

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 our manuscript.

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

Please notice that, motivated by reviewer 1's justified concerns about the data for the chaOM fraction, we have reanalysed 111 samples from that fraction, which has slightly changed some of the study's results. These changes are detailed in the response to reviewer 1.

Specific comments

Abstract:

Please describe the reason for only sampling to 8 cm depth in the introduction and/or M&M, and consider mentioning the depth in the abstract or, alternatively, write “upper topsoil” instead of just “topsoil” in the abstract. I believe there might be arguments for choosing a relatively shallow depth, but the arguments are not mentioned nor discussed (microbial dynamics predominantly occurring in the upper topsoil? No/low tillage, etc.). In terms of the management effects on soil C stocks, the conclusions of the study are limited to the upper 8 cm - which should be discussed.

Answer:

Thank you for this comment. We have added the specific depth in the Abstract line 20 “on topsoil (0-8cm) SOC ...” and in the Introduction, line 117 “The aim of this work is to evaluate the impact of rotational grazing, LRP and grazing exclusion on topsoil (0-8cm) SOC stocks ...”. In the Method section 2.4 we already report the soil depth sampled.

Additionally, we have included the following paragraph in the discussion, after line 423 of the former manuscript, to justify our decision and discuss its limitation:

“Our results are limited to the upper topsoil (the first 8 cm), and it would be necessary to analyze deeper layers to fully understand the processes of SOC formation and stabilization and the effects of management changes. However this top layer is the most important for mediterranean grassland functioning as it contains the majority of roots and therefore most of the microbial, nutrients and water dynamics (Acosta-Gallo et al., 2011; Moreno et al., 2005). Furthermore, changes in management primarily affect the SOC of the topsoil layer, especially in the short term. (Ward et al., 2016)”

Keywords/introduction:

I find the expression “Legume enrichment” slightly unclear/confusing. In my opinion, it connotes with “isotope enrichment experiments”, where the legumes themselves are isotope enriched. Consider if writing “Legume sowing” or “legume inclusion/addition” as keyword and where it is mentioned in the manuscript (e.g., line 58), could be an alternative.

Answer: We appreciate the suggestion and have replaced “enrichment” with “sowing”.

L109-112. Species richness effect on MAOC/POC fractions in managed grasslands have been investigated recently by Mortensen E.Ø., Abalos D., Engedal T., Lægsgaard A.K., Enggrob K., Mueller C.W., Rasmussen J. (2025) Smart mixture design can steer the fate of root derived carbon into mineral-associated and particulate organic matter in intensively managed grasslands. *Global Change Biology* 31: e70117. DOI: 10.1111/gcb.70117. The study observed no effect of species richness *per se*, but effects of plant functional group (grasses vs. legumes). Please consider this in the introduction and/or discussion section, i.e., how that compares with the findings of your study.

Answer:

The article is very interesting, although it focuses solely on the formation of MAOC and POC derived from rhizodepositions, so its comparability with our study is limited. However, we have considered it appropriate to cite in the introduction (line 109-112):

“Other vegetation characteristics such as species richness has been shown to positively influence SOC stocks (Lange et al., 2015; Steinbeiss et al., 2008), but its effects on SOC fractions have been poorly evaluated, with inconclusive results in grasslands (Mortensen et al., 2025). In addition, the relationships between SOM stocks and fractions and plant functional traits have rarely been studied (Manning et al., 2015; Mortensen et al., 2025; Xu et al., 2021) despite the latter being widely used to predict ecosystem functioning and responses (Funk et al., 2017)”

In addition, we have referred to the article in the discussion (lines 477–478 and 484–486 of the previous manuscript).

Material and method:

L162: What is the lower range of time for the legume enrichment/sowing? Does the study also include paddocks where legumes have been sown within the last year or 2, or is there a minimum of “incubation time” for the effects to be observed? E.g. “5-2 years”?

Answer:

Table S1 provides information on the year of sowing in paddocks sown with legumes. Some paddocks were sown only 1 or 2 years before sampling. These sowings are usually carried out in October, and the effects can be observed in the same growing season (the following spring).

