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Interactive comment on “Decreased carbon limitation of litter respiration in a mortality-affected piñon-juniper woodland” by E. Berryman et al.

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Response to Referee 2 comments

General Comments The manuscript provides revealing results about the CO₂ pulses after rewetting in ecosystems characterised by a drought period, and about the effects of the massive death of Pinus-Juniper woodlands on these pulses. However, I have several concerns about how results are presented and interpreted. I also consider that some of the main conclusions should be taken much more carefully. Several methodological constraints also limit enough time resolution to capture the soil flux dynamics.

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Authors' response: Thank you for the time and effort involved in reviewing our manuscript - we appreciate the detailed feedback. We hope that the changes we made in the revised manuscript address your concerns adequately.

Regarding the observed absence of water limitation in mineral soil, in many ecosystems characterised by a drought period, the uneven rain events that occurs during or at the end of the dry period are larger than the simulated here. This would have important implications in the results, since it would enhance the mechanisms involved in the release of labile sources of C and, in consequence, the CO₂ pulses: the disruption of soil aggregates and extrusion of microbial metabolites in response to osmotic stress, the collapse of part of the microbial cells and the release of cytoplasm and metabolites, etc. In addition, this release of labile C could promote a “priming effect” leading to the mineralization of more stable sources of soil organic matter in the soil. Thus, the water and C limitation of soil respiration in dry ecosystems might be coupled, making challenging to disentangle the individual water limitation.

Authors' response: We agree that C limitation depends on water status in this system; that is why we added sucrose in a water solution and measured the response in respiration relative to the water-only application (the same water amount was applied in both the water-only and in the sucrose solution treatments). This allowed us to isolate the effect of additional substrate on respiration in a wetted environment. We also agree that the response to C will depend on how much water was added - the respiration response in the litter declined soon after application due to drying. A larger water application may have delayed the drying rate. However, we stand by our choice of simulated rainfall event (1.2 mm): it is a common rain event size in this ecosystem during dry periods, and our goal was to determine whether respiration following a small rain

event was limited by labile C availability in addition to water. We have clarified key parts of the Discussion in paragraph 1 to address this issue.

We agree that priming is likely happening, but we cannot test for this using this dataset. Further, it was beyond the scope of this study. Future experiments should take both priming and a range of rain event sizes into account.

I also have concerns about how the authors calculated and interpreted time courses of the 'limitation factor'. Observing the figures 2 and 3, one may think the water and C limitation decrease along the time since the treatment applications were performed, whereas it is just the opposite. This ratio just indicates how different the CO₂ fluxes are in treated chambers with respect to the untreated ones. The use of the expression "limitation factor" should not be used in this case. Instead, use the rate of CO₂ increase with respect to the untreated or water-treated chambers (see Kim et al. 2011).

Kim, D.-G., Vargas, R., Bond-Lamberty, B., and Turetsky, M. R.: Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research, *Biogeosciences*, 9, 2459-2483, doi:10.5194/bg-9-2459-2012, 2012.

Authors' response: The water and C limitations in the litter are actually decreasing over time. The rates from the treated chambers and the untreated chambers are becoming more similar over time. We believe this is because the treated areas are drying out and the respiration rate is lowering in response. We have clarified this point in the first and third paragraphs of the Discussion. However, we agree that our calculation of "limitation factor" was counter-intuitive and difficult to relate to previous work in the area, so we have changed the calculation to that in Kim et al. 2012. This is reflected in Eq 1 and in Figures 2,3 and 7 (see below for changes to cumulative limitation). We have removed all references to "limitation factors" and instead define limitation as the percent increase

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in respiration following the water or sucrose applications relative to a control (Section 2.5 “Labile C and water limitation calculations”).

Moreover, the length of the CO₂ pulses can be influenced by several factors included the labile C pools in the soil. Thus, the selection of the ‘time window’ to calculate the accumulated limitations cannot be arbitrary. This impedes the correct interpretation of the accumulated limitation factors.

