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

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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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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

replicates of PCR reactions for each sample were combined. The PCR amplicons from the different samples were pooled at equal molar amounts and used for paired-end sequencing (2 × 250 bp) using an Illumina MiSeq sequencer at the Chengdu Institute of Biology of the Chinese Academy of Sciences.

2.5. Sequence data analysis

The QIIME Pipeline Version 1.7.0 was used for the analysis of sequencing data (Caporaso et al., 2010). All reads were trimmed and assigned to each sample based on unique barcode sequences. Sequences of high quality (length > 300 bp, without the ambiguous base 'N' and average base quality score > 30) were used for downstream analysis. Chimera detection was conducted using the Uchime algorithm Version 8.1.1861 (Edgar et al., 2011). All the samples were randomly resampled to 6800 reads for further analysis. Sequences were clustered into operational taxonomic units (OTUs) at 97% identity. OTUs were taxonomically affiliated using the Ribosomal Database Project classifier (Wang et al., 2007). The original sequence data were stored in the European Nucleotide Archive (www.ebi.ac.uk/ena/PRJEB21670).

2.6. Phylogenetic relatedness

Phylogenetic maximum likelihood–approximation trees were constructed using the generalized time-reversible model in FastTree 2.1.1 (Price et al., 2010). The mean nearest taxon index (mean NTI) was calculated across all samples per treatment and average incubation time using the 'mntd' and 'ses.mntd' functions in the 'picante' R package version 1.1 (Kembel et al., 2011). Briefly, NTI finds the phylogenetic distance between each OTU within a sample and its closest relatives found in the sample (Kembel et al., 2011; Stegen et al., 2012). The abundance-weighted mean was taken across these phylogenetic distances. To evaluate the degree of non-random phylogenetic community structuring, OTUs and their relative abundances were randomized across the tips of the phylogeny (null.model = 'taxa.labels' in the 'ses.mntd' function) (Stegen et al., 2012). A mean NTI taken across all treatments that is significantly different from zero indicates clustering (mean NTI > 2; $p_{\text{null model percentage}} < 0.05$) or overdispersion (mean NTI < −2; $p_{\text{null model percentage}} > 0.05$) (Kembel et al., 2011; Stegen et al., 2013, 2012). Closely related organisms are expected to be phylogenetically structured in same or similar set of niche conditions shaped by deterministic factors (Stegen et al., 2012; Tilman, 2004). On the other hand, less related organisms are considered phylogenetically over-dispersed and controlled by more stochastic factors (Kembel et al., 2011; Stegen et al., 2012).

2.7. Statistical analysis

All data were tested by the Shapiro-Wilk test to check data normality. Filtering of the raw table of OTUs at 0.01% threshold, calculation of alpha-diversity indices (species richness, Simpson index, Shannon index and Chao1 index), principal coordinate analysis, Spearman's correlation coefficient and Per-MANOVA analysis were performed using the 'RAM' and 'vegan' R packages version 1.1.2 (Oksanen, 2015). The effects of dry-rewetting stress on the variability and relative abundances of prokaryotic taxa as well as on greenhouse gas emissions were analyzed by hierarchical ANOVA and linear regression model using basic statistics in R 3.1.2 (www.r-project.org). Weighted UniFrac distances were calculated using the 'phyloseq' package (Lozupone and Knight, 2005; McMurdie and Holmes, 2013). Weighted UniFrac distances to indicate beta-diversity were compared by non-parametric permutational multivariate analysis of variance ('adonis' function of the 'vegan' package), nested by treatment and incubation time (Oksanen, 2015). All statistics and graphics were produced in R 3.1.2 (www.r-project.org).

Table 1
Mean emission rates of greenhouse gases ($nmol \cdot g^{-1} \cdot h^{-1}$) at different treatments in the five months of incubation. SD: short-term drought, LD: long-term drought. Different letters in the same column mean significant difference at $p < 0.05$ based on Kruskal-Wallis test.

