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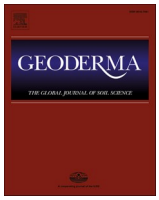

Compost amendment maintains soil structure and carbon storage by increasing available carbon and microbial biomass in agricultural soil – A six-year field study

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ABSTRACT

Soil organic amendments in agricultural production can benefit crop production and a wide range of soil properties, including soil aggregation. Soil aggregate formation is largely driven by microbial activities, and can in-turn influence microbial communities by generating distinct microbial habitats, as well as associated impacts on water and nutrient dynamics. We investigated the long-term effects of two fertilizer management strategies (poultry manure compost vs. mineral fertilizer) and biochar amendment (0 vs. 10 t ha^{-1} walnut shell biochar, 900 °C pyrolysis temperature, by-product of gasification) on soil aggregation, soil organic C, and microbial community dynamics in water-stable aggregate fractions in corn-tomato rotations. Using wet-sieving, soils (0–15 cm) were divided into four size fractions: large macroaggregates (2000–8000 μm), small macroaggregates (250–2000 μm), microaggregates (53–250 μm) and silt and clay (<53 μm) for calculation of mean weight diameter in both 2014 and 2018. The total C and microbial community composition and abundance within each fraction were evaluated in 2018. Across all treatments, six years of continuous compost application maintained soil aggregate stability and C storage by increasing soil microbial biomass and associated dissolved organic C. Bacterial and fungal populations under compost treatments were significantly higher than under mineral fertilizer treatments based on 16S rRNA gene copy number and internal transcribed spacer (ITS) abundance, which likely contributed to the formation and maintenance of macroaggregates in compost treatments. Interestingly, continuous application of manure compost may increase microbial available C sources by increasing the abundance of bacteria with the potential to degrade aromatic C as predicted from 16S sequences. Soil under the mineral fertilizer treatment showed decreases in the proportion of large macroaggregates, bulk soil C, and aggregate-associated C storage compared to the compost treatment. The application of highly recalcitrant walnut shell biochar had limited long-term impacts on soil aggregation and C dynamics, likely due to its lack of microbially-available C and limited interaction with the soil environment. Our results indicate that continuous compost inputs maintained soil structure and associated physical stabilization of SOM by enlarging soil microbial available C pool, higher soil microbial biomass, and increasing aggregate formation. The soil aggregate structure, in-turn, generated diverse habitats and altered soil microbial communities. Compost inputs, in addition to or in partial replacement of mineral fertilizer inputs, can provide valuable microbial-driven ecosystem services, such as carbon storage and soil structure, while still providing fertility for crop growth.

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1. Introduction

Soil aggregation, a frequently overlooked property of agroecosystems, plays many crucial roles: maintaining agricultural productivity, promoting soil C storage, providing habitats for soil biology, and regulating soil water dynamics. Soil water-stable aggregates can contribute to infiltration and water retention (Karami et al., 2012), help control runoff and erosion, and physically protect soil organic matter (SOM) leading to increased soil C storage (Six et al., 2004). Soil aggregate fractions and intra-aggregate pores of different sizes contain distinct physicochemical properties and thus can provide unique habitats for diverse microbial communities (Bach et al., 2018; Bailey et al., 2013; Davinic et al., 2012). Aggregate structure in agricultural soils is influenced by a series of factors, including soil biota (both microbes and macrofauna), plant root growth, soil mineralogy and texture, inorganic binding agents, and environmental conditions (Six et al., 2004).

Soil aggregate structure can help stabilize microbial communities and enhance interactions between microbes (Kuzyakov and Blagodatskaya, 2015; Raynaud and Nunan, 2014). Meanwhile, soil chemical conditions can drastically change over a short distance in soil (Raynaud and Nunan, 2014). Aggregation contributes to heterogeneity in soils by governing oxygen, water and nutrient availability and shapes microbial communities living inside or at the interface of aggregates (Briar et al., 2011). Furthermore, soil microbial communities can quickly respond to local habitat shifts associated with changes in soil aggregation dynamics (Blaud et al., 2012). A better understanding of the interaction between soil amendments, soil aggregate dynamics, and aggregate-associated soil microbial communities can inform management strategies to enhance soil biological activity and a range of desired soil functions.

Most large-scale agricultural systems have moved away from organic amendments with increasing reliance on synthetic fertilizers. Soil degradation, erosion, and soil organic matter loss are unfortunate consequences from the paucity of organic inputs that are common in conventional agricultural production (Lehman et al., 2015). For this reason, a variety of organic amendments such as compost (Diacono and Montemurro, 2011), animal manures (Mikha and Rice, 2004), and biochar (Atkinson et al., 2010) are being considered once again, and studies comparing their impacts on soils managed with synthetic fertilizer that dominate today are critically needed.

Soil organic amendments can enhance soil C storage through multiple mechanisms; these include increasing soil microbial biomass and activity (Liang et al., 2017), enhancing soil water-stable aggregation (Mpeketula and Snapp, 2019), and introducing recalcitrant C (Smith, 2016). Biochar is typically applied only once every few years (Major, 2010), while the application of manure-based compost tends to be more frequent (Larney and Angers, 2012), both to provide sufficient macro- and micronutrients for plants (Diacono and Montemurro, 2011) and support soil microbial communities (Kuzyakov and Blagodatskaya, 2015). Biochar has received considerable attention regarding its potential to increase soil carbon pools (Smith, 2016). Biomass feedstock composition, pyrolysis conditions and soil properties, such as clay content, can impact biochar stability (Wang et al., 2016b). Relatively higher pyrolysis temperatures, longer reaction residence time and slow heating are favorable pyrolysis conditions for generating more recalcitrant biochar with longer half-life (Leng and Huang, 2018), which was considered to have greater potential to increase the stable soil carbon pool. Biochar has been observed to have both positive and negative priming effects in both lab and field-scale studies and previous *meta*-analyses indicated that biochar application commonly induced a relatively short-term positive priming effect initially, followed by a negative priming effect (Joseph et al., 2021). Biochar containing labile C boosted soil microbial activities (Zimmerman et al., 2011) and biochar-induced microbial nutrient mining (Zhu et al., 2021) can generate positive priming effects. Biochar can also cause negative priming effects over a longer time period by substrate switching from biochar labile C to the use of soil organic carbon (Ventura et al., 2019) and substrate dilution (Whitman et al., 2014). Biochar C/N ratio, pyrolysis time, soil clay content, and soil C/N ratio affect the magnitude of the negative priming effect (Ding et al., 2018).

Under field conditions, biochar particles experience a decrease in their bioavailablity through association with clay particles and soil organic matter (Lehmann and Joseph, 2015). However, biochar surface functional groups may become exposed during its aging, which would then increase biochar-microbe interactions (Wang et al., 2020). Unlike highly recalcitrant biochar, compost and cover crops contain a higher variety and concentration of biologically-available nutrients that can be metabolized by microbes (Diacono and Montemurro, 2010). Soil water-stable aggregate dynamics driven largely by soil microbes play an essential role in soil C sequestration (Joseph C Blankinship et al., 2016). In a *meta*-analysis, Islam et al. (2021) reported that biochar properties, soil and environmental conditions all contributed to the impact of biochar on soil aggregation, while wood-sourced and high-temperature biochar were found to have the greatest effect on aggregation. The long-term effects of the combined application of compost and biochar on soil water-stable aggregate dynamics and C sequestration under field conditions remain understudied, especially in annual Mediteranean agroecosystems.

