

Interactive comment on “Fate of rice shoot and root residues, rhizodeposits, and microbe-assimilated carbon in paddy soil: I. Decomposition and priming effect” by Zhenke Zhu et al.

Zhenke Zhu et al.

zhuzhenke@126.com

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Dear Anonymous Referee #2, Thank you very much for your valuable comments. We would like to answer your concerned points one by one (Q, COMMENT, and A, RESPONSE).

Q1. Zhu et al. present an interesting and cleverly designed experiment examining the fate of different ^{13}C -labeled tissues in rice paddy soil. However, in my opinion there are several important deficits in the presentation of the manuscript, one potentially significant error in analysis, and critical caveats of interpretation that should be considered.

C1

A: Thanks for your work on our paper and raising important caveats. We have revised the manuscript accordingly.

Q2. First, the authors use an isotope mixing model based on delta notation (line 216) to partition CO_2 from native SOM vs. ^{13}C -labeled tissues. This is likely to yield biased results, because delta notation becomes highly nonlinear with respect to ^{13}C atom percent away from zero per mil, and thus delta notation should not be used in the context of isotope labeling experiments. This flaw can be readily fixed by using atom percent ^{13}C values, as opposed to delta values, in the mixing model. This should affect the magnitude of calculated C fluxes, but not the direction of the results.

A: We have revised the mixing model equation and recalculated it using atom percent ^{13}C values instead of delta values in the mixing model. See details in P 9-10, L 213-223.

Q3. Second, there appears to be confusion and a misstatement with respect to the total amount of C added in each treatment. If I understand correctly based on the methods, the total C in each treatment is as follows: Shoot C and Root C treatments should have 100 g of bulk soil (1.8 % C) plus 0.6 g tissue with C content of 41 and 29 %, respectively. This yields total mass-weighted C content of 2.04 % and 1.97 % based on the above data, which is in fact substantially greater than the other treatments (1.9%), in contrast to what is claimed without support in the abstract (where it is claimed that the Rhizo C and micro C had greater C).

A: Yes, the total amount of C in the treatment of Shoot C and Root C was 2.04 % and 1.97 %, respectively. However, in the abstract we compared the total C amount of Rhizo-C- and Micro-C-treated soils with the untreated soil, but we did not compare them with Shoot C and Root C treated soil. It reads “the total C contents of Rhizo-C (1.89%) and Micro-C treated soils (1.90%) were higher than those of untreated soil (1.81%)”.

Q4. This difference in C inputs among treatments is important to consider in the context

C2

of priming, one of the main foci of the study. If one assumes that there is a limited and finite capacity for stabilization of fresh C inputs to soil, regardless of source, one might postulate that the priming response to addition of C varies with the amount of C added. Thus, one could potentially observe differences in priming among treatments simply due to C quantity, in addition to the likely impact of biochemical differences among C substrates. This is especially important given that the treatment which exhibited the greatest priming also had the greatest C addition (2.04% for shoot C). I don't think this is necessarily a fatal flaw, but rather an important limitation of interpretation that needs to at least be acknowledged and discussed. It seems odd to me that the experiment was not designed to add a uniform amount of organic matter among treatments.

A: Yes, we acknowledge that the amount of C added to soil is an important factor affect priming effect. In our study, the amount of plant residues C added to soil was based on the fertilizing amount in field, the straw and root were added at a rate of 6 g/kg. As the amount of rhizodeposited C and microbial assimilated C are significantly lower than plant residues C in natural environment, we have also chosen for a smaller rate in our experiment. In order to evaluate the effect of different photosynthetic C sources during the simulated fallow period on native SOC mineralization under realistic conditions, we didn't add a uniform amount of organic matter among treatments. But we got your point that this complicates direct comparison of the carbon sources. In the discussion we addressed this complication at P.14, L.340-342, where we stated the following: "Besides the lower contents of Rhizo-C and Micro-C as compared to Shoot-C and Root-C, this observation is possibly due to the different behaviour of primers in soil".

Q5. Third, estimates of variability around means are typically not presented. These are critically needed to interpret differences (or lack thereof) among treatments. There is also confusion and contradiction in the manuscript about which differences are significant or not, particularly with respect to priming in the root addition treatment (once it is stated that there was a positive priming effect, elsewhere it is stated that this was not significant). These will likely need to be re-evaluated with the new mixing model

C3

results from atom percent data, as discussed above.

