Reviewer 1 Comments

In this manuscript, the authors present a compelling hypothesis of how compost in combination with winter cover crops can lead to accumulation of aromatic-rich subsurface soil carbon. The hypothesis is complex but plausible whereby cover crop roots improve soil structure/porosity facilitating greater transport of soluble C and nutrients derived from the compost directly to the subsurface where this C can be stabilized. While the hypothesis is compelling, unfortunately, I do not think the authors have collected the right data to test this hypothesis.

Response: We would like to thank the reviewer for their well-thought out, fair, and constructive feedback. We will address their concerns by making clearer what evidence we have for the different components of the hypothesis, better clarifying where there are limitations of the data, and drawing more on existing literature to support our synthesis as described below.

Utilizing a long-term field trial should be a great way of trying to address this hypothesis. However, a major limitation of the study is that there is no compost-only treatment, so there is no way to separate the effect of compost alone from the interactive effect of compost and cover crops together. There is nothing the authors can do about this except recognize this as a limitation of the study design.

Response: We will make this limitation of the experimental design clearer, stating that there is no compost-only treatment in the methods, and restructuring the discussion section to make clear that we cannot differentiate between the effects of compost and winter cover crops (WCC) individually.

A major feature of the author's hypothesis is that cover crop roots have created greater porosity that facilitates greater water flow down the soil profile. The data simply do not support this notion. The authors find no difference in saturated hydrologic conductivity at 35 cm (although there was a trend for much greater variability in the compost + cover crop treatment) and no difference in soil aggregates across treatments.

Response: We agree that the discussion needs to be clearer and restructured. We will modify the discussion as follows:
1. We will clarify the non-significant treatment means of the hydraulic conductivity data.
2. We will incorporate data from previous studies at Russell Ranch to support our hypothesis of increased water storage and movement in cover-crop plots.
3. We will clarify that our conclusion of greater porosity due to WCC roots is based on previous observations and not a parameter that we directly measured in this study.

The only significant difference was greater water content in the two treatments with cover crops but the authors did not measure bulk density in the 2018

samples and they did not measure porosity so it is difficult to come up with an explanation for this observation.

Response: The idea that cover crops increase infiltration and hydraulic conductivity in fine-textured soils is not new (see Haruna et al., 2018; Çerçioğlu et al., 2019; Gulick et al., 1994). Previous work carried out at Russell Ranch over the last 30+ years has shown that the cover crop mix of hairy vetch, faba beans and oats used in this study increased infiltration and DOC input into the soil profile (Mailapalli et al., 2012). It also increased soil moisture-holding capacity during saturated conditions (Joyce et al. 2002) with no significant differences in bulk density (Colla et al. 2000) and reduced soil surface strength (Folorunso et al. 1992).

Proposed mechanisms behind the increased infiltration and moisture storage include increased aggregation, reduced crusting, and increased macroporosity. It is important to note that these root-induced soil alterations are highly localized and dependent on the root architecture of the cover crops. Specifically, cover crops with prominent tap roots (faba bean) are effective at creating continuous bio-pores, while fibrous roots (oat and hairy vetch) are effective at forming aggregates (Oglive et al., 2021). Therefore, the mixture of cover crops planted at the site likely resulted in widely variable aggregation and pore connectivity effects.

In the context of these previous observations, we attribute the increased variability of our hydraulic conductivity measurements and the increased moisture storage observed in ORG and CONV+WCC plots to the presence of WCCs.

In addition, the sample size used for Ksat measurements (cross-sectional area of 250 cm2) may be too small to capture the effects of cover crop roots, whose impacts are likely to be detected at a larger scale (Ozelim and Cavalcante, 2017). It is well recognized that hydraulic conductivity measurements can vary widely across fields and landscapes (Rahmati et al. 2018) and often do not account for the presence of macropores (Brooks et al. 2004). Ksat values can span 3 orders of magnitude within a short distance (Øygarden et al. 1997). Thus, although our measurements do not reveal statistically significant differences between the treatments, the scattered high-permeability zones in the cover-crop treatments are likely to contribute to rapid moisture redistribution and may explain the elevated deep moisture.

Other potential, albeit less likely, explanations for increased moisture content include lateral subsurface flow (unlikely due to low slope of field) and differences in runoff and runon (also unlikely due to low slope).

