Responses of Soil Microbial Communities to *Robinia Pseudoacacia* Plantations of Different Ages in A Loess Area

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Abstract: Phospholipid fatty acids (PLFAs) can be used as biomarkers for qualitative and quantitative analyses of soil microbial community diversity. In this study, we collected soil samples at 10-cm intervals to a depth of 1 m from *Robinia pseudoacacia* plantations of four different ages (10, 15, 25 and 40 years) in a loess area and analysed the soil microbial community structure by PLFA analysis. A total of 97 PLFAs were detected in soils of *R. pseudoacacia* plantations of different ages. The individual PLFA contents gradually decreased in the 0- to 40-cm soil layers, with little variation in the 40- to 100-cm soil layers. The individual PLFAs were similarly distributed in the soils of *R. pseudoacacia* plantations of different ages, and there was a clear variation with stand age and soil depth. The individual PLFA contents in the 0- to 20-cm soil layers were highest for the 25-year-old plantation, while those in the 20- to 40-cm soil layers were relatively high for the 25- and 40-year-old plantations; the 16:0 content was the highest among individual PLFAs. The total PLFA content and the PLFA contents of different microbial groups [bacteria, fungi, Gram-positive bacteria (G^+), Gram-negative bacteria (G^-) and actinomycetes] initially increased before decreasing in the soils of *R. pseudoacacia* plantations with increasing stand age, whereas these contents gradually decreased with increasing soil depth; the highest PLFA contents was found in the 25-year-old plantation. The total PLFA content and the contents of fungal, G^- and actinomycete PLFAs in the soils of *R. pseudoacacia* plantations differed significantly among stands of different ages in the 0- to 10-cm, 10- to 20-cm and 30- to 40-cm soil layers, while no significant differences were found in the 20- to 30-cm soil layers; the G^+ and bacterial PLFAs contents in each of the 0- to 40-cm soil layers were significantly different. The PLFA ratios between different microbial groups differed among the stands of different ages. The fungi/bacteria ratio showed a “decrease-increase-decrease” trend with stand age, while the G^+/G^- ratio showed an “increase-decrease” trend. The saturated/monounsaturated PLFA ratio initially decreased before plateauing, while the opposite trend was observed for the cyclopropyl/precursor ratio. The PLFA contents of different microbial groups were ranked as follows: bacteria > G^- > G^+ > actinomycetes > fungi. In the principle component analysis, 18:1ω9c, 10Me18:0, i17:0, a17:0, 18:1ω7c, 18:1ω5c and 18:0 made the greatest contribution to principal component 1, and a14:0, i14:0 3OH, i14:0, i14:1ω7c and 14:0 made the greatest contribution to principal component 2. In conclusion, soil nutrient status and other soil eco-environmental stress factors should be considered in 10- to 25-year-old (particularly ~15-year-old) plots for the management of *R. pseudoacacia* plantations to prevent forest soil degradation and improve forest stand quality, thereby achieving better soil and water conservation and environmental improvement in *R. pseudoacacia* plantations.

Keywords: soil; *Robinia pseudoacacia*; PLFA; stand age; microbial community

1. Introduction

Soil microbes are one of the most active soil constituents, with a decisive influence on the vegetation-soil ecosystem. They actively participate in energy flow, nutrient cycling and organic matter transformation in the

soil, and they play a major role in maintaining ecosystem structure and function [1]. Variations in many soil microbial indicators, such as soil microbial biomass, enzyme activity, community structure and functional diversity, can reflect the health of the soil ecosystem [2]. In particular, the soil microbial community structure and diversity can reflect relatively early variations in soil environmental quality and reveal differences in the ecological functions of microbes; hence, soil microbes are considered important biological indicators [3,4]. The soil microbial community structure is influenced by the soil, climate and environmental factors (e.g., pH, soil moisture, temperature conditions, nutrient contents and availability) [5-8]. Additionally, vegetation is a major influential factor, mainly due to differences in the environment created by plants for microbes, the properties of food provided by plants (e.g., litter) and the chemical composition of root exudates [9,10]. The majority of soil microbes are difficult to culture, making the study of microbial community structures difficult [11]. In recent years, attempts have been made to overcome this difficulty using phospholipid fatty acid (PLFA) analysis [12,13], BIOLOG-ECO analysis [14] and molecular biological methods [15]. However, the BIOLOG method is not suitable for analysing the entire microbial community structure, and the success of molecular biological methods depends on the physiological state of the microbes in the environment. The PLFA analysis can be used to detect microbial community changes in the samples and is less affected by microbial physiology. The main principle of PLFA analysis is that the PLFA composition and content of various microbes show species specificity, which can be used to directly estimate microbial biomass and community structure [16]. Forest ecosystems with different stand ages show substantial differences in nutrient cycling and energy flow. Varying intensities of plant-soil interactions can result in differences in the environment and biological constitution of forest soils. Moreover, soil water and nutrient conditions as well as oxygen concentrations vary with depth, which also influences the microbial biomass and community structure. Therefore, studies examining the characteristics of the microbial community structure at different soil depths with different stand ages have great implications for our understanding of soil environmental conditions and quality.