L163-165: It would be good with a few more words on the legume species that are chosen in those mixtures that are seeding on the farms, instead of only mentioning the broad genus names and referring to another study. Is it always the same seed mixtures, and/or on which criteria are the seeded species selected? Which are the most dominating legumes in the farms that are already established before legume sowing (based on the botanical assessment)? Here, I am thinking specifically on the root systems, whether shallow and/or deep-rooted legumes existed before/are sown. I think this is relevant for the reader,

especially because of the no/negative effects of recent legume sowing on SOC and MAOC stocks. The type/species of sown legumes may have an influence on which effects legume sowing induce/not induce in the upper 8 cm of the topsoil – and which potential effects below 8 cm may not be covered in this study. This caveat should be mentioned when discussing the results.

Answer:

Seed mixtures are mainly marketed by a single private company in Portugal and Spain, and the specific composition remains essentially constant from year to year. In this sense, most farmers sow the same mixtures (it is a widespread practice in the area). To clarify this point, and include the sowed species, we have rewritten lines 162–165 of the previous manuscript:

*“- Recent legume sowing (Lr): Paddocks where pastures have recently (≤ 5 years) been sown with legume mixtures. In dehesa farms, legume sowing consists of sowing a mixture of seeds (at a rate of 20 kg ha^{-1}) from various species of annual legumes (pre-inoculated with *Rhizobium*) such as *Trifolium subterraneum* L., *T. incarnatum* L., *T. michelianum* Savi., *T. resupinatum* L., *T. mutabile* Port. and *Ornithopus sativus* Brot., along with some highly productive annual grass species such as *Lolium multiflorum* Lam. and *Lolium rigidum* Gaud. (Teixeira et al., 2015).”*

It should be noted that all sown species are annuals with relatively shallow root systems. Therefore, the effects of sowing would be expected to be noticeable at 8 cm soil depth. Importantly, several of the species sown can be found naturally in these pastures, or at least species with similar characteristics. These sowings only increase the proportion of these species (more productive and mainly legumes) in the community.

L202: I don't find any results on the aboveground species botanical composition. In line with the comment above, a (supplementary) table with species composition, or at least mentioning the dominant species, would increase our understanding of the system. Does this vary too much between the farms to provide this information, or are there some general species (non-legumes and legumes) that dominate/characterize the farms used in the study?

Answer:

Our vegetation surveys have identified more than 200 species. It should be noted that these Mediterranean grasslands tend to be highly diverse, typically containing 10–20 species per square meter in our study and up to 40 species according to literature. The farms, managements, and climatic regions share most of the species. To clarify this point, we have included a table in the supplementary material listing the common species in each functional group (grasses, forbs, and legumes) and have rewritten lines 147–150 of the previous manuscript:

“The herbaceous layer is composed of species typical of Mediterranean pastures and presents a high diversity and proportion of annual C3 plants (Table S1).”

Table S1. Examples of the most representative species found in the floristic inventories of the study for each functional group (grasses, legumes, and forbs).

| Species | Functional group |
|--------------------------------------|------------------|
| <i>Lolium rigidum</i> Gaud. | Grass |
| <i>Bromus hordeaceus</i> L | Grass |
| <i>Hordeum murinum</i> L | Grass |
| <i>Vulpia geniculata</i> L. | Grass |
| <i>Anthoxanthum aristatum</i> Boiss. | Grass |
| <i>Agrostis pourretii</i> Willd. | Grass |
| <i>Trifolium subterraneum</i> L | Legume |
| <i>Trifolium glomeratum</i> L. | Legume |
| <i>Ornithopus compressus</i> L | Legume |
| <i>Vicia sativa</i> L. | Legume |
| <i>Lathyrus angulatus</i> L. | Legume |
| <i>Hymenocarpus lotoides</i> L. | Legume |
| <i>Anthemis arvensis</i> L. | Forb |
| <i>Echium plantagineum</i> L. | Forb |
| <i>Crepis capillaris</i> L. | Forb |
| <i>Plantago lagopus</i> L. | Forb |
| <i>Sisymbrium officinale</i> L. | Forb |
| <i>Rumex acetosella</i> L. | Forb |

L231: As mentioned above, 8 cm is quite shallow when considering the effects of species on soil C. Please, argue why 8 cm depth was chosen instead of e.g., 15, 20, or 25 cm that would include the effect on a larger part or the entire topsoil layer.

