

Authors' responses to reviewers' and editor comments on:

Tree-microbe-soil interactions affecting soil organic carbon fractions in Mediterranean forest soils

Editor:

Dear authors,

Your manuscript has now been evaluated by two reviewers, who provided generally constructive assessments and identified several points requiring clarification or revision. Your responses address many of these points, and I am pleased to invite you to submit a revised manuscript. In addition to implementing the revisions outlined in your responses to the reviewers, please consider the following points:

- One of the newly suggested sentences is very long. Please split it to improve readability:
"We hypothesize that mixed forests will exhibit higher total SOC stocks, with a greater proportion of MAOC compared to mono-specific forests, due to greater and more diverse plant-derived C inputs and their microbial processing, which may enhance the production of microbial-derived organic compounds that can be stabilized through association with soil minerals."
- Please replace the term capacitance with the established terminology "MAOC capacity" throughout the manuscript
- Please note also that R2 cannot be negative. This needs to be corrected.
- Regarding the reviewer comments on MAOC saturation values exceeding 100% and on the limitations of the MAOC saturation concept, your response explains that values >100% can arise from the empirical procedure used to estimate MAOC capacity. Please expand the revised manuscript to address the broader conceptual point raised by the reviewers. In particular, please explicitly acknowledge the limitations of interpreting the estimated MAOC capacity as a strict physical ceiling and discuss whether values >100% may reflect not only the empirical estimation procedure but also mechanisms such as organo-organic interactions that can lead to >100% MAOC saturation.

I look forward to receiving your revised manuscript.

Kind regards

Sara Vicca

Response: Thank you for your careful evaluation of our manuscript and for the constructive comments and suggestions. We appreciate the opportunity to submit a revised version. We addressed all the points raised, including splitting the long sentence for clarity and replacing “capacitance” with “MAOC capacity” throughout the manuscript. Regarding the negative R^2 we would like to clarify that this was a pseudo- R^2 associated with the quantile regression rather than a conventional R^2 . We agree that its presentation could be misleading, particularly because pseudo- R^2 measures in quantile regression do not have the same interpretation as the coefficient of determination in ordinary least-squares regression. To avoid confusion, we have removed the pseudo- R^2 value from the revised figure. We also expanded the discussion of the conceptual limitations of MAOC capacity and saturation. We clarify that the estimated MAOC capacity should not be interpreted as a strict physical ceiling and discuss how values exceeding 100% may reflect both the empirical estimation procedure and mechanisms such as organo-organic interactions.

We incorporated these revisions, together with the changes outlined in our responses to the reviewers below, into the revised manuscript.

Reviewer1:

This study collected soil samples from different forests (or forest stands), measured soil organic carbon and its fractions as well as related soil properties and analyzed the relationships between soil carbon fractions and soil organic carbon storage, together with the factors influencing soil carbon fractions. Overall, the study provides comprehensive data, employs appropriate analytical methods, and presents clear descriptions across all sections. The manuscript is generally well written and clearly organized. I have no major concerns regarding this manuscript, but only some specific minor comments for the authors to consider.

Response: We thank Reviewer 1 for the valuable comments and insights, which helped improve our manuscript.

L26-49: The title of this manuscript is “Tree–microbe–soil interactions,” but the description of these interactions in the abstract is insufficient. It is suggested that the authors strengthen this aspect in the abstract.

Response: We thank the reviewer for this comment. We agree that the previous version of the abstract did not sufficiently emphasize the interactions among tree species, soil properties, microbial communities, and soil C fractions. We suggest revising the abstract to more explicitly describe these relationships.

L42-44: The abstract should focus on how environmental and microbial factors influence soil organic carbon and its fractions, rather than overemphasizing other indicators such as microbial diversity.

Response: We agree and will revise the abstract accordingly. We reduced the emphasis on microbial diversity and instead focus on the relationships between soil physicochemical conditions, microbial community composition, and SOC fractions.

