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The restoration age of *Robinia pseudoacacia* plantation impacts soil microbial biomass and microbial community structure in the Loess Plateau

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ABSTRACT

Black locust (*Robinia pseudoacacia*, RP) has been widely grown for soil and water conservation in erosion regions. However, the effect on soil microbial profiles after long-term implementation of vegetation plantation was still unclear. In this study, soil samples from RP plantations of 10-, 15-, 30- and 38-year-old in a typical vegetation-recovering region on the Chinese Loess Plateau were investigated for microbial biomass carbon (MBC), nitrogen (MBN), phosphorous (MBP) and microbial community-profiles (revealed by phospholipid fatty acids, PLFAs).

The results showed SOC, total N, MBC and MBP were significant ($P < 0.05$) higher in the older RP (30- and 38-year-old) than those in other younger plantations (10 and 15-year-old). Total viable microbial biomass (indicated by total PLFAs), bacterial PLFAs and MBP increased significantly ($P < 0.05$) with increasing plantation age. Soil microbial communities were distinctly differed in soils of younger- than in older- plantation plots. Redundancy analysis showed that the sampling site with various RP plantations ($P < 0.05$) was the most important factors in structuring various soil microbial communities. Soil C:N ratio, MBP and available P were also significant factors affecting soil microbial communities.

These findings indicate that RP plantation has the potential to transform soil microbial biomass and microbial communities in the direction of improving soil P content in loess soil.

1. Introduction

On a worldwide scale, anthropogenic activities such as land exploitation, deforestation as well as conversion of forest and grassland to cropland gradually brings about the degradation of soil environment in the last century. To protect soil resources, revegetation has been reported as the most effective way to confront accelerating soil degradation (Jia et al., 2012; Sheoran et al., 2010; Zhang et al., 2011; Zhou et al., 2016). In China, for instance, a government project of "Grain for Green" has been initiated to reduce soil degradation and restore degraded land since 1950s on the highly erodible Loess plateau, and large areas of farmlands have been revegetated to grassland or forest (Deng et al., 2012; Zhang et al., 2010). Black locust (*Robinia pseudoacacia*, RP) is considered to be pioneer tree covering > 70,000 ha on the Loess Plateau and has been widely grown as a desirable species in restoration (Lu et al., 2013). Therefore, a comprehensive assessment of the interaction between RP plantation and soil matrix would be of great importance for evaluating the effect of revegetated trees on soil quality. However, those still remain a major challenge.

Previous studies have shown the positive effects of RP plantation in improving soil physicochemical properties (Liu et al., 2012a; Liu et al., 2012b; Vítková et al., 2015), soil N cycling (Akamatsu et al., 2011; Rice et al., 2004; Tateno et al., 2007) and C: nutrients ratios (Li et al., 2013). Along a chronosequence of various RP plantations, soil top layer nutrients were found to be increased steadily until RP reached its maturity (Kou et al., 2016). However, the long-term effect of RP plantation on soil microbial properties is still unclear.

Soil microorganisms play a crucial role in soil C and N cycling. Sensitive microbial indices such as microbial biomass carbon (MBC) and nitrogen (MBN) have been widely investigated in ecosystems of grassland (Wu et al., 2014), agriculture (Purakayastha et al., 2009) and forest (Chen et al., 2015; Foote et al., 2015). In the domain of ecological engineering, MBC and MBN are normally used for assessing the relationships between revegetated species and soil, and for monitoring vegetation-associated variations in soil properties (An et al., 2009; Bolat et al., 2016). For instance, during the process of forest secondary succession, the contents of soil MBC and MBN increased constantly until 17 years and the variations were positively related with changes in soil nutrients such as SOC and total N (Jia et al., 2005).

Microbial biomass phosphorous (MBP), however, had generally

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been ignored by many studies. As pointed out by Buchkowski et al. (2015), the ratio of microbial C:N:P overrides biomass as a regulator of soil nutrients cycling. In consideration of the interaction between soil P and N (e.g. elevated N may aggravate P deficiency in forest; D. Liu et al., 2012; Liu et al., 2012b) as well as closer correlation between soil P and MBP (Tang et al., 2014), MBP therefore should be paid more attention and included as an essential soil quality indicator. Additionally, a comprehensive investigation of MBN, MBN and MBP would help understand biogeochemical features of soil properties along a revegetation chronosequence.

