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Responses of soil microbial biomass, diversity and metabolic activity to biochar applications in managed poplar plantations on reclaimed coastal saline soil

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Abstract

With its relatively high stability, biochar has been suggested as a means to mitigate climate change through carbon fixation and improve the physicochemical properties of soils. However, our understanding of the effects of biochar on soil microbial diversity and their metabolic activity remain unclear. In order to elucidate how the application of biochar to plantation soils influences microbial biomass and functional diversity (using Biolog EcoPlates^{TM}), we conducted an experiment to investigate changes in soil microbial communities at four biochar levels (0, 40, 80 and 120 Mg/ha). We found that biochar application altered the metabolic patterns of microbial communities and accelerated the utilization of amino acids, carboxylic acids, polymers and other miscellaneous plant chemical compounds by microbes. Moreover, compared to the control, soil pH increased by 0.23, 0.24, 0.28 units and microbial biomass carbon to nitrogen ratio (MBC/MBN) by 9.20, 20.99 and 17.74, respectively. Meanwhile, soil moisture decreased from 25.7 to 23.8%, 23.7 and 24.4%, and MBN declined by 42.2, 46.2 and 53.8%. Regression analysis showed that soil pH was the primary factor correlated with reduced MBN. Community physiological profiles revealed that high concentrated biochar (120 Mg/ha) elevated microbial metabolic activity, while biochar application did not alter microbial functional diversity represented by the Shannon diversity index (H') and evenness (E). Furthermore, the application of biochar would affect biogeochemical cycling of carbon and nitrogen through the elevated microbial activity and utilization in different categories of carbon sources (polymers, carboxylic acids etc.) with the reduced MBN.

Keywords: Biochar, Biolog CLPP, carbon fixation, microbial diversity, cycling of carbon and nitrogen, mitigate climate change, soil microbial community

Introduction

The vast imbalance between carbon release and carbon uptake is clearly exemplified by continuous increases in atmospheric CO_2, which has reached 400 ppm in 2012 (Stocker, 2014). Biochar has been evaluated globally as a means to stabilize carbon in soils and improve the physicochemical properties of soils, and thus, may contribute to the mitigation of climate change through carbon fixation. Consequently, biochar has garnered considerable attention over the last few decades, as it is applied to agricultural and forest soils to increase soil carbon storage while serving as a soil amendment (Lehmann *et al.*, 2006; Marris, 2006). Although a number of positive impacts (e.g. increased soil fertility, improved soil structure and permeability, elevated crop yields, enhanced adsorption of heavy metals) of biochar amendment in soil have already been determined in some studies (Cheng *et al.*, 2006; Van Zwieten *et al.*, 2010; Jones *et al.*, 2012), little is known to date as to the effects of biochar on the microbial biomass and functional diversity in soils. In addition, biochar application was shown to accelerate the emission of greenhouse gases (N_2O, CH_4), and the decomposition of organic matter (Spokas *et al.*, 2009;

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Zhang *et al*., 2010) that were clearly counterbalancing the climate mitigation purpose of biochar. Therefore, biochar application has many uncertain and unexplained phenomena awaiting further exploration.

Biochar is the product of the thermal degradation of bio-organic materials in the absence of air. The composition of biochar could be broadly divided into three parts: relatively recalcitrant C, labile or leachable C and ash, in which the recalcitrant C is able to remain in soils for thousands of years without decomposition, therefore carbon may be sequestered within soils over the long term (Lehmann *et al*., 2006; Marris, 2006). To date, most studies of biochar have focused on agricultural soil ecosystems; its effects on tree plantation soils have received much less attention. In China, planted forests have expanded during the past decades; however, the plantation forests are often simple in species composition, their ecological benefit is low, and soil quality in these plantations is often degraded (Lan *et al*., 2017; Liu *et al*., 2017). With its unique porosity and surface properties, biochar can potentially be an effective means to improve soil quality and enhance ecosystem services, in addition to its function as carbon (C) sequester towards the mitigation of global warming. If biochar technologies can be optimized, carbon (C) sequestration might be applied globally to capture as much as 5.5–9.5 PgC/yr by 2100, estimated by Lehmann *et al*. (2006). Yet, very little is known in regards to how biochar affects microbial diversity and activity, or whether biochar might pose risks for soil health and ecological processes (Lehmann *et al*., 2011).

