Soil Biology and Biochemistry 126 (2018) 159–167

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil fungal taxonomic and functional community composition as affected by biochar properties

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ARTICLE INFO

Keywords:
Fungal diversity
Community structure
Saprotroph
Lifestyle
Easily mineralizable C
Pathogen

ABSTRACT

Biochar greatly influences the soil bacterial community and nutrient transformations, while our knowledge of the responses of fungal lifestyles to biochar is still in its infancy. Here, we used experimental pre-treatments (acetone extraction) of nutrient-rich biochars to identify which major biochar properties influence fungi the most: the easily mineralizable C, fused aromatic backbone, or the inorganic nutrients of biochars. Our objective was to investigate how different biochar fractions (easily mineralizable C, fused aromatic C, nutrients) structure the fungal taxonomic and functional communities. The easily mineralizable C of biochars induced greater short-term changes in fungal community composition compared to the fused aromatic C. The easily mineralizable C significantly decreased the relative abundance of *Basidiomycota* by avg. 10.6%, while it had no effects on *Ascomycota* and *Zygomycota*. Co-occurrence network indicated that saprophytic fungi were self-assembled in the easily mineralizable C-sufficient environment, whereas they were the connectors to interact with other groups when lower amounts of energy in form of mineralizable C were present. Thus, the easily mineralizable C in biochar as a microbial C source probably promoted saprotroph growth, caused them to self-assemble and to enhance their competitive capacity, leading to overall diversity decrease and relative decline of fungal pathogens. The inorganic nutrients had no effects on fungal diversity and saprotroph abundance, while they decreased the relative abundance of *Zygomycota*. Our study highlighted the important roles of both mineralizable C and inorganic nutrients in modification of the fungal community, and demonstrated that biochar probably favors the growth of saprotrophs over soil-borne fungal pathogens.

1. Introduction

Biochar is the solid carbonaceous product originating from the pyrolysis of biomass wastes in an oxygen deficient environment. It has been applied to agricultural ecosystems to increase soil carbon sequestration and fertility (Smith, 2016; Bamminger et al., 2017; Li et al., 2018). Previously, biochar has been shown to induce changes in soil microbial activity, biomass, and community composition (Lehmann et al., 2011). The effects of biochar on soil microbial abundance and diversity are mainly caused by it (1) modifying the microbial environment (e.g., nutrients, pH) (Steinbeiss et al., 2009; Lehmann et al., 2011; Gomez et al., 2014); (2) directly supplying energy rich C substrates for microbial growth (Watzinger et al., 2014; Dai et al., 2017a) and (3) providing microbial habitats (Tsai et al., 2009).

Although several studies have focused on the effects of biochar on soil bacterial abundance, diversity, and community composition (Khodadad et al., 2011; Harter et al., 2014; Xu et al., 2016; Yao et al., 2017a), our knowledge of the responses of different fungal lifestyles to biochar remains unclear. Findings vary from decreases in fungal abundance (Chen et al., 2013), declines in alpha-diversity (Hu et al., 2014) to changes in community composition (Jenkins et al., 2017; Yao et al., 2017b). These studies, however, have only focused on fungal taxonomic alterations, which provides a limited interpretation of their role in soil functioning. In general, fungi are a heterogeneous kingdom encompassing three functional groups: saprotrophs, pathotrophs and symbiotrophs (Madigan et al., 2008). Saprotrophs are decomposers that

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https://doi.org/10.1016/j.soilbio.2018.09.001
Received 30 June 2018; Received in revised form 1 September 2018; Accepted 2 September 2018
Available online 05 September 2018

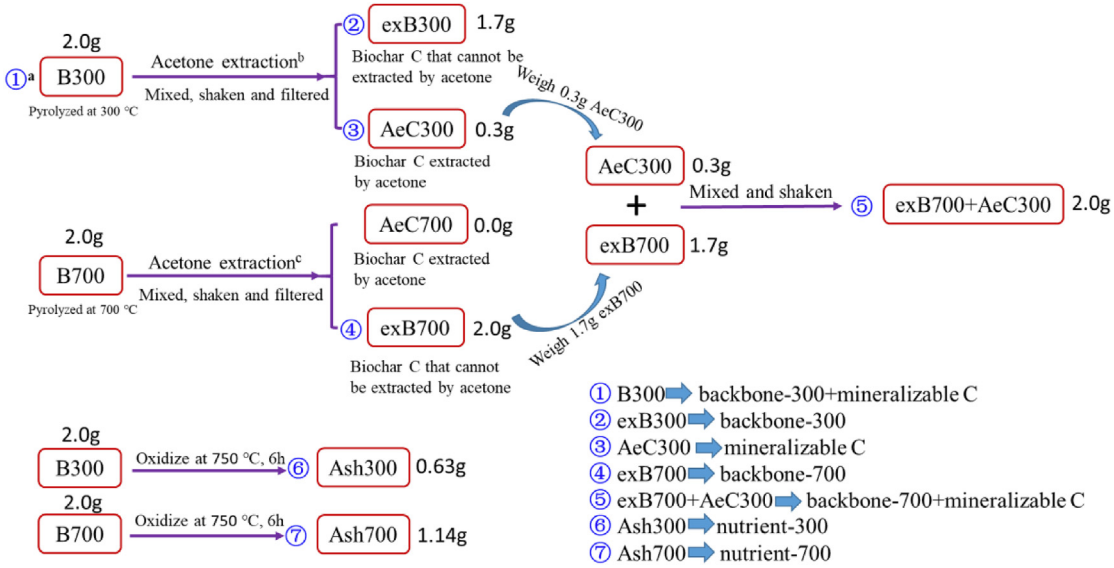

Fig. 1. Detailed information for the procedures used for production of biochar fractions. **a**. Biochars and ashes marked with the numbers (i.e.①-⑦) were added into soil for incubation. **b**. Acetone extraction rate for B300 is 15%.**c**. Acetone extraction rate for B700 is under the detection level.

may influence the persistence and mineralization of biochar and adjacent soil organic matter, while pathotrophs may cause plant diseases in soil ecosystems and symbiotrophs are root-fungal mutualists (Thies et al., 2009). Each of these functional groups has different lifestyles, regulating soil nutrient transformation and soil productivity, and responding differently to biochar addition (Thorn and Lynch, 2007). Thus, it is of great importance to investigate the responses of the fungal functional community to biochar, and so adding to our current understanding of the fungal taxonomic community.

