Full Length Article

Effects of *Suillus luteus* on Soil Microbial Communities of Two Pines in Inner Mongolia, China

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Abstract

Microbial community functional diversity is a sensitive indicator of soil management. This study aimed to determine the influence of ectomycorrhizal fungi [*Suillus luteus* (L. ex Fr.) S.F. Gray] inoculum on soil microbial community functional diversity of two pines (*Pinus tabulaeformis* and *P. sylvestris* var. *mongolica*). The results showed that all the treatments elevated the Average Well Color Development (AWCD) during the first 96 h of incubation, and the differences among the treatments were obvious. The order of AWCD values of different soil layers in the same tree species is 0−20 cm> 20−40 cm. Both ectomycorrhizal inoculated tree species improved the Shannon diversity index (H), Simpson index (D) and Shannon evenness (E) of soil microbial community in comparison with the control treatments and differ significantly (p<0.05). Principal component analysis demonstrated that the carbon source predominantly used by soil microbes in the control plants were carboxylic acids. With mycorrhizal fungi inoculum, these were replaced by carbohydrates; the community composition of soil microbes can be improved by mycorrhizal fungi inoculum. It was concluded that mycorrhizal fungi inoculum had significant affections on the functional diversity of soil microbial community and enhanced microbial metabolism over control. Also mycorrhizal effect of both afforestation tree species was significant, which indicated that it was very valuable to apply mycorrhizal fungi inoculum to afforestation in arid and barren regions © 2018 Friends Science Publishers

Keywords: Soil microbe; Biolog; Functional diversity; *Suillus luteus*; Energy source

Introduction

Soil microorganisms are important parts of the ecosystem, and the diversity of soil microbial communities as a key indicator to describe their ecological characteristics (Wang *et al.*, 2016). In recent years, it has become a hot research topic in the field of ecology. As the major decomposers of litter, soil microorganisms are the most viable component of the soil. The distribution and activity of soil microorganisms are one of the main bases for comprehensive evaluation of production practices, and their characteristics are affected by hydrothermal conditions, seasonal dynamics and elevation, especially the composition of tree species (Zhang *et al.*, 2010). The response mechanisms of soil microorganisms to vegetation and environmental factors vary with the ecosystem. Therefore, the current research mainly focused on the influence of anthropogenic disturbance of environmental change on soil microorganisms such as vegetation type change (Lu *et al.*, 2012), crop serialization (Francisco *et al.*, 2016), environmental impact (Xia *et al.*, 2015).

Distributed widely throughout the world, pines are well-known ectomycorrhizal trees. *P. tabulaeformis* and *P. sylvestris var. mongolica*, two pine species native to China, are the most widely distributed pines in China (An, 2017).

Due to its drought tolerance and capability of growing in poor soil, these two pines are used as a reforestation tree species. In the past two decades, these two pine species have been widely planted in Inner Mongolia for soil and water conservation and ecological environmental improvement. Unfortunately, without ectomycorrhizae, these two pine seedlings survival after out-planting is poor.

The variation of rhizosphere microbes and mycorrhizal fungi community affects the carbon cycle of plant communities and terrestrial ecosystems as well as the change of the entire terrestrial ecosystem (Wu *et al.*, 2014). Studying the diversity, distribution and function of soil microbes in vegetation is important so as to reveal the relationship among plant-soil-microorganisms and restoration of vegetation in the forest ecosystem.

BIOLOG[TM] is used to test the degree of utilization of a single carbon source by microorganisms, to reflect Community Level Physiological Profiles (CLPPs) as well as to analyze methods that describe the functional diversity of microbial communities (Button *et al.*, 2016). Soil physico-chemical properties and microbial community were changed under different utilization patterns (Mekuria, 2013). One of the important effects of plant on soil environment is to change the characteristics of soil microbial community. Different plant species have different impacts on soil