In addition to soil microbial biomass, changes in soil microbial communities are also vital during vegetation recovering (Xiao et al., 2016a, 2016b; Zhang et al., 2016) since variation in microbial population is regarded as an early indicator for soil quality (Schloter et al., 2003; Torsvik and Øvreås, 2002). As for research methods, the quantitative analysis of soil microbial communities via phospholipid fatty acid (PLFA) has been recommended as a desirable approach in the field of soil microbial ecology because i) it is independent of cultivation (Zelles, 1999); ii) relative amounts of certain microbial groups can be indicated by relevant biomarker PLFAs (Kourtev et al., 2002; Zelles et al., 1994); iii) microbial community composition and physiological features can be quantitatively compared between treatments (Liu et al., 2014a). Based on these advantages, the PLFA-based approach has been widely applied to detect the response of soil microbial community-level profiles to vegetation restoration (Zhang et al., 2006; Huang et al., 2011; Wang et al., 2012, Zhang et al., 2006) Soil microbial community structure of forest plantations of different stand ages has been reported and the results are varied between tree species (Wu et al., 2015; Yang et al., 2014) and forest ages (Banning et al., 2011; Wu et al., 2013). For instance, at the early reforestation chronosequence (1 to 5 years) of *Eucalyptus* plantation, soil microbial community changed strongly and characterized by a relative increase of bacterial groups with increasing forest age (Wu et al., 2013). However, soil microbial communities tend to become similar as forest age increased to 18 years (Banning et al., 2011). *Robinia pseudoacacia* (RP), contrastingly, after 30 years of plantation, the soil microbial community composition and structure still differed significantly (Xiao et al., 2016a). It confirmed that the growth of RP had a lasting influence on soil microbial community. However, whether soil microbial communities have distinguished features among the various years of RP plantation (especially after RP reached maturity) is still not elusive. It has been shown that microbial communities are affected by a set of environmental variable/factors such as soil nutrients, pH and C:N ratio (Fierer and Jackson, 2006; Lozupone and Knight, 2005; Rousk et al., 2010a). Among numerous edaphic variables, soil nutrients and C/N ratio contributed significantly to the variation of soil microbial community structure in different stand ages of forest plantations as reported by Wu et al. (2015). Recently, the persistent and strong effect of soil pH and C:N ratio on soil microbial community structure, was even found in 78-year-old tree plantations (Zhou et al., 2017). These results highlighted the importance of soil pH and C/N ratio to microbial communities in afforested soils.

In view of the above, we hypothesized that i) soil nutrients and microbial biomass are higher in older RP plantation plots; ii) microbial communities are different in soils of various PR plantations; iii) soil pH and C:N ratio contribute mostly for variations in microbial communities.

Based on these hypotheses, we chose a typical watershed region-Zhifanggou on the Chinese Loess Plateau. Within the investigated region, the widely planted RP with various years were chosen as representative species to evaluate the effect of vegetation restoration to soil properties.

2. Materials and methods

2.1. Site description

The study area is located in the Zhifanggou watershed, Ansai Research Station of Soil and Water Conservation of the Chinese Academy of Science (CAS) in the northern Shaanxi Province of China (108°5′–109°26′E, 36°30′–37°39′N, altitude: 1010–1400 m a.s.l.). The mean annual air temperature and precipitation was 8.8 °C and 513 mm, respectively. According to the soil classification system of the Food and Agriculture Organization of the United Nations (FAO), the soil is typical of the Loessial soil group (IUSS Working group WRB, 2014) with a soil texture of 62% sand, 25% silt and 13% clay. The study area transited between the warm, temperate deciduous broadleaved forest and the dry grassland belt (Xu et al., 2009). The main land-use types are artificial grassland (*Medicago sativa*), artificial forestland (*Caragana korshinskii Kom*, *Armeniaca sibirica*, *Amygdalus davidiana*, etc.), abandoned land (*Stipa bungeana Trin*, *Thymus mongolicus Ronn* and *Artemisia giraldii Pamp*.) and farmland (*Triticum aestivum*, *Fagopyrum esculentum Moench* and *Zea mays*).

2.2. Experimental design and soil sampling

The government project of ‘Grain for Green’ has been lasted for decades, we therefore selected vegetation-recovering areas with a chronosequence. Except for the years of *Robinnia pseudoacacia* (RP) plantations, the selected four areas were under the same conditions including their topography (hillside field), climate (temperate, semiarid climate), parent soil material (wind-deposited loessial material). In July 2011, we selected sampling areas in the areas of the 10-, 15-, 30-, and 38-year-old *Robinnia pseudoacacia* (RP) plantations (RP10, RP15, RP30 and RP 38) since they are representative in local region with relative larger planting area compared with other RP plantations areas. To avoid the problem of ecological pseudoreplication, we extended the sampling area to 300 m × 300 m. Soil samples were collected from three different slope aspects to minimize subplot effects. At the each slope aspect (within the size of 100 m × 100 m), we used randomized-sampling design to decrease sampling error: 5 randomized soil samples (from the top 0–10 cm with a stainless-steel cylinder (5 cm-inner diam.) were pooled to form a composite sample. Therefore, the three composite samples from different slopes were considered as ecological independent samples. Fresh soil samples were then sealed in plastic bags and transported in iceboxes to the laboratory where the soil samples were homogenized with 2 mm sized mesh sieves to remove discernible roots, stones and macro-fauna. Two aliquots of each sample were stored at 4 °C and −80 °C for microbial biomass and phospholipid fatty acid (PLFA) analysis, respectively. The rest were air dried for soil physicochemical analyses. The geographical and undergrowth characteristics of the sites are shown in Table 1.

2.3. Soil analysis

2.3.1. Soil physicochemical properties

Bulk density (BD) was determined using cutting ring as recommended by the soil agricultural and chemical analysis (Nu, 1999). Soil pH was measured with soil suspension extracted at a 1:2.5 (w/w) soil:water ratio using a Delta 320 pH meter (Mettler-Toledo Instruments (Shanghai, China, Co., Ltd). Soil organic carbon (SOC) was analyzed via wet oxidation using dichromate in an acid medium followed by the $FeSO_4$ titration method (Bao, 2007). Total N and available P (AP) were analyzed by Kjeldahl digestion and distillation azotometry and by $NaHCO_3$ extraction and colorimetry, respectively (Nu, 1999).

