CrossMark

Soil biochemical properties and crop productivity following application of locally produced biochar at organic farms on Waldron Island, WA

Si Gao · Kai Hoffman-Krull · Thomas H. DeLuca

Abstract Biochar (a carbon-rich product from pyrolysis of organic materials) additions to agricultural soils have been shown to often result in neutral to positive influences on soil properties and processes; however, the only a limited number of studies have been conducted on active organic farming systems and of those, none have used multivariate analytical methods to examine the influence of biochar on soil microbial activity, nutrient cycling, and crop performance. In this study, biochar produced from local timber harvest residues on Waldron Island, WA was applied in factorial combination with a poultry litter based fertilizer to replicated plots on six organic farms that were all growing Kabocha squash (*Cucurbita maxima*) in the summer of 2016. A series of soil physicochemical and biochemical properties were examined after 5 months of biochar application; squash samples were evaluated for productivity and nutrient uptake. Factorial multivariate analysis of variance (MANOVA) revealed a significant influence of biochar on soil properties as well as a synergistic effect of biochar and poultry litter during a 5 month field trial. Principle component analysis (PCA) highlighted soil total C content, microbial biomass C, enzyme activities, bioavailable P, and phosphatase enzyme activity as the variables most influenced by biochar incorporation into surface mineral soil. Redundancy analysis (RDA) further indicated that better soil biochemical conditions, particularly soil enzyme activities and available P concentrations, were associated with higher crop productivity in biochar-treated plots. Overall, our study demonstrates that locally produced wood biochar, in addition to improving soil C storage, has the potential to significantly improve soil fertility and crop productivity in organic farming systems on sandy soils.

Keywords Biochar · Crop productivity · Multivariate analyses · Organic farms · Soil available phosphorus · Soil fertility

S. Gao
School of Environmental and Forest Sciences, University of Washington, Seattle, WA, USA
e-mail: gaosi@uw.edu

K. Hoffman-Krull
Forage, Waldron Island, WA, USA
e-mail: kai@forage.so

T. H. DeLuca (✉)
College of Forestry and Conservation, University of Montana, Missoula, MT, USA
e-mail: tom.deluca@umontana.edu

Introduction

The utilization of woody residuals in generating biochar has been widely shown as a carbon negative solution for agriculture management; however, few studies have addressed the potential of biochar for use in organic farming operations. Biochar is a solid material that is generated from the pyrolysis of organic material in an oxygen-limited environment for soil application, is typically enriched in C and associated with fused aromatic ring structures that directly contribute to C sequestration (Brewer et al. 2009). The application of biochar to soils has been found to have few negative effects on soil, yet numerous agronomic benefits, such as enhanced soil nutrient availability and retention, the abundance and structure of microbial communities, as well as crop productivity and minerals uptake (Lehmann and Joseph 2015). A recent field study has reported greater N availability in a temperate sandy topsoil amended with wood chip biochar (Haider et al. 2017); other studies have reported increased availability of soil P and K following biochar incorporation (Olmo et al. 2015; Xu et al. 2015; Gao et al. 2016). Soil microbial biomass and diversity have been found to be indirectly altered by biochar as a result of changes in pH, C availability, bacterial adhesion, or biochar protection; and the effect is related to biochar feedstock and application rate (Lehmann et al. 2011). Improvements in soil fertility by biochar addition have also led to increased crop yields and soil productivity (Graber et al. 2010; Gao and DeLuca 2016). Unfortunately, the majority of the biochar trials have been conducted in the greenhouse, growth chamber, or laboratory environment that limits the validity of the findings; longer-term field trials have often been conducted at agricultural experiment stations using commercial biochar with conventional farming approaches. To date, very few studies have been conducted as a part of a holistic closed loop system that examine the direct link of on-site produced biochar using local feedstocks to on-farm applications associated with organic farming systems, a model that could be replicated by local organic farmers throughout forested regions across the country.

Extensive timber harvest over the last century combined with limited opportunity for economically driven forest regeneration has led to overstocked forest conditions on 80% of the Waldron Island, San Juan County, WA. A lime kiln operation on the island drove the demand for timber, but with the loss of that industry, the high cost of transporting of timber off the island, and the relatively low value of the remaining and regenerated timber on the island, there is no direct economic incentive for thinning. Non-commercial forest thinning has become a prevalent management activity in an attempt to reduce fire hazard and improve forest health, but comes as an incurred cost to landowners. This means that the vast majority of biomass harvested on the islands has no specific value, thus the majority of forest residues are piled and burned thereby degrading air quality (generation of NO_x, CO, CO_2, and particulate matter) and resulting in a net loss of nutrients from the larger ecosystem. Nearly all the residents of Waldron Island are engaged in some form of small scale agriculture or organic farming; soils of the region are dominated by sandy loam soils formed in glacial till and outwash resulting in an inherently high leaching capacity. The growing season on Waldron Island is relatively short given the northern latitude and relatively dry due to the 'rain shadow' effect created by surrounding Olympic Mountains. Given these conditions, the production of biochar from local timber harvest residues on Waldron Island may offer a sustainable means of reducing wildfire hazard fuel loading while potentially improving soil fertility and crop productivity on neighboring organic farms.

In a previous study on three islands in San Juan County, WA, we used univariate, parametric statistical analyses to evaluate the influence of biochar produced from local forest residues on nutrient availability and nutrient leaching in a one year study with replicated trials at ten different farms (Gao et al. 2016). Given that many soil properties and processes are naturally spatially and temporally auto-correlated, we have focused on six farm sites on Waldron Island and applied multivariate analytical methods which allow for the assessment of numerous variables simultaneously allowing for a less biased interpretation of results and an improved understanding of the whole soil ecosystem effect of biochar application to surface soils. Further, in this study we attempt to elucidate the mechanisms for observed changes in soil biochemical properties and processes in the prior study. The purpose of the work reported was to investigate the effect of locally produced wood biochar on soil biochemical properties and processes in small organic

farming operations on Waldron Island. In the following study we combined several univariate and multivariate analytical methods to explore the following objectives: (1) Determine those soil physicochemical and biochemical parameters most greatly influenced by biochar incorporation to organic agricultural surface soils and subsequently investigate several possible mechanisms responsible for these changes in soil variables, particularly soil P availability, with biochar additions; (2) Assess crop response to wood biochar applications to mineral surface soils in an organic farm operation; and (3) Determine the relationship between crop response to biochar and changes in soil physicochemical and biochemical properties following biochar addition to mineral soils.

