Synergy between compost and cover crops leads to increased subsurface soil carbon storage

Daniel Rath[1], Nathaniel Bogie[2], Leonardo Deiss[3], Sanjai Parikh[1], Daoyuan Wang[4], Samantha Ying[5], Nicole Tautges[6], Asmeret Asefaw Berhe[7], Kate Scow[1]

[1]Department of Land, Air and Water Resources, University of California Davis, Davis, CA 95618, USA
[2]Department of Geology, San Jose State University, San Jose, CA 95192, USA
[3]School of Environment and Natural Resources, The Ohio State University, Wooster, OH 44691, USA
[4]Department of Environmental Science and Engineering, Shanghai University, Shanghai, OH 200444, CHN
[5]Department of Environmental Sciences, University of California Riverside, Riverside, CA 92521, USA
[6]Michael Fields Agricultural Institute, East Troy, WI 53120, USA
[7]Department of Life and Environmental Sciences, University of California Merced, Merced, CA 95342, USA

Correspondence to: Daniel Rath (darath@ucdavis.edu)

Abstract. Subsurface carbon stocks are a prime target for efforts to increase soil carbon storage for climate change mitigation and improving soil health. However, subsurface carbon (C) dynamics are not well understood, especially in soils under long term intensive agricultural management. We compared subsurface C dynamics 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). 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 the accumulation of carboxyl rich C in the subsurface (60-100 cm) horizons of all systems. Systems also had greater amounts of aromatic carbon in the order ORG>CONV+WCC>CONV. We identified a potential interaction between cover crops and compost, theorizing that increased macropores from cover crop roots facilitate the transport of soluble C and nutrients into the subsurface, thereby increasing stocks. These results demonstrate the potential for subsurface carbon storage in tilled agricultural systems and highlight a potential pathway for increasing carbon transport and storage in subsurface soil layers.

1 Introduction

Subsurface agricultural soils have the potential to store large amounts of carbon (Rumpel et al., 2012), with longer C residence times (Paul et al., 1997, 2001) relative to surface soils. The line between surface and subsurface soils is often drawn at the average tillage depth (30 cm) (Chen et al., 2018), but studies of microbial carbon processing and soil health indicators identify surface soils as shallow as 15 cm (Wordell-Dietrich et al., 2017). This top 15 cm, due to its high density of microbial biomass, is easy to sample, responds quickly to disturbance, and its outsized perceived role in ecosystem services and carbon processing may be one of the reasons that the average soil sampling depth has shrunk from 53 cm to 27 cm in studies published in the last 30 years (Yost and Hartemink, 2020). However, this shrinking sampling depth ignores decades of research into the role that subsurface soils play in increasing soil carbon stocks (Rapalee et al., 1998; Rumpel and Kögel-Knabner, 2011), and is particularly problematic in agricultural studies given how practices such as cover crops, with

35 rooting depths of up to 85 cm (Fan et al., 2016), can have drastically different effects on surface versus subsurface SOC accumulation (Bernal et al., 2016; Harrison et al., 2011; Tautges et al., 2019). To maximize C storage throughout the entire soil profile, we need to understand how land management influences carbon accumulation and storage in subsurface soils (Chenu et al., 2019).

Not recognizing the different potentials for carbon sequestration in surface and subsurface soils means losing the opportunity
40 to take advantage of their unique biological, physical and chemical differences (Angst et al., 2018; Fierer et al., 2003b; Kautz et al., 2013). Subsurface soils generally have higher clay contents due to illuviation and eluviation processes (Jenny, 1941), as well as greater restrictions on carbon decomposition than in surface soils (Fierer et al., 2003a). The distribution of microbes and carbon are more decoupled in subsurface soils (Dungait et al., 2012) due to lower microbial biomass (Taylor et al., 2002) and more heterogeneous carbon distribution (Chabbi et al., 2009; Syswerda et al., 2011). Subsurface microbes are
45 also less efficient at carbon assimilation than surface microbes, and thus more likely to mineralize SOC to CO_2 (Spohn et al., 2016), possibly due to energy and nutrient limitations (Salomé et al., 2010; Sanaullah et al., 2011). Carbon inputs to subsurface soils are primarily through dissolved organic carbon (DOC) movement (Kaiser and Kalbitz, 2012) and deeper rooting biomass and exudates (Sokol and Bradford, 2019). This contrasts with the greater variety of inputs to surface soils such as compost (White et al., 2020a), plant aboveground biomass/shoots (Liebmann et al., 2020), atmospheric deposition
50 (Mladenov et al., 2012) and abundant shallower root biomass (Poeplau and Don, 2015).

Surface and subsurface SOC also respond differently to agricultural management practices that are concentrated at the soil's surface (Salomé et al., 2010; Syswerda et al., 2011). In a recent study after 19 years of management, cover cropping 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). In the same study, cover cropping combined with composting both increased C stocks
55 above 30 cm by 5%, and increased C over the whole 2m profile by 12.6%. Estimates of whole-profile carbon sequestration based solely on data from surface soils can lead to inaccurate estimates of carbon storage potential in agricultural systems (VandenBygaart et al., 2011).

Agricultural practices can increase or decrease subsurface SOC by modifying the processes that control soil carbon stabilization including occlusion in soil aggregates, sorption to soil minerals, and microbial processing of residues (Rumpel
60 and Kögel-Knabner, 2011). Crop root exudates can be efficiently transformed by microbes into stable soil carbon (Sokol and Bradford, 2019), but the same exudates can also destabilize aggregates and carbon-mineral bonds that are key for long-term stabilization (Keiluweit et al., 2015). Large inputs of dissolved organic carbon and nutrients can prime subsurface microbial biomass to decompose native SOC (Bernal et al., 2016; Kuzyakov, 2010), or can provide favorable nutrient stoichiometry for microbial carbon processing (Coonan et al., 2020; Kirkby et al., 2013). In addition, recent studies have highlighted that
65 subsurface SOC may be vulnerable to loss under changing environmental conditions, including warming (Hicks Pries et al., 2018) and changes in soil moisture status (Min et al., 2020). Taken together, these studies demonstrate that to accurately predict whether a specific farming practice will increase or decrease subsurface SOC storage in a changing climate, it is necessary to perform studies that explicitly examine deeper soils.

Our overarching question asks how agricultural management changes carbon formation and storage processes in subsurface
70 compared to surface soils. We determined the impacts of three land management systems on carbon storage and SOC-related indicators in the microbially active top 15 cm of soil, subsurface soils below 60 cm, and the intervening region (15-60 cm). Our experimental treatments were compost with cover crops (ORG), cover crop with mineral fertilizer (CONV+WCC), and mineral fertilizer application (CONV), and indicators measured include aggregation, carbon chemistry, nutrient availability, and microbial community dynamics down to 1 m. Our specific hypotheses were:
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1. SOC, DOC and nutrient stocks in surface (0-15 cm) and subsurface (60-100 cm) ORG systems will be higher relative to CONV and CONV+WCC stocks.
2. Higher microbial biomass and evidence of microbially processed carbon is found in the subsurface of the ORG and CONV+WCC than of CONV systems.
80
3. Soil aggregation and hydraulic conductivity is higher in ORG than CONV+WCC or CONV systems.