Stress type	CH_4 ± SD	CO_2 ± SD	N_2O ± SD
Control	2.148 ± 0.3a	5601 ± 2328a	12.3 ± 13.2a
Short term drought	1.899 ± 1.1b	1119 ± 883b	4.9 ± 5.2a
Rewetting after SD	3.354 ± 2.4a	6228 ± 1967a	88.9 ± 74.8b
Long term drought	1.745 ± 1.6b	2836 ± 2929b	9.8 ± 9.9a
Rewetting after LD	2.716 ± 0.4a	12812 ± 7326a	274.9 ± 257b
Kruskal-Wallis	$\chi^2 = 21.98$; p-value = 0.002	$\chi^2 = 47.267$; p-value < 0.001	$\chi^2 = 54.474$; p-value < 0.001
Incubation time			
2 days	0.999 ± 1.5a	1823 ± 2441a	0.335 ± 0.5a
1 month	2.154 ± 0.3b	4995 ± 1684b	16.4 ± 13b
2 months	2.041 ± 0.6b	3877 ± 2092ab	23.5 ± 29b
3 months	3.771 ± 2.6bc	7727 ± 2375c	67.6 ± 59ab
4 months	2.257 ± 0.2b	4240 ± 2936b	46.9 ± 75.5ab
5 months	2.717 ± 0.3c	7347 ± 7350bc	125.4 ± 222ab
Kruskal-Wallis	$\chi^2 = 48.9$; p-value < 0.001	$\chi^2 = 31.5$; p-value < 0.001	$\chi^2 = 44.23$; p-value < 0.001

3. Results

3.1. Emission rates of greenhouse gases

Rewetting of soil after short-term/long-term drought led to significantly higher emission rates of CH_4, CO_2 and N_2O compared to control soil, soil under long-term drought or soil under short-term drought (Table 1). Furthermore, our results documented significant temporal changes in greenhouse emissions during the five months of incubation (Fig. 1).

3.2. Diversity and phylogenetic structure of soil prokaryotic community

A total of 2992 OTUs with relative abundances of more than 0.01% were obtained from the 108 soil samples (Table S1). From this dataset, we calculated the following alpha-diversity indices: species richness, Simpson index, Shannon index and Chao1 index (Table 2). Finally, our results revealed also significant temporal changes of alpha-diversity indices (Table 2). All the indices showed higher diversity within the first two months which dropped sharply in the third and fourth months of incubation. Conversely, in the fifth month of incubation, alpha diversity indices increased significantly back to the same level as the start of the experiment.

We observed strong phylogenetic relatedness of soil prokaryotic communities across all treatments and incubation time (mean NTI > 2; $p_{\text{null model percentage}} < 0.05$; Table 3). We found a significant effect of dry-rewetting stress on beta-diversity of soil prokaryotic communities. The pairwise permutational tests of weighted UniFrac distances indicated significant differences between the treatments (Fig. 2). Rewetting after long-term drought significantly differed from the other treatments, but the other treatments did not show any significant differences in beta-diversity. We also found significant changes in the beta-diversity of soil prokaryotic communities with incubation time. Species turnover (beta-diversity) of soil prokaryotes in earlier phases of the incubation differed significantly from that occurring later (Fig. 2).

3.3. Response pattern of soil prokaryotes under dry-rewetting stress

At phylum level, the most abundant phyla were *Acidobacteria*, followed by *Proteobacteria* and *Bacteroidetes* (Fig. S1). Dry-rewetting stress

