Anonymous Referee #1

Received and published: 11 November 2018

The authors have develped a good work about nitrogen and carbon cycling dynamics from the nitrogen and carbon stable isotopes of soil and plant samples along an elevational gradient. Due to the remote African's sites where the work has been carried out the data arise in a very important issue about limitation of N availability in ecosystems C sequestration. Methodologically the work is well developed and results a discussion have a good structure that facilitates the reading. I think more works are needed on the multifactorial analyses that implyies soil data, climatological data, and nitrogen and carbon stable isotopes of soil and plants.

We thank the reviewer for her/his positive comments. We provide our answers in bold font below.

I not totally sure about authors consideration of grasslands and savannas extensivelly managed and semi-natural ecosystems. I think a little bit information about this clasification would be added. However, authors have been there on field seeing the conditions.

The classification we use has been followed by previous research working on the same sites (e.g. Becker and Kuzyakov, 2018; Classen et al., 2015; Ensslin et al., 2015; Gerschlauer et al., 2016; Gütlein et al., 2018; Mganga et al., 2014), and agree with our observation in the field.

These references are on the MS reference list.

As a personal preference, I would like that sites on Lines 162, 166, would be changed by soils.

We replaced sites with soils as suggested, but we felt that the change worsened the reading of the sentences.

Finally, few minor typographics mistakes would be pointed out: Line 96 –> Kilimanjaro doesn't have capital letter.

Revised as suggested

Anonymous Referee #3

Received and published: 1 November 2018

Review of manuscript bg_2018_407: ”Stable carbon and nitrogen isotopic composition of leaves, litter, and soils of various tropical ecosystems along an elevation and land- use gradient at Mount Kilimanjaro, Tanzania” by Gerschlauer et al.

This paper describes the isotopical signature of soils and above ground material in 12 ecosystems at Kilimanjaro. The data obtained is based on a comprehensive sample collection and thus hold a great potential in describing isotopical differences among the ecosystems. And as an isotopical description of the ecosystems the study surely has fine value, but in order to draw some of the conclusions in the paper, my view is that additional data are needed to fully support those statements. In the general comments below I have tried to suggest some additional data, which the authors ought to include strengthening the paper. I advise the editor to ask the authors for a major revision of the manuscript.

We thank the reviewer for her/his comments and value their constructive nature. Our answers and comments are in bold font below.

General comments:

(1) The authors have a strong focus on using differences in 13C and 15N natural abundance to explain how the different ecosystems work. I really lack some information or estimates of biomass production and balances (both C and N) for the ecosystems. Both for C and N, the input and output of matter would have strong effects on the cycling of those elements, and thus this information is needed to understand/justify the conclusions of the paper.

For example, the authors talk about “tight N cycles” for some ecosystem, but 15N natural abundance cannot stand alone to justify such statement. There we need to include both N inputs and input form, and N removals. It is for example well known that animal manure would affect the 15N natural abundance of soil, and thus, if some of the present ecosystems have grazing animals or animal manure is used e.g. in the homegarden, then this would most likely affect the N signature of the soil. Likewise, for C, we would need to know the annual biomass production to really understand the different 13C natural abundances.

Therefore I ask that the authors in the revised manuscript give actual number or estimates of C and N input and output balances, specify any N fertilizer additions, and make use of this information to support the differences in isotopic signatures.

- We have followed the reviewer’s advice and have done our best to provide estimates for biomass production and decomposition rates for all the studied sites.

We have made use of relevant research that has been recently published and that assesses plant material decomposition using tea litterbags along the same elevational and land-use gradient (Becker and Kuzyakov, 2018). While we have used the normalized difference vegetation index (NDVI) calculated for these very sites by Röder et al. (2017) as a proxy for primary productivity (Kerr and Ostrovsky, 2003). These indexes provide relevant information on potential ecosystem productivity and decomposition, and are now shown in the new Fig. S1. While there are some estimates of aboveground litterfall for some of these ecosystems (Becker et al., 2015), there is an

obvious lack of belowground OM inputs, which is a highly significant aspect since they can be up to an order of magnitude larger than aboveground ones. The discussion below has been integrated within the body of the MS, and we include it here for completeness.

Both primary productivity and litter decomposition show a hump-shaped pattern with elevation that resembles that of precipitation. It is interesting to see the close match between the two variables along the elevation range, albeit this trend weakens slightly towards higher elevation sites. Optimum growth and decomposition conditions are shown between 1,800 and 2,500 m.a.s.l.. These locations correspond to low altitude forest ecosystems (Flm and Foc) that do not experience severe seasonal limitations in moisture or temperature as it is otherwise the case in lower as well as higher elevation systems that are moisture and temperature limited respectively (Becker and Kuzyakov, 2018).

It seems reasonable to assume that in the case of natural ecosystems there may be a steady state between SOM inputs and decomposition rates. This should be in contrast with the typically altered nutrient dynamics of disturbed systems, particularly those under agricultural management (Wang et al., 2018). We hypothesized that if carbon inputs and outputs were roughly in balance, then the difference in $\delta^{13}C$ values between plant material and topsoil would be smaller in undisturbed sites compared to managed or disturbed sites. Low fractionation factors in $\delta^{13}C$ are commonly reported between plant material and topsoils in natural systems mainly because of the relatively limited humification of recent organic matter prevalent in topsoils (Acton et al., 2013; Wang et al., 2018). The new Fig. 3 shows relatively small variations in $\delta^{13}C$ enrichment factors (> -1.25 ‰) both in undisturbed semi-natural and extensively managed sites along the elevational gradient, while managed and disturbed sites show higher and more variable $\delta^{13}C$ enrichment factors.

Elevation has a strong influence on the seasonal litterfall dynamics observed in Mt Kilimanjaro, and thus may have significant implications in the SOM cycling across the various ecosystems (Becker et al., 2015). These authors suggest that the large accumulation of particulate organic matter observed at the end of the dry season in low and mid altitude ecosystems may result in the increased mineralization of easily available substrates (Mganga and Kuzyakov, 2014) and nutrient leaching (Gütlein et al., 2018) during the wet season. Therefore, besides the systematic removal of plant biomass characteristic of agricultural systems, annual litterfall patterns may also explain the comparatively lower contents of C and N observed in the topsoils of these managed sites (Table 1). Furthermore, the relationship between $\delta^{13}C$ enrichment factors and soil C/N ratios shown in Fig. 3 may also be quite informative regarding SOM dynamics. As previously mentioned, soil C/N ratios provide a good indication of SOM decomposition processes, typically showing comparatively low values in managed and disturbed systems. These correspond well with sites having large enrichment factors (< -1.25 ‰; i.e. intensively managed and disturbed sites), which agree with the notion of altered SOM dynamics.

- We have also sought the best available information on fertilizer and pesticide use on those sites. We have now included information about the use and isotopic composition of fertilizer and pesticides in a dedicated section in the Supplementary Information.

We would like to acknowledge that contrary to agricultural research stations or purposely-established agricultural field trials, it is extremely difficult to provide reliable estimates of both fertilizers and pesticide rates used in small household farms in sub-Saharan Africa. This is because the actual use of these products is strongly dependent on both its availability in the local/regional market, the economic circumstances of each individual farmer, and individual perceptions about their use (Saiz and Albrecht, 2016). Indeed, a recent study specifically investigating the effect of land use on soil biochemical properties on nearby/comparable sites (Mganga et al., 2016) had to refer to coarse regional estimates of fertilization rates published two decades ago (Giller et al., 1998). Other relevant studies (e.g. Classen et al., 2015; Becker and Kuzyakov, 2018) refer to qualitative estimates compiled by a plant ecologist with long expertise in the region, but no actual amounts of fertilizers or pesticides are provided.

Being well aware of the difficulty to provide accurate numbers on mineral fertilizer and pesticide inputs, we have clearly tagged in the text those sites that receive any of those. These are the two intensively managed systems: Maize (Mai) fields and Coffee (Cof) plantations, and to a lesser extent the homegardens (Hom) sites. In the latter sites Gütlein et al. (2018) report that weed control is mainly done by hand, and the use of mineral or organic N-fertilizers is low or non-existent.

As mentioned earlier, Giller et al. (1998) reported an estimate of ca. 40 kg N ha− inorganic fertilizer use in the Kilimanjaro region. A more recent report (i.e. Senkoro et al., 2017) indicate a generic fertilizer use of 17 kg/ha/yr on a country basis, with about 12% of the national fertilizer share being used in the Kilimanjaro and Arusha regions. Urea (48% N) and diammonium phosphate (18% N) accounted for about half the total volume of fertilizer used in 2010. Nonetheless, the nitrogen isotopic signal of both fertilizers is ~0 ‰ (Bateman and Kelly, 2007), for which it will not provide a significant additional bias on the interpretation of soil $\delta^{15}N$ values. However, the addition of manure ($\delta^{15}N$ ~8 ‰) in Hom systems, albeit used in low quantities (Gütlein et al., 2018), may have well contributed to the high $\delta^{15}N$ values observed in this ecosystem (Fig. 4).

While reliable data on pesticide amounts cannot be provided, we show an indication of two of the most commonly used pesticides as this may serve as a ready reference in future studies. The actual value may strongly depend on the manufacturer, which as in the case of $\delta^{13}C$ can be quite different for glyphosate. Regardless of this, we suggest that the use of pesticides may not pose a strong bias in our isotopic results since their use is limited to intensively managed sites, and the actual isotopic values of pesticides work in the opposite direction to our data (Fig. 4a).

	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Glyphosate	-24 ; -34 [1]	-3.6 [2]
Atrazine	-28.9 ; -27.9 [3]	-0.2 ; -1.5 [3]

[1] Kujawinski, D. M., Wolbert, J. B., Zhang, L., Jochmann, M. A., Widory, D., Baran, N., & Schmidt, T. C. (2013). Carbon isotope ratio measurements of glyphosate and AMPA by liquid chromatography coupled to isotope ratio mass spectrometry. *Analytical and bioanalytical chemistry*, *405*(9), 2869-2878.

[2] Tavares, C. R. D. O., Bendassolli, J. A., Ribeiro, D. N., & Rossete, A. L. R. M. (2010). 15N-labeled glyphosate synthesis and its practical effectiveness. *Scientia Agricola*, *67*(1), 96-101

[3] Meyer, A. H., Penning, H., Lowag, H., & Elsner, M. (2008). Precise and accurate compound specific carbon and nitrogen isotope analysis of atrazine: critical role of combustion oven conditions. *Environmental science & technology*, *42*(21), 7757-7763.

- We have also included information on other ecosystem inputs in our response to a dedicated specific comment about section 2.1.

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

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Gütlein et al (2018). Impacts of climate and land use on N2O and CH4 fluxes from tropical ecosystems in the Mt. Kilimanjaro region, Tanzania. Glob. Change Biol. 24, 1239–1255.

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Röder, J., Detsch, F., Otte, I., Appelhans, T., Nauss, T., Peters, M. K., & Brandl, R. (2017). Heterogeneous patterns of abundance of epigeic arthropod taxa along a major elevation gradient. *Biotropica*, *49*(2), 217-228.