Given that small, cumulative subsurface management impacts may take decades to become detectable, the standard two to three year agronomic experiment is usually not sufficient to observe impacts of agricultural management practices on subsurface soils (Dick, 1992; Johnston and Poulton, 2018; Keel et al., 2019). We address this issue by asking our questions at the Century Experiment at the Russell Ranch Sustainable Agricultural Facility in Davis, CA, where inputs and management history have been tracked in detail over the last 25 years (Wolf et al., 2018). This unique opportunity allows us to draw conclusions relevant to row crops in California's Central Valley, one of the world's most productive agricultural regions (Pathak et al., 2018) and one quite susceptible to negative impacts of climate warming (Medellín-Azuara et al., 2011).

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.

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 the Century Experiment published dataset in Wolf et al. (2018).

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. All plots were irrigated with subsurface drip at the time of sampling, having converted from furrow irrigation to subsurface drip in 2014.

2.2 Field Management - 2018

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 °C 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.

2.3 Historic Carbon and Nutrient Inputs

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 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
125 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 aboveground 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.

2.4 Soil Sampling

130 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). 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
135 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 °C for PLFA analysis within 48 hours of sampling, while the remaining samples were sieved to 8 mm and stored at 4 °C until analyzed.
Bulk density values used in this paper were sampled using a Giddings hydraulic probe to 2m in 1993 and 2012. In 1993, bulk
140 density was collected in 0–25, 25–50, 50–100, and 100–200 cm depth layers with an 8.25 cm diameter probe. In 2012, bulk density was collected in 0–15, 15–30, 30–60, 60–100, and 100–200 cm depth layers, with a 4.7 cm diameter probe. In both 1993 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 and 2012 were adjusted to 2018 depths through the calculation of weighted averages
145 using adjacent depth layers.

2.5 Nutrient and Aggregation Analysis

Dissolved organic carbon was determined using a 0.5 M potassium sulfate extraction. 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
150 frozen at -20°C 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).
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
155 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 °C, and weighed. Mean weight diameter of the
160 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 using the high-throughput PLFA analysis 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,
165 transesterified, and methylated. After methylation, the samples were dried again with N2 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 1).

2.7 Hydraulic Conductivity and Moisture Content

Three 20 cm^3 cores were collected for saturated hydraulic conductivity from each plot that had been under tomato in 2017-2018 (9 plots out of a total of 18). 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, 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 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). All FTIR 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. Spectral subtractions were performed using Omnic 9.8.286 (Thermo Fisher Scientific). 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. Statistically significant 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 carbon 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. Due to compost input and slightly increased cover crop input, ORG systems

received approximately 1.5x more carbon 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 carbon than CONV systems.

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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

215 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.
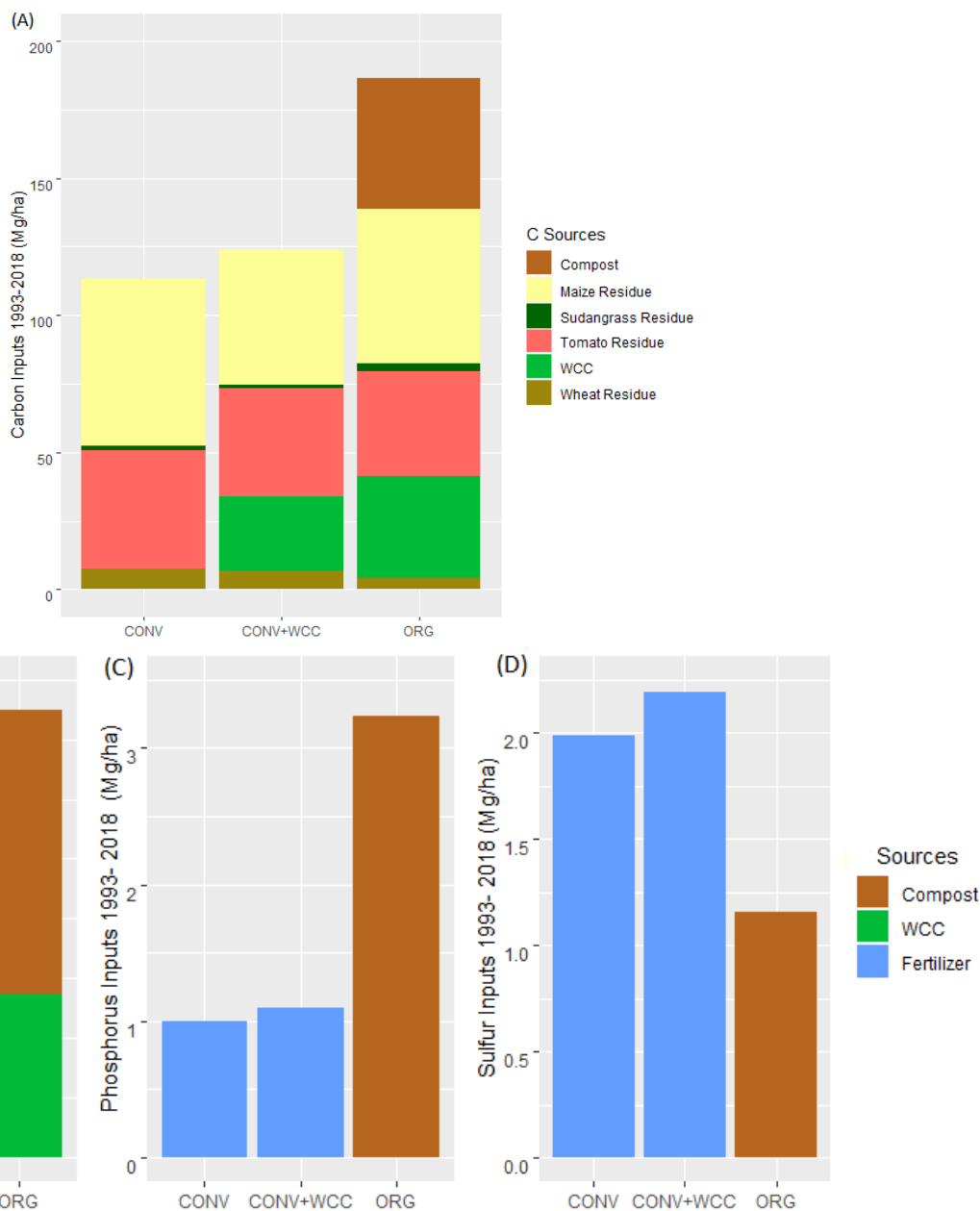
Figure 1a-d. Total aboveground carbon, nitrogen, phosphorus, and sulfur added per plot to ORG, CONV+WCC and CONV systems between 1993-2018. All values are given on a mass basis (Megagrams/hectare).