Materials and methods

Study site description and experimental design

This study was performed in the summer of 2016 at six organic farm sites located on Waldron Island, WA, USA (Fig. 1). These farm sites were located at 48.696, −123.035 (Forage); 48.703, −123.029 (Nootka Rose Home Site); 48.704, −123.032 (Nootka Rose Middle 5 Acres); 48.704, −123.031 (Nootka Rose Middle 5 Acres Hand Till); 48.719, −123.017 (Huntley Farm); and 48.713, −123.011 (Blue Moon Farm). Approximately 80% of Waldron Island is covered by forest consisting mostly Douglas-fir, Western hemlock, and Western red cedar, the remaining arable land on the island is largely used for agriculture. 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 dryer and brighter weather than the surrounding locations. Summers are relatively short, cool and dry, with an average summer temperature of
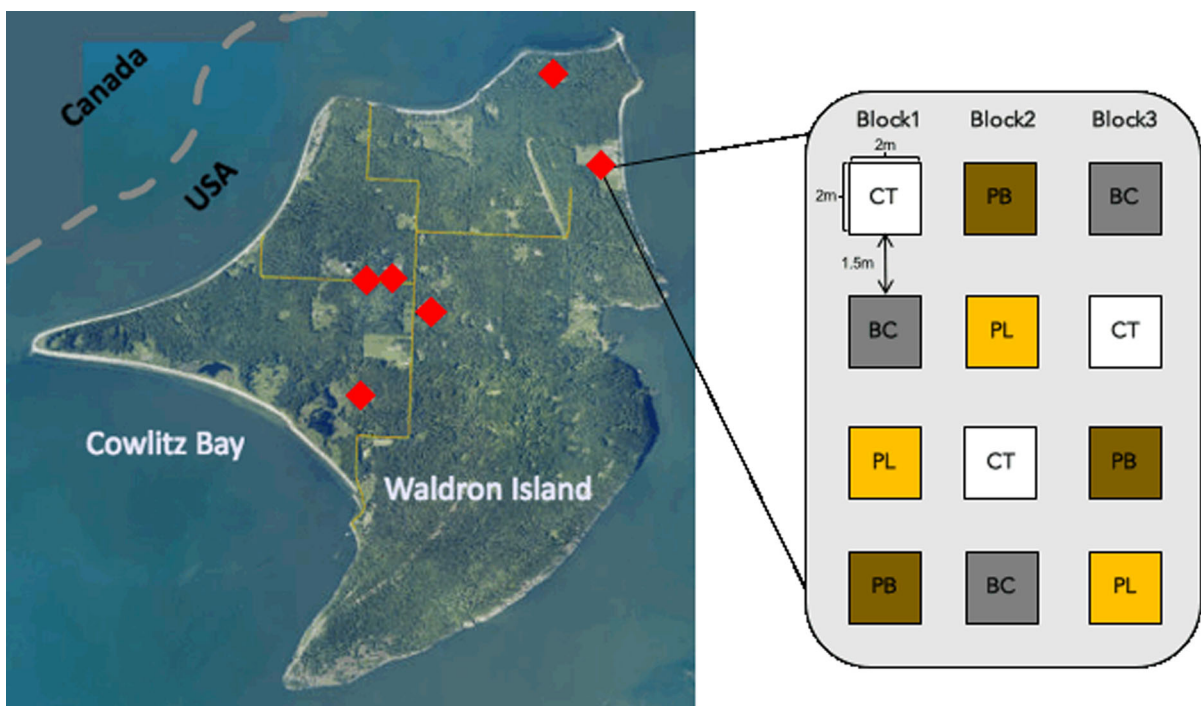

Fig. 1 Map of the location of six organic farms on Waldron Island, WA with example experimental layout for each farm. Each of the six farms received the same four treatments assigned randomly to three blocks and each treatment applied to 4 m^2 plots with a 1.5 m buffer in between plots. Each 4 m^2 plot is an analysis unit. *CT* control, *PL* poultry litter, *BC* biochar, *PB* poultry litter + biochar

15.2 °C; winters are mild and moderately dry when compared to other portions of northern Puget Sound, with an average of 5 °C. Annual precipitation of 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. The farms used in our study are found on gently sloping landscapes and dominated by Dystroxerepts, Haploxerepts, and Haploxeralfs as soil great groups (NRCS, USDA soil survey 2016).

Biochar was produced on-site by using the 'cylinder burn' biochar production method tested by a group of farmers and foresters at Northwest Natural Resource Group and found to be highly efficient (http://restorechar.org/read-me/). The biochar was produced in close proximity to farm sites using logging residues which on average consisted of a mixture of about 80% Douglas-fir (*Pseudotsuga menziesii*), 15% white fir (*Abies concolor*), and 5% Western red cedar (*Thuja plicata*). The cylinder kiln was 1.5 m in height by 1.5 m diameter. Briefly, the cylinder burn operated with an open lid and relied on regular additions of feedstock to fill the cylinder. As the flame wall climbing up and feedstock being added throughout the burning, the material below was kept in a low oxygen environment. Pyrolysis took approximately 7 h with temperature being kept at 450–550 °C. Approximately 55 l of water was used to douse the flame once the fire reached the top of the cylinder. A floating metal lid was then placed on top, and sealed with mineral earth. After 48 h, the charcoal was removed, allowed to dry, ground by crushing under a polyvinyl tarp, and then sieved to 2 cm dia.

In May 2016, orange Kabocha squash (*Cucurbita maxima*) was planted across all test farms. As these organic farms have been applying only organic fertilizers, we added another pair of treatments "poultry litter", and "poultry litter + biochar" to reveal the real biochar effect. Treatments consist of: (1) control (CT): no additional amendment; (2) poultry litter (PL): applied at 70 kg N ha^{-1}; (3) biochar (BC): applied at 20 t ha^{-1}; (4) poultry litter + biochar (PB): a combination of 70 kg N ha^{-1} poultry litter with 20 t ha^{-1} biochar. Three replicated blocks of all four treatments were established at each farm site. The four treatments were randomly applied in each replication block, resulting in a total of 72 treatment plots (Fig. 1). Each treatment was applied to a 2 m by 2 m plot, with 1.5 m buffer in between. Treatments were applied to

the surface soil and incorporated to 15 cm depth in May 2016, prior to planting squash. Biochar used in the study was crushed to create an average particle size of around 5 mm diameter. Composite surface soil samples (0–15 cm, four subsamples taken uniformly at each plot to create one composite sample) were collected on separate occasions using a 1 cm^2 diameter soil core. Each treatment plot was considered as an individual sample unit.

Soil and biochar characterization

Composite surface soil samples (0–15 cm) were collected from each farm prior to biochar incorporation. The soil was thoroughly homogenized and passed through a 2-mm sieve. Soil pH was determined in a 1:1 soil to water suspension. Total C and N of soil, poultry litter, and biochar samples was measured using a CHN analyzer (PE 2400 CHN Analyzer Waltham, Massachusetts, USA). Bulk density was measured using a bulk density core (10 cm in height and 7 cm diameter) that was pressed into the soil. Particle size analysis was conducted by the hydrometer method (Laker and Du Preez 1982). Water holding capacity (WHC) was determined by gravimetry (Loveday 1974). The characteristics of the soil, biochar, and poultry litter are reported in Table 1.

Soil analyses

Composite surface soil samples were collected from each treatment plot (2 m by 2 m) at the end of the growing season (September 2016). Fresh soil samples were returned to the laboratory at the University of Washington and stored at 5 °C, and processed within 3 days of collection. Samples were thoroughly homogenized and passed through a 2-mm sieve. Soil pH, WHC, total C content were measured following the methods mentioned above. Fresh soil samples (5 g) were weighed, shaken with 25 ml of 1 M KCl, filtered through Whatman 42 filter papers, and analyzed for extractable NO_3^-–N, NH_4^+–N by microplate-colorimetric technique using the vanadium method and salicylate-nitroprusside method, respectively (Mulvaney et al. 1996); and reported as mg N kg^{-1} dry soil. Soil P status was determined using the biologically based P (BBP) method recently described by DeLuca et al. (2015). The BBP method is designed to assess a suite of four plant P acquisition

Table 1 Characteristics of (a) soil, (b) poultry litter, and biochar used in a study comparing no treatment, biochar, poultry litter, or biochar and poultry litter in field studies conducted at six organic farms on Waldron Island, WA

(a) Farm site	Total C (%)	Total N (%)	Bulk density (g cm^{-3})	WHC (%)	Cation exchange capacity (meq 100 g^{-1})	PH	Organic matter (%)	Sand (%)	Clay (%)	Texture
NRH	32.3	1.9	0.92	77.5	5.23	6.11	11.77	69.7	10.0	Sandy loam
NRMT	23.0	1.2	0.96	72.5	5.43	6.22	12.28	60.3	15.5	Sandy loam
NRHT	23.5	2.1	0.93	70.2	5.30	6.20	11.30	60.0	14.5	Sandy loam
Huntley	27.4	2.0	1.06	62.5	5.57	6.42	10.25	78.4	13.1	Loamy sand
Blue Moon	25.0	2.3	1.08	62.5	5.50	6.69	8.54	76.8	14.0	Loamy sand
Forage	48.5	2.5	0.60	120	4.73	5.63	18.06	54.1	16.6	Sandy loam