Saiz, G., and Albrecht, A. (2016). Methods for smallholder quantification of soil carbon stocks and stock changes. *In:* Rosenstock TS, Rufino MC, Butterbach-Bahl K, Wollenberg E, Richards M (eds) *Measurement methods Standard Assessment Of Agricultural Mitigation Potential And Livelihoods (SAMPLES)*. ISBN 978-3-319-29792-7. CGIAR Research Program on Climate Change, Agriculture and Food Security. pp 135-162.

Senkoro et al (2017). Optimizing fertilizer use within the context of integrated soil fertility management in Tanzania. *Fertilizer use optimization in Sub-Saharan Africa. CAB International, Nairobi, Kenya*, 176-192.

Wang, C., Houlton, B. Z., Liu, D., Hou, J., Cheng, W., & Bai, E. (2018). Stable isotopic constraints on global soil organic carbon turnover. ***Biogeosciences*****,** ***15*****(4), 987-995**

(2) In the abstract the authors end with a statement regarding "rising temperatures in a changing climate". When I read the manuscript "rising temperatures in a changing climate" is not really clear from the text – please help the reader to understand how this study can say something about "rising temperatures" – many of you ecosystems differ not only in temperature due to the elevation gradient, but also to e.g. management. Thus, I find it hard to directly understand how "rising temperatures" are covered, unless you can specify that the same ecosystem with similar management is studied at two or more points at the elevation gradient.

In fact, please thoroughly consider your statements regarding "temperature". For example in line 357 you state that "we suggest that . . .increasing temperatures in a changing climate may promote C and N losses" – come on folks isn't that common text book knowledge?

As mentioned by the reviewer, our study does not specifically assess the effect of rising temperatures on SOM dynamics. However, our data show strong relationships between temperature and variables directly related to SOM dynamics such as soil δ^{13}C, C, N and C/N ratios. These results agree well with recent findings by Becker and Kuzyakov (2018) who studied SOM decomposition dynamics at these very sites. An important finding revealed by that study is that of seasonal variation in temperature is a major controlling factor in litter decomposition. Their study shows that small seasonal variations in temperature observed at high elevation sites exert a strong effect on litter decomposition rates. Therefore, the authors argue that the projected increase in surface temperature may result in potentially large soil C losses at high elevation sites due to their strong temperature sensitivity to decomposition. This is normally expected since the temperature sensitivity of decomposition is generally higher at higher elevations and at low temperatures (Blagodatskaya et al., 2016; Davidson and Janssens, 2006).

We believe that the data obtained in our study reinforces such view. Please note we use the term 'suggest' to refer to this aspect. In any case, we are ready to remove this statement if the reviewer still has a concern with it.

Blagodatskaya, E., Blagodatsky, S., Khomyakov, N., Myachina, O., & Kuzyakov, Y. (2016). Temperature sensitivity and enzymatic mechanisms of soil organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro. ***Scientific Reports*****, 6, 22240.**

(3) The data from the 12 ecosystems are clustering with the six forest together and the other six ecosystems differing from them. I don't think that all of the statements and comparisons across such clustered data are fair. For example the 13C and 15N natural abundance in forest ecosystems are very alike in spite of quite different temperatures, precipitation, soil C and N contents (Fig. S2 and S3). This to me is interesting – why are they so similar in signature in spite of these differences?

I ask that the authors are more cautious in the data interpretation with such clustered data – as in please don't try to make "correlations across ecosystems", and put some words on where ecosystems have similar isotopic fingerprints. (And why do you forget about the C3 – C4 story in your discussion and presentation of the results?).

Ecosystems dominated by C3 vegetation, such montane tropical forests, usually show a relatively small increase in $\delta^{13}C$ values of about 1.2‰ per 1,000 m elevation (Körner et al., 1988; Bird et al., 1994). Such trend has been graphically depicted in Fig. S2 to allow for direct comparisons with our data. The text in the relevant section (4.1) has been significantly edited to improve the discussion of our results.

Connected to our response to the first comment, explaining the estimates of ecosystem productivity and decomposition, the new figure showing the relationship between $\delta^{13}C$ enrichment factors and soil C/N ratios and soil carbon contents (Fig. 3) further support the contrasting SOM dynamics between semi-natural ecosystems and intensively managed/disturbed systems.

A final comment on the similar $\delta^{13}C$ values in forest ecosystems: work conducted along a comparable elevation range by Bird et al. (1994) in Papua New Guinea shows a negative relationship between soil $\delta^{13}C$ corrected for altitude and SOC contents in C3-only vegetation systems, which roughly resembles our data and relies on similar explanations. Thus, we were not overly surprised with the relatively small variation in soil $\delta^{13}C$ values and the moderate range in SOC contents observed along the environmental conditions encompassed by these semi-natural forest ecosystems.

While it is widely accepted that soil $\delta^{15}N$ provides valuable insights about the N cycle in a given ecosystem, we agree with the reviewer that a number of factors including the nature and balance of N inputs and outputs may significantly affect its isotopic signal, thus rendering it not sufficient to undisputedly draw the conclusion about open and closed nitrogen cycles we had made in section 4.3 (and in the abstract). We do thank the reviewer for having brought this important aspect up. 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 $\delta^{15}N$ 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.

(4) The ”Helichrysum” ecosystem seems to confuse the authors (and thus also the readers of the manuscript). In one place (line 162-163) the sandy nature is used to ”unquestionably” explain soil C and N contents, at another place (line 247-249) lignin is the explaining factor, and in the correlation analysis (Fig. 4, Table S2) also temperature is strongly correlated to the cycling of C and N in this ecosystem. This is confusing, and here I further miss that the authors reflect on their studied ecosystems – the “Helichrysum” ecosystem is a sub-alpine system – where I would guess that temperature play a strong role, not only in C and N turnover processes, but also in biomass production. Thus, I ask the authors to be consistent in their explanation – and please give an estimate of the biomass production in the ecosystems, so that the reader gets a better picture of the production across the ecosystems.

Fig S1 shows that the *Helichrysum* is the only ecosystem where decomposition potential is higher than production. We agree with the reviewer that the limited productivity shown by this ecosystem is strongly influenced by its low temperature. We have amended the text describing general soil characteristics to incorporate such

fact and now reads: "The low temperatures and sandy nature of the *Helichrysum* sites play a strong role in their characteristically low productivity and moderate decomposition potentials (Table 1; Fig. S1), which unquestionably affects the comparatively low soil C and N contents of these alpine systems'.

The above discussion is specifically about soil C and N contents in *Helichrysum* sites. However, the lignin explanation focuses on $\delta^{13}C$ values and connects to the previous point (3) raised by the reviewer. The MS text reads: "Further variations in soil $\delta^{13}C$ values could also be related to the biochemical composition of the precursor biomass. For instance, herbaceous vegetation is pervasive at high elevations, and contains relatively low amounts of lignin – an organic compound characteristically depleted in ^{13}C (Benner et al., 1987). This may contribute to explain the higher $\delta^{13}C$ values observed in plant and soil materials in alpine ecosystems dominated by *Helichrysum* vegetation, compared to forest ecosystems at lower elevations (Fig. 2)".

(5) Table 1 give some basic information regarding the ecosystems. Among other the organic C content, which for the forest ecosystems are at 20-40%. This is quite high. Please clearly specify whether you sampled the O-layer or the upper mineral layer of those soils?

We sampled the upper mineral layer of the soils.

Specific comments:

- Title: I would say it is not tropical ecosystems all the way up Kilimanjaro, therefore I think you should consider removing "tropical" from the title.

Revised as suggested.

- In 2.1. Study sites, please include information regarding variables that can affect the C and N signatures. That could be input of N via biological N2 fixation or animal manure (or other fertilizer) and it could be C via biomass production. For example, was the agroforestry based on N2-fixing trees?

Connected to our response to the first comment, and notwithstanding the obvious practical limitations of a study of such scope and nature, we have now included relevant information on potential ecosystem productivity and decomposition. Moreover, admitting the challenge in providing accurate numbers on mineral fertilizer and pesticide inputs, we have clearly tagged in the text those sites that receive any. These are the two monocultures: Maize (Mai) fields and Coffee (Cof) plantations, and to a lesser extent the homegardens (Hom) sites. Extensively managed sites (i.e. Sav and Gra) receive varying amounts of organic inputs from grazing animals, but again, the actual rates are unknown.

The traditional agroforestry systems (Hom) maintain a forest-like structure consisting of indigenous forest species that includes *Albizia schimperi*, a tree that may potentially fix atmospheric N. This is one of the 5 most abundant species in 2 and 4 of the Hom and Cof sites respectively, making up less than 25% of the vegetation cover in all cases.

- In 2.2. Sampling and Analyses. Please make a statement on whether root fragments were visible in the sieved soil. And please in the discussion reflect upon whether unrecovered root material could have affected the soil isotopic signatures (e.g. by using the enrichment of leaves as a proxy for the enrichment of unrecovered roots).

We have added a specific statement in M&M that reads: "Soil was sieved to 2 mm with visible root fragments being further removed prior to grinding". Furthermore, following the reviewer's advice we have estimated the effect that the removal of visible sieved roots might have caused on soil isotopic values. We re-calculated soil isotope values by mass balance making the following assumptions. In addition to taking leaf isotope values as a proxy for roots as suggested by the reviewer, a non-conservative assumption was made about average root mass (< 2 mm) being ~5% of the total mass in the sample (w/w). This is above double the maximum value observed by Saiz et al (2012) for roots > 2mm contained in soil samples collected from contrasting tropical ecosystems.

Re-calculated soil $\delta^{13}C$ and $\delta^{15}N$ values under the assumptions referred above were on average 0.15 and 0.17‰ higher than the original soil isotopic values, which are even lower than the analytical error (0.2 ‰). We have added a specific mention to this in the discussion.

Saiz, G., Bird, M., Domingues, T., Schrodt, F., Schwarz, M., Feldpausch, T., Veenendaal, E., Djagbletey, G., Hien, F., Compaore, H., Diallo, A., Lloyd, J.: Variation in soil carbon stocks and their determinants across a precipitation gradient in West Africa. Global Change Biology 18, 1670-1683. doi:10.1111/j.1365-2486.2012.02657.x, 2012.

- Line 218-219. Please remove this sentence – it is not justified by the figure – there is too much clustering.

We have deleted this sentence.

- Figures and Table: Please keep the same order of the ecosystems all through, and if possible please add the abbreviations for the ecosystems to the legend inside the figure in Figure 1. Also please consider identifying the C3 and C4 dominate ecosystem when presenting 13C natural abundance data.

We have revised the order of appearance of sites. We have modified Table 1 making sure that all sites appear in the same order both in Figures and Tables. We have we have also included sites' abbreviations in Fig. 1 legend.

- Figure 5: I don't think I understand what I can learn from this figure. Please explain better or delete it.

We have now improved the discussion on this figure (now Fig. 6) in section 4.3.