2.3.2. Microbial biomass C, N and P

The contents of microbial biomass C (MBC), N (MBN) and P (MBP) were determined using the chloroform fumigation–K_2SO_4 extraction

Table 1
Geographical features and vegetation of the different years *Robinnia pseudoacacia* (RP) plantations.
Site information was partly obtained from Liu et al. (2012a) and Liu et al. (2012b).

Sites	Years of plantations	Altitude (m)	Tree density (ind m^{-2})	Canopy density (%)	Litter layer depth (cm)	Latitude/longitude	Undergrowth vegetations
RP10	10	1134	0.21	45	3.0	36°45.553′/ 109°15.704′	*Artemisia gmelinii* *Lespedeza bicolor Turcz.*
RP15	15	1313	0.18	60	4.2	36°44.317′ 109°14.370′	*Artemisia capillaries* *Heteropappus hispidus (Thunb.) Less.*
RP30	30	1185	0.13	74	5.0	36°45.931′/ 109°15.914′	*Artemisia gmelinii* *Lespedeza bicolor Turcz.*
RP38	38	1240	0.08	70	4.9	36°44.251′/ 109°15.831′	*Artemisia gmelinii* *Lespedeza bicolor Turcz.*

method (Brookes et al., 1982; Ocio and Brookes, 1990; Ross, 1990). The content of K_2SO_4-extracted C was determined using a total organic carbon analyzer (Phoenix 8000). The difference in C content between the fumigated and non-fumigated samples was corrected using a K_{EC} factor of 0.45 to estimate MBC (Wu and Brookes, 2005). The MBN was measured as the difference in total N between fumigated and non-fumigated samples using a converting factor $K_{EN} = 0.54$ (Wu and Brookes, 2005). The MBP was determined as the difference in total P of $NaHCO_3$ extracts between fumigated and non-fumigated soils. The P content of the $NaHCO_3$ extracts was measured as previously described (Brookes et al., 1982).

2.3.3. Phospholipid fatty acid (PLFA) analysis

Soil phospholipid fatty acids were extracted and analyzed in triplicate using a modified method by Frostegard et al. (1993). Briefly, 2 g dry weight of soil was extracted with a buffer of chloroform/methanol/citrate mixture at a ratio of 1:2:0.8. Then, neutral lipids, glycolipids, and phospholipids were separated into distinct layers on a silicic acid column. After that, the phospholipids were subjected to a mild alkaline methanolysis. Capillary gas chromatography analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph with a flame ionization detector. The PLFA methyl esters were analyzed using a 50 × 0.32 mm fused silica capillary column coated with a poly-ethylene glycol stationary phase (CPWax 52CB, 0.2 mm film thickness; Chrompack). The initial temperature program was 70 °C for 2 min and increased to 280 °C at 3 °C min^{-1}. An internal standard of methyl nonadecanoate fatty acid (19:0) was added to quantify peak areas. Individual PLFAs were identified using fatty acid methyl ester standard compounds (Bacterial Acid Methyl Esters Mix; Supelco, Bellefonte, PA). To characterize microbial community structure, the individual fatty acids have been used as biomarkers for various microbial groups. Specifically, biomarkers 12:0, 14:0, i15:0, a15:0, 15:0, 16:0, i16:0, 17:0, i17:0, 16:1ω7c, cy17:0, 18:1ω7, cy19:0 were chosen to represent bacteria biomass (Frostegård and Bååth, 1996). Biomarkers 18:1ω9c, 18:1ω9t and 18:2ω 6,9 were used as indicators for fungal biomass (Olsson, 1999). Biomarker 10Me17:0, 10Me18:0, 10Me19:0 were indicators for actinobacteria (Kroppenstedt, 1985). The ratio fungal/bacterial PLFA was calculated by dividing the amount of total bacterial PLFA by the sum of fungal PLFA (Frostegård and Bååth, 1996). The iso- and anteiso-branched saturated fatty acids (i15:0, a15:0, i16:0, i17:0) represent Gram-positive bacteria (Zelles et al., 1994), whereas the monounsaturated 16:1ω7c, cy17:0, 18:1ω7, cy19:0 represent Gram-negative bacteria (Kourtev et al., 2002). Total PLFA concentration was calculated by summing all bacterial-, fungal- and actinobacteria-PLFA and used as an index for total microbial biomass (expressed as n mol·g^{-1} dry soil).

2.4. Statistical analysis

Whole data set was tested for normal distribution using the Shapiro-Wilk test and the homogeneity of variances using Levene's test. Analysis of variance was performed using SPSS 17.0 software. We used Tukey's multiple comparison test (at $P < 0.05$) to compare the significant differences among soil physiochemical and microbial parameters of different years of *Robinnia pseudoacacia* (RP) plantations. A principal components analysis (PCA) was conducted to condense the complexity of the 28 phospholipids fatty acids (PLFA) biomarkers and to identify a subset of PLFA biomarkers that mainly drive the separation of microbial communities. Redundancy analysis (RDA) models were used to determine the relative contributions of soil physiochemical and microbial properties in explaining the variance of soil microbial community. In order to avoid over fitting of the RDA models, the explanatory predictor variables in each RDA model were selected by a forward selection procedure. Variables were incorporated stepwise into the model according to their increasing effect on the variance and their significance tested by Monte Carlo permutation tests (Legendre and Legendre, 2012). The RDA analyses were performed using the CANOCO 5.0 software.

