Topical Editor comments

Dear Authors,

I have read both the reviewer reports and your manuscript carefully, and recommend that some minor revisions be undertaken according to the reviewers' comments. Thank you for your thoughtful responses to those comments, which I feel capture well the revisions that need to be done (especially the response to Reviewer #2). With regard to the reviewer suggestions of putting this work in context of previous work on soil drying-rewetting and CO2 efflux from soils, I recommend including this to the extent possible given the format of the short communication.

Response: Thank you for the clear directions on how to proceed with the revision. We revised the manuscript in response to Reviewer #2's major comments, while briefly putting this work into context as suggested by Reviewer #1. Additionally, we made the line-by-line revisions suggested by both reviewers. More details are below.

Reviewer #1 comments

1. There is considerable research on soil moisture content and biological activity of soils, including dry-down and wet-up. This should be discussed more in Introduction and Discussion.

Response: For the sake of brevity in this "short communication", we did not review the many studies on soil moisture content and CO_2 pulses. However, we have included a more nuanced discussion of the Birch effect in the Introduction (see lines 30 – 36).

There are several studies that show that the drier soils are in the field, they tend to release more CO2 upon re-wetting regardless of dry-down C loss. Thus, there are biophysical mechanisms at play other than dry-down C loss.

Response: In response to your comment, we specifically addressed why these mechanisms are unlikely to be responsible for our results in the Discussion (see lines 166 – 171).

Figure 3 still shows more CO2-C from the 30.

Response: Differences in total C loss among treatments were not significant (one-way ANOVA: $P = 0.28$). More importantly, the proportion of pre-assay loss was substantially smaller (Fig. 3).

2. These findings need to be placed in context of the purpose and practicalities of CO2 Burst test. Drying is often needed, if not necessary, to stabilize microbial activity before analyzing for CO2 production. Whereas accounting for C loss during drying might not be feasible or possible for commercial and even research labs. This would be very onerous. How much more are we gaining by accounting for this C?

Response: We agree that our findings are inconvenient for an assay that requires drying and that calculating a correction factor is an added complexity. As we point out in the discussion (lines 173 – 177 & 187 – 191), a correction factor is not needed if comparing soils of similar moisture contents at collection or perhaps if the dry-down period is fast. However, not correcting for soils at different moistures will likely lead to erroneous conclusions about carbon availability.

This paper has not convinced me that we gain much. Despite finding differences between 30, this paper would be much stronger if it included a comparison of two or more treatments and showed that measuring CO2 Burst at different moisture contents obscured our ability to detect treatment differences.

Response: We agree that pre-assay dry-down effects will likely differ not just by treatment but also by soil type and structure; this is why we discuss the need for calculating correction factors on a case-by-case basis (lines 183 – 185). Little would be gained by adding different treatments to this study because we fully expect correction factors to differ.

There are always experimental artifacts with incubation-based, laboratory measurements of soils. A few studies have shown that using more soil reduces variability. The most important thing is that we treat soils the same across time or space, and that the methodology is not creating confounding effects.

Response: We agree that it is important that methodology not create confounding effects, which is why we chose to conduct this study. Our results reveal that initial soil moisture can confound our interpretation of carbon availability from the CO_2 pulse assay. We want others to be aware of this limitation, and so we provide a correction and a few alternatives for minimizing CO_2 losses during dry-down.

SPECIFIC COMMENTS:

L24. Replace 'the reintroduction of moisture' with 'rewetting'

Response: We have made this change (see line 27).

L33. Delete 'different'

Response: We have made this change (see line 45).

L38. How was it collected? Shovel, or soil probe? More details are needed here.

Response: We collected the soil using a shovel and have added this to the manuscript (see line 50).

L43. So total MAP is 2305? This seems very high.

Response: No, snowfall has an average density of ~10% so 1300 mm represents only ~130 mm of precipitation. Nevertheless "average annual rainfall" should read "average annual precipitation" to include snowfall. Thank you for identifying this issue. We changed the sentence to make it clear that MAP is 1005 mm (not 2305 mm) and that the site receives 1.3 m of snowfall on average (see lines 55 – 56).

L49. What was the initial water content of the field soil when you collected it? Was it below 30

Response: Yes, the initial soil moisture was 11% WFPS (see lines 69 – 70).

L85. This seems overly complicated. Why not use area-under-the-curve to calculate cumulative CO2?

Response: We did in fact calculate the area under the curve, using bootstrapping to define the curve to allow for error estimates. We edited the text for clarification (see lines 103 – 104).

L110. Why does respiration go back up at 8d? This is interesting and looks like there might be treatment effect?

Response: The slight increase in CO_2 respiration at Day 8 may be due to more humid incubation conditions later in the experiment as evidenced by the slight increase in soil moisture (Fig. 1a).

L131. Why use standard deviation in this graph? Fig. 2 uses standard error. I suggest being consistent. Also, use same colors in Fig. 1 and 2 for consistency. Place letters to abbreviate significant differences among means in both Fig. 1 and 2.

Response: SD is more appropriate than SE for Fig 3, which reports error from the results of bootstrapping (see Fig. 3 legend and lines 106 – 114). Thank you for the suggestions about consistent colors and letters. We have updated Fig. 3 to reflect this suggestion (see line 145).