In soil ecosystems, microorganisms play the most active role and contribute to more than 90% of the carbon dioxide that evolves during the decomposition of forest litter (Jenkinson *et al*., 1990). Thus, microbial biomass, as an essential indicator of soil health and the biogeochemical cycling of nutrients and energy flows, requires further in-depth study (Jenkinson *et al*., 1990; Bailey *et al*., 2011). Moreover, microbial community diversity is closely associated with the stability of soil ecosystems (Gross *et al*., 2014). Community-level physiological profiles (CLPP), which allows for the detection of multiple microbial metabolic activities by using Biolog EcoPlates^{TM}, has been widely used to provide data to facilitate the assessment of functional microbial diversity.

The objectives of this study focused on understanding how biochar addition to poplar plantation soils in a coastal area of China influences soil physiochemical properties, microbial biomass nitrogen (MBN) and carbon (MBC), functional diversity and metabolic activity.

Materials and methods

Site description

This study was conducted in a coastal area of the Yellow Sea at the Dongtai Plantation Farm (120°49′E, 32°52′N), Yancheng city, in the Northern Jiangsu Province of Eastern China. The region is under the influence of a monsoon climate. It is located within the transition zone from a northern subtropical to warm temperate climate. The annual mean temperature and rainfall are 14.6 °C and 1051.0 mm, respectively. The frost-free period (225 day/yr) compliments the average hours of sunlight (2169.6 h/yr) (Wang *et al*., 2015). The trial plot soil type is sandy loam, with the primary tree species of this plantation farm including Poplar (*Populus deltoides*), Gingko (*Ginkgo biloba*), Acacia (*Robinia pseudo acacia L*.), Chinese fir (*Cunninghamia lanceolata*), and Paulownia (*Paulownia fortunei*). An eight-year-old stand of a pure poplar plantation was selected as the study site, and the physiochemical properties of the soil within the trial plot is described in Table 1.

Experimental design

This experiment followed a randomized block design, with four treatments replicated in four randomly allocated blocks, resulting in a total of sixteen 2 m × 2 m plots. Four loading levels of biochar unfertilized control (CK, 0), low (T1, 40 Mg/ha), intermediate (T2, 80 Mg/ha) and high (T3, 120 Mg/ha) loadings of biochar were applied to the soil in August 2013. The biochar produced by a Liuhe charcoal manufacturer in Nanjing was from the carbonization of raw wood in a controlled aerobic environment at a temperature of 600 °C. The biochar pH was 9.02, and its carbon content was 60% by weight. The biochar was applied to a depth of 50 cm through trenching, where the identical trench dimensions and pattern were applied in the CK plots to facilitate viable comparative studies.

Soil collection and analysis

Soil samples were collected from a 0–10 cm soil layer depth in each of the 16 plots in March 2015. Immediately after collection, the soil samples were transferred to the laboratory in plastic bags and stored in a refrigerator (4 °C). Subsequently, a portion of the samples was air-dried for seven days, and then was sieved to pass 2 mm mesh. The total organic carbon (TOC) and dissolved organic C (DOC) were measured with a Shimadzu 5050 TOC-VCPN analyzer, while the total carbon (TC), total nitrogen (TN) and total potassium (TK) of the soil samples were measured with an element analyzer (Elementar, Vario ELIII, Elementar Analysen System GmbH, Hanau, Germany). The microbial biomass carbon (MBC) and nitrogen (MBN) were quantified using a fumigation-extraction method (Brookes *et al*., 1985). Each sample was divided into two portions, with 10 g of fresh soil comprising each part, one of which was fumigated for 24 h at 25 °C with $CHCl_3$ (ethanol-free), whereas the other portion was nonfumigated. Subsequently, the soil was extracted with 40 ml of 0.5M K_2SO_4 by

