Author response referee#1

We would like to thank reviewer #1 for the very constructive and thorough feedback on our manuscript. We appreciate all suggestions, which will improve the quality and rigour of our manuscript. Below, we listed the main changes to the manuscript as a reply to the primary concerns raised by the reviewer

- We will enhance the description of the study area:
    - Description of the lateritic zone where we collected all our soil samples
    - Texture of soil and topographical characteristics.
    - Short overview of C3 grasses location and expansion in Madagascar.
- The effect of erosion resulting from vegetation changes will be discussed in more detail
- Background on the use of ^{13}C and ^{14}C on will be added in the introduction.
- We will insert additional supplementary figures and statistical analyses, in particular relating to the difference between $\delta^{13}C$-OC or OC content of the valley and other profile positions.

Reviewer comments are indicated in *italics*, our responses in regular font**.**

In this paper the authors present SOC concentration and stock, 13C, and 14C depth profiles from hillslope transects with forest and grassland vegetation cover in the highlands of Madagascar. The authors use these data to address a debated question – whether current grasslands are grasslands because of bioclimatic and edaphic factors (ie. they are "natural" grasslands") or if they are the consequence of deforestation by humans hundreds of years ago. They argue that 13C depth profiles indicate a shift from C3 (possibly forest) to C4 (current grasses). They further argue that conversion from forest to grassland has caused the sustained loss of SOC since this time as current grasslands store about half as much carbon as intact forests. These data and findings are interesting, but I find that the manuscript could use some improvements and corrections prior to publication.

REPLY: First, we would like to thank referee #1 for his/her positive evaluation and comments on our manuscript. All comments are helpful to improve the presentation of our results and manuscript.

Reviewer #1 General concerns are as follows:

1) *Though there is some consideration of erosion, this could be better explained and addressed in the abstract and discussion sections. This needs to be fully considered as an alternative explanation to the differences in SOC especially considering the presence of gully erosion (lavaka) and lateritic horizons in some grassland areas.*

   REPLY: Indeed, erosion rates are considered to be higher after vegetation change (from forest to grasslands), and this, therefore, could contribute to the higher $\delta^{13}C$ and OC at the valley position compared to the upper hillslope position for the grassland profiles (Top - Upper middle-Middle – Lower middle and Bottom). The effect of erosion will be discussed in more detail in the Discussion of the revised manuscript.

2) There is no other discussion of alternative sources of carbon. At least indicate you've considered carbonate and geogenic OC. What would their presence mean for your findings and conclusions? Why do you think you do not need to consider them?

REPLY: It should first be noted that the data presented only refer to organic carbon: all carbonates were eliminated when preparing our soil for analysis by acidification after weighing subsamples in Ag cups (see Materials & Methods). According to the World Reference Base for Soil Resources, the soils in our study area are classified as ferralsols (WRB, 2006). In addition, the basement rocks on the site we sample our soil are metamorphic and igneous (Du Puy and Moat, 1996). Therefore, we did not consider geogenic OC to be substantial, in contrast to subsoils developed from sedimentary rocks where this might be more important (Graz et al., 2010). We will mention this explicitly in the revised version.

3) Inferring that the conversion to grassland is what caused the large discrepancy in SOC stocks between the grassland and forest transects is interesting but requires consideration of how similar or different the soils are independent of the vegetation cover now – if erosion is a factor now, could it have been before when the vegetation was C3 dominated according to the ^{13}C results? Why do you think they were similar? Are the textures similar?

