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Wood biochar impacts soil phosphorus dynamics and microbial communities in organically-managed croplands

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ABSTRACT

Biochar (a carbon-rich product from pyrolysis of organic materials) addition to agricultural soils has been proposed as a novel technology for enhancing soil C storage and fertility; however, few studies have evaluated the effects of biochar on nutrients from an integrated perspective. Previous studies have demonstrated that biochar has the potential to improve bioavailable phosphorus (P) of sandy soils in organic farming systems; yet the underlying mechanisms remain poorly understood. We hypothesized that the unique characteristics of wood biochar could induce changes in soil microbial communities, which would subsequently drive biotic controls on soil P availability through microbial solubilization and/or mineralization and that this would be reflected in microbial P gene expression. To test this hypothesis, we determined the abundance and diversity of bacterial and fungal communities as related to microbial communities in sandy soils of organically-managed farmlands amended with locally produced wood biochar. A series of soil biochemical properties and genes encoding synthesis of phosphatase and those encoding the production of small molecular weight organic acids (involved in metal chelation and P solubilization) were directly quantified to help understand soil P mobilization following biochar addition. Three months after the application of wood biochar, the bioavailability of soil P was found to be elevated and a shift towards a bacterial dominated community was observed. Contrary to our hypothesis; however, the abundance of genes dictating soil phosphatase synthesis or organic acids production remained unaltered following biochar amendment. We suggest that the shift in P bioavailability could be controlled by abiotic mechanisms such as biochar-induced surface organic matter stabilization or adsorption/desorption of P associated with organo-mineral complexes. Although there was no specific molecular evidence of soil micro-organism-mediated P mobilization, locally produced wood biochar had a positive effect on surface soil P bioavailability which could benefit agricultural soil health and ecosystem service delivery in organic farming systems.

1. Introduction

To date there has been little effort to integrate soil phosphorus (P) availability with enzyme and organic acid production and with biotic P gene expression following biochar application to mineral soils and to our knowledge no such effort has been conducted on organically managed farming systems. Phosphorus is known to be a limiting or co-limiting nutrient in many environments, but plants and microbes have evolved mechanisms to enhance soil P availability including the excretion of phosphatase enzymes and the production of low molecular weight organic acids, that facilitate organic P hydrolysis and inorganic P solubilization, respectively. Biochar is a carbon (C) rich product of pyrolysis or thermochemical decomposition of organic material in an oxygen limited environment under controlled conditions that when applied to soil may alter the soil environment and soil microbial communities resulting in a neutral to positive effect on soil P availability (DeLuca et al., 2015b). Lehmann et al. (2011) illustrated that biochar can induce significant shifts in the size and activity of the soil microbial community chemically by releasing a variety of organic molecules that can induce or inhibit microbial growth and/or physically by increasing surface area, increasing microbial habitat. It has also been suggested that biochar can promote mycorrhizal colonization of plant roots by providing a refugia for mycorrhizal fungi (Warnock et al., 2007) and simultaneously alter soil P availability by enhancing the growth of P-solubilizing bacteria that co-occur with mycorrhiza (Gul and Whalen, 2016). Mineral nutrients contained in biochar were also demonstrated to enhance microbial secretions of P-solubilizing acids that further contribute to the soil bioavailable P pool (Deb et al., 2016; Vassilev

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et al., 2013) and biochar additions have been found to result in short-term increases in soil phosphatase and/or phytase activity yielding a positive impact on soil P mineralization potentially driven by shifts in pH buffering or soil P demand (Al Marzooqi and Yousef, 2017; Gao et al., 2017; Liu et al., 2017). However, there remains uncertainty as to whether the influence of biochar on soil P availability is due to biotic (e.g. enzyme activity) or abiotic factors (e.g. reduced soil bulk density).

Numerous studies have examined the influence of biochar on soil biota and P availability in agricultural soils; however, few have coupled molecular evidence of soil microbial response with P transformations (i.e. quantification of genes dictating phosphatase and organic acids syntheses associated with mineralization and solubilization, respectively). Further, the majority of these studies have been performed as lab incubations, greenhouse experiments, or as short-term field studies in conventional farming systems. There has been little attention paid to the response of organic cropping systems to biochar applications, particularly those associated with on-site biochar generation. The unique situation on Waldron Island, WA afforded the integration of pre-commercial forest thinning with small scale organic farming as an example of a whole cycle sustainable biochar project. By creating a closed-loop system wherein value is added to pre-commercial logging biomass that would otherwise be piled and burned, the production of biochar may offer an innovative means of reducing fire hazard fuel loading while improving soil tilth on neighboring organic farms.