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 d15N, leading to the wrong interpretation of d15 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 δ^{15}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 δ^{15}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 d13C patterns was also unclear to me. The paragraph starting L45 was confusing. C3 plants have lighter d13C 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 $\delta^{13}C$ values on plants. In the referred paragraph we do state that C3 plants do show lighter $\delta^{13}C$ values than their C4 counterparts. The relative abundance of C3 and C4 plants greatly determines the $\delta^{13}C$ 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 C_3-dominated ecosystems. If one just considers those ecosystems (nearly or exclusively) composed by C3 plants ($\delta^{13}C$ values <-24 ‰ ~ –semi-natural ecosystems occurring above 1,800 m.a.s.l.), the effect of increasing $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 C_3 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)".

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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 M ount K ilimanjaro, T anzania?. *Global Ecology and Biogeography*, *24*(6), 642-652.

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Stable carbon and nitrogen isotopic composition of leaves, litter, and soils of various ecosystems along an elevational and land-use gradient at Mount Kilimanjaro, Tanzania

Deleted: tropical

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Abstract

Variations in the stable isotopic composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of fresh leaves, litter and topsoils were used to characterize soil organic matter dynamics of twelve tropical ecosystems in the Mount Kilimanjaro region, Tanzania. We studied a total of 60 sites distributed along five individual elevational transects (860 – 4,550 m a.s.l.), which define a strong climatic and land use gradient encompassing semi-natural and managed ecosystems. The combined effects of contrasting environmental conditions, vegetation, soil, and management practices had a strong impact on the $\delta^{13}C$ and $\delta^{15}N$ values observed in the different ecosystems. The relative abundance of C_3 and C_4 plants greatly determined the $\delta^{13}C$ of a given ecosystem. In contrast, $\delta^{15}N$ values were largely controlled by land-use intensification and climatic conditions.

The large $\delta^{13}C$ enrichment factors ($\delta^{13}C_{litter} - \delta^{13}C_{soil}$) and low soil C/N ratios observed in managed and disturbed systems agree well with the notion of altered SOM dynamics. Besides the systematic removal of plant biomass characteristic of agricultural systems, annual litterfall patterns may also explain the comparatively lower contents of C and N observed in the topsoils of these intensively managed sites. Both $\delta^{15}N$ values and calculated $\delta^{15}N$-based enrichment factors ($\delta^{15}N_{litter} - \delta^{15}N_{soil}$) suggest tightest nitrogen cycling at high-elevation (>3,000 m a.s.l.) ecosystems, and more open nitrogen cycling both in grass-dominated and intensively managed cropping systems. However, claims about the nature of the N cycle (i.e. open/close) should not be made solely on the basis of soil $\delta^{15}N$ as other processes that barely discriminate against ^{15}N (i.e. soil nitrate leaching) have been shown to be quite significant in Mt Kilimanjaro's forest ecosystems. The negative correlation of $\delta^{15}N$ values with soil nitrogen content and the positive correlation with mean annual temperature suggest reduced

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mineralisation rates, and thus limited nitrogen availability, at least in high-elevation ecosystems. By contrast, intensively managed systems are characterized by lower soil nitrogen contents and warmer conditions, leading together with nitrogen fertilizer inputs to lower nitrogen retention, and thus, significantly higher soil $\delta^{15}N$ values. A simple function driven by soil nitrogen content and mean annual temperature explained 68 % of the variability in soil $\delta^{15}N$ values across all sites. Based on our results, we suggest that in addition to land use intensification, increasing temperatures in a changing climate may promote soil carbon and nitrogen losses, thus altering the otherwise stable soil organic matter dynamics of Mt. Kilimanjaro's forest ecosystems.

1 Introduction

Conversion of natural ecosystems to agriculture is a worldwide phenomenon, which is of particular significance in tropical regions where human population growth rates are currently the highest (FAO and JRC, 2012). Changes in climate and land-use significantly alter vegetation composition and biogeochemical cycles, causing a strong impact on carbon (C) and nitrogen (N) turnover and stocks (Smith et al., 2014). Tropical forest biomes are particularly relevant in this context, as they are significant C storages and N turnover hotspots (Bai et al., 2012; Hedin et al., 2009; Lewis et al., 2009; Pan et al., 2011; Vitousek, 1984). Considering the increasing pressure on natural land, it gets even more crucial to understand how anthropogenic interventions affect ecosystem C and N cycling, and gain better knowledge about the main drivers of nutrient cycling, and associated exchange processes with the atmosphere and hydrosphere in tropical environments.

Research exploiting the natural abundance of stable isotopes has proved quite suitable for investigating potential impacts of land-use and/or climate change on C and N cycling in terrestrial systems (Michener and Lajtha, 2007; Pannetieri et al., 2017; Saiz et al., 2015a). Variations in the stable isotopic composition of C ($\delta^{13}C$) and N ($\delta^{15}N$) in plants and soils are the result of fractionation processes occurring during ecosystem exchange of C and N. Thus, $\delta^{13}C$ and $\delta^{15}N$ can serve as valuable indicators about ecosystem state and provide useful insights on how these systems respond to biotic and abiotic factors (Dawson et al., 2002; Högberg, 1997; Ma et al., 2012; Pardo and Nadelhoffer, 2010; Peterson and Fry, 1987; Robinson, 2001).

Plants discriminate against $^{13}CO_2$ (carbon dioxide) during photosynthetic CO_2 fixation depending on plant metabolism (i.e. C_3 and C_4 photosynthetic pathways). Most tropical grasses typically employ the C_4 photosynthetic pathway ($\delta^{13}C$ values >-15 ‰), while trees and shrubs use the C_3 photosynthetic pathway ($\delta^{13}C$ values <-24 ‰) (Bird et al., 1994; Bird and Pousai, 1997; Cernusak et al., 2013; Farquhar et al., 1980). The distribution of C_3 and C_4 vegetation show clear patterns along elevational gradients, with increasing abundance of C_3 species towards high elevations (Bird et al., 1994; Körner et al., 1991; Tieszen et al., 1979). Environmental conditions such as water availability also exert a significant influence on isotopic discrimination during atmospheric CO_2 fixation. Accordingly, compared to optimal moisture conditions, water stress leads to enrichment of ^{13}C in C_3 plants (Farquhar and Sharkey, 1982), while this isotopic fractionation is less obvious or even absent in C_4 plants (Ma et al., 2012; Swap et al., 2004).

The soil organic matter (SOM) pool integrates the isotopic signature of the precursor biomass over different spatiotemporal scales (Saiz et al., 2015a). Variation in soil $\delta^{13}C$ values represents a valuable tool to better assess SOM dynamics, mineralisation processes, or reconstruct past fire regimes (Saiz et al., 2015a; Wynn and Bird, 2007). The $\delta^{13}C$ of SOM in a given ecosystem is greatly controlled by the relative abundance of C_3 and C_4 plants due to their contrasting C isotopic composition. Therefore, strong variations in soil $\delta^{13}C$ can also be used to identify sources of particulate organic matter as well as vegetation shifts such as woody thickening. However, fractionation effects associated to differential stabilisation of SOM compounds, microbial re-processing of SOM, soil physico-chemical characteristics, and the terrestrial Seuss effect preclude a straightforward interpretation of soil $\delta^{13}C$ values (Saiz et al., 2015a).

Plant and soil $\delta^{15}N$ relate to environmental and management conditions controlling N turnover, availability, and losses. $\delta^{15}N$ values of soils are generally more positive than those of vegetation due to the relatively large isotopic fractionation occurring during soil N transformations (Dawson et al., 2002). The N-cycle of a given ecosystem may be characterized as closed, if both efficient microbial N retention and absence of external N-inputs (e.g. atmospheric deposition and fertilizer additions) prevent substantial gaseous and/or leaching N-losses. In contrast, open ecosystem N-cycling is characterized by significant inputs and losses of N. On the one hand, gaseous N losses from soils are strongly depleted in ^{15}N due to the high fractionation factors associated to these processes (Denk et al., 2017). This results in high $\delta^{15}N$ values of the residual substrate, which consequently leaves less importance to impacts of external N additions (Robinson, 2001; Zech et al., 2011). On the other hand, N leaching seems to only discriminate slightly against ecosystem ^{15}N. According to Houlton and Bai (2009) $\delta^{15}N$ values of drained water agree well with those of soils across various natural ecosystems worldwide. Moreover, it is also important to consider that soil $\delta^{15}N$ may also be influenced by other factors including rooting depth, uptake of different N compounds, and symbiotic N_2-fixation (Nardoto et al., 2014). Variations in $\delta^{15}N$ values of plants and soils have been successfully applied to characterize N cycling across a large variety of ecosystems worldwide (Amundson et al., 2003; Booth et al., 2005; Craine et al., 2015a, 2015b; Martinelli et al., 1999; Nardoto et al., 2014). This includes research work that has particularly focused on the study of N-losses derived from land-use changes or intensification (Eshetu and Högberg, 2000; Piccolo et al., 1996; Zech et al., 2011).

Information on ecosystem C and N cycling is still scarce in many tropical ecosystems, particularly in remote regions of Africa (Abaker et al., 2016; 2018; Saiz et al., 2012; Townsend et al., 2011). Furthermore, feedbacks between C and N cycles such as limitations of N availability in ecosystem C sequestration and net primary productivity of tropical forest require urgent investigations (Gruber and Galloway, 2008; Zaehle, 2013). In such context, the Kilimanjaro region in Tanzania offers the rare possibility to study a broad range of tropical ecosystems across contrasting land-use management intensities and varying climatic conditions. This region hosts a large variety of semi-natural and managed ecosystems ass a result of the strong elevational and land-use gradient.

We hypothesized that (i) vegetation composition (C_3/C_4) is the main control for ecosystem δ^{13}C values, whereas (ii) δ^{15}N values are rather controlled by land use management and climatic conditions. The main aim of this study is to evaluate the potential of δ^{13}C and δ^{15}N values in plant and soil material to assess C and N cycling across a broad variety of semi-natural and managed ecosystems under varying climatic conditions.

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2 Materials and Methods

2.1 Study Sites

This study was conducted on the southern slopes of Mount (Mt.) Kilimanjaro (3.07° S, 37.35° E, 5,895 m a.s.l.) in North-East Tanzania. The climate is characterized by a bimodal precipitation pattern with a major rainy season between March and May, and the other peak between October and November. Recently, Appelhans et al. (2016) used a network of 52 meteorological stations strategically deployed in the Kilimanjaro region to measure air temperature and precipitation. They then used geo-statistical and machine-learning techniques for the gap filling of the recorded meteorological time series and their regionalization, which provides the means to calculate the meteorological data used for the complete set of sites (60) used in our work. Please refer to Appelhans et al. (2016) for more details. 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). Variations in air temperature are dominated by diurnal rather than seasonal patterns (Duane et al., 2008). Mean annual temperature (MAT) decreases with increasing elevation, ranging from 24.8 °C at 860 m to 3.5 °C at 4,550 m (Table 1).