REPLY: We agree that our soil should be comparable before testing our hypothesis: soil type, topography, slope gradient, and texture. We will mention in the manuscript that we did not observe significant differences in the texture of soils under grassland and forest. We will add a new figure of texture and gradient as a supplementary Figure. Regarding the possible effect of erosion under C3 vegetation: the $\delta^{13}C$ data collected along our forest transects do not show substantial differences according to the position along the hillslope. This suggests that erosion might not play a major role in the variation of $\delta^{13}C$ in a C3 (in this case, forest) dominated landscape as significant erosion would invariably have led to sign of OC accumulation in the valley position. Water erosion is likely to be more important under grassland: this explains the fact that there is clear accumulation of SOC in the valley positions under grassland. This finding is not surprising: water erosion rates under a dense forest cover are generally very low (see, e.g., Cerdan et al. (2010) who reported an average water erosion rate of 0.14 t $ha^{-1}y^{-1}$ under forest in Europe and Zhao et al., (2021) who reported a median erosion rate of 0.15 t $ha^{-1}y^{-1}$ and an average erosion rates of 1.5 t $ha^{-1}y^{-1}$ under forest in China).

4) is it possible that previous C3 vegetation may not have been forest (possibly savanna or C3 grassland, which is common in other parts of the tropics)?

REPLY: C3 endemic grass species that has been inventoried in Madagascar belong to the "forest shade clade" (Paniceae: Boivinellinea) and bamboos (Hackel et al., 2018). Their diversification since the Miocene is reported to be favored by the expansion of the *Sambirano* rainforest (in the North of Madagascar) (Hackel et al., 2018; Yoder and Nowak, 2006). Therefore, if C3 grasses had existed in our study area, it would have been within a forest ecosystem. We will clarify this in the revised version of our manuscript.

5) The introduction needs some background on the use of 13C and 14C in this context (for vegetation shifts and erosion) as well as context for why these differences in SOC stocks are relevant. There is a lot of good literature on the impacts of agriculture (from the beginning of agriculture, not limited to contemporary studies) on SOC to draw from here.

REPLY: Thank you for this suggestion; a background paragraph on the use of ^{13}C and ^{14}C will be added in the introduction paragraph and we will make sure that the impact of deforestation/conversion to grassland as presented in the literature is included.

6) there are no statistical analyses included in this manuscript. It seems the work could benefit from some relatively simple correlation, regression, and ANOVA to address whether it is appropriate to average all of the hillslope positions, for example. Is there no difference in the valleys or are the valleys just more similar than the other hill slope positions?

REPLY: We agree that statistical analysis will strengthen our conclusions. A summary table showing the result of the statistical analyses will be added in the supplementary files. We will compare data from the valley sites to the other hillslopes positions (in the current grassland) using simple significant differences tests and will thereby concentrate on aggregate measures, such as the total SOC stock down to a specific depth or the average ^{13}C signature. More specifically we will answer/illustrate the following questions using statistical analysis:

- Are the profiles under forest and under grassland significantly different with respect to SOC content and signature ?
- Are the valley positions significantly different from the hillslope positions (i) under forest and (ii) under grassland ?.

Specific comments from referee #1:

-L26: what about geogenic C, which could have a 13C value similar to C3 vegetation. How confident are you this is trees and not C3 savanna or grassland?

REPLY: As outlined in response to previous general comments: in Madagascar, C3 grasses had existed only within a forest ecosystem; all open grasslands are characterized by C4 vegetation. Geogenic OC is not considered as a source of OC because the basement rocks are metamorphic and igneous, and all carbonates were eliminated when preparing our soil for analysis.

-L31: What do you mean by "recent expansion" and why do you think this is 1) recent and 2) expansion? Why not just high productivity in the valleys or erosional deposition of C from the surface up slope? This would also explain why the SOC stocks in the valleys are so high and similar to the forest more so than a recent expansion (I think, but maybe I am missing something?)

REPLY: We do agree with the reviewer that these mechanisms may also be important in explaining the characteristics of the valley profiles and we will change our wording to include these mechanisms as possible explanation for the high carbon content of the valley and the young age of the SOC at the valley floor positions. The sentence then becomes as follows:

"At the valley positions under grassland the upper 80 cm topsoil contains larger amounts of recent, grass-derived OC in comparison to the hillslope positions. This is likely to be related to the higher productivity of the valley grasslands (due to higher moisture and nutrient availability) but deposition of OC that was eroded further upslope may also have contributed".

-L75: a word is missing here "do not allow assess how"

REPLY: We will change to "allow us to assess".

