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Effect of biochar application on microbial biomass and enzymatic activities in degraded red soil

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To assess biochar effect on soil microbial biomass, community and enzymatic activities, degraded acidic soil was amended with three different rates (0.5, 1.0 and 2.0%) of oak wood biochar ($W_{0.5}$, $W_{1.0}$ and $W_{2.0}$) and bamboo biochar ($B_{0.5}$, $B_{1.0}$ and $B_{2.0}$), with control as 0%. The soil and the biochar were mixed thoroughly, wetted and incubated at a constant temperature of 25°C. The amended soil properties were evaluated after the 1st, 8th and 16th weeks of the incubation. It was found that soil pH, total organic carbon (TOC) and urease increased significantly with increasing biochar rate while the activity of acid phosphatase decreased, the reason can be the inverse correlation of this enzyme with soil pH. TOC had positive correlation with urease. The β-glucosidase correlated positively with dissolved organic carbon (DOC) and negatively with C/N, suggesting that mineralization of organic matter provides substrates for this enzyme. The highest microbial biomass C as well as total Phospholipid fatty acid analysis (PLFA) was observed at the lowest rates, particularly the treatment of W0.5 had higher relative abundance of soil bacteria, fungi and gram-positive bacteria. Our results suggest that biochar application improve the fertility of degraded red soil by increasing soil pH, TOC and DOC which, in turn, enhance soil enzymes, microbial biomass and community.

Key words: Biochar, enzymes, microbial biomass, microbial community, phospholipid fatty acids.

INTRODUCTION

Currently, in response to the need of more sustainable agricultural production and in order to tackle global warming, there are attempts to recreate Terra Preta (ancient soils amended with black carbon) (Glaser, 2007) by incorporating biochar to soils as means of increasing soil fertility and carbon sequestration (Lehmann et al., 2006). Biochar is the carbon-rich product obtained when biomass is heated in a closed container with little or no available air with the purpose to amend soil (Lehmann and Joseph, 2009).

Biochar has been widely and increasingly proposed as soil amendment (Lehmann and Joseph, 2009; Sohi et al., 2010). By increasing soil pH, biochar has been proved to ameliorate soil acidity (Yuan et al., 2011). This effect could particularly benefit China where soil acidification is a major problem in soils of intensive agricultural systems such as extremely leached red soils (Argi-Udic Ferrosols) and yellow soils (Ali- Periudic Argosols), the most acidic

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of them in south China are approaching pH values at which potentially toxic metals such as Al and Mn could be mobilized (Guo et al., 2010).

Interactions between biochar, soil, and microbes are known to occur within a short period of time after application to the soil (Lehmann and Joseph, 2009). Dissolution, hydrolysis, carbonation and decarbonation, hydration, and redox reactions are the major process affecting biochar weathering in soil, as well as interactions with soil biota. The rates at which these reactions occur depend on the nature of the reactions, type of biochar, and pedoclimatic conditions.

It is widely recognized that organic matter plays and essential role in a range of soil physical, chemical and biological process and that soil organic carbon is important in maintaining soil quality (Ghosh et al., 2012). Biochar, as a soil amendment, can increase concentrations of soil organic matter, especially water-extractable organic carbon (Lin et al., 2012), increase microbial biomass (Kolb et al., 2009), stimulate soil microbial activity (Lehmann et al., 2011), change microbial community in soil (Pietikainen et al., 2000; Wardle et al., 2011). Biochars application in soil can affect soil microbial community structure due to their high sorption capacity (Lehmann et al., 2011), changing the soil pH (Rousk et al., 2010) as well as modification of microbial environment (Jindo et al., 2012a). Painter (2001) reported that biochars may contain compounds such as polycyclic aromatic hydrocarbons and other toxic carbonyl compounds that can have bactericidal or fungicidal activity. However, Ogawa (1994) has shown that these substances can, and do, serve as C and energy sources for selected microbes.

Due to intensive agricultural activities along with abundant moisture and high temperature in the region, the red soil used in this experiment was low in carbon content (Zhang and Xu, 2005), and soil pH. Hence, we hypothesized that the application of biochar (pyrolyzed at 600°C with high pH value) improve soil organic carbon and soil pH and would have effect on soil enzymes, microbial biomass and community that support many key ecosystem functions essential for soil quality. Therefore, the objective of this study was to evaluate the effect of oak wood and bamboo biochar on soil pH, total organic carbon (TOC) dissolved organic carbon (DOC), soil enzymes, microbial biomass and community of soil.

MATERIALS AND METHODS

Soil sampling

A laboratory incubation was carried in the facilities of the College of Environmental and Resource Science, Soil Laboratory, Zhejiang University, Hangzhou, China. The experimental soil for this study was sampled at Meijiawu, suburban of Hangzhou. The soil is highly weathered (Plinthic Hapli Udic Ferrosols in Chinese Soil Taxonomic Classification System; and Typic plinthustults in Soil Taxonomy), derived from quaternary red clay and characterized by low pH. The Land use was tea plantation.

Biochar preparation and characterization

The biochars used in the incubation were purchased from a company located in Hangzhou city (Linan Yaoshi charcoal production Limited) and obtained from oak wood (*Quercus phillyraeoides*) and bamboo (*Phyllostachy edulis*) after pyrolysis at 600°C for 2 h. These feedstocks (oak wood and bamboo) were chosen to represent woody and grassy biomass. The pH was determined in deionized water at the ratio of 1:10 wt/v (Gaskin et al., 2008) by Orion 720 pH meter. The carbon, hydrogen, and nitrogen contents of the oak wood biochar were determined using a CHN elemental analyzer (Flash EA 1112, Thermo Finnigan). The oxygen content was estimated by mass difference (1000 – C, H, N and ash). The ash content was determined according to ASTM D-1762-84 (2007) by combusting the biochar at 750°C for 6 h in open crucibles on a dry weight basis. The BET (Brunauer-Emmet-Teller) surface areas was measured via N_2 adsorption multilayer theory using a Nova 2200e surface area analyzer (Quantachrome, Boynton Beach, FL) (Chen et al., 2008).

