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Effect of Maize Straw Biochar on Bacterial Communities in Agricultural Soil

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Abstract

Biochar has become a popular soil amendment. However, its effect on soil microbial community is still unclear. In the present study, maize straw biochar was pyrolysed at 300°C, 450°C and 600°C, respectively, and then was added to agricultural soil at the ratio of 0.5%, 1% and 2%. Bacterial dynamics was analyzed in the pot experiments using denaturing gradient gel electrophoresis. The results indicated that the pyrolysis temperature has great impact on the elemental composition, pH and porous structures of biochar. Moreover, pyrolysis temperature was primary factor to drive the variation of bacterial community structure in biochar amended soil. In addition, the results suggested that biochar amendments on agricultural soil would decrease the bacterial diversity, and selectively promote growth of functional bacteria to become the dominant community, which could increase the bacterial community organization and improve the stability of bacteria to counteract effects of perturbation.

Keywords Agricultural soil · Biochar · Bacterial community · Dissipative structures · Dynamics · Pyrolysis temperature

Biochar has received considerable attention as a potential element of 'climate-smart' agricultural practice that could contribute to mitigate climate change (Lehmann et al. 2011; Cernansky 2015). For instance, biochar application could reduce the greenhouse gas (i.e., N_2O) emissions in soil (Van Zwieten et al. 2019). In addition, biochar amendment in soil agro-ecosystems could enhance crop yield and improve physico-chemical characteristics of receiving soils, including pH, cation exchange capacity, soil microstructure, and nutrients (Liu et al. 2017; Bashir et al. 2018; Song et al. 2018). The pyrolysing conditions, i.e., temperature, was regarded as the principal factor to determine biochar properties such as elemental composition, specific surface area, porosity and pH (Gul et al. 2015; Wei et al. 2019). However, knowledge of the effects of biochar on soil biota is rather limited in comparison to their influences on soil physicochemical properties.

Furthermore, the exact effect of biochar addition on microbial community is still controversial (Palansooriya et al. 2019). Some reported that biochar would promote microbial activity, biomass and diversity (Lehmann et al. 2011; Gul et al. 2015; Zhang and Ding 2019), while others reported the opposite (Dempster et al. 2012; Song et al. 2018). For example, Dempster et al. (2012) reported that microbial biomass and activity significantly decreased with Eucalyptus biochar addition in a coarse textured soil. Khodadad et al. (2011) found that biochar amendments would result in lower bacterial diversity in tropical forest soils as well. Since changes in bacterial community structure and functional activities would alter soil ecosystem functions and services (Palansooriya et al. 2019). Therefore, the role of biochar in bacterial community of agro-ecosystems requires further understanding. The objective of the current study was to illustrate how microbial community structure changes in response to biochar amendment in agricultural soil. Three types of maize straw biochars, pyrolysed at 300°C, 450°C and 600°C, were applied into agricultural

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soil at the ratio of 0.5%, 1% and 2%. Denaturing gradient gel electrophoresis (DGGE) was used to analyze bacterial community dynamics.

Materials and Methods

Maize straw was pre-crushed, dried at 80°C, passed through a 2-mm sieve, and then pyrolysed at three different temperatures, i.e. 300°C (BC300), 450°C (BC450), and 600°C (BC600), for 1 h in the oven. Carbon (C), hydrogen (H) and nitrogen (N) content in biochar were measured by an elemental analyzer (Elementar, Vario EL CUBE, Germany). Porous structure of biochar was visualized by scanning electron microscope (SEM) (Hitachi, S-3500N, Japan). Biochar pH measurement was referred to ASTM (2017).

Soil was collected from the top layer (5–20 cm) of farmland in Xiqing District, Tianjin, China. The soil was weathered and passed through a 2-mm sieve. Each kind of biochar (BC300, BC450 and BC600) was mixed fully with screened soil (1.5 kg) in the pots with a final concentration of 0.5%, 1% and 2%. Non-biochar-added soil was set as the blank control. Each treatment had three replicates. The experiment was carried out in a greenhouse. All the pots were ploughed and watered regularly to keep 60% of the water holding capacity. Samples (5 g soil from each pot) were taken at the 1st, 7th, 14th, 21st, 28th, 35th and 42nd day.

