants, notably the availability of nitrogen. A time series of $\delta^{15}N_{pheo}$ covering periods of change in atmospheric pCO_2 could be obtained from an aggrading soil with dated, buried horizons such as in permafrost and used to evaluate the PNL hypothesis (and we have a paper on this in prep).

Second, comparison of the compound-specific $\delta^{15}N_{pheo}$ value with other proxy $\delta^{15}N$ values over the same time period would provide information on processes that cause them to deviate. Common sources of $\delta^{15}N$ proxy values are subaqueous sediment deposits such as from lakes, ungulate tooth enamel, and bulk wood, black carbon, or soil. Deviations in records of $\delta^{15}N$ of pheo and tooth enamel at a single site would highlight changes in factors affecting dietary fractionation, such as animal growth rate. Aquatic signals could be distinguished from terrestrial signals by comparing $\delta^{15}N_{pheo}$ from soil with $\delta^{15}N_{chl}$ of lake

sediment. $\delta^{15}N_{pheo}$ could validate a bulk proxy record such as obtained from wood or black carbon, or highlight diagenetic limitations of the record. In the case of bulk soil, combining records of $\delta^{15}N_{pheo}$ with $\delta^{15}N_{bulk}$ would provide information both on N availability to plants and dominant pathways of loss, hydrologic or gaseous, at a site, allowing for comparison of multiple N cycle dynamics over time.

Technical corrections

1. *Line 4 methods should be "factor"*

We apologize, but we cannot find what is referred to here and need to ask for clarification of the comment.

2. *Figure 3: is there a blue triangle where there shouldn't be in the 2500 mm precipitation category?*

The blue triangle is not aberrant, but we agree that it needs explanation. We will revise the figure legend to indicate that the blue trial corresponds to a sample from soil below 20 cm.

3. *Figure 5: Color code here instead of label? Some of the labels overlap and can be hard to read.*

We agree. We will add color coding and manually move labels that are overlapping.

4. *Table 2: what is "Py Chl a"? I could not find a definition.*

We will provide this definition. Py Chl a is Pyrochlorophyll a, which is likely produced from chlorophyll *a* during laboratory processing (Teng & Chen, 1999) and for this reason is combined with chlorophyll in reporting of compound abundance.

References

Chikaraishi, Y., Matsumoto, K., Ogawa, N. O., Suga, H., Kitazato, H., and Ohkouchi, N.: Hydrogen, carbon and nitrogen isotopic fractionations during chlorophyll biosynthesis in C3 higher plants, Phytochemistry, 66, 911-920, 10.1016/j.phytochem.2005.03.004, 2005.

Kolb, K. J., and Evans, R. D.: Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues, New Phytologist, 156, 57-64, 2002.

Teng, S.S. and Chen, B.H. Formation of pyrochlorophylls and their derivatives in spinach leaves during heating. Food Chemistry, 65:3, 1999.