Comparison of microbial community structures in woody organic amendment soils and traditional local organic amendment soils of Ningxia, Northern China (#34038)

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Comparison of microbial community structures in woody organic amendment soils and traditional local organic amendment soils of Ningxia, Northern China

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Background: Organic amendments have been commonly adopted to restore degraded soils globally. However, the woody organic amendment was newly recognized as a viable materials in soil restoration in recent years, and the effect of woody organic amendment and other traditional local organic amendments on soil microbial community structure was less understood.

Methods: Three local natural, but different qualities of organic materials, i.e. cow manure (CM), corn straw (CS), and poplar branches (PB) were selected as treatments in Ningxia, Northern China. Four microcosms were applied for each treatment containing desertified soil mixed with 3% (w/w) of one of the above named organic materials, respectively. Measurements, including microbial α-diversity, community structure, the relative abundance of microbial phyla, and soil properties were conducted after 7 and 15 months of the start of the experiment.

Results: The bacterial α-diversity indices did not vary consistently with fungal α-diversity among treatments at each sampling time. Similarly, each bacterial and fungal α-diversity index observed after 15 months was also varied inconsistently with after 7 months, among treatments. However, all amendments presented different microbial community composition from the Control; CS and PB also presented different microbial community composition from CM during the whole experimental period. Whereas, CS and PB showed similar microbial community composition to each other after 15 months of experiment, which was consistent with their similar soil properties at that sampling time. Moreover, CS and PB also presented similar effects on the abundance of some microbial taxa during the whole experimental period, but both of them had different effects on microbial taxa from CM.

Conclusion: New local organic amendment with PB tended to affect the microbial community in a similar way to the traditional local organic amendment with CS, but different from the most traditional local organic amendment with CM in Ningxia, Northern China.

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ABSTRACT

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Keywords: Organic amendments; Desertified soils; Cow manure, Corn straw, Poplar branches; Microbial community structure

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INTRODUCTION

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72 The incorporation of locally available organic materials into degraded soil has been regarded as a
73 viable management practice because the amendments not only increase the soil fertility but also
74 stimulate the soil microbial activity (*Randhawa et al., 2005*), leading to enhanced crop growth
75 (*Fernández-Gálvez et al., 2009*). Moreover, soil organic amendments are increasingly recognized
76 for their roles in increasing soil water holding capacity, soil porosity, and water infiltration and
77 percolation while decreasing soil crusting and bulk density (*Thangarajan et al., 2013*). Therefore,
78 lots of soil organic amendments are extensively conducted, based on its advantage of improving
79 soil fertility and water status in arid and semi-arid areas globally. In the arid and semi-arid
80 regions of China and elsewhere, retaining and returning crop residues (e.g. wheat straw, corn
81 straw) and adding livestock manure (e.g. cow manure, chicken manure, pig manure) to the soil
82 have long been regarded as a practical and effective method for improving soil quality and crop
83 productivity (*Fan et al., 2014*). However, woody materials have not been commonly used as soil
84 amendments in these regions, although such kind of materials could be easily collected from
85 more than 2.2 million km^2 of shelterbelt forests areas across China (*Dai and Chu, 2012*).
86 Specifically, rapidly growing poplars (*Populus alba* and other spp.) are the most widespread and
87 available tree throughout China (Jia et al., 2013). Moreover, in the degraded and desertified areas
88 of Northern China, three-quarters of the poplar trees are planted as farmland shelterbelts (*Jing et*
89 *al., 2015*).As for Ningxia Autonomous Region, poplar species rank first among all trees and
90 distribute through an area of 47,000 km^2 in this seriously desertified region (*Sun et al., 2009*).
91 Fortunately, our previous research has demonstrated that incorporation of as little as 2%
92 wood chips (w/w) into the surface soil can effectively capture and retain more precipitation and
93 improve soil physicochemical properties in Ningxia, China (*Li et al., 2018*). Additionally, wood
94 chips also presented several comparable benefits to local traditional amendments crop straw and
95 cow manure, in improving soil health and enhancing crop growth in our previous study.
96 Importantly, the amendments of woody material in severely degraded soils can have valuable
97 and long-lasting benefits due to its low decomposition rate (*Weedon et al., 2009*). However, the
98 effects of wood chips as well as other traditional local organic amendments on soil microbiota
99 were little understood. Microbes play an integral and essential role in all soil processes (*Barrios,*
100 *2007*). For instance, microbial abundance, activity, and composition largely determine the
101 sustainable productivity of agricultural land (*van der Heijden et al., 2008*). Furthermore, some
102 studies have shown the ecological roles which microorganisms are playing in soils (*Fierer et al.,*

2007, 2012; Hartmann et al., 2015; Zhang et al., 2018), based on a 454-pyrosequencing approach for bacteria and fungi. These studies are useful for us to speculate the ecological roles of microorganisms in our soil organic amendments, because wood decomposition process related with microorganisms in the soil (*Sariyildiz et al., 2005*), and microorganisms in turn affecting soil nutrient cycling (*Wutzler et al., 2017*).

Because wood materials and crop straw have more lignin and carbon (C) content, and a higher C: nitrogen (N) ratio than livestock manures which makes them more difficult to decompose, we hypothesized that they also differently affect soil microbes from manures. Besides, some studies indicated that organic amendments with a high C:N ratio resulted in higher microbial biomass and activity, and also displayed differences in microbial composition compared to low C:N ratio materials (*Heijboer et al., 2016*). However, we know little about the effects of all local organic amendments, such as cow manure, corn straw, and poplar branches, on soil microbiota in Ningxia, China. Therefore, in this context, as the overall goal, we employed a 454-pyrosequencing approach of bacterial and fungal ribosomal markers to compare the effect of new woody amendment and traditional local amendments on soil microbiota. The detailed objectives of this study were to (a) compare the impact of new amendment poplar branches and traditional organic amendments cow manure and corn straw, on soil microbial α-diversity and community composition; (b) uncover the different effects of local organic amendments on specific microbial taxa; (c) determine the relationships between soil properties and soil microbial α-diversity, community composition and specific microbial taxa, respectively.

MATERIAL & METHODS

Study Site

A microcosm experiment was conducted for 15 months from April 2015 to July 2016 in a greenhouse in Yinchuan Belly Desert, which is located at the eastern foot of the Helan Mountains in Ningxia Hui Autonomous Region, China (106°08′~107°22′ E, 38°28′~38°42′ N). The study site is a representative area of the desertification occurrence throughout Northern China. Historically, the native landscape was grasslands, but it has been desertified after centuries of agriculture. Currently, the region is characterized by moving dunes with scattered farmland shelterbelt forests and farmlands are supported by extensive irrigation. The study site is located at 1,115 m above mean sea level and characterized by a temperate continental climate with 181.2 mm average annual precipitation and 1882.5 mm mean annual evaporation. The annual average temperature is 10.1 ℃ with the maximum of 37.2 ℃ in July and the minimum of

-27.9 ℃ in January. The average wind speed is 1.6 m s^{-1} and the frostless period is 160-170 days each year. The particle size distribution of soils was 92.5% sand (size < 2 mm), 5.5% silt, and 2.0% clay. The soil pH was 8.74, and the alkali-hydrolyzable N, available P, and available K concentrations were 9.16 mg kg^{-1}, 12.44 mg kg^{-1}, and 102.33 mg kg^{-1}, respectively. The organic matter content of the untreated soil was 0.93 g kg^{-1}.

Material Collection and Preparation

The base soils for this experiment were collected at the study site of Yinchuan Belly Desert. After collection, soils were sifted through a sieve with a mesh diameter of 2 mm and were air-dried before mixing with the selected organic materials. Woody materials were pruned branches from a local poplar (*Populus alba*), while herbaceous materials were consisted of straw residues collected from local corn (*Zea mays*) after harvesting. Poplar branches and corn straws were air-dried, then ground to lengths of about 0.5 cm for use. Cow manure, collected from a local dairy, was also air-dried and sieved through a screen with a mesh diameter of 0.5 cm to remove coarse material. The pH of cow manure, corn straw, and poplar branch were 8.58, 5.65, and 6.67, respectively; their organic C content were 201.25%, 535.00%, and 450.50%, respectively; their total N content were 13.50 g kg^{-1}, 3.65 g kg^{-1}, and 6.51 g kg^{-1}, respectively; their total phosphorus (P) content were 3.70 g kg^{-1}, 0.45 g kg^{-1}, and 1.44 g kg^{-1}, respectively.