A: The variability of replicates was considered in the revised version, and we have added the standard errors. We have also revised the mixing model equation and recalculated PE. We obtained the results that shoot and root addition increased C emission up to 11.4 and 2.3 times higher than that of the control soil by day 20, respectively, the stimulatory effect persisted to the end of incubation period in case of Shoot-C. Over the entire incubation, the priming effect of Shoot-C on CO₂ and CH₄ emission was strongly positive over the entire incubation, however, Root-C failed to exhibit a significant positive priming effect.

Q6. Fourth, the hypothesis that was posed at the end of the introduction was ambiguous and was not further addressed in the discussion. The hypothesis needs to be justified in the Introduction, and evaluated in the context of the data in the Discussion.

A: Thank you very much for this important comment. You are right, the hypothesis is ambiguous and not well prepared for in the Introduction. In the revised version we hope that this has been improved. The hypothesis chapter is rewritten at P.5-6, L.100-105 as follows: "There were only limited studies of estimating the fate of plant residues and rhizodeposits in paddy soils and, to our knowledge, there is no comparative information on (1) the decomposition of different organic C sources, such as rice shoots and roots, rhizodeposits, and microbe-assimilated C; or (2) the effects different organic C sources on the mineralization of native SOC. We hypothesized that depending of the type of the primer both, the decomposition of the primer itself and with that the PEs on native soil organic matter vary. We assume that shoots and roots, entering the soil as unprotected particulate organic residues, are well available for microorganisms and thus also stimulate native organic matter decomposition. In contrast, rhizodeposits and microbial carbon reflect a carbon sources that are rather stabilized and contribute less to priming. We investigated these hypotheses by quantifying the contribution of different organic C sources to CO₂ and CH₄ emission and by analysing their PE, in a 300-d incubation study using ¹³C-labelled rice plant residues, rhizodeposits, and microbe-assimilated C

C4

in paddy soils.”

Q7. Fifth, because there was a different mass of ^{13}C added to each treatment, I cannot see how Figure 1a,b are useful, and these should likely be removed. Figures 1c,d show the normalized data and are much more useful.

A: We have removed Figure 1a, b.

Q8. Sixth, Tables 1 and 2 are confusing and possibly contain errors, as discussed below.

A: We have revised them according to your suggestions. In Table 1, the excess of ^{13}C (not total ^{13}C) in 100 g bulk soil is 0, and the total ^{13}C in 100 g bulk soil was 19.4 ± 0.56 mg. In Table 2, we have added the error bars.

Q9. Finally, although I agree with the authors’ overall interpretation of the data, there are several sentences that are logically inconsistent throughout the manuscripts, where the statement at the beginning of the sentences does not support what follows. There is also substantial speculation and extraneous text that should be revised or removed. These are detailed below.

A: Thank you very much for your valuable detailed comments. We have revised them one by one.

Q10. 32-36: This statement is not logically consistent. An increase in ^{13}C emissions does not imply lower soil organic C decomposition, nor that Rhizo-C and Micro-C soils decrease mineralization of native soil C.

A: Sorry, for not being clear in the original version. Our point is that the total C emission of Rhizo-C and Micro-C was the same as from the control soil, despite (a) the higher carbon contents in the former and (b) the fact that parts of the CO_2 produced in the Rhizo-C and Micro-C variants originated from the label as can be deduced from the $\delta^{13}\text{C}$ ratios. Hence, the mineralization of native SOC appeared to be decreased in case of the Rhizo-C and Micro-C treatments. We modified the sentence to: “Given the

C5

fact that about 0.3% and 0.1% of the cumulative C emission derived from the labeled Rhizo-C and Micro-C, this indicates that the soil organic C-derived emissions were lower in Rhizo-C and Micro-C treated soils than in untreated soil. This indicates that rhizodeposits and microbe-assimilated C could be used to reduce the mineralization of native soil organic carbon and to effectively improve soil C sequestration”. See details at P 2, L 32-36.

Q11. 52: Heterotrophic microbes are typically much more abundant in terms of biomass than autotrophs, and would be expected to be a more important C input to SOM. This distinction is not important here.

A: Yes, heterotrophic microbes are typically much more abundant in terms of biomass than autotrophs, but particularly in paddy soils autotrophic soil microbes assimilate CO_2 and contribute to soil C accumulation. As this has been also addressed in our study, we think that it is valid to mention it in the introduction.

Q12. 58-59: But you just mentioned the importance of microbes. . . green manure and manure are also often used in paddy systems.

