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Soil bacterial diversity in relation to change in altitudinal environment in alpine meadow*

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Abstract Along an elevation gradient(3200 m to 3800 m) on Qinghai-Tibetan plateau, BIOLOG GN2 plates were used to analyze the elevation patterns of bacterial community functional diversity. The redundancy analysis (RDA) method was further used to analyze the relationship between bacterial profiles and the environmental variables. The results indicate that soil variables explain 46.6% of the variation in bacterial community functional diversity. Among the soil variables, soil available phosphorus explains the largest part, which suggests that it might be an important limiting factor for soil bacterial community functional diversity in this area. The soil temperature, which changes with the altitute, also has a profound effect on bacterial community functional diversity.

Key words alpine meadow; BIOLOG; RDA; elevation; available phosphorus
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海拔梯度上高寒草甸土壤细菌功能多样性与环境因子的关系

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摘 要 在青藏高原 3200~3800 m 的海拔梯度上,利用 BIOLOG 和冗余分析的方法,对细菌功能多样性和环境因子的关系进行了研究. 结果表明,碳氮磷等土壤的基本性质可以解释 46.6%的细菌群落功能多样性的变化. 土壤中有效磷的贡献最大,它可能是青藏高原上限制土壤细菌功能多样性的一个重要因子. 由于海拔梯度变化而引起的土壤温度的变化,对细菌群落的功能多样性也有显著的影响.

关键词 高寒草甸; BIOLOG; 冗余分析; 海拔; 有效磷

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Rising global temperature is anticipated to change the function and structure of terrestrial ecosystems[1]. The majority of field experiments have mainly focused on plant communities[2], greenhouse gas emissions[3], soil or ecosystem respiration[4] and nutrient cycling[5]. Only relatively few studies addressed soil microorganisms, especially *in situ* studies[4, 6], although soil microbial communities mediate critical ecosystem processes, such as carbon and nutrient cycles. A deeper understanding of microbial response to warming is of critical significance in predicting terrestrial ecosystem feedback to climate change.

Despite of the potential importance of soil microbial diversity in ecosystem functions, the quantitative linkage between soil microbial diversity and environmental variables is still not well studied. A recent study suggested that microbial diversity is unrelated to site temperature, latitude, or other variables that typically predicted plant and animal diversity[7], in contrast to most other studies[8]. Elevation gradients are powerful systems for testing how ecological properties and processes are affected by changes in temperature and associated variables[9]. They provide a valuable complement to shorter-term experimental studies[10] in analyzing the relationship between environmental variables and microbial community diversity.

The Qinghai-Tibetan Plateau occupies an area of 2.5×10^6 km^2 and features rolling mountains. It has been referred as the third pole of the world. In this study, we used an elevation gradient from 3 200 m to 3 800 m to identify environmental variables responsible for potential elevation patterns of soil bacterial diversity. Given greater temperature fluctuations, stronger UV irradiation and lower nutrient contents at higher altitude, we hypothesized that: 1) bacterial functional diversity decrease with elevation; 2) bacterial functional diversity increase with temperature.

1 Materials and methods

1.1 Site description and soil sampling

Field investigations were carried out at the Haibei Alpine Meadow Ecosystem Station, which is located in the northeast of the Qinghai-Tibetan Plateau. It is a large smooth valley surrounded by the Qilian Mountains at latitude 37°37′N, longitude 101°19′E and altitude approximately 3 200 m. The average altitude of the surrounding mountain area is 4 000 m above sea level and 3 200 m for the valley area. The site is characterized by a typical alpine meadow climate. Annual average temperature is −1.7℃ with a maximum of 27.6℃ and minimum of −37.1℃. Annual average precipitation ranges from 426 mm to 860 mm[11]. Four elevations from the station upwards (3 200, 3 400, 3 600, and 3 800 m) were selected in this study. Samples were collected in August 2008 with the soil depth of 0-10 cm and 10-20 cm. Four samples were taken for each layer at each altitude. The samples used for physical and chemical analysis were air dried and sieved through a 2-mm mesh screen. Samples for microbiological analyses were cooled with ice packs in the field immediately after collection.

1.2 Basic soil property

Soil pH was determined with a compound electrode using a soil-to-water ratio of 1∶2.5. Soil organic matter and total nitrogen were determined by dichromate oxidization and Kjeldahl digestion respectively. Mineral nitrogen was analyzed colorimetrically using an automated procedure after extraction with 0.01 mol/L $CaCl_2$ in a soil-to-solution ratio of 3∶25 for 30 min. Available phosphorus in the soil was measured using the sodium bicarbonate extraction-molybdenum-antimony anti-colorimetry method.

