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Full Length Article

The Soil Bacterial Community and Diversities of *Deyeuxia angustifolia* Population along Different Altitude in Changbai Mountains, Northeastern China

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Abstract

Altitudinal gradients strongly influence vegetation biodiversity, but the microbial composition variation still poorly understands. Therefore, we set an experiment on studying soil bacterial diversity and community along six different altitudinal gradients in Changbai Mountains by using Illumina high through sequencing technology. The results showed that soil physico-chemical properties changed significantly between six different altitudinal gradients. The Soil bacterial alpha diversities of 1690 m and 1800 m were highest than other gradients. Principle coordination analysis (PCoA) indicated that soil bacterial beta diversity significantly changed in six different altitudinal gradients. Moreover, Acidobacteriaand Proteobacteria and Actinobacteria did not significantly change between six different altitudes; but, the Chloroflexi and Bacteroidetes and Gemmatimonadetes were significantly higher at A and B altitudes. The responses of bacterial alpha diversity were mostly associated with soil moisture and NO_3^- and available potassium (AK), whereas the pH and soil moisture were the key factors affect the bacterial composition structures. Overall, our results suggest that soil bacterial diversity and composition of *Deyeuxia angustifolia* population were affected by different soil characteristics and would indicate the *D. angustifolia* population of different altitudes affected by different soil characteristics. © 2020 Friends Science Publishers

Keywords: Altitudinal gradients; Soil microbial community; Soil moisture; Illumina sequencing; Diversity

Introduction

Mountain ecosystems are sensitive areas that respond to climate and environmental changes. In recent years, the vegetation of mountain ecosystems has undergone great changes due to the impact of climate change and human activities. In alpine ecosystems, once an invasion occurs, serious consequences will occur, including extinction of species and degradation of ecosystem function (Li and Hua 2000; Hartmann *et al.* 2017). The invasion of low-altitude plants to high-altitude areas has become a research hotspot; many studies have studies on the aboveground changes (Grace 2002; Wearne and Morgan 2006). However, few studies focus on the understand change.

Soil microorganism is an important part of terrestrial ecosystems and acts a key function in the biogeochemical cycle (Hartmann *et al.* 2017). The composition and diversity of above ground plants can cause changes in the ecological environment, partly affect the changes in soil environmental factors (Frey *et al.* 2016) and thus affect the soil microbial diversity and soil microbial structure composition. Similarly, variations of the structure as well as function of soil microbial communities can also affect plant productivity by regulating plant nutrient availability (Rime *et al.* 2016).

The Changbai Mountain tundra belt is only an alpine tundra belt in China. At present, there are no reports of soil microbial diversity under plant invasion in the tundra belt of Changbai Mountain. The phenomenon that *Betula ermanii* Charm invasive tundra on the northern slope of Changbai Mountain has been recognized and been attributed to global climate warming (Wang *et al.* 2012). In addition, Jin *et al.* (2016) research has proved that the herbaceous plants represented by *Deyeuxia angustifolia* under the *Betula ermanii* forest in western slope gradually invaded into the tundra belt (Jin *et al.* 2016). From the current vegetation distribution, the distribution range of *D. angustifolia* has

covered a large range of the northern slope tundra and the impact on landscape level and community level is very significant. Therefore, research on the soil bacterial diversity of different altitudes alone the invasion of *D. angustifolia* is very necessary, especially to reveal the change of soil bacterial composition structure and the impact on the ecosystem. To test these hypotheses, soil communities sampled at *D. angustifolia* at six elevations and investigated by using the barcoded pyrosequencing technique. The objectives of this study were to elucidate (1) changes in soil physicochemical properties at elevation gradients, (2) differences in bacterial structure and diversity under different altitudes.

Materials and Methods

Research site

Changbai Mountains (126°55'–129°00'E; 41°23'–42°36'N) is located in Jilin Province, of northeast China. The four slops of the Changbai mountains differed, which the average slope of northern slope <3% and the other three average slopes <10%. The local climate is a typical continental temperate monsoon climate. The mean annual precipitations are 700–1400 mm and increases from 600 to 1200 m. The local temperatures are between 2.9–4.8°C and increases along altitudinal gradient from 600 to 1200 m.

The collection of the soil samples was performed on a northern slope belonging to Changbai Mountain on 20 June 2018. 6 elevational gradients that districted *D. angustifolia* population was selected, representing six typical *D. angustifolia* population regions. At each elevation, soil samples from three different plots (25 m × 25 m) were collected to ensure independence among them. In every single plot, samples of the soil organic layer were gathered at 10 arbitrary sites with a sterile blade and assembled into one specimen. Before the homogenization of the soil part contained in every specimen, a removal was conducted to get rid of the residuals as well as the roots that can be seen by naked eyes. Afterwards, sieve was conducted on the newly collected soil specimen with 2 mm meshes to subdivide into two approximately equal parts. One part of fresh soil was stored at 4°C and the remaining fresh soil was stored at -80°C before the DNA extraction.

