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ORIGINAL PAPER

Altered precipitation seasonality impacts the dominant fungal but rare bacterial taxa in subtropical forest soils

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Abstract How soil microbial communities respond to precipitation seasonality change remains poorly understood, particularly for warm-humid forest ecosystems experiencing clear dry-wet cycles. We conducted a field precipitation manipulation experiment in a subtropical forest to explore the impacts of reducing dry-season rainfall but increasing wet-season rainfall on soil microbial community composition and enzyme activities. A 67% reduction of throughfall during the dry season decreased soil water content (SWC) by 17–24% ($P < 0.05$), while the addition of water during the wet season had limited impacts on SWC. The seasonal precipitation redistribution had no significant effect on the microbial biomass and enzyme activities, as well as on the community composition measured with phospholipid fatty acids (PLFAs). However, the amplicon sequencing revealed differentiated impacts on bacterial and fungal communities. The dry-season throughfall reduction increased the relative abundance of rare bacterial phyla (*Gemmatimonadetes*, *Armatimonadetes*, and *Baoacteriodetes*) that together accounted for only 1.5% of the total bacterial abundance by 15.8, 40, and 24% ($P < 0.05$), respectively. This treatment also altered the relative abundance of the two dominant fungal phyla (*Basidiomycota* and *Ascomycota*) that together accounted for 72.4% of the total fungal abundance. It increased the relative abundance of *Basidiomycota* by 27.4% while reduced that of *Ascomycota* by 32.6% ($P < 0.05$). Our results indicate that changes in precipitation seasonality can affect soil microbial community composition at lower taxon levels. The lack of community-level responses may be ascribed to the compositional adjustment among taxonomic groups and the confounding effects of other soil physicochemical variables such as temperature and substrate availability.

Keywords Precipitation change · Soil microorganisms · Enzyme activities · Amplicon sequencing · Phospholipid fatty acids (PLFAs)

Qian Zhao and Shuguang Jian contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00374-016-1171-z) contains supplementary material, which is available to authorized users.

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Introduction

Global precipitation patterns, including the amount, intensity, and timing of rainfall, have been changing in almost all regions of the world over the last decades and are projected to change in the future (IPCC 2013). Changes in precipitation patterns can affect the composition and growth of both plant and soil microbial communities, as well as the biogeochemical processes associated to them, such as litter decomposition and soil C and N cycling (Beier et al. 2012; Carney and Matson 2005). Multiple studies have demonstrated that soil water availability, which is directly influenced by precipitation input, affects soil microbial community composition and

functioning (Atlas and Bartha 1998; Drenovsky et al. 2004; Waldrop and Firestone 2006; Brockett et al. 2012), but the direction and magnitude of soil microbial community responses to precipitation changes remain uncertain (Castro et al. 2010; Landesman and Dighton 2010).

In a microbial community, different functional groups with various physiological and ecological strategies may respond differently to changes in soil water availability. Generally speaking, fungi are more resistant to water limitation than bacteria, because they have the special construction of hypha and more resistant cell walls (Singh et al. 2010). Gram-positive (G^+) bacteria with stronger cell walls and better osmoregulation strategies are also more tolerant to drought than Gram-negative (G^-) bacteria (Landesman and Dighton 2010). As a result, fungi and G^+ bacteria are often more enriched in drier than in wetter soils (Drenovsky et al. 2010; Ma et al. 2015). In drylands, where water is the dominant limiting factor, increased precipitation increased the biomass of both fungi and bacteria (Huang et al. 2015; Maestre et al. 2015), whereas decreased precipitation reduced the abundance and diversity of dominant fungal species (Toberman et al. 2008; Maestre et al. 2015). In a seasonal xeric pineland, neither rainfall addition nor exclusion had significant impacts on the composition of soil microbial community (Landesman and Dighton 2010). In a humid tropical forest, reduced precipitation altered soil microbial community composition by decreasing fungal abundance and the resultant changes in fungal: bacterial ratio (Waring and Hawkes 2015). These examples illustrate how the relationship between soil microbial community and precipitation might vary depending on the ecosystem type and the local climate (Matulich et al. 2015; Waring and Hawkes 2015).

Soil enzymes mainly produced by soil microorganisms are of crucial importance to the release of plant nutrients and the maintenance of soil functions (Burns et al. 2012). Enzyme activities have been extensively studied to evaluate the response of soil functions to climate changes, such as the rise in temperature and atmospheric CO_2 concentration and the shift in precipitation patterns (Nannipieri et al. 2012). However, effects can be contradictory depending on the enzyme of interest, the type of soil and vegetation, and the type of climate (Sinsabaugh 2010; Henry 2012; Nannipieri et al. 2012). For example, rainfall addition was found to suppress the activities of six hydrolases in a grassland ecosystem (Gutknecht et al. 2010) and to stimulate that of chitinase in a heathland ecosystem (Kreyling et al. 2008) but had no impacts on the activities of acid phosphomonoesterase and arylsulfatase in a humid tropical forest (Yavitt et al. 2004). Drought reduced the activities of urease, protease, and β-glucosidase in a Mediterranean forest (Sardans and Peñuelas 2005) and that of polyphenol oxidase in an upland heathland (Toberman et al. 2008). Although many direct and indirect effects make the interpretation of the enzyme activity responses (Nannipieri et al. 2012)

difficult, documenting changes of enzyme activities is suggested to provide mechanistic insights into the ways that climate change impacts the functioning of terrestrial ecosystems (Sinsabaugh et al. 2005, 2008).

Given the diverse responses of microbial community composition and enzyme activity to precipitation changes across the ecosystems, specific manipulative experiments are needed for different ecosystems. Tropical and subtropical forests, which play critical roles in the global C cycle, are considered as a priority region to explore the effects of global climate change (Nepstad et al. 2002; Costa et al. 2010; Bell et al. 2014; Cavaleri et al. 2015). The subtropical evergreen forests in southern China are the largest C sink of the region (Yu et al. 2014), and they are projected to experience a decrease in the dry-season rainfall and an increase in the wet-season rainfall in the coming decades (Zhou et al. 2011). However, very few rainfall manipulation experiments have been conducted in tropical and subtropical forests with rainfall inputs exceeding 1500 mm year^{-1} to address the responses of soil microbial activity and community composition (Beier et al. 2012; Bouskill et al. 2013). Furthermore, most previous precipitation manipulation experiments conducted so far have focused on the quantity, rather than the seasonal pattern of precipitation (Liu et al. 2016a), albeit the dry-wet cycle has been proved important for the composition and functioning of soil microbial communities (Kieft et al. 1987; Shi and Marschner 2015; Fang et al. 2016). Therefore, there is a general lack of knowledge on how soil microbial communities will respond to potential future precipitation changes in warm-humid forests. To contribute filling this gap in our knowledge, we experimentally reduced the dry-season rainfall and increased the wet-season rainfall while keeping the annual rainfall amount unchanged in a subtropical forest of South China and evaluated mainly the responses of soil microbial community composition and enzyme activities. Specifically, we aimed to address the following questions: (i) Do changes in the rainfall seasonality alter soil microbial community composition and enzyme activities in the subtropical forest? We expected that the responses would be significant since soil microbial community composition and functioning have been found sensitive to alterations in soil water availability in various ecosystems. (ii) How do the responses vary among microbial groups and between seasons? We hypothesized that fungi would be more resistant to the dry-season rainfall reduction than bacteria, while both of them would be less sensitive to the wet-season rainfall addition than to the dry-season rainfall reduction. (iii) What are the other soil factors altered by the precipitation seasonality change, and how would they be related to the responses of microbial community composition and enzyme activity? We hypothesized that the precipitation-induced changes in soil C and N availability, pH, and microbial biomass may indirectly shape the composition and enzyme activity responses to the precipitation seasonality changes.

