Authors

Firstly, we would like to thank you for the time and effort you have put into this review. We believe that all your comments have been very valuable in improving the first manuscript submission.

In the this document, we have included your comments in black and our response to each comment immediately below in blue color.

Issues with chaOM fraction:

Figure 3a. The reported mean C/N ratio for the chaOM fraction is 4.41, and several values appear to fall between 0 and 2. These values are exceptionally low for any known pool of organic matter. I recommend that the authors clarify whether the C/N values presented for the soil fractions reflect total N or organic N (i.e., after subtracting inorganic N). If only total N was used, recalculating C/N based on organic N would likely provide a more accurate picture of the biochemical composition of these fractions.

Answer:

We are not aware of the removal of inorganic N prior to soil fractionation in other studies, and in soil, C:N ratios are always calculated as OC:TN, given the inorganic N pool is typically negligible. Additionally, given that the fractionation protocol includes several rinsing steps with water, and that ammonium and nitrate are highly soluble, we would expect most of the inorganic N to be removed during the fractionation process. Therefore, the C/N ratio reported in this study primarily refers to organic C and N contents. That said, we are thankful the reviewer brought our attention to these low values, as we share the concern regarding their being unrealistic. This fraction is largely made of sand with minor amounts of OM, thus it is particularly challenging to get reliable estimates on the Elemental analyzer. We have reanalyzed 30 random chaOM samples (15% of the dataset) on another elemental analyzer (CN802, VELP Scientifica, Italy) that can analyze larger samples with greater precision and accuracy, but to which we did not previously have access. We detected significant deviations from the initial measurements only in samples with very low C and N contents (below 0.3% and 0.09%, respectively) in the chaOM fraction. These values were close to the calibration limit of the elemental analyzer used initially to analyze the samples. To correct this analytical limitation, we have reanalyzed all samples whose C and N contents were below the aforementioned thresholds using the new analyzer. In total, we reanalyzed 111 chaOM samples, making up 59% of the entire dataset and four MAOM samples (2% of the entire dataset). When analyzing the corrected data, we have observed much more plausible C/N ratio values for the chaOM fraction (Figure 1), although they are still relatively low. The MAOM and lPOM C/N ratios have changed slightly compared to those presented in the first manuscript submission (Figure 1) due to the new C and N content values of the four MAOM samples that were reanalyzed, as well as the exclusion of five samples that did not meet the C recovery check (explained in lines 255-256 of the former manuscript) after chaOM C content correction. We are much more confident in our results after these reanalyzes.

This new data has not changed the overall story, but it has resulted in a few changes that we report below, at the end of this document.