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The next major component of the hypothesis is that compost leads to greater soluble C and N. The authors use salt-extractions of soil samples at four time points during the 2018 season to generate supporting data. Salt-extractable C is an interesting carbon pool (a potentially soluble pool of C) but there is ample evidence that this lab-extracted pool has little relationship to DOC when collected in lysimeters in the field.

Response: We recognize that the distinction between dissolved organic carbon (DOC - total organic carbon dissolved in the liquid phase of a bulk soil sample) and extractable organic carbon (EOC - total dissolvable organic carbon in the liquid and solid phases of a bulk soil sample) is important. We will make this differentiation clear by replacing all mentions of "DOC" with "EOC", and also reflect the limitations of our extraction approach in our discussion. We recognize that lysimeters are invaluable for making realistic measurements of DOC in soils, but due to moisture limitations at our study site it was not feasible to use them. Measurements of EOC are commonly used to estimate soluble carbon (Slessarev et. al 2020, Matlou et. al 2007) and we have followed recommended best practices for extraction (Li et. al 2018) using field moist samples with K_2SO_4 extractant at a 1:5 ratio.

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Without direct collection of DOC diffusing and advecting down the soil profile it is difficult to say whether the differences in the extractable pools are actually leading to more DOC flux to the subsoil under compost addition.

Response: We agree that it would have been desirable to directly measure DOC diffusing and advecting down in the soil profile; however it was not feasible to use lysimeters in our study due to the extremely dry conditions at Russell Ranch during the summer months.

Tension lysimeters primarily obtain soil solution from macropores (Chantigny et. al 2003) that may be empty during the summer at our experimental location, while EOC extractions can account for soluble carbon in the solid phase (bound to mineral surfaces) as well as in smaller micropores. Additionally, EOC may be more sensitive to recent carbon and litter inputs, while DOC is more indicative of older, extant soil carbon (Froberg et. al 2007). While DOC has been used in studies of carbon transport in both grassland and forest ecosystems (Sanderman and Amundson 2008), EOC may be more suitable for answering questions on the impacts of carbon input, nitrogen amendment and tillage (Li et. al 2018), such as in our manuscript.

While EOC measurements vary seasonally less than DOC measurements, there is still a significant amount of variation that may occur during a growing season (Li et. al 2018). Our sampling regime at multiple timepoints was meant to account for some of that variation in both winter and summer months, and the EOC extraction method was chosen as the best way to compare soluble C measurements at different timepoints and soil water contents.

Finally, our inference of greater soluble carbon under compost application is supported by the multiple lines of evidence that we present in the manuscript:

1) More soluble C in ORG subsoils,
2) Observations of higher water infiltration and storage under cover crops
3) Greater amounts of soluble organic carbon in compost, and
4) Reduced subsoil microbial stress indicators under ORG systems (attributed to more C availability)

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The third component of the hypothesis relates to the preferential partitioning of DOC chemistry down the soil profile. The evidence here is particularly weak. Mid infrared FTIR spectroscopy is not a quantitative analytical tool for determining abundance of specific compounds. If it were, labs wouldn't spend millions of dollars on more precise equipment. FTIR spectroscopy is good for identifying compounds in simple mixtures but not for quantifying their abundance in simple or complex mixtures (and soil is one of the most complex there is).

Response: First, we want to make clear that we are not quantifying the amount of any specific compound in our samples. In this manuscript we use IR peak locations to demonstrate the presence (or absence) of particular chemical moieties, and the peak height as an indicator of abundance. We fully agree that quantification of specific chemical functional groups in soil organic matter via FTIR is challenging, and that completely accurate methods do not exist. There are some advances being made in this area (e.g., work by Francisco Calderon), but it remains pseudo-quantitative at best (more on this below).

FTIR is limited by the wavelength of IR radiation and thus development of more precise instrumentation is challenging. Since the development of the Michaelson interferometer in 1880 the IR instrument has remained relatively unchanged, with recent advances coming in data processing (such as the Cooley-Tukey algorithm) and sample collection. The ability of computers to conduct a Fourier transform of data, using the Cooley-Tukey algorithm in 1965 was a huge advance, but for data processing. Since that time, there have been many more advances on the software side and for sample collection (e.g., ATR). Some of the more cutting-edge advances are being made using synchrotron sources, which have greater intrinsic brilliances, combined with other techniques such as atomic force microscopy (AFM). However, these advances are most beneficial for reducing the "spot size" or "number of pixels" being probed; going from micron to nanometer scales. When it comes to analysis of complex mixtures, like soil, improvements in quantification will likely arise from more accurate peak fitting/deconvolution, or perhaps the use of internal standards (e.g., Calderon et al. 2013). Unfortunately, due to funding availability in different disciplines, advances in most analytical techniques are developed for other types of samples, such as polymers for industrial purposes, and trickle down for soils.