Suggested revision:

“In contrast, MAOC showed an apparent saturation pattern, reaching approximately 45 g C kg⁻¹ soil, and was strongly constrained by clay and silt content, indicating limited potential for additional mineral-associated C storage in soils approaching saturation. Tree-associated changes in soil C were accompanied by shifts in microbial community composition that depended strongly on microsite conditions. In particular, bacterial communities beneath tree canopies represented subsets of the more diverse communities occurring in forest gaps, suggesting that tree-induced changes in soil conditions act as an environmental filter on microbial assemblages. However, variation in microbial richness itself was not consistently associated with changes in SOC, POC, or MAOC. Together, these results show that tree effects on SOC emerge through interactions among species identity, microsite conditions, soil physicochemical constraints, and microbial community composition, with different controls operating on labile and mineral-associated C pools. Transitioning from monospecific pine stands to mixed forests could increase SOC by approximately 6.1 Mg C ha⁻¹, but this carbon is expected to accumulate primarily in the labile POC pool, particularly where MAOC is already close to saturation.”

L146-148: This is not a complete hypothesis because the underlying mechanism is not stated. In addition, forest gaps receive relatively lower plant carbon inputs and therefore decomposition processes are expected to dominate. Since POC fractions are more easily decomposed, it can be hypothesized that the proportion of MAOC may be higher.

Response: We thank the reviewer for this comment. Rather than retrospectively replacing our original hypothesis, we suggest revising the text to clarify the underlying mechanism and acknowledge the alternative prediction suggested by the reviewer.

Suggested revision:

“We hypothesize that mixed forests will exhibit higher total SOC stocks, with a greater proportion of MAOC compared to mono-specific forests, due to greater and more diverse plant-derived C inputs and their microbial processing, which may enhance the production of microbial-derived organic compounds that can be stabilized through association with soil minerals. We further hypothesize that the capacity for MAOC accumulation will be

constrained by soil mineral properties, particularly clay and silt content, such that soils with greater mineral-associated C storage capacity will support larger MAOC pools. At the microsite scale, we hypothesize that forest gaps, will contain lower total SOC than soils beneath tree canopies because of reduced litter and root-derived C inputs. Lower C inputs in forest gaps may either reduce microbial decomposition and favor POC accumulation, or, if decomposition exceeds C inputs, preferentially deplete POC and increase the relative contribution of MAOC. To test these hypotheses, we used structural equation modeling (SEM) to explore the direct and indirect effects of forest mixing, microsite, tree traits, and soil properties on SOC dynamics (Fig. 1a), providing insights into the complex interactions that drive carbon storage in Mediterranean forests.”

L157-158: What is the mean annual temperature? This is a more important indicator. Please provide it.

Response: Information will be added.

Suggested revision:

“The climate in this area is characterized as hot Mediterranean, with a mean annual precipitation of 510 mm between September and May and mean annual temperature of 20.0 °C, fluctuating between 16.0 °C in February and 25.3 °C in August”.

L217-219: It is usually not appropriate to define particles >50 µm as sand and those <50 µm as silt plus clay, because different soil classification systems use different thresholds (for example, in some systems particles >20 µm are defined as sand). It is recommended to instead define particles >50 µm as POM and those <50 µm as MAOM.

Response: We thank the reviewer for this comment. We agree that the differing size thresholds for sand, silt, and clay across various classification systems can lead to confusion when using this terminology. However, because our study specifically evaluates MAOC capacitance, we believe it is helpful to use the nomenclature commonly used in the cited biogeochemical literature referring to capacitance (e.g., Georgiou et al., 2022, 2025). We would also like to clarify that we are referring to the soil size fractions themselves rather than the organic matter within them, so using the terms POM and MAOM would not fit this specific purpose. To improve clarity and prevent any confusion, we have explicitly defined these terms as operational size classes in the methodology of the revised manuscript.

Suggested revision:

"Wet separation of the soil was performed following Poeplau et al. (2018). The fraction >50 µm is operationally referred to hereafter as “sand” and the fraction <50 µm as “silt + clay”."

L378-528: The Results section is very detailed, but overly so. For example, the description of the structural equation modeling results, which was divided into four subsections and is unnecessary. The authors are encouraged to appropriately condense the text and focus on reporting the most important and central findings.

Response: We thank the reviewer for this suggestion. We suggest to substantially condensing the SEM Results section by removing the separate subsections on model fit, direct, indirect, and total effects, and now focus only on the main pathways explaining variation in MAOC and POC. The complete standardized direct, indirect, and total effects, including non-significant pathways, are retained in Table S4.

