



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; Tatenno 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 RP 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 *Robinia 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 *Robinia 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 FeSO₄ titration method (Bao, 2007). Total N and available P (AP) were analyzed by Kjeldahl digestion and distillation azotometry and by NaHCO₃ 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–K₂SO₄ extraction

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