microbial community (Grayston and Prescott, 2005). The use of BIOLOG to study the effect of different plants on soil microbial biomass under hops was significant. The effects of soybean monoculture on bacterial community were studied to assess the functional diversity of soil microbes in a forest system (Zhu *et al.*, 2013). Research shows that microbial fertilizer also has a strong impact on microbial fertilizer on microbial activity and microbial community diversity in the rhizosphere of wheat growing on the Loess Plateau (Chen *et al.*, 2011b). However, there are few researches on soil microbial communities of afforestation tree species inoculated with mycorrhizal fungi. In this study, Biolog[MT] was used to study soil microbes in *P. tabulaeformis* and *P. sylvestris var. mongolica*. The characteristics of soil microbial community structure in different species of inoculated *S. luteus* were analyzed, providing a scientific basis for vegetation restoration and ecological construction.

Materials and Methods

Experimental Details and Treatments

Site description: The field experiment was conducted at Shengle Economic Development Zone, Horinger County, Inner Mongolia, North China (115°60 E, 42°25 N). Rainfall distribution is uneven, most of which occurred between June and September, annual average precipitation is 415.8 mm, while mean annual temperature is 7−9°C (Shi *et al.*, 2015). The important ecological location and special topography determine the importance of local ecological construction. In recent years, artificial vegetation tree species were dominated by two *Pinus* species mixed with *Armeniaca sibiricat* and *Hippophae rhamnoides* reasonably in order to establish carbon sinks of a high standard for environmental protection. In Shengle Zone, four plots were established by inoculation of ectomycorrhizal fungi preparations in two pines in the nursery.

Soil Sampling

After removing the litter layer, 15 replicate samples were collected in an "S" shape by using a standard soil auger (5 cm inner diameter) at depths of 0−20 cm and 20−40 cm, and then 15 samples of each species were homogeneously mixed on the same layer in an ice box. The samples were sieved through a 2 mm screen and roots and other debris were removed. A portion of each soil sample was immediately transported to the laboratory to determine soil moisture and the soil microbial community diversity was analyzed within 48 h for preservation at 4°C. Soil subsamples were air-dried and stored at room temperature prior to physical and chemical analysis.

Analysis of Soil Physicochemical Properties

Measurements of physicochemical characteristics were carried out on air-dried soil samples as described. Soil pH was measured by a combination glass electrode (soil:water = 1:2.5), and total organic C was determined by dichromate oxidation. Available phosphorus analysis was determined by molybdenum-antimony colorimetry. Available nitrogen was determined by alkali-solution diffusion method (Fontúrbel *et al.*, 2012). The content of invertase (INV) was measured by 3,5-dinitrosalicylic Acid Colorimetric Method and expressed as (mg • g^{-1}). The content of Catalase (CAT) was measured by Potassium Permanganate Titration (mL • g^{-1}). Alkaline Phosphatase activity (ALP) was determined by Kit Method (Comin Biotechnology Co., Ltd., Suzhou, China) and expressed as nmol d^{-1} g^{-1} (Ye *et al.*, 2016).

Community-level Physiological Profile

Biolog[TM] Eco Plates (Biolog Inc., Hayward, CA, USA) were used to study the substrate utilization pattern of soil microbial communities as described. Briefly, 10 g fresh soil was added to 100 mL of distilled water and shaken at 200 r min^{-1} for 10 min and then the 10^{-3} dilution was used to inoculate the Biolog Eco Plates (Chen *et al.*, 2011a). The plates were incubated at 25°C for 10 days and color development was read as absorbance daily with an automated plate reader (GEN Ⅲ, Microstation, USA) at the wavelength of 590 nm, and the data were collected using Microlog 4.20.05 software (Biolog Inc.).

Statistical Analyses

The average well color development (AWCD) value of Biolog data was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates), as described by Garland (1996). The diversity of microbial community was assessed by the Shannon index, the Richness index and evenness index (Moeller *et al.*, 2016). In order to eliminate the differences caused by the inoculation density, we used the optical density of 96 h culture to analyze the principal component analysis and correlation analysis. Statistical analysis using SPSS 20.0. All the data were averaged over three replicates.

