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Tree species composition influenced microbial diversity and nitrogen availability in rhizosphere soil

D. Liu[1,2,3], Y. Liu[1,2], S. Fang[1,2], Y. Tian[1,2]

[1]*Collaborative Innovation Center of Modern Forestry in Southern China, Nanjing Forestry University, Nanjing, P.R. China*
[2]*College of Forestry, Nanjing Forestry University, Nanjing, P.R. China*
[3]*Nanjing Forest Police College, Nanjing, P.R. China*

ABSTRACT

A greenhouse experiment was conducted to evaluate effects of monoculture and mixed planting of three tree species on microbial diversity and nitrogen (N) availability in rhizosphere and bulk soils. Six treatments with poplar, willow, and alder mono- or mixed seedlings were grown in the rhizoboxes and both rhizosphere and bulk soils were sampled and analysed after eight-month growth. Microbial diversity in rhizosphere soil was significantly higher than in bulk soils based on denatured gradient gel electrophoresis (DGGE) fingerprinting of 16S- or 18S-rRNA gene fragments of soil microbial community. Tree species composition significantly influenced microbial diversity index and nitrogen contents in the rhizosphere soil, with the highest values of genotypic richness, Shannon diversity index and inorganic nitrogen contents were observed in rhizosphere soils of poplar-alder mixture. Shannon diversity indices of bacteria and fungi in the rhizosphere soils were positively and significantly correlated with nitrogen contents. Alder addition significantly improved genotypic richness, microbial diversity index and nitrogen availability in the rhizosphere soils, suggesting that adding N-fixing alder into poplar plantations is a good option in the practice.

Keywords: ecosystem; nutrient cycling; *Populus*; *Salix*; *Alnus*

Enhanced microbial quantities and activities by rhizosphere effects are important to ecosystem functioning, nutrient cycling and pollutant degradation in natural ecosystems as well as in contaminated environments (Wenzel 2009). Knowledge of rhizosphere effects on nutrient cycling associated with different species is fundamental for characterizing nutrient acquisition of different tree species and for interpreting the influence of tree species on soil processes. However, only a few attempts have been made to compare the rhizosphere soil microbial diversities and nutrient availability of different tree species and even less for different tree species compositions (Ushio et al. 2010, Liu et al. 2014). Different plant species can promote proliferation of different microbial communities by releasing different amount and types of root exudates (Nguyen 2003), while coexistence of multiple plant species may enhance the complexity of soil microorganism communities by increasing the heterogeneity of root exudates and carbon that are contributed from roots and decomposing litter (Stephan et al. 2000). It is well established that microbial abundance, composition and diversity can fundamentally alter soil processes that lead to changes in soil nutrient availability. Therefore, there is a strong need for more studies on rhizosphere soil microbial diversities and rhizosphere processes under mono- or mixed plantations for providing a more realistic view of rhizosphere effects on soil microbial characteristics and understanding microbial roles in nutrient availability.

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Poplar is one of the most promising short rotation plantation forest species and at present, the area of poplar plantation in China has reached about 8.5 million ha. However, monoculture plantations lead to declines in soil fertility and plant biomass productivity over successive rotations (Mao et al. 2010). Therefore, mixed plantations have been recommended to combat soil degradation and maintain productivity of poplar plantations. In this study, a greenhouse experiment was conducted to evaluate the effect of monoculture and mixed plantations of three tree species on rhizosphere microbial diversity and nitrogen (N) availability. We hypothesized that rhizosphere effects on soil microbial diversity and nitrogen availability would vary with tree species and their compositions due to differences in intrinsic biological characteristics and complementarities of species traits (especially ecological characteristics). The specific objectives were to: (1) assess effects of tree species compositions on diversity of bacteria and fungi and nitrogen availability in rhizospheric and non-rhizospheric soils, and (2) discuss the relationships between soil microbial diversity and nitrogen availability.

MATERIAL AND METHODS

Rhizobox design and the planting experiment. The rhizoboxes were made of two top-open black Plexiglas compartments separated by a polyamide membrane with 30 μm pore-diameters and 75 μm thick (Fang et al. 2013). The plant-root compartment was 250 mm wide and had an internal volume of 22.5 L. The sampling compartment, where rhizosperic and bulk soils were sampled, was 50 mm wide and had an internal volume of 4.5 L. The soil used in the rhizoboxes had pH 7.2 and content of organic carbon 19.0 g/kg, total nitrogen 1.93 g/kg, total phosphorus 0.40 g/kg, and total potassium 6.96 g/kg. The two compartments of the rhizoboxes were packed with the soil at bulk density of 1.05 g/cm^3 after the soil was sieved through a 2 mm mesh.