Soil microbial genomic DNA was extracted with the E.Z.N.A.™ Soil DNA Kit (Omega, USA). The V3 region of bacterial 16S rRNA gene was amplified by nested-PCR with primer sets 63F/1378R and GC-F338/518R (Liu et al. 2019). DGGE analysis was conducted as described in Liu et al. (2019) with modification. Briefly, the range of denaturant agent vertical gradient was adjusted from 45% to 70%. Then, electrophoresis was performed on 8% polyacrylamide gels (acrylamide:bisacrylamide, 37.5:1), and run at 160 V and 60°C for 4 h in TAE buffer (40 mmol/L Tris, 20 mmol/L acetic acid, 50 mmol/L EDTA, pH 8.0).

DGGE fingerprinting patterns were analyzed by Gelcompar II 6.5 (AppliedMaths, Belgium). The microbial community structure indices, including Shannon–Weaver index (*HI*), Simpson index (*DI*), richness index (*RI*) and community organization (*CO*), were calculated as described previously (Marzorati et al. 2008; Liu et al. 2015). In present study, each band was presumed as unique operational taxonomic unit of bacteria species. *HI* is a measure of information entropy, including the relative abundance of bacteria species. *DI* measures the probability that two individuals taken at random from the community will belong to same species. *RI* represents the number of bacteria species within a given community. *CO* describes the degree of evenness within a bacteria community (Read et al. 2011). In detail, *HI* and *DI* were calculated based on the DGGE banding data using the following functions:

$$HI = -\sum(P_i \log P_i);\ \ DI = \sum(P_i)^2$$

where $P_i = n_i / N$, n_i represents the density of a band and N represents the sum of all band densities in each lane.

After the standardization of index data, redundancy analysis (RDA) was performed to illustrate the response of bacterial community structure to biochar pyrolysis temperature, addition ratio and incubation time. The significance of RDA analysis was assessed using Monte Carlo test. Moreover, variation partitioning analysis (VPA) was further conducted to quantify the contributions of biochar pyrolysis temperature, addition ratio and incubation time to the variations of bacterial community structure. All the statistical analyses were performed using R software (v3.4.3) with "ggplot" and "vegan" packages.

Results and Discussion

There were significant changes of elemental composition, pH and porous structure of biochar produced at different pyrolysis temperatures (Table 1; Fig. 1). Molar ratio of hydrogen-to-carbon (H:C) was commonly used to reflect the degree of aromaticity/carbonization of biochar during pyrolysis process. Namely, the higher the molar H:C ratio, the lower the degree of fused aromatic carbon (Dai et al. 2017). The results showed that the higher the pyrolysis temperature, the higher the C content in biochar. In contrast, the hydrogen content and the molar H:C ratio decreased with temperature increasing (Table 1). Consistent with previous study, biochar pyrolysed at low temperature contains more mineralizable aliphatic carbon than those at higher temperatures (Song et al. 2017). While, high pyrolysis temperature produces biochar with low molar H:C ratio, that contains a large fraction of fused aromatic carbon (Dai et al. 2017).

Table 1 Properties of biochar under three pyrolysis temperature conditions

Biochar types	Pyrolysis temperature (°C)	Elemental composition (%)			Molar ratio		pH
		C	H	N	H:C	N:C	
BC300	300	58.79	4.16	2.20	0.85	0.032	8.51
BC450	450	60.75	3.27	2.07	0.65	0.029	10.33
BC600	600	63.65	2.51	2.04	0.47	0.027	10.95