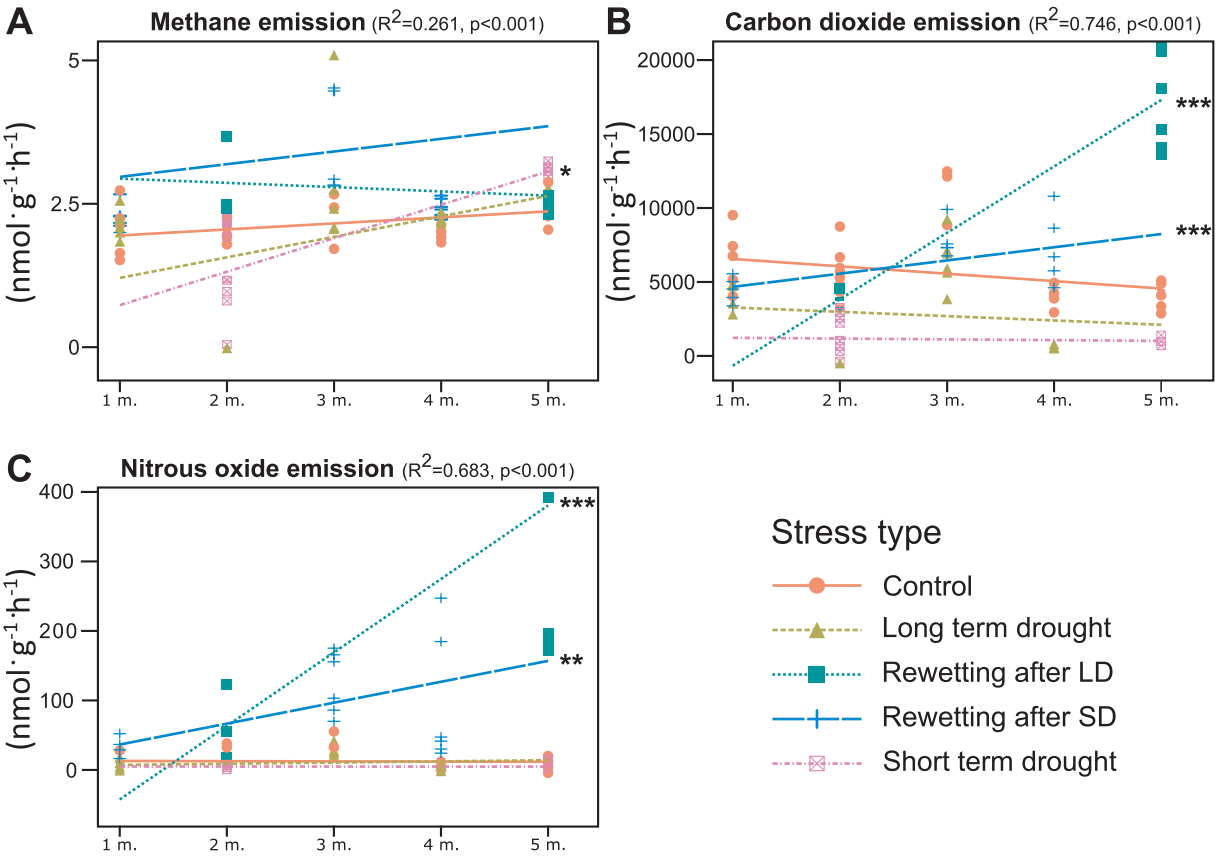

Fig. 1. Temporal dynamics emission rates of methane (A), carbon dioxide (B) and nitrous oxide (C). Asterisks indicate statistically significant differences among various stress types: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

significantly affected the overall relative abundances of *Acidobacteria, Actinobacteria, Firmicutes, Verrucomicrobia, Crenarchaeota*, *Chloroflexi* and *Planctomycetes*. Linear regression model revealed significant ($p < 0.5$) temporal variations in the relative abundances of prokaryotic phyla from soil under various drought and rewetting regimes during the five months of incubation (Fig. S2). The relative abundances of *Acidobacteria*, *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Firmicutes*, *Verrucomicrobia* and *Planctomycetes* differed significantly between the dry treatments as well as between the rewetting treatments over the course of the incubation. (Fig. S2). For example, most abundant phylum *Acidobacteria* (Fig. S2) showed increase in relative abundance in control soils while soil under dry-rewetting treatments showed significant decrease during incubation time.

At genus level, the most abundant genera were DA101, followed by *Candidatus nitrosphaera* and *Rhodoplanes*. Dry-rewetting stress significantly affected the overall relative abundances of DA101, *Candidatus nitrosphaera*, *Candidatus koribacter*, *Alicyclobacillus*, *Candidatus solibacter* and *Flavisolibacter* (Fig. 3). For example, relative abundances of genera DA101, *Candidatus nitrosphaera* and *Alicyclobacillus* increased significantly after both rewetting treatments (rewetting after short term drought as well rewetting after long term drought). In contrast, relative abundance of genus *Candidatus solibacter* decreased significantly after rewetting treatments.

Linear regression model also revealed significant ($p < 0.05$) variations in the relative abundances of prokaryotic genera from soil under various drought and rewetting regimes during the five months of

Table 2
Mean alpha diversity indices at different treatments in the five months of incubation. Different letters in the same column mean significant difference at $p < 0.05$ based on Kruskal-Wallis test.

