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# The soil carbon/nitrogen ratio and moisture affect microbial community structures in alkaline permafrost-affected soils with different vegetation types on the Tibetan plateau

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## Abstract

In the Tibetan permafrost region, vegetation types and soil properties have been affected by permafrost degradation, but little is known about the corresponding patterns of their soil microbial communities. Thus, we analyzed the effects of vegetation types and their covariant soil properties on bacterial and fungal community structure and membership and bacterial community-level physiological patterns. Pyrosequencing and Biolog EcoPlates were used to analyze 19 permafrost-affected soil samples from four principal vegetation types: swamp meadow (SM), meadow (M), steppe (S) and desert steppe (DS). *Proteobacteria*, *Acidobacteria*, *Bacteroidetes* and *Actinobacteria* dominated bacterial communities and the main fungal phyla were *Ascomycota*, *Basidiomycota* and *Mucoromycotina*. The ratios of *Proteobacteria*/*Acidobacteria* decreased in the order: SM > M > S > DS, whereas the *Ascomycota*/*Basidiomycota* ratios increased. The distributions of carbon and nitrogen cycling bacterial genera detected were related to soil properties. The bacterial communities in SM/M soils degraded amines/amino acids very rapidly, while polymers were degraded rapidly by S/DS communities. UniFrac analysis of bacterial communities detected differences among vegetation types. The fungal UniFrac community patterns of SM differed from the others. Redundancy analysis showed that the carbon/nitrogen ratio had the main effect on bacteria community structures and their diversity in alkaline soil, whereas soil moisture was mainly responsible for structuring fungal communities. Thus, microbial communities and their functioning are probably affected by soil environmental change in response to permafrost degradation.

*Keywords:* Alkaline permafrost-affected soil; Microbial community; Soil variables; Tibetan plateau

## 1. Introduction

Permafrost (ground that has been continuously frozen for at least 2 years) covers approximately 25% of the Earth's terrestrial surface and it is estimated to contain approximately 15% of the total global soil carbon pool [34]. Thus, these soils are an important component of the global carbon cycle and their response to climate change will have important consequences for both ecosystem processes and global climate feedback [1]. Climate warming during recent years has caused widespread and rapid degradation of permafrost, which could significantly change soil moisture content, soil nutrient availability and ecosystem species composition [62]. Will permafrost changes lead to the release of massive amounts of carbon (e.g., $CO_2$ and $CH_4$) into the atmosphere? This question can only be answered by understanding how the microbes residing in different permafrost regions will respond to change [52]. Thus, the microorganisms found in permafrost have become a focus of attention [9,11,41,42,57]. However, our

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understanding of the potential effects of permafrost change on shifts in the community structure and functioning of associated microorganisms is limited, because we know little about the factors that influence their distribution.

The Qinghai-Tibet Plateau is the largest geomorphological unit on the Eurasian continent and it is the largest low-latitude permafrost region in the world. The permafrost in this region is estimated to cover approximately $1.5 \times 10^6$ km$^2$, which accounts for 69.8% of the total permafrost area in China. The soil organic carbon reservoirs found in this area comprise $30–40 \times 10^9$ t, which accounts for > 20% of the soil organic carbon storage in China and 2–3% of the global soil carbon reservoirs [59,60]. This permafrost body is warm and thin compared with the high-latitude permafrost in North America and Russia, which makes it more sensitive to climate change [37]. Furthermore, these permafrost soils are alkaline, in contrast to the acid soils in the Arctic [11,42]. The warming climate has accelerated the degradation of permafrost in recent decades, as evidenced by the reduced thickness of permafrost, increased ground temperatures and the depths of the seasonal thawed active soil layer [7,37,62]. Permafrost degradation has resulted in substantial changes in the soil moisture content and vegetation type. For example, swamp meadow (SM) has drained and transformed into alpine meadow (M), followed by steppe meadow (S) and desert steppe (DS), although the soil moisture content has increased with permafrost thawing in Alaska [24,37,58,62]. Permafrost degradation has also resulted in the shrinking of wetlands and grasslands, with the lowering of water tables [7]. The degradation of permafrost and vegetation has important implications for ecosystem functioning and carbon storage [7,59]. For example, it is estimated that vegetation type changes between 1986 and 2000 caused losses of 1.79 Tg of carbon (120 Gg of carbon per year) from the soil organic carbon in the 0–0.30 m soil layer of the Qinghai-Tibet plateau permafrost regions and a concomitant 65% decrease in the bio-available light fraction of organic carbon [59]. In association with widespread changes in the soil chemical nature, there are likely to be parallel changes in the decomposer communities and their functioning, which may play significant roles in changing the patterns of nutrient release and carbon flow. However, the variation in the compositions of microbial communities and their relationships to the soil properties are not clear.