Peak features depending on if they are due to vibrations, wiggles, combinations or overtones all have different relationships between abundance of the specific bonding environment and absorption – basically, you would have to prove that there is a linear relationship between "aromatics" and those two peak features in order to do a spectral subtraction and have any confidence that the difference spectrum represents real differences in chemistry.

Response: We agree that the analysis of heterogeneous samples by mid-FTIR pose many challenges such as the overlapping of peaks and spectral artifacts. However, there is a deep history of the use of FTIR to study the chemical composition of soil organic matter. The reviewer is correct that FTIR cannot be used as a strictly quantitative tool for determining specific compounds in mixed samples, although the basic theory of FTIR does allow for this. As the reviewer mentions, IR absorption occurs when molecular bonds exhibit a dipole moment, and the resulting bands can result from bond stretching, wagging, rocking, etc. However, this absorption that can be quantified as the absorption of IR light by a specific molecular bond at a specific electromagnetic frequency (wavenumber) follows the Beer-Lambert Law (Beer's Law) (e.g., Margenot et al 2016, Smith 2001). Thus, the height and area are proportional to the concentrations of molecules in a sample (linear relationship). While this holds true for some FTIR sampling methods, such as transmission and attenuated total reflectance (e.g., Smith 2011, Parikh et al. 2008, Ge et al. 2014), there is deviation from Beer's Law when using reflectance techniques such as diffuse reflectance Fourier transform (DRIFT) spectroscopy (e.g., Baes and Bloom 1992; Niemeyer et al 1992), as the reviewer suggests. However, it has been shown that > 90% of the photons have shallow penetration and travel short pathlengths when diffusely reflected (Brauns 2014) and thus deviation from Beer's Law is expected to be somewhat minor.

