RESEARCH ARTICLE

Land rehabilitation improves edaphic conditions and increases soil microbial biomass and abundance

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ABSTRACT

Rehabilitation of farmland improves the local eco-environmental conditions. But to what extent this transformation influences soil microbial properties is less known. In our study we compared variations in soil microbial attributes following changes in land-use types to understand the influence of altered soil properties on microbial biomass and their community structure using chloroform fumigation extraction method and phospholipid fatty acid (PLFA) analysis. For this purpose, 3 agricultural (AL) (farmland, apple orchard and 2 years abandoned land) and 4 rehabilitated lands (RL) of various vegetations grassland, shrubland, mixed forest (Amorpha fruticosa and Pinus tabuliformis Carr.) and forest (Robinia pseudoacacia) were selected. Our results showed higher soil organic carbon (SOC) contents in RL soils (forest > mixed forest > grassland > shrub land) than that in AL soils. In RL soils, soil microbial biomass and abundance of group specific PLFA were significantly higher than those in AL soils. Under different land-use types, microbial community was bacteria dominated over fungi. The microbial physiological indices (G^+/G^-) , cyc/prec and S/M) indicated decreased environmental stress in RL soils in comparison with AL soils. In loess soils, SOC and total N correlated positively (p < 0.05) with microbial biomass C, N and P and also with fungal and bacterial PLFA, indicating a positive microbial mediation in improving soil fertility. Taking together, our findings suggest that land rehabilitation, especially Robinia pseudoacacia planation, improves overall edaphic conditions and accelerates soil microbial biomass accumulation in local regions.

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1 Introduction

Land-use transformation implies environmental change [\(van](#page-10-0) [Asselen and Verburg, 2013](#page-10-0)). Globally, land-use changes are occurring primarily due to increasing demand in energy, food and forest products ([Nilsson, 2015](#page-10-0)). Locally, deforestation and agricultural expansion have shown strong negative ecological

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impacts on land-use change [\(Lapola et al., 2014; Graesser](#page-9-0) [et al., 2015;](#page-9-0) [Ordway et al., 2017\)](#page-10-0). Forested land conversions for agricultural purposes and consequently abandonment of previously cultured land due to poor productivity have increased during the 20th century. For example, in 1970s inadequate land management practices and transformation of forests into agricultural fields lead to severe land degradation in southern China (Xu et al., 2010). While in 1980s, drastic ecological impacts of such practices were recognized and led to convert croplands back to forest or grasslands ([Zhang](#page-11-0) [et al., 2011b\)](#page-11-0). These rehabilitation activities mainly involved, i) promotion of vegetation in large areas of poor quality sites ([de Andrés et al., 2007;](#page-8-0) [Wang et al., 2014](#page-10-0); [Kou et al., 2016b\)](#page-9-0); ii) switching from deforestation to stable forest/shrub/grass coverage [\(Holl, 2002;](#page-9-0) [Riitters et al., 2012](#page-10-0); [Tian et al., 2017](#page-10-0); [Liu](#page-10-0) [et al., 2018c](#page-10-0)) and iii) afforestation of abandoned fields to increase forest density ([Poyatos et al., 2003\)](#page-10-0).

The rehabilitation effort usually involves revegetation by introduction of various plant species in neglected lands. For example, in central Spain, leguminous species were commonly used for revegetation of abandoned cropland [\(de](#page-8-0) [Andrés et al., 2007\)](#page-8-0). In Mediterranean middle mountain region, traditional agricultural land practices were abandoned for forest expansion [\(Poyatos et al., 2003](#page-10-0)). While in China, revegetation involves artificial forest plantation (Robinia pseudoacacia (RP) and Caragana korshinskii (CK)) [\(Kou](#page-9-0) [et al., 2016a, 2016b; Liu et al., 2018b](#page-9-0)) and naturally succession in most of the Loess hilly region area.