Figure 3b: It is not clear what the purpose of including the regression line of Díaz-Martínez et al. (2024) is in this context. Please provide an explanation. Also, why is the R^2 value negative? Please also provide an explanation.

Response: We thank the reviewer for highlighting this issue. The data from Diaz-Martinez were intended to provide an estimate of soil capacitance in ecosystems with comparably dry climates, thus serving as a control for climate effects on capacitance. In Discussion Section 4.2, we discuss this, proposing that because a significantly higher capacitance value could be reached under these similar climatic conditions, the cause of lower capacitance observed in our study is likely mineralogy.

We suggest revising the text in Section 4.2 to better explain this (as well as more detailed methodology explanation following a comment by Reviewer 2):

“Georgiou et al. (2025) noted that systems may reach an apparent saturation at values below the mineral capacity to carry MAOC due to ecosystem constraints such as water limitation. To assess whether water was the factor limiting the MAOC capacitance in our site, we examined the MAOC capacitance in a large set of dryland forests using the recently published data of Díaz-Martínez et al. (2024). That dataset yields a significantly higher MAOC capacitance of $70.2 \text{ g C kg}^{-1} \text{ clay + silt}$, supporting the idea that at our site, mineral properties, rather than climate, were the limiting factor on MAOC accumulation.”

Figure 3c: Is it reasonable for MAOC saturation to exceed 100%? Please verify.

Response: We agree with the reviewer that an MAOC saturation level exceeding 100% can appear counterintuitive. However, this is an expected outcome in this type of analysis as noted by Georgiou et al., 2022 - we calculated the saturation limit according to the convention of using empirical quantile regression (Six et al., 2024). Because this limit is

derived statistically from the data distribution using a conservative 0.95 quantile, a subset of samples will mathematically fall above the defined threshold. In the specific case of the *Quercus* stands - these samples had especially high particulate organic matter (POM), constituting a substantial proportion of the total soil mass. This skews the relative mass fraction of the fine minerals downward and artificially lowers the expected capacitance (the denominator).

Because these >100 % values are the result of known statistical and compositional effects rather than analytical errors, we do not believe it is justified to exclude these samples from the analysis or to relate to them differently.

We propose adding the following text to the discussion to clarify this:

“MAOC saturation values, expressed as the percentage of the MAOC capacitance currently filled in each sampling point, exceeded 50% even in the forest gaps. Notably, saturation values exceeding 100% were observed in some *Quercus* stands. Such values are an inherent consequence of defining MAOC capacitance using the 95th percentile, as observations in the upper tail of the distribution can exceed the estimated saturation threshold. Thus, values above 100% do not indicate that the mineral capacity was physically exceeded, but rather reflect the statistical definition of the estimated MAOC capacitance. These values were above the global average for forest soils (Georgiou et al., 2022).”

Figure 4a: A linear mixed model for individual variables is acceptable, but it is not sufficiently comprehensive, because significance (*, **, ***) and R^2 alone cannot identify which factors are the most important in controlling soil carbon fraction storage. The authors are encouraged to further analyze the relative importance of predictors. In addition, the meanings of many indicators on the x-axis are unclear. For example, what do Temperature, Phosphorus, and Magnesium specifically refer to? Are they total nutrients or available nutrients? It would be better if these were clearly specified directly in the figure.

Response: We suggest revising Fig. 4a to more clearly present the relative strength of the relationships between environmental predictors and soil C fractions. Standardized β coefficients are now shown directly as bar heights, indicating both the magnitude and direction of each effect, while R^2 is represented by bar color and statistical significance by asterisks. This revised presentation allows the relative effects of the individual predictors to be compared more directly. We also clarified the predictor labels in the figure, specifying soil temperature, extractable Mg and Cl, inorganic N, available P, and CaCO_3 .

Figure 6: The structural equation modeling (SEM) lacks some connections among variables, such as paths from aboveground biomass and microbial communities to POC

and/or MAOC. In addition, “Above ground biomass” should be changed to “Aboveground biomass”. What does “Soil properties” refer to? This cannot be determined from the figure. Moreover, does soil silt and clay content not belong to soil properties?