Incubation experiment

The soil sample was passed through 5 mm sieve and placed in the plastic pots, and oak wood biochar and bamboo biochar (sieved at 0.25 mm) were added to the soil (toal 2 kg). On basis of soil weight, the biochars were added with three different rates (0.5, 1.0 and 2.0%) of oak wood biochar ($W_{0.5}$, $W_{1.0}$ and $W_{2.0}$) and bamboo biochar ($B_{0.5}$, $B_{1.0}$ and $B_{2.0}$), with control as 0%. After mixing the soil and the biochar thoroughly, they were wetted with deionized water to about saturation of the experimental soil. All pots were covered with plastic film and then a small hole was made to allow gaseous exchange. The pots were incubated at a constant temperature of 25°C. Based on evaporation loss, the soil moisture was kept constant by regular weighing of the pots. For each treatment, triplicate samples were prepared. After Week 1, 8 and 16, the incubated soils were taken and separated into three groups: The first group samples were air dried and sieved with 2 mm and 0.25 mm. These samples used for analysis of chemical properties. The second groups were sieved with 2 mm to determine soil microbial biomass C and N and enzymatic activities. The third group were freeze-dried and preserved at -70°C in refrigerator, these group used to determine phospholipid fatty acids for microbial communities in soil.

Analysis

Soil basic properties

Particle size distribution was determined by pipette method. Soil pH was determined through a suspension sample with a soil (air-dried) to water (w/v) ratio of 1:2.5 and measured with Orion 720 pH meter (Pansu and Gautheyrou, 2006). Soil organic carbon was determined by dichromate oxidation (Nelson and Sommers, 1982). Total nitrogen (TN) in soil was measured using the Kjeldahl method after H_2SO_4 digestion in the presence of K_2SO_4-$CuSO_4$-Se catalyst (Bremner, 1996).

Dissolved organic carbon (DOC)

Soil dissolved organic carbon (DOC) was determined by the method of Jones and Willet (2006). The extracts were analyzed for carbon concentration with a multi N/C analyzer (Flash EA 1112,

Thermo Finnigan).

Enzyme activity

The activities of acid phosphatases were determined by a slightly modified method of Tabatabai and Bremner (1969) at pH 6.5 with p-nirophenyl phosphate (pNPP) solution used as substrate. β-glucosidase activity was measured following the method described by Tabatabai (1982). This method is based on the colorimetric estimation of the p-nitrophenol (PNP) formed by the hydrolysis of the p-nitro-phenyl- β-D-glucopiranoside (PNG) at 37°C for 1 h. The estimation of urease activity was carried out following the method described by Li (1996). Briefly, 10 g soil sample was taken into 100 ml conical flask, and 10 ml of (100 g L^{-1}) urea solution and 20 ml citric acid buffer (pH 6.7) were added into the flask. The soil sample was incubated at 37°C for 24 h. After incubation, the solution was diluted to 100 ml and filtered. Of the filtrate, 1 ml was taken into 50 ml volumetric flask, and 10 ml distilled water, 4 ml of sodium phenolate and 3 ml of sodium hypochlorite were added. Then, it was mixed and made the volume to 50 ml with distilled water, and absorbance of color was checked at 578 nm.

Microbial biomass C and N

Microbial biomass (C and N) were determined by fumigation extraction method (Vance et al., 1987). The extracts were measured for C and N concentration with a multi N/C analyzer (Flash EA 1112, Thermo Finnigan). Microbial biomass C was calculated as follows: microbial biomass C $=E_C/ K_{EC}$, where E_C= [(organic C extracted from fumigated soils) minus (organic C extracted from non-fumigated soils)] and K_{EC}= 0.45 (Wu et al., 1990). Microbial biomass N was calculated as follows: microbial biomass N $=E_N/K_{EN}$, where E_N= [(total N extracted from fumigated soils) minus (total N extracted from non-fumigated soils)] and K_{EN} = 0.54 (Brookes et al., 1985).

Phospholipid fatty acid analysis (PLFAs)

After 8 and 16 weeks of incubation, the incubated soil samples were sieved (<2 mm), freeze-dried and stored at -70°C. Using the freeze-dried soil samples, PLFAs were extracted and identified according to Wu et al. (2009). Lipids were extracted using a single-phase chloroform–methanol–citrate buffer system. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns (Supelco, Inc., Bellefonte, PA). After methylation of the polar lipids, PLFA methyl esters were separated and analysed in an Agilent 6890 N Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE). The fatty acid 19:0 was added as an internal standard before methylation and fatty acid methyl esters were identified automatically by the MIDI peak identification software.

The identified fatty acids were taken to represent different microbial groups: PLFA 18:1ω9c was taken as a fungal biomarker, monounsaturated and cyclopropyl fatty acids as Gram-negative bacteria biomarkers, iso- and anteiso-fatty acids as Gram-positive bacteria biomarkers, straight chain saturated fatty acids as bacteria biomarkers and carboxylic acids with a methyl function on the carbon chain as actinobacteria (Federle et al., 1986; Frostegard et al., 1993a, b; O'Leary and Wilkinson, 1988; Zelles, 1999; Zelles and Bai, 1994; Zogg et al., 1997). The ratio bacteria to fungi were determined. All results are given in nmol g^{-1}.

Statistical analysis

The data collected was subjected to analysis of variance (ANOVA) using SAS statistical analysis software version 9.1. Microbial C and N, soil enzymes, soil pH, total organic carbon, total nitrogen and dissolved organic carbon were analyzed by two-factor ANOVA to compare treatments across time and one-factor ANOVA was deployed to compare treatment effects at any given time. The least significant difference (LSD at 0.05 level of probability) test was applied to assess the differences among the means. Principal component analysis (PCA) was performed on individual fatty acids. Pearson's coefficient analysis was used for correlation.

RESULTS AND DISSCUSION

Biochar effects on soil pH, DOC, total organic C and N

Incorporation of biochar to soils could result in an increase or decrease in soils pH, depending on the pH and liming value of the biochar (Lehmann et al., 2011). The soil used was degraded soil which had an acidic pH of 4.57, whereas the pH of oak wood and bamboo biochar (10.25 and 10.22, respectively) were basic (Table 1). Due to the dissolution of the alkaline minerals, the pH in the amended soils increased with increasing application rate with the highest pH value measured in W2.0 and the lowest in the control for each time of incubation (Table 2). In contrary, the pH decreased with increasing time of incubation. The maximum pH (4.87) was recorded at Week 1 and the lowest (4.46) was measured at Week 16 (Table 2). The reason for a pH decrease through incubation times can be oxidation of C to form acidic carboxyl groups as described by Lehmann et al. (2011).

