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Biogeosciences Discussions

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

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Anonymous Referee #1 (Received and published: 28 April 2016)

Referee1: "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 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 their findings to our current understanding of soil carbon metabolism and how their results can guide future research."

Response: We really appreciate the careful analysis of our findings made by the referee. We also thank the referee for the recommendations formulated with the aim to improve our manuscript during the two stages of the reviewing process.

Referee1: "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."

Response: We agree that EXOMET remains in the hypothesis phase. Therefore, page 16 - line [20-23], our terms were moderated: "Collectively, our results tend to sustain the hypothesis through which soil C mineralization is driven by the well-known microbial mineralization and an EXOMET carried out by soil-stabilized enzymes and by soil mineral and metal catalysts."

Referee1: "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)."

Response: In fact, figure 3b presents the delta 13C of DOC at the beginning of experiment, that is, before the EXOMET might have changed the delta 13C of DOC due to its isotopic discrimination activities (this is specified in the figure caption). Therefore, it is not surprising to see any important difference between treatments. However, we agree that the causal link between the magnitude of fractionation and the DOC content is not certain and deserves other studies. We added two sentences (page 14 line 15, page 15 line 17) conveying this message.

Referee1: “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.”

Response: As explained above we agree with these ideas and we have added two sentences acknowledging the limits of our study and explaining what can be done to progress.

Referee1: "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.”

Response: We have added the following paragraph to discuss this idea: “It is well known that the delta 13C of CO2 emitted from soils shows circadian cycle and seasonal fluctuations that reaches up to 5‰ (Moyes et al., 2010). However, it is difficult to link these fluctuations to a modification of metabolic pathways of soil respiration (living respiration versus EXOMET) in response to environmental changes since numerous other processes can contribute to these fluctuations. Moreover, it is likely that the EXOMET does not induce much C isotope fractionation in non-sterilized soils since the DOC content is typically low (Fig. 3a) (Liu et al., 2015). Therefore, addition of large amount of DOC is necessary to reveal the C fractionation induced by the EXOMET in non-sterilized soils.”

Detailed comments: Referee1: “Page 3 line 28: Aren’t most of these enzymes in soils of cellular origin?”

Response: To avoid confusion we changed the sentence by: “(i) suggest that CO2 emissions from soils are not only dependent to the bio-physicochemical environment provided by the cells”.

Referee1: “Page 4 Line 17: probably want to clarify that the sampling was not made continuously.”

Response: We changed the sentence Page 4 Line 17 by: “The production and the isotope composition (δ13C) of CO2 were monitored in sterilized and non-sterilized soils over 4 periods through 91 days of incubation.”.

Referee1: “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.”

Response: We changed the sentence Page 4 Line 18 by: “We also tested whether the EXOMET in sterilized soils can carry out complex cascade of biochemical reactions (e.g. an equivalent of glycolysis and Krebs cycle) by incorporating 13C- labelled

glucose and by quantifying emissions of 13C-CO2 (Fig 1)."

Referee1: "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."

Response: We changed the sentence Page 5 Line 2 by: "To demonstrate the absence of viable cells in soil after irradiation, . . ."

Referee1: "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?"

Response: We improved this paragraph following your recommendations: "The amount and isotope composition (ðİŻ£ 13C) of CO2 accumulated in flasks during the incubation period were quantified using a cavity ring down spectrometer analyser coupled to a small sample injection module (Picarro 2101-i analyser coupled to the SSIM, Picarro Inc., Santa Clara, CA, USA). A volume of 20 ml of gas was sampled by the analyser. The CO2 concentration in gas samples ranged from 300 to 2000 ppm of CO2 in accordance with the operating range of the analyser. The CO2 concentrations and delta 13C of gas samples were measured at a frequency of 30 mn-1 during 10 mn. Value provided by the analyser is the integrated value during these 10 mn of measurement. A reference gas with a known concentration of CO2 and delta 13C was injected between samples. For each period of incubation, the cumulated amount of CO2 was divided by the duration of the period (in days) to estimate the mean daily CO2 emission rate."

Referee1: 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?"

Response: We have indicated the p-values ranges that we used to test the normal distribution of our values and the equality of the variances: Page 8 Line 20 "Normality was tested using the Shapiro-Wilk test ($p>0.05$). Equality of variances were tested with a Leven's Test ($p<0.05$).".

Referee1: "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)."

Response: There is only one date of measurement, at the beginning of the experiment. We have slightly modified this paragraph in order to clarify the presentation of results: "Both γ-irradiations and autoclaving modified the soil chemistry as revealed by the analysis of the aqueous phase at the beginning of the experiment. The aqueous phase contained much more DOC in irradiated soil than in untreated soil (37 ± 3 μg C.g-1 to 303 ± 17 μg C.g-1 in LS and IS, respectively (Fig. 3a)."

Referee1: "Page 13 line6: I think you mean to say that the "persistence" of emissions or that the emissions were maintained, or something similar."

Response: You are right. We have changed the sentence by: "Moreover, Blankinship et al. (Blankinship et al., 2014) have shown that the persistence of soil CO2 emissions after microbial biomass suppression (or at least reduction) is not specific to irradiated soil but also occurs with other methods of sterilization such as chloroform fumigation and autoclaving."

Referee1: "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)."

Response: In fact, we wanted to present this equation as an example of how this

fractionation coefficient can be calculated. We agree with you that this coefficient can vary across soils and should not be viewed as a generic coefficient (at least at this step of knowledge). We have modified the paragraph to clarify this point.

Referee1: “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).”

Response: Following your recommendations, we have listed the sample size (n=3) in the text of figure 1, 2, 3, 4. We have also showed the differences significance between treatment in figure 3a-b. However, we did not show those last results in figure 3c-d in order to improve the readability of those figures. Standard deviations represent sufficient statistical tools which allow to illustrate the results and the messages described in paragraph 3.1.3.

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