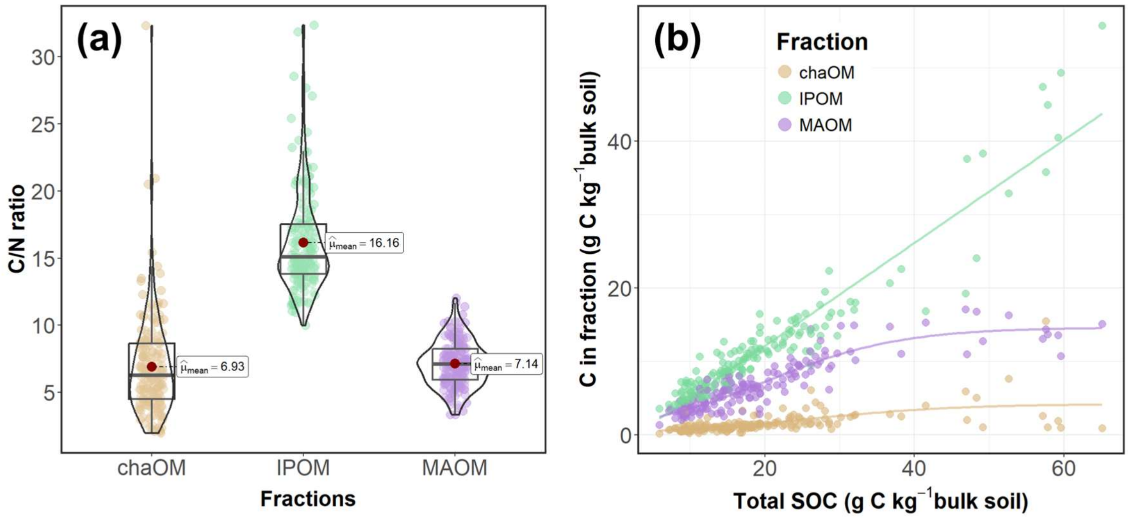

Figure 1. Violin plot and boxplot (with median and quartiles) and mean values (expressed with labels and red dots) for the ratio of carbon to nitrogen content (C/N ratio) in the soil organic matter fractions (a), and relation between carbon content in the soil organic matter fractions (in g of C per kg of soil) and the total soil organic carbon (SOC) content (b). "chaOM" refers to the coarse heavy mineral-associated organic matter, "lPOM" to the light particulate organic matter and "MAOM" to the fine mineral-associated organic matter. In panel "b", a linear regression line depicts the relationship between lPOM and total SOC, while logistic curves illustrate the relationships of chaOM and MAOM with total SOC.

The manuscript reports the use of a combined size-density fractionation protocol following Leuthold et al. (2024), in which the light fraction (<1.85 g cm^{-3}) is separated from the heavier material. The latter is subsequently sieved at 53 µm to yield a coarse heavy-associated organic matter fraction (chaOM; >53 µm) and a fine mineral-associated organic matter fraction (MAOM; <53 µm). The chaOM and MAOM fractions were later combined and reported together as MAOM.

While the decision to include chaOM within MAOM appears justified based on the rationale provided in Leuthold et al. (2024), this approach diverges from the more widely accepted definition of MAOM as material denser than 1.6–1.85 g/cm^3 and smaller than 50–63 µm (Lavallee et al., 2020). Consequently, this choice may limit the generalizability of the findings and their integration into broader syntheses. That said, since the chaOM fraction accounted for less than 5% of total SOM, the implications are likely minor.

Nonetheless, as emphasized by Lavallee et al. (2020), there is a pressing need for greater consistency in the operational definitions of POM and MAOM to facilitate comparability across studies. I recommend explicitly acknowledging that organic matter >53 µm is not typically included in the MAOM fraction.

Answer:

The referee is correct that the chaOM was originally considered distinct from the MAOM and more similar to the POM. In fact, it was initially named heavy POM and included in the POM fraction during size separation. However, more recent work has pointed to the distinct nature of the chaOM fraction from POM, suggesting that it should be considered coarse MAOM. For instance, Samson et al. (2020) found that chaOM characteristics and sensitivity to inputs quality were in transition between both MAOM and POM fractions. In addition, Leuthold et al. (2024) found that, upon a deeper study of the chemical and spectroscopic composition of soil fractions, chaOM and MAOM were more similar to each other than to light POM. In this regard, we believe that, in our study, once we have observed that MAOM and chaOM share similar C/N ratios and relationships with total SOC (Figure 1), it would be inconsistent to mix chaOM and light POM together. Other studies combining chaOM and MAOM in a single fraction after assessing C/N ratios are not rare (Santos et al., 2024; Zhang et al., 2021), and some global meta-analysis combine datasets from both only-density and only-size fractionation methods (Chang et al., 2024; Georgiou et al., 2022). In fact, many studies that only perform size fractionation do not show the C/N ratios of the fractions (Dai et al., 2025; Díaz-Martínez et al., 2024), whereas our study is transparent regarding the success of fractionation and the different properties of the isolated fractions.

In any case, we agree with your proposal to explicitly acknowledge that organic matter >53 µm is not typically included in the MAOM fraction. We have replaced lines 259-261 of the former manuscript with this paragraph (including also a new figure in supplementary material):

"Since the chaOM and the MAOM shared similar C/N ratios, which were lower than POM C/N ratios (Fig.3a), we merged these two mineral associated OM fractions and present them together as MAOM (Santos et al., 2024; Zhang et al., 2021). Thus, in this work, POM and MAOM represented the light (<1.85 g cm $^{-3}$) and heavy (>1.85 g cm $^{-3}$) fractions respectively. This contrasts with other studies based on size fractionation, in which light POM and chaOM are pooled together (Cotrufo et al., 2019a; Dai et al., 2025; Díaz-Martínez et al., 2024). However, our decision is supported by recent findings of Leuthold et al. (2024) who observed greater chemical similarity between chaOM and MAOM than between chaOM and POM. The pooling of the two mineral associated OM fractions is not expected to modify the results or interpretation of this work, as the C content of chaOM only accounts for an average of 8% of the total SOC, and its relative importance is similar across all managements (Fig. S1)"