-L85-6: 13C, 14C, and SOC stock relevance need to be presented earlier in the introduction.

REPLY: A background on the use and relevance of ^{13}C and ^{14}C data, as well as SOC stocks will be added to the introduction.

-L93: again, a word is missing here "allow to assess"

REPLY: We will change this as suggested.

-L105: If at many locations there are lateritic horizons, you need to indicate whether you sampled in any of these areas later. What does this mean for your findings?

REPLY: The lateritic soil horizon is usually between 0.5 to 2m thick (Voarintsoa et al., 2012). Our soil samples have been sampled in the lateritic soil horizon. We will indicate this in the material and methods sections in the revised version. The lateritic soil horizon is considered to be relatively impermeable, thus favoring surface runoff erosion, especially if there is no or little vegetation and if no cracks are present (Wells and Andriamihaja, 1993). However, it should also be pointed out that, while there was a lateritic soil horizon, a true laterite was not present at our sites.

-L108: lavaka need to be better explicitly addressed in the context of erosion in the current grassland areas – what impact can their presence have on your results? How old are they – do they predate human deforestation or are they possibly a consequence of humans using these areas for grazing? Land cover conversion and land use may be conflated here or not independently addressed adequately. They seem used interchangeably.

REPLY: An important point is that we did not sample inside lavaka, we consider the presence of lavaka but on hillslopes outside of the lavaka. By choosing a slope with and without lavaka we wanted to investigate whether soils on slopes that have lavaka development may differ from slopes that do not have them. We found that the OC content and ^{13}C value do not differ significantly (at the surface) and have the same trend with depth for GLP and GLA. A statistical analysis of this will be provided in the supplementary file. We will explain this better in the revised version of the manuscript.

Previous research has shown that some lavaka can be directly associated with human activities such as trenches, tracks, steep fields and the construction of canals and paddies (Riquier, 1954; Wells and Andriamihaja, 1993). However, other lavaka are tens of thousands of years old, predating the permanent settlement of humans in the highlands, which is estimated to have taken place between 1600 and 100 years ago (Douglass et al., 2019; Mietton et al., 2014; Wells and Andriamihaja, 1993). Recent research has shown that lavaka in the Lake Alaotra region are on average ca. 400 years old. Lavaka became far more numerous since ca. 1000 years ago and lavaka formation rates have increased dramatically over the last 200 years. This timing and the rapid increase in lavaka erosion rates has been confirmed by floodplain sedimentation data in the same area (Brosens et al., 2022). Brosens et al. (2022) links this increase in lavaka erosion to increased environmental pressure due to growing human populations and intensified grazing based on scenario modelling and on the absence of significant climatic variations in the period considered. The mechanisms that lead to the initiation of lavaka, which typically occurs at the hillslope between upper middle and lower middle position, are not well understood. Different theories have been developed to explain their initiation, where both surface runoff processes and groundwater sapping are hypothesized to play an important role (Wells and Andriamihaja, 1993). However, the fact that excessive pressure on the land plays a critical role in lavaka initiation suggests that changes in surface properties related to overgrazing/overuse such as soil compaction and the decrease in vegetation cover and the increase of surface runoff caused by these changes play a crucial role. We will briefly re-iterate the main findings of Brosens et al. (2021) in the revised version of the manuscript.

Table 1: Reported errors are > 1 so you should not report decimal places as they are within your uncertainty. Is it appropriate to present the data this way by averaging across landscape position? The presence of a large difference between the forests and grasslands except in the valleys suggests that maybe it is not appropriate as does the statement that the grassland hillslopes may be different from one another. This table is redundant with Figure 4, isn't it? The figure is much more informative, and you provide these values in the text – they do not need to be reported in the main paper so many times. If you find value in the table, move it to the supplement.

REPLY: The table will be presented in the supplementary figure, and the number of decimals will be adjusted. We do indeed average across landscape positions. Given that profiles at different landscape positions are very similar and that their variation is not in any way related to landscape position, we believe this is justified. Also the data are simply used to make a comparison of SOC inventories under forest vs grassland and we believe that by presenting the data in this way this comparison can be most easily made.