3. Results

3.1. Soil physicochemical characteristics

Compared to the younger *Robinnia pseudoacacia* (RP) plantation plots (10-y and 15-y), the content of soil total N, organic C, MBC and MBP in older-aged RP (30-y and 38-y) were significantly increased ($P < 0.05$; Table 2). In detail, the content of soil AP and MBP was the highest in the 38-y-old RP while 30-y-old RP soil had the highest value of OC and MBC ($P < 0.05$; Table 2). Soil C:N ratio was significantly decreased ($P < 0.05$) along RP age while soil bulk density (BD) showed no obvious change ($P > 0.05$; Table 2).

3.2. Soil phospholipid fatty acid (PLFA) profiles

A total of 28 phospholipid fatty acids (PLFAs) were identified: 19 were recognized of microbial origin and 9 could not be assigned to specific groups were not included in further analyses. The relative abundance of all soil PLFAs significantly varied among the years of RP plantations, except for 17:0 (Fig. 1). In addition, relative PLFAs abundance of older-aged RP (30-y and 38-y) was generally higher than those of the younger RP plantation (10-y and 15-y). Therefore, we further compared the PLFAs assigned microbial groups and microbial physiological indicators (Table 3). Specifically, fungal PLFAs and actinomycetes PLFAs were similar among RP plantations of older ages (30- and 38-y). In contrast, bacterial PLFAs and total PLFAs significantly increased ($P < 0.05$) with increasing year of RP plantation (Table 3). The ratio of fungal PLFAs to bacterial PLFAs (F/B ratio) was significantly ($P < 0.05$) decreased with years of plantation showing a pattern of RP 38 < RP 30, RP 15 < RP 10 (Table 3).

In order to further analyze the similarity of microbial communities, the principal component analysis (PCA) of all PLFA biomarkers were conducted. Results showed that the microbial communities from the four RP plantation plots were clearly separated (Fig. 2a). The first and second principal components (PC1 and PC2) explained 76% and 17% of

Table 2
Effect of *Robinnia pseudoacacia* (RP) plantations on selected soil characteristics.

	Years of RP plantations			
	10-y	15-y	30-y	38-y
pH	8.5 ± 0.3a	8.4 ± 0.2b	8.5 ± 0.3a	7.7 ± 0.2c
BD (g cm^{-3})	1.01 ± 0.03a	1.10 ± 0.07a	0.99 ± 0.04a	1.00 ± 0.03a
SOC (g kg^{-1})	4.9 ± 0.2b	3.7 ± 0.2c	7.5 ± 0.2a	5.3 ± 0.6b
TN (g kg^{-1})	0.38 ± 0.04c	0.41 ± 0.03c	0.85 ± 0.03a	0.65 ± 0.06b
AP (mg kg^{-1})	5.5 ± 0.2c	8.9 ± 0.3b	5.8 ± 0.4c	14.5 ± 0.4a
C:N ratio	12 ± 2.1a	9 ± 0.8b	8.5 ± 1.1c	8.1 ± 0.3d
MBC (mg kg^{-1})	276 ± 8c	191 ± 29d	423 ± 46a	307 ± 16b
MBN (mg kg^{-1})	127 ± 7a	67 ± 7b	122 ± 6a	121 ± 18a
MBP (mg kg^{-1})	5.8 ± 0.4d	7.7 ± 0.9c	11.4 ± 0.9b	27 ± 0.8a

Notes: within rows, means and standard deviation followed by the same letter are not significantly different ($P < 0.05$; Tukey's multiple comparison test). n = 3 in all cases; BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus. Date was compiled from Liu et al. (2012a) and Liu et al. (2012b).

total variation in communities (Fig. 2a). The overall loadings for PC1 indicated that major bacterial biomarkers (14:0, 15:0, 16:0, i16:0, 18:1ω7 and cy19:0) and actinobacteria biomarkers (10Me18:0, 10Me19:0) mainly drove the isolation of microbial groups (Fig. 2b). The bacterial biomarkers (a15:0, 17:0) and the fungal biomarker (18:2ω 6,9c) were primary contributors to PC2 (Fig. 2b).

3.3. Relationship between soil properties and microbial communities

Redundancy analysis (RDA) revealed that the x-axis (RDA1, 55.8%) and y-axis (RDA2, 24.5%) together explained 80% of the variation in microbial communities (Fig.3). RDA 1 accounted for the major variability and as the vector "years" was along RDA 1 axis this indicates the trend over the sampling site of various RP plantations (years). When comparing the separation of soil parameters on x-axis, the influence of MBP and 'years' was pronounced and positively correlated to the microbial communities composition. Another important factor explaining alterations of microbial community with time was available P (Fig. 3). As shown in Fig. 3, pH and C/N ratio showed a negative relationship with microbial communities (indicated by opposite directions; Fig. 3) Results from correlation analysis (Table 4) show that bacterial communities (Gram-positive and Gram-negative bacteria) were significantly positively correlated ($P < 0.05$) with MBP, available P and total N. These results were consistent with those from RDA, which indicated the importance of soil P for soil microbial community and abundance. The percentage of microbial community variation explained by soil properties (Table 5) showed that the sampling site (years of RP plantations) had the highest explaining percentage of ~60% ($P < 0.05$), followed by a lower percentage of ~40% for MBP and C:N ratio ($P < 0.05$), and ~35% for Total N and available P ($P < 0.05$).