Authors’ response: We agree that this is an important decision that may affect the outcome of the paper. To assess this, we looked again at the change in response over time and selected multiple time periods following application from which to calculate cumulative responses. We decided to assess cumulative responses during the first 3 cycles following treatment applications (8 hr), during the first 36 hr following treatment application, and during the entire experiment duration (115 hr). We then re-calculated cumulative limitations (per Kim et al. 2012) based on these different time windows and found that our overall conclusions did not change for limitations to respiration (Figure 7 in the revised manuscript). In the case of the response to treatments to the litter surface, using a longer time window would have dampened the differences between the two sites, because the greatest difference in respiration response is seen in the first eight hours. Detecting differences following applications to the mineral soil is less straight-forward, because the peak in respiration is delayed and may not even be fully captured by our time series. It turns out that we were not able to detect significant differences between the two sites for labile C limitation in the mineral soil. This was due to the high among-rep variability in respiration rates following sucrose application to the mineral soil at the Reference site (see error bars in Figure 7); this variability probably would not have declined much after

our measurement period ended, so we think it's unlikely that extending the measurement window would have revealed significant differences between the two sites.

Finally, soils usually experiment a fast response to rewetting (fast mineralization of the labile organic pools that become available by the effect of rewetting) and the observed response CO₂ pulses can last from hours to several weeks. For this reason, high temporal sampling is needed to correctly record the temporal evolution of soil CO₂ pulses. As the magnitude and length of these pulses depend on the amount of water added to the soil and the amount of water added in this experiment was quite small, it may have happened that an important part of the pulse have not been registered, thus underestimation the response to the application treatments.

Authors' response: We agree that this is a possibility. Our first measurement post-treatment was 20 minutes following application - it is possible that respiration may have peaked before the first measurement. However, we think that 20 minutes is an acceptable amount of time to wait before taking the first respiration measurement - even if we missed an initial peak, the effects would linger long enough to detect treatment differences. As a reference, here are some previous studies examining respiration responses to water and C additions. Ekblad and Nordgren (2002) waited 1 hr following sucrose application to measure respiration in a temperate forest, and Schaeffer et al. (2003) waited 24 hr following dextrose application to desert soils. Considering our small water application and the faster drying in our system, we decided to sample less than an hour following applications. We did not want to sample respiration immediately following application because we were concerned that disruptions to the boundary layer from removing and replacing the chamber may have artificially elevated respiration rates.

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Ekblad, A. and Nordgren, A. 2002. Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability? *Plant and Soil* 242:115-122.

Schaeffer, S.M., Billings, S.A. and Evans, R.D. 2003. Responses of soil nitrogen dynamics in a Mojave Desert ecosystem to manipulations in soil carbon and nitrogen availability *Oecologia* 134:547-553.

Specific Comments

Introduction

Page 14477, Line 6: It would have helped to add that the predominant heterotrophic contribution to these pulses happen because, at these point, most of the annual grasses is dried and the respiration of the roots is not expected to have a great contribution as long as the deep roots of the trees are not willing to access immediately to the water that stays in the superficial soil layers.

Authors' response: Agreed, and we have added this clarification of the importance of small rain events for net C loss.

Page 14477, Line 14 and along the manuscript: Temperature plays a more important role when moisture and C are not limiting factors, but this does not happen always when moisture and C are in high levels (soil pores saturation and reduction of diffusion within the soil matrix, anaerobic conditions, recalcitrant C, etc). I would change 'high levels' by 'not limiting conditions'.

Authors' response: We have changed "at higher moisture levels" to "when moisture is not restrictive" added a note that high moisture levels may suppress respiration by filling pores and slowing gas diffusion.

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Page 14477, Line 23: I would add at the beginning of this paragraph a sentence that highlights the relevance of these CO₂ pulses for the net annual CO₂ emissions or for the net C balance.

Authors' response: We have added a sentence indicating this.

Material and methods 2.2 Soil litter and properties

Page 14480, Line 18: Where the piñon canopies different at each site?

Authors' response: Piñon canopy cover differed slightly between the two sites - we have added this to the first paragraph of Methods.

Page 14480, Lines 15-18: Where the soil moisture measurements coupled to soil respiration, inside the chambers? Differences could be also due to the microstructure and porosity in each site, which affect to the desiccation and time courses of soil moisture

Authors' response: Gravimetric litter moisture was not measured from within the chambers because it would have required a destructive sample. Soil moisture sensors were also not located within the soil chambers themselves. We have added a sentence clarifying both of these points.

2.3 Experimental treatments

Page 14480, Line 26 and along the manuscript: Substitute the expressions 'mineral soil applications and litter applications' by 'solution applications to litter or mineral soil'.

