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Extreme drought slightly decreased soil labile organic C and N contents and altered microbial community structure in a subtropical evergreen forest

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ABSTRACT

It is predicted that climate extremes such as drought will become more frequent and prolonged by the end of this century, so it is vital that we improve our understanding of the effects of extreme drought on the dynamics of soil labile C and N contents and microbial communities in terrestrial ecosystems. Using a 70% rainfall reduction manipulation experiment to simulate extreme drought in a subtropical evergreen forest of eastern China, we collected soil samples over all four seasons and analyzed the dynamic changes in soil labile organic C and N contents and microbial communities via high-throughput sequencing during the period from June 2016 to February 2017. Soil labile organic C and N contents were represented by soil extractable organic C (EOC) and extractable organic N (EON) contents, microbial biomass C (MBC) and microbial biomass N (MBN) contents. We also measured soil potential CO_2 and N_2O emissions by using laboratory incubation under drought conditions. The results showed that drought significantly decreased soil pH, while it slightly decreased soil EOC and EON contents, and MBC and MBN contents by 13.4%, 15.7%, 11.1% and 15.2% respectively, though it had no significant effects on these compared with the control plots. The dominant bacterial phyla across all soils were Proteobacteria (32.52–41.30%), Acidobacteria (34.47–42.64%) and Actinobacteria (6.52–8.16%). Drought greatly altered the soil microbial community structure underlying soil C and N cycling: (1) drought significantly increased the relative abundance of Acidobacteria, which was associated with lower soil pH in the drought plots; (2) drought had a significantly lower relative abundance of Proteobacteria, which was supported by the lower soil labile organic C and N contents under drought. Redundancy analysis showed that the indirect drought-induced effects of soil EOC and EON contents from two sampling times played a weightier role in influencing the patterns of soil microbial communities than the direct drought-induced effects of soil moisture. We found that drought significantly decreased soil potential CO_2 and N_2O emissions, confirming that drought decreased soil microbial activity. Overall, our results suggest that extreme drought slightly decreased soil labile C and N contents, and altered the microbial community structure, mainly through indirect drought-induced pathways.

1. Introduction

Many regions of the world will experience more frequent and prolonged droughts as it is predicted that climate extremes such as drought will occur with greater frequency and duration by the end of this century (IPCC, 2013). Drought coupled with high temperature has dramatic impacts on soil biogeochemical cycling in forest ecosystems, and it can even induce tree mortality and reduce forest ecosystem productivity (Schlesinger et al., 2016). In particular, soil N cycling directly relates to soil N availability for tree growth. It is known that soil C and N cycling in forest ecosystems is mediated by soil microbial communities (Falkowski et al., 2008). Until now, however, little has been known about the effects of drought on soil C and N cycling and the underlying microbial mechanisms involved in these processes in forest ecosystems. It is necessary to increase our understanding of how drought affects soil C and N pools and the associated microbial communities in forest ecosystems, which will help us manage our forest ecosystems better under drought in the future.

Abbreviations: ANOVA, analysis of variance; EOC, extractable organic C; EON, extractable organic N; H, Shannon–Wiener microbial diversity index; MBC, microbial biomass C; MBN, microbial biomass N; OTU, operational taxonomic unit; PCR, polymerase chain reaction; RDA, redundancy analysis; TC, total C; TN, total N

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In general, it is difficult to detect significant rapid changes in soil organic C and total N contents under different management practices (Haynes, 2005; Shahid et al., 2017). Soil labile organic C and N contents such as the extractable organic C (EOC) and extractable organic N (EON) pools, and microbial biomass C (MBC) and microbial biomass N (MBN) contents are active fractions of soil organic matter, and they can respond rapidly to different forest management practices and provide a short-term reservoir of N availability for plants and microorganisms (Haynes, 2005; Shahid et al., 2017; Yuan et al., 2017; Zhou et al., 2016). It has been reported that drought can alter soil labile organic C and N contents (Li et al., 2016; Schaeffer et al., 2017). Most of these studies focus on grassland ecosystems, agricultural ecosystems and some peatland ecosystems, but only a handful of studies focus on the effects of drought on soil C and N cycling in forest ecosystems (Canarini and Dijkstra, 2015; Kwon et al., 2013; Schaeffer et al., 2017; Wang et al., 2017). Therefore, more studies are needed to clarify how soil C and N cycling respond to drought stress to improve our knowledge of the effects of drought in forest ecosystems.