Experimental Design

Four treatments were tested in this study, including 1) the Control with desertified soil only, and soil treatments incorporated with: 2) 3% (w/w) cow manure (CM), 3) 3% corn straw (CS), 4) 3% poplar branch (PB). 3% (w/w) organic material was added to the base soil and then mixed fully to be the soil mixture. For example, the soil mixture of 3% corn straw was obtained by adding 300 g corn straw to 9700 g base soil and mixing completely. Within the continuum of amendments, these proportions represented the minimum requirements for soil water retention which was tested in a previous set of our experiments (*Li et al., 2018*).

Control soil and soil mixtures were placed in buckets with the same size, who's top and bottom diameters were 31.5 cm and 26.0 cm, respectively, and the height was 31.5 cm. All buckets had small drainage holes at the bottom. Twelve kilograms of each soil mixture (base soil + 3% incorporated material) was packed in each bucket, after which 5 g urea ($CO(NH_2)_2$) and 2 g monopotassium phosphate (KH_2PO_4), dissolved into solution, were added to each bucket at the beginning of the experiment. The required amounts of urea and monopotassium phosphate added to the bucket were based on the results of our previous studies (*Li et al., 2018*). Initially, 12 plump alfalfa seeds were sowed in each bucket, however, only the six most robust seedlings

were kept growing after the first month for the rest of the experiment. Alfalfa was selected to sow in buckets of this experiment as it is the indicator plant in our study region. Totally, 4 treatments × 4 replications (buckets) × 2 times (7 months and 15 months) = 32 buckets were set up and arranged in a randomized block design in a greenhouse. Throughout the whole experiment, soil moisture of all buckets was monitored based on weight. All buckets were watered once every ten days and were maintained at an approximately constant water content equal to 70% of the water holding capacity of the Control. The indoor greenhouse temperature was maintained at 15-20℃ from November to the following March, and 20-35℃ from April to October during the experimental period.

Soil sampling and nutrients measurements

All 32 buckets were divided into two successive batches, thus 16 buckets including four treatments with four replicates in each batch. However, the actual time of measurements was based on the precise alfalfa flowering stage that occurred in the greenhouse during this study. The analyses of the first batch of 16 buckets were conducted when the alfalfa first flowered and it was harvested in October 2015 (seven months after the start of the experiment), while the second set of measurements were conducted in July 2016 (15 months after the beginning of the experiment) when the alfalfa flowered again and was harvested for a second time. After the plants were harvested, soil from each entire bucket was mixed with a sterile shovel. Then a portion was collected, air dried, and sieved through a sieve with the mesh diameter of 0.5 mm for chemical analysis, while another portion was placed in a sterile plastic bag, and was transported to the laboratory on ice, and then sieved (2-mm-mesh sieve) and stored at -80℃ for DNA extraction and other biological property analysis.

Soil organic C (SOC) was measured with the $K_2Cr_2O_7$–H_2SO_4 oxidation method of Walkley-Black (*Nelson et al., 1982*); total N (TN) was analyzed using the Kjeldahl procedure (*ISSCAS, 1978*); Alkaline-hydrolyzable (AN) was determined by an alkaline diffusion method (*ISSCAS 1978*); total P (TP) was determined using H_2SO_4+$HClO_4$ digestion (*Olsen et al., 1982*); available P (AP) was extracted with 0.5 mol l^{-1} $NaHCO_3$ (pH 8.5) (*ISSCAS, 1978*); total K (TK) was determined following the Cornfield method (*Kundsen et al., 1982*); and available K (AK) was determined using a CH_3COONH_4 extraction method (*Tran and Simard, 1993*). Microbial C (MBC) and N (MBN) were estimated by the fumigation-extraction method (*Vance et al., 1987*). Above data were listed in **Table S1**.

DNA extraction, PCR amplification, and pyrosequencing

Samples were separately tested at each sampling date. The total DNA was extracted from 0.5 g homogenized soil per sample using a Power Soil™ DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. A total of 16 extracted DNA samples, at each sampling date, were quantified using a Nanodrop-1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA) and diluted to a final concentration of 10 ng μL^{-1}. We used the universal primers 515F 5′- GTGCCAGCMGCCGCGGTAA -3′ and 909R 5′- CCCCGYCAATTCMTTTRAGT -3′ that target the V4-V5 regions of the 16S rRNA bacterial gene and produce the accurate phylogenetic information (Weisburg et al. 1991). For fungi, the ribosomal ITS region was amplified using primers ITS1F: 5′- CTTGGTCATTAGAGGAAGTAA-3′ (Gardes and Bruns 1993) and ITS2R: 5′- GCTGCGTTCTTCATCGATGC-3′ (*White et al., 1990*). To simultaneously analyze several samples in a single sequencing run, the 5' end of the forward primer was fused with a 12 bp different barcode sequence to a single sample. Each 25 μL PCR reaction containing 10 ng extracted DNA as a template, Mg^{2+}-free PCR buffer, 3 mM $MgCl_2$, 200 mM dNTP, 200 nM forward primer, 200 nM reverse primer, and 1 unit of PrimeSTAR Max DNA Polymerase (Takara, Dalian, China). The thermal cycling protocol was 95 °C for 2 min as the first step, followed by 30 cycles of PCR at 95 °C for 15 s, at 57 °C for 15 s, and at 72 °C for 40 s, and a final 10 min extension at 72 °C. All the amplicons were run in a 1% agarose gel (w/v), and the separated bands of the appropriate length were excised from the gel and purified using a QIAquick Gel Extraction kit (QIAGEN GmbH, Hilden, Germany). Finally, high-throughput sequencing of the 16S rDNA and ITS rDNA genes were conducted using Illumina MiSeq, and 300 bp paired-end reads were generated (Nanjing Tree & Cloud InfoTech Ltd., Nanjing, China). All the sequences have been deposited in the Sequence Read Archive (SRA) of NCBI under the accession number of SRP149652 for 16S rDNA and the accession number of PRJNA503750 for ITS rDNA.

Post-run analysis for 16S rDNA

The raw data were processed using the UPARSE pipeline with default parameters for each of the following steps: (1) to sort those exactly matching the specific barcodes into different samples; (2) to merge the paired-end reads with FLASH; (3) to trim the adapters, barcodes, and primers, and to remove sequences shorter than 200 bp; (4) to perform a quality filter using E (the sum of the error probabilities) > 1; (5) to cluster operational taxonomic units (OTUs) using a 97% identity threshold, discard singleton reads, and perform chimera filtering (http://drive5.com/uparse/) (Edgar, 2013).

Statistical analysis

Indices of α-diversity analysis (observed OUT richness, Chao1, and Shannon indices) were carried out in Quantitative Insights Into Microbial Ecology (QIIME) on rarefied OTU tables, which were obtained from the results of the high-throughput sequencing. Then, all data analyses were computed using the Vegan package in R. In all tests, a p-value < 0.05 was considered statistically significant. The differences of soil properties, microbial α-diversity, and relative abundances of microbial taxa among amendments were tested using one-way ANOVA followed by Tukey's post-hoc test. The statistical significances of the dissimilarities in the microbial community composition between every two amendments were analyzed with permutational multivariate analysis of variance. Principal coordinate analysis (PCoA) based on the Bray-Curtis distance was used to visualize the distribution patterns of the microbial community. Distance-based redundancy analysis (dbRDA) (based on the Bray-Curtis distance) was used to estimate the proportion of variability in the microbial community composition caused by each selected soil properties and marginal tests were performed to test the significance of each test. Pearson's correlation analysis was used to detect the relationships between soil properties and the means of α-diversity, as well as soil properties and relative abundance of microbial taxa.