In a previous study on ten farms located on three islands of San Juan County, WA, USA, we demonstrated that locally produced wood biochar applied alone or in combination with an organic fertilizer had the potential to increase soil C storage, nitrogen (N) and P availability over one growing season (Gao et al., 2016). Establishment of long-term trials on Waldron Island (Gao et al., 2017) allowed us to test the hypothesis that wood biochar application to sandy soils of organically-managed croplands influences microbial community biomass, abundance, and diversity, which in turn stimulates microorganism-mediated solubilization or mineralization of soil P. The purpose of the work reported herein was to examine the impact of wood biochar application on soil microbial community characteristics and explore the mechanisms responsible for the observed shift in soil P bioavailability by using a series of biochemical and microbial analyses. To our knowledge, this is the first study adopting a molecular approach in evaluating soil biotic P mobilization processes following biochar addition in an organic farming systems thus providing essential insights into the soil biological P transformation and availability in response to biochar addition in agricultural ecosystems.

2. Materials and methods

2.1. Site description and study design

The study was performed in the summer of 2016 at two adjacent organic farm sites (Huntley Farm: 48.719, −123.07; Blue Moon Farm: 48.713, −123.011) located on Waldron Island, WA, USA (Figure S1). The climate of the region is influenced by the Olympic Mountains and Vancouver Island, creating a “rain shadow” effect producing less rainfall and experiencing significantly drier and brighter weather than the surrounding locations. Summers are relatively short, cool and dry, with an average temperature of 15.2 °C; winters are mild and moderately dry when compared to other portions of northern Puget Sound, with an average winter temperature of 5 °C. Average annual precipitation on the island is 650–750 mm. The soils of this region are predominately sandy loam soils formed in glacial till and outwash with a naturally high leaching capacity (see Gao et al., 2017). Replicated treatment plots (n = 3) were laid out in a randomized block pattern at each farm. All plots were seeded to winter squash (*Cucurbita maxima*) for the 2016 growing season. Since the soils of these organic farms have been receiving poultry litter for years, we included this organic fertilizer as a full factorial design in our field trial. The four treatments employed in this study were: 1) Control with no additional amendment; 2) Organic fertilizer: a poultry litter based organic fertilizer (8:4:2 Nutri-rich chicken litter) applied at 70 kg N ha^{-1}; 3) Wood biochar applied at 20 t ha^{-1}; 4) A mix of organic fertilizer and biochar (70 kg N ha^{-1} + 20 t ha^{-1}). Local pond water was used to create a slurry of dry organic fertilizer and biochar in treatment 4, while the same volume of pond water was also applied with the control, the poultry litter in Treatment 2 and the biochar in Treatment 3 (see Gao et al. (2016) for more detail and Table S1 for the nutrient concentrations of the pond water). Each treatment plot was 2 m by 2 m in size with 1.5 m buffer in between. Treatments were applied to the surface soil and incorporated to approximate 10 cm depth before planting crops. Biochar was generated on-site by ‘cylinder burn’ method using local timber harvest residues consisting of 80% Douglas-fir, 15% white fir, and 5% western red cedar; and was crushed under a polyvinyl tarp and sieved to 2 cm diameter. Charcoal generation temperatures were observed to be in the range of 450–550 °C (www.restorechar.org). The four treatments were applied in early May 2016 prior to planting with each treatment being randomly assigned to plots within each replication block, resulting in a total of 24 treatment plots across both farms. Further details of study site and biochar generation process are provided in Gao et al. (2017). Nutrient levels of the treatments are summarized in Table S2. Two farms used in this study share similar background properties: loamy sand in texture; bulk density was 1.06–1.08 g cm^{-3}; water holding capacity (WHC) was 62.5%; 6.42–6.69 in pH (H_2O); 25.0–27.4% of total C; 8.6–10.2% of organic matter content; and soil cation exchange capacity was 5.50–5.57 meq 100 g^{-1}. Both farms used in our study were found on gently sloping landscapes (3%–10% slope) and dominated by Inceptisols with Xerepts as suborders (NRCS, USDA soil survey, 2017).