The soil used in this experiment was highly degraded with low amount of C (5.5 g kg^{-1}), therefore amendment of this soil with organic matter is unquestionable. Biochar, pyrogenic organic matter (PyOM) (Santos et al., 2012) contains a considerable organic matter (Schmidt et al., 1999). Recent research findings also showed that biochar increase concentrations of soil organic matter (Lin et al., 2012). As shown in Table 2, there was a sharp increase in total organic C (TOC) with increasing biochar application, which was due to the high C content of the oak and bamboo biochars. Due to its sensitivity to heating, the N content of the biochars used in this experiment was low (Tyron, 1948). Hence, the C/N ratio kept the trend of total organic C, increased with increasing the application rate. As to time effect, both TOC and total N (TN) decreased with increasing time of incubations and the reverse was observed in C/N.

DOC represents a small proportion of soil organic matter, but is of significant importance in the soil ecosystem due to its mobility and reactivity (Lin et al., 2012). The bamboo biochar treated soils had higher DOC than the oak wood biochar (Table 2). The reason could be the higher labile organic carbon content in bamboo biochar than oak wood biochar. The DOC was higher in B1.0 (46.57 mg kg^{-1}) followed by B0.5 (43.14 mg kg^{-1}) at Week 1. Similarly, at Week 8, DOC was higher in B1.0

Table 1. Basic property of soil, oak wood and bamboo biochars.

Property	Soil	Oak wood biochar	Bamboo biochar
Sand %	22	ND	ND
Silt%	40	ND	ND
Clay%	38	ND	ND
Texture	Clay Loam	ND	ND
pH	4.57	10.25	10.22
Total C (g kg^{-1})	5.50	758.10	759.20
Total N (g kg^{-1})	0.90	6.40	11.60
Total P (mg kg^{-1})	881.28	897.90	1098.33
Total K (g kg^{-1})	12.70	9.94	19.76
Hydrogen (g kg^{-1})	ND	11.20	21.10
Oxygen (g kg^{-1})	ND	104.90	64.50
Ash (g kg^{-1})	ND	119.40	143.60
Surface area (m^2g^{-1})	ND	154.6	137.7

ND- not detected, Oxygen =1000- (C+N+H+ Ash).

Table 2. Soil pH, DOC, total organic C and N at different rate of biochar applications.

Treatment	pH	DOC (mg kg^{-1})	Total organic C (g kg^{-1})	Total N (g kg^{-1})	C/N
			Week 1		
Con	4.56±0.01^{f}	27.65±1.10^{e}	5.50±0.10^{g}	0.94±0.05^{d}	5.86±0.42^{g}
$W_{0.5}$	4.69±0.01^{c}	37.60±1.04^{c}	8.71±0.04^{e}	0.95±0.00^{cd}	9.17±0.04^{e}
$W_{1.0}$	4.74±0.01^{b}	36.33±0.76^{c}	15.21±0.02^{c}	0.95±0.02^{cd}	16.01±0.32^{c}
$W_{2.0}$	4.87±0.01^{a}	33.32±1.07^{d}	23.95±0.04^{a}	0.99±0.00^{c}	24.19±0.04^{a}
$B_{0.5}$	4.59±0.03^{e}	43.14±0.31^{b}	7.71±0.05^{f}	0.95±0.04^{cd}	8.13±0.40^{f}
$B_{1.0}$	4.66±0.02^{d}	46.57±0.04^{a}	11.21±0.01^{d}	1.06±0.02^{b}	10.58±0.19^{d}
$B_{2.0}$	4.68±0.01^{d}	42.34±0.34^{b}	20.55±0.04^{b}	1.14±0.02^{a}	18.03±0.28^{b}
			Week 8		
Con	4.50±0.13^{a}	18.37±0.23^{d}	5.47±0.02^{g}	0.94±0.01^{d}	5.82±0.04^{g}
$W_{0.5}$	4.56±0.10^{a}	19.10±0.60^{cd}	8.69±0.00^{e}	0.94±0.00^{d}	9.24±0.00^{e}
$W_{1.0}$	4.59±0.23^{a}	18.80±1.03^{cd}	15.19±0.02^{c}	0.94±0.00^{d}	16.16±0.02^{c}
$W_{2.0}$	4.67±0.08^{a}	15.40±0.45^{e}	23.95±0.04^{a}	0.98±0.01^{c}	24.44±0.29^{c}
$B_{0.5}$	4.52±0.07^{a}	20.43±0.28^{ab}	7.69±0.03^{f}	0.94±0.02^{d}	8.18±0.21^{f}
$B_{1.0}$	4.56±0.09^{a}	21.41±1.38^{a}	11.15±0.03^{d}	1.05±0.04^{b}	10.63±0.38^{d}
$B_{2.0}$	4.60±0.11^{a}	19.88±0.34^{bc}	20.54±0.01^{b}	1.12±0.00^{a}	18.34±0.01^{b}
			Week 16		
Con	4.46±0.08^{c}	21.95±0.05^{a}	5.45±0.01^{g}	0.93±0.01^{d}	5.86±0.07^{g}
$W_{0.5}$	4.50±0.04^{bc}	20.83±0.78^{b}	8.67±0.03^{e}	0.93±0.00^{d}	9.32±0.03^{e}
$W_{1.0}$	4.53±0.02^{b}	19.70±0.28^{c}	15.17±0.05^{c}	0.94±0.04^{cd}	16.16±0.74^{c}
$W_{2.0}$	4.65±0.03^{a}	16.36±0.42^{e}	23.94±0.03^{a}	0.97±0.01^{c}	24.68±0.29^{a}
$B_{0.5}$	4.47±0.03^{bc}	22.28±0.47^{a}	7.67±0.01^{f}	0.93±0.02^{d}	8.25±0.17^{f}
$B_{1.0}$	4.51±0.04^{bc}	18.30±0.40^{d}	11.10±0.05^{d}	1.03±0.01^{b}	10.78±0.15^{d}
$B_{2.0}$	4.53±0.01^{b}	18.24±0.70^{d}	20.51±0.01^{b}	1.11±0.00^{a}	18.48±0.01^{b}

DOC: dissolved organic carbon. All values were expressed as mean ± standard deviation n=3). Different letters in the same column for each of sampling time indicate significant differences ($p < 0.05$).