A: We revised the statement as “The aboveground biomass and root systems of rice plants represent one of the most important inputs of available organic C to paddy SOC”.

Q13. 75-77: This may be statistically significant but autotrophic microbial C fixation is equivalent to a rounding error in the total C budget of these systems. . .

A: Yes, although the amount of autotrophic microbial fixed C is relatively small, it might be worth to investigate it, as the fate of this is different than that of e.g. particulate plant-derived organic matter (roots or shoots). Microbial carbon can be particularly stabilized in soil. The wording in the paper with this respect is more careful in the revised version.

Q14. 81-83: This is a false dichotomy, as plant residues decompose to yield low molecular weight substances. 88: Contradicts the above statement, where you asserted that straw leads to priming.

C6

A: Thanks for your comment. We have rewritten this part of the introduction.

Q15. 97: How do you define complexity here? It is unclear whether fresh plant tissue or microbial biomass would be more complex than the other in terms of biochemical composition. This hypothesis needs to be introduced and justified in the context of the literature.

A: Thank you very much for this important comment. You are right, the hypothesis is based on a not well defined and partly wrong prerequisite. Indeed microbial carbon is having a complex structure, and there is increasing evidence in literature that microbial residues are stable in soil. Hence, Microbe-C added to the soil might be relatively stable as well (which is actually also shown in the paper). In contrast, rhizodeposits exist of low-molecular and easily available organic substances. But as shown by Lu et. al. (2002) and Kuzyakov (2002), a large part of this carbon is uptaken by microorganisms and undergoing microbial metabolism. Hence, the carbon ends up partly as microbial carbon, and it is expected that Rhizo-C is having a similar fate as Microbe-C. As we quoted in our reply to Q6, we have revised the hypothesis chapter.

Q16. 127: I disagree with this statement, microbes were definitely exposed to the $^{13}\text{CO}_2$ label given that root respiration would have been enriched in ^{13}C . Even heterotrophic microbes assimilate CO_2 via anapleurotic fixation. This does not matter in the context of your treatments, and this text could be removed.

A: We have removed this text in the updated version.

Q17. 215: Delta notation should not be used for ^{13}C -enriched samples because it is highly nonlinear away from 0 permil. The mixing analysis should be repeated using the atom percent data.

A: In the present version, the mixing analysis is based on atom percent data.

Q18. 235: This contradicts what was stated in the abstract with respect to trends in ^{13}C in the Rhizo C and Micro C treatments. A: Thanks for your comment. Since we

C7

stated “but with lower percentages”, this means less labeled substrate was mineralized. Therefore, it does not contradict the abstract.

Q19. 232-237: Because there was a different mass of ^{13}C label added to each treatment I think that Figure 1 a, b is misleading. Figure 1 c, d normalize the $^{13}\text{CO}_2$ fluxes to the amount of label added, thus the treatments can be readily compared. I recommend removing Fig. 1a, b and the associated text in the Results.

A: We have removed Fig. 1a, b and the associated text in the Results.

Q20. 250-255: Standard errors associated with these percentages are needed. 259-260: Standard errors needed

A: We have added the standard errors.

Q21. 273-274: Isn't it trivial that the cumulative $^{13}\text{CO}_2$ respired increased over the experiment? Discussing rates of change would be more informative.

A: Yes, it is certainly trivial that a substrate is mineralized with time. We deleted this phrase.

Q22. 278: Do you mean “no” effect?

A: Yes.

Q23. 304-310: This claim cannot be supported by the present data, and should be couched as speculation or removed. A: We added the term “presumable” to this sentence, emphasizing that this is an assumption. However, there is a wealth of literature showing that such substances can get well sorbed to minerals and thereby stabilized. Some of the references are cited in the text.

Q24. 319-321: Unsupported speculation

A: Thanks also for this comment. However, here we kindly disagree. If an organic substrate consisting of a mixture of distinct organic compounds is decomposed, the

C8

more stable compounds get selectively enriched during decomposition. And it is also logically, and shown by e.g. Lu et al. (2003) and Brant et al. (2006) that a less available carbon source is used in a more conservative way, i.e. meaning that relatively less organic carbon is respired.

Q25. 322-323: But you saw PE decrease over time, right?

A: Yes, the PE decreased over time but it was still positive PE.

Q26. 328: But in natural systems, Rhizo C and Micro C typically accompany root and shoot C, they are not present on their own, unless roots and shoots are manually removed. One implication of your results might be that soil C would disproportionately benefit from shoot removal by farmers, is this correct?

