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Functional and phylogenetic response of soil prokaryotic community under an artificial moisture gradient

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ABSTRACT

Moisture is recognized as a key factor shaping the structure of soil microbial community and its function in soil ecosystem. However, the temporal response patterns of soil microbes under various moisture regimes remain poorly understood. Therefore, the main objective of our study was to reveal how moisture regulates prokaryotic community structure, diversity, phylogenetic structure and finally how moisture regulates greenhouse gas emissions, as an indicator of microbial community function. We monitored prokaryotic community in soil incubated under an artificial moisture gradient for three months. We observed robust effects of both moisture gradient and incubation time on increased greenhouse gas emissions (methane, carbon dioxide and nitrous oxide). Furthermore, the moisture gradient as well as the incubation time exerted significant effects on species turnover of the soil prokaryotic community. In contrast, the artificial moisture gradient did not show any significant effects on prokaryotic alpha diversity. Alpha diversity of the soil prokaryotic community decreased significantly with incubation time. Different community assembly patterns were observed (based on both the mean nearest relatedness index (NRI) and nearest taxon index (NTI)). The mean NRI exhibited the dominance of stochastic factors, while the NTI indicated the dominance of deterministic factors. The prokaryotic communities in soils with less moisture tended to be controlled by stochastic factors, while prokaryotes in soils with higher moisture (60%) were controlled by deterministic factors. Relative abundances of oligotrophs and copiotrophs did not change significantly along the artificial moisture gradient, while the relative abundances of some prokaryotic taxa did vary significantly along the artificial moisture gradient.

1. Introduction

Soil microorganisms are important for ecosystem services such as organic matter mineralization, carbon sequestration and soil fertility (de Vries et al., 2013; Fierer, 2017; Wagg et al., 2014). They are also important contributors to emissions of greenhouse gases (Eisenlord et al., 2013; Trivedi et al., 2013). The direct impact of greenhouse gases released during the decomposition of soil organic matter on global climate change has been widely studied and discussed (Ward et al., 2013). Despite increasing evidences, little effort has been paid to incorporate soil microorganisms into the predictive models of future climatic changes (e.g. extreme rainfall event, drought) (Evans et al., 2011; Ward et al., 2013).

Alterations in precipitation may significantly impact the structure and function of an ecosystem, especially in semi-arid grassland regions (Chen et al., 2014; Yao et al., 2017). Some studies report the sensitivities of microbial structure and diversity to the alterations of water regimes along precipitation gradients and other environmental factors (Evans et al., 2014; Evans and Wallenstein, 2014, 2011; Yao et al., 2017). However, the phylogenetic responses and ecological processes driving microbial community assembly along the moisture gradient are not fully understood.

It has been recognized that microbial communities are simultaneously driven by both deterministic (moisture, pH, and nutrient flow)

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and stochastic processes (speciation, extinction, and ecological drift) (Chase, 2007; Evans and Wallenstein, 2014; Stegen et al., 2013, 2012). Traditional niche-based theory suggests that community assemblies are mainly determined by a set of environmental conditions (e.g., moisture), which the species prefer the niches that they adapt to (Chase, 2007; Tilman, 2004). The stochastic model assumes that community dynamics are the sum total of individual stochastic events such as selection, drift, dispersion, speciation, mortality, and migration of individuals over time (Nemergut et al., 2013; Stegen et al., 2013; Vellend, 2010).

Organisms affected by deterministic factors display strong phylogenetic relatedness, and they are affected by environmental filtering (e.g., moisture stress) (Kembel et al., 2011), while organisms shaped by stochastic factors display reduced phylogenetic relatedness, and they are affected by stochastic factors such as selection, drift, dispersion and speciation (Nemergut et al., 2013; Stegen et al., 2013; Vellend, 2010). Most widely used approaches to evaluate phylogenetic relatedness are direct analyses of phylogenetic tree using nearest relatedness index (NRI) and nearest taxon index (NTI). The NRI is generally more sensitive to the tree-wide patterns of phylogenetic clustering and evenness, while the NTI is more sensitive to the patterns of evenness and clustering closer to the tips of the phylogeny (Kembel et al., 2011).

Based on the metabolic potential and growth rates, soil prokaryotes can be classified into two ecological categories. Members of phyla Actinobacteria and Acidobacteria and class Deltaproteobacteria are considered as oligotrophs (k-stategists) whereas phylum Bacteroidetes and class Alphaproteobacteria and Gammaroteobacteria are copiotrophs (r-strategists) (Fierer et al., 2007; Trivedi et al., 2013). The responses of different ecological groups of microbes vary under increased environmental stress such as extreme drought (Evans et al., 2011; Williams, 2007). Oligotrophs are recognized as slow growing microorganisms, which are adapted to poor nutrient substrates and low moisture content (de Vries and Shade, 2013; Fierer et al., 2007). In contrast, copiotrophs are recognized as fast growing microorganisms that prefer rich nutrient substrates, and they are sensitive to low moisture content (Evans and Wallenstein, 2014; Fierer et al., 2007).