Table 1 Physical and chemical properties of soil in a poplar plantation in a coastal region of Northern Jiangsu, Eastern China

Soil layer (cm)	TOC(g/kg)	TN(g/kg)	TP(g/kg)	C/N	pH	Soil moisture (%)	Bulk density (g/cm^3)
0–10	12.70	0.87	0.54	14.6	8.2	23.9	1.24
10–25	12.32	0.55	0.43	22.4	8.5	22.9	1.38

C/N, carbon-nitrogen ratio; pH, determined with a glass electrode in 1:2.5 soil: water solution (w/v); TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus.

oscillating for 30 min at 180 rpm and filtered (Whatman No. 42). The MBC and MBN were calculated as described by Vance *et al.* (1987). The soil pH measured by using a glass electrode in a 1:2.5 (the ratio of soil and water solution). The total soil moisture was measured as the difference between the field moisture and the dried (at 105 °C for 24 h) soil weight.

Community-level physiological profiles

The Biolog EcoPlate was composed of 96 wells, which included a triplicate set of 31 carbon sources. The main steps of the Biolog assay were identical to those described by Liu *et al.* (2015). The inoculated plates were incubated in the dark at 28 °C for 10 days, and the microbial development was documented by monitoring the optical density (OD) at 595 nm every 24 h using a Biolog Microstation™ reader (Biolog Inc., Hayward, CA, USA). The overall rate of carbon source utilization by microorganisms was measured by calculating the average well colour development (AWCD) by using the equation as follows:

$$\mathrm{AWCD} = \sum(C_i - R)/31$$

where C_i is the OD value in each well of the 31 carbon sources, and R is the OD value of the corresponding control well. The development curve of AWCD over the incubation time of 240 h was documented.

The microbial functional diversity was assessed by the richness index S (calculating the numbers of wells with the OD > 0.15) and the Shannon diversity index (H') and evenness (E) were calculated using the following equations:

Shannon diversity: $H' = -\sum p_i \times (\ln p_i)$

Shannon evenness: $E = H'/\ln S$

where p_i, calculated by each relative absorbance value (the OD value in each well of 31 carbon sources minus the OD value of the corresponding control well), then divided by the total relative absorbance value, which was recorded for all 31 substrates; S refers to the numbers of wells that exhibited colour changes. When diversity was calculated, only positive OD values were considered and the negative values were set to zero.

According to their biochemical properties, the carbon substrates in the Biolog EcoPlate could be divided into six categories: amines/amides, amino acids, carbohydrates, carboxylic acids, polymers, and miscellaneous (Xu *et al.*, 2015). The average absorbance in each category was calculated via the following equation:

$$f_j = \sum_{i=1}^{n_j}(C_i - R)/n_j$$

where $C_i - R$ refers to the relative absorbance of the carbon substrate i, which belongs to the biochemical category j; n_j

Table 2 Physiochemical parameters of soil (mean ± SD) as influenced by different levels of biochar application

Parameter	0	40	80	120
pH	7.81 ± 0.12b	8.04 ± 0.08a	8.05 ± 0.03a	8.08 ± 0.05a
Soil moisture (%)	25.7 ± 0.01a	23.8 ± 0.002b	23.7 ± 0.01b	24.4 ± 0.01b
DOC (mg/kg)	294.61 ± 48.34a	273.10 ± 33.85a	248.33 ± 50.43a	276.68 ± 50.22a
A-P (mg/kg)	13.87 ± 7.16a	6.65 ± 1.60a	11.7 ± 7.16a	7.07 ± 2.46a
MBC (mg/kg)	978.47 ± 322.29a	888.81 ± 241.12a	1156.9 ± 239.65a	977.29 ± 314.51a
MBN (mg/kg)	61.02 ± 15.00a	35.29 ± 8.58b	32.83 ± 7.22b	28.21 ± 2.05b
MBC/MBN	16.45 ± 6.35b	25.65 ± 5.61ab	37.44 ± 14.01a	34.29 ± 9.61a

