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Long Term Impacts of Farming Practices on Carbon, Water and Microbes in a Mediterranean Ecosystem

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Long Term Impacts of Farming Practices on Carbon, Water and Microbes in a Mediterranean Ecosystem

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Abstract

This dissertation investigates how management with different combinations of practices, namely mineral N fertilizer, cover cropping, compost amendment, furrow irrigation and subsurface drip irrigation have impacted soil carbon, nutrients, and microbial communities in a 25-year long term experiment. First, I investigated how subsoil carbon stocks have increased under a combination of compost and cover crops after 25 years. I found that a potential synergistic effect between soluble C, soluble N and soil pores created by cover crop roots that allowed for greater C and N transport to subsoils. This transport of soluble C and N was associated with reduced microbial stress levels, and potentially longer C residence times. Secondly, I looked at how soil microbial community structure and microbial functional potential changed after 20 years of management practices intended to improve soil health. I found significant changes in functional potential for C breakdown and N transformation, but no significant changes in microbial community structure. These changes in functional potential were associated with changes in soil health measurements and may indicate the potential impact of practices intended to improve soil health. Finally, I examined how soils under a combination of compost and cover crops changed carbon and nutrient distribution under 5 years of subsurface drip irrigation (SDI) and furrow irrigation. I found that soils under SDI and furrow irrigation only differed in carbon and nutrient distribution in the top 30 cm, and that furrow irrigation was associated with a loss of surface organic matter after 5 years while SDI was not. The differences in spatial distribution and amount of water under SDI vs furrow irrigation may not significantly reduce the amount of carbon and nutrients stored in subsoils but may have an impact on surface organic matter content. In conclusion, the impacts of management practices on soils with similar climate and mineralogy may be difficult to measure over the course of a single season, but these impacts may accumulate over multiple years to result in significant shifts in soil edaphic and microbial properties.

Introduction

As the impacts of climate change have continued to grow across the globe, so too have our expectations of the soil. Our agricultural production systems are relying heavily on soils to help us mitigate and adapt to the extreme weather events, reduced precipitation, and disruption in climate patterns expected in the next several decades (Pathak et al., 2018). Because of this increased reliance, our management of soils has expanded from a focus on yield (Ortiz-Bobea et al., 2021) to incorporate concepts such as agroecology, soil health, and ecosystem services.

In the search for management practices that drive more sustainable, productive, and resilient agricultural systems, there are many important unanswered questions: How do we increase the amount of carbon stored in soils, and ensure that carbon is retained for longer timespans? How do we leverage the enormous potential of the soil microbial community to improve soil processes such as carbon and nutrient cycling, soil structure formation and pollutant degradation? And how can we most effectively use water (rapidly becoming the world's most valuable resource) to both grow crops and preserve soil function? Finding answers to these questions and translating those answers into practical recommendations for land management is a momentous task, made more difficult by the complex interactions between soil depth, soil type, climate, time and management (Manoharan et al., 2017; Yang et al., 2020). Given the heterogenous nature of soils, research also needs to be tailored for specific agricultural regions.

Long term experiments such as the Russell Ranch Century Experiment - located in Davis, California, provide an excellent opportunity for research into the impacts of land management on soils and the microbes living in them. The Century Experiment's location in one of the most productive agricultural regions for vegetables, fruits and nuts in the world (California Department of Food and Agriculture, 2021), its ability to control for the impacts of climate and soil type, and its long term nature make it ideal for isolating the impact of management on a variety of soil health indicators. This agricultural region is quite susceptible to negative impacts of climate warming due to seasonal rainfall patterns, increased fire risk, and potential for soil C losses (Medellín-Azuara et al., 2011).

Through a combination of field measurements and genomic analysis, this dissertation evaluates the impacts of different combinations of farming practices on soils in the California Central Valley in the context of the Russell Ranch Century Experiment.

Chapter 1 explores how a combination of compost and cover crops increased subsoil (>60cm) carbon stocks after 25 years. Using a combination of carbon, nutrient, hydraulic conductivity, carbon chemistry and microbial measurements down to 1m, we were able to identify patterns in extractable organic carbon and soluble nutrients in soil profiles under a variety of management practices (mineral fertilizer, cover crops, compost). We showed that cover crop root channels increase the amount of water that makes it into subsoils, and that the soluble carbon and nutrients carried along with that water result in decreased microbial stress levels, changes in carbon structure, and increases in subsoil carbon stocks. These results highlight the potential synergistic effects of compost and cover crops to increase subsoil carbon stocks, which in turn are likely to extend that carbon's residence time in the soil.

Chapter 2 investigates how different management systems have impacted microbial communities and functional potential, and how those changes are related to soil health indicators using a combination of shotgun metagenomic measurements and the substantial body of research on soil health from the Russell Ranch experiment. We observed no significant changes in overall microbial community structure after 20 years but did find significant changes in several specific microbial functions that were related to some degree to changes in related soil health indicators. We conclude that practices intended to improve soil health do result in small improvements to microbial functional potential but are not necessarily related to significant changes in overall microbial community structure.

Chapter 3 investigates the impact of a switch from furrow irrigation to subsurface drip irrigation (SDI) under compost and cover crops on the distribution of carbon, nutrients and microbial stress indicators in a 2m profile. We compared soils in a 2m profile under 5 years of SDI and furrow irrigation in the California Central Valley and found that the impacts of SDI on carbon and nutrient distribution after a single growing season were limited to the top 30 cm compared to furrow irrigation. We also compared surface organic matter content after 7 years and found that furrow irrigation was associated with greater organic matter loss at the surface compared to SDI after 5 years. Our results suggest that the spatial variation in water distribution under SDI may be associated with reduced organic matter loss at the surface compared to furrow irrigation without significantly reducing the amount of carbon and nutrients stored in subsoils.

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Chapter 1: Synergy between compost and cover crops in a Mediterranean row crop system leads to increased subsoil carbon storage

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Abstract

Subsoil carbon (C) stocks are a prime target for efforts to increase soil C storage for climate change mitigation. However, subsoil C dynamics are not well understood, especially in soils under long term intensive agricultural management. We compared subsoil C storage and soil organic matter (SOM) composition in tomato-corn rotations after 25 years of differing C and nutrient management in the California Central Valley: CONV (mineral fertilizer), CONV+WCC (mineral fertilizer + cover crops) and ORG (composted poultry manure + cover crops). The cover crop mix used in these systems are a mix of oat (*Avena sativa* L.), faba bean (*Vicia faba* L.) and hairy vetch (*Vicia villosa* Roth). Our results showed a ~19 Mg/ha increase in SOC stocks down to 1m under ORG systems, no significant SOC increases under CONV+WCC or CONV systems, and an increased abundance of carboxyl rich C in the subsoil (60-100 cm) horizons of ORG and CONV+WCC systems. Our results show the potential for increased subsoil C storage with compost and cover crop amendments in tilled agricultural systems, and identify potential pathways for increasing C transport and storage in subsoil layers.

1 Introduction

Agricultural subsoils (>60cm) have the potential to store large amounts of C (Rumpel et al., 2012), for a longer period of time (Paul et al., 1997, 2001) relative to surface soils (<15cm). Surface soils are much easier to sample than subsoils, and respond more quickly to management, which makes them the focus of most studies of how soil organic C (SOC) is formed and stored. However, an increased focus on interrogating the surface soil to answer questions about processes in the entire soil profile exacerbates the risk of subsoils being treated merely as "more dilute surface soils" (Salomé et al. 2010) and ignores decades of research into the unique role that subsoils play in increasing soil C stocks (Rapalee et al., 1998; Rumpel and Kögel-Knabner, 2011). A focus on surface soils is particularly problematic in agricultural studies, given how practices such as cover cropping can have drastically different effects on surface versus subsoil SOC accumulation (Bernal et al., 2016; Harrison et al., 2011; Tautges et al., 2019) depending on the cover crop used. In addition, recent studies have highlighted that subsoil SOC may be vulnerable to loss under changing environmental conditions, such as warming (Hicks Pries et al., 2018) and drought (Min et al., 2020). To maximize C stored in the entire soil profile, we need to understand and capitalize on the numerous and interacting physical, chemical and biological changes throughout the profile (Angst et al., 2018; Fierer et al., 2003b; Kautz et al., 2013).

The fact that the average soil sampling depth has decreased from 53 to 27 cm in studies published in the last 30 years (Yost and Hartemink, 2020) may be based on ease of sampling and a focus on surface microbiological processes, but also due to the lack of agreement on where surface soils end and subsoils begin. Depending on the goals of the study, the lower limit of surface soils may be anywhere between the top 7.5 and top 30 cm of the profile, while the upper limit of subsoils can range anywhere from 20-100cm (Soong et al. 2021, Chen et al. 2018, Lorenz and Lal 2005, Whitmore et al. 2015). There may also be an "intermediate" or "transition" zone that is operationally defined, often corresponding to the maximum tillage depth (Mobley et al. 2015). In this study, we define surface soils as the top 0-15 cm of the profile, and subsoils as the lower 60-100 cm, with the intervening 15-60 cm as an intermediate zone based on previous work carried out at our study site, and the relative lack of horizon formation in these young soils.

The unique role that subsoils play in storing SOC is due in part to the extensive, site-specific changes that happen across the soil profile. Often, there are changes in bulk density and mineralogy due to clay accumulation, but the exact magnitude and direction of this change varies depending on depositional environment and soil forming factors (Brady and Weill 2015, Soil Survey Staff 2014, Jenny 1941). Subsoils also experience much less disturbance than surface soils, with lower fluctuations in temperature and moisture content (Smitii 1932, Cole and Matthews 1939, Zeynoddin et al. 2019, de Quieroz et al. 2020) and less mechanical disturbance such as tillage (though it is important to note that tillage events deeper than 30 cm are not altogether rare in many systems). Inputs of oxygen, water, C and nutrients are usually lower to subsoil than surface soils and mostly occur via transport through the soil pore network constructed from intra-aggregate pore spaces, root channels, and cracks that form as the soil dries (Pagliai 2004, Sanderman and Amundson 2008). The types of C input are much less varied in subsoils, mostly coming from biomass and exudates of plants with deep roots (Sokol and Bradford, 2019) and transport of dissolved organic carbon (DOC). This downward transport of DOC is described by the “cascade theory” (Kaiser and Kalbitz, 2012), where subsoil DOC inputs undergo a series of successive sorption, desorption, microbial processing, and transport steps. This results in a gradual increase in the age of C as we move through the soil profile, with subsoil C molecules as old as 10^3-10^4 years (Rumpel et al. 2012) compared to younger C_{14} ages of 10^2-10^3 years in the top 30 cm. The transport of C into the subsoil via both roots and DOC movement leads to more heterogeneous C distribution (Chabbi et al., 2009; Syswerda et al., 2011) that is closely associated with the soil pore network.

Since the soil pore networks responsible for dissolved carbon transport are also hotspots of microbial activity (Banfield et al. 2017), C molecules found in the subsoil have often undergone extensive microbial transformation and processing (Peixoto et al. 2020, Dwivedi et al. 2017). However, once that C does enter the subsoil, it is less likely to undergo further microbial processing due to the combination of heterogeneous C distribution, increased mineral surface area, decoupled microbial-carbon presence (Dungait et al., 2012), greater metabolic and physical restrictions on C decomposition (Fierer et al., 2003a) and lower microbial biomass (Taylor et al., 2002). This leads to higher concentrations of simpler, microbially-derived carbohydrates, aliphatics and carboxylates in subsoils, in contrast to the more complex aromatic structures in cellulose and lignin present in surface soils (Roth et al. 2019). These simple microbial products may preferentially associate with mineral surfaces (Samson et al. 2020, Williams et al. 2018), driving the formation of mineral-associated organic matter and further rendering that C inaccessible to microbes. Subsoil microbial communities have adapted to this relative scarcity of C and nutrients (Salomé et al., 2010; Sanaullah et al., 2011) by increasing the proportion of Gram+ bacteria, whose thicker cell walls make them more resilient to adverse environmental conditions. These subsoil microbes may also optimize for survival rather than population growth, being less efficient at C assimilation than surface microbes (Spohn et al., 2016), and thus more likely to mineralize SOC to CO_2. Low C use efficiency would also be expected in soils with unfavourable carbon-nutrient stoichiometry for biomass production (Ng et al. 2014, Coonan et al. 2020).

Using existing methods to examine subsoils under different land management practices can help explain how the size and concentration of soil C stocks are related to types of C input and the status of the soil microbial community (Sradnick et al. 2014). While it is difficult to accurately estimate whether the C, nutrient and water status of a particular subsoil will promote or hamper microbial SOC decomposition (Soong et al. 2020); some insight can be obtained by looking at microbial stress levels. Phospholipid fatty acid analysis (PLFA) targets metabolically active cells in soil (Zhang et al. 2019) and is effective in measuring rapid changes in active microbial cell walls and membranes (Frostegård et al. 2010). Measurements of Gram negative : Gram positive ratios via PLFA agree with those obtained via the more recent 16s rRNA metabarcoding (Orwin et al. 2018) and are useful as an indicator of microbial nutrient limitation under different land management practices. Understanding how these practices then affect the molecular composition of soil organic matter (SOM) is more difficult, as the most accurate method for quantifying specific C functional groups in soil (nuclear magnetic resonance, NMR) is sensitive to C concentrations, the presence of iron oxides, and requires extensive sample preparation if samples contain low C concentrations or a high abundance of paramagnetic species (Fe, Mn) (Bleam 1991, Smernik and Oades 2022). However, Fourier transform infrared spectroscopy (FTIR) presents a rapid, lower-cost method that allows pseudo-quantification of the relative abundance of certain carbon functional groups (Margenot et al. 2016). While FTIR may also be used as a high-throughput method to predict soil properties (Dangal et al. 2021, Deiss et al. 2020), it is particularly useful when comparing changes in SOM structure over time via spectral subtractions (Margenot et al. 2019).

Agricultural practices can increase or decrease subsoil SOC by modifying the physical, chemical and biological processes that control microbial mineralization of soil C including occlusion in soil aggregates, sorption to soil minerals, microbial processing of residues and C transport into the subsoil (Rumpel and Kögel-Knabner, 2011). Crop root exudates can be efficiently transformed by microbes into stable soil C (Sokol and Bradford, 2019), but the same exudates can also destabilize aggregates and carbon-mineral bonds that are key for protecting C from mineralization (Keiluweit et al., 2015). Large inputs of dissolved organic C and nutrients can prime subsoil microbial biomass to decompose native SOC (Bernal et al., 2016; Kuzyakov, 2010), or provide the nutrients needed for microbes to process soil C (Coonan et al., 2020; Kirkby et al., 2013) and promote the formation of mineral-associated organic matter (MAOM) (Lavallee et al. 2020). Cover crops may not only increase soluble organic C inputs (Steenwerth and Belina, 2008) but can also influence C dynamics indirectly by increasing soil macroporosity and pore connectivity (Scott et al., 1994, Haruna et al., 2018; Çerçioğlu et al., 2019; Gulick et al., 1994), as well as increasing topsoil disturbance due to the processes of planting, mowing, and residue incorporation. These indirect cover crop effects can lead to increases in both infiltration and hydraulic conductivity in fine textured soils and potential increases in soluble C transport, particularly over longer time scales. It is clear that to accurately predict whether a specific farming practice will increase or decrease subsoil SOC storage in a changing climate, it is necessary to perform studies that explicitly examine deeper soils.

Given that small, cumulative subsoil management impacts may take decades to become detectable, impacts of agricultural management practices may not be detectable in the two to three year focus of most agronomic field studies (Dick, 1992; Johnston and Poulton, 2018; Keel et al., 2019). Additionally, measurements of soil C and available nutrients may be highly variable throughout the year (Wuest 2014, Drenovsky et al. 2004), necessitating sampling at multiple timepoints. We conducted our study at the Century Experiment at the Russell Ranch (RR) Sustainable Agricultural Facility in Davis, CA, where inputs and management history have been tracked over the last 25 years and are representative of row crop systems of the California Central valley (Wolf et al., 2018). This agricultural region is quite susceptible to negative impacts of climate warming due to seasonal rainfall patterns, increased fire risk, and potential for soil C losses (Medellín-Azuara et al., 2011). A previous study at the Century Experiment found that after 19 years of management cover cropping (oats, fava beans and vetch) combined with mineral fertilizer application increased C stocks above 30 cm by ~3.5%, but decreased C over the entire 2 m profile by 7% (Tautges et al., 2019). The same mix of cover crops combined with compost both increased C stocks above 30 cm by 5%, and increased C over the whole 2 m profile by 12.6%. At the same time, these systems had similar tomato and corn yields (Scow et al. 2012). Other studies have also demonstrated that surface and subsoil SOC respond differently to agricultural management practices that are primarily concentrated at the soil's surface (Chenu et al., 2019; Syswerda et al., 2011). Estimates of whole-profile C sequestration based solely on data from surface soils can lead to inaccurate estimates of C storage potential in agricultural systems (VandenBygaart et al., 2011).

The goal of this study was to explore some of the potential mechanisms behind the observed differences in carbon storage in different RR management systems, and to see how these carbon stores have changed after an additional 7 years. In particular, we focus on the role of cover crops in promoting hydraulic conductivity, and how those hydraulic changes impact water, C chemistry, nutrient distribution, microbial biomass and community composition in the subsoil under the addition of additional C (compost) and N (nitrogen fertilizer). We hypothesized that the combination of cover crops and additional C input would result in large amounts of soluble C and nutrients being transported deeper via hydraulic transport and the cascade process, leading to more microbially processed carbon and increased carbon stocks in the subsoil. We also hypothesized that these differences are not due to seasonal variation, and that increased soluble C and nutrient stocks will be consistent at multiple timepoints throughout the year.

2 Methods

2.1 Field Site and Historical Management

The experiment was conducted at the Century Experiment at the Russell Ranch Sustainable Agricultural Facility in Davis, CA, in the southern region of the Sacramento Valley at an elevation of 16 m. A detailed description of management history at the Century Experiment is provided in Tautges and Chiartas et al.(2019) and is described here only briefly. Davis experiences hot summers and cool winters, with a 2018-2019 average temperature of 16°C from November to March when cover cropping occurs, and 29°C during the normal vegetable production period of April to September. Average annual rainfall for the 2018-2019 year was 812 mm, most of which fell between December - April in keeping with the xeric moisture regime in this area. (Supplementary Figure A5) (http://atm.ucdavis.edu/weather/uc-davis-weather-climate-station/).

The site has two soil types: (a) Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent) and (b) Rincon silty clay loam (fine, smectitic, thermic Mollic Haploxeralf). Detailed soil horizon information (classification, texture and depths) can be found in Supplementary Table A3 and the Century Experiment published dataset in Wolf et al. (2018). Abbreviations used in this paper (CONV, CONV+WCC, ORG) correspond to the abbreviations used in Wolf et al. 2018 (CMT, LMT, OMT), and are identical to those used in Tautges & Chiartas et al. 2019 for ease of comparison.

The experimental design is a randomized complete block design (RCBD) with three blocks and nine systems. Two blocks are placed on the Rincon silty clay loam, and the third block is on the Yolo silt loam. Experimental plots were 64 m x 64 m (0.4 ha). Only three systems of the nine described in Tautges and Chiartas et al. (2019) were measured in the current paper: CONV (mineral fertilizer), CONV+WCC (mineral fertilizer + cover cropped) and ORG (composted poultry manure + cover cropped). All plots are in a two-year maize-tomato rotation, with three replicate plots of each crop in any given year. Each treatment sampled in this manuscript consisted of 3 plots under tomato and 3 plots under corn, to give a total of 9 corn plots and 9 tomato plots in total. All plots were irrigated with subsurface drip at the time of sampling, having converted from furrow irrigation to subsurface drip in 2014. All plots also received 4 tillage passes to a depth of 20.5cm, and ORG and CONV+WCC plots received additional tillage passes to 6.5cm to incorporate cover crop and compost residue (Supplementary Table A5). While the lack of a compost-only treatment at Russell Ranch precludes conclusions about the impact of compost application alone, comparing the CONV+WCC treatment to the ORG treatment allows us to highlight how adding compost to a cover cropped plot impacts surface and subsoil C stocks, and provides insight into why these impacts occur.

2.2 Historic Carbon, Nutrient and Bulk Density Values

Historical cover crop shoot, compost, and crop residue inputs were calculated based on the Century Experiment published dataset in Wolf et al. (2018). Total C and N of composted manure, aboveground cover crop biomass, and crop residues were determined on a CS 4010 Costech Elemental Analyzer (Costech Analytical Technologies). Total aboveground C and N incorporated was calculated by multiplying percent C and N of residues by total harvest biomass. Due to compost nutrient analysis not being performed every year, estimates from 1993-2000 used %C, N, P and S values averaged for that 7-year period, while estimates from 2000-2018 used %C, N, P and S values averaged for that 18-year period. Total aboveground C, N, P and S inputs were calculated by summing above ground crop residue, WCC, mineral fertilizer and compost inputs per plot per year. Calculated N inputs represent the total N content of the aboveground added WCC and crop residue biomass, and do not differentiate between fixed N and N uptake from the soil in the case of cover crop legumes. Measurements do not include estimates of belowground biomass due to a lack of data.

Soil % C and nitrogen (N) values for 0-15, 15-30, 30-60 and 60-100 cm in 1993 and 2012 were taken from Tautges & Chiartas et al. (2019), while values for the same depths in 2003 were taken from the Century Experiment published dataset in Wolf et al. (2018). C and N analyses used in this paper were all performed using the same methods (Tautges and Chiartas et al. 2019, Wolf et, al 2018) on ball-milled, air dried samples in a CS 4010 Costech Elemental Analyzer (Costech Analytical Technologies). Total C and N values for 15-60 cm in 1993 were calculated by performing a weighted average of C and N % values from 15-30 and 30-60 cm.

Bulk density values used in this paper were sampled using a Giddings hydraulic probe to 2m in 1993, 2007 and 2012 (2007 values taken from Wolf et al. 2018 and 1993, 2012 values taken from Tautges et al. 2019). In 1993, bulk density was collected in 0–25, 25–50, 50–100, and 100–200 cm depth layers with an 8.25 cm diameter probe. In 2007 and 2012, bulk density was collected in 0–15, 15–30, 30–60, and 60–100 cm depth layers, with a 4.7 cm diameter probe. In 1993, 2007 and 2012, cores were collected from four random locations within each plot. Bulk densities were determined using mass of oven-dried soil (105°C, 24 hr.) and total volume of the core averaged for each depth increment (Blake and Hartge, 1986). Bulk density depths from 1993, 2007 and 2012 were adjusted to 2018 depths through the calculation of weighted averages using adjacent depth layers for comparison. Historical C stocks from 0-100 cm for 1993, 2003 and 2012 were calculated via depth weighted sum (Tautges and Chiartas et al. 2019) using bulk density values taken in 1993, 2007 and 2012 respectively. Depth-adjusted 2012 bulk density values were then used to calculate 2018 C and nutrient stocks due to the lack of more recent bulk density measurements for all plots. Bulk density values below 30 cm were assumed to have not undergone large changes between 2012-2018 (Tautges and Chiartas et al. 2019), while bulk density sampling from 0-20 cm in select Century Experiment plots indicated a limited difference in bulk density (less than 3%) from 2012-2019 (Wang, unpublished data).

2.3 Field Operations

Cover crop planting and incorporation in ORG and CONV+WCC systems in 2017-2018 followed the trend of previous years, being planted onto 15 cm raised beds 1.5 m apart with a mixture of oat (*Avena sativa* L., 42.0 %C, 2.5 %N), faba bean (*Vicia faba* L., 44.1 %C, 3.5 %N) and hairy vetch (*Vicia villosa* Roth, 44.5 %C, 5.2 %N), and terminated by mowing plus 2-3 disking passes in March. Cover crop biomass was sampled by cutting aboveground biomass from one 4.5 m^2 area in each plot prior to termination. Corn and tomato biomass residues were measured by cutting aboveground biomass at two 1.5 m^2 locations per plot after harvest. Biomass samples were oven dried at 65 ℃ for 4 days and ground to 2 mm prior to total C and N analysis.

Fertilization during the 2017-2018 growing season was also similar to previous years, with CONV and CONV+WCC plots receiving 325 kg/ha 8-24-6 (26 kg N/ha, 78 Kg P/ha, 19.5 kg K/ha) starter fertilizer at the time of planting. Tomato CONV plots also received ammonium sulfate at a total rate of 200 kg N/ha, while maize CONV plots received ammonium sulfate at a total rate of 235 kg N/ha.

From 1993-2018, ORG plots normally received a spring application (February 2018) of composted poultry manure at a rate of 3.6 Mg/ha (24.9 % C, 3.5 % N, 1.6 %P, 1.47 %S). However, during the 2018 season, these plots switched from spring to fall compost application, resulting in an additional application of 3.6 Mg/ha compost in September 2018.

2.4 Soil Sampling

Soil sample collection took place in the 2018-2019 growing season. Plots were sampled at 4 timepoints: February 2018 (Pre-CC Incorporation), June 2018 (Mid-Season), September/October 2018 (Post-Harvest), and February 2019 (Pre-CC Incorporation). A substantial amount of variation in both extractable organic carbon (EOC) and mineral N measurements can occur during the growing season (Li et al. 2018). Our sampling regime at multiple timepoints was meant to account for that variation in both winter and summer months to give a more accurate snapshot of C and nutrient availability during the growing season. All sampling took place in the raised beds between furrows. Samples in February 2018, September/October 2018 and February 2019 were taken using a tractor-mounted Giddings probe with a diameter of 3 cm from all replicate plots of each system (n = 6 plots per treatment). Samples taken in June 2018 were taken using an auger to 100 cm and were only taken in the experimental plots planted with tomato (n = 3 plots per treatment). Three replicate cores were taken per plot, sectioned into 0-15, 15-60 and 60-100 cm depths, composited, and then subsampled. Aliquots of each soil were frozen at -20 ℃ for PLFA analysis within 48 hours of sampling, while the remaining samples were sieved to 8 mm and stored at 4 ℃ until analyzed.

2.5 Carbon, Nutrient and Aggregation Analysis

All analyses described below were carried out on samples taken during the 2018-2019 growing season. Extractable organic carbon was determined using a 0.5 M potassium sulfate extraction within 48 hours of sampling. For each sample, 6 g of soil were extracted with 0.5 M K_2SO_4 in a 1:5 ratio, shaken for one hour, filtered through Q5 filter paper and analyzed within 48 hours on a Shimadzu TOC-L Total Organic Carbon analyzer according to Jones and Willett (2006). Due to the moisture limited conditions present during summer at our study site, we chose an EOC extraction method as opposed to DOC sampling via tension lysimeters in order to compare soluble C measurements at different timepoints and soil water contents. Measurements of EOC are commonly used to estimate soluble C (Slessarev et al. 2020, Matlou et al. 2007) and may be more sensitive to recent C and litter inputs, making them more suitable for answering questions on the impacts of C input, N amendment and tillage (Li et al. 2018).

Aliquots of the K_2SO_4 extract were immediately frozen at -20℃ and later analyzed for nitrate by reacting with vanadium(III) chloride according to Doane and Horwath (2003); and ammonium via the Berthelot reaction as laid out in Rhine et al. (1998). Available calcium, phosphorus and sulfur were measured on 2 mm sieved air-dried samples using the Mehlich-3 soil test (Mehlich, 1984). Total soil C and N values were measured on a CS 4010 Costech Elemental Analyzer (Costech Analytical Technologies) using air-dried, ball milled samples. The 2018 C and nutrient stocks were calculated using depth-weighted sums (Tautges et al. 2019) with bulk density values from 2012.