Soil microorganisms play vital roles in soil C and N cycling and ecosystem functions, such as soil C stocks and N_2O emissions (Falkowski et al., 2008; Xiao et al., 2015; Zhang et al., 2011). Previous findings have suggested that the activities and community structure of soil microorganisms can be greatly affected by water availability (Kaisermann et al., 2013; Uhlířová et al., 2005). In general, drought can influence soil microbial communities through two pathways. On one hand, drought can directly influence the physiological status and activities of soil microorganisms through osmotic stress and then cause a considerable shift in community structure (Hueso et al., 2012; Uhlířová et al., 2005). For example, drought stress can induce a higher abundance of Gram-positive bacteria, as they have rigid cell walls and form spores when subjected to drought, which make them more tolerant to drought stress than Gram-negative bacteria (Chodak et al., 2015; Hueso et al., 2012; Uhlířová et al., 2005). On the other hand, drought can influence soil microbial communities indirectly through the regulation of substrate availability such as labile organic C and N contents (Chodak et al., 2015; Fierer, 2017; Fierer et al., 2007; Uhlířová et al., 2005) and soil pH (Kwon et al., 2013). It is well known that soil pH is a major factor driving the patterns of soil microbial communities in terrestrial ecosystems (Chodak et al., 2015; Fierer, 2017; Zhou et al., 2017). In particular, some researchers have found a close relationship between the relative abundance of Acidobacteria and soil pH (Jones et al., 2009). Usually, the direct and indirect pathways influence soil microbial communities in combination. To our knowledge, little information is available about quantifying the relative contributions of the two pathways to soil microbial communities under drought in forest ecosystems.

Subtropical evergreen forests are a distinct form of forest vegetation type compared with other arid and semi-arid ecosystems at similar latitudes worldwide (Song and Da, 2016). To date, numerous studies have focused on aboveground plant communities and soil respiration in subtropical evergreen forests (Wang et al., 2007; Gao et al., 2014). However, few studies are available about the effects of drought on soil labile organic C and N contents and the underlying microbial communities in subtropical evergreen forests. Here, we took advantage of a rainfall reduction manipulation experiment platform after 3 years of treatment to investigate the effects of extreme drought on soil labile organic C and N contents and microbial communities in a subtropical evergreen forest in Eastern China. In particular, we collected soil samples over all four seasons between June 2016 and February 2017 to address the effects of drought on soil labile organic C and N contents and microbial taxa in a comprehensive way. The objectives of this study were to (1) quantify the responses of soil labile organic C and N contents including soil EOC and EON contents as well as MBC and MBN contents to drought; (2) quantify the responses of soil microbial communities and N_2O emissions as an ecosystem function to drought; (3) quantify the relative contributions of the direct and indirect effects of drought on the patterns of soil microbial communities.

2. Materials and methods

2.1. Experimental site

The rainfall reduction manipulation experiment site was established in July 2013 at Tiantong National Forest Park, Ningbo City, Zhejiang Province, Eastern China (29°52′N, 121°39′E, 200 m above sea level). This region belongs to a typical subtropical monsoon climate with a hot, humid summer and a drier, cold winter. The mean annual temperature and mean annual precipitation are 16.2 °C and 1374.7 mm, respectively (Wang et al., 2007). The dominant tree species at the study site are *Castanopsis fargesii* Franch., *Schima superba* Gardner and Champ., *Castanopsis carlesii* (Hemsl.) Hayata and *Lithocarpus glaber* (Thunb.) Nakai.

We manipulated the site with three treatments (Fig. S1): a 70% rainfall reduction to simulate an extreme drought scenario in the future (hereafter referred to as the “drought” treatment) using large plastic plates, a plate disturbance treatment (hereafter referred to as the “disturbance” treatment) and an ambient control treatment. Each treatment had three replicates, resulting in nine plots in total. The size of each plot is 25 m × 25 m, with at least 5 m spacing between adjacent plots. To minimize the effects of disturbance, buffer regions with a 2.5-m width were set around each plot. In the drought plots, transparent concave polycarbonate plates were uniformly fixed at a height of 1.5–3.5 m above the ground to reduce rainfall. In order to ascertain the effects of the shadow produced by these plates on soil (i.e. the disturbance), we installed identical plates in the disturbance plots. However, the grooves of these plates were placed face down in the disturbance plots to allow rainfall to penetrate, whereas these plates were placed face up in the drought plots (see Fig. S1). For each plot, a trench about 2 m deep was excavated around the plot’s perimeter and a PVC segregation board was placed inside to prevent lateral runoff to the plots.

