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Characterization of Microbial Community Structure in Rhizosphere Soils of Cowskin Azalea (*Rhododendron aureum* Georgi) on Northern Slope of Changbai Mountains, China

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Abstract: The vegetation and soil are mutual environmental factors, soil characteristics, such as chemical properties and microorganism that affect the vegetation occurrence, development and succession speed. In this study, we evaluated the structure of microbial communities of rhizosphere of Cowskin Azalea (*Rhododendron aureum* Georgi) populations and compared with non-rhizosphere soils at four sample sites of the Changbai Mountains, China, and analyzed the correlation between chemical properties of soil and microbial communities. The results showed that microbial structure and soil chemical properties are significant superior to non-rhizosphere at all four sample sites. The rhizosphere microorganisms are mainly composed of bacteria, actinomycetes, followed by fungi least. The principal component analysis (PCA) biplot displayed that there are differences between rhizosphere and non-rhizosphere soils for microflora; Through correlation analysis, we found that the bacteria is clearly influenced by pH on the Changbai Mountains, besides pH, other soil features such as NO_3^--N. These data highlight that *R. aureum* as the dominant vegetation living in the alpine tundra is a key factor in the formation of soil microorganism and improving soil fertility, and is of great significance for the maintenance of alpine tundra ecosystem.
Keywords: *Rhododendron aureum* Georgi; microbial community structure; rhizosphere; Changbai Mountains

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1 Introduction

The Changbai Mountains is the highest mountain in the northeastern China and is the head of three large rivers (the Songhua River, Yalu River and Tumen River) (He *et al.*, 2005). The vertical distribution of vegetation is known to mirror the vegetation horizontal zonation from temperate to frigid zones on the Eurasian continent (Xu *et al.*, 2004; Zhang *et al.*, 2011). In particular, a typical alpine tundra belt was above 2000 m where the majority of plants are of polar origin from the Quaternary period glacier retreat (Xu *et al.*, 2004), and these soils may be expected to harbor relatively unique bacterial comm.-unities. In addition, the Changbai Mountains is a very well-known dormant volcano in the northeastern China, which experienced four large-scale eruptions. Alpine tundra ecosystem have been ever suffered several seriously natural interferences at different geologic period. Vegetation living in alpine tundra was mass destruction. After hundreds of years of evolution, alpine tundra has been covered with vegetation once again. All the aforementioned provide an optimal environment to investi-

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gate microbial patterns change, and further understand the interaction between plant and microbe during revegetation of alpine tundra ecosystem.

Rhododendron aureum Georgi is a perennial evergreen dwarf shrub inhabiting alpine regions of the northeastern Asia that can tolerate cold climate (Kudo, 1993). In China, it is native to the alpine tundra and the *Betula ermanii* population ecotone of the Changbai Mountains, ranging from 1000 m to 2506 m a.s.l. (Hu and Fang, 1994). The record of recent years shows that it distributed in altitude of 2600 m in the Changbai Mountains (Gong *et al.*, 2010). The *R. aureum* is one of the constructive and dominant species on the alpine tundra ecosystem, and it plays an important role in maintaining the ecological balance and preventing and controlling soil erosion.

Soil microorganism is the very important for nutrient cycling and plant growth. The results of some researches showed that the trend of microbial community structure characteristics with increasing elevation height, and that the results did not mean the same. The results thought that the content of Phospholipid fatty acids (PLFA) in soil fungi and bacteria was decline with increasing elevation height (Wagai *et al*., 2011), but another result demonstrated that this trend was not found under alpine environment (Djukic *et al*., 2010). Thus it can be found that the researches on the trend of microbial community structure characteristics with increasing elevation height are insufficient and it will be necessary to do more researches. However, more researches were focused on rhizosphere microorganism of plant. The rhizosphere is the habitat for a vast interactive microorganism community where soil microbes transform organic substrates, release mineral, and hence strongly influence the establishment of plants (Jin *et al.*, 2009). On the other hand, plants supply energy for the soil subsystem in the form of litter and root exudate and specifically influence microbial community (Miethling *et al.*, 2000). Accordingly, the rhizosphere microbial communities can be regarded as a subsystem of the soil microbial community (Foster, 1988; Duineveld *et al.*, 2001), and influence on the plant colonization, growth and succession of community (Lambers *et al.*, 2009). Recently, some researches showed that there is a strong interaction between plants and rhizosphere microbial communities (Marilley and Aragno, 1999; Marschner *et al*, 2002).

In our previous study, we revealed that the rapid change of topography associated with the elevation had greatly contributed to the genetic structure and genetic diversity of this species by inter-simple sequence repeat (ISSR) and random amplified polymorphic (RAPD) markers (Du *et al.*, 2011; Liu *et al.*, 2012). Based on genetic analysis, we found that high genetic diversity is one of the intrinsic factors driving expansion of *R. aureum* population. However, it is inadequate to understand expansion and ecological functions of *R. aureum* populations. In the view of importance of rhizosphere microbial communities, we selected as study subjects *R. aureum* populations distributed in four altitudes zone to understand the change of microbial communities in the rhizosphere.

The main objectives of this study were: 1) to explore the elevational diversity gradient of microbial communities of rhizosphere soils of *R. aureum* at four different altitudes in the Changbai Mountains, China; 2) to determine key factors controlling the distribution of microbial communities. These results may improve our knowledge of microbial composition in the Changbai Mountains and can then be used to gain further understanding of the population expansion of *R. aureum*. Furthermore, it can provide a theoretical basis and scientific basis for the research to protect plants in the Changbai Mountains.

