

Feedback from the reviewers is written in italic, while our responses are written in in green. When changes were made to the manuscript, we included a screenshot of this part using track changes.

Reviewer 2

The study described by Van de Broek et al capitalizes on a long term ISFM field experiment in central Kenya (Embu and Machanga) and western Kenya (Sidada and Aludeka) Kenya. The study builds on extensive SOC research performed at the same sites and published in multiple papers by Mortiz Laub et al.

This study adds information to the previous work by measuring MAOM and POM fractions. d13C and D14C radioisotopes on a selection of the ISFM treatments.

This study is a valuable addition to the previous publications as it gained new insights on the fractions of MAOC and POC and the stabilisation of amended carbon in MAOC.

Variable topsoil MAOC stocks in the topsoil as an effect of nutrient or organic amendment application (*Tithonia diversifolia* or Farm Yard manure addition)

Clear differences in MAOC stocks between in clayey and sandy sites.

No significant differences in SOC fractions in the subsoil (15-50cm).

Previous studies (Laub et al 2023) showed that organic amendments reduced native SOC losses from these field sites. This study showed that the prevented losses in the clayey sites were not caused by the formation of newly formed MAOC from added organic amendments but originated from prevented losses of native SOC while in the sandy soils the lower losses were partly caused by new MAOC stabilisation.

We thank the reviewer for taking the time to read our manuscript and for providing detailed and constructive feedback. This is greatly appreciated. Please find our responses to the feedback below.

More specific comments on the manuscript:

Line 11: Given the distinct stable carbon isotopes signatures of C3 and C4 substrates of Maiz and *Tithonia* we calculated.. etc.

Thanks for this suggestion. The start of this sentence is now changed to: “Using the distinct stable carbon isotopic signature ($\delta^{13}\text{C}$) of the maize crop (C4) and the *Tithonia* amendments (C3), we calculated that [...]”.

N fertiliser did not affect MAOC stocks at any site. Using ~~stable carbon isotopes~~ the distinct stable carbon isotopic signature ($\delta^{13}\text{C}$) ~~, we found of the maize crop (C4) and the *Tithonia* amendments (C3), we calculated~~ that the portion of topsoil MAOC

Line 96: You now name the treatments nutrient management strategies. However before you this term was not used and only organic amendments and mineral fertilizer addition was used to indicate the different treatments. I would replace “nutrient management strategies” with “amendments”.

Thanks for this suggestion, we agree that it’s better to stick to one term. “Organic resources” is now used throughout the manuscript, as this term is generally used in research concerning integrated soil fertility management.

Line 98: why a question on the 15-50cm layer and measurements in two layers 15-30, 30-50 cm? See also line 134.

In our study, we made use of previously collected soil samples (as described in Laub et al., 2023; <https://doi.org/10.5194/soil-9-301-2023>). We wanted to get insights in the distribution of C fractions along the depth profile, initially analysing the layers 0-15, 15-30, and 30-50 separately. However, the results were similar for the 15-30 and 30-50 layers, and to simplify the presentations of the results we chose to present them together (i.e., as being the subsoil). At the same time, we want to give the reader the opportunity to see how the depth profiles of C fractions looked like, which is relevant, for example, if one later aims to simulate these depth profiles.

To make this clear to the reader, this sentence is changed to: “In the present study, the 0-15 cm depth layer is referred to as topsoil or plough layer, while the samples collected in the 15-30 and 30-50 cm depth intervals are referred to as the subsoil. Depth profiles of the analysed variables are presented for all layers, while statistical results are presented for the topsoil and the combined subsoil layers.”

15–30 cm and 30–50 cm depth (Laub et al., 2023a). In the present study, the 0–15 cm depth layer is referred to as topsoil or plough layer, while the ~~15–50 cm depth layer is~~ samples collected in the 15–30 and 30–50 cm depth intervals are referred to as
145 ~~subsoil~~ the subsoil. Depth profiles of the analysed variables are presented for all layers, while statistical results are presented
for the topsoil and the combined subsoil layers. Samples were collected using a half open gauge auger (60 mm diameter,

Why then use alle layers in figure 1 and in text for instance Line 324.

The reason for referring to these layers separately in the text, and showing them separately in the figures, is to provide the reader with this additional level of detail, rather than discussing only the results for both layers combined. As the results for the two subsoil layers were identical, these are used together to formulate the conclusions. We think this is the most straightforward way to discuss while showing the detailed data.

Line 117: For the reader it is difficult to assess what the influence is of the intense topsoil erosion throughout the experiment at the Machanga site. As the site is included in all the analysis the erosion is not strong enough to exclude this site from this study? No bias expected. This could be explained a bit more.

