

**EFFECTS OF HUMAN ACTIVITIES ON SOIL PROPERTIES AND
BACTERIAL COMMUNITY IN YAOQUANSHAN OF THE
WUDALIANCHI SCENIC AREA, CHINA**

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Abstract

Traditional microbial culture was used to qualitatively and quantitatively analyze the bacterial community which was sampled from the active, buffer and background regions of the top, side and foot of the Yaoquanshan mountain to investigate the effects of human activities on the soil properties and bacterial community structure, response of bacterial community structure to the soil chemical properties and enzyme activity. The results showed that altitude was the main reason in differential bacterial population distribution in Yaoquanshan soil, followed by human activity disturbance. The bacterial community structure showed little difference at the same altitude. The isolation frequencies of *Bacillus*, *Micrococcus* and *Proteus* decreased with increased human activities, while those of the *Enterobacter*, *Lactobacillus*, *Aeromonas* and *Azotobacter* presented an elevating trend. *Bacillus* was the dominant genera in the soil from all the sampling points, having an isolation frequency of 17.78 - 68.97%. Activities of total phosphorus and catalase had relatively greater effect on the bacterial community distribution, followed by sucrase, cellulase and organic matter. The *Proteus* was positively correlated with the total phosphorus content when it was in the range of $1.49 \pm 0.39 \sim 1.80 \pm 0.26$ g/kg. The *Kluyvera* in the soil from active area of mountain-top was positively correlated with the sucrase. This study demonstrates that massive human activities to some extent might have effects on soil properties, bacterial population and bacterial community structure in Yaoquanshan mountain, China.

Introduction

Tourism has become one of the world's largest industry, with its negative impact on the ecological environment has become one of the research priorities in the field of environment and tourism (Davenport and Davenport 2006, Reyes-Martinez *et al.* 2015, Zhang *et al.* 2012). In recent years, with the rise of mountain tourism, its impact on mountain ecosystems has gradually attracted attention (Cheng and Zhang 2005, Geneletti and Dawa 2009, Nepal and Chipeniuk 2005, Zhang *et al.* 2012). Human activities in tour may have significant impacts on soil ecosystem in the mountain soil, thereby changing the soil's chemical structure and its properties (Shi 2006). The micro-organisms constituting the soil ecosystem plays a key role in energy flow, nutrient cycling, regulating biogeochemical cycle and maintaining ecosystem functions (Bell *et al.* 2005, Bing-Ru *et al.* 2006, Fierer and Jackson 2006). Microbial diversity has an effect on the structure, function and process of the soil ecosystem. It is an important component in maintaining soil's productivity as well as serves as an important index for evaluating the changes of soil quality induced by natural or human disturbance. The accelerated decomposition of organic matter in the soil can be attributed to the increase in microbial population producing decomposing enzymes (Guan *et al.* 1986). Therefore, the soil's chemical properties, enzyme activity and microbial diversity are interrelated and interacted communities in the soil ecosystem.

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The chemical properties and enzyme activity may vary due to anthropogenic activities, as bacteria may evolve community structure component adaptive to these disturbances due to their strong adaptability. Therefore, the present study is aimed to analyze the changes in the bacterial structure as well as the response of bacterial community structure to the soil's chemical properties and enzyme activities under varying degrees of anthropogenic activation, so as to provide basis for assessing the effects of tourism development on natural environment of the Yaoquanshan, the Wudalianchi Scenic area in Heilongjiang province, China.

Materials and Methods

Yaoquanshan mountain was divided into three levels: top (T), side (Y) and foot (J), and 3 experimental regions were set up at each level: (1) active area (severe trampling area by tourists, Z), i.e. roads, with severe trampling and absence of leaves, weeds and other shrubs; (2) buffer area (mild trampling area by tourists, M), which was 3 - 8 m away from the roads, with relatively less human activities and covered with fallen leaves and dry branches, where the ground was partially covered by shreds and weeds; (3) background area (no trampling, Q), which was 20 - 30 m away from the roads, with almost no tourists invasion, and was covered with large amount of dry branches and fallen leaves (about 10 cm), and the ground was completely covered by shreds and weeds.

At each experimental region, 5-point sampling method was adopted to collect samples from topsoil (0~15 cm). In an area of $1 \times 1 \text{ m}^2$, the diagonal center was considered as the central sampling point with the other four corners as the rest of the sampling points. After removing the fallen materials, the soil was mixed, placed into a valve bag and taken to laboratory for analysis. The collected soil was divided into two parts, one was dried naturally and filtered using 1 mm sieve for enzyme activity detection and chemical properties analysis. Another part was preserved at 4°C for quantitative and qualitative analyses of bacteria.