(b) Amendment	Total C (%)	Total N (%)
Poultry litter	40.0	8.0
Biochar	69.5	0.1
Poultry litter + biochar	68.9	0.5

strategies to evaluate P availability in dynamic agricultural systems. Briefly, soil samples were extracted in parallel with 0.01 M CaCl$_2$, 0.1 M citric acid, 0.2 EU ml^{-1} phosphatase enzymes, and 1 M HCl and analyzed for orthophosphate using the Malachite green method and reported as µg P g^{-1} soil (DeLuca et al. 2015). Soil total P, K, S, Ca, Fe, Mn, Zn, Cu, and Ni were measured using a handheld X-ray fluorescence (Handheld XRF Spectrometer, SI TITAN, Bruker, Germany) (McLaren et al. 2012).

Soil potentially mineralizable N (PMN) was measured using 14d anaerobic incubation method, and was calculated by subtracting initial NH$_4^+$–N (0 day) from that determined at the end of the incubation (14 days) (Bundy and Meisinger 1994). Soil microbial biomass C was determined by fumigation extraction method with amino-N determination by reaction with ninhydrin (Brookes et al. 1985). Soil basal respiration was measured using a 3d incubation method described by Anderson 1982. Briefly, samples were incubated in glass jars containing a gas septum, adjusted to 60% WHC, incubated at room temperature, and sealed to trap respired CO$_2$ (Anderson 1982). Headspace gas was taken after 3 days of incubation and analyzed for CO$_2$ by a Gas Chromatography Analyzer (Shimadzu GC, Japan) with a flame ionization detector (FID). Acid phosphatase, β-glucosidase, dehydrogenase, and urease activities were examined to investigate soil P, C, and N cycling processes following Tabatabai and Bremner 1969, Eivazi and Tabatabai 1988, Klein et al. 1971, and Tabatabai and Bremner 1972, respectively; and were expressed as µg PNP g^{-1} h^{-1} for

phosphatase and β-glucosidase activities, µg TPF g^{-1} 24 h^{-1} for dehydrogenase activities, and µg NH$_4^+$–N g^{-1} h^{-1} for urease activities. Phosphorus solubilizing bacteria abundance was examined using a culture based approach. Briefly, 5 g fresh soil samples were serially diluted (10^{-1} to 10^{-6}) and spread plated on National Botanical Research Institute's Phosphate (NBRIP) media to enumerate culturable PSB (Nautiyal 1999). Media contained 100 µg ml^{-1} of cycloheximide to inhibit fungal growth. Plates were incubated at 28 °C for 8 days prior to counting the numbers of colony forming units (CFU).

Crop response

Squash (*Cucurbita maxima*) fruits were harvested at the end of growing season (September 2017). Total fresh weight fruit yield data was determined for each farm. Fruit water content was determined by weighing the fruits fresh, drying samples at 105 °C in conventional drying oven, and weighing the fruits dry. Total fruit dry mass was calculated using the total fresh weight corrected to total dry weight by multiplying by (1—the fraction of water in the squash fruit). Squash N concentration was analyzed using a CHN analyzer (PE 2400 CHN Analyzer Waltham, Massachusetts, USA), squash P concentration was determined using an inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific 6300, Waltham, MA) following a dry-ashing and nitric acid procedure (Santos et al. 2008). Other macro (K, S, Ca) and micro (Fe, Mn, Zn, Cu, Ni) nutrient concentrations

were measured using a handheld X-ray fluorescence (Handheld XRF Spectrometer, SI TITAN, Bruker, Germany) after pulverizing plant samples.

Statistical analyses

Each test plot (4 m^2) was considered as an analysis unit in this study. Farm sites served as replication in the following analyses given the fact that: (1) The land use structure of Waldron Island is relative simple, with very small proportion of the land being used for agricultural cultivation; (2) The background properties of the soils in each test farms are quite similar, together representing the agricultural soil characteristics of the entire island (NRCS, USDA soil survey 2016; Table 1). Analyses were conducted using three data matrices: 'plots × soil physicochemical parameters'; 'plots × soil biochemical parameters'; and 'plots × crop productivity variables' (parameters were all listed below). All matrices were column standardized to zero mean and unit variance to account for differences in units between variables. Data were then log transformed to reduce skewness and the influence of outliers. Variables in each matrix are continuous. All matrices were screened for outliers, missing data, and insufficient variables before any analysis. Soil P, K, S, and Ni concentrations were dropped from the first matrix, as these four variables had too little variation among plots (cv < 5).

Principle component analysis (PCA) was selected as the appropriate ordination method where dissimilarity was calculated as Euclidean distance (Kessell and Whittaker 1976; Legendre and Legendre 1998; Dray et al. 2003). PCA was performed on the first two data matrices to assess the dominant patterns in soil characteristics across treatments and investigate those components that drive the differentiation in soil properties and processes following biochar incorporation. A total of 14 soil physicochemical parameters (pH, WHC, NH_4^+–N, NO_3^-–N, $CaCl_2$ extractable P, citrate extractable P, enzyme extractable P, HCl extractable P, total C, Ca, Fe, Mn, Zn, and Cu) were introduced as the analysis variables in the first PCA; eight soil biochemical parameters (microbial biomass C, potentially mineralizable N, P solubilizing bacteria abundance, basal respiration, β-glucosidase activity, dehydrogenase activity, phosphatase activity, and urease activity) were introduced as the analysis variables in the second PCA. The significance of each

principle component (PC) was evaluated using a Monte Carlo randomization test. The loadings of variables to each PC was examined by converting eigenvector coefficients to structure correlations. Pearson correlation test was next conducted on specific extracted soil variables that are of interest to us to examine the dependent relationship between soil physicochemical and biochemical parameters. A factorial multivariate analysis of variance (MANOVA) was carried out for all soil variables to examine the pure biochar effect and the synergistic effect between the poultry litter and biochar (model = poultry litter × biochar). Together with PCA, Pearson correlation test and MANOVA may help elucidate potential mechanisms responsible for the changes in soil variables following biochar addition.

Crop yield and specific nutrient concentrations were compared among treatments using Tukey-HSD test following ANOVA. A permutation of analysis of variance (PERMANOVA) was conducted to test for differences in crop response variables among four treatments; a subsequent test of multivariate homogeneity of group dispersions (DISPER) was also conducted to calculate the significant multivariate dispersions in crop response variables among four treatments. Euclidean distance was chosen for both PERMANOVA and DISPER; significance of the *Pseudo-F* value was tested via 999 random permutations.