Materials and methods

Site description

This study was conducted at the Heshan National Field Research Station of Forest Ecosystem (112° 50′ E, 22° 34′ N), which is located in Guangdong province, southeastern China. The climate in this region is subtropical monsoon with a distinct wet season from April to September and a dry season from October to March. The mean annual precipitation is 1800 mm, and the mean annual air temperature is 21.7 °C. The soil is classified as Ultisol according to the USDA's soil taxonomy (Soil Survey Staff 2010). The mean slope of the experimental area is 15°. Vegetation is dominated by two evergreen broad-leaved tree species, *Schima superba* and *Michelia macclurei*. The average tree height and diameter at breast height are approximately 14.0 m and 22.0 cm, respectively.

Experimental design and soil sampling

Eight experimental 12×12-m^2 plots were established within the study area. Four plots were assigned for the precipitation change treatment (hereafter PC treatment), and four paired plots were designed as controls. In each PC plot, a set of steel frames was set up on 16 standing steel pillars to support the throughfall reduction shelters and water addition sprinklers. Each clear exclusion shelter was fixed on two 12-m-length stainless steel beams and hanged on the supporting frames 1.5 m above the ground surface (Fig. S1). The exclusion shelters were pitched consistent with the slope of the plots and connected to PVC troughs located at the lower slope so that the excluded throughfall could be drained out of the plots. Depending on the distribution of trees in the plots, the width of each shelter ranged from 0.5 to 1 m. Therefore, 10 to 12 shelters, covering67% of the total plot area, were used in each PC plot to exclude throughfall during the dry season. Due to the lack of quantitative projections on the reduction of future dry-season rainfall in the region, we used a similar throughfall reduction rate (i.e., 67%) as those had been used in throughfall reduction experiments of tropical forests (Brando et al. 2008; Costa et al. 2010) in order to facilitate the comparison between similar ecosystems. The water addition sprinklers were also hanged on the supporting frames. Each PC plot had 25 automated sprinklers with a spraying diameter of 2.5 m and showering 50 L water per hour. The control plots were set up without throughfall reduction and water addition facilities, but we trenched the four sides of each plot to 60–80-cm depth using 1-m height PVC boards to prevent the lateral water flow. Automatic rain gauges (Davis Instrument, MD, USA) were installed under the canopy of plot to record the amount of throughfall.

The PC treatment was applied to four plots from October to March with throughfall reduction and from June to September with water addition. During the dry season, 67% of the total throughfall was excluded by opening the exclusion shelters. During the wet season, the clear sheets were folded and an equivalent amount of water to that excluded during the dry season was added to each PC plot in one large rainfall event (50 mm per day) per month (depending on the amount excluded during the previous dry season). The added water was pumped from a pond about 800 m away from the experimental site. In general, the pond water had lower organic C and N contents than those from throughfall. For example, the total organic C and inorganic N contents were 8.89 and 4.85 mg/L, respectively, in the throughfall, while they were 4.19 and 2.05 mg/L in the pond water. The pH was similar in both throughfall (6.42) and pond (6.19) waters. The experimental treatments lasted for 2 years from October 1, 2012, to September 30, 2014. We divided the 2-year experimental period into two hydrological years: 2013 (October 1, 2012, to September 30, 2013) and 2014 (October 1, 2013, to September 30, 2014) in the reporting of our experimental results.

Soil samples were taken three times in each hydrological year: during the dry season (October–March) when throughfall was excluded, in spring (April–May) when ambient precipitation was permitted, and in summer (June–September) when water was added. During each sampling date, six soil cores (4.5-cm diameter × 10-cm depth) were sampled randomly within each plot. After the removal of litter, stones, and other dead soil animals, soil samples were sieved through a 2-mm mesh immediately in the field and the six soil cores in the same plot were homogenized into a composite sample. Each composite sample was further divided into four subsamples and stored for analyzing soil chemical properties (air-dried and stored at 4 °C), microbial biomass, and enzyme activities (stored at 4 °C), PLFAs (stored at −20 °C after freeze drying at −80 °C), and amplicon sequencing (stored at −80 °C), respectively. It is noted here that all the 2013 and 2014 samples (48 in total = 2 year × 3 sampling seasons × 2 precipitation treatments × 4 replicate plots) were analyzed for soil chemical properties, microbial biomass, and PLFAs, but only the 2014 samples were analyzed for enzyme activities and microbial sequences, since after a preliminary check of the PLFA and microbial respiration data in the first year of the experiment (i.e., 2013), we did not find any significant responses.

Analyses of soil physicochemical properties and microbial biomass

Soil water content (SWC) was measured by oven drying 10 g of fresh soil sample at 105 °C for 24 h. Soil pH was measured in a 1:2.5 air-dried soil/water suspension using a pH meter

(Mettler-Toledo GmbH, Greifensee, Switzerland). Total organic C (TOC) was determined by the $K_2Cr_2O_7$ titration method. Dissolved organic C (DOC) in filtered 0.5-M K_2SO_4 extracts of 10-g fresh soil sample was measured with a TOC auto-analyzer (Shimadzu, Kyoto, Japan). Total N (TN) was measured by the indophenol blue colorimetric method and total P (TP) by the molybdenum antimony blue colorimetric method (Fang et al. 2008). Inorganic N was measured in filtered 2 M KCl-soil extracts by the indophenol blue colorimetric method for exchangeable NH_4^+-N and the cadmium reduction method for NO_3^--N with a modified Berthelot reaction (Fang et al. 2008). Soil microbial biomass was measured using the fumigation extraction method (Vance et al. 1987). A subsample of 10 g was fumigated by chloroform and incubated in the dark for 24 h. Another paired sample of 10 g was prepared as non-fumigated. The K_2SO_4 extracted for both the fumigated and non-fumigated samples was analyzed using a TOC auto-analyzer (Shimadzu, Kyoto, Japan). The conversion factor used to calculate the microbial biomass C (MBC) and N (MBN) was 0.45 and 0.54, respectively (Brookes et al. 1985; Joergensen et al. 2011).

PLFA analysis

The method for analyzing microbial PLFAs was modified from Frostegård and Bååth (1996). Total lipids were extracted overnight from 8-g freeze-dried soil in a mixture of chloroform/methanol/phosphate (1:2:0.8, *v/v/v*) and separated into neutral lipids, glycol-lipids, and polar lipids by chloroform, acetone, and methanol solutions in solid-phase extraction tubes. After separated, the polar lipid fraction was transesterified into fatty acid methyl esters using 1 mL 0.2 M methanolic KOH. Fatty acid methyl esters were analyzed on a gas chromatograph mass spectrometry (Agilent Technologies, CA, USA).

Based on the fatty acid nomenclature described by Frostegård and Bååth (1996), the sum of fatty acids as bacterial PLFAs was presented by 17:0, i14:0, i15:0, i16:0, i17:0, a15:0, a17:0, 16:1ω7, 16:1w7c, cy17:0, and 18:1w7c, while the sum of 18:2w6, 9c, and 18:1w9c was considered as non-mycorrhiza fungi. The sum of saturated unsubstituted fatty acids represented G^+ bacteria, while the sum of monounsaturated fatty acid represented G^- bacteria. The PLFAs 10Me16:0, 10Me17:0, and 10Me18:0 represented actinobacteria. The ratio of fungal to bacterial PLFAs (F/B) and the ratio of G^+ to G^- bacterial PLFAs (G^+/G^-) were also calculated to indicate microbial community composition. All PLFAs were calculated as nanomole per gram dry soil.