RUSULTS

Effects of local organic amendments on microbial α-diversity

The bacterial α-diversity did not vary consistently with fungal α-diversity among treatments at each sampling time (**Table 1**). Similarly, each bacterial and fungal α-diversity index observed after 15 months was also varied inconsistently with after 7 months, among treatments. After organic materials were incorporated after seven months, CM and PB decreased OTUs and Chao1 index compared to the Control, but there was no difference between amendments for these indices ($p > 0.05$). However, CS increased the bacterial Shannon index but did not affect OTUs and Chao1. Similar to bacteria, CS also increased fungal Shannon index and did not affect OTUs and Chao1, too, when compared to the Control. However, CM decreased all fungal α-diversity indices, but PB decreased OTUs and Chao1 index, yet it increased Shannon index. After 15 months, CM decreased all bacterial α-diversity indices, but PB increased Chao1 and Shannon index when compared to the Control. However, there was no difference between CS and the Control ($p > 0.05$). Different from bacteria, CM increased all fungal α-diversity indices, yet CS decreased those indices, compared to the Control and PB increased fungal OTUs only.

Effects of local organic amendments on microbial community composition

All amendments altered bacterial and fungal community composition compared to the Control at

each sampling times ($p < 0.05$) (**Table 2**). Moreover, both CS and PB also presented different bacterial and fungal community compositions from CM at each sampling time. However, though CS had different bacterial and fungal community composition from PB after seven months, they showed similar microbial community composition after 15 months ($p > 0.05$). Similarly, PCoA analysis also showed that treatments CS and PB were clustered together both for the bacterial and fungal community after 15 months, but they were separated from CM and the Control respectively, and CM was also separated from CM (**Fig. 1**). However, all treatments were weakly separated from each other after seven months, represented by PCoA coordination (**Fig. 1**).

Effects of local organic amendments on relative abundance of microbial phyla

In this study, nine major bacterial phyla (relative abundance > 1%), while all fungal phyla were included for analysis, as only five fungal phyla were detected. The composition of bacterial phyla was different among treatments between different sampling times (**Fig. 2a**). CM increased the relative abundance of Actinobacteria compared with the Control after seven months of experiment. However, CM decreased Planctomycetes abundance compared to CS and PB, while with no difference compared to the Control ($p > 0.05$).

Similarly, CM also decreased Chloroflexi compared to CS, with no difference compared to the Control and PB ($p > 0.05$), respectively. Differently, PB decreased Gemmatimonadetes abundance compared to CM, yet there was no difference compared with the Control and PB ($p > 0.05$), respectively. After 15 months, both CS and PB increased relative abundances of Proteobacteria compared with CM and the Control, but they decreased relative abundance of Bacteroidetes compared with CM and the Control. Similarly, both CS and PB also decreased Cyanobacteria abundance, compared with the Control. However, higher values of Acidobacteria abundance were also detected in both CS and PB, than in CM and the Control, respectively.

The compositions of fungal phyla were also different among treatments between different sampling times (**Fig. 2b**). After seven months, compared with the Control, the highest level of Zygomycota was observed in the treatment PB, while the highest level of Basidiomycota was found in the treatment CS, among all treatments. Moreover, PB also presented higher value in the level of Ascomycota than CM. After 15 months, only PB was observed the increased abundance of Basidiomycota when compared with the Control.

Relationships between soil properties and α-diversity, microbial community composition, and relative abundance of microbial phyla

After 7 months, as for bacterial α-diversity indices, Chao1 was negatively correlated with both SOC and TN, while OTUs was only negatively correlated with TN; as for fungal α-diversity indices, there were only negatively correlations between Chao 1 and soil pH, and also between

Shannon and AN (**Table 3**). After 15 months, however, bacterial α-diversity indices were mainly negatively correlated with TP, AN, and AP, while fungal α-diversity indices were mostly positively correlated with TP, AN, AP, and AK.

Distance-based RDA analysis showed that bacterial and fungal community compositions were also affected by different soil properties (**Table 4**). After seven months, the bacterial community composition was significantly influenced by pH, SOC, and TN, which explained 20.45, 9.73, and 13.98% of the total variance, respectively, whereas fungal community compositions were significantly influenced by pH, SOC, TN, and AK, which explained 13.05, 9.20, 11.44, and 9.34% of the total variance, respectively. On the other hand, after 15 months, bacterial community compositions were significantly influenced by pH, SOC, TN, and TP, which explained 12.83, 10.12, 13.74, and 14.30% of the total variance, respectively, while the fungal community composition was significantly influenced by pH, SOC, TN, TP, and AK, which explained 8.96, 9.84, 9.53, 10.49, and 15.07% of the total variance, respectively. As a whole, pH, SOC, and TN were factors which affected both bacterial and fungal community compositions during the whole experimental period. However, AK was the sole factor which impacted on the fungal community composition, rather than the bacterial community composition, during the entire experimental period, while TP was the only factor which affected both bacterial and fungal community compositions after 15 months but influenced neither the bacterial nor the fungal community composition after seven months.

The significantly different abundance of bacterial phyla among treatments were differently affected by soil properties as well (**Table 5**). Thus, after seven months, Actinobacteria were negatively correlated with pH, while positively correlated with AN, AP and AK, whereas Planctomycetes was negatively correlated with AN, AP, AK, and TP. Similarly, Chloroflexi was also negatively correlated with AN, and AP. However, Gemmatimonadetes was positively correlated with AN, AP, and AK, but positively correlated with MBN. After 15 months, Proteobacteria were positively correlated with SOC and MBC but negatively correlated with TP and AN. Cyanobacteria were negatively correlated with pH, while positively correlated with TK and MBC. Acidobacteria were negatively correlated with TP, AN, and AP. However, Bacteroidetes was not correlated with any selected soil properties in this study. Besides, though some other bacterial phyla were not detected significant among treatments, they were also correlated with soil properties.

Similarly, the significantly changed fungal phyla in abundance among treatments were also differently affected by soil properties (**Table 6**). After seven months, Ascomycota was negatively correlated with AN but positively correlated with MBC. Similarly, Basidiomycota was also negatively correlated with AN but positively correlated with MBC and MBN. However, no selected soil properties were correlated with Zygomycota after seven months and

Basidiomycota after 15 months in this study. Nevertheless, the relative abundances of some other fungal phyla were also correlated with some properties, though they were not significantly different among treatments during the whole experimental period.

DISCUSSION

Effects of local organic amendments on microbial α-diversity

It seems that previous studies have not achieved an agreement on the effect of organic amendments on soil microbial α-diversity. Some studies reported an increase (*Francioli et al. 2016; Sharma et al. 2017*), but some studies found a decrease (*Montiel-Rozas et al., 2018*), and some others found no effect on soil microbial α-diversity after organic amendment (*Zhang et al., 2018*). Interestingly, all the above cases were found in our study. Particularly, some α-diversity indices decreased in some treatments at the early stage, but they increased at the later stage, and some other α-diversity indices increased at first but then decreased. These inconsistent results were also reported by others (*van Diepeningen et al., 2006*). Hence, it seems difficult to draw a robust conclusion from the effect of organic amendments on soil microbial diversity, in part because these metrics often have little power in explaining differences in community structure (*Hartmann and Widmer, 2006*). Particularly, for instance, bacterial Shannon index did not vary in CM and CS compared to the Control after seven months, but both OUTs and Chao1were found to decrease in these two treatments. Similarly, *Calleja-Cervantes et al*. (2015) did not observe any changes in bacterial Shannon diversity after 15 days of organic fertilization, too.

Though variations in the α-diversity among treatments were complicated in this study, we assume that different soil properties, caused by different organic amendments, have effects on the α-diversity. For instance, the higher content of SOC and TN, in both CM and PB soils than other treatment soils after seven months (**Table S1**), should be also related to decreased α-diversity in soils of CM and PB, since correlation analysis also showed that the bacterial α-diversity was negatively correlated with SOC and TN after seven months. However, the higher pH value in PB, and higher value of AN in CM (**Table S1**) were also the factors which caused a decreased α-diversity in these two treatments after seven months, and our correlation analysis also supported this assumption. Similarly, after 15 months, the microbial α-diversity was also affected by some soil properties, such as TP, AN, AP, and AK. Nevertheless, we should acknowledge that some other soil properties might impact on α-diversity as well, though we did not determine in this study. Meanwhile, we should also acknowledge that alfalfa growth affected microbial α-diversity.

