



EGUsphere, author comment AC2  
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## Reply on RC2

Alexa K. Byers et al.

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Author comment on "Soil depth as a driver of microbial and carbon dynamics in a planted forest (*Pinus radiata*) pumice soil" by Alexa K. Byers et al., EGU sphere,  
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*This study explored the changes in SOC dynamics and soil microbes in the Puluki Experimental Forest (New Zealand) down to a soil depth of 1 m. ITS and 16S rRNA sequencing and quantitative real-time PCR were used to measure changes in soil microbial diversity, composition, and abundance. Stable ( $\delta^{13}\text{C}$ ) and radioactive ( $^{14}\text{C}$ ) C analyses were performed to assess depth-driven changes in SOC stability and age. It has to be said that it is very important to use these methods to explore the dynamic changes of deep SOC, but it is not the most innovative. The sentences are smooth and comfortable to read. To my surprise, the total C stocks of deep soil accounted for only 35%, which was not more than 50% as previously reported. The Introduction is like science fiction, lacking clear questions and hypotheses. Moreover, few links were made between changes in soil microbes and soil carbon stocks in the Discussion. Other suggestions are as follows:*

**Response: As with Reviewer 1, we would like to thank Reviewer 2 for contributing their time and expertise to review our manuscript. We value your comments on our work and will take them on board when revising the manuscript. Regarding the comment that "The Introduction is like science fiction". Nothing we have written is fictional and is entirely based on current scientific knowledge.**

**Reviewer 2 was surprised to read that 35% of total C stocks were allocated to the subsoil considering we previously reported the value of 50%. This 50% value was mentioned in the Introduction and was a reference to a different study (lines 36 to 39). At no point did we suggest this was a finding of our own. Furthermore, deep C allocation will vary depending on a range of factors such as soil type, soil age, vegetation, climate, and management history. Thus, deviations in our findings from previous research are expected and build on the limited knowledge available in the scientific literature.**

**Additionally, Reviewer 2 commented that few links were made between changes in soil microbes and soil carbon stocks in our Discussion. We agree; our balanced our discussion against the methods we undertook and took care in not drawing too many direct conclusions on soil-microbial relations as we examined microbial DNA only. No need to present the readers with fiction. Whilst DNA-based NGS targeting SSU rRNA genes provide a wealth of valuable information, they provide limited information on the functional role or activities of soil microorganisms.**

*Title: Microbial communities are not suitable to be described as big and old.*

**Response: "Large, old pools..." was in reference to soil carbon, not microbial communities.**

*L16: Why must it be an incremental change?*

**Response: we used the term 'incremental change' as we analyzed changes in SOC and the microbiome of the soil cores at 10cm increments down the soil profile. Many previous studies have analyzed depth-driven changes in soil carbon at broader horizons i.e. 0 to 10, 10 to 50, and 50 to 100cm. For our study, divided soil core into smaller increments to identify at which point large shifts in SOC and the microbiome take place.**

*L22: Does soil carbon refer to carbon storage or carbon concentration or stability or others?*

**Response: Upon review of our manuscript, we understand how this sentence is confusing to the reader. We were referring to soil carbon concentration (or calculated soil carbon stock). We are happy to clarify this ambiguity in the revised manuscript. Thank you to Reviewer 2 for identifying this.**

*L23-L25: This study is only a sample study, why do you say "These research findings highlight the importance of quantifying subsoil C stocks for accurate systems-level global and local C budgets and modeling"? Moreover, this study does not address climate change.*

**Response: We added this sentence in to address the wider implications of our research findings- that subsoil C can contribute to total forest soil C stocks. Consequently, accurate quantification of subsoil C should be considered more by the wider research community. However, we understand including this sentence in the Abstract may be misleading as our study did not directly research climate change. This sentence may be better suited to the Discussion/Conclusion where we can fully expand upon its intent and meaning. Thus, we are happy to remove this sentence from the Abstract of the revised manuscript and add it to a more appropriate section in the Discussion and ask the Editor for clarification as to if this is necessary. Thank you.**

*L28-31: These three sentences all emphasize the importance of forest soil carbon, which can be simplified and combined with the next paragraph.*

**Response: Thank you to Reviewer 2 for identifying this. Whilst lines 28 to 31 provide valuable information, we agree the 3 sentences are unnecessarily repetitive. We are happy to make this section of the manuscript more concise for the reader.**