2.2. Soil sampling and measurements of soil physicochemical properties

Soil samples were collected over all four seasons (i.e., in spring on 1 June 2016, in summer on 6 August 2016, in autumn on 3 December 2016 and in winter on 25 February 2017) following a diagonal sampling pattern (i.e., one point at each corner and one in the center of each plot) with a soil auger (2.5 cm in diameter) at a depth of 0–10 cm within each plot. The soil cores were immediately mixed thoroughly and kept in a cooler at 4 °C. The samples were then passed through a 2-mm sieve to remove roots and stones, and stored at 4 °C before further analysis. Subsamples were stored at –20 °C for DNA extraction. Soil moisture was determined gravimetrically after the samples were oven-dried at 105 °C overnight. Soil pH was measured at a 1:2.5 dry soil-to-water ratio. Soil total C (TC) and N (TN) contents were determined on a Vario MICRO cube elemental analyzer (Elementar, Germany). We also calculated the ratio of TC to TN (C:N ratio) to represent the quality of the soil.

2.3. Measurement of soil labile organic C and N contents

Soil EOC and EON contents, and MBC and MBN contents were measured, as they both act as indicators of soil labile organic C and N contents.

Soil EOC and EON contents were determined in hot water extracts (Zhou et al., 2013). Briefly, field soil samples (5 g) were extracted with 50 mL of hot water and incubated at 70 °C for 16 h in Falcon tubes. After that, the Falcon tubes were rotated in an end-to-end shaker at 120 rpm for 1 h and then the supernatant was filtered through Whatman No. 42 paper. The inorganic N contents (sum of NH_4^+–N and NO_3^-–N) were measured with a Smartchem Discrete Auto Analyzer (Smartchem 200, AMS, Italy). Soil EOC and total soluble N in hot water

extracts were determined on a Multi N/C 3100 total organic carbon analyzer fitted with a total N unit (Analytik Jena, Germany). Soil EON was calculated by subtracting extractable inorganic N from total soluble N for every soil sample.

Soil MBC and MBN contents were measured by the chloroform fumigation–extraction method with a conversion factor of 2.64 for MBC (Vance et al., 1987) and 2.22 for MBN (Brookes et al., 1985). Briefly, two 10-g samples of moist field soil were weighed. One portion of each was fumigated with chloroform for 24 h, and soil MBC and MBN contents were extracted with 0.5 M K_2SO_4 in an end-to-end shaker for 1 h, then filtered through Whatman No. 42 paper. The other portion of soil was extracted directly as above. The amount of total soluble organic C and N in the fumigated and non-fumigated extracts was determined on a Multi N/C 3100 total organic C analyzer fitted with a total N unit (Analytik Jena, Germany).

We also calculated the ratio of EOC to EON (EOC:EON ratio) and the ratio of MBC to MBN (MBC:MBN ratio) to represent the quality of the soil labile organic C and N contents.

2.4. Measurements of soil potential CO_2 and N_2O emissions and microbial quotient

Soil samples in December 2016 were incubated in the laboratory to calculate soil potential CO_2 and N_2O emissions, and the metabolic quotient (qCO_2, in μg CO_2–C mg^{-1} biomass-C h^{-1}). About 10 g (dry weight equivalent) of field-moist soil was incubated in a 1-L sealed flask in the dark at 22 °C for 7 days. Gas samples of 10 mL from the headspace of the flasks were taken before and after the incubation. The CO_2 and N_2O concentrations in the gas samples were analyzed on a gas chromatograph (Agilent 7890A GC USA). Soil potential CO_2 and N_2O emissions were calculated from the differences in their concentrations in the gas samples between the two sampling times. Soil qCO_2 was calculated as the ratio of microbial respiration to MBC (Wardle and Ghani, 1995).