2.2. Soil sampling and analyses

Composite surface soil samples (four samples taken uniformly) were collected at each treatment plot at mid-growing season (three months after biochar application, early August 2016). Fresh soil samples were thoroughly homogenized and passed through a 2-mm sieve before being analyzed for a series of physicochemical and biochemical variables. Soil pH was determined on field-moist soil (1:1 w/w soil-to-distilled water). Water holding capacity (WHC) was determined by gravimetry (Loveday, 1974). Extractable NO_3^- -N and NH_4^+ -N were determined by shaking fresh soil samples in 1M KCl for 30 min, filtering through Whatman 42 filter papers, and the extractants analyzed by microplate-colorimetric technique using the vanadium method and salicylate-nitroprusside method, respectively (Mulvaney et al., 1996). Soil P status was determined using the biologically based P (BBP) method which is designed to assess a suite of four plant P acquisition strategies to evaluate P availability in dynamic soil systems (DeLuca et al., 2015a). Briefly, 0.01 M $CaCl_2$, 0.1 M citric acid, 0.2 EU ml^{-1} phosphatase enzyme, and 1 M HCl were used as extractants to emulate soluble P, citrate extractable inorganic P that is weakly clay-sorbed or bounded in inorganic precipitates, labile organic P readily attacked by phosphatase enzymes, and moderately stable active inorganic P present in precipitates (DeLuca et al., 2015a). Soil total P, Ca, and Fe were measured using a handheld X-ray fluorescence (Handheld XRF Spectrometer, Bruker, Germany). Potentially mineralizable N (PMN) was measured using a 14 d anaerobic incubation method (Bundy and Meisinger, 1994). Microbial biomass C was determined by fumigation extraction method with amino-N determination by reaction with ninhydrin (Brookes et al., 1985). Each composite soil sample is considered as an analysis unit (n = 24). Oven dried (70 °C) soil samples were ground, sieved and analyzed for total C and N using a CHN analyzer (PE 2400 CHN Analyzer Waltham, MA, USA).

2.3. Soil DNA extraction and droplet digital PCR

Bulk soil DNA was extracted from 0.25 g fresh weight soil samples

using Powersoil® DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. The quality of the extracted DNA was checked using electrophoresis in agarose gels (1% w/v in TAE buffer) with DNA mass standards and molecular weight markers. DNA concentration was determined using a 96-well UV-spectrophotometer by measuring the absorbance at 260 nm (A_{260}) and calculated as $A_{260} \times 50\ ng\ \mu l^{-1}$ x dilution factor. Extracted soil DNA was then stored at −20 °C prior to further manipulation.

Individual primers were used to assess specific P mineralization and solubilization genes in soil samples. Information on the primers used to target specific genes using a droplet digital PCR (ddPCR) of this study are given in Table S3. Each ddPCR reaction mixture (20 µl) contained 1x EvaGreen ddPCR Supermix (Bio-Rad, Hercules, CA, USA), 1000 nM gene-specific primers, and 3 µl of DNA template. Each reaction was mixed with 70 µl Bio-Rad droplet generator oil and partitioned into 15,000–20,000 droplets in Bio-Rad QX200 droplet generator (Bio-Rad). The droplets of individual samples were separately transferred to each well of a 96-well PCR reaction plate and sealed. PCR was performed in a C1000 deep well Thermocycler (Bio-Rad) with the following conditions: 10 min at 95 °C for enzyme activation, 40 cycles of denaturation for 30 s at 95 °C and 1 min at the optimal annealing temperature of each primer set with a ramp rate of 2.5 °C s^{-1}, followed by 10 min at 98 °C for enzyme inactivation and an infinite hold at 4 °C. The optimal annealing temperature for each assay was obtained by the thermal gradient optimization test of C1000 thermal cycler, where the optimized temperature resulted in the largest fluorescence amplitude difference between the positives and negatives (Bio-Rad). The plate was transferred to the Bio-Rad QX200 droplet reader following PCR amplification. QuantaSoft software 1.3.2.0 (Bio-Rad) was used to quantify the copies of target DNA in μl^{-1}. The threshold for a positive signal was determined according to the software instructions. Any droplet beyond the fluorescence threshold was counted as a positive event. Blanks included in the assay showed negative results for DNA copies. All samples were run in triplicate and were averaged for further analysis (averaged value = analysis unit). Values for gene quantification are then expressed as gene copies per gram dry weight of soil.