Aggregation measurements were carried out using the method outlined in Wang et al. (2017), adapted from the wet-sieving method outlined in Elliott (1986). Soils were gently passed through an 8mm sieve, and a 50g representative sample was submerged in room temperature water on top of a 2 mm sieve. This sieve was moved up and down for 2 min (50 submersions per minute) using an audio metronome to keep track of the number of submersions. The soil and water passed through the 2mm sieve were gently transferred by rinsing onto a 250 μm sieve and submerged again. The process was repeated using a 53 μm sieve to generate 4 aggregate size fractions (8 mm-2 mm, 2 mm-250 μm, 250 μm-50 μm, >50 μm) which were rinsed into pre-weighed aluminum pans, oven-dried at 60 ℃, and weighed. Mean weight diameter of the aggregate fractions was calculated as the weighted average of the four aggregate size fractions (van Bavel, 1950).

2.6 Phospholipid Fatty Acid (PLFA) Analysis

PLFA analysis was carried out on 2018 samples using the high-throughput method outlined in Buyer and Sasser (2012). Briefly, freeze-dried aliquots were extracted using Bligh-Dyer extractant. Phospholipid fractions were separated from the neutral lipid and glycolipid fractions using solid phase extraction columns. Phospholipids were then dried under N2 gas, transesterified, and methylated. After methylation, the samples were dried again with N_2 gas and redissolved in hexane containing a known concentration of an internal standard (19:0) (Microbial ID, Newark, DE, USA). PLFAs were identified using the Sherlock software from Microbial Identification Systems and quantified using a gas chromatograph equipped with a flame ionization detector. A total of 56 different PLFAs were identified. PLFAs were assigned to Gram-positive, Gram negative, Cyclopropyl precursors, Saturated and Monounsaturated groups as outlined in Bossio and Scow (1998) (Supplementary Table A1).

2.7 Hydraulic Conductivity and Moisture Content

Three 20 cm^3 cores were collected in September 2018 for saturated hydraulic conductivity from each plot that had been under tomato in 2017-2018 (a total of 9 plots). Cores were taken from a depth of 35 cm. Unfortunately, two cores were damaged during measurement, giving a total of 25 cores measured from the three treatments. Care was taken to transport the cores in foam holders to avoid creating compaction or preferential flow paths in transit. Cores were stored at 5 ℃ until measurement. A KSAT device was used to measure the cores with a falling head technique per the manufacturers manual and conductivity data was normalized to 20 ℃ using the Ksat software from the manufacturer (Meter Group, Pullman, Washington USA).

Soil moisture content was measured with a multi-depth profile capacitance probe in carbon fiber access tubes that were installed according to the manufacturer’s recommendations with great care taken to avoid air gaps along the tube (PR 2/6, Delta-T Devices, Cambridge, UK). The factory calibration of the profile probe was used with an accuracy of ± 0.04 m^3 m^{-3}. Volumetric soil moisture was measured at six depths (10, 20, 30, 40, 60, 100cm) (PR 2/6, Delta-T Devices, Cambridge, UK). Access tubes were installed in the field with a custom auger taking care to make the holes smooth and straight according to the manufacturer’s recommendations. A total of 27 tubes were installed, with 3 tubes per subplot for a total of n = 9 per treatment (ORG, CONV+WCC, CONV). The measurements were made on 8 dates between January 12 - March 1, 2019. Data was processed using R (R Core Team 2014), and soil moisture depth from 10-100 cm was calculated using trapezoidal integration.

2.8 Fourier transform infrared Spectroscopy

Fourier transform infrared (FTIR) spectra of soil samples from 1993 and 2018 were collected using diffuse reflectance infrared Fourier transform spectroscopy (DRIFT; PIKE Technologies

EasiDiff) with soil (air dried) diluted to 10% with KBr (Deiss et al., 2020). Spectra from 1993 samples were collected from air-dried, homogenized, archived soils from the Century Experiment Archive, while 2018 spectra were collected from air-dried, homogenized samples taken in 2018. 1993 spectra from 15-30 cm and 30-60 cm were combined into a single 15-60 cm spectra via weighted average for comparison with 2018 samples. The variation between these averaged 15-30 and 30-60 cm soils was found to be negligible for all three systems (Figure A6). All DRIFT spectra were collected using a Thermo Nicolet 6700 FTIR spectrometer (Thermo Scientific) using 256 scans, 4 cm^{-1} resolution, and a DTGS detector. Three replicate samples were used, and average spectra were created for analysis. Peak intensity ratios of aromatic to carboxyl moieties [v(C=C):vas(COO^{-}) (1662 cm^{-1}:1631cm^{-1})] were calculated using peak areas.

While FTIR is not a strictly quantitative tool for identifying specific compounds in mixed samples, it can be used pseudo-quantitatively due to the fact that the absorption of IR light by a specific molecular bond at a specific electromagnetic frequency follows the Beer-Lambert Law (Beer's Law) (e.g., Margenot et al.2016, Smith 2001). Therefore, the height and area of a spectral peak are proportional to the abundance of molecules in a sample (linear relationship), and comparing the presence and absence of peaks and the relative differences in spectral contributions from each peak in a subtraction can suggest differences in C chemistry. However, it is important to note that spectral reflectance can lead to some non-linearity in concentration and absorbance, and thus pseudo quantification. 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 C concentration and absorbance at specific frequencies (depicted as peak height or area of single peaks or peak ratios). Spectral subtractions were performed using Omnic 9.8.286 (Thermo Fisher Scientific) and corrected for non-linearity of concentration and absorbance by using the Kubelka-Munk (KM) function. Plots of FTIR spectra were made using Origin 2018b (OriginLab Corporation). Subtractions were performed in two ways: 1) mean spectra, for each treatment and depth, of the 1993 spectra were separately subtracted from the corresponding 2018 spectra to reveal C chemistry changes over this period; and 2) the 2018 mean spectra, for each depth, were subtracted (ORG-CONV, ORG-CONV+WCC, CONV+WCC-CONV) to show the difference in C chemistry by treatment.

2.9 Statistical Analysis

All data analysis and graph production were done using R v. 4.0.2, (R Core Team, 2020) using the tidyverse package (Wickham et al., 2019). Analysis of variance (ANOVA) was conducted using a linear model to determine the effects of management system, depth, and time point. We first checked for normality and assumptions of the linear model prior to ANOVA, then fit a mixed effect model with “block” as a random effect. Since “block” was not significant for any of the variables measured, we removed it from the model. Statistical differences between management systems were analyzed separately for each depth using paired t-tests with Bonferroni correction for multiple tests at 5% significance level. Data and code used for this paper are archived at https://zenodo.org/badge/latestdoi/181972884.

3 Results

3.1 Nutrient Inputs

The cumulative estimated aboveground C input over 25 years was 186 Mg ha^{-1}, 123 Mg ha^{-1} and 113 Mg ha^{-1} for ORG, CONV+WCC and CONV systems respectively. Averaged per year over 25 years, C inputs to each system were 7.44 Mg ha^{-1}, 4.92 Mg ha^{-1} and 4.52 Mg ha^{-1} for ORG, CONV+WCC and CONV systems respectively (Supplementary Table A4). Due to the combination of compost and cover crop residue and root inputs, ORG systems received approximately 1.5x more C than CONV+WCC. Although CONV+WCC produced similar amounts of tomato residue and less maize residue than CONV, the presence of cover crops meant that CONV+WCC systems received 1.1x more C than CONV systems.

Due to combined N inputs from cover crop and compost, ORG systems received 1.4x as much external N inputs (7.5 Mg ha^{-1}) as CONV+WCC systems (5.4 Mg ha-1), and 1.65x as much N as CONV systems (4.5 Mg ha^{-1}). External N inputs to CONV+WCC systems were close to 1 Mg ha^{-1} higher than CONV systems over 25 years, with ~40% of the external N inputs to CONV+WCC systems coming from the decomposition of cover crop residue, and the other ~60% from mineral fertilizer application, compared to 100% of total N inputs in the CONV coming from mineral fertilizer application. ORG systems received over 3x as much phosphorus via compost (3.23 Mg ha^{-1}) as CONV+WCC (1.09 Mg ha^{-1}) and CONV (0.99 Mg ha^{-1}) did from P fertilizer. ORG systems also received 1.15 Mg ha^{-1} of sulfur from compost, approximately 0.5x as much as CONV+WCC (2.19 Mg ha^{-1}) or CONV (1.98 Mg ha^{-1}) systems received.

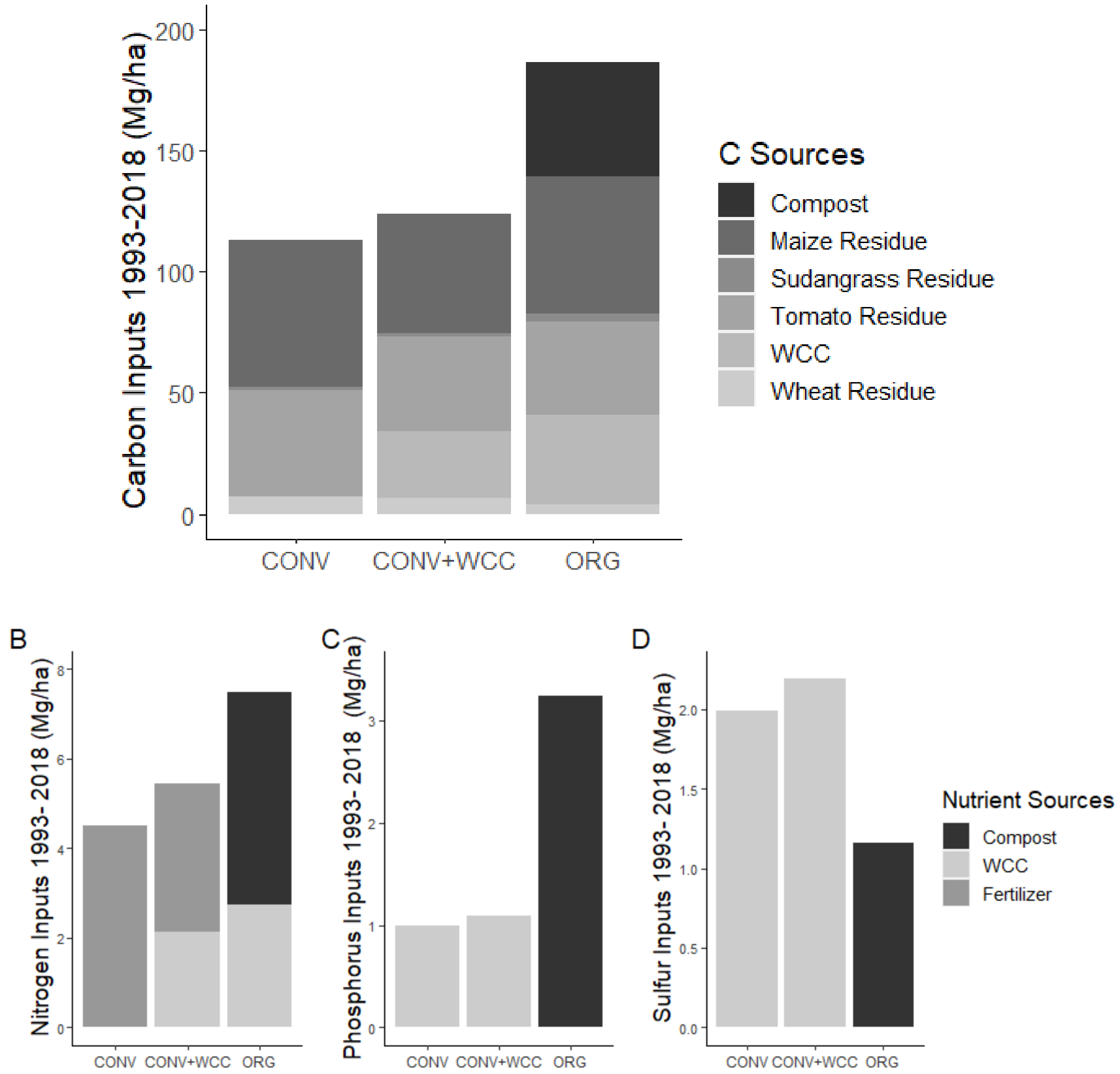

Figure 1.1a-d. Total aboveground C, N, phosphorus, and sulfur added per plot to ORG, CONV+WCC and CONV systems between 1993-2018. All values are given on a mass basis (Megagrams/hectare).

3.2 Soil Carbon Content Changes over 25 years

Carbon stocks in the 1 m profile of ORG systems showed an increase of ~19 megagrams/hectare from 1993-2018 (p=0.06) (Figure 2). Most of this C gain was concentrated in the 0-15 cm (~5 Mg ha$^{-1,}$ p<0.01) and 15-60 cm depths (~10 Mg ha^{-1}, p=0.1). Due to the large amount of variation present in these observations and the limited number of replicates, it was difficult to spot strong trends in C stock changes, as shown in the lack of significant change in the bottom 60-100 cm (~3 Mg ha^{-1}, p=0.26). No significant changes in C stocks in the 1 m profile were noted in CONV or CONV+WCC systems from 1993-2018 (p=0.47, p=0.51). When depth intervals were considered separately, only CONV systems showed a decrease in C stocks (~ -3 Mg ha^{-1}), at the 0-15 (p<0.01) depth (Figure 3). CONV+WCC systems did not show a clear trend of C decrease at any individual depth with the significance testing used.

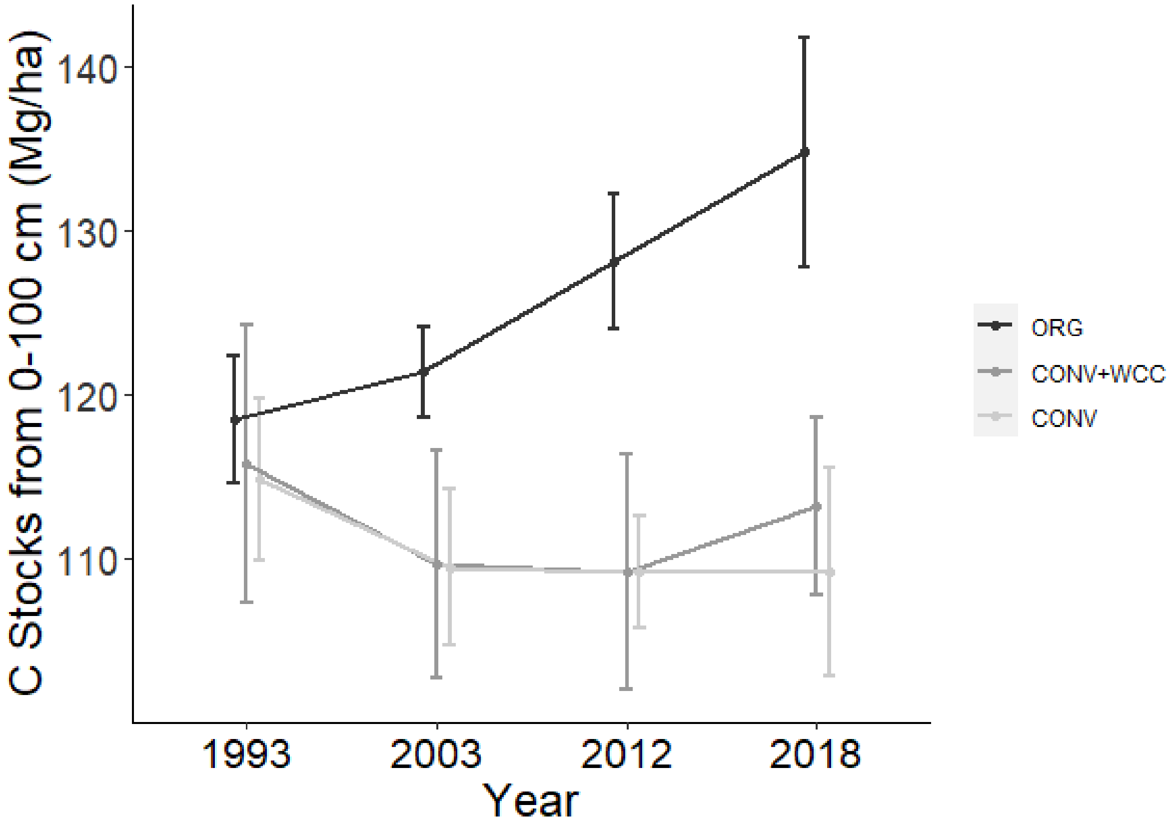

Figure 1.2. Carbon stocks of the 1m profiles of ORG, CONV+WCC and CONV systems from 1993 to 2018. Carbon stocks are given in Mg ha^{-1}. Error bars denote standard error. Please note that all systems transitioned from furrow to drip irrigation in 2014.

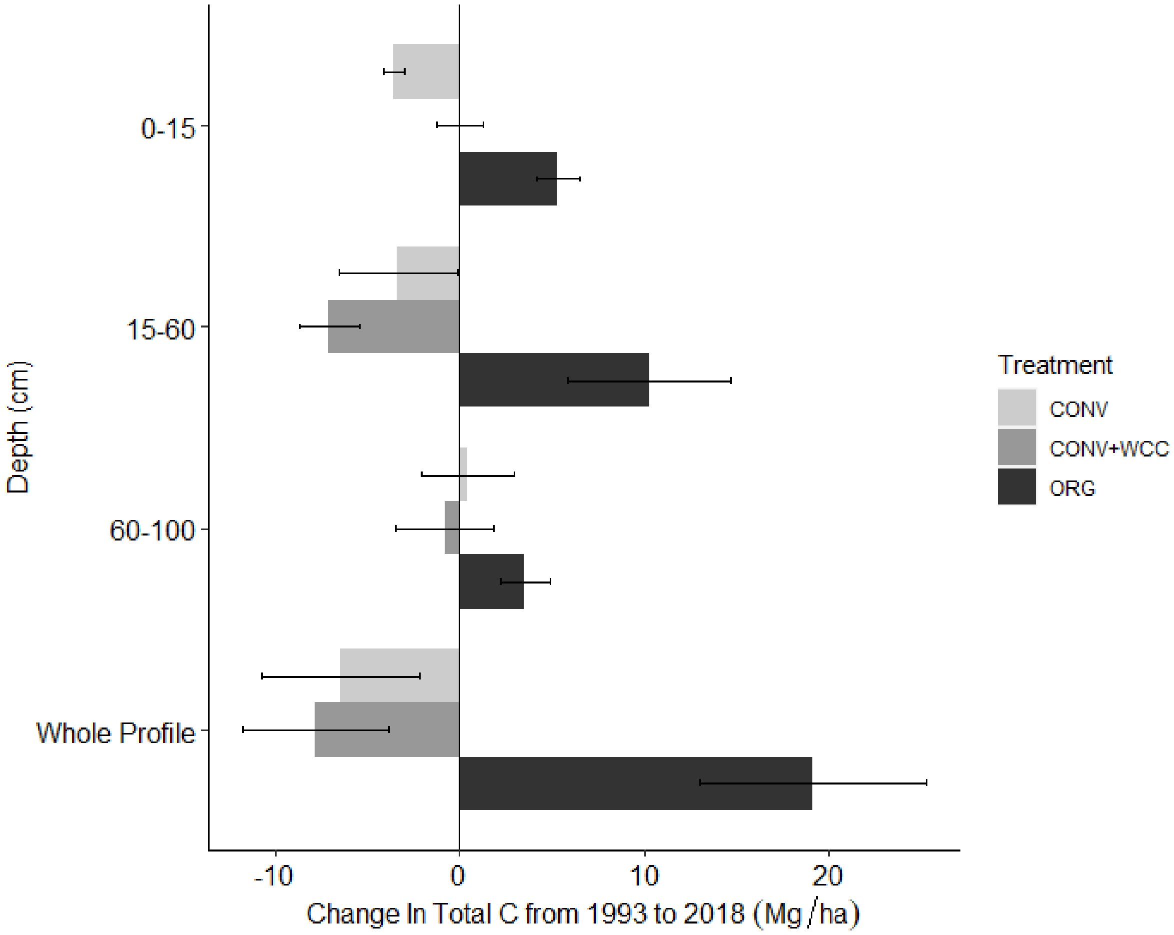

Figure 1.3. Change in C stocks of ORG, CONV+WCC and CONV systems from 1993-2018 by depth. Values were obtained by subtracting C stocks in 1993 from 2018 stocks for individual systems, and then averaging by management system. Error bars denote standard error. (* = significantly different from 0, p-value <0.05, + = significantly different from 0, p-value 0.05<x<0.1)

3.3 Moisture Content, Hydraulic Conductivity, and Aggregation

Cover cropped systems (ORG and CONV+WCC) stored approximately 10% more water than non cover cropped systems (CONV) in the upper 1m of the soil profile during the 2019 winter (Fig 4). There was no difference in moisture content between ORG and CONV+WCC systems. Averaged hydraulic conductivity measurements showed differences among all three systems, but treatments with cover crops (ORG and CONV+WCC) had values that spanned 3 orders of magnitude compared to treatments without cover crops (CONV) (Fig. 5). There was no significant difference in MWD of aggregates between all three systems at any depth (Supplementary Figure A2).

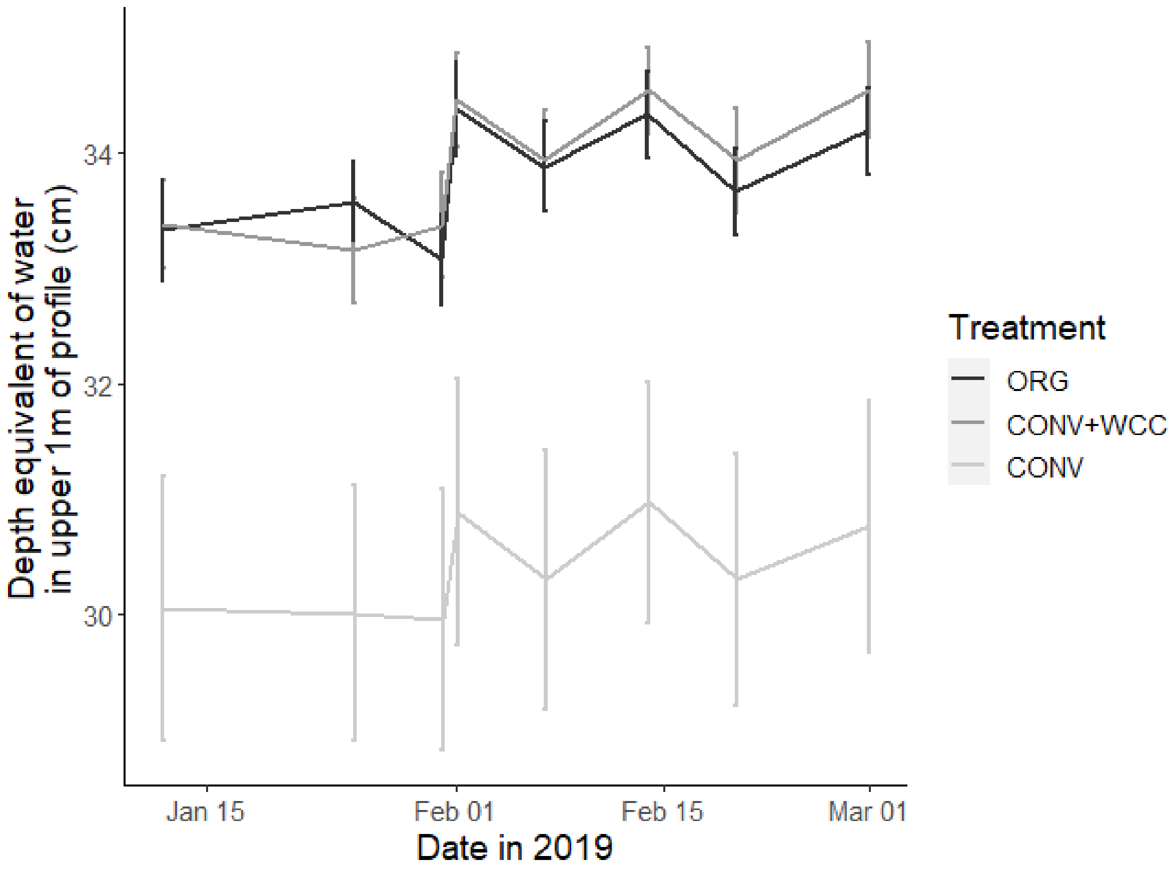

Figure 1.4. Depth equivalent of water (in cm) in the upper 1m of ORG, CONV+WCC and CONV profiles during the Jan -Mar 2019 winter season. Error bars represent standard error.

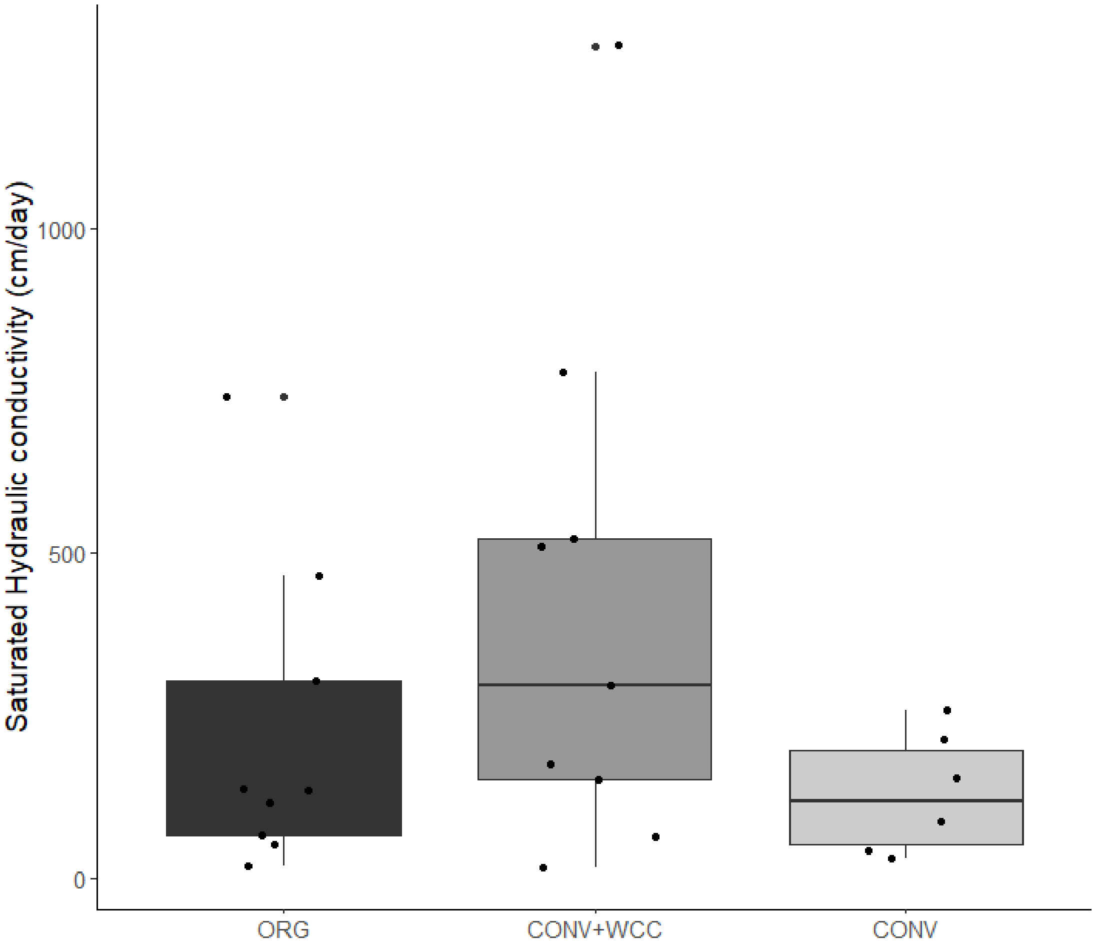

Figure 1.5. Saturated hydraulic conductivity (cm/day) in ORG, CONV+WCC and CONV systems taken in August 2018.

