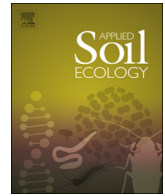




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Effects of permafrost thaw-subsidence on soil bacterial communities in the southern Qinghai-Tibetan Plateau

Xiaodong Wu^a, Lin Zhao^{a,*}, Guimin Liu^b, Haiyan Xu^b, Xiaolan Zhang^b, Yongjian Ding^{a,c,d}

^a Cryosphere Research Station on the Qinghai-Tibetan Plateau, State Key Laboratory of Cryospheric Science, Northwest Institute of Eco-Environment and Resource, Chinese Academy of Sciences, Lanzhou, Gansu 730000, China

^b School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou 730070, China

^c Key Laboratory of Ecohydrology of River Basin Sciences, Chinese Academy of Sciences, 320 West Donggang Road, Lanzhou 730000, China

^d University of Chinese Academy Sciences, 19(A) Yuquan Road, Shijingshan District, Beijing 100049, China

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ABSTRACT

Permafrost thaws cause ground subsidence as the ground ice melts and drains away. Little is known about the effects of this permafrost thaw subsidence on bacterial communities. In this study, using Illumina sequencing methods, we investigated the structure of bacterial communities in the upper 50 cm of the soil in a typical permafrost thaw subsidence area on the southern Qinghai-Tibetan plateau. The micro topographies in the study area were classified as control, collapsing, and subsided types. Results showed that the organic carbon content in the collapsing areas was slightly lower than that in the control areas, while there was a substantial decrease in the subsided areas, with a loss of $23.6 \pm 13.2\%$ of organic carbon. The microbial carbon contents showed the highest values in collapsing areas. For all three types of soils, the most abundant microbial groups were Proteobacteria, Acidobacteria, and Bacteroidetes. The Non-metric multidimensional scaling (NMDS) results showed that the bacterial communities were different in the subsided areas than in the control and collapsing areas. In the control and collapsing areas, the soil bacterial communities showed a clear vertical distribution pattern with depth, which was not apparent in the subsided areas. The bacterial communities also correlated with soil variables such as carbon, moisture, nitrogen contents, and the C:N ratio. The ground subsidence can greatly change these variables. The results suggested that permafrost thaw subsidence had important effects on microbial communities via the changes of soil properties.

1. Introduction

The permafrost ecosystems have received worldwide attention because climate warming could potentially cause changes of carbon balance in these areas and affect global carbon cycle (Hugelius et al., 2014; Ping et al., 2015; Ping et al., 2008). Microbial decomposition is the basic pathway by which soil organic carbon (SOC) is converted into greenhouse gases, and understanding of soil microbial communities and their relationship to environmental factors is of great importance to evaluate the organic matter decomposition. Under frozen conditions, the microbes are relatively inactive. As permafrost thaws, the microbes will be reactivated. The thawing of permafrost can lead to changes in soil properties, including soil water content, temperature, and pH values, and subsequently, changes in land cover types (Mu et al., 2017; Wu et al., 2017a). These factors have been recognized as important causes of changes in the soil microbes (Brockett et al., 2012; Feng et al., 2014; Kim et al., 2014; Shi et al., 2015). Therefore, soil microbes are

sensitive to permafrost thaws although they respond differently to permafrost thaws at different depths (Deng et al., 2015).

Permafrost degradation not only means a soil temperature increase but also landform changes. Permafrost thaw subsidence is one of the typical landforms of thermokarst terrains due to the ground ice melting and water draining away. The change in landforms due to ground subsidence greatly affects the soil properties, including the SOC content and greenhouse gas fluxes (Abbott and Jones, 2015). It has been suggested that the effects of ground subsidence should be taken into consideration when evaluating permafrost carbon feedback (Schuur et al., 2015). However, there are few reports about the bacterial communities in the different micro topographies in the subsided areas that result from permafrost degradation, and the determinants of the bacterial communities in these areas are unknown.

The Qinghai-Tibetan Plateau (QTP) is the largest low-latitude permafrost area in the world. The permafrost area on the QTP accounts for approximately 8% of the global permafrost area and approximately

* Corresponding author at: Northwest Institute of Eco-Environment and Resource, Chinese Academy of Sciences, 320 west Donggang Road, Lanzhou, Gansu 730000, China.
E-mail address: linzhao@lzb.ac.cn (L. Zhao).

three quarters of the mountain permafrost area (Zhang, 2012). On the QTP, the ground temperatures are relatively higher than those in circum-arctic regions and just slightly lower than 0 °C (Qin et al., 2016; Zhao et al., 2010). In addition, the rate of warming can be amplified in high-mountain regions (Kang et al., 2010). Thus, the permafrost on the QTP is extremely sensitive to climate change. Similar to the circum-arctic regions, the permafrost regions on the QTP store a large amount of SOC, which has been estimated at up to 160 Pg for the upper 25 m soils (Mu et al., 2015). The permafrost degradation can potentially produce emissions of large amounts of greenhouse gases on the QTP (Mu et al., 2016b; Wu et al., 2014). To understand the possible roles of microbial community in organic matter decomposition on the QTP, several studies have been conducted to investigate the microbial communities and their relationship to environmental factors. With the samples collected from several sites with gradients of environmental factors, it was found that pH, SOC, soil moisture content, and even soil depths were determinants of the soil microbial communities (Chu et al., 2016; Zhang et al., 2014).