(21.41 mg kg^{-1}) followed by B0.5 (20.43 mg kg^{-1}). However, at Week 16, DOC decreased in increasing the biochar rate. The reason could be sorption of DOC into the biochar. Our previous study also showed that fixation of labile organic carbon with increased biochar application rates (Zhang et al., 2012). This is because

Table 3. Correlations between soil pH, DOC, C, N, microbial biomass and soil enzymes at different incubation times (n = 3).

Property	MBC	MBN	Urease	Acid Phosphatase	β-glucosidase
			Week 1		
pH	0.41	0.04	0.37	-0.88***	-0.88***
DOC	0.21	0.46*	0.66**	0.07	0.44*
TOC	0.04	-0.16	0.62**	-0.76***	-0.73***
TN	-0.37	-0.30	0.60**	-0.08	0.11
C/N	0.13	-0.11	0.56**	-0.82***	-0.81**
			Week 8		
pH	0.11	0.03	-0.00	-0.52*	-0.25
DOC	-0.16	0.09	0.53*	0.66**	0.79***
TOC	0.15	-0.09	0.25	-0.81***	0.71***
TN	-0.38	-0.35	0.75***	-0.10	0.06
C/N	0.23	-0.04	0.14	-0.85***	-0.77***
			Week16		
pH	0.37	0.13	0.25	-0.74***	-0.52*
DOC	-0.25	0.01	-0.50*	0.65**	0.52*
TOC	0.27	0.10	0.53*	-0.74***	-0.77***
TN	-0.16	-0.28	0.74***	-0.17	-0.22
C/N	0.33	0.15	0.45*	-0.77***	-0.80***

DOC: dissolved organic C; TOC: total organic C; MBC: microbial biomass C; MBN: microbial biomass N; ns: non significant. *, **, and *** are significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

high-temperature pyrolysis (>550°C) produces biochars that generally have high surface areas (Downie et al., 2009; Keiluweit et al., 2010), are good adsorbents (Mizuta et al., 2004). Time of incubations had significant effect on DOC. The addition of biochars to the soil increased DOC in the first week but decreased in later part of incubation. This is because DOC, biologically easily available form of carbon, may be consumed by microorganisms in early time of incubation.

Biochar effects on microbial biomass and enzymatic activities

Microbial biomass is responsible for organic matter decomposition in terrestrial ecosystems, and thus ultimately responsible for maintenance of nutrient release in soil and soil fertility (Guo et al., 2012). There were significant differences among the treatment in the microbial biomass C (MBC) and microbial biomass N (MBN) (Figures 1 and 2). The maximum microbial biomass C (Figure 1) and N (Figure 2) were measured in W0.5 and B0.5, respectively. The lowest MBC and MBN were measured in control. Both the MBC and MBN decreased with increasing time of incubations. Jindo et al. (2012b) also reported decrease of microbial biomass carbon after 150 days of composting biochar blended poultry manure compared to 35 days. DOC is labile form of soil organic matter, easily available for microorganisms. Thus, the less availability of DOC at Week 8 and 16 as compared to Week 1, could result in decrease in microbial biomass along with time of incubations.

Enzymes are the main mediators of soil biological processes, such as organic matter degradation, mineralization and nutrient cycling (Marx et al., 2001). In this study, there were significant effect of biochar on β-glucosidase, acid phosphatase and urase activities. Moreover, there was significant correlation among the soil properties (soil pH, TOC, DOC, C/N) that changed due to biochar amendment and enzymatic activities at different times of incubation (Table 3). As reviewed by Lehmann et al. (2011) application rates between 1 and 12 t h^{-1} will likely show significant decreases in the activity of some C-mineralizing enzymes. The application rate between 1 and 12 h^{-1} is in the range used in this study. The activity of β-glucosidase (one of C-mineralizing enzyme) was higher in B1.0 (61.05 µg PNP g-1 soil h^{-1}) followed by B0.5 (60.94 µg PNP g-1 soil h^{-1}) at Week 1, similarly at Week 8, it was higher in B1 (46.69 µg PNP g-1 soil h^{-1}) followed by B0.5 (45.78 µg PNP g-1 soil h^{-1}) whereas, at Week 16 the control (58.27 µg PNP g^{-1} soil h^{-1}) showed the highest value (Figure 3). However, at Week 16, no significant difference among the treatments except W1.0 (43.55 µg PNP g-1 soil h^{-1}) and W2.0 (35.62 µg PNP g^{-1} soil h^{-1}) which were lower than the rest of treatments. The reason can be a co-location of C and microorganisms on biochar surfaces that may improve efficiency and reduce the need for enzyme production as described by Lehmann et al. (2011).

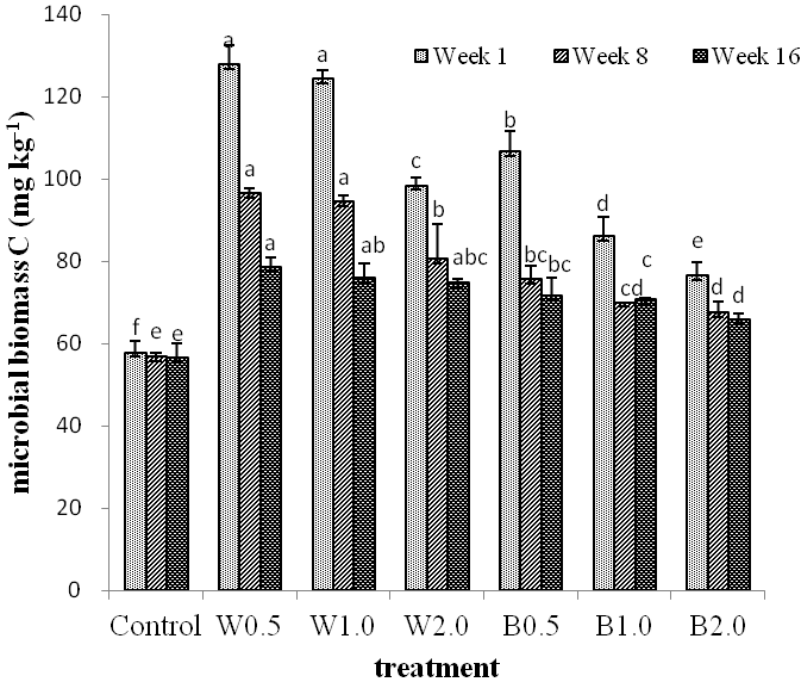

Figure 1. Soil microbial biomass C at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