2 Materials and Methods

2.1 Study area

Sample sites were established at the northern slope of the Changbai Mountains, located in the Changbai Mountains National Nature Reserve (41°41′49″–42°25′18″N, 127°42′55″–128°16′48″E). Mean altitude ranges from 500 m to 2691 m above sea level (Yang and Wu 1998). Vegetation presents obvious altitudinal zonation spectrum, and there has a clearly defined vertical zones of vegetation. From foot to peak it runs as follows: mixed coniferous and broad-leaved forest, dark coniferous forest, *Betula ermanni* forest, and alpine tundra, which harbors over 2277 species of plants and a notable richness of endemic species (Bai, 1988).

The Changbai Mountains National Nature Reserve is a typical mountain climate. The climate conditions are also distinct in different altitudes. The average annual temperature is generally 3℃–7℃, and annual precipita-

tion is over 600 mm. With relatively high altitude above sea level, the annual precipitation is over 1400 mm (Yang and Wu, 1998).

The *R. aureum* is one of the constructive and dominant species on the alpine tundra ecosystem, and it plays an important role in maintaining the ecological balance and preventing and controlling soil erosion. So *R. aureum* populations, i.e., three populations living in high altitude (2000 m, 2300 m and 2614 m) and one population living in low altitude (1580 m), were selected. All sample sites are located at different altitudes on the northern slope in the Changbai Mountains (Table 1).

2.2 Soil sampling

Two soil samples, about 500 g each, were collected from each of four different sites. Rhizosphere soils were collected from the fine roots of *R. aureum* in growing season (July) in 2009. Fine roots at the depth of 0–10 cm were severed and brought to lab. Excess bulk soils were flaked away and those attached to roots were rhizosphere soils (Berg *et al.*, 2006). Then the rhizosphere soils were washed off with sterile 0.85% NaCl solution (Schmalenberger and Tebbe, 2003). Non-rhizosphere samples (mineral soil) were collected from the same areas 1 m from the *R. aureum* communities where no roots existed. Samples were placed in a sterile bucker and stored at 4℃ to determine the physical and chemical properties, and the other was stored at –20℃ until required for analysis soil microbial community.

2.3 Sample analyses

2.3.1 Chemical properties of soil

Chemical properties of soil for all locations were analyzed by Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. We determined total phosphorus (TP), quick avail phosphorus (QAP), total kalium (TK), quick avail kalium (QAK), slowly avail kalium (SAK), total nitrogen (TN), nitrate nitrogen (NO_3^--N), ammonium-nitrogen (NH_4^+-N), total sulphur (TS), humic matter (HM), organic matter (OM), and pH.

2.3.2 Isolated soil microbe

Populations of selected components of the soil microbial community were measured with the Pour Method and the Surface Spread Plate Count Method (Xu and Zheng, 1986). Table 2 shows the isolated microbial groups and the type of selective media used. Three soil sub-samples (5 g) were processed with two replicates of each treatment. This gave six repetitions per treatment, three dilutions per sample, and four pour plates per dilution. Soil dry weight was determined by oven drying 1 g of soil at 105℃ for 24 h. The results were expressed as Colony Forming Units (CFU) per gram of dry weight.

Table 1 Location and characteristics of study area

Altitude (m a.s.l.)	Ecosystem type	Coordinate
1580	Mountain frigid-temperate coniferous forest (MFTCF)	42°05′24″N, 128°04′08″E
2000	Alpine krummholz (AK)	42°03′38″N, 128°04′00″E
2300	Alpine tundra (AT)	42°02′23″N, 128°04′05″E
2614	Alpine tundra (AT)	42°01′45″N, 128°04′07″E

Table 2 Microbial groups isolated from soil, selective medium used, 10-fold dilution plated, and days of incubation

Microbial group	Selective medium	Dilution	Days and temperature	Reference
Total bacteria	Plate count agar	10^{-3}–10^{-5}	2 (37℃)	(Xu and Zheng, 1986)
Total fungi	Martin′s Bengal rose agar	10^{-1}–10^{-3}	5 (30℃)	(Xu and Zheng, 1986)
Actinomycetes	Starch-casein agar	10^{-1}–10^{-3}	7–10 (30℃)	(Xu and Zheng, 1986)
Ammonifier	Peptone agar	10^{-2}–10^{-4}	2–3 (30℃)	(Xu and Zheng, 1986)
Silicate bacteria	Potassium aluminum silicate agar	10^{-2}–10^{-4}	3–4 (30℃)	(Xu and Zheng, 1986)
Sulfur bacteria	Sulfur bacteria agar	10^{-1}–10^{-3}	2 (25℃–28℃)	(Xu and Zheng, 1986)
Azotobacter	Ashby agar	10^{-2}–10^{-4}	7 (30℃)	(Xu and Zheng, 1986)
Nitrobacteria	Nitrobacteria agar	10^{-1}–10^{-3}	12–14 (37℃)	(Xu and Zheng, 1986)
Cellulolytic microbe	Cellulolytic microbe agar	10^{-1}–10^{-3}	10–14 (30℃)	(Xu and Zheng, 1986)

2.4 Statistical analysis

All data were log transformed, and averages per treatment were subsequently determined. A one-way ANOVA was used to analyze the effect of colony count results on the number of CFU collected from rhizosphere and non-rhizosphere soils of the *R. aureum*, principal component analysis (PCA) and pearson correlation matrix were analyzed using SPSS version 16.0. Significance values are for $P < 0.05$.

