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Biochar additions can enhance soil structure and the physical stabilization of C in aggregates

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1. Introduction

Demand for agricultural products continues to increase with a growing population and shifting diets (Lotze-Campen et al., 2008). Global change, driven in part by the intensification of agriculture, is one of the major challenges of our time and, in turn, threatens the long-term sustainability of agricultural production (Vrochidou et al., 2013). Development of innovative agricultural technologies to address these challenges has thus become a major research priority due the need to achieve food security, protect soil resources, and both mitigate and adapt to global change.

Biochar is a C-based byproduct of biomass pyrolysis in an oxygen-depleted environment (Lehmann et al., 2011). The production of biochar and its use as a soil amendment is regarded as a promising strategy for soil carbon (C) sequestration (Lehmann, 2007) and has been shown to offer a range of agricultural benefits, such as reduced nutrient leaching (Laird et al., 2010), increased soil cation exchange (Liang et al., 2006) and water holding capacity (particularly in sandy soils) (Kammann et al., 2012). Biochar can also influence soil greenhouse gas (e.g., nitrous oxide and methane) emissions (Sagrilo et al., 2015; Verhoeven et al., 2017) and crop yields (Major et al., 2010; Schnell et al., 2012). Due to its impact on soil chemical properties, such as soil pH (Aciego Pietri and Brookes, 2008) and soil organic matter (SOM) composition (Mitchell et al., 2015), biochar can also induce shifts in soil microbial communities with important implications for a range of soil processes. Additionally, labile components of biochar can potentially serve as a microbial C source (Wang et al., 2016).

Soil aggregation, largely responsible for soil structure, is fundamental for soil functioning and agricultural productivity. Soil water stable aggregates can physically stabilize SOM and protect it from decomposition (Six et al., 2002). Aggregation and associated soil structure also has important consequences for the movement of water and energy in soils (Bronick and Lal, 2005; Franzluebbers, 2002) as well as microbial activities (Navarro-García et al., 2012). Soil aggregate dynamics are influenced by a number of factors, including: (1) soil biota (both microorganisms and macrofauna); (2) root growth; (3) soil mineralogy and texture (4) the availability of inorganic binding agents; and (5) environmental conditions (Six et al., 2004).

The interactions between soils and biochar depend on both soil and biochar properties, as well as environmental conditions. The biochemical stability of biochar can vary considerably depending on biochar pyrolysis production conditions, feedstock and application environment (Ameloot et al., 2013; Mukome et al., 2013). Similarly, soils differ substantially in structure and texture, SOM content and composition, and soil biological communities. Thus, interactions between soils and biochar can be diverse and difficult to predict. Previous studies of biochar impacts on soil aggregation have focused on relatively short-term impacts of biochar amendments and the results have been variable. For example, Liu et al. (2014) found a wheat straw biochar (350–550 °C) significantly increased water-stable aggregation and crop productivity in a subtropical red soil in China. At the same time, rice straw biochar (250–450 °C) had no effect on aggregate stability in a subtropical Ultisol, though it did increase maize biomass (Peng et al., 2011). Such results suggest that the impacts of biochar on soil properties, and on aggregation in particular, require further study to better understand the potential implications of biochar on SOM dynamics and a range of key soil processes.

The objective of our study was to investigate the influence of biochar on soil water-stable aggregate dynamics, C storage in aggregates, soil microbial community composition and their interactions. We hypothesized that: 1) biochar amendments affect soil chemical properties and microbial community composition; 2) biochar induced changes to microbial communities and soil properties will enhance soil aggregation and the physical stabilization of SOM in soil aggregates; and 3) low-temperature biochar (i.e., greater labile C) will have more pronounced impacts on microbial activity and aggregation. To address these hypotheses, we compared the impacts of two chemically different biochars on aggregation in agricultural soils of different textures for 60 weeks.

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2. Materials and methods

2.1. Soil and biochar

In February 2013, the surface layer (0–15 cm) of two soils in the Central Valley of California was sampled for this study in order to better understand how generalizable biochar impacts are across distinct, agricultural soils in the region. The first was a Yolo silt loam (Yolo soil; Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent; 29.5% sand, 42.5% silt and 29.0% clay; 9.2 g C kg^{-1}C content; 1.45 g cm^{-3} bulk density) at the Russell Ranch Sustainable Agricultural Research Facility (http://asi.ucdavis.edu/rr), managed by the University of California, Davis. The other soil is classified as a Vina fine sandy loam and was taken from the Lockeford Plant Materials Center of the Natural Resources Conservation Service (Vina soil; Coarse-loamy, mixed, superactive, thermic Cumulic Haploxeroll; 68.8% sand, 16.2% silt and 15% clay; 11.2 g C kg^{-1}C content; 1.35 g cm^{-3} bulk density). Upon sampling, soils were air-dried, sieved to pass through a 2 mm mesh, sealed in plastic bags, and stored at room temperature until use. The soils are typical agricultural soils in California and have contrasting soil texture and mineralogy.