The effects of such rehabilitation were scientifically evaluated by assessing, i) spatial variations in soil organic carbon [\(Wang et al., 2012;](#page-10-0) [Almagro et al., 2013\)](#page-8-0); ii) differences in soil nutrients such as nitrate, ammonium, available P, and soil pH [\(Zhang et al., 2011b](#page-11-0); [Xu et al., 2014\)](#page-11-0); and iii) soil microbial activities [\(Zhang et al., 2011b;](#page-11-0) [Li et al.,](#page-9-0) [2014](#page-9-0), [2018](#page-9-0)). Following the establishment of several soil health indicators such as physical, chemical and nutritional properties [\(Torsvik and Øvreås, 2002; Schloter et al., 2003\)](#page-10-0), the assessment of biological properties such as soil microbial biomass [\(Xiao et al., 2016](#page-11-0); [Guo et al., 2018\)](#page-9-0) and changes in community structure ([Xiao et al., 2016](#page-11-0); [Yang et al., 2018](#page-11-0)) have attracted more attention recently.

As part of ecological conservation program Grain-to-Green initiated by the Chinese government, to restore highly eroded Loess Plateau soils, large areas of farmlands have been revegetated to grassland, shrub land, and forest zones ([Deng](#page-8-0) [et al., 2012](#page-8-0); [Zhao et al., 2013](#page-11-0); [Fazhu et al., 2015](#page-8-0)). During this conservation effort, soil quality is shown to greatly improve under rehabilitated land soils (RL) than agricultural use soils (AL) ([Guo et al., 2018\)](#page-9-0). However, how microbial community structure changes under different land-use types especially following the rehabilitation efforts is still unclear. Our central scientific question was whether soil microbial biomass and physiological status will improve in the direction of benefitting nutrients accumulation after land rehabilitation. Therefore, our aim in this study was to, i) assess variations in the soil microbial taxa and their physiological status within two groups of land-use types AL (farmland, orchard and abandoned land) and RL (grassland, shrubland, mixed forest and forest) in the Loess Plateau, and ii) determine the effects of land-uses on individual microbial groups and their associations.

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 Sciences (CAS) in Northern Shaanxi Province of China (108°5′–109°26′E, 36°30′–37°39′N). The altitudinal range of the area is 1010–1400 m above sea level (m a.s.l.), mean annual temperature (MAT) 8.8°C and the mean annual precipitation (MAP) 513 mm. The soil type is silt loam (USDA classification), typically classified as Loessi Orthic Primosols in China.

Corn is usually cultivated as the main crop on farmland and an increasing number of previously cultured lands have been abandoned due to poor productivity. The abandoned land normally treats as a control (2 years abandoned land) in the studied region characterizing by its minor disturbance and low soil nutrient contents (lowest organic carbon and total nitrogen; Table 1). Large areas of apple orchards have been planted for around 30 years. Grassland is dominated by three types of herbaceous plant species, while the shrub land mainly shows presence of Caragana korshinskii. Vegetations in the mixed forest zone have N-fixing plant species such as 10-year old Amorpha fruticosa and 25-year old Pinus tabuliformis Carr. grown with afforestation purposes. The forest-to-grassland vegetation belt encompass transitional environment between warm temperate deciduous broadleaf forest and dry grassland [\(Xu et al., 2009\)](#page-11-0). Detailed geographic information and vegetation characteristics at the investigated sites are given in Table 1.

2.2 Soil sampling

Soil samples from 0 to 10 cm depth were collected in July 2011 from seven different land-use types (sites) at each 100 m \times 100 m plot. In each plot, three replicate subplots of 10 m \times 10 m were selected, and 6 soil replicates were collected from each subplot to get a total of 18 random samples. A composite sample of 18 samples was prepared to minimize the spatial variability in soils. Fresh samples were then sealed in plastic bags and transported to laboratory in ice boxes. Samples were sieved (< 2 mm) to remove discernible roots, stones and macro-fauna and later air-dried to measure the soil physicochemical properties. Microbial biomass was analyzed from the aliquots of the samples stored at 4°C.