Results

Soil Characteristics

Table 1 shows the physical and chemical properties and enzyme activities of two *Pinus* species. Soil pH values were from 8.30 to 8.51. SWC contents were 6.6% ~ 12.4%. The magnitude is small. After inoculation mycorrhizal fungi different tree species showed *P. sylvestris var. mongolica+S. luteus* (ZD)> *P. sylvestris var. mongolica* (No *S. luteus*) (ZM)> *P. tabulaeformis+S. luteus* (YD)> *P. tabulaeformis* (No *S. luteus*) (YM). ALP, INV and CAT activity showed ZD> ZM> YD> YM. Compared with non-inoculated mycorrhizal fungi, the physical and chemical indexes and enzyme activities of *P. tabulaeformis* and *P. sylvestris var. mongolica* increased after inoculated with mycorrhizal fungi.

Table 1: Soil physicochemical properties and enzyme activities

Species	Soil layer cm	pH value	SWC (%)	TOC g/kg	AN mg/kg	AP mg/kg	ALP nmol/d/g	INV mg/g	CAT mL/g
YD	0-20(T)	8.44a	9.41c	23.46c	38.60c	0.94c	3604c	5.50c	7.34c
YM		8.30d	7.46d	21.81d	33.25d	0.75d	3295d	5.00d	6.65d
ZD		8.40b	12.4a	30.29a	44.80a	1.87a	5361a	7.54a	8.59a
ZM		8.32c	10.28b	27.66b	36.40b	0.98b	4004b	6.77b	7.52b
YD	20-40(L)	8.51a	8.30c	24.55c	26.62c	0.75c	2349c	3.62c	7.19c
YM		8.39c	6.65d	23.42d	23.00d	0.71d	2138b	2.92d	6.63d
ZD		8.43b	9.36a	27.54a	34.12a	0.97a	3555a	4.74a	8.24a
ZM		8.39c	8.77b	21.34b	29.05b	0.81b	2912b	3.86b	7.45b

Notice:YD-*P. tabulaeformis*+*S. luteus*YM-*P. tabulaeformis* (No *S. luteus*)

ZD-*P. sylvestris var. mongolica*+*S. luteus*ZM-*P. sylvestris var. mongolica* (No *S. luteus*)

Microbial Metabolic Activity

The average color change rate (AWCD) of Biolog-ECO micropore plate indicated the C source utilization rates of the microbial community, which is an important indicator of the ability of the soil microbial community to utilize a single C source, reflecting the activity of soil microorganisms, the diversity of physiological functions of microbial communities. The AWCD value increased with the incubation time, and the changes of different tree species within 0–24 h were minor, indicating that C sources were basically not used within 24 h and the metabolic activity of microorganisms was very low and their utilization of C sources. Microbial metabolic activity and its ability to utilize C sources increased sharply within 24–96 h and vary slowly after 96 h (Fig. 1).

Through the inoculation of ectomycorrhizal fungi, in the same soil layer, the AWCD values of different tree species by the same time showed similar variation rules, and the order of their sizes was ZD> ZM> YD> YM. The AWCD values of 0–10 cm and 10–20 cm soil layers were the highest at the 168th h. It can be seen that the order of AWCD values of different soil layers of the same species is 0–10 cm > 10–20 cm (Fig. 1).

Carbon Source Utilization by Soil Microbial Community

There was clear difference in the use of six C sources of soil microorganisms such as amino acids, carbohydrates, carboxylic acids, polymers, amines/amines and other compounds. Different species of fungi treatment showed roughly the same laws on the utilization of C sources in six major categories, the order of their sizes was LZD> LYD>LYM> LZM (P <0.05). The ZD soil carbon utilization rates for amino acids, sugars, carboxylic acids, polymers, amines/amines and other compounds are 280, 110, 300, 134, 140 and 150%, respectively (Table 2).