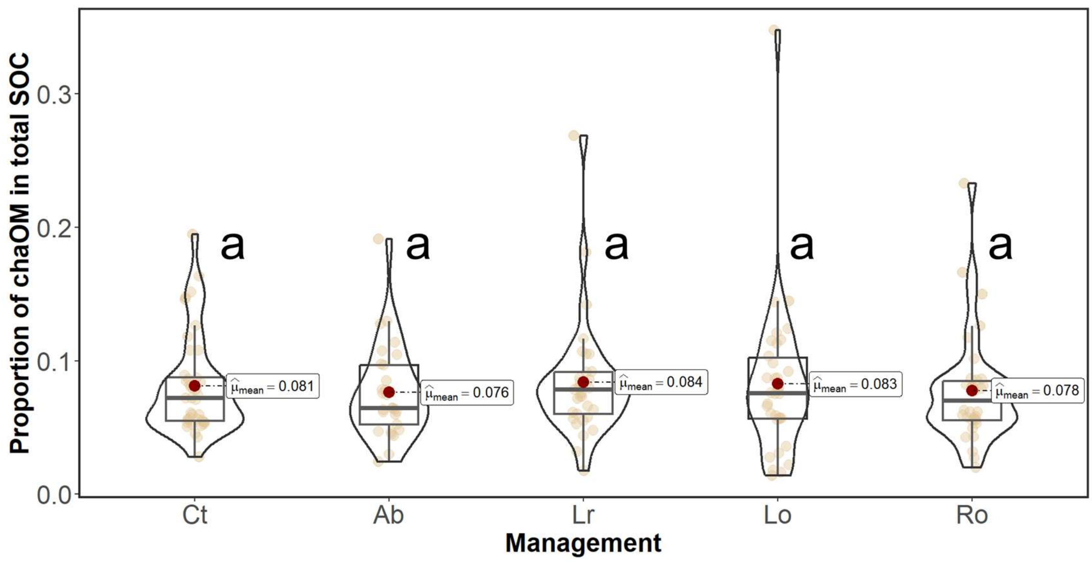

Figure S1. Violin plot and boxplot (with median and quartiles) for the ratio between coarse heavy mineral associated organic carbon (chaOC) and soil organic carbon (SOC in each management (Ct = Continuous grazing; Ab = grazing abandonment; Lr = Recent legume sowing; Lo = old legume sowing; Ro = Rotational grazing).

Labels and red dots indicate mean value. Lower case letters indicate significant differences ($p < 0.05$) between managements according ANOVA testing.

L184-187. 2.2. Climatic variables. This section consists of a single sentence, which may not warrant a standalone subsection. I suggest either expanding it with additional context or incorporating it into a broader methodological section.

Answer:

We agree with your proposal. Instead of expanding the section, we have decided to remove it entirely and include a reformulation of lines 149–152 of the former manuscript:

"The region has a continental Mediterranean climate, but on a local scale, in relative terms, farms can be grouped into three main climatic regions (Fig.1b, c) according to the average climate for the period 1980–2018 (García Bravo et al., 2023). A cold-dry region [12.9 ºC mean annual temperature (MAT) and 445 mm mean annual precipitation (MAP)] in the north; a warm-wet region (17.3 ºC MAT and 603 mm MAP) in the middle of the latitudinal gradient; and a warm-dry region (17.0 ºC MAT and 510 mm MAP) in the south."

L248. SOC was already defined.

Answer: Thanks for noticing, we deleted the SOC definition here.

L249-250. It would be useful to specify whether inorganic carbon (e.g., carbonates) was measured or removed prior to C analysis.