1.3 Biolog experiment

The 10 g soil was mixed with 100 mL autoclaved 0.85% NaCl solution and glass beads (2 mm in diameter). The suspensions were shaken for 30 min. A 1∶1000 dilution in 0.85% NaCl was used to inoculate Biolog GN2 plates (150 μL per

well). The plates were incubated for up to 7 days at 25℃ and the color development was measured every 24 h with a microtitre plate reader at 590 nm.

Average well color development (AWCD) was calculated according to Garland and Mills[12],

$$AWCD = \sum_{i=1}^{n} (C - R)/n,$$

where C was color production within each well, R was the absorbance value of the control wells, and n was the number of carbon substrates ($n = 95$). AWCD values were calculated at 120 h incubation time. Except calculating the value of the whole plate, the substrates was also assigned to six groups of carbon sources, including amines/amides (6), amino acids (20), carbohydrates (28), carboxylic acid (24), miscellaneous (12), and polymers (5)[13]. AWCD of each group was calculated using the average absorbency fraction in the same way as the whole plate.

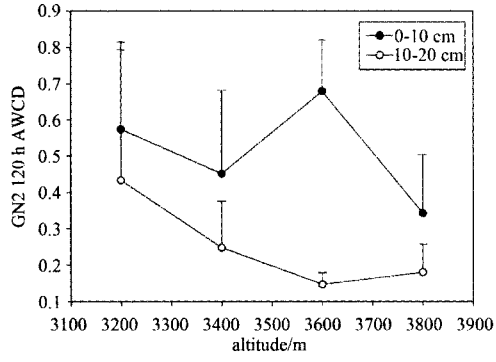
1.4 Statistical analysis

One-way ANOVA was used to determine statistically differences in soil properties and bacterial assays on each soil layer among different altitudes. The relationship between elevation and other parameters were analyzed using linear regression model. All above were calculated using SPSS® 11.5 (SPSS Inc.). Twelve environmental parameters were used to analyze multivariate relationships between bacterial properties and these variables, including altitude (Alti), growing season average temperature (T), growing season average soil water content (SWC), soil depth (Dept), pH, nitrate content (NN), ammonium content (AN), total nitrogen content (TN), soil organic matter (SOM), available phosphorus content (AP), C∶N and N∶P ratio. 95 quantitative variables from individual carbon substrate data of Biolog GN2 microplate, was also used in analysis. Considering all 32 samples as a whole, Detrended Correspondence Analysis (DCA) was run first. Redundancy Analysis (RDA) was then used to quantify and test effects of explanatory variables on the Biolog variations since the gradient length was low. The statistical significance of the relationships was tested using the Monte Carlo permutation method. All these analyses were performed with CANOCO 4.5[14].

2 Results

2.1 Soil bacterial community functional diversity along the elevation gradient

Despite 120 h AWCD values showing a general declining trend from 3200 m to 3800 m elevation in 10-20 cm soil layer, no significant relationships between elevation and the 120 h AWCD values were found using linear regression models ($P > 0.1$). ANOVA analysis also indicated that there is no significantly different of bacterial 120 h AWCD among different altitudes of the same soil layer, except a significantly higher value for bacteria in the upper layer at 3600 m than 3800 m. Higher AWCD values were also found in upper soil layer than lower soil layer at the 3600 m sites (Fig. 1).



Standard deviation were shown ($n = 4$).

Fig. 1 120 h average well color development (AWCD) of GN2 Biolog plates

2.2 Relationships between bacterial community functional diversity and soil temperature

Regression analysis between bacterial community functional diversity (120 h AWCD) and soil temperature in the growing season were shown in Table 1. Among different carbon groups, the bacterial utilization of polymers, carbohydrates, carboxylic acid and miscellaneous all increased with temperature significantly; only the utilization of

Table 1　Regression analysis between 120 h AWCD of six groups of carbon sources and soil temperature of the growing season

Carbon sources	Regression equation	R^2	F	P
AWCD	$y = 0.052x - 0.013$	0.127	5.455	0.027
Polymers	$y = 0.044x - 0.087$	0.138	4.626	0.04
Carbohydrates	$y = 0.06x - 0.13$	0.152	5.207	0.03
Carboxylic acid	$y = 0.054x + 0.071$	0.137	4.615	0.04
Miscellaneous	$y = 0.043x - 0.005$	0.135	4.544	0.042
Amino acids	$y = 0.054x + 0.053$	0.109	3.562	0.069
Amines/Amides	$y = 0.027x + 0.006$	0.1	3.209	0.084

amino acids and Amines/Amides groups were not strictly significantly correlated with soil temperature ($0.05 < P < 0.1$). Nevertheless, regression analysis indicated soil bacterial carbon catabolic activity significantly increased with temperature.