4. Discussion

4.1. Effect of RP plantation on selected soil properties

Soil chemical and microbial properties of *Robinnia pseudoacacia* (RP) plantation of various years were significantly different (Table 2). These variations were related to the year of RP plantation. In line to our first hypothesis, we found older RP plantation (30- and 38-y-old RP) had higher contents of soil nutrients and of microbial biomass compared to those of the younger RP plantation (10- and 15-y-old RP). Relative higher values of the soil indicators at the older RP plantation could be attributed to the stronger accumulation of aboveground leaf litter as indicated by thicker litter layers in those of older RP soils (Table 1). Further, mineralization of leaf litter by decomposer communities generates C fluxes that accelerate the release of organic

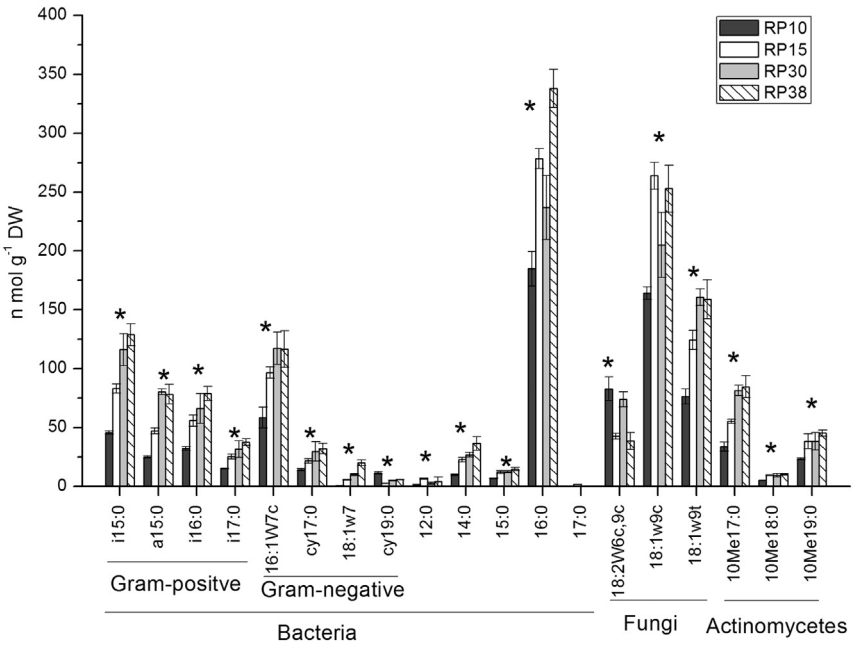

Fig. 1. Relative abundance of fatty acids from microbial origin (bacterial, fungal and Actinometeys) as affected by years of *Robinnia pseudoacacia* (RP) plantations. RP10, 15, 30 and 38 refer to years of *Robinnia pseudoacacia* (RP) plantations. Bars refer to mean values and error bar represent SD, n = 3. Asterisks show significant differences between RP plots ($P < 0.05$; Tukey's multiple comparison test).

Table 3
Relative abundance of phospholipid fatty acids assigned microbial groups and changes of community physiological indicators.

	Total PLFAs	Bacterial PLFAs	Fungal PLFAs	Actinomycetes PLFA	F/B ratio	S/M ratio	cy/pre ratio	G+/G− ratio
RP10	850 ± 40d	411 ± 19d	329 ± 16b	63 ± 3b	0.8 ± 0.04c	0.8 ± 0.03a	0.44 ± 0.02a	9.8 ± 0.5d
RP15	1287 ± 67c	670 ± 35c	438 ± 23a	114 ± 12a	0.65 ± 0.03a	0.78 ± 0.04a	0.24 ± 0.01c	20 ± 1.4b
RP30	1393 ± 77b	746 ± 42b	447 ± 24a	131 ± 7a	0.61 ± 0.06a	0.69 ± 0.04b	0.27 ± 0.02b	26 ± 1a
RP38	1606 ± 87a	906 ± 49a	458 ± 25a	142 ± 8a	0.51 ± 0.04b	0.87 ± 0.04a	0.28 ± 0.01b	13 ± 0.6d

Notes: F/B, ratio of fungal to bacterial PLFAs; S/M, ratio of normal saturated PLFAs to monounsaturated PLFAs; cy/pre, ratio of cyclopropyl/precursors (cyc/prec) as calculated by (cy17:0 + cy19:0) / (16:1ω7 + 18:1ω7); G+/G−, ratio of Gram-positive bacterial to Gram-negative bacterial PLFAs.