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Five altitudinal transects ranging from 860 to 4,550 m a.s.l. were established along the mountain slopes. At each transect, twelve ecosystems occurring over a strong land use gradient encompassing intensively managed cropping systems and semi-natural stands were investigated. Hence, the total number of plots studied was 60 (5 transects x 12 ecosystems; Table 1 and Fig. 1). The cropping systems comprised multi-layer and multi-crop agroforestry homegardens (Hom), monoculture coffee plantations (Cof) with dispersed shading trees, and maize fields (Mai) subject to regular albeit moderate fertilizer and pesticide applications. Plant litter is regularly removed from Cof and Mai sites. Homegardens are manually ploughed, while combustion engine machinery is used for ploughing coffee plantations and maize fields. Coffee plantations are irrigated with drip irrigation systems. Both Hom and Cof sites still host indigenous forest trees that include *Albizia schimperi*, a species that may potentially fix atmospheric N. This is one of the 5 most abundant species in 2 and 4 of the Hom and Cof sites respectively, making up less than 25% of the vegetation cover in all cases. Grasslands (Gra) and savannas (Sav) are

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extensively managed by means of domestic grazing and occasional grass cutting, thus having significantly lower anthropogenic disturbances than cropping systems. Semi-natural ecosystems include several montane forest stands. These include lower montane (Flm), *Ocotea* (Foc), *Podocarpus* (Fpo), *Erica* (Fer), and alpine shrub vegetation *Helichrysum* (Hel). Even though lower montane forests are currently under protection they are still subject to sporadic illegal logging. In addition to sampling undisturbed forest ecosystems of *Ocotea* and *Podocarpus*, we purposely studied sites that had been affected by logging activities and fire events prior to the establishment of the Kilimanjaro National Park (Soini, 2005): *Ocotea* (Fod) and *Podocarpus* (Fpd) (Table 1). Erica forests represent Africa's highest forests in the subalpine zone. Higher above is the alpine zone, the realm of Helichrysum vegetation that is dominated by cushion plants and tussock grasses (Ensslin et al., 2015; Hemp, 2006). Potential ecosystem productivity and decomposition rates show a hump-shaped pattern resembling that of precipitation (Fig S1). It is interesting to see the close match between the two variables along the elevation range, albeit this trend weakens slightly towards higher elevation sites. Optimum growth and decomposition conditions are shown between 1,800 and 2,500 m.a.s.l.. These locations correspond to low altitude forest ecosystems (Flm and Foc) that do not experience severe seasonal limitations in moisture or temperature as it is otherwise the case in lower as well as higher elevation systems that are moisture and temperature limited respectively (Becker and Kuzyakov, 2018).

Detailed physico-chemical characteristics of the dominant soils are listed in Table 1. Soils in the Mt. Kilimanjaro region are mainly derived from volcanic rocks and ashes. The wide array of climatic conditions present along the elevational gradient influence soil genesis, which results in the occurrence of andosols at high elevations, and soils of more advanced genesis at lower elevations (e.g. nitosols) (Majule, 2003).

It is extremely difficult to provide reliable estimates of both fertilizers and pesticide rates used in small household farms in sub-Saharan Africa. This is because the actual use of these products is strongly dependent on both its availability in the local/regional market, the economic circumstances of each individual farmer, and individual perceptions about their use (Saiz and Albrecht, 2016). The only sites receiving fertilizer are the two monocultures: Maize (Mai) fields and Coffee (Cof) plantations, and to a lesser extent the homegardens (Hom) sites. In the latter sites Gütlein et al. (2018) report that weed control is mainly done by hand, and the use of mineral or organic N-fertilizers is low or non-existent. Extensively managed sites (i.e. Sav and Gra) receive varying amounts of organic inputs as a result of grazing activities, but again, their actual rates are unknown. A more detailed explanation on fertilizer and pesticides inputs used in the region is provided in the Supplementary Information.

2.2 Sampling and Analyses

Fieldwork took place in February and March in 2011 and 2012. Sampling was conducted on 50 x 50 m plots established at each of the 60 studied sites (12 ecosystems x 5 transects). Surface litter and mineral topsoil (0-5 cm) were sampled at five locations (four corners and the central point) at each plot. Additionally, fresh mature leaves of the five most abundant plant species covering 80% of total plant biomass per site were collected (Schellenberg Costa et al., 2017). All sampled materials (leaves, litter and soil) were air-dried until constant weight, and leaf material was subsequently oven-dried at 70 °C for 60 hours prior to grinding. Soil was sieved to 2 mm with visible root fragments being further removed prior to grinding with a mixer mill (MM200, Retsch, Haan Germany). Soil pH was determined with a pH meter (Multi Cal SenTix61, WTW, Weilheim, Germany) in a 0.01 M $CaCl_2$ solution, with a $CaCl_2$ to soil ratio of 2:1. Particle size distribution was determined gravimetrically using the pipette method (van Reeuwijk, 2002).

All soil, litter, and leaf samples were analysed with a dry combustion elemental analyzer (Costech International S.p.A., Milano, Italy) fitted with a zero-blank autosampler coupled to a ThermoFinnigan DeltaPlus-XL using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) for determination of abundance of elemental C and N, and their stable isotopic composition ($\delta^{13}C$, $\delta^{15}N$). Precisions (standard deviations) on internal standards for elemental C and N concentrations and stable isotopic compositions were better than 0.08 % and 0.2 ‰ respectively.

Natural ^{13}C or ^{15}N abundances are expressed in δ units according to Eq. (1):

$$\delta\,(‰) = (R_{sample} - R_{standard} / R_{standard}) \times 1000, \quad (1)$$

where R_{sample} denotes the ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample, and $R_{standard}$ denotes the ratios in Pee Dee Belemnite or atmospheric N_2 (international standards for C and N, respectively). The average values for the plant samples were weighted considering their relative abundance at each site. Individual values for soil, litter, and leaves were averaged for each plot.

In addition, both $\delta^{13}C$- and $\delta^{15}N$-based enrichment factors (ε) were calculated following Eqs. 2 and 3:

$$\varepsilon_C = \delta^{13}C_{litter} - \delta^{13}C_{soil}, \quad (2)$$

$$\varepsilon_N = \delta^{15}N_{litter} - \delta^{15}N_{soil}, \quad (3)$$

These were used as indicators for SOM decomposition dynamics and ecosystem N status (Garten et al., 2008; Mariotti et al., 1981). Note that we use the stable isotopic values values of litter material rather than fresh leaves from various species to calculate enrichment factors, since litter provides a more unbiased representation of the quality, quantity, and spatiotemporal dynamics of organic inputs entering the SOM pool (Saiz et al., 2015a).

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2.3 Statistical Analysis

Normal distribution of the data was confirmed with the Shapiro-Wilk test. One-way ANOVA was performed to test for significant differences between ecosystems, while Tukey's HSD was used as post hoc procedure to test for significant differences across sites ($P \leq 0.05$). Correlation analyses were performed to identify soil, foliar, and climatic variables influencing soil δ^{15}N values. Subsequently, a principal component analysis (PCA) was conducted to reveal relationships between the main variables affecting soil δ^{15}N values. The PCA was based on a correlation matrix including soil (C and N concentrations, C/N ratio, δ^{13}C, pH values, sand and clay contents) as well as climatic parameters (MAT and MAP). A stepwise multiple regression was used to identify the main driving parameters determining soil δ^{15}N across the elevational transect. All statistical analyses were conducted with R (version 3.2.2; R Core Team, 2015).

3 Results

3.1 General soil characteristics

Soil C and N contents were the highest in forest ecosystems and showed a decreasing trend towards managed sites (i.e. homegardens, grasslands, coffee and maize fields) (Table 1). Also, natural savannas and *Helichrysum* ecosystems had lower soil C and N values compared to forest ecosystems. The low temperatures and sandy nature of the *Helichrysum* sites play a strong role in their characteristically low productivity and moderate decomposition potentials (Table 1; Fig. S1), which unquestionably affects the comparatively low soil C and N contents of these alpine systems.

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An opposite trend to that of soil C and N abundance was observed for soil C/N ratios, whereby managed sites showed significantly lower values compared to those of semi-natural ecosystems. Soil pH values revealed acidic conditions at all sites, with the lowest values observed in forest sites having comparatively higher MAP (Table 1).

3.2 Variation of δ^{13}C values along the elevational and land-use gradient

There were large variations in δ^{13}C values along the elevational and land-use gradient, with distinct differences between managed and semi-natural ecosystems (Fig. 2). Compared to soils and litter, leaves invariably showed the lowest δ^{13}C values in all the studied ecosystems, with the exception of grasslands and savannas that exhibited lower soil δ^{13}C values than plant material.

The δ^{13}C values of semi-natural ecosystems ranged between -32.8 and -24.1 ‰ (mean ± SE: soil -26.0 ± 0.2 ‰; litter -27.2 ± 0.2 ‰; leaves -29.3 ± 0.3 ‰), showing a progressive reduction with decreasing elevation (i.e. from 4.500 to 1,750 m a.s.l.; Fig. S2). The variation in δ^{13}C values was much higher (-29.7 to -13.3 ‰) in managed ecosystems located at lower elevations (i.e. between 860 and 1,750 m a.s.l.; Fig. S2). The highest δ^{13}C values were observed in C$_4$-dominated ecosystems (i.e. savannas, maize fields, and grasslands; soil -16.8 ± 0.6 ‰, litter -19.3 ± 0.8 ‰, leaves -18.8 ± 1.1 ‰); while lower δ^{13}C values were obtained for coffee plantations and homegardens (soil -24.8 ± 0.5 ‰, litter -27.2 ± 0.4 ‰, leaves -27.3 ± 0.4 ‰). Coffee plantations showed a slight influence of C$_4$ vegetation in the soil data as a result of grasses growing between the rows of coffee plants. No significant variations were observed between δ^{13}C values of soils and those of litter and leaves in the

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ecosystems with predominance of C_4 vegetation (savannas, maize fields and grasslands). Exploratory data analyses revealed that in most cases, soil, litter, leaf, and climatic variables cross-correlated with each other (Table S1).

Figure 3 shows relatively small variations in δ^{13}C enrichment factors (> -1.25 ‰) both in undisturbed semi-natural and extensively managed sites along the elevational gradient, while managed and disturbed sites show higher and more variable δ^{13}C enrichment factors.

3.3 Variation of δ^{15}N values along the elevational and land-use gradient

Significantly higher δ^{15}N values were observed for all sampled materials in the intensively managed (cropping) systems compared to semi-natural and grass-dominated ecosystems (Fig. 4a). The δ^{15}N values for managed systems ranged between -2.6 and 7.8 ‰ (mean ± SE: soil 5.6 ± 0.3 ‰, litter 1.7 ± 0.5 ‰, leaves 2.0 ± 0.5 ‰). By contrast, semi-natural ecosystems had considerably lower δ^{15}N values, which ranged from -5.0 to 3.6 ‰ (soil 1.5 ± 0.2 ‰, litter -2.1 ± 0.2 ‰, leaves -1.3 ± 0.3 ‰). Soil δ^{15}N values were significantly higher than those of leaves and litter across all the ecosystems studied, with the only exception of agroforestry homegardens (Fig. 4a). δ^{15}N values of leaves and litter did not show significant differences within any given ecosystem.

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Calculated δ^{15}N-based enrichment factors showed high variability across all ecosystems with values ranging from -7.5 to -1.6 ‰ (Fig. 4b). A differentiation between managed and natural ecosystems was less clear than for δ^{15}N values. The most negative enrichment factors (< -4.0 ‰) were observed for *Helichrysum, Erica, Podocarpus* disturbed, and grass-dominated ecosystems (savannas and grasslands). These enrichment factors were significantly less negative for montane forests at lower elevations (*Podocarpus*, *Ocotea* and lower montane) and intensively managed (cropping) systems (i.e. homegarden, coffee, and maize; Fig. 4b).