The soil microbial diversity of different soil layers in the same tree species was also significantly different with the inoculation of ectomycorrhizal fungi. The general trend was 0–20 cm soil layer > 20–40 cm soil layer except miscellaneous. The soil carbon utilization of ZD in 0−10 cm soil layer was 97.66, 140.94, 94.12 and 125.69% higher than that in 20−40 cm soil layer respectively for sugars,
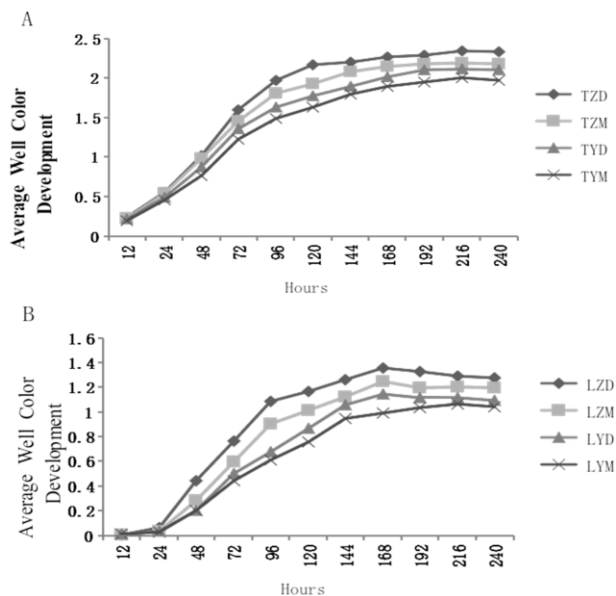

Fig. 1: Averrage Well color development (AWCD) obtained by Biolog-EcoPlate[TM] incubationof all treatments Treatments: YD-*P. tabulaeformis*+*S. luteus*; YM-*P. tabulaeformis* (No *S. luteus*); ZD-*P. sylvestris var. mongolica*+*S. luteus*; ZM-*P. sylvestris var. mongolica* (No *S. luteus*). T: 0-10cm; L: 20-40cm

polymers, amino acids, carboxylic acids, amines/ammonia and other compounds (Table 2).

In different tree species, a highest utilization of carbohydrates, carboxylic acid, followed by the utilization of polymer; upper soil microbial carbon utilization rate was higher than the lower soil (Fig. 2).

Soil Microbial Functional Diversity

The soil microbial community function was affected by the inoculation treatments. Based on the AWCD value at 96 h (Fig. 1), the Shannon's species richness index (H), Shannon evenness index (E), Simpson dominance index (D) and McIntosh index (U) of soil microorganisms were calculated (Table 3). The results showed that significant inoculation treatment was observed for all ECO microplate variables except McIntosh.

Table 2: Carbon source utilization by soil microbial community at different tree species of inoculant

Tree Species	Soil layer (cm)	Carbohydrate	Polymer	Amino acid	Carboxylic acid	Amine/amide	Miscellaneous
YD	0-20	5.71b	5.58b	4.38b	4.22b	1.98a	0.88a
YM		5.29d	4.61c	3.92c	3.14c	1.06c	0.63c
ZD		6.27a	5.68a	4.48a	8.71a	1.55b	0.81b
ZM		5.68c	4.24d	1.60d	2.90d	1.11c	0.54d
YD	20-40	4.53b	4.27b	3.63b	4.00b	1.64a	1.55b
YM		3.87d	2.75c	3.31c	2.97c	0.86d	1.53c
ZD		4.82a	4.49a	3.76a	6.93a	1.40b	1.61a
ZM		4.10c	2.24d	1.39d	2.51d	0.98c	1.43d

Table 3: Functional diversity index for soil microbial community

Tree Species	Soil layer (cm)	Shannon richness(H)	Shannon evenness (E)	Simpson(D)	McIntosh(U)
YD	0-20	4.46a	0.98b	6.50a	0.988a
YM		4.40c	0.99a	5.48c	0.986c
ZD		4.45b	0.97c	6.46b	0.987b
ZM		4.38d	0.99a	5.14d	0.986c
YD	20-40	4.38a	0.95c	6.75a	0.986a
YM		4.24c	0.97a	4.83c	0.982d
ZD		4.33b	0.95c	5.15b	0.985b
ZM		4.18d	0.96b	4.63d	0.983c

H: Shannon species richness index; E: Shannon evenness index; D: Simpson dominance index; U: McIntosh index. Different letters within thesame column meant significant difference at 0.05 level

In the same soil layer, Shannon's species richness index (H) indicated the number of C sources utilized, with the highest being YD and the lowest ZM. The Shannon evenness index (E) reflects the species variation and difference degree of microbial communities. E of YM and ZM was higher. The Simpson dominance index (D) showed YD> ZD> YM> ZM. The McIntosh index (U) reflects the difference in the number and utilization of C sources. U of YD was the highest that showed the soil microbial species were rich, C source utilization was higher.

