

Dear Anonymous Referee #3, thank you very much for your valuable comments. We would like to answer your concerned points one by one (Q, plain, and A, blue font).

Q1. This paper presents an incubation experiment using rice tissues and soils labeled with ^{13}C . Labeled shoots and roots were directly added to soil. Rhizodeposits were added by shaking soil from roots, and microbe-fixed C was added by using soils that were sunlit and treated with ^{13}C but had no plants.

This study addressed important issues related to priming effects in rice agriculture, and is appropriate for this journal. The results have important implications for C emissions, soil carbon storage, and potential strategies for mitigating greenhouse gas emissions from agriculture. The isotope labeling procedure and the incubation were sound.

A: Thank you for the positive comment.

However, there were some issues with the analysis and interpretation that should be addressed.

Q2. In general, the root and shoot amendments seem sound. However, the amount of labelled carbon added in the Rhizo-C and Micro-C treatments was much smaller than in the root and shoot treatments, and it's not clear whether the different treatments can be directly compared with each other. Judging from Table 2, the amount of ^{13}C added to the soil in the Rhizo-C and Micro-C treatments was very small relative to the ambient ^{13}C content of the soil, and I am not convinced that the emissions from these small additions were enough to be detectable in this experiment. It's difficult to tell how much labeled substrate was actually added in those two treatments, and in my opinion it calls the interpretation of results related to those additions into question. I think there should be more discussion of why the ^{13}C emissions from these treatments can be interpreted as resulting from the amendments rather than just mineralization of ambient ^{13}C that was already present in the soils.

A: As these ^{13}C -labeled microbial biomass or rhizosphere exudates are attached to soil minerals, it is not possible to obtain them without an associated soil matrix, unless obtained in an artificial environment. Hence, it would not make much sense to produce these substances in vitro. Of course, our approach leads to the complication that we obtained the rhizodeposits and microbial assimilated C embedded in a matrix of mineral soil with native SOM. But we still feel that our approach is appropriate to comparatively investigate the priming effects of particulate plant-derived materials and plant and microbial-derived substances sorbed or being attached to minerals within one experiment.

The total C contents of the soils containing rhizodeposited C (1.89%) and microbial assimilated C (1.90%) were larger than that in bulk soil (1.81%), though we have to admit that this difference is not statistically. But despite there was no significant difference in the total C emission between the treatments of Rhizo-C, Micro-C and CK, there was a considerable amount of label-derived $^{13}\text{CO}_2$ emitted during incubation. This indicates that the rhizodeposits and microbial assimilated C contributed to total CO_2 emission and, hence, the mineralization of native SOC appeared to be smaller at addition of these two primers. We do think that our approach does make sense, as the allocation pathway of rhizodeposition and autotrophic synthesis of microbial biomass is to get instantly associated with minerals.

Q3. There are issues with the equations. Most of them have typographical errors or confusing notation.

A: Thanks, we have revised all the equations. See details in L 194-223, P 9-10.

Q4. The calculation of priming effects is problematic. They are defined using cumulative emissions. However, they are then interpreted as changes over time with statements like “a positive priming effect was observed until the end of the incubation.”

If calculated using cumulative emissions, any short-term priming effect would appear to last for the entire experiment, because the additional emissions at the beginning would not be cancelled out by any negative emissions later in the experiment (unless there are negative priming effects later on). Cumulative emissions could be used to calculate a total priming effect over the entire experiment in terms of extra carbon lost from SOC, but a time series of fractional priming effects like the results presented here would make much more sense if it were calculated using emission rates rather than cumulative emissions

A: We have recalculated the PE.

Q4. Line 32-36: I don't follow the logic of this statement. According to Fig. 1, emission rates from Rhizo-C and Micro-C were decreasing over most of the incubation. Cumulative ¹³C emission increased over the experiment, of course, but this only means that emission rates were greater than zero.

A: Thanks. As reviewer 2 raises the same issue in his query 10, we kindly refer to our response to Q10 of reviewer 2. See details at P 2, L 32-36.

Q6. Line 75-77: These are tiny fractions. Are they really detectable in this kind of experiment? It's a factor of 100 less than rhizo-deposits.

A: Yes, we detected that phototrophic soil microbes assimilate CO₂ using ¹⁴CO₂ labeling method in upland soil and paddy soil (Ge et. al., 2013).

Ge, T. D., Wu, X. H., Chen, X. J., Yuan, H. Z., Zou, Z., Li, B. Z., Zhou, P., Liu, S. L., Tong, C. L., Brookes, P., Wu, J. S.: Microbial phototrophic fixation of atmospheric CO₂ in China subtropical upland and paddy soils, *Geochim. Cosmochim. Acta.*, 113, 70-78, 2013.