3 Results

3.1 Chemical properties of rhizosphere and non-rhizosphere soil

Soil chemical property is one of the major quality evaluation indictors. Nutrient characteristics in rhizosphere and non-rhizosphere soil from different *R. aureum* populations are shown in Table 3. Results showed that most of the parameters of rhizosphere soil were significant superior to non-rhizosphere soils. The contents of TN, TS, HM and OM were higher in rhizosphere soil compared to non-rhizosphere soils. The QAP, QAK, NO_3^--N and SAK were higher in rhizosphere soil besides the sample site of 1580 m; the TP and MC of rhizosphere soils were higher than non-rhizosphere soils except the site of 2000 m; the TK and pH of rhizosphere soils were lower than those of non-rhizosphere soils; the content of NH_4^+-N at the site of 1580 m was higher in non-rhizosphere soils, but the sites of 2000 m and 2614 m were higher in rhizosphere soils, and it was similar at 2300 m.

The change of soil properties are related with the change of altitude (Fig. 1). Such as in the rhizosphere and non-rhizosphere soil, TP, TN, HM and OM reduced with increasing elevation height, and TK and pH increased with the increasing elevation, but other chemical properties of soil change irregularly with the altitude.

3.2 Microbial community structure

At all sample sites, the number of microbe for almost all in rhizosphere soil of *R. aureum* is higher than that in non-rhizosphere (Fig. 2), and there are some differences (Table 4). However, only a few microbes are not significantly differences (Table 4).

3.2.1 Total bacteria

The number of total bacteria in the rhizosphere is higher than that in the non-rhizosphere, especially total bacteria of 2300 m (R) are significantly higher compared to that of 2300 m (B). Among rhizosphere soils, the counts of 1580 m (R) are the highest and 2614 m (R) is the lowest. For all non-rhizosphere soils, the total bacteria of 1580 m (B) are the highest, while that of 2614 m (B) is the lowest.

Table 3 Chemical properties of rhizosphere and non-rhizosphere soils from different *R. aureum* populations

Sample site (m)	1580		2000		2300		2614	
Position	R	B	R	B	R	B	R	B
TP (g/kg)	1.1714Aa	0.6900Bb	0.5336Aa	0.5541Bb	0.4569Aa	0.3835Bb	0.3635Aa	0.3367Bb
QAP (g/kg)	0.0116Aa	0.0240Bb	0.0054Aa	0.0028Bb	0.0046Aa	0.0036Ab	0.0094Aa	0.0022Bb
TK (g/kg)	13.04Aa	17.03Bb	28.00Aa	31.61Bb	35.38Aa	36.38Bb	38.46Aa	49.51Bb
QAK (g/kg)	0.25Aa	0.30Bb	0.15Aa	0.58Bb	0.16Aa	0.10Bb	0.14Aa	0.04Bb
SAK (g/kg)	1.13Aa	1.24Bb	0.85Aa	0.64Bb	0.87Aa	0.73Bb	0.87Aa	0.53Bb
TN (g/kg)	11.12Aa	9.36Bb	3.99Aa	3.33Bb	3.21Aa	3.15Bb	2.65Aa	2.08Bb
NO_3^--N (g/kg)	0.0025Aa	0.3530Bb	0.0037Aa	0.0015Bb	0.0039Aa	0.0030Bb	0.0342Aa	0.0012Bb
NH_4^+-N (g/kg)	0.55Aa	0.66Ab	0.23Aa	0.05Bb	0.23Aa	0.23Aa	0.14Aa	0.01Bb
TS (g/kg)	1.11Aa	0.78Bb	1.89Aa	0.51Bb	0.39Aa	0.36Aa	0.47Aa	0.37Aa
HM (%)	5.01Aa	3.91Bb	1.60Aa	1.20Bb	1.19Aa	0.83Bb	0.85Aa	0.09Bb
OM (%)	36.68Aa	21.34Bb	10.51Aa	6.68Bb	9.01Aa	5.18Bb	7.18Aa	0.26Bb
pH	4.53Aa	5.29Bb	5.07Aa	5.2Aa	5.02Aa	5.5Ab	6.03Aa	6.31Bb

Notes: R, rhizosphere soil; B, non-rhizosphere soil. TP, total phosphorus; QAP, quick avail phosphorus; TK, total kalium; QAK, quick avail kalium; SAK, slowly avail kalium; TN, total nitrogen; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium-nitrogen; TS, total Sulphur; HM, humic matter; OM, organic matter. Different capital letters mean significant level at 0.01; different small letters mean significant level at 0.05