2.5. Soil DNA extraction and bacteria-specific 16S rRNA gene amplification

Soil genomic DNA in August 2016 and December 2016 were extracted from 0.5 g of each sample by using procedures described previously (Zhou et al., 2010). In brief, soil was placed in a 2-mL screwcap tube containing a mixture of ceramic and silica particles (Bio101, Carlsbad, CA); the mixture was homogenized for 30 s in a FastPrep bead beater cell disrupter (Bio101). After the nucleic acids had been precipitated and washed twice in 75% (vol/vol) ethanol, the final DNA was re-suspended in 100 μL of double-distilled water. Furthermore, the crude extract was purified with the Qiagen Gel Extraction Kit (Qiagen Inc, Shanghai, China). DNA concentrations were measured with a Biospec-mini spectrophotometer (Shimadzu, Kyoto, Japan).

The bacteria-specific 16S rRNA primers of 515F and 907R were used to amplify the bacterial 16S rRNA gene (Chen et al., 2016). Briefly, the oligonucleotide sequences included a 12-bp barcode fused to the forward primer in the form of barcode + forward primer. The final concentration of different components in the polymerase chain reaction (PCR) amplification mixture was as follows: 0.4 μM of each primer, 200 μM of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 1× thermophilic DNA polymerase and 10× reaction buffer ($MgCl_2$-free), 1.25 U per 50 μL of Taq DNA polymerase (Promega, Madison, WI), and DNAse- and RNAse-free filter-sterilized water. All reactions were performed in a PTC-200 thermal cycler (MJ Research Co., New York, NY). The PCR cycle conditions were: an initial denaturation or activation of the hotstart polymerase at 98 °C for 30 s, followed by 30 cycles of denaturation at 98 °C for 5 s, annealing at 53 °C for 20 s and elongation at 72 °C for 20 s, and then a final elongation step at 72 °C for 5 min. The PCR products were run on a gel and the appropriate fragments were cut and purified with the Qiagen Gel Extraction kit (Qiagen Inc, Shanghai, China). The amplicons were sequenced on an Illumina Miseq machine (Illumina, Nanjing, China).

2.6. High-throughput sequencing data analysis

The sequence data were processed mainly with QIIME (Caporaso et al., 2010) and UPARSE (Edgar, 2013). The following filter settings were used: no ambiguous bases were allowed (Huse et al., 2007) and the minimum read length was 150 bp. The raw sequences were demultiplexed by using unique barcodes that allowed no mismatches. Paired-end data for each sample were joined with FLASH (Magoč and Salzberg, 2011) using the default parameters. Reads with average quality scores below 20 were discarded. The high-quality reads were clustered at a similarity of 97% to generate operational taxonomic units (OTUs) using UPARSE. Simultaneously, chimeras were filtered. For each OTU, one representative sequence was picked. A taxonomic annotation was assigned to each representative sequence via the assign_taxonomy.py tool in QIIME, using SILVA 123 (Quast et al., 2013) as a reference database. To avoid any potential uncertainty caused by sampling error, each sample was refined to the same sequence number: 32,000 reads for 16S rRNA. These sequences have been deposited in DDBJ biosample database with an accession number of PRJDB7138.

2.7. Statistical analysis

Repeated-measure analysis of variance (ANOVA) was used to determine the effects of drought and sampling time on soil moisture content, pH, TC and TN contents, C:N ratios, EOC and EON contents, EOC:EON ratios, NH_4^+–N and NO_3^-–N contents, MBC and MBN contents, and MBC:MBN ratios, as well as the Shannon–Wiener microbial diversity index (H), and the OTUs, bacterial phyla and genera. Given that there were no significant interactive effects between drought and sampling time on soil labile organic C and N contents or on most microbial phyla and genera, we put the values of these properties across the four sampling times together and compared the mean values of these properties in the drought plots relative to the control plots. For the incubation experiment, one-way ANOVA was used to examine the effects of drought on soil potential CO_2 and N_2O emissions and qCO_2 among the treatments. After statistical analysis via ANOVA, Tukey's HSD test was used to compare the significant differences among the treatments.

For soil microbial community structure, Venn diagram analysis was used to calculate the shared and unique OTUs in August 2016 and in December 2016 among the treatments. To calculate the relative contributions of drought effects via direct and indirect pathways, redundancy analysis (RDA) was used to elucidate the relationships between soil moisture content and other physicochemical properties, and the patterns of bacterial communities based on the OTU level across the plots. The differences were considered significant at $P < 0.05$. Pearson's correlation coefficients among the soil properties were also calculated. All ANOVA and correlation analyses were performed in R (R Core Team, 2014).