Black locust (*Robinia pseudoacacia* Linn.) is characterised by tolerance to drought and barren soil, a developed root system, rapid growth and a high yield. Thus, *R. pseudoacacia* has become one of the major types of plantations for vegetation restoration in semi-arid and sub-humid areas of the Loess Plateau [17,18]. Over the past few decades, *R. pseudoacacia* plantations have been extensively constructed in large areas of this region. *R. pseudoacacia* has also been widely planted in Yan'an, which plays a major role in improving the eco-environment and preventing soil and water loss in this region, even regulating the hydrological conditions in the Yellow River Basin [19]. *Robinia pseudoacacia*, which originates in the Appalachian Mountains of North America, has been introduced and cultivated in China. This tree is most common in North China and the Yellow River Basin, with the best growth documented at 400-1200 m above sea level in North China [20]. The construction of *R. pseudoacacia* plantations uses mainly pure stands, while local species slowly invade during the late growth stage. The tree layer comprises *Acer stenolobum*, *Pyrus betulaefolia*, *Populus davidiana* and *Ulmus pumila* L.; the shrub layer comprises *Syringa oblata* Lindl., *Cotoneaster acutifolius* Turcz., *Rosa hugonis* Hemsl. and *Rhamnus erythroxylon* Pall.; and the herb layer comprises *Artemisia gmelinii*, *Artemisia giraldii* Pamp., *Stipa bungeana* Trin., *Bothriochloa ischaemum* (L.) Keng, *Cirsium setosum* (Willd.) MB. and *Plantago depressa* Willd. [21]. In recent years, numerous studies have been conducted to examine *R. pseudoacacia* plantations in terms of reasonable planting density, intensity of thinning improvement, species diversity and biomass [19,22-27]. However, few studies have investigated differences in microbial biomass and microbial community structure between stands of different ages. In the present study, we selected 10-, 15-, 25- and 40-year-old *R. pseudoacacia* plantations and used PLFA analysis to investigate changes in soil microbial biomass and community structure under the near-natural management of *R. pseudoacacia* plantations. This study provides a theoretical reference for the long-term sustainable management of *R. pseudoacacia*

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plantations.

2. Materials and Methods

2.1. Study Area

The study area is located in the urban and suburban areas of Baota District, Yan'an City, Shaanxi Province, China. This area is part of the hilly-gully region of the Loess Plateau, lying at the geographic location of 36°24′-38°47′N and 109°28′-110°22′E, with an average elevation of 987.4 m above sea level. The area has a continental monsoon climate in the temperate zone. There are four distinct seasons, with rain and heat over the same period. The average annual rainfall is 550 mm, the annual frost-free period lasts 170 days, and the annual average temperature ranges from 7.7-10.6°C. The vegetation in the study area consists of deciduous broad-leaved forests in the warm temperate zone. Owing to the serious destruction of the original vegetation, this area has experienced serious soil and water losses. Plantations including mainly *R. pseudoacacia*, *Platycladus orientalis* (L.) Franco and *Pinus tabuliformis* Carrière have been planted for many years to rebuild and restore healthy forest ecosystems. The major soil type is loessial soil, containing 63-73% silt and 17-20% clay. The soil has a loose texture and poor resistance to erosion and scouring, with serious soil and water loss.

2.2. Sample Collection and Processing

We selected 10-, 15-, 25- and 40-year-old *R. pseudoacacia* plantations with a relatively consistent slope (30° and 35°) and aspect (shaded slope) among other site conditions to form a chronosequence of *R. pseudoacacia* plantations. Soil samples were collected in June 2016. Four sampling points were selected at 5-m intervals from the upper to the lower slope in plantations of each age. Soils were collected using a soil auger at each point to a depth of 1 m, and one sample was obtained every 10 cm. A total of 160 soil samples were obtained.

In the field, the soil samples were placed in sealed bags and stored in a cooler box with periodic replacement of the ice packs. The samples were transported to the laboratory, immediately freeze-dried and stored in a -70°C refrigerator for PLFA analysis.

2.3. Sample Determination and Analysis

Soil microbial PLFAs were extracted following the modified Bligh-Dyer method [28,29], using esterified C19:0 as the internal standard. The extraction, purification and analytical determination procedures were as follows. A 5-g soil sample was oscillated with a mixture of chloroform:methanol:citric acid buffer (volume ratio = 1:2:0.8) to extract the total lipids. The obtained PLFAs were separated on an SPE silica gel column (CNWBOND Si SPE Cartridge, 500mg, 3ml; Art Number: 2.CA1353.0001). After alkaline esterification, the PLFA composition was analysed using an Agilent 7890N gas chromatographer. The PLFA profile was obtained for statistical analysis of the types and quantities of soil microbes. The chromatographic conditions were as follows: HP-5 capillary column (25 m × 200 μm × 0.33 μm); sample volume, 2 μl; split ratio, 100:1; carrier gas, H_2 (2 ml·min^{-1}); makeup gas (30 ml·min^{-1}). The content of the PLFA fractions was analysed using the MIDI Sherlock Microbial Identification System (MIDI, Inc., Newark, DE, USA).

2.4. Naming and Calculation of Soil PLFAs

In this study, we used the method reported by Frostegård [30] with the formula (i/a/cyc)X: YωZ(c/t), where "X" is the number of carbon atoms in the main chain, "Y" is the number of unsaturated olefinic bonds, "ω" is the distance between the olefinic bond and the carboxyl group, "Z" is the position of the olefinic bond or cyclopropane chain, "i" is the isomeric methyl branch, "a" is the former isomeric methyl branch, "cyc" is the

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cyclopropyl group, and suffixes "c" and "t" are cis and trans isomers, respectively. "Me" denotes methyl branches. In general, saturated PLFAs were calculated as the sum of 12:0, 13:0, 14:0, 16:0 and 18:0; monounsaturated PLFAs were calculated as the sum of 16:12OH, 16:1ω5c and cy17:0. Isomeric PLFAs were calculated as the sum of i14:0, i15:0, i16:0 and i17:0; trans-isomeric PLFAs were calculated as the sum of a15:0 and a17:0. Cyclopropyl PLFAs were calculated as the sum of cy17:0 and cy19:0; precursor PLFAs were calculated as the sum of 16:1ω7c and 18:1ω7c [31]. Microbial communities were classified based on the PLFA biomarkers listed in Table 1 [32-35]. The PLFA content was calculated for bacteria, fungi, actinomycetes, Gram-positive (G^+) bacteria and Gram-negative (G^-) bacteria.

The contents of total and individual PLFAs were calculated based on the molar amount of the internal standard C19:0. The absolute content of PLFAs (C/nmol·g^{-1}) was calculated as follows: $C = A_i m_s/(As \cdot m)$, where A_i is the peak area of the i-th PLFA fraction, A_s is the peak area of the internal standard, m_s is the mass of the internal standard, and m is the mass of the soil sample. The relative percentage of PLFAs was obtained as the percentage of the peak area of the i-th PLFA to the total peak area.