A redundancy analysis (RDA) was conducted to investigate the dependent relationship between soil variables and crop response variables, as well as the pattern in crop productivity across treatments. RDA was selected as the appropriate approach as crop productivity variables would be expected to respond linearly to soil parameters (Legendre and Legendre 1998). 'Plots × crop productivity variables' dataset includes seven variables: squash water content, total yield, dry mass, N and P concentrations, other macro and micro nutrient concentrations. Soil physicochemical and biochemical parameters were together introduced as explanatory variables in RDA. It should be noted that several soil variables were grouped or released to address our study interest and reduce the large number of explanatory variables for RDA model: soil NH_4^+-N and NO_3^-–N were grouped as inorganic N; Soil K, S and Ca were grouped as 'other macronutrients;' Fe, Mn, Zn, Cu, and Ni were grouped as soil micronutrients; soil total P, $CaCl_2$-P, HCl-P,

Table 2 Structure correlation coefficients (only list variables with loading > 0.75 on either PC) between measured parameters and the first two principal components (PC1 and PC2) for (a) soil physicochemical and (b) biochemical properties in field studies conducted at six organic farms on Waldron Island, WA comparing no treatment, biochar, poultry litter, or biochar and poultry litter

(a) Principle Component (variance explained, significance value)	PC1 (40.1%, p < 0.001)	PC2 (20.4%, p < 0.001)
WHC	0.94	
Citrate extractable P (Active inorganic P)	0.76	
Enzyme extractable P (Potential available organic P)	0.88	
Total C	0.79	
Fe		−0.88
Mn		−0.78
(b) Principle component (variance explained, significance value)	PC1 (50.1%, p < 0.001)	PC2 (16.3%, p = 0.56)
Microbial biomass C	0.84	
PSB abundance	0.82	
Basal respiration	0.81	
Dehydrogenase activity	0.85	
Phosphatase activity	0.82	

PMN, and basal respiration were released as they either had too little variation or had small structure correlation in previous PCA results; and the geometric mean of four enzyme activities (phosphatase, β-glucosidase, dehydrogenase, and urease) were used as an overall enzyme indicator. Significance for the RDA model, each axis, and each variable was tested using Monte Carlo randomization tests. Afterwards, a partial RDA was conducted to identify the variance explained by soil physicochemical variables, biochemical variables, and their overlap, respectively. All statistical tests were performed using R studio (0.99.903).

Results

Soil response to biochar application

Biochar application to soils of Waldron Island resulted in significant shifts in both nutrient availability and microbial activity. Using PCA, we observed that much of the variability in soil data matrices could be explained by two PCs (Table 2). In the first PCA (Fig. 2a physiochemical variables), soil WHC, total C content, citrate extractable P, and enzyme extractable P were highly correlated with PC1 where soil Fe and Mn concentrations were highly correlated with PC2. In the second PCA (Fig. 2b, biochemical variables), soil microbial biomass C, dehydrogenase activity, phosphatase activity, and P solubilizing bacteria abundance were positively correlated with PC1. The dissimilarities between biochar-treated plots (biochar, poultry litter + biochar) and untreated plots (control, poultry litter) were clearly demonstrated in the ordination space of both PCAs, indicating that soil characteristics were largely altered by biochar incorporation (Fig. 2). Overall, soil total C, Fe, Mn concentrations, P related variables, and biochemical parameters were identified as the parameters most sensitive to shifts in soil characteristics following biochar incorporation. Vector arrows of these variables nearly all point to biochar-treated plots, suggesting that these soil variables tend to be greater in biochar-treated plots compared to untreated plots. The result of Pearson correlation coefficients between specific soil physicochemical and biochemical variables indicated significant positive correlations between: (1) Soil WHC and most soil biochemical parameters; (2) Citrate extractable P and P solubilizing bacteria abundance; (3) Enzyme extractable P and soil phosphatase activity (Table 3). A significant effect of biochar and a synergistic effect of biochar and poultry litter were observed with many soil physicochemical and biochemical variables using MANOVA (e.g. WHC, enzyme extracted P, microbial biomass C, basal respiration, etc.) (Table 4, S1 and S2).

Fig. 2 Principle component analysis (PCA) ordination of **a** soil physicochemical variables, and **b** soil biochemical variables following biochar incorporation in field studies conducted at six oganic farms on Waldron Island, WA. *WHC* water holding capacity, *PMN* potentially mineralization N, *MBC* microbial biomass C, *PSB* P solubilizing bacteria abundance, *BR* basal respiration, *CT* control, *PL* poultry litter, *BC* biochar, *PB* poultry litter + biochar
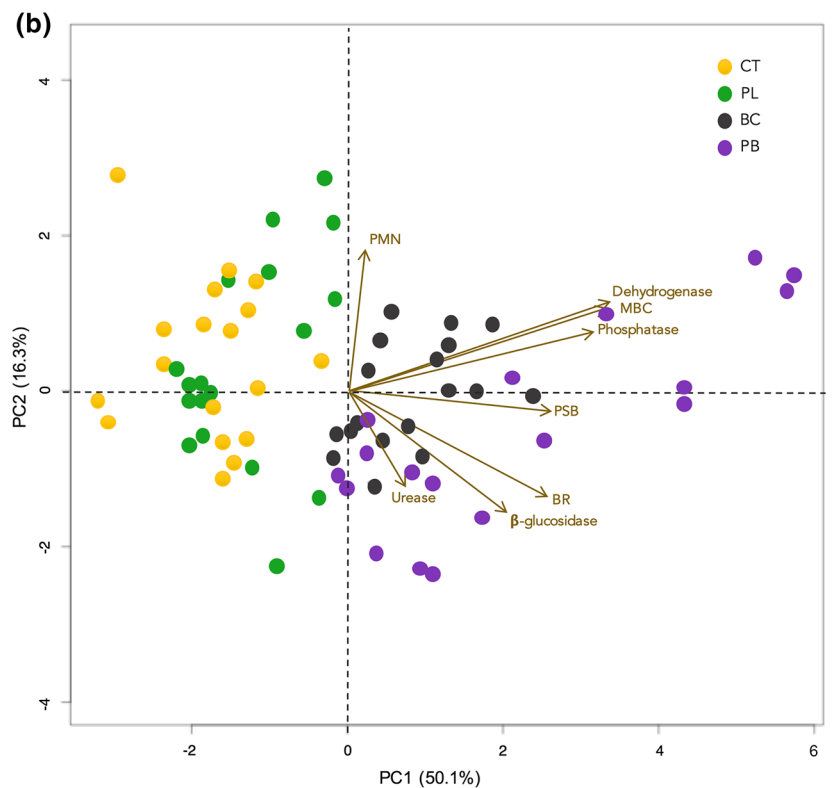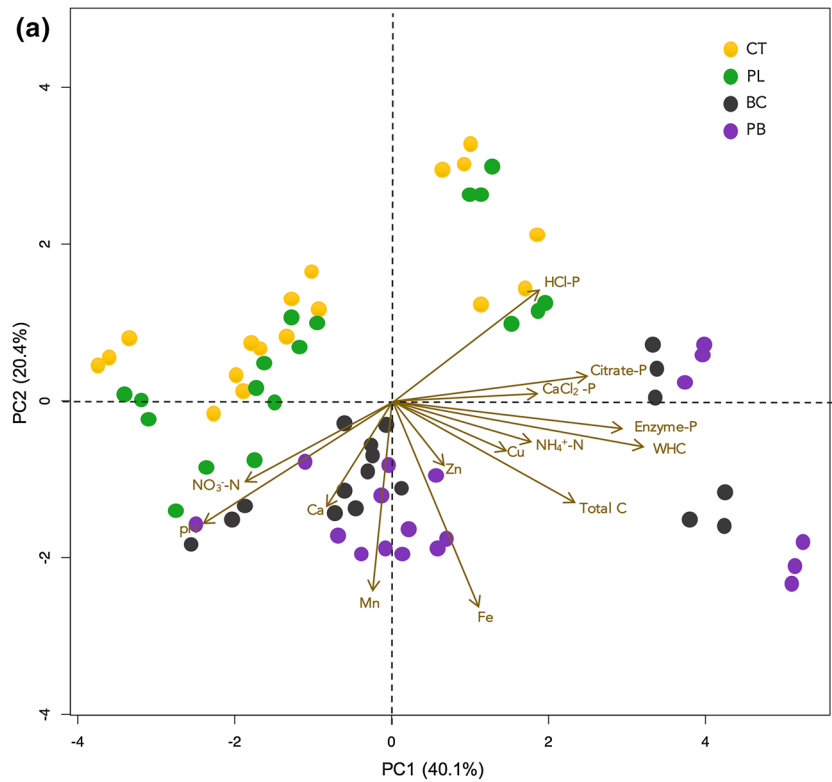
Table 3 Pearson correlation coefficient (r) between selected soil physicochemical and biochemical parameters in field studies conducted at six organic farms on Waldron Island, WA