Amplicon sequencing

Soil microbial DNA was extracted using Power Soil DNA Kit (MoBio, CA, USA) according to the manufacturer's instructions. The initial concentration of DNA was detected using a Nanodrop spectrophotometer (Thermo Fisher Scientific, CA, USA) to ensure that the concentration and total amount of DNA were suitable for the future analysis. The primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) were used for amplifying the bacterial 16 s V4 region (Caporaso et al. 2012), while the primers ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) were used for amplifying the fungal ITS1 region (White et al. 1990). For both bacteria and fungi, the polymerase chain reaction (PCR) mixture included 15 μL of Phusion® High-Fidelity PCR Master Mix (Biolabs Inc., CA, USA), 0.2 μM of forward and reverse primers, and about 10 ng template DNA. The PCR reactions were run under the following procedure: a predenaturation at 98 °C for 1 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s with a final extension at 72 °C for 5 min. The PCR was run three times to reduce PCR bias for each sample.

Three replicated PCR products for each sample were pooled before running amplicons on 2% agarose gel. Samples with a bright main strip between 300 and 450 bp were purified with the Qiagen Gel Extraction Kit (Qiagen, Dusseldorf, Germany). Sequencing libraries were generated using the Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, CA, USA) following the manufacturer's instructions, and index codes were added. The library quality was assessed on the Qubit ® 2.0 Fluorometer (Thermo Fisher Scientific Inc., CA, USA) and Agilent Bio-analyzer 2100 system (Agilent Technologies, CA, USA). After that, the library was sequenced on an Illumina HiSeq 2500 platform (Illumina, CA, USA).

Sequence analysis

Paired end reads were assigned to each sample according to the unique barcode and were assembled by using FLASH v1.2.7 (Lozupone et al. 2011). The splicing sequences were filtered by QIIME (v1.7.0, http://qiime.org/) to obtain high-quality clean tags then using the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) to detect and remove chimera sequences to obtain the effective tags (Edgar et al. 2011). The effective tags were assigned to the same operational taxonomic units (OTUs) using UPARSE software (Uparse v7.0.1001; http://drive5.com/uparse/) at the 97% similarity level (Edgar 2013). The number of sequences in each sample was rarefied to 58,933 and 27,575 in bacteria and fungi, respectively, to ensure the identical sequencing coverage for all the samples. The taxonomic identity of OTUs was assigned to bacteria using the RDP classifier in Greengenes Database (Castro et al. 2010)

and to fungi using the Blast algorithm in the UNITE database (Kõljalg et al. 2013).

Soil enzyme activities

The potential activities of five hydrolytic enzymes (acid phosphomonoesterase, β-glucosidase, cellubiosidase, xylosidase, and chitinase) were determined by fluorimetric assays in 96-well black microplates (Sinsabaugh et al. 2005; German et al. 2011). The substrates used to determine the activities of the five enzymes are listed in Table S1. Soil suspensions were prepared by adding 1-g soil to 100 mL 50 mM acetate buffer at pH 4 and homogenized with magnetic stirrers for 3 min in a beaker. The pH of the buffer was chosen to match the initial pH of the soil. Fluorescence was measured using a spectrofluorometer (TECAN, Salzburg, Austria) set to 365 nm for excitation and 450 nm for emission. After correcting for quenching and negative control, the enzyme activity ($nmol^{-1}$ g^{-1} h^{-1}) was calculated using the method described in Saiya-Cork et al. (2002).

The potential activities of two oxidase enzymes, polyphenol oxidase and peroxidase, were determined using colorimetric assays in 96-well clear microplates. The functions of these enzymes are also listed in Table S1. The reaction of the oxidase enzymes was assessed by the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA), which caused quantitative color change. Soil (2 g) was suspended in 100 mL 50 mM acetate buffer at a reference pH 5.0 and homogenized by magnetic stirrers for 3 min. Their activities were assayed as in the study by Sinsabaugh and Follstad Shah (2012) and Saiya-Cork et al. (2002). Plates were incubated in the dark for 4 h at 20 °C. At the end of each incubation period, absorbance was detected using a spectrofluorometer (TECAN, Salzburg, Austria) at 450 nm. After correcting for negative and blank controls, the activity was expressed in nanomole per gram per hour. The specific enzyme activity, i.e., the ratio of enzyme activity and microbial biomass carbon, was also calculated to relate the contribution of the extracellular enzyme activity with that of the intracellular enzyme activity (Nannipieri et al. 2012).

Statistical analysis

The effects of the precipitation manipulation on microbial biomass, PLFA structure, enzymatic activities, and soil physicochemical properties were analyzed using ANOVA. The effects of season, year, and their interactions with the precipitation treatment effect were analyzed using the general linear model (GLM) univariate analysis. Data were normalized where necessary using the log or square transformation. Spearman's correlation analysis was used to test the relationship between soil chemical properties and enzyme activities. The ANOVA, GLM univariate analysis, and Spearman's

correlation analyses were performed in the SPSS 16.0 software (SPSS, Chicago, USA). Redundancy analysis (RDA) combined with Monte Carlo test (CANOCO, Ithaca, USA) was used to find out the relationship between microbial abundance and soil chemical properties (Leps and Smilauer 2003).

Metastats analysis was used to detect fungal or bacterial diversity features based on the OTU taxon (Segata et al. 2011; http://metastats.cbcb.umd.edu/detection.html). We calculated Chao 1 to estimate the species richness, observed species to estimate the number of the observed OTUs, and Shannon diversity index to estimate the diversity within a sample. These metrics indicated the α-diversity within a sample and were calculated using the QIIME software (V1.7.0, http://qiime.org/). We calculated the UniFrac distance and Bray-Curtis distance matrices to estimate the β-diversity for bacterial community. Since it is impossible to generate accurate alignments for the ITS genes using the UniFrac distance (Delgado-Baquerizo et al. 2016), we only calculated the Bray-Curtis distance to estimate the β-diversity for fungal community. Principal coordinate analysis (PCoA) based on the UniFrac distance (Lozupone et al. 2011) was used to show the patterns of bacterial community. Non-parametric permutational multivariate analysis of variance (perMAVON) was conducted to detect the effects of the treatment, season, and their interaction on the bacterial community based on the UniFrac distance (adonis function of vegan packages in R software; Barnard et al. 2013). Non-metric multidimensional scaling (NMDS) analysis based on the Bray-Curtis distance was conducted in PRIMER-E (Plymouth, WA, USA) to detect the difference between samples for composition of both fungal and bacterial communities. The effects of the treatment, season, and their interactions on the composition of fungal community were also detected based on the Bray-Curtis distance (PERMAVON+ and ANOSIM function in PRIMER-E). It is noted here that the higher R statistic in the ANOSIM analysis represents greater dissimilarity between two groups (Rees et al. 2004).

Data accession numbers

All the HiSeq sequencing data (.fq files) were uploaded to the National Center for Biotechnology Information (NCBI) database, and it is possible to access these sequences by the BioProject PRJNA338826 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA338826).

