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Bacterial communities in the upper soil layers in the permafrost regions on the Qinghai-Tibetan plateau

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ABSTRACT

The Qinghai-Tibetan plateau (QTP) is the largest middle-low latitude permafrost region on earth, while little is known about the microbial community in this area. Here, we investigated the bacterial community in the upper 30 cm soils in the permafrost regions on the central QTP using Illumina sequencing technology. In these soils, the most abundant phyla were Acidobacteria, Proteobacteria, and Bacteroidetes. The depth was significantly correlated with Acidobacteria, Proteobacteria, Nitrospirae, and Gemmatimonadetes. The soil pH and the gravel content were significantly positively correlated with Bacteroidetes. The active layer thickness was significantly correlated with Bacteroidetes and Arabinonates. Although these factors were closely correlated with the relative abundances of specific bacterial phyla, the overall bacterial community structure was mainly affected by pH, soil organic carbon content, and the mean annual precipitation, while the community structure had no significant relationship with the active layer thickness. Our results suggested that the permafrost region on the QTP had greatly heterogeneous environmental conditions, and the responses of microbial communities to permafrost degradation would also be affected by other factors such as precipitation, soil texture and vegetation.

1. Introduction

Soils are complex systems including interactions of biotic and abiotic processes. Soil microorganisms regulate biogeochemical processes in soils and play vital roles in organic matter (SOM) cycling (O'Donnell et al., 2007). SOM is mainly originated from the partial decomposition and transformation of plant debris and litter input by soil microbes (Cindye, 2010). The microbial decomposition of SOM is also the main pathway for the transfer of greenhouse gases from the terrestrial ecosystem to the atmosphere (Chauvin et al., 2015). The soil microbes also play fundamental functions for the maintenance of soil ecosystem integrity. For example, ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are involved in ammonia oxidation and nitrification and thus are important drivers of soil nitrogen cycle (Isobe et al., 2012).

During the past centuries, human activities greatly changed the global carbon cycle and the climate. The climate warming definitely changed soil temperature, moisture and thus have both direct and indirect effects on the soil microbial communities (Joshi and Shekhawat, 2014). It is of great importance to understand the changes of soil microbes in a warming world because they are vital to many basically ecological processes (Bardgett et al., 2008).

The circum-arctic permafrost regions store approximately 1330–1580 Pg organic carbon, which is almost twice as the carbon in the atmosphere (Hugelius et al., 2014; Schuur et al., 2015). The SOC pools in the permafrost regions have received worldwide attention because global warming could potentially cause emissions of greenhouse gases (Hugelius et al., 2014; Ping et al., 2015, 2008). In permafrost regions, a shift in the microbial community would greatly change the organic matter decomposition (Xue et al., 2016), and the release of greenhouse gases from the organic matter decomposition is closely associated with soil microbial abundance (Mackelprang et al., 2011). Therefore, investigation of soil microbial community could shed light on the relationship between soil microbes and environmental factors thus improve our knowledge of the effects of climate change on ecosystems (Herold et al., 2014; Nannipieri et al., 2003; Tardy et al., 2014).

Many biotic and abiotic factors such as pH (Feng et al., 2014; Kim et al., 2014), soil moisture content (Brockett et al., 2012), nutrient levels (Cleveland and Liptzin, 2007; Stark et al., 2011), organic carbon

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(Zhang et al., 2014), and vegetation cover (Shi et al., 2015) can affect the soil microbial community structure. In the permafrost regions, it has also been found that temperature and the freeze-thaw cycle affected the microbial community (Jansson and Tas, 2014; Stres et al., 2010). These studies summarized the factors that control the soil microbial community. However, these conclusions were mainly based on statistical analyses of measured soil parameters, but little consideration was given to the factors that had no significant relationships with the microbial community. In fact, soil variables usually interacted with each other. For example, the permafrost conditions can affect the soil microbial community since permafrost is associated with many soil variables, such as soil moisture content (Wang et al., 2008), pH, and soil texture (Mu et al., 2016a,b).

The low temperature in the permafrost region favors the perseverance and accumulation of organic matter (Mu et al., 2014; Zimov et al., 2006), and the organic matter at different depths usually has a high proportion of labile fractions (Wu et al., 2014). So far, most studies for the soil microbial community have mainly been focused on the upper 10 cm layer (Bowles et al., 2014; Chu et al., 2016; Yue et al., 2015). Although the soil microbes below 0–10 cm soils were abundant and active (Fenchel, 2003), and these microbes at the subsurface and even the deep soil doubtless play an important role in soil biogeochemical cycling and other processes (Fierer et al., 2003; Mackelprang et al., 2011), little is known about the soil microbial community below the upper 10 cm layer.