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3.4 Impacts of soil and climatic variables on soil δ^{15}N values

Two principal components (PC) explained 78.3 % of the total soil δ^{15}N variation (Fig. 5). The first component explained 55.8 % of the variability, and included soil chemistry and climatic variables (soil C and N concentrations, soil C/N ratio, soil pH, soil δ^{13}C, MAP and MAT). Highly significant correlations ($P < 0.001$) were obtained between PC 1 and the above factors (r = 0.93, 0.93, 0.61, -0.87, -0.76, 0.87, and -0.63, respectively; Table S2). The second component explained an additional 22.5 % of soil δ^{15}N variability and included soil texture (clay and sand contents) and MAT. These variables were highly correlated with PC 2 (r = -0.84, 0.82, and -0.65; Table S2). The principal component bi-plot showed a strong grouping between managed and semi-natural ecosystems (Fig. 5). Managed sites clustered around MAT, soil δ^{13}C, and soil pH, while C_4-dominated ecosystems (grassland, savannas, and maize fields) were preferentially influenced by the latter two variables.

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In contrast, semi-natural montane forest ecosystems, rather grouped around soil chemical properties such as C and N contents, C/N ratio, as well as MAP, while alpine *Helichrysum* ecosystems clustered around soil sand content.

In addition to PCA, multiple regression analyses were performed using a stepwise procedure that identified soil N content and MAT as the main driving variables explaining the variation in soil δ^{15}N. A paraboloid model explained 68 % of this variability ($P < 0.05$; Fig. 6). The combination of relatively high soil N contents (1 to 3 %), and low MAT (up to 14 °C), invariably corresponded to low soil δ^{15}N values (< 2 ‰) characteristic of semi-natural ecosystems. Conversely, the relatively high soil δ^{15}N values (> 2 ‰) observed in managed ecosystems corresponded to low soil N contents (<1 %) and comparatively high MAT (17 to 25 °C).

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The relationship between soil δ^{15}N values and climatic and edaphic variables provided valuable information about potentially different SOM dynamics in the various ecosystems studied, with data showing a clear differentiation between semi-natural and managed ecosystems (Fig. S4). The former is characterized by comparatively higher C/N ratios and lower δ^{15}N values (averaging 15.5 and 1.5 ‰ respectively), while the latter showed lower C/N ratios and higher soil δ^{15}N values (averaging 11.9 and 3.5 ‰ respectively). Managed ecosystems further grouped into intensively cropped (homegardens, maize fields, and coffee plantations) and extensively managed grass-dominated ecosystems (savannas and grasslands).

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4 Discussion

4.1 Factors influencing the variation of δ^{13}C values along the elevational and land-use gradient

The δ^{13}C values of leaves in C_3-dominated (semi-natural) ecosystems in Mt. Kilimanjaro increased with elevation (Figs. 1 and S2), which is in agreement with findings from other mountainous ecosystems in the tropics, Europe, and North America (Bird et al., 1994; Körner et al., 1991; Ortiz et al., 2016; Zhou et al., 2011; Zhu et al., 2009). The wider scatter of δ^{13}C values observed in leaves relative to soils is most certainly due to the inherently large (inter- and intra- specific) variability of δ^{13}C in plants (Bird et al., 1994). Different tissues within the plant can present widely divergent δ^{13}C values as a result of fractionation processes associated with the C compounds involved in their construction (Dawson et al., 2002). Moreover, other factors including light intensity, humidity, and the re-utilization of previously respired low ^{13}C-CO_2 within the canopy may further contribute to the variability of δ^{13}C in leaf tissues (Ometto et al., 2006; van der Merwe and Medina, 1989).

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While fractionation effects preclude a straightforward interpretation of δ^{13}C of SOM, this variable provides an integrated measure of the isotopic composition of the precursor biomass at the ecosystem level (Bird et al., 2004; Saiz et al., 2015a). Mass balance calculations that assume (i) 5% (w/w) average root mass (< 2 mm) in soil samples, and (ii) leaves having similar isotopic signals as roots, show that the removal of visible sieved roots might cause a very small effect on soil isotopic values. This would amount to values ~0.15‰ higher than the original soil isotopic values, with such discrepancy being even smaller if root samples were considered having values 0.5-1‰ higher than leaves as is commonly reported in the literature (calculations not shown). Besides the natural variability of soil δ^{13}C values observed in C$_3$-dominated semi-natural ecosystems, there were distinct patterns in δ^{13}C values of soil samples collected in extensively managed, low-elevation ecosystems where woody and grass vegetation coexist (i.e. grasslands and savannas), which indicate the strong influence exerted by C$_4$ vegetation on the C isotopic composition of all sampled materials (Fig. 2). The results obtained in semi-natural ecosystems at Mt. Kilimanjaro fit well within the interpretative framework for elevational soil δ^{13}C data proposed by Bird et al. (1994). These authors suggest that besides temperature and atmospheric pressure, other primary factors influencing soil δ^{13}C values are the age and degree of decomposition of SOM, as well as variables related to the characteristics of the canopy, including the proportion of respired CO$_2$ that is recycled during photosynthesis, the relative contribution of leaf and woody litter to SOM, and soil moisture.

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Besides the factors explained above, soil δ^{13}C values are strongly influenced by the balance between ecosystem C inputs and outputs. It seems reasonable to assume that in the case of natural ecosystems there may be a steady state between SOM inputs and decomposition rates. This should be in contrast with the typically altered nutrient dynamics of disturbed systems, particularly those under agricultural management (Wang et al., 2018). Low fractionation factors in δ^{13}C are commonly reported between plant material and topsoils in natural systems mainly because of the relatively limited humification of recent organic matter prevalent in topsoils (Acton et al., 2013; Wang et al., 2018). Thus, we hypothesized that if C inputs and outputs were roughly in balance, then the difference in δ^{13}C values between plant material and topsoil would be smaller in undisturbed sites compared to managed or disturbed sites. The results shown in Fig. 3 agree well with this notion.

Soil δ^{13}C values decreased with increasing MAP and decreasing MAT, which also corresponded with higher SOC contents (Fig. S3). This suggests that the relatively cooler and wetter conditions of high elevation semi-natural forest ecosystems (i.e. Foc, Fpo) promote the accumulation of SOM, which is similar to previous findings of work conducted along elevational gradients (Bird et al., 1994; Kohn, 2010). Compared to high-elevation locations, the climatic conditions of mid-elevation ecosystems are more favourable for the activities of SOM decomposers, as these sites are consistently warmer and drier than the characteristically cool and occasionally waterlogged high-altitude ecosystems (Fig. S1; Becker and Kuzyakov, 2018; Borken and Matzner, 2009; Garten et al., 2009; Kirschbaum, 1995; Leirós et al., 1999). The comparatively high soil δ^{13}C values observed in the disturbed *Podocarpus* (Fpd) and *Erica* forest (Fer) plots may have been partly caused by recurrent fire events (Hemp, 2005) leading to reduced SOC contents and higher C/N ratios (Saiz et al., 2015a). Further variations in soil δ^{13}C values could also be related to the biochemical composition of the precursor biomass. For instance, herbaceous vegetation is pervasive at high elevations, and contains relatively low amounts of lignin – an organic compound characteristically depleted in ^{13}C (Benner et al., 1987). This may contribute to explain the higher δ^{13}C values observed in

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plant and soil materials in alpine ecosystems dominated by *Helichrysum* vegetation, compared to forest ecosystems at lower elevations (Fig. 2).

Elevation also has a strong influence on the seasonal litterfall dynamics observed in Mt Kilimanjaro, and thus may have significant implications in the SOM cycling across the various ecosystems (Becker et al., 2015). These authors suggest that the large accumulation of particulate organic matter observed at the end of the dry season in low and mid altitude ecosystems may result in the increased mineralization of easily available substrates (Mganga and Kuzyakov, 2014) and nutrient leaching (Gütlein et al., 2018) during the following wet season. Agricultural practices such as the removal of biomass or ploughing deplete SOM, particularly in the intensively managed systems (i.e. maize, homegardens and coffee plantations), thus leading to lower SOC contents and C/N ratios, and slightly higher soil $\delta^{13}C$ values than those observed in semi-natural ecosystems at comparable elevations (e.g. lower montane forests; Fig. S3). Indeed, the relationship between $\delta^{13}C$ enrichment factors and soil C/N ratios shown in Fig. 3 is quite informative regarding SOM dynamics. As previously mentioned, soil C/N ratios provide a good indication of SOM decomposition processes, typically showing comparatively low values in managed and disturbed systems. These correspond well with sites having large enrichment factors (< -1.25 ‰; i.e. intensively managed and disturbed sites), which agree with the notion of altered SOM dynamics. Therefore, besides the systematic removal of plant biomass characteristic of agricultural systems, annual litterfall patterns may also explain the comparatively lower contents of C and N observed in the topsoils of intensively managed sites (Table 1; Figs. S3, S4). Moreover, low-elevation ecosystems contain a variable mixture of C$_3$ and C$_4$ vegetation, which have been shown to have differential mineralization dynamics as demonstrated by incubation experiments (Wynn and Bird, 2007), and field-based research (Saiz et al., 2015a).

Our data show strong relationships between temperature and variables directly related to SOM dynamics such as soil $\delta^{13}C$, C, N and C/N ratios (Table S1). These results agree well with recent findings by Becker and Kuzyakov (2018) who studied SOM decomposition dynamics at these very sites. An important finding revealed by that study is that of seasonal variation in temperature being a major factor controlling litter decomposition. Their study shows that small seasonal variations in temperature observed at high elevation sites exert a strong effect on litter decomposition rates. Therefore, the authors argue that the projected increase in surface temperature may result in potentially large soil C losses at these sites due to the comparatively strong temperature sensitivity to decomposition that is commonly observed at low temperatures and at high elevations sites (Blagodatskaya et al., 2016).

Savannas and grasslands are subject to recurrent fire events, and thus the soils of these ecosystems may potentially contain significant amounts of fire-derived (pyrogenic) C (Saiz et al., 2015b). This can be partly demonstrated by the higher soil C/N ratios observed in these ecosystems compared to C$_4$-dominated agricultural systems protected from fire (e.g. maize plantations; Fig. S3d). Moreover, the $\delta^{13}C$ values of soils in grasslands and savannas were lower than those of leaves, which

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may be due to the savanna isotope disequilibrium effect (SIDE) (Bird and Pousai, 1997; Saiz et al., 2015b). The latter concept explains the difference in C isotopic composition between the precursor vegetation and pyrogenic C compounds produced during the combustion of biomass. Saiz et al. (2015b) have demonstrated that savanna fires produce pyrogenic C that is relatively ^{13}C depleted with respect to the precursor biomass. Furthermore, the combustion of C_4 vegetation produces finer pyrogenic C particles than woody biomass, resulting in the preferential export of grass-derived pyrogenic particles from the site of burning, which further enhances the depletion of ^{13}C in these soils (Saiz et al., 2018).