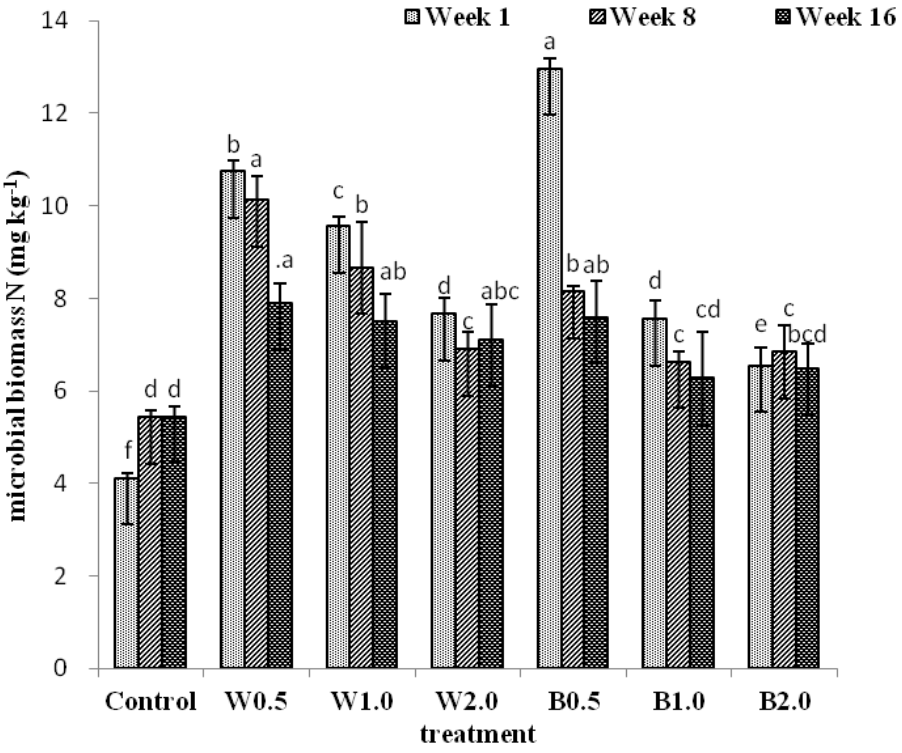

Figure 2. Soil microbial biomass N at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

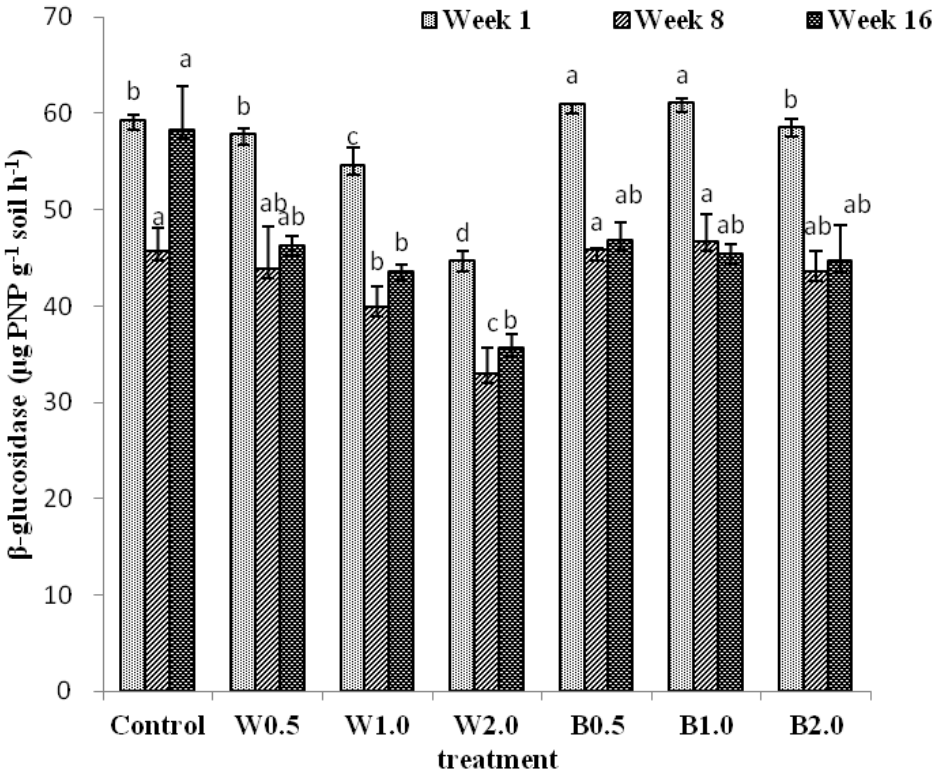

Figure 3. β-glucosidase activity at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

Our results agree with the results described by Paz-Ferreiro et al. (2012) who reported β-glucosidase activity decreased in biochar treated soil in comparison with the enzyme activity of the control soils. As to time effect, β-glucosidase showed the maximum activity at Week 1. The decrease of β-glucosidase to last two incubation times could be related to the decrease of DOC. This context is supported by the positive correlation of this enzyme with DOC (r=0.44*, r=0.79***, r=0.52* at Week 1, 8 and 16, respectively). Jindo et al. (2012b) also reported the decrease of the enzyme towards the end of the composting process. Moreover, the negative correlation of this enzyme with C/N (r=-0.81***, r=-0.77***, r=-0.80*** at Week 1, 8 and 16, respectively) implies mineralization of organic matter provides substrates for this enzyme.

Acid phosphatase play vital role in P cylces. This enzyme hydrolysis organic phosphourus compounds to different inorganic forms. Acid phosphatase is predominant in acidic soils (Eivazi and Tabatabai, 1977). In this study, at Week 1, acid phosphatase activity was higher in control (136.03 µg PNP g^{-1} soil h^{-1}) compared to biochar treated pots, however no significant difference with B0.5 (132.73 µg PNP g^{-1} soil h^{-1}) and B1.0 (130.14 µg PNP g^{-1} soil h^{-1}) was found (Figure 4). At Week 8, B1.0 (149.11 µg PNP g^{-1} soil h^{-1}) had higher activity followed by B0.5 (148.95 µg PNP g^{-1} soil h^{-1}), but they were not significantly different from the control. The control was higher at Week 16, however, it had no significant difference with the treatments except W1.0 (132.64 µg PNP g^{-1} soil h^{-1}), B2.0 (131.68 µg PNP g^{-1} soil h-1) and W2.0 (115.14 µg PNP g-1 soil h-1) which were lower than the rest of treatments. Due to the inverse correlation of acid phosphatase (r=-0.88***, r=0.52*, r=-0.74*** at Week 1, 8 and 16 respectively) with soil pH, its activity could decrease with increased rate of biochar application.

