

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Rachael Akinyede et al.

Author comment on "Temperature sensitivity of dark CO₂ fixation in temperate forest soils" by Rachael Akinyede et al., Biogeosciences Discuss.,
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We would like to thank Reviewer 1 for the insightful comments on our manuscript, "bg-2022-90". We are glad that the reviewer finds our study on dark CO₂ fixation and its temperature sensitivity, to be interesting and that suggestions are given to improve the manuscript.

To meet the reviewer's concerns, we have addressed the question on why the temperature sensitivity of dark CO₂ fixation differs for rates reported per gram of soil and per gram of microbial biomass carbon and explained the rationale behind the quantification of the carbon allocation. We would also clarify working of all confusing text and remove Figure 7. Our point-by-point response to each of the reviewer's comments is given below (see italicized bold text).

Reviewer 1: Comments to Author:

This study explores the temperature sensitivity of microbial non-photosynthetic CO₂ fixation in temperate forest soils. The manuscript is interesting but some aspects are not clear and require improvement. Particularly, the authors should explain why the temperature sensitivity of CO₂ fixation differs depending on whether it is reported per soil mass or per microbial biomass C (see below).

Main comments

- Figure 2 Why does Q₁₀ for CO₂ fixation differ between the rates per MBC and soil? I assume this is due to differences in the MBC in the two soil subsamples that have been exposed to different temperatures. It is rather surprising that the MBC concentrations differ so strongly, and it would be good to see the values (in a table).
The difference in Q₁₀ based on rates per MBC and soil dry weight is not caused by differences in the MBC content as the MBC content were similar between the beech and spruce soils with hardly any changes with temperature (this is given in Table S2, supplementary information). Instead, it is caused by the difference of the rates being assessed. For calculating CO₂ fixation rates per gram dry soil, we measured the excess ¹³C in the soils and for rates per gram microbial biomass, we measured excess ¹³C extracted in MBC (lines 183–195). Hence, we are looking at two different uptake rates: the rate of incorporation in total soil carbon (living and dead microbial biomass plus soil organic matter), and the rate of incorporation of the label into microbial biomass (lysable cells). The Q₁₀ values for both rates would be the same, if, for example, all ¹³C in the soils was still in the living biomass at 4 and 14 °C.

However, for the beech soil, we found 23% more ^{13}C label incorporated into the MBC pool at 14 °C than at 4°C. In contrast, we found a 70 - 90% increase in ^{13}C label incorporation at the higher temperature in the SOC pool. This led to larger calculated temperature dependence of fixation rates expressed per gram of soil and hence, higher Q_{10} values, compared to those calculated for rates expressed per gram MBC. This information would be clarified in the revised manuscript.

- Section 3.3 The rationale behind the quantification of the “C allocation” is not clear. Given that the incubation lasted only a few days, it is unrealistic that a lot of the microbial biomass C already turned into microbial necromass during the incubation. Thus, what the authors report here is probably rather the result of differences in the efficiency of the chloroform fumigation.

We disagree with the reviewer that significant amounts of microbial biomass carbon cannot be transferred to SOM within the 21-day time frame of our experiment based on the different rates we measured. Our results show differences in the proportion of fixed ^{13}C in the SOC and MBC pools for the beech and spruce soils (see reply to comment 1). Since CO_2 fixation is a microbial process, we assume that the excess ^{13}C label measured in the soils after 21 days originates from CO_2 fixed by microbial biomass which has been transferred as microbial residues into the soil. Other studies found that microbial biomass can turn over quite rapidly as fast as 18 – 33 days in soils (Cheng, 2009; Spohn et al., 2016) and the transfer of microbial residues (both as turnover of necromass and formation of extracellular products from living biomass) into SOM can occur in as little as hours (Geyer et al., 2020). Since we don't just expect microbial turnover via necromass production but also transfer of extracellular metabolites from living biomass, we would introduce the broader term, “microbial residues” in the revised manuscript which is by definition, any non-living organic material of microbial origin including necromass and extracellular metabolites (Geyer et al., 2020).