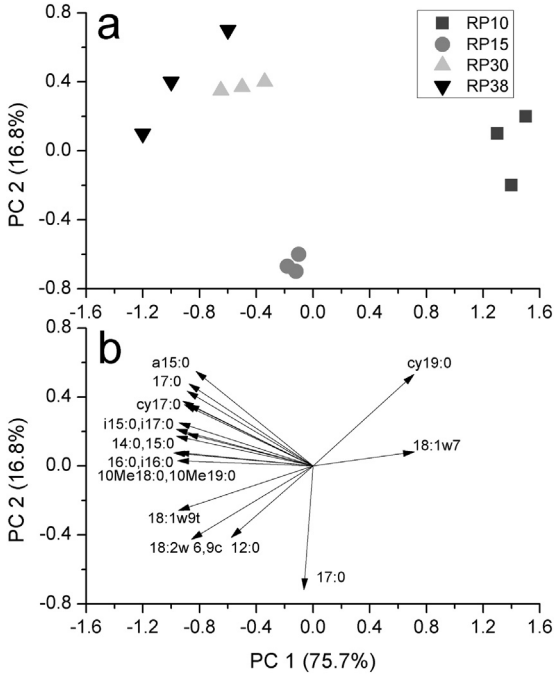

Fig. 2. Ordination plots of the soil microbial community composition (indicated by phospholipids fatty acids - FA) as determined by principal component analysis (PCA). a, plots of different *Robinnia pseudoacacia* (RP) plantations (10-, 15-, 30- and 38 years). b, position of the FA in the axes referring to the loading values of the PCA.

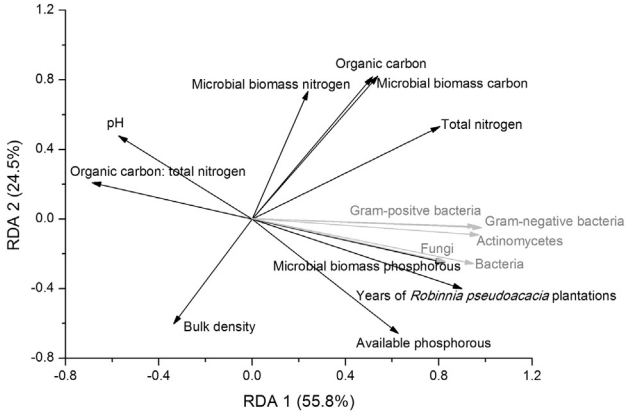

Fig. 3. Redundancy analysis (RDA) of the correlations between soil microbial groups and soil properties. The length of arrows refers to the strength relative to other variables. The intersection angle between vectors represents the affinity degree of their relationships (smaller angle with closer correlation). For all vectors, values on the x and y axes represent the percentage change explained by RDA 1 and RDA 2 respectively.

material into soils and thus return nutrients back into soils (Schlesinger and Andrews, 2000). However, the contents of SOC and total N were not constantly increased over time but decreased in the 38-year-old RP soil suggesting at the later stage of growth (relatively mature RP

Table 4
Correlation coefficients among soil PLFAs, physicochemical properties and microbial biomass.

	Total PLFAs	Bacterial PLFAs	Gram+ bacteria	Gram− bacteria	Fungi	Actinomyetes
Organic C	0.31	0.31	0.50	0.35	0.30	0.47
Total N	0.61	0.61	**0.78***	0.50	0.55	**0.74***
Available P	0.69	**0.73***	0.60	**0.73***	0.51	0.59
Bulk density	−0.18	−0.20	−0.32	−0.49	−0.08	−0.28
C:N ratio	−0.54	−0.51	−0.53	−0.47	−0.54	−0.55
pH	−0.69	−0.66	−0.63	**−0.83***	−0.48	−0.61
MBC	0.22	0.23	0.43	0.36	0.15	0.39
MBN	0.02	0.05	0.15	0.57	−0.09	0.11
MBP	**0.74***	**0.79***	**0.75***	**0.92***	0.51	**0.72***

Note: MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorous. Bold numbers indicate significant changed coefficients.
* Indicates significant correlation at P = 0.05.

Table 5
Constrained factors from soil properties as simple term effects to microbial communities.

Name	Explains %	Pseudo-F	p
Years of RP plantation (year)	63.8	17.7	0.002
C:N ratio	42.1	7.3	0.01
Microbial biomass phosphorous (MBP)	40.5	6.8	0.01
Total nitrogen (TN)	35.3	5.5	0.024
Available phosphorous (AP)	33.8	5.1	0.022
pH	24	3.2	0.074
Soil organic carbon (SOC)	21	2.9	0.156
Microbial biomass carbon (MBC)	13	1.5	0.232
Microbial biomass nitrogen (MBN)	11.7	1.3	0.278
Bulk density (BD)	6	0.6	0.558

stands), the RP physiological function and growth rate may decline due to soil water deficiency (Jia et al., 2017). The results were similar to the study conducted on the same region, where SOC and TN were higher in RP growth of 30-year than those of 40-year (Wang et al., 2012). As exotic nitrogen-fixing trees, the soil C:N ratio decreased evidently with increasing RP plantation (Table 2), indicating the accumulation of soil nitrogen is stronger than SOC due to the powerful root system as well as inhabiting nitrogen-fixing root nodules in soils of old RP plantations (Malcolm et al., 2008; Rice et al., 2004) In contrast, with the consideration of low productivity in earlier RP plantation, contributions of aboveground leaf litter decomposition to SOC increase might be stronger than soil N fixation via belowground root system, leading to higher soil C:N ratios. In addition, the gradually narrowed soil C:N ratio accompanied by RP growth is an indirect reflection of the increase in nutrient availability for soil microorganisms and the accelerating of nutrient fixation/convergence into microbial biomass(Liu et al., 2016; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015).