The results showed that there was no significant difference among different tree species, implying that the soil microbial species and the utilization degree of C source were basically similar in four tree species, while 0−20 and 20−40 cm showed similar characteristic.

The diversity index was the highest in YD, followed by ZD. In 0−20 cm soil layer, the Shannon's species richness index, Simpson dominance index and McIntosh index of YD were respectively 1, 18 and 0.2% higher than that of YM. Similarly, the diversity index of ZD were respectively 1.8, 26 and 0.1% higher than that of ZM, indicating that the different species of inoculation of mycorrhizal fungi caused the differences in functional diversity of soil microbes due to the treatment of ectomycorrhizal fungi and the characteristics of tree species.

Relationship between Soil Microbial Communities and Soil Factors

In order to further reveal the inherent relationship between functional diversity of microbial communities and soil environmental factors, the correlation analysis was performed between the index of soil microbial metabolic diversity and the soil physical and chemical properties.
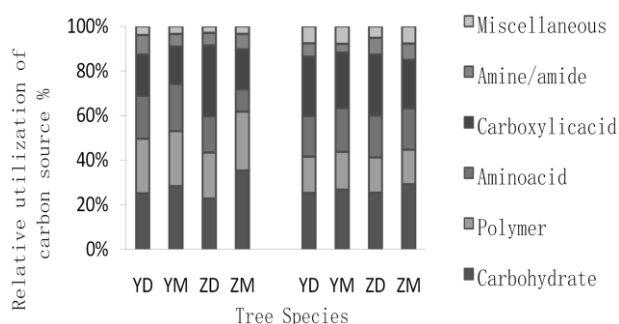

Fig. 2: Relative utilization of carbon sources (%) incubation of all treatments. Treatments: YD-*P. tabulaeformis*+*S. luteus*; YM-*P. tabulaeformis* (No *S. luteus*); ZD-*P. sylvestris var. mongolica* + *S. luteus*; ZM-*P. sylvestris var. mongolica* (No *S. luteus*)

The results showed that Shannon richness (H), Simpson (D), McIntosh (U) and CAT were positively correlated with INA (p<0.01). CAT was positively correlated with Shannon evenness (E). It can be seen that soil physical and chemical indicators and functional diversity of soil microbial communities are closely related (Table 4).

Principal Component Analysis

Soil microbial diversity reflects the overall community changes, but fails to reflect the detailed information of microbial community metabolism, and to study the differences in the utilization of soil carbon sources between different carbon sources, which helps to understand more about the metabolic functional characteristics of microbial communities.

Table 4: Correlation coefficients between soil microbial metabolic activity, functional diversity indices and physico-chemical properties, enzyme activities

Variables	SWC	STOC	AN	AP	ALP	INV	CAT	H	E	D	U
PH	0.399	-0.433*	-0.375	-0.644**	-0.870**	-0.014	-0.454*	0.072	-0.805**	0.576**	0.119
SWC		0.511*	0.518**	-0.186	-0.101	0.737**	0.171	0.707**	-0.097	0.700**	0.702**
STOC			0.931**	0.258	0.453*	0.735**	0.557**	0.481*	0.385	0.010	0.367
AN				0.098	0.342	0.688**	0.378	0.332	0.284	-0.017	0.263
AP					0.844**	0.270	0.726**	0.343	0.668**	0.020	0.235
ALP						0.290	0.643**	0.342	0.931**	-0.135	0.276
INV							0.683**	0.722**	0.274	0.542**	0.529**
CAT								0.384	0.514*	0.058	0.132
H									0.38	0.800**	0.947**
E										-0.074	0.314
D											0.791**