The urease activity is involved in the hydrolysis of C–N bonds of some amides and urea (Bremner and Mulvaney, 1978). Increasing biochar rates increased urease activities (Figure 5). At Week 1, the maximum urease activity was observed by W2.0 (943.63 µg NH_3-N g^{-1} soil 24 h^{-1}), however it had no significant difference with B2.0 (938.80 µg NH_3-N g^{-1} soil 24 h^{-1}) and B1.0 (930.21 µg NH_3-N g^{-1} soil 24 h^{-1}). The highest activity was observed in B2.0 (901.38 µg NH3-N g^{-1} soil 24 h^{-1}, at Week 8 and 797.72 µg NH_3-N g^{-1} soil 24 h^{-1}, at Week 16). The TOC could have contribution for increase of urease activity with increasing biochar rates. The positive correlation of urease activity with TOC (r=0.62**, r=0.25, 0.53*) support this context. This is because soil organic matter plays a vital role in protecting soil enzymes since they form complexes with clay and humus (Tabatabai, 1994).

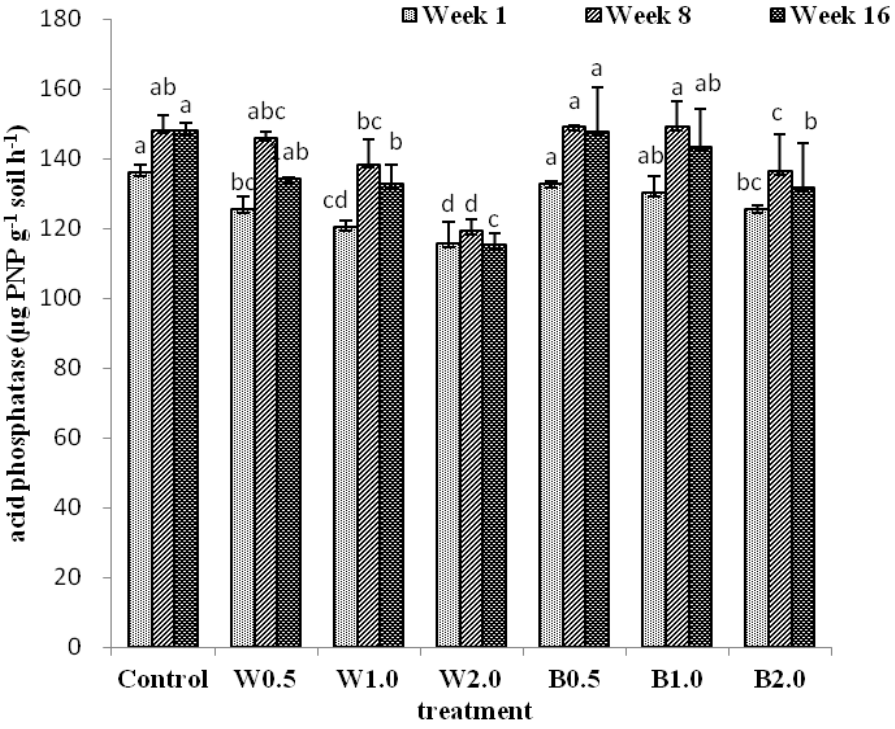

Figure 4. Acid phosphatase activity at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

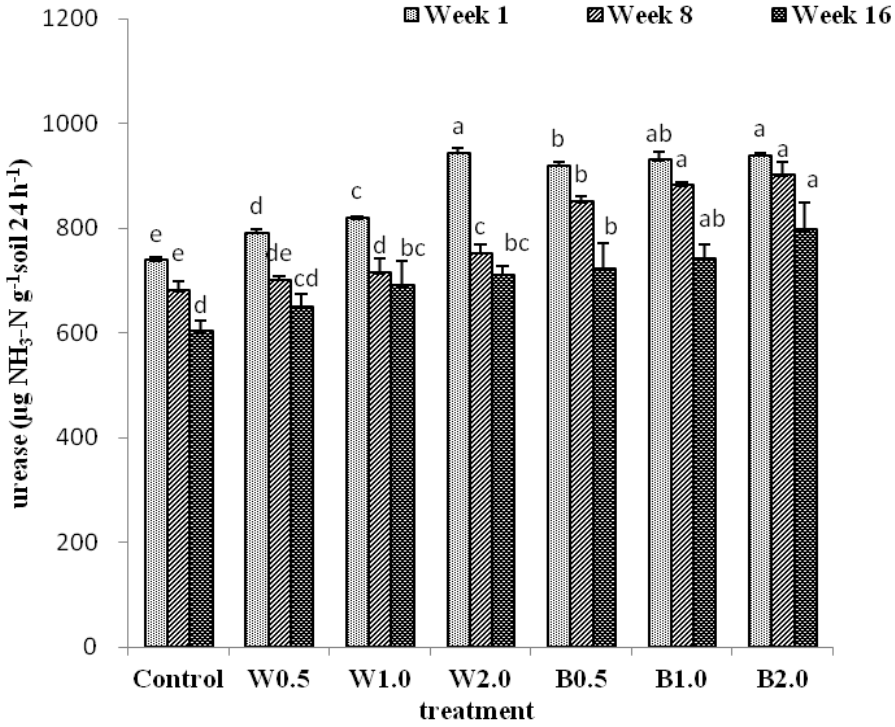

Figure 5. Urease activity at different rate of biochar applications. Bars represent the standard deviation of the mean (n=3). Different letters over the bars for each sampling time indicate significant differences ($p < 0.05$) among treatments.

Table 4. The microbial biomass of the different PLFA groups in the soils incubated at the different biochar application rates, and their respective ratios.