2.3　Main environmental variables explaining bacterial diversity patterns

There were complex changes for many soil variables along the elevation gradient Table 2. ANOVA analyses showed that most soil properties were significantly different among elevations.

Table 2　Basic soil properties (mean value ± SD, $n = 4$)

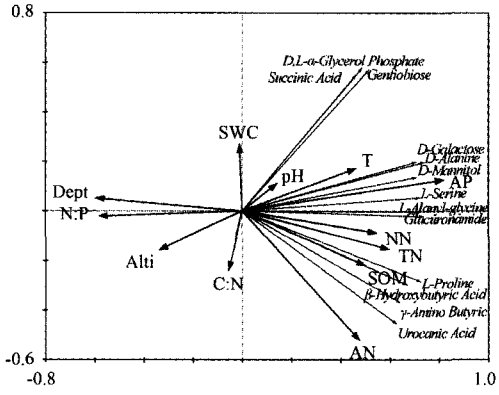
soil depth/ cm	altitude/ m	pH	inorganic N		total N/ (g/kg)	available phosphorus/ (mg/kg)	soil organic matter/ (g/kg)	soil moisture content/ %
			NH_4^+-N/ (mg/kg)	NO_3^--N/ (mg/kg)				
0-10	3200	7.76 ± 0.24a	2.70 ± 3.66a	14.14 ± 8.09b	5.86 ± 0.82b	15.57 ± 2.82a	126.96 ± 24.88b	32.57 ± 8.44c
	3400	6.94 ± 0.26b	3.16 ± 1.43a	10.18 ± 2.59b	4.91 ± 0.31b	9.57 ± 1.40b	110.94 ± 9.34b	39.65 ± 3.06bc
	3600	6.32 ± 0.20c	9.64 ± 9.54a	38.36 ± 13.56a	7.56 ± 0.22a	11.35 ± 1.65b	179.82 ± 9.73a	41.18 ± 3.35c
	3800	6.68 ± 0.39bc	0.86 ± 0.75a	17.60 ± 4.06b	4.92 ± 1.08b	5.16 ± 0.80c	109.08 ± 27.20b	53.38 ± 7.75b
10-20	3200	8.08 ± 0.04a	1.53 ± 1.56a	9.06 ± 5.00a	4.08 ± 0.70b	10.04 ± 0.91a	81.91 ± 10.86c	31.68 ± 2.96d
	3400	7.01 ± 0.22b	1.90 ± 0.78a	10.54 ± 3.80a	4.51 ± 0.40b	6.96 ± 0.32b	96.61 ± 4.44bc	41.18 ± 3.35c
	3600	6.46 ± 0.03c	1.98 ± 0.51a	10.94 ± 3.93a	5.86 ± 0.21a	5.33 ± 1.28bc	134.68 ± 7.51a	64.08 ± 0.93a
	3800	6.54 ± 0.18c	0.81 ± 0.46a	5.35 ± 3.58a	4.69 ± 0.46b	5.14 ± 1.23c	106.03 ± 13.48b	50.79 ± 8.63b

Note: Significant difference ($P < 0.05$) among different altitudes in the same layer is indicated by different letters.

RDA ordination diagrams (Fig. 2(a)) showed relationships between bacterial profiles and environmental variables. 54.9% of the variances in bacterial profiles was explained by environmental variables ($F = 1.732$, $P = 0.024$). The canonical axis 1 explains 31.6% of the total variance of soil bacterial diversity, whereas axis 2 explains only 5.6%. The first axis was clearly dominant and statistically significant ($F = 7.843$, $P = 0.026$). We set a threshold of a combination of axis 1 and 2 greater than 50% to select individual carbon sources. Only 13 GN2 carbon sources met this criterion and were showed in Fig. 2(a). All 13 carbon compounds were closely correlated with the first RDA axis, and also soil properties AP, T, TN, NN, AN and SOM. They were negatively correlated with soil depth and N: P ratio.