As soil erosion took place across the field, and treatments were distributed in blocks, it is unlikely that erosion differently affected the treatments. However, soil erosion was unfortunately not quantified. Nevertheless, we chose to include the results in our manuscript as they are still useful to the reader. Based on this comment and a comment by the other reviewer, we now describe this in more detail in the methods section where the sites are described (section 2.1) as follows: “The sandy site at Machanga had a gentle slope (< 1 %) and therefore experienced topsoil erosion throughout the experiment. Because treatments were randomized within horizontal blocks following the contour of the slope—and erosion affected the field broadly—it is unlikely that any treatment was disproportionately impacted. However, the erosion may have removed part of the topsoil and brought subsoil closer to the surface, causing some of the original subsoil (i.e., below 15 cm depth) to be included in the 0–15 cm samples.”.

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125	<u>erosion throughout the experiment. Because treatments were randomized within horizontal blocks following the contour of the slope—and erosion affected the field broadly—it is unlikely that any treatment was disproportionately impacted. However, the erosion may have removed part of the topsoil and brought subsoil closer to the surface, causing some of the original subsoil (i.e., below 15 cm depth) to be included in the 0–15 cm samples.</u>

Table 1 Could the SOC stocks from Laub et al 2023 be added to this table to make a clear distinction between what is new and what is already published in previous papers?

Table 1 describes general characteristics per site, while the SOC stocks differed per treatment at each site. This results in 16 SOC stocks (4 treatments at 4 sites), which cannot be mentioned in this table without expanding greatly. In addition, Laub et al. (2023; <https://doi.org/10.5194/soil-9-301-2023>) calculated SOC stocks on an equivalent soil mass basis, while we report stocks of POC and MAOC down to a common depth (0.5 m, as explained in section 2.2). These different ways of reporting mean that both results are not directly comparable. Therefore, we prefer not to mention the SOC stocks by Laub et al. (2023) in the manuscript.

However, to make it clear that these results have been reported by Laub et al. (2023), we now added the following sentence to section 2.1, after we noted that Laub et al. (2023) found no significant differences in the SOC stocks below 15cm depth at any of the sites: “The initial topsoil OC content and SOC stocks across depth, as measured in 2021, were reported by Laub et al. (2023a).”.

(Laub et al., 2023a). <u>The initial topsoil OC content and SOC stocks across depth, as measured in 2021, were reported by (Laub et al., 2023a).</u>
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Why was the HCL, NaOH procedure used for the D14C radioisotopes while this was skipped for the OC procedures (Line 203-205)

We did not acidify the samples prior to OC analysis as all samples had a pH < 6.5, and we found no presence of CaCO₃ in a subset of samples to which HCl was added (as described in section 2.4). However, the addition of HCl and neutralization with NaOH is standard practice in the lab performing the ¹⁴C analysis which happens irrespective of the possibility of CaCO₃ being present. Therefore, these steps were performed and reported in the manuscript, although it is highly unlikely that it had any effect on the results.

Line 214-215: Not very clearly explained here. What is meant with equivalent soil masses of the respective soil layer, no bulk density of the layers used, Why not? What is meant with a common depth.

Thanks for pointing out that this has not been described properly. We now changed this part to the following and hope to answer your question with this modification: “Stocks of particulate OC (POC) and mineral-associated OC (MAOC) were calculated by multiplying the OC % of each fraction with the mass of the respective soil layer. It has been observed that treatments in field trials can lead to changes in bulk density over time. When this occurs, it is preferable to report SOC stocks down to an equivalent soil mass rather than to

a fixed depth, to avoid bias caused by collecting more (or less) soil in certain treatments at the same sampling depth (Ellert and Bettany, 1995). In our study, however, bulk density differences between treatments within each site were minimal (see above) indicating that the same amount of soil was present in every depth layer at the same site for the different treatments. Therefore, POC and MAOC stocks were reported down to a common depth, rather than for an equivalent soil mass.”

Stocks of particulate OC (POC) and mineral-associated OC (MAOC) were calculated ~~for each sample~~ by multiplying the OC % of ~~these fractions each fraction~~ with the mass of the respective soil layer. ~~As the differences in soil bulk density between-~~ It has been observed that treatments in field trials can lead to changes in bulk density over time. When this occurs, it is preferable to report SOC stocks down to an equivalent soil mass rather than to a fixed depth, to avoid bias caused by collecting more
235 (or less) soil in certain treatments at the same sampling depth (Ellert and Bettany, 1995). In our study, however, bulk density differences between treatments within each site were minimal (see above) ;no equivalent soil masses were calculated, and SOC stocks are indicating that the same amount of soil was present in every depth layer at the same site for the different treatments. Therefore, POC and MAOC stocks were reported down to a common depth, rather than for an equivalent soil mass.

Line 223: Can at least a range of $\delta^{13}\text{C}$ be mentioned for the Farm yard manure?

We understand that this information could be interesting for the reader. However, given that this has not been measured at any time during the experiment, and because the $\delta^{13}\text{C}$ depends on what the animals producing the manure were fed (C3, C4, or a combination of both), there is likely a large range in potential $\delta^{13}\text{C}$ values of the manure. To not bias the reader with uncertain or potentially incorrect estimates, we prefer not to include speculative values.