In general, biochar comprises aliphatic C, fused aromatic C and ash (containing inorganic nutrients and altering the soil pH), regardless of pyrolysis conditions and feedstock type (Enders et al., 2012). The fused aromatic C fraction is considered to be mineralized more slowly by microorganisms and provides the physical backbone of biochars with its pores and surfaces, while the other C fraction (mainly aliphatic C) can be readily utilized as a C source (Dai et al., 2017a; Luo et al., 2017). Although different biochars have been compared regarding their effects on the microbial community (Thies et al., 2009), a comparison of different biochars alone, made from different feedstocks at different pyrolysis temperatures, alters the amount of mineralizable C, surface area, pore sizes, the inorganic nutrient content, the pH, and the proportion of fused aromatic ring structures (Enders et al., 2012). This makes attribution of any one of these properties to an effect on the microbial community composition challenging, as so many different soil and biochar properties may be confounded.

As a heterogeneous group, the fungal community is closely associated with soil C in biochar amended soils (Dai et al., 2016; Lucheta et al., 2016; Yao et al., 2017b). Thus, it is likely that different fungal members will respond differently to organic C availability. Saprophytic fungi, as decomposers with high C dependence and hyphae, probably more readily obtain C substrates compared to pathotrophs or symbiotrophs. Thus, biochars that contain both aliphatic C and aromatic C may alter fungal diversity and community composition, change the interactions between fungal groups and selectively stimulate certain specific group over others. However, little is currently known about how fungal lifestyles respond to different biochar C properties. In contrast, the ash in biochars only provide mineral nutrients for fungal growth, so it is considered to have less effects on fungal community change compared to biochar organic C availability.

Here, we investigated the effects of different biochar properties (i.e. easily mineralizable C, fused aromatic C backbone and inorganic nutrients) on fungal taxonomic and functional community composition. The co-occurrence network that has been widely used to explore the interactions between microbial species or OTUs (Deng et al., 2012) was employed to assess the fungal responses to different biochar properties. We asked the following question: do easily mineralizable C, inorganic nutrients or physical properties in form of the fused aromatic C change the patterns of fungal taxonomic and functional community composition differently and in what way(s)? We tested the hypotheses that (1) the easily mineralizable C of biochar selects a more saprotroph-dominant community than the fused aromatic C backbone, (2) the saprotroph growth stimulated by the easily mineralizable C leads to the reduction of overall fungal diversity and the suppression of other fungal growth, (3) the ash fraction, by supplying inorganic nutrients, will have less effects on fungal diversity change and saprotroph growth compared to the easily mineralizable C, due to the high dependence of fungal metabolism on energy rich substrates.

2. Materials and methods

2.1. Biochar preparation

In order to identify the responses of the soil fungal community to biochar properties, i.e. easily mineralizable C, fused aromatic C backbone and inorganic nutrients, biochars were conceptually separated into: (1) the easily mineralizable C mainly composed of aliphatic C; (2) the persistent C fraction mainly composed of fused aromatic C that provides pores and surfaces; and (3) the ash content that contains inorganic nutrients, as based on previous experimentation (Güereña et al., 2015).

The details of biochar preparation procedures are shown in Fig. 1. Two original biochars (B300 and B700) were produced by pyrolyzing autoclaved manure at 300 and 700 °C in an argon atmosphere. The low pyrolysis temperature resulted in an aliphatic C-dominated biochar (B300) with a low surface area and pores, while the high pyrolysis temperature resulted in a fused aromatic C-dominated biochar (B700) with high surface area and pores (Spokas, 2010). The heating rate was 2.5 °C min^{-1} to reach the highest treatment temperature which was held for 0.5 h. To extract easily mineralizable carbon (C) from B300, anhydrous acetone (100%) was mixed with B300 (biochar:acetone ratio = 1 g:10 ml) and the mixture shaken at room temperature for 24 h (Dai et al., 2017b). The C extracted by acetone, herein termed acetone extractable C (AeC), was separated from biochar particles by vacuum filtration. The AeC extracted from B300 was termed AeC300 and

considered to be the C that can be relatively easily mineralized by microorganisms, as acetone would not extract fused aromatic C. The residual biochar particles from B300 (i.e. biochar without AeC300) was termed exB300 and considered to be the more recalcitrant C backbone that provides pore structure and surface. The same procedure was performed on B700, but there was no gravimetrically detectable C (AeC700) extracted from B700, indicating that the C structure of B700 did not change after acetone extraction. Thus, we used exB700 (i.e. B700 after acetone extraction) as a treatment instead of B700, as any extractable C (AeC700) may influence the following incubation experiments although its content was below the detection level. Thus, the exB700 was also considered to be more recalcitrant C that provides pore structure and surfaces, similar to exB300. To account for (i) artifacts created during isolation procedures (e.g., whether the removal and addition of the easily mineralizable fraction had similar effects as when not removed), and (ii) for interaction effects (e.g., whether the identical composition and amount of easily mineralizable fraction had similar effects regardless of ash and fused aromatic ring structure), the AeC300 was collected and added back to exB700, mixed thoroughly for 24 h, resulting in a new sample: exB700 + AeC300 that was considered as a mixture of aromatic backbone C (i.e. recalcitrant C) and easily mineralizable C.

All the samples including biochar materials were then dried at 40 °C in the oven at ambient pressure for 12 h and then under vacuum for 1 h to thoroughly remove excess acetone residues. To investigate the fungal response to the inorganic nutrients (ash) of biochars, B300 and B700 were oxidized by heating at 750 °C for 6 h, resulting in ash samples: Ash300 and Ash700, respectively. Ash- and organic nutrient-rich biochars, i.e. swine manure biochars in this study, were used to provide measurable responses, as biochars with lower ash contents (such as those made from woody material) would result in a lower response to this fraction. In total, seven different fractions with conceptual definitions in brackets were used for the following incubation experiment, i.e. B300 (backbone C-300 + easily mineralizable C), exB700 (backbone C-700), exB300 (backbone C-300), exB700 + AeC300 (backbone C-700 + easily mineralizable C), AeC300 (easily mineralizable C), Ash300 (inorganic nutrient 300) and Ash700 (inorganic nutrient 700). Dai et al. (2017b) describe the methods for biochar characterization and its basic properties.