Soil variables	WHC	Citrate-P	Enzyme-P
Microbial biomass C	0.70***	0.45***	0.64***
PSB abundance	0.78***	0.64***	0.63***
Basal Respiration	0.56***	0.36**	0.54***
Dehydrogenase activity	0.70***	0.45***	0.64***
Phosphatase activity	0.84***	0.69***	0.94***

** $p < 0.01$, *** $p < 0.001$

Crop response to biochar application

The nutrient concentration of ten elements (total N, P, S, K, Ca, Fe, Mn, Zn, Cu, and Ni) and total yield (in kg ha^{-1}) in harvested squash as influenced by treatments are reported in Table 5. Biochar treatment significantly increased squash P, S, and Fe concentrations after one growing season, reflecting enhanced

soil nutrient concentrations following woody biochar application. Total yield across six organic farms was also significantly enhanced by biochar additions when comparing 'biochar' treatment to 'control' (Table 5). PERMANOVA results demonstrated noted differences in crop productivity and nutrient uptake in comparing the four treatments ($F = 44.67$, $R^2 = 0.67$, $p < 0.001$). The results of subsequent DISPER tests ($F = 4.95$, $p < 0.01$) and pairwise comparisons revealed significant difference between 'control' and 'biochar' ($p < 0.05$), as well as 'poultry litter' and 'poultry litter + biochar' ($p < 0.05$) in crop productivity.

Relationship between soil properties and crop response

The productivity of squash grown in test plots was clearly influenced by biochar and poultry litter additions. The RDA results showed that soil physical and chemical characteristics explained a significant

Table 4 Statistical results of the factorial MANOVA on soil (a) physicochemical and (b) biochemical variables in field studies conducted at six organic farms on Waldron Island, WA

(a) Source of variation	PH		WHC		NH$_4^+$–N		NO$_3^-$–N		CaCl$_2$–P		Citrate-P		Enzyme-P	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Poultry litter	0.40	NS	0.002	NS	0.20	NS	0.46	NS	3.89	0.05	0.06	NS	2.14	NS
Biochar	4.07	*	8.46	**	0.82	NS	0.32	NS	2.35	NS	4.18	*	10.74	**
Poultry litter × biochar	0.51	NS	0.06	NS	0.07	NS	0.15	NS	5.33	*	0.22	>0.05	4.68	*

Source of variation	HCl-P		Total C		Ca		Fe		Mn		Zn		Cu	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Poultry litter	0.25	NS	2.58	NS	4.35	*	0.98	NS	0.11	NS	0.08	NS	0.01	NS
Biochar	0.01	NS	20.89	***	15.57	**	51.00	***	8.06	**	2.14	NS	0.33	NS
Poultry litter × biochar	0.16	NS	0.18	NS	0.55	NS	0.01	NS	0.11	NS	0.02	NS	0.13	NS

(b) Source of variation	MBC		PMN		PSB		BR		β-glucosidase		Dehydrogenase		Phosphatase		Urease	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Poultry litter	4.94	*	3.27	0.07	0.01	NS	0.01	NS	0.01	NS	4.94	*	2.78	NS	282	***
Biochar	36.5	***	1.43	NS	5.54	*	92.5	***	86.6	***	36.6	***	8.58	**	34.5	***
Poultry Litter × biochar	0.01	NS	0.60	NS	0.37	NS	5.06	*	5.12	*	0.01	NS	4.14	*	4.43	*

WHC water holding capacity, *MBC* microbial biomass C, *PMN* potentially mineralization N, *PSB* P solubilizing bacteria abundance, *BR* basal respiration

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS indicates $p > 0.1$

Table 5 Total fresh fruit yield and nutrient concentrations of harvested squash fruit under no treatment, poultry litter, biochar, or poultry litter and biochar in field studies conducted at six organic farms on Waldron Island, WA. Data are presented as mean ± standard error. Data were compared among treatments using Tukey-HSD 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

Treatment	Total yield (kg ha^{-1})	Nutrient concentrations (mg kg^{-1})									
		N	P	K	S	Ca	Fe	Mn	Zn	Cu	Ni
Control	1390.70^a ± 106.67	37300 ± 1058	5628.2^a ± 399.6	15481 ± 1006	3501.4^a ± 392.0	511.29 ± 23.57	84.01^a ± 19.20	21.93 ± 1.11	75.45 ± 17.18	25.22 ± 18.19	4.96 ± 0.56
Poultry litter	1552.92^{ab} ± 164.67	44700 ± 1465	6765.9^b ± 204.1	22848 ± 1806	5947.4^c ± 501.3	551.10 ± 48.84	118.02^b ± 13.78	23.39 ± 2.72	72.06 ± 12.68	32.03 ± 14.42	5.35 ± 1.33
Biochar	1729.32^b ± 70.59	41800 ± 1689	6977.8^{bc} ± 409.0	20941 ± 790	5143.1^b ± 1096.1	503.21 ± 79.99	106.98^b ± 12.80	21.95 ± 1.31	61.02 ± 5.69	20.11 ± 9.00	5.30 ± 1.12
Poultry litter + Biochar	1845.87^b ± 119.35	45400 ± 2346	7259.3^c ± 646.0	17987 ± 879	4104.9^{ab} ± 444.1	543.41 ± 33.61	134.29^c ± 14.28	25.50 ± 1.38	81.35 ± 3.88	20.96 ± 3.01	5.73 ± 1.22

portion of squash productivity (Fig. 3). Subsequent permutation tests on the RDA axes and variables indicated that RDA axes 1 and 2 were significant and together explained 84.8% of crop productivity variance; while soil citrate extractable P, enzyme extractable P, and the geometric mean of enzyme activities were the strongest drivers of the constrained variability (Table 6). Nearly all soil related vectors were roughly aligned with crop vectors indicating a highly positive relationship between the two. Biochar-treated plots tended to have higher crop yield, P concentration, and micronutrient concentrations, whereas crop N concentration and water content did not show much response to biochar additions. Soil microbial biomass C and the geometric mean of soil enzyme activity had the most positive correlation with squash fruit dry mass and total yield; crop other macronutrient and micronutrient concentrations were positively correlated with soil other macronutrient and micronutrient concentrations, respectively; crop P concentration was positively correlated with soil citrate and enzyme extractable P, as well as WHC. Crop N concentration showed no dependent relationship with soil inorganic N. Poultry litter treated plots were likely to have squash plants with higher water content and possibly N content. The results of the partial RDA showed that soil physicochemical and biochemical variables had a large overlap when explaining the variation of crop productivity, which is consistent with the findings in the Pearson correlation results between soil physicochemical and biochemical variables (Fig. 4; Table 3).