Stress type	Species richness ± SD	Simpson index ± SD	Shannon index ± SD	Chao index ± SD
Control	552 ± 93 ns	0.986 ± 0.032a	5.736 ± 0.716 ns	1050 ± 164 ns
Short term drought	581 ± 68 ns	0.996 ± 0.001b	6.008 ± 0.223 ns	1087 ± 152 ns
Rewetting after SD	517 ± 116 ns	0.973 ± 0.047a	5.418 ± 1.034 ns	999 ± 192 ns
Long term drought	537 ± 112 ns	0.983 ± 0.043a	5.661 ± 0.929 ns	1078 ± 193 ns
Rewetting after LD	576 ± 32 ns	0.996 ± 0.001b	5.948 ± 0.093 ns	1081 ± 89 ns
Kruskal-Wallis test	χ^2 = 5.465; p-value = 0.242	χ^2 = 9.918; p-value = 0.041	χ^2 = 7.951; p-value = 0.093	χ^2 = 4.49; p-value = 0.343
Incubation time				
2 days	586 ± 75a	0.996 ± 0.001a	6.039 ± 0.244a	1142 ± 148a
1 month	588 ± 64a	0.99 ± 0.021a	5.935 ± 0.504ab	1131 ± 134a
2 months	582 ± 52a	0.995 ± 0.0016ab	5.943 ± 0.196ab	1115 ± 99a
3 months	517 ± 129b	0.976 ± 0.051b	5.491 ± 1.09b	993 ± 221b
4 months	470 ± 124b	0.959 ± 0.058b	5.047 ± 1.17b	961 ± 199b
5 months	555 ± 45b	0.995 ± 0.001a	5.905 ± 0.17ab	1001 ± 96ab
Kruskal-Wallis test	χ^2 = 29.37; p-value < 0.00001	χ^2 = 33.39; p-value < 0.00001	χ^2 = 34.94; p-value < 0.00001	χ^2 = 25.16; p-value < 0.00001

Table 3
Mean nearest taxon index (NTI) and null model at different treatments in the five months of incubation.

Stress type	NTI ± SD	P value of null model percentage
Control	8.47 ± 1.2 ns	0.001
Long term drought	7.67 ± 1.4 ns	0.001
Rewetting after LD	8.14 ± 1.4 ns	0.001
Short term drought	7.93 ± 1.09 ns	0.001
Rewetting after SD	8.19 ± 1.36 ns	0.001
Kruskal Wallis test	χ^2 = 4.010; p-value = 0.404	
Incubation time		
2 days	8.7 ± 1.07 ns	0.001
1 month	7.34 ± 1.27 ns	0.001
2 months	7.99 ± 1.32 ns	0.001
3 months	7.44 ± 1.37 ns	0.001
4 months	7.82 ± 1.18 ns	0.001
5 months	7.97 ± 1.42 ns	0.001
Kruskal Wallis test	χ^2 = 4.078; p-value = 0.538	

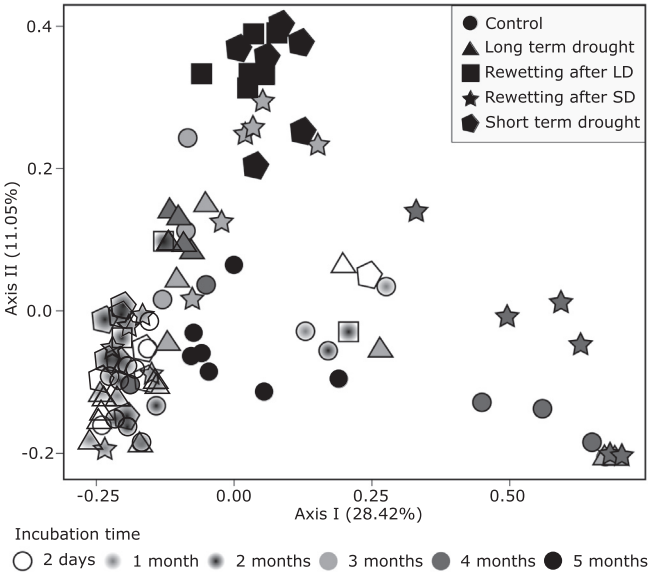

Fig. 2. Principal coordinates analysis (PCoA) of the weighted UniFrac dissimilarities of the soil prokaryotic communities. PerMANOVA of UniFrac distance matrix dissimilarity: stress type: R^2 = 0.089; F = 2.195; P = 0.001. incubation time: R^2 = 0.138; F = 3.534; P = 0.001. Stress Type * incubation time: R^2 = 0.122; F = 1.962; P = 0.001.

