Diversity And Distribution Of CO₂-Fixing Microbial Community Along Elevation Gradients In Meadow Soils On The Tibetan Plateau

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Abstract

Soil CO₂-fixing microbes play a significant role in CO₂-fixation in the terrestrial ecosystems, particularly in the Tibetan Plateau. To understand carbon sequestration by soil CO₂-fixing microbes and the carbon cycling in alpine meadow soils, microbial diversity and their driving environmental factors were explored along an elevation gradient from 3900m to 5100m, on both east and west slopes of Mila Mountain region on the Tibetan Plateau. The CO₂-fixing microbial communities were characterized by high-throughput sequencing targeting the *cbbL* gene encoding the large subunit for the CO₂-fixing protein ribulose 1, 5-bisphosphate carboxylase/oxygenase. The overall OTU abundance is concentrated at an altitude between 4300m~4900m. The species richness and distribution uniformity on the east slope is better than those on the west slope. In terms of microbial community composition, Proteobacteria is dominant, and the most abundant genera are *Cupriavidus*, *Rhodobacter*, *Sulfurifustis* and *Thiobacillus*. The CO₂-fixing microbial community structure dramatically shifted along the elevation. It was jointly driven by vegetation coverage, soil moisture content, and soil organic carbon and soil particle size, and most environmental factors are positively correlated. Our results are helpful to understand the variation in soil microbial community and its role in soil carbon cycling along elevation gradients.

Introduction

Microorganisms have large biomass, numerous species, diverse metabolism, and complex interactions¹. As an important biological component of the soil, soil microbes are the main driving force of soil organic matter and nutrient cycles and are regulating the biogeochemical cycling²⁻⁴ and maintaining ecosystem functions^{5,6}. Autotrophic microorganisms are widely distributed in different ecosystems, and assimilation of CO₂ is a key microbial process in the carbon cycle of global ecosystems and plays an extremely important role in regulating the concentration of carbon dioxide in the atmosphere⁷. The autotrophic microorganisms in global terrestrial soils can capture 0.5%~4.1% of the atmospheric CO₂ and total 0.6~4.9 Gt C each year⁸.

The Calvin-Benson-Bassham (CBB) cycle is a CO₂ fixation pathway commonly found in plants and algae in nature⁹. It is also a pathway for photoautotrophic microorganisms and almost all aerobic autotrophic microorganisms to fix CO₂. The global terrestrial soil ecosystem can fix 0.68~4.9 pg of carbon annually through the CBB cycle, accounting for about 4% of the total annual CO₂ fixation in terrestrial ecosystems¹⁰. At present, the research on microorganisms with carbon-fixing function genes is mostly concentrated in aquatic ecosystems^{11,12}, and most of the researches on carbon-fixing microorganisms in terrestrial ecosystems are also concentrated in areas with specific ecological conditions. The most common ones are soils in large farmland areas^{13,14}. In extreme environments such as low temperature, drought, and high UV, soil carbon-fixing microbial communities may play an important role in CO₂ fixation but have rarely been investigated.

The composition, distribution, and diversity of soil microorganisms are the keys to studying soil ecological functions. At the same time, the diversity of soil microorganisms is also restricted by the physical and chemical properties of soil in different environments¹⁵. Altitude gradient changes integrate the gradient effects of multiple environmental factors⁴. They are an important means to test the interaction between autotrophic microbial communities and environmental factors¹⁶. Some studies have shown that with the increase of altitude, most mountain soil microbial diversity shows a monotonous decreasing trend^{17–19}. However, the soil microbial diversity in some mountainous areas has a nonlinear relationship with the altitude gradient, which is manifested as a unimodal/inverted unimodal or bimodal/inverted bimodal distribution pattern^{20–22}. The Tibet Plateau is the highest altitude area globally, with an average altitude of more than 4000 meters. It is called the third pole of the world²³. Its grassland area is about 1.2×10^6 km², accounting for about 48% of the plateau's land area¹⁶. It is a sensitive area and ecologically fragile zone of climate change and is very sensitive to climate change and disturbance of human activities. Although the Tibet Plateau is a hotspot in global biodiversity research, few studies on its soil functional microbial community and its response to environmental changes, especially CO₂-fixing microbial communities. In this study, high-throughput sequencing methods are employed to study the characteristics of CO₂-fixing microbial communities in alpine meadows along an altitude gradient, which is helpful to understand the function of soil microbial community and its role in the process of soil carbon cycling and provides a scientific basis for a more accurate evaluation of the process of soil carbon cycling in alpine meadow.