3.4 Soil Nutrient Content: Extractable Organic Carbon, Mineral Nitrogen, Phosphorus, Sulfur

Composted systems (ORG) had higher amounts of extractable organic carbon (EOC) ($p<0.01$), plant available phosphorus ($p<0.01$) and sulfur ($p<0.01$) in the 1m profile than non-composted systems (CONV+WCC and CONV) averaged across all dates the 2018-2019 year (Fig. 6). These differences were most pronounced in the upper 15 cm, where ORG systems had approximately 2x more EOC ($p<0.01$), 3x more phosphorus ($p<0.01$) and 1.75x more sulfur ($p<0.01$) than CONV+WCC or CONV systems (Supplementary Figure 1).

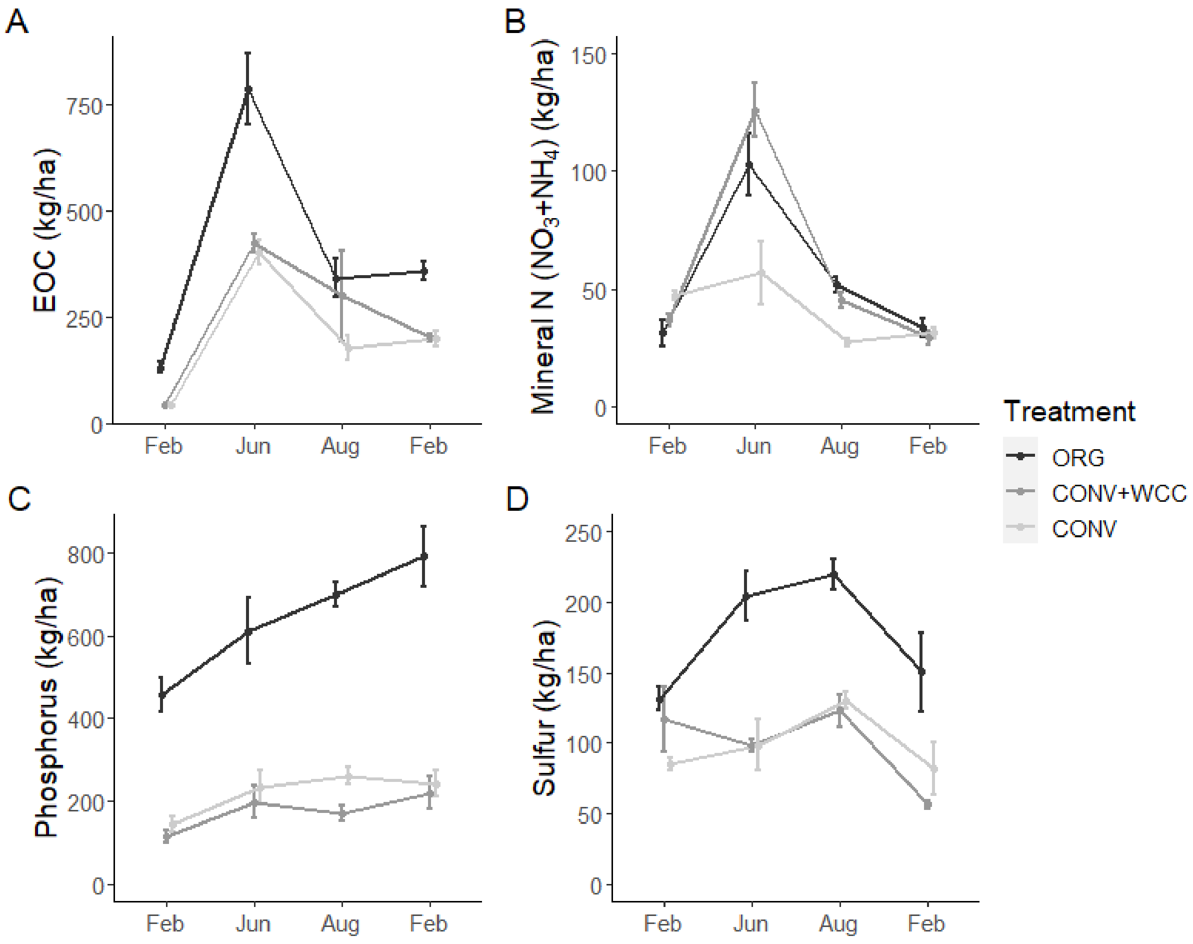

Figure 1.6a-d. Extractable organic C, mineral N (NO_3^-+ NH_4^+), phosphorus and sulfur in 0-100cm profiles of ORG, CONV+WCC and CONV systems over the Feb 2018- Feb 2019 season. All values are given on a mass basis (kilograms/hectare). Error bars represent standard error.

CONV+WCC systems had more mineral N (NO3+NH4) than CONV systems during the June and August timepoints (p=0.04), with up to 3.5x more mineral N than CONV systems mid-season, and 1.6x more mineral N at harvest. ORG systems trended towards higher mineral N during the April - September growing season but the magnitude of this difference was small (p=0.17).

Nutrient values showed large seasonal variation, with the highest levels of C and N observed during the June timepoint and highest sulfur levels at the August timepoint. EOC, mineral N, and sulfur values were lowest during the winter (Nov - Feb), which coincided with the period of highest rainfall. Phosphorus levels increased slightly throughout the 2018-2019 year.

Differences among systems and seasonal variation were also noted at a depth of 60 cm. ORG systems had more EOC ($p<0.01$), phosphorus ($p<0.001$), and sulfur at 60-100 cm than CONV+WCC or CONV systems. Mineral N values did not show significant differences between any of the three systems at 60-100 cm, though ORG and CONV+WCC systems trended higher during the growing season (Figure 7).

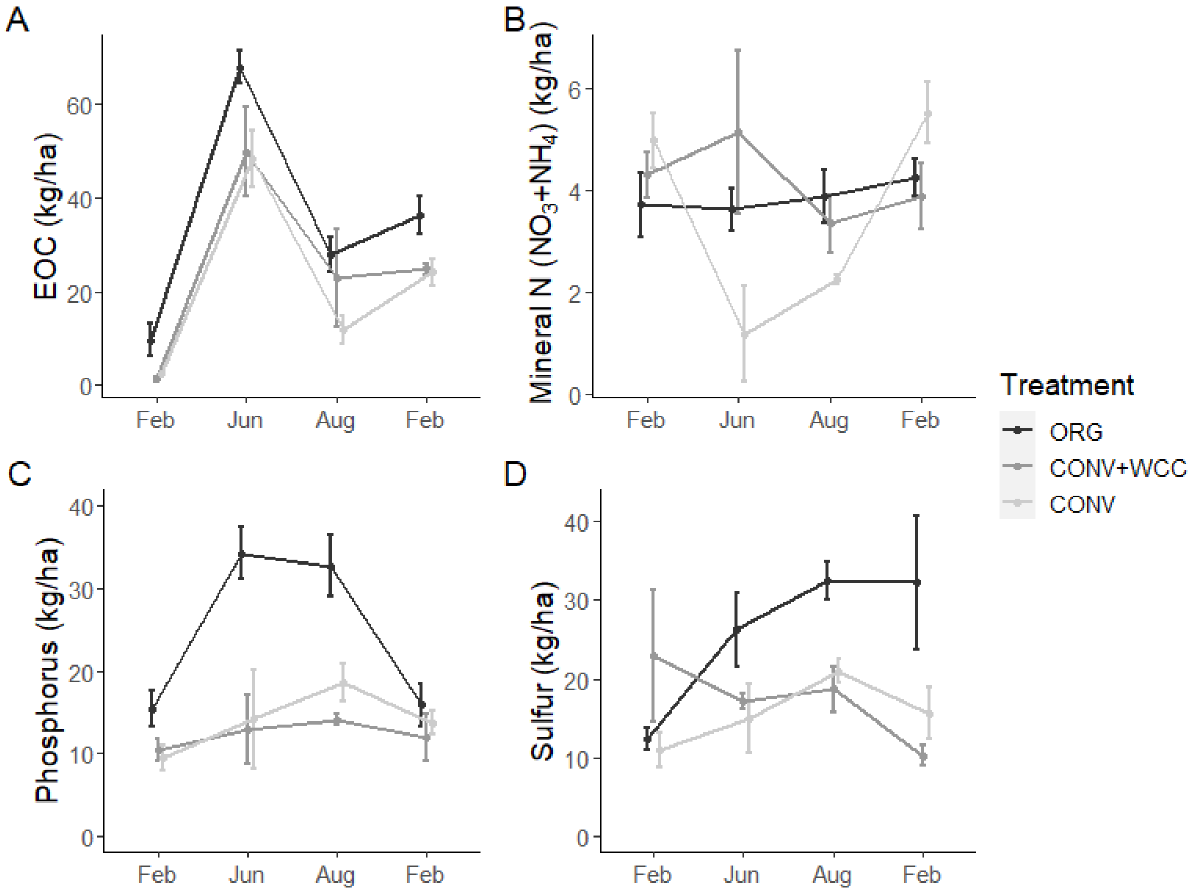

Figure 1.7a-d. Extractable organic C, mineral N, phosphorus, and sulfur stocks at 60-100 cm in ORG, CONV+WCC and CONV systems over the Feb 2018- Feb 2019 season. All values are given in kg/ha. Error bars represent standard error.

3.5 SOM Composition via FTIR

Spectral subtractions of 1993 from 2018 FTIR spectra revealed positive peaks (increased absorbance) from 1900 to 1200 cm^{-1} in all systems, indicating an increase in C functional groups within this region (e.g., aromatic, carboxyl) (Fig. 8A). FTIR band assignments are presented in Supplementary Table A2. All treatments showed positive peaks indicating an increase in carboxylate functional groups between 1993 and 2018, as denoted by bands at 1625 cm^{-1} and 1400 cm^{-1} (Fig 8a). However, ORG and CONV+WCC showed these distinct peaks at 15-60 and 60-100 cm depths, while CONV systems showed distinct peaks only at the 0-15 and 15-60 cm depths. CONV systems also showed a lower aromatic:carboxylate peak intensity ratio at all depths than ORG and CONV+WCC systems from 1993-2018 (Table 1).

ORG and CONV+WCC systems showed distinct positive peaks associated with carboxylate functional groups at the 60-100 cm depths in 2018 when compared with CONV systems (bands at 1631 cm^{-1}), and slightly higher peaks associated with aromatic functional groups from 0-15 cm for CONV+WCC and 15-60 cm for ORG (bands at 1662 cm^{-1}) (Figure 8b). ORG also showed positive peaks associated with aromatic functional groups from 0-15 and 15-60 cm when compared to CONV+WCC (bands at 1662 and 1602 cm^{-1}) in 2018. Aromatic:carboxylate ratios provide an indication of the intensity of carboxyl peaks relative to aromatic peaks, which can be related back to concentrations of these functional groups in the sample. A lower aromatic:carboxyl ratio can indicate either more carboxyl or less aromatic functional groups, while a higher ratio can mean increased aromatic or decreased carboxyl groups. Aromatic:carboxylate peak intensity ratios decreased with depth for ORG and CONV systems when looking at changes from 1993-2018, but CONV+WCC ratios increased with depth (Table 1).

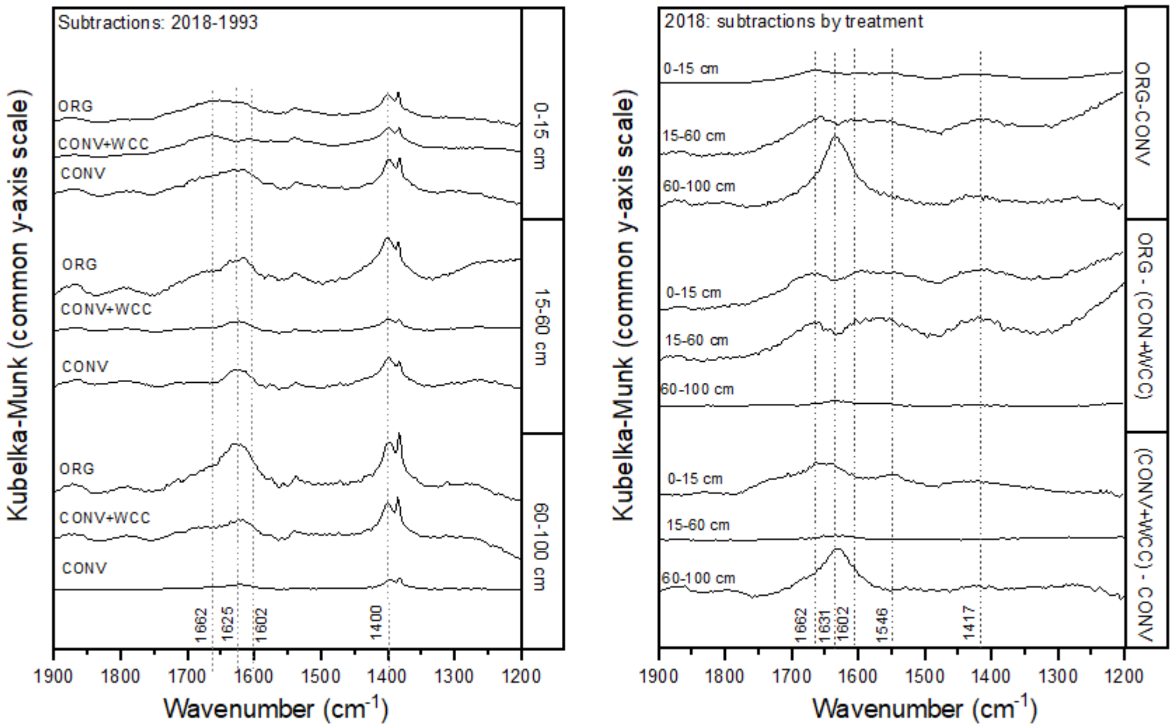

Figure 1.8a,b. DRIFT spectral subtractions for the 1900-1200 cm^{-1} range comparing (A) 2018-1993 spectra for ORG, CONV+WCC, and CONV, and (B) ORG, CONV+WCC, and CONV spectra in 2018. Spectra are plotted with Kubelka-Munk units on a common y-axis scale, and are offset from one another for ease of comparison.

	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

Table 1.1. Peak Intensity Ratios for aromatic (1662 cm^{-1}) to asymmetric carboxyl (1631 cm^{-1}) groups in spectral subtractions.

3.6 Microbial Biomass and Stress Indicators - July 2018

Microbial biomass decreased with depth in all systems. ORG and CONV+WCC systems had more microbial biomass at 0-15cm than CONV systems (p=0.04 & p=0.04 respectively), while ORG systems had more microbial biomass at the 15-60 cm depth than CONV+WCC or CONV systems (p=0.03 & p=0.06 respectively). Saturated: Unsaturated fatty acid ratio and Cyclopropyl 19: precursor ratio increased with depth, with CONV systems showing a weaker trend of higher Cy19: pre (p=0.12) and saturated: unsaturated fatty acid ratios (p=0.07) than ORG at 60-100 cm. Gram+: Gram- ratio also increased with depth, with CONV systems having a higher ratio than ORG or CONV+WCC systems at 60-100 cm (p=0.01 and p=0.01 respectively).

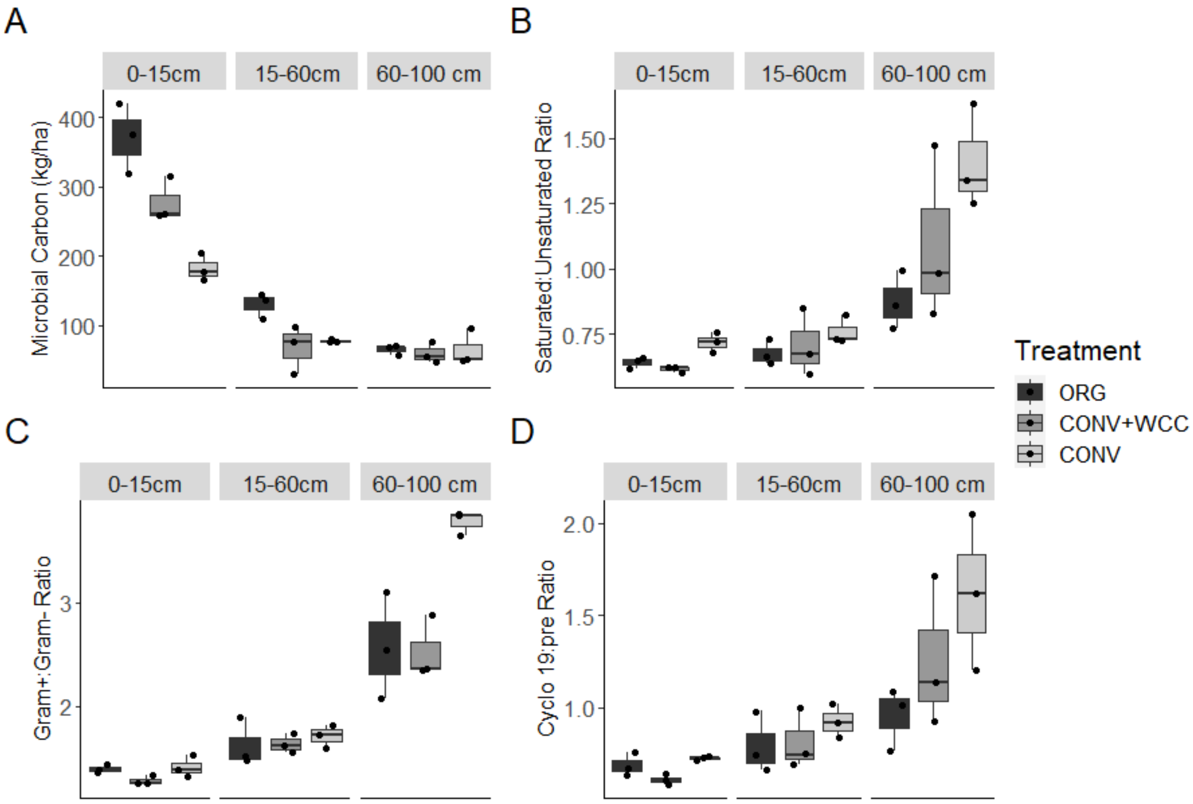

Figure 1.9a-d. Microbial biomass and PLFA stress indicators measured in ORG, CONV+WCC and CONV systems during mid-season (July 2018). Ratios are unitless, while microbial biomass is given in kg/ha.

4 Discussion

The ~19 Mg/ha increase in SOC over the 1m ORG profile after 25 years was attributed to a synergistic effect between cover crops and compost, which resulted in the movement of mobile C and nutrients deeper into the soil profile. We believe that high concentrations of mobile C and essential nutrients for microbial activity provided by the compost, combined with the easier movement of water downward associated with a history of cover-cropping, helped transport the material needed to build C in the subsoil.

4.1 Cover crop roots increase water storage and movement into subsoils

The higher moisture contents noted in CONV+WCC and ORG than CONV systems during the winter growing season are likely due to the presence of more water-filled spaces from cover crop roots (Figure 4). Cover crops increase both soil macroporosity and pore connectivity in fine textured soils, leading to an increase in both infiltration and hydraulic conductivity over longer time scales (Scott et al., 1994, Haruna et al., 2018; Çerçioğlu et al., 2019; Gulick et al., 1994). A similar impact of cover crops have been noted in previous work done in Russell Ranch soils: a cover crop mix of purple vetch (*Vicia benghalensis* L.), common vetch (*Vicia sativa* L.) and oats (*Avena sativa* L.) increased soil moisture-holding capacity during saturated conditions (Joyce et al. 2002); a cover crop of common vetch produced no changes in bulk density after 10 years (Colla et al. 2000) and a wheat (*Triticum aestivum* L.) cover crop increased infiltration by 43% and decreased DOC export by 54% in a furrow irrigated system, causing the soil profile to become a DOC sink (Mailapalli et al., 2012). Proposed mechanisms are that cover crops increase infiltration and hydraulic conductivity by altering soil structure through aggregate formation, reduced soil crusting, and reduced soil compaction due to increased organic matter content and formation of root channels (Chen and Weil, 2010; Franzluebbers, 2002). Other potential, albeit less likely, explanations for increased moisture content could include lateral subsurface flow (unlikely due to the <1% slope of this field) and differences in runoff and runon (also unlikely due to low slope). Given that our cover crop mix is known to have extensive root networks that extend deeper than the 30cm plow layer (Fan et al., 2016), the observed differences in moisture content during the winter were attributed to increased biopores created by roots (Hangen et al., 2002) at the time of sampling.

Despite the presence of cover crops, we found no significant difference in aggregate MWD among systems (Supplementary Figure A2). This may be because root-induced soil alterations, such as aggregation, are highly localized and dependent on the root architecture of the cover crops. Specifically, cover crops with prominent tap roots, such as fava bean, are effective at creating continuous bio-pores, while fibrous roots such as in oats and hairy vetch, are particularly effective at promoting soil aggregate formation (Ogilvie et al., 2021). Therefore, the mixture of cover crops planted at the site likely resulted in a large amount of variation in aggregation and pore connectivity and may have resulted in the non-significant aggregate MWD values.

While mean hydraulic conductivity values were also not significantly different between treatment systems, hydraulic conductivities were more variable in the two systems with, than without, cover crops (Figure 5). Roots may increase macroporosity by opening up channels as they decay (Ghestem et al., 2011), and increased water movement through these macropores can result in hydraulic conductivity values that can range over three orders of magnitude (Øygarden et al., 1997) similar to what we observed. In addition, the sample size used for K_{sat} measurements (cross-sectional area of 250 cm^2) 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 reflect the presence of macropores (Brooks et al. 2004). Though our measurements do not reveal statistically significant differences between treatments, the scattered high-permeability zones in the cover-crop treatments likely play a role in rapid moisture redistribution and may explain the elevated deep moisture contents in ORG and CONV+WCC plots compared to CONV plots.

We therefore attributed the more variable hydraulic conductivity and increased moisture content in ORG and CONV+WCC than CONV systems to the deeper, more abundant root-derived macropores from cover crops.

4.2 Compost + Cover Crops increased the amount of EOC and carboxylate functional groups in subsoils

Increased EOC levels (Figure 6a) in ORG plots and relatively more oxidized carboxylate C in the bottom 60-100 cm of ORG and CONV+WCC plots relative to CONV plots (Figure 8b) point to an accelerated cascade process (Kaiser and Kalbitz 2012) in these systems relative to CONV systems. The presence of elevated EOC levels in ORG plots are due to the higher amounts of soluble C found in compost (Wright et al., 2008; Zmora-Nahum et al., 2005), the increase in dissolved and water extractable organic C expected from cover crop residue (Singh et al. 2021 - cereal rye and hairy vetch), and the larger amounts of C added to ORG plots (Figure 1a). Compost also contains a large proportion of aromatic functional groups derived from lignin and other biomolecules (Leifeld et al., 2002) which tend to be rapidly removed from the soil solution at the surface (Leinemann et al., 2018). This preferential removal at the soil surface may be a function of the relatively low solubilities of non-polar aromatic functional groups (Maxin and Kogel-Knabner, 1995), as well as their tendency to partition into other non-polar, insoluble organic matter (Pignatello, 1999). When these aromatic functional groups are eventually oxidized by microbes, the higher solubility of carboxylate and other O-rich functional groups may allow for greater C transport. Carboxylate functional groups' ability to form mineral-associated organic matter through association with charged surfaces or cation bridging (Aquino et al., 2011) would also promote MAOM formation and increase C storage times (Cotrufo et al., 2013; Leinemann et al., 2018) though our results do not provide sufficient support to determine the stability of the increased C stocks at 60-100 cm. As the C cascade is triggered by fresh C inputs which are preferentially sorbed within the top 30 cm (Liebmann et al. 2020), the regular application of soluble C-rich compost and WCC residue, combined with increased hydraulic conductivity due to WCC roots can accelerate the process leading to greater subsoil C transport.

Cover crops are associated with elevated EOC levels and more aromatic functional groups in topsoil SOC (Ding et al. 2005, Zhou et al. 2012). Application of cover crops in CONV+WCC did not increase EOC content deeper than 15 cm compared to CONV (Figure 7a) but did have an impact on carboxylate functional group presence at the 60-cm depth (Figure 8b), possibly indicating that cover crop residues are associated with smaller inputs of soluble C that are not as easily detected as the larger soluble C inputs coming from the compost. Additionally, the trend of increasing aromatic:carboxylate ratio with depth in CONV+WCC systems (Table 1) indicates that CONV+WCC systems may be accumulating more aromatic C relative to carboxylate C in deeper soil layers. This accumulation of aromatic C in CONV+WCC subsoils may be due to cover crop root residue introducing lignin and cellulose directly into the subsoil, while the carboxylate-C obtained from the decomposition of surface residues can be potentially mineralized before being transported deeper as DOC (Chantigny, 2003; White et al., 2020a).

4.3 Compost + Cover crops increased nutrient availability and decreased microbial stress in subsoils

The higher P and S values noted in ORG subsoils (Figure 7c,d) can be attributed both to the higher organic P and S inputs associated with compost (Preusch et al., 2002), as well as the increased mobility of these inputs. Differences in crop uptake also play a potential role, but the lack of significant treatment effects at RR on crop nitrogen use efficiency (Kong et al. 2009), or P cycling (Maltais-Landry et al. 2014) in these plots make crop uptake less likely to be a significant factor in nutrient availability. Phosphorus-rich organic matter can be more mobile than mineral P (Laos et al., 2000; Sharpley and Moyer, 2000), and mineralization of organic S into more soluble sulfate could also facilitate its movement (Edwards, 1998). Though our results were not able to detect significant differences in mineral N in the measured subsoils, the higher amount of organic N added in compost was also likely mineralized into more soluble nitrate (Vinten et al., 1994) (Figure 7b). Although soil microbial communities are primarily water and C limited (Soong et al. 2020), the addition of N, P and S in ratios similar to that found in soil organic matter may increase transformation of C inputs into SOM by up to 52% by promoting microbial anabolism (Coonan et al. 2020).

Greater C and nutrient inputs were associated with the lower Gram +: Gram - ratios observed in subsoil ORG soils (Fig 6b, d). Higher values for these ratios, such as those observed in CONV plots, have been associated with nutrient and energy limitation (Bossio and Scow, 1998; Petersen and Klug, 1994). Increases in these ratios 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 C (Fanin et al. 2019, Fierer et al. 2003b, Bossio et al. 1998), while an increase in the saturated: unsaturated ratio and cy17:pre ratios has been associated with lower water potentials (−1.3 to −0.9 MPa) and potential dehydration (Moore-Kucera et al. 2007). The stress indicator trends in our data support our observations of increased soluble C and water content in ORG systems.

Adding compost and cover crop residue increases microbial biomass at the 0-15 and 15-60 cm but not 60-100 cm depth relative to CONV systems. This greater biomass increase in ORG than CONV plots was attributed to compost providing a favorable nutrient stoichiometry for biomass formation (Kirkby et al., 2011; Richardson et al., 2014). Increased microbial biomass in surface layers of soil is an important potential source of C and other nutrients to subsoil layers through cell lysis from predation and wet-dry cycles (Bonkowski, 2004; Xiang et al., 2008). It is associated with increased C storage through microbial necromass formation (Buchmann and Schaumann, 2018; Jilling et al., 2020), and we hypothesize that transport of microbial products downwards through the profile could have contributed to the SOC increase in the subsoil observed in ORG systems (Figure 2b).