incubation (Fig. 4). The relative abundances of DA101, *Candidatus nitrosphaera*, *Candidatus koribacter*, *Alicyclobacillus*, *Candidatus solibacter*, *Rhodoplanes*, *Bradyrhizobium* and *Flavisolibacter* differed significantly between the dry treatments as well as between the rewetting treatments over the incubation time (Fig. 4). For instance, relative abundances of genera *Rhodoplanes* and DA101 increased significantly in soils under long term drought during incubation time. Conversely, *Candidatus solibacter* showed significant decrease across all treatments during the whole incubation course.

Finally, we found different responses of various genera belonging to same phylum. For example, phylum *Acidobacteria* showed increase in relative abundance in control soils while soils under dry-rewetting treatments showed significant decrease during incubation time. Genera *Candidatus solibacter* and *Candidatus koribacter* belonging to *Acidobacteria* showed different responses to dry-rewetting treatments. *Candidatus soliacter* showed decrease in relative abundance in control soil as well as in soil under dry-rewetting treatments while the relative

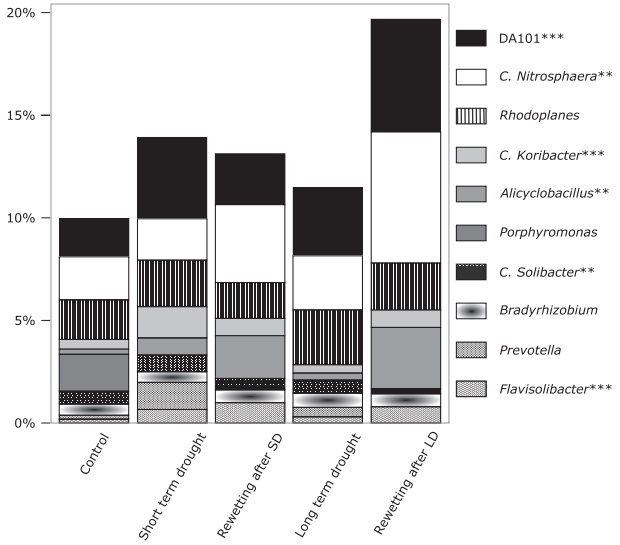

Fig. 3. The relative abundances of prokaryotic genera under different dry-rewetting stress. Asterisks indicate statistically significant differences: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

abundance of genus *Candidatus koribacter* increased its relative abundance in soil under short term drought only.

Both positive and negative correlations were observed between the relative abundances of prokaryotic genera and the emission rates of greenhouse gases. *Candidatus Nitrosphaera*, *Candidatus koribacter*, *Alicyclobacillus* and *Flavisolibacter* showed positive correlations with the emission rates of methane (Table 4). *Candidatus nitrosphaera* and *Alicyclobacillus* showed positive correlations with carbon dioxide emission rates while *Candidatus solibacter* showed negative correlation with the emission rates of carbon dioxide (Table 4). *Candidatus nitrosphaera*, DA101 and *Alicyclobacillus* showed positive correlations with nitrous oxide emission rates (Table 4). In contrast, *Candidatus solibacter* indicated negative correlation with emission rates of nitrous oxide.

4. Discussion

4.1. Effect of dry-rewetting stress on prokaryote diversity and processes controlling community assembly

Several studies demonstrate that the frequency of rewetting and the duration of desiccation significantly affect the diversity, relative abundance and the phylogenetic structure of soil microbial communities (Evans et al., 2011; Evans and Wallenstein, 2011). In this study, the diversity indices in the soils under short-term drought and soils rewetted after long-term drought showed higher diversity than control soils, soil rewetted after short-term drought and soil under long-term drought. One explanation could be that greater frequency of disturbances promotes more extensive niche partitioning and thus allows colonization by a more diverse array of organisms (Dini-Andreote et al., 2014). Furthermore, we argue that the increased diversity in soil rewetted upon long-term drought resulted from rapid physiological activation of an inactive microbial community during desiccation (Aanderud et al., 2015). Alpha diversity increased within the first two months and then decreased sharply in the third and fourth months of incubation. This may suggest that dispersal mechanisms and connectivity are important factors in the maintenance of local diversity (Lindstrom and Langenheder, 2012).