Table 1. PLFA biomarker for calculating the soil microbial biomass

Microbe type	PLFA biomarker
Bacteria	12:0,13:0,14:0, a14:0, i14:0, 15:0, i15:0, a15:0, 16:0, a16:0, i16:0, i17:0, 16:1ω5c, 16:1ω7c, cy17:0, a17:0, 18:0, i19:0, cy19:0,20:0
Actinomycetes	10Me16:0, 10Me17:1ω7c, 10Me18:1ω7c, 10Me18:0, 10Me20:0
G^+	i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, i18:0
G^-	16:1ω5c, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω5c, 18:1ω7c, cy17:0, cy19:0
Fungi	16:1ω5c, 18:2ω6c, 18:2ω9c, 18:1ω9c

2.5. Data Processing

Data processing, statistical analyses and mapping were performed using Excel 2007 and SPSS 20. The difference between different data sets was evaluated using one-way analysis of variance (ANOVA) and the least significant differences (LSD) test. The raw data for PLFAs were subjected to principal component analysis (PCA).

3. Results

3.1. Variation Trends in Microbial PLFA Content with Depth in Soils of R. Pseudoacacia Plantations of Different Ages

The microbial PLFAs content showed distinct variation trends with depth in soils of *R. pseudoacacia* plantations of different ages. Fig. 1 shows the trends in the contents of 10 individual PLFAs with relatively high levels in the 1-m soil profile. In the soil layers above 40 cm, the content of individual PLFAs markedly decreased with increasing depth for *R. pseudoacacia* plots of different ages. In the soil layers below 40 cm, the variation trend in the content of individual PLFAs tended to be minimal. Thus, we focused on the analysis of variations in PLFA content within the 0- to 40-cm soil layers in the following sections.

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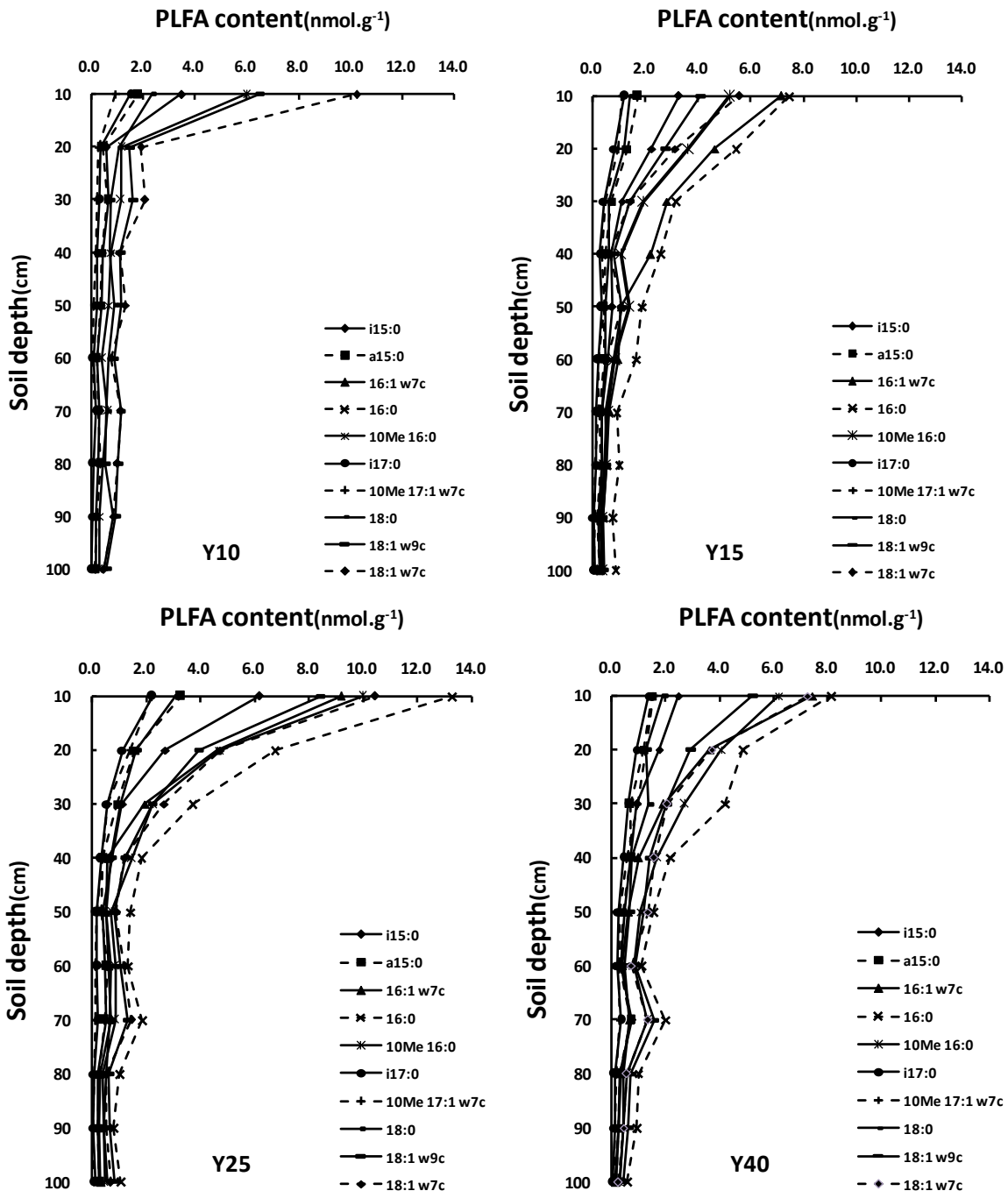

Fig. 1. Soil PLFA content profiles of *Robinia pseudoacacia* plantations of different ages

Note: Y10, Y15, Y25 and Y40 denote 10-, 15-, 25- and 40-year-old *R. pseudoacacia* plantations, respectively

3.2. Microbial PLFA Composition in Soils of R. Pseudoacacia Plantations of Different Ages

A total of 97 PLFAs were detected in soils of *R. pseudoacacia* plantations of different depths and ages. Specifically, 77, 72, 83 and 85 PLFAs were found in soils of the 10-, 15-, 25- and 40-year-old plantations, respectively, including saturated straight fatty acids, saturated branched fatty acids, cyclopropyl fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. Further analysis of the 28 PLFAs present in the soils of *R. pseudoacacia* plantations at relatively high levels (Fig. 2) revealed similar patterns and variations in PLFA contents; the content of 16:0 was the highest and accounted for 12.76-14.01% of the total PLFA content.