2.3 Analysis of soil physical and chemical properties

The soil moisture was determined gravimetrically on oven dry basis (105°C, 24 h). The bulk density was determined using cutting ring as recommended in standard methods of soil and

Land-use type	Altitude	Latitude	Longitude	Main species ^b
	(m a.s.l. ^a)	$(°$ from N)	($^{\circ}$ from E)	
Farmland	1035	36°46.234'	109°16.102'	Zea mays
Orchard	1384	36°43.696'	109°15.251'	Malus pumila
Abandoned land	1420	36°43.491'	109°14.290'	Heteropappus altaicus, Salsola collina, Artemisia capillaris,
Grassland	1361	36°43.880'	109°14.237'	Artemisia gmelinii, Heteropappus altaicus,
Shrub land	1232	36°45.116'	109°14.998'	Caragana korshinskii
Mixed forest	1136	36°46.027'	109°15.963'	Pinus tabulaeformis, Amorpha fruticosa
Forest	1185	36°45.931'	109°15.914'	Robinia pseudoacacia

Table 1 Geographic and vegetative characteristics of the different land-use types.

a m a.s.l.: meters above sea level. ^b Dominated species are shown in bold. Understorey vegetation are Artemisia gmelinii, and Lespedeza dahurica in shrub land; rtemisia giraldii, Artemisia gmelinii and Heteropappus altaicus in mixed forest; Artemisia giraldii, Artemisia gmelinii and Syringa vulgaris in forest.

chemical analysis ([Nu, 1999](#page-10-0)). The soil pH was determined at a soil to water ratio of 1:2.5, W/V using a Delta 320 pH meter (Mettler-Toledo Instruments (Shanghai, China) Co., Ltd.). Soil organic carbon (SOC) was determined by wet oxidation using dichromate followed by $FeSO₄$ titration [\(Bao, 2007\)](#page-8-0). Available P (AP) was colorimetrically measured after extraction with NaHCO₃. Total N (TN) was measured via Kjeldahl digestion and distillation azotometry. Flow injection analysis determined nitrate-N (NN) and ammonium-N (AN). Mineralizable nitrogen (MN) was determined by the aerobic culture method [\(Nu,](#page-10-0) [1999](#page-10-0)). Total soluble N was determined from K_2SO_4 extracts and dissolved organic nitrogen (DON) was calculated as difference of total soluble N and inorganic N in amount.

2.4 Determination of soil microbial biomass

The microbial biomass C (MBC) and microbial biomass N (MBN) were determined in fresh soil using chloroform fumigation extraction method ([Joergensen and Mueller,](#page-9-0) [1996](#page-9-0)). The separate 5 g fumigated and non-fumigated soil samples were extracted using 20 mL 0.5 M K_2SO_4 solution and followed by horizontal shaking and filtering. The organic C and total N in the extracts were determined using automatic analyzer (Phoenix 8000). MBC was calculated as E_C/K_{EC} , where E_C = difference between organic C extracted from fumigated and non-fumigated soils and K_{EC} = 0.45 [\(Wu et al.,](#page-11-0) [1990](#page-11-0)). While MBN was calculated as E_N/K_{EN} , where $E_N =$ difference between total N extracted from fumigated soils and non-fumigated soils and K_{EN} = 0.54 ([Joergensen and Mueller,](#page-9-0) [1996\)](#page-9-0). The microbial biomass phosphorous (MBP) was calculated as the difference between total P in fumigated and non-fumigated samples extracted with $NaHCO₃$ ([Brookes, Powlson and Jenkinson, 1982](#page-8-0)).

2.5 Phospholipid fatty acid (PLFA) analysis

The phospholipid fatty acids were extracted from 2 g fresh soil with a chloroform/methanol/citrate (1:2:0.8 ratio) buffer as described previously in triplicate ([Frostegard et al., 1993\)](#page-9-0). Later, neutral lipids, glycolipids, and phospholipids were eluted separately on a silicic acid column. The eluted