Even taking in consideration those potential limitations, previous studies with DRIFTS in both the near-infrared (Dalal and Henry, 1986) and mid-infrared regions (Demyan et al., 2012; Margenot et al., 2015; West et al., 2020; Deiss et al., 2021) have shown direct associations between soil organic carbon concentration and absorbance at specific frequencies (depicted as peak height or area of single peaks or peak ratios). The non-linearity of concentration and absorbance arising from DRIFT can be partially corrected using the Kubelka-Munk (KM) function. However, since a KM correction does not fully correct spectra (e.g. Clark and Roush, 1984) we have still chosen to include the spectra uncorrected, but those are now in the appendix on a non-common scale with the spectral amplification factor noted for each spectrum. The Kubelka-Munk corrected spectra, on a common y-axis scare, will be placed in the main document.
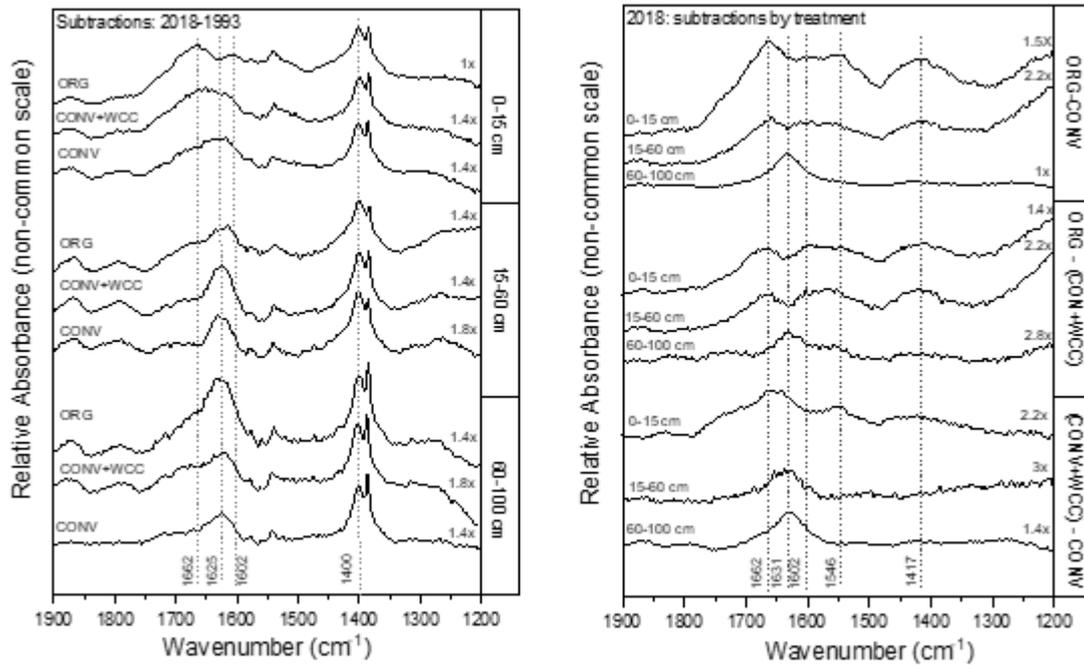
Figure A3. DRIFT spectral subtractions for the 1900-1200 cm^{-1} range comparing (A) 2018-1993 spectra for ORG, CONV+WCC, and CONV, and (B) ORG, CONV+WCC, and CONV spectra in 2018. Spectra are plotted with absorbance units on a non-common y-axis scale. The spectral amplification factor is noted on the right-hand side of each spectrum.
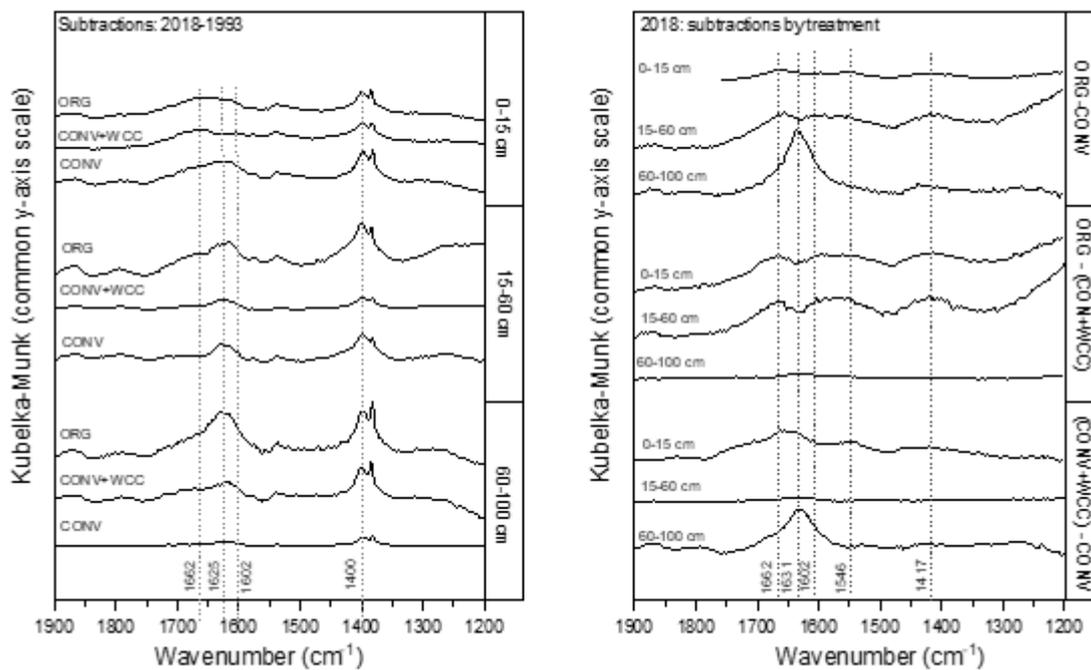

Figure 8. DRIFT spectral subtractions for the 1900-1200 cm^{-1} range comparing (A) 2018-1993 spectra for ORG, CONV+WCC, and CONV, and (B) ORG, CONV+WCC, and CONV spectra in 2018. Spectra are plotted with Kubelka-Munk units on a common y-axis scale.