L345-6: this suggests the surface young C has been eroded, which would explain why the valley has more SOC and younger C but this does not seem adequately discussed as an important part of the story for the grassland transects.

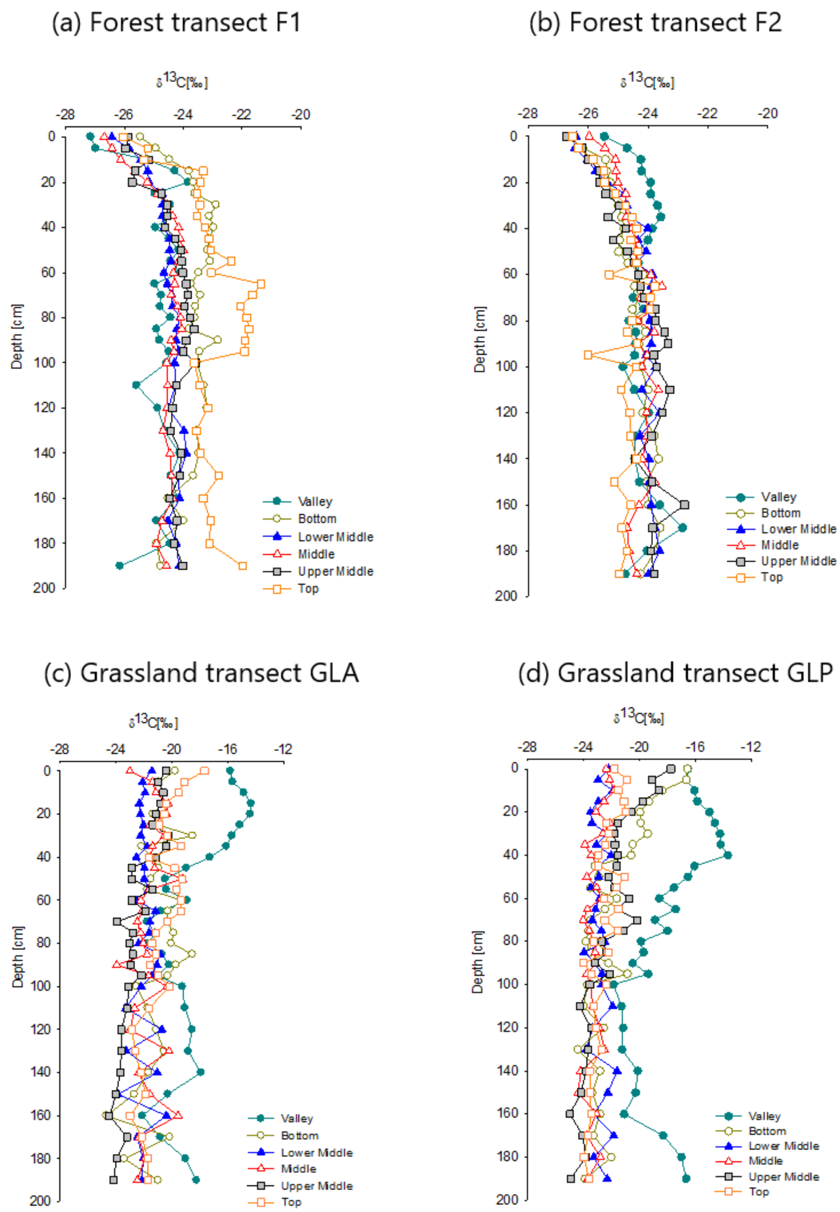
REPLY: As suggested, a section of the effect erosion that follows the vegetation change will be added to grassland transect discussions and we will refer to the effects of erosion as a possible explanation.