4.2 Variation of δ^{15}N values along the elevational and land-use gradient

The δ^{15}N values of leaves, litter, and topsoil presented here (Fig. 4a) agree well with the range of data reported from earlier investigations in the same study region (Amundson et al., 2003; Zech et al., 2011), but with our study involving more ecosystems, replicate sites and a far larger spatial sampling domain. Overall, the δ^{15}N values for montane tropical forest ecosystems in Mt. Kilimanjaro are considerably lower than the mean values reported for a broad variety of tropical lowland forests worldwide (soil values ranging from 3 to 14 ‰; de Freitas et al., 2015; Martinelli et al., 1999; Nardoto et al., 2014; Piccolo et al., 1996; Sotta et al., 2008). Rather, the δ^{15}N values observed in the montane forests investigated are in the same range of temperate forest ecosystems reported in a comprehensive literature review by Martinelli et al. (1999). These authors argue that, compared to tropical lowland forests, the lower δ^{15}N values of temperate and montane tropical forests result from their lower N availability and thus lower ecosystem N losses. However, this hypothesis may not completely hold for the montane forest ecosystems of our study, since Gütlein et al. (2018) reported elevated soil NO_3^- and DON concentrations at deep soil solution (80 cm) and significant nitrogen leaching rates of 10 - 15 kg N ha^{-1} y^{-1}. The relatively low δ^{15}N-based enrichment factors observed in the lower montane, *Ocotea* and undisturbed *Podocarpus* forest (Fig. 4b) were probably due to the prevalence of biological di-nitrogen fixation (BNF) at these ecosystems. The assumption of significant BNF is supported by leaf δ^{15}N values close to 0 ‰ (Fig. 4a) and is in line with previous works (Craine et al., 2015a; Nardoto et al., 2014; Robinson, 2001). Furthermore, sporadic measurements of N-compounds in rainfall and throughfall conducted at our forest sites showed substantial input of N via atmospheric deposition, which may be in the order of N leaching losses (unpublished results). This agrees well with findings from Bauters et al. (2018) reporting 18 kg N ha^{-1} y^{-1} N inputs via wet deposition into tropical forests of the Congo Basin, which are predominantly derived from biomass burning and long-range atmospheric transport. High N inputs into these forest ecosystems are likely to be in a similar range as N outputs (prevailed by leaching losses particularly where MAP is highest; Gütlein et al., 2018), and therefore, they would not translate to strong effects on ecosystem δ^{15}N values. The significantly more negative enrichment factors observed in the disturbed *Podocarpus* and *Erica* forests (Fig. 4b) may be related to past fire events (Hemp, 2005; Zech et al., 2011). Burning of vegetation may

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cause losses of ^{15}N-depleted NO$_{\chi}$ gas and N leachate, resulting in higher soil δ^{15}N values, thus producing variations in δ^{15}N-based enrichment factors (Zech et al., 2011).

Previous studies have shown that δ^{15}N values generally increase with land-use intensification (Martinelli et al., 1999; Stevenson et al., 2010), which corresponds well with the more positive δ^{15}N values observed in the intensively managed agricultural systems occurring at the mountain's foot slope (Fig. 4a). Indeed, agronomic practices such as fertilization, removal of plant material after harvest, or ploughing, are factors known to affect N turnover processes that strongly affect δ^{15}N values (Bedard-Haughn et al., 2003; Saiz et al., 2016). However, our values are in the lower range of published data for other land-use gradients (Aranibar et al., 2008; Eshetu and Högberg, 2000; Traoré et al., 2015), and may partly be the result of comparably low to moderate organic and inorganic N fertilization rates currently applied in the region (anecdotal evidence gathered by the authors and SI). Additionally, the nitrogen isotopic signal of mineral fertilizers commonly used in the region is ~0 ‰ (Bateman and Kelly, 2007), and thus, it may not exert a significant additional bias on the interpretation of soil δ^{15}N values. However, the addition of manure (δ^{15}N ~8 ‰) in Hom systems, albeit used in low quantities (Gütlein et al., 2018), may have well contributed to the high δ^{15}N values observed in this ecosystem (Fig. 4). Also, we suggest that the use of pesticides may not pose a strong bias in our isotopic results since their use is limited to intensively managed sites, and the actual isotopic values of pesticides work in the opposite direction to the observed data (Fig. 4; SI).

Compared to other low-elevation managed stands such as homegardens and coffee plantations, the higher δ^{15}N-based enrichment factors observed in maize fields and in grass-dominated ecosystems (grasslands and savannas) (Fig. 4b) may be related to both the organic inputs resultant from grazing activities and the influence of C$_4$ vegetation. Both Aranibar et al. (2008) and Wang et al. (2010) have suggested that variations in δ^{15}N values within a given ecosystem could be due to C$_3$ and C$_4$ plants preferentially absorbing chemical forms of N with differing ^{15}N abundances. Moreover, recurrent fires characteristic of tropical grasslands and savannas may have also influenced their comparatively high soil δ^{15}N, causing the relatively high δ^{15}N-based enrichment factors.

4.3 Factors controlling soil δ^{15}N along the elevational and land-use gradient

The strong controlling effects exerted by climatic and edaphic factors on soil δ^{15}N values agree well with numerous previous works (Amundson et al., 2003; Conen et al., 2013; Eshetu and Högberg, 2000; Martinelli et al., 1999; Stevenson et al., 2010). The principal component analysis of factors controlling soil δ^{15}N revealed a strong clustering between managed and semi-natural ecosystems (Fig. 5), which was also reflected in the multiple regression analysis and graphical representation depicting soil δ^{15}N as a function of soil N concentration and MAT (Fig. 6). Semi-natural ecosystems were characterized by relatively low soil δ^{15}N values, and occurred across a broad range of soil N contents in locations with low to medium MAT. By contrast, intensively managed ecosystems had higher soil δ^{15}N values and corresponded to locations with low soil N

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contents and high MAT. The negative correlation of δ^{15}N values with soil nitrogen content and the positive correlation with mean annual temperature suggest reduced mineralisation rates, and thus limited nitrogen availability, at least in high-elevation ecosystems.

The sharp contrast observed both in soil C/N ratios and δ^{15}N values between managed and semi-natural ecosystems offers additional useful information about their potentially contrasting SOM dynamics (Fig. S4d). Intensively managed sites consistently showed low soil C/N ratios and high soil δ^{15}N values, which may initially suggest a more open N cycle and potentially greater N losses as reported by Gerschlauer et al. (2016) for some of these ecosystems. This may due to C-limitation of heterotrophic microbial N retention under low C/N ratios (Butterbach-Bahl and Dannenmann, 2012). However, nitrate leaching is quite a relevant process that discriminates only slightly against ^{15}N (Denk et al., 2017), which may confound the interpretation of soil δ^{15}N values. Indeed, Gütlein et al. (2018) have recently shown that nitrate leaching may be quite significant in Mt Kilimanjaro's semi-natural forests. Therefore, at least in these ecosystems, claims about the nature of the N cycle (i.e. open/close) should not be made solely on the basis of soil δ^{15}N.

Grass-dominated ecosystems (grasslands and savannas) were noticeably different to the intensively managed croplands, as demonstrated by the higher soil C/N ratios and lower soil δ^{15}N of the former, which suggest a lower degree of decomposition of organic matter and potentially lower N turnover rates (Saiz et al., 2016). Within the intensively managed sites, the stands under maize cultivation show an interesting case of enhanced SOM dynamics. These sites are under an intensive management regime that involves the removal of aboveground vegetation after harvest. This fact combined with the faster decomposition rates reported for C_4-derived SOM (Saiz et al., 2015a; 2016; Wynn and Bird, 2007) may invariably lead to their characteristically low SOC and N contents (Table 1; Figs. S3, S4). Furthermore, low soil C/N ratios have been reported to enhance gaseous losses in semi-arid systems, which leads to increased soil δ^{15}N values (Aranibar et al., 2004) and may explain why maize stands showed the highest soil δ^{15}N values of all the land uses studied.

Semi-natural ecosystems showed rather high soil C/N ratios and low soil δ^{15}N values compared to managed sites (Fig. S4d). The more humid and cooler conditions prevalent in forest ecosystems may limit decomposition processes, thereby contributing significantly to their higher SOM abundance (Table 1). A small variation range in soil δ^{15}N values was also reported by Zech et al (2011) for semi-natural ecosystems (Foc and Fpo) when working along the same land-use and elevation gradient. Like us, these authors also observed a strong significant correlation of soil δ^{15}N with MAT, but not with MAP (Table S1). Additionally, site-specific soil characteristics, and the structural composition of vegetation have a strong influence on ecosystem nutrient dynamics (Saiz et al., 2012; 2015a). Ecosystem disturbances (e.g. fire, selective logging, etc.) cause changes in vegetation cover that affect SOM cycling and may translate into variations in soil C/N ratios (Saiz et al., 2016). Both *Ocotea* and *Podocarpus* forests contain disturbed (Fod, Fpd) and undisturbed stands (Foc, Fpo), though only

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the *Podocarpus* ecosystems allow for a general overview of disturbance impacts on SOM-related properties. While changes in the isotopic composition of C and N were not significant, soil C/N ratios were heavily influenced by disturbance (Fig. S4). Compared to non-disturbed sites, the lower C and N contents observed in the soil of disturbed ecosystems indicate reduced OM inputs to the soil and/or enhanced decomposition of SOM (Table 1). The higher soil C/N ratios observed in the *Podocarpus* disturbed and *Erica* forests may well be the result of fire, which may preferentially promote N losses while accruing relatively recalcitrant C forms (i.e. pyrogenic C). Woody biomass combustion produces pyrogenic C that accumulates preferentially close to the site of production (Saiz et al., 2018), thus likely contributing to the higher soil C/N ratios observed at these disturbed ecosystems. The lowest soil C/N ratios among all semi-natural ecosystems were observed at the alpine *Helichrysum* sites, which may relate to their characteristically sparse vegetation and extremely low MAT. Under such circumstances soil development, biomass inputs, decomposition processes, and thus, soil N turnover may be strongly limited, as it was confirmed by a recent study conducted at one of these sites (Gütlein et al., 2017).

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5 Conclusions

The variations in δ^{13}C and δ^{15}N values combined with interpretation of other indices such as δ^{13}C- and δ^{15}N-based enrichment factors and soil C/N ratios, enabled a qualitative characterisation of regional differences in C and N dynamics as affected by vegetation characteristics, environmental conditions, and management activities.

Our data show that SOM contents are higher in cold and wet high-elevation ecosystems than at low-elevation managed sites. Management practices such as tillage, harvest, and vegetation burning promote the loss of OM, with SOM decomposition being further enhanced by the warm and moderately wet conditions of the mountain's foot slope. Based on our results, we suggest that besides management, increasing temperatures in a changing climate may promote C and N losses, thus altering the otherwise stable SOM dynamics of Mt. Kilimanjaro's forest ecosystems. Moreover, the current situation of low N inputs in managed systems of sub-Saharan Africa is likely to change, since national efforts aim to increase fertilizer use are currently <10% of recommended rates (Hickman et al., 2014). Therefore, our data may also be valuable as a generic reference for low-elevation tropical agrosystems managed under low N inputs, while it may also allow the monitoring of expected changes in agricultural management, and associated impacts on ecosystem N cycle through the study of the variation in δ^{15}N values.

