Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effect of dry-rewetting stress on response pattern of soil prokaryotic communities in alpine meadow soil

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ARTICLE INFO

Keywords:
Dry-rewetting
Prokaryotic community
Alpinemeadow soil
Microbial diversity
Greenhouse gas

ABSTRACT

Soil microorganisms are recognized as key players in all biogeochemical cycles. However, little effort has been paid to incorporate them in predictive models for future climate change. Here, we investigated the variation of prokaryotic community composition in alpine meadow soil from the Qinghai-Tibet Plateau under dry-rewetting stress using MiSeq sequencing approach. We incubated soils treated by various frequencies of rewetting and durations of desiccation. Emission rates of methane, carbon dioxide and nitrous oxide were measured every week during five months of incubation, and soil samples were taken each month for community composition analysis. Our results revealed that soil prokaryotic community showed different response patterns to dry-wetting cycles. Diversity indices significantly increased in soils under short-term drought and soils rewetted after long-term drought. Higher niche partitioning was promoted by higher frequencies of disturbance and rapid physiological activation of inactive microbial communities during desiccation, allowing colonization by a diverse array of organisms. Null model percentage of NTI revealed a strong phylogenetic relatedness of soil prokaryotic communities across all treatments and incubation times, suggesting that desiccation and rewetting events were strong biological filters shaping community assemblies. Our results also indicated different responses of various genera belonging to same phylum. These results suggest that prokaryotes that are well adapted to extremely stressful conditions such as long-term desiccation may release more greenhouse gasses in a positive feedback loop and that this prospect should be considered when modeling climate change.

1. Introduction

Soil microorganisms are recognized as important players in the emissions of greenhouse gases through their metabolic activities (Trivedi et al., 2013). The direct impact of greenhouse gases released during the decomposition of organic matter on global climate change has been widely studied and discussed (Barnard et al., 2013; Göransson et al., 2013; Ward et al., 2013). For example, a significant increase in basal soil respiration via augmented microbial activity after rewetting of dried soil has been observed repeatedly (Barnard et al., 2013; Evans and Wallenstein, 2011). Atmospheric emissions of greenhouse gases, such as methane and carbon dioxide, are responsible for approximately 20% of Earth's warming since pre-industrial times (Ward et al., 2013).

Recent predictions warn about an increased frequency of extreme drought events followed by heavy rainfall (Barnard et al., 2013; Evans et al., 2014; Evans and Wallenstein, 2014; Jensen et al., 2003; Sorte et al., 2013). However, emission rates of greenhouse gases under various frequencies of dry-rewetting stress stay to be determined (Yu et al., 2014). Mountain meadows are of great interest as they are vulnerable to the variation of the drought events (Gao et al., 2013). Moreover, even though these particular habitats cover only 5–8% of the Earth's land surface, they store 20–30% or more of the world's terrestrial soil organic carbon pool (Gao et al., 2013; Kou et al., 2017).

Soil prokaryotes are major drivers of key ecosystem processes such

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https://doi.org/10.1016/j.apsoil.2018.02.015
Received 30 October 2017; Received in revised form 24 January 2018; Accepted 13 February 2018

as carbon sequestration or transformation of organic matter (Bardgett and van der Putten, 2014; Fierer, 2017; Trivedi et al., 2013). The investigation of factors shaping microbial community assemblies has become the search for the Holy Grail in microbial ecology (Barberán et al., 2014; Dini-Andreote et al., 2015; Chase, 2007; Lee et al., 2013). Microbial community composition and structure appear to be sensitive to experimental alterations of precipitation regimes (Evans and Burke, 2012). However, until the last decade, methodological hurdles do not allow an accurate insight into the functioning of soil microbial ecosystems. Next-generation sequencing methods provide a complete insight into the hidden diversity, structure and functioning of soil microbial communities even at the level of functional genes (Caporaso et al., 2011; Fierer et al., 2013, 2012; Li et al., 2014; Seppey et al., 2017; Zhou et al., 2016).

The next step is to determine the key ecological factors that significantly influence microbial communities. These factors are either deterministic (e.g. moisture, pH and nutrient flow) or stochastic (speciation, extinction and ecological drift) (Nemergut et al., 2013; Stegen et al., 2012; Tilman, 2004; Vellend, 2010). On the one hand, the theoretical framework for studying species/community dynamics, traditional niche-based theory, assumes that species abundance and distribution are mainly driven by a set of niche conditions that species tolerate and resources that species utilize (Tilman, 2004). On the other hand, the stochastic model, originally based on Hubbell's neutral theory (Hubbell et al., 2001), assumes that community dynamics are the sum of individual stochastic events such as natality, mortality and migration of individuals over time (Nemergut et al., 2013; Stegen et al., 2012; Vellend, 2010).