Authors' response: We have changed this here and in a few other places in the manuscript.

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Page 14481, Line 4: Was the litter replaced or removed? This would have different implications for the interpretation of the results. If it was removed, why this was done after the treatment applications?

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Authors' response: The litter was replaced immediately after the mineral soil applications. This is indicated in page 14480, lines 26-27 in the original manuscript.

2.5 C and water limitation calculations

Page 14482, Lines 4-5: This detail is not relevant for this manuscript. In this case is not possible to ensure that the treated chambers are in absence of water and labile C limitations

Authors' response: We agree, especially in light of the change we made to the limitation calculations. We have removed this.

Page 14482, Lines 6-7: Even if a small amount of water is added, physical displacement of soil CO₂ cannot be avoided. Water always would provoke a displacement of the gas that is in the soil pores.

Authors' response: This is true, although we argue that the amount of CO₂ displaced by such a small wetting event is minor, and is completed before the respiration rate measurement.

Page 14483, lines 11-14: A more detailed explanation about the selection of time window for the calculation of cumulative limitations.

Authors' response: We have made this change in the revised manuscript.

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2.6. Statistical analyses

Page 14484, lines 20-21: Did you try to improve normality and homoscedasticity of the data by transformation before applying a non-parametric Mann-Whitney rank sum test?

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Authors' response: We did not apply any transformations, because we considered the retention of heuristic power due to untransformed data more valuable than the minor improvement in statistical validity due to transformation.

Discussion

References to the figures and tables would help to follow the discussion of most relevant results. The effects of rewetting can decline with successive drying and rewetting cycles. This could be an explanation to the more accentuated soil respiration responses observed on July (Experiment 1) compared to August (Experiment 2). To exclude for this effect, the experiment should have been performed in different experimental areas for each of these two experiments.

Authors' response: The experiment was in fact performed on distinct areas for the two experiments. We added a statement clarifying this in the Methods (page 3 line 202-205).

Page 14486, Line 17: I only see a clear limitation of C availability in the case of the litter but not in the case of mineral soil, this has to be specified (Table 1, Fig 1, Fig 7)

Authors' response: When we expanded our time window to more fully capture the respiration pulse in response to the solution applications to the mineral soil, we revealed labile C limitation in the mineral soil at both sites (new Fig 3, Fig 7). In light of this, we have kept this statement.

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Page 14486, Lines 20-21: See general comments, paragraph 2, about coupling effects between water and C limitations

Authors' response: We agree that water and labile C limitations are likely coupled. We have added a sentence clarifying that the initial response of respiration to water was probably substrate-related.

Page 14486, Line 21: The stimulation of respiration of water additions is not significant for mineral soil in August. Here, it must be specified that water additions stimulated litter respiration. The effect of the previous irrigation treatment in the same sites in July can be a possible explanation for the absence of response observed in August (Table 1)

Authors' response: Thank you for this correction; we have changed it accordingly. As noted above, the irrigation treatments were not applied to the same precise location, so this is not an explanation for the absence of response observed.

Page 14487, line 1: The reduction of the labile C limitation is restricted to the litter. This cannot be extrapolated to the mineral soil.

Authors' response: We have corrected this accordingly.

Table 1: The discussion or interpretation of figures must be in discussion section and not in footnotes. Instead, use this space to specify the meaning of each parameter.

Authors' response: We have deleted the discussion from the Table 1 caption.

Table 2: It would be appropriate to see the results of the statistical test in the table.

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Authors' response: We have added this as suggested.

Fig. 1: Change the colour of symbols for water and water + sucrose treatments. They cannot be visualized clearly. Changing the range of the x axes (up to 8) would also help

Authors' response: Based on yours and the other reviewer's comments, we have changed this Figure to display mean respiration rates for each treatment group and their standard error bars.

Fig. 7: The explanation given in the foot note about the negative values of the limitation factors must be wrong. Should not be 'negative proportion represent a treatment where the water addition yielded minor respiration than untreated treatments'?

Authors' response: The explanation is correct; in the case of the water application to the mineral soil surface at the Girdled site, the respiration rate was reduced below that of the untreated areas. We speculate in the Discussion about the influence of carbonate dissolution and precipitation reactions on measured soil CO₂ fluxes.