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In addition to climatic and edaphic factors, δ^{15}N values of plant and soil material can largely depend on both the amount and δ^{15}N signal of atmospheric deposition and BNF, which highlights the importance of conducting additional measurements of site specific N cycling, when comparing ecosystem δ^{15}N values across different biomes and regions. The combination of qualitative isotope natural abundance studies at a large number of sites (this study) with more elaborated quantitative process studies using enriched isotope labelling and N losses on a lower number of selected sites represent an ideal approach to characterize ecosystem C and N cycling of the larger Mt. Kilimanjaro region with its diverse ecosystems, climate, and management.

Author contribution

FG contributed to design, performed the study, and co-wrote the paper; GS contributed to analyses and co-wrote the paper; DSC and MK provided plant samples and contributed to writing; MD contributed to writing; and RK designed the study and contributed to analyses and writing.

Competing Interests

The authors declare no competing interests.

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Table 1 General characteristics of ecosystems investigated at Mt. Kilimanjaro, Tanzania.

Ecosystem	Land-use type	Elevation (m a.s.l.)	MAP (mm)	MAT (°C)	Soil properties						
					Soil type	pH ($CaCl_2$)	Clay (%)	Sand (%)	Organic carbon (%)	Total nitrogen (%)	C/N ratio
Savanna (Sav)	(M) extensive grazing, grass cutting	971 (40)	764 (50)	23.7 (0.3)	Leptosol	6.6 (0.3)	27.3 (4.0)	39.3 (8.7)	3.5 (0.4)	0.2 (0.0)	13.5 (0.2)
Maize field (Mai)	(M) cropped agriculture	938 (25)	674 (34)	23.6 (0.4)	Nitosol	5.6 (0.3)	37.4 (4.5)	20.3 (7.7)	1.6 (0.2)	0.1 (0.0)	11.8 (0.1)
Coffee plantation (Cof)	(M) cropped agriculture	1,349 (78)	1,393 (96)	19.8 (0.7)	Vertisol	4.5 (0.3)	45.2 (8.0)	17.8 (4.5)	4.2 (0.4)	0.4 (0.0)	10.5 (0.2)
Homegarden (Hom)	(M) cropped agroforestry	1,478 (112)	1,656 (177)	18.7 (0.8)	Andosol	5.4 (0.4)	45.4 (8.0)	16.5 (5.8)	6.7 (1.3)	0.6 (0.1)	11.5 (0.4)
Grassland (Gra)	(M) extensive grazing, grass cutting	1,506 (84)	1,610 (135)	18.9 (0.7)	Umbrisol	5.1 (0.4)	48.1 (8.1)	16.0 (5.1)	5.3 (2.1)	0.4 (0.2)	12.6 (0.2)
Lower montane forest (Flm)	(S-N) montane forest	1,806 (71)	2,201 (33)	15.5 (0.3)	Andosol	4.7 (0.3)	47.3 (5.2)	14.5 (2.2)	22.7 (4.9)	1.6 (0.2)	13.3 (1.5)
Ocotea forest (Foc)	(S-N) montane forest	2,464 (106)	2,388 (73)	11.5 (0.4)	Andosol	3.5 (0.2)	52.3 (4.5)	10.4 (2.3)	40.2 (1.5)	2.7 (0.1)	14.9 (0.7)
Ocotea forest disturbed (Fod)	(S-N) montane forest	2,378 (56)	2,334 (35)	11.9 (0.4)	Andosol	3.6 (0.2)	53.9 (3.4)	10.1 (2.5)	32.0 (1.8)	2.2 (0.2)	15.1 (1.3)
Podocarpus forest (Fpo)	(S-N) montane forest	2,856 (41)	2,036 (27)	9.6 (0.2)	Andosol	3.8 (0.1)	48.7 (1.1)	9.4 (1.3)	37.0 (1.0)	2.4 (0.1)	15.5 (0.8)
Podocarpus forest disturbed (Fpd)	(S-N) montane forest	2,904 (48)	2,056 (29)	9.7 (0.3)	Andosol	4.0 (0.2)	45.8 (3.4)	12.6 (3.3)	33.8 (2.3)	1.7 (0.0)	19.9 (1.4)
Erica forest (Fer)	(S-N) montane forest	3,716 (77)	1,517 (54)	6.2 (0.6)	Andosol	3.9 (0.2)	29.5 (5.1)	24.1 (6.2)	28.1 (2.4)	1.5 (0.1)	18.9 (0.7)
Helichrysum vegetation (Hel)	(S-N) alpine scrub vegetation	4,250 (100)	1,293 (31)	4.2 (0.4)	Andosol	5.7 (0.3)	7.9 (1.4)	69.9 (9.5)	6.1 (3.3)	0.3 (0.2)	12.0 (1.1)

Land uses are generically classified as managed (M) and semi-natural ecosystems (S-N). MAP and MAT stand for mean annual precipitation and temperature respectively. Climatic values are according to Appelhans et al. (2016). Data represent mean values (n = 5 ± SE) for different ecosystems. The most representative soil type is shown for each ecosystem. Soil properties are given for topsoil (0 – 10 cm for pH and soil texture, 0 – 5 cm for soil organic carbon and total nitrogen).

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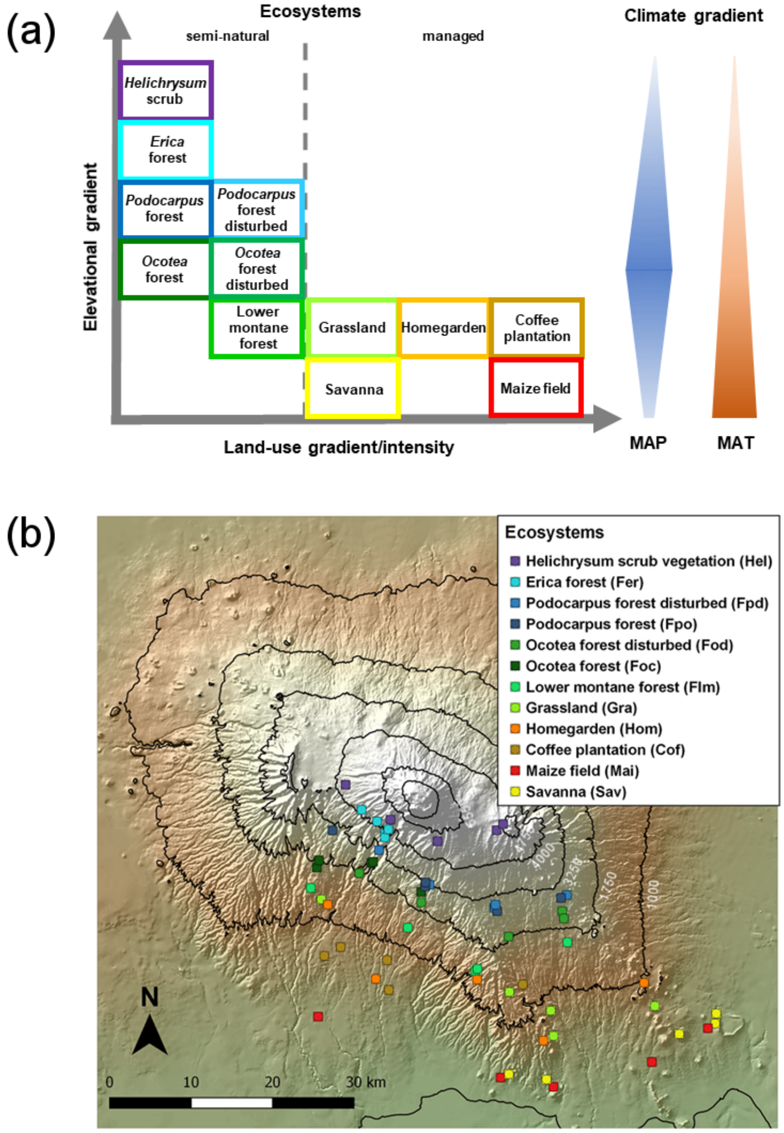

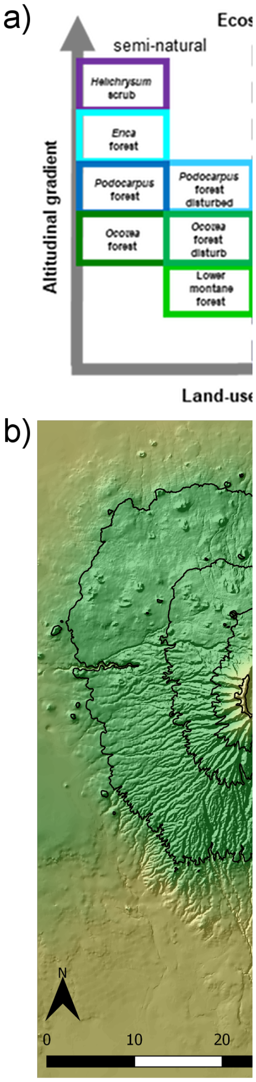

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Figure 1: Geographical distribution of investigated ecosystems: a) along the elevational and land-use gradient. MAP denotes mean annual precipitation and MAT mean annual temperature. Colours of boxes framing ecosystems' names match colours of symbols in the GeoTIFF panel below; b) along the southern slope of Mt. Kilimanjaro. Symbols represent individual ecosystems (12) replicated 5 times (60 study sites in total).

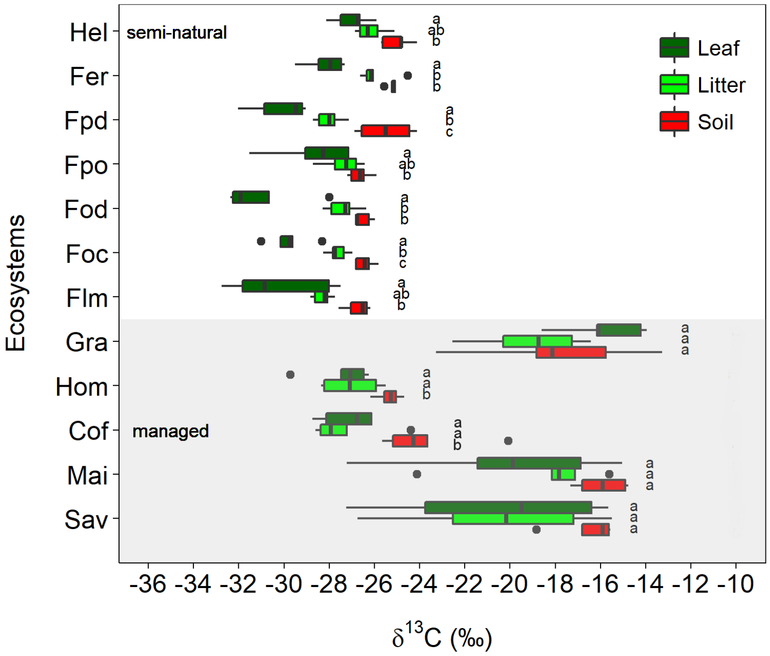
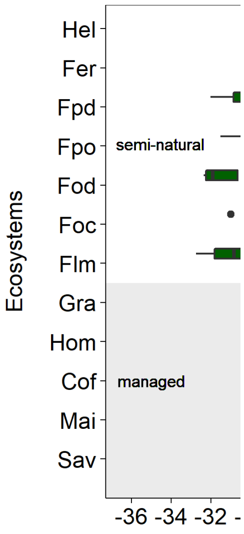


Figure 2: Variation in δ^{13}C values for leaves, litter, and soil along the Kilimanjaro elevational and land-use gradient. Ecosystem data represent the average values of five sites (one per each transect), with each site being composed of five samples (n = 5). Boxplots show median values per ecosystem with whiskers representing 1st and 3rd quartiles. Dots represent outliers. The shaded region represents managed ecosystems (both intensively and extensively), while those un-shaded indicate semi-natural ecosystems. Lower case letters show significant differences between sampled materials within each ecosystem (one-way ANOVA followed by Tukey's HSD test as a post hoc procedure, $P \leq 0.05$). The ecosystem acronyms used are as per Table 1. Mai, Cof, and Hom are managed cropping sites, Gra and Sav are extensively managed grasslands and savannas, while the rest represent semi-natural ecosystems. Sites are ordered by increasing altitude.