4.4 Compost + Cover Crops increased profile C stocks after 25 years, but Cover Crops alone did not

We found evidence that the SOC increases under the ORG system after 25 years (Figure 2) were due to the increased mobility of compost-added C and nutrients combined with increased infiltration due to cover crop roots, as well as the larger amounts of C added to ORG plots. Larger SOC increases under yard waste compost + cover crops relative to cover crops alone have also been noted in other California long term experiments on a loamy sand soil (White et al. 2020a - rye, fava bean, pea, common vetch, purple vetch), indicating that C input from cover crops alone may not play a large role in increasing subsoil C. These experiments also note the importance of belowground carbon inputs on SOC stocks, a factor that was not included in this analysis. While cover crop biomass does represent significant C and N input to surface soils, the channels their roots create for mobile nutrients (either organic or mineral) to move downwards may be as important as their C and N inputs to subsoil SOC dynamics.

We noted significant seasonal variation in EOC, mineral N, P and S levels throughout the 2018-2019 growing season, though ORG plots consistently had higher EOC and P than CONV+WCC or CONV plots at all timepoints (Figures 6a,c) and higher S during the growing season. These soluble C and nutrient inputs peaked during the growing season likely due to the influence of compost application, root exudates and fertigation. Since the months of April-September are the driest months of the year at the study site, the large C and nutrient inputs during the growing season may have depended on irrigation water to be transported into subsoil layers, highlighting the importance of irrigation amounts and types (drip, furrow) to understanding changes in subsoil C stocks. The shifts in soluble C:N:P:S ratios during the course of the year may also indicate that C:nutrient stoichiometry is more suitable for microbial biomass growth in these row cropped plots during the growing season than it is during the winter rainy season.

In contrast to the ORG system, SOC stocks did not significantly increase in the CONV+WCC plots after 25 years. While our FTIR results suggest that cover crop residues have an impact on subsoil C by increasing the proportion of carboxylate-C relative to the CONV system (Figure 8b), they do not suggest a clear reason behind the lack of an increase in SOC stocks. A possible hypothesis is that small inputs of C and N over time from cover crop roots primed decomposition of native SOC, potentially by stimulating phosphatases and accelerating MAOM breakdown (Cui et al., 2020; Mise et al., 2020). Additionally, common root exudates such as oxalic acid may have dispersed organomineral complexes (Keiluweit et al., 2015), making that C more accessible for decomposition. While any priming of SOC due to cover crop root exudates would likely also be occurring in the ORG systems, we believe this was counteracted by the higher EOC inputs and more favorable nutrient stoichiometry for microbial biomass provided by the compost.

We also observed a continual decline in SOC in subsoil in the conventional with cover crops treatment (CONV + WCC) as observed in Tautges and Chiartas et al. (2019); however, the rate of decline was lower over the last 7 years than in the first 19 years of study. This slower decline in subsoil C stocks from 2012-2019 may be due to the switch from furrow to drip irrigation in 2014. Lower water inputs with drip reduces microbial activity and C and N cycling enzyme activities (e.g. beta-glucosidase and N-acetyl-glucosaminidase) in a large part of the bed in the surface of these same plots (Schmidt et al., 2018). The shift in irrigation and reduced water inputs potentially increased the prevalence of complex SOM by reducing microbial mineralization, and may have facilitated greater DOC transport during the winter rainy season.

5 Conclusion

The combination of growing cover crops and compost amendment created a unique set of conditions conducive to C transport and accumulation in the subsoils of a tilled row crop rotation. This was, in part, likely due to hydraulic conductivity facilitated by cover crop roots leading to higher rates of transport of soluble C and nutrients from the surface to subsoil. In turn, higher transport led to increased C stocks, reduced levels of microbial stress and higher available C, P and S values throughout the year in ORG systems. The accumulation of oxygen-rich carboxylate C in subsoil horizons under all treatments, attributed to an increase in microbially-processed C, provides support for the “cascade theory” of C transport. These results demonstrate the potential for subsoil C storage in tilled agricultural systems, and highlight a potential pathway for increasing C transport, storage, and sequestration in subsoil layers.

6 Appendices

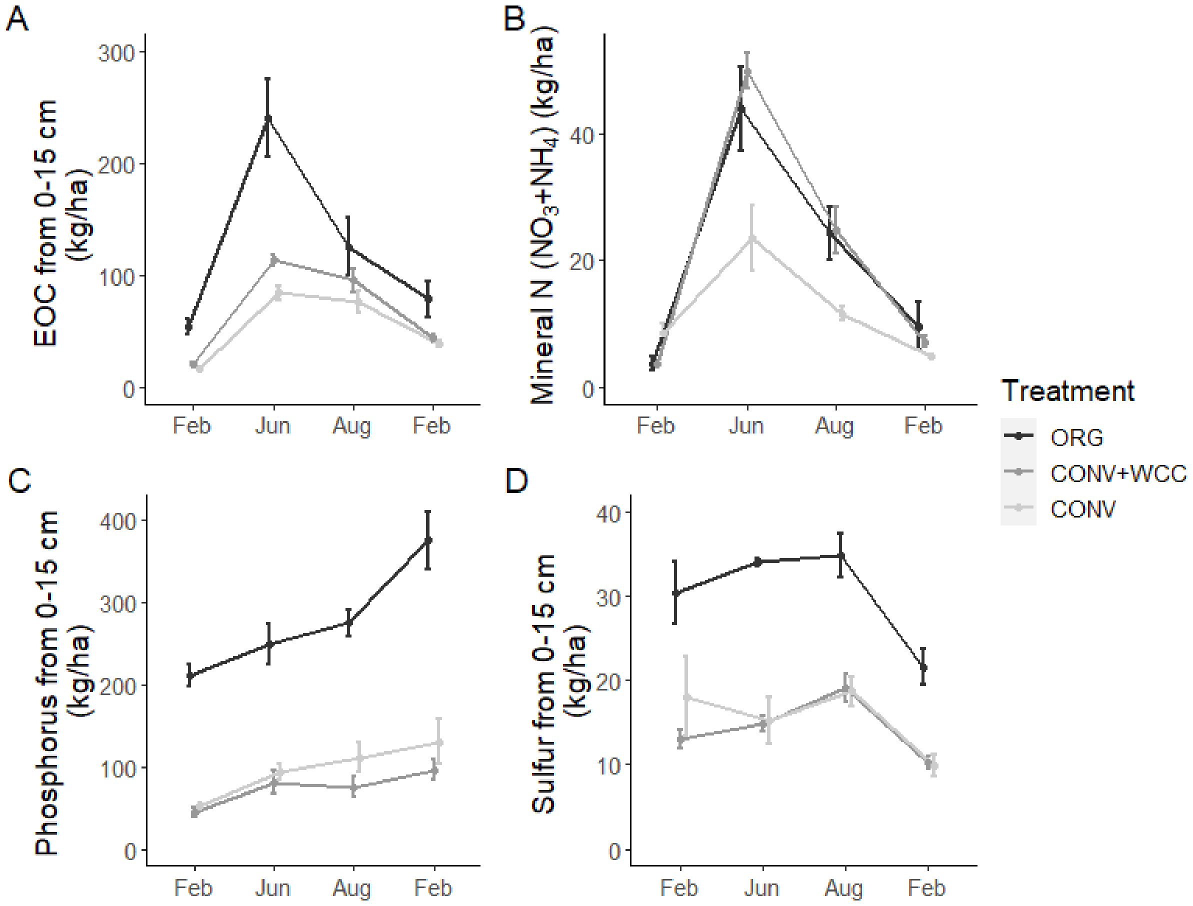

Figure 1.A1. Extractable organic C, mineral N, phosphorus, and sulfur stocks at 0-15 cm in ORG, CONV+WCC and CONV systems over the Feb 2018- Feb 2019 season. All values are given in kg/ha. Error bars represent standard error.

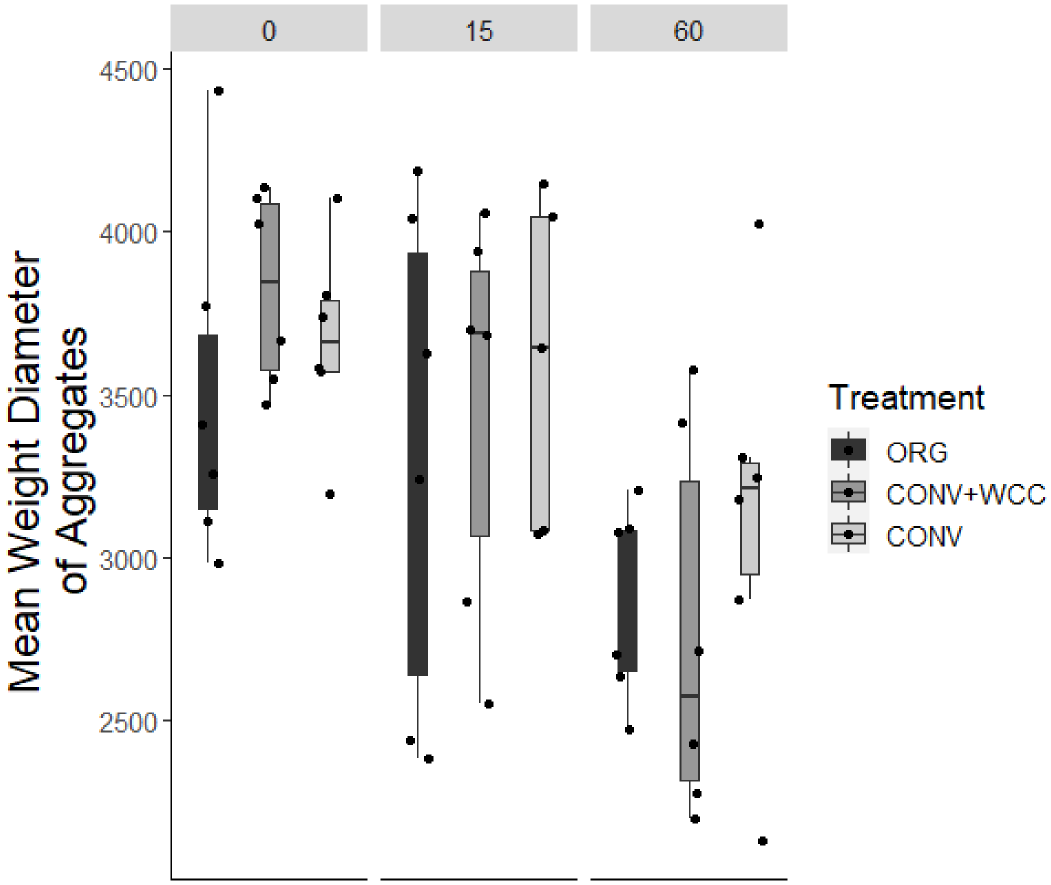

Figure 1.A2. Mean weight diameter of aggregates obtained by wet sieving for 0-15, 15-60 and 60-100 cm depth intervals in ORG, CONV+WCC and CONV systems.

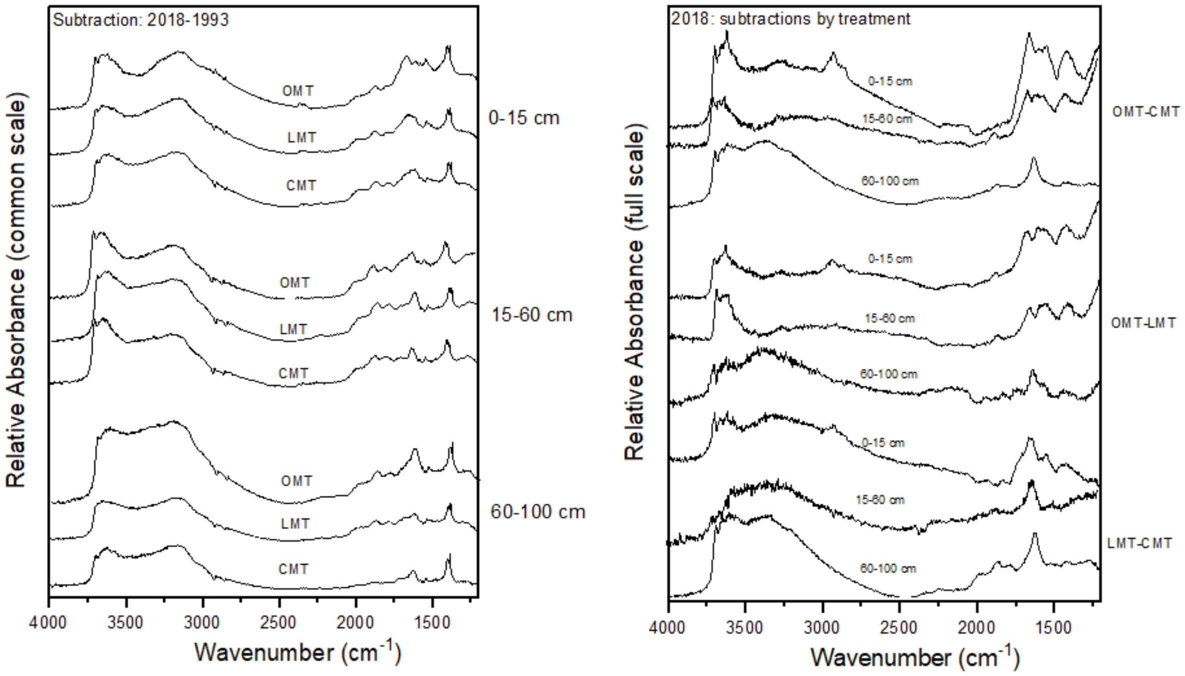

Figure 1.A3a,b. FTIR spectral subtractions for the 4000-1200 cm^{-1} range comparing (A) 2018 -1993 spectra for ORG, CONV+WCC and CONV, and (B) ORG, CONV+WCC and CONV spectra in 2018.

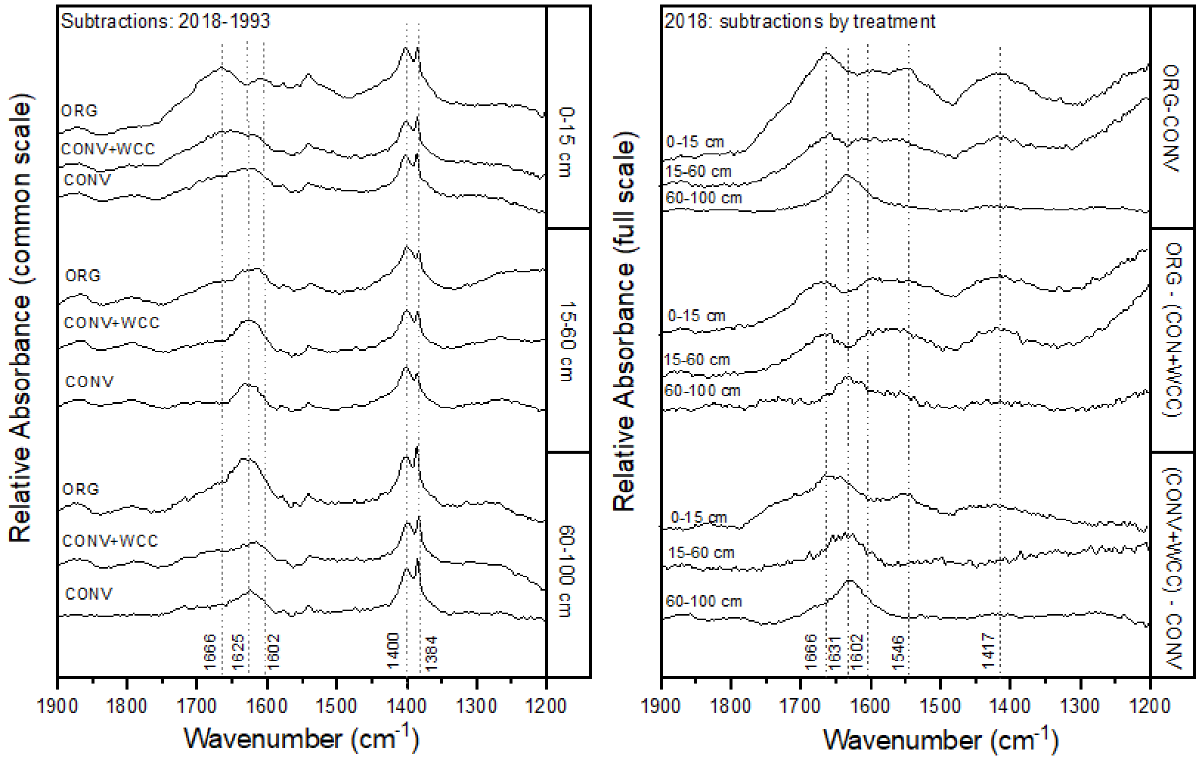

Figure 1.A4a,b. FTIR spectral subtractions for the 1900-1200 cm^{-1} range comparing (A) 2018 -1993 spectra for ORG, CONV+WCC and CONV, and (B) ORG, CONV+WCC and CONV spectra in 2018.

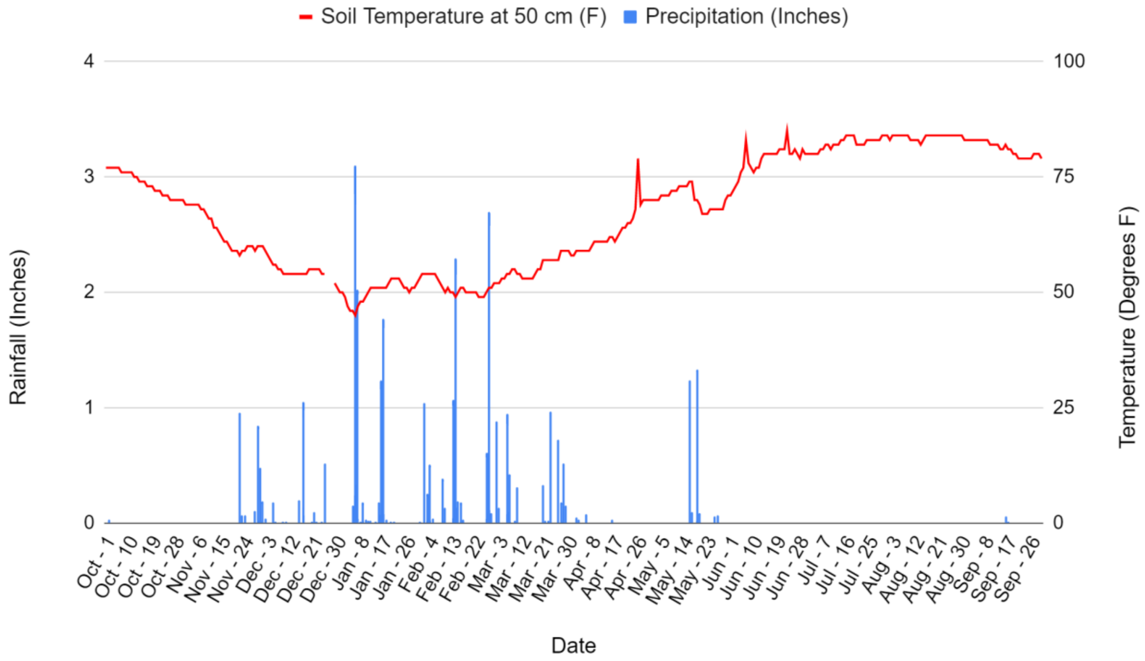

Figure 1.A5. Rainfall and soil temperature at 50 cm at Russell Ranch from Oct 2018 to Oct 2019. Data taken from http://atm.ucdavis.edu/weather/uc-davis-weather-climate-station

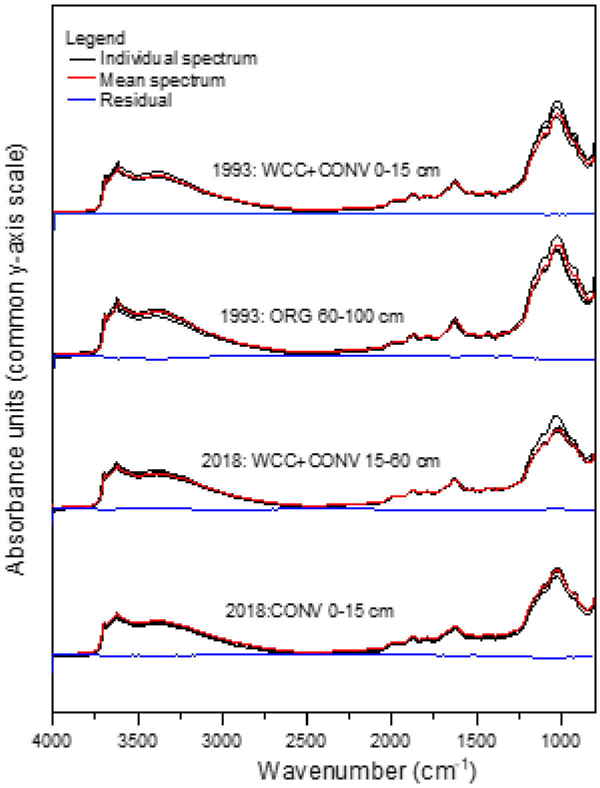

Figure 1.A6. Example DRIFT spectra showing three replicate spectra, the mean spectra and the residual spectra for 1993 and 2008 samples.

Table 1.A1 – PLFA (Phospholipid Fatty Acid) Assignments taken from Bossio and Scow (1998).

Gram Positive	15:1 iso w6c; 15:0 iso; 15:0 anteiso; 16:0 iso, 17:1 iso w10c, 17:1 iso w9c; 17:1 anteiso w9c; 17:0 iso, 17:0 anteiso; 18:0 iso
Gram Negative	16:1 w9c; 16:1 w7c; 17:1 w8c; 17:0 cyclo w7c; 18:1 w7c; 19:0 cyclo w7c; 20:1 w9c; 21:1 w3c
Saturated	12:0; 14:0; 15:0; 16:0; 17:0; 20:0
Monounsaturated	16:1 w5c; 16:1 w7c; 18:1 w9c; 18:1 w7c
Cyclopropyl Indicator	19:0 cyclo w7c / 18:1w7c

Table 2.A2 - FTIR Peak Assignments* used for analysis of spectra

Wavenumber (cm^{-1})	**IR Assignment**
2800-3100	aliphatic $v_s(CH_2)$, $v_{as}(CH_2)$, $v_s(CH_3)$, $v_{as}(CH_2)$
1700-1765	v(C=O)
1666	aromatic v(C=C)
1620-1631	$v_{as}(COO)$
1602	skeletal v(C=C)
1546	aromatic v(C=C)
1417	δ(C-H)
1400	$v_s(COO)$
1384	v(C-O) vibration aromatic and δ(C-H) vibrations in CH_3 and CH_2

*Assignments taken from

Baes, A.U., Bloom, P.R., 1989. Diffuse reflectance Fourier transform infrared (DRIFT) of humic and fulvic acids. Soil Sci. Soc. Am. J. 53, 695–700. doi:10.2136/sssaj1989.03615995005300030008x**;**

Hesse, M., Meier, H., & Zeeh, B. (2005). Spektroskopische Methoden in der Organischen Chemie. (In German.) Georg Thieme Verlag, Stuttgart. doi:10.1002/pauz.19960250417

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Orlov, D.S., 1986. Humus acids of soil. Rotterdam: Balkema. doi:10.1002/jpln.19871500116

Table 2.A3. Texture, pH, OM, and clay content for the Rincon and Yolo soil series found at Russell Ranch.

Soil Series	**Rincon**	**Yolo**
Soil Taxonomic Class	Fine, smectitic, thermic Mollic Haploxeralfs	Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents
Horizon Designation	AP	AP
Depth (cm)	0-10	0-5
Texture	SiCL	SiL
pH	6.5	6.7

Organic Matter Content (%)	2.4	2.4
Clay Content (%)	31	30
Horizon Designation	A12	AP2
Depth (cm)	10-41	5-20
Texture	SiCL	SiL
pH	6.5	7.1
Organic Matter Content (%)	2.4	2.4
Clay Content (%)	31	30
Horizon Designation	B21t	A1
Depth (cm)	41-64	20-48
Texture	SC	SiL
pH	7	7.2
Organic Matter Content (%)	0.75	1.8
Clay Content (%)	40	30
Horizon Designation	B22t	A2
Depth (cm)	64-79	48-66
Texture	SC	SiL
pH	7.9	7.3
Organic Matter Content (%)	0.75	1.3
Clay Content (%)	40	30
Horizon Designation	B3tca	C1
Depth (cm)	79-102	66-84
Texture	SCL	SiL
pH	8	7.4
Organic Matter Content (%)	0.75	1
Clay Content (%)	40	28

Table 2.A4. Average annual inputs of Carbon, Nitrogen, Phosphorus and Sulfur to ORG, CONV+WCC and CONV plots between 1993-2018. Please note that Sudangrass and Wheat C inputs were excluded from this table, as they were only grown for a limited amount of time.

Input (Mg/Ha/yr)	**CONV**	**CONV+WCC**	**ORG**
Tomato Residue	1.73	1.57	1.54
Corn Residue	2.43	1.96	2.25
Compost	0	0	1.9
WCC Residue	0	1.086	1.48
WCC Nitrogen	0	0.09	0.11
Compost Nitrogen	0	0	0.19
Mineral N	0.18	0.13	0
Mineral Phosphorus	0.04	0.04	0
Organic Phosphorus	0	0	0.13
Mineral Sulfur	0.08	0.09	0
Organic Sulfur	0	0	0.05

Table 1.A5. Management summary for ORG, CONV+WCC and CONV plots for the 2018-2019 year.

Practices - 2018-2019	**CONV**	**CONV+WCC**	**ORG**
Compost Application	NA	NA	Apr 2018 and Oct 2019
Corn Harvest	Sept 10th	Sept 10th	Sept 10th
Tomato Harvest	Aug 2nd	Aug 2nd	Aug 2nd
Total Amount of Irrigation (mm/hectare)	245.13	239.43	538.27
Number of tractor passes/yr (7.5cm deep)	4	13	16
Number of tractor passes/yr (20.5cm deep)	4	4	4

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Chapter 2: Changes in microbial functional potential but not overall community structure after 20 years of practices intended to improve soil health

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Abstract

Increasing the potential for agricultural soils to cycle nutrients, store carbon and form soil structure under the purview of soil health can help us adapt to the impacts of climate change. However, it is not clear how soil functional potential changes under different types of land management, and how changes in functional potential are related to changes in soil health measurements. We compared how soil microbial (bacterial + archaeal) communities in a California Central Valley soil have changed after 2 decades of management under different types of C and nutrient input: CONV (mineral fertilizer), CONV+WCC (mineral fertilizer + cover crops) and ORG (composted poultry manure + cover crops). We found that the overall taxonomic and functional composition of microbial communities did not change significantly after 2 decades, but that there was significant changes in specific and important microbial functional potential (C transformation, N transformation) that were related to changes in soil health measurements. Our results show that practices intended to improve soil health may not

be associated with significant microbial community changes but may increase the potential for specific soil functions such as C transformation to occur.