L269: This paragraph is correct but the way the logic is presented is a little confused in my opinion. Important to this explanation but only implied, is that respiration would be depleted in ^{13}C relative to the organic matter because the light isotope is preferentially converted to CO2 and diffused to the surface – this is based on mass-dependent fractionation and is why the heavy isotope remains behind in the microbial biomass and byproducts. This is why the leaves that are taking up CO2 in soil respiration may be depleted relative to leaves taking up CO2 from well mixed air higher in the canopy. Also important is that mass-dependent fractionation causes the light isotope to be transported within the plants, so roots and root respiration are also quite depleted in 13C relative to the classic values for C3 plant leaf tissue of -25 permil. Similarly within a tree leaves growing closer to the ground may be more depleted that leaves in the upper canopy.

REPLY: As the reviewer explains, the understory effect or canopy effect found in tropical forest is mainly related to the gradient in $\delta^{13}C$ of ambient CO_2 along the vertical gradient: $\delta^{13}C$-CO_2 is low close to the ground, due to elevated CO_2 concentrations via the contribution of soil respiration. Higher up in the canopy, the $\delta^{13}C$-CO_2 values are closer to the average atmospheric CO_2 composition. We will try to express this more clearly in the revised version.

L278: Figures 3a and 3c look the same. Only 3 d looks like it may be different. Is this a mistake? There are no statistics again to assess what differences are statistically significant, making ecological significance questionable.

REPLY: We apologize for this error; the right figure will be corrected (see below). Statistical analyses will be provided in the supplementary file in the revised version.



L296: Could this be because of deposition from soil that originated upslope via erosion? Could this explain why topsoils don't have more enriched 13C values on the slopes?

REPLY: We consider all alternatives that might explain the higher value of $\delta^{13}C$ in the valley. We suggested that the value of $\delta^{13}C$ of the top position could be due to vegetation change, which induces more erosion in the upper slope positions. In addition, there is a higher vegetation density in the valley that we observe compared to other profiles positions. We will clearly express this in the revised version of the MS.

L355: Rephrase for clarity – something like "Surface erosion is expected to be variable across topographic positions along the hillslope transect, with minimal…."

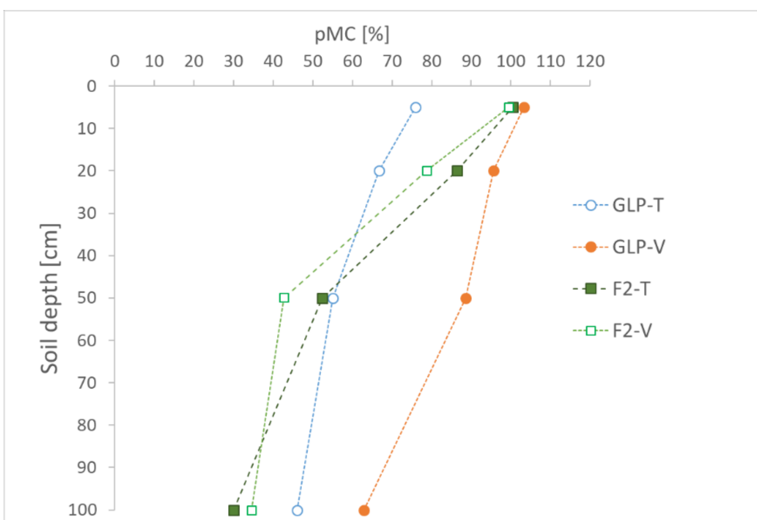
REPLY: Thank you for this suggestion; the sentences will be changed as suggested.

L357: what does "10Be in-situ topsoil samples" mean? I am more familiar with "in situ 10Be" which means cosmogenic formation of 10Be when surfaces are exposed in rock or sediment. This is an analysis so again this phrasing does not make sense to me. Try "erosion rates from in-situ 10Be analysis of the topsoil samples" perhaps? Also, please clarify what you would expect in terms of variation in the pMC and ^{13}C values based on the erosion rates indicated by the ^{10}Be analyses.

REPLY: We apologize for the confusion; the sentences will be modified as suggested. With the data that we have now, we could not really have a specific expectation for pMC and ^{13}C values based on the erosion rates indicated by the 10Be analyses. However, the fact that we saw an increase in OC, $\delta^{13}C$ and pMC values at the valley-position under grasslands seems to indicate that at this position soil that has been eroded from upslope position is deposited. This is not observed in the forest transect, which is consistent with low erosion rates, with minimal deposition taking place at the valley position.