phospholipids fractions were subjected to mild alkaline methylation and analyzed by gas chromatographically using a Hewlett Packard 5890 Series II chromatograph equipped with flame ionization detector. In this analysis the PLFA methyl esters were run through a 50 mm \times 0.32 mm fused silica capillary column coated with a poly-ethylene glycol as stationary phase (CPWax 52CB, 0.2 mm film thickness; Chrompack). Peak areas were quantified using nonadecanoate fatty acid (19:0) added as an internal standard to the methylated PLFA extracts. Individual PLFA were identified using fatty acid methyl ester standard compounds (Bacterial Acid Methyl Esters (BAME) Mix; Supelco, Bellefonte, PA). Group specific PLFA biomarkers were used to characterize the microbial community structure in the collected soils. PLFA 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 are biomarker for bacteria ([Frostegård and Bååth, 1996](#page-9-0)). The PLFA 18:1ω9c, 18:1ω9t and 18:2ω6,9 were used as indicators for fungal biomass ([Olsson, 1999](#page-10-0)). PLFA 10Me17:0, 10Me18:0, 10Me19:0 as indicators of actinobacteria ([Zelles et al., 1994\)](#page-11-0). The ratio fungal/bacterial PLFA was calculated by the amount of total fungal PLFA divided by the sum of bacterial PLFA ([Frostegård](#page-9-0) [and Bååth, 1996](#page-9-0)). The iso- and anteiso-branched saturated fatty acids (i15:0, a15:0, i16:0, i17:0) represent Gram-positive bacteria ([Zelles et al., 1994\)](#page-11-0), whereas the monounsaturated 16:1ω7c, cy17:0, 18:1ω7, cy19:0 represent Gram-negative bacteria [\(Frostegard et al., 1993](#page-9-0); [Zelles et al., 1994\)](#page-11-0). Total PLFA was calculated by the sum of all bacterial, fungal and actinobacterial PLFA biomarkers (expressed as n mol g^{-1} soil).

2.6 Statistical analysis

Shapiro-Wilk and Levene's tests were used to examine normality and homogeneity of variances in the data sets. One-way analysis of variance (ANOVA) followed by Tukey HSD (at $p < 0.05$) was used to compare significant differences in soil and microbial biomass attributes, microbial group assigned PLFA and physiological indicators in SPSS 20.0 (SPSS Inc., USA) software. Principal component analysis (PCA) was used to differentiate the microbial communities of

soils from different land-use types. Redundancy analysis (RDA) is advantageous of assessing the explanatory power of each defined variable by parsing out other terms as constraints to calculate its proportion of total variance (O'[Connor, 1988](#page-10-0)).

3 Results

3.1 Nutritional properties of soils under various land-uses

The content of soil organic carbon (SOC) ranked in the order of farmland > forest > mixed forest > orchard > grassland > shrub land > abandoned land. SOC content was significantly lower in the abandoned land than other land-use types $(p < 0.05$; Table 2). Soil bulk density, C/N ratio and pH values were higher in the agricultural land-use types (AL) (farmland, orchard, and abandoned land) than in the rehabilitated lands of various vegetations (RL) (grassland, shrub land, mixed forest, and forest) (Table 2). The concentration of nitrate-N (NN) changed in the order farmland > forest > shrub land > orchard > abandoned land > grassland > mixed forest. Unlike NN, the concentration of ammonium-N (AN) varied in the order forest > mixed forest > shrub land > grassland > abandoned land > orchard > farmland. In general, the content of total N and various N fractions (AN, ON, MN and DON) were all significantly lower in AL than those in RL ($p < 0.05$; Table 2). MBC contents in RL soils (ranged from 295.97 to 418.28 mg kg $^{-1}$) were higher than AL soils (ranged from 86.52 to 135.27 mg kg^{-1}) ($p < 0.05$; Table 2). Among AL, MBN content was lowest in orchard soil than in other soils ($p < 0.05$; Table 2). Whereas, the contents of MBC, MBN, and MBP were significantly lower in abandoned land (Table 2).

Table 2 Selected soil properties of the investigated land-use types.

3.2 Phospholipid fatty acid (PLFA) profiles under different land-use types

Land-uses have significantly affected the abundance of various microbial groups (Table 3, Fig. 1). All individual PLFA of the grassland was significantly more abundant than those of other land-use types (Fig. 1). Whereas, the lowest values of individual PLFA was observed in soils of abandoned land. Among all PLFA biomarkers, the concentrations of PLFA 16:0 and 18:1ω9c were higher than the rest (Fig. 1). Regarding bacterial PLFA biomarkers, Gram-positive bacterial PLFA dominated in all land-use types (Table 3). The abundance of all microbial PLFA significantly varied among all land-use types and ranked in the order of grassland > forest > mixed forest > shrub land > orchard > farmland > abandoned land (Table 3). Therefore, in comparison with the abandoned land (treated as a control treatment), the total PLFA (sum of the bacterial and fungal PLFA) content increased by 6.6 (in grassland), 5.2 (forest), 4.7 (mixed forest), 3.7 (shrub land), 3.1 (orchard) and 2.4 times (farmland), respectively. Moreover, all the PLFA concentrations of soils in RL were about 1 fold higher than those of soils in AL (Table 3). Compared with the other land uses, the abandoned land had the lowest $G^{\dagger}/G^$ ratio and the highest cy/pre ratio. Regarding AL soils, the ratios of G⁺/G⁻, F/B and S/M did not change significantly (Table 3).