1 Introduction

We are increasingly reliant on soils to help us in mitigating and adapting to both the impacts of climate change and the legacy of our own agricultural practices (Sanderman et al., 2017). Our management of soils, frequently focused on improving yield (Ortiz-Bobea et al., 2021), has expanded its focus to include multiple microbially-driven functions such as nutrient cycling (Soong et al., 2020), soil structure formation (Rabot et al., 2018), pollutant breakdown (Alkorta et al., 2017), and carbon (C) storage (Lal, 2004). Together, these functions are integral to the concept of soil health (Lehmann et al., 2020), both when assessing the impacts of agricultural management and developing and testing potential soil health indicators.

Improving soil health through land management is strongly coupled with promoting beneficial microbial processes (Bünemann et al., 2018; Doran and Zeiss, 2000). Recommended management practices for improving soil health often include increasing inputs of microbial energy sources such as organic C in the form of compost or cover crop residues, or reducing disturbance of microbial habitats by reducing tillage (Mitchell et al., 2017; Schmidt et al., 2018; Wick et al., 2017). Many soil health indicators also reflect impacts of microbial processes in some way, even if they are categorized as physical or chemical measurements (Stott, 2019). Examples include soil aggregation, which is driven by microbial exudation of extracellular polymeric substances (Costa et al., 2018), or potentially mineralizable C, which is associated with microbial enzyme activity (Zhang et al., 2021). However, it is difficult to find clear, direct associations between these soil health indicators and changes in microbial community composition because of the complex interactions between microbes, soil depth, soil type, climate and management (Chen et al., 2020; Manoharan et al., 2017; Yang et al., 2020). This is compounded by the lack of data on subsoil (>60cm) microbial communities, as most investigations of soil health and soil microbial communities limit themselves to the top 0-30 cm of soil, overlooking the significant potential impact of subsoils on carbon storage (Chenu et al., 2019). In order to manage for healthier soils that are more sustainable, productive and resilient, we need to better understand how soil microbial communities and soil health indicators are connected in their response to land management.

Soil DNA analyses provide powerful and diverse tools for analyzing soil microbial communities. DNA-based methods such as shotgun metagenomics and 16S rRNA analysis have already demonstrated the potential to predict soil physicochemical variables (Hermans et al., 2020). The decreasing price of DNA analysis, increase in availability of computational tools for analyzing the resulting data (Shaffer et al., 2020) and the sensitivity of soil microbial community responses (Carini et al., 2020) have only served to make these analyses more attractive to researchers. These analyses do have limitations, including the influence of relic DNA (Carini et al., 2016), and the gap between the functional potential measured by DNA and expressed phenotype (Jansson and Hofmockel, 2018). Despite these potential limitations, soil DNA analyses have the potential to provide a detailed description of how the microbial community of a particular soil has changed under land management, especially when variables of depth, climate and soil type are controlled for.

The Century Experiment at the University of California, Davis, provides one such unique opportunity - a 20+ year experiment established in 1993 to investigate the long-term effects of wheat- and maize-based crop rotations on productivity, profitability, resource use efficiency, soil conditions, and ecosystem services in a Mediterranean climate (Tautges et al., 2019; Wolf et al., 2018). The Century Experiment's location in one of the most productive agricultural regions in the world (California Department of Food and Agriculture, 2021), its ability to control for the impacts of climate and soil type, and its long term nature make it ideal for isolating the impact of management on a variety of soil health indicators. Three of the most well-documented management systems include maize-tomato rotations: 1. conventional (mineral fertilizer & standard tillage) control (CONV), 2. conventional with addition of a winter cover crop (CONV+WCC), and 3. certified organic (no mineral fertilizer, no pesticides & standard tillage) with compost and a winter cover crop (ORG).

There is a substantial body of research from the Century Experiment contrasting how decades of different management practices have impacted soil health indicators. ORG soils have shown higher total C stocks, available C, organic N, phosphorus, sulfur, and microbial biomass relative to CONV or CONV+WCC systems to a depth of 1m (Kong et al., 2011; Rath et al., 2022; Tautges et al., 2019). ORG soils also have higher rates of C and N mineralization (Lazicki and Geisseler, 2021), more permanganate oxidizable C (POXC) (Hurisso et al., 2016; Wade et al., 2016), higher aggregate stability (Kong et al., 2009, 2005; Lazicki et al., 2021), more potential for nitrification, and less potential for denitrification (Kong et al., 2010) than CONV systems. CONV+WCC and CONV systems have higher amounts of mineral N, and release larger amounts of N_2O during the growing season compared to ORG systems (Kallenbach et al., 2010; Kong et al., 2009). ORG systems also showed greater tomato yield, stability and yield resilience, but lower corn yield, stability and yield resilience compared to CONV+WCC and CONV systems (Li et al., 2020). It is clear that over 20 years of cover crop and compost amendment in the ORG system have produced a soil with improved soil health and fertility metrics compared to the control (CONV), but whether this improvement is also associated with significant changes to the soil microbial community is unknown.

We used this unique opportunity at the Century Experiment to determine whether the observed improvement in soil health indicators under intensively managed, tilled cover crop and cover crop+compost application was associated with changes in the taxonomic and functional potential of the soil bacterial + archaeal community (hereafter referred to as the soil microbial community) relative to the control. Soils were analyzed using shotgun metagenome sequencing (MGS), and applied a trait-based approach for new insights into the links between bacterial and archaeal community composition and changes in soil conditions (Malik et al., 2019). We hypothesized that ORG and CONV+WCC systems would support higher potential for C degradation, carbohydrate-active enzymes (CAZymes) and organic nitrogen cycling relative to CONV systems both at the soil surface and in the subsoil (>60cm). We also hypothesized that increased inputs of mineral nitrogen in CONV+WCC and CONV systems would result in increased potential for nitrification and denitrification relative to ORG systems.

2 Methods

2.1 Field Site

Soils in this study were taken from the Century Experiment at the Russell Ranch Sustainable Agricultural Facility in Davis, CA (38° 32' 48.79" N, -121° 52' 16.89" W). Details on this experimental system are further outlined in Tautges and Chiartas et al. (2019), Rath et al. (2022), and Wolf et al. (2018). Briefly, all plots are in a two-year maize-tomato rotation, with three replicate plots of each crop in any given year. Systems included in this study are: (a) CONV (synthetic fertilizer, pesticides, and winter fallow), (b) ORG (certified organic maize–tomato with composted poultry manure and WCC), and (c) CONV+WCC (synthetic fertilizer, pesticides, and WCC). This combination of practices was chosen based on a suite of practices employed by farmers in the California Central Valley. A summary of nutrient, water, and tillage inputs to each system is presented in Table 1. The two soil types present were (a) Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent) and (b) Rincon silty clay loam (fine, smectitic, thermic Mollic Haploxeralf). Abbreviations used (CONV, CONV+WCC, ORG) correspond to the abbreviations used in Wolf et al. 2018 (CMT, LMT, OMT), and are identical to those used in Tautges & Chiartas et al. (2019) and Rath et al. (2022) for ease of comparison.

2.2. Sampling Method

Soil samples for metagenome sequencing were taken at the 20-year post-harvest sampling in September 2012 (Tautges and Chiartas et al. 2019). Soil cores to a depth of one meter were sampled from five CONV plots, five CONV+WCC plots and six ORG plots using a Geoprobe. Each core was collected within an individual plastic lined tube, split into four depth intervals (0-15 cm, 15-30 cm, 30-60 cm, 60-100 cm) in the field, placed into plastic bags, and placed on ice. Aliquots were extracted and frozen at -80 ℃ within 24 hrs.

Sampling for carbon use efficiency (CUE) measurement took place in the 2018-2019 growing season, 26 years after establishment. Plots were sampled at 3 timepoints: March 2018 (Pre-CC Incorporation), September 2018 (Post-Harvest), and February 2019 (Pre-CC Incorporation). All sampling took place in the raised beds between furrows using a tractor-mounted Giddings probe with a diameter of 3 cm from the same plots sampled for metagenome sequencing. Three replicate cores were taken per plot, sectioned into 0-15, 15-60 and 60-100 cm depths, composited, and then subsampled. Aliquots of each soil were transported on ice to the Lawrence Berkeley National Lab and stored at 4 ℃ before analysis.

2.3 DNA Extraction and Sequencing

Wet soils were weighed out into 2ml tubes and stored at -80C until analysis. DNA extraction was performed in 2017 with the MoBio PowerSoil kit using 0.25 mg of soil following manufacturer's instructions and eluted in 50 µl of TE buffer. Genomic DNA was quantified using Qubit dsDNA High Sensitivity assay and DNA quality was assessed using agarose gel electrophoresis. Library preparation and sequencing were performed at the California Institute for Quantitative Biosciences' (QB3) Genomics Sequencing Laboratory. Libraries were prepared with target insert sizes of 400-600 bp and sequenced using 150 bp paired-end reads on an Illumina NovaSeq 6000 System with a read depth of ~12 Gbp per sample.

2.4 Carbon Use Efficiency Measurement

Carbon use efficiency was estimated using a substrate-independent method based on measuring ^{18}O incorporation from labelled water into microbial DNA (Spohn et al. 2016). Soil water content was determined gravimetrically prior to incubation. Dilutions of ^{18}O–enriched water (97 atom%) were added to 500 mg subsamples to reach an enrichment of 20 atom% ^{18}O at 80% water holding capacity in the final soil water. Paired samples were prepared using equivalent volumes of unlabelled water as controls for background heavy oxygen incorporation. Samples were then sealed in gas-tight vials and incubated at room temperature for 48 h. The CO_2 produced during incubation was measured using a gas chromatograph

(Shimadzu GC-2014). DNA was extracted from labelled and unlabelled samples using the FastDNA SPIN Kit for Soil (MP Biomedicals) and quantified using PicoGreen (Invitrogen). DNA extracts were dried and measured for at% ^{18}O and total oxygen using TC/EA-IRMS (Delta V Advantage, Thermo Fisher, Germany). The amount of DNA produced during the incubation was calculated for each sample based on the abundance ^{18}O in the labelled DNA, unlabelled DNA, and in the soil water of the labelled sample. CUE and microbial biomass turnover times were calculated as described in Spohn et al. (2016) and calibrated to carbon values using microbial biomass carbon measurements obtained by chloroform fumigation. Two samples showed negative ^{18}O-atom% excess resulting in negative growth values and were therefore excluded from the analysis.

2.5 Bioinformatic Analyses

Soil samples were analyzed using shotgun metagenome sequencing (MGS). This method simultaneously describes microbial functional potential and taxonomic composition, while reducing the potential issues of primer bias (Brooks et al., 2015). We would like to highlight that functional and taxonomic diversity of fungal communities was not included in these analyses.

Community diversity analysis. Microbial community diversity was assessed using single-copy ribosomal protein gene rpS3 as in (Diamond et al., 2019). Open reading frames (ORFs) were predicted from contigs longer than 1Kb using Prodigal V2.6.3 (Hyatt et al., 2010) in metagenome mode with the parameters “-m -p meta”. rpS3 marker sequences were identified across all metagenome assemblies using a hidden markov model (HMM) based on the alignment of rpS3 (rpsC) sequences from the tree of life data set from Hug et al. (Hug et al., 2016). Scanning the translated ORFs with the rpS3 model resulted in 8199 sequences. Sequences shorter than 100 amino acids in length were removed that resulted in 5220 sequences. Sequences were clustered at 99% identity using USEARCH [10.1093/bioinformatics/btq461] with the following options: “-cluster_fast -sort length -id 0.99 -

maxrejects 0 -maxaccepts 0” to define species groups (SG). For each species group, the longest contig for the corresponding cluster was identified. Reads from each sample was mapped to these contigs using Bowtie2 in sensitive mode (--sensitive). The resulting bam files were used to generate a read count table using coverm contig command (version 0.5.0) [https://github.com/wwood/CoverM] with the following options: “--proper-pairs-only --min-read-percent-identity 0.99 --min-read-aligned-percent 0.9 --methods count”. Normalized per base pair coverage for each contig was calculated with the following formula: (reads mapped from sample/ (contig length x reads sequenced in sample)).

Metagenomic assembly. Paired reads from each sample were assembled separately (64 assemblies) with JGI’s metagenome assembly pipeline (Clum et al., 2021) which includes the following steps. Reads went through quality control removing reads matching a standard set of contaminants, trimming adapters and linkers using BBTools RQCFilter (Bushnell et al., 2017) . Quality controlled reads are corrected for sequencing errors with BFC (version r181) with parameters “-1 -k 21 -t 10”. Any orphan reads from the paired end library after quality filtering and error correction were removed with seqtk’s dropse command [https://github.com/lh3/**seqtk**]. The resulting paired reads are assembled with SPAdes (version 3.12.0) (Bankevich et al., 2012) in metagenome assembly mode (--meta) with the following options: “—only-assembler -k 33,55,77,99,127 -t 32 -m 2000).

Genome binning and dereplication. For each assembly, contigs were filtered by length to remove sequences shorter than 1Kb using seqkit (Shen et al., 2016). Length-filtered contigs were binned into metagenome assembled genomes (MAGs) as follows. For each sampling depth, quality-controlled error-corrected reads from all the samples from that depth (16 samples per depth) were mapped to the length-filtered contigs using Bowtie 2 in sensitive mode (--sensitive). The differential coverage profiles for the contigs across 16 samples were used as input to the following differential coverage binners: CONCOCT (Alneberg et al., 2014), MaxBin2 (Wu et al., 2016), MetaBAT (Kang et al., 2019). Bins from the three binners were consolidated to select the highest quality bins using DasTool (Sieber et al., 2018). Bins obtained from 64 samples were dereplicated with dRep (Olm et al., 2017). DasTool consolidated bins from all 64 assemblies were pooled together and inputted to dRep (dereplicate command) with the following parameters: "--P_ani 0.9 --S_ani 0.99 --S_algorithm ANImf --completeness 50 --contamination 25." DasTool uses CheckM (Parks et al., 2015) to assess bin completeness and contamination.

2.6 Statistical Analyses

All data analysis and graph production were done using R v. 4.0.2, (R Core Team, 2020) using the tidyverse package (Wickham et al., 2019). NMDS ordination and UniFrac calculations were done using Phyloseq v1.38.0, while PCA ordination of trait coverage was done using the prcomp function from the R/stats package. Analysis of microbial taxa coverage, carbon use efficiency and microbial biomass turnover data was conducted using a linear model to account for the effects of management system, depth and time point. Statistical differences between management systems were analyzed separately for each depth using paired t-tests with Bonferroni correction for multiple tests at 5% significance level.

Assignment of sequence data to microbial fitness traits was done using the R/microtrait package (https://github.com/ukaraoz/microtrait). The microtrait workflow uses hidden markov models (HMM) and logical operations to predict and map proteins predicted in genome sequence data to a hierarchy of fitness traits. Comparison of trait coverage between systems output from microtrait was done via the Mann-Whitney test using the wilcox.test function from the R/stats package on weighted % coverage values. Trait comparisons were then filtered using p=0.01 as the criteria. Heatmap colors represent the median of the difference between a sample from system 1 and a sample from system 2, and are labelled using the format "System 1 - System 2".

3 Results

3.1 Cumulative Inputs from 1993-2012

ORG plots received an estimated 135.14 Mg/ha of C, 6.15 Mg/ha of N, 2.62 Mg/ha of Phosphorus, 0.94 Mg/ha of sulfur, and 3.69 Mg/ha of potassium from 1993-2012 in the form of compost and tomato, corn, and cover crop residues. CONV and CONV+WCC plots received similar amounts of C (73.2 Mg/ha and 78.1 Mg/ha) during the same time period, approximately 55% of the C inputs to ORG plots. CONV and CONV+WCC plots received 3.6 Mg/ha and 4.4 Mg/ha of N respectively (59% and 71% of ORG inputs), 0.77 and 0.86 Mg/ha of P (30% and 32% of ORG inputs), 1.61 and 1.86 Mg/ha of S (170% and 197% of ORG inputs) and 1.61 and 1.86 Mg/ha of K (44% and 50% of ORG inputs) respectively.

Table 2.1. Cumulative inputs to each system from 1993-2012. WCC - Winter Cover Crop. CONV = synthetic fertilizer, pesticides, and winter fallow, ORG = certified organic with composted poultry manure and WCC, CONV+WCC = synthetic fertilizer, pesticides, and WCC, CONV = synthetic fertilizer, pesticides.

Input (Mg/Ha Cumulative to 2012)	**CONV**	**CONV+WCC**	**ORG**
Tomato Residue Carbon	26.63	22.53	22.94
Corn Residue Carbon	45.5	33.18	41.11
Compost Carbon	0	0	39.12
Winter Cover Crop (WCC) Residue Carbon	0	22.39	31.97
Winter Cover Crop (WCC) Nitrogen	0	1.76	2.31
Compost Nitrogen	0	0	3.84
Mineral N	3.61	2.64	0
Mineral Phosphorus	0.77	0.86	0
Compost Phosphorus	0	0	2.62
Mineral Sulfur	1.61	1.86	0
Compost Sulfur	0	0	0.94
Compost Potassium	0	0	3.69
Fertilizer Potassium	1.61	1.86	0

3.2 Relative Abundance of Microbial Taxa

Taxonomic assignment of our sequence data showed the presence of 22 phyla. The most abundant phylum was Actinobacteria, which dominated up to 60% of the relative coverage over all samples, followed by Chloroflexi, Thaumarcheota, Acidobacteria and Betaprotebacteria (Figure 1). Actinobacterial coverage remained above 50% of the total coverage for all treatments and depths.

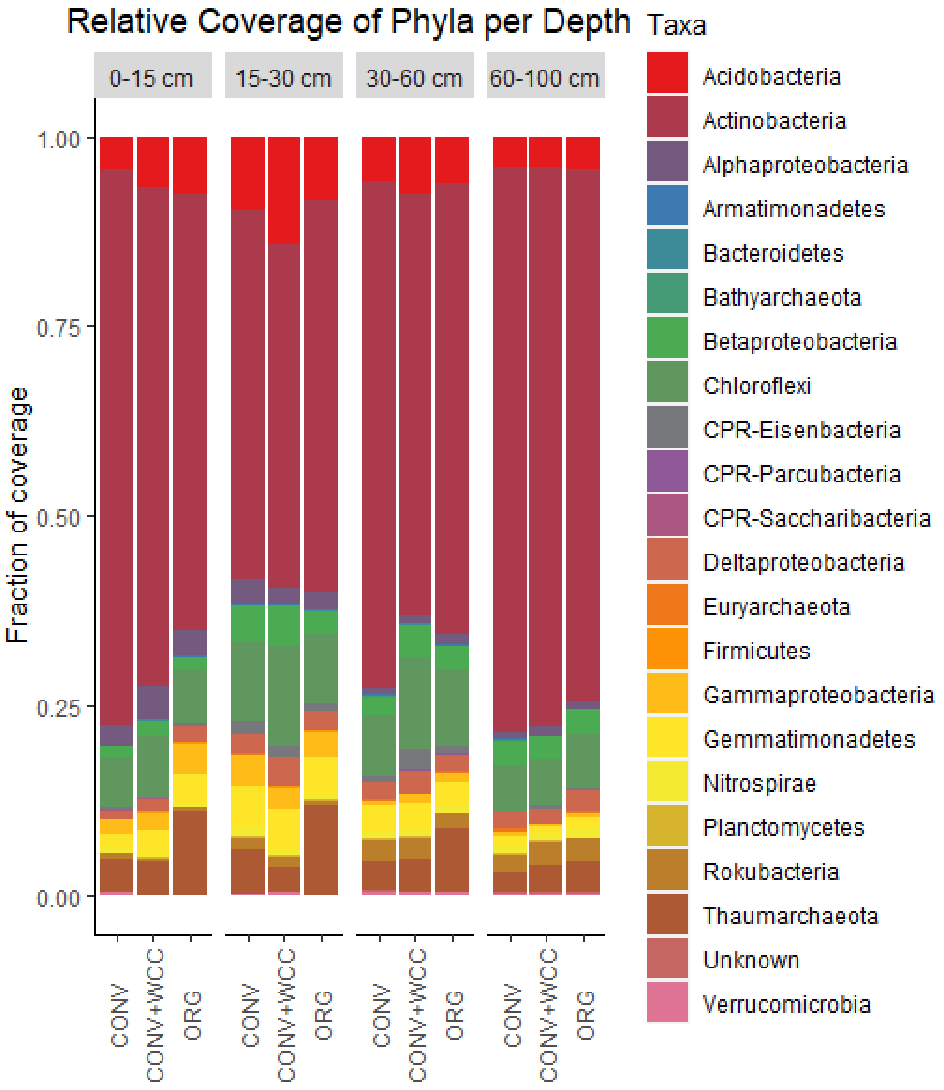

Figure 2.1. Relative abundances of microbial phyla across sampling sites and depths. ORG = certified organic with composted poultry manure and WCC, CONV+WCC = synthetic fertilizer, pesticides, and WCC, CONV = synthetic fertilizer, pesticides.

3.3 Bacterial and Archaeal Taxonomic and Functional Community Ordination

Taxonomic ordination showed that depth ($p=1x10^{-4}$) had a significant effect on sample clustering. Depth accounted for nearly 20% of the variation in community structure (Figure 2). Treatment did not have a significant effect on taxonomic ordination when comparing the entire profile from 0-100cm or when each depth was considered separately (Table A1).

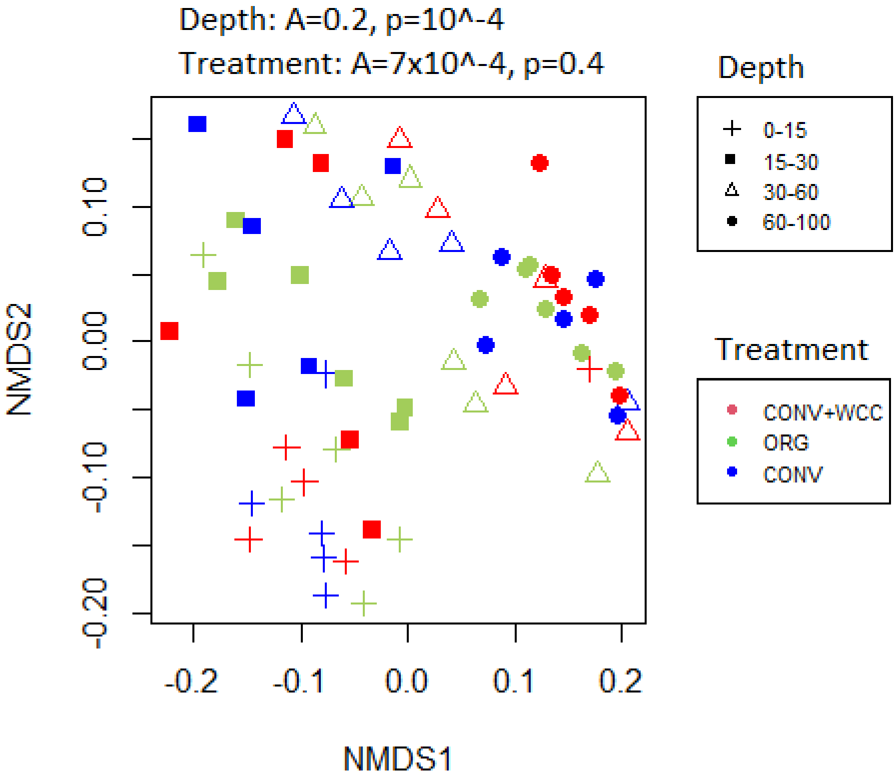

Figure 2.2. NMDS ordination of bacterial and archaeal taxonomy from 0-100 cm using weighted Unifrac distances. Points are color coded to represent treatments, while shapes represent depths. Significance testing and proportion of distances explained by group identity (A) were calculated using the mrpp (Multi Response Permutation Procedure) method in R

PCA ordination of functional trait coverage (proportions) obtained using the R/microtrait package showed a similar trend: a significant effect of depth ($p<0.01$) on sample clustering, but no significant effect of treatment ($p>0.1$). No effect of treatment on functional clustering was observed when all samples from 0-100 cm were combined, or when each depth was considered separately ($p>0.1$ for all depths).

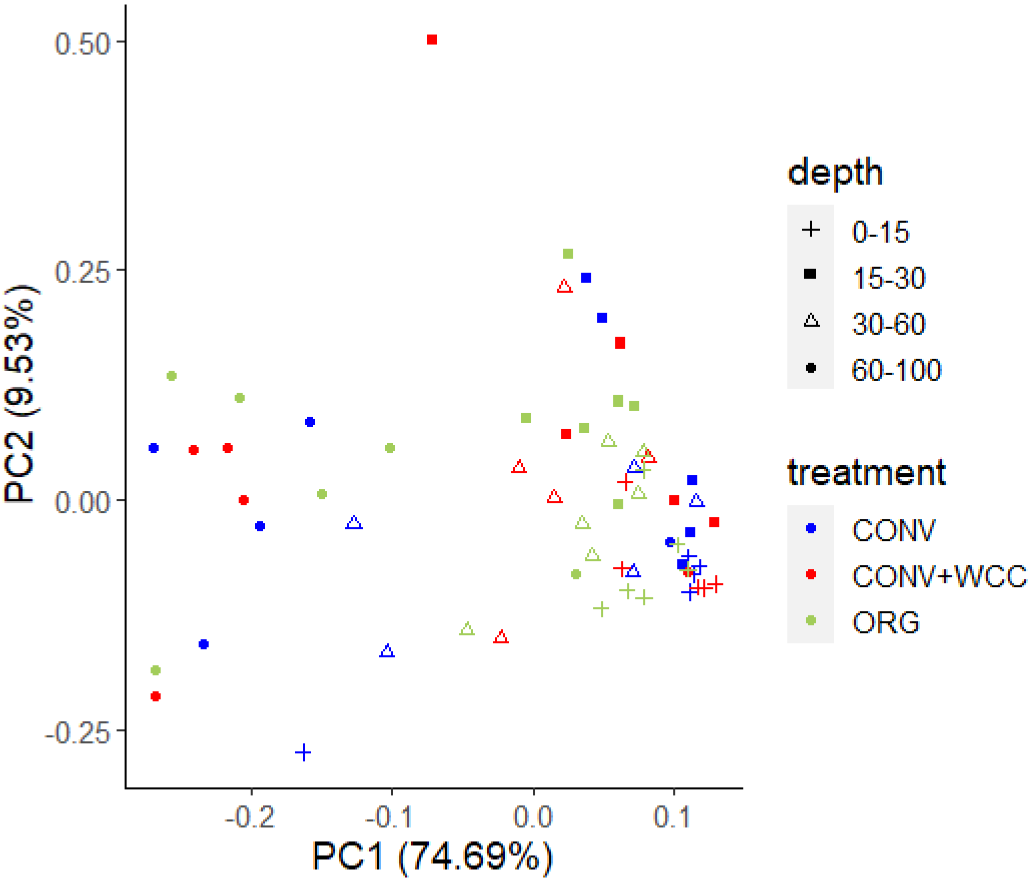

Figure 2.3. PCA ordination of microbial functional trait coverage. Points are color coded to represent treatment, while shapes represent depth. Significance testing was done using Monte Carlo permutation in R.

3.4 Comparison of Functional Trait Abundance among Systems

When the relative abundance of functional traits was compared among systems, an overall trend of significantly higher trait abundances associated with carbon, nitrogen, phosphorus and sulfur cycling were observed in ORG and CONV+WCC systems relative to CONV systems (Figures 4a-c). ORG and CONV+WCC systems had higher relative abundance of traits coding for substrate degradation, extracellular enzymes and polysaccharide production associated with biofilms (Figure 5a), as well as traits coding for organophosphate transport, nitrite oxidation and thiosulfate, arsenate and iron transformations. ORG systems also had higher relative abundance of ammonia assimilation and assimilatory sulfate reduction compared to CONV systems (Figures 5b,c). In contrast, CONV+WCC and CONV systems had significantly higher relative abundance of ammonia oxidation to nitrite traits relative to ORG plots, but only at the surface (Figure 5b).