Of course, quantification is incredibly difficult in complex mixtures, and thus the concept of pseudo-quantitation is used by comparing spectral features within, and between, samples. However, in this case we expect a minor deviation in the linear relationship between aromatic C=C and COO^- with a change in concentration due to data collection via DRIFTS as some unknowns remain regarding path length and scattering effects. Most important, for our study, is the presence and absence of peaks and the relative differences in spectral contributions from each peak. Assuming that band assignments are correct (they are consistent with literature), the presence of a peak at 1666 cm^{-1} suggests greater abundance of aromatics in the surface horizons, especially for the ORG treatment. We will also update the manuscript to provide peak intensity ratios of aromatic to carboxyl moieties [$v(C=C):vas(COO^-)$ (1662 cm^{-1}:1631cm^{-1})] to show the relative difference within each spectrum. Thus, we believe that the FTIR data collection and subtractions are appropriate to show semi-quantitative results of changes in soil chemical functional groups as affected by land use and soil depth.

Table 1. Peak Intensity Ratios for aromatic (1662 cm^{-1}) to asymmetric carboxyl (1631 cm^{-1}) groups in spectral subtractions.

	Depth	Peak Intensity Ratio (1662 cm^{-1} : 1631 cm^{-1})		
		ORG	CONV+WCC	CONV
Subtraction: 2018-1993	0-15 cm	1.38	1.22	0.45
	15-60 cm	1.21	1.40	0.77
	60-100 cm	1.17	2.50	0.014
		ORG-CONV	ORG-(CONV+WCC)	(CONV+WCC) -CONV
Subtraction by treatment: 2018	0-15 cm	1.39	1.18	0.46
	15-60 cm	1.20	1.42	0.47
	60-100 cm	0.38	0.64	0.45

I also find it problematic that all treatments have showed the same increase in carboxylate functional groups over 25 years – wouldn't we expect the conventional treatment to be more or less at steady state, so we shouldn't see the same changes as seen in the cover crop and compost + cover crop treatments?

Response: The subtraction spectra do not represent the total amount of COO etc, they represent the change in that functional group. But the reviewer is correct that they appear very similar when plotted using absorbance units, and we thank them for pointing this out. However, when the spectra are shown with the KM correction the spectral contributions for the change in carboxyl groups are no longer similar. We believe that these spectra along with the peak intensity ratio table will help make this clear. Additionally, the band at 1384 cm^{-1} represents both aromatic and aliphatic carbon; however, we have determined that the intensity of that peak in the 2018-1993 spectra is impacted by an artifact of spectral reflection in the original spectra that is amplified during subtraction. It is possible that the artifact is a Restsrahlen band (e.g., carbonate), but it is more likely that trace amounts of CH_2 and CH_2 on the KBr beamsplitter (which are always present) resulted in a spectral reflectance artifact at 1384 cm^{-1} when the soil samples diluted with KBr were analyzed and amplified via spectral subtractions. Although the intensity of the 1400 cm^{-1} band is still meaningful, we focus on the asymmetric COO band at 1633 cm^{-1} for pseudo quantification.

Lastly, what is the actual magnitude of the "increase" in aromatic features in the compost treatment over the conventional treatment? There are no units on the y-axis. The authors have replicates so they could run statistics to see if this increase was significant.

Response: In our original Figure 8a, FTIR spectra are shown on a common scale (displaying 0.07 absorbance units for each spectrum). The y-axis does not have units as the spectra are stacked. Overlaying the spectra would make observing differences very difficult. In the original Figure 8b, FTIR spectra are shown on a full scale (amplified) to maximize the observable differences between spectra. We felt this necessary as the differences between treatments in 2018 are much less than the differences between treatments from 1993 to 2018. However, in response to the reviewers concerns we will now use a) the spectra with KM units on a common y-axis scale and b) the spectra with absorbance units with a non-common y-axis scale labeled with their spectral amplification factor. Again, we believe the peak intensity ratios help to elucidate the relative changes in aromatic carbon between treatments and years.