3.3 Shifts in soil microbial community structure

Principal component analysis (PCA) of all PLFA biomarkers indicated a substantial difference in soil microbial community structure of soils under different land-use types (Fig. 2). The first principal component (PC1) axis explained 83.65% of the variance while the second axis (PC2) explained only 6.05%.

^a The units are g kg⁻¹; SOC, soil organic carbon; Total N, total nitrogen. ^bBD, bulk density (g cm⁻³). ^c The units are mg kg⁻¹; AP, available phosphorus; NN, nitrate-N; AN, ammonium-N; MN, mineralizable nitrogen; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; Within rows, means values and standard deviation in parentheses of 3 replicates ($n = 3$ in all cases), followed by the same letters are not significantly different ($p < 0.05$; Tukey HSD test).

Fig. 1 Relative abundance of phospholipid fatty acid (PLFA) biomarkers in soils across various land-use types (farmland, orchard, abandonment land, natural grassland, shrub land, mixed forest and forest). Values refer to mean \pm SD, $n = 3$. Asterisks show significant differences between land use types ($p < 0.05$; Tukey's multiple comparison tests).

The PLFA profile of abandoned land soil was completely different from those of soils of other land-use types.

Most of the PLFA biomarkers (bacteria, actinobacteria and fungi) loaded parallelly along PC1 while only bacterial biomarkers (18:1ω7, cy19:0, 17:0) were primary contributors along PC2, which causes the observed distribution pattern of various land-use type soil samples (Fig. 2).

3.4 Relationships between microbial group specific PLFA and soil attributes

Redundancy analysis (RDA) considered group specific PLFA and soil attributes overall land-use types. The first axis RDA explained 96.80% variations of the PLFA and the second axis RDA explained 1.08% variations (Fig. 3). This variation was mainly driven by variations in soil C/N ratio and bulk density (BD) having pronounced negative correlation with soil microbial groups. While other soil attributes consisted of soil carbon fractions (SOC and MBC), nitrogen fractions (ON, MN, MBN and NN) and microbial phosphorous fractions (MBP) were positively related to microbial communities. Bacterial communities (Gram-positive and Gram-negative bacteria), actinobacteria, G⁺/G⁻ PLFA ratio and fungi highly positively correlated ($p < 0.05$) with MBC, ON, SOC and MN. In contrast, the microbial physiologic indicator (S/M and cy/pre ratios) showed negative correlation. While soil pH, AP and NN had weak correlation with the microbial groups (Fig. 3).

4 Discussion

4.1 Land-use effect on basic soil physicochemical properties

In Loess Plateau of China, changes in land-use reflected great variations in soil C and N dynamics [\(Wang et al., 2011](#page-10-0); [Deng et al., 2013](#page-8-0)). In present work, we also observed significant high SOC contents (6.36 g kg^{-1}) in rehabilitated lands of various vegetations against the low SOC contents (2.51 g kg^{-1}) in soils of abandoned lands (control), indicating SOC accumulation in vegetative alkaline loess soils. These results are in consistent with the previous study considering physical and chemical changes in soils during revegetation on abandoned farmland in Zhifanggou watershed ([Wang et al.,](#page-10-0) [2011](#page-10-0)). In abandoned land, the absence of above-ground input plus the continued easily-available C consumption by growing weed could have substantially declined SOC concentration. Another presumable reason could be the lack of rainfall which lowers vegetation productivity and the return of weed root biomass, resulting in low turnover of soil carbon pool ([Wang](#page-10-0) [et al., 2016](#page-10-0)). In tropical region of the southern Ecuador, abandoned fields were found to be poor in nutrient contents and ecological value ([Knoke et al., 2014\)](#page-9-0). Also, in a semi-arid ecosystem of the Central Iran, abandoned lands were also characterized by both low C turnover [\(Raiesi, 2012b\)](#page-10-0) and weak N availability to microbes [\(Raiesi, 2012a\)](#page-10-0).