2.4. Terminal-restriction fragment length polymorphism analysis of bacterial 16s rRNA and fungal 18s rRNA

Regular PCR for terminal-restriction fragment length polymorphism (T-RFLP) analysis was performed for 16s rRNA and 18s rRNA in a total volume of 50 µl reaction mixture containing 10 ng template DNA, 1x Taq Mastermix, and 0.2 µM of each primer. The 16s rRNA forward primer was modified with a 5′ 6-FAM and the 18s rRNA reverse primer was modified with a 5' HEX. Post-PCR amplicons were purified using QIAquick PCR purification kits (Qiagen, Netherlands). Profiles for the T-RFLPs were constructed on purified 16s and 18s rRNA samples. Restriction enzyme *HhaI* was used to generate bacterial 16s fragments and *MboI* was used to generate 18s fragments (Edel-Hermann et al., 2004). Digestions were carried out in a total volume of 10 µl containing 5 µl of PCR product, 2 units of each restriction enzyme (New England Biolabs, Ipswich, USA) in 1x NEB CutSmart buffer. Restriction reactions were incubated for 3 h at 37 °C and 30 min at 65 °C. Incubations were purified before mixing with ROX500 internal size standard for fragment analysis on Applied Biosystems 3730S DNA Analyzer equipped with a 50 cm capillary and POP-y polymer. Peak signals were converted to numeric data for fragment size and peak height by GeneMapper software (Applied Biosystems, USA).

2.5. Statistical analysis

Factorial ANOVA followed by Tukey's post-hoc test was applied to soil biochemical and microbial variables with 'biochar' and 'poultry litter' serving as fixed factors. 'Farm site' and 'replication block' served as random factors and were removed whenever significant effect was not observed. A redundancy analysis (RDA) was performed to elucidate the relationship between soil biochemical properties and microbial responses following biochar incorporation. Significance for the RDA model, each axis and each variable, was tested using Monte Carlo randomization tests (Legendre and Legendre, 1988). To illustrate relationship between soil biochemical properties and microbial responses, inter-set correlations between the weighted average scores for soil biochemical and microbial attributes were calculated from the RDA model (Legendre and Legendre, 1988). For the results obtained from T-RFLP analysis, a presence-absence matrix was created by binary transforming peak profiles after removing T-RF peaks with an area less than 5%. The Shannon index of diversity (H) was then calculated using the formula $H = -\Sigma pi\ (lnpi)$, where pi is the proportion of individual T-RFs (Blackwood et al., 2007). Data were tested for homogeneity of variance and normality of residuals before analyses, and were log transformed when necessary. All statistical analyses were performed in R Version 1.1.

3. Results

3.1. Soil biochemical properties

Soil physicochemical and biochemical properties are presented in Table 1. Soil pH was slightly higher (from 6.5 to 6.9) three months following biochar incorporation compared to the control. Biochar additions also resulted in significantly enhanced soil WHC and total C content, suggesting an improved hydrological function and C storage potential in the sandy soils of Waldron Island. While soil NO_3^- -N was not significantly altered, biochar incorporation caused a significant increase in PMN ($p < 0.001$) and NH_4^+ -N ($p < 0.001$). Among the four fractions of BBP that were measured in this study, enzyme extractable P (labile organic P) and citrate extractable P (active inorganic P) were observed to be both significantly increased by biochar additions ($p < 0.001$ and $p < 0.05$, respectively). It is important to note that the total BBP contained in biochar accounted for only 2.3% of total BBP in soil prior to this field trial (Table S2). Biochar applications to soil also resulted in a slight increase in soil iron (Fe) and calcium (Ca) concentrations compared to the control over the short-term.

3.2. Soil bacterial, fungal communities, and genes dictating soil P mobilization

Three months following incorporation of biochar to sandy surface soils, both microbial biomass C and abundance of soil bacterial 16s rRNA were found to have increased significantly compared to the control (Fig. 1(a)(b)). While fungal abundance was not significantly altered by biochar additions, Shannon's H diversity index of fungal 18s rRNA was significantly higher in biochar treated soils compared to controls (Fig. 1(c)). The bacteria to fungi ratio was elevated with biochar additions, but copy numbers of the P genes, *phoC, gcd,* and *pqqC,* were unaltered by biochar (Table 1).