L358-9: This is very hard to see in figure 5. It is much easier to see in figure 3 for the ^{13}C. Please provide a similar figure as Figure 3 to show the 14C value. If it is only useful for this statement, put it in the supplement. I would very much appreciate seeing this figure along with the depth profiles for SOC and 13C as you have shown.

REPLY: Thank you for this suggestion, we will include a new figure (see below) in the supplementary figures:



New supplementary Figure: Depth profile of pMC for GLP-T, GLP-V, F2-T and F2-V.

L360: Again, some statistics would be great and could strengthen your story. Correlation or regression would be very simple but quantify the relationship you see between the isotopes in the grassland transects. On figure 5, drawing a regression line on this plot for the grasslands (and also perhaps for the forests) would also drive home your point about how the grassland values converge with the forest ones at depth and make it easier to identify the depth labels on the different datapoints, which are quite difficult to read. Also, figure 5 would be easier to digest if the grassland points had the same symbols and color, with one open and one closed like the forests. This would make the figure feel less cluttered and make it easier to *pick out the labels for the depths and transects. I am unsure why the hillslopes and valleys are marked using different symbols – I do not see a pattern. Is there one? If so this plot should be further improved to make it easier to see. I see the grasslands falling on one regression line and the forests on another.*

REPLY: Thank you for this suggestion. We used different symbols for the valley to show the difference between pMC value found in the valley position of forest and grassland transect. In this figure 5, we highlighted that the valley of grassland at the upper 50 cm is composed of modern OC (pMC≥100%), which is not the case of the OC in the valley position of the forest. In addition, we also highlighted that there is a pMC trend; the grassland is reaching the pMC value of the forest SOC at the subsoil. Therefore, this figure will be improved as suggested, and a regression line will be added.

L365-378 This section should be significantly shortened to just a few sentences about how your findings are similar to other similar studies. There is no introduction or context about why the stocks or distribution of stocks are important so it is very out of place in a manuscript so focused on vegetation shifts and erosion across hillslopes. What about how similar these soils were prior to when humans may have deforested the current grasslands? What else could explain your results? What about the laterite? What about the lavaka – when did it form and what influence does it have on your findings? What other things may explain your findings other than human deforestation? I very much like the suggestion in this section that there may be long sustained loss of C and I think this is consistent with what long term global evidence for a massive loss of C since the dawn of agriculture has been. But this needs to be better substantiated in the paper through consideration of alternative explanations.

REPLY: Thank you for this suggestion, as outlined in response to other comments, we will (i) introduce the importance of SOC stocks in the introduction, and (ii) add more context on the laterite depth and lavaka.

Figure 6: Is averaging the grassland profiles like this valid? There is no effect of the lavaka? Are some of these sites influenced by laterite?

REPLY: We collected all our soil samples on the hillslope and did not find any significant difference between the hillslope with and without lavaka: we will demonstrate this statistically in the revised version of the Material and Methods sections. The two grassland transects that we analyzed do not show any statistical difference in terms of $\delta^{13}C$ and OC content at the surface, and they show the same trend with depth. We therefore think it is justified to combine the data here.

Figure 7: This is redundant with figure 3, no? chose which one best shows your results (I think figure 3 but it is difficult to tell as it seems to have a mistake). If you like both plot types, move the less impactful one to the supplement.

REPLY: Figure 3 mainly compares the value in each transect. Figure 7 compares $\delta^{13}C$ of forest and grassland for each position and shows that $\delta^{13}C$ become similar at a lower depth. We agree that the underlying results we present are the same – but feel it is still useful to present them both ways; we will therefore move Figure 7 to the supplement as suggested.