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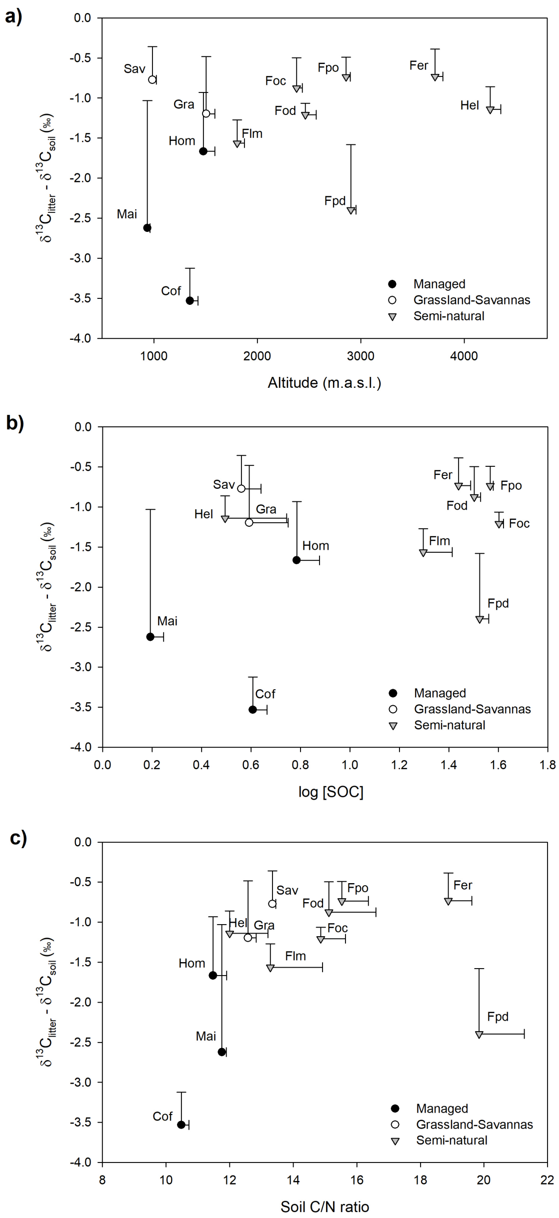

Figure 3: a) Variation in δ^{13}C-based enrichment factors ($\delta^{13}C_{itter-soil}$) with elevation; b) Relationship between δ^{13}C-based enrichment factors ($\delta^{13}C_{itter-soil}$) and SOC concentration (log SOC); and c) Relationship between δ^{13}C-based enrichment factors ($\delta^{13}C_{itter-soil}$) and soil C/N ratios. Note: A savanna site with large C_3 influence was removed from the figure for clarity.

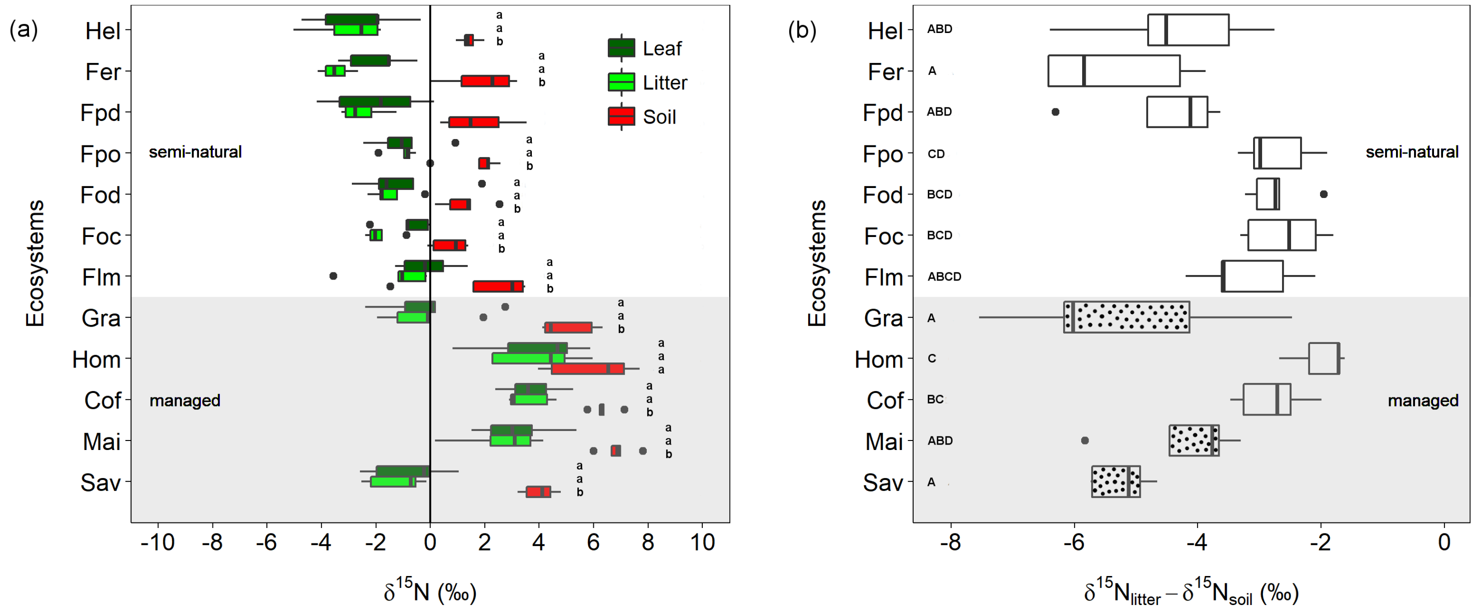

Figure 4: Variation in $\delta^{15}N$ values and $\delta^{15}N$-based enrichment factors along the Kilimanjaro elevational and land-use gradient. a) Variation in $\delta^{15}N$ values for leaves, litter, and soil material sampled along the Kilimanjaro elevational and land-use gradient. Boxplots show median values per ecosystem with whiskers representing 1st and 3rd quartiles. Dots represent outliers. Ecosystem data represent the average values of five sites (one per each transect), with each site being composed of five samples. Lower case letters show significant differences between sampled materials within each ecosystem (one-way ANOVA followed by Tukey's HSD test as a post hoc procedure, $P \leq 0.05$); b) Variation in $\delta^{15}N$-based enrichment factors ($\delta^{15}N_{litter-soil}$) calculated for the different ecosystems along the elevational and land use gradient. Dotted boxplots indicate ecosystems dominated by C_4 vegetation. Capital letters indicate significant differences between ecosystems (one-way ANOVA followed by Tukey's HSD test as a post hoc procedure, $P \leq 0.05$). The ecosystem acronyms used are the same as those in Table 1. Sites are ordered by increasing altitude.

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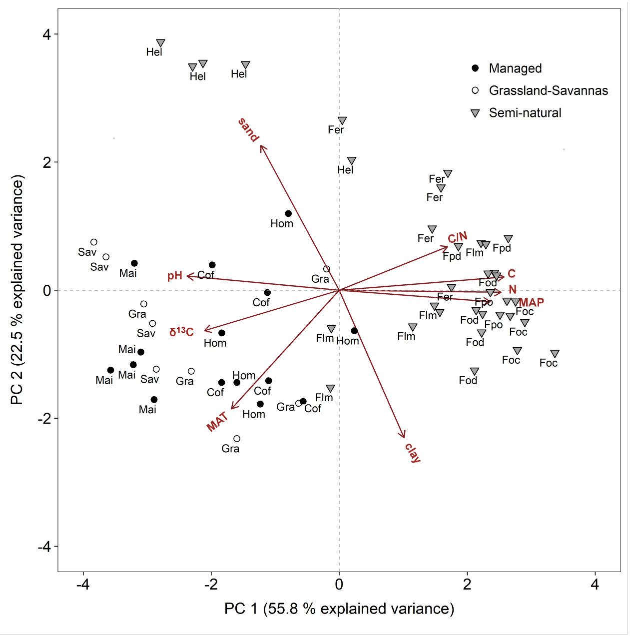

Figure 5: Principal component analysis bi-plot for soil and climate variables potentially controlling soil δ^{15}N. Symbols are as per all previous figures. Acronyms are as per Table 1. C/N = soil C/N ratio, C = soil carbon content, N = soil nitrogen content, MAP = mean annual precipitation, clay = soil clay content, MAT = mean annual temperature, δ^{13}C = soil δ^{13}C, and pH = soil pH.

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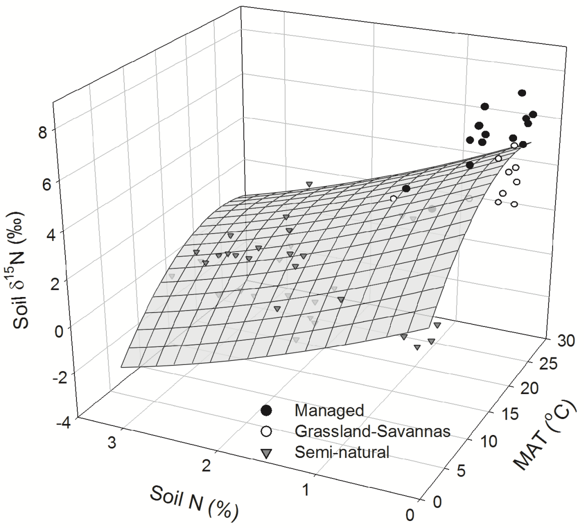

Figure 6: Measured and modelled soil δ^{15}N values predicted as a function of soil N abundance and mean annual temperature (MAT). Data points are classified by generic land uses (i.e. intensively managed cropping sites, extensively managed grassland and savannas, and semi-natural ecosystems) observed along the elevational and land use gradient. The regression takes the following form: soil δ^{15}N = 1.10 + 0.49 (MAT) – 1.86 (soil N) – 0.01 (MAT)^2 + 0.14 (soil N)^2; (r^2 adj= 0.68, $P <$ 0.05, n = 60).

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