Comparison of weighted UniFrac distance matrices is a good approach to investigate beta-diversity (Lozupone and Knight, 2005). Pairwise permutational tests of weighted UniFrac distances indicated significant differences between treatments as well as between incubation times. We showed that dry-down and wetting-up significantly

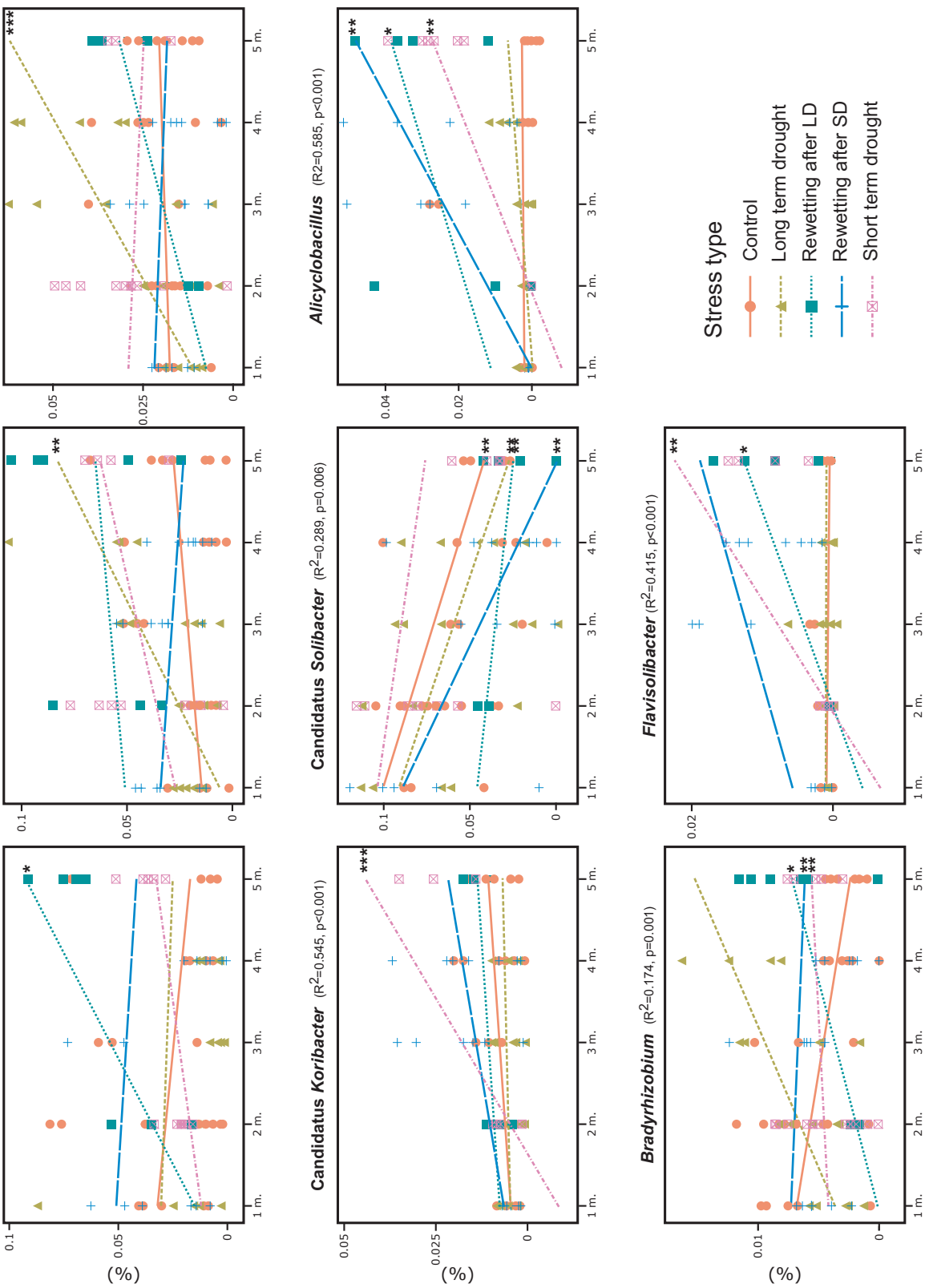

Fig. 4. Temporal effects of dry-rewetting stress on the relative abundances of prokaryotic genera (Linear regression model). Asterisks indicate statistically significant differences among various stress types: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 4
Spearman's correlation coefficients between relative abundances of most abundant prokaryotic genera and the emission rates of greenhouse gases.

Genera	CH_4	CO_2	N_2O
DA101	0.173	0.111	0.288**
Candidatus *Nitrososphaera*	0.354***	0.496***	0.454***
Rhodoplanes	0.204*	0.054	0.054
Candidatus *Koribacter*	0.399***	0.028	0.102
Alicyclobacillus	0.332***	0.447***	0.506***
Porphyromonas	−0.015	−0.023	−0.046
Candidatus *Solibacter*	−0.185	−0.292**	−0.304**
Bradyrhizobium	0.192	0.094	0.028
Prevotella	−0.074	−0.137	−0.059
Flavisolibacter	0.404***	0.170	0.208*

Asterisks indicate significantly differences after Bonferonni's correction (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

impact soil prokaryotic communities. Indeed, different moisture content significantly affects the composition of soil prokaryotic communities (Yao et al., 2017). We suggest that dry-rewetting events can substantially affect microbial communities by environmental filtering of various taxa over time. Certain prokaryotic phyla (e.g. *Actinobacteria* and *Verrucomicrobia*) are resistant to water stress, possibly due to their unique physiological traits that allow them to survive in the habitat of desiccation and highly moisture fluctuations (Barnard et al., 2013). Furthermore, in agreement with Dini-Andreote et al., (2014), we propose that increased frequency of disturbances promotes extensive niche partitioning and thus allow higher species turnover (beta-diversity).