Results

Soil physicochemical properties

The annual precipitation was 2100 and 1576 mm during the hydrological years 2013 and 2014, respectively. The total
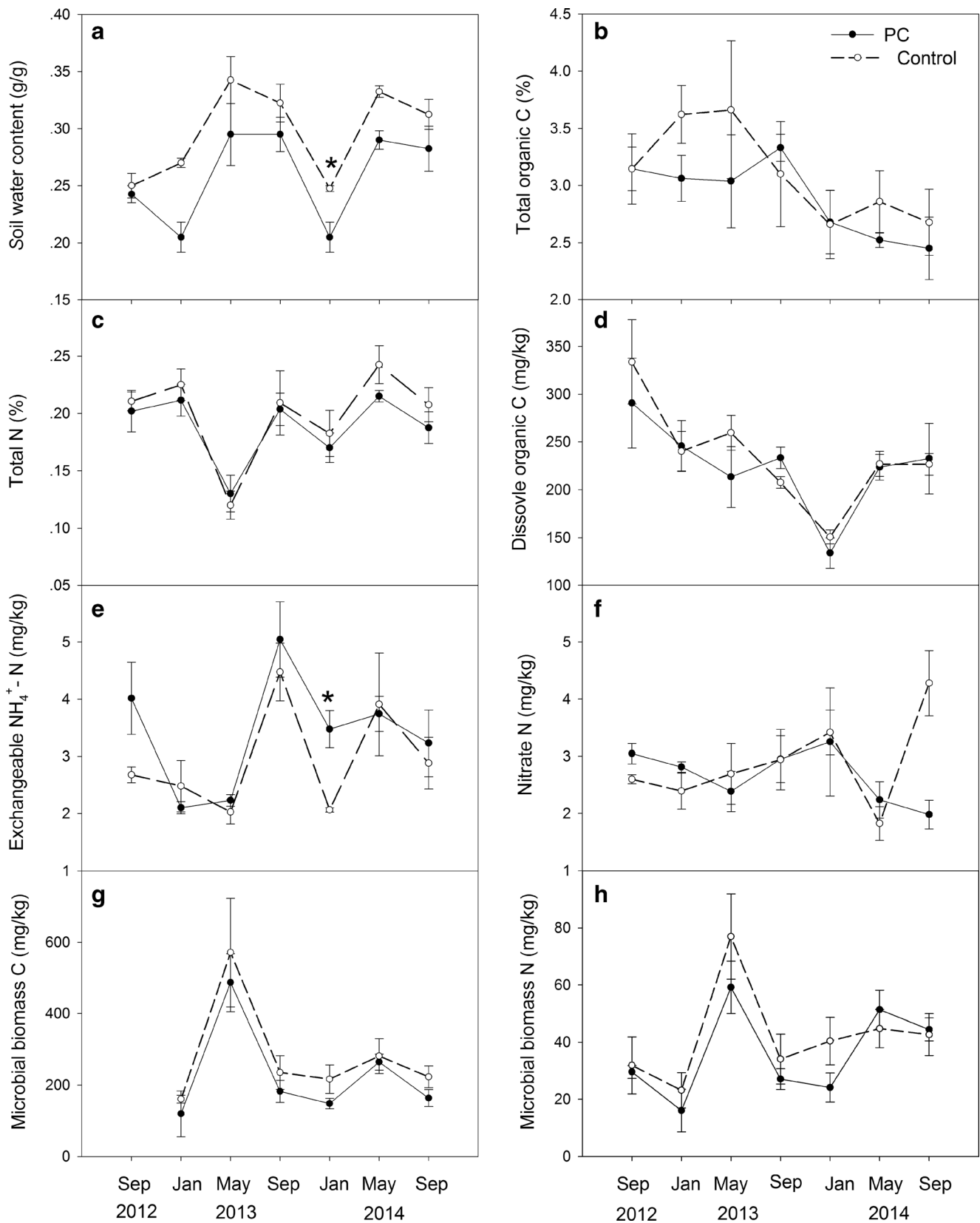
Fig. 1 Responses of soil physicochemical properties and soil microbial biomass to the precipitation changes. *Bars* represent standard errors of the mean (*n* = 4). *Open circles* represent the control and *solid circles* the precipitation change (PC) treatment. The *asterisk* marks significant differences between the PC treatment and the control (*P* < 0.05)
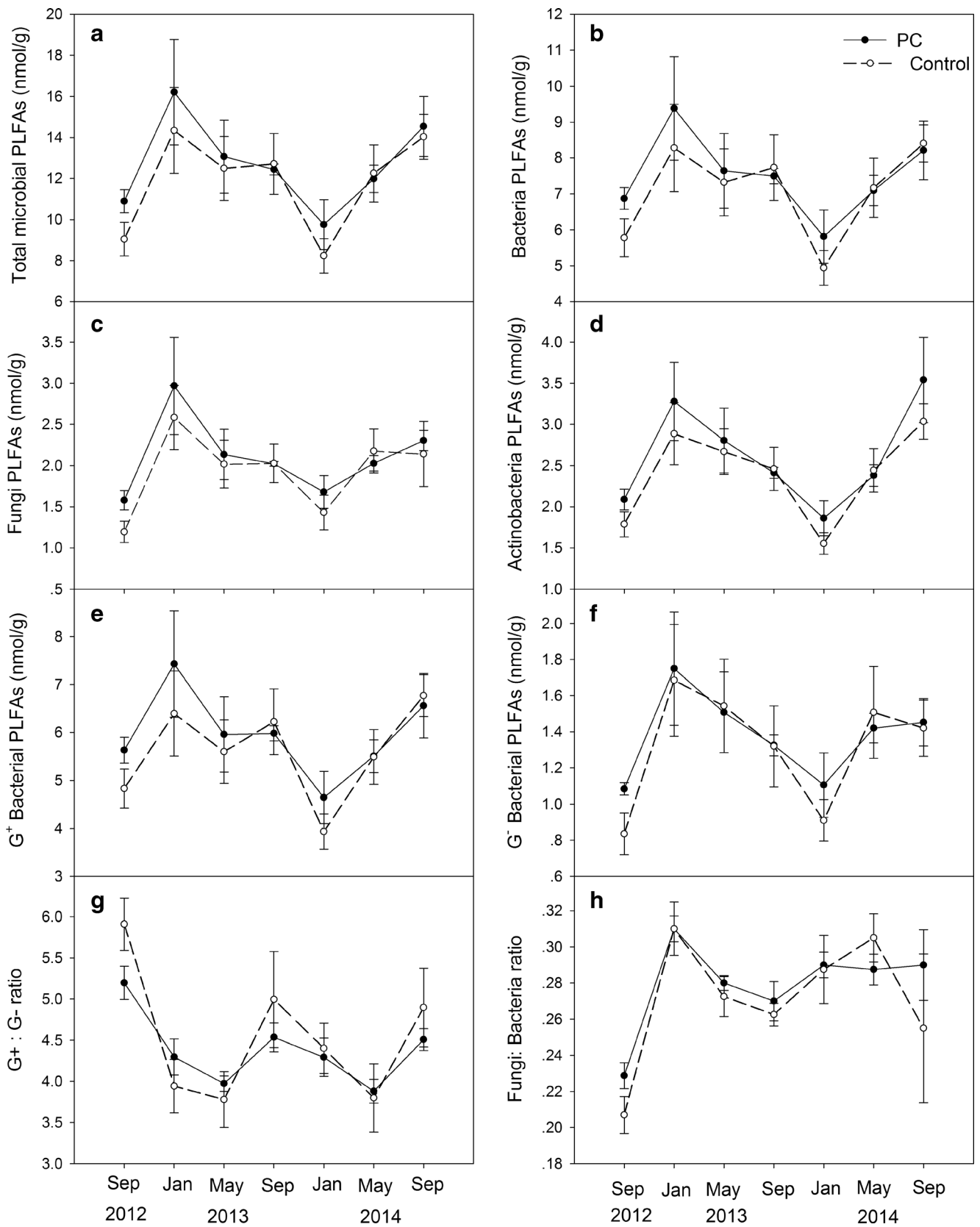
Fig. 2 Responses of soil microbial phospholipid fatty acids (PLFAs) to the precipitation changes. *Bars* represent standard errors of the mean (*n* = 4). *Open circles* represent the control and *solid circles* the precipitation change (PC) treatment
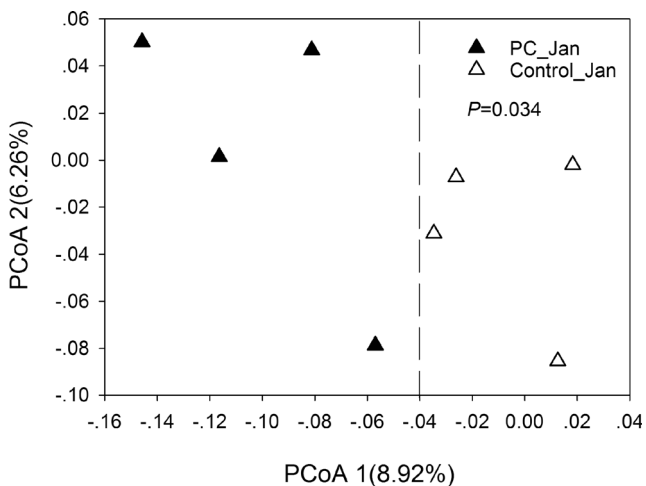
Fig. 3 Principal coordinate analysis (PCoA) of soil microbial community composition based on the unweighted UniFrac distance of bacterial OTUs. *Solid* and *open triangles* stand for the precipitation change (PC) treatment and the control in January, 2014, respectively. The *P* value represents the significance of the throughfall exclusion effect on the bacterial community as revealed by the non-parametric permutational multivariate analysis of variance