2.2. Incubation experiments

Soil was taken from the 0–0.2 m profile (after the soil O horizon was removed) in a forest hillside, without fire history, (42°27′46.4″N, 76°23′10.6″W), which was located in Freeville, NY, USA. The soil was air-dried, crushed (in order to reduce aggregate sizes to < 1 mm, without reducing the size of primary particles), then sieved < 1 mm and thoroughly homogenized. Then, the soil was pre-incubated for 7 days at 30 °C and at 40% of the water holding capacity before adding the biochars and fractions. Prior to addition, the pH of all the biochar materials were adjusted to 7.6 to minimize the biochar effects related to pH. Then, biochars (i.e. B300, exB700 + AeC300, exB300, exB700) and AeC were added at 50 g kg^{-1} (i.e. 2 g of each biochar was added to 40 g soil) and 7.5 g kg^{-1} (i.e. 0.3 g of each biochar was added to 40 g soil) into soil and mixed thoroughly. The soil without added biochar materials served as the control (i.e. CK). Here, the addition of AeC alone identified the effects of the easily mineralizable C fraction of biochar on the soil fungal community without any influences of biochar properties, e.g. fused aromatic C backbone. Because the acetone extraction rate of AeC300 from B300 was 15%, the samples of B300, exB700 + AeC300 and AeC300 all contained the same mass of AeC300, i.e. 0.3 g, while other biochar properties varied. Overall, 0.3 g AeC300 was introduced into soils with the treatments of B300, exB700 + AeC300 and AeC300, while no AeC300 was introduced with the treatments of exB300, exB700 and CK. To address the question "What is the maximum ash effect of the biochar on the soil fungal community if all the mineral nutrients were to be released from biochar?", we set up two treatments where soil received a mass of ash equivalent to the mass of ash contained in B300 and B700. Thus, 0.63 g Ash300 and 1.14 g Ash700 were added to 40 g soil, respectively. The resulting eight incubation treatments were: (1) Soil (CK); (2) Soil + B300; (3) Soil + exB300; (4) Soil + exB700; (5) Soil + (exB700 + AeC300); (6) Soil + AeC300); (7) Soil + Ash300; and (8) Soil + Ash700. We classified these treatments into three groups, i.e. AeC group (B300, exB700 + AeC300 and AeC300), non-AeC group (exB300, exB700 and CK) and Ash group (Ash300 and Ash700).

Soils mixed with biochars, AeC300 and ashes were then incubated in darkness at 30 °C for 150 days with three replicates, and maintained at 50% water holding capacity (WHC), to maximize microbial activity. Soil samples were collected on day 3 and the final incubation at day 150 to investigate the dynamics of fungal taxonomic and functional community change after the incorporation of biochar and fractions. The collection times were chosen because the microbial respiration rate had the highest peak near day 3 and was constant at day 150, respectively (Fig. S1). The cumulative CO_2 emission was measured at day 150 (Strotmann et al., 2004) where CO_2 production was measured by converting the electrical conductivity (EC) of the traps (i.e. bottles containing 0.09 M KOH) to CO_2 volume. This measurement aimed to test whether the C extracted by acetone from B300 (i.e. AeC300) was microbially easily mineralizable fraction. The experimental equipment and reagents for CO_2 emissions measurement was described by Whitman et al. (2014). The evidence that the AeC fraction was relatively easily mineralizable C is presented in Fig. S2.

2.3. Molecular analyses

After incubation, all soil samples were collected for DNA extraction on the same day and maintained at −80 °C until extraction. Total soil DNA was extracted using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The ITS1 region was amplified by the polymerase chain reaction with the following forward ITS1 (5′-CTTGGTCATTTAGAGGA AGTAA-3′) and reverse ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) primers. Sequencing raw data were pre-processed to remove low-quality reads by using the same procedures as Fadrosh et al. (2014). Paired-end sequences were merged to tags by FLASH (Magoč and Salzberg, 2011) using the following settings: minimal overlapping length of 15 bp and mismatching ratio of overlapped region < or = 0.1. Tags were clustered to operational taxonomic units (OTUs) by USEARCH (Edgar, 2013) as follows: (1) clustering at ≥ 97% identity level by UPARSE and (2) chimera elimination by UCHIME. The taxonomy for each fungal OTU was classified using the Ribosomal Database Project (RDP) Classifier (Cole et al., 2013) based on the UNITE database (Abarenkov et al., 2010), using a cutoff value of 0.8. OTU richness was calculated from the R package "vegan" (Oksanen et al., 2015). To parse fungal OTUs by ecological categories, we used an annotation tool, FUNGuild, to categorize fungal taxonomic groups into functional groups, i.e. saprotroph, pathotroph and symbiotroph (Nguyen et al., 2016). A large proportion of the fungal OTUs was assigned into saprotroph, pathotroph or symbiotroph, while a proportion of fungal OTUs that could not be assigned (i.e. unidentified fungal species) was called "unknown". The "unknown" group was included for analysis as it may provide valuable information for future studies. All sequences have been deposited in the GenBank short-read archive under the accession number SRP129692.

2.4. Statistical analysis

Non-metric multidimensional scaling plots (NMDS) of taxonomic similarity (Bray–Curtis) were generated to investigate the similarities and differences in the fungal community composition between different treatments using the "vegan" R package (Oksanen et al., 2015). The statistical analysis was conducted by using Nonparametric

PERMANOVA (permutational multivariate analysis of variance) (Anderson, 2001). The student T test tested whether the AeC fraction can induce significantly higher CO_2 emission compared to the treatments without AeC. The Pairwise Wilcoxon rank-sum test compared the relative abundance of major fungal phyla between different treatments, with the criterion based on p values adjusted using the correction of Benjamini and Hochberg. One-way analysis of variance (ANOVA) investigated significant differences ($p < 0.05$) between (1) alpha diversity (OTU richness) and (2) relative abundance of saprotrophs. The relationship between the OTU richness and saprotroph abundance was conducted using Spearman's Rank correlations. The R package 'DESeq2' calculated differential abundances of each OTU for AeC amended sample compared with the soil control at 3 and 150 days (Love et al., 2014). The fungal OTUs whose relative abundance increased or decreased significantly by more than doubling in response to AeC addition were defined as the 'responders'. The co-occurrence network of fungal communities was conducted by Gephi software (Jacomy et al., 2009) using the method MENA (Deng et al., 2012). The network parameters, i.e. centralization of betweenness, maximal stress centrality, modularity and connector were calculated to represent the fungal interactions induced by biochar and the topological roles of fungal functional groups in the network. Higher centralization of betweenness and maximal stress centrality represented the higher network complexity and microbial interactions (Newman, 2003). The modularity is the number of the modules where a group of species that interact strongly among themselves, but have few interactions with species from other modules. The connectors in a certain module are the fungal species that strongly interact with species from other module units (Deng et al., 2012).