Results

Soil physicochemical factors along the elevation gradient

Most tested soil parameters significantly changed with increasing elevations (Table S1). The organic carbon content, total nitrogen, total carbon, available phosphorus, moisture content of the soil samples showed a trend of first increasing and then decreasing with the increase of altitude. The overall trend was more pronounced on the eastern slope compared with the western slope. Soil pH showed no significant change with altitude. The sand content and clay content of the western slope are higher than that of the eastern slope, and their contents do not vary greatly with altitude.

Diversity of CO₂-fixing microbes

According to the same elevation gradient, the samples of the same elevation on the east and west slopes were grouped. They numbered MLS1-5, corresponding to 3900~4100 meters, 4100~4300 meters, 4300~4500 meters, 4500~4700 meters, 4700~4900 meters, respectively. MLS6 was separately corresponding to the peak. The number of OTU in the MLS4 is significantly higher than that in the other groups, with the corresponding altitude of 4500~4700 meters. The eastern slope is mainly concentrated in MLSD4 and MLSD5 groups, corresponding to the elevation of 4500~4900 meters, while the western slope has a large number of OTU in 4300~4500 meters (Figure 1).

Comparing the chao1 index of the east and west slopes of Mila Mountain, it is obvious that the eastern slope chao1 index of the MLS4 group is much higher than that of other altitude groups. Chao1 index of the eastern slope is generally higher than that of the western slope. Moreover, the east slope exhibited the highest species abundance on the 4500~4700m zone, while the lowest is in the 3900-4100m zone on the western slope (Table 1).

Variation in Shannon index was similar to that of Chao1 index. In addition, the average Shannon index of the eastern slope is about twice that of the western slope (Table 1).

Table 1
Chao1 index and Shannon index of CO₂-fixing microbes on Mila Mountain (Mean±SD)

	Sampling sites	Chao1	Shannon index
Eastern slope	MLSD1	99.76±35.54	3.37±0.52
	MLSD2	97.00±6.06	2.40±0.98
	MLSD3	96.31±26.78	2.60±0.76
	MLSD4	273.13±39.50	4.87±1.00
	MLSD5	106.13±66.44	1.88±0.39
Mountaintop	MLS6	90.08±22.46	2.05±0.78
Western slope	MLSX5	81.96±76.60	1.6110±0.85
	MLSX4	63.88±26.3686	1.5500±1.20
	MLSX3	129.55±80.63	3.1878±1.31
	MLSX2	87.86±23.67	2.4345±0.99
	MLSX1	183.30±72.18	2.6901±0.83

Community structure of CO₂-fixing microbes

The composition of different microbial groups can be visually observed on the horizontal histogram (Figure 2). The relative abundance of *Cupriavidus* was the largest in the MLSD1 group, and the relative abundance of *Rhodobacter* was the absolute dominant in the MLSD2 and the MLS6 groups. The MLSD3 and MLSD5 group is mainly composed of *Cupriavidus* and *Thiobacillus*, while CO₂-fixing microorganisms are evenly distributed in the MLSD4 group, and it is difficult to identify the dominant bacteria. *Rhodobacter* in the MLS6 group has the highest proportion, the MLSX5 group is composed of *Cupriavidus* and *Thiobacillus*. *Sulfurifustis* gradually decreases from MLSX4 to MLSX2 groups along the elevation. MLSX4, MLSX3, MLSX2 group also contain different proportions of *Cupriavidus*. MLSX1 group contains a large proportion of the unique *Thiorhodococcus*.

The representative sequence corresponding to the top 30 abundant OTUs was selected to build the evolutionary tree in the unit of the genus (Figure 3). In the figure, it could be found that the abundant OTU can be divided into four phyla. Proteobacteria was predominant. Cyanobacteria, Actinobacteria, and Streptophyta, constitute a small proportion. The most abundant bacteria are *Cupriavidus*, *Rhodobacter*, *Sulfurifustis* and *Thiobacillus*.

Correlation between soil carbon fixing microbial community composition and environmental factors

The microbial community structure of soil CO₂-fixing microorganisms changed significantly with the altitude (Figure 4). RDA1 and RDA2 components accounted for 58.2% of the variation in community structure, and RDA1 alone accounted for 32.5% of the variation, indicating that soil physical and chemical factors such as soil moisture content, soil organic carbon, and sand particle size caused by elevation were the main factors driving the change in community structure of carbon fixing microorganisms. RDA2 accounted for 25.7% of the variation in the community structure of carbon sequestration microorganisms, and clay, phosphorus, and pH were the main driving factor of RDA2. In conclusion, the most important factor affecting the diversity of soil CO₂-fixing microorganisms is altitude, followed by coverage, soil moisture content, soil organic carbon and sand particle size, etc.