Identification for the soil chemical characteristics

A pH meter was implemented to identify the soil pH, where prior to the dissolution of a 1:2.5 (wt./vol.) soil proportion within 0.01 *M* $CaCl_2$ solution, a 30 min shake was performed. The total nitrogen (TN) as well as soil total carbon (TC) were measured by an elemental analyzer (VarioEL III, Germany). An extraction was performed on soil weighted as 10 g through 2 *M* KCl solution and the NH_4^+-N as well as NO_3^--N contained in the soil were obtained. The $NaHCO_3$ extraction and $HClO_4$-H_2SO_4 digestion approaches were implemented to identify the available phosphorus (AP) as well as total phosphorus (TP), independently. A measurement on the concentrations of the NH_4^+-N, NO_3^--N, TP and AP contained within the soil were performed by the continuous flow analytical system (SKALARSan ++, Skalar, Holland). The samples digesting by concentrated hydrofluoric acid was implemented to estimate the total potassium (TK) contained in the soil and ammonium leaching technique as well as acetic acid were utilized to extract the available potassium (AK), followed by the quantification by inductively coupled plasma atomic emission spectrometry (ICPS-7500, Shimadzu, Japan) on the obtained compounds. The chloroform fumigation, which is obtained by 0.35 (KC) as well as 0.4 (KN) correction factors, was adopted to analyze the biomass N (MBN) as well as the Microbial Biomass C (MBC).

Soil DNA extraction

For all specimen, through a MOBIO Power Soil Extraction Kit (Mo Bio Laboratories, Carlsbad, CA, USA), an extraction was performed on the newly collected soil specimen weighted as 0.5 g (reserved in a −80°C freezer) to obtain the microbial DNA contained in the soil following the manufacturing protocols. Afterwards, a dilution was conducted on the obtained DNA in DES buffer (DNA Elution Solution-Ultra Pure Water), after which a Nano Drop 2000 spectrophotometer (Thermo Scientific, U.S.A.) was adopted to count the DNA quantity and the obtained DNA was reserved at −20°C aimed at downstream investigation.

Illumina MiSeq sequencing

DNA obtained from every specimen was utilized as a prototype to amplify bacterial 16S rRNA fragments adopted within MiSeq sequencing. The V3-V4 hyper variable parts of the 16S rRNA gene were taken to amplify by primers 338F (5′-ACTCCTACGGGAGGCAGCA3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). A 6 bp barcode sequence exclusive to every specimen was introduced to the primers to distinguish various specimens. The followings are the PCR conditions: 94°C over 5 min, 28 cycles of 94°C over 30 s, 55°C over 30 s, 72°C over 60 s, ending with an extension at 72°C over 7 min. The PCR reacts inside a triplicate within a 25 μL mixture containing 12.5 μL 2x Taq Plus Master Mix，1 μL (5 umol.L^{-1}) of each primer, 3 μL BSA, 30 ng prototype DNA and double-distilled water. Purification and pooling were performed on the PCR products, which were taken with equimolar concentrations, and paired-end sequenced (2 × 300 bp) was conducted by the Illumina MiSeq platform (Allwegene BioPharm Technology Co., Ltd., Beijing, China).

Analysis on the sequencing data

De-multiplexation and quality filtration were performed on the unprocessed FASTQ data by QIIME software and the

paired reads were joined by FLASH software. A removal was performed on the sequences less than 200 bp with ambiguous base 'N' as well as an average base quality score less than 20 for later exploration. Chimera was eliminated from trimmed sequences by a Uchime algorithm. By CD-HIT, sequences with high quality were clustered into Operational Taxonomic Units (OTUs) at 97% sequence similarity (Li and Godzik 2006).

Alignment was performed on the representative sequences from every phylotype by the Python nearest alignment space termination (PyNAST) tool (Caporaso *et al.* 2010) and a relaxed neighbor-joining tree was constructed with Fast-Tree software (Price *et al.* 2009). Taxonomic information contained in every 16S rRNA gene sequence was identified by the Ribosomal Database Project (RDP) classifier, where the confidence threshold is 0.80 (Cole *et al*. 2014). A random extraction was performed on every specimen sequences for α-diversity as well as β-diversity analyses, where the minimum sequencing number was arbitrarily chosen for every specimen aimed at following community analysis.

Statistical analysis

The assessment on the soil characteristics was performed for differences manipulation through one-way analysis of variance (ANOVA) and Pearson correlation analysis was adopted to investigate potential correlations a midst bacterial community, bacterial variety, and soil characteristic. The SPSS version 22.0 software was implemented to perform the ANOVA and Pearson analyses. The Venn diagrams were constructed by R software (v.3.2.5, R Development Core Team 2015) presenting the quantity of shared OTUs. The bacterial community constitution diversity was investigated through principal component analysis (PCA). Variations in relative abundance of phyla and genus for various altitudes were performed by kruskal-wallis method using SPSS 22.0. Mantel's test was implemented to identify the statistical significance for the relationships between bacterial communities and soil characteristic, where soil characteristics that exhibits significant influences ($P < 0.05$) were chosen for canonical correlation analysis (CCA). The "vegan" package of R software (v.3.2.5, R Development Core Team 2015) was implemented to perform the analyses.

Results

Soil physico-chemical properties and soil enzymes along the different altitudes of *D. angustifolia* population

Except for soil Acid protease and sand, all other parameters of soil physio-chemical properties and enzymes analyzed differed significantly ($P < 0.05$) among the six *D. angustifolia* population at six altitudes (Table 1). Soil moisture (SMC) was higher for the low altitude than for the high altitude and ranged from 44.0% (A) to 33.4% (D) (Table 1). Soil pH was between 5.5 (A) and 4.6 (B) (Table 1). Soil organic carbon was between 14.3(C) and 9.0(E) (Table 1). Ammonium nitrogen was between 2.0 (B) and 0.7 (A) (Table 1). Nitrate nitrogen was between 1.9 (A) and 0.2 (F) (Table 1). Total nitrogen was between 19.3 (B) and 9.9 (A). Total soil potassium was between 4.8 (E) and 3.1 (A). Soil total phosphorus was between 0.1 (A) and 0.0 (B). Available potassium was between 24 (B) and 13.9 (D). Available phosphorus was between 20.6 (C) and 10.3 (F). Silt was between 1.6 (F) and 0.5 (D). Clay was between 5.2 (E) and 4.3 (A). Soil microbial biomass carbon was between 675.5 (D) and 281.8 (C). Soil microbial biomass nitrogen was between 89.3 (B) and 69.0 (A).