*L41-42: What is the meaning of "having a physical and chemical nature"?*

**Response: the physical and chemical properties of the soil environment i.e. reactive mineral surfaces, soil redox state, and access by soil microorganisms. This will be clear to most readers.**

*L44-46: Although these are all factors affecting the stability of SOC, they are not addressed in this study, and the preamble should introduce more advances in microbes, isotopes, etc.*

**Although we appreciate Reviewer 2's comment, we think this is an important section of information to include in the introduction of the manuscript. Providing**

the reader with information on environmental factors governing SOC sequestration is relevant when we are quantifying subsoil carbon stocks. Furthermore, the paragraph following this section (lines 50 to 62) details the importance of studying soil microbes. Lines 67 to 72 outline why we performed isotopic measurements.

*L46: delete ", thereby,"*

**Response: this will be deleted in the revised manuscript**

*L51: delete "fundamental"*

**Response: this will be deleted in the revised manuscript**

*L64: How to understand the meaning of the word "fundamental"?*

**Response: In this context, we were referring to baseline or foundational. We understand how this term may be confusing and are happy to remove or replace fundamental with a more suitable term (or just remove it from the sentence).**

*L65: How to understand "at a highly refined spatial scale"?*

**Response: Here we were referring to the fact that we measured depth-driven changes at 10cm increments. We can see how "at a highly refined spatial scale" is non-specific and not helpful for the reader's understanding. We are happy to rephrase the sentence to "we aimed to examine the depth-driven variability in soil C dynamics in 10cm increments down a soil profile" (or words to such effect).**

*L87: delete the second "(".*

**Response: this will be deleted in the revised manuscript**

*L100: To avoid DNA degradation, soil samples are usually stored at -20 or -80 °C before DNA determination. How long will this study complete DNA determination after sampling?*

**Response: Soils were sampled from Puruki Forest located in the North Island of New Zealand (NZ). After sampling soils were immediately stored at 4°C for a brief period (~1 week). Soil DNA extractions were performed in a laboratory in Christchurch (South Island of NZ). Consequently, after sampling soils and storage at 4°C, soils required for DNA extraction were transported down to the South Island based laboratory. Once at the laboratory soils were then placed in the -20 freezer prior to DNA extraction. Apologies, this information should have been added to the manuscript. It will be included in the revised manuscript as "Once transported to the laboratory, soils required for DNA extraction were stored at -20°C".**

*L103: What are the "Mehlich 3 extractable elements"? How are fractions equal to 2 mm treated?*

**Response: The Mehlich 3 extractable elements were Potassium (K), Calcium (Ca), Magnesium (Mg), and Sodium (Na). The second question is not clear. Is Reviewer 2 asking how soil fractions that were exactly 2 mm in size were treated? If so, I cannot answer this question as to the best of my knowledge such soils were not found. Soils that passed through a 2 mm sieve were regarded as < 2 mm soils, and those which did not were classified as > 2 mm. My**

**apologies if I have misinterpreted the question. We have detailed in Section 2.2 exactly what forms of analyses were tested for each soil fraction.**

*L150: How to calculate soil carbon storage, it is recommended to list a formula.*

**Response: Soil carbon stock was calculated using the formula ' $SOC_{stock} = BD \times SOC_{conc} \times D$ ' (Gattinger et al., 2012; Jones et al., 2008). Here BD is bulk density ( $g/cm^3$ ),  $SOC_{conc}$  is Total C %, and D is thickness of the soil layer (cm). Following this, soil carbon stocks were slope corrected by multiplying  $SOC_{stock}$  by the slope ratio. We are happy to add this information to the revised manuscript and thank Reviewer 2 for the suggestion.**

- **Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fließbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N.E.H. and Niggli, U., 2012. Enhanced topsoil carbon stocks under organic farming. *Proceedings of the National Academy of Sciences*, 109(44), pp.18226-18231.**
- **Jones, H. S., Garrett, L. G., Beets, P. N., Kimberley, M. O., & Oliver, G. R. (2008). Impacts of harvest residue management on soil carbon stocks in a plantation forest. *Soil Science Society of America Journal*, 72(6), 1621-1627.**