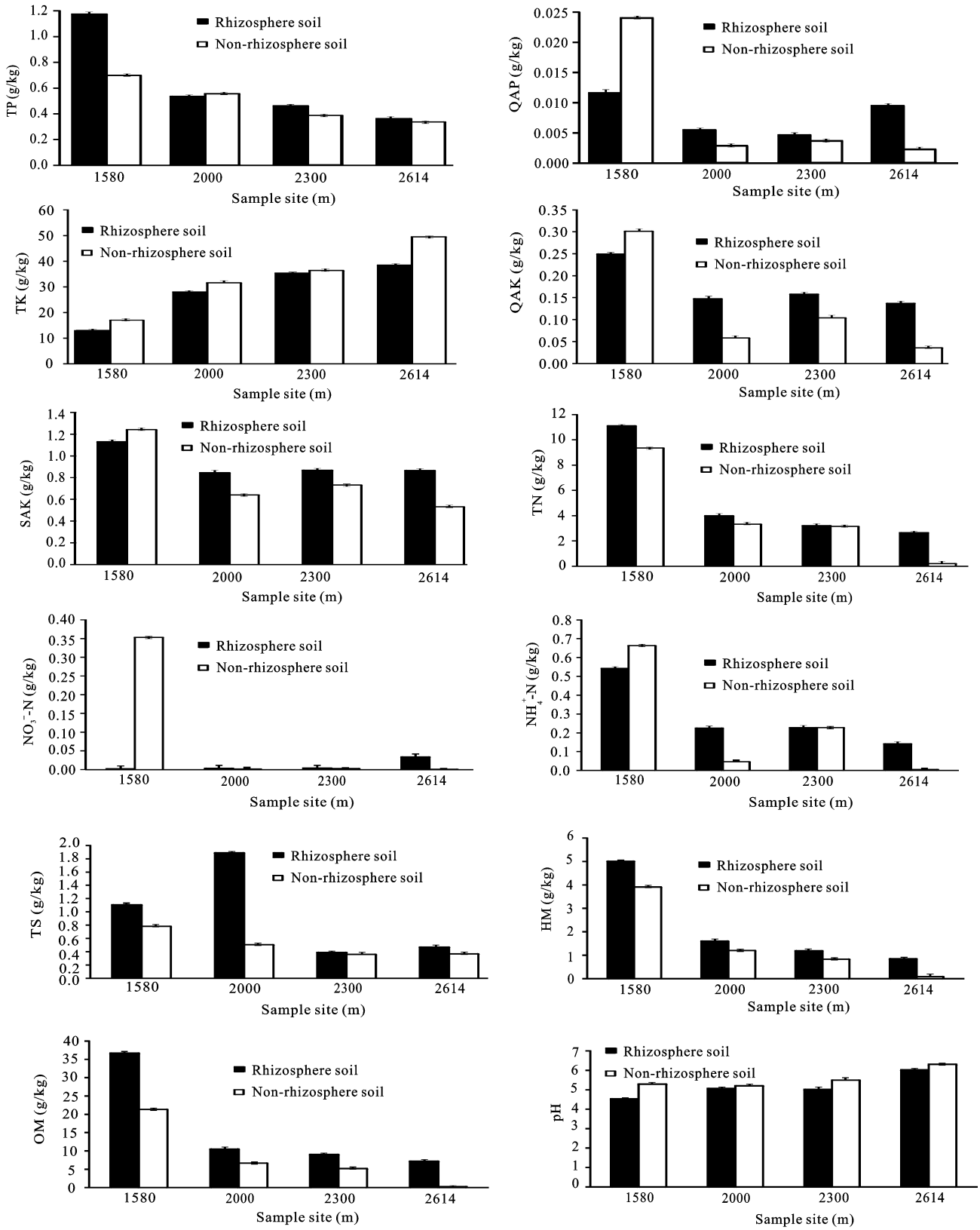

Fig. 1 Chemical properties of rhizosphere and non-rhizosphere soils along an altitudinal gradient on northern slope of Changbai Mountains. TP, total phosphorus; QAP, quick avail phosphorus; TK, total kalium; QAK, quick avail kalium; SAK, slowly avail kalium; TN, total nitrogen; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonium-nitrogen; TS, total Sulphur; HM, humic matter; OM, organic matter