Depending on the phylogenetic structure, organisms affected by a sets of niche conditions exhibit strong phylogenetic relatedness, whereas organisms shaped by stochastic factors exhibit lower phylogenetic relatedness (Kembel et al., 2011). Despite all that have been done so far, there is still no general framework that would enable us to integrate community assembly rules and changing environmental factors. Another fundamental question in microbial ecology is to understand how microorganisms respond to global climate change at various taxonomic levels (Fierer, 2017). For example, the increase of *Actinobacteria* and the decrease of *Acidobacteria* with dry-down reflect a differential response conserved at the phylum level (Barnard et al., 2013). However, dynamics of response pattern of soil prokaryotes at larger temporal scale remains unclear.

To assess the effects of changing environmental factors on the response patterns of soil prokaryotes at different taxonomic level, we conducted a microcosm experiment using natural soil from an alpine meadow in Qinghai-Tibet plateau. Our experimental design consisted of five months of long incubation experiments under various duration of drought followed by rewetting events. We measured greenhouse gases (CH_4, CO_2 and N_2O) every week and sampled DNA every month. We analyzed the prokaryote community composition using Miseq sequencing of 16S rRNA gene (Yao et al., 2014). We aimed to answer the following questions: (i) How do different frequencies of dry-rewetting stress affect the diversity and structure of the soil prokaryotic community? (ii) How do different frequencies of dry-rewetting stress shift the phylogenetic relatedness of the soil microbial community? (iii) how do various frequencies of dry-rewetting stress affect the emission rates of greenhouse gases?

2. Material and methods

2.1. Site descriptions and sampling

The sampling site is located in a natural alpine meadow in Hongyuan County, Sichuan Province, China, which is at the eastern edge of the Qinghai-Tibetan Plateau (33° 05′ N, 102° 35′ E). The average elevation of the study area is 3462 m above sea level. The region is characterized by the average annual temperature of 1.4 °C and annual rainfall of 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 over 90% (Liu et al., 2013). The soil type is Mat-cry-gelic-cambisols according to the Chinese soil classification system (Liu et al., 2013). Soil samples were collected from the depths of 0–15 cm and then stored at 4 °C before experiment set-up. The soil was sieved through a 2 mm mesh to separate visible stones and plant residuals. Original soil moisture (measured gravimetrically) was 35%, pH 6.8, conductivity 35 $cm \cdot s^{-1}$ and soil organic matter (SOM) 14.2% (measured by the titration method according to Jenkinson and Powlson (1976)).

2.2. Dry-rewetting experiment setup

For the incubation experiments, fresh sieved soil (50 g) was added to each of 18 glass bottles (310 ml) with sealing caps, pre-incubated for one week in a dark room at 25 °C to avoid priming effect. Six bottles were incubated under the original moisture level (35%, by adding of equivalent amount of distilled water) for five months, serving as controls. Six bottles were incubated for one month under extreme drought (5% moisture). These bottles were rewetted to reach normal moisture (by adding equivalent amount of distilled water) and incubated for 2 weeks. In total, these bottles under short-term drought were subjected to three rewetting cycles during five-month incubation. The last six bottles were incubated under extreme drought for 2 months, then they were rewetted to reach normal moisture, and incubated for 2 weeks. In total, these bottles under long-term drought were subjected to two rewetting cycles during five-month incubation. Soil moisture was reduced using nylon bags (2 mm mesh size) with silica gel placed on the bottom of the sealing caps for two days and thereafter dried by air for twelve days. Sampling for DNA (1 g of soil) analysis was performed 2 days after the start of the incubation and then every month over the five months of incubation. The bottles were incubated at 25 °C for five months in a dark room under stable conditions. To allow gas exchange, the bottles were opened and slowly shaken every second day of incubation. Soil properties measured after five months of incubation did not differ among the treatments (Tukey test, $F = 0.014$; $p = 0.987$).

2.3. Measurement of greenhouse gases

Methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions from the incubated soil were measured at the second day after the start of incubation, and then every week over the course of the incubation period. One milliliter of gas sample was taken using a glass syringe from the headspace of each bottle and used for measuring CH_4, CO_2, N_2O by a gas chromatography (Shimadzu GC 2013, Shimadzu Inc., Japan). Emission rates of greenhouse gases were expressed as $nmol \cdot g^{-1} \cdot h^{-1}$.

2.4. DNA extraction and Miseq sequencing

The soil genomic DNA from each of the six bottles per treatment was extracted once per month using 0.5 g of fresh soil with the Power Soil extraction kit (MOBIO Inc., Carlsbad, USA) according to the manufacturer's instructions. The PCR amplification was conducted using primers 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 (Yao et al., 2014). 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, 0.5 U of ExTaq polymerase (TaKaRa, Dalian) and 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 of 94 °C for 40 s, 56 °C for 60 s and 72 °C for 60 s, and a final extension at 72 °C for 10 min (Li et al., 2014). To minimize PCR bias, two technical