3. Results

3.1. Effects of drought on soil moisture content and pH

Variations in daily mean air temperature and precipitation, soil temperature and moisture at the study site during January 2016 and March 2017 are shown in Fig. S2. The figure shows that although drought markedly decreased soil moisture content ($P < 0.05$), it had no effects on soil temperature. As expected, we found that drought significantly decreased soil moisture content (Table 1 and Fig. 1a), which was supported by the 1-year field recorder (Fig. S2). Soil pH was significantly lower in the drought plots (4.25 ± 0.09) than in the control plots (4.45 ± 0.09) (Table 1 and Fig. 1b).

Table 1
F-values showing the effects of drought (D), sampling time (S) and their interactions on soil moisture, pH, EOC, EON, EOC:EON ratios, NH_4^+–N and NO_3^-–N in a subtropical evergreen forest during the period between June 2016 and February 2017.

	Moisture	pH	EOC	EON	EOC:EON	NH_4^+–N	NO_3^-–N
D	21.98***	17.56***	0.84	0.10	6.22**	6.88**	8.15**
S	15.34***	1.54	5.15**	6.28**	1.70	45.83***	2.58
D × S	1.53	2.52	0.48	0.45	0.48	1.21	0.97

Significance level: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
EOC, extractable organic C; EON, extractable organic N.

Table 2
F-values showing the effects of drought (D), sampling time (S) and their interactions on soil MBC, MBN, MBC:MBN ratios, operational taxonomical units (OTUs) and the Shannon–Wiener diversity index (H) in a subtropical evergreen forest during the period between June 2016 and February 2017.

	MBC	MBN	MBC:MBN	OTUs	H
D	1.86	2.70	1.07	1.63	0.13
S	44.32***	12.47***	41.13***	5.67*	0.04
D × S	1.23	0.76	0.45	2.40	1.54

Significance level: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
MBC, microbial biomass C; MBN, microbial biomass N.

3.2. Effects of drought on soil labile organic C and N contents

Drought had no significant effects on soil EOC, EON, MBC and MBN contents, and MBC:MBN ratios, whereas sampling time had significant effects on all of them (Table 1 and Table 2). Overall, changes in soil EOC and EON contents showed similar patterns among the treatments across the sampling times, with higher mean values in June 2016 and August 2016 than in December 2016 and February 2017 (Fig. 2), whereas the mean values of soil MBC and MBN contents were higher in August 2016, lower in February 2017 and similar in June 2016 and December 2016 (Fig. 3). We found that there were significantly positive relationships among soil EOC, EON, MBC and MBN contents (all $P < 0.01$, Table S2) across the plots, indicating that they were good indicators of soil labile organic C and N contents across the plots. Additionally, there were no significant interactive effects between drought and sampling time on soil EOC, EON, MBC and MBN contents; EOC:EON ratios or MBC:MBN ratios (Table 1 and Table 2).

Generally, the form of soil inorganic N was dominated by NH_4^+–N at the study site, which had much higher values (one to two orders of magnitude) than NO_3^-–N contents (Fig. 2d, e). Drought significantly decreased NH_4^+–N contents across all the sampling times (Table 1 and Fig. 2d), but it produced similar soil NO_3^-–N contents to the control plots except in August 2016 (Table 1 and Fig. 2e). Sampling time had significant effects on NH_4^+–N contents (Table 1). There were no significant interactive effects between drought and sampling time on soil NH_4^+–N and NO_3^-–N contents (Table 1).

3.3. Effects of drought on soil microbial diversity and community structure

We obtained 829,640 quality sequences from 18 soil samples collected in August 2016 and December 2016 with an average of 46,091 sequences per sample. The majority of these sequences belonged to bacterial taxa and only a small proportions was unclassified or from Archaeal taxa (Table S3). In general, drought increased soil microbial OTUs and H by 10.6% and 0.5% respectively, although there were no significant differences in soil microbial OTUs and H among the treatments (Table 2 and Fig. S3). Sampling time had significant effects on soil microbial OTUs but it had no effect on H (Table 2). There were no significant interactions between drought and sampling time on soil microbial OTUs and H (Table 2).