Response: Figure 6 presents only the direct effects retained in the final SEM, whereas all hypothesized pathways, including links from aboveground biomass and microbial variables to MAOC and POC, are shown in the conceptual model in Fig. 1. Paths not retained in the final model were not displayed in Fig. 6 to improve clarity.

Suggested revision: correcting “Above ground biomass” to “Aboveground biomass.” In addition, clarifying that “Soil properties” represents PC1 summarizing the measured soil physicochemical variables, excluding clay + silt content. Clay + silt was treated separately because of its specific importance for mineral-associated C storage. These definitions are now stated more explicitly in the figure caption and are described in detail in the Methods.

“Fig. 6. Structural equation model showing the direct effects retained in the final model. Boxes represent measured variables and arrows represent direct paths, with arrow width scaled to the magnitude of the standardized regression coefficient. yellow arrows indicate positive effects and purple arrows negative effects; solid and dashed lines indicate significant ($p \leq 0.05$), marginally significant ($p \leq 0.1$) and n.s. ($p \geq 0.05$) paths, respectively. “Soil properties” represents PC1 summarizing the measured soil physicochemical variables, excluding clay + silt content, which was treated separately because of its importance for MAOC formation. All hypothesized pathways are shown in Fig. 1, and complete direct, indirect, and total effects are reported in Supplementary Table S5.”

Reviewer 2:

Overall comments: This manuscript represents a study on dryland soils with a comprehensive suite of analyses and an interesting study design. Authors examined how monospecific versus mixed forest stands affect soil and microbial characteristics with an emphasis on the idea of mineral saturation. Strengths of this paper included the interesting study design, and further, the writing and presentation of results are well done. I have no major concerns with the study overall, however, I do have some misgivings surrounding how afforestation and mineral saturation are presented. I think these are both fraught concepts, with afforestation presented as a possible natural climate solution when gains can be transient. Further, the limitations of mineral saturation can be observed in this study itself, where >100% saturation was reported for monospecific quercus stands. I think an interesting discussion could be had about where the idea of mineral saturation falls apart. I included more specific comments below.

Response: We thank Reviewer 2 for the thoughtful and constructive comments, which helped us improve the quality and clarity of our manuscript.

Specific comments:

Lines 102-115: What about fire and transient gains? I feel that this discussion of afforestation gets tricky when we don't take into account altered fire regimes that can release a lot of forest carbon, and the fact that gains from afforestation can be transient. Regarding fire regimes, there is evidence that drylands dominate the globally burned area and that this will be exacerbated by climate change. See:

Ermitão, T., Gouveia, C.M., Bastos, A. and Russo, A.C. (2024), Recovery Following Recurrent Fires Across Mediterranean Ecosystems. *Glob Change Biol*, 30: e70013. <https://doi.org/10.1111/gcb.70013>.

Pellegrini, A.F.A., Reich, P.B., Hobbie, S.E. et al. Soil carbon storage capacity of drylands under altered fire regimes. *Nat. Clim. Chang.* **13**, 1089–1094 (2023). <https://doi.org/10.1038/s41558-023-01800-7>

Response: We thank the reviewer for raising this important point. We agree that altered fire regimes can strongly affect forest carbon stocks and may reduce or reverse carbon gains associated with afforestation, particularly in dryland and Mediterranean ecosystems. Importantly, our study site has not experienced fire for at least the past 50 years, and we have now explicitly stated this in the revised Methods section. Thus, fire does not represent a direct disturbance affecting the SOC patterns observed in our study.

We nevertheless acknowledge that fire is an important consideration when evaluating the long-term carbon consequences of dryland afforestation more broadly, as demonstrated by the studies highlighted by the reviewer (Pellegrini et al., 2023; Ermitão et al., 2024). However, the paragraph in Lines 102-115 is specifically intended to introduce the documented effects of afforestation on SOC and the mechanisms underlying variation in SOC accumulation. We therefore feel that expanding this paragraph to discuss altered fire regimes and the potential transience of afforestation-derived carbon gains would broaden its scope and interrupt the flow of the introduction. Instead, we suggest clarifying the absence of recent fire at our study site in the Methods, while recognizing that the persistence of afforestation-derived carbon stocks under changing disturbance regimes remains an important consideration when extrapolating these effects over longer timescales and to other dryland forests.