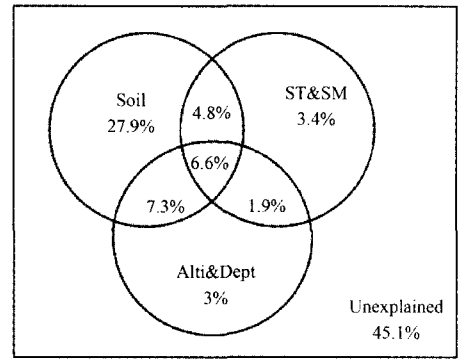
When using single environmental factor as explaining variables, available phosphorus (AP) had the highest explaining power for soil bacterial carbon source utilization profiles. AP explained 22.1% of bacterial carbon source utilization diversity ($P = 0.002$). Other environmental variables, except altitude, pH, C: N and soil water content, also significantly explained bacterial diversity variance ($P < 0.05$) (Fig. 2, Table 3). The environmental variables could be divided into three groups: soil variables (excluding soil temperature and moisture) explained 44.6% of the variations in the community functional diversity; soil temperature and moisture directly explained 16.7%, altitude and soil depth explained 18.8% of the

variation. Partial RDA analysis for variation partitioning (Fig. 2 (b)) indicated that the soil variables shared 13.9% with Alti and Dept in explaining variance in bacterial diversity. This indicates that altitude and depth contributed fundamentally to the effect of soil properties. Taken together, it implied that the functional activity of the bacterial communities was strongly controlled by soil environmental conditions with a large unexplained part (45.1%).



Relatively thin descriptors are GN2 plate carbon sources,more than 50% of which can be explained by two axes,and thick descriptors are environmental variables.

(a) Redundancy analysis (RDA) of bacterial functional diversity data and environmental variables



Numbers in the main circles show the variations explained by each group of environmental variables with the others as covaria-bles,and the numbers in the small circles show the interactions between two or three variables.

(b) RDA-based variation partitioning analysis which shows the relative proportions of bacterial community function data that can be explained by different types of environmental variable

Fig. 2 Constrained ordination analysis

Table 3 Power and significance of individual or subsets of the environmental variables in explanation of the variations in bacterial community carbon utilization data

variable	marginal effects		
	Lambda1	*F*	*P*
Soil	0.466	2.287	0.006
AP	0.221	7.941	0.002
TN	0.12	3.832	0.008
NN	0.115	3.642	0.018
N: P	0.117	3.717	0.02
SOM	0.09	2.775	0.042
AN	0.095	2.95	0.05
pH	0.035	1.015	0.336
C: N	0.016	0.467	0.91
Alti & Dept	0.188	3.117	0.006
Alti	0.059	1.748	0.092
Dept	0.121	3.85	0.008
ST & SM	0.167	2.705	0.01
ST	0.09	2.772	0.04
SM	0.034	0.974	0.362

3 Disscussion

There was no significant correlation between elevation and 120 h AWCD values in this study. RDA analysis also demonstrated that the explanatory effect of elevation alone was not significant for individual C substrates data according to the Monte Carlo permutation test ($P > 0.05$). These results were in contrast with those reported by Bryant et al.[15] and Singh et al.[16], in which bacterial diversity was significantly correlated with elevation. There are some possible reasons for the above discrepancy. 1) the smaller altitudinal gradient and data set in this study. Soil bacterial diversity was highly various in each elevation, therefore, the R^2 values for regression with elevation were low despite of statistically significant in both studies[15-16]. The R^2 was even lower in Bryant et al.[15] than this study, yet it spanned 2 400 m, much larger than the 600 m here. Nevertheless, a decreasing trend was

still shown for soil bacterial diversity across the elevation gradient in this site. 2) The higher elevation here. The elevation gradients were from 3 200 m to 3 800 m, which exceeded those in the other two studies[15-16]. Other variables may become more important in shaping the elevation pattern of soil bacterial diversity at much lower or higher altitudes, as presented in the study by Fierer et al. [7], which covered the elevation from 200 m to 3 450 m, and found no trend with elevation of bacterial diversity. The obviously different trend at the lower end of the gradients distorted the elevation relationships in some phylotypes. 3) The larger influence of local conditions. Körner[17] proposed that local environments may confound the elevational trend of ecosystem properties and processes, which could also explain the absence of an elevation trend in the study by Fierer et al. [7]. In the current study we found AP as the best predictor of bacterial functional diversity, indicating soil properties may have more important effects on bacterial functional diversity than elevation. 4) The spatial heterogeneity within each of elevation sampling site may be high, which obscured any inter-site differences in functional diversity levels across the gradient[18].

It is not surprising that upper soil layers in this study had larger AWCD values than the lower soil layers. Many studies showed that soil bacterial community composition is profoundly influenced by plant root exudates. For example, applying synthetic plant exudates in an *in vitro* experiment significantly stimulated bacterial densities and modified the oxidation pattern of Biolog GN2 plates[19]. Another study at this site indicated that 70% of root biomass in the top 20 cm soil layer was distributed within the 0-10 cm soil layer[20], and a higher portion was expected for higher elevation sites[21].

