SOIL Discuss.,
https://doi.org/10.5194/soil-2020-55-RC2, 2020

Interactive comment on “Short Communication: Quantifying and Correcting for Pre-Assay CO_2 Loss in Short-Term Carbon Mineralization Assays” *by* Matthew A. Belanger et al.

Anonymous Referee #2

Received and published: 14 November 2020

The manuscript by Belanger et al. describes a soil incubation experiment designed to quantify the effect of antecedent soil moisture on the amount of CO2 released from soil after drying and rewetting. The authors find that wetter soils emit more CO2 during dry-down and less CO2 after wet-up. In the soils studied here the additional C respired during drying approximately equaled the CO2 that was not respired after wet-up. The authors conclude that the C respired represents a fixed pool of available C, and hence soil tests that include respiration measurements from wetting of dried soil can be corrected to account for C lost during drying.

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.

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 persay. 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.

Line-by-line comments:

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).

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

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

References:

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