Rarefaction curve

The rarefaction curve reflects the sample depth and can be used to evaluate whether the sequencing volume is sufficient to cover all groups. Fig. 1 shows the dilution curve for all samples in this test under the condition of similarity of 0.97. As shown in Fig. 1, all soil sample dilution curves tended to flatten, indicating that sampling was reasonable, and the confidence in the bacterial community structure in the actual environment was high, which could reflect the bacterial community of a soil sample in a relatively real way.

Analysis of soil bacterial alpha diversity

From the Fig. 2, both the Chao Index and Observed_species and Shannon-Wiener index and PD_whole_tree differed significantly (one-way ANOVA, $P < 0.01$) among the six altitudes (Fig. 2). For Chao Index, A and B did not differ significant (one-way ANOVA Tukey test, $P > 0.05$) and C, D, E, F did not differ significant (one way ANOVA Tukey test, $P > 0.05$), but the A and E differed significantly (one way ANOVA Tukey test, $P < 0.05$) and B differed with D, E and F significantly (one way ANOVA Tukey test, $P < 0.05$). For Observed_species, A and B did not differ significant (one-way ANOVA Tukey test, $P > 0.05$) and differ significant with C, D, E, F (one way ANOVA Tukey test, $P < 0.05$); B differ significant with C, D, E, F (one way ANOVA Tukey test, $P < 0.05$); C did not differ significant with D, E, F (one way ANOVA Tukey test, $P > 0.05$); D did not differ significant with E and F (one way ANOVA Tukey test, $P > 0.05$); E and F differ significant (one way ANOVA Tukey test, $P < 0.05$). For PD_whole_tree index, A and B did not differ significant (one-way ANOVA Tukey test, $P > 0.05$), and differ significant with C, D, E, F (one way ANOVA Tukey test, $P < 0.05$); B differ significant with C, D, E, F (one way ANOVA Tukey test, $P < 0.05$); C did not differ significant with D, E, F (one way ANOVA Tukey test, $P > 0.05$); D did not differ significant with E and F (one way ANOVA Tukey test, $P > 0.05$); E and F did not differ significant (one way ANOVA Tukey test, $P > 0.05$). For

Table 1: Soil physico-chemical characteristics of *D. angustifolia* population at the different altitude in Changbai Mountains, Northeastern China

Properties[1]	A	B	C	D	E	F
SMC	44.0 ± 1.17^{a}	37.6 ± 0.89^{b}	35.9 ± 1.03^{b}	33.4 ± 1.06^{b}	34.4 ±1.17^{b}	36.0 ± 5.29^{b}
pH	5.5 ± 0.15^{a}	4.6 ± 0.15^{b}	4.6 ± 0.08^{b}	4.6 ± 0.09^{b}	4.7 ± 0.09^{b}	4.7 ± 0.08^{b}
NH_4^+	0.7 ± 0.13^{d}	2.0 ± 0.06^{a}	1.3 ± 0.06^{bc}	0.8 ± 0.03^{d}	1.1 ± 0.09^{c}	1.4 ± 0.12^{b}
NO_3^-	1.9 ± 0.07^{a}	0.4 ± 0.02^{b}	0.2 ± 0.02^{d}	0.2 ± 0.01^{d}	0.3 ± 0.01^{c}	0.2 ± 0.00^{d}
SOC	10.3 ± 0.63^{b}	10.1 ± 0.36^{b}	14.3 ± 1.02^{a}	9.4 ± 0.60^{b}	9.0 ± 1.24^{b}	9.5 ± 0.67^{b}
TN	9.9 ± 0.08^{c}	19.3 ± 0.86^{a}	15.0 ± 0.74^{b}	11.0 ± 0.51^{c}	14.6 ± 0.53^{b}	14.6 ± 0.83^{b}
TK	3.1 ± 0.28^{b}	4.7 ± 0.19^{a}	3.5 ± 0.29^{b}	4.6 ± 0.40^{a}	4.8 ± 0.32^{a}	3.3 ± 0.13^{b}
TP	0.1 ± 0.01^{a}	0.0 ± 0.01^{b}	0.1 ± 0.00^{a}	0.0 ± 0.01^{b}	0.1 ± 0.00^{b}	0.1 ± 0.00^{b}
AK	17.7 ± 0.63^{b}	24.0 ± 0.60^{a}	16.6 ± 0.33^{c}	13.9 ± 0.60^{e}	14.0 ± 0.21^{e}	15.1 ± 0.33^{d}
AP	11.7 ± 0.31^{d}	17.0 ± 0.22^{b}	20.6 ± 0.61^{a}	15.1 ± 0.22^{c}	15.1 ± 0.45^{c}	10.3 ± 0.53^{e}
Sand (%)	94.7 ± 4.50^{a}	96.7 ± 6.24^{a}	94.8 ± 4.08^{a}	95.2 ± 4.92^{a}	94.5 ± 4.92^{a}	95.2 ±4.92^{a}
Silt (%)	1.1 ± 0.08^{c}	0.6 ± 0.05^{d}	1.3 ± 0.05^{b}	0.5 ± 0.03^{d}	1.3 ± 0.05^{b}	1.6 ±0.04^{a}
Clay (%)	4.3 ± 0.22^{c}	4.8 ± 0.25^{ab}	4.3 ± 0.21^{c}	5.1 ± 0.08^{a}	5.2 ± 0.12^{a}	4.5 ± 0.34^{bc}
MBC	516.5 ± 4.90^{b}	569.4 ± 5.73^{b}	281.8 ± 4.55^{d}	675.5 ± 3.68^{a}	573.4 ± 4.11^{b}	527.1 ± 4.11^{c}
MBN	69.0 ± 2.05^{d}	89.3 ± 2.94^{a}	80.6 ± 3.30^{bc}	84.7 ± 2.05^{ab}	77.1 ± 2.87^{c}	76.8 ± 2.45^{c}