Poplar (*Populus* × *euramericana* cv. Nanlin-895), willow (*Salix* × *jiangsuensis* cv. J799), and alder (*Alnus trabeculosa*) were selected in this study and six planting patterns were designed as poplar monoculture (P); willow monoculture (W); alder monoculture (A); poplar-willow mixture (P × W, 1:1); poplar-alder mixture (P × W, 1:1); and poplar-willow-alder mixture (P × W × A, 1:1:1). The unrooted cuttings of poplar and willow, and 1-year-old bare-rooted seedlings of alder were used in the experiment and 12 trees were planted in two rows with spacing of 5 cm × 10 cm in the plant-root compartment of each rhizobox (Fang et al. 2013). For treatments that employed more than one species, different tree species were placed in alternative spots within rows. Each treatment was replicated three times. Plants were grown in a greenhouse with night and day temperatures of 10°C and 35°C and soil water content maintained at 60% water holding capacity.

Soil sampling and preparation. After eight months of growth in the rhizoboxes, the two compartments of each rhizobox were separated carefully. Based on the results from Fang et al. (2013), in the sampling compartments, soils in contact with root mats were sliced vertically approximately at 4 mm width and sampled as rhizosphere soils, while the soils at the distance of 10–50 mm to the root mats were sampled as bulk soils (non-rhizosphere soil). Each sample was mixed thoroughly, sieved with a 2 mm mesh and then divided into two portions. One was air-dried for chemical analysis, and the other was stored at –80°C before subsequent analysis of microbial diversity.

DNA extraction, polymerase chain reaction (PCR) amplification and denatured gradient gel electrophoresis (DGGE) analysis. A protocol modified by Solaiman and Marschner (2007) was used in soil total DNA extraction. Briefly, the DNA was liberated from the microbial cells by homogenization with freezing-thawing in the presence of phosphate buffer and sodium dodecyl sulfate (SDS). A successful DNA extraction was detected by electrophoresis on 1.0% (w/v) agarose gels.

PCR amplification of bacterial 16S rRNA gene was performed using the universal primers pair 341F containing a GC clamp and 907R (Muyzer et al. 1998). To amplify specific 18S rRNA of fungi from soils for DGGE, nested PCR was performed (Solaiman and Marschner (2007). The PCR reaction mixture was set up in 50 μL, containing 1 μmol of $MgCl_2$, 200 μmol of each dNTP, 0.5 μmol of each primer, 5 μL of template DNA, 5 μL of 10 × PCR buffer and 2.5 U of Ex Taq^{TM} polymerase (TaKaRa Inc., Dalian, China). PCR reactions were performed on a MyCycler Thermal Cycler (Bio-Rad, Hercules, USA). The cycling conditions of bacteria were as follows: 94°C for 4 min, then 94°C for 40 s, then 46°C for 40 s, then

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72°C for 40 s, for 38 cycles, followed by 72°C for 10 min, and finally hold at 4°C constant. A modified touch-down PCR procedure was used for fungal cycling amplification (Solaiman and Marschner 2007). All PCR products were electrophoresed on 1.5% (w/v) agarose to verify their size and quality.

The PCR amplicons were separated by DGGE using a D-Code universal mutation detection system (Bio-Rad, Hercules, USA) according to the manufacturer's instructions. Briefly, 20 mL of each PCR product was loaded onto an 8% (w/v) polyacrylamide gel (acrylamide:bisacrylamide = 37.5:1) with a denaturant gradient of 35–65% for bacteria and 18–38% for fungi (100% denaturant contains 7 mol/L urea and 40% deionized formamide). Electrophoresis was then conducted at 60°C in 1 × TAE buffer (40 mmol Tris-acetate, 1 mmol Na-EDTA, pH 8.0) at 75 V for 16 h. After DGGE, the gels were stained with ethidium bromide for 30 min and then scanned with a Bio-Rad image scanner.