Effects of local organic amendments on microbial community composition

Though α-diversity varied complicatedly among treatments, the variation of microbial community composition was evident. Thus, all local organic amendments changed the microbial community composition compared with the Control at any sampling times. Moreover, both CS and PB also presented different bacterial and fungal community compositions from CM at each sampling time, which was probably due to the different soil properties among these treatments (**Table S1**). On the other hand, though CS showed different bacterial as well as different fungal community composition from PB after seven months, they had the similar microbial community compositions after 15 months. These similar and different microbial compositions between CS and PB were also probably because the soil properties were dissimilar after seven months, but they became more similar after 15 months. Furthermore, the soil properties determined in this study indeed supported the above hypothesis, since there was no significant difference for any soil properties between CS and PB after 15 months, but AK was higher in CS than that in PB after seven months (**Table S1**). Similar findings were also documented by others (*Zhang et al., 2018*). However, dbRDA revealed that the effects of each soil property on microbial community composition were different. After seven months, pH, SOC, and TN were the factors which drove changes in both soil bacterial and fungal community compositions. This result was similar to *Hartmann et al*. (*2015*). However, AK also presented strong effect on fungal community composition, which explained 9.34% of the total variance. But among all soil parameters, pH was the strongest factor that drove varies in both bacterial and fungal community compositions in the soil; this was similar to the findings of other studies (*Lauber et al. 2009; Rutigliano et al. 2014*). Even after 15 months, pH, SOC, TN, and TP affected both bacterial and fungal community compositions in the soil. Importantly, AK was still another, but the most influential factor (explaining 15.07%) in changing the fungal community composition. Therefore, we assume that fungal community, in desertified soils in our study, reacted more sensitively to AK than bacterial community. Moreover, we also noted that TP became a stronger factor affecting soil microbial community composition at the later stage.

In all, compared with the Control, though all local organic amendments altered the microbial community composition, the new organic amendment PB tended to change microbial community composition similarly to the traditional amendment CS at the later stage. However, both CS and PB represented different effects on microbial community composition from the most common and traditional organic amendment CM during the whole experimental period.

Effects of local organic amendments on relative abundance of microbial phyla

Though local organic amendments were applied for short-term in this study, we found that they changed some dominant bacterial and fungal phyla abundance. However, changes in those

dominant bacterial and fungal phyla among treatments were different between the two sampling times. This finding was also similar to *Zhang et al.* (*2018*), and we assume that these various changes were related to qualities of incorporated organic materials, changes in soil properties and sampling season. Also, similar to other studies (*Zeng et al. 2016; Lladó et al. 2017; Zeng et al. 2017*), the dominant bacteria phyla of Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes were detected in our amended soil. Whereas, among all bacterial phyla, Proteobacteria was most dominant and had higher abundance in treatments with CS and PB than in CM and the Control. This result might due to the reason that Proteobacteria are copiotrophic microorganisms (*Fierer et al., 2012*) and the decomposer of lignin (*Tian et al., 2014*). However, Proteobacteria was negatively correlated with some soil nutrients, but positively correlated with SOC and MBC after 15 months, which was probably because CS and PB enhanced the microbial activity and fixed more nutrients to microbial growth in a microcosm bucket after 15 months since we found much higher MBC in CS and PB than in CM (**Table S1**). Moreover, scientists also documented that organic amendments with a high C:N ratio resulted in a higher microbial biomass and activity (*Heijboer et al., 2016*).

Similarly, Actinobacteria is also putatively identified as copiotrophic taxa which thrive in the condition of high C availability and exhibit relatively rapid growth rates (*Fierer et al., 2012*). Our study confirms this finding since treatment CM not only increased SOC and other soil nutrient contents (**Table S1**), but also increased abundance of Actinobacteria compared with control, after 7 months. Besides, Actinobacteria was also positively correlated with AN, AP and AK after seven months. Bacteroidetes are another group of typically copiotrophic texa (*Fierer et al., 2007*) and have been found to be positively correlated with soil total P (*Tian et al. 2015*) and soluble P (*Yashiro et al. 2016*). In our study, Bacteroidetes had higher abundance in CM than CS and PB after 15 months, whereas we did not found any soil nutrient which was correlated with Bacteroidetes. Hence, we speculate that Bacteroidetes are slow-growing copiotrophic microorganisms (*Fierer et al., 2007*), which reacted slowly to soil nutrients in our short-term study.

Also, we speculate that Cyanobacteria are slow-growing copiotrophic microorganisms since they also showed comparatively higher abundance in the treatment CM than CS and PB (though not significantly different from each other), after 15 months. Nevertheless, Acidobacteria is regarded to be more adapted to nutrient-limited soil environment (*Ward et al., 2009; Fierer et al., 2012*). Some scientists also viewed that Acidobacteria generally prefer soil environments with high carbon resource (*Větrovský and Baldrian, 2013*). Our study also confirms above viewpoint due to the fact that Acidobacteria was found in higher abundance in treatments CS and PB after 15 months, which contained lower available N, P, and K at that sampling time, too (**Table S1**). Moreover, Acidobacteria was also negatively correlated with TP, AN, and AP in this study.

Gemmatimonadetes also showed copiotrophic features in this study, because the greater relative abundance was detected in a higher nutrient content treatment CM. Correlation analysis also revealed that the relative abundance of Gemmatimonadetes was positively correlated with AN, AP, and AK. In contrast, Planctomycetes and Chloroflexi showed oligotrophic features because they presented lower abundance in CM than in CS and PB, after 7 months. Similarly, correlation analysis indicated that those two bacterial phyla were negatively correlated with some soil nutrients.

Though we know little about the ecological function of detected fungal phyla, some studies reported that the cellobiohydrolase gene is unique to fungi, broadly distributed in Ascomycota and Basidiomycota in forest soils, and encodes for an enzyme which is critical to cellulose breakdown (*Edwards et al., 2011*). Therefore, we assume that the higher relative abundance of Ascomycota, Basidiomycota, and Zygomycota detected in treatments PB and CS should be correlated with the process of wood decomposition in our study. Furthermore, we consider those fungi play an essential role in incorporated organic material decomposition at the early stage, since some studies indicated that fungi were dominant litter decomposer at the early stage (*Voříšková and Baldrian, 2013; Žifčáková et al., 2016*), while bacteria were dominant decomposer at the later stage (*Berg, 2014*). However, it is essential to acknowledge that we can only speculate on the ecological role of the detected taxa based on what has been previously described in other studies. Also, we only discovered several organic amendment-sensitive bacteria and fungi in phyla level in this study. Therefore, the in-depth analysis should also be conducted based on our data to find more information about the microbial ecological role. Moreover, long-term field study should also be considered as the next step. At last, we should acknowledge that different alfalfa growth status also affected the microbial community, though our preference was to discover the effects of organic materials.

CONCLUSIONS

The effect of local organic amendments on soil microbial α-diversity was complicated. The bacterial α-diversity indices were not varied consistently with fungal α-diversity among treatments at each sampling time. Moreover, each bacterial and fungal α-diversity index observed after 15 months was also varied inconsistently with after 7 months, among treatments. However, all local amendments varied bacterial and fungal community compositions compared to the Control at each sampling time. The local new organic amendment PB also presented different microbial community compositions from that of local traditional organic amendments CM and CS, respectively, after seven months. However, PB and CS had the similar microbial

community compositions after 15 months, which was consistent with the result that they have similar soil properties after 15 months. Soil pH, SOC, and TN were the factors which affected both bacterial and fungal community compositions during the whole experimental period. But AK was the factor which influenced only fungal community composition, rather than bacterial community composition, during the entire experimental period; while TP was the factor which affected both bacterial and fungal community compositions after 15 months, rather than seven months.

Moreover, both CS and PB presented different effects on microbial taxa from CM. Thus, some high C/N-sensitive and wood decompose-related microbial phyla, such as Proteobacteria, Ascomycota, Basidiomycota, and Zygomycota, might increase their abundance in CS and PB compared to CM. Simultaneously, the oligotrophic microorganisms, such as Acidobacteria, Planctomycetes, and Chloroflexi, also increased their abundance in CS and PB, compared to CM. By contrast, some copiotrophic microbes, such as Actinobacteria, Gemmatimonadetes, Bacteroidetes, and Cyanobacteria, increased in CM, compared to CS and PB. In summary, both the different qualities of local organic materials and soil properties, affected the microbial α-diversity, community composition, and specific taxa differently. The new organic amendment PB tended to change the microbial community in a similar way to the local traditional amendment CS, but different from CM.