The objective of our study was to investigate the effects of two fertiliser treatments, namely poultry manure compost and mineral fertilizer, each with and without biochar, on soil water-stable soil aggregation, aggregate-associated C storage, and soil microbial community composition and abundance. We hypothesized that: (1) annual compost addition will increase soil C storage, (2) one-time highly recalcitrant biochar-C input will increase soil C storage, (3) multiple and continuous compost addition will increase soil microbial biomass in bulk soil and alter microbial community composition across aggregate fractions, and (4) compost amendments will increase soil aggregation and associated soil C storagecompared tounamended soil receiving only mineral fertilizer. To test these hypotheses, we compared the impacts of different fertility management practices and biochar amendment in a 6-year tilled, row crop field trial in northern California, representative of an annual Mediterranean agroecosystem.

2. Material and methods

2.1. Long-term field trial setup

The field site is located at the Russell Ranch Sustainable Agricultural Research Facility, University of California, Davis (Davis, California, USA; 38°32′47″N 121°52′28″W). The region has a Mediterranean climate characterized by dry arid summers and wet winters. The soil is a Rincon silty clay loam (fine, smectitic, thermic Mollic Haploxeralfs, 20% sand, 49% silt, and 31% clay; 11 g C kg^{-1}C content; 1.30 g cm^{-3} bulk density).

A field experiment was initiated in May of 2012 to investigate the impacts of soil amendments (poultry manure compost, biochar, and mineral fertilizer) on soil aggregation, C storage, and microbial communities in aggregate size fractions. The field was kept fallow for 10 years before 2012, except for a season of Montezuma oats grown between October 2009 and March 2010. The cropping system consisted of a 2-yr rotation of processing tomatoes (*Lycopersicon esculentum* Mill.) and corn (*Zea mays* L.). The farm management was based on practices and equipment similar to that used by local commercial growers. Biochar was applied once to half of the plots at the start of the experiment at a rate of 10 Mg ha^{-1} and disked in to a depth of 15 cm. The applied biochar was a by-product of gasification (pyrolysis temperature of 900 °C) derived from walnut shells and produced by Dixon Ridge Farms in Winters, CA (with 57.5 m^2 g^{-1} surface area, 40.4% ash content, 33.4 cmol g^{-1} cation exchange capacity, pH of 9.7, 55.3 wt% C, 0.47 wt% N, 0.89 wt% H, 1.6 wt% O, 0.64 wt% PO_4-P, 9.32 wt% K; see Mukome et al. (2013) for details). Additionally, two fertility management systems were tested with equivalent total N inputs, based on either: i) mineral

fertilizer (27.6 kg N ha^{-1} as urea-ammonium-nitrate 32 (UAN-32), 36.2 kg P ha^{-1}P as phosphorus pentoxide, 17.2 kg K ha^{-1} as potassium oxide, and 1.7 kg ha^{-1} of zinc chelate as starter fertilizer applied before planting each season; UAN-32 was applied at a rate of 134.5 kg N ha^{-1} three weeks after tomato transplanting and at a rate of 207.4 kg N ha^{-1} at the four-leaf growth stage in corn), or ii) poultry manure compost (8.97 Mg ha^{-1} applied yearly, adding on average 225.4 kg ha^{-1} total N, 119.5 kg ha^{-1} total P, and 155.4 kg ha^{-1} total K; including an incorporated winter cover crop for the first four years) (Griffin et al., 2017). This resulted in four treatments: 1) mineral fertilizer without biochar; 2) mineral fertilizer with biochar; 3) compost without biochar; and 4) compost with biochar, arranged in a randomized complete block design with four replicate blocks per treatment and one treatment replicate per block, making a total of 16 plots (Fig. S1). Each replicate plot was 4.6 m wide and 50 m long.

The average annual above-ground C input in the mineral fertilizer treatment without biochar was 4.30 Mg C ha^{-1} $year^{-1}$ as crop residue. Based on calculations for the adjacent Century Experiment at Russell Ranch that includes crop rotations with identical compost and cover crop management, the compost without biochar treatment received an average annual input of 7.27 Mg C ha^{-1} $year^{-1}$ for the first 4 years and 6.52 Mg C ha^{-1} $year^{-1}$ for the remaining duration of the experiment, of which approximately 2.22 Mg C ha^{-1} $year^{-1}$ was accounted for by the compost amendment (Tautges et al., 2019). The biochar-amended treatments both received 5.53 Mg C ha^{-1} as biochar-C (only in Year 1) in addition to the carbon inputs above.

2.2. Soil water-stable aggregate analysis

Water-stable aggregates were separated by size using a wet-sieving method adapted from (Elliott, 1986). In March 2014 and March 2018, three soil sub-samples were taken to a depth 15 cm from each field replicate using a soil knife and combined into a single representative soil sample. The field-moist soils were passed through an 8 mm sieve by gently breaking the soil clods by hand along natural planes of weakness. A 50 g sample of the moist, 8 mm sieved soil was then submerged in deionized water (at room temperature) on top of a 2000 μm sieve for 5 min. The sieve was then moved up and down (~3 cm) for 2 min (50 repetitions min^{-1}). The soil and water passing through the sieve were transferred by gently rinsing the material with deionized water onto the next smaller size sieve, and the same sieving procedure was repeated. Three sieve sizes (2000 μm, 250 μm and 53 μm) were used to generate four aggregate size fractions: 1) > 2000 μm (large macroaggregates); 2) 250–2000 μm (small macroaggregates); 3) 53–250 μm (microaggregates); 4) < 53 μm (silt and clay fraction). Two independent rounds of sieving were performed. First, one set of samples were obtained to quantify water-stable aggregates and conduct physicochemical analyses, in which all the aggregate fractions retained on each sieve were rinsed off the sieve in pre-weighed aluminum pans, oven-dried at 60 °C, and then weighed. The other set of samples was obtained for analysis of microbial community composition and abundance. The large and small macroaggregates and microaggregates retained on each sieve were rinsed off the sieve into sterile 50 mL polypropylene tubes. The silt and clay fraction was allowed to settle for a few minutes, and then subsamples of both sediment and supernatant were collected in a 50 mL sterile tube. All the aggregate size fractions were immediately stored at −80 °C until DNA extraction.

Mean weight diameter (MWD), an index of aggregate stability based on a weighted average of the four aggregate size classes, was calculated according to the following equation (Van Bavel, 1950):

where P_i is the weight percentage of the fraction in the whole soil and S_i is the average diameter (μm) for particles in its fraction.

2.3. C content and its change over time

The C content of bulk soils and of each aggregate size fraction was analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility.