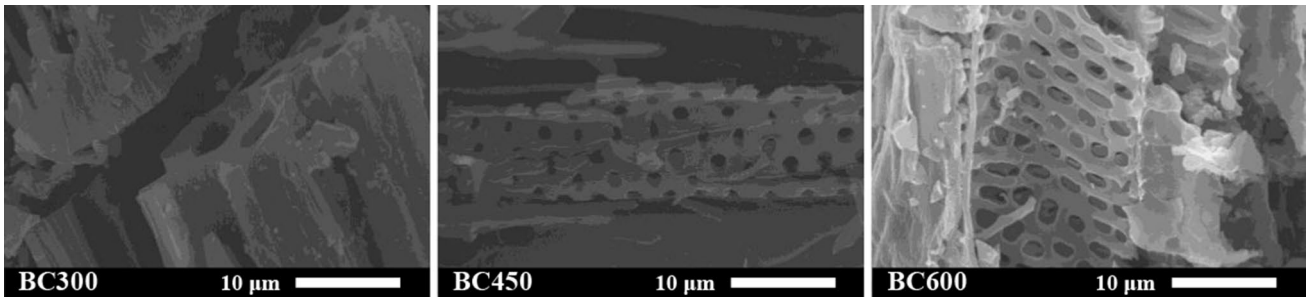

Fig. 1 SEM images of biochar under three pyrolysis temperature conditions. White scale bars represent 10 μm

The low molar H:C ratio indicates the increased aromaticity of biochar, which promoted their stability when added to soil (Van Zwieten et al. 2010). Higher pyrolysis temperature produced biochar with higher alkaline pH (Table 1). pH values indicate the level of acidic or alkaline composition in biochar. With higher pyrolysis temperature, more mineral substances that contain rich base-cations, such as calcium, magnesium and potassium can form, which increase the pH value of biochar. Soil pH increase following biochar application would be contributed to the enhancement of soil fertility (Van Zwieten et al. 2010). There was distinct surface porous structure on biochar pyrolysed at different pyrolysis temperature (Fig. 1). SEM showed that biochar produced at 300°C were tube-shaped, while those produced at 450°C and 600°C were more hole-structured, which have greater porosity and thinner wall. Highly porous structure suggested that the volatile component filled in the biochar materials are released more efficiently at higher temperature (Mukherjee et al. 2011).

Bacterial community structure and diversity information was obtained from DGGE fingerprint analysis. There was an obvious shift among different treatments (Fig. 2a). RDA analysis indicated that, *HI* and *RI* decreased with the increase in biochar addition ratio and incubation time, while increased with the increase in pyrolysis temperature (Monte Carlo test, $F = 3.145$, $p = 0.01$, Fig. 2b). Besides, *DI* and *CO* increased with the increase of biochar addition ratio and incubation time. The VPA analysis further revealed that biochar pyrolysis temperature was primary factor to affect bacterial community structure, which could account for 17.6% of all community variations, then followed by addition ratio (6.2%) and incubation time (4.2%) (Fig. 2c). It suggested that the effect of biochar pyrolysis temperature exerted on bacterial community structure was stronger than that of addition ratio and incubation time. This could attribute that, biochar produced at high temperature had a more porous structure and a greater surface area than that at low temperature (Fig. 1). This porous structure can absorb more soluble organic matter and nutrients, and provide a favorable habitat for diverse bacteria to colonization and protection against predators in soil (Saito and Marumoto 2002). Moreover, high-pyrolysis-temperature biochar has higher mineral substance (e.g., P, K, etc.) than low-pyrolysis-temperature biochar, which is essential element for bacterial metabolism and growth (Song et al. 2018; Van Zwieten et al. 2019). Graphite-like structure of high-pyrolysis-temperature biochar can more efficiently promote the electron transfer in soil biogeochemical process as well (Yu et al. 2015). Similar to previous study, Song et al. (2017) found that the changes of bacterial community structure was mainly attributed to biochar type, such as pyrolysis-temperature, after 12 weeks of amendment, while bacterial abundance and diversity failed to enhance further with the increase of biochar addition in soil. The increased biochar addition ratio would decrease soil N bioavailability or mineralization due to the low molar N:C ratio of biochar (Table 1) and the sorption of inorganic N to biochar (Dempster et al. 2012). Such processes may be indirectly affect bacterial community. Biochar addition could destabilize the nitrifying-bacteria community and inhibit nitrification process in soil (Muhammad et al. 2014). However, it was also reported the adsorption of N rich organic molecules onto the biochar surface would stimulate ammonification and nitrification (Gundale and DeLuca 2006). These discrepancies could be resulted from the multiple roles that biochar played in soil. It implies that the priming effect of biochar addition on soil bacterial community might be long-term lasting. Therefore, further study should be devoted to address longer temporal scale effects of biochar addition to soil.