Our results showed the decrease in soil pH toward neutral

cy19:0)/(16:1ω7 + 18:1ω7). ⁴ S/M, ratio of normal saturated PLFAs to monounsaturated PLFAs. Within columns, means and standard deviation followed by the same letter are not significantly
different (ρ<0.05; Tukey HSD tes cy19:0)/(16:1ω7). ^d S/M, ratio of normal saturated PLFAs to monounsaturated PLFAs. Within columns, means and standard deviation followed by the same letter are not significantly different (p < 0.05; Tukey HSD test), n = 3 in all cases.

Fig. 2 Principal component analysis (PCA) of soil microbial communities considering phospholipids fatty acid (PLFA) biomarkers. Colors represent different land-use types while arrows represent loadings for individual PLFAs separately.

Fig. 3 Redundancy analysis (RDA) showing relationships between soil microbial groups and soil attributes. The length of arrows represents the association strength of the respective variable with the microbial groups. Angle between vectors demonstrates the degree of their interactions (smaller angle means high correlation). Values on the x and y-axes illustrate the percentage change explained by RDA 1 and RDA 2, respectively. Abbreviations used in the figure were described in Tables 2 and 3.

range, i.e., turning soils less alkaline followed by decrease in bulk density and also soil C/N ratio in the four rehabilitated lands of various vegetations (RL) (grassland, shrub-land, mixed forest and forest). This trend was similar to the one observed previously ([Liu et al., 2018a\)](#page-10-0) and demonstrated an improvement in soil physical and chemical properties upon revegetation as also seen in North Indian sodic soils ([Singh](#page-10-0) [et al., 2012\)](#page-10-0). The possible explanations of the positive effect of RL soil physicochemical properties may be that, i) organic acids exuded by roots lower soil pH ([Corti et al., 2005; Aoki](#page-8-0) [et al., 2012](#page-8-0)); ii) soils are less bulked in presence of plant fine roots [\(Volkmar, 1993; Stirzaker et al., 1996\)](#page-10-0); and iii) in vegetated plots soil C gets allotted more to plant roots due to enhanced competition by plants for essential nutrients in comparison to the C assimilation by soil microorganisms [\(Van](#page-10-0) [Wijk et al., 2003](#page-10-0)). However, this C upon turnover of fine roots become more available in soil and consequently increases SOC contents. This is also true in case of enhanced total organic N and easily available N concentrations (i.e., mineralizable N (MN), dissolved organic N (DON) and ammonium-N (AN)) in RL land-use types. However, the variation N forms within RL land-use types might be related to differential plant and microbial species composition further differing in N uptake capabilities ([Weigelt et al., 2005](#page-10-0)).

The higher amount of soil organic C and nitrate-N found in farmland compared to those of forest and mixed forest, can be mainly attributed to the local crops planting and cultivation custom: i) the high nitrate-N might be related to the legacy effect of N fertilizer application during corn vigorous growing period on the farmland; ii) widespread artificial land management practices such as reduced tillage and corn stubble retention [\(Zhang et al., 2011a\)](#page-11-0) could increase soil C sequestration via positively regulates soil C cycling ([Havlin](#page-9-0) [et al., 1990;](#page-9-0) [Martens, 2000\)](#page-10-0). The differences between the content of microbial biomass C, N and P in different land-use types reflect the contents of total storage pool in soils. Microbial biomass responds quickly to changes in soil management practices and often signifies soil quality ([Syers, 1997](#page-10-0); [Hoyle and Murphy, 2011](#page-9-0)). Our results demonstrated significant increase of microbial biomass C (MBC) content in RL in comparison with AL. This result indicates less disturbance in soils of land-use types with natural vegetation succession. Among agricultural land-use types (AL), the significant low microbial biomass N (MBN) contents in orchard suggested low active N level resulting from quick turnover ([Iyyemperumal et al., 2007](#page-9-0)) to maintain soil N supply ([Kooijman et al., 2009](#page-9-0)). Although available P was significantly lower in soils of RL than that of soils in AL, MBP was significantly higher in soils of RL than that of soils in AL. This is similar to MBC which was higher in soils of RL. This suggests that microbial P incorporation was predominantly dependent on MBC pool in RL soils ([Achat et al., 2012\)](#page-8-0). Our findings in microbial biomass agree with those reported by [An et al.](#page-8-0) [\(2012\)](#page-8-0) and [Liu et al. \(2018a\)](#page-10-0) in Loess soils.

