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RESEARCH ARTICLE

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Diversity of soil microorganisms in natural *Populus euphratica* forests in Xinjiang, northwestern China

Abstract To better understand the distribution of soil microorganisms in *Populus euphratica* forests in Xinjiang, northwestern China, we studied and compared the populations and numbers of bacteria, fungi and actinomycetes in the soil at four different age stages of natural *P. euphratica* forests, i.e., juvenile forests, middle-aged forests, over-mature forests and degraded forests. Results showed that there were clear differences in the amount of microorganism biomass and composition rates across the four forest stages. Dominant and special microorganisms were present in each of the four different soil layers. The vertical distribution showed that the microorganism biomass decreased with increasing soil depth. The population of microorganisms was the lowest at 31–40 cm of soil depth. The microorganisms consisted of bacteria, actinomycetes, as well as fungi. Bacteria were the chief component of microorganisms and were widely distributed, but fungi were scarce in some soil layers. *Aspergillus* was the dominant genus among the 11 genera of fungi isolated from the soil in different age stages of *P. euphratica* forests.

Keywords soil microorganism, fungi, *Populus euphratica*, Xinjiang

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1 Introduction

Populus euphratica Oliv., as one of the most primitive tree species, is characterized by genetic traits of tolerance to hot, cold, salty, arid and sandy environmental conditions. It can survive in arid areas, especially in deserts, and can constitute forest communities in dry, nutrient-depleted deserts and the Gobi (Cheng and Liu, 2004). This poplar exists on three continents: Europe, Asia and Africa. It is largely found in the arid and semi-arid areas of Mediterranean, in northwestern China and Mongolia. The area of *P. euphratica* forests in Xinjiang Province accounts for 91% of the total natural *P. euphratica* forests in China. About 95% are well centralized in the Tarim River Basin in Xinjiang. *P. euphratica* forests play an important role in protecting biodiversity, controlling desert expanse and improving environment in the arid landscape of northwestern China. Therefore, it has been regarded as one of the most valuable tree species to be protected, as urged by the FAO (Wang et al., 1995). However, the ecology and environment of *P. euphratica* natural reserves are deteriorating annually due to factors such as anthropogenic activities and pests. With the exploitation of oil and gas fields in the Tarim Basin, the number of pests imported into the area by means of wooden packing material has increased and escalated risk in the disaster-prone stands of *P. euphratica*. It is an important task to protect the healthy development of *P. euphratica* forests, in particular, to protect the microorganisms in these forests. To provide an essential and scientific basis, as well as to reveal changes in soil fertility and nutrients in *P. euphratica* forests, we studied the composition, number and diversity of soil microorganism communities in this region.

2 Study area

The study site was located in the Nature Reserve of *P. euphratica* in the Bayingolin Mongolia Autonomous Region of Xinjiang. The region (40°55′–41°17′N, 84°15′–85°30′E) is on the northern edge of the Tarim

Basin. The average annual temperature ranges from 10.6°C to 11.5°C, while the amount of annual precipitation is about 25 to 75 mm. The average amount of annual transpiration is 2000 to 3000 mm, while the relative air humidity is only from 40% to 50%. The maximum wind speed is 27 m/s throughout the year, and the number of days with an over 8-grade wind can range from 41 to 46 per year. *P. euphratica* is the main vegetation species in this area. Other vegetation species in the area include *P. pruinosza*, *Tamarix ramosissima*, *Apocynum venetum*, *Halostachys caspica*, *Halimodendron halodendron*, *Alhagi pseudalhagi* and *Glycyrrhiza inflate*. Xerophiles, sand plants and halophytes are dominant species among the vegetations. Our study site consisted of a typical and secondary salinized soil type (Wang et al., 1995; Cheng and Liu, 2004).

3 Materials and methods

3.1 Data collection

Four age stages of natural *P. euphratica* forests in the Tarim Basin (Luntai County, Korla City and Yuli County), consisting of juvenile forests, middle-aged forests, over-mature forests and degraded forests, were selected for the study. Two sample plots were established for each age class. The area of each sample plot was 30 m × 30 m. Three soil profiles were marked randomly in each sample plot. The soil samples were collected from four vertical layers of soil, i.e., at 0–10 cm, 11–20 cm, 21–30 cm and 31–40 cm depths (Shao et al., 1995). The soil samples of the same layer in each soil profile were mixed and analyzed in our laboratory. The four age stages of *P. euphratica* forests were described as follows (Wang et al., 1995):

1) Juvenile forests: The trees were from the seedling stage of a 10-year old, about 3 m tall, and with diameter of about 4 cm. The leaf shape was lanceolate.