Q7. Line 79-81: There is a balance between microbial decomposition and mineral sorption of these substrates, and there's a lot of uncertainty about how much is respired vs sorbed over various time scales. This balance probably depends on soil physical and chemical factors, and might be different in frequently flooded soils.

A: Yes, this is certainly true. Of course, as a result of decomposition, water soluble intermediate products of decomposition are produced that can sorb on minerals. We modified the respective sentence. However, the difference between the four substrates tested is that Shoot-C and Root-C are first particulate, and just after formation of water soluble substances or resynthesis in microbial biomass, this C will get attached to minerals. In contrast Rhizo-C can be immediately bound to minerals (if not mineralized) and Micro-C is attached to minerals from the beginning. This difference is having an important impact on the mineralization rate of the substrates, as is shown by the cumulative mineralization.

Q8. Line 97-98: There aren't really any measurements of the "complexity of substrate composition" (which isn't clearly defined either)

A: Thank you very much for this important comment. As reviewer 2 raises the same issue in his query 6, we kindly refer to our response to Q6 of reviewer 2. The hypothesis chapter is rewritten at P.5-6, L.100-105.

Q9. Line 98-99: This sentence isn't very clear. Is "their relatively higher quantity and stability in soil" referring to plant residues or rhizodeposits and microbe-assimilated C? Shouldn't substrates with higher stability in soil cause weaker priming effects, because they are more resistant to decomposition?

A: Yes, the hypothesis chapter was not clear. We hope that this is clear in the revised version, which is cited in our reply to Q6 of reviewer 2.

We also expected that substrates with higher stability in soil, either due to their

inherently higher stability or due their stabilization by e.g. sorption to minerals, cause weaker priming effects because they are more resistant to decomposition. This is actually also one of our major results.

Q10. Line 144-145: The procedures for collecting rhizodeposits and microbe-assimilated ^{13}C sound like they include a little bit of labeled material mixed with a lot of soil, which means these additions were quite different from the plant tissue amendments, which were pure labeled tissue. This raises questions about whether the rhizodeposit and microbe-assimilated C additions are directly comparable to the root and biomass additions.

Line 164-167: The amount of carbon in these two treatments is not well known, and likely very different from the other two treatments, making direct comparison tricky.

A: Thanks for raising these two critical points. But as these ^{13}C -labeled microbial biomass or rhizosphere exudates are attached to soil minerals, it is not possible to obtain them without an associated soil matrix, unless obtained in an artificial environment. Of course, our approach leads to the complication that we obtained the rhizodeposits and microbial assimilated C embedded in a matrix of mineral soil with native SOM. However, we do think that our approach does make sense, as the allocation pathway of rhizodeposition and autotrophic synthesis of microbial biomass is to get instantly associated with minerals. Hence, we were feeling worth to test the priming capabilities of rhizodeposition and microbial biomass together with roots and shoots within the same experiment, and our approach is appropriate to comparatively investigate the priming effects of particulate plant-derived materials and plant- and microbial-derived substances sorbed or being attached to soil minerals within one experiment.

Q11. Equation 2: I'm not an expert on isotope labeling math, but this equation looks a little strange. What is $(\delta^{13}\text{C}+100)$ doing? I think it should be $(\delta^{13}\text{C} + 1000)$, which

equals RS/RPDE. Either way, it seems needlessly confusing to convert RS into per-mil units and then convert that into atomic percent, instead of just using $RS/(RS+1)$, which as far as I can tell is mathematically equivalent. Equation 3: The notation of this equation (with all the brackets and commas) is confusing. It would be easier to read with some different notation (subscripts or something).

Line 207: This is labeled equation 2 but should be equation 4. It also doesn't make sense relative to the description on lines 208-210. If y is a percentage of ^{13}C emission, then all of the terms in the equation should be percentages, while in fact they are pools. If y_0 is the pool of labeled C remaining in the soil, then it should be decreasing with time. The description of a is basically the same as y_0 . This equation would make more sense (relative to the description of the terms) if it were $y_0 = a(1 - e^{-bx})$.