4.2 Shifts in PLFA profile among different land-uses

Soil microbial indices often reflect changes in microbial communities [\(Jangid et al., 2011](#page-9-0); [Guan et al., 2013](#page-9-0); [Lauber](#page-9-0) [et al., 2013](#page-9-0)). Such variations can be monitored briefly via phospholipid fatty acid (PLFA) profiles [\(Drenovsky et al.,](#page-8-0) [2004;](#page-8-0) [Grayston et al., 2004](#page-9-0); [Wu et al., 2012](#page-11-0)). Among the

rehabilitated lands of various vegetations, almost all individual PLFA were significantly higher in grassland compared with that of forest and mixed forest. It can be closely related to the significant highest dissolved organic N (DON) content in grassland (Table 2) as the vegetation type (grass vs. forest) mediated dissolved nutrient availability was an important predictor of PLFA profile [\(Cookson et al., 2007](#page-8-0)). As one of the most active components of soil nitrogen, the higher amount of DON in grassland might increase cell-specific activity ([Menyailo et al., 2008\)](#page-10-0) which would lead to an increase in the all microbial phospholipid fatty acid profiles. These results were similar to the earlier study ([Jangid et al., 2011](#page-9-0)) demonstrating significant high microbial rRNA gene copy number and clones in grassland than in forest and cropland soils.

Further, amounts of fungal PLFA were nearly two fold higher than those of Gram-positive bacterial PLFA. This indicates stronger occupying of fungi in soils is in line with the critical review on abundance of microbial PLFA in natural soils ([Joergensen and Wichern, 2008](#page-9-0)). Generally, soil pH is main driver of microbial community [\(Bååth and Anderson, 2003](#page-8-0)) and fungi are more acid tolerant than bacteria and leads to higher F/B ratio in acidic soils ([Strickland and Rousk, 2010\)](#page-10-0). However, we observed nearly twofold higher F/B ratio (0.60- 0.76) in our alkaline soils of Loess Plateau, in contrast to highly acidic Hapludalfic soils under various land-use types in the United States (0.26-0.39) ([Jangid et al., 2011\)](#page-9-0). A possible explanation for this could be greater competitiveness of fungi compared to bacteria in highly alkaline soil pH of Loess Plateau due to wider pH growth optima ([Wheeler et al., 1991\)](#page-11-0). In general, the higher PLFA assigned to different groups followed the order grassland > forest > mixed forest > shrub land in RL than in AL where the order occurred as orchard > farmland > abandoned land (Fig. 1 and Table 3). These results are in line with our earlier study in Loess soils considering abundance of different microbial groups [\(Liu](#page-9-0) [et al., 2014a](#page-9-0)). This means that RL soils positively affect microbial growth along with better nutritional conditions possibly driven by low soil C/N ratio (Table 2). Considering changes in physiological indices, the increase in pH shifted bacterial community toward Gram-negative bacteria and lowered Gram-positive bacterial PLFA [\(Wu et al., 2009;](#page-11-0) [Shen et al., 2013\)](#page-10-0). This may be the reason for observed higher G⁺/G⁻ ratios of soils in RL than those of soils in AL. This further reflected microbial growth strategies in two different land-uses in Loess plateau. Whereas, significantly high cyc/ prec ratio in abandoned land soil may be due to C and oxygen limitation [\(Liu et al., 2014a](#page-9-0)).