*was significantly correlated at 0.05 level (bilateral); ** significantly correlated at .01 level (bilateral)

Using principal component analysis (PCA), the two principal component factors was extracted from six kinds of carbon sources of the tree species inoculated with mycorrhizal fungi (Fig. 3). The cumulative contribution rates of the two principal components are respectively 81.6 % (A) and 86.47 % (B). Among them, carbohydrate (Fig. 3A) was the major component of two pines inoculated with ectomycorrhizal fungi. In contrast, most of the soil microorganisms in the uninoculated were mainly carboxylic acid in the utilization of carbon sources (Fig. 3B).

Discussion

Through inoculating mycorrhizal fungi in this experiment, the metabolic activity of all carbon sources in different tree species of soil microorganisms showed an increasing trend with time (Lin *et al.*, 2012; Fan *et al.*, 2013). In the process of culturing, the metabolic capacities of soil microbial were significantly higher than those of the controls. AWCD values of *P. tabulaeformis* and *P. sylvestris var. mongolica* inoculated with mycorrhizal fungi were higher than those in the control. The soil microbes' metabolic activity of the inoculated trees was higher than that in the non-inoculated ones, which was consistent with the previous studies (Sun *et al.*, 2010; Xu *et al.*, 2015). Therefore, the soil factors affect the microbiological diversity, and the metabolic activity of microbial carbon sources. The soil biological factors have a positive impact on the soil microbial community. Related studies have shown that soil moisture (SWC) and total organic carbon (TOC) are important factors affecting soil microbial metabolic activity and functional diversity. First of all, an increase of SWC was conducive to the optimization of community structure and increase in the aboveground biomass. With an increase in aboveground biomass, the nutrients input to the soil also gradually increased, thereby increasing the soil enzyme activity and affecting the soil microbial propagation. In addition, SWC not only has a direct impact on soil microorganisms, but also can promote the microbial activities and functional diversity of soil by accelerating the cycling of soil C and soil mineralization by affecting TOC, TN and other soil physical and chemical properties (Burke, 2008).
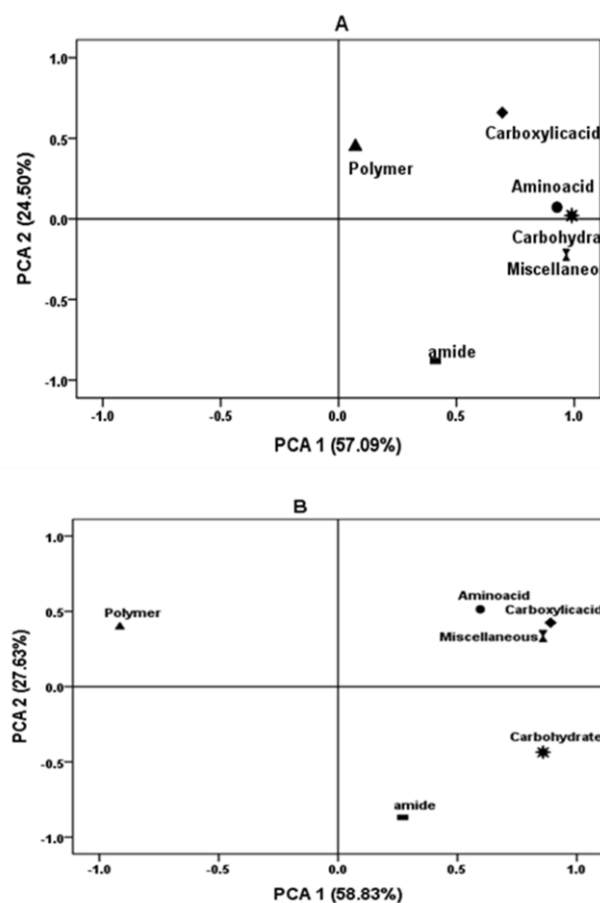

Fig. 3: The principal component analysis of soil microbial carbon source diversity. A: *P. tabulaeformis* and *P. sylvestris* inoculated with mycorrhizal fungi; B: *P. tabulaeformis* and *P. sylvestris* not inoculated with mycorrhizal fungi

In this study, SWC was positively correlated with metabolic activity and functional diversity indexes of soil microorganisms (P <0.01), indicating that SWC and TOC had a great influence on the C source utilization ability of soil microorganisms.