Chemical analysis. Soil total nitrogen was determined by the Kjeldahl method (Bremner and Mulvancey 1982), and measured using an autoanalyzer (Auto Analyzer III, Bran + Luebbe, Hamburg, Germany), while soluble organic nitrogen (SON) was determined using alkaline persulfate oxidation and measured by dual-wavelength ultraviolet spectrophotometry (Cabrera and Beare 1993, Liang et al. 2011). Soil inorganic N (NH_4^+-N and NO_3^--N) was extracted with 2 mol/L KCl (1 g of soil to 5 mL KCl solution ratio), and quantified colorimetrically with an autoanalyzer (Auto Analyzer III, Bran + Luebbe GmbH, Hamburg, Germany) (Liu et al. 2014).

Data analyses. The banding patterns of the DGGE profile were analysed with the Quantity One software (version 4.5, Bio-Rad, Hercules, USA). The position and intensity of each band were determined automatically. The density value of each band was divided by the average band density of the lane to minimize the influence of loaded DNA concentrations among samples. Based on DGGE fingerprints, bacterial and fungal diversity was valued by scoring the presence (1) or absence (0) of individual bands in all lanes in a binary matrix. Genotypic richness and Shannon diversity index were used for characterizing genotypic diversity of soil microbial communities. Similarity coefficient was calculated using unweighted pair group method with arithmetic means by the Quantity One software.

Statistical analyses were performed using the SPSS 13.0 statistical software program (SPSS Inc., Chicago, USA). A two-way analysis of variance (ANOVA), followed by the Duncan's multiple-range test, was conducted to compare the effects of tree species compositions on genotypic richness, Shannon diversity index and nitrogen contents among the treatments. All statistical analyses were carried out at $P < 0.05$. Relationships between microbial diversity indices and selected soil parameters were evaluated using the Pearson's correlation analysis.

RESULTS AND DISCUSSION

Effects of tree species compositions on microbial diversity. Overall bacterial and fungal diversity in rhizosphere soils was significantly higher than in non-rhizosphere soils for all treatments tested

Table 1. Genotypic richness (S) and Shannon diversity index (H) of bacterial and fungal communities in the rhizosphere and bulk soils under planting patterns with different tree species composition

Microbial community	Index	Planting pattern						Sampling position	
		PAW	P	PA	W	PW	A	rhizosphere	bulk soils
Bacteria	S	16^{c}	19^{b}	24^{a}	16^{c}	14^{d}	17^{bc}	18^{A}	11^{B}
	H	2.726^{c}	2.851^{b}	2.891^{a}	2.732^{c}	2.672^{d}	2.754^{b}	2.771^{A}	2.530^{B}
Fungi	S	11^{b}	11^{b}	13^{a}	10^{bc}	9^{c}	10^{bc}	11^{A}	7^{B}
	H	2.594^{ab}	2.660^{a}	2.668^{a}	2.548^{b}	2.398^{c}	2.567^{b}	2.573^{A}	2.319^{B}

PAW – poplar-willow-alder mixture; P – poplar monoculture; PA – poplar-alder mixture, W – willow monoculture; PW – poplar-willow mixture; A – alder monoculture. Data for sampling positions represent the mean values of six planting patterns. For each index, significant differences among the rhizosphere soils of six tree species compositions are indicated by different lowercase letters ($P < 0.05$), while significant differences between rhizosphere and bulk soils are indicated by different capital letters ($P < 0.05$)

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Table 2. Similarity coefficient (%) of bacterial and fungal communities in the rhizosphere soil among different tree species compositions (n = 18)

Microbial community	Planting pattern	PAW	P	PA	W	PW
	P	54.1				
	PA	63.7	78.5			
Bacteria	W	38.5	57.1	46.8		
	PW	67.1	63.2	62.1	75.5	
	A	61.2	62.4	79.6	49.7	57.9
	P	68.7				
	PA	75.6	77.6			
Fungi	W	52.4	48.3	39.8		
	PW	53.2	63.4	47.5	59.8	
	A	68.7	39.6	68.9	39.7	41.2

PAW – poplar-willow-alder mixture; P – poplar monoculture; PA – poplar-alder mixture, W – willow monoculture; PW – poplar-willow mixture; A – alder monoculture

(Table 1). Compared to the non-rhizosphere soils, mean genotypic richness (S) and Shannon diversity index (H) of bacteria in the rhizosphere increased by 63.6% and 9.5%, respectively, while the values of fungi in the rhizosphere were enhanced by 85.7% and 10.9%.