Line 165-168: I understand that contrasting the C inventory of the pinus species versus annual plants is important for justifying the lack of focus on the transient annual plants, but is there a citation or methodology that can be included here for how this was done?

Response: Thank you for this comment. We suggest adding the missing information:

“Additionally, a variety of annual plants flourish from winter to spring in gaps between trees at the forest floor. These smaller plants have biomass of $\sim 8.22 \times 10^{-5} \text{ kg C m}^{-2}$, compared with 61.8 kg C m^{-2} of e.g., the Pinus trees, and hence a disproportionally smaller contribution to the forest C cycle (for additional information on the tree species, see Table S1). Annual plant biomass was measured on the total plant amount sampled from each plot at the same locations used for forest-gap sampling, in the spring of 2023. Plants were collected within a $0.3 \text{ m} \times 0.3 \text{ m}$ frame, brought to the lab, dried in an oven at 60.0°C for three days and then weighed”.

Line 194-198: Some greater clarity here regarding soil sampling is needed. In the sentence: "Next to each tree, we collected two samples at 0-1m from the trunk in opposite directions." - are the samples here soil samples?

Response: See our comment below.

Line 201: The use of sampling points is a bit vague here, after reading the whole section, it seems that it is a combination of a soil pit for undisturbed samples, and nearby sites for litter collection and soil samples for carbon and chemical analysis. It could be helpful to clarify this earlier in this section

Response: We thank the reviewer for identifying this ambiguity. We suggest revising the description of the sampling methods to clarify that the two samples collected adjacent to each tree were soil samples and to define the soil sampling points before describing the individual measurements. We also clarify that the undisturbed soil samples and litter collections described subsequently were collected at, or adjacent to, these same sampling locations.

Suggested revision:

“To account for the effect of tree diversity, we chose five individual representative trees in each plot. At each tree, two soil sampling points were established 0-1 m from the trunk in opposite directions. At each sampling point, soil was collected for carbon, physicochemical and microbial community analyses, together with the corresponding measurements and samples described below. In addition, we sampled three soil samples from a forest gap (2-3 m from the canopy) in each plot. All soil samples were collected at a

depth of 0-10 cm, representing the thin soil layer at the site. Across the four microsites, beneath *Pinus*, *Quercus*, and *Pistacia* canopies and in forest gaps within each plot, a total of 104 soil samples were collected.”

“In September 2022, sampling was conducted across all designated plots (For the complete description of the experimental design see Fig. S1). . Plant litter was collected adjacent to the same soil sampling points using a frame measuring 0.3 m × 0.3 m. The collected litter was then placed in individual paper bags. Subsequently, the plant litter was dried at 60°C for several days until it reached a constant weight. At each sampling point, an adjacent soil core was used to collect undisturbed samples for bulk density measurements.”

Line 208-209: Was bulk density calculated after cores were sieved to 2mm? More specifically, was this just fine earth bulk density or total bulk density? Please clarify this.

Response: We thank the reviewer for highlighting this omission. The reported values represent the fine earth bulk density (< 2 mm). To calculate this, coarse fragments (rocks > 2 mm) were separated from the cores, and both their mass and volume were subtracted from the total core measurements to isolate the fine earth fraction

We suggest the following revision to address this point:

“After the drying period, samples were sieved to < 2 mm. The mass and volume of coarse fragments (rocks) were determined and subtracted from the total core measurements, to calculate the bulk density specifically for the fine earth fraction (< 2 mm).”

Line 223-224: For determining SOC separately from SIC, there is usually some acid treatment to dissolve inorganic C. Was this performed on these samples? To be fair, the acid treatment can be fraught, see:

Apestegua, Marcos, Alain F. Plante, and Iñigo Virto. "Methods assessment for organic and inorganic carbon quantification in calcareous soils of the Mediterranean region." *Geoderma Regional* 12 (2018): 39-48.

Response: We appreciate the reviewer highlighting this point – we performed organic carbon measurement using the ramped combustion available with the SoliTOC elemental analyzer but omitted this detail from the methodology. The reference the reviewer provided recommends this method as the least biased option currently available.

We suggest revising the methods section accordingly:

“SOC, total nitrogen and soil inorganic carbon in the separate fractions and bulk soil samples were determined using ramped combustion (EA; Elementar, Cheadle, UK, Model: soli TOC cube). SOC values reported include the total organic carbon combusted up to 600 °C.”