3.3. Relationships between soil biochemical properties and microbial responses

Soil microbial attributes determined in this study were largely and significantly explained by soil biochemical properties (RDA model $p < 0.001$, Table S4) and were clearly influenced by biochar additions (Fig. 2). Subsequent permutation tests showed significant trends on axes 1 and 2 and together explained 83.7% of microbial attributes; soil BBP (all four fractions), PMN, total C, and WHC were the strongest drivers of the constrained variability (Table S4). Biochar treated plots tended to have higher WHC, BBP, PMN, total C, NH_4^+ -N, and these variables were roughly aligned with bacterial abundance, bacteria to fungi ratio, and microbial biomass C, indicating a highly positive relationship among these attributes (Fig. 2). Bacterial abundance was

Table 1
Soil physical and biochemical properties and selected microbial attributes in response to biochar, organic fertilizer, and biochar + organic fertilizer amendments at two adjacent farms on Waldron Island, WA. Data are presented as mean ± standard error (n = 6). Data were compared among treatments using Tukey-HSD test following ANOVA. Numbers with the same letter are not significantly different at p = 0.05. No letters following the numbers indicate no significant difference at p = 0.05 among treatments. Variables with significant biochar effect are in bold. *Abbreviation: WHC – water holding capacity, PMN – potentially mineralizable N.*

Soil properties	pH	WHC	Total C	Total N	Total P	NH_4^+-N	NO_3^--N	PMN	Bacteria to fungi ratio
Unit		ml ml^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1} $14d^{-1}$	
Control	6.45 ± 0.07	**0.62^{a} ± 0.01**	**26.19^{a} ± 0.79**	2.08 ± 0.19	5.64 ± 0.06	**0.62^{a} ± 0.11**	3.16 ± 0.72	**2.27^{a} ± 0.69**	**20.74^{a} ± 3.60**
Biochar	6.92 ± 0.03	**0.75^{b} ± 0.01**	**35.47^{b} ± 1.98**	2.17 ± 0.19	5.47 ± 0.06	**1.76^{b} ± 0.38**	2.50 ± 0.441	**4.84^{b} ± 0.52**	**18.66^{a} ± 4.30**
Organic fertilizer	6.55 ± 0.07	**0.64^{a} ± 0.01**	**27.69^{a} ± 2.29**	2.39 ± 0.17	5.88 ± 0.13	**2.65^{bc} ± 0.44**	2.47 ± 0.42	**4.62^{b} ± 1.01**	**32.29^{b} ± 1.93**
Biochar + Organic fertilizer	6.83 ± 0.02	**0.74^{b} ± 0.01**	**37.30^{b} ± 1.78**	2.02 ± 0.17	5.76 ± 0.05	**5.27^{d} ± 0.80**	2.15 ± 0.30	**7.08^{c} ± 1.30**	**38.08^{c} ± 3.41**
Soil properties	$CaCl_2$-P	Citrate-P	Enzyme-P	HCl-P	Fe	Ca	*phoC*	*gcd*	*pqqC*
Unit	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	log (copies g^{-1})	log (copies g^{-1})	log (copies g^{-1})
Control	3.17 ± 1.24	**338.01^{a} ± 87.47**	**15.42^{a} ± 1.55**	864.87 ± 173.87	**91.2^{a} ± 1.5**	**13.41^{a} ± 0.02**	5.89 ± 5.56	5.12 ± 4.58	4.72 ± 3.97
Biochar	4.23 ± 1.69	**428.31^{b} ± 105.83**	**22.19^{b} ± 2.21**	881.63 ± 174.68	**104.5^{b} ± 0.9**	**15.81^{b} ± 0.50**	5.99 ± 5.70	5.06 ± 4.56	4.58 ± 3.89
Organic fertilizer	3.95 ± 1.47	**337.95^{a} ± 97.46**	**22.55^{b} ± 3.42**	943.05 ± 167.95	**96.7^{a} ± 2.9**	**14.40^{a} ± 0.22**	5.91 ± 5.43	5.10 ± 4.60	4.72 ± 3.94
Biochar + Organic fertilizer	3.47 ± 1.26	**445.30^{b} ± 119.98**	**27.73^{b} ± 3.91**	933.52 ± 157.44	**106.0^{b} ± 1.3**	**16.59^{b} ± 0.48**	5.92 ± 5.44	5.20 ± 4.39	4.51 ± 3.96

negatively correlated with fungal abundance in the ordination space. Soil $CaCl_2$-P, citrate-P, enzyme-P and HCl-P were all negatively correlated with *phoC* or *gcd* gene abundance, but showed little relationship with *pqqC* gene abundance and was weakly negatively correlated with fungal abundance in the first two dimensions (Fig. 2).