3. Results

3.1. Fungal community composition

The addition of biochar, fractions and ash changed the soil fungal taxonomic community composition in different ways (Fig. 2). Fungal communities associated with additions of B300 (backbone-300 + mineralizable C), exB700 + AeC300 (backbone-700 + mineralizable C) and AeC300 (mineralizable C) clustered closely, whereas the communities with additions of exB700 (backbone-700), exB300 (backbone-700) and CK were different ($p < 0.05$) and also clustered (Fig. 2). Fungal communities associated with additions of Ash300 (nutrient-300) and Ash700 (nutrient-700) were closer ($p < 0.05$) to the treatments of exB700 (backbone-700), exB300 (backbone-300) and CK (no biochar materials addition) than B300 (backbone-300 + mineralizable C), exB700 + AeC300 (backbone-700 + mineralizable C) and AeC300 (mineralizable C) as shown by PERMANOVA (Fig. 2).

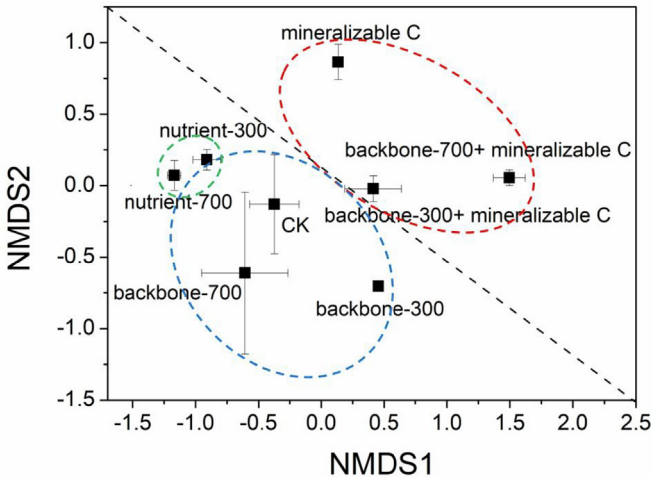

Fig. 2. Nonmetric multidimensional scaling plots (NMDS) of fungal taxonomic community in the soils incorporated with biochars, AeC300 and ashes, and soil control, based on the Bray-Curtis metric. backbone-300 + mineralizable C = B300, backbone-700 = exB700, backbone-300 = exB300, backbone-700 + mineralizable C = exB700 + AeC300, mineralizable C = AeC300, nutrient-300 = Ash300, nutrient-700 = Ash700, CK = soil control.

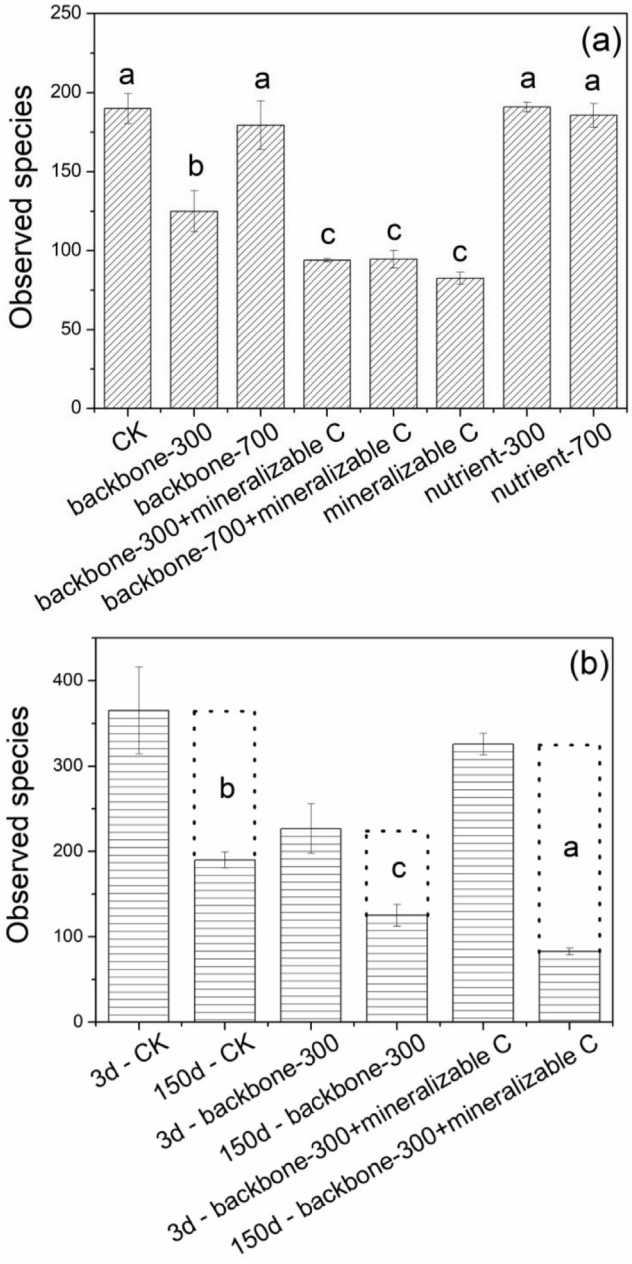

Fig. 3. Number of observed fungal species (i.e. alpha-diversity) in the soils following biochar addition after incubation of 150 days (a) and 3 days (b). Different lower letters in (a) represent significant differences ($P < 0.05$) in variables between treatments. Different lower letters in (b) represent significant differences ($P < 0.05$) in the changes in observed fungal species between treatments. backbone-300 + mineralizable C = B300, backbone-700 = exB700, backbone-300 = exB300, backbone-700 + mineralizable C = exB700 + AeC300, mineralizable C = AeC300, nutrient-300 = Ash300, nutrient-700 = Ash700, CK = soil control.