Answer:

Carbonates concentration was measured in all bulk soil samples, with a value of 0 in all cases. We have included a brief explanation in this regard after line 248 of the previous manuscript, in section '2.5. Soil organic matter fractionation":

"First, 10 g aliquots of all samples were ground in a ball mill and the Bernard's calcimeter method (Sherrod et al., 2002) was used to test for the presence of inorganic C. Only four samples (0.02 % of the database) contained traces of carbonates, with $CaCO_3$ contents between 0.2 % and 0.8 %. These samples were excluded from subsequent analyses."

Figure 4. Please add the slope of the regression line. This figure should be in the results section.

Answer:

Suggestion followed. We have moved Figure 4 to the Results section, right after line 354 of the original manuscript, and included the slope of the regression line in the plot: "MAOC = (0.86 ± 0.9) * CS"

L299. This sentence should be included in another paragraph.

Answer: We have moved it to the end of the section.

Figure 5

- The PCA plots refer to the components as "Dim", whereas the figure legend refers to them as "Axes". For consistency and clarity, I recommend using a single term.

Answer:

Thanks for the suggestion, we have changed all component names in the PCA to “Axes” for consistency.

- Axis 1 is labeled “C/N axis”. However, C/N is a single measured variable, and a PCA is not required to assess it alone. This axis also includes strong loadings from other traits such as ABG cellulose and CWM hemicellulose. Therefore, the axis appears to represent a broader concept, perhaps related to litter quality or decomposition potential, rather than just the C/N ratio. I suggest reconsidering the label to better reflect the multidimensional nature of the trait composition.
- A similar issue applies to Axis 3, labeled “Lignin axis”. Lignin is again only one of several traits contributing to the axis. Since this component also captures variation in ABG hemicellulose and CWM LNC, among others, it likely reflects a broader litter chemistry or quality gradient rather than lignin content per se.

Answer:

We agree with the reviewer that the axis labels do not capture their multivariate nature, and their names can be confused with those of variables included in the PCA. We have changed the first axis name to “N-C stoichiometry”, because it largely reflects the C/N ratio of plant inputs, which is obviously positively correlated with the cellulose and hemicellulose content (the source of C in plant tissues) and negatively correlated with the N content of vegetation and its leaves. With regard to the third axis, we have changed its name to “productivity-lignification (P-L) axis” to highlight that the axis is negatively correlated with the plant ABG production and positively correlated with lignin content.

- In panel (h), ABG hemicellulose is more negatively correlated with the axis than CWM SLA, yet only CWM SLA is shown in the schematic.

Answer: Sorry about this omission, we have included ABG hemicellulose in the schematic.

- Finally, I recommend reducing the number of figure panels by integrating each schematic (panels e–h) below the corresponding PCA biplot (a–d). This would streamline the presentation while preserving the valuable visual summary of trait syndromes.

Answer: That is a very good idea, we have changed the figure accordingly. Attached below is the figure with all the proposed corrections (Figure 2).