The Qinghai-Tibet Plateau (QTP), accounts for approximately three quarters of the high-latitude permafrost on earth. Under global warming scenarios, the soil environment in the permafrost region on the plateau has become a major concern since its role in maintaining ecosystem function and services (Mu et al., 2015; Wu et al., 2017; Yang et al., 2014). It is well-known that pH, SOC, and C:N ratios are the most important factors that determine the soil bacterial community, and these factors vary considerably at different depths of the upper soil layer (Shang et al., 2016). Therefore, we hypothesized that depth could have a great effect on the microbial community in this area. Based on the interactions of environmental conditions in the permafrost region, we also hypothesized that active layer thickness, soil moisture, soil texture, vegetation cover, and climatic conditions could also affect the soil bacterial community. To test these hypotheses, six plots in the permafrost region of the central QTP were selected and the soil bacterial community and its relationship with soil parameters was investigated.

2. Material and methods

2.1. Soil sampling and analysis

The soils at 6 sites in the permafrost region of the central QTP were sampled during July, 2014 (Fig. 1). The climatic, permafrost and vegetation conditions for the sampling sites are shown in Table 1. The thawing of frozen ground on the QTP is expected to start in April, and the maximum thaw depths, which equal to the active layer thicknesses, are largely recorded in late September. The thaw depths in July are about 1 m in the central QTP (Zhao et al., 2000, 2010). To exclude the possibility that soil properties would differ considerably within the same site, 1.5 m × 1.5 m plots with relatively uniform microrelief conditions (i.e., without a patchy distribution of vegetation and flat areas) were selected. There is still a possibility that soils have heterogeneities in the same plots. Therefore, to examine the spatial distribution of bacterial community structure and its relationship to environmental variables, we collected five subsamples at each depth, and then pooled into a single soil sample to represent the sample at the certain depth. The soil variables and DNA sequencing analysis were all performed using this sample and thus we can examine their relationships. Samples from the surface 30 cm were collected at a depth interval of 10 cm. The soil samples were collected aseptically using an ethanol-disinfected soil auger and placed in clean, sealable plastic bags. The soil samples were immediately transported to the laboratory in a cooler with ice packs and stored at −80 °C until genomic DNA extraction was carried out.

We used a square-meter quadrant and took five samples at each site to investigate the vegetation cover. Additionally, we excavated a shallow soil pit (approximately 50 cm) and measured the soil bulk density using a bulk soil sampler (i.e., a 5-cm diameter and 5-cm high stainless steel cutting ring). We collected soil samples at different depths to calculate the total water content, which was determined by drying the soil at 105 °C for 8 h. The pH values of the air-dried soils were measured using the 1:5 mixture of soil:water. The SOC and total nitrogen (TN) were measured using a Vario EL elemental analyzer (Elementra, Hanau, Germany). To measure the SOC, we pretreated 0.5 g dry soil samples with HCl (10 mL, 1 mol l^{-1}) for 24 h to remove the carbonate. We calculated the mass ratio of C/N. The percent of gravel was measured by weight of rock fragments (> 2 mm) using the oven-dried samples, and soil particle distribution was measured using a combination of wet sieving (Chaudhari et al., 2008) and a laser diffraction instrument (Malvern Mastersizer 2000, Malvern, UK). The proportions of sand, silt and clay were classified by the respective size intervals of 2 mm–50 μm, 50–2 μm, and < 2 μm.

2.2. Soil DNA extraction

We extracted the total soil DNA from each soil sample using a MoBio PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) per the manufacturer's instructions, using 0.3 g soil. The concentration of the extracted DNA was determined using a QuBit DNA quantification system (Invitrogen) with the QuBit high sensitivity assay reagents. Then, all the soil DNA samples were stored frozen at −20 °C until used.

2.3. PCR amplification

We performed PCR amplification, purification, and sequencing of a region of the 16S rRNA gene (Fierer and Jackson, 2006). The V4 and V5 hypervariable regions of the bacterial 16S ribosomal RNA gene were amplified by PCR. The PCR conditions were 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 s, and then a final extension step at 72°C for 10 min. We used the primer set F515 (5′-GTGCCAGCMGCCGCGG-3′) and R907(5′-CCGTCAATTCMTTTRAGTTT-3′) (Bates et al., 2011). The PCR amplification was performed in 50 μL reactions containing about 10 ng template DNA, 25 μL of PCR Pre-Mixture (TaKaRa) and 0.3 μM of forward and reverse primer. The same volume of 1X loading buffer (contained SYB green) was mixed with the PCR products and visualized with 1.2% agarose gel electrophoresis. We chose samples with a bright strip between 350 and 450 bp for further analysis. The PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA, USA). We prepared a single composite sample for sequencing by combining approximately equimolar amounts of PCR products from each sample. Sequencing was performed using a Miseq PE250 platform (Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China).

2.4. Processing of sequencing data

Paired-end reads from the original DNA fragments were merged with FLASH, which was designed to merge paired-end pairs of reads when the original DNA fragments were shorter than twice the length of the reads. The sequences were analyzed using QIIME 1.17 (http://qiime.org/scripts/pick_otus.html). Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered. To compute Alpha Diversity, we rarified the OTU table and calculated four metrics, i.e., the Chao1 metric, the Observed OTUs, which was a count of unique OTUs in the sample, the Ace Shannon, and the Simpson index. We performed the mantel test, redundancy analysis (RDA), and prepared figures using the