2.4. Soil dissolved organic C and microbial biomass C

The fresh soil collected in March 2018 was also used to evaluate the impacts of different soil management treatments on soil dissolved organic C and soil microbial biomass-C. A representative bulk soil sample (8 g) was mixed with 40 mL 0.5 mol L^{-1} potassium sulfate in polypropylene tubes and placed on an orbital shaker (250 rev min^{-1}, 30 min). After shaking, samples were centrifuged (relative centrifugal force of 7969 × g for 15 min) to remove suspended solids. Supernatant solutions were retained for dissolved organic C concentrations (mg L^{-1}). The total microbial biomass-C was measured by chloroform fumigation (Joergensen, 1996; Yang et al., 2016). Both soil dissolved organic C and microbial biomass-C were measured using a TOC analyzer (Shimadzu TOC-VCSH analyzer, Kyoto, KYT, Japan).

2.5. Soil DNA extraction from aggregate fractions and amplicon sequencing

DNA from each soil aggregate size fraction was extracted using the Powerlyzer PowerSoil DNA Isolation kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The extracted DNA was quantified using the Qubit dsDNA HS Assay kit (Life Technologies, Carlsbad, CA, USA). The V4 hypervariable region of the bacterial 16S rRNA gene was amplified from each sample in duplicate using the primer pair 505F/816R (Caporaso et al., 2012), which was designed to include Illumina adaptors and 12 bp barcode sequences. The resulting amplicons were inspected by gel electrophoresis on a 1% agarose gel, quantified by fluorimetry as above, pooled in equimolar concentrations, and sequenced on an Illumina MiSeq platform (paired-end 250 bp) at the UC Davis DNA Technologies core facility. The raw reads were processed using DADA2 (Callahan et al., 2016) implemented in R v.3.4.4. Briefly, paired-end fastq files were processed by quality-trimming forward and reverse reads to 190 and 150 bp lengths, respectively. After sequence dereplication, merging, error-correction, and chimera removal, Exact Sequence Variants (ESVs) were inferred and taxonomy was assigned using the SILVA database v. 132. After quality control, the number of sequences per sample varied from 14,596 to 44,786, with an average of 28,724. The resulting ESV abundance table was rarified to 14,000 sequences per sample to ensure equal sampling depth for statistical analysis. The Functional Annotation of Prokaryotic Taxa (FAPROTAX) pipeline (Louca et al., 2016) was used to predict the functional potential of bacterial taxa identified in our dataset. Raw sequences were deposited at the NCBI sequence read archive (SRA) under BioProject accession number PRJNA644905.

2.6. Quantitative PCR

To assess prokaryotic and fungal gene copy number as a proxy for absolute abundance, quantitative PCR (qPCR) was performed on each DNA sample using the universal primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for the 16S rRNA gene (Rubin et al., 2014) and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) for the fungal Internal Transcribed Spacer (ITS) region (De Beeck et al., 2014). qPCR was performed in 20 μL reaction mixtures containing 10 μL SsoAdvanced Universal SYBR Green Supermix (Biorad, Hercules, CA, USA), 0.5 μM each primer, and 10 ng sample DNA. Reactions were carried out on a BioRad CFX Connect System (Biorad, Hercules, CA, USA) and amplification of the 16S rRNA gene consisted of an initial denaturation of 95 °C for 3 min, followed by 39 cycles of 95 °C for 10 s and 60 °C for 30 s. Amplification of the ITS region consisted of an initial denaturation of 95 °C for 3 min, followed

by 39 cycles of 95 °C for 10 s, 50 °C for 30 s, and 72 °C for 10 s. Quantification was performed by comparing unknown samples to a standard curve (ranging from 10^2-10^9 copies for 16S rRNA; 10^1-10^8 copies for ITS) generated with the pCR Blunt II-TOPO vector (Invitrogen, Carlsbad, CA, USA) containing a PCR-amplified fragment of each target. R^2 values for the standard curves ranged from 0.982 to 0.986 and 0.991–0.994 for the 16S rRNA gene and ITS region, respectively. Triplicate reactions were performed for each target per sample, and a melting curve analysis was performed after each assay to ensure specificity of the amplified products. The abundance of total 16S rRNA and ITS were normalized as copies per gram of soil aggregate fraction.

16S rRNA and ITS abundances of the bulk soil were estimated based on soil aggregation and copy numbers according to the following equation:

where is the copy number of the target for each soil aggregate fraction, and P_i is the weight percentage of the soil aggregate fraction in the whole soil, respectively, as above.

2.7. Statistical analyses

All soil physicochemical data were analyzed in Microsoft Excel for Windows 2010 with XLSTAT Version 2019.1 (Addinsoft, 2019) and were tested for assumptions of normality and homogeneity of variance. Statistically significant differences between treatments were analyzed using a mixed model analysis of variance (ANOVA) with biochar and fertility management considered as fixed effects and block was a random effect followed by a Tukey's range test. Statistical analyses were performed seperately for years 2014 and 2018 to avoid the potential confounding effects of soil moisture on the soil aggregation results and other results associated with aggregate fractions.

Non-metric multidimensional scaling (NMDS) plots were generated based on Bray-Curtis dissimilarities to visualize differences in bacterial community composition from our 16S rRNA sequences. Differences in bacterial community composition were tested by permutational multivariate analysis of variance (PERMANOVA) with the 'adonis' function (Oksanen et al., 2007) in R v.3.4.4 using Bray-Curtis dissimilarities and management practice, biochar treatment, and aggregate size fraction as predictor variables. All other microbial data, including FAPROTAX counts and qPCR values were tested for assumptions of normality and homogeneity of variance before performing ANOVA and a Tukey HSD post-hoc test to identify significant differences between treatments, with biochar and fertility management considered as main effects, block included as a random variable and aggregate fractions nested within each plot replicate. Natural log transformations were applied to meet the assumptions of ANOVA (normality, homoscedasticity) when necessary. For all analyses, statistically significant differences were defined at $P < 0.05$.

3. Results

3.1. Soil water-stable aggregates

Compost amended soil had significantly higher aggregate stability compared to mineral fertilizer treatments after six years, such that in March 2018, the MWD of soils managed with compost was 140% higher than those receiving mineral fertilizer (Fig. 1). Compost application maintained water-stable aggregation, in which the soil MWD in March 2018 remained the same as that in March 2014, while the mineral fertilizer treatments significantly decreased by ~48% over the four years (Fig. 1). The observed loss in aggregation under mineral fertilizer was primarily due to a significant loss in large macroaggregates (Table 1). Neither fertility management nor biochar amendment had a significant short-term (two years, in March 2014) effect on soil structure (Fig. 1a). Biochar amendment had no long-term effect on soil MWD under either fertilizer treatment (Fig. 1b).

3.2. Soil C content and C in aggregate fractions

Compost application resulted in significantly higher bulk soil C content compared to the mineral fertilizer treatment. After six years of compost amendment in 2018, the C content in the top 15 cm of soil was 12.4 g C kg^{-1}whole soil, which was 17% higher than the mineral fertilizer treatment (Fig. 2) and due largely to an increase in the large macroaggregate associated C (Table 2). This was in contrast to early in the trial, where two years of compost addition did not significantly affect bulk soil C content in 2014 (Fig. 2a). Surprisingly, the 10 Mg ha^{-1} biochar amendment had no long-term effect on soil C content or C distribution across aggregate fractions under either fertility management practice after six years (Fig. 2 and Table 2).