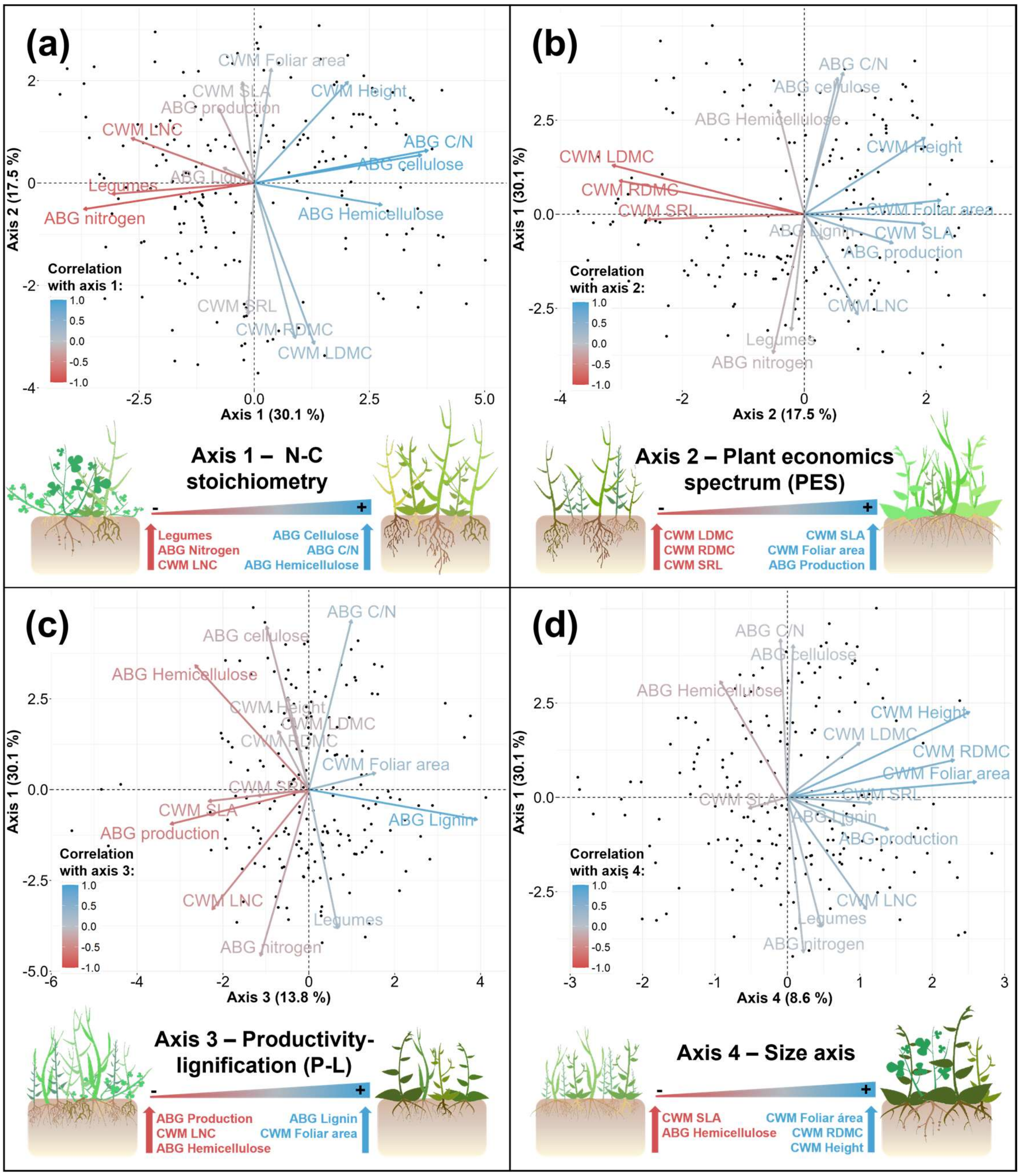

Figure 2. (Figure 5 in the manuscript) Representation of the 4 main axis of variation in the principal component analysis (PCA) summarizing the vegetation characteristics variables. Panels a, b, c, and d illustrate the correlation between the different variables included in the PCA and the new axis, with a graphical representation of the characteristics of the plant communities at the end of each axis. Representative species of each axis are shown in Fig.S1.

L322. ... bulk density (BD). What was the range of BD? Was it similar across treatments? If not, equivalent mass corrections should be considered.

Answer:

The bulk density values in our data range from 0.66 to 2.1 g/cm^3, with some variation between the treatments. However, the bulk density used in equation 1 to calculate carbon (C) stocks is the bulk density of the fine soil (less than 2 mm) after excluding coarse material (gravel and large roots). This fine soil bulk density ranges from 0.43 to 1.66 g/cm^3. Since the gravel content in the soil differs between treatments, the distribution of fine soil bulk density across treatments does not exactly match that of normal bulk density (more information below). To limit the influence of our approach, we also analyzed the POC, MAOC, and SOC contents (Figures S4 and S5 of the former supplementary material). The POC, MAOC and SOC content analysis is briefly discussed in lines 547-548. The results of the content and stock analyses were very similar. To be clearer and more transparent about this issue, we have included the following lines in section "2.6: Data Analysis," below line 343 of the original manuscript:

"Given that average bulk density values partly differed among managements (Figure S3), and that this variation may affect carbon stocks, the same analysis procedure used for POC, MAOC and SOC stocks was used to analyze the change in POC, MAOC and SOC contents."

In this study, where samples were taken at a fixed depth, mass equivalent correction of C stocks would be equal to analyzing C content, as we have done.