amount of throughfall excluded was 220 and 171 mm during the dry seasons of 2013 and 2014, respectively. There were significant differences between years and seasons in the physicochemical variables measured, depending on the amount of precipitation registered (Table S2). For example, soil TOC was lower in the drier 2014 than in the wetter 2013 (Fig. 1b; Table S2); MBC and MBN were higher in spring than in the other seasons (Fig. 1g, h; Table S2).

Immediately prior to establishing the PC treatment, the average SWC of the PC and control plots was very similar (0.24 ± 0.01 vs. 0.25 ± 0.01 g/g; Fig. 1a). Following the initiation of the PC treatment, SWC was significantly reduced by 24.1% (2013 dry season) and 17.2% (2014 dry season) relative to the control plots (Fig. 1a; Table S3). The differences were due to the fact that the exclusion treatment reduced throughfall by 67% during the dry season. During the wet seasons, when water was added, the SWC of the PC plots remained below that of the control plots, although the differences were smaller than during the dry seasons (Fig. 1a). Soil TOC, DOC, MBC, and MBN were not significantly affected by changes in the rainfall patterns (Fig. 1; Table S3). However, soil-exchangeable NH_4^+-N was increased significantly during the throughfall reduction period; NO_3^--N was decreased with water addition in the relatively drier 2014, but not in the wetter 2013 wet seasons (Fig. 1e, f; Table S3).

Microbial community composition

The amounts of PLFA biomarkers for the different microbial groups were markedly higher (Fig. 2; Table S4; $P < 0.05$) in the dry season of 2013 (507 mm rainfall) than in that of 2014

(382 mm rainfall). However, neither of the precipitation regime changes had significant effects on fungal, bacterial, G^+ or G^- biomass, the G^+/G^- ratio, or the F/B ratio (Fig. 2; Table S3).

Based on the high-throughput sequencing, the seasonal precipitation changes affected particular taxonomic groups of both bacterial and fungal communities. A total of 4484 bacterial OTUs were characterized from a total of 1,899,457 high-quality sequences. Dominant bacterial phyla were the *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*, followed by *Planctomycetes*, *Verrucomicrobia*, *Chloroflexi*, *Crenarchaeota*, and *Gemmatimonadetes*. These phyla accounted for 94% of the total bacterial OTUs observed (Table S5). At the whole bacterial community level, the precipitation alterations had no significant impact on bacterial α-diversity throughout the year 2014 (Table S6), while the throughfall reduction caused a differentiation in the composition of bacterial communities as revealed by the PCoA analysis using UniFrac distance that considers the presence or absence of OTUs ($P = 0.034$; Fig. 3; Tables S7 and S8). At the phylum level, the throughfall reduction significantly increased the relative abundance of *Gemmatimonadetes*, *Bacteroidetes*, and *Armatimonadetes* by 15, 42, and 26% during the dry season, respectively ($P < 0.05$; Fig. 4a, b, d). The water addition during the wet season significantly increased the relative abundance of *Firmicutes* by 49% ($P < 0.05$; Fig. 4c). The RDA analysis showed that the relative abundances of *Proteobacteria*, *Bacteroidetes*, and *Gemmatimonadetes* were positively related to exchangeable NH_4^+-N, while those of *Firmicutes* and *Armatimonadetes* were positively related to pH (Fig. 5a).

