

Anonymous Referee #4

Received and published: 2 November 2018

The authors infer nitrogen and carbon cycling dynamics from the nitrogen and carbon stable isotopes of soil and plant samples along an elevational gradient. The gradient in the Mt Kilimanjaro area has a number of variables, including water availability, plant type (C3 and C4) and changes to soils. There are also differences referred to as “ecosystems”, where the authors divide the altitudinal gradient into areas as disparate as a ‘maize field’ versus relatively undisturbed forests. The authors classify these ecosystems and have sufficient samples to examine relationships. The spatial scale of the study is admirable.

While there is much data here to examine relationships between habitat features and C and N stable isotopes, the relations are correlative. They also rely on inferring what is likely a dynamic process with underlying fluxes from static data. What the authors are relying on is that the isotopes integrate the processes with integrity.

We thank the reviewer for her/his positive comments. We also appreciate the criticism, which we address in bold font below.

There were several instances where I was concerned about the assumptions and the links the authors were making. First, fertilizers and pesticides could change the $\delta^{15}\text{N}$, leading to the wrong interpretation of $\delta^{15}\text{N}$ differences across ecosystems. Is there anything known about this potential artefact? Statements that then follow these N analyses such as “N cycles are tighter” (e.g. L 354) seem too strong.

We agree that the use of fertilizer and pesticides may pose a bias on the results and their subsequent interpretation. As explained in our answers to reviewer #3, we have clearly tagged and discussed those sites that have had external applications of fertilizers (both organic and mineral) as well as pesticides. We have also included information about the use and isotopic composition of fertilizer and pesticides in a dedicated section in the Supplementary Information, and included information on N-fixing trees.

We trust that the reader has now sufficient information to critically assess the limitations that the study contains on external nutrient additions.

The discussion on the N cycle as supported by soil $\delta^{15}\text{N}$ values, was also a criticism shared by reviewer #3. We also thank this reviewer for having raised this important aspect. Indeed, after considering the water concentrations of soil nitrate provided by Gütlein et al (2018), it appears that forest ecosystems have significant N losses through this pathway, which would go unnoticed if one relies exclusively on soil $\delta^{15}\text{N}$ values as was the case in the study by Zech et al (2011). Consequently, we have modified our statements regarding the open and close N cycles in the abstract, discussion and the conclusions.

Second, the a priori expectations for $\delta^{13}\text{C}$ patterns was also unclear to me. The paragraph starting L45 was confusing. C3 plants have lighter $\delta^{13}\text{C}$ values but water stress increases the value? How do we think these differences are integrated in Figure 2.

I don't have much in the way of minor edits, etc because I think these broader issues need to be addressed first.

The paragraph starting in Line 45 is a general introduction about the variation of $\delta^{13}\text{C}$ values on plants. In the referred paragraph we do state that C3 plants do show lighter $\delta^{13}\text{C}$ values than their C4 counterparts. The relative abundance of C3 and C4 plants greatly determines the $\delta^{13}\text{C}$ of a given ecosystem, which greatly explains the large variation exhibited by managed sites with mixed C3/C4 vegetation located at lower elevations.

Our sites have been categorized according to land use intensities (i.e. managed and semi-natural) following a similar classification used by Classen et al. (2015) and Schellenberger Costa et al. (2017), which employed factors as land use, vegetation structure, annual biomass removal, input of fertilizers and pesticides.

We see pertinent to reiterate (as it has been explained in the MS text), that all semi-natural sites are C₃-dominated ecosystems. If one just considers those ecosystems (nearly or exclusively) composed by C3 plants ($\delta^{13}\text{C}$ values $<-24\text{‰}$ ~ –semi-natural ecosystems occurring above 1,800 m.a.s.l.), the effect of increasing $\delta^{13}\text{C}$ values with altitude is quite noticeable (Fig. S2), and corresponds with a decreasing trend in MAP (Fig. S3 b). Fig. 2 shows the variation in $\delta^{13}\text{C}$ values of plants, litter and soil samples along the elevational and land use gradient. As such, the figure does not directly show the variation in $\delta^{13}\text{C}$ values with precipitation. Rather, this is shown in Fig. S3 b.

Finally, we would also like to state that it is abundantly clear that water deficits may cause the enrichment of ^{13}C in C₃ plants (Farquhar and Sharkey, 1982; Kohn, 2010; Körner et al., 1991). Therefore, we do not see any discrepancy with the referred introductory statement and our results.

Note: The MS text (and to a lesser extent Fig. 1) explain the distribution of precipitation along the elevation gradient “Maximum mean annual precipitation (MAP) of 2,552 mm occurs at an elevation of around 2,260 m a.s.l., decreasing towards lower as well as higher elevations, reaching 657 and 1,208 mm y⁻¹ at 871 and 4,550 m respectively (Table 1)”.

References:

- Classen, A., Peters, M. K., Kindeketa, W. J., Appelhans, T., Eardley, C. D., Gikungu, M. W., ... & Steffan-Dewenter, I. (2015). Temperature versus resource constraints: which factors determine bee diversity on Mount Kilimanjaro, Tanzania?. *Global Ecology and Biogeography*, 24(6), 642-652.
- Farquhar, G.D., Sharkey, T.D.: Stomatal Conductance and Photosynthesis. *Annu. Rev. Plant Physiol.* 33, 317–345. doi.org/10.1146/annurev.pp.33.060182.001533, 1982.
- Kohn, M.J. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate. *Proc. Natl. Acad. Sci.* 107, 19691–19695. doi.org/10.1073/pnas.1004933107, 2010.
- Körner, C., Farquhar, G.D., Wong, S.C.: Carbon Isotope Discrimination by Plants Follows Latitudinal and Altitudinal Trends. *Oecologia* 88, 30–40, 1991.
- Schellenberger Costa, D., Gerschlauser, F., Pabst, H., Kühnel, A., Huwe, B., Kiese, R., Kuzyakov, Y., Kleyer, M. and Kühn, I.: Community-weighted means and functional dispersion of plant functional traits along environmental gradients on Mount Kilimanjaro, *J. Veg. Sci.*, 28(4), 684–695, doi:10.1111/jvs.12542, 2017.