We did not run statistics because of the very low variability between the spectral replicates of the same experimental treatments. We provide below an example of spectral replicates and residual spectra for some selected samples to demonstrate the low variability between spectra of the same treatment and depth (on a common y-axis scale).
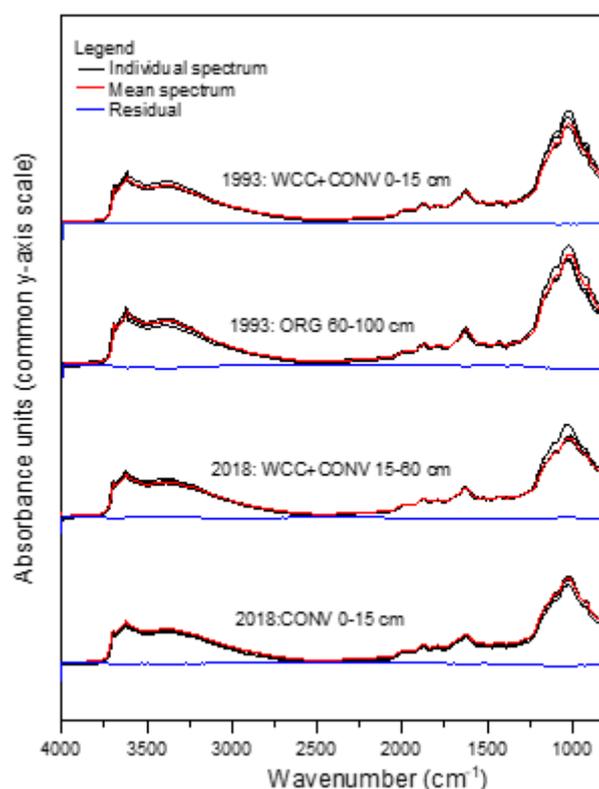
Figure AX. Example DRIFT spectra showing three replicate spectra, the mean spectra and the residual spectra for 1993 and 2008 samples.

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Finally, the microbial data is not well integrated into the hypothesis. Would lower microbial stress result in greater carbon stabilization via increased carbon-use efficiency or would it result in greater priming and potential loss of older SOM? Regardless of what microbial stress means for carbon cycling, the data were non-significant across treatments. The only significant difference was in Gram+:Gram- ratio but the ecological significance of this difference was not described.

Response: We acknowledge that our manuscript did not describe the importance of these microbial stress indicators in detail, and will modify our discussion and introduction accordingly. We have also noted that our measurement of Sat:Unsat ratios is not significant with a p of 0.07, and will change our wording accordingly. We believe that the trends displayed in the sat: unsat ratio and cy17:pre ratios are indicative of a greater trend in microbial stress levels with depth despite their non-significance, and so have chosen to continue including them in the results section.

Phospholipid Fatty Acid analysis (PLFA) is a useful tool for looking at living cells in the soil environment (Zhang et. al 2019). Measurements of microbial stress responses via PLFA agree with those obtained via the more recent 16s rRNA metabarcoding (Orwin et. al 2018). PLFA stress ratios are useful as an indicator of limited carbon and water availability, as they represent an overall shift away from the thinner, more permeable cell membranes associated with Gram - bacteria and monounsaturated fatty acids; towards more tightly packed, less permeable cell membranes associated with Gram+ bacteria and saturated fatty acids (Silhavy et. al 2010). An increase in the Gram+:Gram- ratio has been associated with a decrease in easily available water and carbon (Fanin et. al 2019, Fierer et. al 2003, Bossio et. al 1998), while an increase in the saturated: unsaturated ratio and cy17:pre ratios are associated with dehydrated conditions (Moore-Kucera et. al 2007). The stress indicator trends in our data agree with and support our observations of increased soluble carbon and water content in ORG systems.

Using PLFA to make predictions of functional or structural change in microbial communities is difficult, as many PLFAs are not specific to distinct species and instead distributed across taxa (Ruess et. al 2010). Rather than providing detailed information on taxonomy, PLFA permits evaluation of rapid changes in the cell walls and membranes of metabolically active soil microbes (Frostegård et. al 2010). As such, we have used the PLFA data to support the inferences drawn from our nutrient and moisture measurements. These results do indicate that a more detailed investigation into the carbon use efficiency and functional potential of these subsoil microbial communities may yield interesting results, and is the focus of another paper currently being drafted by the first author.

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Just to reiterate, I think the hypothesis laid out here for subsoil C accumulation under compost and cover crops is entirely plausible but the evidence in this study to support the hypothesis is not particularly strong.

Response: We would like to again thank the reviewer for the time and energy they put into this review. These comments are extremely helpful. We hope that we have provided a more convincing argument with our responses to both reviewers' comments.