Two biochar types were tested: 1) a walnut shell (WS) biochar produced by Dixon Ridge Farms in Winters, CA., and 2) a commercially available softwood-based "enhanced biochar" (EB) produced by Algae Aqua Culture in Whitefish, MT. The WS biochar was produced at 900 °C (BioMax 50 gasifier), yielding a product with 227.1 m^2 g^{-1} surface area, 517.3 g C kg^{-1} C content, 40% ash content, 33.4 cmol g^{-1} cation exchange capacity and pH of 9.7. The EB biochar was produced from a mix of conifers species (ponderosa pine, Douglas fir, larch, lodgepole pine, spruce, and alpine fir) via pyrolysis between 600 and 700 °C and mixed with algal digestate. This biochar has a 2.0 m^2 g^{-1} surface area, 360.0 g C kg^{-1}C content, 6.4% ash content, 67.0 cmol g^{-1} cation exchange capacity and pH of 6.8. Both biochars were sieved to pass through a 2 mm mesh and additional information on characteristics and methods of analysis is available in (Mukome et al., 2013). The two biochars were chosen due to their contrasting feedstocks, pyrolysis conditions and surface characteristics.

2.2. Incubation experiment setup

A laboratory incubation experiment was conducted from February 2013 to March 2014 (60 weeks). Soil alone or soil and biochar mixtures totaling 200 g were placed in 500 mL Mason jars. Soil moisture was adjusted to 80% of field capacity (i.e, 0.23 g g^{-1} dry soil for Yolo and 0.18 g g^{-1} dry soil for the Vina soil), as determined from a pressure plate measurement at 1/3 bar. The jars with soil and biochar mixture were equilibrated for two weeks before initiating the incubation. The temperature was kept constant at 23 ± 1 °C throughout the entire incubation period. The soil moisture content was maintained at a relatively constant level by weighing the jars and adding distilled water as needed. Biochar treatments consisted of 0, 0.5, and 1.0 g of biochar per 100 g dry soil (equivalent to approximately 0, 10, 20 Mg ha^{-1}) with three replicates jars for each soil-biochar treatment combination. Destructive sampling was conducted at the end of 60 weeks. This relatively long-term incubation was conducted to provide an idea about the impacts of biochar on soil aggregation dynamics in the medium to long-term that is often not addressed in laboratory studies.

2.3. Soil water-stable aggregate analysis and calculation

Water-stable aggregates were separated by a wet-sieving method adapted from Elliott (1986). At the end of the incubation, the moist soils were removed from each Mason jar and passed through an 8 mm sieve by gently breaking the soil clods by hand along the natural planes of weakness. A 50 g representative 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 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). The aggregate fractions retained on each sieve were rinsed into pre-weighed aluminum pans, oven-dried at 60 °C, and then weighed. 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):

$$MWD = \sum_{i=1}^{4} P_i{}^{*}S_i \quad (1)$$

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

2.4. Soil chemical properties

Soil pH (1:1) and electrical conductivity (EC) were measured in the bulk soil at the end of the incubation with a Mettler SevenGo Duo™ pH/Conductivity meter SG23 (Mettler Toledo, Switzerland).

A representative soil sample (8 g) from incubated soils was extracted with 40 mL of 0.5 mol L^{-1} potassium sulfate solution in 50 mL polypropylene tubes and placed on an orbital shaker (250 rev min^{-1}) for 1 h. Colorimetric methods were used to determine nitrate and ammonia concentrations in the supernatant solutions (Doane and Horwáth, 2003; Verdouw et al., 1978).

2.5. C content and recovery calculations

Carbon contents of the soils, biochars and aggregate samples were 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.

The theoretical average C content of each treatment (following biochar additions) was calculated according to the following equation:

$$Theoretical\ C\ content = C_1W_1 + C_2W_2 \quad (2)$$

where C_1 is the biochar C content (g C kg^{-1}), W_1 is proportion of biochar mass in the soil-biochar mixture of each treatment, C_2 is initial soil C content (g C kg^{-1}), W_2 is proportion of soil in the soil-biochar mixture of each treatment.

The C content in of each water stable aggregate fractions was calculated and these four values were summed to determine overall C recovery for each treatment according to the following equation:

$$C\ recovered = \sum_{i=1}^{4} C_i{}^{*}P_i \quad (3)$$

where C_i is C content of each aggregate fraction (g C kg^{-1}), P_i is proportion of the whole soil mass represented by each aggregate fraction.

The difference between theoretical average C content and the sum of C in the aggregate fractions was defined as C loss during the incubation, calculated according to the following equation:

$$C\ loss = Theoretical\ C\ content - C\ recovered \quad (4)$$

2.6. Phospholipid-derived fatty acid (PLFA) analyses

The microbial communities were characterized using PLFA analysis, an indicator of soil microbial biomass and community composition, following methods reported previously (Bossio et al., 1998; Cordova-Kreylos et al., 2006). Briefly, a representative soil sample was taken at

the end of the incubation, frozen (− 80 °C), and then freeze-dried, yielding 8 g of dry material for lipid extraction. After initial extraction, solvents of increasing polarity were used to separate the phospholipid fraction from the neutral lipid and glycolipid fractions using solid phase extraction columns (0.58 Si; Supelco, Bellefonte, PA, USA). Fatty acids were then dried under N_2 gas, transesterified, and methylated. After methylation, the samples were dried again with N_2 gas and redissolved in hexane containing a known concentration of the internal standard 19:0. Fatty acids were identified using the Sherlock software from Microbial Identification Systems (Microbial ID, Newark, DE, USA).