Treatment	Total PLFA (nmol g^{-1})	Bacteria (nmol g^{-1})	Fungi (nmol g^{-1})	Actinomycetes (nmol g^{-1})	Gram+ (nmol g^{-1})	Gram- (nmol g^{-1})	Fungi/ Bacteria	Gram+ /Gram-
					Week 8			
Con	32.92±1.17^{b}	11.99±0.30^{b}	3.28±0.30^{a}	2.26±0.04^{ab}	5.81±0.59^{ab}	5.88±0.17^{c}d	0.27±0.03^{a}	0.99±0.13^{ab}
$W_{0.5}$	36.56±1.17^{a}	12.94±0.30^{a}	3.48±0.27^{a}	2.37±0.21^{ab}	6.30±0.04^{a}	6.31±0.23^{ab}	0.27±0.01^{a}	1.00±0.03^{ab}
$W_{1.0}$	33.06±1.28^{b}	12.23±0.43^{ab}	2.92±0.17^{ab}	2.19±0.05^{ab}	6.00±0.35^{a}	5.96±0.08^{bc}	0.23±0.01^{ab}	1.00±0.05^{ab}
$W_{2.0}$	29.35±0.68^{c}	10.35±0.25^{c}	2.60±0.18^{b}	1.88±0.04^{c}	5.10±0.05^{bc}	5.05±0.16e	0.25±0.01^{ab}	1.01±0.02^{ab}
$B_{0.5}$	34.33±0.24^{ab}	12.81±0.59^{ab}	2.67±0.26^{b}	2.17±0.17^{b}	6.24±0.29^{a}	5.67±0.28^{cd}	0.21±0.01^{b}	1.10±0.00^{a}
$B_{1.0}$	33.17±0.26^{b}	12.67±0.09^{ab}	3.08±0.34^{ab}	2.46±0.06^{a}	5.92±0.06^{ab}	6.47±0.16^{a}	0.24±0.03^{ab}	0.91±0.03^{b}
$B_{2.0}$	28.69±0.78^{c}	10.61±0.58^{c}	3.00±0.09^{ab}	2.18±0.15^{ab}	4.81±0.55^{c}	5.47±0.05d^{e}	0.28±0.02^{a}	0.88±0.09^{b}
					Week 16			
Con	31.22±2.76^{b}	11.41±1.24^{b}	2.96±0.07^{a}	2.22±0.04^{a}	5.64±0.90^{b}	5.51±0.33^{a}	0.26±0.02^{a}	1.02±0.10^{b}
$W_{0.5}$	36.11±0.23^{a}	13.66±0.22^{a}	3.25±0.15^{a}	2.32±0.00^{a}	7.34±0.04^{a}	5.99±0.05^{a}	0.23±0.01^{ab}	1.22±0.02^{a}
$W_{1.0}$	32.33±1.04^{ab}	11.93±0.10^{b}	2.95±0.04^{a}	2.16±0.05^{a}	6.35±0.06^{b}	5.37±0.12^{a}	0.25±0.00^{a}	1.18±0.04^{ab}
$W_{2.0}$	31.54±0.76^{b}	11.72±0.19^{b}	2.94±0.07^{a}	2.19±0.08^{a}	5.90±0.33^{b}	5.59±0.12^{a}	0.25±0.00^{a}	1.05±0.08^{ab}
$B_{0.5}$	32.11±0.13^{ab}	11.59±0.13^{b}	2.39±0.08^{b}	2.11±0.02^{a}	5.65±0.12^{b}	5.57±0.25^{a}	0.20±0.00^{b}	1.01±0.07^{b}
$B_{1.0}$	32.19±3.49^{ab}	11.72±0.95^{b}	3.03±0.32^{a}	2.25±0.21^{a}	5.94±0.22^{b}	5.54±0.68^{a}	0.25±0.01^{a}	1.07±0.09^{ab}
$B_{2.0}$	29.70±1.43^{b}	11.65±0.12^{b}	3.05±0.32^{a}	2.13±0.26^{a}	5.78±0.26^{b}	5.68±0.34^{a}	0.26±0.03^{a}	1.02±0.11^{b}

Gram+/Gram-: Gram-positive to Gram-negative bacteria PLFA ratio; Fung/Bacteria: Fungal to bacterial PLFA ratio. All values were expressed as mean ± standard deviation. Different letters in the same column for each of sampling times indicate significant differences ($p < 0.05$).

Biochar effects on microbial communities

Phospholipids are the key components of cellular membranes of all living cells, their composition are an important criterion to classify microbial groups and to evaluate their physiological conditions (Zelles, 1999). Microbial abundance in biochar amended soil has been determined by various methods, of which PLFA extraction is the one (Birk et al., 2009). As shown in Table 4, bacteria, fungi, and gram-positive bacteria were higher in W0.5 than the rest of the treatments at both times of incubation. This resulted in higher total PLFA (36.56 nmol g-1 soil at Week 8 and 36.11 nmol g^{-1} soil at Week 16) in W0.5 than the rest of treatments. Aromatic C and aliphatic C that are found in biochar as well as in soil are consumed by gram-positive bacteria (Fierer et al., 2003; Bird et al., 2011) which are the largest PLFA microbial groups present, resulted in higher bacteria and total PLFA in W0.5. This confirms reports of Farrell et al. (2013) who suggested bacteria are capable of rapidly metabolizing biochar-C, and increasing their community size relative to other microbial groups. The microbial groups were analyzed by principal component analysis and the contributions of the first two principal components (PCs) were 44.44 and 26.29%, respectively at Week 8 (Figure 6) and 38.75 and 28.88%, respectively at week 16 (Figure 7). The first two principal components accounted for 70.73% (at Week 8) and 67.63% (at Week 16) of the total variation. Treatment W0.5, to the right of PC 1, had higher relative abundance of bacteria, fungi, and gram-positive bacteria than the other treatments at Week 8 (Figure 6) and Week 16 (Figure 7). Moreover, the maximum total PLFA was measured at the lowest rates (W0.5 and B0.5) and the minimum at the highest rates (W2.0 and B2.0), indicating the optimum rate of biochar application for higher microbial biomass, at this specific soil is 0.5%. This implies that biochar at rate of 0.5% has more effect on microbial community. Labile components of biochar (DOC) and adsorbed volatile organic compounds, along with pH, are likely to be the major drivers of soil microbial community structure change when biochar is applied to soil (Farrell