3.2 Soil Carbon Content Changes over 25 years

225 Carbon stocks in the 1 m profile of ORG systems showed a marginally significant increase of ~19 megagrams/hectare from 1993-2018 (p=0.06) (Figure 2). Most of this carbon 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), but the bottom 60-100 cm also showed a non-significant increase (~3 Mg ha^{-1}, p=0.26). No significant changes in carbon 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 (~ -
230   3 Mg ha^{-1}), at the 0-15 (p<0.01) depth (Figure 3). CONV+WCC systems did not show significant C decreases at any
individual depth.
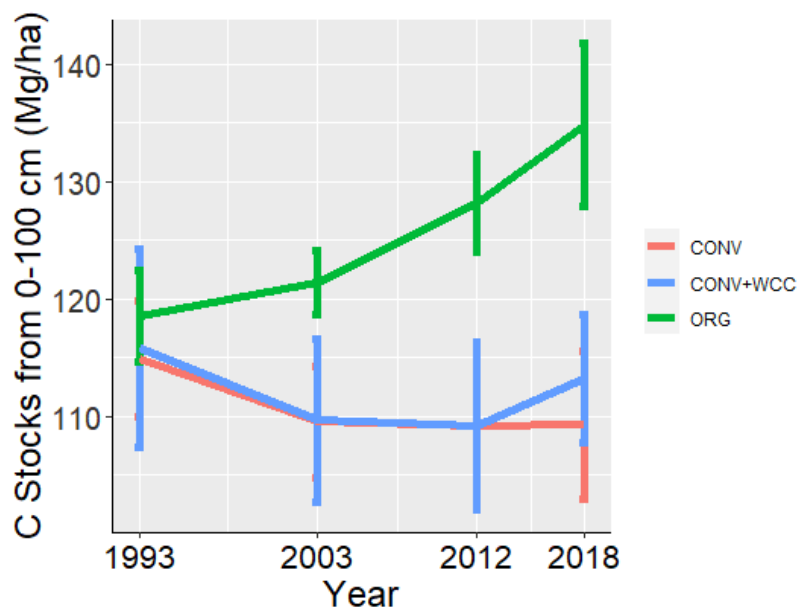
Figure 2. Carbon stocks of the 1m profiles of ORG, CONV+WCC and CONV systems from 1993 to 2018. Carbon stocks are given
235   **in Mg ha^{-1}. Error bars denote standard error.**
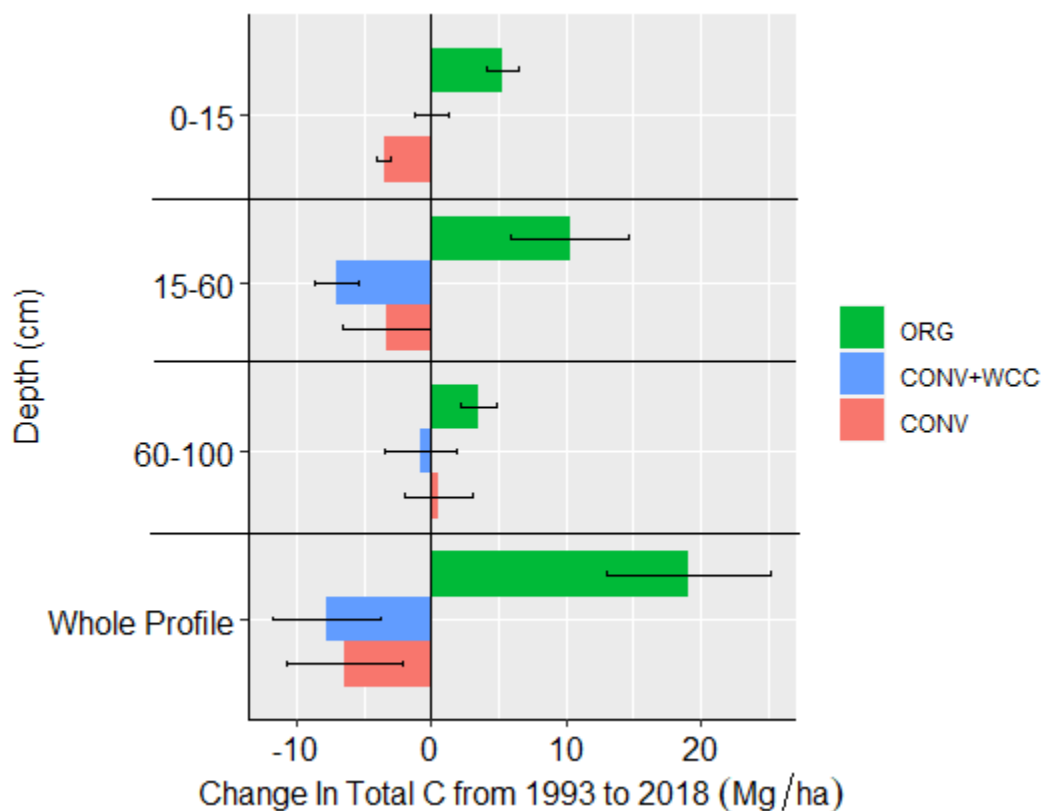
Figure 3. Change in carbon stocks of ORG, CONV+WCC and CONV systems from 1993-2018 by depth. Values were obtained by subtracting carbon stocks in 1993 from 2018 stocks for individual systems, and then averaging by management system. Error bars
240 **denote standard error.**

3.3 Moisture Content, Hydraulic Conductivity, and Cover Crop Roots

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 no significant difference
245 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).
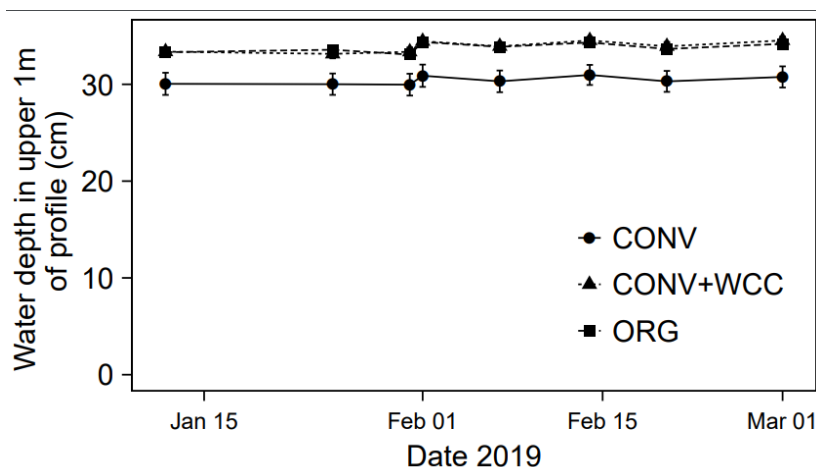
Figure 4. Sum of moisture content (in cm) in the upper 1m of ORG, CONV+WCC and CONV profiles during the Jan -Mar 2019 winter season. Error bars represent standard error.
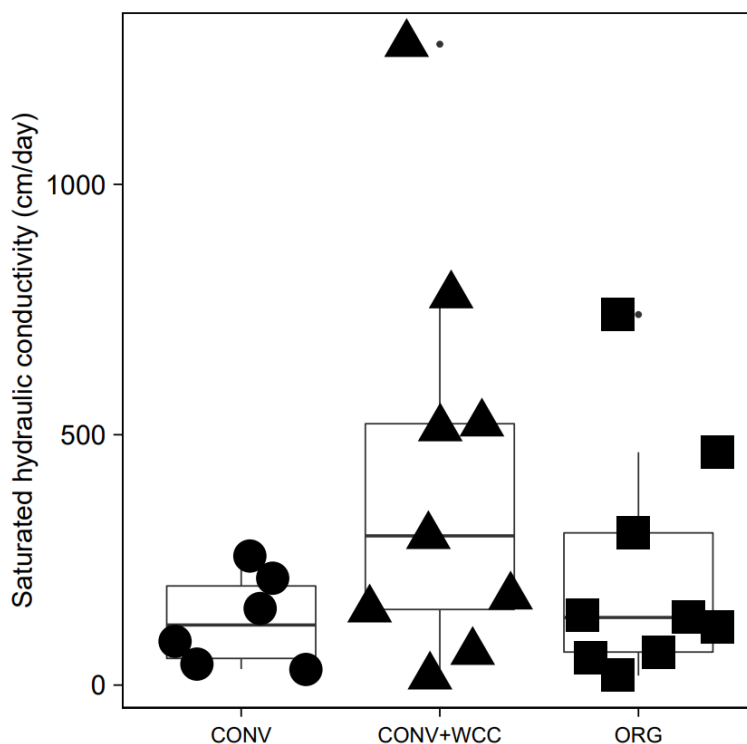
Figure 5. Saturated hydraulic conductivity (cm/day) in ORG, CONV+WCC and CONV systems taken in August 2018.