4.3 Relationships between the microbial PLFA and crucial soil properties

Redundancy analysis indicated soil nutrients and microbial groups varied greatly on six land-use types in Loess Plateau. In comparison to this, variation in soil bacterial communities based on high throughput sequencing (HTS) using these soils ([Liu et al., 2018d](#page-10-0)) explained only up to 40% of variation mainly

driven by soil properties such as pH, SOC, total N and total P in addition to available P, NH_4^+ -N, MBC and MBN [\(Yang et al.,](#page-11-0) [2018\)](#page-11-0). In different soil matrixes (Black soil, with high SOC content of \sim 25 g kg⁻¹), soil variables explained even lower (37.52%) variations in bacterial communities ([Liu et al.,](#page-10-0) [2014b\)](#page-10-0). The varying high percentage of explanation between HTS and PLFA-based assessment of microbial community structure could be due to low resolution in the latter method. While also the appropriate screening of most reliable soil attributes covering main physicochemical and nutritional parameters from different land-use type soils could be another potential reason. In our study, soil fertility indicators (SOC, C/N ratio, MBC, MBN, organic N and NH_4^+ -N) were highly correlated with individual microbial groups which further had high correlation with soil physiochemical indicators such as BD and soil pH. Similar relationships were observed by [Buckeridge et al. \(2013\)](#page-8-0) and [Delgado-Baquerizo et al. \(2017\)](#page-8-0) suggesting key role of soil microbial groups in available nutrient transformation and turnover processes. Positive relationship between fungi and soil nutrients is well documented ([Lauber et al., 2008; Liu et al., 2016](#page-9-0)). This relationship is closely related to the fungal life style and ecological roles in providing nutrients to host plants [\(Güsewell and Gessner,](#page-9-0) [2009](#page-9-0); [McGuire et al., 2010\)](#page-10-0) and also to the bacterial communities in soil ([Starke et al., 2016](#page-10-0)). Among the aforementioned soil fertility indicators, close relationship among MBC, organic N and bacterial communities (Grampositive and Gram-negative bacteria) might be because of general preference of Gram-negative bacteria to nutrients in the form of organic N. Whereas, the close relationship between SOC and MBN with actinobacteria and fungi could be due to immobilization of C and/or N from organic polymers to these microbial groups ([Courty et al., 2010;](#page-8-0) [Kim et al.,](#page-9-0) [2014\)](#page-9-0). We have to admit that, in addition to the measured parameters, soil humidity is also a very important factor in the microbial biomass and community structure. By investigating over one thousand soil samples at large spatial extents, Drenovsky et al. (2010) concluded that the main factor driving composition and microbial biomass was land-use type, especially as related to water availability. As the seasonal soil humidity variation is a common physiological stress influencing microbial community composition and metabolic functions ([Fierer et al., 2003](#page-9-0); [Liu et al., 2019](#page-9-0)). Therefore, temporal dynamics of soil microbial communities should also be considered in our further work.

Together, our results implicate, land rehabilitation improves soil conditions and increases soil microbial biomass and abundance in Chinese loess plateau. In the short-term, returning farmland to forest or grass land is recommended in order to maintain soil fertility in local areas. Soil microbial profiles should also be monitored during middle-term implementation of vegetation plantation. In a long run, a holistic evaluation approach that integrated with ecological and socioeconomic indicators, is required for quantifying and assessing the ecosystem service functions and benefits of land rehabilitation.

5 Conclusions

The accumulation of microbial biomass and abundance attributes in rehabilitated lands were stronger than those in the agricultural land-use in Loess Plateau of China. Specifically, black locusts (Robinia pseudoacacia) plantation is the best rehabilitated vegetation comparing to the worst of the agricultural corn planting. These results demonstrated that vegetation played significant role in augmenting microbial attributes in severely eroded soils. The increased fungal/ bacterial PLFA ratio of soils in rehabilitated lands in comparison with those of soils in agricultural lands indicate combined but different contribution of fungal and bacterial abundance to microbial biomass formation. The observed differences in microbial community and abundance were mainly driven by soil bulk density and soil pH. The close associations among SOC, total N, microbial biomass and microbial groups indicated that improved soil fertility conditions contributed to enhanced microbial biomass, community composition and also their differentiation. These fertility conditions also reduced the environmental stress on soil microbial communities as indicated by reduced microbial physiological indices (G⁺/G⁻, cyc/prec and S/M) in rehabilitated soils. Finally, our results showed that rehabilitation in Loess Plateau soils led to overall improvement of soil health.

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Conflict of interest

Dong Liu, Baorong Wang, Parag Bhople, Fayzmamad Davlatbekov and Fuqiang Yu declare that they have no conflict of interest.

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