In this study, 45. 1% of variations in bacterial data across the elevation gradients were not explained by all the measured soil variables. Plants also play an important role in the bacterial functional diversity[8]. Although plant diversity may have different elevation patterns from soil bacterial diversity[7], variations in vegetation could influence the soil bacterial community differently across the elevation gradient. In these sites the relative predominance of the dominant species, mainly *Kobresia humilis*, *Potentilla fruticosa*, and *P. nivea*, gradually changed along the slope of this study[22]. In addition, the grazing period was not uniform along the altitudinal gradient[22]. Although difference in grazing intensity was difficult to precisely determine due to free grazing, it may create additional variations on soil bacterial diversity among these sites.

The temperature at the 3 200 m site is 4℃ higher than that at 3 800 m. Bacterial carbon utilization potential dramatically decreased with the decrease of soil temperature, which indicated that soil temperature had a profound effect on bacterial community functional diversity on the Qinghai-Tibetan Plateau. This supported our second hypothesis that temperature had a positive effect on soil bacterial community diversity on the Qinghai-Tibetan Plateau due to the high altitude and low temperature. Similarly, a 4℃ increase in soil temperature strongly increased microbial metabolic activity in a temperate mountain forest soil[23] and 2℃ warming increased the rate of microbial utilization of some carbon sources in a tall grass prairie[24]. This was not surprising as the decomposition of organic matter was a temperature-dependent biochemical process. Experimental warming tended to increase soil biomass at the colder and wetter site (cold limitation)[25], but reduced soil biota abundances at the colder and drier sites as revealed by a meta-analysis[26]. Annual average precipitation at the 3 200 m site ranged from 426 mm to 860 mm and annual average temperature was −1. 7℃[11]. Precipitation increases and temperature decreases with elevation. In contrast to the medium-altitude grassland and forest site, the low temperature rather than soil moisture was a main factor limiting the growth of soil microorganisms in the high-altitude meadow[27]. The results implied

that warming in Qinghai-Tibetan Plateau (wet site) was likely to promote functional diversity of soil bacteria. Soil water content did not correlate with soil bacterial functional diversity along the elevation gradient in this study (Fig. 2), consistent with the high rainfall and consequently high surface soil moisture content (Table 2). Therefore, soil moisture was unlikely to be a limiting factor for soil microbes. Our results agreed with a greenhouse experiment with a ryegrass monocrop and mixture of ryegrass and clover[28]; however, in water-limited situation, the number of substrates utilized by the cultivable microbial community tended to be greater in continuously wetted soil[29]. Therefore, the effect of soil moisture on soil bacterial diversity is not likely to be uniform and perhaps varies in response to the original soil humidity which is dependent on the balance of precipitation and evapotranspiration.

Lastly, we attempted to identify the environmental variables which controlled soil bacterial communities in order to understand the ecosystem nutrient cycling. pH was previously shown to be the best predictor of variation in microbial diversity[30]. However in the present study it was a weak predictor, partly because the pH range was narrow in these sites. Instead, available phosphorus explained the largest variation of bacterial substrate utilization among the 12 soil parameters, suggesting that P availability was a limiting factor for bacterial community functional diversity on the Qinghai-Tibetan Plateau (Fig. 2). Soil available P is derived primarily from rock weathering, which means that even very small amounts of P losses from ecosystems may not readily be replenished. P is a common limiting nutrient in tropical forest, due to high sorption and low input from such highly weathered soils[31]. P release from organic matter decomposition is by far the most important source of soil available P for mature ecosystems, particularly in cold ecosystems such as tundra[32]. Therefore, P was also found to be a limiting factor for primary production in ecosystems where organic matter decomposition was low, such as tundra, grassland and freshwater systems[33]. On the Qinghai-Tibetan Plateau, available P was usually regarded as one of the limiting variables for vegetation because of the slow weathering rates of parent material and slow decomposition rate of soil organic matter under low temperature[34]. Despite the recognition of P limitation for primary production, few studies have investigated the effect of P limitation on soil microbes. One study showed that bacterial community in a coastal ecosystem was limited by phosphorus[35] and another one found that P availability was a limiting factor for bacterial growth and related processes in tropical forest[36]. According to our results, available P may also be an important limiting factor on soil bacterial community functional diversity in Qinghai-Tibetan Plateau.

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