A total of 3221 fungal OTUs were characterized from a total of 1,291,618 high-quality sequences. Dominant fungal phyla included the *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Chytriomycota*, and *Chytridiomycota*, which together accounted for 82% of the total fungal OTUs observed (Table S9). At the community level, both the throughfall reduction and water addition decreased the number of observed OTUs that represented α-diversity (Table S6; $P < 0.05$). Based on the NMDS analysis using the Bray-Curtis distance, the fungal community in PC plots was separated from that in control plots with the throughfall reduction in the dry season ($R = 0.75$; Tables S10 and S11; Fig. 6a). Specifically, the throughfall reduction significantly decreased the relative abundance of *Ascomycota*, the dominant phylum accounting for 41.2% of the total fungal OTUs (Table S9), by 33% during the dry season ($P < 0.05$; Fig. 4e). Contrastingly, the relative abundance of *Basidiomycetes*, which accounted for 27.8% of the total fungal OTUs, was increased by 27% ($P < 0.05$; Fig. 4f). However, the addition of water during the wet season did not affect the relative abundance of these phyla (Fig. 4). The RDA analysis showed
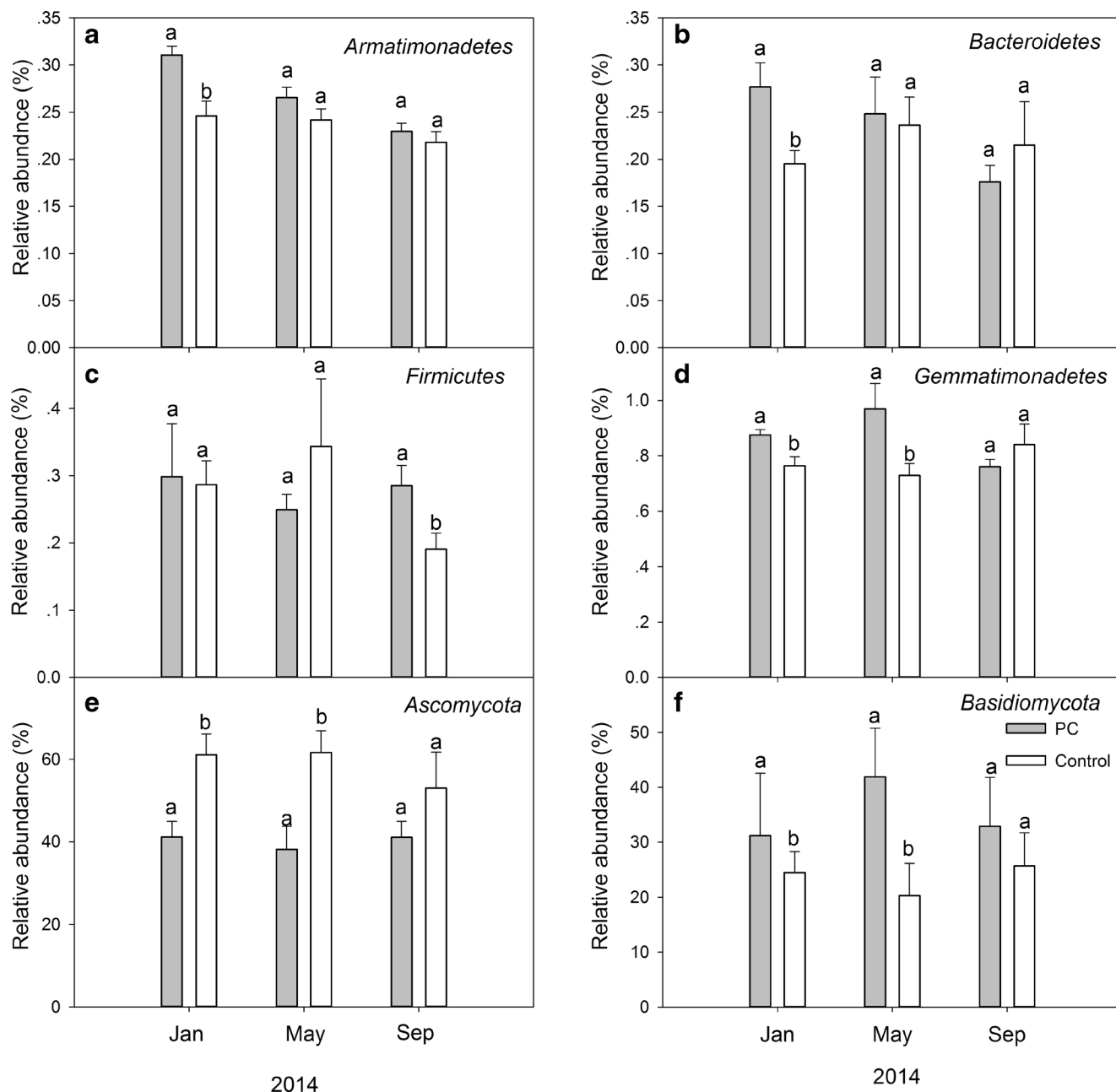
Fig. 4 Relative abundance of bacterial and fungal phyla in response to the precipitation changes. *Bars* represent standard errors of the mean ($n = 4$). *Gray bars* represent the precipitation change (PC) treatment while *white bars* the control. *Letters above the bars* represent significant difference between the PC treatment and the control ($P < 0.05$)

that the relative abundance of *Ascomycota* was positively related to DOC, TP, or SWC, whereas the relative abundance of *Basidiomycota* was negatively related with these soil physicochemical properties (Fig. 5b).

Enzyme activities

Most enzyme activities did not respond to the dry-season throughfall reduction (Table 1). Only β-1,4-xylosidase and β-1,4-glucosidase activities increased after the addition of water during the wet season (Tables 1 and S12). In addition, neither the dry-season throughfall reduction nor the wet-season rain addition had significant impacts on the specific enzyme activities (Table S13). All enzyme activities were positively correlated with TOC or DOC (Table 2; $P < 0.05$). The activities of lignin-degrading (polyphenol oxidase and peroxidase) and chitin-degrading (β-1,4-*N*-acetyl-glucosaminidase) enzymes were lowest in winter
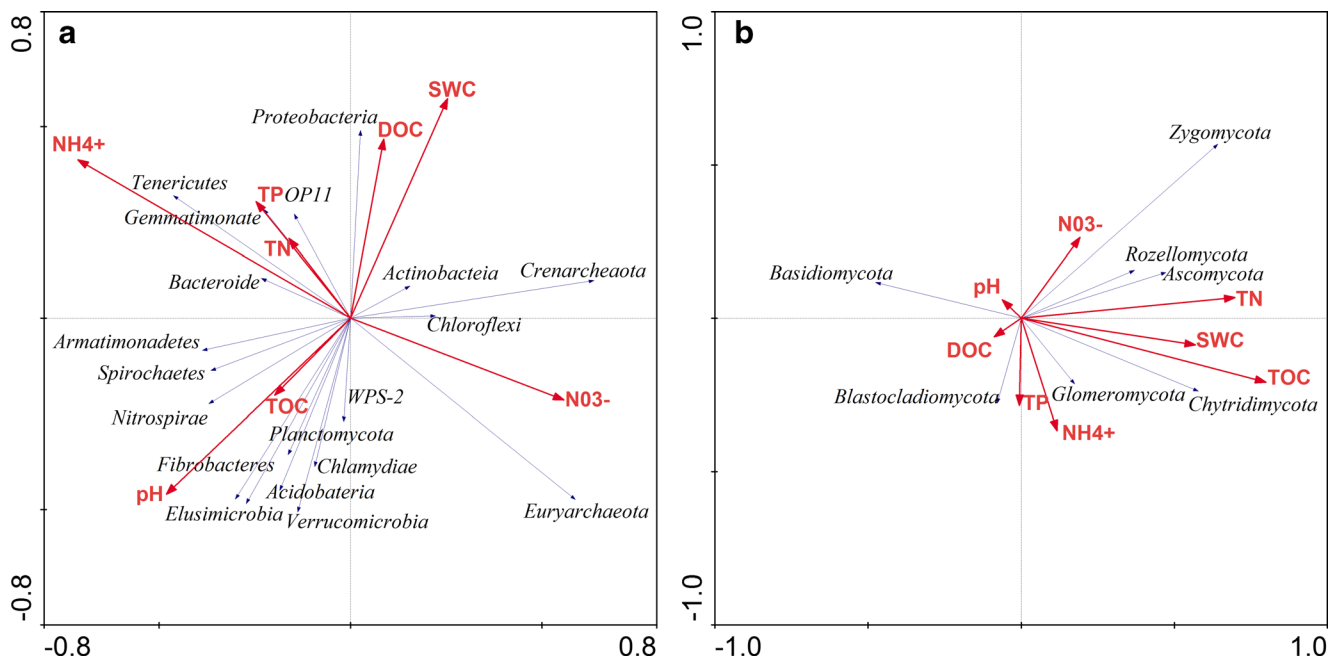
Fig. 5 The biplot of redundancy analysis illustrating the relationships between the dominant bacterial (**a**) or fungal (**b**) phyla and the soil physicochemical properties. *SWC* soil water content, *TOC* total organic C, *DOC* dissolved organic C, *TN* total N, *TP* total P

(Table S12) and positively correlated with SWC (Table 2; $P < 0.05$), indicating that the substrate availability and SWC were two important factors influencing enzyme activities.

