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 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. Indeed, we have taken the simple soil health path in our discussion of effects, and agree that acknowledging alternatives would be helpful and appropriate. To that end, we propose:

a) **In the Introduction, note why this index is often used as an indicator of C availability for soil health tests. In particular, we will note Franzleubbers (2000) wherein assay responses were correlated with microbial biomass carbon, soil organic carbon, and particulate organic carbon. We will also note the potential mechanisms behind the Birch effect (Jarvis et al. 2007).**

b) **In the Discussion, we will make it clear 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. We will therefore adapt our language to represent this method from a soil health perspective rather than as a direct indication of available C, acknowledging as suggested that the relationship between C-respired-after-wetting and the available C pool is not straightforward.**

c) **Additionally we will discuss the potential effects of depolymerization, microbial stress, and extracellular C sources along with other Birch effect mechanisms to point out that while they are unlikely responsible for the results we observed (i.e., more loss during dry-down results in a lower CO_2 pulse after re-wetting), that using a correction factor may be complicated by them. For example, as referenced, depolymerization rates increase with higher levels of soil moisture, in turn resulting in higher rates of C replenishment in wetter soils (Wild, et al., 2014). Therefore, we would expect wetter soils with higher depolymerization rates to result in higher flushes of C, which is not consistent with our finding that wetter soils had a smaller CO_2 flush upon re-wetting (Fig. 2), a result most likely due to CO_2 loss during drying (Fig. 1b).**

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 will incorporate them throughout the Introduction and Discussion.

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 will clarify this in the figure caption.

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

Response: Agreed, we will clarify.

References

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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.

Wild, B., Ambus, P., Reinsch, S., & Richter, A. (2018). Resistance of soil protein depolymerization rates to eight years of elevated CO 2, warming, and summer drought in a temperate heathland. *Biogeochemistry*, *140*(3), 255-267.