However, as for individual elements, C and N contents in microbial biomass (MBC and MBN) did not show an even increase with time (Table 2) except for microbial biomass P (MBP). Furthermore, we observed that both MBP and soil available P content were significantly increased with time (Table 2). Similarly, an increase in MBP and phosphorus availability has also been reported by Tang et al. (2014). It

should be noted that loess soils are suffered from P deficiency (~0.5 g kg^{-1}, Liu et al., 2013) and under P-limiting situations soil microbes tend to immobilize P into their biomass as a soil P reservoir (Kulaev and Kulakovskaya, 2000). Meanwhile, in order to maintain self-metabolism during RP growth, belowground soil microbes tend to simulate organic P mineralization (Richardson and Simpson, 2011; Spohn and Kuzyakov, 2013) and accelerate the secretion of alkaline phosphatase (Wang et al., 2012), which explains strong accumulations of soil AP and MBP in the old RP plantation soils (Table 2).

4.2. Soil microbial community level shifts as influenced by RP plantations

Robinnia pseudoacacia (RP) currently is common in Europe, temperate Asia, northern Africa Australia and temperate South America (Pysek, 2004). Climate change is likely to enlarge the range of RP habitats on various soil textures (Kleinbauer et al., 2010). In the subtropical zone in China, RP occurs on Cambisols (Lv et al., 2013). In the temperate, semi-arid climate of our sites, RP stands dominate on Loessial soil. Under this soil texture, we found a significant increase of soil total viable microbial biomass (total PLFA) with increasing RP plantation (Table 3). In a humid climate in southern Appalachians and in a Mediterranean climate in Central Spain, RP stands dominate mainly on Luvisols (Castro-Díez et al., 2009; Montagnini et al., 1991). However, no comparable soil microbial parameters are measured in these studies instead the authors mainly focused on the effect of RP growth on soil N cycling. In view of soil texture difference in aforementioned climatic regions and the microbial mediation/involvement in soil N cycling, soil microbial profiles (as sensitive soil indicator) is likely to change. Previous studies have shown that the changes of soil microbial profiles are affected by soil texture (Bach et al., 2010; Chodak and Niklińska, 2010; Müller and Höper, 2004). In this study, the soil is silt loam (USDA classification) with the higher fraction of silt (~25%). The significant increase of the total PLFA across the chronosequence of RP plantation (Table 3) may be related to abundance of moderate-sized fraction (2–50 mm) of silt that provides suitable micro-environment for microbial growth (Liu et al., 2014a; Liu et al., 2014b). In a similar soil texture of silty clay loam, soil microbial PLFA concentrations increased steadily during vegetation restoration chronosequence; whereas, in a different soil texture of loamy fine sand, no microbial groups varied positively with chronosequence (Bach et al., 2010). Soil nutrients in a given soil texture seem to be a factor influences soil microbial profiles. Chodak and Niklińska (2010) found that the loamy sands had higher contents of SOC and TN exhibited higher microbial activities than the sandy soil (low nutrients). In the present study, the total PLFAs significantly increased with years of RP plantations (Table 3) and this can be attributed to the obvious increase of soil nutrients during RP plantations (Table 2). As previously reported by Ding et al. (1992) and Mendham (2002), increased soil fertility (i.e. SOC, TN and available P) accelerates the growth of soil microbes in forest soils. As for the concept of microbial biomass, the results were inconsistent between the chloroform fumigation extraction (FE) and the PLFAs approach. Since the FE method extracts the elements (i.e., C, N and P) from lysed cells and its obtained microbial biomass is the living part of soil organic matter, while PLFA is a constituent of the living cells (cell membrane; Kaur et al., 2005). Therefore, we recommend a closer investigation of the both indicators in order to gain a deep understanding of how viable (Total PLFAs) and FE-based microbial biomass response to experimental treatments.

In consistent with our second hypothesis, the relative abundance of PLFAs significantly varied with the restoration year of the RP (Fig. 1). This indicates that soil microbial communities are sensitive to RP plantation, and this was supported by Wang et al. (2012) who reported RP have long-term benefits on the improvement of soil microbial properties such as biomass and enzyme activities.

Specifically, soil microbial composition (as measured by phospholipid fatty acid profiles-PLFA) had minor difference between the old-aged RP soils (Fig. 2), suggesting that changes in microbial composition were not evident after RP reached a mature stage of growth (30- and 38-y). However, the microbial community of mature RP soils distinctively separated from that of the two young-aged RP soil (10 y and 15 y) (Fig. 2). The main attributes for this separation were a set of representative bacterial biomarkers. This could be the reason of the significant increased abundance of bacterial communities (Table 3) drives the divergence of the microbial composition along time. In an ecological restoration study of a similar N-fixing forest species - Eucalyptus, a significant variation of bacterial PLFAs and vegetation plantation age was also identified (Cao et al., 2010).