4. Discussion

Sandy soils at organic farms of Waldron Island exhibited increased P availability three months' following biochar amendment and particularly in the active inorganic P (citrate extractable P) and labile organic fraction of soil P (enzyme extractable P) (Table 1 and Fig. 2). Contrary to our hypothesis, however, the abundance of genes tested that relate to phosphatase synthesis (*phoC*) or low molecular organic acids production (*gcd*, *pqqC*) were not significantly altered following biochar incorporation. This may indicate that the shift in surface soil bioavailable P was the result of abiotic processes such as the direct surface adsorption and desorption of P (Zhang et al., 2016), shifts in redox potentials (Joseph et al., 2015), or the development of organo-mineral complexes (DeLuca et al., 2015b) surrounding biochar particles rather than a direct influence on organic acid synthesis in soil microorganisms (He et al., 2014). Our results showing no specific relationship between P-availability and P-gene abundance is likely a function of which genes were actually tested and if they were expressed when being detected. Molecular evidence of functional genes and soil nutrient cycling processes are often not correlated simply because a large fraction of the soil microorganisms are metabolically inactive (Lennon and Jones, 2011). Numerous studies have found little relationship between soil P availability and putative controlling genes (Fraser et al., 2015; Lidbury et al., 2017), except in those instances where the two processes are tightly coupled as in rhizosphere soil (Fraser et al., 2017). This is likely due to the broad array of genes coding for the production of organic acids (Rodríguez et al., 2006) and the fact we only tested two in this study. The abundance of genes coding for alkaline phosphatase (*phoD*) in soils collected in this study was also determined along with *phoC*, *gcd*, and *pqqC*; however, its copy number across all soil samples was much lower than that of *phoC* (approximately 0.5% of the copy number of *phoC*), and was also shown not significantly affected by biochar treatment in our study (data not shown). The lack of relationship between phosphatase gene production and P mineralization rates in soil is partially a function of the fact that phosphatase is a constitutive rather than induced enzyme and can be adsorbed onto clay and organic matter particles (Tabatabai, 1994). This results in a ubiquitous presence of phosphatase in soils and therefore a lack of clear connection between gene abundance and enzyme activity is not particularly surprising (Nannipieri et al., 2002). Long-term, simultaneous tracking of soil P availability, enzyme activity and functional gene expression in response to biochar might ultimately yield greater insight into inherent mechanistic connections.

Similar to our findings, Jones et al. (2010) reported that municipal green waste biochar applied to sandy soils resulted in only a minor effect of biochar on soil phosphatase. Further, Weng et al. (2017) recently provided spectroscopic evidence for a biochar induced increase in the formation rate of microaggregates via organo-mineral interactions and subsequently resulted in a stabilization and accumulation of organic matter over time. Their arguments could be partially seen in our observations of significantly enhanced PMN and potentially mineralizable P (enzyme extractable P) along with a higher total C content following biochar incorporation (Table 1 and Fig. 2); as well as higher microbial biomass C and bacterial abundance in biochar treated soils (Fig. 1(a)(b)). Biochar and its stabilized aggregates may have created an additional surface to which phosphatase enzymes could adsorb (Swaine et al., 2013) resulting in a net increase in phosphatase that led to an altered P availability but no reflectance in its production detected by the gene abundance. Although we did not perform phosphatase enzyme assays at the same time as this current study, we detected both higher