3.3. Soil dissolved organic C and microbial biomass-C

Compost addition significantly increased both soil dissolved organic C and soil microbial biomass C compared to the mineral fertilizer treatment. Soil dissolved organic C contents in compost treatments were 174.6 mg C kg^{-1} in the whole soil after 6 years, which was 54% higher

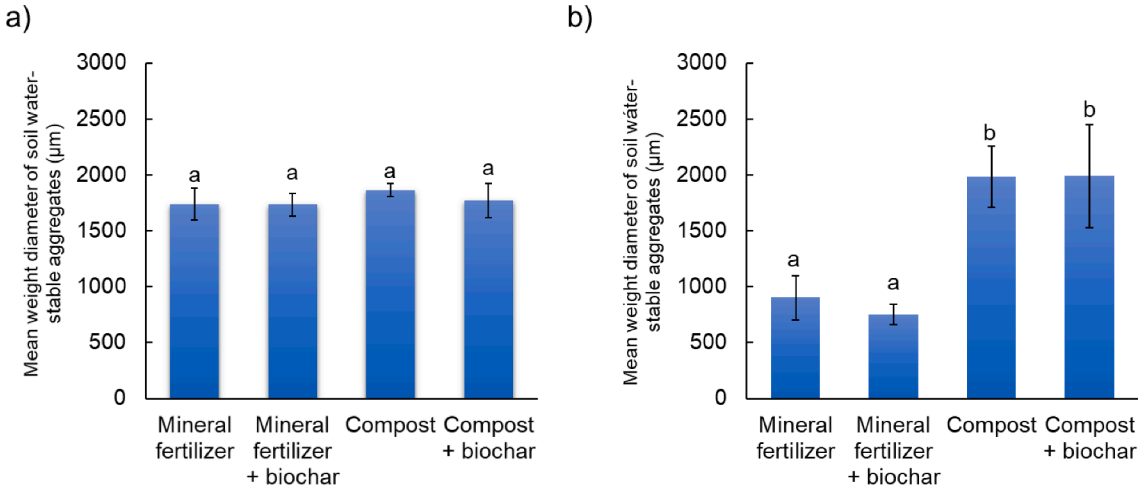

Fig. 1. (a) Soil aggregate stability (mean weight diameter) after 2 years (March 2014) and (b) soil aggregate stability (mean weight diameter) after 6 years (March 2018) under different management practices (compost and mineral fertilizer), with and without walnut shell biochar amendment (0 and 10 ton ha^{-1}); The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

Table 1
Distribution of water stable aggregate size fractions (% of whole soil mass) under mineral fertilizer and compost management, with and without biochar amendment (0 and 10 Mg ha^{-1}) after 2 (March 2014) and 6 years (March 2018). The numbers to the right of each value represent the standard error of the mean. Significant differences based on Tukey test between two different time points are indicated by different letters in parentheses to the right of each value.

Time	Treatment	Large macroaggregates (2000–8000 μm)	Small macroaggregates (250–2000 μm)	Microaggregates (53–250 μm)	Silt and clay (<53 μm)
		% of whole soil mass			
March 2014	Mineral Fertilizer	26.56 ± 2.39 (a)	33.52 ± 3.24 (a)	17.95 ± 1.15 (a)	21.97 ± 4.84 (b)
	Mineral Fertilizer + Biochar	26.87 ± 2.69 (a)	31.6 ± 3.78 (a)	18.01 ± 0.72 (a)	23.53 ± 2.55 (b)
	Compost	28.11 ± 1.68 (a)	37.61 ± 3.27 (ab)	19.92 ± 0.94 (b)	14.36 ± 2.39 (a)
	Compost + Biochar	25.39 ± 3.58 (a)	41.38 ± 2.59 (b)	21.06 ± 1.39 (b)	12.17 ± 1.91 (a)
	ANOVA	P-values			
	Fertilizer	0.985	**0.019**	**0.010**	**0.004**
	Biochar	0.552	0.646	0.530	0.879
	Fertilizer × Biochar	0.211	0.292	0.286	0.976
March 2018	Mineral Fertilizer	6.40 ± 3.75 (a)	46.80 ± 1.74 (a)	33.80 ± 3.36 (b)	13.01 ± 1.86 (ab)
	Mineral Fertilizer + Biochar	3.92 ± 1.34 (a)	44.02 ± 2.66 (a)	37.72 ± 4.11 (b)	14.33 ± 0.38 (b)
	Compost	29.80 ± 6.64 (b)	41.01 ± 4.95 (a)	18.53 ± 1.85 (a)	10.66 ± 1.12 (a)
	Compost + Biochar	30.27 ± 10.32 (b)	39.29 ± 5.13 (a)	19.98 ± 4.59 (a)	10.46 ± 1.59 (a)
	ANOVA	P-values			
	Fertilizer	**0.003**	**0.039**	**0.002**	**0.034**
	Biochar	0.822	0.484	0.187	0.279
	Fertilizer × Biochar	0.689	0.804	0.550	0.410

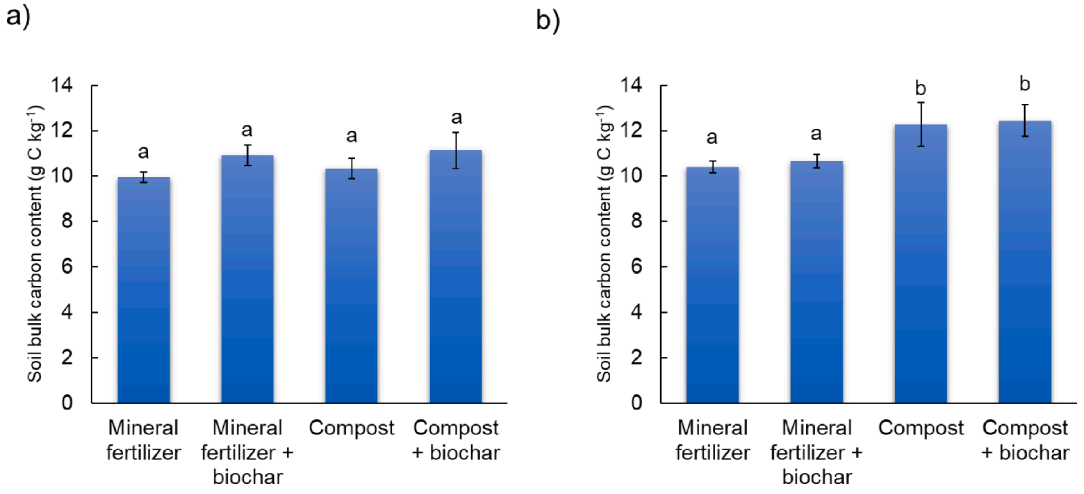

Fig. 2. (a) Soil bulk carbon content after 2 years (March 2014) and (b) soil bulk carbon content after 6 years (March 2018) under different management practices (compost and mineral fertilizer), with and without walnut shell biochar amendment (0 and 10 ton ha^{-1}); The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

Table 2
Soil C distribution in soil aggregate fractions (g C kg^{-1} whole soil) after 6 years (March 2018) under mineral fertilizer and compost management, with and without biochar amendment (0 and 10 Mg ha^{-1}). The numbers to the right of each value represent the standard error of the mean. Significant differences based on Tukey test between all four treatments are indicated by different letters in parentheses to the right of each value.