The dominant bacterial phyla (relative abundance > 5%) across all soils were Proteobacteria (32.52–41.30%), Acidobacteria (34.47–42.64%) and Actinobacteria (6.52–8.16%), followed by the groups of Bacteroidetes, Planctomycetes, Candidate Division WPS-2, Chloroflexi, Firmicutes, Candidate Division WPS-1, Armatimonadetes and Cyanobacteria, which were less abundant (relative abundance > 0.5%) but were still found in all soils (Fig. 4). Overall, drought significantly decreased the relative abundance of Proteobacteria and Armatimonadetes, but increased the relative abundance of Acidobacteria (Table 3 and Fig. 4). We also found significant differences in Chloroflexi and Candidate Division WPS-1 among the treatments (Table 3). Sampling time had significant effects on the relative abundance of Proteobacteria, Candidate Division WPS-2, Chloroflexi, Firmicutes, Candidate Division WPS-1 and Cyanobacteria (Table 3 and Fig. 4). There were no significant interactive effects between drought and sampling time on dominant soil bacterial phyla except for Proteobacteria (Table 3).

Bacterial taxa mainly came from 16 genera, which were concentrated in Acidobacteria and Proteobacteria, with 14 genera shared by the August 2016 and December 2016 sampling times (Table 4 and Fig. S4). Among them, unclassified taxa of *Sinobacteraceae* (Gammaproteobacteria) and *Rhizomicrobium* (Alphaproteobacteria) were mainly found in August 2016 (Fig. S4b), whereas *Actinoallomurus* (Actinobacteria) and *Actinomycetales* (Actinobacteria) were mainly found in December 2016 (Fig. S4d). Drought significantly increased the relative abundance of Subgroup 2 (Acidobacteria), but it decreased the relative abundance of unclassified (Gammaproteobacteria) and *Phenylobacterium* (Alphaproteobacteria) (Table 4 and Fig. S4). Almost half of the genera were significantly affected by the sampling time. There were

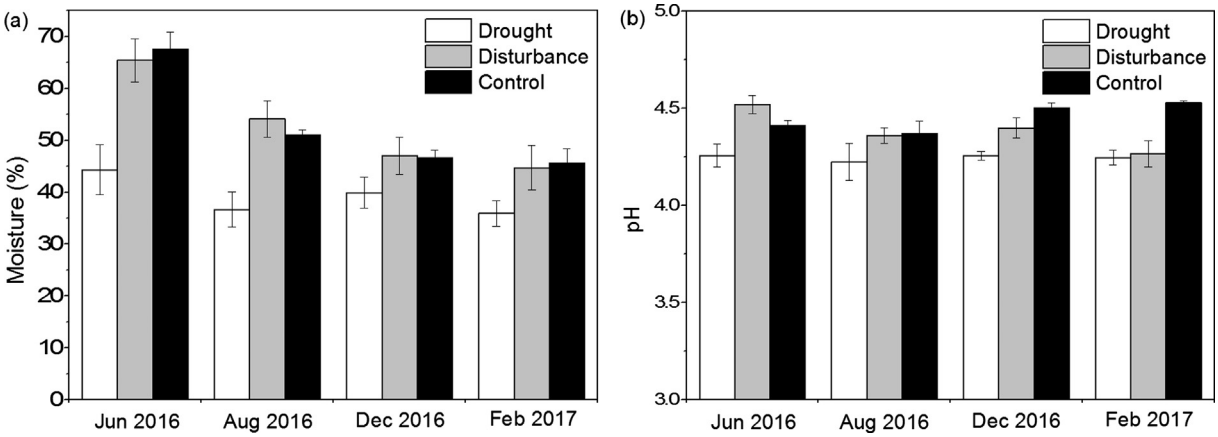

Fig. 1. Variations in soil moisture (a) and pH (b) under drought in a subtropical evergreen forest during the period between June 2016 and February 2017. Data represent the means and standard errors ($n = 3$).