Discussion

Soil response to biochar application

Biochar applications to agricultural soils of Waldron Island resulted in significant shifts in soil nutrient status and microbial activity. Soil total C content, parameters related to P cycling processes and microbial activities (WHC, dehydrogenase activity, microbial biomass C, active inorganic P, phosphatase activity, potential available organic P, PSB abundance) were identified as those variables most responsive to biochar incorporation. The significant enhancement of surface soil total C content following biochar addition is logical and consistent with prior

Fig. 3 Redundancy analysis (RDA) ordination of crop productivity constrained by soil physicochemical and biochemical parameters in field studies conducted at six oganic farms on Waldron Island, WA. Response variables (crop variables) are shown in red vectors; explanatory variables (soil variables) are shown in brown vectors. *Geomean(Enzyme)* geometric mean of four enzyme activities (phosphatase, dehydrogenase, β-glucosidase, and urease activities), *IN* inorganic N, *CT* control, *PL* poultry litter, *BC* biochar, *PB* poultry litter + biochar
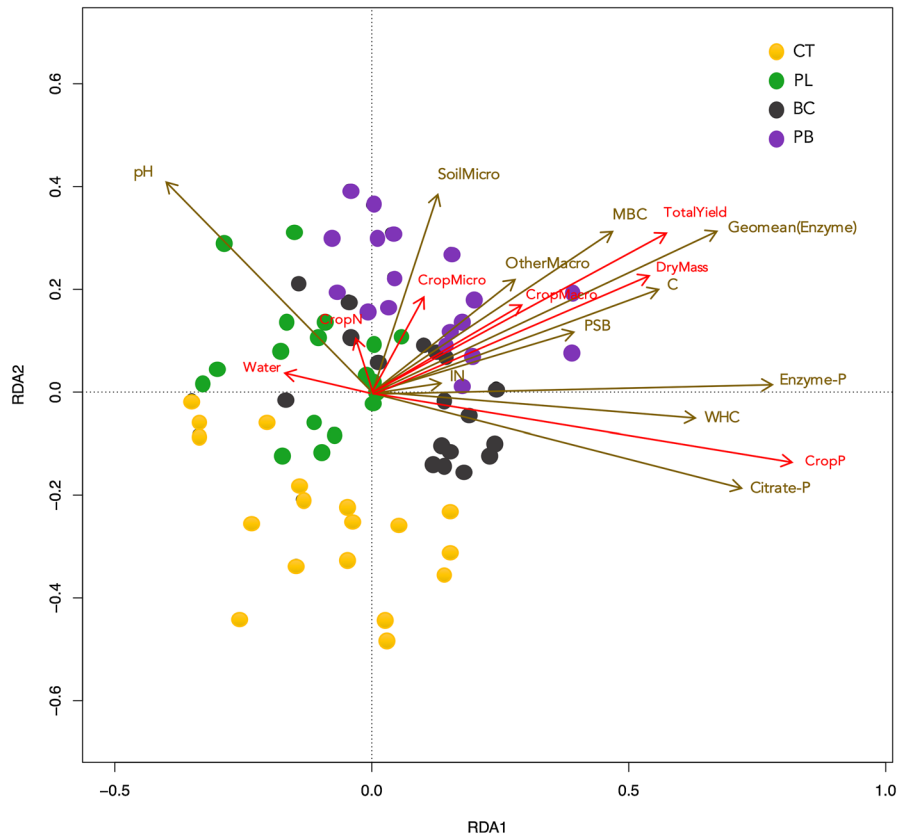
Table 6 Inter-set correlations between the weighted average scores for the crop productivity data and soil variables from the RDA for axis 1 and axis 2

Soil variable	RDA model: p < 0.001	
	RDA1 (61.9%, p < 0.001)	RDA2 (22.9%, p < 0.001)
pH	−0.50	0.42
WHC	0.78	−0.06
Available N	0.21	−0.03
Citrate extractable P (active inorganic P)	0.87	−0.23
Enzyme extractable P (potential available organic P)	0.95	−0.02
Total C	0.69	0.21
Other macronutrients	0.35	0.23
Micronutrients	0.16	0.40
Basal respiration	0.54	0.17
Microbial biomass C	0.58	0.33
Enzyme activities (geometric mean)	0.84	0.33

findings where researchers evaluated biochar influence on soil C storage (Wang et al. 2016; Laird et al. 2017). Numerous other studies cited by Lehmann and

Joseph (2015) had also indicated that a large portion of biochar consists of a certain level of organic C forms, namely fused aromatic ring structures, that is
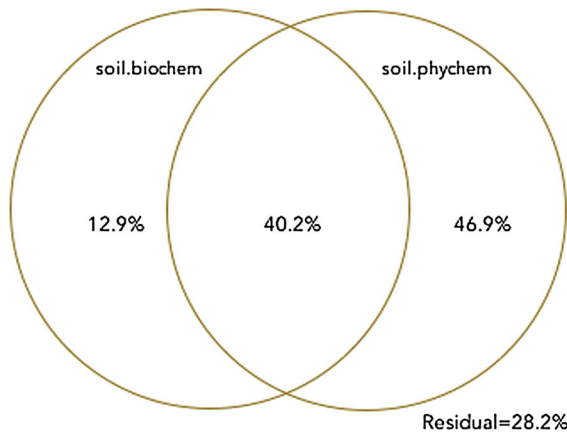
Fig. 4 Results of partial RDA showing effects of soil variables on crop productivity. Venn diagram shows unique and shared explained variance between soil physicochemical and biochemical parameters for plots receiving no treatment, biochar, poultry litter, or biochar and poultry litter in field studies conducted at six organic farms on Waldron Island, WA

relatively resistant to microbial decay and has slower return of terrestrial organic C as carbon dioxide (CO_2) back to the atmosphere, contributing directly to long-term soil C sequestration (Lehmann et al. 2011). This particularly works with biochar generated from woody materials such as that used in our study, rather than those generated from agricultural wastes such as crop residues and animal manures. (Lehmann and Joseph 2015). Total C content has a relatively high loading (0.79) on PC1, suggesting that C sequestration and storage was one of the major changes and a potential benefit for soils in Waldron Island following biochar incorporation.

Soil P solubilization and mineralization processes were readily influenced by biochar addition to surface soils in this study. Citrate and enzyme extractable P represented these two potential available P pools by emulating the corresponding mechanisms of P acquisition strategies. This suggests that wood-based biochar added to sandy soils in Waldron Island can potentially increase the phytoavailability of both organic and inorganic P pools through stimulating the P solubilizing bacterial communities and plant and microbial phosphatase activity. The labile C in biochar could potentially stimulate metabolic demands of the soil microbial community and thus drive enhanced the P demand in the surrounding soil organisms (Lehmann et al. 2011). Dehydrogenase plays a key role in the biological oxidation of soil organic matter by

transferring electrons from substrate to acceptors, and it occurs intracellular in all living cells, thus can be used as an indicator of overall soil microbial activity (Wolinska and Stepniewsk 2012). High loadings of dehydrogenase activities (0.85) and microbial biomass C (0.84) on PC1 also suggested that biochar greatly stimulated the activity of soil microbial communities (Fig. 2b; Table 2). This effect is consistent with the observation of higher extractable P, that is related to the labile C inside biochar that provided metabolic substrate for the surrounding microorganisms in a short-term (Jones et al. 2011); it could also be related to the structure of biochar itself (Lehmann et al. 2011).

Soil WHC is another variable that was clearly influenced by biochar applications and one that contributed greatly to variation across all soil characteristics (structure correlation = 0.94). It is likely that the internal pores of the biochar particles directly improved soil WHC, providing improved soil moisture conditions and potentially a more favorable habitat for microorganisms. Further, it is likely that this porous structure functions as to attract and aid in the transport of dissolved organic matter needed for microorganisms to metabolize. This argument is supported by the high correlation coefficients between WHC and multiple soil biochemical indicators (Table 3). It is also possible that biochar indirectly influenced soil microbial activity and P cycling by absorbing otherwise inhibitory organic compounds (i.e. chlorinated hydrocarbons, cyanogenic glycosides, polycyclic hydrocarbons) or transition state metals that can otherwise inhibit microbial activity (Zhang et al. 2013). However, such a "negated inhibition hypothesis" for explaining the biochar effect on microbial activity holds limited weight in organic farming systems which by definition do not use synthetic pesticides and should have natural soil metal concentrations. Increased soil P availability associated with higher microbial activity following biochar addition can be further accelerated by synergistic effects of biochar and poultry litter (Table 4) indicating that the char may induce a priming effect that yields greater bioavailable C and nutrients from soil organic matter and poultry litter (Cheng 2009).