3.2. Fungal diversity

The additions of B300 (backbone-300 + mineralizable C), exB700 + AeC300 (backbone-700 + mineralizable C) and AeC300

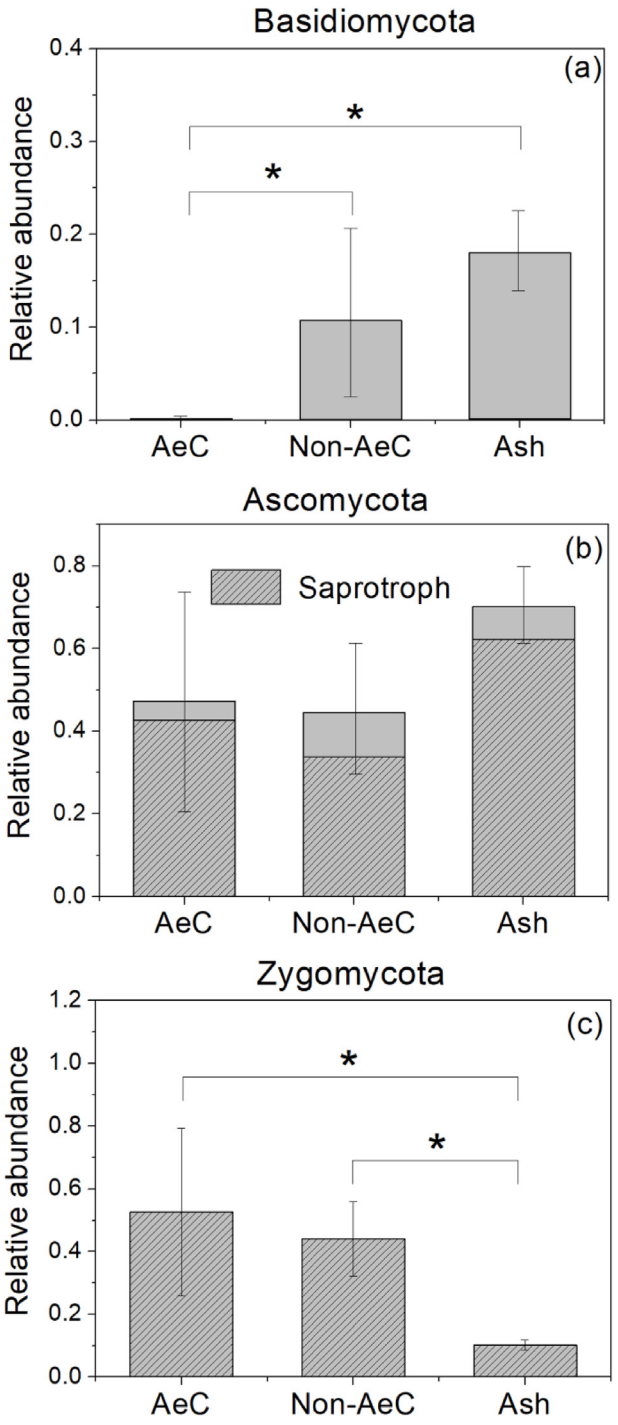

Fig. 4. Relative abundances of fungal phyla, *Basidiomycota* (a), *Ascomycota* (b) and *Zygomycota* (c) in the soils following biochar addition. The symbol "*" represents significant differences ($P < 0.05$) in the relative abundance of fungal phylum between treatments. The AeC group included the treatments of B300, exB700 + AeC300 and AeC300. The Non-AeC group included the treatments of exB300, exB700 and CK. The Ash group included the treatments of Ash300 and Ash700.

(mineralizable C) to soil decreased ($p < 0.05$) the number of observed fungal species (i.e. species richness) (Fig. 3a). In contrast, the addition of Ash300 (nutrient-300), Ash700 (nutrient-700) and exB700 (backbone-700) did not change the number of observed species (Fig. 3a). The number of fungal species in the soils with exB300 addition was higher ($p < 0.05$) than in the soils with B300, exB700 + AeC300 and AeC300 addition (Fig. 3a). From day 3 to day 150, the decrease in the alpha diversity in the soil with AeC300 (mineralizable C) was the highest ($p < 0.05$), followed by CK (no biochar materials addition) ($p < 0.05$) and that in exB300 (backbone-300) was lowest ($p < 0.05$) (Fig. 3b).

3.3. Fungal taxonomy at phylum level

The relative abundance of *Basidiomycota* (avg. 10.8%) in non-AeC amended soils, i.e. exB300 (backbone-300), exB700 (backbone-700) and CK (no biochar materials addition), was higher ($p < 0.05$) than that in AeC amended soils, i.e. B300 (backbone-300 + mineralizable C), exB700 + AeC300 (backbone-700 + mineralizable C) and AeC300 (mineralizable C) (avg. 0.16%) (Fig. 4a). Most fungal OTUs from *Basidiomycota* were assigned as non-saprotrophs (Fig. 4a), especially in the non-AeC amended soils. Absence of significant differences ($p > 0.05$) in the relative abundance of *Ascomycota* and *Zygomycota* was observed between the non-AeC amended and AeC amended soils (Fig. 4b and c). The relative abundances of *Basidiomycota* in ash amended soils were higher ($p < 0.05$) than in AeC amended soils (Fig. 4a), while the relative abundance of *Zygomycota* showed the opposite trend ($p < 0.05$) (Fig. 4c).

3.4. Fungal saprotrophs

The addition of B300 (backbone-300 + mineralizable C), exB700 + AeC300 (backbone-700 + mineralizable C) and AeC300 (mineralizable C) to soil increased ($p < 0.05$) the relative abundance of saprotrophs compared to addition of exB300 (backbone-300), exB700 (backbone-700), Ash300 (nutrient-300) and Ash700 (nutrient-700) (Fig. 5a). From day 3 to day 150, the increase in the relative abundance of saprotrophs in the soil with AeC300 (mineralizable C) addition was the highest ($p < 0.05$), followed by CK (no biochar materials addition) ($p < 0.05$) and that in exB300 (backbone-300) was the lowest ($p < 0.05$) (Fig. 5b). From day 1 to day 150, the proportion of positive saprotroph responders that were sensitive to biochar addition increased from 67% to 95% (Fig. S3). The relative abundance of saprotrophs was ($p < 0.05$) negatively correlated with fungal alpha diversity (Fig. 6).