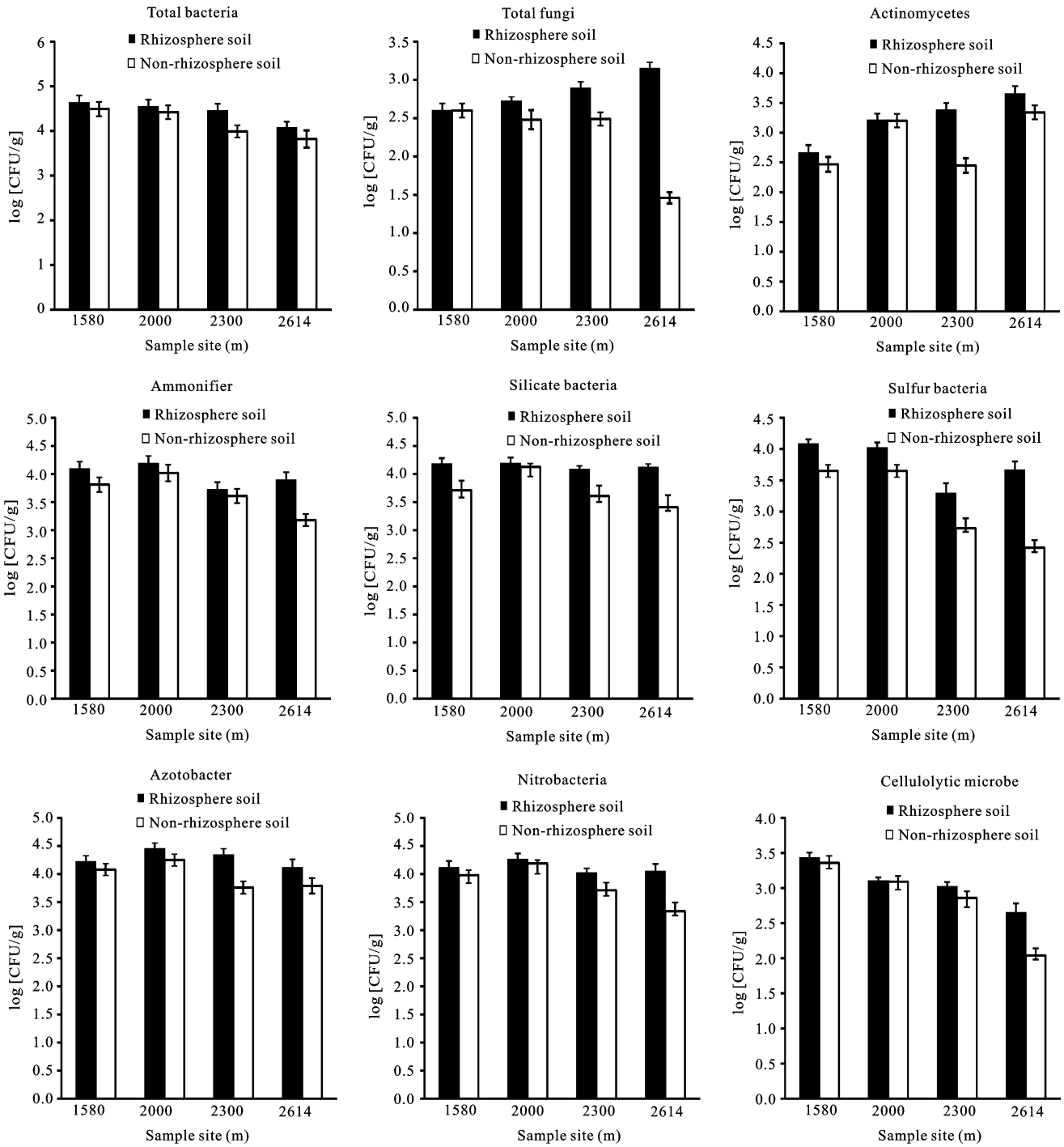

Fig. 2 Change trend of microbial community composition of rhizosphere and non-rhizosphere soils under different *R. aureum* populations (CFU, colong-forming units)

3.2.2 Total fungi

Total fungi in the rhizosphere are higher than that in the non-rhizosphere, but total fungus of 2000 m (R) are significantly higher compared to that of 2000 m (B). Similar results are obtained for 2300 m (R) and 2300 m (B), 2614 m (R) and 2614 m (B). Among rhizosphere soils, the counts of 2614 m (R) are the highest and are significantly higher compared to others. For all non-rhizosphere soils, 2614 m (B) is significantly lower compared to others.

3.2.3 Actinomycetes

The numbers of actinomycetes in the rhizosphere is

Table 4 Microbial community composition of *R. aureum* in rhizosphere and non-rhizosphere soil under different *R. aureum* populations (log[CFU/g])

Samples site (m)	1580		2000		2300		2614	
Position	R	B	R	B	R	B	R	B
Total bacteria	4.63a	4.49a	4.54a	4.42a	4.45a	3.99b	4.07b	3.82b
Total fungi	2.60ab	2.60ab	2.72ab	2.48b	2.89ab	2.49b	3.15a	1.46c
Actinomycetes	2.66b	2.47b	3.21a	3.20a	3.38a	2.45b	3.65a	3.34a
Ammonifier	4.09a	3.81a	4.19a	4.02a	3.72a	3.61a	3.89a	3.18b
Silicate bacteria	4.18a	3.71b	4.19a	4.13a	4.08a	3.61b	4.12a	3.41c
Sulfur bacteria	4.08a	3.65ab	4.02a	3.65ab	3.29b	2.73c	3.66ab	2.42c
Azotobacter	4.22ab	4.08b	4.45a	4.25ab	4.34ab	3.76c	4.11b	3.79c
Nitrobacteria	4.11a	3.98ab	4.26a	4.19a	4.02ab	3.71b	4.05ab	3.34c
Cellulolytic microbe	3.43a	3.36a	3.10ab	3.09ab	3.02ab	2.86ab	2.65b	2.04c

Notes: R, rhizosphere soil; B, non-rhizosphere soil. Different capital letters mean significant level at 0.01; different small letters mean significant level at 0.05

higher than that in the non-rhizosphere, but actinomycetes of 2300 m (R) are significantly higher compared to 2300 m (B). There are no significant differences for other sample sites between rhizosphere and non-rhizosphere soils. Among rhizosphere soils, the counts of 2614 m (R) are the highest and are significantly higher compared to 1580 m (R). Among non-rhizosphere soils, 2614 m (B) is significantly higher compared to 1580 m (B) and 2300 m (B).

3.2.4 Ammonifier

The numbers of ammonifier in the rhizosphere is higher than that in the non-rhizosphere, but the ammonifiers of 2614 m (R) are significantly higher compared to 2614 m (B). There are no significant differences for other altitudes between rhizosphere and non-rhizosphere soils. Among rhizosphere soils, 2000 m (R) is the highest, and there are no significant differences compared to others. Among non-rhizosphere soils, 2614 m (B) is significantly lower compared to others.

3.2.5 Silicate bacteria

The number of silicate bacteria in the rhizosphere is higher than that in the non-rhizosphere, but the silicate bacteria of 1580 m (R) are significantly higher compared to 1580 m (B). Similar results are obtained for 2300 m (R) and 2300 m (B), 2614 m (R) and 2614 m (B). Among rhizosphere soils, the counts of 1580 m (R) are the highest, and there are no significant differences compared to others. Among non-rhizosphere soils, 2614 m (B) is the lowest and significantly lower compared to others.

3.2.6 Sulfur bacteria

The number of sulfur bacteria in the rhizosphere is higher than that in the non-rhizosphere, but the sulfur bacteria of 1580 m (R) are significantly higher compared to 1580 m (B). Similar results are obtained for others. Among rhizosphere soils, 1580 m (R) is significantly higher compared to 2300 m (R) and 2614 m (R). Among non-rhizosphere soils, 1580 m (B) is significantly higher compared to 2300 m (B) and 2614 m (B).

3.2.7 Azotobacter

The number of azotobacter in the rhizosphere is higher than that in the non-rhizosphere, but the azotobacter of 2614 m (R) are significantly higher compared to 2614 m (B). Similar results are obtained for other altitudes. Among rhizosphere soils, 2000 m (R) is significantly higher compared to others. Among non-rhizosphere soils, 2300 m (B) is significantly lower compared to 1580 m (B) and 2000 m (B).

3.2.8 Nitrobacteria

The number of nitrobacteria in the rhizosphere is higher than that in the non-rhizosphere, but the nitrobacteria of 1580 m (R) are significantly higher compared to 1580 m (B). Similar results are obtained for 2300 m (R) and 2300 m (B), 2614m (R) and 2614 m (B). Among rhizosphere soils, 2300 m (R) is significantly lower compared to 1580 m (R) and 2000 m (R). Among non-rhizosphere soils, 2614 m (B) is significantly lower compared to others.