[1]Values represent means ± standard deviations (n=3). Different letters indicate significant ($P < 0.05$) differences between individual means assessed by one-way factorial analysis of variance (ANOVA) followed by Tukey's HSD post-hoc testing. Abbreviations: SMC, soil moisture content; NH_4^+, Ammonium nitrogen; NO_3^-, Nitrate nitrogen; SOC, soil organic cabon; TN, total nitrogen; TK, Total potassium; TP, Total phosphorus; AK, Effective potassium; AP, Effective phosphorus; MBC, Microbial biomass carbon; MBN, Microbial biomass nitrogen. Note: A is 1690 m altitude; B is 1800 m altitude; C is 1860 m altitude; D is 1910 m altitude; E is 1950 m altitude; F is 2020 m altitude. The same below

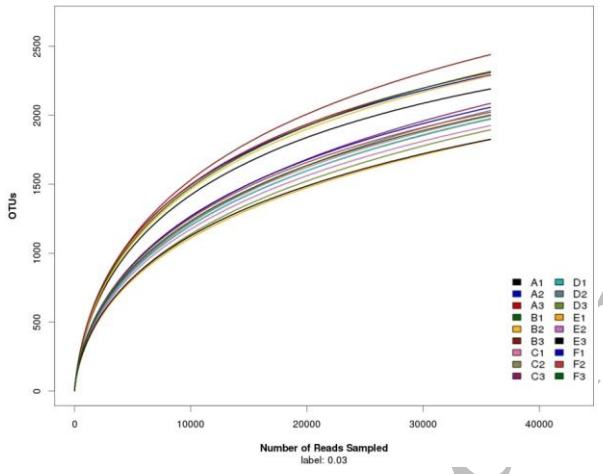

Fig. 1: Rarefaction curve of *D. angustifolia* population along different altitude in Changbai Mountains, Northeastern China

Note: A (1-3) is 1690 m altitude; B (1-3) is 1800 m altitude; C (1-3) is 1860 m altitude; D (1-3) is 1910 m altitude; E (1-3) is 1950 m altitude; F (1-3) is 2020 m altitude. The same below

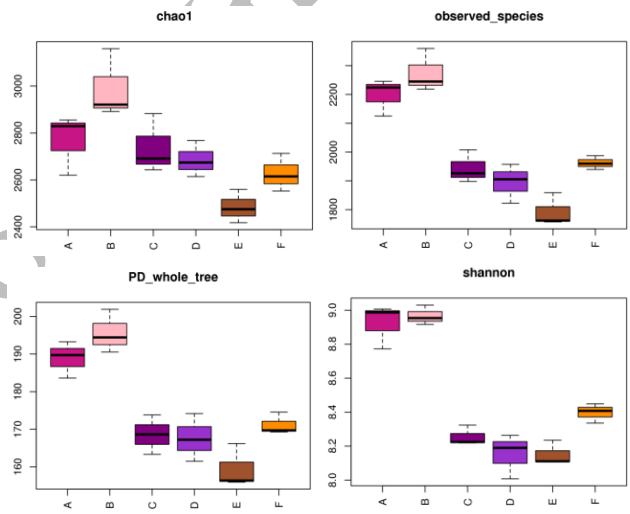

Fig. 2: Bacterial alpha diversity of *D. angustifolia* population along different altitude in Changbai Mountains, Northeastern China

Note: A is altitude of 1690 m; B is altitude of 1800 m; C is altitude of 1860 m; D is altitude of 1910 m; E is altitude of 1950 m e; F is altitude of 2020 m. The same below

Shannon index, A and B did not differ significant (one way ANOVA Tukey test, $P > 0.05$) and differ significant with C, D, E, F (one-way ANOVA Tukey test, $P < 0.05$); B differ significant with C, D, E, F (one way ANOVA Tukey test, $P < 0.05$); C did not differ significant with D, E, F (one way ANOVA Tukey test, $P > 0.05$); D did not differ significant with E and F (one way ANOVA Tukey test, p>0.05); E and F did not differ significant (one way ANOVA Tukey test, p>0.05).

Analysis of soil bacterial beta diversity

The bacterial diversities of the whole soil specimens are depicted as a PCA plot in Fig. 3, which apparently exhibit that the whole soil specimens were partitioned into three collections in terms of the location of sampling, where the altitude is the first and second principal components coordinate (PC1, 41.74% and PC2, 16.63%) (Fig. 3). A and B differed significant with C, D, E, F along with the first principal components coordinate and the F differed significant with A, B C, D, E (Fig. 3). Anosim result is R = 0.97, $P = 0.001$.

Composition of soil bacterial community

At the level of the phylum, bacteria are distributed in 13 known bacterial phyla except of the unclassified population. As shown in Fig. 4, the relative abundance of Proteobacteria, Bacteroidetes, Acidobacteria and Actinobacteria is relatively high, and the relative abundance more than 95% of the total amount of soil bacteria.

