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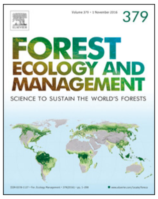

Effects of tree species and soil properties on the composition and diversity of the soil bacterial community following afforestation

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ABSTRACT

Afforestation with single and mixed tree species on cropland alters soil properties and the composition and diversity of soil bacterial community. We investigated changes in soil properties and the soil bacterial community composition in the topsoil (0–20 cm) following afforestation on cropland on the Loess Plateau. Forests of six types ranging from 23 to 25 years in age were chosen in this study: *Robinia pseudoacacia* (RP), *Platycladus orientalis* (PO), *Pinus tabuliformis* (PT), *Robinia pseudoacacia* + *Platycladus orientalis* (RPPO), *Robinia pseudoacacia* + *Pinus tabuliformis* (RPPT), and *Platycladus orientalis* + *Pinus tabuliformis* (POPT). Afforestation with different tree species altered the soil nutrient contents, with total nitrogen (TN) and ammonium nitrogen (NH_4^+) contents increased by 18.28–87.10% and 13.97–141.34%, respectively, and available phosphorus (AP) content decreased by 56.09–82.13% compared to cropland sites. Cropland afforestation reduced the soil bacterial richness and core bacterial genera abundance (relative abundance ≥ 0.1%), and significantly altered the bacterial diversity. Soil properties explained 73.41% of the total variation in the core soil bacterial community composition at the genus level, and soil organic carbon (SOC), TN, nitrate nitrogen (NO_3^-), NH_4^+, AP, pH and the carbon:phosphorus (C:P) ratio were significantly correlated with first two RDA (redundancy analysis) axes. The results of hierarchical clustering indicated that the soil bacterial community composition in the RPPO, RPPT and POPT sites was largely affected by *Platycladus*, *Pinus tabuliformis* and *Platycladus*, respectively. Our observations suggest that soil bacterial community composition and diversity following afforestation were mainly affected by tree species, followed by soil parameters. The effect of afforestation on the soil bacterial diversity was larger than its effect on bacterial richness.

1. Introduction

Afforestation, which is the conversion of non-forestlands to plantation forests, is one of the most commonly used techniques to prevent soil degradation and restore the ecological integrity of disturbed ecosystems (Nunez-Mir et al., 2015). The establishment and development of plantation forests with different tree species drive alterations in soil properties and the composition and diversity of soil microbial community (Ren et al., 2016; Deng et al., 2017; Gunina et al., 2017). A variety of culture based studies have examined the effects of tree species and soil parameters on the structure and composition of microbial communities; however, these studies have mainly focused on the effects of a single tree species and soil nutrients on the soil microbial community (Zhalnina et al., 2015; Nacke et al., 2016; Pei et al., 2016; Ding et al., 2017).

Due to their drought tolerance, *Robinia pseudoacacia*, *Platycladus orientalis* and *Pinus tabuliformis* have been widely planted as afforestation species on the Loess Plateau (Qiu et al., 2010; Qin et al., 2017). *Robinia* afforestation increases soil nitrogen (N) availability due to the N-fixing ability of symbiotic rhizobial bacteria in the roots (Buzhdygan et al., 2016). Berthrong et al (2009) reported that afforestation by *Pinus* species causes a 15% and 20% decrease in the soil carbon (C) and N contents, respectively, at the global level. The total phosphorus (TP) stock following afforestation decreased by 12% in the top 20 cm of mineral soil, but no change in available phosphorus (AP) was observed at the global level (Deng et al., 2017). However, studies addressing changes in soil chemical properties following *Platycladus* afforestation are scarce.

Soil properties play important roles in shaping the soil bacterial community structure and diversity in terrestrial ecosystems. Soil

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Table 1
General condition of six forest type sites.

Tree stand levels	Afforestation age (years)	Meandiameter at breast height (cm)	Mean height (m)	Tree composition	Main herbaceous composition	Soil type
RP	25	20.83	12.8	*Robinia pseudoacacia* (100%)	*Humulus scandens*	Ferralsols
PO	23	7.85	7.0	*Platycladus orientalis* (100%)	*Carex parva Nees*	Ferralsols
PT	25	12.87	11.2	*Pinus tabulaeformis* (100%)	*Potentilla chinensis*	Ferralsols
RPPO	23	RP:6.33 PO:11.32	RP:8.45 PO:7.30	*Robinia pseudoacacia* (30%) *Platycladus orientalis* (70%)	*Potentilla chinensis*	Ferralsols
RPPT	25	RP:12.56 PT:14.00	RP:13.28 PT:12.83	*Robinia pseudoacacia* (60%) *Pinus tabulaeformis* (40%)	*Humulus scandens*	Ferralsols
POPT	25	PO:8.19 PT:13.50	PO:6.18 PT:7.26	*Platycladus orientalis* (50%) *Pinus tabulaeformis* (50%)	*Artemisia gmelinii* *Potentilla chinensis*	Ferralsols

Robinia pseudoacacia (RP); *Platycladus orientalis* (PO); *Pinus tabuliformis* (PT); *Robinia pseudoacacia* and *Platycladus orientalis* (RPPO); *Robinia pseudoacacia* and *Pinus tabuliformis* (RPPT); and *Platycladus orientalis* and *Pinus tabuliformis* (POPT).

bacterial communities obtain their energy from soil organic matter via decomposition and mineralization, and bacterial diversity is significantly affected by the soil C, N and P supply (Zechmeister-Boltenstern et al., 2015; Zhang et al., 2016). Fresh C and labile soil C inputs alter the abundance and structure of the soil microbial community in forest soils (De Graaff et al., 2010; Garcia-Pausas and Paterson, 2011; Wang et al., 2014a). Li et al (2015) observed that N addition decreased soil bacterial biomass and that P addition increased soil bacterial and fungal biomass and fungi:bacteria ratio in a secondary forest. P availability affects the soil bacterial community composition and the turnover of C and P in the microbial biomass in forest soils (Bergkemper et al., 2016; Spohn and Widdig, 2017).