Reviewer #2 comments

1. The basic finding of this paper seems well supported by the data: antecedent moisture history affects the release of CO2 from dry soil following wetting. This observation seems generally consistent with previous studies: for instance, both the duration of drying (Miller et al. 2005; Meisner et al. 2015) and the severity of drying (Meisner et al. 2017) influence respiration after wetting. In this case, soil moisture during the wet period was varied and found to affect the respiration rate after wetting. The novelty of this short note is that it raises this point specifically in the context of soil health testing.

Response: We appreciate the evaluation that the basic findings are sound and consistent with prior observations.

2. The specific interpretation advanced in this study–that respiration prior to drying affects the post-wetting respiration pulse specifically by reducing C availability–is only indirectly supported by the data and might need more thought. This interpretation seems to rest on an assumption that there is a fixed pool of available C at sampling, and that any losses of C between sampling and drying/rewetting reduce the size of this pool–resulting in a proportionately smaller pulse. Strictly speaking this is assumption is true of the bulk organic C pool, but it may not apply to the small fraction of that bulk pool that is actually available at any given moment (e.g. the soluble C pool). The apparent balancing of C fluxes observed in this experiment (Fig 3) does seem consistent with the idea of a fixed available C pool–but several factors could make things more complicated:

(1) Depolymerization of soil organic matter may at least partly replenish the soluble C pool after sampling, even as microbial uptake and respiration deplete it. High respiration rates in the wetter soil samples are likely accompanied by higher rates of enzyme production/diffusion and depolymerization–consequently it is not obvious what the short-term net effect of soil moisture on available C should be.

(2) The CO2 released after wetting of dry soil may come from multiple sources–both endo- and extra-cellular. To the extent that respiration after wetting represents a microbial stress-response or a side effect of microbial stress physiology, the link between available C and respiration is not direct. For instance, if this C represents microbial osmolytes, the size of the pulse might depend more on the propensity of the microbial community to allocate C to osmolytes than C availability per se. Microbes acclimated to dry soil might accumulate more osmolytes, thus releasing more C after wetting regardless of overall C availability.

(3) Similarly, to the extent that C respired following wetting is derived from extracellular sources, it is unclear whether those sources represent the same C that is readily available under moist conditions versus some more occluded form that is only made available by the physical effects of drying and wetting (see for instance Homyak et al. 2018).

These concepts are really broader critiques of the use of short-term CO2 emissions after wetting as a general metric of soil C availability in the first place. The phenomenon in question is very complex and still not totally understood on a mechanistic level. In the soil-health realm, the relationship between the pulse and C availability is taken as a given. This is appropriate at some level, as it seems plausible that soils that exhibit larger respiration pulses after wetting likely have more microbial biomass, and possibly a more active microbial biomass. However, it would be good to acknowledge that the relationship between C-respired-after-wetting and “available C” (defined as a pool) is not straightforward. I would advocate for a brief but well referenced consideration of the possible mechanisms that might influence the post-wetting respiration pulse: depolymerization, synthesis of osmolytes, and release of occluded C on wetting. Some combination of these mechanisms might explain the findings of this study–but from the perspective of soil health testing the main point is that antecedent soil moisture matters.

Response: We agree that our statement that the pulse effect is (by implication exclusively) a C availability response is an over-simplification that deserves an expanded explanation. As helpfully noted, there are a number of potential mechanisms, all difficult to parse in these sorts of assays (and not exclusive to this study). These are excellent comments. To that end, we revised the manuscript as follows:

a) In the Introduction, we noted why this index is often used as an indicator of C availability for soil health tests (see lines 21 – 24). We also included the potential mechanisms behind the Birch effect as reviewed by Jarvis et al., 2007 (see lines 30 – 36).

b) In the Discussion, we clarified that the correction factor is not intended to imply that there is a fixed C pool, rather to demonstrate the way antecedent conditions could affect soil health tests and offer some potential solutions (see lines 181 – 183). We adapted our language to represent this method from a soil health perspective rather than as a direct indication of available C (see throughout entire manuscript).

c) Additionally, we discussed the potential Birch effect mechanisms in the introduction (see lines 30 – 36). We then pointed out in the discussion why they are unlikely responsible for the results we observed (i.e., more loss during dry-down resulting in a lower CO_2 pulse after re-wetting; see lines 166 – 171).

3. Lines 24-25: This remains an area of active research. Some studies suggest significant microbial mortality on wetting (Blazewicz et al. 2015, 2020); others suggest that the CO2 is derived from osmolytes, but that they might be processed endo-cellularly and that lysis isn't a big player (Slessarev et al. 2020; Warren 2020); yet more studies emphasize the role of wetting in liberating soluble components of (extracellular) soil organic matter (Homyak et al. 2018).

Response: Agreed, and suggested references are duly noted and helpful. We incorporated them into the introduction (please see lines 31 – 36).

4. Line 131: In the figure caption, the "standard deviation" referred to here is based on the bootstrap error propagation? Please clarify.

Response: Yes, standard deviation is based on bootstrapping the total amount of CO_2 loss during dry-down 10,000 x. We clarified this in the figure caption (see lines 150 – 151).

5. Line 153: ". . .moisture contents sufficient to oxidize. . .". Clarify that the microbes do the oxidizing, not the moisture itself

Response: We clarified this in line 183.