Table 3 shows the bacterial species that have

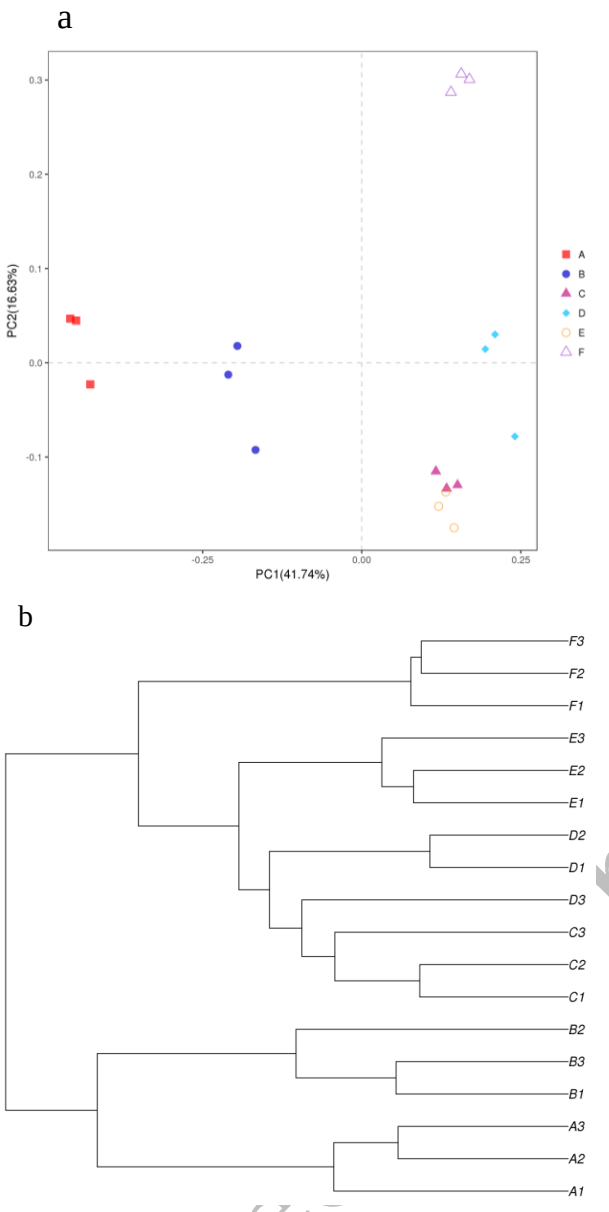

Fig. 3: Bacterial beta diversity of *D. angustifolia* population along different altitudes in Changbai Mountains, Northeastern China. β-Diversity indexes was calculated at the OTU level (97%)
Note: A is altitude of 1690 m; B is altitude of 1800 m; C is altitude of 1860 m; D is altitude of 1910 m; E is altitude of 1950 m; F is altitude of 2020 m

significant differences at different altitudes (relative abundance >1%). It can be seen from Table 3 that there are 6 soil bacterial species relative abundance changes under different altitude gradients, namely the dominant species Chloroflexi, Verrucomicrobia and Planctomycetes, Bacteroidetes, Firmicutes, Gemmatimonadetes. The difference between Acidobacteria, Proteobacteria and Actinobacteria was not significant. Among them, the relative abundance of Chloroflexi is the highest in A, F is the lowest; Verrucomicrobia is the highest in E, A is the lowest; Planctomycetes is the highest in E, A is the lowest;

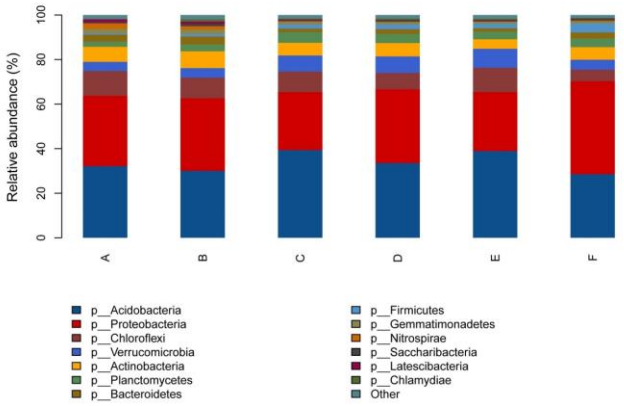

Fig. 4: Relative abundance of the dominant bacterial phyla under different altitudes in Changbai Mountains, Northeastern China

Bacteroidetes is the highest in B, the lowest in E; Firmicutes is the highest in F, the lowest in A Gemmatimonadetes are the highest in B and the lowest in F.

Table 3 shows the bacterial genus (relative abundance > 1%) with significant differences at different altitudes. It can be seen from Table 3 that in addition to the unclassified genus, there are 6 soil bacterial genus relative abundance changes under different altitude gradients, which are dominant genus *RB41*, *Bryobacter*, *Haliangium*, *Acidothermus*, *Reyranella*, *Rhizomicrobium*. The difference between *Candidatus_Solibacter* and unidentified was not significant. Among them, the relative abundance of *RB41* was the highest in A, B was the lowest; *Bryobacter* was the highest in F and A was the lowest; *Haliangium* was the highest in B and lowest in D; *Acidothermus* was the highest in D and lowest in A; *Reyranella* was the highest in D and lowest in C. *Rhizomicrobium* is the highest in F and lowest in A.

