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 would be happy to include a more nuanced introduction and discussion if we are permitted to increase this manuscript's length. We also thank Reviewer #1 for pointing us towards these sources.

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: While we recognize that biophysical mechanisms influence CO_2 pulses after re-wetting dry soil (reviewed in Jarvis et al. 2007), they are not likely to differ between treatments because soil samples were treated identically except for initial water content. All soil samples were sieved and air-dried, thus destroying aggregates equally, and then re-wet to 50% WFPS for the 24-hr CO_2 pulse assay (Fig. 2). The only difference in moisture content between treatments occurred before air-drying. Additionally, these biophysical mechanisms do not account for the drier soil releasing the largest CO_2 pulse after re-wetting (Fig. 2). If anything, wetter soils should have released higher CO_2 pulses due to greater microbial activity (e.g., Linn and Doran 1984), more microbial biomass (e.g., Franzluebbers 1999), or a greater release of osmolytes due to the increased risk of lysis. However, the 30% WFPS treatment had the largest CO_2 pulse (Fig. 2), and dry-down loss in wetter soils is the most plausible explanation (Fig. 1).

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 145-166), 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 151-155). 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: Agreed.

L33. Delete ‘different’

Response: Agreed.

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

Response: We collected the soil using a shovel and will add this to the manuscript.

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 will change 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.

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 (please see lines 56-57). We will move this to the collection section to clarify.

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 will edit the text to clarify.

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. Thank you for the suggestions about consistent colors and letters.

<u>**References**</u>

Borken, W., Davidson, E. A., Savage, K., Gaudinski, J., & Trumbore, S. E. (2003). Drying and wetting effects on carbon dioxide release from organic horizons. *Soil Science Society of America Journal*, *67*(6), 1888-1896. https://doi.org/10.2136/sssaj2003.1888

Fierer, N., & Schimel, J. P. (2002). Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, *34*(6), 777-787. https://doi.org/10.1016/S0038-0717(02)00007-X

Franzluebbers, A. J. (1999). Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. *Applied Soil Ecology*, *11*(1), 91-101. https://doi.org/10.1016/S0929-1393(98)00128-0

Jarvis, P., Rey, A., Petsikos, C., Wingate, L., Rayment, M., Pereira, J., ... & Manca, G. (2007). Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: the "Birch effect". *Tree physiology*, *27*(7), 929-940. https://doi.org/10.1093/treephys/27.7.929

Liang, L. L., Grantz, D. A., & Jenerette, G. D. (2016). Multivariate regulation of soil CO2 and N2O pulse emissions from agricultural soils. *Global change biology*, *22*(3), 1286-1298. https://doi.org/10.1111/gcb.13130

Linn, D. M., & Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, *48*(6), 1267-1272. https://doi.org/10.2136/sssaj1984.03615995004800060013x