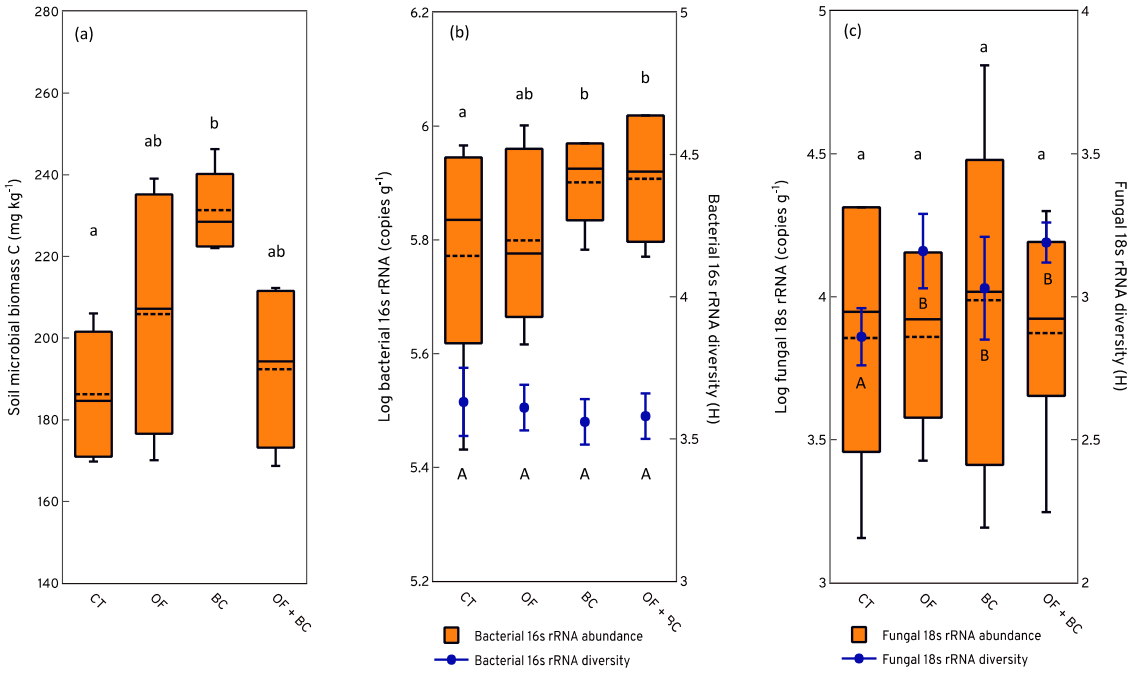

Fig. 1. Soil (a) microbial biomass C, (b) bacterial 16s rRNA and (c) fungal 18s rRNA abundance and diversity (Shannon' H index) as influenced by biochar, organic fertilizer, and biochar + organic fertilizer amendments at two adjacent farms on Waldron Island, WA. Data were compared among treatments using Tukey-HSD test following ANOVA. The solid line represents the median and dashed line represents the mean in box and whisker plots. Shannon's H index is presented as mean ± standard error (n = 6). Numbers with the same letter are not significantly different at p = 0.05 (lowercase indicates abundance, uppercase indicates diversity). *Abbreviation: CT – control, OF – organic fertilizer, BC – biochar, OF + BC – organic fertilizer and biochar.*

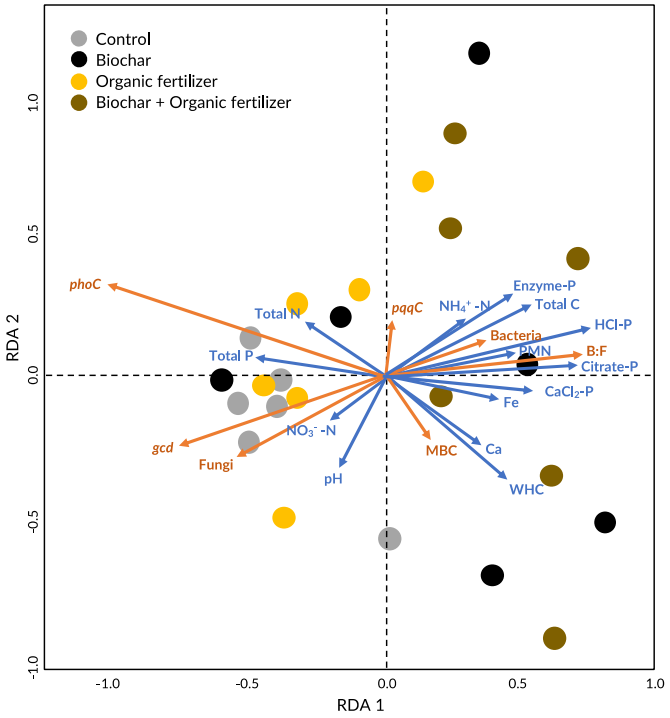

Fig. 2. Redundancy analysis (RDA) ordination of soil microbial attributes (orange) constrained by soil physiochemical and biochemical parameters (blue) following amendments of biochar, organic fertilizer, and biochar + organic fertilizer on two adjacent organic farms of Waldron Island, WA, USA (n = 24). *Abbreviation: Bacteria – bacterial 16s rRNA abundance, Fungi – fungal 18s rRNA abundance, B:F – bacteria to fungi ratio, MBC – microbial biomass C, PMN – potentially mineralizable N, WHC – water holding capacity.* (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