Despite recent findings showing simultaneous effects of deterministic and stochastic factors controlling community assembly (Chase, 2007; Lee et al., 2013; Stegen et al., 2012), our results did not indicate any significant effects of dry-rewetting stress or incubation time on the magnitude of mean NTI. However, the null model expectation of the mean NTI revealed a strong phylogenetic relatedness of soil prokaryotic communities across all treatments and incubation times. Therefore, the frequency of desiccation and rewetting events could be strong environmental filters shaping species assemblies and driving the phylogenetic turnover (beta-diversity). Phylogenetic relatedness of prokaryotic communities in all the treatments implicated that dry-rewetting stress likely resulted in the dominance of closely related taxa.

4.2. Response patterns of soil prokaryotic community

This study demonstrated that dry-rewetting stress triggered a consistent change of community structure, mainly characterized by large changes in the relative abundances of only a few bacterial groups, e.g. DA101, *Candidatus nitrosphaera*, *Candidatus koribacter*, *Alicyclobacillus*, *Candidatus solibacter* and *Flavisolibacter*. For example, rewetting stimulated the growth of rare bacteria (e.g. *Flavisobacter* or *Verrucomicrobia*). Rewetting causes rapid changes in soil conditions that drive the dynamic shifts in relative abundances of specific taxa, and maintain high levels of biodiversity in soil (Aanderud et al., 2015).

Barnard et al. (2013) showed that the increase of *Actinobacteria* and the decrease of *Acidobacteria* with dry-down reflect a differential response conserved at the phylum level. We observed same pattern of soil prokaryotes at general level. Our results indicated the increase of relative abundances of genera DA101, *Candidatus nitrosphaera* and *Alicyclobacillus* at rewetting after both short term drought and long term drought. In contrast, relative abundance of genus *Candidatus solibacter* decreased significantly after rewetting treatments.