Equation 5: Should the denominator have $\delta^{13}\text{C}_{\text{shoot}}$ and $\delta^{13}\text{C}_{\text{soil}}$ rather than $\delta^{13}\text{CO}_{2\text{shoot}}$ and $\delta^{13}\text{CO}_{2\text{soil}}$? $\delta^{13}\text{CO}_{2\text{soil}}$ in the equation doesn't seem to be a thing that was actually measured. Equation 6: Priming effects are defined here as the difference in total C emissions between the amended experiments (C_{shoot} or C_{root}) and the control experiment (CK). This includes the C emissions from the decomposition of the added material as well as extra decomposition of native SOC. This is not how priming effects are usually defined, or discussed in the introduction. Usually, priming effects are defined as extra decomposition of just the native SOC, excluding emissions from the added material. If that's the case, then this equation should be isolating emissions derived from native SOC rather than using total emissions. Also, I think it would make more sense to compare emission rates rather than cumulative emissions in this ratio. If the priming effect occurs as a short pulse effect, then using this equation will exaggerate how long the priming effects last, because the increase in cumulative emissions will slowly decline as it's divided by increasing total emissions, even after increases in emissions due to priming have ceased.

A: We apologize for the mistakes in the equations, we have revised them in the revision as follows:

“The $\delta^{13}\text{C}$ values of plant residues, rhizodeposits, microbe-assimilated C, soils, CO_2 , and CH_4 were converted in δ (‰) relative to the Pee Dee Belemnite (PDB, 0.0111802) standard and further expressed in atom% as following

$$\text{atom}\% = \frac{100 * 0.0111802 * (\frac{\delta}{1000} + 1)}{1 + 0.0111802 * (\frac{\delta}{1000} + 1)} \quad (1)$$

and the incorporation of ^{13}C (^{13}C excess) in plant residues, rhizodeposits, microbe-assimilated C, bulk soils, CO_2 , and CH_4 was calculated as follows:

$$\text{excess } ^{13}\text{C}_{\text{sample}} = [(\text{atom}\% ^{13}\text{C})_{\text{L}} - (\text{atom}\% ^{13}\text{C})_{\text{UL}}] \times \text{C}_{\text{sample}} / 100 \quad (2)$$

Where $(\text{atom}\% ^{13}\text{C})_{\text{L}}$ and $(\text{atom}\% ^{13}\text{C})_{\text{UL}}$ are the *atom* ^{13}C in labelled and unlabelled samples, respectively, and C_{sample} are the C contents of each sample.

The $^{13}\text{CO}_2$ and $^{13}\text{CH}_4$ efflux (%) were calculated as the increases in excess of ^{13}C - CO_2 and ^{13}C - CH_4 within each sampling interval,, respectively, as percentages of the ^{13}C input. The mineralization percentage of the input ^{13}C was calculated as the sum of total ^{13}C in CO_2 and CH_4 , at each sampling day, relative to the initially added total ^{13}C .

The kinetics of the mineralization were described by fitting a first order single exponential function:

$$y = a (1 - e^{-bx}) \quad (3)$$

where a describes the amount of bioavailable labelled-substrate pool; b is the mineralization rate of substrate; and x is time (d). Obtained parameters were used to calculate the mean residence time as $1/b$ and half-life as $\ln(2)/b$.

The end-member mixing model was used to calculate the fractions of SOC- (C_{SOC}) and plant residue-derived C (C_{shoot} and C_{root}), as described by Phillips et al. (2005) and Wild et al. (2014). This model allows the combination of mass spectrometric and efflux measurements. The shoot-derived $^{13}\text{CO}_2$ emission ($^{13}\text{CO}_{2\text{shoot-derived}}$) was calculated as follows:

$$^{13}\text{CO}_{2\text{shoot-derived}} = \frac{\text{atom}\% \text{CO}_{2\text{shoot}} - \text{atom}\% \text{CO}_{2\text{CK}}}{\text{atom}\% \text{C}_{\text{shoot}} - \text{atom}\% \text{C}_{\text{soil}}} \times \text{CO}_{2\text{shoot-C}} \quad (4)$$

where $\text{atom}\% \text{CO}_{2\text{shoot}}$ and $\text{atom}\% \text{CO}_{2\text{CK}}$ are the $\text{atom}\% \text{}^{13}\text{C}$ values of CO_2 derived from shoot treated soil and untreated soil (CK), respectively; $\text{atom}\% \text{C}_{\text{shoot}}$ and $\text{atom}\% \text{C}_{\text{soil}}$ are the $\text{atom}\% \text{}^{13}\text{C}$ values of shoot and bulk soil respectively; and $\text{CO}_{2\text{shoot-C}}$ is the total CO_2 derived from shoot treated soil; and the shoot-derived $^{13}\text{CH}_4$ emission ($^{13}\text{CH}_{4\text{shoot-derived}}$) and the root-derived $^{13}\text{CO}_2$ and $^{13}\text{CH}_4$ emission ($^{13}\text{CO}_{2\text{root-derived}}$ and $^{13}\text{CH}_{4\text{root-derived}}$, respectively) were calculated similarly (Phillips et al., 2005; Ye et al., 2015).