Discussion

This study found that Proteobacteria dominate in carbon-fixing microorganisms. Proteobacteria has also been found to be dominant in different habitats^{24–28}; this may be due to the low habitat specificity of Proteobacteria, and strong ability to adapt to different environments²⁹. Among them, the most abundant bacterial genera are *Cupriavidus*, *Rhodobacter*, *Sulfurifustis* and *Thiobacillus*. We also found a small number of cyanobacteria, Actinobacteria. As primary producers and predators in the ecological food chain, cyanobacteria are widespread in the natural environments^{30–32}. Actinobacteria are mainly involved in the decomposition of organic matter³³, which plays an important role in accelerating the decomposition of animal and plant residues in the soil, and also promotes the soil nitrogen cycle³⁴.

We found that the diversity of carbon-fixing microorganisms in Mila Mountain showed a mid-peak similar to flora and fauna as the altitude increased. At MLS4 on the east slope (4500~4700m), the Chao 1 index and Shannon diversity index of soil carbon-fixing microorganisms are much higher than those of other altitude gradient groups, and the overall OTU abundance first increases and then decreases with the elevation and mainly concentrated in the altitude gradient of 4300~4900m. However, in many previous studies soil microbial diversity showed a monotonous decreasing trend with increasing altitude^{35–38}. The reason why our results are inconsistent may be related to the content of soil organic carbon. Studies have shown that soil organic carbon has a significant impact on the carbon-fixing microbial community structure³⁹. In addition, the Chao 1 index and Shannon diversity index of the east slope as a whole is

generally higher than that of the west slope. Due to the differences in temperature and precipitation in different slopes, the microbial diversity also exhibited different altitude distribution patterns²².

As the altitude gradient rises, environmental factors such as soil and climate change, causing changes in the diversity of soil microbial communities. Therefore, changes in altitude gradients are an ideal natural laboratory for studying the characteristics of soil carbon-fixing microbial communities and their driving factors¹⁶. Soil parameters have a strong correlation with altitude, and the most significant correlation with altitude gradient is soil moisture content. Relevant studies have shown that soil water content is very important in regulating microbial activity and diversity. It directly affects the physiological state of microorganisms, restricts the ability of microorganisms to decompose certain compounds, and regulates soil enzymes and soil physical and chemical properties, thereby affecting microbial composition and activity⁴⁰. Guo et al. (2015) found that RubisCO enzyme catalyzing the Calvin cycle was promoted by SWC and NH_4^+ and inhibited by temperature¹⁶. Autotrophic organisms are usually controlled by temperature, plateau region is rich in microorganisms, this may be the region of microbial growth in this environment for a long time, can endure or adapt to low temperature. At the same time, the soil water content may counteract the inhibition of low temperature on microbial and enzyme activities. Mila Mountain connects the high mountain valleys of southeast Tibet and southwest Tibet. The climate on the east and west slopes are quite different. The eastern part is rich in precipitation and belongs to a warm and semi-humid climate type; the western part is dominated by a warm and semi-arid climate. Previous research indicated that the slope aspect markedly affects soil and microbiological properties in micro-ecosystem environments. We also found that there are differences in microbial communities on the eastern and western slopes. It may be that the slope aspect changes the microclimate of the study area, which determines the amount of solar radiation absorbed by the soil, thus affecting the soil water content and soil temperature and then affecting the soil microbial content and community structure. Similarly, Sidari et al. (2008) also found that the difference of soil microbial biomass in different slope aspects may be caused by the different microclimate under this condition⁴¹.

Materials And Methods

Site description and sample collection

The study area is located in the Mila Mountain of the Tibetan Plateau, connecting the high mountain valleys of southeast and southwest Tibet. The eastern part is rich in precipitation and belongs to a warm and semi-humid climate type; the western part is dominated by a warm and semi-arid climate. Vertically, Mila Mountain includes mountain temperate, subalpine cold temperate, and alpine cold temperate climate zones. The structure and distribution pattern of vegetation communities are affected by different climate types and soil types on the east and west slopes.

