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Temporal response of soil prokaryotic communities to acidification and alkalization under laboratory conditions

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ABSTRACT

Soil pH plays an important role in shaping the structure and diversity of prokaryotic community. Altered pH regimes may change prokaryotic community composition by selecting species or groups with different ecological strategies to optimize their fitness. However, it remains unresolved whether prokaryotic communities exhibit deterministic (phylogenetically conserved) or stochastic (phylogenetically overdispersed) responses to pH. In this study we investigated the responses of greenhouse gas emissions and prokaryotic community structure to pH using three-month incubation experiments by adjusting an artificial pH gradient from 4.5 to 8.5. We found decreasing OTUs richness after three months of incubation. Phylogenetic clustering of the prokaryotic community was observed at earlier incubation times whereas greater phylogenetic distance of the prokaryotic community was found at later incubation time. Our results evidenced differential responses of various soil bacterial taxa to the changes in pH. Relative abundances of bacterial phyla and classes of main ecological groups of soil prokaryotes, oligotrophs and copiotrophs, changed significantly along an artificial pH gradient at various incubation times. Relative abundance of *Acidobacteria* significantly increased with pH at the start of experiment, while opposite trend was observed after 90 days of incubation. In contrast, the relative abundance of *Bacteroidetes* showed opposite response as *Acidobacteria* to elevated pH gradient during various incubation time. Methane emissions increased with pH as well as with incubation time, but carbon dioxide and nitrous oxide only increased with incubation time.

1. Introduction

Soil microbes are important contributors to greenhouse gases [1,2]. The direct impact of soil microbes on the emission rates of greenhouse gases released during the decomposition of soil organic matter has been widely studied and discussed [3–5]. Soil microbial communities and their associated functions, e.g. methane oxidation or increased emission of carbon dioxide, are sensitive to various aspects of environmental changes such as the changes of moisture regime [6], temperature [7] and soil acidification or alkalization [8–10]. However, little effort has been paid to incorporate soil microorganisms into the predictive models of future climatic changes and their consequences on ecosystem structure and function.

Many environmental factors are identified to shape the structure and composition of soil microbiota [2]. Climate factors, e.g. temperature and water, are considered the primary drivers influencing soil microbial communities because they directly affect physiological functions of soil microbes [6]. On other hand, soil properties, in particular pH, also play an important role in shaping soil microbial community [10,11]. Soil pH is often recognized as a key factor shaping the community structure and composition of soil prokaryotes [9,12–14]. Soil microbes may be sensitive or tolerant to pH changes [15]. Altered pH regimes may change microbial community composition by exerting selection pressure on those species or functional groups with different

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ecological strategies and thus optimizing their fitness. The effect of pH on the soil microbiota is widely studied and discussed at the continental [9], regional [13] or local scale [8]. However, the spatio-temporal dynamics and the response patterns of soil microbiota to altered pH remains unexplored [9,12–14]. Therefore, it is important to identify which microbes or functional groups are sensitive to pH changes, to describe their dynamics and to unravel their relationships to greenhouse gas emissions under pH change condition.

Based on the metabolic potential and growth rates, soil prokaryotes are divided into two ecological categories. Phyla *Actinobacteria, Acidobacteria* and class *Deltaproteobacteria* are considered as oligotrophs (K-stategists), whereas phylum *Bacteroidetes* and classes *Alphaproteobacteria* and *Gammaroteobacteria* are copiotrophs (r-strategists) [1,16]. The responses of different ecological groups may vary under increased environmental stress such as soil acidification or alkalization [10,17]. Oligotrophs are recognized as slow growing microorganisms that are adapted to poor nutrient substrates, while copiotrophs are recognized as fast growing microorganisms that prefer rich nutrient substrates [1,16].

Microbial communities are simultaneously shaped by deterministic (moisture, pH and nutrient flow) and stochastic factors (speciation, extinction and ecological drift) [18–25]. Traditional deterministic theory assumes that species abundance and distribution are mainly driven by a set of conditions that species tolerate and resources that species utilize [19,26]. The stochastic model, originally based on Hubbell's neutral theory [27], assumes that community dynamics are the sum of individual stochastic events such as natality, mortality and migration of individuals at spatio-temporal scale [21–23]. Depending on the phylogenetic structure, organisms shaped by deterministic factors are assumed to be strongly phylogenetically related and affected by environmental filtering (moisture, pH) whereas organisms shaped by stochastic factors are assumed to exhibit lower phylogenetic relatedness and affected by stochastic factors such as ecological drift [23,28].