Bacterial communities with statistically significant differences

Beyond the identification of a- as well as b-diversities, an additional main objective of bacterial community's comparison is to determine specific communities within specimens. Therefore, a LEfSe tool (33) was utilized, which enables the analysis on microbial community data at any clade. Note that the computation required for analyzing the OTUs obtained in the current investigation is time-consuming, limiting only statistical analysis from the domain to the genus level. Cladograms showed the groups and LEfSe (Fig. 5) was implemented to confirm the LDA scores which are three and beyond. In A, seven groups of bacteria were significantly enriched, namely Chloroflex (from phylum to genus), Actinobacteria (from order of Thermoleophilia to family of Glaiellales and from phylum of Subgroup6 to genus of unidentified), Subgroup 17 (from phylum to family), Blastocatellis (from phylum to family), Betaproteobacteria (from

Table 2: Differences in relative abundance of phyla (>1%) and genus (>1%) between the different altitudes (means)

Phylum	A	B	C	D	E	F	*P*_value
Acidobacteria	32.1%	30.0%	39.4%	33.6%	39.0%	28.5%	0.09^{ns}
Proteobacteria	31.6%	32.6%	26.0%	33.0%	26.4%	41.7%	0.09^{ns}
Chloroflexi	11.2%	9.3%	9.3%	7.4%	11.0%	5.2%	0.01^{**}
Verrucomicrobia	4.1%	4.3%	7.2%	7.4%	8.5%	4.5%	0.02^{*}
Actinobacteria	6.7%	7.5%	5.7%	6.0%	4.3%	5.6%	0.05^{ns}
Planctomycetes	2.3%	3.1%	4.8%	4.1%	3.4%	3.9%	0.02^{*}
Bacteroidetes	3.1%	3.5%	1.6%	2.1%	1.5%	2.7%	0.02^{*}
Firmicutes	0.6%	1.0%	1.8%	2.1%	2.0%	4.1%	0.01^{**}
Gemmatimonadetes	1.9%	2.1%	1.2%	1.0%	0.8%	0.8%	0.02^{*}
Genus							
g__unidentified	74.0%	72.3%	74.3%	69.8%	75.0%	62.7%	0.09^{ns}
g__Candidatus_Solibacter	3.1%	2.5%	3.8%	3.8%	3.8%	4.8%	0.06^{ns}
g__RB41	2.1%	0.3%	0.5%	0.3%	0.5%	0.3%	0.01^{**}
g__Bryobacter	1.7%	2.7%	2.2%	2.7%	2.2%	3.7%	0.02^{*}
g__Haliangium	1.4%	1.5%	0.8%	0.7%	0.7%	1.1%	0.01^{*}
g__Acidothermus	1.3%	2.1%	2.5%	2.6%	1.7%	1.9%	0.03^{*}
g__Reyranella	1.0%	0.9%	0.6%	1.2%	0.9%	1.6%	0.02^{*}
g__Rhizomicrobium	0.8%	1.6%	1.1%	1.2%	1.0%	1.9%	0.01^{*}

Note: The level of significance determined by kruskal-wallis method is listed (**$P < 0.01$, *$P < 0.05$, ns: not significant). Italics indicate minimal abundance and bold indicates maximum abundance

Table 3: Pearson correlation analyses of physiochemical properties and soil bacterial community structure

	SWC	pH	NH_4	NO_3	SOC	TN	TK	TP	AK	AP
Chao1	0.312	-0.014	0.452	0.189	0.288	0.358	0.115	0.012	0.769^{**}	0.260
Observe species	0.615^{**}	0.346	0.366	0.545^{*}	0.095	0.238	-0.137	0.151	0.840^{**}	-0.066
PD	0.593^{**}	0.317	0.359	0.531^{*}	0.042	0.233	-0.083	0.097	0.827^{**}	-0.078
Shannon	0.698^{**}	0.482^{*}	0.318	0.649^{**}	-0.030	0.200	-0.175	0.179	0.818^{**}	-0.196

***** meant significant difference at 0.05 level among treatments

****** meant significant difference at 0.01 level among treatments

phylum to family), Nitrospira (from phylum to family) (Fig. 5). In B, nine groups of bacteria were significantly enriched, namely Bacteroidetes (from class to genus), Chlamydiae (from family of Chlamydiae to genus of genus of Chlamydiales), Ktedonobacteria (from phylum to family), Actinobacteria (from family to genus), Verrucomicrobia (from phylum to genus), class of HSB_OF53_F07, Haliangiaceae (from phylum to class), Burkholderiales, Xanthobacteraceae, Gemnatimocadaceae (from class to family).

In C, three groups of bacteria were significantly enriched, namely Planctomycetes (from phylum to genus), subgroup2 (from plylum to family) and DA111. In D, three groups of bacteria were significantly enriched, namely Acidothermaceae and unidentified. In E, three groups of bacteria were significantly enriched, namely Holophagae, EJG37_AG_4, Spartobacteria (from phylum to genus). In F, seven groups of bacteria were significantly enriched, namely Clostridia, Caulobacterales, Roseiarcaceae, Acetobacteraceae, Rhodospirillales, Xanthomonadales_Incertae_Sedis and Gammaprobacteria.

Relationship between bacterial community structure and environmental characteristics

RDA exhibited that the formation of microbial community structure was the result of main environment properties (such as pH, NO_3^--N, NH_4-N, moisture, organic carbon, total nitrogen, and AK, AP, TK, TP). As shown in Fig. 6, NO_3^--N ($P < 0.01$), pH ($P < 0.01$), AK ($P < 0.01$), AP ($P < 0.05$) and soil moisture ($P < 0.01$) significantly affected the bacterial community structure.