Biochar treatments appeared to improve the concentrations of soil macro and micronutrients, with a highly visible response for Fe and Mn. One possible explanation for increased alkaline and transition state metal concentrations is related to the increased cation

exchange capacity in soils amended with biochar (Novak et al. 2009). Evidence of near edge X-ray absorption fine structure (NEXAFS) and scanning transmission X-ray microscopy (STXM) from a study of Liang et al. (2006) suggested that the oxidized surface associated with the aromatic C originated from biochar itself was responsible for its higher surface charge compared with non-biochar (Liang et al. 2006); the surface oxidation was created by the adsorption of surrounding non-biochar substances, and could increase with time in soil (Chan et al. 2008). It has also been reported that the organic ligands (carboxyl, phenol, alcohol, or enol groups) in biochar can form complexes with soil metals, subsequently increasing the concentration of metals in soil solutions (Graber et al. 2010). The increased exchange capacity would simply increase the capacity of the soil to retain metals in surface soils. Alternatively, Graber et al. (2014) indicated that biochar can participate in a variety of chemical and biological redox reactions, which would further lead to the solubilization of Fe and Mn. These two metals, Fe and Mn, oxides have higher reduction potentials than most other metals and therefore would be likely to be solubilized under microaerophilic conditions (Graber et al. 2014).

Biochar had limited influence on N related parameters (soil NH_4^+–N, NO_3^-–N, potentially mineralizable N, and urease activity). This departs somewhat from our previous findings on the San Juan Islands (Gao et al. 2016), but is consistent with many studies (Griffin et al. 2017), particularly the result of a meta-analysis that investigated biochar effect and soil inorganic N using 33 studies (Biederman and Harpole 2013) and another recent study that specifically examined the effect of wood biochar on soil inorganic N (Nguyen et al. 2017). Agricultural soils already have highly active nitrifying communities and rapid net N mineralization rates, which may not further respond to biochar addition (DeLuca et al. 2006). Soil N related parameters also showed no patterns with the poultry litter treatment as well (Fig. 2a). However, it should be noted that our soil samples were collected at harvest season (end-growing season), it is highly likely that soil inorganic N was enhanced by poultry litter treatment and subsequently used by plants during the growing season, as supported by the RDA result that crop N concentration tended to be higher in poultry litter-treated plots (Fig. 3).

Crop response and relationship to soil properties following biochar application

Biochar treatments significantly increased squash P, S, and Fe concentrations, as well as total yield after one growing season (Table 5). Using PERMANOVA and DISPER to test differences and significant dispersions among all crop variables, we found that biochar-treated plots had significantly different crop response compared to non-biochar plots, reflecting a positive overall effect.

A large number of soil properties and processes were reflected in crop productivity in this 5-month field trial (Fig. 3; Tables 5, 6). Improved soil biochemical conditions (particularly citrate extractable P, enzyme extractable P and enzyme activities) in biochar-treated plots were associated with higher crop productivity. One of the most common hypotheses for the observed beneficial effect of biochar on crop productivity, is related to a shift in mycorrhizal fungal activity following biochar application (Warnock et al. 2007; LeCroy et al. 2013). Joseph et al. (2010) indicated that plant roots or root hairs could enter the water-filled macro-pores or bond onto the biochar surface, causing a wide range of reactions that help the uptake of nutrients (Joseph et al. 2010). Although mycorrhizal fungal abundance or activity was not examined in our study, it could represent a factor responsible for the highly positive correlation of soil nutrient concentrations, particularly soil available P and associated phosphatase activity, as well as the improved squash nutrients in char-treated plots at the end-growing season. It is important to note that squash (C. maxima) is considered as a "P efficient" crop that releases organic acids to increase P solubility and meet the high P demand of its life cycle (Reinbott and Blevins 1999). This would help explain the observation that soil P was a major explanatory variable in RDA (Table 6).

Besides soil nutrients and corresponding crop nutrient uptake, soil enzyme activity was observed to be effective explaining crop total yield and dry mass (Table 6). To date, there is little strong evidence to directly relate soil enzyme activity to crop production, therefore, the positive relationship may be indirect, considering nutrient mineralization to plant available forms is accomplished through enzymatic activity. The addition of biochar with available substrate and nutrients may have increased the activity of

microorganisms degrading cellulose either by promoting the growth of microbial species with constitutive enzymes, or by stimulating inductive enzyme synthesis in microbial cells (Dilly and Nannipieri 2001). Biochar may influence the induction of enzymes by adsorbing the catalysis product thereby creating a positive feedback, where more enzyme is produced in the absence of product which has adsorbed to the "charosphere." Alternatively, substrate adsorption to biochar may create a concentration of material that stimulates enzyme production. Given that aromatic, organic acid anions adsorb to biochar via proton exchange with water and subsequent H-bonding with carboxylate or phenolate biochar surface groups (Ni et al. 2011), it is possible that products produced during the enzyme assay adsorbed to the charcoal. Soil inorganic N concentration was not correlated with crop N concentrations, implying a complex mechanism of crop N uptake. It has been reported that crop N uptake could be regulated by soil N concentration based on multiphasic kinetics, and individual kinetic parameters can differ among crop species (Laine et al. 1993). It is likely that the soil inorganic N levels at end-growing season exceeded the crop associated 'critical N dilution curve', which defines the minimum amount of N needed for maximal crop growth rate (Greenwood et al. 1990), thereby rendering soil N a non-limiting factor for crop N uptake late in the growing season (Devienne-Barret et al. 2000). Again, it is highly likely that the regulation of crop N uptake by soil N availability has functioned during the growing season. Continuous temporal in situ sampling techniques will be needed for future research in monitoring soil nutrient status or rates of pool turnover. The use of a partial RDA indicated that a large portion (40.2%) of the total variance observed was jointly explained by soil physicochemical and biochemical parameters, as is supported by the Pearson correlation test result following both PCAs (Fig. 4; Table 3).

Conclusion

The sandy soils that dominate Waldron Island are of a glacial origin leading to a naturally high leaching potential and relatively low WHC creating a need for soil tilth improvements that enhance nutrient and water retention in surface soils. Concurrently, the island has an urgent need for forest health or fuel reduction treatments to reduce fire risk on this isolated dry-forest ecosystem, but the left over logging residues represent a problem for residents. Herein we studied the outcomes of wedding the utilization of logging residues with the need to improve soil fertility on organic farms. The multivariate analysis of results from this short-term field study on six organic farms in Waldron Island suggest that biochar produced from local fuel reduction treatments and applied alone or in combination with poultry litter has the potential to induce positive benefits in overall fertility of these sandy, glacial origin soils. The primary benefits of the biochar applications being a significant increase in total soil C storage, active inorganic P (citrate extractable P), potential available organic P (enzyme extractable P), microbial biomass C and several specific enzyme activities. In particular, we suggest that the biochar induced enhancement of bioavailable P from inorganic and organic P sources might be attributed to an increased presence of P solubilizing bacteria abundance and generation of phosphatase enzyme. In organic farming systems, organic P fertilizer resources (i.e. manure) dominate P application in these systems, therefore the enhanced enzyme activity (partially through adsorbing enzymatic inducing agents by biochar amendment) may potentially play a key role in supplementing the bioavailable P through mineralization processes. The input of metabolic C in the presence of biochar might also induce the abundance of P solubilizing bacteria, and thus aid on the P solubilization process.