3.4 Soil Nutrient Content: Dissolved Carbon, Mineral Nitrogen, Phosphorus, Sulfur

Composted systems (ORG) had significantly higher amounts of dissolved organic carbon (DOC) (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) during the 2018-2019 year (Fig. 6). These differences were most pronounced in the upper 15 cm, where ORG systems had

approximately 2x more DOC (p<0.01), 3x more phosphorus (p<0.01) and 1.75x more sulfur (p<0.01) than CONV+WCC or
260 CONV systems (Supplementary Figure 1).
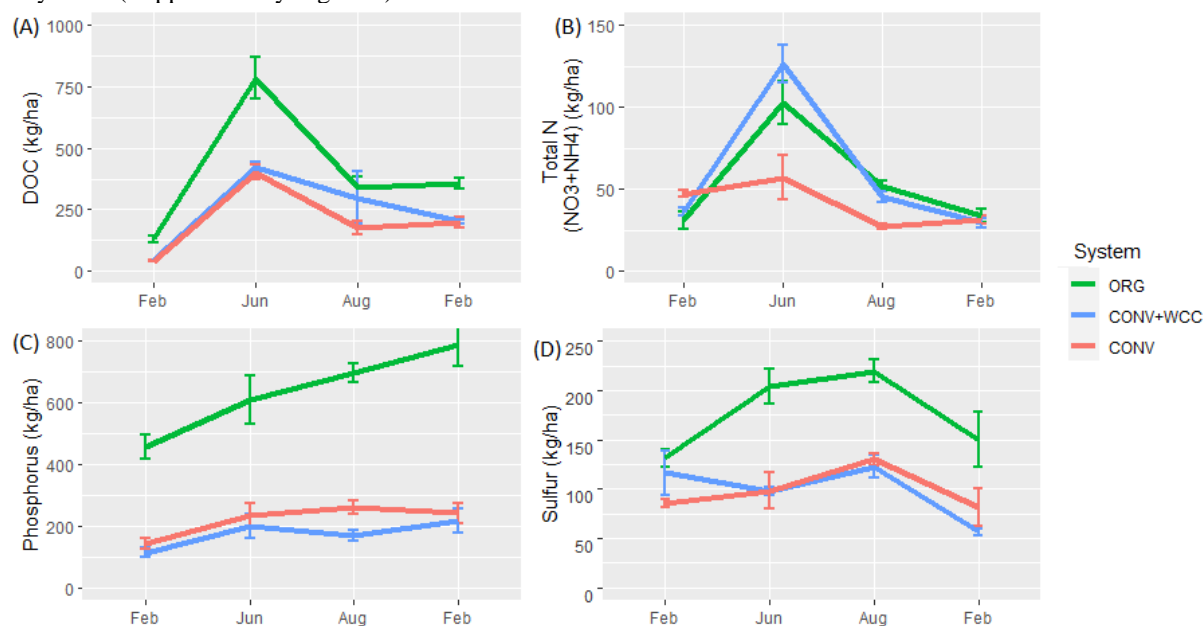
Figure 6a-d. Dissolved organic carbon, mineral N (NO$_3^-$+ NH$_4^+$), phosphorus and sulfur in 0-100cm profiles of ORG, CONV+WCC and CONV systems over the Feb 2018- Feb 2019 season. All values are given on a mass basis (kilograms/hectare). Error bars represent standard error.

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CONV+WCC systems had significantly more mineral nitrogen (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 nitrogen during the April - September growing season but were not significantly different from CONV (p=0.17).

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Nutrient values showed large seasonal variation, with the highest levels of carbon, nitrogen and sulfur observed during the June timepoint. DOC, mineral nitrogen, 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.