In this work, we studied the responses of soil prokaryotic communities along an artificial pH gradient (4.5–8.5) during three-months of incubation. We focused on the following questions: (i) Do acidification and alkalization alter the diversity and phylogenetic structure of soil prokaryotic communities during three-months of incubation? (ii) What is the response pattern of the soil prokaryotic community to acidification and alkalization during three-months of incubation? (iii) How do acidification and alkalization affect the emission rates of methane, carbon dioxide and nitrous oxide?

2. Material and methods

2.1. Soil sample

Soil was collected from the depth of 0–15 cm in a natural mountain meadow in Hongyuan County, Sichuan Province, P. R. China, located at the eastern edge of the Qinghai-Tibetan Plateau (33° 05′ N, 102° 35′ E). The average altitude of the study area is 3462 m a.s.l. The region is characterized by an average annual temperature of 1.4 °C and annual rainfall of 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 cover of over 90% [29]. The soil type is Mat-cry-gelic-cambisols, according to the Chinese soil classification system [29]. The soil was sieved through a 2-mm mesh to separate visible stones and plant residues. The water content (measured gravimetrically) was 40%, pH 6.8 (measured using a pH meter at the soil:water ratio of 1:5), conductivity 35 cm s^{-1} (measured simultaneously as pH at the soil:water ratio of 1:5) and soil organic matter (SOM) 12.2% (measured by the titration method according to Jenkinson et Powlson [30]). Soil properties were measured once again by the end of experiment. We didn't find any significant differences in SOM and conductivity among treatments at the start and the end of the experiment.

2.2. Incubation experiment setup and measurements of greenhouse gasses

Fifty grams of soil were weighed into a glass bottle (310 ml) and sealed with a cap. The bottle with the soil was pre-incubated for a week to avoid a priming effect caused by sample handling. To adjust the pH of the soil, we first tested how much HCl (0.1 M) or NaOH (0.5 M) solution was needed [31], then we diluted the solution to the final volume of 15 ml with H_2O and added it to the soil to achieve a final 40% of water holding capacity. Soil pHs were adjusted to 4.5, 5.5, 6.8, 7.5 and 8.5 whereas untreated soil with a pH 6.8 represented the control treatment. The pH was further adjusted each month to achieve an accurate pH gradient. Each pH treatment was conducted in quadruplicates. 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. The amounts of methane, carbon dioxide and nitrous oxide emitted from the incubated soil were measured on the second day of the incubation and then every week throughout the course of the incubation period. Gas sample (1 ml) was taken using a glass syringe from the headspace of each bottle. The emissions of greenhouse gases (methane, carbon dioxide and nitrous oxide) were analysed by a gas chromatography (Shimadzu GC 2013, Shimadzu Inc., Japan), and were expressed as nmol g^{-1} 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. DNA was extracted using the Power Soil extraction kit (MOBIO Inc., Carlsbad, USA) according to the manufacturer's instructions. The extracted DNA was checked using a NanoDrop2000 spectrophotometer (Thermo Scientific Inc., USA), diluted to 10 ng/μl and stored at −20 °C for downstream analysis. The PCR amplification was conducted using the universal primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 909R (5′- CCCCGYCAATTCMTTTRAGT -3′) with a 12 nt unique barcode at the 5′-end of the 515F primer to amplify the V4-V5 hypervariable region of the 16S rRNA gene [17]. The PCR mixture (25 μl) contained 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM deoxynucleoside triphosphate, 1.0 μM primers and 0.5 U ExTaq polymerase (TaKaRa, Dalian) and 10 ng of soil genomic DNA. The PCR amplification programme 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 [32]. Two technical replicates of PCR reactions for each sample were combined together. The DNA bands were separated on 1.5% agarose gels using the gel electrophoresis method. Correct size PCR bands were purified using the Sangon Gel Extraction Kit (Sangon Biotech, Shanghai, China). The PCR amplicons of the 16S rRNA gene from different samples were pooled using equimolar amounts and used for paired-end sequencing (2 × 250 bp) using an Illumina MiSeq sequencer at the Chengdu Institute of Biology, Chinese Academy of Sciences.

2.4. Sequence data analysis

QIIME Pipeline–Version 1.7.0 was used to analyse sequencing data [33]. All reads were trimmed and assigned to each sample based on unique barcode sequences. High-quality sequences (length > 300 bp, without ambiguous base 'N', average base quality score > 30) were used for downstream analysis. The presence of chimeras was checked using the Uchime algorithm [34]. All the samples were randomly resampled to 7600 reads. The sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence identity. OTUs were assigned using the Ribosomal Database Project classifier [35]. Maximum likelihood approximation phylogenetic trees were constructed using the generalized time-reversible model in FastTree 2.1.1 [36]. Rare sequences that occurred in less than three reads per sample were