Discussion

The soil bacterial community, which is the representative of every altitudinal site, can be explained by the variation of soil as well as plant properties, alongside the gradients of the altitude (Siles and Margesin 2017). The alpha diversity of the bacterial varies alongside the gradients of the altitude, which was demonstrated by our outcomes (Fig. 2), since the variations of soil moisture content, pH and NO_3^- and AK as a function of the gradients of the altitude considerably influence bacterial (Table 3). Several reports made a suggestion on the bacterial response (Nie *et al*. 2009; Shen *et al*. 2013; Siles and Margesin 2017). Altitude changes usually cause changes in climatic environmental factors and dramatic changes in material turnover over a short geographic distance (Yu *et al*. 2005). As a sensitive indicator of soil ecosystem, soil microbial community structure will respond very quickly to changes in the surrounding environment. In this paper, the soil bacterial diversity of different leaflet populations also changed with the elevation. As the altitude increases, the soil bacteria Chao1, Observed_species, PD_ whole_tree and Shannon indicas all show a downward trend, that is, high altitude causes the bacterial alpha diversity to decrease. Similarly, soil bacteria alpha has been shown to vary

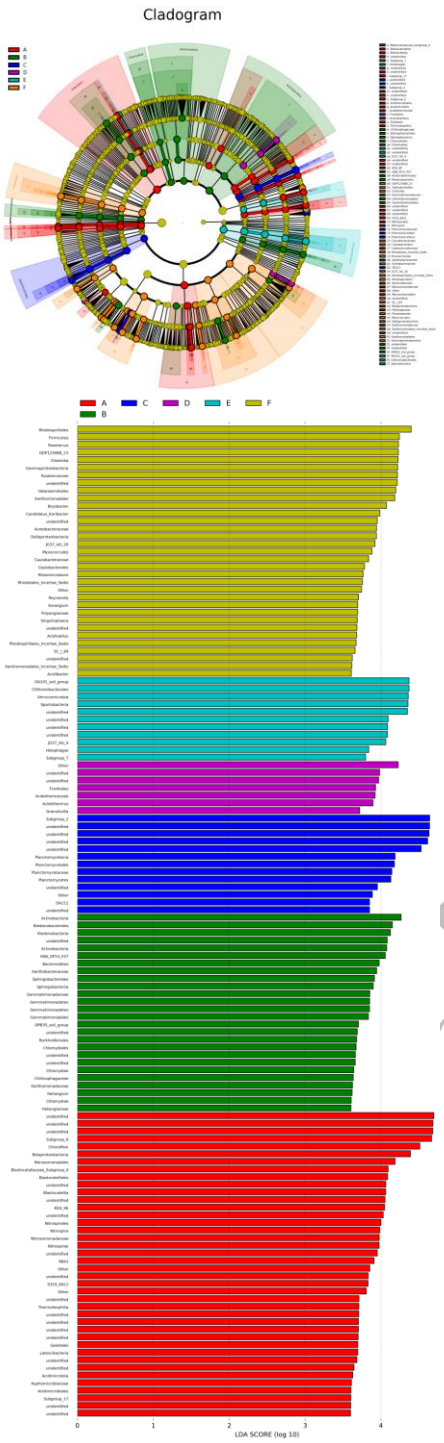

Fig.5: Cladogram exhibiting the phylogenetic distribution of the bacterial lineages linked to soil from six *Deyeuxia angustifolia* populations along with different altitude in Changbai Mountains, Northeastern China (**a**). Indicator bacteria havingLDA scores no less than three in bacterial communities linked to soil from the six *Deyeuxia angustifolia* populations with different altitude in Changbai Mountains, Northeastern China (**b**). The phylogenetic levels from domain to genus is indicated by circles, of which the radius is positively linear dependence on the group abundance

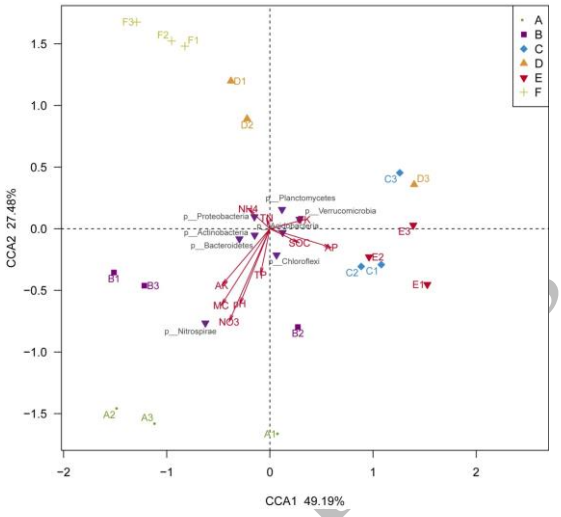

Fig.6: Redundancy analysis (RDA) of Miseq data (symbols) and environmental characteristics (arrows)

significantly along the elevation gradient (Han *et al*. 2018). Previous studies have also observed that some alpine soil bacterial community structures are not significantly different between different altitude gradients (Li *et al*. 2017). For example, Zhang *et al*. (2014) used pyrosequencing to study the soil bacterial diversity along the four forest types of evergreen broad-leaved forest, deciduous broad-leaved forest, subalpine dark coniferous forest and subalpine shrub in Shennongjia Nature Reserve. The distribution pattern shows that the bacterial diversity shows a distinct monotonous decreasing trend with the increase of altitude; Margesin *et al*. (2009) and Bryant *et al*. (2008) also found that the bacterial diversity appeared with the elevation. The downward trend; Singh *et al*. (2012) PCR amplification and sequencing of soil bacteria at an altitude of 1000~3700 m in Mount Fuji showed that the variation of bacterial diversity showed a single-peak curve with elevation, at an altitude of 2500 m. The diversity of bacteria is the highest; Wang and Liu (2012) and others in the Laojun Mountain in Yunnan at an altitude of 1820~4050 m, along a rocky stream, using high-throughput sequencing technology to obtain the diversity of bacteria as the altitude decreases first and then rises. It has a concave curve; Fierer *et al*. (2011) and Shen *et al*. (2013) even believe that soil bacterial diversity does not show obvious changes in altitude gradient.