Fertilizer treatment	Biochar application rate (Mg ha^{-1})	Large macroaggregate C (2000–8000 μm)	Small macroaggregate C (250–2000 μm)	Microaggregate C (53–250 μm)	Silt and clay C (<53 μm)
		— g C kg^{-1} whole soil —			
Mineral fertilizer	0	0.80 ± 0.40 (a)	5.10 ± 0.15 (a)	2.88 ± 0.28 (bc)	1.61 ± 0.23 (ab)
	10	0.55 ± 0.17 (a)	4.92 ± 0.46 (a)	3.27 ± 0.41 (c)	1.91 ± 0.25 (b)
Compost	0	3.64 ± 0.82 (b)	5.26 ± 0.58 (a)	1.88 ± 0.14 (a)	1.49 ± 0.11 (a)
	10	3.63 ± 1.25 (b)	5.16 ± 1.02 (a)	2.24 ± 0.65 (ab)	1.42 ± 0.17 (a)
	ANOVA	P-values			
	Fertilizer	**0.004**	**0.005**	**0.008**	0.070
	Biochar	0.777	0.251	0.122	0.320
	Fertilizer × Biochar	0.769	0.698	0.953	0.063

than the mineral fertilizer treatment (Fig. 3a). Similar differences were also observed in soil microbial biomass C, which was 315.2 mg C kg^{-1} whole soil in compost, approximately two times as in mineral fertilizer treatments (Fig. 3b). Similar to total soil C, dissolved organic C and microbial biomass C were not influenced by an initial application of biochar under either treatment in 2018.

3.4. Soil microbial community composition and abundance in water-stable aggregate fractions

Compost management significantly increased both bacterial 16S rRNA gene and fungal ITS abundances across soil water-stable aggregate fractions, while biochar had no effect in year 2018 (Figs. 4 and 5). The weighted average gene abundance in bulk soil also indicated that the abundance of the 16S rRNA gene was 76% higher under compost than in soils receiving mineral fertilizer (Fig. 6a). The weighted average ITS copy number in bulk soil under compost addition was two orders of magnitudes higher than in soil under mineral fertilizer treatment (Fig. 6b). Nonmetric multidimensional scaling (NMDS) analysis of the 16 s rRNA gene at the ESV level revealed that soil prokaryotic community composition was significantly distinct in aggregate fractions of different sizes (Fig. 7). These differences were reflected by higher proportions of bacteria from the orders *Micrococcales*, *Streptomycetales*, *Propionibacteriales*, and *Sphingomonadales* in the microaggregate and silt and clay fractions, and higher proportions of the *Gaiellales*, *Gemmatimonadales*, *Nitrososphaerales*, and *Nitrospirales* in the large and small aggregate fractions. Across all aggregate size fractions, both compost and mineral fertilizer treatments had significant but limited effects on bacterial community composition, whereas biochar had no effect (Fig. S2).

Predictive assignment of soil microbial functions based on the 16S rRNA gene sequencing results revealed that the abundance of bacteria potentially capable of degrading aromatic C compounds was higher in compost than mineral fertilizer treatments in 2018 (Fig. 8). No differences were detected for predicted functions related to many major C (methanotrophy, methylotrophy, chitinolysis, cellulolysis, xylanolysis, non-methane aliphatic hydrocarbon degradation, hydrocarbon degradation) and N (aerobic ammonia oxidation, aerobic nitrite oxidation, nitrification, nitrate reduction, ureolysis) cycling pathways between the two treatments.

4. Discussion

4.1. Impacts of external C inputs on soil C storage

Our findings suggest that continuous compost application is more effective at increasing soil C sequestration by maintaining soil structure and stabilizing SOM than in soils not receiving compost. In contrast, without compost inputs, there were significant decreases in aggregate structure, which in turn was associated with significant C loss. Poultry manure compost is rich in multivalent ions, such as Ca and P (Griffin et al., 2017), which can generate a bridging effect to enhance sorption of SOM to clay minerals (Feng et al., 2005) and increase soil aggregation (Bronick and Lal, 2005). The lack of sufficient C input and associated aggregate structure in the mineral fertilizer treatments could lead to greater exposure and more rapid decay of native SOM (Dungait et al., 2012).

Surprisingly, walnut shell biochar had little effect on soil C in the top 15 cm. Previous research has hypothesized that recalcitrant C compounds are the major contributors to C sequestration from biochar (Cheng et al., 2008; Harvey et al., 2012), especially high-temperature biochars like the type applied in our study, which has a high proportion of recalcitrant to labile C (Zimmerman, 2010). The O/C and H/C atom ratios of the walnut shell biochar were 0.0217 and 0.193, respectively (Mukome et al., 2013a), which indicated that the walnut shell biochar-C was relatively stable and potentially had a longer than 1000-year half-life (Spokas, 2010). A large amount of biochar particles (with a diameter of around 2–4 mm) was easily observed and recovered in the surface soil at the end of the field trial. We did not detect soil bulk C content difference between no-biochar and biochar-amended treatments under the same management practice in either year 2 or year 6 (Fig. 2), which indicated a positive priming effect at the beginning of the field trial. The walnut shell biochar was high in potassium ion (Mukome et al., 2013b). The increase in soil monovalent ion concentration can enhance mobility of soluble SOM and dispersion of organic matter and clay particles (Chow et al., 2006), which can both result in a short-term positive priming effect due to increased microbial available substrates and microbial activities (DeCiucies et al., 2018). A 14-month lab incubation study using similar soil and the same biochar (with application rates equivalent or doubled as in the field study) also showed evidence of positive priming effect and potential soil native organic C loss within the timeframe of the incubation (Wang et al., 2017). Additionally, we speculate that in the field, some biochar particles may migrate from the point of application and leave the surface soil through irrigation, wind