Line 367 not correct see also other reviewer

Thanks for pointing this out, this has been corrected in the manuscript.

390 dynamics at these field trials. This is limited to results for the top 15 cm, as no significant differences in subsoil (15–50 cm) SOC stocks (Laub et al., 2023a) and MAOC (this study) were detected, thereby ~~rejecting~~ confirming our third hypothesis.

Line 388 not OC stocks but SOC stock?

As we mention “topsoil OC” stocks here, we prefer not to repeat the ‘soil’ in SOC, and stick to topsoil OC. Otherwise, one would read it as topsoil soil organic carbon.

Line 398: would you expect to find differences with higher numbers of replications? Be more explicit here and a bit less

Thanks for pointing this out. We now added the following sentence: “Although it is uncertain whether more replications would have led to the detection of more significant differences, we encourage future studies to aim for the collection of more replicates, whenever possible.”.

415 replicate measurements, which had to be limited to minimize disturbance to the field trials. Although it is uncertain whether more replications would have led to the detection of more significant differences, we encourage future studies to aim for the collection of more replicates, whenever possible.

Line 402: Which are in the lower range. 0,2%-4.9% is a lot lower than the average 8,2%!

Thanks for pointing this out. It seems Fujisake et al. (2018; <https://doi.org/10.1016/j.agee.2017.12.008>) reported standard errors, not standard deviations (they do not clarify this for the value of $8.2 \pm 0.8 \%$ they report, but they note for similar means in their manuscript that the spread is reported as a standard error). This would mean that the mean \pm standard deviation is $8.2 \pm 8.1 \%$ ($n = 102$). Therefore, our values of 0.2 and 4.6 % are within one standard deviation of their mean. That our values are in the lower range of values they report, but not against the lower limit, also appears from their Fig. 5 (see below).

Therefore, we prefer to keep the statement in our manuscript, but clarify it as follows: “These translated into apparent carbon storage efficiencies (CSE_a) of 0.2 and 4.9 %, respectively, which are in the range of values reported for tropical cropland by Fujisaki et al. (2018), with an average of $8.2 \pm 8.1 \%$ (mean \pm standard deviation).

calculated values of 0.5 % and 11.9 % of MAOC consisting of *Tithonia*-derived OC at Embu and Sidada, respectively. These translated into apparent carbon storage efficiencies (CSE_a) of 0.2 and 4.9 %, respectively, which are in the range of values reported for topical cropland ~~(with an average of $8.2 \pm 0.8 \%$; Fujisaki et al., 2018)~~ by Fujisaki et al. (2018), with an average of $8.2 \pm 8.1 \%$ (mean \pm standard deviation). These values were lower than the ones calculated using the temporal trends in

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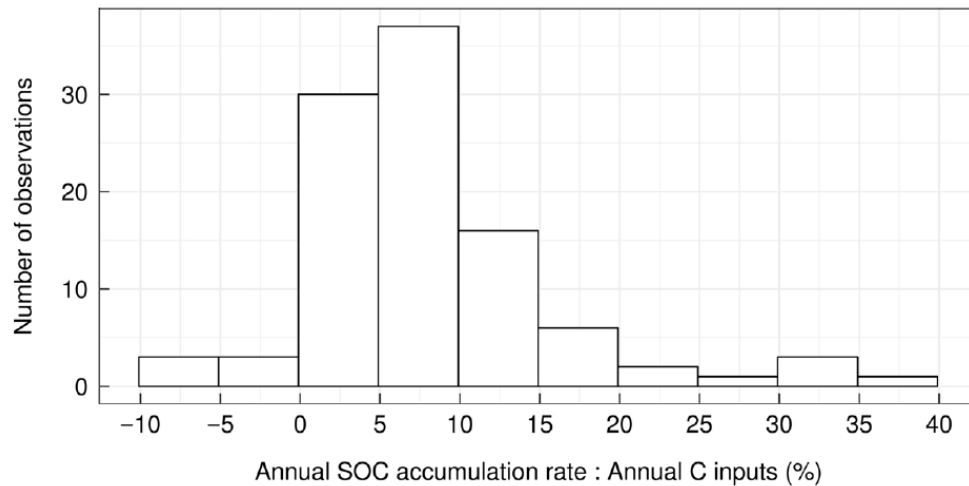


Fig. 5. Histogram of the distribution of the conversion rate of C inputs to Δ SOC, i.e. the ratio between Δ SOC and C inputs ($n = 102$).

Fig. 5 from Fujisake et al. (2018; <https://doi.org/10.1016/j.agee.2017.12.008>)

There is a pressing need for more studies closing the knowledge gap on SOC stabilisation in tropical ecosystems. However, more long-term field trials are needed in tropical ecosystems also in other regions and countries to get representative data.

We fully agree, and hope that future research project can complement our results with more data on SOC stabilization from other tropical regions around the world.