The PE of SOM on CO_2 and CH_4 emission was calculated as follows:

$$\text{PE}_t(\%) = \frac{\text{Gas} - \text{Gas}_{\text{CK}}}{\text{Gas}_{\text{CK}}} \times 100 \quad (5)$$

where PE_t is the PE at time t (d); Gas the total amount of CO_2 and CH_4 derived from native SOC mineralization in the treatment of Shoot-C and Root-C, Gas_{CK} is the SOC mineralization in the CK treatment (Hu et al., 2012).”

Q12. Lines 239-241: These units don't make sense for emission rates, unless they are percent of initial ^{13}C lost over a specified time period (% per day or something). It's hard to interpret this without knowing what the initial ^{13}C was for each treatment. Those values are in Table 1, and it would help to discuss those before going into percentage losses.

A: We revised the units as ^{13}C efflux (% of initial ^{13}C) d^{-1} . We illustrated the initial ^{13}C values (in Table 1) of each treatment before the ^{13}C loss efflux.

Q13. Lines 245-246: Based on Table 1, the initial ^{13}C in Rhizo-C and Micro-C treatments is nearly indistinguishable from the unlabeled bulk soil value. Are these measurements sensitive enough to determine how much of these ^{13}C emissions was from the labeled amendments of those treatments and how much was from the ambient ^{13}C content of the soil?

A: Yes, we acknowledge that the initial ^{13}C in rhizodeposited C and microbial assimilated C in soil was relatively small. However, we could determine the ^{13}C emissions from the labeled C sources by setting up a control, by which we calculated the amount of ^{13}C emissions derived from ^{13}C in rhizodeposited C or microbial assimilated C by subtracting the ^{13}C emissions from control.

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Q14. Line 248-249: Why is this figure in supplemental material instead of main text?

A: Thanks. The Fig. S1 was the cumulative ^{13}C emissions (% initial ^{13}C) of soils treated with different ^{13}C -labelled carbon substrates over a 300-d incubation. The cumulative ^{13}C emissions was represented the sum of $^{13}\text{CO}_2$ and $^{13}\text{CH}_4$ emissions, however the $^{13}\text{CO}_2$ and $^{13}\text{CH}_4$ emissions have already shown in Fig. 2. If we added Fig. S1 to main text it could be a bit repeated.

Q15. Line 259: The methods don't describe exactly how SOC-derived C emissions were calculated. Is this the root or shoot-derived CO_2 emission from equation 5 subtracted from total emission?

A: We have revised the equation 5. Using this mixing-model equation we could directly calculate the C emission derived from SOC or added C source, and we could also calculate the C emission derived from SOC by subtracted C emission from added C (root or shoot) from total C emission.

Q16. Line 269: Of course the total C emissions increased. It would be impossible for them to decrease unless C emissions were negative.

A: Yes, this is a kind of trivial. In the revised version we just mention the total CO_2 emission.

Q17. Line 271-273: The Rhizo-C treatment included an addition of soil that was shaken off roots, so there was extra carbon. This might explain the greater total C emission.

A: Yes, surely we added some additional extra soil carbon with our method, but as the overall CO₂ emission did not differ, and in addition the $\delta^{13}\text{C}$ signature of the CO₂ shows that part of the CO₂ derived from the labeled Rhizo-C or Micro-C, this indicates that less indigenous organic matter was mineralized.

Q18. Line 278 should say “had no effect on the mineralization...”. Also, why do section 3.3 and the associated figure only address two of the four treatments?

A: Yes, it had no effect on the mineralization.

In our experiment we can't measure the original ¹³C abundance and amount of rice rhizodeposited C and soil microbial assimilated C, because the rhizodeposited 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 CO₂-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 that there was a negative effect of both primers on the mineralization of native SOC.

Q19. Line 302-303: There is no evidence in this study to support this statement about mineral-associated organic matter. It's a possible explanation, but shouldn't be presented as a finding supported by this experiment.

Line 305-310: There also doesn't seem to be any evidence to support any of these statements. There weren't any measurements of ¹³C incorporated into microbial biomass, or any of the compounds listed in line 309, so it shouldn't be stated as something found in this experiment.

A: Thanks. We revised the text in order to emphasize that this was not found in our experiment. Rather, this is information from literature that was used to explain the findings of our experiment.