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EFERENCES

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5):335–336 DOI 10.1038/nmeth.f.303

Dai G and Chu W. 2010. Analysis on the status of farmland shelterbelt resources in the three-north regions and corresponding strategies. Forest Resources Management 1: 27-32.

Edgar RC. 2013 Uparse: Highly accurate otu sequences from microbial amplicon reads. *Nature Methods* 10(10): 996–998 DOI 10.1038/NMETH.2604

Edwards IP, Zak DR, Kellner H, Eisenlord SD, Pregitzer KS. 2011. Simulated Atmospheric N Deposition Alters Fungal Community Composition and Suppresses Ligninolytic Gene Expression in a Northern Hardwood Forest. *PLoS ONE* 6, e20421. DOI:10.1371/journal.pone.0020421

Fan J, Yu G, Wang Q, Malhi SS, Li Y. 2014. Mulching effects on water storage in soil and its depletion by alfalfa in the loess plateau of northwestern china. *Agricultural Water Management* 138: 10-16 DOI 10.1016/j.agwat.2014.02.018

Fernández-Gálvez J, Gálvez A, Peña A, Mingorance MD. 2012. Soil hydrophysical properties resulting from the interaction between organic amendments and water quality in soils from southeastern spain-a laboratory experiment. *Agricultural Water Management* 104:104-112. DOI 10.1016/j.agwat.2011.12.004

Fierer N, Bradford MA, Jackson RB 2007. Toward an ecological classification of soil bacteria. *Ecology* 88(6):1354–1364 DOI 10.1890/05-1839

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J 6(5):1007–1017 DOI 10.1038/ismej.2011.159

Francioli D, Schulz E, Lentendu G, Wubet T, Buscot F, Reitz T. 2016. Mineral vs. Organic amendments: Microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Frontiers in Microbiology* 7(289): 1446 DOI 10.3389/fmicb.2016.01446

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2 (2): 113–118 DOI 10.1111/j.1365-294X.1993.tb00005.x

Hartmann M, Widmer F. 2006. Community structure analyses are more sensitive to differences in soil bacterial communities than anonymous diversity indices. *Applied & Environmental Microbiology* 72: 7804–7812 DOI 10.1128/AEM.01464-06

Hartmann M, Frey B, Mayer J, Mäder P, Widmer F. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal* 9: 1177–1194. DOI 10.1038/ismej.2014.210

Heijboer A, Berge HFMT, Ruiter PCD, Jørgensen HB, Kowalchuk GA, Bloem J. 2016. Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes; a 15N tracer-based approach. *Applied Soil Ecology* 107: 251–260 DOI 10.1016/j.apsoil.2016.06.009

Institute of Soil Science, Chinese Academy of Sciences (ISSCAS). 1978. Physical and Chemical Analysis Methods of Soils. China Shanghai Science Technology Press, Shanghai, pp 7-59.

Jia L, Liu S, Zhu L, Jianjun HU, Wang X. 2013. Carbon storage and density of poplars in china. *Journal of Nanjing Forestry University* 37:17.

559 Jing DW, Xing SJ, Du Z. 2015. Effects of water stress on physiological and biochemical characteristics of
560 *Populus* × *euramericana* cv. '*Neva*' seedlings. *Journal of Arid Land Resources and Environment*
561 29(1): 54-58.
562 Kundsen D, Peterson GA, Pratt PF. 1982. Lithium, sodium, and potassium. Methods of soil analysis. Part
563 2. Chemical and microbiological potassium. Methods of soil analysis. Part 2. Chemical and
564 microbiological.
565 Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing-based assessment of soil pH as a
566 predictor of soil bacterial community structure at the continental scale. *Applied and*
567 *Environmental Microbiology* 75(15): 5111–5120 DOI 10.1128/AEM.00335-09
568 Li Z, Schneider RL, Morreale SJ, Xie Y, Li C, Li J. 2018. Woody organic amendments for retaining soil
569 water, improving soil properties and enhancing plant growth in desertified soils of Ningxia, China.
570 *Geoderma* 310: 143–152 DOI 10.1016/j.geoderma.2017.09.009
571 Lladó S, López-Mondéjar R, Baldrian P. 2017. Forest soil bacteria: diversity, involvement in ecosystem
572 processes, and response to global change. *Microbiology and Molecular Biology Reviews*
573 81:e00063-00016 DOI 10.1128/MMBR.00063-16
574 Montiel-Rozas MM, Domínguez MT, Madejón E, Madejón P, Pastorelli R, Renella G. 2018. Long-term
575 effects of organic amendments on bacterial and fungal communities in a degraded Mediterranean
576 soil. *Geoderma* 332, 20–28. DOI 10.1016/j.geoderma.2018.06.022
577 Nelson DW, Sommers LE, Sparks DL, Page AL, Helmke PA, Loeppert R H. 1996. Total carbon, organic
578 carbon, and organic matter. Methods of Soil Analysis Part-chemical Methods, 961-1010.
579 Olsen SR, Sommers LE, Page AL. 1982. Methods of soil analysis. Part 2. Chemical and microbiological
580 properties of Phosphorus. ASA Monograph. 9: 403- 430.
581 Randhawa PS, Condron LM, Di HJ, Sinaj S, Mclenaghen RD. 2005. Effect of green manure addition on
582 soil organic phosphorus mineralisation. *Nutrient Cycling in Agroecosystems* 73: 181-189 DOI
583 10.1007/s10705-005-0593-z
584 Rutigliano FA, Romano M, Marzaioli R, Baglivo I, Baronti S, Miglietta F, Castaldi S. 2014. Effect of
585 biochar addition on soil microbial community in a wheat crop. *European Journal of Soil Biology*
586 60(2): 9–15 DOI 10.1016/j.ejsobi.2013.10.007
587 Sariyildiz T, Anderson JM, Kucuk M. 2005. Effects of tree species and topography on soil chemistry,
588 litter quality, and decomposition in northeast turkey. *Soil Biology Biochemistry* 37(9): 1695–1706
589 DOI 10.1016/j.soilbio.2005.02.004
590 Sharma A, Kachroo D, Hardev Ram RP, Pooja Gupta Soni DJ, Malu Ram Yadav TY. 2017. Impact of
591 different transplanting dates and nutrient sources on soil microbial population and grain yield of
592 basmati rice (*Oryza sativa* L.) grown under SRI. *International Journal of Current Microbiology*
593 *&* Applied *Sciences* 6(3): 778–782.
594 Sun Y, Wang D X, Zhang H, Li ZG, Wei YF, Hu TH. 2009. Forest ecosystem services and their valuation

595 of Ningxia area. *Journal of Northwest A & F University* 37: 91-97.
596 Thangarajan R, Bolan NS, Tian G, Naidu R, Kunhikrishnan A. 2013. Role of organic amendment
597 application on greenhouse gas emission from soil. *Science of the Total Environment* 465: 72-96
598 DOI 10.1016/j.scitotenv.2013.01.031
599 Tian W, Wang L, Li Y, Zhuang K, Li G, Zhang J, Xiao X, Xi Y. 2015. Responses of microbial activity,
600 abundance, and community in wheat soil after three years of heavy fertilization with manure-
601 based compost and inorganic nitrogen. *Agriculture, Ecosystems & Environment* 213:219–227
602 DOI 10.1016/j.agee.2015.08.009
603 Tran TS, Simard RR. 1993. Mehlich III-extractable elements. In: Carter, M.R. (Ed.), Soil Sampling and
604 Methods of Analysis. Canadian Society Soil Science. Lewis Publishers, CRC Press, Boca Raton,
605 FL, pp 43-49.
606 van der Heijden MGA, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as
607 drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters* 11: 296–310.
608 van Diepeningen AD, de Vos OJ, Korthals GW, van Bruggen AHC. (2006). Effects of organic versus
609 conventional management on chemical and biological parameters in agricultural soils. Appl Soil
610 Ecol 31: 120–135 DOI 10.1111/j.1461-0248.2007.01139.x
611 Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass
612 C. *Soil Biology Biochemistry* 19:703–707 DOI 10.1016/0038-0717(87)90052-6
613 Voříšková J, Baldrian P. 2013. Fungal community on decomposing leaf litter undergoes rapid
614 successional changes. *The ISME journal* 7: 477 DOI 10.1038/ismej.2012.116
615 Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho PM, Wu M. 2009. Three genomes from
616 the phylum acidobacteria provide insight into the lifestyles of these microorganisms in soils.
617 *Applied Environmental Microbiology* 75: 2046–2056 DOI 10.1128/AEM.02294-08
618 Weedon JT, Cornwell WK, Cornelissen JH, Zanne AE, Wirth C, Coomes DA. 2009. Global meta-
619 analysis of wood decomposition rates: a role for trait variation among tree species? *Ecology*
620 *Letters* 12: 45–56 DOI 10.1111/j.1461-0248.2008.01259.x
621 Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for
622 phylogenetic study. *Journal of Bacteriology* 173(2): 697–703 DOI http://dx.doi.org/
623 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal rna
624 genes for phylogenetics. In: Innis MA, D.H. Gelfand, Sninsky JJ, White. TJ (eds) PCR protocols:
625 A guide to methods and applications. Academic Press, New York, pp315–322
626 Wutzler T, Zaehle S, Schrumpf M, Ahrens B, Reichstein M. 2017. Adaptation of microbial resource
627 allocation affects modelled long term soil organic matter and nutrient cycling. *Soil Biology*
628 *Biochemistry* 115: 322–336. DOI 10.1016/j.soilbio.2017.08.031
629 Yashiro E, Pintofigueroa E, Buri A, Spangenberg JE, Adatte T, Niculita Hirzel H, Guisan A, van der
630 Meer JR. 2016. Local environmental factors drive divergent grassland soil bacterial communities