The reviewer is correct that differences in CFE efficiency might affect the calculated carbon allocated to MBC due to possible effects on MBC measurements. However, previous studies using a K_{EC} of 0.45 to account for the CFE extraction efficiency (Wu et al., 1990; Joergensen et al., 2011) as used in our study (lines 138 - 142), show that this factor does not strongly vary between soils irrespective of differences in soil properties (Martens, 1995; Joergensen et al., 2011) and would not be incubation-temperature dependent. Hence, we argue that the difference in turnover described now as residue formation between beech and spruce soils and also with temperature are caused by factors differentially affecting either the lifespan of microbial cells or the formation of microbial residues and this would be clarified in the discussion of the revised manuscript. Additionally, the relationships of rates to MBC previously found in other soils (Akinyede et al., 2020; 2022a) suggest that the CFE efficiency does not differ dramatically between the two soils. However, we cannot exclude possible effects resulting from differences in CFE extraction efficiency on our results. We will thus add a sentence in the revised manuscript to the effect that: While previous studies do not show that the CFE extraction efficiency factor of 0.45 varies strong between soils or temperatures of incubation, the assumption that this is constant between depths may affect our results, especially in comparisons of rates between different soil depths, or between rates expressed per MBC or gram soil.

- Lines 459-461 I agree with this sentence. In addition, the authors should also mention that changes in primary production and root exudation might completely change the response of the studied processes to changes in soil temperature, which adds further to the uncertainty to the predictions. Given these uncertainties, I strongly recommend to remove Fig. 7 from the manuscript.

We agree and we would include this statement in the revised manuscript and remove Figure 7.

- Section 2.4 For how long were the soils explored to the $^{13}\text{CO}_2$?
In this study, all soils were exposed to the ^{13}C -labelled CO_2 for a period of 21 days. we would modify the manuscript to clarify this.
- L. 25-27 Based on the determined parameters (respiration and CO_2 fixation) no conclusion about microbial biomass turnover can be drawn.
In reference to our reply to comments 1 and 2, we still wish to speculate about the microbial biomass turnover which we now describe as a part of microbial residue formation. But following the suggestion from reviewer 2 comment 1, this speculation would be limited to the discussion section of the revised manuscript.
- L. 42 Remove "which also affects CO_2 emissions from other soils"
This would be modified as suggested.
- L. 52 Do you mean SOC concentration or quality?
We meant both SOC content and quality. This would be modified in the revised manuscript.
- L. 71 replace second "by" by "until"
This would be modified as suggested.
- L. 77-80 This statement cannot be drawn from the cited studies since they measured both processes at only one temperature
Thank you for this comment. Our assumption is not only based on the findings from past studies showing that dark CO_2 fixation rates correlate linearly with net soil respiration (Miltner et al., 2005; Santruckova et al., 2018). We also considered that both soil respiration and CO_2 fixation rates increase with temperature as stated in lines 58 – 62 and lines 66 – 68. this section would be modified accordingly in the revised manuscript.
- L. 83 are there other forest types in the temperate zone besides coniferous and deciduous forests?
For simplicity, we would rephrase the sentence in the revised manuscript to reflect deciduous and coniferous forests as the two temperate forest types based on vegetation as reported in past studies.
- Table 1 Please indicate the depths of the soil horizons
This table would be modified as suggested.
- L. 441/442 Remove "derived"
This would be modified as suggested.
- L. 462-464 These two sentences are not clear, at all.
We apologise for the confusion; these sentences would be clarified in the revised manuscript.
- L. 492-494 There seems to be some confusion here, and the process of microbial biomass turnover and microbial necromass turnover get mixed up. I think what the authors actually refer to is the rate at what C turns over in the living soil microbial biomass. It would be good to separate these two process more cautiously in the text.
Thank you for this comment. We refer to the transfer or turnover of carbon from the living microbial biomass into the soil which we now describe more accurately as microbial residues (see reply to comment 2). Hence, we would remove the wording on necromass stability in the revised manuscript.