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Additionally, the 18:1ω7c, 10Me16:0, 16:1ω7c, 18:1ω9c, cy19:0 and i15:0 contents were relatively high. Variations in the contents of individual PLFAs exhibited a certain regularity. In the 0- to 20-cm soil layers, the contents of different PLFAs remained the highest for the 25-year-old plantation, while there were no significant differences in the contents of individual PLFAs between the remaining plots of other ages. In the 20- to 40-cm soil layers, the contents of different PLFAs were relatively high for the 25- and 40-year-old plantations. Additionally, Fig. 2 shows that the contents of different PLFAs markedly decreased with increasing soil depth, in agreement with the results shown in Fig. 1.

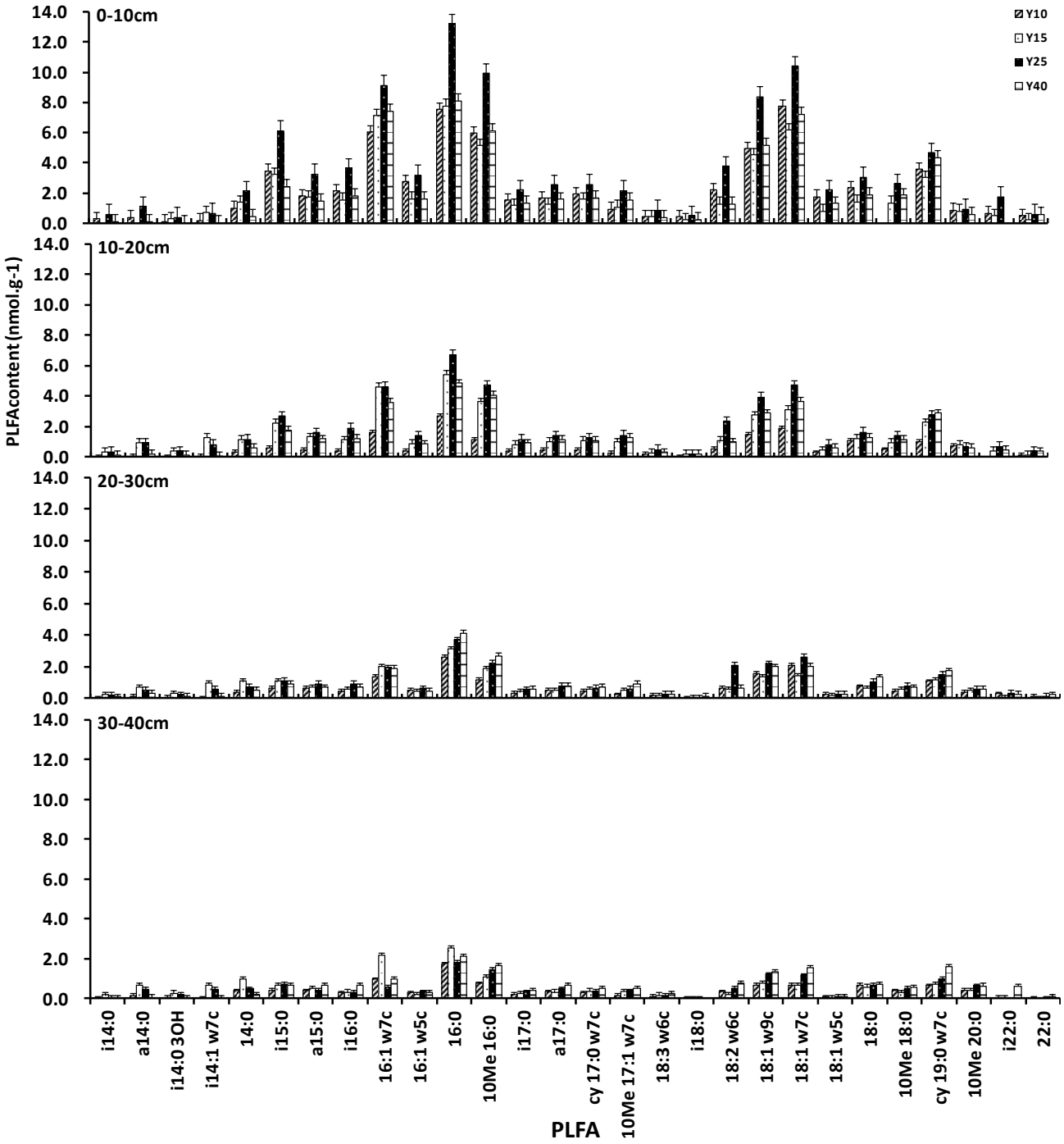

Fig. 2. Distribution of different PLFAs at different soil depths in *Robinia pseudoacacia* plantations of different ages