Treatments with the same letters in the same row were not significantly different (ANOVA, the homogeneity of variance with LSD-t method, missing variance with Dunnett-t method, $P < 0.05$). Means ± standard deviations are listed. A-P, available phosphorus; CK, control group (0 Mg/ha); DOC, dissolved organic carbon; T1, low level of biochar (40 Mg/ha); T2, intermediate level of biochar (80 Mg/ha); T3, high level of biochar (120 Mg/ha).

refers to the number of carbon substrates in specific biochemical category *j*.

Statistical analysis

For each level of biochar, we determined the means of microbial biomass and other parameters across the four replicates. The effect of the four different loadings of biochar was tested by one-way analysis of variance (ANOVA). We assessed the assumptions of normality and homogeneity to make sure that they meet the condition for all analyses. Regression analysis and principal component analysis (PCA) were used to assess univariate and multivariate relationships for continuous variables. All analysis was conducted using SPSS 17.0 statistical analysis software. Most of the reported measurements refer to the 96-h time point (Liu *et al*., 2012, 2015).

Results

Effects of biochar on soil microbial biomass

Following 20 months of biochar incubation, significant changes ($P < 0.05$) in the physiochemical properties and microbial biomass occurred in soils loaded with biochar (Table 2). Mean soil pH values increased significantly by 0.23, 0.24, and 0.28 units, as compared with the control, in the 40, 80 and 120 Mg/ha biochar addition treatments, respectively, but soil pH did not differ among biochar addition levels. Soil moisture decreased significantly, from 25.7% in the control to 23.8, 23.7, and 24.4%, in the biochar treatments from low to high levels, respectively, and did not differ among biochar addition levels.

Biochar additions did not affect microbial biomass carbon (MBC). However, microbial biomass nitrogen (MBN) reduced by 42.2, 46.2, and 53.8% from low to high biochar addition levels, respectively, and did not differ among biochar addition levels. Furthermore, ratios of MBC/MBN increased by biochar additions. The values of the T2 (37.44) and T3 (34.29) increased by 131.2 and 108.5%, respectively, than the control group (16.45).

We used regression analysis to investigate which independent variables were significantly related to the reduction in MBN. We found that pH is significantly ($P < 0.01$) related to MBN and accounted for the reduction in MBN by 87.3%. Then, we used a linear regression function to investigate how pH quantitatively impacts MBN, the function between them being built up as follows:

$$y = -216.63x + 1810.4\ (\mathrm{R}^2 = 0.7969 \text{ and } P < 0.01, \text{ Figure 1}) \quad (1)$$

The result suggested that when soil pH increases by a 0.1 unit, soil MBN would decrease by 21.663 mg/kg.

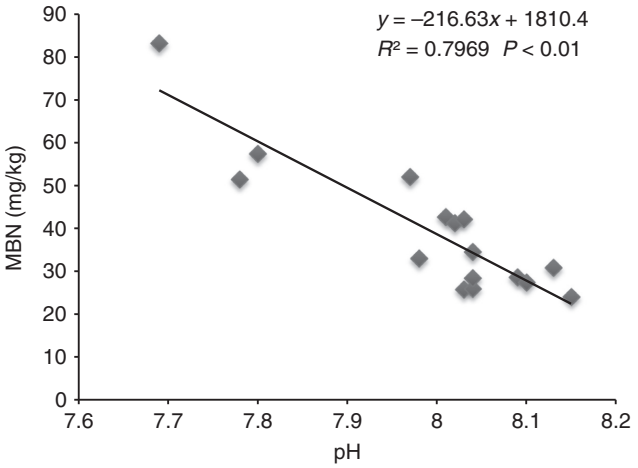

Figure 1 The relationship between MBN and pH. Equation and line represent linear relationship between MBN and pH. *P*-values <0.05 represent a statistically significant correlation relationship.