Line 315: The use of the word "microsite" for what is essentially more specific forest positions might create some confusion for biogeochemists reading this - it did for me. For example, there is a rich and growing lit on the importance of anoxic microsites for C cycling - something occurring on micrometer scale. Authors could consider using a different word or clarifying this distinction earlier in the manuscript (especially in the abstract).

Response: We understand the confusion; we suggest clarifying the use of the term “Microsite” at the beginning of the abstract, introduction, and in the methods. We also suggest adding Figure S1 describing the full experimental design to better clarify the use of the different microsites.

Suggested revision:

From the abstract: “We further examined how these relationships varied among forest microsites (i.e., beneath the tree canopies and adjacent forest gaps).”

From the introduction: “At the microsite scale, we hypothesize that forest gaps, will contain lower total SOC than soils beneath tree canopies because of reduced litter and root-derived C inputs.”

From the methods: “Across the four microsites, beneath *Pinus*, *Quercus*, and *Pistacia* canopies and in forest gaps within each plot, a total of 104 soil samples were collected.”

Line 422: I think the methodology for computing MAOC capacitance should be included in the methods. The paper that is cited by Six et al. also suggests that special consideration should be taken for calcium rich soils (they mention that this could lead to high uncertainty in MAOC - which I believe is present in this study). I think the limitations of this MAOC capacitance estimation in this particular soil type should be addressed

Response: We accept the reviewer's recommendation and have moved the details regarding the MAOC capacitance computation to the Methods section, where we now provide a more detailed explanation of how it was performed.

The suggested revised text for the methodology section:

“MAOC capacitance

MAOC capacitance (i.e., the specific capacity of the silt and clay fraction to hold MAOC)

was estimated using the methodology described by Six et al. (2024). To represent conditions closest to physical saturation, we selected data from under-canopy locations in forest stands and plotted their MAOC concentrations against the silt and clay content. A 95th percentile regression line was then computed, forcing the intercept to 0, to yield the capacitance value. As a reference value for dryland forests, we applied the same analysis to the dataset published by Diaz-Martinez et al. (2024). Within their dataset, we similarly selected data representing MAOC concentrations strictly from under tree canopies in forest sites. This provided 67 independent measurements spanning precipitation levels of 266 to 891 mm, all of which fell within an aridity index (annual precipitation/potential evapotranspiration) < 0.5. MAOC saturation values were expressed as the percentage of the MAOC capacitance currently filled in each sampling point.”

Regarding the issue highlighted by Six et al., 2024 for calcareous soils - this follows the reviewer's earlier request for clarification on organic carbon determination. The high uncertainty highlighted by Six et al. is primarily associated with the acid pretreatment typically required to remove carbonates, which can alter the soil matrix and interfere with accurate size fraction determination. Because our study utilized ramped combustion for organic carbon determination, we did not need to subject the samples to acid pretreatment. Therefore, our methodology avoids the specific size-fractionation artifacts that typically complicate MAOC estimations in calcareous soils.

Section 3.5, starting at line 487: I agree with the first reviewer's comment that this section needs to be condensed

Response: We agree; see our response to reviewer 1 above.

Section 4.2, starting at line 568: What about the very high saturation values reported for mono-specific quercus stands? How should the reader interpret such a high value, and wouldn't over 100% saturation suggest that there are important limitations to the idea of mineral saturation, even within this dryland context?

Response: We agree with the reviewer that an MAOC saturation level exceeding 100% can appear counterintuitive. However, this is an expected outcome in this type of analysis as noted by Georgiou et al., 2022 - we calculated the saturation limit according to the convention of using empirical quantile regression (Six et al., 2024). Because this limit is derived statistically from the data distribution using a conservative 0.95 quantile, a subset of samples will mathematically fall above the defined threshold.

In the specific case of the Quercus stands – these samples had especially high particulate organic matter (POM), constituting a substantial proportion of the total soil mass. This

skews the relative mass fraction of the fine minerals downward and artificially lowers the expected capacitance (the denominator).

Because these >100 % values are the result of known statistical and compositional effects rather than analytical errors, we do not believe it is justified to exclude these samples from the analysis or to relate to them differently.