2) Middle-aged forests: The trees were 11–30 years old. The tree height was about 3–8 m (even up to 10 m) with diameter of 4–10 cm. Leaf shapes were variable.

3) Over-mature forests: The trees were 31–80 years old. The tree height ranged from 14–20 m, with diameter of 30–70 cm. The trunk was crude, while the bark was thick and part of them peeled off automatically. Heart wood-rot could be found randomly over the whole trunk.

4) Degraded forests: The trees were 80–100 years old. Leaves in the upper parts of trees had faded or died. Fallen trunks and standing dead trees could be always found.

3.2 Methods

3.2.1 Soil water content

Soil samples weighing 10 g were dried at 105°C for eight hours. The baked soil samples were stored in a desiccator until subjected to refrigeration and weighed. Water content of the soil samples was calculated by the following formula: soil water content = ((fresh soil weight − dry soil weight)/fresh soil weight) × 100% (Department of Microbiology, Institute of Soil Science, Chinese Academy of Sciences, 1985).

3.2.2 Soil microorganisms

Preparation of culture media: Bacteria, actinomycetes and fungi were cultured using a medium of beef extract peptone, a No. 1 Gause synthetic medium and a Martin medium respectively.

Method of diluted separation of soil microorganism: A 10 g soil sample was mixed with 90 mL of sterile water. Then it was vibrated for 10 min in a beater. Next, the concentration of the diluted soil suspension of 10^{-2} was made by mixing 1 mL suspended liquid soil with 9 mL sterile water (Tian and Liang, 1996; Barnett and Hunter, 1997; Luo et al., 2002; Chen 2004; Feng et al., 2005; Liu et al., 2006). 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} diluted solutions were obtained respectively according to the same method. The diluted 0.2 mL suspensions for each solution of 10^{-3}, 10^{-4} and 10^{-6} were respectively cultured on media for obtaining fungi, actinomycetes and bacteria. Each diluted solution was replicated two to four times and cultured at 28°C. The number of colonies was counted after 5 to 10 days. The number of soil microorganisms was calculated by the formula: number of colonies per 1 g dry soil (cell/g) = average number of colonies × dilution density/(1 − soil water content). Fungi, cultured in a PDA medium, were identified by their morphological characteristics (Gu et al., 2000).

4 Results and analysis

4.1 Distribution of soil microorganisms

The number sequence of soil microbes at the different age stages of *P. euphratica* forests was: bacteria > actinomycetes > fungi. The number of soil microorganisms in juvenile forests (1.7×10^8 cell/g) was the largest. Fewer soil microbes were found in middle-aged forests, accounting for 50% of those in juvenile forests. The number of soil microorganisms in degraded forests was the smallest, less than 6.8×10^7 cell/g. In the four age stages of the forests, the number sequence of bacteria and actinomycetes was: juvenile forests > over-mature forests > middle-aged forests > degraded forests. For the number of fungi, the order from high to low was: middle-aged forests > juvenile forests > over-mature forests > degraded forests (Table 1).

Table 1 Distribution of soil microorganisms at different age stages of *P. euphratica* forests (unit: cell·g^{-1})

stages of forests	bacteria		actinomycetes		fungi		total number ($\times 10^7$)
	number ($\times 10^7$)	proportion/%	number ($\times 10^5$)	proportion/%	number ($\times 10^4$)	proportion/%	
juvenile forests	16.81	97.34	46.37	2.69	10.88	0.63	17.27
middle-aged forests	8.07	97.70	14.95	1.81	52.17	6.32	8.26
over-mature forests	11.81	98.01	24.27	2.01	0.77	0.06	12.05
degraded forests	6.76	99.85	1.29	0.19	0.13	0.02	6.77

4.2 Vertical distribution of soil microorganisms

In juvenile and degraded forests, the total number of soil microorganisms decreased with increasing soil depth. The number of microorganisms was the lowest at a soil depth of 31–40 cm in all four age stages. The sequence in terms of the number of soil microbes in different soil profiles at the same age stages of *P. euphratica* forests was: bacteria > actinomycetes > fungi (Table 2).