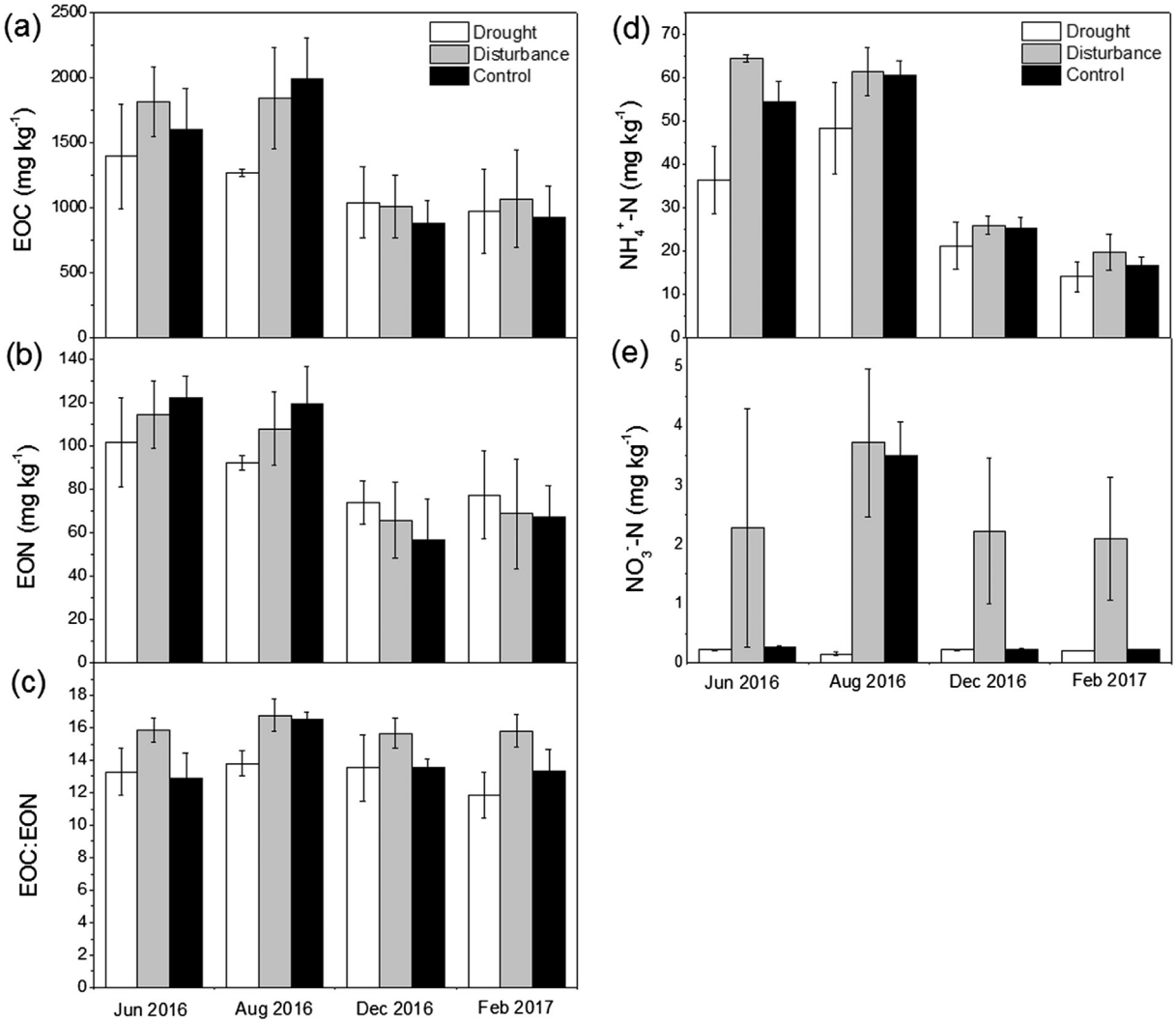

Fig. 2. Variations in soil extractable organic C (EOC) (a) and extractable organic N (EON) (b) contents, ratios of EOC to EON (c), NH_4^+–N contents (d) and NO_3^-–N (e) contents under drought in a subtropical evergreen forest during the period between June 2016 and February 2017. Data represent the means and standard errors (n = 3).

no significant interactive effects between drought and sampling time on the dominant bacterial genera except for unclassified taxa of *Rhodospirillales* (Alphaproteobacteria) (Table 4).