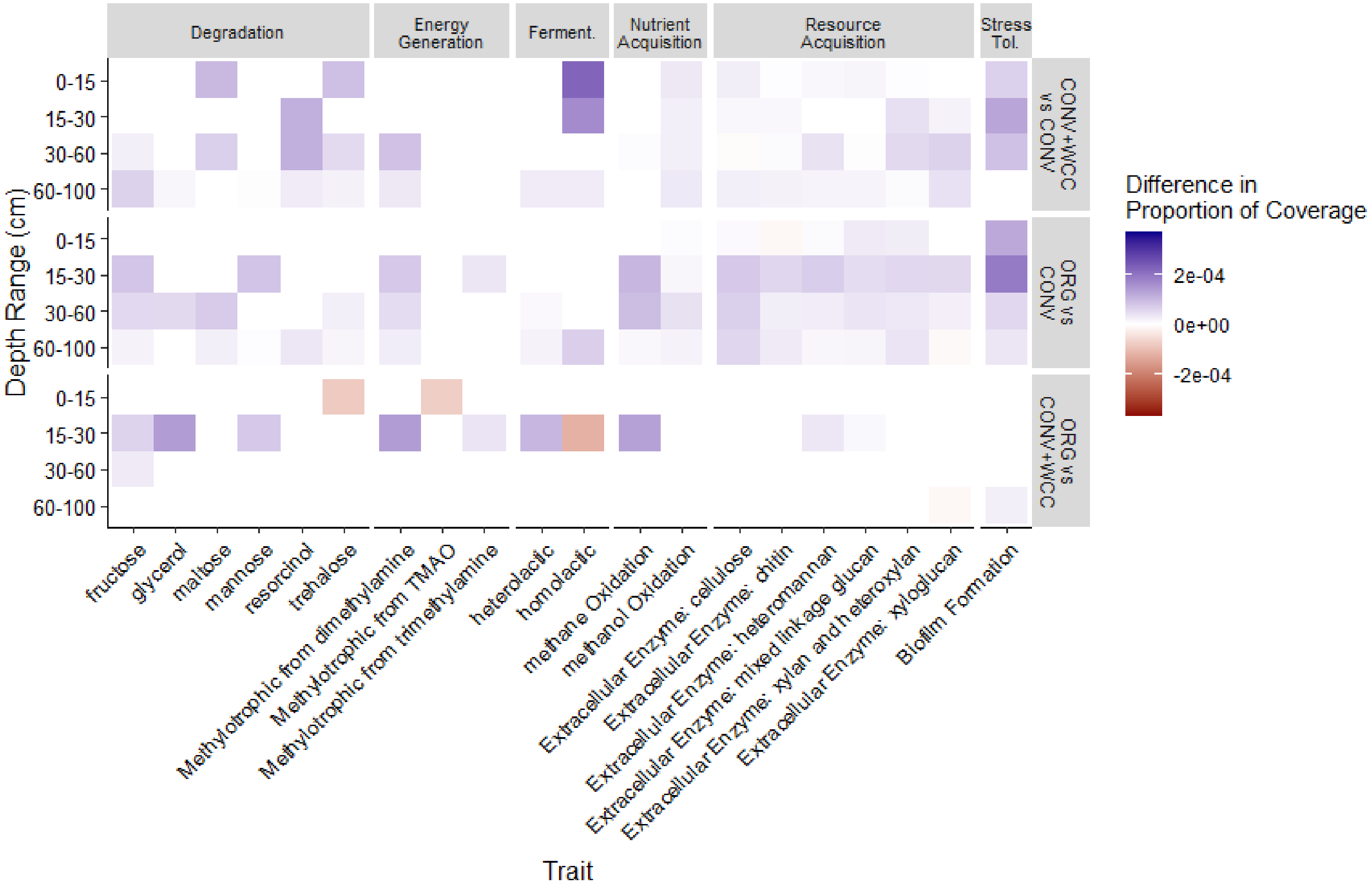

Figure 2.4 a. Selected bacterial and archaeal traits associated with carbon cycling. Colored boxes indicate a significant difference in trait abundance at p<=0.05, while a white box indicates no sig. difference. Intensity of the color shading represents the size of the median difference between the two systems compared. Eg. A blue box in the CONV+WCC vs CONV section indicates higher trait abundance in the CONV+WCC system (p<=0.05) when compared to the CONV system. Significance testing and median estimation was done using the Mann-Whitney test. ORG = compost, cover crops, organic certified. CONV+WCC = cover crops, mineral fertilizer. CONV = mineral fertilizer.

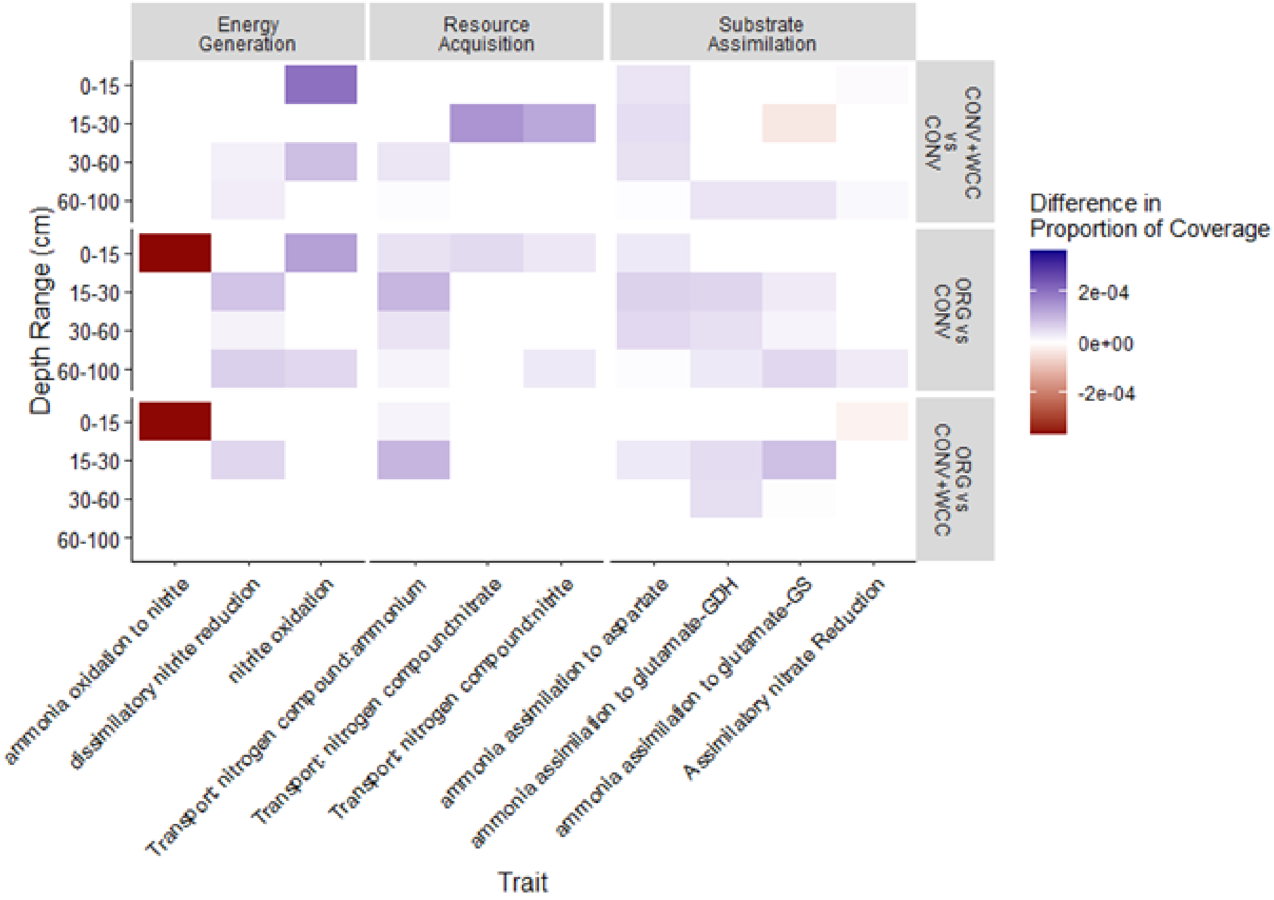

Figure 2.4 b. Selected microbial traits associated with nitrogen cycling. Colored boxes indicate a significant difference in proportion of trait coverage at p=0.05, while a white box indicates no sig. difference. Intensity of the color shading represents the size of the median difference between the two systems compared. Eg. A blue box in the CONV+WCC vs CONV indicates higher trait abundance in the CONV+WCC system when compared to the CONV system. Significance testing and median estimation was done using the Mann-Whitney test. ORG = compost, cover crops, organic certified. CONV+WCC = cover crops, mineral fertilizer. CONV = mineral fertilizer.

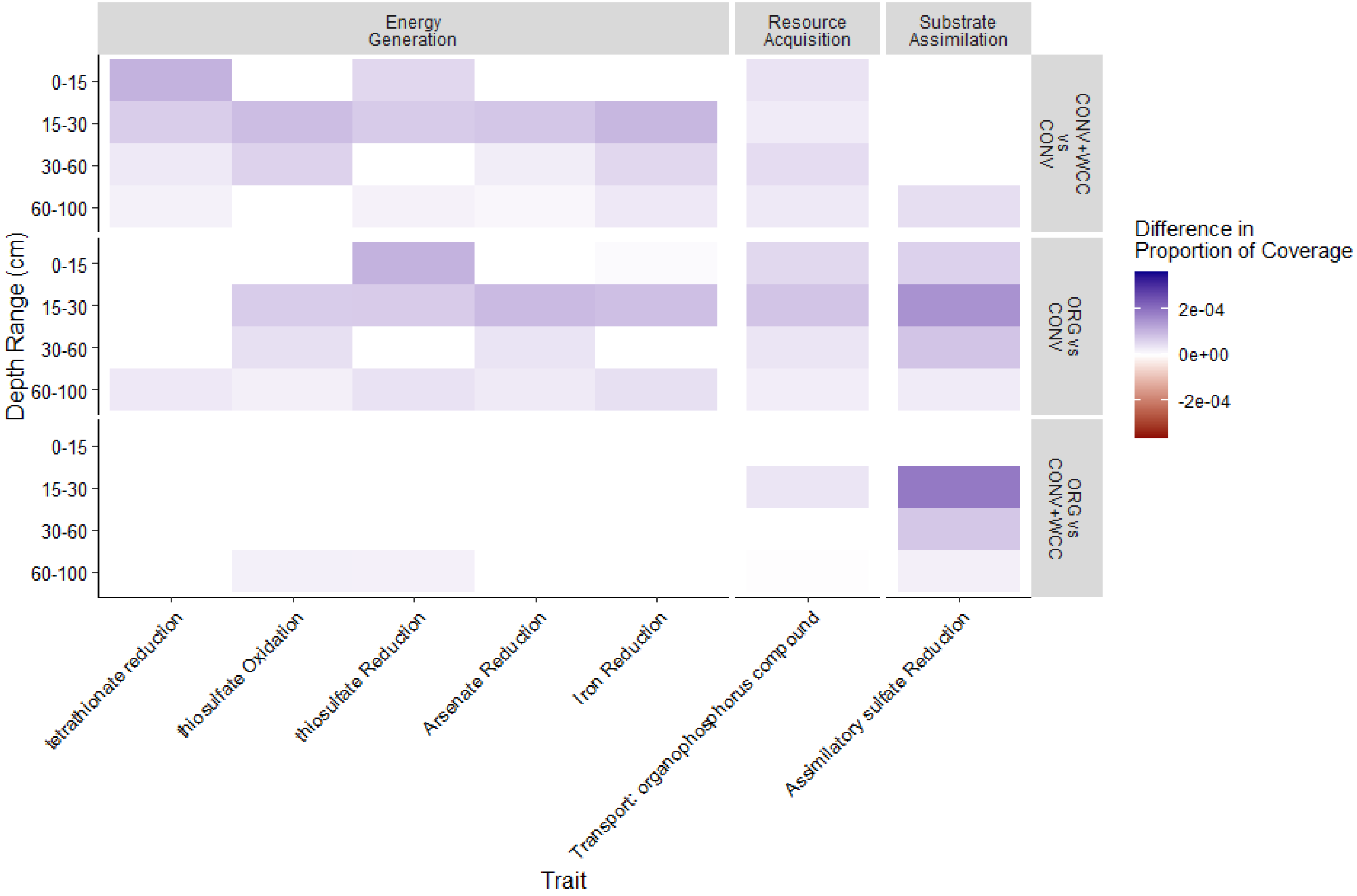

Figure 2.4 c. Selected bacterial and archaeal traits associated with nutrient (P,S,etc.) cycling. Colored boxes indicate a significant difference in proportion of trait coverage at p=0.05, while a white box indicates no sig. difference. Intensity of the color shading represents the size of the median difference between the two systems compared. Eg. A blue box in the CONV+WCC vs CONV indicates higher trait abundance in the CONV+WCC system when compared to the CONV system. Significance testing and median estimation was done using the Mann-Whitney test. ORG = compost, cover crops, organic certified. CONV+WCC = cover crops, mineral fertilizer. CONV = mineral fertilizer.

3.5 Carbon Use Efficiency: 2018-2019

Carbon use efficiency (CUE) measurements taken during the 2018-2019 year showed an impact of both soil depth and sampling time on CUE. We observed a trend of lower CUE at deeper depths ($p<0.001$), though the difference between surface and deeper soils was more pronounced at the March timepoints. CUE measurements made in March 2019 did show a significant treatment effect of lower surface (0-15 cm) CUE in CONV+WCC and CONV systems compared to ORG systems ($p=0.001$), and a nonsignificant treatment effect in March 2018 ($p=0.08$).

Microbial biomass turnover time did not show a significant effect of depth or sampling time when all dates were considered together, but did show a treatment effect at 60-100 cm for the October 2018 timepoint, where it was lower in ORG systems ($p=0.08$).

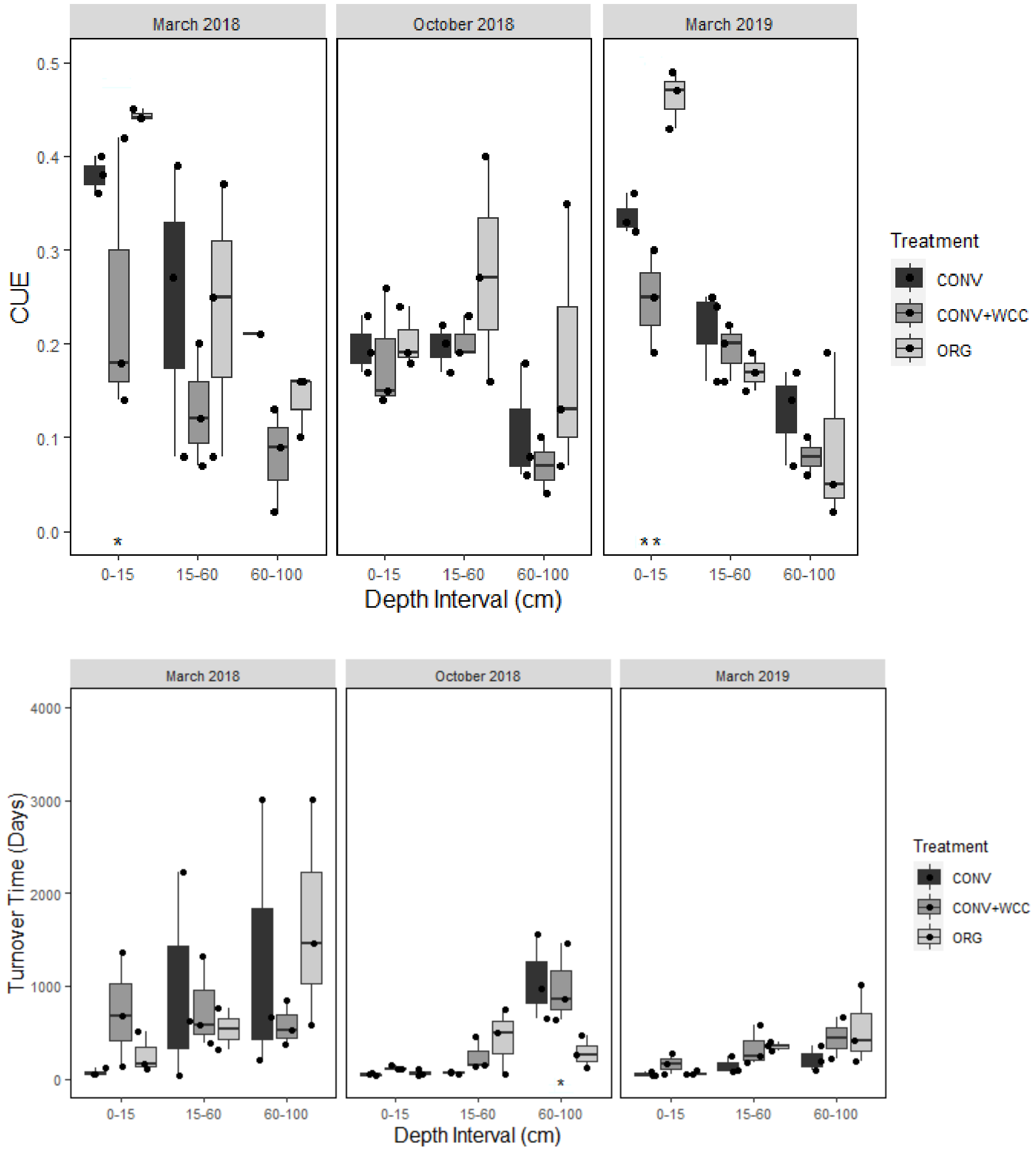

Figure 2.5 A and B. Carbon use efficiency and microbial biomass turnover times measured in 2019 for Russell Ranch plots at the depths 0-15, 15-60 and 60-100 cm. Samples were taken over the course of 1 year in March 2018, October 2018 and March 2019. The presence of a treatment effect is denoted above the depth label by ** ($p<0.05$) and * ($p<0.1$).

4 Discussion

We found that despite multiple lines of evidence that management practices – including cover cropping and compost amendment – have improved soil health indicators in intensively managed row crops at the Century Experiment in the long term, there were no associated significant changes in overall microbial community composition and function. We did, however, find significant changes in several specific microbial functions that were related to some degree to changes in related soil health indicators. This may indicate that significant changes in soil health indicators are not necessarily associated with significant shifts in soil microbial community structure in similar soils.

4.1 20 years of compost and cover crop input did not significantly change overall microbial taxonomic and functional composition

The lack of significant difference in microbial taxonomic or functional clustering with treatment after 19 years of different management practices (Fig. 3, 4) was at first surprising given the well-documented impacts of these practices on soil health indicators, many of which are known to be strongly governed by soil microbial processes. These results also contrast previous investigations using 16S rRNA and PLFA analysis which did observe significant impacts of similar management practices on soil microbial communities in the Century Experiment and nearby experimental plots (Bossio and Scow, 1998; Devarajan et al., 2021; Kong et al., 2011, R. Schmidt et al. - unpublished data).

Other MGS studies have reported significant treatment effects of tillage and crop rotation on overall taxonomic community structure, but not functional community structure (Carbonetto et al., 2014; de Queiroz et al., 2020; Miura et al., 2016; Nelkner et al., 2019). The potential for discrepancies between 16S and MGS sequencing on the same soils have also been highlighted in a limited number of studies: under biochar amendment (Yu et al., 2020, 2019), mine restoration efforts (Miralles et al., 2021; Rodríguez-Berbel et al., 2021) and in natural ecosystems (Steven et al., 2012). Difficulties in connecting the MGS-derived taxonomy and potential function of the bulk soil microbial community to land management may be due to a number of reasons: microbial functional redundancy (Jia and Whalen, 2020), issues with MGS assembly and binning, and the need to correct for the larger false positive rate introduced by the large size of MGS datasets (Korthauer et al., 2019).

Rather than focusing on broad profiling of microbial community structure, it may be more productive to focus on targeted analyses if we are comparing soil health and microbial community responses to land management (Fierer et al., 2021). The fact that our samples were only taken at one time point, which is one of the driest times of year, may represent such a targeted analysis. Limiting the experimental scope, either by targeting specific microhabitats such as aggregates, targeting specific organisms through stable isotope probing (SIP) or targeting specific functional genes or traits may better highlight differences between agricultural treatments, especially in soils that share edaphic and climatic conditions.

4.2 Organic C amended systems showed more potential for C decomposition and biofilm formation in both surface and subsoils

Soil health indicators such as potentially mineralizable C (PMC) and enzyme activity reflect how soil microbes make use of available soil organic carbon (SOC), which in turn can power processes such as biomass formation and nutrient cycling. In a previous study of same plots at the Century Experiment, average mineralizable C (PMC - 1-day incubations) was approximately two times higher, and enzyme activity (FDA hydrolysis) approximately 2.5x higher in ORG than CONV surface soils (Lazicki and Geisseler, 2021). Based on this we hypothesized that treatments receiving higher organic matter inputs would have a higher genomic potential for C breakdown than CONV soils. Indeed, we found that ORG and CONV+WCC systems both displayed increased CAZyme and C degradation potential relative to the CONV system (Figure 5). This greater relative abundance of traits associated with extracellular enzyme production and simple carbohydrate breakdown were attributed to microbial adaptation to the increased C inputs (Table 1) and diversity of organic compounds present in both compost (Leifeld et al., 2002) and cover crop residue (Ding et al., 2006; Zhou et al., 2012).

However, the higher CUE efficiency noted in ORG surface soils compared to CONV (Figure 5a,c) may indicate that microbes in the ORG system were more likely to convert added C into microbial biomass. At the same time, lower CUE in CONV+WCC than CONV systems may indicate that more of the available C in the CONV+WCC system had the potential to be converted into CO_2. These significant treatment effects on CUE were observed only during the March timepoints at the end of the California winter, indicating that seasonal variation may play a strong role in CUE trends. Treatment differences in carbon use efficiency were likely due to improved C:N:P:S stoichiometry in ORG systems due to the large amounts of organic N, P and S added in compost (Table 1) (Coonan et al., 2020; Preusch et al., 2002), and the lack of those same organic nutrient inputs in the CONV+WCC system. These CUE treatment differences could also potentially be attributed to a shift in microbial traits, but our lack of trait data at the CUE sampling timepoints do not allow us to eliminate this possibility. This potential for increased C availability and preferential nutrient stoichiometry may also have been reflected in the lower subsoil turnover times observed in ORG systems (Figure 5e) (Spohn et al., 2016).

Soil aggregates play an important role in protecting soil C from decomposition through occlusion (Six et al. 2004), and are an important soil health indicator that can be related back to functions such as infiltration, structure formation and resilience (Trivedi et al., 2018). Microbes are thought to play an important role in aggregate formation through the exudation of extracellular polysaccharides (often to form biofilms) that bind soil particles together (Redmile-Gordon et al., 2020, 2014). These polysaccharides may also function as a method of excess electron dissipation and C storage for later use (Costa et al., 2018). The ORG system has shown aggregate stability index scores approximately 20% higher than the CONV system (Lazicki and Geisseler et al. 2021). This observed aggregate stability may be associated with the increased relative abundance of biofilm traits (which are comprised of genes associated with polysaccharide secretion in the microtrait pipeline) noted in both CONV+WCC and ORG plots relative to CONV systems (Figure 5a).

While the majority of soil health measurements are focused on surface soils, the relatively carbon-limited subsoil (>60cm) plays a vital role in long term carbon storage (Rumpel et al., 2012). The increased potential for carbon storage in the subsoil is associated both with lower C saturation levels, and lower co-occurrence of C and microbes (Dungait et al., 2012). Microbes may adapt for survival rather than growth in the relatively more C- and nutrient-limited environment of the subsoil (Spohn et al., 2016), which is supported by our observations of decreasing CUE with depth (Figure X). Previous observations of increased inputs of soluble C to subsoils noted in ORG and CONV+WCC systems (Rath et al., 2022) indicate that these soil health practices may have an impact on soils as much as 1m deep. ORG and CONV+WCC soils did show greater relative potential for anaerobic processes as evidenced by the increase in traits associated with iron, arsenate, and thiosulfate reduction (Figure 5c) relative to CONV systems. While it is unclear whether subsoils experience sufficient oxygen limitation to limit C decomposition relative to surface soils (Christel et al., 2016; Kirschbaum et al., 2021), it is possible that the increased soluble carbon inputs may have resulted in localized oxygen depletion due to microbial activity, and the formation of anaerobic microsites. These anaerobic microsites also play a large role in protecting otherwise decomposable C from microbial breakdown (Keiluweit et al., 2017).

4.3 Mineral N amended systems showed more potential for NH_4 oxidation, while organic N amended systems showed more potential for N assimilation

Nitrogen input, whether in mineral form or with C in the form of organic residues, impacts microbial life history strategies (Fierer et al., 2012) in a manner dependent on organic C and pH levels (Geisseler and Scow, 2014). Soil N availability is highlighted by soil health indicators such as potentially mineralizable nitrogen (PMN) and other indicators such as nitrate+ammonium availability. We have observed 2x higher potentially mineralizable N values as well as higher nitrate and ammonium levels in ORG and CONV+WCC systems compared to CONV systems (Lazicki and Geisseler 2021, Rath et al. 2022). Higher levels of these N availability indicators have been connected to a number of factors: larger amounts of microbial biomass in these systems (Kong 2010), higher N inputs (Table 1), and increased potential for the breakdown of the organic N contained in compost and cover crop residue. This increased N availability may also be connected to the higher relative abundance of glutamate and aspartate synthesis traits seen in ORG and CONV+WCC systems compared to CONV systems (Figure 5b). The primary pathway of ammonia assimilation to amino acids in most cells is glutamate synthesis, after which it is converted to aspartate via the transamination of oxaloacetate (Reitzer, 2014). More potential for ammonia assimilation may indicate that ORG and CONV+WCC systems are more likely to retain added N as a part of the microbial biomass.

At the same time, we observed increased relative abundance of ammonia oxidation to nitrite traits in the surface of CONV+WCC and CONV systems relative to ORG plots (Figure 5b, Supp Figure). These systems receive the majority of their N inputs in the form of ammonium nitrate (Table 1), and the oxidation of ammonia to nitrite is the rate limiting step in the nitrification process (Lehtovirta-Morley, 2018). Increased potential for ammonia oxidation may indicate greater potential for nitrite accumulation in these systems. While nitrite may be converted to either nitrate or N2 via the nitrification or denitrification pathways, nitrite accumulation and nitrification is associated with increased N_2O production (Sahrawat, 2008) and CONV and CONV+WCC systems have shown increased N_2O production compared to ORG soils (Kallenbach et al., 2010; Kong et al., 2010).