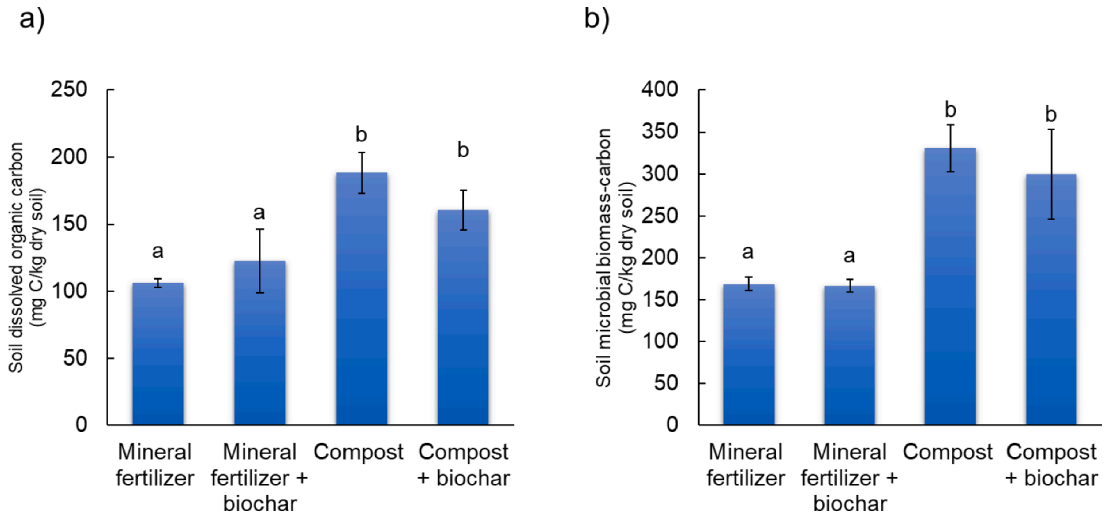

Fig. 3. Soil dissolved organic carbon and soil microbial biomass carbon after 6 years (March 2018) under different management practices (compost and mineral fertilizer), with and without walnut shell biochar amendment (0 and 10 ton ha-1). The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

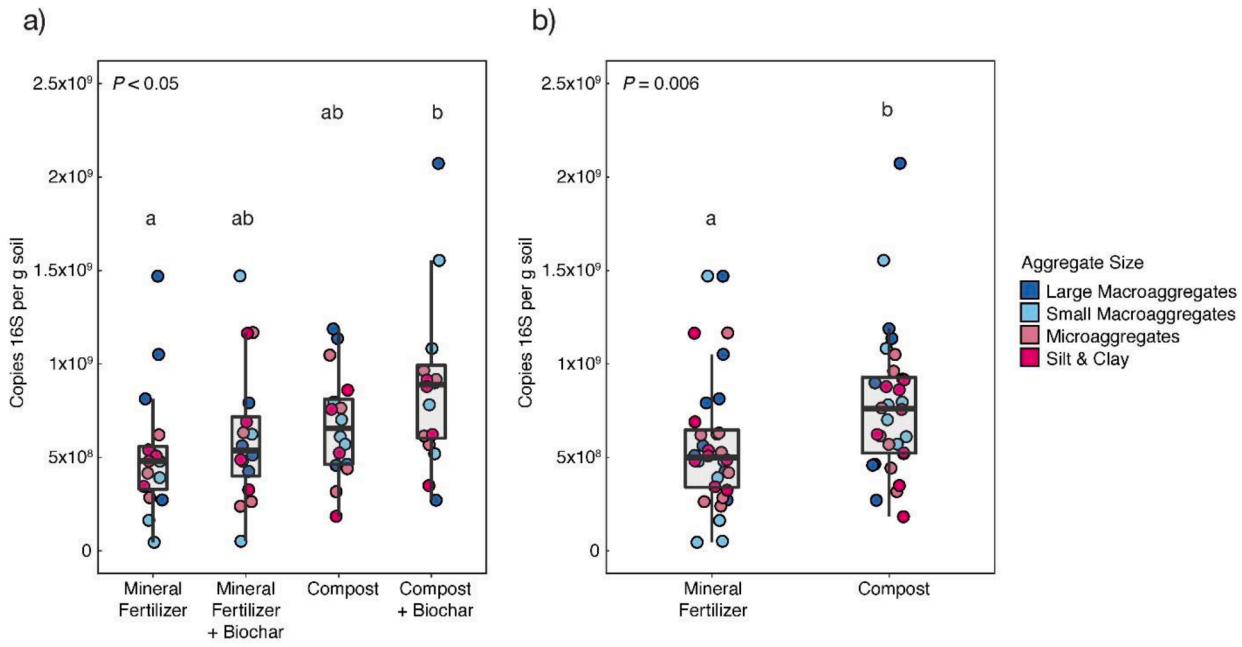

Fig. 4. 16S gene copy number after 6 years (March 2018) under different management practices. In Fig. 4(b), the with/without biochar treatments under the same fertilizer treatment were combined and compared since there was no significant difference between with/without biochar treatments and there was no interaction between fertilizer treatment and biochar treatment.The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

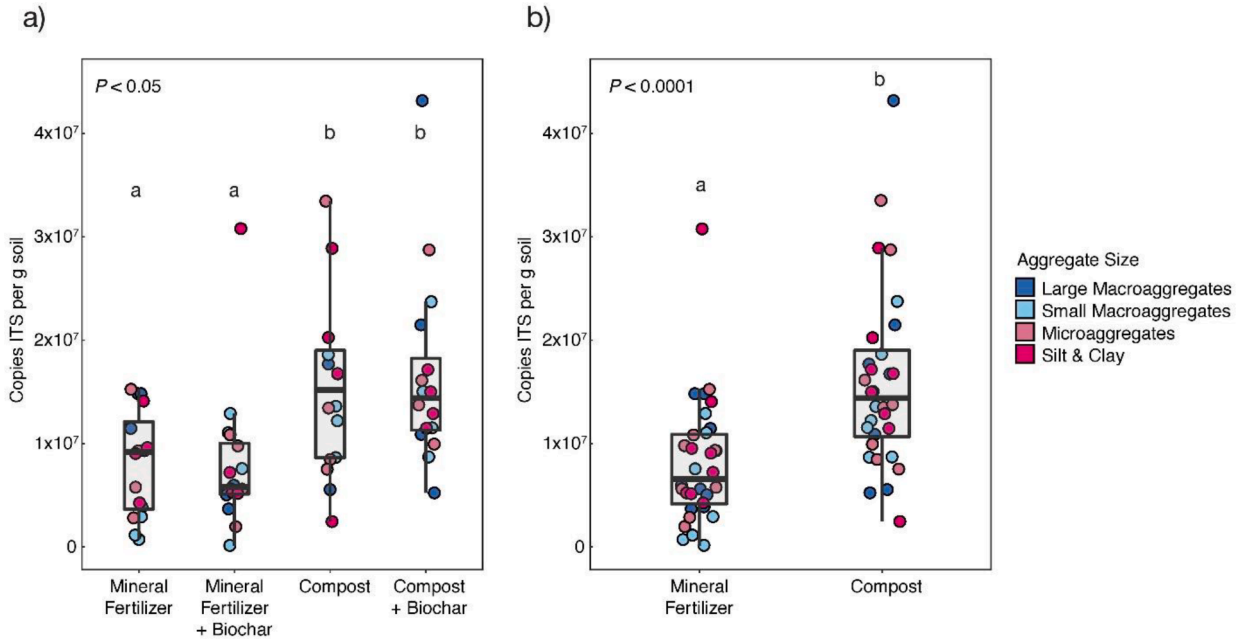

Fig. 5. Internal transcribed spacer (ITS) copy number after 6 years (March 2018) under different management practices. In Fig. 5b, the with/without biochar treatments under the same fertilizer treatment were combined and compared since there was no significant difference between with/without biochar treatments and there was no interaction between fertilizer treatment and biochar treatment. The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

erosion (Gelardi et al., 2019), or vertical movement (Singh et al., 2015), thus decreasing their impact on soil properties. The walnut shell biochar contains some fine particles, which can be readily mobilized during irrigation events in the first growing season. We observed some fine biochar particles in the surface runoff during furrow irrigation at the beginning of the field trial.