Answer: As we mentioned above, we have added a sentence in the discussion arguing why we chose to sample 8 cm depth and the limitations of our study as compared to sample deeper soil layers.

L281: Is the reference (“as shown in eq. 1”) referring to the same equation that are used to calculate OC in soil C fractions? If so, please make this clear, as it does not seem obvious that microbial biomass stocks can be calculated by PLFA via the same equation.

Answer: We have clarified this point by rephrasing lines 280–281 of the former manuscript: “Further, we estimated microbial biomass stocks (in mol ha⁻¹) by substituting the OC_{content} by the total PLFAs concentration in equation 1.”

L304: Does “the latter being considered a good proxy for the lignin content (Van Soest et al., 1991).” refers to ADL – or to “ADF minus ADL”? If it’s the first, I suggest clarifying it by writing: “... ADF minus ADL, **ADL** being considered a good proxy for the lignin content (Van Soest et al., 1991)”.

Answer: You are right, we rephrased the sentences as you suggest.

Results:

L358: Please check if the mentioned negative correlation between the Fungi/Bacteria ratio and MOAC stock was statistically significant? It does not look like that in the Figure 7b annotation, and in that case, you could argue for a tendency while just stating that they are negatively correlated is wrong if it is not statistically significant. Same comment for line 455 where this correlation is mentioned again.

Answer:

There were inconsistencies between the confidence intervals and the significance of the relations provided by the linear mixed models that compose the SEM (Figure 6) and the total effects estimation using the "semEff" package (Figure 7). The relationship between the fungi/bacteria ratio and MAOC was significant in the linear mixed model estimation, but not significant when using the "semEff" package. This discrepancy was due to the way the bootEff() function in the "semEff" package estimates variable effects and intervals, using nonparametric bootstrapping by default. We changed the bootstrapping method to "parametric" in this function to ensure more consistency with the linear mixed models estimates (lmer models). We have updated all the graphs according to the new analysis.

L358 and line 359: For both sentences here, you would ease the readability by adding an explanatory sentence in both places, e.g. such as "... meaning that a higher proportion of fungi over bacteria correlated with higher MAOC stock" – or what direction the correlation may have. Especially when ratios are negatively correlated, it becomes a lot of twisting to interpret the direction of the effects, unless you are very familiar with the parameters.

Answer: We appreciate the suggestion. We have included that explanation.

Discussion:

L417: I recommend writing something like "... long-term carbon storage in the upper soil layer/topsoil", or in other ways mentioned the depth restriction of the conclusions already here.

Answer: Done, we have rephrased the sentence as you suggest.

L437-443: This is an important point and paragraph! Good.

Answer: Thanks for the positive comment.

L477-478 and 484-486: These are two places where it may be relevant to discuss whether your findings align with Mortensen et al., 2025, mentioned above (doi.org/10.1111/gcb.70117).

Answer: We have cited that study on those lines, as we believe it is well-aligned with what we discussed there.

L541-551: In this section (the effects of legume sowing), it is particularly relevant to discuss the shallow sampling depth of 8 cm, and making us aware of the limitations of the conclusions. Sowing new species will most likely also affect carbon and nitrogen dynamics below 8 cm, and thus the total SOC/POC/MAOC stocks of the soil profile. This aspect could also be discussed in a separate paragraph for all management effects, but at for the effect of legume sowing.

Answer: As mentioned above, sowed legumes have shallow root systems and main impact would be expected to occur in the topsoil layer. A paragraph discussing the study limitations have been already provided in a previous comment.