631 in the western Swiss Alps. *Applied Environmental Microbiology* 82(21):6303–6316. DOI
632 10.1128/AEM.01170-16
633 Zeng Q, An S, Liu Y. 2017. Soil bacterial community response to vegetation succession after fencing in
634 the grassland of China. *Science of The Total Environment* 609: 2-10 DOI
635 10.1016/j.scitotenv.2017.07.102
636 Zeng Q, Dong Y, An S. 2016. Bacterial community responses to soils along a latitudinal and vegetation
637 gradient on the Loess Plateau, China. *Plos One* 11: e0152894 DOI 10.1371/journal.pone.0152894
638 Zhang Y, Hao X, Alexander TW, Thomas BW, Shi X, Lupwayi NZ. 2018. Long-term and legacy effects
639 of manure application on soil microbial community composition. *Biology and Fertility of Soils* 54,
640 269–283. DOI 10.1007/s00374-017-1257-2
641 Žifčáková L, Větrovský T, Howe A, Baldrian P. 2016. Microbial activity in forest soil reflects the
642 changes in ecosystem properties between summer and winter. *Environmental microbiology* 18:
643 288-301 DOI 10.1111/1462-2920.13026

644 **Table 1** Effects of organic amendments on α-diversity of soil bacteria and fungi at different times (mean ± SE)

Time	Treatment	Bacteria			Fungi		
		OTUs	Chao1	Shannon	OTUs	Chao1	Shannon
7 months	Control	1054.54±48.65a	1800.50±66.09a	7.60±0.16b	170.10±3.65a	304.41±5.36a	4.24±0.04c
	CM	870.17±38.87b	1485.51±54.40b	7.55±0.11b	148.00±3.34b	271.76±5.08b	4.05±0.01d
	CS	1038.67±45.57a	1735.63±63.96a	8.48±0.13a	165.21±3.13a	283.83±4.87ab	5.17±0.03a
	PB	846.69±36.81b	1437.08±53.83b	7.80±0.12b	123.80±2.24c	188.33±2.94c	4.53±0.01b
15 months	Control	1761.08±29.41a	2815.17±36.32b	8.83±0.03b	204.03±3.58c	308.70±4.83b	4.36±0.03b
	CM	1647.99±27.77b	2665.08±35.05c	8.59±0.04c	320.84±5.55a	432.92±5.49a	5.42±0.03a
	CS	1815.81±30.14a	2949.82±37.78a	9.00±0.03a	180.24±3.71d	287.95±5.38c	3.29±0.07c
	PB	1814.24±29.92a	2948.10±37.70a	8.97±0.03a	238.11±6.09b	342.58±6.36b	4.74±0.06b

645 CS = corn straw, CM = cow manure, PB = poplar branch. Means with different letters are significantly different with $p < 0.05$ assessed by Tukey's
646 HSD test.

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657 **Table 2** Pairwise comparison of soil microbial community composition under different organic amendments at different times

Pairwise comparison	7 months	15 months

	Bacterial		Fungi		Bacterial		Fungi	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Control vs CM	3.21	0.030	2.99	0.035	3.81	0.032	4.55	0.034
Control vs PB	3.24	0.031	3.64	0.032	2.04	0.033	3.79	0.024
Control vs CS	2.58	0.030	4.14	0.029	2.60	0.033	4.01	0.023
CM vs PB	7.97	0.024	3.57	0.023	3.17	0.031	6.77	0.025
CM vs CS	7.83	0.038	2.91	0.028	3.57	0.034	3.96	0.032
PB vs CS	2.87	0.037	3.36	0.036	1.42	0.185	1.69	0.062

658 Pairwise comparisons were analyzed by multivariate permutational analysis of variance (PERMANOVA). Values represent the pseudo-F ratio (F)
659 and the level of significance (*p*).

660 **Table 3** Coefficients between microbial α-diversity and soil properties

Time	Microbiome	α-diversity	pH	SOC	TN	TP	TK	AN	AP	AK
7 months	Bacteria	OTUs	0.025	-0.479	**-0.533***	0.136	0.271	0.205	0.230	0.159
		Chao1	-0.019	**-0.511***	**-0.546***	-0.196	-0.334	-0.126	-0.305	-0.288
		Shannon	0.280	0.191	-0.095	-0.072	-0.091	-0.427	-0.237	0.001
	Fungi	OTUs	-0.280	-0.307	-0.316	-0.176	-0.379	-0.030	-0.020	-0.026
		Chao1	**-0.509***	-0.459	-0.266	-0.040	-0.288	0.274	0.168	0.140
		Shannon	0.244	0.186	-0.204	-0.406	-0.253	**-0.528***	-0.393	-0.131
15 month	Bacteria	OTUs	0.270	-0.207	-0.248	-0.389	0.204	-0.399	-0.438	-0.293
		Chao1	0.159	-0.019	-0.177	**-0.540***	0.230	**-0.525***	**-0.555***	-0.317
		Shannon	0.306	-0.239	-0.190	-0.313	0.124	-0.284	-0.353	-0.234
	Fungi	OTUs	-0.281	0.059	0.332	0.448	-0.034	**0.531***	**0.569***	**0.725****
		Chao1	-0.293	0.077	0.371	**0.515***	0.019	**0.577***	**0.611***	**0.708****
		Shannon	-0.026	-0.110	0.153	0.467	-0.255	0.416	0.460	**0.601***

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662 **Table 4** Relationships between soil microbial community composition and soil properties

Soil properties	7 months		15 months	
	Bacteria	Fungi	Bacteria	Fungi
pH	**20.45(0.001)**	**13.05(0.005)**	**12.83(0.008)**	**8.96(0.021)**
SOC	**9.73(0.024)**	**9.20(0.038)**	**10.12(0.012)**	**9.84(0.040)**
TN	**13.98(0.012)**	**11.44(0.012)**	**13.74(0.002)**	**9.53(0.041)**
TP	5.49(0.195)	5.31(0.420)	**14.30(0.003)**	**10.49(0.009)**
TK	3.42(0.554)	4.38(0.648)	6.86(0.138)	4.43(0.538)
AN	6.18(0.152)	6.62(0.209)	5.34(0.221)	5.07(0.374)
AP	6.07(0.139)	5.37(0.413)	3.72(0.572)	4.01(0.712)
AK	6.43(0.130)	**9.34(0.038)**	3.86(0.579)	**15.07(0.011)**
MBC	3.93(0.419)	4.30(0.681)	3.85(0.550)	3.58(0.737)
MBN	3.92(0.496)	4.52(0.670)	3.19(0.733)	4.21(0.625)

663 Values represent the estimation variance component (VC) that explained the distribution of microbial community composition are shown out of
664 the bracket, and the corresponding levels of significance (p) are shown in the bracket. Values at $p < 0.05$ are shown in bold.
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666 **Table 5** Correlations between relative abundance of major bacterial phyla and soil properties

