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Interactive comment on "Soil carbon dioxide emissions controlled by an extracellular oxidative metabolism identifiable by its isotope signature" *by* B. Kéraval et al.

Anonymous Referee #1

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Review of " Soil carbon dioxide emissions controlled by an extracellular oxidative metabolism identifiable by its isotope signature" by Kéravak and colleagues.

This MS presents interesting data on CO2 released from non-cellular origin in soil. The MS follows up on the previous paper by Maire et al., published in this journal in 2013. The primary goal of this MS is to provide further evidence of the extracellular oxidative metabolism by comparing CO2 released from soil that has undergone different levels of sterilization. An additional goal was to observe whether or not the extracellular metabolic mechanism can break down a relatively complex organic molecule using isotopically labeled glucose.

The MS has improved immensely since the first iteration, especially with the addition

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Discussion paper

of figure 1 and other clarifications made throughout the text. The methods are appropriate for the questions asked and they have been meticulously carried out. The statistical component is easier to understand, but a few details need to be attended to (see below). The discussion addresses the hypotheses and goals described in the introduction and the author's have pointed out the relevance of the their findings to our current understanding of soil carbon metabolism and how their results can guide future research.

I find the study novel and the results to be very interesting. I think, however, there are a few questions remaining within the results that highlight that the extracellular metabolism is still in the hypothesis phase and that the conclusions the authors draw should reflect this.

My first question concerns the isotope results. From figure 3d, we see CO2 that is very depleted in the heavy isotope (-40 to -55 ‰ at the beginning of the experiment that becomes even more depleted (-50 to -75 ‰, before returning to the beginning values. The authors suggest that this is related to the DOC concentration associated with each autoclave level; however, what is curious to me is that there were no significant differences between the DOC 13C, if the logic is that a low concentration leads to higher fractionation, then we should expect DOC enriched in 13C, but we actually see the opposite (the value in the first bar of fig 4b is about 1‰ depleted relative to the other treatments).

Along this line of reasoning, it seems that a change in the isotopic fractionation should shift linearly only within a treatment, but because there is only a total sample size of 3 and the within treatment DOC concentration variability was small, this cannot be tested. What was done instead, was a comparison across the treatments and I don't entirely agree with this interpretation, simply because the relationship presented in figure 3E is not simply a matter of DOC concentration but also whatever effects (biotic and abiotic) resulted from the treatments.

Thus, I feel the concentration effect as an explanation to the isotopic fractionation effect to be unsatisfying. The precise mechanism seems to still lie within a black box and this study has provided evidence for the extracellular metabolic breakdown of glucose, but much more research remains to fully clarify the processes behind it. Lastly, I think the readers would appreciate it if the authors could put their results in context with what we know already about the isotopic signature of soil respiration. For example, we know that the range extends (normally) from -30 to -23‰ in C3 dominated systems. If the non-cellular breakdown of carbon in soil was significant then shouldn't we expect these values to be much more depleted? Furthermore, how does this theory fit within the diel and seasonal understanding that we have of soil respiration? Perhaps this phenomenon will only be relevant in certain types of soils or climates.

Detailed comments: Page 3 line 28: Aren't most of these enzymes in soils of cellular origin? Page 4 Line 17: probably want to clarify that the sampling was not made continuously. Line 18: maybe reference a biological analog to the "complex cascade of biochemical reactions" to give the reader an idea about what you are describing.

Page 5 Line 2: The beginning of this sentence is confusing – are you trying to make sure that cells were there or were not there.

Section 2.2 I am not aware that picarro sells an injection system for gas samples. Is this a customized unit? Can you also describe how the data were used from the analyzer? For example, normally an injection will have distinct tails as the sample moves through the system, did you take the peak value, integrate, or average over this pulse? Can you also describe the concentration range of your samples and whether or not calibration was necessary?

Page 8 Section 2.9: It is written that the data were tested for normality, but I couldn't find the test results in the results section- is ANOVA justified or should a non-parametric test be used instead?

Page 9 Section 3.12 Were there treatment differences in DOC concentration and the

isotopic signature (not simply between dates as indicated in the text)?

Page 13 line6: I think you mean to say that the “persistence” of emissions or that the emissions were maintained, or something similar.

Page 15 Section 4.4: This section is a fine theoretical example of how to use isotopic information to calculate the contribution of CO2 from the extracellular respiration. The only difficulty is the empirical equation derived from figure 3e. This should be removed for the reasons discussed previously and also to avoid others using the equation under the impression that it might be universal (despite any caveat written in the text).

Figure 1: List the sample size in the figure text. Figure 3a-d: show which treatments are significantly different from each other. In the figure heading list the sample size (n).

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