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Changes of soil microbial community under different degraded gradients of alpine meadow

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ABSTRACT

Changes in soil bacterial and fungal composition and their diversity with degradation degrees (i.e. non-degraded (ND), moderately degraded (MD) and severely degraded (SD) meadows) were investigated in a Tibetan alpine meadow using the Illumina MiSeq. *Proteobacteria*,*Actinobacteria*, and *Acidobacteria* were predominant bacteria in the studied meadow soils, regardless of degradation. ND and MD exhibited no significant differences in bacterial species composition and diversity, while SD significantly altered bacterial composition and increased their diversity compared with ND and MD. *Sordariomycetes* was predominant fungal class in ND, however, a shift in the fungal class from it to *Dothideomycetes* was found with increasing degradation level. Moreover, SD apparently increased the relative abundance of pathogenic fungi compared with ND. Degradation significantly shifted fungal species composition and increased their diversity. Soil nutrient conditions could explain 33.8 and 35.6% of the variance in bacterial and fungal composition, respectively. In addition, 25.3 and 21.7% of the variance in bacterial and fungal composition, respectively, were explained by plant properties. However, neither bacterial nor fungal diversity paralleled plant diversity with degradation. Soil silt to sand ratio was the best predictor of shifts in bacterial α-diversity with degradation degrees ($R^2 > 0.46$), while fungal α-diversity was most closely associated with changes in soil available potassium ($R^2 > 0.66$). Together, these results suggest that changes of microbial diversity and plant diversity was decoupled under degradation process, and degradation could increase the potential risk of plant diseases and decrease health of the alpine ecosystem.

1. Introduction

Overgrazing causes degradation of alpine meadows on the Tibetan Plateau (Zhou et al., 2005; Zhao, 2009; Wang et al., 2012), which has resulted in severely environmental problems (Zhao, 2009; Harris, 2010) due to 2.95 Pg carbon loss from the 0.7 m top soil with the degradation over the last 30 years on the Plateau (Wang et al., 2002). Previous studies found the meadow degradation alters plant composition and reduces plant diversity, above- and below-ground biomass (Wang et al., 2002; Zhou et al., 2004; Zhou et al., 2005; Wu et al., 2014). However, little is known about how the soil microbial composition and their diversity are impacted by meadow degradation.

Soil microbial communities mediate many biogeochemical processes that are central to ecosystem functioning, including carbon cycles, and their composition and diversity are sensitive to disturbances (Balser and Firestone, 2005; Allison and Martiny, 2008). Different microbes exhibit different ability/strategy to efficiently utilize soil organic matter (SOM) and the composition of microbial decomposers directly influence a variety of ecosystem processes, such as CO2 flux and litter decomposition (Strickland et al., 2009; Allison et al., 2010; Allison et al., 2013). In particular, Olff et al. (2000) studied the effects of soil microorganisms on growth of plant species by sterilization. They found that the soil microorganisms from a site where plant species were being degraded were more harmful to growths of the plants than the soil microorganisms from a nearby site where the plant species were increasing in abundance. Therefore, the negative effect of microbes (e.g. fungal pathogens) on plant growth may outweigh their positive effect (e.g. mycorrhizal fungi) during plant degradation. Soil substrate availability (Myers et al., 2001), heterogeneity

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(Ranjard and Richaume, 2001), and plant attributes (i.e. composition and biomass) (Wardle et al., 2004) are important factors responsible for changes in microbial communities. Therefore, the apparently changed soil nutrient availability, plant composition and biomass in the process of grassland degradation (Wu et al., 2014) would inevitably alter the composition and diversity of soil microbial communities. Understanding how microbial community responds to degradation in composition and diversity and their key affecting factors can provide important insights for the alpine meadow health assessment and management.

In this study, alpine meadows with different degradation conditions (i.e. non-degraded (ND), moderately degraded (MD) and severely degraded (SD) (i.e. desertization)) were selected to investigate changes in edaphic and plant properties, soil bacterial and fungal composition and their diversity using Illumina sequencing technology. Our objectives were to determine (1) how the changes of soil bacterial and fungal communities vary with changes in specific edaphic and plant properties; and (2) to assess the potential risk of microbial pathogens under the alpine meadow degradation.

2. Materials and methods

2.1. Study area

This study was conducted during summer in 2013 at the Naqu Ecological and Environmental Observation and Research Station, China (31°17' N, 92° 06' E; 4501 m a.s.l.) which is located in the center of the major distribution of *Kobresia pygmaea* on the Tibetan Plateau and is about 22 km in the southeast of Naqu City and 270 km northeast of Lhasa (Fig.S1). The local climate is characterized by strong solar radiation with long, cold winters, and short, cool summers. The annual mean air temperature and annual mean precipitation is −2.1 °C and 406 mm, respectively. Most of the precipitation falls between June and September.