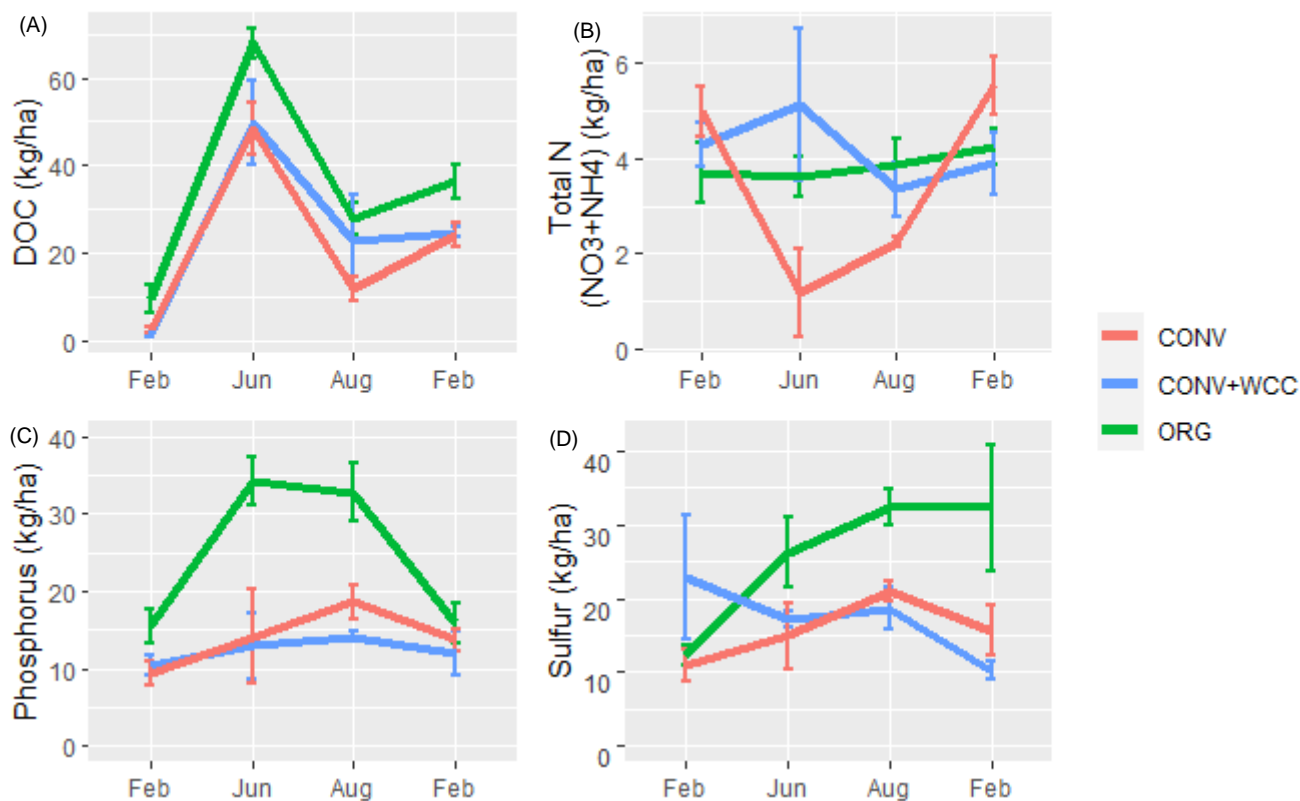
275 Differences among systems and seasonal variation were also noted at a depth of 60 cm. ORG systems had significantly more DOC (p<0.01), phosphorus (p<0.001), and sulfur at 60-100 cm than CONV+WCC or CONV systems. Mineral N values were not significantly different between any of the three systems at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

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Figure 7a-d. Dissolved organic carbon, 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 Aggregation

285 There was no significant difference in MWD of aggregates between all three systems at any depth (Supplementary Figure 3).

3.6 Soil Carbon Composition via FTIR

Spectral subtractions of 1993 and 2018 FTIR spectra revealed positive peaks 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
290 are presented in Supplementary Table B.

A distinct increase in aromaticity in ORG systems at all depths between 1993 and 2018 was denoted by bands at 1666 cm^{-1} and at 1602 cm^{-1} (Fig 8A). There is little noticeable difference in aromaticity in both the CONV+WCC and CONV spectra over this time period. All treatments and depths showed an increase in carboxylate functional groups between 1993 and
295 2018, denoted by bands at 1625 cm^{-1}, 1400 cm^{-1} and 1384 cm^{-1}.

Comparison of 2018 spectra showed higher amounts of aromaticity (1666 and 1602 cm^{-1}) for the top 0-15 cm in the order ORG>CONV+WCC>CONV (Fig 8B). ORG systems also showed more aromaticity at 15-60 cm than CONV+WCC or CONV, but this trend did not apply to the bottom 60-100 cm. Instead, higher amounts of carboxylate (1631 cm^{-1}) functional
300 groups were noted from 60-100 cm in the order ORG>CONV+WCC>CONV.
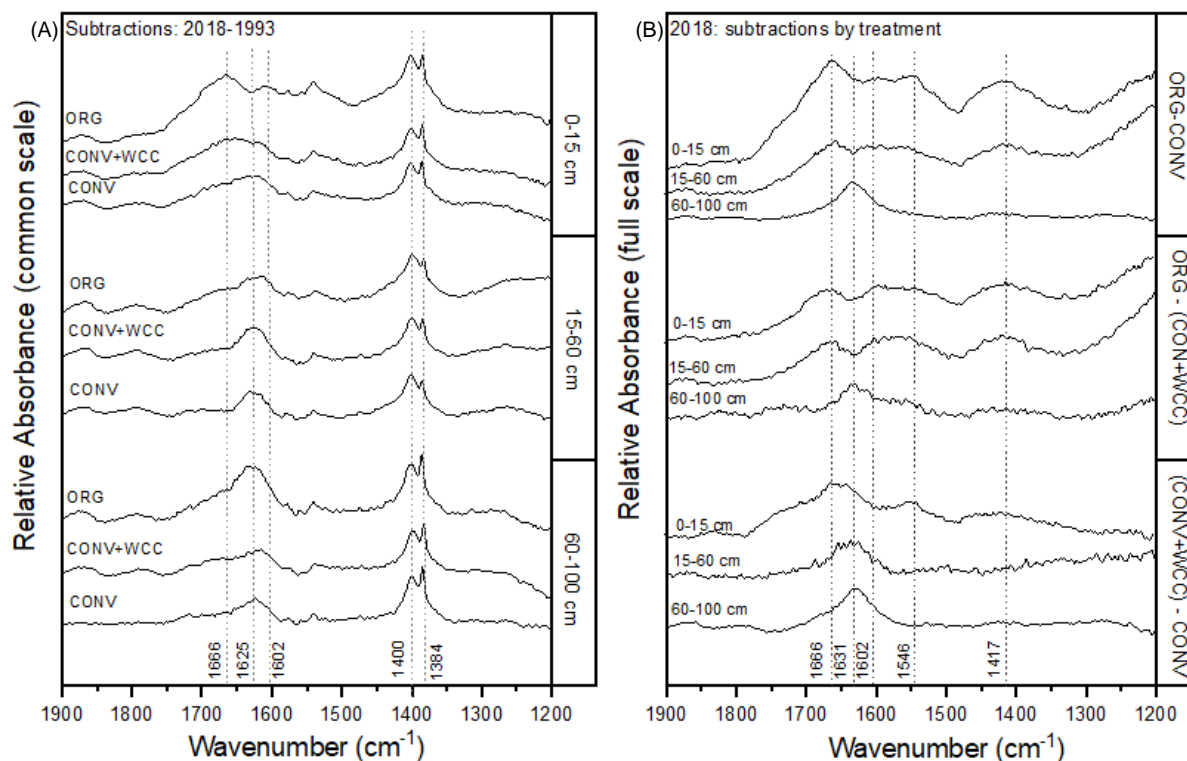
Figure 8a,b. FTIR spectral subtractions for the 1900-1200 cm^{-1} range comparing (A) 2018 -1993 spectra for ORG, CONV+WCC and CONV, and (B) ORG, CONV+WCC and CONV spectra in 2018.