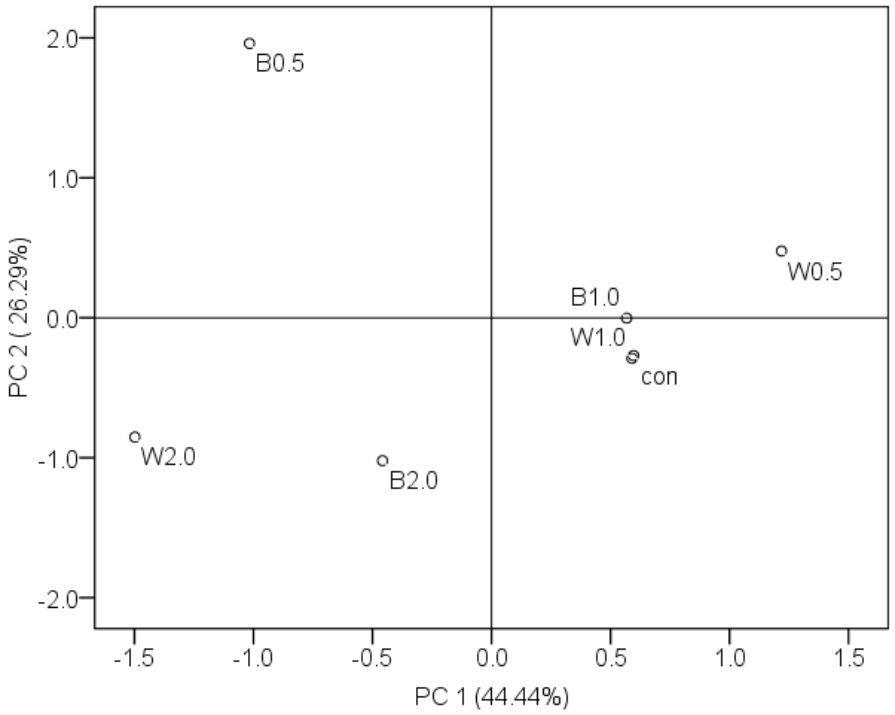

Figure 6. Principal component analysis (PCA) of microbial phospholipid fatty acids extracted from different treatments at week 8. Con= control, oak wood biochar at 0.5, 1.0, and 2.0% ($W_{0.5}$, $W_{1.0}$, and $W_{2.0}$, respectively) and bamboo biochar at 0.5, 1.0, and 2.0% ($B_{0.5}$, $B_{1.0}$, and $B_{2.0}$, respectively).

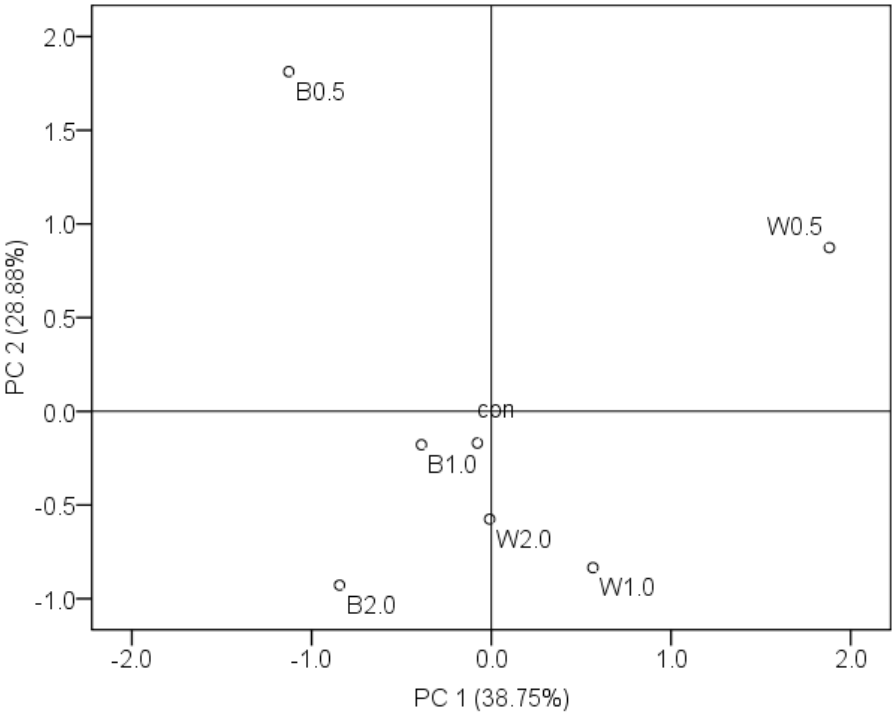

Figure 7. Principal component analysis (PCA) of microbial phospholipid fatty acids extracted from different treatments at week 16. Con= control, oak wood biochar at 0.5, 1.0, and 2.0% ($W_{0.5}$, $W_{1.0}$, and $W_{2.0}$, respectively) and bamboo biochar at 0.5, 1.0, and 2.0% ($B_{0.5}$, $B_{1.0}$, and $B_{2.0}$, respectively).

et al., 2013). In treatment B0.5, the fungi as well as fungi/ bacteria ratio was lower than the rest of treatments in both time of incubations, which could be related to the highest DOC which may favored bacteria than fungi, because bacteria are capable of metabolizing biochar-C than the other groups (Farrell et al., 2013). Incubation times had significant effect on microbial biomarkers gram-negative and gram-positive bacteria. Gram-positive bacteria increased at Week 16 compared to Week 8, whereas gram-negative bacteria decreased (Table 4).The decrease of gram-negative bacteria at Week 16, indicate that the soil microorganisms may have been energy-limited (due to the decrease of DOC) during the latter part of the incubation.

Conclusion

Biochar application significantly increased soil pH, organic C and dissolved organic C which created conducive environment and add substrates for microbial biomass and for enzyme production. Due to the low C/N of the soil, the lowest rates (W0.5 and B0.5), particularly W0.5, had higher microbial biomass and abundance than the highest rates. The inverse correlation of acid phosphatase with soil pH, the positive correlation of β-glucosidase with dissolved organic C as well as the positive correlation of total organic carbon with urease activities indicates the indirect effect of biochar on soil enzymes. The results from this study confirm that biochar application improve fertility of red soil by increasing the soil pH, organic carbon and dissolved organic carbon which, in turn, enhance the soil microbial biomass, soil enzymes and microbial community.

Conflict of Interest

The authors have not declared any conflict of interest.

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