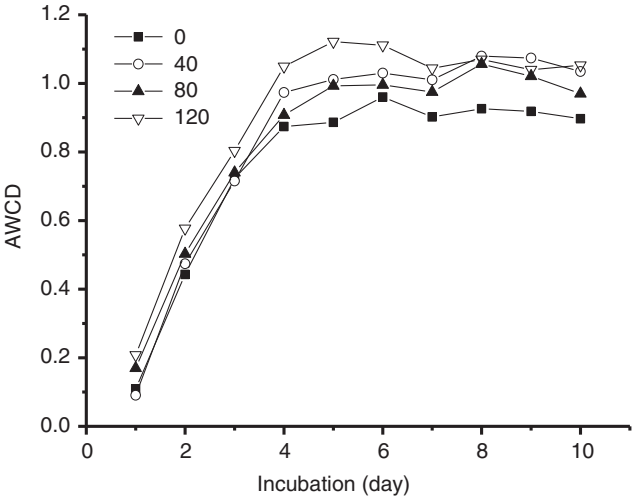

Figure 2 Overall average well colour development (AWCD) curve of different treatments. AWCD is derived from the average absorbance values of four replications. CK: control group (0 Mg/ha), T1: low level of biochar (40 Mg/ha), T2: intermediate level of biochar (80 Mg/ha), T3: high level of biochar (120 Mg/ha).

Effects of biochar on microbial metabolic activity

In the Biolog assay, the metabolic rates of T3 and T1 were more rapid than the CK and T2 treatments, and the inflection point of the growth curve occurred on the fourth day, which suggested that it was reasonable to select the time point of the fourth day to undertake further analysis, such as diversity and PCA analysis (Figure 2).

Biochar addition treatments on average had higher AWCD values than the control group; however, neither richness, Shannon's diversity index nor evenness changed with biochar addition (Table 3).

Different treatments had different performance in the utilization of the six categories of carbon substrates (Figure 3). For example, there were significant differences in the amino acids, carboxylic acids, polymers and miscellaneous substrates between the various treatments. T3 had a more robust metabolic capacity than did CK in these four categories of carbon substrates, whereas T2 and T1 had higher utilization rates than CK, only in the miscellaneous category.

PCA of the effects of biochar on microbial metabolic patterns

The PCA showed that the first two-dimensional PC of CLPP explained only 34.54% of the total variance; therefore, an additional two PCs were used to assist with the extraction of additional information (Figure 4). In the PC1–PC2 plot, T3 was completely distinct from the other treatments and was located in an area that was in the positive direction of both PCs, whereas CK, T1 and T2 clustered in the area that was close to the bottom left. This result indicated that high biochar concentration levels altered microbial communities on utilizing carbon substrates, represented by PC1 and PC2 (Table 4). Although not complete, CK was separated from the other treatments by PC4, and its score was higher than the others in PC4, indicating that biochar imparted negative effects on the utilization of carbon substrates represented by PC4 (Table 4).

Discussion

Effect of biochar on soil biomass

The impact of biochar on soil moisture content is contingent on its structural features and the conditions of the host soil.

Table 3 ANOVA result of AWCD, richness (*S*), Shannon diversity index (*H*′) and evenness (*E*) under different treatments

Biochar application	AWCD	Richness (*S*)	Shannon diversity (*H*′)	Evenness (*E*)
CK	0.902 ± 0.097b	28.5 ± 0.577a	3.297 ± 0.023a	0.960 ± 0.005a
T1	1.005 ± 0.064ab	28.5 ± 1a	3.302 ± 0.034a	0.966 ± 0.015a
T2	0.937 ± 0.029b	28.75 ± 0.5a	3.312 ± 0.025a	0.969 ± 0.011a
T3	1.083 ± 0.082a	29.5 ± 1a	3.329 ± 0.012a	0.969 ± 0.003a

Means ± standard deviations are listed. Treatments with the same letters in the same row were not significantly different (ANOVA, the homogeneity of variance with LSD-*t* method, missing variance with Dunnett-*t* method, $P < 0.05$).