In late July 2017, soils were sampled from both slopes and mountaintop of Mila Mountain region with five replicates at six altitude gradients (3900~4100m; 4100~4300m; 4300~4500m; 4500~4700m;

4700~4900m; 5000~top). Vegetation distribution on the eastern and western slopes of Mila Mountain with different elevations as shown in the figure 5. Five sampling plots are arranged symmetrically on the east, and west slopes along the altitude gradient, and a single sampling plot are set on the top of the mountain. The five-point sampling method is adopted platform sampling. At each plot, five surface soil samples (0–20 cm) were collected, and a total of 55 samples were collected. After the samples were collected, they were stored in a refrigerator with blue ice at -80°C and then transported back to the laboratory for DNA extraction and subsequent experiments. The remaining soils were air-dried for physicochemical analyses.

Analysis of soil physicochemical properties

The soil moisture content was determined by the drying method. Soil pH was determined with PHS-3C by the potentiometric method. The soil particle size was measured with Mastersizer 3000 laser particle size analyzer. Soil total carbon and organic carbon are measured by multi-C/N310 host and HT1300 solid module.

DNA extraction

Soil microbial DNA was extracted by E.Z.N.A.[™] Soil DNA Kit. The soil sample was homogenized and then processed in a specially formulated buffer containing detergent. Humic acids, proteins, polysaccharides and other contaminants were subsequently precipitated after the thermal freezing step. Extraction was performed, the binding conditions were adjusted, and the sample was applied to the HiBind[™] DNA spin column. Trace contaminants were removed by two rapid washing steps, and pure DNA was eluted in water or a low ionic strength buffer. Purified DNA can be used directly for subsequent analysis.

PCR amplification and MiSeq library

After the genomic DNA extraction is completed, the sample is melted on ice, centrifuged and mixed thoroughly; Nanodrop detects the quality of the sample and takes 30 ng for PCR amplification. According to the designated *cbbL* 2 amplification region, specific primers with barcode or synthesize fusion primers with misplaced bases were synthesized. KAPA 2G Robust Hot Start Ready Mix and *cbbL* 2 primers were adopted for PCR amplification. 1% agarose gel electrophoresis was used to detect the extracted genomic DNA.

According to the pooling ratio, it was pooling a certain volume of the PCR product of the fusion primer without misplaced bases into a computer library and using 2% agarose gel to screen the library fragments. The selected library fragments were detected and quantified by Qubit. A certain amount of library was added to 10µl Endrepair&Add A for end repair and A tailing Then 33.5µl Adaptor Ligation Mix was added for connection with sequencing adapters, and the library was purified and recovered. Then add linker primers, enzymes, and Mix for PCR enrichment to complete library construction. Finally, the library was purified by the magnetic bead method. Use Nanodrop to roughly check library concentration, Agilent 2100 to detect library fragments. The library was sequenced on the Illumina Miseq platform.

Statistical analysis

In MEGA 6.0 software, representative sequences of the top 30 OTUs corresponding to abundance were selected by genus to construct phylogenetic trees. The formula of the Vegan package in R (V.3.5.2) was used to conduct analyze community structure and community diversity. They were drawing using Adobe Illustrator 2020 software.

Conclusion

Our results revealed the community structure of carbon-fixing microorganisms and their influencing factors along an altitude gradient. In the Mila Mountain area, the overall soil OTU abundance was concentrated at 4300~4900m, showing a mid-peak trend. Proteobacteria dominated the soil carbon fixing microbial community and might play an important role in the CO₂ fixation in alpine meadow soils. The relatively abundant bacteria belong to *Cupriavidus*, *Rhodobacter*, *Sulfurifustis*, and *Thiobacillus*. Within the elevation gradient in this study, the abundance and community structure of carbon-fixing microorganisms in meadow soil was mainly affected by the elevation change, among which soil moisture content, soil organic carbon, and sand particle size were important influencing factors.

Declarations

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Author contributions

All co-authors have provided intellectual content of critical importance to the work presented and approve this manuscript. J.P.L. and W.Z analyzed data and drafted the article, F.H.Y., G.J.P., C.B.D. and W.S.K. designed the study, collected data and revised the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Venn diagram of OTU distribution along elevation gradients in Mila Mountain (A, eastern slope; B, western slope; C, whole Mila Mountain)

Figure 2

Community structure of CO₂-fixing microorganisms along different altitude gradient zones

(A, eastern slope; B, western slope; C, whole Mila Mountain)

Figure 3

Horizontal evolutionary tree of the top 30 genera of OTU abundance of CO₂-fixing microbes along altitude gradient on the Mila Mountain

Figure 4

Redundancy analysis between soil microbial diversity and environmental factors

Figure 5

Schematic diagram of vegetation distribution with different altitude gradients on the eastern and western slopes of Mila Mountain

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)