305   3.7 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 having higher trending Cy19: pre (p=0.12) and 310   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 repsectively).
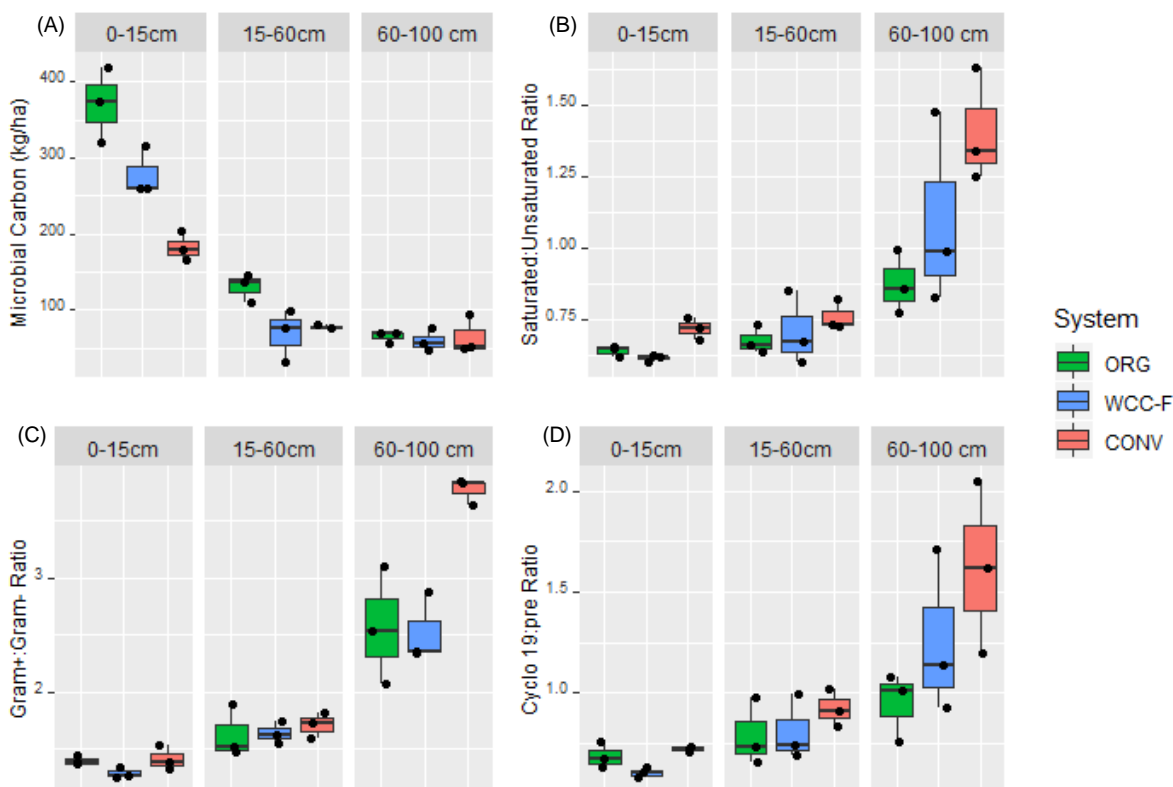
Figure 9a-d. Microbial biomass and PLFA stress indicators measured in ORG, CONV+WCC and CONV systems during mid-season (July 2018). Ratios are unitless, while microbial biomass is given in kg/ha.

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 carbon 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 subsurface.

4.1 Cover crop roots increased water storage and movement in subsurface soils

Cover crops increase both infiltration and hydraulic conductivity by increasing soil macroporosity and pore connectivity, particularly over longer time scales (Scott et al., 1994). This increase occurs due to an improvement in soil structure through aggregate formation, and a reduction in compaction due to increased organic matter content and formation of root channels (Chen and Weil, 2010; Franzluebbers, 2002). We measured a higher moisture content in CONV+WCC and ORG than CONV systems during the winter growing season, likely due to the presence of more water-filled spaces (Figure 4). However, we found no significant difference in aggregate MWD among systems (Supplementary Figure 4), potentially ruling out increased pore space due to aggregation as the cause of increased water content. We did find, however, more variable hydraulic conductivity in the two systems with cover crops (ORG and CONV+WCC, Figure 5). Roots may increase

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

335 Due to the fact that tillage in all systems would likely eliminate differences among them in the top 30cm, we would expect any differences in macroporosity and infiltration among treatments to be most affected by those roots that extend below the 30 cm plow layer (Hangen et al., 2002). Indeed, the mixture of cover crops in ORG and CONV+WCC included oats (~65-85 cm), fava beans (~52-70cm) and vetch (~30-85 cm), all of which have extensive rooting deeper than 30 cm (Fan et al., 2016).

340 **4.2 Compost + Cover Crops increased DOC and aromatic carbon in subsurface soils**

Compost application is associated both with high levels of mobile DOC (Wright et al., 2008; Zmora-Nahum et al., 2005), as well as a large proportion of aromatic functional groups, attributed to high amounts of lignin and other biomolecules (Leifeld et al., 2002). Research into the transport of organic molecules, such as described by the "cascade theory", has shown that "fresh" carbon inputs are preferentially retained within the top 30 cm (Liebmann et al., 2020) and that aromatic moieties are 345 rapidly removed from the soil solution at the surface (Leinemann et al., 2018). This removal 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. In the "cascade theory", carbon is transported downwards via a series of successive sorption, 350 desorption, processing, and transport steps resulting in highly processed, O-enriched carbon accumulating in the subsoil (Kaiser and Kalbitz, 2012). As this stepwise process is triggered by fresh DOC inputs, the regular application of DOC-rich compost, combined with increased hydraulic conductivity due to WCC roots, can accelerate this "cascade", leading to greater subsurface C transport. Increased DOC levels (Figure 6a), more aromatic carbon in the top 60 cm (Figure 8a), and relatively more oxidized carboxylate carbon in the bottom 60-100 cm of ORG plots point to an accelerated "cascade" 355 process in ORG systems relative to CONV+WCC and CONV systems.

CONV+WCC systems received more C than CONV systems, but the application of cover crops did not increase DOC or aromatic carbon content deeper than 15 cm when compared to CONV (Figure 7a). This may be because microbial processing of cover crop and crop residues need to occur at the surface before these residues can be transported deeper as DOC (Chantigny, 2003; White et al., 2020b). The increase in carboxylate carbon in CONV+WCC and CONV plots, 360 particularly in the bottom 60-100 cm after 25 years, does point to a "trickle" of soluble carbon inputs over time, albeit less than in ORG plots. Though our results do not provide sufficient data to determine the stability of the carbon at 60-100 cm, carboxylate functional groups would be more likely to form mineral-associated organic matter through cation bridging (Aquino et al., 2011) or association with charged surfaces, which could lead to C stabilization and increased storage times (Cotrufo et al., 2013; Leinemann et al., 2018).

365 **4.3 Compost + Cover crops increased nutrient availability and decreased microbial stress in subsurface soils**

The higher N, P and S values noted in ORG subsurface soils (Figure 7a-d) can be attributed both to the higher organic N, P, and S inputs associated with compost (Preusch et al., 2002), as well as the increased mobility of these inputs. Organic phosphorus is more mobile than mineral P (Laos et al., 2000; Sharpley and Moyer, 2000), and mineralization of organic N and S into their soluble forms of nitrate and sulfate could also facilitate their movement (Edwards, 1998; Vinten et al., 370 1994).