3.2.9 Cellulolytic microbe

At all sample sites, the number of cellulolytic microbes in the rhizosphere is higher than that in the non-rhizosphere, but the cellulolytic microbe of 2614 m (R) are significantly lower compared to 2614 m (B). There are

no significant differences for other altitudes between rhizosphere and non-rhizosphere soils. Among rhizosphere soils, 2614 m (R) is significantly lower compared to others. Among non-rhizosphere soils, 2614m (B) are significantly lower compared to others.

3.3 Integrated analysis by principal component analysis

The principal component analysis (PCA) biplot of micro-flora in the rhizosphere and non-rhizosphere soil are shown in Fig. 3. For rhizosphere soil, *R. aureum* populations, the *X*-axis explains 73.71% of the variability, and the *Y*-axis explains an additional 15.60% of the original variability. Nitrobacteria, sulfur bacteria, total bacteria and fungi are important factors for rhizosphere soils.

The biplot of the PCA for micro-flora in the non-rhizosphere soil indicated that the *X*-axis explains 78.06% of the variability, while the *Y*-axis explains an additional 18.01% of the original variability. Actinomycetes, cellulolytic microbe, nitrobacteria and azotobacter are important factors for non-rhizosphere soils.

3.4 Coefficients of pearson′s correlations analysis

In rhizosphere soils, coefficients of pearson′s correlations between the soil parameters and microbes suggested that total bacteria had significantly negative effects on PH and NO_3^--N; actinomycetes abundances had significantly negative effects on total P, total N, NH_4^+-N, humic matter and positive effects on total K; ammonifier abundances had significantly negative effects on total S; and cellulolytic microbe abundances had significantly negative effects on pH. In non-rhizosphere soils, total bacteria had significantly positive effects on total P, fungi abundances had significantly negative effects on pH, ammonifier abundances had significantly negative effects on PH and cellulolytic microbe abundances had significantly negative effects on total K and pH (Table 5).

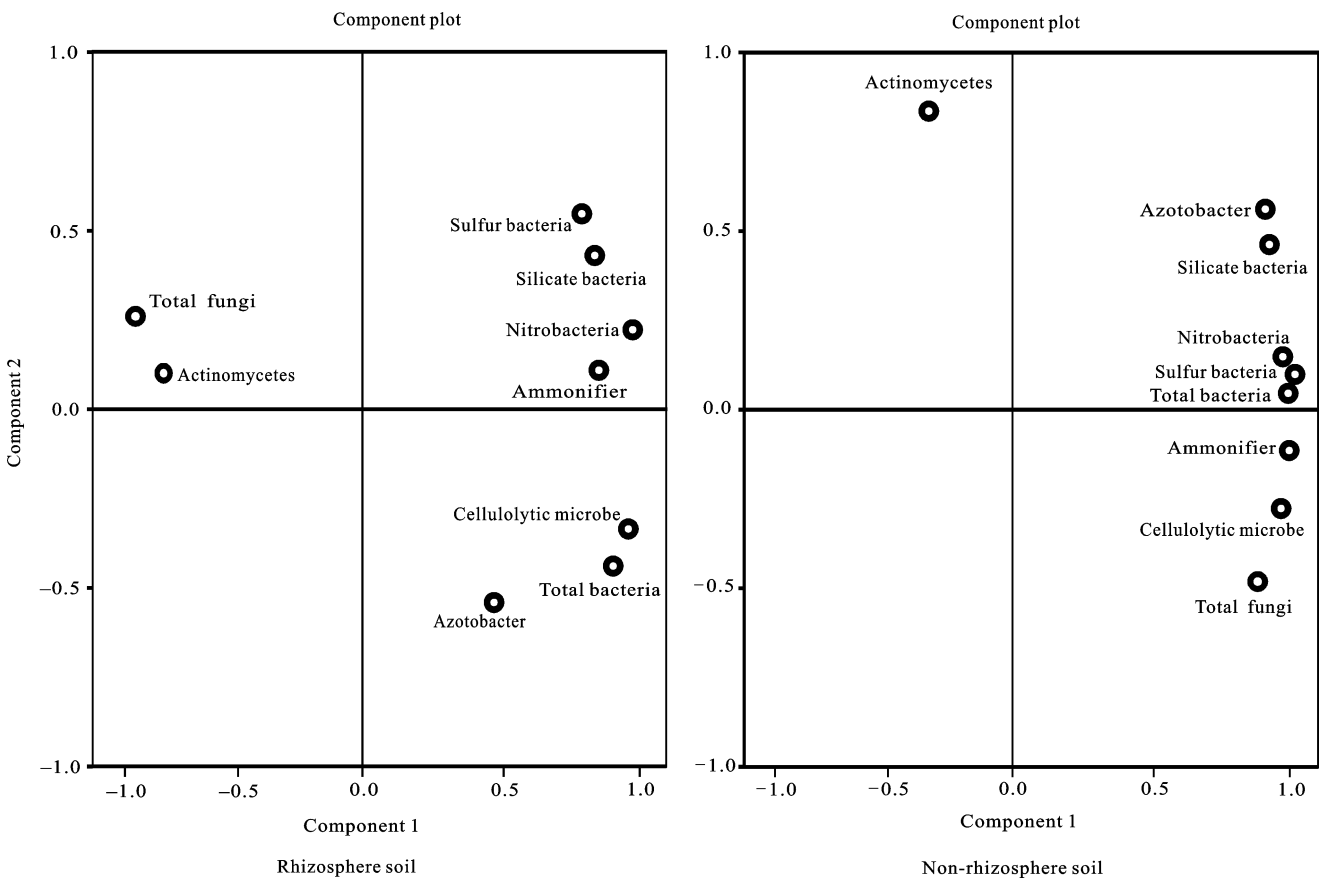

Fig. 3 Principal component analysis (PCA) biplot based on data of microbial communities of *R. aureum* populations in rhizosphere and non-rhizosphere soil. The first two principal components (PCs) accounted for over 89.95% and 97.82% of the total variance, respectively