Our results also indicated different responses of various genera belonging to same phylum. For example, phylum *Acidobacteria* showed the increase in relative abundance in control soils while soils under dry-rewetting treatments showed significant decrease during incubation time. Genera *Candidatus solibacter* and *Candidatus koribacter* belonging to *Acidobacteria* showed different responses to dry-rewetting treatments. This results partly corroborated with the study of Rousk et al., (2010) showing different response patterns of subgroups of phylum *Acidobacteria* to increased pH gradient. This also indicates the roles of various ecological strategies at genera level, which in turn strongly affect community compositions of soil microbiota.

Various ecological strategies (resistant or tolerant to stress) of soil prokaryotic phyla were observed in response to different durations of desiccation and frequencies of rewetting. For instance, the relative abundances of genera *Rhodoplanes* and DA101 increased significantly in soils under long term drought during incubation time. In contrast, *Candidatus Solibacter* showed significant decrease across all treatments during the whole incubation course. The adaptation of soil bacteria to extreme drought and rapid rewetting is often explained through different bacterial life-strategies, conferring a spectacular resilience to potentially active bacterial communities (Barnard et al., 2014, 2013; Evans and Wallenstein, 2014). In addition, we argue that such variations of response strategies, tolerance or resistance, can vary during incubation time.

The mechanisms of these response patterns to dry-wetting stress are multiple. Recent studies show that some bacterial phyla (e.g. *Actinobacteria*) possess a high tolerance to extreme drought (de Vries and Shade, 2013; Evans et al., 2014; Kakumanu et al., 2013). Physiological traits as well as morphological adaptations of soil microbes play significant roles in the response to dry-wetting stress. For example, the filamentous structure of *Actinobacteria* may facilitate the tolerance to desiccation.

Soil properties are another important factors influencing the response of microbial composition to dry-rewetting stress, for example, carbon substrates that are accumulated during desiccation periods may affect the mineralization pulses upon soil rewetting (Barnard et al., 2014; Göransson et al., 2013; Yu et al., 2014). In contrast, community composition may respond differently by dry-down and wet-up, even if the soil properties at various sites do not differ significantly in their dry-down or wet-up. The activity and survival potential of some prokaryotes to extreme stress conditions are likely influenced by the interactions of various mechanisms of adaptation to stressful conditions which can vary during incubation time.

4.3. Dry-wetting effects on greenhouse gas emission

Previous studies show that soil microorganisms are important contributors to the emissions of greenhouse gases (Eisenlord et al., 2013; Trivedi et al., 2013). Soil water content influences microorganisms directly and indirectly through changes in oxygen concentrations and nutrient availability (McHugh and Schwartz, 2016). Rewetting of soil after short-term drought as well as its rewetting after long-term drought induced significantly higher emissions of greenhouse gases. It is possibly a result of increased physiological activity of soil microbial communities (Jensen et al., 2003). On the other hand, the accumulation of labile organic material (e.g. cell debris) during dry period may trigger high metabolic activity of soil microbes when water is available (Barnard et al., 2013).

Moreover, non-biological sources of soil gases upon rewetting are expected to contribute to the amount of carbon dioxide released immediately after the rewetting. We also suggest that in extremely desiccated soil with higher porosity, gases displaced from pores by rewetting could also significantly contribute to greenhouse gas emissions (Barnard et al., 2014). Our analyses indicated that soil under long-term desiccation emitted larger amounts of carbon dioxide and nitrous oxide than soil under short-term desiccation. The activity and survival potential of some prokaryotes to extreme stress conditions seems to result from a broad variety of mechanisms of adaptation to stressful conditions. Soil physico-chemical properties are strong traits that best explain the potential methane oxidation rate, and its influence might be associated mainly with its effects on methanotrophic communities (Kou et al., 2017). Thus, direct effect of dry-wetting stress on microbial

community is possibly the key to control the greenhouse gas emission rates.

5. Conclusions

We found significantly increased alpha diversity in soil under short-term drought and soil rewetted after long-term drought due to rapid reactivation of inactive microbial communities during desiccation. The mean NTI indicated a strong phylogenetic relatedness of soil prokaryotic communities across all treatments and durations of incubation, showing that desiccation and rewetting events constitute strong biological filters shaping species assemblies. We also found different response patterns of various genera belonging to same phylum. Our results suggest that prokaryotes that are well adapted to extreme conditions such as dry-rewetting stress may release more greenhouse gases when water is available.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2018.02.015.

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