Tree species affect soil microbial communities in several ways, including through the production of litter, rhizodeposition, root-symbiotic microorganisms, as well as the microclimate (Prescott and Grayston, 2013; Ushio et al., 2013). Litter chemistry variables, such as the initial substrate and some specific chemical components released via litter decomposition, play prominent roles in shaping the soil microbial community composition (Bray et al., 2012; Prescott and Grayston, 2013). For example, the high N content in *Robinia* leaves and some specific chemical components in conifer leaves, such as condensed tannins, affect the soil microbial community composition and diversity (De Marco et al., 2013; Ushio et al., 2013). Root exudates, which contain low-molecular-mass compounds, polymerized sugars, root border cells and dead root cap cells, association with root-symbiotic shape the rhizosphere bacterial community and diversity (Broeckling et al., 2008; Philippot et al., 2013; Okubo et al., 2016). The taxonomic, phylogenetic, functional trait distributions and genotype of tree also have a large influence on soil microbial community composition and structure in a forest ecosystem (Schweitzer et al., 2008; Lau and Lennon, 2011; Barberán et al., 2015). Therefore, some scholars suggest that the soil microbial community structure and composition are predominantly influenced by the tree species in forest ecosystem (Scheibe et al., 2015; Urbanova et al., 2015).

In a previous study, the effects of afforestation with single and mixed tree species on the soil microbial community composition was analyzed using phospholipid fatty acid analysis (PLFAs), but this method could not provide enough information at the genus and species levels (Gunina et al., 2017). Therefore, an understanding of the effects extent of afforestation using single and mixed tree species on the soil bacterial community at different taxonomic levels is lacking. In this study, a 16S rRNA high-throughput gene sequencing technique was used to analyze the soil bacterial community composition and structure at different taxonomic levels. We hypothesized that the soil bacterial community composition and diversity following afforestation with single and mixed tree species are mainly affected by tree species composition and tree traits. In particular, our study focused on the following: (i) changes in the composition, diversity and richness of the soil bacterial community following afforestation with different tree species and (ii) the effects of tree species and soil properties on the soil bacterial community composition at different taxonomic levels on the Loess Plateau.

2. Materials and methods

2.1. Study site and sampling

The study area was located at the Maliantan valley of Yongshou County in the southern region of the Loess Plateau of China (108°05′–108°10′E, 34°47′–34°51′N). The climate in this region is considered as temperate semiarid; the mean annual temperature is 10.8 °C, and the mean annual precipitation is 605 mm. The elevation ranges from 1123 m to 1417 m, and the soil type is loessial (Ferralsols). The mean temperature and total precipitation in 2016 were 12.8 °C and 605.8 mm, respectively. Homogeneity in the geographical features, soil types and climatic conditions ensured that changes in the soil properties and bacterial communities were mainly driven by the establishment and development of different forest types.

Six types of forest stands afforested on cropland were chosen for this study: *Robinia pseudoacacia* (RP); *Platycladus orientalis* (PO); *Pinus tabuliformis* (PT); *Robinia pseudoacacia* and *Platycladus orientalis* (RPPO); *Robinia pseudoacacia* and *Pinus tabuliformis* (RPPT); and *Platycladus orientalis* and *Pinus tabuliformis* (POPT) (Table 1). The stand ages of the forests ranged from 23 to 25 years, and the slope gradients of cropland and forestland sites were less than 7°. The averages of soil bulk density, water content and pH among the cropland and six forest types ranged from 1.19 to 1.44, from 8.42% to 17.94% and from 8.17 to 8.65, respectively (Table S1). The main crops on the cropland prior to afforestation were maize and wheat.

The borders among forest sites were clear due to the high landscape fragmentation of the cropland. Three replicated plots (20 m × 20 m) per forest type and cropland were chosen for measuring vegetation and soil properties in August 2016. The typical distance between single and mixed forests was more than 1 km, and the distance between replicates of the same tree species exceeded 100 m. The diameter at breast height (DBH) and height were measured for all the trees with a DBH > 5 cm in the plots. Five 1 m × 1 m subplots were randomly established to measure the herbaceous plant composition. Nine soil cores, which were collected using a stainless steel corer that was 5 cm in diameter, were collected along an S-shaped pattern and mixed together into one soil sample. After removing the plant roots, stones and litter, each sample was divided into three subsamples: one was immediately stored at −80 °C for DNA analysis; one was stored at 4 °C for nitrate nitrogen (NO_3^-), ammonium nitrogen (NH_4^+), microbial biomass C (MC) and pH analysis; and the last one was air dried, ground and passed through a 2-mm sieve for soil organic C (SOC), total N (TN), TP and AP contents analysis.