The analytical agents were purchased from Harbin Haitian Biotechnology Co., Ltd. (Heilongjiang province, China). The pH value, the organic matter content, total nitrogen, total phosphorus content and available phosphorus were measured using potentiometry, potassium dichromate heating method, Kjeldahl's method, Mo-Sbeolorimetric method, and Bray's method, respectively (Kemper and Rosenau 1986, Shidan 2000). The enzyme activities of soil was determined according to the soil enzyme and its research methods (Guan *et al.* 1986). Analyses were carried out in triplicates.

The bacteria was quantitatively measured by spread plate method and it was cultured on a beef extract peptone medium at 37°C for 24 hrs. Isolation, purification, and qualitative analysis of bacteria for morphologic identification (Gram's staining, flagellum staining, capsular staining and spore staining) and physiological-biochemical tests of oxidant enzyme, catalase, amylolytic enzyme, cellulose, etc. were carried out. Bacterial identification was carried out following manual of common bacterial identification and Bergey's manual of systematic bacteriology.

Isolation frequency was determined by the following equaton. Isolation frequency (%) = The total number of one isolate/the total number of all isolates $\times 100$. Data analyses were performed using SPSS17.0 and Origin 8.0 software. The chemical properties of soil, enzyme activity and bacterial population were compared among the soil from different sampling points using one way ANOVA. The correlation of chemical properties and enzyme activities with the bacterial amount was assessed using Pearson correlation coefficient. Cluster analyses of the bacterial community structure in different soil samples was performed using Primer 5 software. The detrended correspondence analysis (DCA) and redundancy analysis (RDA) were performed to analyse the correlation between the microbial community structure with soil chemical properties and enzyme

activity in the CANOCO 4.5 software. RDA diagram can be used to describe the changes in species diversity across the samples in ordination space, with optional interpretation using environmental variables.

Results and Discussion

Fig. 1 shows that the chemical properties and enzyme activities, the total nitrogen (TN), total phosphorus (TP), available phosphorus (AP) and pH contents did not show statistically significant differences among soil from top, side and foot of the mountain, while the organic matter (OM) content was 97.14 ± 14.07 g/kg in the mountain-top soil, which was statistically significant different among mountain-side soil and mountain-foot soil ($p < 0.05$). The cellulose activity showed a statistically significant difference between the mountain-top and mountain-foot soil ($p < 0.05$), with activities of 10.03 ± 1.01 mg glucose g⁻¹ d⁻¹ and 5.34 ± 1.08 mg glucose g/d, respectively, while the activities of other enzymes did not show statistically significant differences. Correlation analysis of chemical properties and enzyme activities revealed that the OM, TN, catalase and cellulase were significantly correlated at a level of 0.01 (bilateral) and the correlation coefficients are shown in Table 1.

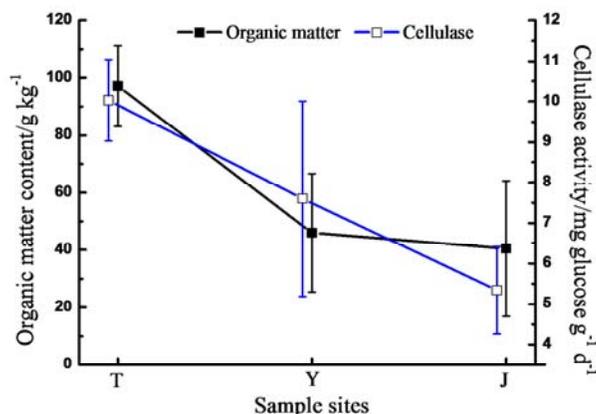


Fig. 1. Organic matter content and cellulase activity in the soil from different altitudes. T: Mountain-top; Y: Mountain-side; J: Mountain-foot; Z: Active area; M: Buffer area; Q: Background area.

Table 1. Correlation analysis of bacterial number, soil chemical properties and enzyme activity.

Index	Bacteria No.	OM	TN	catalase	cellulase
Bacteria No.	1				
OM	0.167	1			
TN	0.168	0.897**	1		
Catalase	-0.052	0.962**	0.889**	1	
Cellulase	0.200	0.861**	0.850**	0.866**	1

**Refers to significantly correlated at a level of 0.01 (bilateral); OM = Organic matter; TN = Total nitrogen.

The cultivable bacterial population in the soil ranged from $1.13 \pm 0.1 \times 10^7$ - $5.90 \pm 0.8 \times 10^7$ cfu/g (Fig. 2). The bacterial population had no statistically significant difference among TZ, TM and TQ. There was no statistically significant difference among the YZ, YM and YQ, indicating that human activities do not have any significant effect on bacterial population on

mountain-top and mountain-side soil of the Yaoquanshan. While the bacterial population showed significant difference among the soil at the mountain-foot where the bacterial population in JZ ranged $1.13 - 5.90 \times 10^7$ cfu/g, which is comparatively lesser than those in JM and JQ ($p < 0.05$) (Fig. 2), indicating that the bacterial population at the mountain-foot is significantly negatively correlated with the degree of human activities, where it is significantly decreased with increased human activities (Fig. 2). The bacterial population in the soil from background areas of mountain-top and mountain-side showed significant difference when compared with that of mountain-foot soil ($p < 0.05$), hereby presenting a decreasing trend with increased altitude.