3.5. Interactions between fungal groups

The centralization of betweenness and maximal stress centrality, both of which represent the degree of microbial interactions, were 0.154 and 2184, respectively, in the AeC group, i.e. B300 (backbone-300 + mineralizable C), exB700 + AeC300 (backbone-700 + mineralizable C) and AeC300 (mineralizable C). These two parameters were lower than those (0.196 and 6447, respectively) in the non-AeC group, i.e. exB700 (backbone-700), exB300 (backbone-700) and CK (no biochar materials addition) (Fig. 7). The modularity that represents the number of the modules in which a group of species interact strongly among themselves in the network of AeC group was 3, whereas that in the network of the non-AeC group was as high as 13 (Fig. 7). The top 15 OTUs with the highest connections in the AeC group were all saprotrophs, except for some OTUs whose functional classification was unknown (Fig. 7a). However, the top 15 OTUs in the non-AeC group comprised saprotroph, symbiotroph and pathotroph fungi (Fig. 7b). The non-AeC group had 9 connectors representing the fungal OTUs that strongly interacted with species from other module units (Fig. 8). A large proportion of these (six connector) were saprotrophs (Fig. 8). However, there was no connector observed in the AeC group, and all the nodes were from peripherals that interacted strongly with the OTUs within their own modules (Fig. 8).

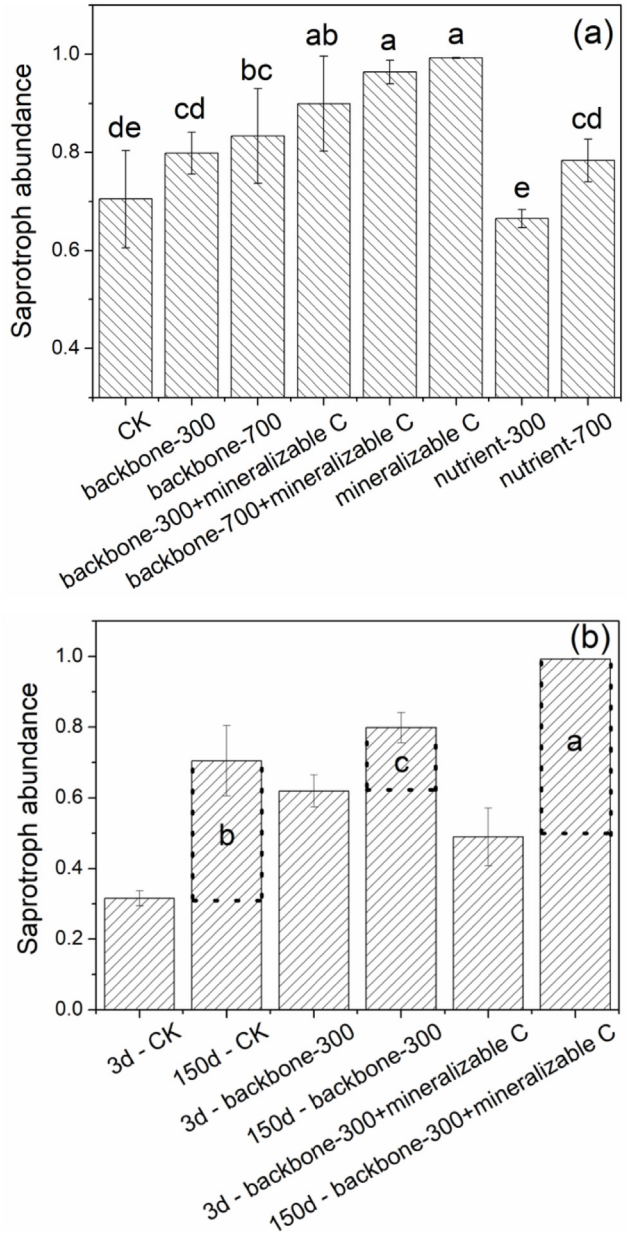

Fig. 5. Relative abundances of the saprotroph in the soils following biochar addition after the incubation of 150 day (a) and 3 day (b). Different lower letters in (a) represent significant differences ($P < 0.05$) in saprotroph abundance between treatments. Different lower letters in (b) represent significant differences ($P < 0.05$) in the change of saprotroph abundance between treatments. backbone-300 + mineralizable C = B300, backbone-700 = exB700, backbone-300 = exB300, backbone-700 + mineralizable C = exB700 + AeC300, mineralizable C = AeC300, nutrient-300 = Ash300, nutrient-700 = Ash700, CK = soil control.

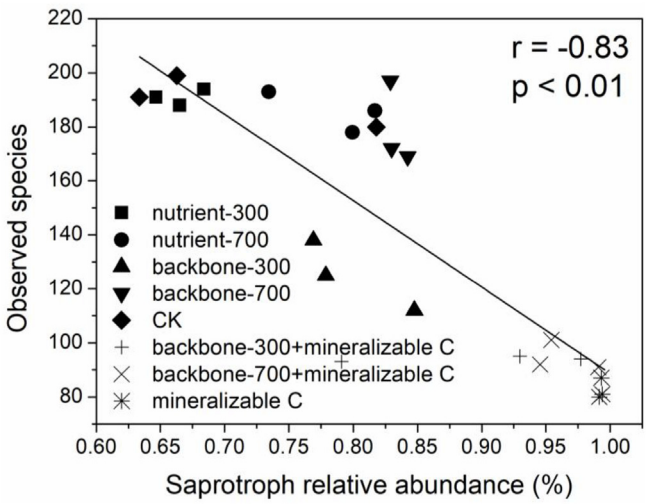

Fig. 6. Correlations between observed fungal species (i.e. alpha diversity) and the relative abundance of saprotrophs in the soils following biochar addition at 150 day. backbone-300 + mineralizable C = B300, backbone-700 = exB700, backbone-300 = exB300, backbone-700 + mineralizable C = exB700 + AeC300, mineralizable C = AeC300, nutrient-300 = Ash300, nutrient-700 = Ash700, CK = soil control.

4. Discussion

4.1. Fungal taxonomic community affected by biochar properties

Our study explored the responses of the fungal taxonomic and functional community composition to biochar application, and the roles of different properties of biochars in regulating fungal community were investigated. With the fungal taxonomic community, changes in the soil fungal community with biochar additions corroborated other studies. For instance, the fungal community composition was highly influenced by three years of biochar amendment in an upland soil (Yao et al., 2017b). An alteration of the soil fungal community composition caused by biochar additions was also found in a paddy soil (Chen et al., 2013). The fungal community composition in Amazonian Dark Earths which contain biochars from three different sites was more similar to each other than the adjacent soils without biochar (Lucheta et al., 2016).