In present study, community organization (*CO*) reflected the functionality of the community to organize in an adequate distribution of dominant bacteria and assure the potentiality of counteracting the effects of a sudden perturbation exposure (Read et al. 2011). Our result showed that biochar addition could reduce bacteria diversity to some extent, while selectively promote certain bacterial species to become the predominant population in soil (Fig. 2b). The results indicate that specific biochar can facilitate growth of functional bacteria populations (Muhammad et al. 2014; Dai et al. 2017), and biochar addition can increase the relative

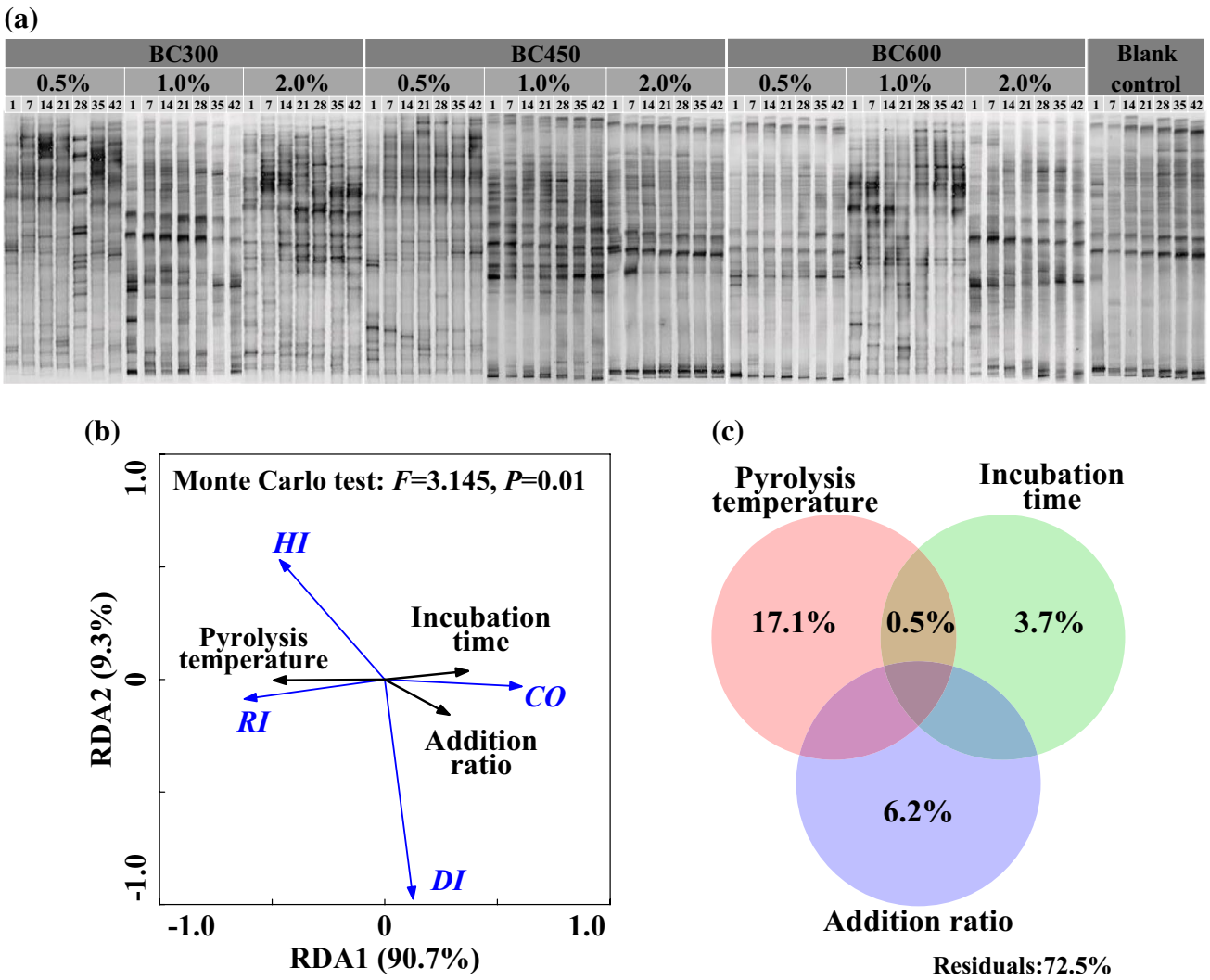

Fig. 2 DGGE pattern (**a**), redundancy analysis (**b**), and variation partitioning analysis (**c**) of bacterial community structures respond to the changes of biochar pyrolysis temperature, addition ratio and incubation time in soil. Blank control indicated non-biochar-added soil, and bottom-labeled numbers indicated the sampling day in subgraph (**a**). *HI* Shannon–Weaver index, *DI* Simpson index, *RI* richness index, *CO* community organization

abundance of rare soil bacteria species (Imparato et al. 2016; Song et al. 2017). For instance, it was reported that *Chloroflexi* and minor species of *Nitrospira* became dominant under the biochar treatment condition (Chen et al. 2013; Liu et al. 2018). Dai et al. (2017) also detected that *Chloroflexi*, specifically utilize aromatic carbon as energy source, was more prevalent in high pyrolysis-temperature biochar added soil. Khodadad et al. (2011) found that the relative abundance of the phyla *Gemmatimonadetes* and *Actinobacteria* increased during oak and grass biochar-amended soil. On the contrary, acidophilic bacteria, e.g., *Acidobacteria*, *Hydrogenophilaceae* and *Methylophilaceae*, were inhibited because of the increased alkalinity resulted from biochar application to soil (Chen et al. 2013; Palansooriya et al. 2019). Meanwhile, the degree of cell adsorption also depends on the structure and pore size of biochar. It was reported that the optimal adsorbent pore diameter was 2 to 5 times larger than the cells for the maximum immobilization of dividing microbial cells, and habitable capacity of biochar would reduce in both oversize and undersize conditions (Samonin and Elikova 2004). Therefore, biochar would selectively adsorb and promote the growth of bacteria with the fitted size (Gul et al. 2015).

