

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Johanna Pihlblad et al.

Author comment on "The influence of elevated CO₂ and soil depth on rhizosphere activity and nutrient availability in a mature *Eucalyptus* woodland" by Johanna Pihlblad et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-145-AC1>, 2022

Dear Reviewer #1

The authors would like to thank the anonymous referee #1 for the thorough attention and skill with which the referee read and suggested edits as well as general comments to the manuscript titled "The influence of elevated CO₂ and soil depth on rhizosphere activity and nutrient availability in a mature *Eucalyptus* woodland" by Johanna Pihlblad et al., Biogeosciences Discuss (bg-2022-145). We are confident that the edits and comments suggested by anonymous reviewer #1 has made the manuscript into a more clear and higher quality paper.

Briefly we decided to adhere to the reviewers' main general comment to strengthen the link to previous results as well as introduce the enzyme data in the main text along with their methodology. However, though we agree with the reviewer #1 that the transition layer is not the main discussion point of the paper, we do think there is value in presenting the transition layer data and statistical testing of the transition layer data in the main text given it is not a common endeavor to look at the deeper layers of soil (specifically clay related properties at depth) and the microbial usage of nutrients in this sphere under elevated CO₂ or indeed in any large-scale ecological experiments. Though the knowledge gained is based on negative results the novel information is of value for further studies informing on an area of soil affected by climate change factors that have previously not been included in the scientific cannon. For this reason, we want to keep it in the main text though we deeply appreciate the attention to detail and skill by which the reviewer has considered the manuscript as a whole.

Below we have detailed the changes made to the manuscript and if when not changed as suggested the reasons for not doing so.

Kind regards on behalf of all listed authors,

Dr Johanna Pihlblad

Specific comments

Lines 142-143: Freezing the soil samples prior to enzyme analysis is not ideal, as freezing and thawing could kill the microbes. Why was this approach chosen? I recognize that this cannot be changed for the publication, but I recommend the authors to write a few sentences about the caveats of this and perhaps only discuss their results in terms of the differences between treatments, since the absolute values could be well underestimated. Another option is to make a quick comparison with other studies, perhaps in similar areas/conditions, to show that the absolute values were not affected by the soil preparation method chosen.

Suggested references for the debate:

https://www.sciencedirect.com/science/article/pii/S0038071712004476?casa_token=_VQgFC080-sAAAAA:0OipU3cTduPiXo0_g6QWb8HCel8rb2lO_jxEYQR6uWx_tIg38yq4T_IS8IxXmeqw4vbbRdSoMg

https://www.sciencedirect.com/science/article/pii/S0038071709004441?casa_token=yD92bxS-vEQAAAAA:c3l8X3LT_9bRJ84K2fZ6g5P0P3E1abvweiZlv0u_ok17lYxNs4eix5oZw9vAombmMqdVI_Vnpg

Why not include the enzyme methodology into the main manuscript document?

The reason for freezing soil after harvesting prior to the analysis of potential enzyme activity was mainly due to two reasons; firstly, the time restrictions during the field harvest, making sure the soil was processed no longer than 7 days was crucial to maintain the validity not just for the enzyme analysis but also for the other microbial pools and rates of transformation as well as C and nutrient pools. Collecting the field samples was done over 5 days followed by a day of sieving and processing leaving no time to mauer around analysing potential enzyme activity on fresh soil. Secondly, to not cause the enzymes to break down due to hot temperatures often experienced on the eastern coast of NSW, Australia. If the samples were kept fresh or air dried and stored for the analysis the sometimes high temperatures experienced in this region can degrade the enzymes faster than in the milder temperate or Mediterranean climates of the northern hemisphere. To minimize temperature degradation of the soil enzymes, which can happen in hotter climates, aliquots of soils were frozen in -20 °C. Additionally, a standard dry sample was included on every plate prepared for analysis allowing for inter plate comparison showing similar levels of enzyme activities as the frozen and thawed soils (data not shown).

Other studies from the same region have all used frozen soils for their potential enzyme activity including but not limited to:

Hasegawa, S., Macdonald, C.A. and Power, S.A. (2016), Elevated carbon dioxide increases soil nitrogen and phosphorus availability in a phosphorus-limited Eucalyptus woodland. *Glob Change Biol*, 22: 1628-1643. <https://doi-org.ezproxy.uws.edu.au/10.1111/gcb.13147>

Additionally, the enzyme data table and methodology was included in the main paper as suggested by the reviewer.

Line 188: How did you deal with it? Data was transformed to log as described in lines 194-195?

It's not uncommon for data describing gross rates to miss values and have skewed distributions between treatments, for this reason we are removing the current description and instead writing the following for clarity: "For gross N mineralization rate in the deepest layer (10 to 30 cm depth) ammonium concentrations in most samples were below detection limit."

Lines 273-274: It seems that this sentence is contradicting the previous ones in this paragraph. If there was more P available with depth (because you argue there are less roots and microbial activity in those deeper layers), why do you state that "P became limiting at depth"? I would understand that overall P is more limiting than N, as supported by your enzyme results, but the depth argument is not very clear to me in this section. Could you clarify this, please?

The section was clarified by adding the sentence: "Hence, without the influence of roots, N and P both declined at a similar rate, while keeping the total magnitude of N larger than P as both decreased with depth." And changing the last sentence to be more explicit: "Furthermore, inorganic P decreased with depth more resources were invested to access it, supported by the consistently higher P targeting enzyme activity than N enzyme activity".