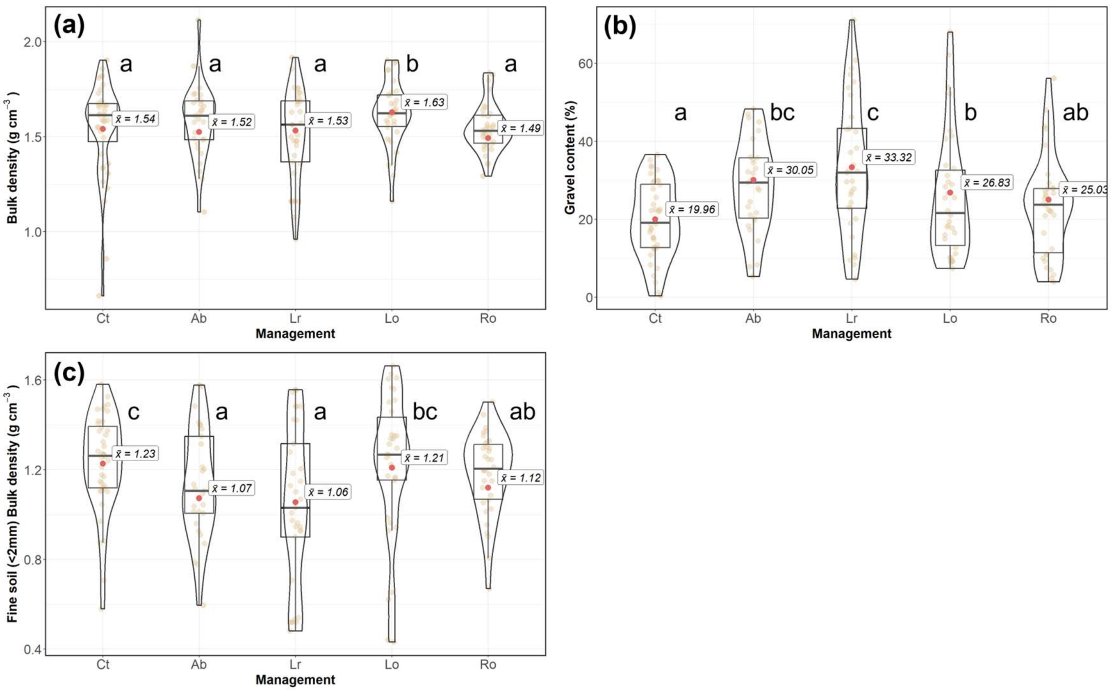

Figure S2. Violin plot and boxplot (with median and quartiles) for the (a) soil bulk density, (b) soil gravel content and (c) fine soil (<2mm) bulk density, as used in equation 1 in the main text, in each management (Ct = Continuous grazing; Ab = grazing abandonment; Lr = Recent legume sowing; Lo = old legume sowing; Ro = Rotational grazing). Labels and red dots indicate mean value. Lower case letters indicate significant differences ($p < 0.05$) between managements according ANOVA testing.

L340. Could you please clarify what is a direct and indirect effect?

Answer:

Lines 329-331 specify the direct and indirect (through effects on other variables) paths through which the effects of the variables can be transmitted.

L364-365. "…informing on lignin content and vegetation productivity, was negatively correlated with the POC, MAOC and SOC stocks and the microbial biomass". Lignin content was negatively correlated with vegetation productivity. Therefore, those variables were negatively correlated with lignin, but positive correlated with vegetation productivity.

Answer:

That is correct, but in this case, we are explaining that the third axis of the PCA (now the "productivity-lignification (P-L) axis") is negatively correlated with POC, MAOC, and SOC stocks and microbial biomass. Figure 5 shows that this PCA axis is positively correlated with lignin and negatively correlated with ABG productivity.

L430-432. "The silvopastoral character of our farms could explain the low MAOC/SOC ratios, as litter from scattered trees increases carbon stocks in woody grasslands, especially in the POM fraction". This explanation is plausible; however, your results show that lignin and C/N were negatively correlated with POC. How do you reconcile this apparent inconsistency?

Answer:

To avoid this inconsistency, we have decided to remove the interpretation of low MAOC:SOC ratios due to the silvopastoral character of the study area (lines 430-432 of the former manuscript), maintaining the explanation based on sampling depth, which is more robust.