Studies in the Arctic and Antarctic have shown that vegetation type and associated soil properties affect microbial community structure. For example, the vegetation types may regulate microbial communities by determining the substrate supply (e.g., litter and root exudates) and by modifying the physical environment [8,9,63]. Changes in plant cover and morphology may also affect the transmission of biologically damaging UV-B to the soil surface, thereby influencing microbial diversity [27,64]. The bacterial and fungal communities found in the Arctic tundra differed among vegetation types and their variation was correlated with the bio-available carbon fraction, as well as the soil carbon/nitrogen ratio as determined using total carbon and total nitrogen (C/N), moisture, pH and available soil nitrogen [9,57]. In contrast, other studies found that the soil pH, but not the vegetation type, was the major factor that affected community composition and diversity, which might be linked to the correlation between soil pH and the relative abundances of dominant bacterial groups, such as *Acidobacteria* [11,35,42]. Other studies have suggested that the water regime affects microbial communities, such as ammonium-oxidizing bacteria and methane-oxidizing bacteria, by controlling the dominant vegetation and the soil redox potential [26,63]. Shifts within the bacterial community structure and functions in terms of both the carbon utilization and turnover rates are also correlated with variations in the redox potential, which are determined by the water content [56]. These observations suggest that microbial communities can respond to the soil environment.

In the present study, we compared soil microbial community structures and function in a range of vegetation types (permafrost-affected soil environmental gradients) to develop a framework for predicting the responses of microbial communities to changes in soil properties across the Tibetan plateau. This survey may facilitate a better understanding of the effects of permafrost and vegetation degradation on microbial communities by using a spatial-temporal substitution policy. Our study focuses on SM, M, S and DS vegetation types, which are the main types in the soil moisture gradients determined by permafrost. The study area extended 700 km from south to north (Fig. 1) and we addressed the following questions. (1) How do bacterial and fungal communities change across the gradients in different soil habitats? (2) What are the key factors that drive the community distributions of bacteria and fungi? (3) Do bacteria and fungi respond in a similar manner to these environmental drivers?

## 2. Material and methods

### 2.1. Soil sampling and analysis

Soil samples were collected from 19 sites between Xidatan (XDT) and Liangdaohe (LDH) along the Tibetan highway in August 2009 (altitude: 4520–5100 m), including four SM, five M, five S and five DS sites (Table 1 and Fig. 1). These sites are underlain by permafrost and an active layer developed during the thawing season, which reached a maximum depth of 3 m [37]. The annual air temperature was between −7.1 and −1.8 °C and annual precipitation was approximately 300–500 mm year$^{-1}$ [37]. Four soil cores were collected at each site (20 cm deep and 5 cm in diameter) within a 50 m$^2$ area and were combined to form a single sample [8,9,63]. All samples were transported immediately to the laboratory with ice packs and were stored at −80 °C before extraction of genomic DNA. The physicochemical properties of the soil samples were determined using standard methods [9].

### 2.2. Soil DNA extraction and barcoded pyrosequencing

DNA was extracted from 2.0 g soil samples using a MoBio PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad,