

## 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.

## **Anonymous Referee #3**

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This paper presents an incubation experiment using rice tissues and soils labeled with 13C. 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 13C 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.

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

- 1. 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 13C added to the soil in the Rhizo-C and Micro-C treatments was very small relative to the ambient 13C 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 13C emissions from these treatments can be interpreted as resulting from the amendments rather than just mineralization of ambient 13C that was already present in the soils.
- 2. There are issues with the equations. Most of them have typographical errors or confusing notation.
- 3. 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.

Specific comments follow below:

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.

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.

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.

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

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?

Line 144-145: The procedures for collecting rhizodeposits and microbe-assimilated 13C 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 microb-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.

Equation 2: I'm not an expert on isotope labeling math, but this equation looks a little

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strange. What is  $(\delta 13C+100)$  doing? I think it should be  $(\delta 13C+1000)$ , which equals  $R_S/R_{PDE}$ . Either way, it seems needlessly confusing to convert  $R_S$  into per-mil units and then convert that into atomic percent, instead of just using  $R_S/(R_S+1)$ , 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 13C 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  $\delta 13C_{shoot}$  and  $\delta 13C_{soil}$  rather than  $\delta 13CO2_{shoot}$  and  $\delta 13CO2_{soil}$ ?  $\delta 13CO2_{soil}$  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 (Cshoot or Croot) 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.

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

Lines 245-246: Based on Table 1, the initial 13C 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 13C emissions was from the labeled amendments of those treatments and how much was from the ambient 13C content of the soil?

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

Line 259: The methods don't describe exactly how SOC-derived C emissions were calculated. Is this the root or shoot-derived CO2 emission from equation 5 subtracted from total emission?

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

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.

Line 276-278: This could just be because the amount added was very small compared to the amount of root and shoot material added.

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?

Line 295-297: It's hard to compare these directly since different amounts of labeled

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substrate were added. Based on Table 1, most of the 13C in the Rhizo and Micro treated soil could have been native SOC, and so it's not surprising that the total emissions would be small relative to the initial total.

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.

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.

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.

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.

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

Line 333-334: Since cumulative emissions are being shown, an increase in 13C 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 13C, I'm not sure this result says anything about the treatment effect on SOC.

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

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

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

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.

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