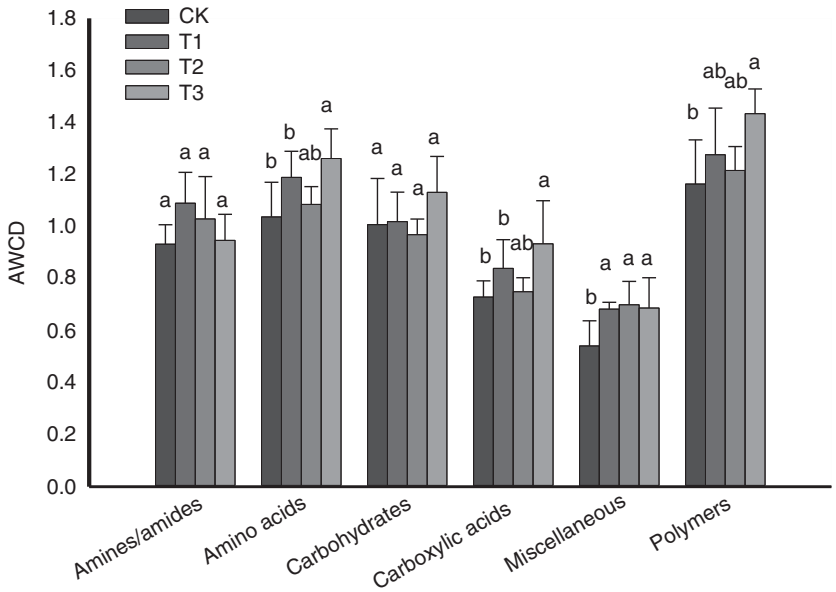

Figure 3 The average well colour development (AWCD) values of different treatments on six categories of carbon substrates. Treatments with the same letters in the same category were not significantly different (ANOVA, the homogeneity of variance with LSD-*t* method, missing variance with Dunnett-*t* method, $P < 0.05$). CK: control group (0 Mg/ha), T1: low level of biochar (40 Mg/ha), T2: intermediate level of biochar (80 Mg/ha), T3: high level of biochar (120 Mg/ha).

The biochar-amended treatments in this study exhibited a statistically significant decrease in soil moisture content, which is similar to the results of Zeng *et al.* (2013). The result may be because of biochar surfaces have not been completely oxygenated, and its porous construction might lead it to be hydrophobic, which reduces soil moisture content (Zeng *et al.*, 2013). Verheijen *et al.* (2010) found that biochar could increase the moisture content of sandy soils, while decrease moisture in clay soils. So different types of soil may have different responses to biochar addition, and our trail basing on loam showed a decline in soils moisture. Previous studies have reported an increase in pH with the addition of biochar; this result confirms that biochar might serve as a liming agent resulting in increased pH for a number of different soil types (Cheng *et al.*, 2006; Van Zwieten *et al.*, 2010; Jones *et al.*, 2012). The observation for soil pH might be explained by the progressive surface oxidation of biochar. Cheng *et al.* (2006) have showed that the oxidation causes an increase in the CEC (cation exchange capacity) of the system, therefore the soil pH is increased.

However, biochar treatment had no effects on soil MBC in this study, and the unchanged MBC with increasing biochar application rates was consistent with Castaldi *et al.* (2011), who found no changes in total microbial biomass in the first 3 months and after 14 months after biochar incorporation. This might be because the biochar has been added for almost 20 months, where its labile carbon was almost completely decomposed (Bamminger *et al.*, 2016).