Due to permafrost degradation, thermokarst terrains, including thermokarst lakes, ground subsidence, permafrost collapse, and thaw slumps occur over much of the permafrost areas (Mu et al., 2016a; Niu et al., 2012). The landform changes can also redistribute the SOC and affect the quantities of organic matter (Mu et al., 2016d). Since the microbial community is closely associated with soil variables (Goenster et al., 2017), we hypothesize that 1) thaw-subsidence changed the soil variables and further affected the bacterial community; 2) the vertical distribution of the soil bacterial community in the thaw-subsided area has been changed due to the land form deformation. To test these hypotheses, we investigated the bacterial communities in soils with different microfeatures and their relationships with soil variables within a typical thaw subsidence area on the southern QTP. The results examined bacterial community and its relationship to soil physico-chemical variables in a typical thaw-subsidence area and thus would be beneficial toward understanding the microbial mechanisms of changes of soil biogeochemical cycles in permafrost regions under future global warming scenarios.

2. Materials and methods

2.1. Site description and soil sampling

An area with ground subsidence in the permafrost regions (91.76°E, 32.00°N, 4744 m) in the southern QTP was studied. From 1980 to 2010, the mean annual precipitation (MAP) is approximately 350 mm (<https://data.cma.cn/> or <http://www.cma.gov.cn/2011qxw/2011qsjgx/>). The sampling area is located in a mountain valley with poorly drainage class. The slope of the sampling area is 1.5°, and the aspect is 90°. The parent materials of the soils are colluvial deposits, and the land cover type is alpine meadow. According to the Soil Taxonomy (ST) (Soil Survey Staff, 2014), the soil is classified as a Glacial Histoturbels (ABAB). From the soil pits, which were excavated in the late September in 2013, the active layer thickness was 2.5 m because the ground ice was present at this depth.

The subsidence of the surface ground resulted from melting of the ground ice (Fortier et al., 2010). According to the microfeatures, the subsidence was defined in three stages: control, collapsing and subsided (Fig. 1). The dominant vegetation in the three areas is shown in Table 1. The sampling sites for the control area were collected from the plots approximately 10 m from the boundary of the subsided area (soil samples hereafter abbreviated as AC). The soils from the collapsing areas exposed to the air were also collected (soil samples hereafter abbreviated as AE). The subsided area was abbreviated as AD. For all the three stages, six subsamples of each soil were randomly collected from each area.

The surface 50 cm soil layers were collected with increments of 10 cm, and the soil samples from the control areas were abbreviated as

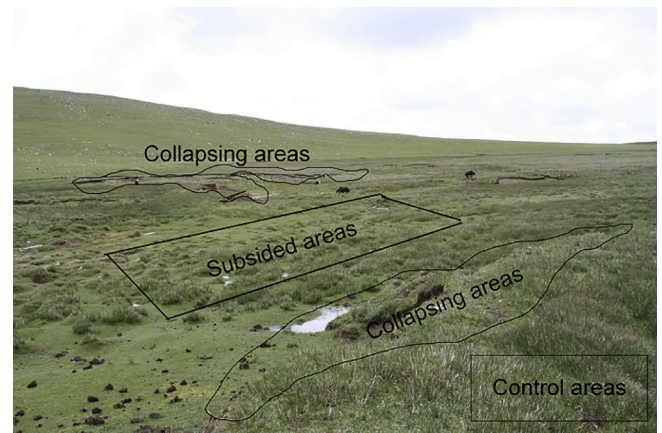


Fig. 1. Sampling sites for the three micro topographies (control, collapsing, and subsided).

AC0_10, AC10_20, AC20_30, AC30_40, and AC40_50. The soil samples from the collapsing and subsided areas were abbreviated similarly, i.e., AE0_10 (0–10 cm soil from the collapsing area), AD0_10 (0–10 cm soil from the subsided area). The soils were collected aseptically using ethanol-disinfected soil augers. We placed the soil samples in sealable, clean plastic bags, and preserved in a car refrigerator at –4 °C. The samples were brought to the laboratory immediately. For soil moisture determination, another 5 soil samples were collected and stored in aluminum boxes and carefully sealed to prevent changes in soil moisture. For the DNA analysis, soils were stored at –80 °C until genomic DNA extraction.

2.2. Laboratory experiments and DNA extraction

The soil moisture content was determined using oven-dried method (105 °C for 8 h). The soil pH and conductivity values were measured using soil suspensions (1:5 soil:water ratio). The total soil carbon (TC) and SOC of were measured using a TOC analyzer (Vario TOC cube, Elementra). The total inorganic carbon (TIC) content was calculated as: SIC = TC – SOC. Total nitrogen (TN) was measured using the micro-Kjeldhal procedure. The mass ratios of SOC and TN were calculated as C:N ratios. The light-fraction organic carbon was measured from the SOM that was separated by flotation method (1.8 g cm⁻³ NaI solution). Microbial carbon (MBC) was determined using the chloroform fumigation–extraction method (Shang et al., 2016). Soil particle distribution was analyzed by a laser diffraction instrument (Mastersizer 2000, Malvern, UK).

We extracted total soil DNA from 0.3 g soils using the MoBio PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA). For each sample, we performed three replicate extractions, and then pooled the extractions for further analyses. The extracted DNA was measured using a QuBit DNA quantification system (Invitrogen) with QuBit high sensitivity assay reagents. The soil DNA samples were stored frozen at –20 °C pending further analysis.

2.3. PCR amplification

We conducted the PCR amplification, purification, and sequencing of a region of the 16S rRNA gene (Fierer and Jackson, 2006). The primer set of F515 and R907 was chosen to amplify the V4 and V5 hypervariable regions of the bacterial 16S ribosomal RNA gene (Bates et al., 2011). Template DNA (10 ng) and PCR Pre-Mix (TaKaRa, 25 µL) were mixed with reverse and forward primers (0.3 µM) (Fang et al., 2016). The PCR products were mixed with an equal volume of 1X loading buffer (containing SYBR green) and analyzed by electrophoresis on agarose gels (1.2%). Samples with bright areas between 350 and

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