Q20. Line 315: It's misleading to say that a positive PE was observed until the end of the incubation, because the PE was calculated using cumulative values. The only way the PE could stop being positive would be if there were a negative effect on total emissions later in the incubation that reduced the cumulative emissions of the amended soils. Also, it's misleading to say "with the exception of the Root-C-treated soils", since only two things are being compared. "Exception" implies that only one thing was different out of a larger group.

A: Actually, we were calculating the PE separately for all time increments during the incubation. With that we could identify that for both substrates, Shoot-C and Root-C, the PE was more pronounced at the beginning of the incubation, when more primer was available. At later stages, when the primer was having a smaller concentration and was probably microbially transformed, the stimulating effect on the native organic matter mineralization decreased. In case of Root-C the PE effect was significant at early stages of the incubation while this was not the case anymore during the later stages. We have revised the sentence as follows: "For Shoot-C, a positive PE was observed over the entire incubation period, while for Root-C this was significant only for early stages of the incubation".

Q21. Line 322-323: Again, this statement isn't supported by PE calculated using cumulative emissions. If there is a PE observed using instantaneous emissions, it might be a more reasonable explanation.

A: We have recalculated the PE using instantaneous emissions. The PE was significant positive at initial stages of shoot- and root-C decomposition, while the PE was slowed

down at later stages, this might be the extracellular enzymes generated to degrade recalcitrant C, and promote the decomposition of SOC.

Q22. Line 327: Those differences were not statistically significant and very small, so I don't think this statement is really supported by the evidence. Certainly not enough to make such a strong statement about using them to increase SOC and mitigate global warming without stronger evidence.

A: We discussed this issue now more carefully and removed the strong statements on mitigation of global warming. We discussed the part as follows: "Both, Rhizo-C and Micro-C augmented the C content of paddy soil (1.89 and 1.90%, respectively) over that of the untreated soil (1.81%). At the same time we found that the C emissions of Rhizo-C and Micro-C treated soils were similar to those of untreated soil. As about 0.3% and 0.1% of the substrate C, respectively, were mineralized, this suggests that rhizodeposits and microbe-assimilated C input did not stimulate native SOC mineralization but rather shows a negative priming. 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)." See details in L 329-335, P 13-14.

Q23. Line 331-333: This was true for this study, but the amendments were very small. Maybe larger amendments would cause stronger effects?

A: Thanks. Yes, we agree with your assessment. The amount of ^{13}C -rhizodeposits and microbe-assimilated C was relatively small input into soil during only 18 days continues labeling in our experiment, and might underestimate the PE.

Q24. Line 333-334: Since cumulative emissions are being shown, an increase in ^{13}C is guaranteed as long as emissions are greater than zero, so this doesn't prove much. I don't follow the connection with suppression of native SOC mineralization. Given

that SOC contained some amount of ambient ^{13}C , I'm not sure this result says anything about the treatment effect on SOC.

A: The total C contents of the soils containing rhizodeposited C (1.89%) and microbial assimilated C (1.90%) were larger than that in bulk soil (1.81%), though we have to admit that this difference is not statistically. But despite there was no significant difference in the total C emission between the treatments of Rhizo-C, Micro-C and CK, there was a considerable amount of label-derived $^{13}\text{CO}_2$ emitted during incubation. This indicates that the rhizodeposits and microbial assimilated C contributed to total CO_2 emission and, hence, the mineralization of native SOC appeared to be smaller at addition of these two primers. We do think that our approach does make sense, as the allocation pathway of rhizodeposition and autotrophic synthesis of microbial biomass is to get instantly associated with minerals.

Q25. Line 336-340: These statements are not really supported by any evidence from the experiment.

A: Thanks. We have revised these statements.

Q26. Table 1: Total ^{13}C should be in mg per some mass of soil, not just mg. Bulk soil total ^{13}C shouldn't be zero – those soils have 1% atomic ^{13}C and nonzero C content, so they must contain some ^{13}C as well. In fact, based on the numbers the amount of ^{13}C in the Micro-C and Rhizo-C should be very difficult to distinguish from the amount of ^{13}C in unlabeled soil.

A: Thanks. We are sorry for this mistake, the total ^{13}C in 100 g bulk soil was 19.4 ± 0.56 mg, the excess of ^{13}C (not total ^{13}C) in 100 g bulk soil was 0.

Q27. Figure 1: Panels (c) and (d) show emissions in units of % of initial ^{13}C . These units don't make sense for emission rates, unless they are percent of initial ^{13}C lost over a specified time period (% per day or something).

A: We have revised the units.

Q28. Figure 3: The legend is confusing because it uses "Total C in . . ." and "C derived from . . ." to refer to the same thing (i.e. cumulative C emissions). Also, "derived" is misspelled.

A: We have revised the legends.