Many previous studies indicate the role of soil moisture on the composition and structure of soil microbial communities at both continental (Fierer et al., 2012; Lauber et al., 2009) and regional scales (Evans et al., 2014). A microbial community can be either sensitive, tolerant, and/or opportunistic to various levels of moisture (Evans and Wallenstein, 2014). Soil microbial communities that are dominated by oligotrophs show resistance to moisture stress (de Vries and Shade, 2013; Evans and Wallenstein, 2014). In contrast, microbial communities dominated by copiotrophs are considered to be sensitive to moisture stress (de Vries and Shade, 2013). As a consequence, the changes of a microbial community in response to moisture would significantly alter soil function, such as emission of greenhouse gases.

In this study, we investigated the functional and phylogenetic response of soil prokaryotic community under an artificial moisture gradient. We aimed to answer following questions: (i) how moisture regulates prokaryotic community structure and diversity; (ii) how moisture regulates microbial community function indicated by greenhouse gas emissions; (iii) how moisture affect ecological processes (deterministic or stochastic) driving the response of soil prokaryotic community to moisture.

2. Material and methods

2.1. Study site and sampling description

Soil used in the incubation experiment was collected from a natural mountain meadow soil (0–15 cm in depth) in Hongyuan County of China located at the eastern edge of the Qinghai-Tibetan Plateau (33° 05′ N, 102° 35′ E) in May 2014. The average altitude of the sample area is 3462 m a.s.l. The region is characterized by an average annual temperature of 1.4 °C, and the annual rainfall is approximately 752 mm. The dominant plant species in this region are *Clinelymus nutans* and *Roegneria nutans*, accompanied by *Koeleria litwinowii*, *Agrostis schneideri*, *Kobresia setchwanensis*, and *Anemone rivularis*, with an average vegetation coverage of over 90% (Gao et al., 2013). The soil type is Mat crygelic cambisols according to the Chinese soil classification system (Gao et al., 2013). The soil was sieved with 2 mm mesh to remove visible stones and plant residuals. The soil pH is 6.8 (measured using a pH meter with a soil:water ratio of 1:5), conductivity 35 s cm^{-1} (measured simultaneously as the pH with a soil:water ratio of 1:5), and soil organic matter (SOM) 12.2% (measured by dichromate digestion method).

2.2. Incubation experiment setup and measurement of greenhouse gases

Fifty gram of air-dried soil was weighed into each glass bottle (310 ml), and adjusted soil moisture to 5%, 20%, 40%, 60%, and 80% and sealed with a cap for each bottle; whereas 40% moisture represented the control treatment. The soil was pre-incubated for a week to avoid priming effect caused by sample handling and wetting. The moisture was further adjusted each month to achieve an accurate moisture gradient as outlined above. Each moisture treatment was conducted in quadruplicate. The bottles were incubated at 25 °C for three months in a dark room. To allow gas exchange, the bottles were opened and slowly shaken every second day before and after sampling.

Greenhouse gases, methane, carbon dioxide, and nitrous oxide in the bottle were measured on the second day after the start of incubation, and then performed each week throughout the entire incubation period. Samples (1 ml) were taken using glass syringe from headspace of each bottle. Methane, carbon dioxide, nitrous oxide were analyzed using a gas chromatograph (Shimadzu GC 2013, Shimadzu Inc., Japan). Emissions of the greenhouse gases were expressed as nmol g^{-1} dry soil h^{-1}.

2.3. DNA extraction and MiSeq sequencing

Two grams of soil samples from the incubation bottles were obtained on day 2, 30, 60, and 90. The DNA was extracted using a Power Soil extraction kit (MOBIO Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The quality of the extracted DNA was checked using a NanoDrop2000 spectrophotometer (Thermo Scientific Inc., USA). The DNA samples were diluted to 10 ng μl^{-1}, and stored at −20 °C for downstream analysis. The PCR amplification was conducted using primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) with a 12 nt unique barcode at the 5′-end of 515F, to amplify the V4-V5 hypervariable region of the 16S rRNA gene. The PCR mixture (25 μl) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 1.0 μM primers and 0.5 U ExTaq polymerase (TaKaRa, Dalian), as well as 10 ng of soil genomic DNA.

The PCR amplification program included the following steps: initial denaturation at 94 °C for 3 min, followed by 30 cycles at 94 °C for 40 s, 56 °C for 60 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min (Li et al., 2014). Two technical replicates of PCR reactions were conducted for each sample and PCR products were pooled for purification. The DNA bands were separated on 1.5% agarose gel using the gel electrophoresis method. The correct size PCR bands were purified using a Sangon Gel Extraction Kit (Sangon Biotech, Shanghai, China). The purified PCR products from different samples were pooled using equal-molar amounts, and then used for paired-end sequencing (2 × 250 bp) using an Illumina Miseq sequencer (Chengdu Institute of Biology, Chinese Academy of Sciences).

2.4. Sequencing data analysis

The QIIME 1.7.0 was used to analyze the sequencing data (Caporaso et al., 2010). All reads were trimmed and assigned to each sample based