Lines 300-301: I would suggest adding another argument here at the end of this paragraph, to put P availability of your site/plots into perspective, by comparing it to other studies. Although you state that this is a both P and N poor site, your results indeed point to perhaps more inorganic P being cycled than organic P. Comparing with other studies could strengthen your discussion.

The end of the paragraph has been edited to strengthen the argument as suggested by the reviewer: "...microbes in the rhizosphere as an alternative to high energy cost enzyme production. Although soil P accumulates in the soil organic fraction with increasing soil age (Crews et al., 1995) this soil is also rich in metal oxides with large surfaces capable of adsorbing phosphate ions (Achat et al., 2016) which root activity in the rhizosphere can release with the help of organic acids (Adeleke et al., 2017)."

Lines 308-310: Can you expand a bit more on how you can extrapolate your findings to turnover? I suggest bringing a bit the discussion from lines 337-339 (reference from Pineiro et al 2020) here as well.

The paragraph was amended to include: "Because we did not find a significant increase in potential enzyme activity in the rhizosphere (Table 5) this effect can instead be driven by microbial biomass turnover and a strong recycling of nutrients without large decomposition of SOM requiring enzyme activity. Although we can show that deep rhizosphere has an impact on available nutrients our study cannot assess if plants are utilising the increased availability though increased root turnover (Piñeiro et al., 2020) has been reported suggesting that is the case."

Lines 346-347: I suggest that this reference

<https://www.sciencedirect.com/science/article/abs/pii/S0016706181900240> could strengthen your argument.

We agree that the McGill and Cole (1981) reference is a good addition to strengthen the argument. The sentence has been edited to the following: "Mineral adsorbed P forms are however sensitive to root derived changes in pH (Jones and Darrah, 1994), representing a different mechanism for affecting the P cycle separate from SOM decomposition (McGill and Cole, 1981)."

Lines 378-379: It could be useful to add a bit of the short-term versus long-term responses, as perhaps, the system might not be able to keep this faster cycling for too long under nutrient limitation.

The short term versus long term effect is briefly discussed in the section starting on line 345. One additional point was added to increase the contextual importance of new C on

soil C stocks at the end of that same paragraph: "Tough a recent meta-analysis assigning short- and long-term effect of newly fixated C on soil C stocks could show that any short-term gains of C into SOM was gone after one to four years (van Groenigen et al., 2017).".

Technical corrections

Line 38: Reference style should be revised.

The reference style was corrected throughout the manuscript.

Line 47: "thus promote" should be "thus promoting".

The sentence has been changes to "thus promoting" as suggested by reviewer #1.

Line 74: Add hyphen: depth-dependent.

Hyphen was added as suggested.

Line 124: Remove ; after Londonderry clay and perhaps add a parenthesis.

The ";" was removed and the sentence was changed to: "...clay layer called Londonderry clay (Atkinson, 1988) found...".

Lines 135-137: I suggest to revise this sentence to: "Although the depth of the transition layer differed throughout the site, the chemical properties are assumed to be similar within this zone across the plots, as the water periodically builds up above the clay before it drains, creating conditions for podzolification."

The sentence was changed as suggested by reviewer 1.

Line 168: From "mineralization, rate" to mineralization rate,"

The comma was removed as suggested by reviewer 1.

Line 172: Should read "added in duplicate to fresh and..."

The sentence was changes as suggested by reviewer 1.

Line 185: Shouldn't it be the effect of eCO₂ and depth on roots, and not the other way around?

Sentence was changed as suggested by reviewer 1.

Line 192: "analysis all CO₂" should read "analysis of all CO₂".

Sentence was changed as suggested by reviewer 1.

Line 198-199: Reverse the results for better flow. Since you report a decrease with depth, state the 0-10cm results first, followed by the deeper layers.

Section was edited to reflect the comments of reviewer 1 as follows: "Fine root biomass density significantly decreased with depth and ranged from 0.12 mg·g⁻¹ in the 0-10 cm depth to 2.75 mg·g⁻¹ in the transition depth (Figure 2)."

Line 205: Was the 24% increase for both 0-10 and 10-30 cm together (averaged) or the

magnitude of change was the same for both depths separately?

The 24 % is referring to the magnitude change between bulk and rhizosphere soil as an average of the 0-10 cm and 10-30 cm depth (soil type as single factor). The sentence was edited to clarify this in the following way: "The DOC was significantly higher (by 24 %) in rhizosphere soil than bulk soil (Figure 2 and Table 1) when averaged for 0-10 and 10-30 cm depths."

Line 614: Remove the italics format.

The italics format was removed as suggested.

Lines 221-222: Could you point to where (table, figure) we could see those results?

Figure reference were added as suggested by reviewer: "...ambient 10-30 cm rhizosphere (Figure 5), though..."

Line 680: Parenthesis missing after 10-30 cm.

Parenthesis was added as suggested by reviewer.

Line 627: Instead of "for of a mature" it should read "for a mature".

Edited as suggested by reviewer.

Line 323: Initial caps missing in "rather the...".

Typo corrected.

Line 326; PO₄⁺ or PO₄³⁻?

The correct compound here is: "PO₄⁻³", which has been corrected in text.