A positive relationship between soil properties and crop productivity as influenced by biochar additions was revealed in this study. Squash productivity and squash nutrient uptake were found to correlate well with available P concentrations and enzyme activities as explanatory soil variables. While soil microbial parameters are often considered responsible for soil physicochemical properties, alterations in the physicochemical conditions of soils might also lead to shifts in microbial community composition and changes in microbial function. This natural relationship between soil physicochemical properties and soil biochemical properties is frequently observed upon amending soils with fertile organic residues or biochar. Producing biochar from local timber harvest residues, converting them to biochar, and applying them in neighboring agricultural soils resulted in a net positive effect on

both agronomic conditions and forest health and is a strategy that could be exported to other ecosystems with sandy soils and fuel reduction demands.

References

Anderson JPE (1982) Soil respiration. Methods of soil analysis: Part 2—microbiological and biochemical properties. Soil Science Society of America, Madison, pp 831–871

Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5:202–214

Brewer CE, Schmidt-Rohr K, Satrio JA, Brown RC (2009) Characterization of biochar from fast pyrolysis and gasification systems. Environ Prog Sustain Energy 28:386–396

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Bundy LG, Meisinger JJ (1994) Nitrogen availability indices. Methods of soil analysis: Part 2—microbiological and biochemical properties. Soil Science Society of America, Madison, pp 951–984

Chan KY, Van Zwieten L, Meszaros I et al (2008) Agronomic values of greenwaste biochar as a soil amendment. Soil Res 45:629–634

Cheng W (2009) Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. Soil Biol Biochem 41:1795–1801

DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly influences nitrogen cycling in Ponderosa Pine forests. Soil Sci Soc Am J 70:448

DeLuca TH, Glanville HC, Harris M et al (2015) A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol Biochem 88:110–119

Devienne-Barret F, Justes E, Machet JMM, Mary B (2000) Integrated control of nitrate uptake by crop growth rate and soil nitrate availability under field conditions. Ann Bot 86:995–1005

Dilly O, Nannipieri P (2001) Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biol Fertil Soils 34:64–72

Dray S, Chessel D, Thioulouse J (2003) Co-inertia analysis and the linking of ecological data tables. Ecology 84:3078–3089

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biol Biochem 20:601–606

Gao S, DeLuca TH (2016) Influence of biochar on soil nutrient transformations, nutrient leaching, and crop yield. Adv Plants Agric Res 4:00150

Gao S, Hoffman-Krull K, Bidwell AL, DeLuca TH (2016) Locally produced wood biochar increases nutrient retention and availability in agricultural soils of the San Juan Islands, USA. Agric Ecosyst Environ 233:43–54

Graber ER, Harel YM, Kolton M et al (2010) Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant Soil 337:481–496

Graber ER, Tsechansky L, Lew B, Cohen E (2014) Reducing capacity of water extracts of biochars and their solubilization of soil Mn and Fe. Eur J Soil Sci 65:162–172

Greenwood DJ, Lemaire G, Gosse G et al (1990) Decline in percentage N of C_3 and C_4 crops with increasing plant mass. Ann Bot 66:425–436

Griffin DE, Wang D, Parikh SJ, Scow KM (2017) Short-lived effects of walnut shell biochar on soils and crop yields in a long-term field experiment. Agric Ecosyst Environ 236:21–29

Haider G, Steffens D, Moser G et al (2017) Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study. Agric Ecosyst Environ 237:80–94

Jones DLL, Murphy DVV, Khalid M et al (2011) Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. Soil Biol Biochem 43:1723–1731

Joseph SD, Camps-Arbestain M, Lin Y et al (2010) An investigation into the reactions of biochar in soil. Aust J Soil Res 48:501–515

Kessell S, Whittaker R (1976) Comparisons of three ordination techniques. Vegetatio 32:21–29

Klein DA, Loh TC, Goulding RL (1971) A rapid procedure to evaluate the dehydrogenase activity of soils low in organic matter. Soil Bio Biochem 3:385–387

Laine P, Ourry A, Macduff J et al (1993) Kinetic parameters of nitrate uptake by different catch crop species: effects of low temperatures or previous nitrate starvation. Physiol Plant 88:85–92

Laird DA, Novak JM, Collins HP et al (2017) Multi-year and multi-location soil quality and crop biomass yield responses to hardwood fast pyrolysis biochar. Geoderma 289:46–53

Laker M, Du Preez C (1982) Investigation into the accuracy of hydrometers for soil particle size determinations. Agroplantae 14:17–22

LeCroy C, Masiello CA, Rudgers JA et al (2013) Nitrogen, biochar, and mycorrhizae: alteration of the symbiosis and oxidation of the char surface. Soil Biol Biochem 58:248–254

Legendre P, Legendre L (1998) Numerical ecology. Elsevier, Amsterdam

Lehmann J, Joseph S (2015) Biochar for environmental management: science, technology and implementation. Routledge, Abingdon

Lehmann J, Rillig MC, Thies J et al (2011) Biochar effects on soil biota—a review. Soil Biol Biochem 43:1812–1836

Liang B, Lehmann J, Solomon D et al (2006) Black carbon increases cation exchange capacity in soils. Soil Sci Soc Am J 70:1719

Loveday J (1974) Methods for analysis of irrigated soils. Commonwealth Agricultural Bureaux, Wallingford

McLaren TI, Guppy CN, Tighe MK et al (2012) Rapid, non-destructive total elemental analysis of vertisol soils using portable X-ray fluorescence. Soil Sci Soc Am J 76:1436

Mulvaney RL, Sparks DL, Page AL et al (1996) Nitrogen-inorganic forms. Methods of soil analysis: Part 3—chemical methods. Soil Science Society of America, Madison, pp 1123–1184

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270

Nguyen TTN, Xu CY, Tahmasbian I et al (2017) Effects of biochar on soil available inorganic nitrogen: a review and meta-analysis. Geoderma 288:79–96

Ni J, Pignatello JJ, Xing B (2011) Adsorption of aromatic carboxylate ions to black carbon (biochar) is accompanied by proton exchange with water. Environ Sci Technol 45:9240–9248

Novak JM, Busscher WJ, Laird DL et al (2009) Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Sci 174:105–112

Olmo M, Villar R, Salazar P, Alburquerque JA (2015) Changes in soil nutrient availability explain biochar's impact on wheat root development. Plant Soil 399:333–343

Reinbott TM, Blevins DG (1999) Phosphorus nutritional effects on root hydraulic conductance, xylem water flow and flux of magnesium and calcium in squash plants. Plant Soil 209:263–273

Santos WPCD, Gramacho DR, Teixeira AP et al (2008) Use of Doehlert design for optimizing the digestion of beans for multi-element determination by inductively coupled plasma optical emission spectrometry. J Braz Chem Soc 19:1–10

Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301–307

Tabatabai MA, Bremner JM (1972) Assay of urease activity in soils. Soil Biol Biochem 4:479–487

Wang J, Xiong Z, Kuzyakov Y (2016) Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenerg 8:512–523

Warnock DD, Lehmann J, Kuyper TW, Rillig MC (2007) Mycorrhizal responses to biochar in soil—concepts and mechanisms. Plant Soil 300:9–20

Wolinska A, Stepniewsk Z (2012) Dehydrogenase activity in the soil environment. Dehydrogenases. InTechOpen, Croatia

Xu CY, Bai SH, Hao Y et al (2015) Peanut shell biochar improves soil properties and peanut kernel quality on a red Ferrosol. J Soils Sediments 15:2220–2231

Zhang X, Wang H, He L et al (2013) Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ Sci Pollut Res 20:8472–8483