Time	Bacterial phyla	pH	SOC	TN	TP	TK	AN	AP	AK	MBC	MBN
7 months	Proteobacteria	0.442	-0.047	-0.260	-0.336	-0.291	-0.325	**-0.515***	-0.432	0.461	0.334
	Actinobacteria	**-0.544***	0.198	0.409	0.349	0.009	**0.448**	**0.589***	**0.629****	-0.449	-0.187
	Firmicutes	**-0.530***	0.202	0.400	0.432	0.221	**0.540***	**0.673****	0.411	-0.453	-0.407
	Bacteroidetes	0.393	0.326	0.363	0.360	0.457	0.154	0.304	**0.604***	0.036	0.256
	Cyanobacteria	-0.129	-0.325	-0.222	-0.001	0.181	0.032	-0.089	-0.298	-0.198	-0.308
	Acidobacteria	**0.554***	-0.017	-0.321	**-0.616***	-0.239	**-0.800****	**-0.718****	-0.396	**0.680****	**0.571***
	Planctomycetes	0.352	-0.192	-0.452	**-0.540***	-0.352	**-0.566***	**-0.670****	**-0.501***	0.416	0.348
	Chloroflexi	0.354	0.051	-0.282	-0.319	-0.372	**-0.566***	**-0.576***	-0.333	0.459	0.400
	Gemmatimonadetes	-0.483	-0.130	0.135	0.343	0.063	**0.648****	**0.615***	**0.498***	-0.495	**-0.601***
15 months	**Proteobacteria**	-0.406	**0.700****	0.342	**-0.563***	0.218	**-0.466**	-0.469	-0.072	**0.680****	0.000
	Actinobacteria	0.259	**-0.587***	-0.412	0.302	0.011	0.247	0.248	-0.078	**-0.566***	-0.031
	Firmicutes	-0.158	-0.123	0.232	**0.823****	0.217	**0.817****	**0.819****	0.491	-0.400	0.255
	Bacteroidetes	0.238	-0.235	-0.060	0.440	-0.404	0.356	0.393	0.181	-0.304	-0.135
	Cyanobacteria	**0.578***	-0.400	-0.467	0.172	**-0.569***	-0.048	0.022	-0.216	**-0.585***	0.015
	Acidobacteria	-0.031	0.164	-0.119	**-0.814****	0.104	**-0.718****	**-0.753****	-0.419	0.478	-0.003
	Planctomycetes	0.089	0.052	0.163	-0.149	0.137	-0.104	-0.164	0.048	0.303	0.111
	Chloroflexi	-0.329	0.178	0.179	**-0.502***	0.299	-0.243	-0.339	-0.218	0.489	-0.199
	Gemmatimonadetes	**0.652****	**-0.644****	**-0.646****	-0.125	**-0.665****	-0.307	-0.270	-0.393	-0.480	-0.438

667 Bolded bacterial phyla were detected significantly different in relative abundance among treatments. *$p < 0.05$, **$p < 0.01$

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671 **Table 6** Correlations between relative abundance of fungal phyla and soil properties

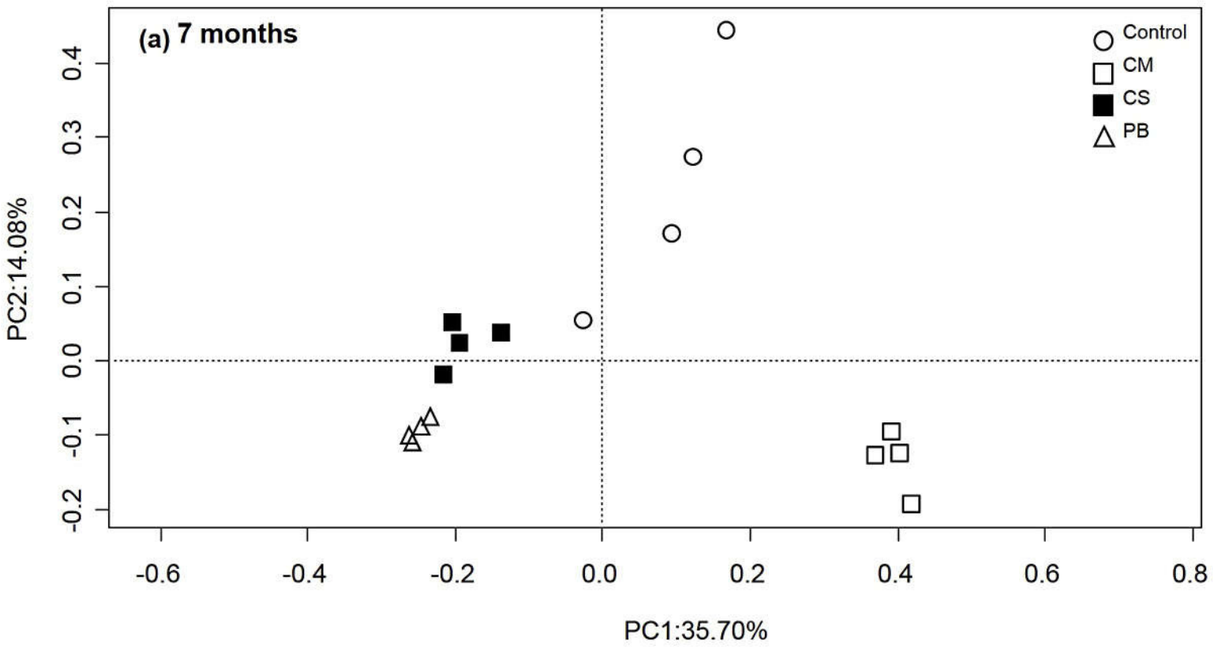
Time	Fungal phyla	pH	SOC	TN	TP	TK	AN	AP	AK	MBC	MBN
7 months	**Ascomycota**	0.425	0.279	-0.205	-0.284	0.025	**-0.515***	-0.483	-0.454	**0.665****	0.382
	Basidiomycota	0.479	0.323	0.020	-0.090	-0.013	**-0.588***	-0.384	0.055	**0.674****	**0.610***
	Zygomycota	0.322	0.214	-0.090	-0.225	-0.052	-0.421	-0.342	-0.383	0.408	0.458
	Glomeromycota	0.257	**-0.527***	-0.411	-0.214	-0.146	-0.320	-0.421	-0.427	-0.107	-0.172
	Chytridiomycota	0.284	-0.028	-0.260	-0.284	0.025	**-0.636****	-0.483	-0.454	0.394	0.494
15 months	Ascomycota	0.096	0.183	-0.137	-0.408	-0.023	-0.463	-0.492	**-0.598***	0.234	0.200
	Basidiomycota	-0.155	0.423	0.153	-0.231	-0.172	-0.223	-0.241	0.062	0.331	-0.167
	Zygomycota	-0.140	0.065	0.133	-0.013	-0.081	0.070	0.083	**0.541***	0.070	-0.217
	Glomeromycota	0.356	**-0.725****	**-0.634****	-0.129	-0.179	-0.238	-0.216	-0.412	**-0.509***	-0.321
	Chytridiomycota	0.175	-0.188	-0.243	-0.332	-0.441	-0.300	-0.280	0.142	-0.026	-0.482