Fatty acids were summed into biomarker groups as described by Buyer and Sasser (2012), briefly: Gram-positive (Gram +) bacteria, iso and anteiso saturated branched fatty acids; Gram-negative (Gram −) bacteria, monounsaturated fatty acids and cyclopropyl 17:0 and 19:0; fungi, 18:2 ω6 cis and ratio of saturated to unsaturated PLFA.

2.7. Statistical analyses

All data were subjected to statistical analysis with Microsoft Excel for Windows 2010 add-ins with XLSTAT Version 2014.6 (Addinsoft, New York, NY, USA). Statistically significant differences between biochar treatments were analyzed separately for each soil type using analysis of variance (ANOVA) and Duncan's multiple range tests at 5% significance level.

Canonical Correspondence Analysis (CCA) was also applied using Microsoft Excel for Windows 2010 add-ins with XLSTAT Version 2014.6 to evaluate the relationship between soil properties (e.g. pH, EC, nitrate concentration, ammonium concentration, soil type, soil aggregate fractions, biochar type and biochar dose) and soil microbial community structure (all the PLFA lipids detected). Monte Carlo tests were applied using the R package Vegan to explore multivariate differences in the PLFA data and understand which factors best contributed to explaining variation in microbial communities (Jari Oksanen et al., 2015).

3. Results

3.1. Soil pH and EC

The impact of biochar amendment on soil pH depended on the type of biochar applied, biochar application rate, and soil type. As shown in Table 1, both EB and WS biochar significantly increased pH for both soil types, with greater biochar amendment rates resulting in larger changes in soil pH. The impact of WS biochar on soil pH was much greater than that of EB biochar, with average increases in pH of 1.13 vs. 0.19 in the WS and EB treatments, respectively. Similarly, the impact of biochar on soil EC depended on biochar type and application rate. The EB biochar treatments had little impact on soil EC (except for the 1% EB biochar amendment which increased EC in Yolo soil), while a significant increase in soil EC was observed in both soils with WS biochar additions (Table 1).

Table 1
Soil pH and electrical conductivity after 60 weeks incubation with two soil types (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar) at varying application rates (doses). The numbers to the right of each value represent the standard error about the mean. Significant differences between treatments are indicated by different letters in parentheses to the right of each value.

Soil	Biochar	Biochar dose (%)	Soil pH (1:1)	Soil EC (μs cm^{-1})
Vina	–	–	5.70 ± 0.01 (a)	368.0 ± 14.1 (a)
	EB	0.5	5.83 ± 0.01 (a)	383.0 ± 6.9 (a)
		1	5.96 ± 0.04 (b)	357.7 ± 24.9 (a)
	WS	0.5	6.79 ± 0.02 (c)	456.0 ± 13.1 (b)
		1	7.37 ± 0.05 (d)	489.7 ± 10.5 (b)
Yolo	–	–	7.08 ± 0.02 (a)	392.3 ± 77.7 (a)
	EB	0.5	7.15 ± 0.01 (a)	310.0 ± 2.7 (ab)
		1	7.37 ± 0.10 (b)	242.9 ± 62.1 (b)
	WS	0.5	7.88 ± 0.07 (c)	528.7 ± 19.2 (c)
		1	8.04 ± 0.01 (d)	632.3 ± 20.5 (c)

3.2. Soil water-stable aggregates

No significant differences in water-stable aggregation were observed between Vina and Yolo soils in the absence of biochar following 60 weeks of incubation. However, when biochar was added, the impact on soil aggregation differed markedly between the two soils, with biochar dramatically improving aggregate stability in the Yolo soil and having no significant impacts in the Vina soil. In the Yolo soil both types of biochar significantly increased MWD, with a 217% and 126% average increase observed for EB and WS biochar treatments, respectively (Fig. 1). The 1% EB biochar treatment resulted in the largest increase, with a MWD of 1304 μm vs. 412 μm in non-amended control. Observed impacts on aggregate stability for the EB biochar treatment are largely due to a significant increase in large macroaggregates at both the 0.5% and 1.0% application rates (Table 2). While WS biochar did not significantly influence the formation of large macroaggregates, it did increase the formation of small macroaggregates at both application rates. As previously mentioned, differences were not significant among Vina soil treatments; however, we note that both types of biochar appeared to have subtle impacts at the 0.5% biochar application rate in the Vina soil, suggesting a 32% and 46% increase in MWD over the unamended control for WS and EB biochars, respectively (Fig. 1).