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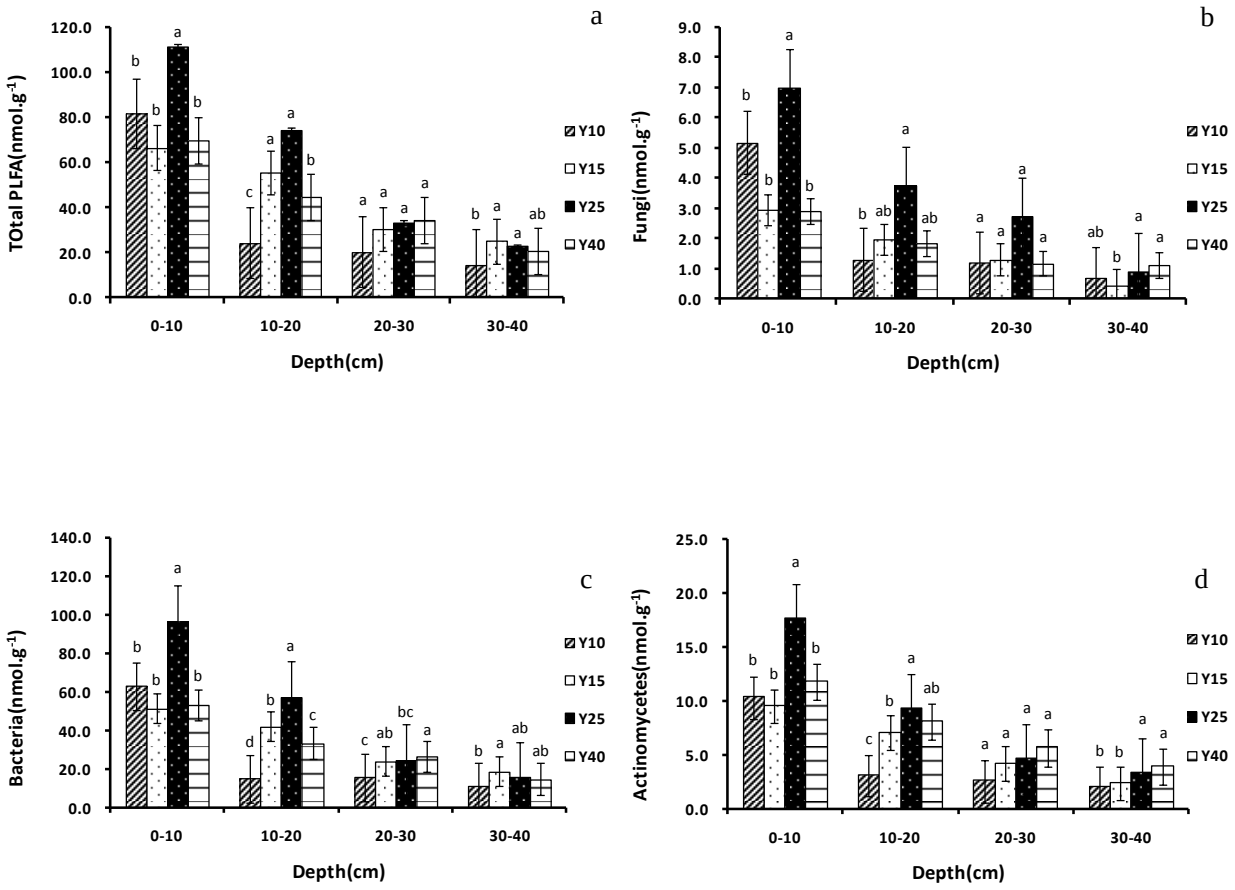
3.3. *PLFA Content of Various Microbial Groups in Soils from R. Pseudoacacia Plantations of Different Ages*

The biomass of various microbial groups and the total microbial biomass in soils of *R. pseudoacacia* plantations showed significant variations among the different stand ages and soil depths (Fig. 3). As shown in Fig. 3a, in the 0- to 10-cm and 10- to 20-cm soil layers, the total PLFA soil contents in the *R. pseudoacacia* plantations initially increased and then decreased with the stand age, and the highest content was found in the 25-year-old plantation. In the 20- to 30-cm and 30- to 40-cm soil layers, the total PLFA content did not differ significantly in soils among plantations of various ages ($P > 0.05$). The total PLFA content in soils of all plantations varied with depth: 0-10 cm > 10-20 cm > 20-30 cm > 30-40 cm, in line with the variations in individual PLFA contents with depth. A comparison of Figs. 3b-f with Fig. 3a revealed that the PLFA contents of various microbial groups, including fungi, bacteria, actinomycetes, G^+ and G^-, generally followed the same trends as the total PLFA content in the soils. Based on ANOVA, we found significant differences in total PLFA content as well as the contents of fungal, actinomycete and G^- PLFAs among the stands of different ages in the 0- to 10-cm, 10- to 20-cm and 30- to 40-cm soil layers ($P < 0.05$); however, no significant differences were detected in the 20- to 30-cm soil layer ($P > 0.05$). The contents of bacterial and G^+ PLFAs significantly differed between stand ages in each soil layer ($P < 0.05$). Additionally, the results showed that the PLFA contents of microbial groups varied in the same soil layer and could be ranked as follows: bacteria > G^- > G^+ > actinomycetes > fungi.



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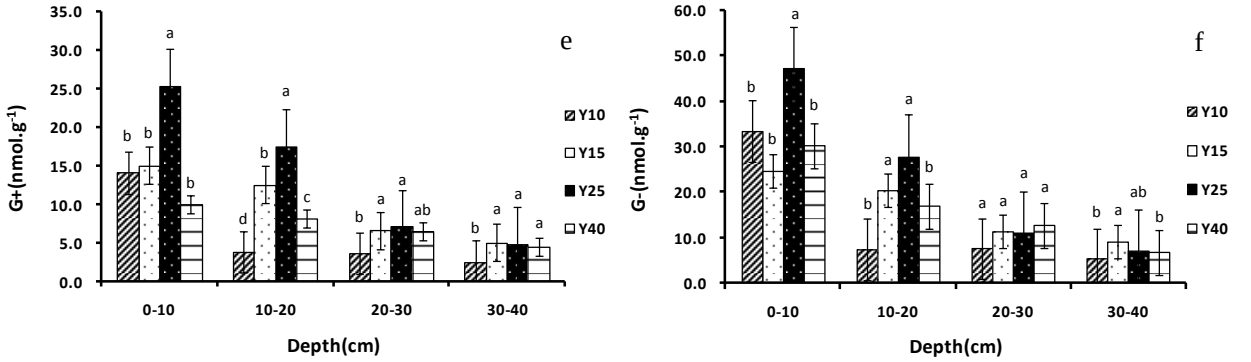

Fig. 3. Microbial PLFA contents in different soil depths in *Robinia pseudoacacia* plantations of different ages

3.4. *Variations in Microbial Community Structures in Soils of R. Pseudoacacia Plantations*