Discussion

Changes in soil moisture have been identified as a key uncertainty in projections of global and regional climate change (IPCC 2013). This study showed that a manipulative 67% reduction of rainfall caused a 17–24% decrease of the SWC (Fig. 1a), and the percentage of SWC decline was greater in the relatively wetter 2013 (507 mm of rainfall) than in the drier 2014 dry season (382 mm) (Fig. 1a). This result, consistent with previous studies conducted in dryland and temperate forest ecosystems (Gimbel et al. 2015; Liu et al. 2016b), indicates that soil moisture responds disproportionally to precipitation changes, which may result from the non-linear interactions between previous and current soil water status and between soil water availability and evapotranspiration (Shen et al. 2016; Tuttle and Salvucci 2016). In contrast, water addition during the wet season did not affect SWC in this study (Fig. 1a), which differs from many studies on dryland ecosystems where rainfall amendment often increases SWC (Sorensen et al. 2013; Reichmann et al. 2013). The unaffected soil moisture by the wet-season water addition in our study might result from two reasons. First, SWC in the wet season was already high or close to saturation (on average 30.2% across all the plots); excessive water, especially the water

added in the manner of large events (50 mm/day), might had been lost through surface runoff. Second, changes in SWC were not captured by our sampling scheme that took place 2 weeks after the watering, when the added water might have already been lost via evapotranspiration into the atmosphere or percolation into deeper soil layers.

Contrary to our first hypothesis, neither the dry-season throughfall reduction nor the wet-season water addition significantly impacted microbial biomass and microbial community composition characterized by PLFAs (Figs. 1 and 2). These results differ from what have been found in water-limited ecosystems where drought often decreased microbial biomass and fungal and bacterial PLFAs (Hueso et al. 2012) while the enhanced soil water availability increased them (Williams and Rice 2007; Huang et al. 2015). The decrease of soil microbial biomass under drought conditions may not only be ascribed to the death or the reduced microbial growth by allocating more resources to accumulate protective solutes to avoid dehydration, but also to the reduced microbial mobility, solute diffusion, and therefore substrate availability (Harris 1981; Kieft et al. 1987; Ciardi et al. 1993; Schimel et al. 2007). Reversely, water/rainfall addition may eliminate the water stress or substrate limitation for microorganisms and therefore benefit microbial growth. However, rewetting of a dry soil may also result in a large release of biomass C that could account for 17–70% of the total microbial biomass (Kieft et al. 1987), which could cause an overall decrease of microbial biomass during a drying-rewetting cycle. Consistent with our field experimental results, the bacterial and fungal counts were also not influenced by the remoistening of air-
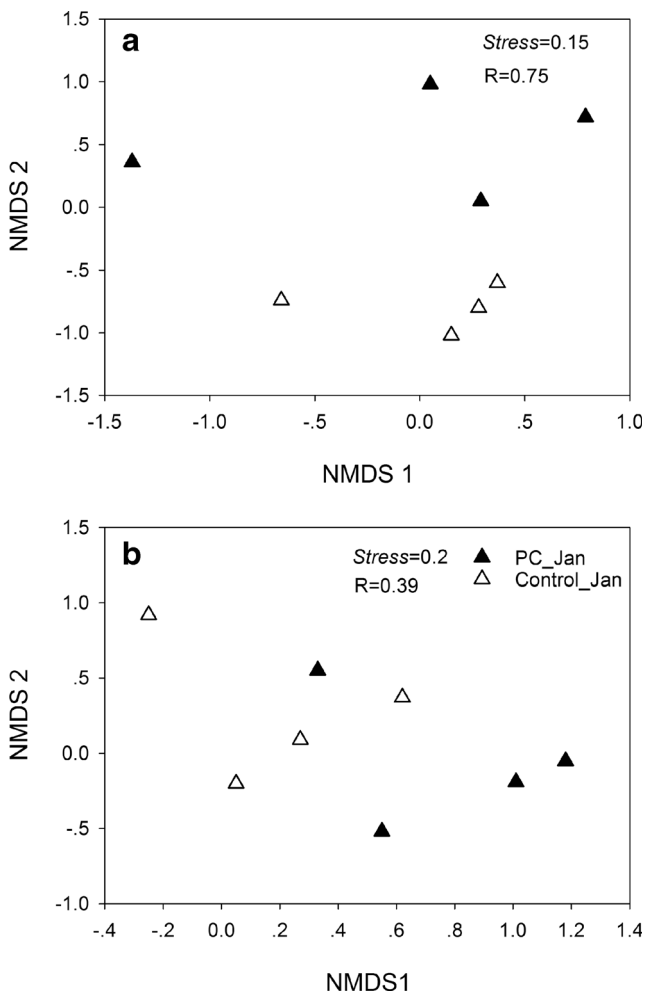
Fig. 6 Non-metric multidimensional scaling (NMDS) analysis based on the Bray-Curtis distance for the fungal (**a**) and bacterial (**b**) communities. *Solid* and *open triangles* stand for the precipitation change (PC) treatment and the control in January, 2014, respectively. The *R*-value represents the dissimilarity between the fungal and bacterial communities in the PC and control plots during the dry season

dried soils in Ciardi et al. (1993). Therefore, the influence of precipitation changes on soil microbial biomass and community composition is a function of not only the absolute magnitude but also the manner of the precipitation change (Kieft et al. 1987; Waldrop and Firestone 2006; Manzoni et al. 2012). In our system, the 67% of the throughfall reduction significantly decreased SWC from 0.26 to 0.21 g/g, which were about 47 and 37% of the field capacity (FC), respectively. For the same forest soil, Zhou et al. (2014) reported that 40–60% FC was the optimal moisture condition within which soil microbial respiration and PLFAs did not change markedly with soil moisture. We therefore consider the mild soil moisture change without reaching the threshold as one of the reasons why soil microbial communities did not show significant responses to the precipitation manipulation (Fang et al. 2016). In addition, non-significant responses of microbial community composition to the moisture changes could also be due to the low resolution of the detecting method used (i.e., the PLFA method) in our study (Marschner 2007) and the plate count method in Ciardi et al. (1993).

Besides microbial community composition, most enzyme activities assayed also did not show significant responses to the precipitation manipulation (Fig. 2; Table 1). Both the mild changes in soil moisture and the non-significant changes in the microbial community composition may be responsible for that. The unaffected specific enzyme activities (Table S13) indicate that it was also not due to the enzyme enrichment of soil microflora. However, it has been reported that the fluctuation of functional activities may not concert with that of the composition or diversity under environmental perturbations (Griffiths et al. 2001; Rocca et al. 2015). This is true in our case during the wet season, when the two enzyme activities (β-1,4-glucosidase and β-1,4-xylosidase) were significantly suppressed by the water addition treatment (Table 1) in spite of the unchanged microbial composition (Fig. 2). Water addition in the wet season might have reduced the enzyme

Table 1 Soil enzyme activities (means ± se, $n = 4$) in the PC and control plots

Enzyme activity (nmol g^{-1} h^{-1})	January, 2014		May, 2014		September, 2014	
	PC	Control	PC	Control	PC	Control
Acid phosphomonoesterase	364.76 ± 62.72	297.48 ± 14.13	338.85 ± 36.44	269.37 ± 13.95	351.78 ± 62.18	462.01 ± 34.32
β-D-1,4-cellubiosidase	21.69 ± 4.09	16.23 ± 3.79	16.02 ± 4.07	13.05 ± 2.39	17.41 ± 4.45	17.69 ± 1.60
β-1,4-Xylosidase	40.88 ± 7.29	28.80 ± 6.56	27.87 ± 4.22	25.57 ± 2.49	*26.61 ± 2.49* a	*50.41 ± 1.09* b
β-1,4-N-acetyl-glucosaminidase	8.63 ± 1.22	9.76 ± 0.73	11.43 ± 1.61	18.16 ± 4.07	11.79 ± 2.62	12.64 ± 0.39
β-1,4-glucosidase	81.40 ± 8.00	75.97 ± 1.74	103.06 ± 4.40	107.37 ± 6.20	*71.42 ± 2.06* a	*97.79 ± 3.64* b
Peroxidase	0.91 ± 0.09	1.15 ± 0.09	1.24 ± 0.06	1.59 ± 0.35	1.63 ± 0.03	1.45 ± 0.16
Polyphenol oxidase	0.44 ± 0.02	0.50 ± 0.02	0.69 ± 0.03	0.85 ± 0.20	0.82 ± 0.09	0.82 ± 0.14