3.3. C in aggregate fractions and overall C losses

The impacts of biochar on aggregate-associated C storage largely mirrored overall effects on soil structure. Soil carbon contents in no-biochar control treatments were 10.7 g C kg^{-1} whole soil for Vina soil and 8.5 g C kg^{-1} whole soil for Yolo soil after 60 weeks incubation. Both types of biochar increased C storage in macroaggregates of the Yolo soil (Table 3). No significant differences were observed in microaggregate-associated C storage for any of the treatments. While effects on soil structure were not significant for the Vina soil, WS biochar influenced C distribution in aggregate fractions of this soil, such that the 1% application rate of WS biochar significantly decreased C stored in small macroaggregates and increased C storage in the silt and clay fraction relative to the non-amended control. When examining overall C loss during the incubation, the Vina soils with biochar additions all demonstrated significantly higher loss of C than the non-biochar control (20.2% average loss of initial C for biochar treatments vs. 4.6% in the control; Fig. 2). In the Yolo soil, biochar treatments also tended to lose more C than the control (9.9% and 7.6% in the biochar vs. control treatments; respectively), but these differences were not significant ($P > 0.05$).

3.4. Soil microbial communities

While biochar treatments indicated a trend of increased total PLFA (an indicator of total soil microbial biomass) in both soils, differences were significant only in the 1% EB biochar treatment in Yolo soil (Table 4). Biochar also appears to have altered soil microbial community structure, as indicated by changes in ratios of fungi to bacteria, Gram + to Gram − bacteria, and saturated to unsaturated PLFA. For example, the 1% WS biochar treatment significantly decreased fungi to bacteria ratio in Yolo soil, while both WS biochar levels significantly increased the ratio of Gram + to Gram − bacteria in Yolo soil. Additionally, the 0.5% WS biochar amended treatment significantly decreased the ratio of saturated to unsaturated PLFAs in Vina soil. All biochar-amended treatments significantly increased the ratio of saturated to unsaturated PLFA in Yolo soil.

Using CCA (Fig. 3), microbial community composition clustered

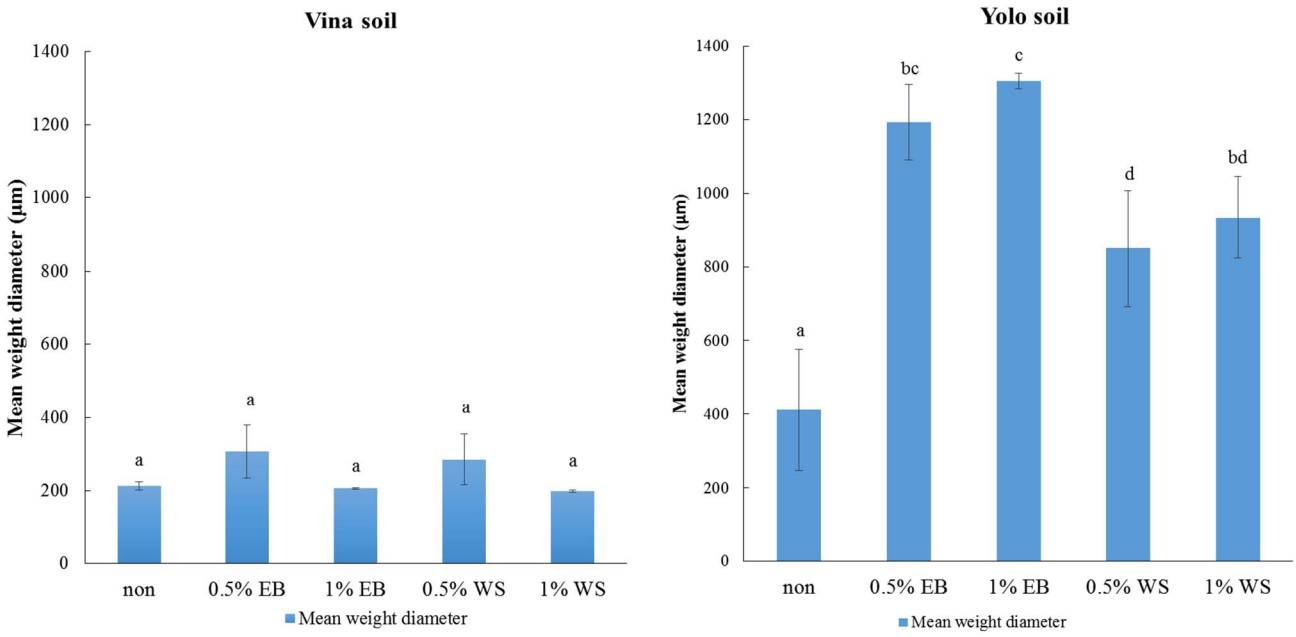

Fig. 1. Soil aggregate stability (mean weight diameter) after 60 weeks incubation in two soil types (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar) at varying application rates (doses). The error bars represent standard errors and bars with different letters indicate statistically significant ($P < 0.05$) differences.

Table 2
Soil aggregate fractions (% mass) after 60 weeks incubation with two soil types (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar) at varying application rates (doses). The numbers to the right of each value represent the standard error about the mean. Significant differences between treatments are indicated by different letters in parentheses to the right of each value.