L439. I don't see a saturation curve in Fig. 3 b. Figure 4 shows a better way to illustrate the C-saturation deficit. It would be interesting to compare the results with the maximum C-loading found by Georgiou (~ 86 mg C g silt+clay).

Answer:

Following the reviewer's suggestion, we have included a saturation curve in Figure 3b (Figure 1 on this document). On the other hand, in line 439, we point out that even though our soils were far from the MAOC saturation capacity according to the results of Georgiou (as shown in Figure 4), we still observed a limit to MAOC accumulation at high total SOC concentrations. This is shown in Figure 3b, where POC increases linearly with increasing SOC, but MAOC seems to saturate and stay stable above SOC contents of 30 g/kg^{-1}. To clarify this point, we have rewritten lines 437-440 of the former manuscript:

"Carbon concentrations in the fine soil fraction (clay + silt) were well below the saturation point observed in previous studies (Cotrufo et al., 2019b; Georgiou et al., 2022). However, we observed a certain limit to MAOC accumulation in our system, as its content remained stable above SOC contents of 30 g/kg^{-1} and stayed below 20 g kg^{-1} even when SOC reached values above 60 g kg^{-1}, following a saturation curve (Fig. 3b)."

L497-498. However, roots promote MAOM formation not from root biomass but from rhizodeposition (see Sokol, N.W., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. Nature Geosci 12, 46–53. https://doi.org/10.1038/s41561-018-0258-6; Villarino, S.H., Pinto, P., Jackson, R.B., Piñeiro, G., 2021. Plant rhizodeposition: A key factor for soil organic matter formation in stable fractions. Science Advances 7, eabd3176.)

Answer:

We agree that this positive relationship between rhizodeposition and MAOC formation somewhat contradicts our discussion on this point. Without data on rhizodeposition or root biomass, we can only propose and discuss possible explanations for the relationship between the size axis and MAOC stock, pointing out their shortcomings. Therefore, we will reword the paragraph from lines 502 to 510 of the former manuscript as follows, including the references proposed by the reviewer on the role of rhizodeposition:

"We also observed that taller and larger plants (high values on the size axis) were associated with higher MAOC stocks and MAOC/SOC ratios, although the mechanism driving this correlation was unclear. Generally, plant height is positively correlated with shoot:root ratio (Li et al., 2008), and several studies have found a higher contribution of shoots, rather than roots, to the MAOC fraction due to the higher recalcitrance of root tissues (Huang et al., 2021; Lavallee et al., 2018; Ridgeway et al., 2022). However, rhizodeposition, which is closely linked to root biomass, has been shown to promote MAOC formation over POC (Berenstecher et al., 2023; Villarino et al., 2021). On the other hand, a greater accumulation of standing litter, rather than surface litter, might be expected in communities with bigger plants, and some studies in semi-arid grasslands have observed higher rates of microbial degradation and release of soluble compounds (thus contributing more to MAOC) in standing litter, compared to surface litter, due to greater retention of night-time moisture in the former (Gliksman et al., 2018; Wang et al., 2017)."

L546. In line with my previous comment, bulk density values and changes should be reported. Previously, the acronym BD was used for apparent density.

Answer:

Please see our answer above about BD.

L590. Mineral-associated organic carbon was previously defined as MAOM.

Answer:

Thanks for noticing, we have removed the definition here.

Additional changes:

The changes made to the database following the reanalysis of the samples mentioned in the response to the first comment have resulted in some slight changes to the study results, which are outlined below.

The effects of rotational grazing have become significant and positive for both POC and MAOC (Figure 2.a & b). In the previous analysis, these effects were only significant in the case of MAOC and were close to being significant for POC (Figure 7 in former manuscript). On the other hand, the negative effect of recent legume sowing on the MAOC/SOC ratio is no longer significant, and the positive effect of abandoning grazing has become significant (Figure 2.d). The rest of the relationships have remained the same as those reported in the previous manuscript.