4.2. Interactions between agricultural management, soil structure and soil aggregate-associated microbial communities

Our findings suggest that continuous compost addition can potentially generate a positive feedback loop for enhancing soil C storage by increasing microbially-available substrates, in turn increasing and maintaining soil microbial biomass, and thereby increasing aggregation through microbial activities. Soils received ~60% more C in the

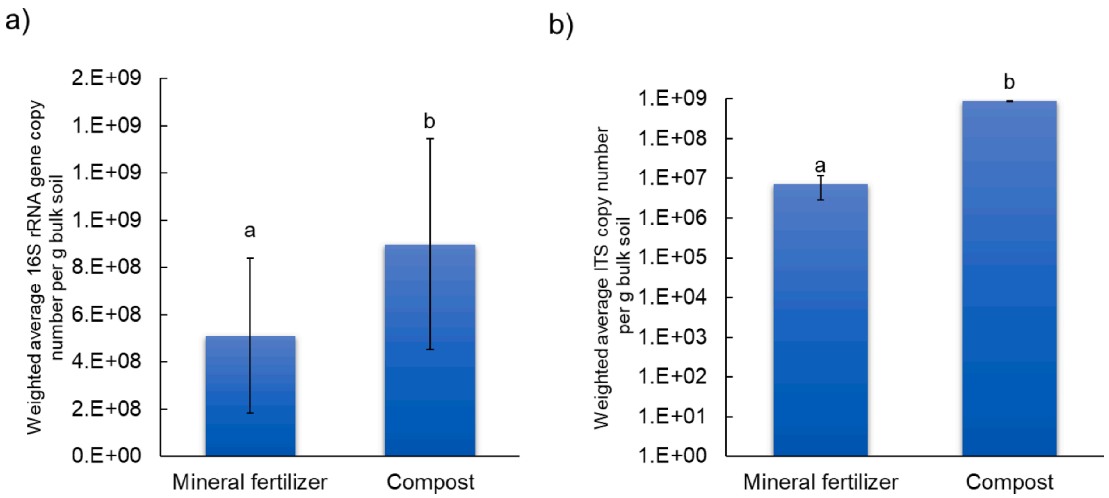

Fig. 6. Weighted average 16S rRNA gene (a) and internal transcribed spacer (ITS) copy numbers (b) in bulk soil after 6 years (March 2018) under different management practices. The with/without biochar treatments under the same fertilizer treatment were combined and compared since there was no significant difference between with/without biochar treatments and there was no interaction between fertilizer treatment and biochar treatment. The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

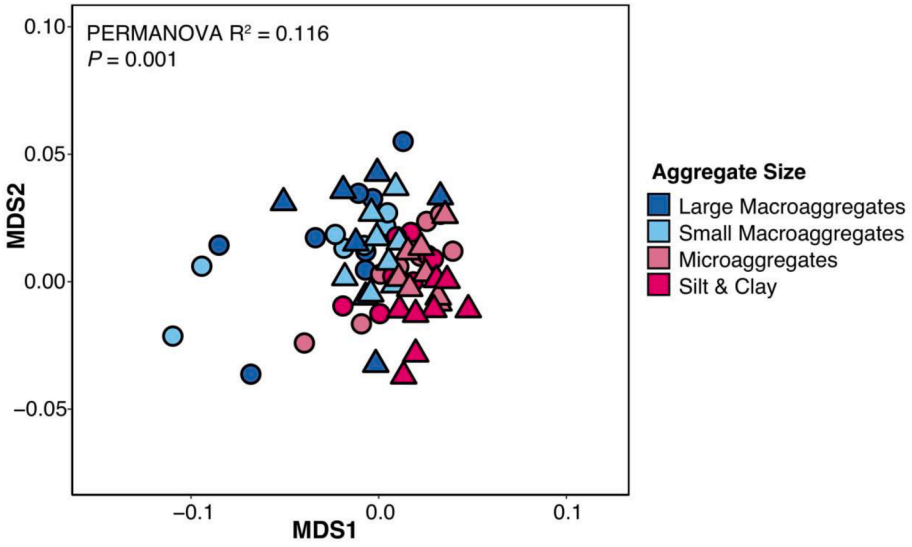

Fig. 7. Nonmetric multidimensional scaling (NMDS) analysis of soil microbial communities with ESVs (triangles indicated samples from mineral fertilizer treatments, circles indicated samples from compost treatments) after 6 years (March 2018). Different colors and symbols represent different water-stable aggregate fractions.

compost-amended than mineral fertilizer treatments. Compost also contains a diverse range of C sources for soil microbes (Barker, 1997), and its addition helped maintain higher soil labile C (Fig. 3a) and microbial biomass (Fig. 3b) compared to in the mineral fertilizer treatments. The compost-induced increase in soil microbial biomass also corroborated our findings of increased 16S rRNA gene and ITS copy numbers across aggregate size fractions (Figs. 4 and 5) and in weighted bulk soil averages (Fig. 6). Compost amendment enhanced soil aggregation by providing a large amount of labile C (Amlinger et al., 2003) as feedstock for microbes to produce extracellular polymeric substances, which can serve as binding agents for soil aggregates (J C Blankinship et al., 2016; Miltner et al., 2012). The increased fungal biomass (Figs. 5 and 6) can also promote soil aggregation by binding and entangling soil particles to form macroaggregate structure (Van Der Heijden et al., 2006). Enhanced soil aggregation provides more diverse habitats for organisms, which can lead to increases in soil microbial diversity (Briar et al., 2011). Furthermore, the soil aggregate structure can enhance the interactions within microbial consortia responsible for metabolizing complex organic compounds (Wilpiszeski et al., 2019). The shift we observed in bacterial communities in different aggregate size fractions (Fig. 7) provides evidence for the paradigm that unique aggregate microenvironments can provide niches to support the development of distinct microbial communities, which can further benefit soil and ecosystem properties (Bach et al., 2018; Wilpiszeski et al., 2019).