Soil	Biochar	Biochar dose (%)	Large macroaggregates (%)	Small macroaggregates (%)	Microaggregates (%)	Silt and clay (%)
Vina	–	–	0.0 ± 0.0 (a)	13.7 ± 1.7 (a)	38.7 ± 6.0 (a)	47.5 ± 4.6 (a)
	EB	0.5	0.0 ± 0.0 (a)	24.0 ± 5.1 (b)	30.5 ± 1.1 (a)	45.5 ± 4.3 (a)
		1	0.0 ± 0.0 (a)	13.3 ± 0.6 (a)	37.7 ± 3.9 (a)	49.1 ± 3.4 (a)
	WS	0.5	0.0 ± 0.0 (a)	20.8 ± 6.2 (ab)	33.5 ± 2.3 (a)	45.7 ± 6.1 (a)
		1	0.0 ± 0.0 (a)	12.8 ± 0.6 (a)	36.3 ± 6.2 (a)	50.9 ± 5.6 (a)
Yolo	–	–	0.03 ± 0.1 (a)	28.7 ± 15.8 (a)	54.4 ± 9.5 (b)	16.8 ± 6.7 (b)
	EB	0.5	12.1 ± 2.1 (b)	47.6 ± 0.7 (ab)	32.4 ± 1.7 (a)	7.9 ± 0.3 (a)
		1	12.5 ± 0.5 (b)	57.0 ± 1.3 (b)	24.8 ± 1.2 (a)	5.7 ± 0.3 (a)
	WS	0.5	3.4 ± 3.1 (a)	55.8 ± 0.9 (b)	33.9 ± 3.0 (a)	6.9 ± 0.9 (a)
		1	4.1 ± 2.2 (a)	60.7 ± 0.5 (b)	29.6 ± 2.2 (a)	5.6 ± 0.5 (a)

distinctly dependent upon soil type, biochar type and application rate. The first two CCA axes explained 67.4% and 16.9% of the variation in composition. Soil macroaggregate fractions were associated with axis 1, indicating its greater proportion of the total soil mass in the Yolo vs. Vina soil and a higher soil pH. According to the Monte Carlo permutation test, microbial community composition was most strongly influenced by soil type, followed by soil pH, nitrate concentration, the proportion of small macroaggregates, WS biochar rate, soil EC, large macroaggregates, and EB biochar application rate. The WS biochar had a greater impact than did EB biochar on microbial communities in both soils. Also, microbial community composition was altered more by biochar amendments in the Yolo than in the Vina soil.

4. Discussion

4.1. Biochar impacts on aggregation and aggregate-associated C dynamics

Our findings suggest a novel mechanism by which biochar addition stimulates soil C sequestration by improving aggregation and stabilization of SOM with aggregates. This finding is in contrast to a more passive role that is more typically attributed to biochar where it contributes to soil C sequestration simply due to its recalcitrance. Much of the previous research on biochar has focused on increased soil C storage brought about directly from addition of biochar itself: many types of biochar are relatively stable (particularly those produced at high temperatures) and persistent in soil for extended periods of time (Mašek et al., 2013; Yu et al., 2016; Zimmerman, 2010). In contrast, our results suggest that biochar can play a more active role, by facilitating the physical protection of C (both biochar- and native-derived SOM) in aggregates, at least in the finer textured Yolo soil. Both types of biochar enhanced C storage in macroaggregates, and this can facilitate the longer-term stabilization of SOM in stable microaggregates which form within macroaggregates (Six et al., 2002). While others have shown biochar to affect aggregation, this is the first study to our knowledge linking such changes in soil structure to C storage and stabilization. Furthermore, given that aggregate stability was stimulated more by EB than WS biochar, this biochar-induced physical stabilization of C may be associated with the presence of incompletely carbonized, more degradable organic residues more characteristic of low-temperature biochars (Novak et al., 2009b; Zimmerman, 2010).

The concept of biochar-induced physical protection of SOM is further supported by the results of total C loss from each treatment, including both native and biochar-derived C, at the end of the incubation. In general, biochar resulted in a lower relative loss of C in Yolo soil where biochar additions substantially increased both aggregation and C storage in macroaggregates. In contrast, biochar

