Comment on bg-2022-145
Anonymous Referee #1

Referee comment on "The influence of elevated CO$_2$ 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-RC1, 2022

General comments

This study is part of the EucFACE experiment, aiming to understand the effects of elevated CO$_2$ on belowground soil parameters and aspects of nutrient cycling. Such study becomes even more important when considering that terrestrial ecosystems might be limited by nitrogen (N) and/or phosphorus (P) as is potentially the case for this study site in Australia. Understanding the role of nutrient limitation on the so-called "CO2 fertilisation effect" is therefore crucial. The authors investigated rhizosphere and bulk soils in terms of C, N and P availability, enzyme activities and root stocks in deeper (>30cm) soil layers.

The manuscript does fit into the scope of this journal and it is overall very well written, organised and also concise. Figures and tables are clear and are sufficient. This study presents novel data regarding the responses of this mature Eucalyptus forest to elevated CO$_2$, but following other studies in the same area, none or weak responses were found, even after 5 years of manipulation. Stronger differences between the studied parameters were found between soil types (rhizosphere and bulk soil) and soil depth, rather than with elevated CO$_2$. The authors then argue that their results indicate a faster recycling of nutrients rather than increasing nutrient availability derived from organic matter decomposition. Although I see the point made here, I think that the discussion and conclusions of the manuscript could benefit from integrating a bit more of previous results from EucFACE studies. Some references to past work are made along the text, but I suggest (see specific comments below) that such references and comparisons could be expanded and come in different parts of the text (for example, expanding on root productivity/turnover and litter decomposition previous results). Only when making such stronger links I would think that the conclusions are then justified.

Although I understand and agree that understanding soil-plant feedbacks in deeper soils is important when talking about climate change scenarios, I think that the deeper layer (below 30 cm depth, called “transition layer”) is not really discussed and perhaps the authors should consider if it really needs to be included in the manuscript. A suggestion could be to include it in the supplementary material. This could give the authors space to
bring the methods regarding enzymes to the main text as well.

And finally, a bit about short versus long-term impact of these results could add to the discussion and future perspectives in the conclusions.

**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 recognise 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=_VQgFO80-sAAAAA:0OiPU3cTduXi0o_g6QWb8HHe8r2bQjxjYQR6uWx_tIg38yq4T_IS8IxXmeqw4vbbRDSoMg

https://www.sciencedirect.com/science/article/pii/S0038071709004441?casa_token=yD92bxS-vEQAAAAA:c3Il8X3LT_9bRJ84K2fZ6g5P0P3E1abvweiZl0u_0k17lYxNs4eix5oZw9vAo mbmMqdvII_VnpG

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

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

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?

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.

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.

Lines 346-347

I suggest that this reference https://www.sciencedirect.com/science/article/abs/pii/0016706181900240 could strengthen your argument.

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.

**Technical corrections**

Line 38

Reference style should be revised.

Line 47

“thus promote” should be “thus promoting”.

Line 74

Add hyphen: depth-dependent.

Line 124

Remove ; after Londonderry clay and perhaps add a parenthesis.

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.”
From “mineralization, rate” to mineralization rate,”

Should read “added in duplicate to fresh and...”

Shouldn’t it be the effect of eCO2 and depth on roots, and not the other way around?

“analysis all CO2” should read “analysis of all CO2“.

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

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?

Remove the italics format.
Could you point to where (table, figure) we could see those results?

Parenthesis missing after 10-30 cm.

Instead of “for of a mature” it should read “for a mature”.

Initial caps missing in “rather the...”.

PO$_4^{+}$ or PO$_4^{3-}$?