Greater C and nutrient input can also explain the lower microbial stress ratios observed in subsurface 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). While adding compost did not appear to increase microbial

biomass in ORG plots at 60-100 cm, it did increase biomass at 0-15 and 15-60 cm depths, attributed to more favorable
375   nutrient stoichiometry for biomass formation (Kirkby et al., 2011; Richardson et al., 2014). Increased surface microbial
biomass is an important potential source of C and other nutrients to subsurface layers through cell lysis from predation and
wet-dry cycles (Bonkowski, 2004; Xiang et al., 2008) and is associated with increased stabilized carbon storage through
microbial necromass formation (Buchmann and Schaumann, 2018; Jilling et al., 2020), contributing to the SOC increase
observed in ORG systems (Figure 2b).

380   **4.4 Cover crop carbon was not as effective as compost carbon in increasing subsurface SOC**

The SOC increases noted under the ORG system after 25 years are attributed to the increased mobility of compost-added
carbon and nutrients, as well as more microbial processing at the soil surface. Larger SOC increases under compost + cover
crops relative to cover crops alone have also been noted in other long term experiments (Brennan and Acosta-Martinez,
2017), indicating that carbon input from cover crops alone may not play as large of a role in increasing subsurface C. While
385   cover crop biomass does represent significant C and N input to surface soils, the "paths" their roots create for mobile
nutrients (either organic or mineral) to move downwards may be as important as their C and N inputs to subsurface SOC
dynamics.

There is evidence that root-associated C inputs appear to be more effective than aboveground biomass in building SOC
(Sokol and Bradford, 2019). The lack of an observed C increase in the CONV+WCC system was attributed to small inputs of
390   C and N over time from cover crop roots priming 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 destabilized organomineral complexes (Keiluweit et al., 2015), making that carbon more accessible for
decomposition. While any priming and destabilization of SOC due to cover crop roots would also be occurring in the ORG
systems, we believe this was counteracted by the higher DOC inputs and more favorable nutrient stoichiometry for microbial
395   biomass provided by the compost.

The trend of a SOC decrease over 25 years in CONV+WCC profiles is consistent with the results observed in Tautges and
Chiartas et. al (2019), but we observed a smaller decrease in SOC stocks from 1993-2018 below 60 cm. This difference may
be attributed to the high variability of soil C measurements (Kravchenko and Robertson, 2011; Wuest, 2014), but may also
be due to the switch from furrow to drip irrigation in 2014. The reduction in water inputs that occurs when switching from
400   furrow to drip can cause changes in C and N cycling enzymes (Schmidt et al., 2018), potentially increasing the prevalence of
complex SOM, and reducing microbial mineralization. The potential for complex interactions of tillage, cover cropping,
compost application and irrigation strategies in our experimental system indicate that care should be taken when applying
these results to different soil types and climates.

5 Conclusion

405   We believe that the combination of growing cover crops and compost amendment created a unique set of conditions
conducive to carbon transport and carbon accumulation in the subsurface. This was facilitated by increased soil macropores
created by cover crop roots leading to higher rates of transport of soluble C and nutrients from the surface to subsurface
soils. In turn, higher transport led to increased C stocks and reduced levels of microbial stress. The accumulation of oxygen-
rich carboxylate carbon in subsurface horizons under all treatments, attributed to an increase in microbially processed
410   carbon, and the accumulation of aromatic carbon under compost application, attributed to the lower solubility of aromatic
functional groups, provide support for the "cascade theory" of carbon transport. These results demonstrate the potential for
subsurface carbon storage in tilled agricultural systems, and highlight a potential pathway for increasing carbon transport,
storage, and sequestration in subsurface soil layers.

6 Appendices

6.1 Appendix A
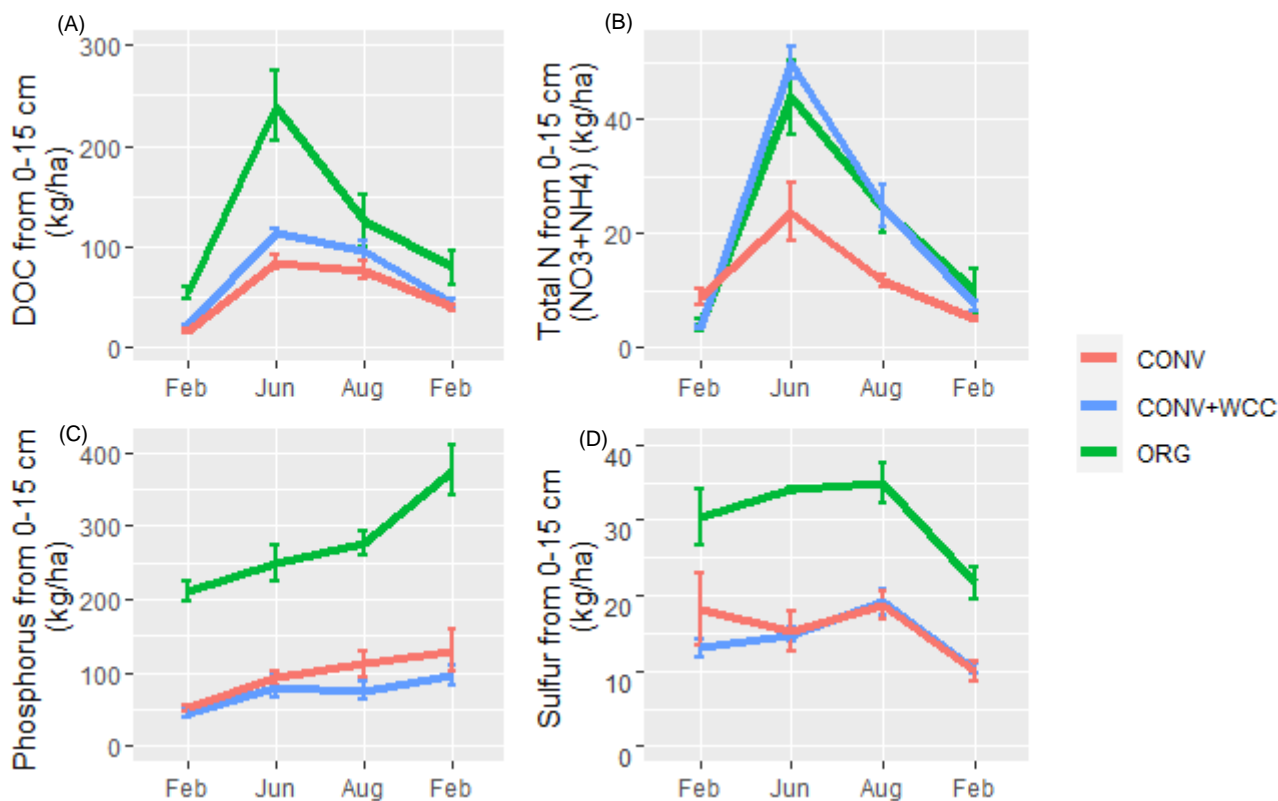
Figure A1. Dissolved organic carbon, mineral N, phosphorus, and sulfur stocks at 0-15 cm in ORG, CONV+WCC and CONV systems over the Feb 2018- Feb 2019 season. All values are given in kg/ha. Error bars represent standard error.
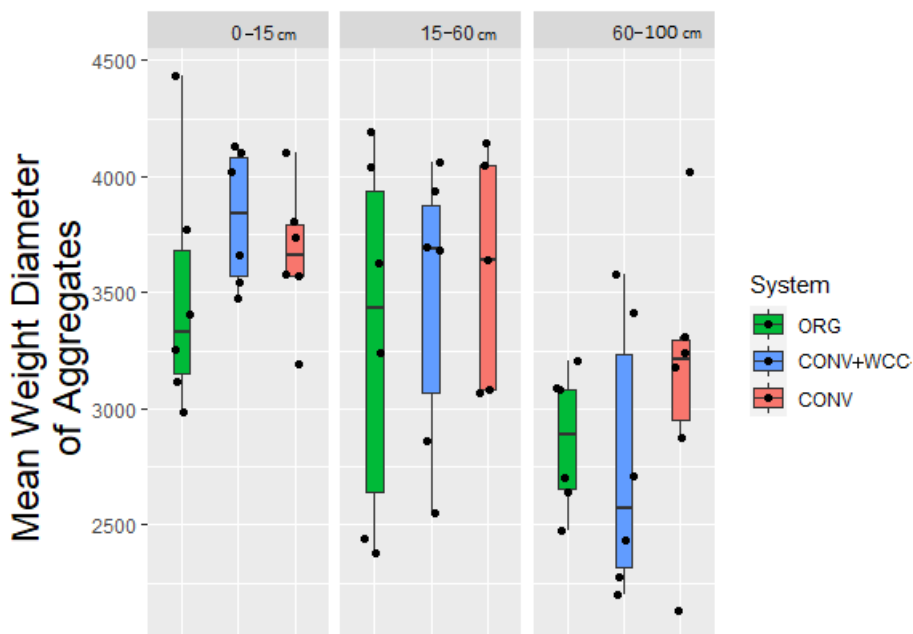
Figure A2. Mean weight diameter of aggregates obtained by wet sieving for 0-15, 15-60 and 60-100 cm depth intervals in ORG,
425   **CONV+WCC and CONV systems.**