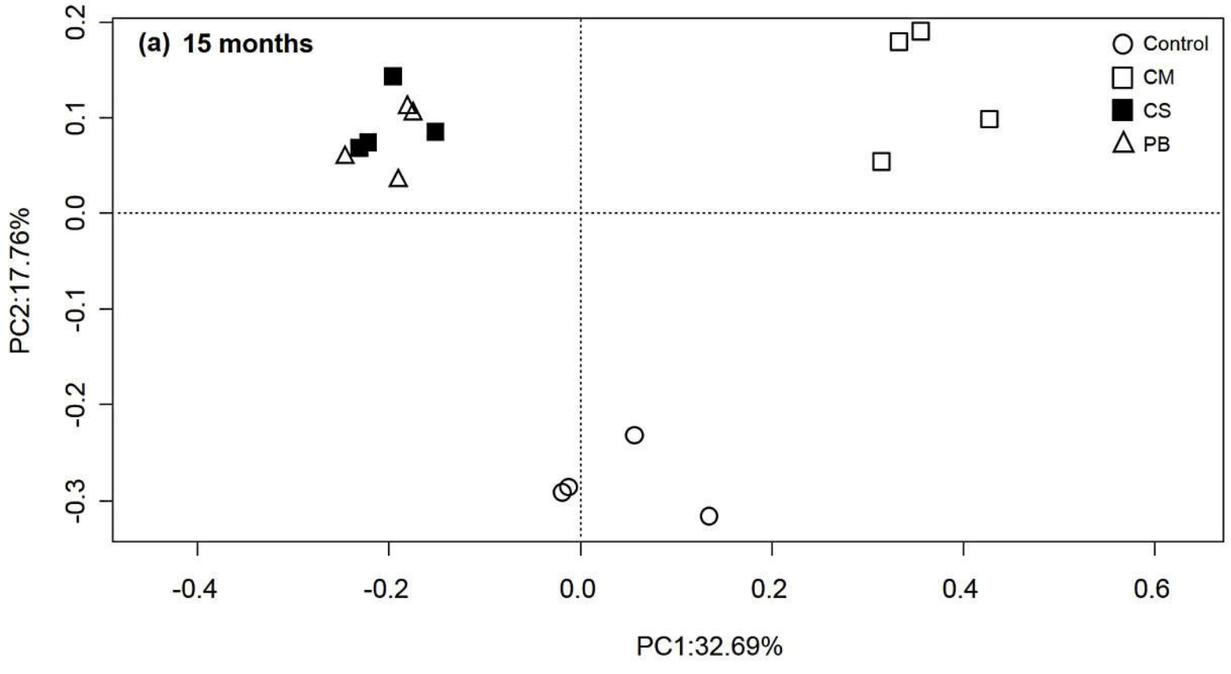
672 Bolded fungal phyla were detected significantly different in relative abundance among treatments. *$p < 0.05$, **$p < 0.01$

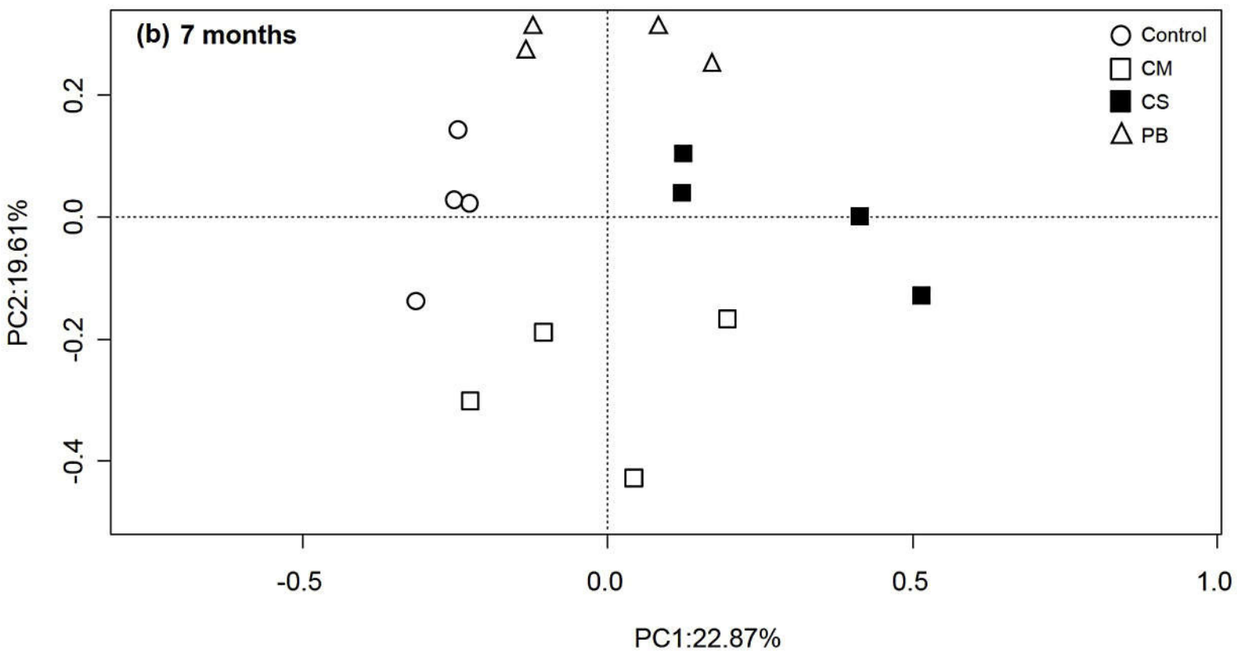
Figure 1(on next page)

Principal coordinate analysis (PCoA) of bacterial (a) and fungi (b) community composition.

CS = corn straw, CM = cow manure, PB = poplar branch.


(a) 7 months
Control
CM
CS
PB
PC2:14.08%
PC1:35.70%
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4


(a) 15 months
Control
CM
CS
PB
PC2:17.76%
PC1:32.69%
-0.4
-0.2
0.0
0.2
0.4
0.6
-0.3
-0.2
-0.1
0.0
0.1
0.2


(b) 7 months
Control
CM
CS
PB
PC2:19.61%
PC1:22.87%
-0.5
0.0
0.5
1.0
-0.4
-0.2
0.0
0.2

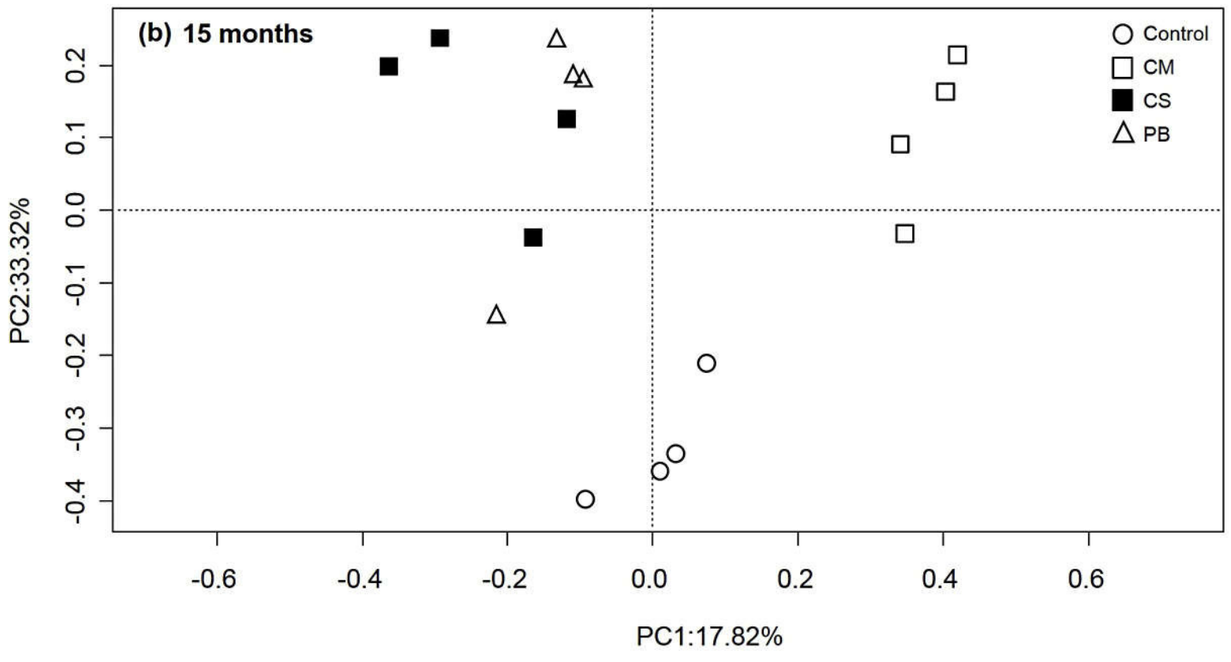

(b) 15 months
Control
CM
CS
PB
PC2:33.32%
PC1:17.82%
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2

Figure 2

Relative abundance of the dominant bacterial phyla (a) and fungal phyla (b).

Different letters indicate significant differences based on Tukey's HSD test ($p < 0.05$). CS = corn straw, CM = cow manure, PB = poplar branch. Only the phyla with significantly different relative abundance among amendments were labelled with letters. Error bars represent standard error (n = 4).