ND, MD and SD alpine meadows were chosen based on plant coverage and proportion of *Kobresia pygmaea* as described by Liu et al. (2003). The dominant plant species is *Kobresia pygmaea* with intact turf and more than 90% canopy coverage for ND meadow. MD meadow remains about 40% native *Kobresia pygmaea* vegetation with crusts of Cryptogams. SD meadow is completely desertization due to removal of the native vegetation with dominant plant species of forbs (e.g. *Heteropappus hispidus*, *Przewalskia tangutica Maxim*, *Chenopodium glaucum* L., and *Axyris prostrate*).

2.2. Plant measurements and soil sampling

Seven quadrats (50 cm × 50 cm) with a pairwise distance of more than 50 m were randomly selected for each type of meadow. The distance between plots (more than 50 m) exceeded the spatial dependence of microbial variables (Franklin and Mills, 2003), thus they were independent replicates. Plant cover, height and composition of each quadrat were measured using point-intercept sampling, employing a 50 cm × 50 cm square frame, with 100 sampling points spaced equidistantly within the frame (Wang et al., 2012). The plant species richness was recorded and the Shannon–Wiener index was calculated according to an established protocol (Klein et al., 2007).

At each quadrat, three surface soil cores (the upper 10 cm) of 5-cm diameter were randomly collected in August 12, 2013 and were mixed, homogenised, and sieved (<2 mm) to remove root or other plant materials. Then each soil sample was immediately transported to the laboratory and divided into two sub-samples. One part was stored at 4 °C for measuring soil characteristics and the other was stored at −80 °C for total DNA extraction and molecular analyses. The above- and below-ground plant biomasses of the soil samples were separated, dried in an oven set at 60 °C for 48 h and weighted.

2.3. Soil characterization and DNA-extraction

Soil temperature and soil moisture at depth of 10 cm were continuously recorded for each meadow type using data loggers (Em50 Decagon Devices Inc., Pullman, Washington, USA) from July 1 to August 31, 2013. Both soil temperature and soil moisture were measured every minute and mean values per hour were stored. In addition, mean soil temperatures and moisture of July and August were calculated. The soil structure was measured by hydrometer method (Ashworth et al., 2001). Soil total carbon (TC), total nitrogen (TN), total phosphorous (TP) and total potassium (TK) were analyzed using an isotope ratio mass spectrometer with a Eurovector Elemental Analyzer (Isoprime-EuroEA 3000, Milan, Italy). Total organic carbon (TOC) was measured by a TOC-5000A analyzer (Shimadzu Corp., Kyoto, Japan). The pH value was measured by using 5 g soil and 10 ml of deionized water. Soil available nitrogen (AN), available phosphorous (AP), and available potassium (AK) content were measured by the methods of Wu et al. (2011). Microbial biomass carbon (MBC) was estimated by the fumigation-extraction method described by Wu et al. (1990).

Total soil DNA was extracted from 0.5 g soil using FastDNA spin kit for soil (MP Biomedical, Carlsbad, CA, USA) following the manufacturer's instructions. DNA quality assessment and quantification was conducted by using a Nano-Drop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA).

2.4. Bar-coded pyrosequencing of bacterial and fungal communities

The DNA extractions were diluted to 10 ng/μl to serve as PCR template. For Illumina sequencing analysis, individual bacterial and fungal DNA was amplified from each soil sample. For bacterial 16SrRNA, the forward primer used was F515 combined with the reverse primer, R907 (Zhou et al., 2011a), which targeted the region V4/V5 of the 16S rRNA (Table S1). Fungal ITS2 region was amplified with a nested PCR approach as recommended by Berry et al. (2012). The entire ITS region of fungal DNA was first amplified using the fungal-specific primers ITS1-F and ITS4, and then a second PCR using the primers ITS3 and ITS4 targeted the ITS2 region (Table S1). Forward primers contained barcodes to allow subsequent position assignment of all amplicons.

A PCR mix of 25 μl containing 1 × PCR buffer (Fermentas, Vilnius, Lithuania), 0.2 μM of each primer, 0.2 mM deoxynucleoside triphosphates (dNTPs), 2.75 mM $MgCl_2$, 300 ng/μl BSA, 10 ng of template, and 2.5 Units of the Pfu polymerase (BioVision, Mountain View, CA, USA) was prepared for each amplification. Reactions, performed in triplicate, were combined and purified by using gel electrophoresis followed by the QIAquick gel extraction kit and the Qiagen PCR purification kit and quantified using QuantiFluor™-ST (Promega, USA). Purified barcode tagged amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Majorbio, Shanghai) according to the standard protocols. The pyrosequencing datasets of soil bacteria and fungi have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (accession no. SRP068608).

2.5. Sequence analysis

All sequence processing and diversity estimates were performed using the QIIME V. 1.3.0 (Caporaso et al., 2010) as described by Davey et al. (2012). Raw sequences were quality-filtered with the following criteria: (i) sequence reads were truncated at any site