We propose adding the following text to the discussion to clarify this:

Notably, saturation values exceeding 100 % were observed in the Quercus stands. This is, , an inherent statistical artifact of defining the saturation limit based on the 95th percentile, which mathematically dictates that 5 % of the data will exceed the threshold.

Section 4.3, starting at line 590: I found this section informative, but it was hard to follow the logical flow with all the previous work done at the site. Consider editing for clarity and breaking up the section

Response: Thank you for this comment. Following up on your suggestions, we suggest splitting this section into two paragraphs: the first with interpretation and synthesis of the soil microbiota results, and the second paragraph with wider interpretation considering the existing knowledge in the literature. In the second paragraph, we replaced some of the references to previous work done at the site with a new, more relevant reference.

The second paragraph opens with these revised sentences: “Previous studies have shown that tree roots actively recruit specific soil bacteria, as demonstrated in *Cupressus sempervirens* (Oppenheimer-Shaanan et al. 2022). Further, increased root exudation rates during the dry season have been observed in the tree species studied at the mixed plots which may promote bacterial growth (Jakoby et al. 2020, Obersteiner et al. 2026). This aligns with Yuste et al., (2014), who found more pronounced drought responses in summer, but contrasts with Yuste et al., (2011), who did not find a correlation between bacterial biomass and SOC. Our study revealed that soil bacterial community composition was significantly shaped by microsite conditions, whereas forest type and season alone had no overall significant effects. Similarly, the bacterial communities growing on the roots of the trees in the mixed plots changed with host tree species but not with season (Obersteiner et al. 2026)”.

Obersteiner, S., Oppenheimer-Shaanan, Y., Yalin, D., Sade, D., Zavaro, V., Reich, Z., & Klein, T. (2026). Pine root exudate terpenoids associate with *Mycobacterium* sp. under summer drought in a mixed Mediterranean forest. *Environmental Microbiome*.

Line 597-599: There is a growing body of work on quantitative stable isotope probing (qSIP) which suggests that it's not just what microbial communities are present, but which ones are active that is impactful for SOC cycling. I understand that qSIP is highly specialized and difficult to employ, but could this possibly affect your interpretation of the microbial community results? This could also be brought up as a limitation

Response: We agree that microbial community composition and richness do not necessarily reflect the metabolically active fraction of the community. qSIP can identify actively growing taxa and therefore provide a more direct link between specific microbial groups and C cycling than community composition alone. We have added this as a limitation in the Discussion.

Suggested revision:

“...Finally, our microbial analyses characterize community composition and richness rather than microbial activity. Consequently, active taxa involved in SOC formation and turnover may not be fully represented by these metrics, and approaches such as quantitative stable isotope probing (Hungate et al. 2015) could provide a more direct assessment of microbial contributions to SOC cycling.”

Figure 4 comment: Were vectors that were excluded from this figure (loading magnitudes <0.4) excluded just for readability? It creates a discrepancy between what is described in the text versus presented in the results. A full analysis could be included in supplemental materials. Red/blue distinction not color blind friendly

Response: Thanks. Vectors with loadings < 0.4 were excluded from Fig. 4 only to improve readability. To provide the complete PCA results and avoid any discrepancy between the text and figure, we have will add the full PCA loadings for the soil physicochemical variables in Table S4. We also revised the figure colors to use a color-blind-friendly scheme.

Figure 5 comment: there is no explanation of part e in the caption (or maybe it got cut off for me). The pinus logo also got lost and pushed towards panel d

Response: Thank you for catching this; we revised the figure caption to include panel e and fixed the appearance of the figure.

Suggested revision for Fig. 5 caption:

“(e) Log fold change in the relative abundance of the top 10 bacterial taxa beneath tree canopies compared with open forest gaps for *Pistacia*, *Quercus*, and *Pinus*. Positive values (green) indicate taxa with higher relative abundance beneath tree canopies, whereas negative values (grey) indicate taxa with higher relative abundance in open gaps.”

Figure 6 comment: Red/blue distinction not color blind friendly. Also, something not explained by the caption are the box colors, some are a more saturated yellow than others, what is the reason for this?

Response: Thank you. We removed the confusing color boxes and revised the figure colors to use a color-blind-friendly scheme.