The microbial PLFA composition in soil reflects the physiological characteristics of the soil microbes. Analyses of the fungi/bacteria (F/B), saturated/monounsaturated PLFA (sat/mono), cyclopropane:precursor PLFAs (cy/pre) and G^+/G^- ratios (Fig. 3) showed that the PLFA ratios between different microbial groups exhibited various trends among the stands of different ages, while these trends were subject to slightly different effects of depth. The F/B ratio significantly differed among the stands of various ages ($P < 0.05$) and showed a "decrease-increase-decrease" trend with stand age; this ratio was relatively high in the 10- and 25-year-old plantations. The sat/mono ratio initially decreased before levelling off with the change in stand age; this ratio was highest in the 10-year-old plantation, with no significant differences among the stands of various ages ($P > 0.05$). The cy/pre ratio followed a trend opposite to that of the sat/mono ratio, with the highest ratio observed in the 40-year-old plantation; no significant differences were observed in the cy/pre ratio between the 10-, 15- and 25-year-old plantations ($P > 0.05$). The G^+/G^- ratio showed an "increase-decrease" trend with stand age; this ratio was highest in the 25-year-old plantation and lowest in the 10-year-old plantation.

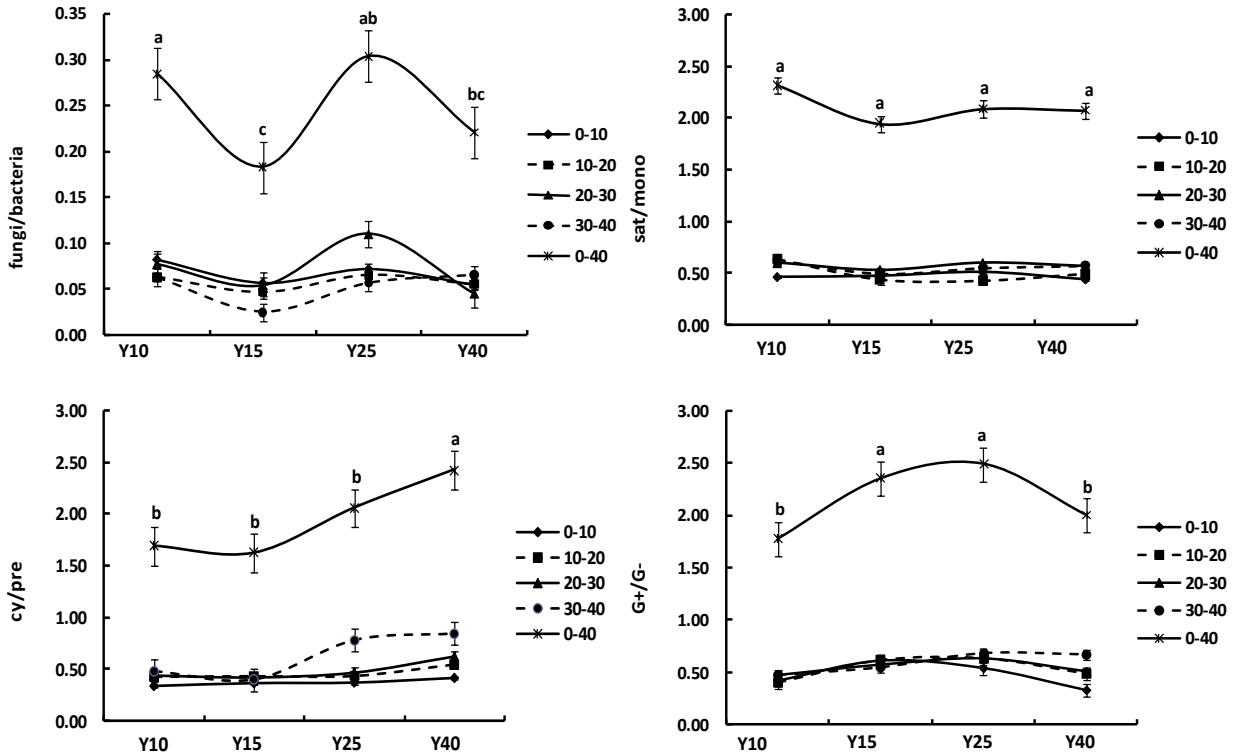

Fig. 4. Ratios among different microbial PLFAs in the soil of *Robinia pseudoacacia* plantations of different ages

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3.5. PCA Analysis of Microbial PLFAs in Soils of R. Pseudoacacia Plantations

PCA analysis was performed on microbial PLFAs in soils of *R. pseudoacacia* plantations of different ages (Fig. 5A). Two principal components (PC1 and PC2) were obtained, which explained 62.35% and 32.22% of the total variation in microbial community structure in soils of *R. pseudoacacia* plantations of different ages. The results revealed significant differences between various stand ages (F = 4.883, P = 0.028). The 10- and 40-year-old *R. pseudoacacia* plantations were influenced mainly by PC2, whereas the 15- and 25-year-old *R. pseudoacacia* plantations were influenced mainly by PC1.

The eigenvector loadings of different PLFAs on the principal components reflect the relationship between PLFAs and the principal factors; the higher the loading is, the closer is the relationship [36](Sun *et al*., 2011). The analysis of initial eigenvector loadings of different PLFAs (Table 2) showed that five PLFAs had original eigenvector loadings greater than 0.95 for factor 1; four PLFAs had original eigenvector loadings between 0.90 and 0.95 for factor 1; and i17:0 had higher original eigenvector loadings than did the remaining PLFAs. To better explain the relationship between PLFAs and the principal factors, we performed a varimax rotation on the factor matrix (Table 2). For factor 1, 18:1ω9c and 10Me18:0 had loadings greater than 0.9, and i17:0, a17:0, 18:1ω7c, 18:1ω5c and 18:0 had loadings between 0.90 and 0.95; these PLFAs made the greatest contribution to PC1. For factor 2, a14:0 and i14:0 3OH had loadings greater than 0.90, and i14:0, i14:1ω7c and 14:0 had loadings between 0.80 and 0.90; these PLFAs made the greatest contribution to PC2. Further analysis of the loading diagram of PLFAs after rotation (Fig. 4B) revealed three PLFAs (a14:0, 14:0 3OH and i14:1ω7c) in the second quadrant and five PLFAs (10Me20:0, a22:0, i18:0, 18:1ω5c and 18:1ω7c) in the second quadrant. All the remaining PLFAs were distributed in the first quadrant. Additionally, a14:0, 14:0 3OH, i14:1ω7c and 10Me20:0 were relatively distant from the PLFA-dense area, indicating a poor correlation between these four PLFAs and other PLFAs.