The bacteria are cosmopolitan and accounts for 70 - 90% of the soil microbial population. The bacterial population of Yaoquanshan soil presented a decreasing trend with increased human activity. DUAN Qing-qian *et al.* (2014) investigated the effect of tourism disturbance on microbial population in Wutaishan soil, and found significant effect on bacteria, actinomycelial, fungi as well as the total number of microbes in 0 ~ 20 cm topsoil. Wang (2014) found that the microbial population in Jiugongshan scenic area was significantly positively correlated with degree of tourism, and the degree of tourism disturbance was the most significant factor in affecting the microbial population in the soil.

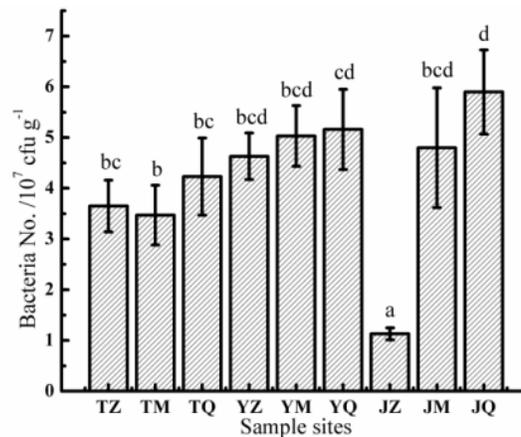


Fig. 2. Bacterial amount in the soil from different sampling points. Different lowercase letters refer to significant difference between data in the columns ($p < 0.05$, $n = 3$). The meaning of the letters as shown in Fig. 1.

A total of 95 isolated belonging to 14 genera, such as *Alphaproteo bacteria*, *Gammaproteo bacteria*, *Bacillum*, *Deinococcus* and *Actino bacteria* etc. were isolated and purified from Yaoquanshan (Fig. 3). Similarity among bacteria in the soil was analyzed by cluster analysis and found to be 34.10% (Fig. 4). *Bacillus* was a common in the soil from different sampling points, having a proportion ranged from 17.78 - 68.97% and belonging to the dominant cultivable species (Fig. 3). The similarities among cultivable bacteria in the soil from each sampling point at mountain-top was 57.14%, and at mountain-side and mountain-foot were 57.27 and 56.41%, respectively suggesting that the bacterial community structure different narrowly in the soil from different sampling points at the same altitude. The similarity of cultivable bacteria was 16.67, 33.33 and 30.76% in the soil from sampling points TZ, YZ and JZ from sampling points TM, YM and JM, as well as from sampling points TQ, YQ and JQ, respectively suggesting that the bacterial community structure had large difference in the soil from different altitudes (Fig. 4). Fig. 3 shows that the isolation frequencies of TQ *Bacillus* and *Micrococcus* were 68.97 and 4.93%, respectively

and were reduced to 17.78 and 0% at TZ, indicating that the isolation frequency decreases with increased human activities. The isolation frequency of *Enterobacter* was 4.93% at TQ and was elevated to 17.78% at TZ, indicating that the isolation frequency of enterobacter was elevated with increased human activities. The isolation frequency of *Lactobacillus* in the soil from mountain side increased from 0 to 15.62% with increased human activities. The isolation frequencies of *Aeromonas* and *Azotobacter* was 0% at JQ and increased to 16.07 and 3.57% at JZ, suggesting that the isolation frequencies of *Aeromonas* and *Azotobacter* was elevated with increased human activities. While the isolation frequency of *Proteus* was contrary, which were 16.05 and 0% at JQ and JZ.

The bacterial community structure had small difference in the soil from the same altitude in Yaoquanshan, while had large difference at different altitudes, suggesting that spatial differentiation was the main reason causing difference of microbial community structures. With the increased human activities, the isolation frequencies of *Bacillus*, *Micrococcus* and *Proteus* were decreased, while those of the *Enterobacter*, *Lactobacillus*, *Aeromonas* and *Azotobacter* presented an increasing trend, indicating that the tourism disturbance affected the composition of bacterial community structure to certain extent. The tourism disturbance might change the soil porosity (XiangLi *et al.* 2008), organic matter content (Gong *et al.* 2009) and other physicochemical properties, but also might destroy the composition of ground flora and fallen materials (Peng *et al.* 2012), hereby indirectly affecting the composition of bacterial community structure, further leading to increasing or decreasing trend of isolation frequency of some bacterial communities in the soil.