A: Yes, we agree with your assessment, that in reality the C sources are not added separately into soil. However, in our study we intended to study the effect of the different C sources on the soil C mineralization and sequestration in order to identify their potential role in priming. This can be only done, when their separate effect on priming is studied.

Q27. 330-332: Better support for this claim would come from the isotope mixing model.

A: We agree with your assessment, however, in our experiment we can't measure the original ^{13}C abundance and amount of rice rhizodeposits C and soil microbial assimilated C, because the rhizodeposits C and soil microbial assimilated C were bound to soil mineral or mixed with unlabeled SOC during the labelling period. Hence, we could not partition the amount of $\text{CO}_2\text{-C}$ derived from native SOC and from rhizodeposited C and soil microbial assimilated C bound to soil mineral or mixed with unlabeled SOC during the labelling period. So we couldn't calculate their priming effect. But we could infer from the data (see also our response on Q 10) that there was a negative effect of both primers on the mineralization of native SOC.

Q28. 333: I assume you mean ^{13}C of CO_2 ? Need to specify here and elsewhere. , it

C9

was ^{13}C of CO_2 .

A: Thanks, we have revised it.

Q29. 333: Unnecessary to include "rice-growing season" given that this is not a field study.

A: We have deleted it.

Q30. 333-336: This conclusion does not follow from the premise. This sentence is confusing and not logically consistent.

A: Thanks, we have revised it as follows (P 14, L 333-344): "Hence, it seems that Rhizo-C and Micro-C protects native SOC, increase the organic carbon storage of paddy soil (Ge et al., 2012; Li and Yagi, 2004; Gunina et al., 2015). Besides the lower contents of Rhizo-C and Micro-C as compared to Shoot-C and Root-C, this observation is possibly due to the different behaviour of primers in soil. Roots and shoots enter the soil as particulate and unprotected organic matter, which is to a large part well available for microorganisms; i.e. 31.9% and 45.4% where mineralized within the 300 days of incubation (Fig. S1). Rhizodeposits consist mostly of low molecular sugars and acids that are highly bioavailable (Lu et al., 2002). The relatively long MRTs of Rhizo-C (Table 2) suggests a stabilization process of this carbon, either by sorption or by microbial metabolism and recycling during the incubation (Lu et al., 2002; Gunina et al., 2014; Schurig et al., 2013). Also Micro-C has a long MRT in the incubation (Table 2), which fits well to the observation that microbial residues are accumulating in soil (Schurig et al., 2013)"

Q31. 336-340: That is one hypothesis; another would be that these tissues are selectively stabilized due to interactions with minerals or aggregate formation. This uncertainty should be acknowledged.

A: Yes, you are definitively right. In the right version we consider stabilization via interactions with minerals in the introduction (in order to prepare the hypotheses) as well as

C10

in the discussion.

Q32. 346: You stated before that the PE for root treated soils was insignificant. Need to be consistent in the text, is it significant or not? If not, PE is not positive.

A: At early stages, there was also a positive priming in case of roots, while after loss of easily available parts of the Root-C the decomposition rates decreased and as a consequence priming was less, and considering the whole period of incubation it was not significant. We have revised this sentence as follows: "By the end of 300-d incubation, both Shoot-C treated soils exhibited higher total mineralization and positive PEs, while Root-C failed to exhibit a significant priming effect".

Q33. 350: Should mention as a caveat that different amounts of C were added in each treatment, and it is uncertain whether this contributed to differences in the results. Table 1: The third row is unclear, why does bulk soil have 0 mg total ^{13}C , when it is 1.08 atom percent ^{13}C ? You need to clarify or account for natural abundance ^{13}C . Table 2: "Size" of the pools is unclear here, is this the proportion of ^{13}C that was respired over the experiment?

A: Yes, you are right. And, as mentioned in our reply on Q4, now we consider this fact in the discussion. In Table 1, the excess of ^{13}C (not total ^{13}C) in 100 g bulk soil was 0, and the total ^{13}C in 100 g bulk soil was 19.4 ± 0.56 mg. And total ^{13}C of the four photosynthesized C substrates input to 100 g bulk soil after 18 d of ^{13}C -labelling were 11.43 ± 0.52 , 5.75 ± 0.41 , 1.61 ± 0.06 and 0.49 ± 0.05 mg, respectively. In Table 2, the "Size" describes the proportion of bioavailable labelled-substrate mineralized relative to initial ^{13}C amount.

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