While availability of nutrients other than N are not often considered as indicators of soil health, their availability can impact processes such as carbon mineralization and biomass formation (Coonan et al., 2020). Our observations of higher organic P and S inputs in ORG systems

(Table 1) supported the potential for a treatment effect on P and S transformations. ORG and CONV+WCC systems showed higher relative trait abundance associated with organophosphate transport (Figure 5c), thiosulfate and tetrathionate transformation, and assimilatory S reduction relative to CONV systems. While this increased organophosphate transport potential may be related to higher external organic P inputs in the ORG system, it may also be due to the uptake and redistribution of phosphorus to the surface by cover crops (Maltais-Landry et al., 2014). Increased potential for S transformations may have been associated with increased inputs of organic S in the form of compost in ORG systems and increased C inputs in ORG and CONV+WCC systems (Preusch et al., 2002). Sulfur mineralization and transformation in soils is closely linked to the amount of available C, while S assimilation in plants is closely tied to N availability (Reuveny et al., 1980). The decomposition of sulfur-bearing amino acids in organic matter and their eventual oxidation to sulfate is a key factor in sulfur availability in soils (Fuentes-Lara et al., 2019).

4.4 Combining metagenomic and soil health measurements–what insights have we gained?

Viewed through the combined lens of functional trait abundance and soil health measurements, ORG (and to a lesser extent, CONV+WCC) systems have shown potential for increased soil functionality in a way that CONV systems have not. While previously measured soil health indicators provided some indication that this was the case, metagenomic analysis has both supported and expanded on how this increased potential is related to land management in several ways.

Firstly, the changes occurring in ORG and CONV+WCC systems were difficult to visualize without making direct comparisons to the CONV system. Given that there is no ideal soil microbial community that can be used as a template for success under different land management practices, describing changes in our systems relative to the business-as-usual control allowed us to give a more accurate description of how this particular soil has improved under two decades of differing management. Site-specific comparisons such as this one are among the most promising methods for clearly identifying microbial impacts of farming practices.

Secondly, we found that measurements of the relative abundance of specific functional traits described the differences between these systems better than overall community structure, and better complemented soil health indicators such as aggregation and mineralizable carbon. The need to use more targeted questions and measurements to better describe changes in soil microbial communities (Fierer et al. 2021) has been highlighted as one of the reasons why finding relevant, practical and informative biological indicators of soil health has been difficult (Lehmann et al., 2020).

Finally, these increases in functional potential were related to the types of inputs added, such that plots with increased organic carbon inputs had more potential to break that carbon down, while plots with increased inputs of mineral fertilizer had more potential to oxidize the ammonia in that fertilizer. It seems likely that microbial adaptation to take advantage of these inputs is at least partly responsible for the increased potential for nutrient cycling and carbon breakdown.

It is possible that the microbial communities in this experiment have experienced greater changes than we observed at this particular time point. While microbial communities may exhibit significant temporal variation over years and decades (Carini et al., 2020), it is unclear how much variation occurs over a single season. We chose to sample the metagenome at post-harvest in September to limit the impact of disturbances associated with the growing season including watering, crop growth, tillage and fertigation. However, this time point is also one of the drier months in our Mediterranean ecosystem, with low water availability across all treatments leading to a convergence of communities with selection for taxa adapted to drought. The higher abundance of Actinobacteria in dry soils may also help explain why over

60% of the coverage in our samples was assigned to Actinobacteria (Barnard et. al 2015, Blazewicz et. al 2020).

While our results preclude management recommendations, they do provide some insight into the types of microbial measurements that may bear fruit as soil health indicators. In this water- and C- limited California soil, changes in microbial community structure may not be as visible or relevant as the potential for increased aggregation and improvement in soil structure, or the potential for increased carbon and nitrogen mineralization indicated by biofilm traits or enzyme production traits. Therefore, identifying specific microbial indicators through the use of targeted, context-specific DNA assays may prove most useful when describing how microbial communities have changed due to practices that promote soil health.

5 Conclusion

Nineteen years of increased organic C, nitrogen (N), phosphorus (P) and sulfur (S) input in the form of compost (ORG plots) and/or cover crops (CONV +WCC plots) increased the potential for carbon, nitrogen, phosphorus and sulfur cycling, but did not significantly change microbial community structure compared to plots with mineral fertilizer application alone (CONV). These results confirmed our hypotheses, which were generated from previous work on soil health indicators carried out on these plots. However, the lack of a significant change in overall community composition or functional profiles under these soil health practices was surprising, and may indicate that moving towards healthier soils is not necessarily associated with significant shifts in bacterial and archaeal community structure.

6 Appendices

Table 2.A1 - P and A values for the effect of treatment on taxonomic clustering at separate depths

Depth	A Value (Treatment)	P Value (Treatment)
0-15	6.3×10^{-3}	0.34
15-30	-8.9×10^{-3}	0.55
30-60	-1×10^{-2}	0.52
60-100	-3.1×10^{-3}	0.97

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Chapter 3: Furrow irrigation is associated with higher surface organic matter losses compared to subsurface drip irrigation in a Mediterranean row cropped soil

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Abstract

Subsurface drip irrigation (SDI) is a widely adopted climate-smart practice in the California Central Valley due to its potential to increase water use efficiency, increase tomato yields, and reduce N_2O emissions. However, the potential impacts of SDI on carbon and nutrient distribution in subsoils (>60cm) and surface soils over multiple growing seasons is not well understood. We compared soils in a 2 m profile under 5 years of SDI and furrow irrigation in the California Central Valley amended with a combination of composted poultry manure + cover crops. We found that the impacts of SDI on carbon and nutrient distribution after a single growing season were limited to the top 30 cm compared to furrow irrigation, and that furrow irrigation was associated with greater organic matter loss at the surface compared to SDI after 5 years. Our results suggest that the spatial variation in water distribution under SDI may reduce organic matter loss at the surface compared to furrow irrigation without significantly reducing the amount of carbon and nutrients stored in subsoils.

1 Introduction

Subsurface drip irrigation (SDI) is an important tool for climate-smart agriculture with the potential to increase adaptation to some of the impacts of climate change such as water scarcity (Wang et al., 2022). Implementing SDI not only potentially reduces water use but is also associated with a number of benefits including increased crop yields, reduction in weed density, and reduction in N_2O emissions (Gao et al., 2019; Kallenbach et al., 2010; Sutton et al., 2006). SDI is now widely used in irrigated high-value crops throughout California, with close to 15% (592,000 acres) of California's approximately 4,000,000 acres of irrigated farmland under SDI in 2018 (National Agricultural Statistics Service, 2018). SDI was the primary form of irrigation in over 85% of processing tomato acreage in California in 2012 and micro-irrigation strategies including SDI accounted for up to 70% of almond irrigation in 2010 (Taylor and Zilberman, 2017; Tindula et al., 2013). Both tomato and almond crop acreages have rapidly expanded in the past 20 years (California Department of Food and Agriculture, 2021), and SDI usage in California is expected to expand along with them due to mandates to decrease agricultural water usage such as the passing of the Sustainable Groundwater Management Act (California Department of Water Resources, 2014).

Subsurface drip irrigation differs from furrow irrigation in both the amount of water applied to a plot (17-36% less water than furrow irrigated plots, Schmidt et al., 2018) and how that water is distributed across the soil surface. SDI is associated with a saturated zone around the drip emitter, large amounts of spatial variation in moisture content above the drip line (usually set at 6 in/15cm) (Amali et al., 1997), and declining moisture content in surface soils 8-16 inches (20-45cm) away from the drip line (Hanson and May, 2004). In contrast, furrow irrigation is associated with more uniform distribution of water along furrows, with lower spatial variability in water content both at the surface and below 60 cm (Amali et al.1997). These patterns of water distribution also translate to patterns in the distribution of soluble salts, with drip irrigation having higher salinity at the edges of beds, and furrow irrigation having higher salinity in the center of beds (Hanson et al. 1996). While there is limited data on the transport of soluble nutrients in drip vs. furrow irrigation, there is evidence that drip irrigation is associated with increased lateral transport of available N, causing the accumulation of nitrate at the edge of the wetting front (Badr, 2007), and the accumulation of nutrients applied via the drip line at the soil surface (Raina et al., 2012). Differences in soluble N transport may also indicate potential for differences in the lateral and vertical transport of soluble C under drip vs furrow irrigation, but there has been limited research to date on this topic.

Despite the widespread adoption of SDI and its potential for modifying nutrient and salinity distribution in soils relative to furrow irrigation, very little is known about the impacts of SDI on soil microbial communities and soil health properties. Switching to SDI from furrow irrigation (FI) has been shown to result in reduced carbon mineralization and aggregation at the soil surface (Rath et al., 2017; Schmidt et al., 2018). Reduced water availability under SDI has been shown to reduce amounts of microbial biomass at the edge of planting beds, increase microbial PLFA stress indicators and modify microbial community composition (Griffin, 2018). Changes in microbial communities may, in turn, alter functions and physical properties of soils such as aggregation, carbon mineralization, microbial biomass and microbial activity and thus overall soil health (Lehmann et al., 2020). Therefore, the potential exists for the agronomic benefits of SDI to be offset in part by reductions in soil health, presenting a potential conflict between California’s efforts to increase agricultural water use efficiency, and efforts to improve soil health and carbon storage (California Department of Food and Agriculture, 2022)

Lower volumes of water applied under SDI than furrow can also impact carbon and nutrient storage in subsoils (e.g. > 60cm), given that water movement via the soil pore network is one of the main pathways linking surface soils and subsoils (Sanderman and Amundson, 2008). The potential reduction in microbial activity due to water limitation in the surface of drip irrigated soils may reduce the release of soluble C and N from the breakdown of organic inputs (Manzoni et al., 2012), and therefore reduce the amount of C and N available for transport. Subsoils are potentially important reservoirs of stored carbon, with longer residence times than

C in surface soil (Dynarski et al., 2019). These longer residence times are due, in part, to higher potential for mineral-organic complex formation, and reduced microbe-carbon co-occurrence (Dungait et al. 2012, Samson et al. 2020). A reduction in C transport to subsoils may therefore represent reduced potential for carbon storage in that soil over longer timescales, which is at odds with soil health goals.

To isolate differences between SDI and furrow irrigation, it is important to control for the impacts of soil type, climate, and management over a multi-year timescale. The Russell Ranch experiment at UC Davis provides one such unique opportunity - a 20+ year experiment established in 1993 to investigate the long-term effects of wheat- and maize-based crop rotations on productivity, profitability, resource use efficiency, soil conditions, and ecosystem services in a Mediterranean climate (Tautges et al., 2019; Wolf et al., 2018). Within this long-term experiment, the organic (compost and cover crops, no synthetic pesticides, ORG) treatment has demonstrated higher levels of carbon and nutrients with improved soil health and fertility metrics compared to a control (Lazicki and Geisseler, 2021). However, it is not clear how irrigation management has influenced the distribution of these elevated carbon and nutrient values in the ORG system. We took advantage of a 5-year comparison of split-plot furrow and drip irrigation in ORG systems to investigate differences in carbon, nutrient and microbial measurements down to 2m.

We hypothesized that there would be higher concentrations of soluble carbon and nutrients in surface soil in SDI than furrow irrigation. Conversely, we also hypothesized there would be lower concentrations of soluble carbon and nutrients in drip irrigated soils deeper than 60cm due to decreased water inputs relative to furrow irrigated soils. We also hypothesized that the reduction in water associated with SDI would result in decreased microbial biomass and increased microbial stress indicators measured via PLFA at the soil surface relative to furrow irrigation.

2 Methods

2.1 Field Site and Management

The Russell Ranch Sustainable Agricultural Facility is located in Davis, CA, at an elevation of 16m above sea level. This area experienced an average temperature of 15°C from November to March, and 29°C during the normal vegetable production period of April to September. The site has two soil types: (a) Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent) and (b) Rincon silty clay loam (fine, smectitic, thermic Mollic Haploxeralf), and due to its xeric moisture regime, received 812 mm of rainfall for the 2019-2020 year primarily during the winter. A description of management history at the Century Experiment is outlined in Tautges and Chiartas et al.(2019) and Rath et al. (2022) and will be described here only briefly. The experimental design is a randomized complete block design (RCBD) with three blocks and nine systems. Two blocks are placed on the Rincon silty clay loam, and the third block is on the Yolo silt loam. The water table is located at approximately 300 feet (I. Herrera, personal communication).

The plots sampled in this paper (n=6) were 64mx64m (0.4ha) and received approximately 4 tonnes of composted poultry manure per year plus a winter cover crop mixture of oat (*Avena sativa* L., 42.0 %C, 2.5 %N), faba bean (*Vicia faba* L., 44.1 %C, 3.5 %N) and hairy vetch (*Vicia villosa* Roth, 44.5 %C, 5.2 %N). No mineral N, commercial fertilizers or commercial pesticides were applied to any plots. All plots were in a 2-year tomato maize rotation, with 3 plots in tomato and 3 plots in corn in 2019. Plots received 4 tillage passes per year to a depth of 20.5cm for cultivation and bed formation, and an additional 8 tillage passes to 6.5cm to incorporate cover crop and compost residue.

Forty eight raised planting beds approximately 152 cm wide were cultivated in each plot, with half of these beds under SDI and half of these beds under furrow irrigation. SDI had been in place since the end of the 2014 season, when plots were converted from furrow irrigation. SDI treatments (ORGD) received water via a drip line placed 6 inches below the soil surface centered in the bed. In furrow irrigated treatments (ORGF), alternate beds were irrigated twice a week with no runoff leaving the field. Watering amounts were determined via evapotranspiration estimates using an in-field sensor (Tule Technologies Inc. CA, USA).

2.2 Sampling

Soil sample collection took place during the 2019-2020 growing season. Plots were sampled in September/October (post-harvest) to reduce the impact of disturbance on soil edaphic conditions. All sampling took place using a Geoprobe over the course of 2 days from all replicate half-plots of the same treatment. Three samples were taken per half-plot approximately 35 cm from the center of the raised bed using a 1.5in diameter probe. Samples were immediately removed from the plastic sleeves, sectioned into 0-15, 15-30, 30-60, 60-100 and 100-200 cm increments, composited by plot and subsampled prior to being placed on ice. 25 g of soil were immediately weighed and dried at 100C for 8 hours to determine moisture content. Aliquots of each soil were extracted for EOC, NO3 and NH4+ analysis and frozen at -20C for PLFA analysis within 48 hours of sampling, while remaining samples were sieved to 8mm and air-dried.

2.3 Carbon and Nutrient Analysis

Extractable organic carbon (EOC) was determined using a 0.5 M potassium sulfate extraction within 48 hours of sampling. For each sample, 6 g of soil were extracted with 0.5 M K_2SO_4 in a 1:5 ratio, shaken for one hour, filtered through Q5 filter paper and analyzed within 48 hours on a Shimadzu TOC-L Total Organic Carbon analyzer according to Jones and Willett (2006). Aliquots of the K_2SO_4 extract were immediately frozen at -20℃ and later analyzed for nitrate by reacting with vanadium(III) chloride according to Doane and Horwath (2003); and ammonium via the Berthelot reaction as laid out in Rhine et al. (1998).

Total nutrient analysis (Ca %, Mg %, K %, Zn ppm, Fe ppm, Mn ppm, Cu ppm, P %, S %, Na %, Mo ppm, B ppm) was carried out via acid digestion using inductively coupled plasma spectroscopy as outlined in Campbell and Plank (1991) and Kovar (2003). Briefly, 1g air-dried samples were acid digested using 3ml HCl, 6ml HNO_3 and 2ml 30% H_2O_2 at 140 deg. C for 3 cycles of 15 min each. Acid-digested extracts were then brought to a final volume of 50ml, filtered and quantified using ICP-OES against a set of internal standards (Ward Laboratories, Kearny, NE).

Available calcium, phosphorus, potassium, and sulfur were measured on 2 mm sieved air-dried samples using the Mehlich-3 soil test (Mehlich, 1984). Total soil C and N values were measured on a CS 4010 Costech Elemental Analyzer (Costech Analytical Technologies) using air-dried, ball milled samples. Organic matter (OM) content was determined using the loss on ignition (LOI) method on 5 g of soil heated to 385 deg C overnight (Schulte and Hopkins, 1996). Potentially mineralizable carbon (PMC) was measured by a 24-hr incubation of 10g of air-dried soil wet to 50% water holding capacity (Franzluebbers et al., 2000). Autoclaved Citrate Extractable soil protein (ACE Soil Protein) was determined according to (Hurisso et al., 2018) via extraction of 3g of air-dried soil with 20 mM sodium citrate, followed by autoclaving at 121 deg. C for 30 min. Permanganate oxidizable carbon (POXC) was measured via oxidation of 2.5 g of soil with 0.02M potassium permanganate prior to being analyzed via spectrophotometer (Weil et al., 2003). CEC and base saturation were determined using ammonium acetate and quantified using atomic emission spectroscopy (AES) (Shuman and Duncan, 1990).

2.4 Phospholipid Fatty Acid (PLFA) Analysis

PLFA analysis was carried out on 2018 samples using the high-throughput method outlined in Buyer and Sasser (2012). Briefly, freeze-dried aliquots were extracted using Bligh-Dyer extractant. Phospholipid fractions were separated from the neutral lipid and glycolipid fractions using solid phase extraction columns. Phospholipids were then dried under N2 gas, transesterified, and methylated. After methylation, the samples were dried again with N_2 gas and redissolved in hexane containing a known concentration of an internal standard (19:0) (Microbial ID, Newark, DE, USA). PLFAs were identified using the Sherlock software from Microbial Identification Systems and quantified using a gas chromatograph equipped with a flame ionization detector. A total of 56 different PLFAs were identified. PLFAs were assigned to Gram-positive, Gram negative, Cyclopropyl precursors, Saturated and Monounsaturated groups as outlined in Bossio and Scow (1998) (Supplementary Table A1).

2.5 Historical Data Comparison

Soil % C and nitrogen (N) values for 0-15, 15-30, 30-60, 60-100 and 100-200 cm in 2012 were taken from Tautges & Chiartas et al. (2019), while OM, P (ppm), S (ppm) and K (ppm) values were taken from the Century Experiment published dataset in Wolf et al. (2018). C and N analyses used in this paper were all performed using the same methods (Tautges and Chiartas et al. 2019) on ball-milled, air dried samples in a CS 4010 Costech Elemental Analyzer (Costech Analytical Technologies). Historical OM values were determined using loss on ignition (LOI) while P (ppm), S (ppm) and K (ppm) values were determined using the Mehlich-3 soil test.

2.6 Statistical Analysis

All data analysis and graph production were done using R v. 4.0.2, (R Core Team, 2020) using the tidyverse package (Wickham et al., 2019). CCA ordination was performed using the vegan package (Oksanen et al. 2020). Significant changes in soil variables from 2012-2019 were analyzed using one-sample t-tests. Statistical differences in soil measurements for 2019 were conducted using an Analysis of variance (ANOVA) to determine the effects of management system and depth. We first checked for normality and assumptions of the linear model prior to ANOVA, then fit a mixed effect model with “block” as a random effect. Since “block” was not significant for any of the variables measured, we removed it from the model. Statistical differences between management systems were analyzed separately for each depth using paired t-tests with Bonferroni correction for multiple tests at 5% significance level.

3 Results

3.1 Irrigation Amounts

Cumulatively, the ORGF system received approximately 1.6x as much water as the ORGD system since the introduction of SDI in 2014.

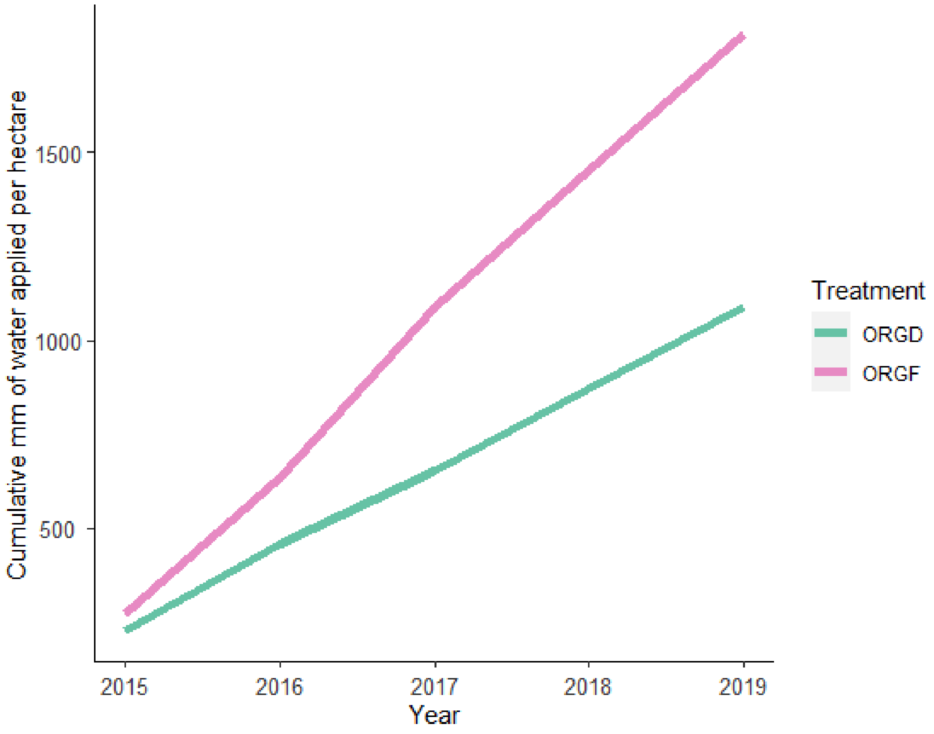

Figure 3.1. Cumulative water application in organic furrow irrigated (ORGF) and organic subsurface drip irrigated (ORGD) plots from 2015-2019.

3.2 Differences in C, Nutrient Content and Microbial Measurements in the 2m Profile in 2019

Differences between the organic furrow irrigated system (ORGF) and organic drip irrigated system (ORGD) were visible only in the upper 30 cm of the soil profile. The ORGF system had lower amounts of potentially mineralizable carbon (PMC) (p=0.07), mineral N (NO_3+NH_4) (p=0.02), organic matter (p=0.08), available phosphorus (p=0.03) and soil moisture (p=0.05) in the top 0-15 cm compared to the ORG at the time of sampling. At the same time, the ORGD system had lower calcium (Ca, p=0.06) and magnesium (Mg, p=0.07) base saturation and a higher pH (p=0.02) than the ORGF systems in the top 0-15 cm.

ORGD and ORGF systems did not differ significantly at any depth in extractable organic carbon (EOC) concentration, permanganate oxidizable carbon (POXC), ACE Soil Protein content, cation exchange capacity (CEC), potassium base saturation, or in concentrations or total percentage of a number of elemental analyses (%C, %N, %S, %P, ppm Zn, ppm Mn, ppm Cu, ppm B, ppm Mo) (Figure A1). No significant impact of SDI or furrow irrigation was noted on PLFA community structure, microbial biomass, or microbial stress indicators throughout the 2-m profile.

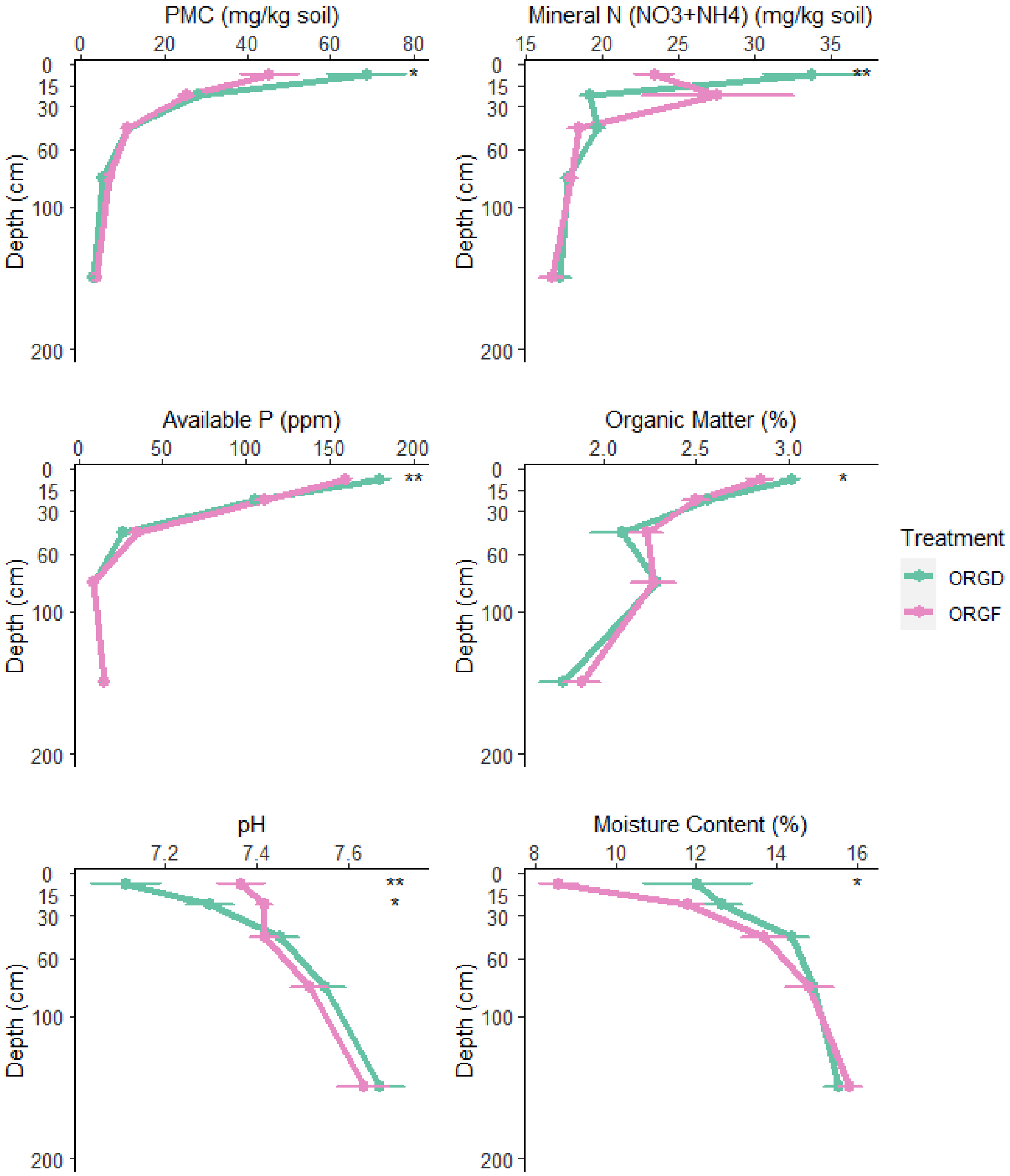

Figure 3.2. Potentially mineralizable carbon (PMC), Mineral N, available P, OM%, pH and moisture content of the 0-200 cm profiles of ORGD and ORGF systems post-harvest in August 2019. Error bars represent standard error. Significance testing is portrayed by * = 0.05<p<0.1, **=p<0.05.