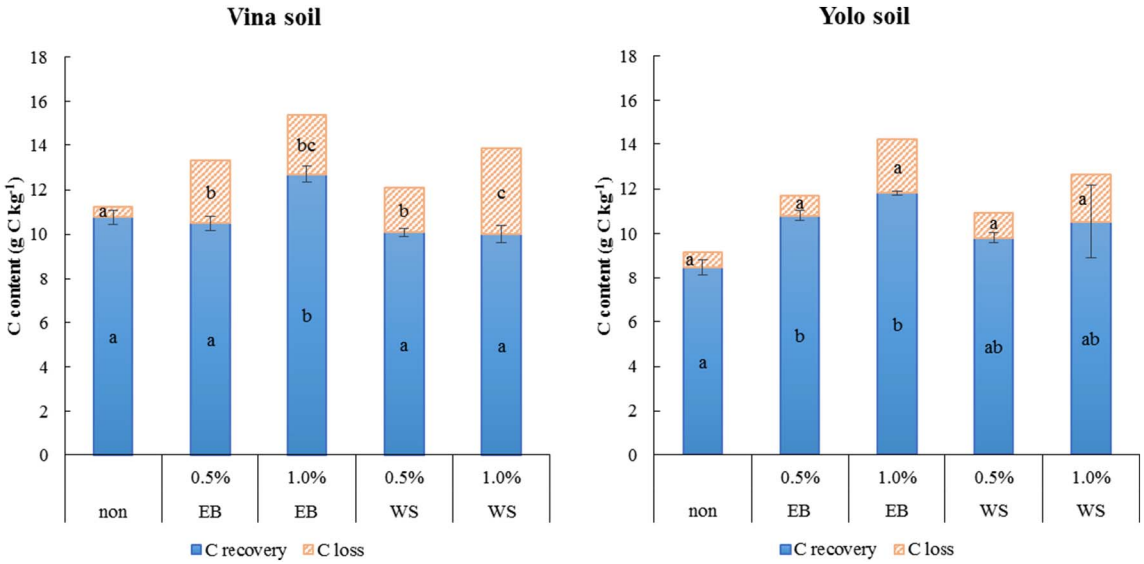

Fig. 2. C recovery (blue bars) and C loss (orange bars) based on theoretical average C content of the treatments (using initial soil and biochar C content) after 60 weeks incubation in two soil types (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar) at varying application rates (doses). Error bars represent standard errors for recovered C and bars with different letters indicate statistically significant ($P < 0.05$) differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stimulated a greater C loss in the Vina soil in which there were only subtle effects on aggregation (Fig. 2). The absence of substantial aggregate structure in Vina soil could result in greater accessibility of SOM, nutrients and oxygen to microbes, which is likely to stimulate SOM decay (Dungait et al., 2012). Though SOM is generally more susceptible to mineralization in coarser than finer textured soils due to the lower surface area of mineral binding sites that can stabilize organic particles, our results indicate that biochar may have further enhanced C mineralization in the Vina soil due to a priming effect. This finding is corroborated by Fang et al. (2015) who reported that two *Eucalyptus saligna* wood biochars increased mineralization of native organic C in sandy soil, but not in a high clay soil. Similarly, addition of slow pyrolysis biochars (like the EB biochar) to a grassland soil led to stimulation of mineralization of native soil organic C due to positive short-term priming effects (Singh and Cowie, 2014). While we cannot discern whether the higher relative loss of C in Vina soil represents mineralization of biochar- or native-derived soil C, both scenarios have important implications for biochar management, since the priming effect of either SOM pool under such conditions could potentially negate the C sequestration potential of biochar in certain soils. Thus, potential trade-offs of biochar serving as a promoter of both soil aggregation and C priming need to be better understood if the intent of adding biochar is to increase soil C sequestration.

4.2. Soil chemical, physical and biological drivers of structural change

Several mechanisms may be involved in the biochar-induced improvements in soil aggregation in Yolo soil. Previous research indicates that biochar can influence soil aggregation by altering soil pH and enhance aromaticity of soil organic C pool (Chan et al., 2008; Novak et al., 2009a), both important factors for aggregate formation. Higher soil pH can increase the flocculation of clay particles (Haynes and Naidu, 1998), thus facilitating the formation of water-stable aggregates (Boix-Fayos et al., 2001). However, a large increase of soil pH can also cause clay to disperse due to a dominance of repulsive forces between clay minerals and in turn result in decreased aggregation (Roth and Pavan, 1991). Thus, we speculate that a slight increase of soil pH due to biochar amendment can benefit soil aggregation, while a large increase in pH may actually inhibit soil aggregation. Biochars are usually C-rich and highly aromatic (Kookana et al., 2011). Biochar in soil occurs not only as free particles, but can also become intimately associated with water-stable aggregates (Brodowski et al., 2006). Piccolo and Mbagwu (1999) reported that hydrophobic components of organic matter contribute more to soil aggregate stability than hydrophilic components. Thus, the highly aromatic C structure in biochar may improve aggregation by helping to bind native SOM, enhancing the resistance of soil aggregates to water and making aggregates more resistant to physical disturbance (e.g., wet-dry cycles).

Biochar can also influence soil aggregation by altering the ionic

Table 3
Soil C distribution in soil aggregate fractions (g C kg^{-1} whole soil) after 60 weeks incubation with two soil types (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar) at varying application rates (doses). The numbers to the right of each value represent the standard error about the mean. Significant differences between treatments are indicated by different letters in parentheses to the right of each value.