soil phosphatase activity and enzyme-P availability two months later at the end of the growing season following crop harvest (five months following biochar addition) in biochar treated soils compared to controls (same treatment plots) (Gao et al., 2017). Further, the adsorption of chelating organic molecules via surface binding or ligand exchange during the formation of organo-mineral-biochar complexes could have modified soil P solubility and the pool of bioavailable P, since the citrate extractable P pool in biochar-treated soils was larger than that of controls (Table 1). It has been widely reported that the biochar-soil interface could efficiently catalyze a variety of abiotic redox reactions, where the soil redox potential (Eh) was often observed to drop following biochar addition (Joseph et al., 2015, 2010). Therefore, it is possible that the biochar served as a reducing agent that altered soil Eh in our study yielding a net release of soluble P as iron-associated compounds were chemically reduced (Table 1). Alternatively, soil P retention ability could also be exerted by the equilibrium of sorption and desorption of P mediated by biochar additions (Bornø et al., 2018; Xu et al., 2014). Organic farming systems tend to have more efficient P recycling machinery over a growing season (due to a less direct leaching of inorganic P) compared to conventional farming systems (Möller et al., 2017) with all else equal, and soils in this study were not considered deficient in P given the high P content of soils from past organic fertilizer applications. The exudation of phosphatase and small molecular weight organic acids have commonly been documented to be stimulated only in P-deficient soils (Jones and Oburger, 2011; Nannipieri et al., 2011; Yao et al., 2018), thus phosphatase enzymes and organic acids might not further respond to biochar addition in the Waldron Island system. It is also important to note that other phosphatases not measured in this study (i.e. phytases, phosphodiesterases) may also be responsible for hydrolyzing P compounds to the extractable fraction.

Our study demonstrated a shift towards a bacterial dominated microbial community three months following wood biochar addition to sandy soils (Table 1 and Fig. 2). This finding is consistent with Jones et al. (2012) who also observed a lower fungi to bacteria ratio in the

second year after biochar application in an agricultural soil; Chen et al. (2013) who detected a higher bacterial abundance under 20 t ha^{-1} biochar application on surface soils of a rice paddy; and Nguyen et al. (2018) who demonstrated a short-term positive response of bacteria to biochar in a field trial where wood biochar was added. Fungal abundance did not respond significantly to biochar additions over short term in our study, which partially supported our argument that soils with or without biochar amendment in our study system did not exhibit P deficiency, thus fungal associations were not further developed to promote P availability (Warnock et al., 2007). Soil WHC was shown to be significantly elevated following biochar addition (mainly due to an increased soil pore space and surface area), which would also likely reduce the need for fungal associations to acquire extra moisture (Fig. 2). Elevation in soil pH by alkaline metal oxides (i.e. Ca^{2+}) in wood biochar could play some role in controlling the relative abundance of bacteria and fungi, where it is documented that neutral soils favor the growth of bacteria rather than fungi (Rousk et al., 2009). Although the change in bulk soil pH in our study was rather small, microsite pH effects associated with the biochar could be notably larger. Soil microorganisms target simpler compounds (more labile C) upon the initial decomposition process, followed by a subsequent degradation of more complex polymers for energy (Ritz, 2005). The shift towards a bacterial dominated community over short term as observed in our study could be related to the release of labile C from biochar (Nguyen et al., 2018) or biochar-stabilized aggregated-associated organic matter (Rousk et al., 2013). This shift to bacterial dominance was shown to be the greatest in biochar-poultry litter mixture treatment potentially suggesting an inherent positive interaction between biochar and poultry litter (Table 1). The addition of poultry litter was generally found to increase soil microbial activity, therefore, adding poultry to biochar likely increased surface area for bacteria adhesion compared to poultry litter alone (Lehmann et al., 2011). The slight increase in fungal diversity with biochar or poultry litter additions was paralleled with an increase in total fungal biomass (Fig. 1(c)). Volatile organic compounds (VOCs) produced by soil microorganisms have been observed to influence microbial community structure and function and some VOCs produced by bacterial species can either inhibit or increase the growth rates of some fungi (Mackie and Wheatley, 1999; Wheatley, 2002). Some quantity of VOCs can be formed during biochar production (i.e. carbonization process) and subsequently adsorbed onto biochar (Spokas et al., 2011) which may alter fungal diversity in biochar amended soils.

Organic farming has been documented to be relatively efficient in nutrient recycling within the system compared to conventional farming systems (Goulding et al., 2008). In this study, biochar produced from local fuel reduction treatments and application to neighboring organic farming systems was shown to promote nutrient recycling and particularly soil P bioavailability. Although we found no molecular evidence of microorganism-mediated P mobilization following biochar incorporation using a limited set of P mineralization and organic acid production primers, it is possible that the applied biochar increased net adsorption of phosphatase in surface soils which could likely be reflected in enzyme assays, but not in gene abundance. Our results also suggest that biochar application concentrates more labile C in surface soils resulting in a short-term shift towards a more bacterial dominated community. Overall, this study illustrates the role of locally produced wood biochar in modifying available nutrient supplies in organic farming systems associated with sandy soils.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.09.002.

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