For individual microbial consortia, the positive response of soil bacteria was confirmed by the increased bacterial PLFAs abundance with RP plantation (Fig. 3). It is commonly know that soil bacteria have higher nutrients requirements (Güsewell and Gessner, 2009; Keiblinger et al., 2010) and high quality substrates (low C/N ratio) favor bacterial communities (Bossuyt et al., 2001). Therefore, it was not surprising to find the increased soil bacterial PLFAs in the older RP plantation soils with lower soil C/N ratios (Table 2).

Using PCR-DGGE and PLFA, Xiao et al. (2016a, 2016b) observed a significant increase of soil bacterial PLFAs of four restoration forest species till 30 years. Additionally, in a study using high-throughput sequencing of the 16S rRNA gene, a various bacterial species richness and diversity were also reported even in a natural way of farmland undergoing succession for 30 years (Zhang et al., 2016). Further, the significant relationship between bacteria and soil nutrients (i.e. total nitrogen and available phosphorous; Table 4) provide evidence for its essential role of bacteria in soil nutrient supply during vegetation restoration. This was previously confirmed by studies conducted on the Loess Plateau (An et al., 2012; Zhang et al., 2016). In contrast, the relative abundance of fungal PLFAs and actinomycetes PLFAs were stable with non-significant increase after the RP plantation of 15-y (Table 3). This dissimilarity among the microbial groups may be related to microbial physiological characteristic/growth patterns: fungi and actinomycetes were not obviously restricted by nutrient availability (Vos et al., 2013) therefore showed dull responses to vegetation restoration.

In order to understand the physiological alteration of the whole microbial community along RP plantation, we further investigated the indicators that reflect the change of microbial community physiological changes: i) nutrient stress was lowest in 30-y-old PR as indicated by the significant lowest S/M (the ratio of normal saturated- to mono-unsaturated-PLFAs; Bossio et al., 1998) in RP30 soil and this was confirmed by the highest soil C and N contents in the RP30 soil (Table 2); ii) environmental stress (reflected by the ratio of cyclopropyl PLFAs (cy17:0 and cy19:0) and its precursor PLFAs (16:1ω7 and 18:1ω7; Kieft et al., 1997) tend to decrease with increasing RP plantation. It was reported that increased ratio of Gram-positive bacteria to Gram-negative bacteria (G+/G−) was related with an enlarged C input and a shift from a chemoautotrophic- (G+) to heterotrophic-dominated (G− bacteria) communities (Rinnan et al., 2009). We found the highest G+/G− ratio (Table 3) in RP 30 soil and this was consistent with highest aboveground litter accumulation (Table 3). Taking together, our results showed the changes of microbial physiological index were consistent with the variations in selected soil parameters and the aboveground litter layer. In future, deeper investigations are recommended to reveal detailed interactions among leaf litter, soil matrix and soil microbes during forest plantation.

4.3. Interrelationship between microbial biomass, microbial communities and soil properties

RDA analysis helps us to explore the variation in soil microbial community along both sites and measured edaphic variables. It was not surprising that the year of RP planation and soil C:N ratio were important environmental factors in the PLFA ordination (Fig.3). The

patterns of relative abundance of PLFAs and soil C/N ratio along the year of RP plantation verified the result. (Figs. 1 and 2; Table 2). Soil pH has been documented as a major factor shaping soil microbial communities (Rousk et al., 2010a, 2010b). In this study, contrary to the third hypothesis, we did not observe a prevailing influence of soil pH on microbial communities (Table 5). It has been shown that fungal communities are less strongly correlated with soil pH (Lauber et al., 2008). Rousk et al. (2010b) proposed that the apparent direct influence of pH on bacterial community composition is probably due to the narrow pH ranges (4–8.3) for optimal growth of bacteria. But, the strong alkaline condition (> 8.3) in our loess soils beyond this favoring pH range and therefore may weaken the influence of pH on microbial community. This is in agreement with pervious study from the same loess soils (Xiao et al., 2016a, 2016b). However, as compared with other soil types such as black soils and arctic soils, soil pH was still reported as a predominating factor in shaping soil microbial communities (Chu et al., 2010; Liu et al., 2014a; Liu et al., 2014b).

Previous studies showed that P is dynamically exchanged between soil solution and microbial biomass (Achat et al., 2012; Oehl et al., 2001). Our finding showed the available P and MBP significantly affected microbial communities as indicated by the significant and higher explaining percentage (30–40%) (Table 5). MBP constitutes a significant component of total soil P dynamic and cycling (Xu et al., 2013) and replenishes soil solution P and contributes to P nutrition of forest trees (Achat et al., 2012). The importance of MBP to overall viable microbial biomass was convinced by a closer relationship between soil MBP and soil total PLFAs (Table 4). Besides, the influence of MBP on soil microbes was also identified in microbial community composition (Bünemann et al., 2011) and the growth stage of microorganisms (Elser et al., 2003).

5. Conclusions

A set of representative soil nutrients and physicochemical properties respond sensitively to black locusts plantations of various years. For alkaline soil, bacterial communities appear to respond positively with forest plantation and this process may be propelled by the accumulation of phosphorous within microbial biomass. Black locusts have long-term but uneven effects on elements (C, N and P) distribution within soil microbial biomass and on individual microbial groups. Whether the diverging trend within microbial communities will continuously increase with even longer plantation is yet to be seen. However, our results still indicate that, on the Loess Plateau, black locusts plantation has the potential to transform soil microbial biomass and microbial communities in the direction of improving soil P content.

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