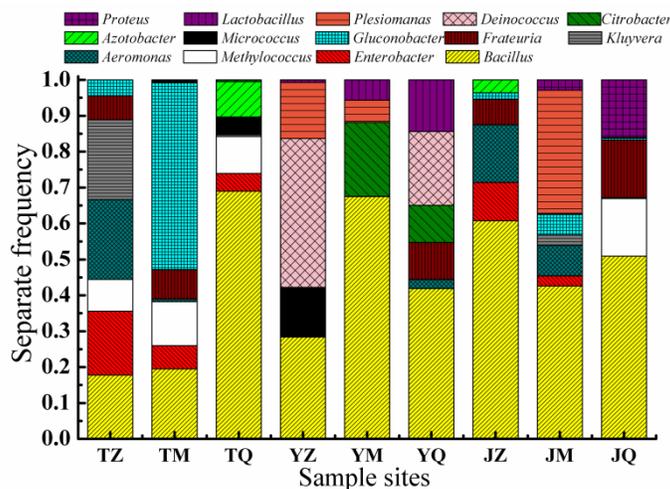


Fig. 3. Isolation frequencies of cultivable bacteria in the soil from different sampling points. The meaning of the letters as shown in Fig. 1.

The bacterial amount was not significantly correlated with OM, pH, TN, TP and AP, and also was not significantly correlated with enzyme activities of catalase, cellulase, sucrase, urase and phosphatase (Table 1). RDA method was used to analyze the correlation of bacterial community structure with environment factor, in which the Eigen values of the first two ordination axes were 0.327 and 0.172, respectively which could be used to explain the bacterial community changes. The correlation coefficients of bacterial community-environment corresponded to 35.2 and 18.6%

of the community-environment relationship, where the selected 5 chemical factors and enzyme activity factors accounted for 92.9% of the total eigenvalues (Table 2). Fig. 5 shows that the five chemical factors and activity of five enzymes had varying degrees of response relationship with the bacterial community distribution, among which the TP and catalase had relatively large effect, followed by sucrose, cellulase and OM. *Bacillus* is the widely distributed as well as a common bacterial genera in Yaoquanshan soil with a TP content ranged $1.49 \pm 0.39 \sim 1.80 \pm 0.26$ g/kg (Fig. 5). The *Proteus* was positively correlated with TP content. Meanwhile, the isolation frequency of *Proteus* as well as TP content in the soil from background area of mountain-foot were higher than those from buffer area, and both were negatively correlated with human activities. In the soil from active area of the mountain-top, the *Kluyvera* was positively correlated with sucrose, and its isolation frequency was as high as 22.22%, belonging to the dominant species in that area. Meanwhile, the sucrose activity was also high in that area, which was 1.38 ± 0.02 ml 0.1 N $\text{Na}_2\text{S}_2\text{O}_3/\text{g}$.

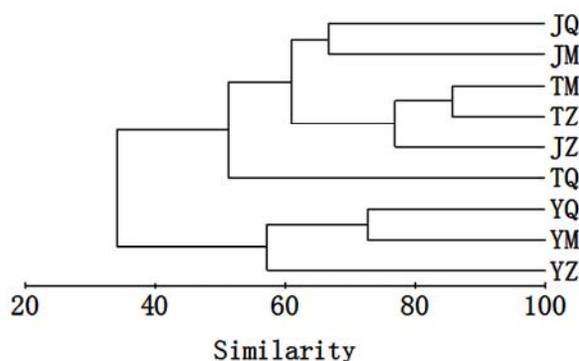


Fig. 4. Cluster analyses of bacterial community structure in the soil from different sampling points. The meaning of the letters as shown in Fig. 1.

Table 2. The results of axis and species-environment correlation from RDA analysis.

Axes	1	2	3	4
Eigen values	0.327	0.172	0.149	0.121
Species-environment correlations	1.000	1.000	1.000	1.000
Cumulative percentage variance of species data	32.7	50.0	64.9	77.0
Cumulative percentage variance of species-environment relation	35.2	53.8	69.9	82.9
Sum of all canonical Eigen values		0.929		
Sum of all Eigen values		1.000		

The changes in soil environment (such as chemical properties and enzyme activity, etc.) might directly affect the microbial community structure (Lemenih and Itanna 2004, Templer *et al.* 2005, Yu *et al.* 2011). GuoYa-fen (Yafen and Pengwei 2009) found that with the tourism disturbance, the soil nutrient was closely correlated with the soil enzyme activity, where there was significant correlation between the organic matter and catalase, as well as between the total nitrogen and catalase, which were consistent with the present study. However, we found that the bacterial population was not significantly correlated with the soil chemical properties and enzyme activity

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