We found that the use of biochar decreased the MBN, and increased the MBC/MBN ratio, which was similar to some studies (Bruun, 2011; Zavalloni *et al.*, 2011). This result may be explained by changes in the composition of the microbial communities subsequent to the addition of biochar, where the environment was more suitable for fungi than bacteria (Zhang *et al.*, 2018). In our study, we found that soil pH value was the primary factor associated with the decreasing MBN in soils after the addition of biochar. The reason might be that changes in pH may dramatically alter the composition of microbial communities in soils, and therefore, change the MBN and MBC/MBN. Lauber *et al.* (2009) suggested that changes in soil pH had impacts on the composition of microbial communities, and bacterial populations decrease with the increase in pH when the soil pH was > 6.5.

However, MBN did not continuously decline as biochar addition increase, which suggested that there was no simple linear relationship between biochar levels and MBN. As the resource of active nitrogen in soils, MBN is closely associated with other nitrogen compounds, such as NO_3^- and NH_4^+, and plays an essential role in the biogeochemical cycling of nitrogen. Therefore, the decrease in MBN following the addition of biochar may have further negative effects on soil ecosystems.

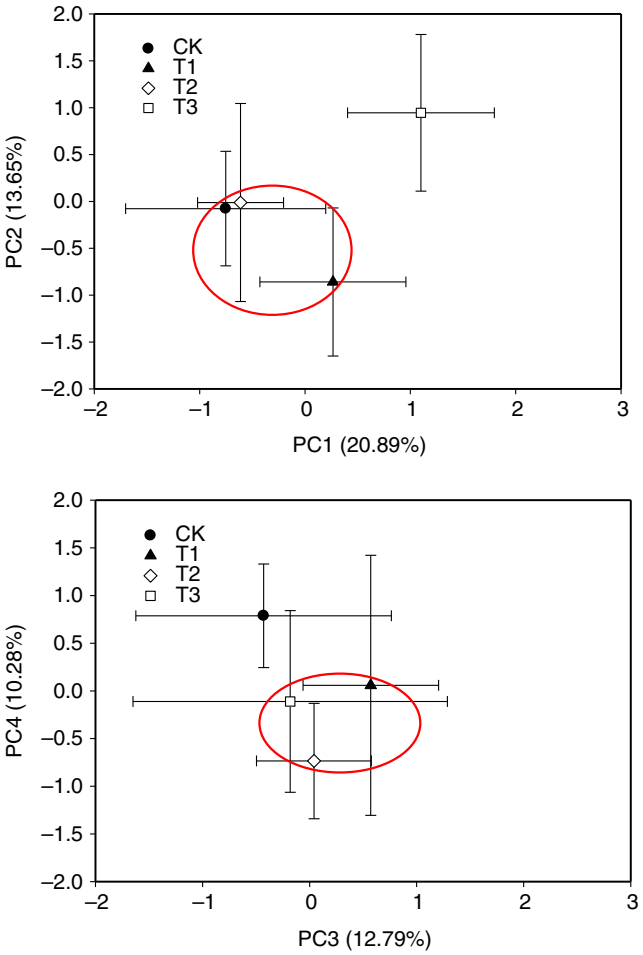

Figure 4 Principle component analysis of 31 carbon substrates of all treatments for Biolog Ecoplates. Error bars represent the standard error of the mean of four replicates ($n = 4$). CK: control group (0 Mg/ha), T1: low level of biochar (40 Mg/ha), T2: intermediate level of biochar (80 Mg/ha), T3: high level of biochar (120 Mg/ha).

Community-level physiological effects of biochar

Through a continued incubation, biochar-amended groups had higher AWCD values than did the control group overall. The value of T3 was significantly higher than the control group in this study, while other biochar-amended treatments exhibited no differences from the control. In this case, the results indicated that the microbial metabolic activity did not improve until the concentration of biochar attained a specific threshold. A similar result obtained by Wardle *et al.* (2008), who found that microorganisms had a higher decomposition capacity for soil carbon when biochar was added. This result might be because the labile biochar carbo stimulates microorganisms to utilize additional carbon substrates.