Tree species compositions significantly affected the values of S and H in rhizosphere soils (Table 1). The highest values of S and H in the rhizosphere soils were observed in the planting pattern of poplar-alder mixture for both soil bacterial and fungal communities, followed by poplar monoculture, and the lowest values of S and H were detected in poplar-willow mixture. However, similarity coefficients of soil bacterial community were 78.5% between P and PA, 63.2% between P and PW, and 53.1% between P and PAW, respectively, whereas similarity coefficients of soil fungal community were 77.6% between P and PA, 63.4% between P and PW, and 68.7% between P and PAW (Table 2). Alder addition significantly improved S values of bacteria and fungi as well as H value of bacteria in rhizosphere soils (Table 1). Compared with poplar monoculture, S values of bacteria and fungi in the rhizosphere soils of poplar-alder mixture increased by 26.3% and 18.2%, respectively. However, willow addition significantly decreased the S and H values of bacteria and fungi in rhizosphere soils. Compared to poplar monoculture, S values of bacteria and fungi in the rhizosphere of poplar-willow mixture reduced by 26.3% and 18.2%, respectively, while the H values of bacteria and fungi turned down by 6.3% and 9.9%.

Results from this present study illustrated that composition and complementarity of species traits are important factors influencing rhizosphere microbial community, in agreement with the result from Stephan et al. (2000). Our results also showed that there existed no close relationship between plant diversity per se and microbial diversity in rhizosphere soils, supporting the idea that a highly productive or keystone plant species present in the community results in greater influence over soil functions than the contribution of additional diversity per se (Ladygina and Hedlund 2010).

Based on DGGE fingerprinting of the whole microbial diversity, the number of electrophoretic bands, strength and mobility varied among different treatments (Figure 1). However, several studies have shown that microbial genes with less than 1% of the total DNA are not amplified sufficiently to visualize as the bands on a DGGE gel (Marzorati et al. 2008). Therefore, the bands evaluated from a DGGE gel in this study have their intrinsic limitation to elucidate only the dominant members in the microbial communities in the studied soils.

Effects of tree species compositions on nitrogen availability. Contents of total N, soluble organic N and inorganic N (NH_4^+-N and NO_3^--N) in the rhizosphere soils showed a significant variation among the different planting patterns (Table 3, $P < 0.05$). At the end of the 8-month growing period, the highest contents of total N and soluble organic N were not found in the rhizosphere soils of mixed planting patterns but in monoculture. For example, the content of soluble organic N in rhizosphere soil of poplar monoculture was 15.4, 7.4 and 8.6% higher than these of the poplar-alder mixture, poplar-willow mixture and poplar-alder-willow mixture, respectively. However, the greatest content of NO_3^--N was observed in the rhizosphere soil of the poplar-alder mixture (Table 3), which is 1.8, 1.2 and 2.3 times greater than in the soils of poplar monoculture, poplar-willow mixture and poplar-alder-willow mixture, respectively. NH_4^+-N accounted for more than 88% of inorganic N in the rhizosphere soils after the 8-month growing period, while the content of inorganic N (NH_4^+-N and NO_3^--N) in the rhizosphere soils by planting

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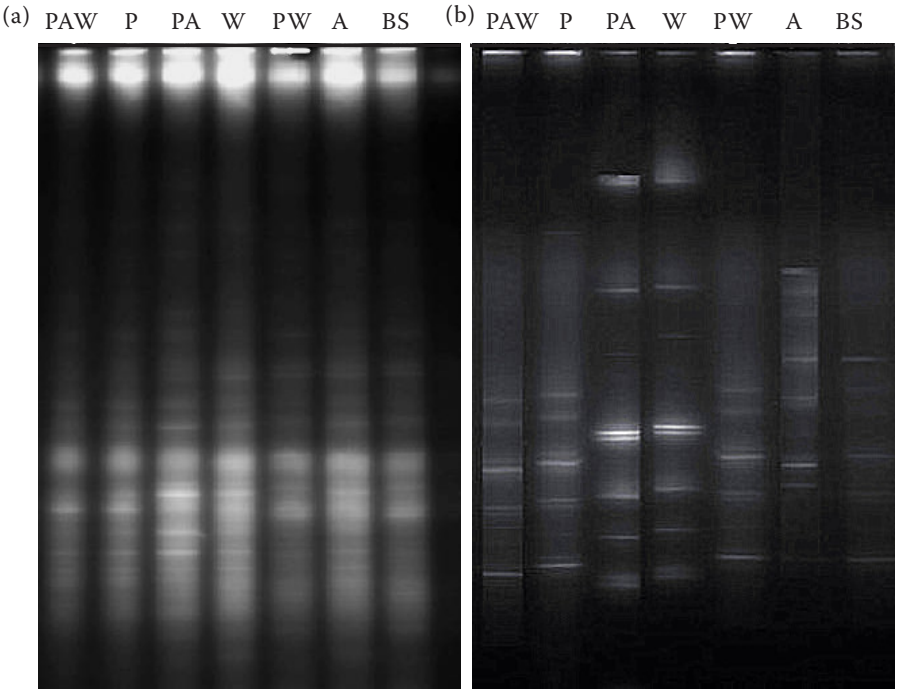