Across all aggregate size fractions, we also observed distinct differences in prokaryotic community compositions between compost and mineral fertilizer treatments (Fig. S2). Previous research investigating a similar soil (Yolo silt loam) from a field adjacent to this trial indicated that the dissolved organic matter from the soil in our field trial was highly aromatic (Wang et al., 2016a). Our FAPROTAX predictive results also indicated a higher abundance of bacteria potentially capable of degrading aromatic C compounds in compost treatments (Fig. 8), which suggests that microbial communities under continuous compost application are potentially capable of utilizing a wider range of C sources from both soil amendments and native soil organic matter, which further enriched soil microbially-available nutrients. Such feedback can

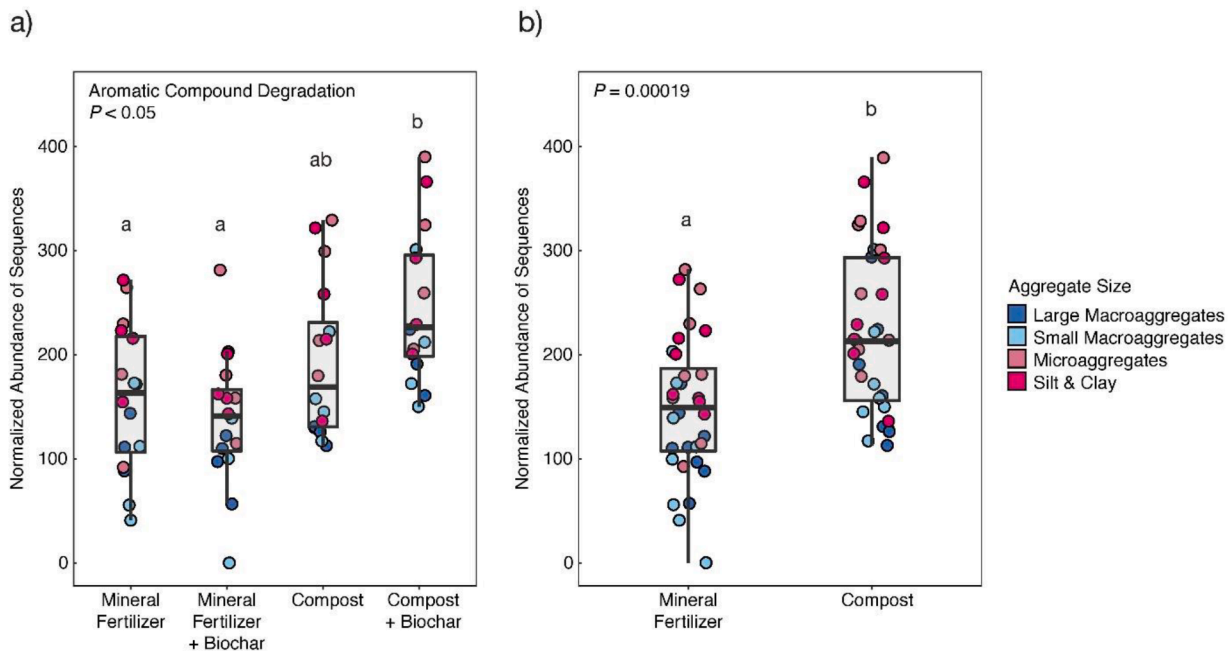

Fig. 8. Faprotax predictive assignment of abundance of bacteria capable of degrading aromatic C compounds based on the 16S sequencing after 6 years (March 2018). In Fig. 8(b), the with/without biochar treatments under the same fertilizer treatment were combined and compared since there was no significant difference between with/without biochar treatments and there was no interaction between fertilizer treatment and biochar treatment. The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

potentially enlarge the active C pool in the agroecosystem which, in turn, could maintain a higher level of soil aggregation associated microbial activities. Other soil C and N cycling pathways did not show differences since the sampling time was 300 days after the yearly compost amendment.

Compost application may have increased macroaggregate formation by maintaining higher fungal populations across aggregate fractions. Although both bacteria and fungi populations were higher in the compost than mineral fertilizer treatments (Figs. 5 and 6), the difference in fungal abundance was two orders of magnitudes higher in compost than mineral fertilizer treatments (Fig. 6b). Higher fungal biomass density has been linked with increased soil aggregate formation (Lehmann et al., 2020) and fungi have been suggested to contribute more than prokaryotic communities to macroaggregate formation due to the enmeshing properties of their hyphae (Lehmann et al., 2017). The major difference in aggregation between the mineral fertilizer and compost treatments was due to the loss of large-macroaggregates under mineral fertilizer (Table 1), which suggested that the differences in fungal community abundance could have been a major contributor to the observed differences in soil structure. Similar to our finding, Li et al. (2019) in a long-term field study in Guizhou, China, reported that soil fungal abundances across aggregate fractions were maintained when mineral fertilizer was supplemented with manure but decreased in the absence of amendments; aggregate-associated C content also declined. Increased macroaggregate structure in compost treatments can, in turn, enhance the resistance of soil microbial habitats to environmental disturbances (Rillig et al., 2017). Our results support the hypothesis that impacts of management practices on soil C are dependent on changes in soil aggregation and aggregate-associated microbial communities (Trivedi et al., 2017). As shown in Table S1 and summarized in Griffin et al. (2017), despite the benefits associated with soil aggregation and C dynamics, crop yield in compost treatments were significantly lower than those in mineral fertilizer treatments due to the uncertainty of nitrogen availability in compost amended soil and the asynchronous nitrogen supply and demand, which is a common tradeoff for similar practices (Seufert et al., 2012).

Interstingly, we found no effect of walnut shell biochar on soil microbial community composition or any soil parameters in our study, unlike what has been observed in previous studies investigating at other types of biochar (Jiang et al., 2016; Khodadad et al., 2011; Zhu et al., 2019). This may be due to the differences in biochar feedstock, pyrolysis conditions, soil and other environmental factors, or the fact that biochar under field conditions behaves differently than in the lab (Islam et al., 2021). It is possible that biochars produced at high temperatures (900 °C) contain little labile organic C compared to low temperature biochar and hence are not capable of supporting growth of microbial populations. Another explanation for the lack of impact may be the limited accessibility of biochar pores to microbes, despite its relatively high surface area and pore volume (Mukome et al., 2013a). The interparticle pore structure in biochar is rapidly filled by soil particles after addition to soil (Lehmann and Joseph, 2015). Joseph et al. (2010) found that biochar internal pores started to fill in with organic and mineral matter after 1 year and most pores were filled 2 years after application in a field experiment. Ameloot et al. (2014) also found that biochar stopped serving as microbial substrate soon after application (~60 days) and had little long-term impact on soil microbial biomass and activities under field conditions. While previous studies have shown that biochar can alter soil microbial communities and potentially increase microbial interactions, most of these studies have been conducted in highly weathered oxisols with limited impacts observed in soils with high fertility (Yu et al., 2018). We speculate that soil amendments with higher amounts of non-pyrolyzed and/or microbially-available organic C can help achieve agricultural management targets, such as enhancing soil aggregation and C storage more effectively.

5. Conclusion

Our findings indicate that continuous compost amendment can potentially generate a positive feedback loop for soil aggregate formation and associated C storage by maintaining higher dissolved organic C content, increasing microbial biomass, and supporting large-macroaggregate formation. These processes, in turn, promote the ability of the microbial community to utilize more diverse C sources. In fine-textured soil, biochar had a limited impact on the soil microbial

community, soil aggregation, and C dynamics, likely due to low microbial available C and limited interaction with the environment. Long-term continuous diversified C source amendment, such as adding compost, could be an effective agricultural management practice to not only maintain but increase soil microbial biomass, aggregate formation, and soil C storage, and also replace some mineral fertilizer which could reduce greenhouse gas emissions associated with fertilizer production. An integrated management practice that carefully balances compost and mineral fertilizer composition can potentially balance the benefits and tradeoffs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.116117.

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