Furthermore, the porous structure of biochar may reduce competitive pressures between microorganisms, which

Table 4 Carbon substrates that are highly relative (correlation coefficients <−0.5 or >0.5) with PC1 to PC4 in principal component analysis

Chemical guild	Substrate	PC1	PC2	PC3	PC4
Amines/amides	Phenylethylamine		−0.62		0.535
	Putrescine			0.576	−0.66
Amino acids	Glycyl-l-glutamic acid				
	l-Arginine		0.66		
	l-Asparagine		−0.76		
	l-Phenylalanine			0.568	
	l-Serine			0.77	
	l-Threonine				
Carbohydrates	α-d-Lactose	0.554			
	β-Methyl-d-glucoside		0.669		
	d-Cellobiose		−0.62		
	d-Mannitol			−0.5	
	d-Xylose	0.55			
	i-Erythritol			0.64	
	N-Acetyl-d-glucosamine	0.555			
Carboxylic acids	γ-Hydroxy butyric acid	0.631			
	α-Keto butyric acid		0.509		
	2-Hydroxy benzoic acid				
	4-Hydroxy benzoic acid			0.638	
	d-Galactonic acid γ-lactone	0.668			
	d-Galacturonic acid	0.9			
	d-Glucosaminic acid	0.821			
	d-Malic acid				
	Itaconic acid			0.722	
Miscellaneous	d,l-α-Glycerol phosphate	0.713			
	Glucose-1-phosphate				−0.63
	Pyruvic acid methyl ester				
Polymers	α-Cyclodextrin	0.602			
	Glycogen				
	Tween 40	0.594			
	Tween 80	0.585			

improves their ability to utilize carbon substrates. The functional diversity of microorganisms assessed by richness, the Shannon Index and evenness, revealed no obvious differences between the various treatments. This might have been attributed to the fact that the stability of the functionality of microorganisms is difficult to change during the short biochar incubation period, following a long period of adaptation to a specific type of soil environment, as found by Calbrix *et al.* (2007).

In addition, although previous studies have shown that the microbial composition will be altered subsequent to the addition of biochar, changes in functional diversity were not found in this study (Warnock *et al.*, 2007; O'Neill *et al.*, 2009; Jin, 2010). This may be explained by modifications in the microbial composition of biochar-amended soils, which were limited to those species that possessed similar functions with the previous species of soils; therefore, these changes had negligible effects on functional diversity.

According to their biochemical properties, the carbon substrates of the Biolog EcoPlate could be divided into six categories as mentioned before. Different performance was observed between the biochar amendments and the control in amino acids, carboxylic acids, polymers, miscellaneous substrates. This result demonstrated that biochar could improve the growth of microorganisms who thrive on these types of substrates, which means that the addition of biochar may have reshaped the soil environment for microorganisms, where some species may spread more rapidly than others, and vice versa (O'Neill *et al.*, 2009; Jin, 2010; Verheijen *et al.*, 2010).

We found that biochar altered microbial metabolic patterns when its concentration attains a certain threshold. Through PCA, it has been shown that T3 had a higher score in utilization of d-Galacturonic acid, d-Galacturonic acid, d, l-α-Glycerol phosphate, which are presented by PC1, and of β-Methyl-d-glucoside, α-Keto butyric acid, which are

presented by PC2. This result indicates that the use of biochar could lead to a changed metabolic pattern of microbes, which could further affect the cycling of carbon.

Conclusion

We found that biochar increased the pH value of saline soil, which meant that increases in pH through the addition of biochar was not limited to acidic soils. Meanwhile, the reduction in MBN with the application of biochar has been also shown in this study, which demonstrated that the biogeochemical cycling of nitrogen would be disturbed with the subsequent conveyance of negative effects on soil ecosystems. In addition, the metabolic activities of microorganisms were improved and microbial metabolic patterns (especially in the utilization of carbons substrates belonging to polymers, carboxylic acids etc.) were altered via biochar addition, although its functional diversity, as assessed by the Shannon index and evenness, showed no significant changes.

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