The distribution of three kinds of soil microorganisms was different in the four age stages of forests. In juvenile forests, the numbers of bacteria and fungi at a depth of 0–10 cm were larger than those in the other three soil layers. The number of actinomycetes (19.29×10^5 cell/g) was the largest at 21–30 cm of soil depth. In middle-aged forests, the sequence of soil depth in terms of the number of bacteria was: 11–20 cm > 0–10 cm > 21–30 cm > 31–40 cm. Actinomycetes were distributed widely at 21–30 cm of depth and existed the least in the 31–40 cm soil layer. Fungi were concentrated in the 0–10 cm soil layer (49.83×10^4 cell/g). In over-mature forests, the number of fungi was less than 0.5×10^4 cell/g. The number of bacteria in the 0–10 cm and 31–40 cm soil layers were less than in the other soil layers. Actinomycetes were concentrated in the 11–20 cm soil layer (22.98×10^5 cell/g). In degraded forests, the number of all soil microorganisms was small and in some layers actinomycetes and fungi could not even be found.

4.3 Species composition of soil microorganisms

More species of soil microorganisms were found in juvenile and middle-aged forests than those in over-mature and degraded forests (Fig. 1). For bacteria, the number of species in juvenile forests was similar to that in middle-aged forests. The smallest number of species was found in over-mature forests, accounting for 33% of the number in middle-aged forests. For actinomycetes, the number of species was the largest in juvenile forests and the smallest in degraded forests. For fungi, the largest number of species was found in middle-aged forests. Only one fungi species was found in degraded forests.

4.4 Composition and relative density of soil fungi

Fungi belong to the most important components in soil microbial biomass of forests (Tian and Liang, 1996). *Aspergillus* was the dominant genus among the 11 genera of fungi isolated from soils at the different age stages of *P. euphratica* forests (Table 3). In juvenile forests, five genera of fungi were isolated and identified from the soil; *Coprinus*, *Aspergillus* and *Alternaria* were the dominant genera with relative densities of 41.28%, 29.36% and 16.51% respectively. In middle-aged forests, 11 genera of fungi were obtained from the soil. *Aspergillus*, *Candida*, *Penicillium* and *Hyalodendron* were the dominant genera. In particular, the relative density of *Aspergillus* was the

Table 2 Vertical distribution of soil microorganisms at different age stages of *P. euphratica* forests (unit: cell·g^{-1})

stages of forests	soil layer/cm	bacteria ($\times 10^7$)	actinomycetes ($\times 10^5$)	fungi ($\times 10^4$)	total number ($\times 10^7$)
juvenile forests	0–10	5.57	10.38	4.27	5.67
	11–20	3.31	9.54	2.08	3.40
	21–30	4.64	19.29	1.97	4.83
	31–40	3.29	7.16	2.56	3.37
middle-aged forests	0–10	2.47	2.71	49.83	2.54
	11–20	3.01	3.69	1.98	3.05
	21–30	2.14	7.10	0	2.21
	31–40	0.45	1.45	0.36	0.46
over-mature forests	0–10	2.22	0.38	0.38	2.23
	11–20	4.98	22.98	0.13	8.82
	21–30	3.57	0.39	0.26	7.44
	31–40	1.04	0.52	0	1.05
degraded forests	0–10	3.07	0.90	0	3.08
	11–20	1.08	0.39	0	1.08
	21–30	1.99	0	0.13	1.99
	31–40	0.62	0	0	0.62

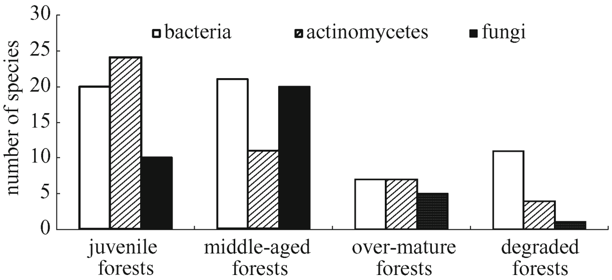

Fig. 1 Species composition of soil microorganisms at different age stages of *P. euphratica* forests

largest. In over-mature forests, four genera of fungi were isolated from the soil and in degraded forests only one species was identified.

Table 3 Composition and relative density of soil fungi at different age stages of *P. euphratica* forests (unit: %)

genera	juvenile forests	middle-aged forests	over-mature forests	degraded forests
Aspergillus	29.36	30.82	33.33	100
Alternaria	16.51	7.53	33.33	–
Penicillium	–	14.38	16.67	–
Paecilomyces	–	1.37	–	–
Phoma	–	2.74	–	–
Meria	–	2.05	–	–
Trichoderma	–	1.37	–	–
Candida	–	18.49	16.67	–
Coprinus	41.28	2.74	–	–
Hyalodendron	10.09	17.81	–	–
no-identification	2.75	0.68	–	–

Note: Relative density = number of each species of microbes/total number of microbes × 100%.