Soil	Biochar	Biochar dose (%)	Large macroaggregate C	Small macroaggregate C	Microaggregate C	Silt and clay C
Vina	–	–	0.0 ± 0.0 (a)	1.7 ± 0.2 (a)	4.0 ± 1.4 (a)	5.0 ± 0.03 (a)
	EB	0.5	0.0 ± 0.0 (a)	2.2 ± 0.3 (a)	2.9 ± 0.2 (a)	5.4 ± 0.7 (a)
		1	0.0 ± 0.0 (a)	2.0 ± 0.3 (a)	4.5 ± 0.6 (a)	6.2 ± 0.2 (a)
	WS	0.5	0.0 ± 0.0 (a)	1.7 ± 0.6 (a)	3.0 ± 0.4 (a)	5.4 ± 0.7 (a)
		1	0.0 ± 0.0 (a)	0.6 ± 0.2 (b)	2.9 ± 0.8 (a)	6.5 ± 0.5 (a)
Yolo	–	–	0.0 ± 0.0 (a)	2.7 ± 1.4 (a)	4.2 ± 1.2 (a)	1.6 ± 0.6 (a)
	EB	0.5	1.3 ± 0.3 (b)	5.1 ± 0.2 (ab)	3.4 ± 0.2 (a)	1.0 ± 0.0 (a)
		1	1.4 ± 0.1 (b)	5.9 ± 0.2 (b)	3.4 ± 0.2 (a)	1.1 ± 0.1 (a)
	WS	0.5	0.3 ± 0.3 (a)	5.4 ± 0.1 (b)	3.2 ± 0.4 (a)	0.9 ± 0.1 (a)
		1	0.4 ± 0.2 (a)	6.2 ± 1.4 (b)	3.0 ± 0.3 (a)	0.9 ± 0.0 (a)

Table 4
Soil microbial biomass and microbial biomarkers, (a) Total PLFA (in nmol g^{-1}), (b) Fungal PLFA (in nmol g^{-1}), (c) Ratios of fungal to bacterial biomass, (d) Ratios of Gram-positive (Gram +) to Gram-negative (Gram −) bacteria of soil microbial community in different treatments and (e) Ratio of saturated to unsaturated PLFA after 60 weeks incubation with two soil types (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar) at varying application rates (doses). The numbers to the right of each value represent the standard error about the mean. Significant differences between treatments are indicated by different letters in parentheses to the right of each value.

Soil	Biochar	Biochar dose (%)	Total PLFA	Fungal PLFA	Ratio of fungal to bacterial biomass	Ratio of Gram + to Gram − bacteria	Ratio of saturated to unsaturated PLFA
Vina	–	–	23.1 ± 1.7 (a)	0.60 ± 0.22 (a)	0.09 ± 0.02 (a)	1.8 ± 0.01 (a)	2.5 ± 0.2 (b)
	EB	0.5	23.4 ± 1.1 (a)	0.70 ± 0.28 (a)	0.10 ± 0.02 (a)	1.6 ± 0.0 (a)	2.1 ± 0.1 (ab)
		1	25.2 ± 2.9 (a)	0.63 ± 0.26 (a)	0.10 ± 0.02 (a)	1.6 ± 0.2 (a)	2.1 ± 0.3 (ab)
	WS	0.5	24.3 ± 2.9 (a)	0.46 ± 0.11 (a)	0.09 ± 0.02 (a)	1.7 ± 0.2 (a)	1.9 ± 0.1 (a)
		1	26.2 ± 4.3 (a)	0.65 ± 0.49 (a)	0.10 ± 0.03 (a)	1.8 ± 0.1 (a)	2.0 ± 0.1 (ab)
Yolo	–	–	21.0 ± 1.9 (a)	0.20 ± 0.02 (ab)	0.07 ± 0.00 (b)	1.5 ± 0.1 (a)	1.2 ± 0.04 (a)
	EB	0.5	24.5 ± 1.8 (ab)	0.33 ± 0.11 (b)	0.07 ± 0.01 (b)	1.6 ± 0.1 (ab)	1.3 ± 0.01 (b)
		1	25.6 ± 1.0 (b)	0.33 ± 0.05 (b)	0.07 ± 0.00 (b)	1.6 ± 0.1 (ab)	1.3 ± 0.02 (b)
	WS	0.5	22.7 ± 1.0 (ab)	0.17 ± 0.03 (ab)	0.06 ± 0.00 (ab)	1.7 ± 0.0 (b)	1.3 ± 0.04 (b)
		1	22.1 ± 2.0 (ab)	0.06 ± 0.10 (a)	0.04 ± 0.01 (a)	1.7 ± 0.1 (b)	1.3 ± 0.03 (b)