Further, we provided evidence in this study that different C components of biochar lead to distinct differences in fungal community composition (Fig. 2). Similarly, Dai et al. (2016) demonstrated that the biogenic resource parameters (e.g. total C content) in biochar-amended soils explained 50.1% of the total variation in fungal community composition, which was greater than the 7.0% explained by soil acidity parameters (e.g. pH), indicating that the C content of biochar likely plays an important role in structuring the fungal community composition. In our study, the acetone extractable C fraction induced a significant change in fungal community composition compared to the fused aromatic C fraction (Fig. 2). In addition, the acetone extractable C fraction was supported to be microbially easily mineralizable C (Fig. S2). These results indicate that the fungal community was affected to a greater extent by the easily mineralizable C rather than any effects of biochar itself (Lehmann et al., 2011) over the relatively short period studied here. The effects of ash on fungal community composition were more similar to those of fused aromatic C than of easily mineralizable C, as shown by NMDS analysis and alpha diversity trends (Fig. 2 and Fig. 3). Given that the maximum ash content of biochar only causes a slight change in the fungal community and only a small part of the minerals can be released from biochar during biochar mineralization, we conclude that the inorganic nutrients of biochar has a very limited effect on fungal community composition. This is supported by the report that fungi rely more on organic C (not mineral nutrients) for metabolism as a heterogeneous group (Paul, 2014).

The observed decrease in soil fungal species caused by AeC of biochar (Fig. 3) is consistent with the decrease in fungal alpha-diversity (Chao 1 and Shannon index) with short-term additions of biochar pyrolyzed at 400 °C using forest litter (Hu et al., 2014). However, in other work, no differences in fungal alpha-diversity between biochar-amended soil and adjacent soils were observed (Lucheta et al., 2016). We attribute these different results to the mineralizable components of biochar. The biochar used by Hu et al. (2014) was made at low pyrolysis temperature and contained more mineralizable C while the biochar reported by Lucheta et al. (2016) had been undergoing long-term

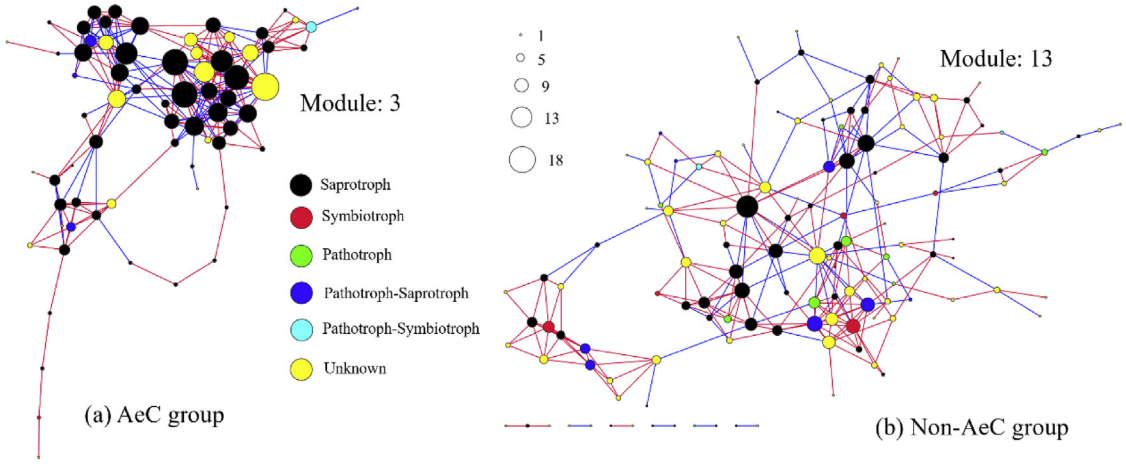

Fig. 7. The co-occurrence network of the fungal functional community in the AeC group (a) and Non-AeC group (b). The module is a group of species that interact strongly among themselves, but little with species in other modules. The higher the module number, the higher the connections among the nodes within the whole network. The AeC group included the treatments of B300, exB700 + AeC300 and AeC300, while the Non-AeC group included the treatments of exB300, exB700 and CK.

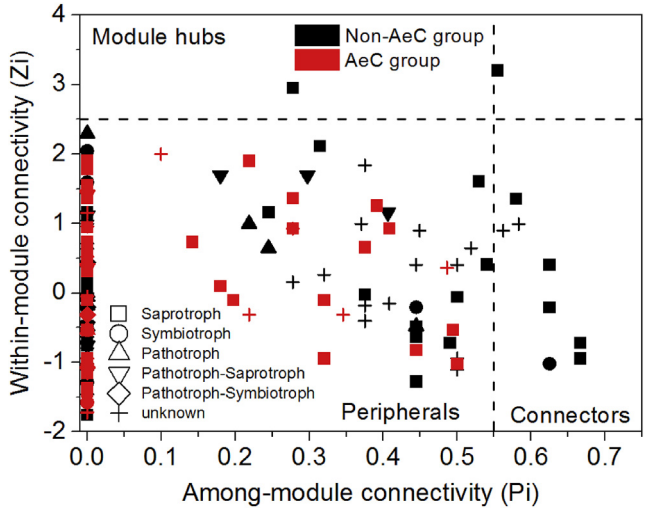

Fig. 8. The topological roles of nodes (fungal OTUs) in the AeC group and Non-AeC group. Higher Pi means the nodes (fungal OTUs) had higher connections outside their own modules. Higher Zi means the nodes (fungal OTUs) had more neighbors within their own modules. The AeC group included the treatments of B300, exB700 + AeC300 and AeC300, while the Non-AeC group included the treatments of exB300, exB700 and CK.

mineralization. In our study, we found that even when easily mineralizable C was added to a biochar dominated by fused aromatic C, i.e. backbone-700, the fungal diversity was significantly decreased by 50%, due to the selective growth of saprotrophs (Fig. 3). We therefore consider that the easily mineralizable C of biochars is one of the most important factors inducing fungal diversity changes over monthly time periods. pH has been verified to be important in determining microbial diversity in soil ecosystems (Rousk et al., 2010), but in our study the pH of biochars was adjusted to the same value and therefore the changes in fungal diversity can be attributed to the easily mineralizable fraction of biochar.