Table 5 Coefficients of pearson′s correlations between soil parameters and microbial flora across four sites

	TP	QAP	TK	QAK	SAK	TN	NO_3^--N	NH_4^+-N	TS	HM	OM	pH
Rhizosphere												
Total bacteria	0.690	–0.076	–0.776	0.654	0.509	0.648	–0.969*	0.719	0.579	0.664	0.623	–0.973*
Fungi	–0.709	–0.108	0.881	–0.725	–0.615	–0.756	0.888	–0.798	–0.664	–0.770	–0.730	0.945
Actinomycetes	–0.968*	–0.469	0.988*	–0.935	–0.877	–0.953*	0.703	–0.969*	–0.448	–0.959*	–0.941	0.894
Ammonifier	0.471	0.058	–0.651	0.322	0.257	0.445	–0.543	0.419	–0.980*	0.458	0.404	–0.526
Silicate bacteria	0.570	0.378	–0.712	0.425	0.417	0.563	–0.306	0.496	0.888	0.570	0.530	–0.393
Sulfur bacteria	0.637	0.533	–0.749	0.505	0.521	0.639	–0.214	0.558	0.794	0.642	0.611	–0.358
Azotobacter	–0.105	–0.760	–0.069	–0.184	–0.341	–0.160	–0.734	–0.080	–0.646	–0.139	–0.197	–0.464
Nitrobacteria	0.777	0.414	–0.890	0.661	0.625	0.764	–0.527	0.728	0.815	0.772	0.735	–0.652
Cellulolytic microbe	0.887	0.245	–0.926	0.858	0.762	0.860	–0.855	0.906	0.471	0.870	0.844	–0.975*
Non-rhizosphere												
Total bacteria	0.959*	0.641	0.904	0.630	0.576	0.805	0.633	0.583	0.846	0.803	0.804	–0.866
Fungi	0.690	0.467	–0.845	0.575	0.397	0.733	0.430	0.593	0.540	0.646	0.673	–0.967*
Actinomycetes	–0.381	–0.601	0.648	–0.734	–0.585	–0.698	–0.563	–0.795	–0.407	0.613	–0.641	0.513
Ammonifier	0.684	0.267	–0.742	0.338	0.183	0.566	0.239	0.328	0.470	0.497	0.517	–0.985*
Silicate bacteria	0.814	0.461	–0.598	0.359	0.416	0.515	0.479	0.272	0.738	0.577	0.558	–0.512
Sulfur bacteria	0.933	0.572	–0.866	0.559	0.504	0.750	0.565	0.508	0.802	0.747	0.748	–0.869
Azotobacter	0.776	0.303	–0.614	0.247	0.237	0.465	0.309	0.175	0.618	0.488	0.480	–0.706
Nitrobacteria	0.709	0.2230	–0.697	0.265	0.146	0.510	0.211	0.235	0.490	0.465	0.478	–0.939
Cellulolytic microbe	0.866	0.640	–0.951*	0.705	0.573	0.859	0.613	0.698	0.746	0.805	0.822	–0.962*

Notes: *, correlation is significant at the 0.05 level (2-tailed); **, correlation is significant at the 0.01 level (2-tailed)

4 Discussion

4.1 Chemical properties in rhizosphere and non-rhizosphere soil

The succession of vegetation is a process that plant is constant adaption and transformation to soil, and the growth and development of plant effect the changes of soil properties (Qi *et al.*, 2007). In the previous study, many researchers have found that plant can improve the content of organic matter, humic matter and mineral elements (Hassan and Majumder, 1990). In this study, the results showed that the chemical properties of rhizosphere soil from different *R. aureum* populations living in the alpine tundra (2000 m, 2300 m and 2614 m) were excel compared to non-rhizosphere, particularly mineral elements, humic matter and organic matter.

In all chemical properties of soil, the distribution of NO_3^--N is very interesting. At the sample site of 1580 m, NO_3^--N of rhizosphere soil is significantly lower than that of non-rhizosphere soil, but NO_3^--N is very nearly both the sample sites of 2000 m and 2300 m, and it is obviously higher than non-rhizosphere at the sample site of 2614 m (Table 2 and Fig. 2). The results of some researches have indicated that plants get the NO_3^--N affected by many environmental factors, such as temperature, pH and CO_2 concentration *etc*., lower temperature, higher pH and lower CO_2 concentration can slow down NO_3^--N absorption (Johnson *et al.*, 2000; Chen *et al.*, 2005). The four sample sites are located at different altitudes, and 1580 m has the highest temperature and CO_2 concentration, and the pH is the lowest, but it will still accelerate the absorption of NO_3^--N in the rhizosphere soil of *R. aureum* populations. Furthermore, the habitat of *R. aureum* population living in 1580 m is located at Mountain frigid-temperate coniferous forest (MFTCF), and there are lots of fir, spruce and shrubs around *R. aureum*, so NO_3^--N of non-rhizosphere soil may be went up. Therefore, there is higher level of NO_3^--N in non-rhizosphere soil. The site of 2614 m has the highest pH, the lowest temperature and CO_2 concentration, which will result in slow down the NO_3^--N absorption in the rhizosphere soil of *R. aureum* populations. Additionally, the non-rhizosphere soil of 2614 m is a desert region and the lack of NO_3^--N input. As a result, there is higher level of NO_3^--N in the rhizosphere

soil of *R. aureum* populations, which indicated that *R. aureum* can increase soil fertility, especially rhizosphere soil.