January represents the dry season with throughfall reduction; May represents the early wet season with ambient precipitation; September represents the main wet season with water addition; Different lowercase letters (a, b) behind the italic numbers indicate significant difference between the PC and control treatments ($P < 0.05$)

PC precipitation change treatment

Table 2 Spearman's correlation coefficients between soil enzyme activities and soil physicochemical properties

Soil enzyme	SWC	TOC	DOC	TP	TOC/TN
Acid phosphomonoesterase	−0.015	0.454*	−0.004	0.210	0.596**
β-D-1,4-Cellubiosidase	0.051	0.365	−0.044	0.501**	0.423**
β-1,4-Xylosidase	−0.078	0.428*	−0.080	0.275	0.573**
β-1,4-N-acetyl-glucosaminidase	0.761**	0.341	0.468*	0.291	0.058
β-1,4-Glucosidase	0.574**	−0.015	0.457*	0.000	−0.398
Peroxidase	0.624**	0.555**	0.639*	−0.016	0.163
Phenol oxidase	0.734**	0.517**	0.805**	0.194	0.142

SWC soil water content, *TOC* total organic C, *DOC* dissolved organic C, *TP* total P, *TN* total N

The asterisks "*" and "**" indicate significance at $P < 0.05$ and $P < 0.01$, respectively

activities due to the increased enzyme efficiency by facilitating the diffusion across water films (Henry et al. 2005; Gutknecht et al. 2010). In a soil microbial community, more than one microbial taxon can carry out a process under the same environmental condition (Allison and Martiny 2008). For example, *Basidiomycota* and *Ascomycota* are both claimed to be responsible for the production of lignocellulose-degrading enzymes in soils (Baldrian and Valaskova 2008; Burns et al. 2012). *Bacteroidetes* and *Actinobacteria* are important enzyme producers that contribute to C cycling in the soil (Goodfellow and Williams 1983; Fazi et al. 2005). Similarly, a target enzyme activity (e.g., phenol oxidase activity) may be associated with several processes (e.g., the synthesis of secondary compounds, decomposition, defense, and humification) and a single process (e.g., the degradation of lignin) may involve the activity of several enzymes (lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccases) (Sinsabaugh 2010; Nannipieri et al. 2012). Functional redundancy might therefore be another reason why enzyme activities did not significantly respond to the precipitation manipulation, since the functioning of one microbial group or enzyme activity might have been compensated by another under altered precipitation conditions. Furthermore, many direct and indirect effects confound the response of the enzyme activity to changes in environmental conditions (Nannipieri et al. 2012). Combining the detection and expression of enzyme-encoding genes with the classical measurement of enzyme activities is an important future approach for a better understanding of the linkages between the function and the composition of microbial communities (Pathan et al. 2015; Nannipieri et al. 2012).

However, the amplicon sequencing analysis revealed that the relative abundance of some bacterial and fungal phyla was affected by the throughfall reduction in the dry seasons. The throughfall reduction significantly increased the relative abundance of three rare bacterial phyla (*Gemmatimonadetes*, *Armatimonadetes*, *Bacteroidetes*), which were also found to be more abundant in arid soils (DeBruyn et al. 2011; Fierer et al. 2012). The relative abundance of these three bacterial

phyla only accounted for 1.5% of the total bacterial abundance; increase in their abundance therefore had ignorable impacts on the composition of the whole soil microbial community. Supporting to our second hypothesis, the relative abundance of two dominant fungal phyla (*Basidiomycota* and *Ascomycota*) responded significantly to the throughfall reduction in the opposite directions: the *Basidiomycota* abundance decreased, whereas that of *Ascomycota* increased. Such compositional adjustment between different fungal groups might be another reason why the abundance of the whole soil microbial community was not significantly altered. Our results also confirm that the high-throughput sequencing is a more informative approach to target the response of microbial community composition to the environmental changes compared to the PLFA approach (Jackson et al. 2007).

Although the community-level measures such as PLFA composition and microbial biomass did not show significant responses to the precipitation manipulation, they co-varied closely with the seasonal variations in SWC (Table S14). This result indicates that SWC and other co-varying environmental factors such as temperature and soil nutrient availability can be influential on microbial community composition (Fang et al. 2016). Our results showed that the microbial biomass peaked in spring (Fig. 1g, h), which is the start of the warm wet-season after a 6-month cold dry season. Therefore, seasonal variations in microbial biomass may be influenced not only by SWC, but also by the confounding factors such as temperature and microbial phenology. Such confounding effects may also shape the precipitation treatment effects. For example, the relative abundance of *Gemmatimonadetes* and *Bacteroidetes* increased with the throughfall reduction (Fig. 4b, d) and was also positively correlated with exchangeable NH_4^+-N content (Fig. 5a). Previous studies have shown that increasing N availability stimulated the relative abundance of *Gemmatimonadetes* and *Bacteroidetes* (Nemergut et al. 2008; Högberg et al. 2014). We therefore argue that the throughfall reduction-induced increases in ammonium availability might be responsible for the increases in the relative abundance of the two bacterial phyla after the throughfall

reduction. Moreover, the relative abundance of the two fungal phyla that responded significantly to the throughfall reduction was also correlated with DOC and TP (Fig. 5b). It is therefore possible that the throughfall reduction-induced changes in the substrate availability may be responsible for the changes in fungal abundance. Being supportive to our third hypothesis, these evidences demonstrate that precipitation regime changes may also indirectly affect soil microbial composition through the interactions with other soil physicochemical variables such as temperature, substrate availability, and microbial physiological and phenological traits.

Conclusions

Neither the dry-season throughfall reduction nor the wet-season water addition had significant impacts on the soil microbial biomass, most enzyme activities, and the community composition characterized by PLFAs. However, the relative abundance of certain bacterial and fungal phyla characterized by the high-throughput sequencing showed significant responses to the precipitation manipulation, especially to the dry-season throughfall reduction. The lack of the response of soil microbial community at the community level may be ascribed to the mild soil moisture alterations by the precipitation manipulation, the compositional adjustment and functional redundancy of different microbial groups, and the confounding effects by other soil physicochemical properties. Our results suggest that the DNA-based sequencing method is more sensitive and informative in detecting soil microbial community responses to precipitation changes than PLFA analysis. Combining the classical measures (e.g., PLFAs, gene abundance, and enzyme activity assays) with the measurements of the presence and expression of the relative enzyme-encoding genes using omics approaches (e.g., metagenomics, transcriptomics, and proteomics) is needed in order to better detect and interpret the responses of soil microbial composition and function to future climate changes (Nannipieri et al. 2012; Pathan et al. 2015). This study reports the response behaviors of soil microbial communities in the first 2 years of the precipitation experiment. Long-term responses may be different from the short-term reactions and therefore are suggested to be further studied to achieve a deeper understanding of the responsive behaviors and feedback pathways.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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