References

Brosens, L., Broothaerts, N., Campforts, B., Jacobs, L., Razanamahandry, V. F., Van Moerbeke, Q., Bouillon, S., Razafimbelo, T., Rafolisy, T. and Govers, G.: Under pressure: Rapid lavaka erosion and floodplain sedimentation in central Madagascar, Sci. Total Environ., 806, 150483, doi:10.1016/j.scitotenv.2021.150483, 2022.

Cerdan, O., Govers, G., Le Bissonnais, Y., Van Oost, K., Poesen, J., Saby, N., Gobin, A., Vacca, A., Quinton, J., Auerswald, K., Klik, A., Kwaad, F. J. P. M., Raclot, D., Ionita, I., Rejman, J., Rousseva, S., Muxart, T., Roxo, M. J. and Dostal, T.: Rates and spatial variations of soil erosion in Europe: A study based on erosion plot data, Geomorphology, 122(1–2), 167–177, doi:10.1016/j.geomorph.2010.06.011, 2010.

Douglass, K., Hixon, S., Wright, H. T., Godfrey, L. R., Crowley, B. E., Manjakahery, B., Rasolondrainy, T., Crossland, Z. and Radimilahy, C.: A critical review of radiocarbon dates clarifies the human settlement of Madagascar, Quat. Sci. Rev., 221, doi:10.1016/j.quascirev.2019.105878, 2019.

Graz, Y., Di-Giovanni, C., Copard, Y., Laggoun-Défarge, F., Boussafir, M., Lallier-Vergès, E., Baillif, P., Perdereau, L. and Simonneau, A.: Quantitative palynofacies analysis as a new tool to study transfers of fossil organic matter in recent terrestrial environments, Int. J. Coal Geol., 84(1), 49–62, doi:10.1016/j.coal.2010.08.006, 2010.

Hackel, J., Vorontsova, M. S., Nanjarisoa, O. P., Hall, R. C., Razanatsoa, J., Malakasi, P. and Besnard, G.: Grass diversification in Madagascar: In situ radiation of two large C 3 shade clades and support for a Miocene to Pliocene origin of C 4 grassy biomes, J. Biogeogr., 45(4), 750–761, doi:10.1111/jbi.13147, 2018.

Mietton, M., Cordier, S., Frechen, M., Dubar, M., Beiner, M. and Andrianaivoarivony, R.: New insights into the age and formation of the Ankarokaroka lavaka and its associated sandy cover (NW Madagascar, Ankarafantsika natural reserve), Earth Surf. Process. Landforms, n/a-n/a, doi:10.1002/esp.3536, 2014.

Du Puy, D. J. and Moat, J.: A refined classification of the primary vegetation of Madagascar based on the underlying geology: using GIS to map its distribution and to assess its conservation status, Biogéographie de Madagascar, 205–218, 1996.

Riquier, J.: Etude sur les Lavaka, Mem. l'Institut Sci. Madagascar, D(VI), 169–189, 1954.

Voarintsoa, N. R. G., Cox, R., Razanatseheno, M. O. M. and Rakotondrazafy, A. F. M.: Relation between bedrock geology, topography and lavaka distribution in Madagascar, South African J. Geol., 115(2), 225–250, doi:10.2113/gssajg.115.225, 2012.

Wells, N. A. and Andriamihaja, B.: The initiation and growth of gullies in Madagascar: are humans to blame?, Geomorphology, 8(1), 1–46, doi:10.1016/0169-555X(93)90002-J, 1993.

WRB: World Reference Base for Soil Resources (WRB), in Encyclopedia of Environmental Change, SAGE Publications, Ltd., 2455 Teller Road, Thousand Oaks, California 91320., 2006.

Yoder, A. D. and Nowak, M. D.: Has Vicariance or Dispersal Been the Predominant Biogeographic Force in Madagascar ? Only Time Will Tell, , doi:10.1146/annurev.ecolsys.37.091305.110239, 2006.

Zhao, M., Jacobs, L., Bouillon, S. and Govers, G.: Rapid soil organic carbon decomposition in river systems: effects of the aquatic microbial community and hydrodynamical disturbance, Biogeosciences, 18(4), 1511–1523, doi:10.5194/bg-18-1511-2021, 2021.