In this study, INV activity was significantly positively correlated with soil microbial activity and functional diversity indexes, which was consistent with the results of many scholars (Zhao *et al.*, 2010). Soil microbial diversity indexes and soil ALP and CAT reached a significant positive correlation, indicating that soil enzyme activity and microbial metabolism together affect the diversity of microbial communities. Related studies have shown that the diversity of soil microbial community is the result of combined effects of soil nutrients, water and heat conditions, litters and root exudates (Eisenhauer *et al.*, 2017). The results showed that through inoculation of ectomycorrhizal fungus (*S. luteus*), soil microorganisms and soil qualities continued to improve, which in turn increased the functional diversity of soil microorganisms. At the same time, it is worthwhile to inoculate ectomycorrhizal fungi during afforestation.

The diversity of soil microbial communities can be analyzed synthetically by combining different functional diversity indices of microbial diversity, such as richness, evenness and Simpson, to a certain extent reflecting the differences in soil microbial community composition. The results showed that the Simpson diversity and richness of soil microbial community of two pines inoculated with ectomycorrhizal fungi (*S. luteus*) were significantly higher than those of non-inoculated (Table 3). Through inoculating ectomycorrhizal fungi (*S. luteus*), the soil microbial community structure was changed and the soil microbial activity was improved. Comparing two pines, soil microbial diversity and abundance of *P. tabulaeformis* were higher than *P. sylvestris var. mongolica*. The inoculation of ectomycorrhizal fungi (*S. luteus*) reduced the soil microbiological species.

After inoculation of ectomycorrhizal fungi (*S. luteus*), soil microbial diversity of two pines had significant difference in the utilization of different types of carbon sources. In the 96 h cultivation period of Biolog microliter plate, soil microbes of *P. tabulaeformis* and *P. sylvestris var. mongolica* inoculated with mycorrhizal fungi had stronger utilization of carbohydrate and polymer media. The utilization intensity of these two types of carbon sources accounted for about 50% of the six types of carbon sources. It can be inferred that soil microorganisms use carbon sources as carbohydrates and polymers. However, *P. tabulaeformis* and *P. sylvestris var. mongolica*, inoculated with ectomycorrhizal fungi (*S. luteus*), were more preferred to carbohydrates than uninoculated treatments**.** By inoculating ectomycorrhizal fungi (*S. luteus*), it may change the rhizosphere micro-ecological environment and would correspondingly affect the plant metabolism, resulting in plant rhizosphere secretion quantity and quality changes. Therefore, the pines may be more conducive to produce large amounts of high-quality root exudates, promoting rhizosphere microbial assimilation of carbon and enhance the functional diversity of microbial communities (Aghili *et al.*, 2014). At the same time, by inoculating ectomycorrhizal fungi (*S. luteus*), it is helpful to root growth of pines and also to growth of microbial population with carbohydrates as carbon source. The conversion of carbon source types from the control to the carbohydrates may be due to changes in the dominant population of ectomycorrhizal fungi, affecting the results of soil microbial community structure.

Conclusion

Ectomycorrhizal fungi (*S. luteus*) can enhance soil biological activity. Microbial functional diversity can be used an indicator of management induced, changes to soil quality. *S. luteus* increased microbial biomass and had a positive impact on soil microbial functional diversity. The results highlight higher microbial activity in soils inoculating ectomycorrhizal fungi (*S. luteus*). Therefore, the results not only provided a theoretical basis for the cultivation and planting of our future vegetation, but also to apply high-throughput techniques to genomic analysis of soil microbial communities so as to further reveal the structure and function of soil microbial communities inoculated with as a next step.

Acknowledgements

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