In this study, the soil bacterial diversity index differed significantly at different altitude gradients and increased with increasing altitude between AB m. After reaching B, it began to decline (Table 1), reaching the lowest E. It then rises to a concave curve, similar to the findings of Wang *et al*. (2008). The variation of bacterial diversity is closely related to soil water content, and the changes of altitude with altitude are basically the same. In Pearson correlation analysis, the Shannon diversity index of bacteria is significantly positively correlated with water content (Table 3).

Furthermore, analysis on the community structure response to the gradients of altitude showed that the microbial beta diversity of soil varied considerably with the gradients of altitude (Fig. 3), which was significantly associated with soil moisture amidst the environment factors (Fig. 6). This might be due to either variation of ecological strategy or limited oxygen available for aerobic microbes, eventually leading to variations in the microbial community (Allen 2011; Fierer *et al.* 2011; Meng *et al.* 2013). Additionally, the variation of soil pH alongside the gradients of altitude contributed to the changes of beta diversity of the bacteria (Table 2), which was confirmed by us and was also demonstrated by the reported results (Shen *et al.* 2013). Generally, the aforementioned outcomes confirmed that the gradients of altitude showed primary influences on the beta diversity of the soil bacteria, where there is a dependence of the differential responses on climatic variables (soil moisture).

At the level of the phylum, the composition of soil bacteria in different altitudes is basically the same, which is different from soil types of different regions (Italy, Norway, Germany, Russia, the United States and the Netherlands) by Janssen (2006) (farming farmland, no-till farmland, abandoned farmland). The results of bacterial community structure in organic soil, mineral soil, forest, grassland, tundra and desert are similar, in which Proteobacteria and Acidobacteria are dominant, accounting for 39 and 20%, followed by Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes and Firmicutes, etc., it can be seen that at the level of the door, different ecosystems, different soil types

The dominant flora of the type is basically the same (Janssen 2006; Green and Bohannan 2013). However, at different altitudes, soil bacterial gate level abundance is different (Fig. 6 and Table 3). The relative abundance of other bacterial gates was significantly different except for the difference in acid bacteria, proteobacteria, and actinomycetes. The reason for the analysis may be due to differences in soil physical and chemical factors at different altitudes. It can be seen from CCA that the effects of physicochemical factors on acid bacteria, proteobacteria, and actinomycetes are not significant, while other bacteria mainly receive soil pH, AK, MC and NO_3^-. Shen *et al.* (2013) analyzed the bacterial community at different altitudes and found that pH is the main reason affecting the distribution of soil bacterial communities. Blaž *et al.* (2008) found that soil bacterial communities are mainly related to soil water content. Therefore, these results suggested that differential responses of soil bacterial community composition to the altitudinal gradients were largely dependent on climate factors (soil moisture) and on the dynamics of soil physical-chemistry properties (*e.g.*, pH, SOC, AP, TP, NO^{3-}, NH_4^+).

At the genus level, there are certain differences in the species composition of bacteria at each altitude. The total genus accounts for 65.69% of the total genus, accounting for 96.48% of the total relative abundance; the main genus with a relative abundance of more than 1% The species were basically the same, but the relative abundance of 29.30% of the common genus was significantly different between altitudes. Janssen (2006) also suggested that although the dominant populations of bacteria differed among different soil types, the abundance was significantly different, and the abundance may be affected by soil environmental conditions including biological, chemical and physical factors. In this study, soil moisture content and pH as a function of altitude are key factors that have a significant impact on the relative abundance of dominant species (see Fig. 2–6), which is similar to most people's findings (Shen *et al.* 2013; Zhang *et al.* 2013). In addition, differences in habitat composition at different altitudes are also important factors influencing the abundance of the flora, as evidenced by the full sample similarity analysis (Fig. 2–5). The soil nutrient content, water content and pH value of CCA (Fig. 2–7) have a high contribution rate to soil microbial community structure differences at different altitude gradients. Therefore, soil microbial abundance can directly respond to changes in ecological conditions (Zhang *et al.* 2013) and is closely related to soil nutrient content, water content and pH value (Shen *et al.* 2013).

Conclusion

Large-scale altitudinal gradients result in variations of microbial biomass, diversity of bacteria, and community constitutions. The nitrogen as well as carbon content inside a plant-soil system alongside the altitudinal gradients, though responding differently to altitude gradients, can be reflected by the dynamics of soil microbial biomass. The analysis on the microbial diversity patterns exhibit that more alpha diversity of bacteria was involved in the sites at A and B altitude alongside the altitudinal gradients, where the differential responses were primarily as a consequence of the soil physical-chemistry properties dynamics. Proteobacteria and Acidobacteria are most abundant and dominant phyla in all six altitudes; the aboveground vegetation may the key factor influence the soil bacterial composition. The soil moisture of different variations as a result of altitudinal gradients could be implemented as the potential variables to predict the beta diversity of microbial though without the same influence involved, due to the constitution variations of bacterial community. In addition, the soil pH and soil NO_3^-, AK, TP were positive with low altitudes (A and B) as well as NH_4^+, AP, SOC and TK were positive with high altitudes (C, D, E, F). The driving environmental factors on soil bacterial community structures were different. There still need to reveal the response of bacterial community to plant invasion and climate change in Changbai Mountains. Our results accentuated that the altitudinal gradients could shape several of bacterial community's patterns, revealing the microbial biodiversity as well as their ecological effects on the climate change.

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