*L153: What are the "Bray P sequential 1"? What does slope corrected mean?*

**Response: Bray P was measured using the Bray 2 extraction method. Three sequential extractions were performed (sequential extraction 1, 2, and 3) to measure the amount of P extracted from the soil. We used the value of the 1<sup>st</sup> sequential extraction in our data analysis. We will revise the manuscript and instead write "Bray P (sequential extraction 1)" to be clearer.**

**'Slope corrected' refers to the slope factor applied to values for total C & N stocks, as well as Bray P1 and exchangeable cations. Our data was slope corrected because the sampling transect from which our soils were sampled was along a 12° slope. This detail should have been included in the Methods section; it will be added to the revised version of the manuscript. Apologies for this omission.**

*L209: It is recommended to merge 3.1 and 3.2.*

**Response: Thank you for the suggestion, we are happy to merge these two sections in the revised manuscript.**

*L213: Is the result reliable if the variation of SOC stocks in the coarse soil fraction is so large? Some similar results can be described together to avoid redundancy. For example, the results of most indicators decrease with the soil layer. Where is the Table?*

**The variability in SOC stocks between soil cores is consistent with previous research also conducted at Puruki Forest. This is something we discussed in detail in the Discussion, lines 306 to 318. Apologies, the Table referred to in line 215 needs to be corrected to Table A3 (note: an appendix, not located in the main text). Any incorrect table numbers referred to in-text will be corrected in the revised manuscript.**

*L220: delete "this trend was variable. In particular,".*

**Response: this will be deleted in the revised manuscript**

*L225: It is suggested to delete some results in 3.4 and 3.5. Not all results need to be*

*described, but they should be targeted.*

**Thank you for the suggestion, we agree these too sections are detailed unnecessarily, and we are happy to make these sections more concise in the revised manuscript or create a supplementary results file for secondary findings.**

*L251-257: Generally,  $|R| < 0.3$  is considered a weak correlation, where  $R$  is generally lower than 0.3. I doubt the reliability of the results*

**Response: The reliability of the correction was tested using a formal statistical test. The results should be interpreted as 'there is a statistical association between the response of the two variables, however the association strength is moderate (or weak, depending on your view). i.e., there is an association between the variables, the 'explain' some the variation in each other's response, but a lot remains unexplained. We agree that an element of subjectivity comes into if particular R values are weak, moderate, strong etc, but this in no way affect the reliability of calculating that particular R value.**

*L264: Microbial classification names need to be italicized.*

**Response: We followed the convention of not italicising classifications above genus level which is why the names in this section are not in italics.**

*L333: neatly to? Or nearly to.*

**Response: here neatly referred to corresponded well or closely. We are happy to change this to a less ambiguous term in the revised manuscript.**

*L350: What does "quantity" mean here?*

**Response: the soil carbon concentration (Total C %). We will reword this sentence in the revised manuscript to "...correlated with the concentration (Total C %) and radiocarbon age..."**

*L365: How to calculate the microbial density?*

**Response: Here we were referring to microbial biomass, or DNA abundance. Microbial DNA abundance (16S and ITS rRNA gene abundance) were outlined in the Methods section 2.5. We will reword this in the revised manuscript as 'density' may be confusing and is inconsistent with the terminology we have otherwise used.**

*L367-368: The abundance of bacteria significantly declined with soil depth. Is it contradictory to say that the relatively large community abundance?*

**Response: Reviewer 2 is correct; bacteria did decline with depth. However, what we were stating in this sentence is that bacterial communities had larger abundances relative to fungal communities- which is true. Our results show the diversity and taxonomic coverage of subsoil bacterial communities was greater than that of fungal communities. However, we understand the point Reviewer 2 to making here, so we are happy to remove or reword this sentence to be less suggestive.**

*L408: How to understand that the abundances of Myxomycetes, Thelephorales, and Tremells were related to soil C quantity and age?*

**Response: The methods for this were outlined in lines 192 to 194- “log adjusted abundances of microbial taxa obtained from ANCOM-BC analysis were correlated to soil chemical properties using pairwise Spearman’s rank correlation tests...”. The results of these tests were presented in the Results section (lines 284 to 288). NOTE: the manuscript refers the reader to Table A15 for more detailed supplementary results however this is incorrect, it will be updated to Table A14 in the revised manuscript.**

*The red line in Figure 1 is not obvious, so it is recommended to consider other colors.*

**Response: We thank Reviewer 2 for the suggestion. We are happy to change it to a more contrasting colour in the revised manuscript.**