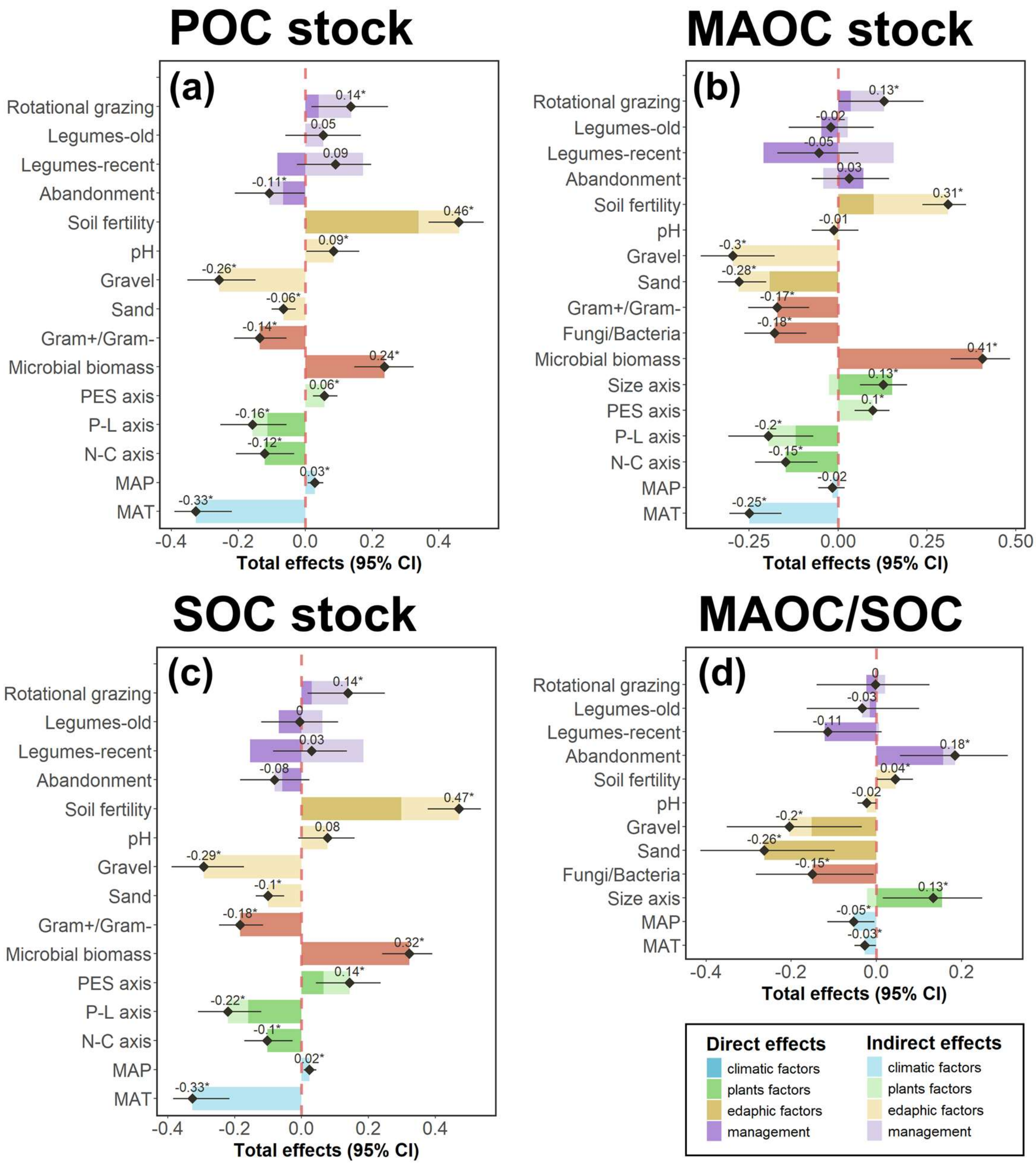

Figure 2. Direct, indirect and total standardized effects of all studied variables included in the structural equation model (Figure6) over the (a) particulate organic carbon (POC), (b) mineral-associated organic carbon (MAOC), and (c) soil organic carbon (SOC) stocks and (d) the relative MAOC abundance (MAOC/SOC). Bars indicate direct (dark colors) and indirect (light colors) effects, and the black points-ranges indicate the total (i.e. direct + indirect) effect (with its 95% confidence interval). Stars over the total effect values indicate significant effects at a level of 0.05.

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