4.2 Microbial community structure in rhizosphere and non-rhizosphere soil

The composition and quantity of soil microorganism is affected by vegetation types (Lipson and Monson, 1998; Stephan *et al.*, 2000; Rajaniemi and Allison, 2009). The results displayed that the composition of microorganism between rhizosphere and non-rhizosphere soil of *R. aureum* populations is not difference, while the quantity of *R. aureum* populations have significant differences between rhizosphere and non-rhizosphere soil. At all sample sites, the number of microbe for almost all in rhizosphere soil of *R. aureum* is higher than that in non-rhizosphere. The rhizosphere microorganisms of *R. aureum* are mainly composed of bacteria, actinomycetes, followed by fungi least, similar to the study of Govaerts *et al.* (2008). The principal component analysis (PCA) biplot displayed that nitrobacteria, sulfur bacteria, total bacteria and fungi were important factors for rhizosphere soils, and actinomycetes, cellulolytic microbe, nitrobacteria and azotobacter were important factors for non-rhizosphere soils. This indicates that there were differences between rhizosphere and non-rhizosphere soils for microflora.

The nutrient sources of soil microorganism are mainly plant residues (litters), root exudates and root exfoliation (Lipson and Monson, 1998; Sun *et al.*, 2009), and the quality and quantity of nutrient sources is different between rhizosphere and non-rhizosphere soil of *R. aureum* populations, which will inevitably lead to the non-uniform distribution of soil microorganism in rhizosphere and non-rhizosphere.

In addition, we observed that soil microbial community exhibit no apparent altitudinal gradient in the Changbai Mountains. These results suggest that microbial distribution may not follow the patterns of *R. aureum* among the selected altitude. However, microbial distribution were sharply different between the selected elevations (1580 m, 2000 m, 2300 m) and the highest elevation (2614 m) in this study. Maybe there are not any other plant could grow at altitude of 2614 m except for *R. aureum*, so there is the least microorganism at altitude of 2614 m. It indicates that *R. aureum*, a cold-resistant alpine plant, can grow and adapt to such an adverse ecological environment, so it may play an important role in soil improvement.

4.3 Correlations between soil chemical properties and microbial community structure

Plants regulate bacterial communities by determining the quantity and quality of the litter substrate supply (such as C or N source) and by modifying the soil physical environment which has been observed that vegetation type has a strong influence on the distribution of the dominant bacterial communities in Arctic tundra soil (Wallenstein *et al*., 2007; Chu *et al*., 2011). The *R. aureum*, as the dominant vegetation living in the alpine tundra, can also regulate the rhizosphere microorganism structure by changing the chemical properties of the soil.

For rhizosphere soils of *R. aureum*, the total bacteria were significantly negatively correlated to pH and NO_3^--N. In the sample sites of 2614 m, it has the highest pH and NO_3^--N, while the total bacteria were the lowest (Fig. 2 and Fig. 3). These results indicate that bacteria abundances is clearly influenced by soil pH at the Changbai Mountains, and it is similar to previous studies on the Changbai Mountains (Chu *et al*., 2010; Shen *et al*., 2013). However, in this study, we found that NO_3^--N is also a better predictor of bacteria abundance across an altitudinal gradient. Whereas at a landscape scale, Nielsen *et al.*, (2010) observed that soil bacterial community composition was not directly associated with plants, but selected by soil pH and C/N ratio.

Actinomycetes population has been identified as one of the major groups of the soil population. They are widespread in nature and found to be more in dry than wet soils (Thangapandian *et al.*, 2007). Actinomycetes are significantly negatively correlated to total P, total N, NH_4^+-N, humic matter and positive correlations with total K. The study of various factors (soil acidity, the barley variety, and their developmental phases) on the rhizosphere actinomycete complex showed that it is soil acidity that substantially influences the population of rhizosphere actinomycetes. The effect of soil acidity was most likely due to the different tolerance of rhizosphere actinomycetes to high concentrations of hydrogen ions. In the acid soil, the total number of actinomycetes was lower (Shirokikh *et al*., 2002). In this study, pH of 2614 m in rhizosphere soil is the highest among all altitudes, and the number of actinomycetes is

the most. Additionally, ammonifier is significant negatively correlated to total S, and cellulous microbe is significant negatively correlated to pH; meanwhile the change of total S and pH in the same sites is coincident.

For non-rhizosphere soils of *R. aureum*, only fungi, ammonifier and cellulose microbe are negatively correlated to pH, and total bacteria is positively correlated to total P (Table 5). These results showed that pH is the major factor effecting microorganism structure in non-rhizosphere soil.

5 Conclusions

In this study, we evaluated the structure of microbial communities of rhizosphere of *R. aureum* populations located at different altitudes on the northern slope of the Changbai Mountains, China, and analyzed the correlation between chemical properties of soil and microbial communities. The microbial population of the four sample sites is more in the rhizosphere soils compared to non-rhizosphere soils; the rhizosphere microorganisms are mainly composed of bacteria, actinomycetes, followed by fungi least. Moreover, microbial distribution has convergent characteristics and may not follow the patterns of *R. aureum* among the selected elevation. Bacteria abundance is clearly influenced by pH on the Changbai Mountains, and besides pH, other chemical properties of soil like NO_3^--N. These results demonstrate that *R. aureum* as the dominant vegetation living in the alpine tundra is a key factor in the formation of soil microorganism and improving soil fertility, and is of great significance for the maintenance of alpine tundra ecosystem. Furthermore, alpine tundra located at the Changbai Mountains is built by the accretion of tephra, and many areas is lack of vegetation, combined the role of *R. aureum* effecting on rhizosphere soil should provide a useful guide for the revegetation of alpine tundra.

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