Moreover, our study suggested that biochar amendment would promote bacterial community to be more stable in soil. In nature, under a nonequilibriurn conditions, the disturbances could cause biological community evolving to a new spatiotemporal ordered state through the spontaneous self-organization processes, the so-called dissipative structures (Prigogine et al. 1974). Community organization

reflects the microbial flexibility and adaptability which organize the distribution of dominant microorganisms to resist environmental stress (Read et al. 2011). Our result together with previous study suggested that minority community members may become dominant in a short period of time under perturbation condition which assures to preserve the specific community functionality (Marzorati et al. 2008; Wittebolle et al. 2009). Besides, initial reductions in species richness at low extents are unlikely to significantly alter soil ecosystem function related to carbon cycling dynamics (Nielsen et al. 2011). In the present study, although the biochar addition reduced the bacterial diversity to some extent, it promoted certain predominant species, e.g., *Nitrospira* and *Chloroflexi*, to form and improved the microbial community organization, which resulted in higher resistance to the changes of environment. Nevertheless, it should be kept in mind that, due to the limited resolution of DGGE, high-throughput sequencing techniques should be applied to access more bacterial taxonomic information and community changes in biochar amended soil in future study.

In summary, the present study indicated that pyrolysis temperature primarily drive the variation of bacterial community structure in biochar amended soil. Our results demonstrates that biochar addition would reduce the bacterial diversity, while promote growth of functional bacteria to become the dominant population, and improve the microbial community organization, which in turn would result in a more stable bacterial community in the soil. The study presented here was carried out under laboratory experimental condition, further field study is needed to reveal the long-term ecological influences of biochar amendment in agricultural soil.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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