L593: Please change from “are” to “were” (saying that your study found this - not common knowledge), or if generalizing this statement to more than your study, at least define the limit of this conclusion to the upper topsoil layer and to semi-arid grazed grasslands. As it stands now it is a very strong generalization, which may not necessarily be the same in other regions, agricultural systems, nor if a deeper soil profile was investigated.

Answer: You are right, we have replaced it with ‘were’.

Wording and typos:

L97: Typo. Change from “MENS” to “MEMS”

L119: Consider writing “... **bio**chemical and morphological traits”

L213: I assume the last word of the sentence should be in past tense: “...measured...”

L302: Change to “These analyses...” (plural)

L322: Check grammar. I would write “Nutrient concentrations in each soil were...”

L330: Insert comma: “SOC, POC and MAOC”

L372: I assume this should be in past tense to align with the rest of the results (“increased”) not indicating general knowledge which would be in present tense.

L385: Delete “it”, writing just “*As expected, being negatively*”

L559: Change to “Our results are in line with...”

L617-621: Please check the use of “” signs. There seem to be lacking some "" around some parts. Also align first names in this section to be either full first names or just the first letter.

Answer: Thank you for this thorough review. We have corrected all the errors you have pointed out.

Figures:

Fig. 2: In the figure caption, do you mean to write “References embedded in the figure or Fig. 2....?”

Answer: We meant to write “References embedded in the figure”, we have corrected it.

Fig. 4: There might be missing a word in the end of the first line: “... in the studies ____? ____ soils and... ”. Or split the sentence into two. It does not make sense how it is currently reading.

Answer: You are right, it is a typo, we meant to write “....in the soil samples....”

Fig. 5: Please increase the font size of most elements in the figure. As it is now it's not possible to read all variable names in the PCA's in a 1:1 printout, tricky to read the legends, and very tricky to read the blue/red text in the gradient explanations/axis explanation (in the lower part of the figure). It is a shame since the figure contains a lot of good information. Although it is nice that the text coloring follows the legend/gradient in the PCA's, it also makes it harder to read the text. Maybe the coloring can be kept if the text size increases – or vice versa if text is darker.

Answer: We have increased the font size as much as possible while avoiding overlap. Below is the new Figure 5, with some modifications also proposed by reviewer 1.