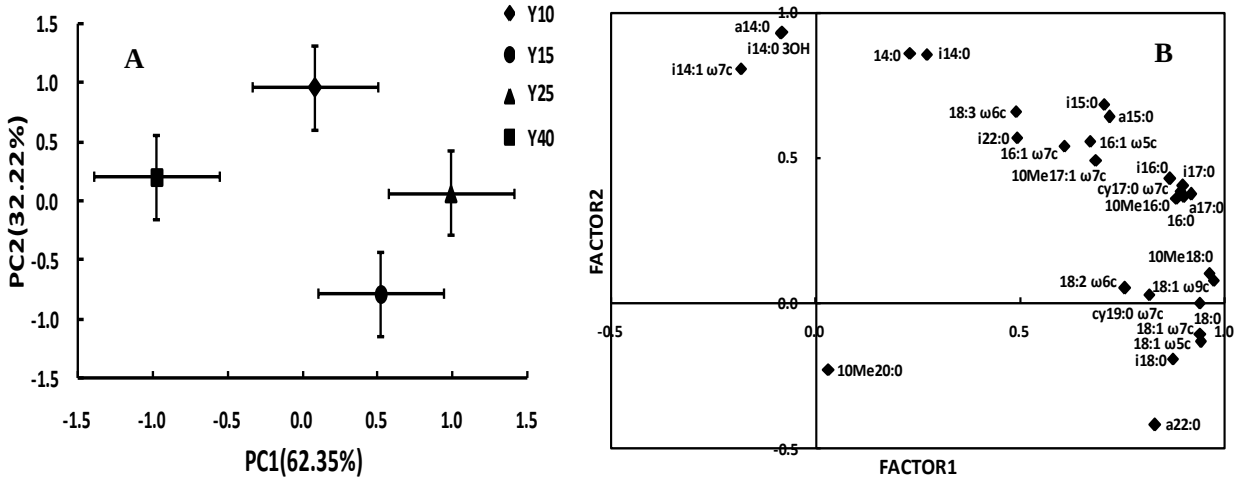

Fig. 5. Principal component analysis (A) and eigenvector loadings (B) of PLFAs in the soil of *Robinia pseudoacacia* plantations of different ages

Table 2. Eigenvector loadings of different PLFAs in the soil of *Robinia pseudoacacia* plantations of different ages

PLFA	Original eigenvector loadings		Eigenvector loadings after rotation*	
	Factor 1	Factor 2	Factor 1	Factor 2
i14:0	0.602	0.666	0.271	0.856
a14:0	0.313	0.884	-0.083	0.934
i14:0 3OH	0.308	0.881	-0.087	0.929
i14:1ω7c	0.169	0.810	-0.183	0.807
14:0	0.568	0.686	0.231	0.861
i15:0	0.926	0.329	0.705	0.684

a15:0	0.919	0.284	0.718	0.641
i16:0	0.985	-0.006	0.898	0.405
16:1ω7c	0.778	0.239	0.608	0.541
16:1ω5c	0.842	0.227	0.671	0.557
16:0	0.971	-0.021	0.891	0.385
10Me 16:0	0.966	0.029	0.867	0.429
i17:0	0.990	-0.040	0.917	0.375
a17:0	0.971	-0.041	0.900	0.367
cy17:0ω7c	0.952	-0.040	0.882	0.360
10Me 17:1ω7c	0.827	0.162	0.685	0.491
18:3ω6c	0.719	0.398	0.489	0.661
i18:0	0.714	-0.538	0.873	-0.192
18:2ω6c	0.709	-0.266	0.756	0.053
18:1ω9c	0.917	-0.334	0.973	0.078
18:1ω7c	0.800	-0.511	0.941	-0.132
18:1ω5c	0.811	-0.486	0.940	-0.104
18:0	0.854	-0.390	0.939	0.001
10Me 18:0	0.918	-0.305	0.962	0105
cy19:0ω7c	0.754	-0.313	0.815	0.029
10Me 20:0	-0.066	-0.219	0.031	-0.227
i22:0	0.685	0.312	0.493	0.569
22:0	0.580	-0.725	0.829	-0.417

* Varimax rotation.

4. Discussion

In the present study, we analysed the soils from *R. pseudoacacia* plantations of different ages in Yan'an, northern Shaanxi Province. A total of 97 PLFAs were detected, including 77, 72, 83 and 85 PLFAs in the soils of 10-, 15-, 25- and 40-year-old *R. pseudoacacia* plantations. These numbers were slightly higher than the numbers of PLFAs detected by Hu [37] in soils of single *R. pseudoacacia* plantations on a slope in Yangjuangou, Yan'an (66 PLFAs in the 0- to 10-cm soil layer and 68 PLFAs in the 10- to 20-cm soil layer). On the one hand, the *R. pseudoacacia* plantations examined in the present study included stand ages of 10, 15, 25 and 40 years. Due to the increase in stand age, the succession of vegetation could change, resulting in variations in the soil eco-environment and further influencing the microbial community structure. On the other hand, the soil depth interval tested in the present study was 0-100 cm. With increasing soil depth, the moisture, temperature and oxygen concentrations could change, thus influencing the types and quantities of microbes. Based on the analysis of 10 PLFAs that changed with depth, we found that the content of different PLFAs gradually decreased in the 0- to 40-cm soil layers, while little difference occurred in the 40- to 100-cm soil layers. Soil microbial communities directly and sensitively reflect the changes in soil biological activity and soil environmental quality [38]. The contents of different PLFAs showed similar structural patterns in the soils of *R. pseudoacacia* plantations of different ages, and dominant PLFAs were distinct. This finding suggests that the structural pattern of individual PLFA contents in soils of *R. pseudoacacia* plantations was influenced mainly by vegetation type. Vegetation type has a significant effect on soil microbial composition and diversity [39]. However, owing to the vegetation-soil interaction, there are inconsistent mechanisms and outcomes for the effect of various factors on soil microbial groups [40]. In the present study, stand age had little effect on the structural pattern of individual PLFA contents in soils of *R. pseudoacacia* plantations; however, the content of individual PLFAs showed obvious variation trends with stand age. In the 0- to 20-cm soil layers, the contents of the different PLFAs were highest in the 25-year-old plantation. In the 20- to 40-cm soil layers, the contents of individual PLFAs were relatively high for both the 25- and 40-year-old plantations. The content of 16:0, indicative of bacteria, was highest among various PLFAs, in agreement with the results of most studies, such as those examining *Phyllostachys heterocycla* [41], *Averrhoa carambola* L. and *Litchi chinensis* Sonn. [42].

