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Comment on soil-2021-19

Daniel Rath et al.

Author comment on "Synergy between compost and cover crops in a Mediterranean row crop system leads to increased subsoil carbon storage" by Daniel Rath et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-19-AC1, 2021

Please find an updated methods section below, outlining:

1) Clearer delineation between novel and historic analyses

2) Citations for methods used in historic analyses

Due to the small reorganizational changes, I have reuploaded the entire methods section.

2 Methods2.1 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 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 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.

Soil % carbon and nitrogen 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). Carbon and nitrogen 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 carbon and nitrogen 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 carbon 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 carbon 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 changed significantly between 2012-2018 (Tautges and Chiartas et. al 2018), while bulk density sampling from 0-30 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 Management

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 m2 area in each plot prior to termination. Corn and tomato biomass residues were measured by cutting aboveground biomass at two 1.5 m2 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). 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. Dissolved organic carbon was determined using a 0.5 M potassium sulfate extraction. 6 g of soil were extracted with 0.5 M K2SO4 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 K2SO4 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 carbon and nitrogen values were measured on a CS 4010 Costech Elemental Analyzer (Costech Analytical Technologies) using air-dried, ball milled samples. 2018 carbon 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 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, 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 cm3 cores were collected in September 2018 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 m3 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 in 2019 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. 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.