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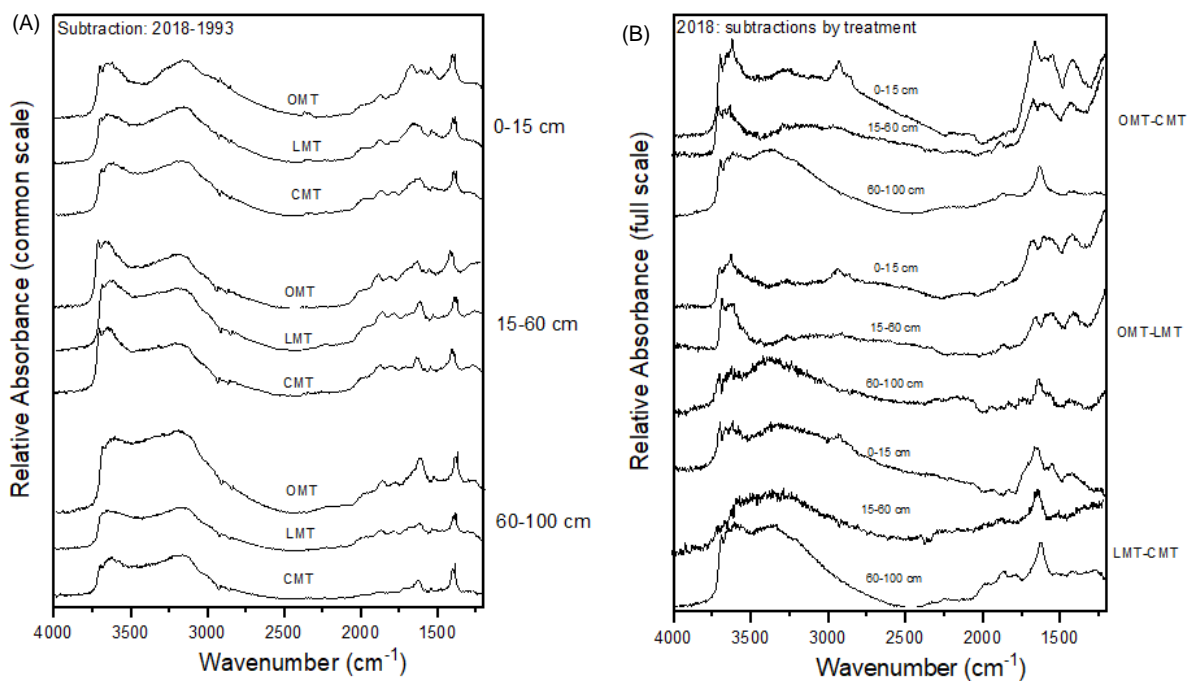
Figure A3a,b. FTIR spectral subtractions for the 4000-1200 cm⁻¹ range comparing (A) 2018 -1993 spectra for ORG, CONV+WCC and CONV, and (B) ORG, CONV+WCC and CONV spectra in 2018.

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

Wavenumber (cm⁻¹)	IR Assignment
2800-3100	aliphatic $\nu_s(CH_2)$, $\nu_{as}(CH_2)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$
1700-1765	$\nu(C=O)$
1666	aromatic $\nu(C=C)$
1620-1631	$\nu_{as}(COO)$
1602	skeletal $\nu(C=C)$
1546	aromatic $\nu(C=C)$
1417	$\delta(C-H)$

1400	ν_s(COO)
1384	ν(C-O) vibration aromatic and δ(C-H) vibrations in CH$_3$ and CH$_2$

Table A2 - FTIR Peak Assignments* used for analysis of spectra
*Assignments taken from

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7 Code availability

The code for the graphs and analyses in this manuscript is available at
https://github.com/danrath/2018_RRCARBON_DEPTH (DOI: https://zenodo.org/badge/latestdoi/181972884)

8 Data availability

The data included in this manuscript is part of the Russell Ranch long term dataset and is available at
https://github.com/danrath/2018_RRCARBON_DEPTH. (DOI: https://zenodo.org/badge/latestdoi/181972884)

9 Author contribution

NB, AAB, SY, KS and SP acquired funding for this project. DR, NT, NB, KS and DW designed the experiment and DR,
NB, DW, LD, SP, NT collected the data. DR, NB, LD, and SP performed data analysis and interpretation with assistance
from DW, NT, KS, SY and AAB. DR prepared the manuscript with contributions from all co-authors.

10 Competing interests

The authors declare that they have no conflict of interest.

11 Acknowledgements

Funding for this research was made possible by the University of California Office of the President, and the USDA National
Institute of Food and Agriculture (NIFA) through the UCOP Catalyst Grant, Hatch Formula Funding CA 2122-H and
multistate regional project W-2082. All opinions, findings, conclusions, and recommendations expressed in this publication
are those of the authors and do not necessarily reflect the view of the National Institute of Food and Agriculture (NIFA), nor
the United States Department of Agriculture (USDA). Authors would also like to express gratitude to Israel Herrera and the

Russell Ranch staff for field assistance during the project, and Danielle Gelardi and Oren Hoffman for offering comments on
465 the draft version of the manuscript.

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