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The PLFA content in soil is closely related to microbial biomass and can be used to measure microbial groups and biomass [43]. In the present study, the total PLFA content and the PLFA content of different microbial groups in soils of *R. pseudoacacia* plantations showed a "decrease-increase-decrease" trend with stand age in the 0- to 20-cm soil layer. This finding is consistent with the results reported by Yang [44]. regarding different years of artificial grassland in the Sanjiangyuan region. At a consistent stand age and soil depth, the PLFA content of different microbial groups can be ranked as follows: bacteria > G^- > G^+ > actinomycetes > fungi. With increasing stand age, the relative abundance of fungi compared with bacteria first decreased and then increased, followed by a decrease in the soils of *R. pseudoacacia* plantations. This trend reflects the changes in organic matter content and system stability in soils of *R. pseudoacacia* plantations. The relatively high F/B ratio in soils of the 10- and 25-year-old *R. pseudoacacia* plantations may be associated with soil structure and nutrition status. The sat/mono ratio can indicate the activity level of soil organic matter [45]. In the present study, we found that the sat/mono ratio initially decreased and then increased before plateauing; however, no significant differences were found among the *R. pseudoacacia* plantations of different ages. The cy/pre ratio gradually increased with increasing stand age, indicating that the characteristics of the microbial community structure in soils of *R. pseudoacacia* plantations were influenced by nutrient factors and other environmental factors. It has been reported that water stress [46], heavy metals and organic pollutants [47] can increase the cy/pre ratio.

The microbial community structure revealed differences in the soils of *R. pseudoacacia* plantations of different ages (Fig. 5A). In the PCA analysis, PC1 and PC2 could well explain the variation by 62.35% and 32.22%, respectively. 18:1ω9c, 10Me18:0, i17:0, a17:0, 18:1ω7c, 18:1ω5c and 18:0 made the greatest contribution to PC1, whereas a14:0, i14:0 3OH, i14:0, i14:1ω7c and 14:0 made the greatest contribution to PC2. Additionally, a14:0, 14:0 3OH, i14:1ω7c and 10Me20:0 correlated poorly with the remaining PLFAs.

Soil microbes are an important part of forest ecosystems, and microbial community diversity is of great significance to the stability of forest ecosystems. In this study, we analysed by PLFA analysis the variations in microbial community structure with stand age in soils of *R. pseudoacacia* plantations from Yan'an. We found that the PLFA content of different microbial groups initially increased before decreasing with stand age. Both the individual PLFA contents and the PLFA content of the microbial groups reached relatively high or the highest level in the 25-year-old *R. pseudoacacia* plantation. This finding has implications for guiding the reasonable management of *R. pseudoacacia* plantations. During the management of *R. pseudoacacia* plantations, attention should be paid to soil nutrient status and other soil eco-environmental stress factors in 10- to 25-year-old (particularly ~15-year-old) plots to prevent forest soil degradation and improve forest stand quality, thereby achieving better soil and water conservation in *R. pseudoacacia* plantations and improving the environment.

5. Conclusions

(1) A total of 97 PLFAs were detected in soils of *R. pseudoacacia* plantations of different ages in Yan'an. The individual PLFA contents gradually decreased in the 0- to 40-cm soil layers, with little change in the 40- to 100 cm soil layers. Individual PLFAs were similarly distributed in the soils of *R. pseudoacacia* plantations with different ages, and the 16:0 content was the highest among the different PLFAs.

(2) The total PLFA content and the PLFA content of the different microbial groups (bacteria, fungi, G^+, G^- and actinomycetes) initially increased and then decreased with increasing stand age, while they gradually decreased with increasing soil depth. The highest contents were observed for the 25-year-old *R. pseudoacacia* plantation. The PLFA ratio between microbial groups showed varying trends among the different stand ages. The F/B ratio showed a "decrease-increase-decrease" trend, while the G^+/G^- ratio showed an

"increase-decrease" trend. The sat/mono ratio initially decreased before plateauing, demonstrating an opposite trend compared with that of the cy/pre ratio. The PLFA content of the different microbial groups can be ranked as follows: bacteria > G^- > G^+ > actinomycetes > fungi.

(3) The content and structure of microbial PLFAs in soils of the 25-year-old *R. pseudoacacia* plantation were significantly different compared with those of *R. pseudoacacia* plantations of other ages. In the PCA, 18:1ω9c, 10Me18:0, i17:0, a17:0, 18:1ω7c, 18:1ω5c and 18:0 made the greatest contribution to PC1, whereas a14:0, i14:0 3OH, i14:0, i14:1ω7c and 14:0 made the greatest contribution to PC2. Therefore, in the management of *R. pseudoacacia* plantations, soil nutrient status and other soil eco-environmental stress factors should be noted in 10- to 25-year-old (particularly ~15-year-old) plots to prevent forest soil degradation and improve forest stand quality, thereby achieving better soil and water conservation in *R. pseudoacacia* plantations and improving the environment.

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