Consistent with our results showing that initial biochar pyrolyzed at 300 °C decreased the relative abundance of *Basidiomycota* by 19.9%, a large proportion of genera from *Basidiomycota* responded negatively to biochar additions (Dai et al., 2016). By contrast, incorporation of biochar produced at a higher pyrolysis temperature (400 °C) slightly increased the relative abundance of soil *Basidiomycota* (Hu et al., 2014). We attribute these contradictory results to the higher mineralizable C contents of biochars in our study. The lower relative abundance of *Basidiomycota* with AeC additions suggested that the easily mineralizable C fraction contributed greatly to the decrease in *Basidiomycota* abundance, which was consistent with the finding that few saprotrophs were observed in *Basidiomycota* without AeC additions. Since ITS sequencing only provides the relative abundance of each species, we cannot conclude that the easily mineralizable C of biochar suppressed *Basidiomycota* growth and proliferation. However, our results support the conclusion that the *Basidiomycota* were more sensitive to the easily mineralizable fraction of biochar than the *Ascomycota* and *Zygomycota*, as both of them did not change in response to AeC additions.

4.2. Fungal functional community affected by biochar properties

The observation that the relative abundance of saprotrophs increased with greater availability of easily mineralizable C (Fig. 5 and Fig. 6) supports previous findings regarding the great dependence of saprotrophs on mineralizable C substrates. In addition, saprotrophs often have hyphae and invasive growth habits (Wessels, 1999), which enable them to be effective colonizers of the interior of biochar porous structures. Thus, the morphological advantage of saprotrophs likely enhanced the C use efficiency of easily mineralizable C inside otherwise not easily accessible pore spaces.

Co-occurrence network analysis has recently been used to decipher the fungal assemblages across spatial or temporal gradients, or responses of the fungal community to environment disturbances (Barberán et al., 2012; Ma et al., 2016). Network complexity reflects the potential interaction of OTUs within the whole community. In comparison, the module in a network is a group of OTUs that interact strongly among themselves but have weak connections with OTUs outside the module (Deng et al., 2012). The network complexity and the number of modules in the AeC group were much lower than those in the non-AeC group (Fig. 7). This suggests that most fungal OTUs regulated by AeC connected internally in their own assemblage and had less interactions with other species outside. Most OTUs in the module from the AeC group were classified as saprotrophs (Fig. 7) and no connectors were classified as saprotroph in the AeC group (Fig. 8). This indicates that this group might be leaders (dominant species) in their own assembled group and respond to the presence of easily mineralizable C resources. By contrast, the higher module number and network complexity in the non-AeC group indicated that greater interactions among ecological OTUs occurred in the entire community (Fig. 7). The saprotrophs connected strongly with other groups such as pathotrophs and symbiotrophs (Fig. 7) from different modules. In addition, the non-

AeC group exhibited six saprotroph connectors, indicating that the saprotrophs became more active and strongly interacted with OTUs from other modules. We argue that the low food supply may be a potential reason for saprotrophs to compete with other OTUs. Our network analysis provides new insights into the response of fungal interactions under the different C components of biochar (aliphatic C and fused aromatic C) addition. Lack of C substrates would induce stronger interactions among fungal functional groups. Our results are different from those of a study showing high C inputs (e.g. organic C fertilizer) into agro-ecosystems resulted in an increase in phylogenetic and functional complexity of microbial networks (Xue et al., 2018). We considered that organic amendments can also cause changes in soil abiotic properties, e.g. pH (Wang et al., 2013). Thus, changes in network complexity may not be caused by C resource supply, but other soil property changes. In our study, the pH values of biochar were adjusted to the same pH. This experimental design allowed us to support the conclusion that easily mineralizable C of biochar led to a simplification of fungal interactions while a lack of easily mineralizable C, and only the presence of fused aromatic C, induced higher network complexity and strong fungal interactions.

4.3. Unbalanced competition theory

The finding of significant negative correlations between fungal diversity and relative abundance of saprotrophs (Fig. 6) illustrates the unbalanced competition between saprotrophs and other fungal groups investigated here. When easily mineralizable C was introduced, the relative abundance of saprotrophs (generally decomposers) was increased dramatically over time, as shown by the increased proportion of positive saprotroph responders from day 3 to day 150 (Fig. 5 and Fig. S3). As a result, saprotrophs were more competitive compared to other groups, and gradually became the dominant group in the fungal community. Other functional groups such as soil-borne fungal pathogens declined relatively, which was shown by the increased proportion of non-saprotroph negative responders (Fig. S3). We call this phenomenon “unbalanced competition” theory. It describes a phenomenon that the increase in saprotroph abundance triggered by the easily mineralizable C of biochar can lead to decreased fungal diversity and the potential suppression of other fungal groups (Fig. 6). Here, we should point out that a soil incubation may naturally result in the growth of saprotrophs rather than pathotrophs. This was supported by the increase in relative abundance of saprotrophs in the soil control (CK) from day 3 to day 150 (Fig. 5b). However, the increase in saprotroph abundance with additions of backbone −300 was significantly lower than that in CK, indicating the difference in saprotroph abundance changes between CK and backbone-300 were mainly attributed to a biochar effect, and not due to the incubation alone.

In conclusion, we linked the fungal taxonomic community with functional traits, showing that the fungal diversity decreased as saprotroph abundance increased, which was mainly regulated by biochar C components. The observed unbalanced competition between saprotrophs and other functional groups was induced by easily mineralizable C of biochar. Our work provides a new perspective for soil fungal community modification and plant pathogen control by biochar application to soil, while more work should be conducted to explain whether the community changes were a result of spatially occluded C resources or specific C components eliciting differential fungal responses. Also, the studies based on the absolute abundance of fungal functional groups affected by biochar addition should be included. Overall, as the fungal community is important for soil ecosystem functioning, we can modify it to meet different agricultural purposes by application of biochar with different C components or produced at different pyrolysis temperatures, which also has implications for understanding natural cycles of pyrogenic organic matter as a result of vegetation fires. The community changes identified here should then be verified in field experiments.

Acknowledgements

This research was jointly supported by the National Natural Science Foundation of China (41520104001, 41721001), China Postdoctoral Science Foundation (2017M620249, 2018T110598) and the Fundamental Research Funds for the Central Universities in China.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.09.001.

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