Figure 1. Typical denaturing gradient gel electrophoresis (DGGE) fingerprinting of bacteria (a) and fungi (b). PAW – poplar-willow-alder mixture; P – poplar monoculture; PA – poplar-alder mixture; W – willow monoculture; PW – poplar-willow mixture; A – alder monoculture; BS – bulk soil

pattern were in the order: A > PA > PAW > W > PW > P. The high inorganic N content detected in this study was under alder mono- or mixed culture, indicating that alder could play a vital role in inducing higher N availability in the rhizosphere soil.

Most researches on plant N uptake in terrestrial ecosystems have focused on ammonium and nitrate (Blumfield et al. 2006, Chen and Xu 2008). However, over 80% of N in soil is present in organic form (Schulten and Schnitzer 1998), and soluble organic N (SON) has received little attention due to the uncertainty in its availability for direct uptake by plants and its ecological significance (Chen and Xu 2008). This study indicated that alder addition obviously reduced the SON in the rhizosphere soils (Table 3), suggesting that alder addition may facilitate transformation of SON into inorganic N. However, the evidence of direct utilization of soil SON by forest plants is still lacking, and the extent of the contribution of SON to forest plants is far from certain (Chen and Xu 2008).

Correlation between soil microbial indices and nitrogen contents. A significant correlation between microbial diversity indices and N contents

Table 3. Nitrogen (N) contents in the rhizosphere soil (sampled at 0–4 mm) among different tree species compositions

Planting pattern	Total N (g/kg)	Soluble organic N	NH_4^+-N	NO_3^--N
		(mg/kg)		
PAW	1.99 ± 0.11^{ab}	31.02 ± 2.84^{cd}	10.94 ± 0.93^{ab}	0.62 ± 0.12^{c}
P	2.06 ± 0.12^{a}	33.68 ± 3.24^{b}	9.67 ± 0.89^{b}	0.79 ± 0.13^{b}
PA	1.91 ± 0.17^{bc}	29.19 ± 2.59^{de}	10.93 ± 1.25^{ab}	1.46 ± 0.07^{a}
W	2.03 ± 0.16^{a}	35.74 ± 2.72^{a}	11.03 ± 0.81^{ab}	0.44 ± 0.17^{d}
PW	1.95 ± 0.12^{b}	31.36 ± 2.96^{c}	9.88 ± 1.03^{b}	1.16 ± 0.09^{ab}
A	1.94 ± 0.15^{b}	28.09 ± 2.11^{e}	12.87 ± 1.02^{a}	1.22 ± 0.12^{a}

PAW – poplar-willow-alder mixture; P – poplar monoculture; PA – poplar-alder mixture, W – willow monoculture; PW – poplar-willow mixture; A – alder monoculture. Data represent the means ± standard deviation. The same letters within each column indicate no significant differences at the 0.05 level of the mean values

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in the rhizosphere soils was detected. Overall, Shannon diversity indices of bacteria and fungi in the rhizosphere soils were positively correlated with total N ($r > 0.69$; $P < 0.01$), SON ($r > 0.66$, $P < 0.01$), NH_4^+-N ($r > 0.79$; $P < 0.01$) and NO_3^--N ($r > 0.64$; $P < 0.05$), indicating that soil N availability was associated with active microorganisms in the soil. Microbial characterisation of tree rhizosphere provides vital information relating to evaluating soil nutrient status (Sinha et al. 2009, Liu et al. 2012), and soil enzymes play a fundamental role in establishing biogeochemical cycles and facilitate the development of plants.

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Corresponding author:

Prof. Dr. Shengzuo Fang, Nanjing Forestry University, College of Forest Resources and Environment, 210 037 Nanjing, P.R. China; e-mails: fangsz@njfu.edu.cn; fangsz@njfu.com.cn