We analyzed the distribution frequency of fungi in the different soil layers. Results showed that 11 genera of fungi were isolated from the 0–10 cm soil layer. *Aspergillus*, *Alternaria* and *Hyalodendron* were the most frequently occurring species in this layer, while *Candida* was found only in this layer. Analysis of the frequency of fungi in the other soil layers showed that *Aspergillus*, *Penicillium* and *Coprinus* were the dominant genera in the 11–20 cm soil layer. *Hyalodendron* and *Coprinus* were frequently isolated in the 21–30 cm soil layer. Three genera of fungi were found at 31–40 cm of depth, where *Coprinus* was the dominant genus (Table 4).

Table 4 Vertical distribution of soil fungi in *P. euphratica* forests

genera	0–10 cm	11–20 cm	21–30 cm	31–40 cm
Aspergillus	+++	++++	++	+
Alternaria	++	+	–	–
Penicillium	+	++	+	–
Paecilomyces	+	–	–	–
Phoma	+	–	–	–
Meria	+	–	–	–
Trichoderma	+	+	–	–
Candida	++	–	–	–
Coprinus	+	++	+++	++++
Hyalodendron	++	+	+++	++
No-identification	+	–	++	–

Note: "++++"represents isolation frequency over 50%; "+++" a frequency from 30% to 50%; "++" a frequency from 10% to 30%; "+" a frequency of less than 10%.

5 Conclusions

1) The natural *P. euphratica* forests in the dry desert areas of northwestern China are located on the edge of the Taklimakan Desert. Soil in these forests belongs to the transition zone between oasis and desert regions. Results showed that the number of soil microorganisms in these forests was greater than that of desert regions, due to less water and organic soil matter that limited the activity of microorganisms in the desert regions of the Tarim Basin. However, vegetation and ox and sheep droppings in *P. euphratica* forests were abundant enough for soil microorganisms to survive. Furthermore, the sequence of soil microorganism biomass in the forests and desert regions was similar in terms of their numbers: bacteria > actinomycetes > fungi. Microorganisms in desert regions are concentrated in the lower soil layers. In contrast, microorganisms in the soils of *P. euphratica* forests are largely concentrated in the upper soil layers. This occurrence will improve the level of productivity and prevent soil desertification.

2) Our results showed that the total number of soil microorganisms in natural *P. euphratica* forests, $6.77 - 17.27 \times 10^7$ cell/g, is 6 to 150 times as large as that of *P. euphratica* forests planted in 1982 (Fan et al., 2005). We should not only protect biodiversity of soil microorganisms in natural *P. euphratica* forests, but also strengthen the management of soil fertility in *P. euphratica* plantations to maintain a balanced ecosystem.

3) The number of bacteria accounted for over 97% of the total number of soil microorganisms in natural *P. euphratica* forests. Actinomycetes accounted for about 2% and fungi for less than 1%. Fungi, as heterotrophic microbes, apparently can survive in acidic soils. However, in the alkalescent soil of *P. euphratica* forests the number of fungi is far less than that of the other two soil microorganisms. The number of soil microorganisms in juvenile and over-mature forests was clearly larger than that in middle-aged and degraded forests. The vertical distribution showed that the biomass of microorganisms decreased with increasing depth. Bacteria were the most widely distributed microorganisms. In some layers actinomycetes and fungi did not occur in some age stages of *P. euphratica* forests, owing to different soil water contents and ground vegetations.

4) *Aspergillus* was the dominant genus among the 11 genera of fungi isolated from the soil in different age stages of *P. euphratica* forests. The numbers of species and soil fungi in middle-aged forests were clearly larger

than those of the other age stages. The reason may be that our experimental site was located near the Tarim River and the soil was desalinized by flood. The soil salinity of middle-aged forests was low and soil fungi survived there easily. The function and characteristics of soil fungi still need to be studied.

5) The number of soil microorganisms correlated with richness of plant communities and groundwater level at different age stages of *P. euphratica* forests. While the number of shrub and herbal species were much more abundant in juvenile forests than in the other age stages, the species and number of soil microorganisms were also abundant there. In degraded forests, serious soil erosion, weak trees and extremely scarce ground vegetation have led to an extremely poor habitat for most soil microorganisms.

6) The number of soil microorganism and soil fertility correlated with the health of the forest. Microorganism biomass and soil fertility degraded over forest age. Ground vegetation of degraded *P. euphratica* forests was extremely scarce, far less than in other age stages of *P. euphratica* forests. However, more abundant herbs and shrubs were found in the younger age forests. Therefore, the health of these younger forests was superior to that of the degraded forests, which can be used as an important index to evaluate the condition of the ecosystem and soil degradation of *P. euphratica* forests.

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