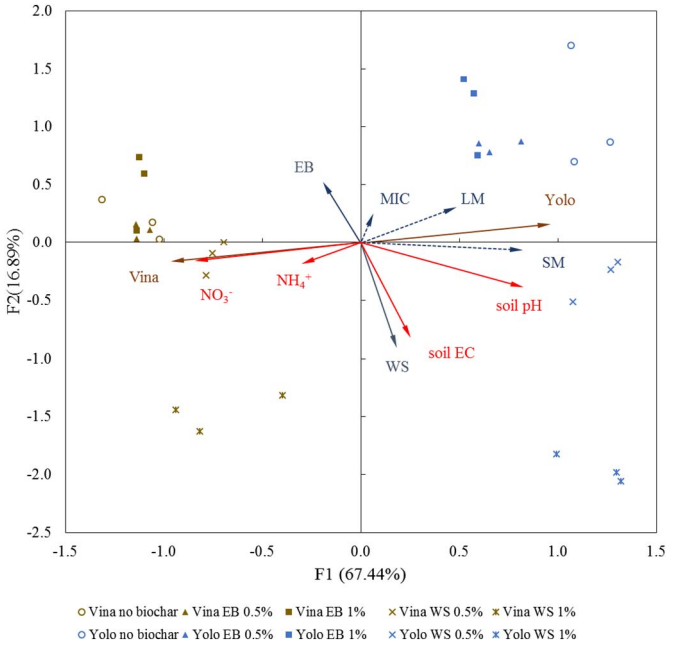

Fig. 3. Canonical-correlation analysis map of PLFA data showing separation microbial communities by treatments after 60 weeks incubation. Arrows represent the vectors for key soil chemical properties and aggregation (Vina = fine sandy loam, Yolo = silty loam), with and without the addition of two biochar types (EB, softwood biochar; WS, walnut shell biochar, 0.5% for 0.5% (w:w) biochar amendment, 1% for 1% (w:w) biochar amendment, LM for large-macroaggregates, SM for small-macroaggregates, MIC for microaggregates).

composition of the soil solution. Monovalent and multivalent ions released from biochar would differ in their influence on soil aggregation (Le Bissonnais, 1996). The WS biochar, for instance, is high in K^+ (Mukome et al., 2013) which could significantly increase soil EC (Table 1). Biochar associated increases in monovalent ion concentration can cause dispersion of organic matter and clay particles and at the same time enhance mobility of soluble SOM (Chow et al., 2006). Multivalent ions associated with biochar may play a positive role through interactions with negative charged surface functional groups on SOM (e.g., R-COO^-) and soil minerals (e.g., Al-O^-, Si-O^-). The EB biochar has a high Fe^{3+} content (Mukome et al., 2013) and it improved soil aggregation and associated C storage more than the WS biochar (Figs. 1 and 2). The bridging effects of multivalent ions, such as Fe^{3+}, can enhance sorption of SOM to clay minerals (Feng et al., 2005) and thus enhance soil aggregation (Bronick and Lal, 2005; Six et al., 2000). While the role of monovalent and multivalent ions is somewhat speculative, we suggest that these mechanisms for biochar impacts on aggregations may be important and require further examination.

Biochar also impacted soil microbial communities. While only significant for the 1% EB biochar treatment, microbial biomass (as indicated by total PLFA; Table 4) tended to be higher in biochar treatments, a phenomenon also observed in previous studies (Chan et al., 2008; Kolb et al., 2009). We note that the EB biochar, produced at a lower temperature, still contained a relatively large amount of non-pyrolyzed organic residue (Mukome et al., 2013), which could potentially increase both microbial activity and soil aggregation, by providing feedstock for production of extracellular polymeric substances that act as cementing agents for soil aggregates (Le Guillou et al., 2012).

While effects on microbial biomass were not especially pronounced, changes in microbial community composition were sensitive to biochar type, concentration and the soil tested (Fig. 3; Table 4). The WS caused shifts in community composition in both soils. The physical and chemical changes, e.g., in pH and EC, brought on by WS biochar may have contributed to these microbial changes and vice versa. Soil microbial community composition is known to be sensitive to soil pH (Bååth and Anderson, 2003; Lauber et al., 2009). Increases in soil aggregation from both biochars in the finer textured Yolo soil could also reduce microbial activity, and hence community composition, by increasing physical protection of both native SOM and biochar-C, thus decreasing accessibility of C pools to microbes (Dungait et al., 2012). Thus, reasons for shifts in microbial community composition with biochar amendment may be both partially responsible for, as well as potentially result from, observed effects on soil aggregation, especially the formation of large and small macroaggregates.

Biochar additions were much more effective in improving soil structure and changing microbial communities in the higher clay Yolo soil than the coarser textured Vina soil. While other differences in these soils' properties may be responsible for soil-specific effects of biochar, previous studies point to the importance of soil texture. Similar to our results, Soinne et al. (2014) reported that amendments with biochar produced by mixed spruce and pine (550–600 °C) have greater impacts on aggregate stability of two higher clay content soils than in a sandy soil. Biochar application also improved soil aggregate formation and stability in silt loam, but not sandy loam soils (Liu et al., 2012). While the specific chemical and/or microbial driving factors responsible for the biochar induced changes in aggregation cannot be clearly determined here, we speculate that the relatively higher clay content and associated surface area in finer textured soils may provide more nucleation sites for organic matter (or biochar) to react with clay and thus facilitate aggregate formation processes.

5. Conclusion

Our results suggest that biochar can enhance the physical-protection

of SOM in Yolo soil by increasing the proportion of C stored within macroaggregates and thus offers a novel mechanism by which biochar may contribute to soil aggregation and C sequestration. This mechanism appears to be dependent on soil texture as biochar had minimal impacts on aggregation and microbial communities in a coarser textured soil. Better understanding of these drivers of aggregation and identifying soil conditions that determine whether biochar will physically protect SOM vs. stimulate soil C loss under different environmental scenario and agricultural practice deserves more research and it must be considered in managing agroecosystems for both mitigation of, and adaptation to, climate change.

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