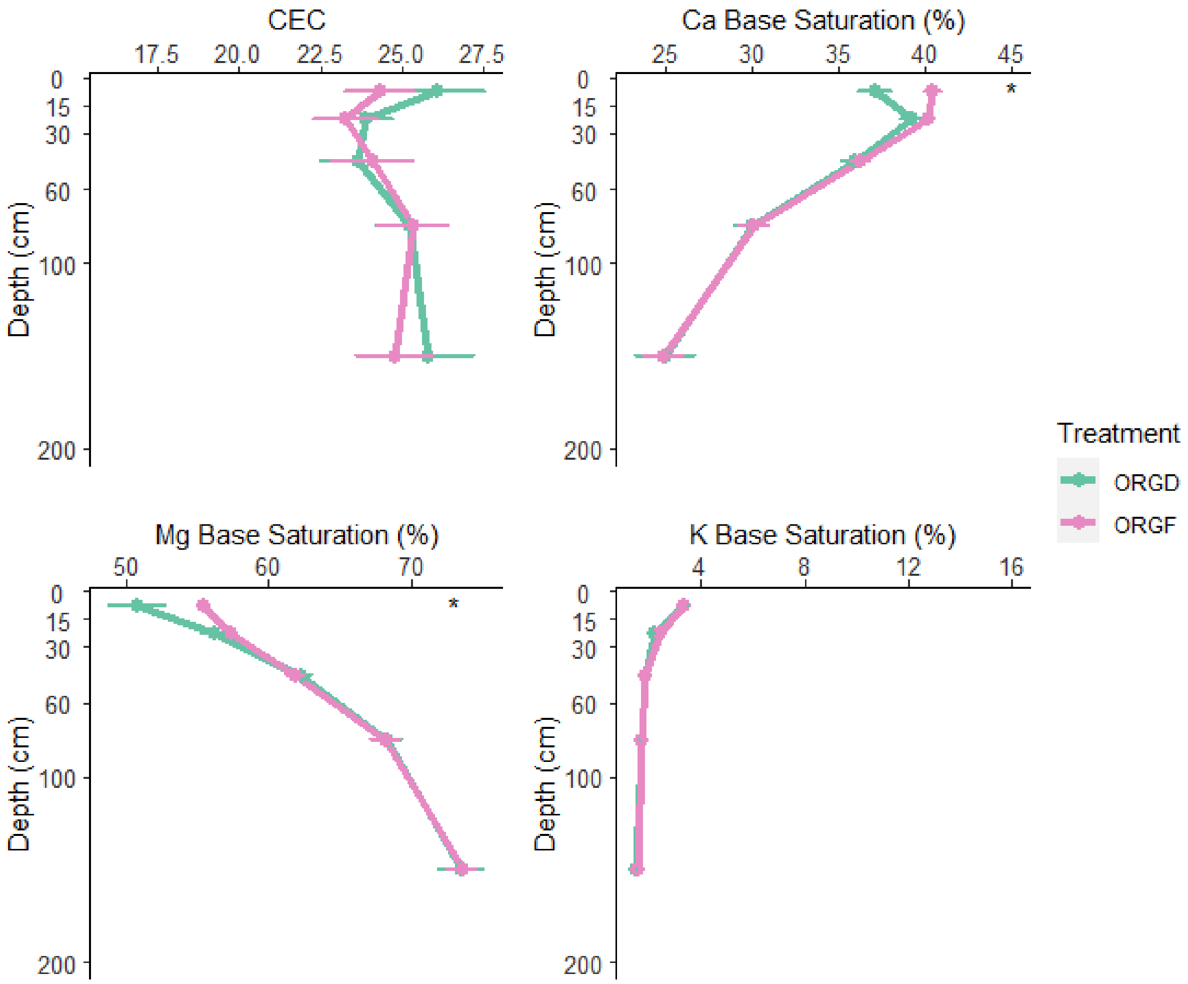

Figure 3.3. Cation exchange capacity (CEC), Calcium, Magnesium and Potassium base saturation of the 0-200 cm profiles of ORGD and ORGF systems post-harvest in August 2019. Error bars represent standard error. Significance testing is portrayed by * = 0.05<p<0.1, **=p<0.05.

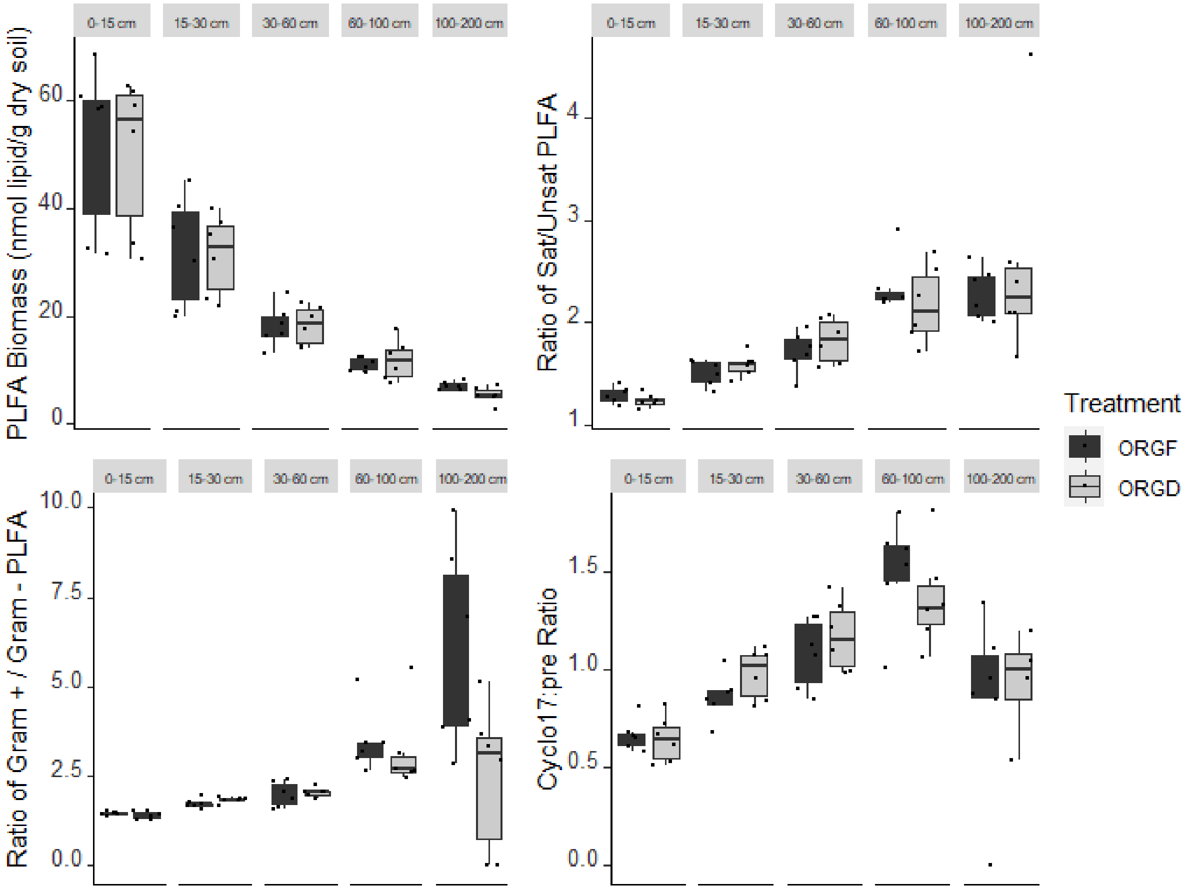

Figure 3.4. Microbial biomass (PLFA), and microbial stress indicator ratios (Gram +:Gram-, Saturated:Unsaturated Fatty Acids, and Cyclopropyl 17:precursor ratio) of the 0-200 cm profiles of ORGD and ORGF systems post-harvest in August 2019. Ratios are unitless, while microbial biomass is given in nmol lipids/g dry soil.

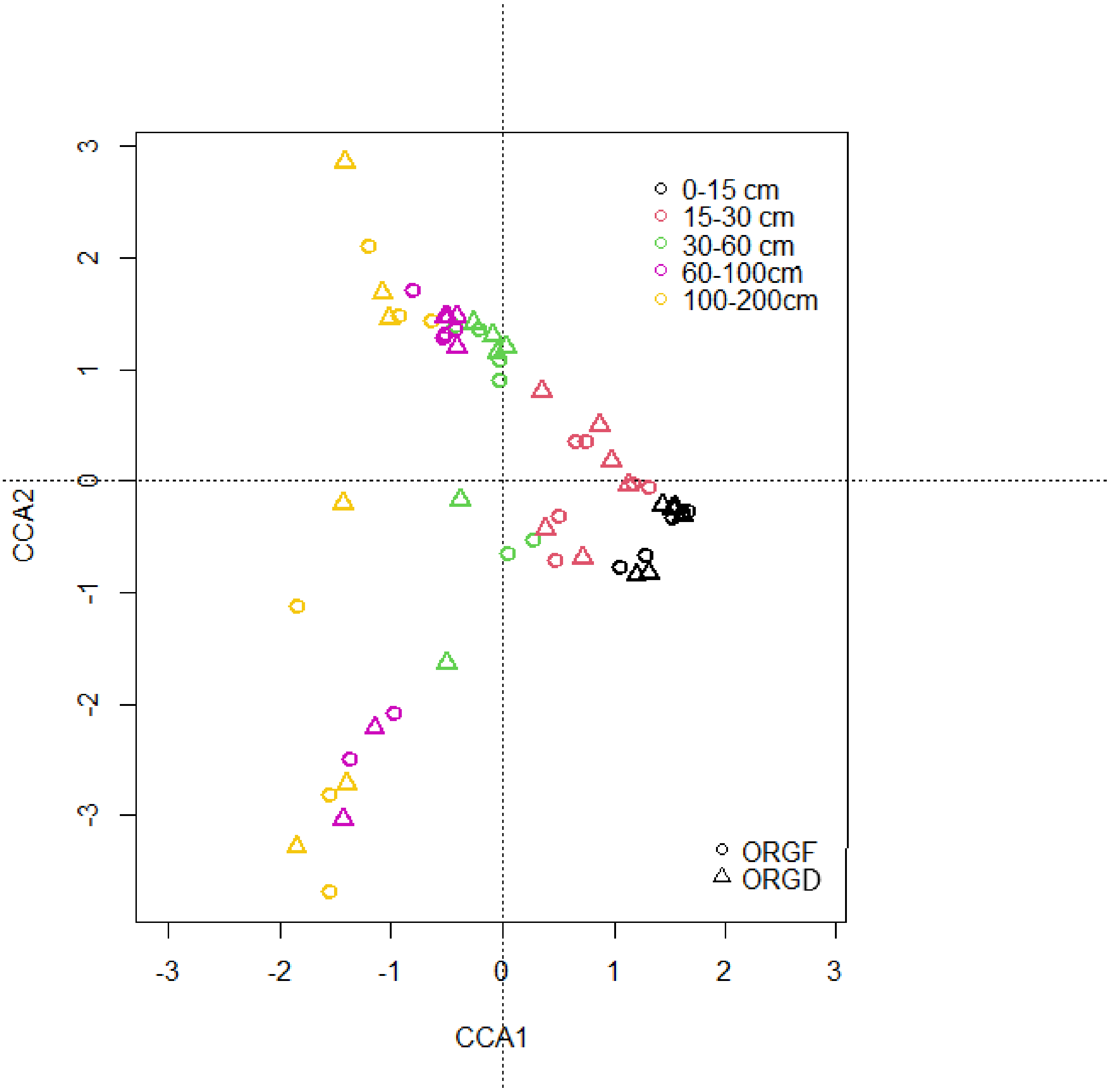

Figure 3.5. CCA of microbial community composition measured via PLFA analysis for the 0-200 cm profiles of ORGD and ORGF systems post-harvest in August 2019.

3.3 Organic Matter and Nutrient Content Changes from 2012-2019

Our observations of the impact of SDI on the top 30 cm in 2019 led us to investigate how organic matter and nutrient content have changed in the past 5 years in that upper 30 cm between SDI and furrow irrigation. ORGD and ORGF systems showed an increase in available P ($p<0.01$) and K ($p<0.01$) values from 2012-2019, and a decrease in available S ($p<0.01$). At the same time, the ORGF system showed a decrease in % organic matter (%OM) values from 2012-2019 ($p=0.05$), while the ORGD system showed no significant changes during the same time period.

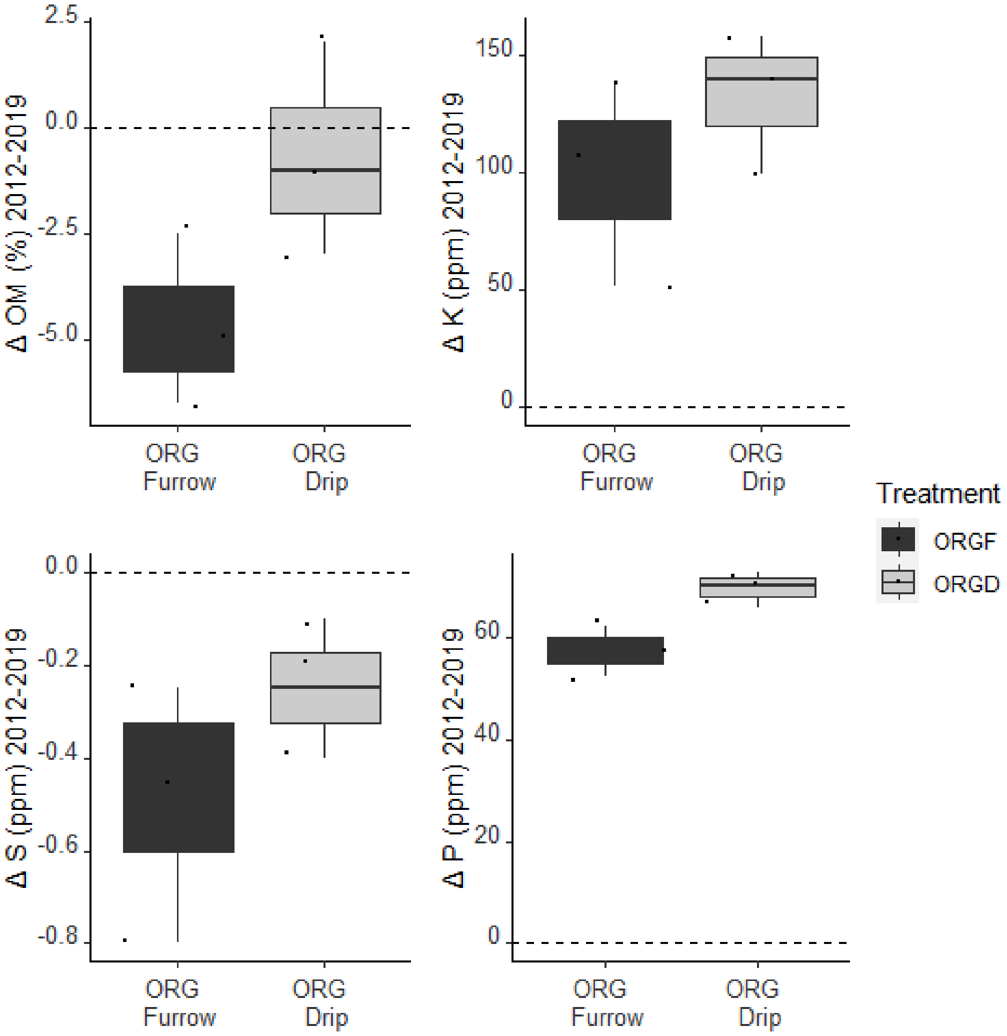

Figure 3.6. Changes in percent organic matter (OM%), available K (ppm), available S (ppm) and available P (ppm) from 2012-2019 in the top 30 cm of ORGD and ORGF systems (Data taken from Wolff et al. 2018).

4 Discussion

We expected that, due to differences in amount and distribution of water under SDI compared to furrow irrigation, we would see much larger differences in carbon, nutrient concentration and microbial measurements deeper in the soil. However, we only observed significant differences in the top 30 cm of soil, with a clear trend of lower mineral N, P and higher pH in furrow irrigated systems. Additionally, the furrow irrigated system appeared to have lost more organic matter at the soil surface than the SDI system since 2012 despite having no differences in microbial biomass (Figure 4). This may be due to either increased potential for microbial carbon mineralization and transport from increased water availability, greater biomass turnover, or reduced microbial carbon use efficiency. This may indicate that significant changes in carbon and nutrient distribution under drip irrigation are limited to the top 30 cm of the soil after 5 years, but that these impacts may include reduced organic matter loss compared to furrow irrigation.

The decreased pH, decreased Ca and Mg saturation, and increased mineral N in the surface of SDI irrigated plots can be attributed to the differences in wetting patterns between surface and drip irrigation (Figures 1 and 2). The movement of water outwards from the drip line during irrigation can result in the accumulation of soluble N at the soil surface (Raina et al., 2012), especially between 20-40cm from the drip line (Hanson and May, 2004). Our samples, taken approximately 35 cm from the drip line, fall within this zone of potential N accumulation under SDI. This soluble N likely comes from the applied poultry manure and release of ammonia from OM mineralization and production of nitrate via nitrification (Gale et al., 2006), given that mineral fertilizer was not applied to these plots via the drip line. In addition, the acidification relative to furrow irrigated plots at the same location may have been due to the formation of nitric acid from the conversion of accumulated nitrate to nitric oxide (Dal Molin et al., 2020). This pH change may also be attributed to the reduction in Ca and Mg saturation noted in surface SDI samples, which may represent the soil’s reduced ability to buffer this pH change under SDI due to lateral transport of these cations to the edge of the bed (Schmidt et al., 2018; Selim et al., 2013).

The lack of a significant impact of SDI on microbial community, biomass, or microbial stress indicators throughout the 2-m profile is surprising and may be due to the time of sampling. The lower moisture content in furrow irrigated plots at the time of sampling indicates that the smaller amount of water applied to SDI plots (approximately 2/3rd of the water applied to furrow irrigated plots - Figure 1) did not impact soil moisture at our sampling location at the end of the season. Irrigation, especially of tomato crops, often stops during the last 1-2 weeks of the growing season to allow the soil to dry out in preparation for harvest (Lu et al., 2019). While previous research has shown a significant impact of drip irrigation on microbial communities across the surface of the bed (Griffin, 2018), the sensitivity of PLFA analysis to changes in environmental conditions may have meant that the extended dry period these plots underwent resulted in similarly elevated microbial stress levels in all systems, and a convergence of PLFA measurements.

Soil water availability has strong impacts on carbon and nutrient distribution as well as microbial activity (Schimel 2018). Uneven water distribution, such as that observed in surface soils under SDI, represents not just a potential resource limitation (Morgano et al. 2013), but a limitation placed on solute availability (Cleveland and Yavett 1997) and transport (Bailey et al. 2017) in localized areas across the surface of the bed. The water limitation experienced by these dry spots during the growing season under SDI may result in reduced OM mineralization and respiration for a number of reasons, including microbial dormancy, reduced enzyme mobility, and shifts in microbial community structure (Carbone et al. 2011, Wu et al. 2011). In contrast, the more even distribution of water along furrows under furrow irrigation represents conditions more conducive to microbial OM mineralization and solute transport during the growing season. This difference in water availability under SDI vs furrow irrigation may explain the significant decrease in OM% in the surface of the furrow irrigated system from 2012-2019.

The frequency of wetting events in furrow vs SDI may play a further role in increasing OM loss in furrow irrigated surface soils. Approximately 25% of a planting bed near the drip tape under SDI experiences relatively continuous water availability during the growing season (Griffin 2018). Though water distribution across the surface of a bed may be more uniform under furrow irrigation, these soils also experience more frequent wet-dry cycles due to the fact that furrows are flooded and left to dry regularly. These wet-dry cycles can increase C availability through lysis of the microbial biomass, as well as reducing physical SOM protection by destabilizing aggregates (Mikha et al. 2005, Fierer et al. 2003). Subsequent furrow irrigation events may both encourage mineralization of that newly available C, as well as transport that C away from the surface via infiltration (Tecon and Or 2017). Our observations of a weak trend of lower PMC and OM values in the surface of ORGF plots in 2019, corroborate other observations of lower PMC in furrow irrigated plots (Schmidt et al. 2018, Griffin et al. 2018), and support the idea of increased C loss at the surface under furrow irrigation due to wet-dry cycling over the course of a growing season.

This loss of OM from 2012-2019 was not echoed by a significant change in total C or EOC in the surfaces or subsoils of these plots over the same time period. The similarity in total C between SDI and furrow irrigation may reflect the reduced sensitivity of measurements of the total C pool compared to other types of C measurement such as potentially mineralizable carbon and organic matter content (Xu et al., 2011). Total C measurements may also vary by up to 10% over the course of a growing season (Wuest, 2014), introducing additional variability that may obscure treatment effects. Likewise, the similarity in EOC between SDI and furrow irrigation in both surface and subsoils may indicate that differences in vertical transport of soluble C were not sufficiently large to be detected by our sampling regime. ORGD plots have shown EOC values approximately 2x as high as conventionally managed drip plots at the surface, and 1.2x as high below 60 cm (Rath et al., 2022). Given that both SDI and furrow irrigated plots received the same amount of compost annually, differences in vertical transport of soluble C due to water availability may not have been visible against the backdrop of existing EOC transport after only 5 years of drip.

It is possible that the time when we sampled may have had an impact on the absence of large differences in irrigation systems. The sampling of soil every ten years at the Russell Ranch experiment specifically targets a time in the growing season when differences in crops, management practices and rainfall/irrigation across systems is minimal. However, for our study, we may have seen greater impacts during the growing season when differences in water availability are more evident. We anticipated that because our study was almost 20 years old, differences in many of the parameters, perhaps with the exception of soil moisture, would have been additive and detectable at the end of the growing season. However, potential differences attributed to water stress may have been masked by the dry period at the end of the growing season, or because our sampling regime did not have sufficiently high resolution to differentiate between localized dry spots and the bulk soil under SDI. Additionally, the cumulative impact of differences in moisture stress due to SDI or furrow irrigation over several years would likely be reduced by the annual onset of winter rains.

With increased periods of drought and reduced water availability predicted as impacts of climate change in California (Pathak et al., 2018), the use of SDI will likely become more common both in the state and the wider US. The reduction in soil water availability under SDI may reduce loss of OM in surface soils relative to furrow irrigation in addition to other benefits such as increased water use efficiency and decreased N_2O production. However, it is unclear whether the higher levels of OM at the surface under SDI come at the cost of potential subsoil carbon inputs and storage under furrow irrigation over periods of time longer than 5 years. Carbon stored at the soil surface is more susceptible to the extreme weather conditions predicted under climate change, whether to erosion or fire (Williams et al., 2019), while subsoil carbon is a prime target for carbon sequestration efforts due to its increased residence time (Paul et al., 1997, 2001). Therefore, the potential impacts of this reduced surface OM loss under SDI and its consequences for long-term subsoil carbon storage warrant further investigation.

5 Conclusion

The comparison of SDI and furrow irrigation was only associated with minor differences in carbon, pH and nutrient distribution in the top 30 cm during the 2019 season. Furrow irrigated plots were also associated with OM losses in the top 30 cm from 2012-2019. In contrast to what we expected, there were no differences between SDI and furrow irrigation with regard to the spatial distribution of water, carbon, and other nutrients deeper in the soil profile. Therefore, our results suggest that the spatial variation in water distribution under SDI at the surface may reduce organic matter loss compared to furrow irrigation without significantly reducing the amount of carbon and nutrients stored in subsoils over 5 years.

6 Appendices

Table 3.A1 – PLFA (Phospholipid Fatty Acid) Assignments taken from Bossio and Scow (1998).

Gram Positive	15:1 iso w6c; 15:0 iso; 15:0 anteiso; 16:0 iso, 17:1 iso w10c, 17:1 iso w9c; 17:1 anteiso w9c; 17:0 iso, 17:0 anteiso; 18:0 iso
Gram Negative	16:1 w9c; 16:1 w7c; 17:1 w8c; 17:0 cyclo w7c; 18:1 w7c; 19:0 cyclo w7c; 20:1 w9c; 21:1 w3c
Saturated	12:0; 14:0; 15:0; 16:0; 17:0; 20:0
Monounsaturated	16:1 w5c; 16:1 w7c; 18:1 w9c; 18:1 w7c
Cyclopropyl Indicator	19:0 cyclo w7c / 18:1w7c

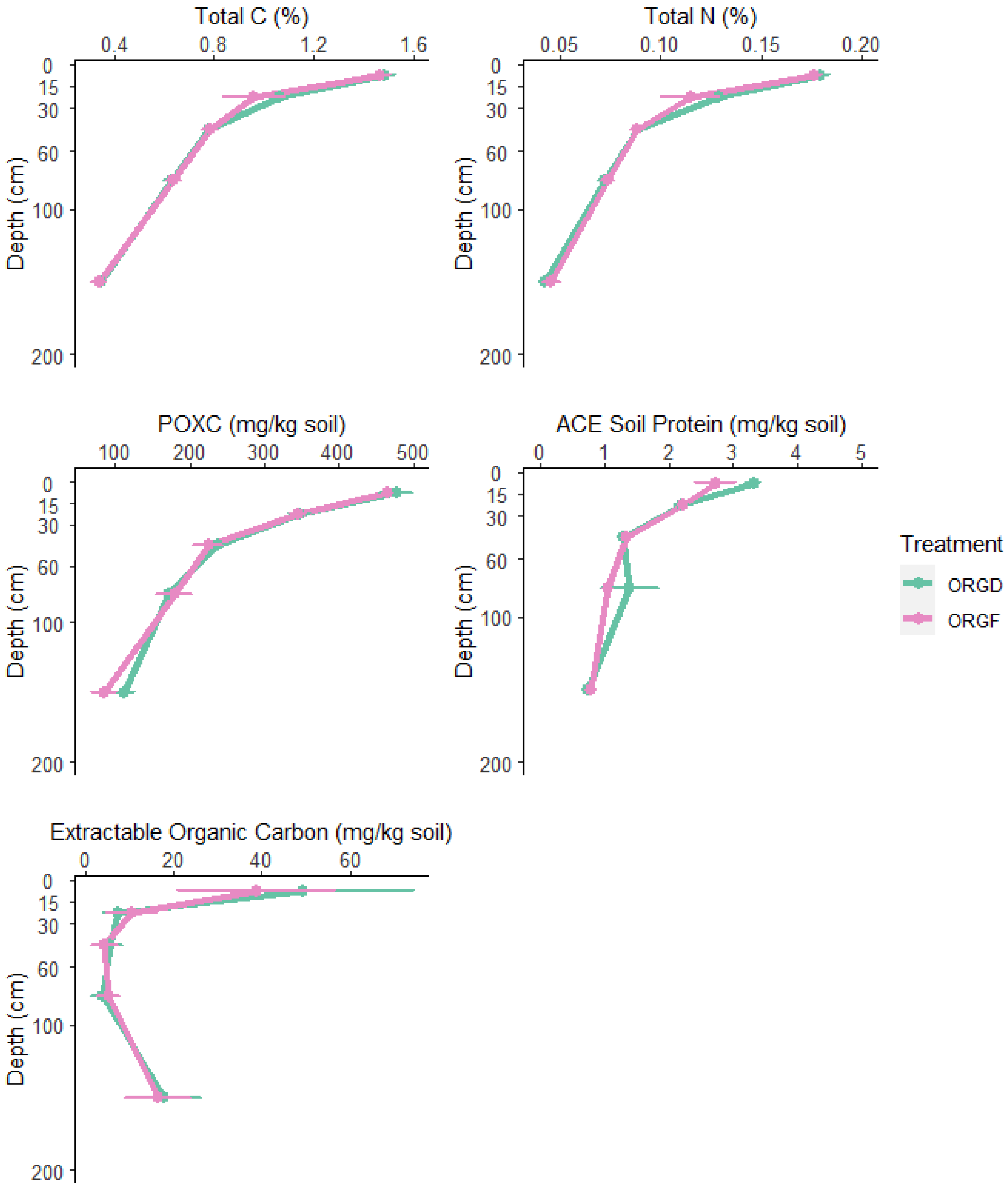

Figure 3.A1. Total Carbon (%), Total Nitrogen (%), permanganate oxidizable carbon (POXC), Autoclaved Citrate Extractable soil protein (ACE Soil Protein), and extractable organic carbon (EOC) content of the 0-200 cm profiles of ORGD and ORGF systems post-harvest in August 2019. Error bars represent standard error.

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