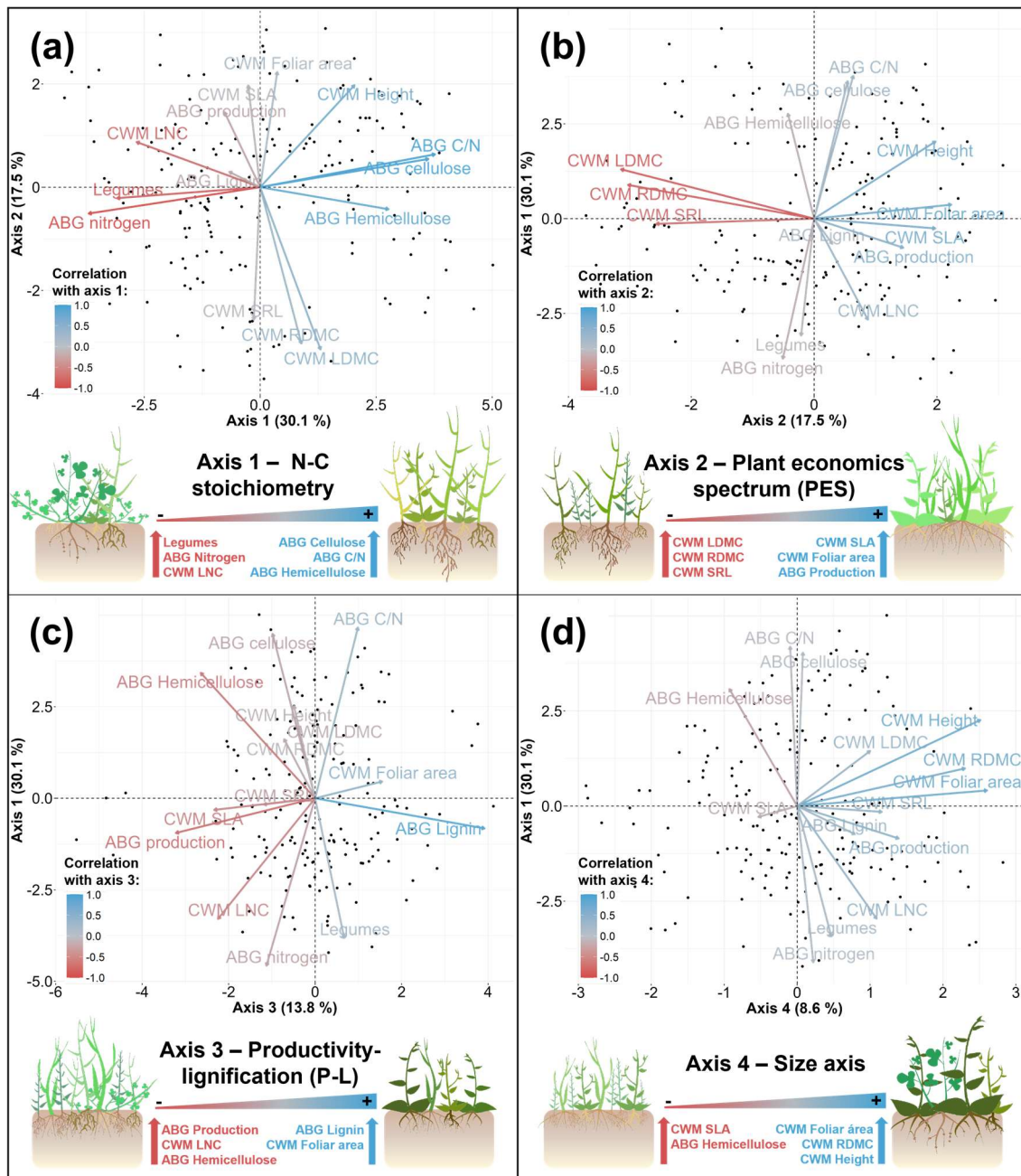


Figure 5. Representation of the 4 main axis of variation in the principal component analysis (PCA) summarizing the vegetation characteristics variables. Panels a, b, c, and d illustrate the correlation between the different variables included in the PCA and the new axis, with a graphical representation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

Fig. 6: Axes font size within legends is very small, making it impossible to read certain words in a 1:1 size. Also, consider changing “R2” to R^2 in the center circle (stock changes). Other than that, it is a key useful figure. In the figure text, you can delete one “s”, thus writing “...by factor type...”.

Answer: We have made the requested corrections and increased the font size as much as possible (Figure 6). However, due to the amount of information included in the figure, the font size is still relatively small. We hope that this will be acceptable to the reviewer.

Answer: Suggestion followed, we have increased font size.

Figure S3: Increasing font size of labels would make it possible to read them.

Answer: Suggestion followed, we have increased font size of labels.

Figure S5: Please provide the unit for the content in the figure caption - or if it is centered and scaled / indexed, mention this in the figure caption as well.

Answer: As indicated in the caption, the figure shows standardized effects, which are unitless.

References:

Acosta-Gallo, B., Casado, M. A., Montalvo, J., and Pineda, F. D.: Allometric patterns of below-ground biomass in Mediterranean grasslands, *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, 145, 584–595, <https://doi.org/10.1080/11263504.2011.578836>, 2011.

Funk, J. L., Larson, J. E., Ames, G. M., Butterfield, B. J., Cavender-Bares, J., Firn, J., Laughlin, D. C., Sutton-Grier, A. E., Williams, L., and Wright, J.: Revisiting the Holy Grail: using plant functional traits to understand ecological processes, *Biological Reviews*, 92, 1156–1173, <https://doi.org/10.1111/brv.12275>, 2017.

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