3.4. Relative contributions of direct and indirect drought effects on the patterns of soil microbial communities

The RDA was used to quantify the relative contributions of the factors of direct and indirect effects of drought on the patterns of soil microbial communities. Axes 1 and 2 explained 33.13% and 21.57% of the total variance in soil microbial communities in August 2016, respectively (Fig. 5a), whereas Axes 1 and 2 explained 35.06% and 16.44% of the total variance in soil microbial communities in December 2016, respectively (Fig. 5b). We found that the arrows of soil EOC and EON contents were longer than those of the other properties in August 2016 (Fig. 5a), suggesting that the patterns of soil microbial communities were mainly influenced by the indirect drought-induced changes in soil EOC and EON contents. Similar patterns of soil microbial communities were also observed in December 2016 (Fig. 5b), further confirming the importance of soil EOC and EON contents in shaping soil microbial communities at the study site. Besides, soil pH was also found to be correlated with the control plots for both sampling times, again highlighting the importance of indirect drought-induced effects of soil pH on soil microbial communities (Fig. 5).

3.5. Effects of drought on soil potential CO_2 and N_2O emissions and qCO_2

The drought treatment had significantly lower soil potential CO_2 and N_2O emissions than the control treatments (Fig. 6a, c), though there were no significant differences in soil potential CO_2 and N_2O emissions between the disturbance and the control treatments. Additionally, drought significantly decreased qCO_2 in comparison with the control treatments (Fig. 6b).

4. Discussion

In this study, we examined how extreme drought, (i.e., a 70% rainfall reduction) affected soil labile organic C and N contents, microbial communities and ecosystem functions such as N_2O emissions comprehensively in a subtropical evergreen forest ecosystem. We found that there were no significant differences in soil TC and TN contents, soil labile organic C and N contents, soil microbial communities and its ecosystem functions between the disturbance and control treatment (Fig. S5 and Fig. S6). However, we found that drought slightly decreased soil labile C and N contents, and soil potential CO_2 and N_2O emissions, and greatly altered the microbial communities underlying

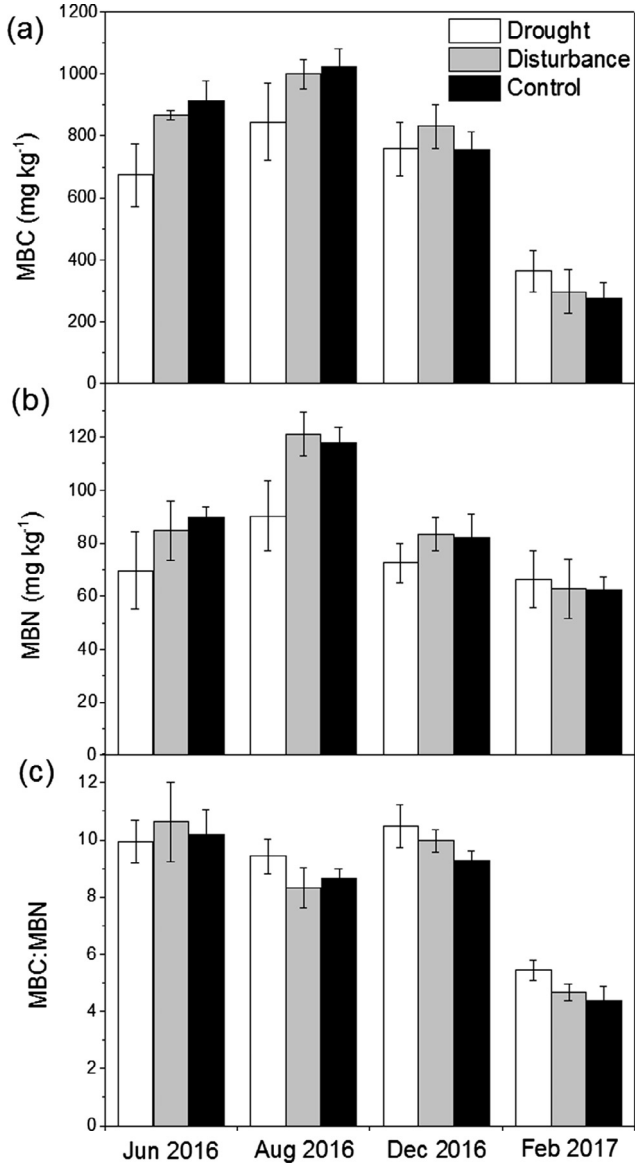

Fig. 3. Variations in soil microbial biomass C (MBC) (a) and microbial biomass N (MBN) (b) contents and ratios of MBC to NBN (c) under drought in a subtropical evergreen forest during the period between June 2016 and February 2017. Data represent the means and standard errors (n = 3).

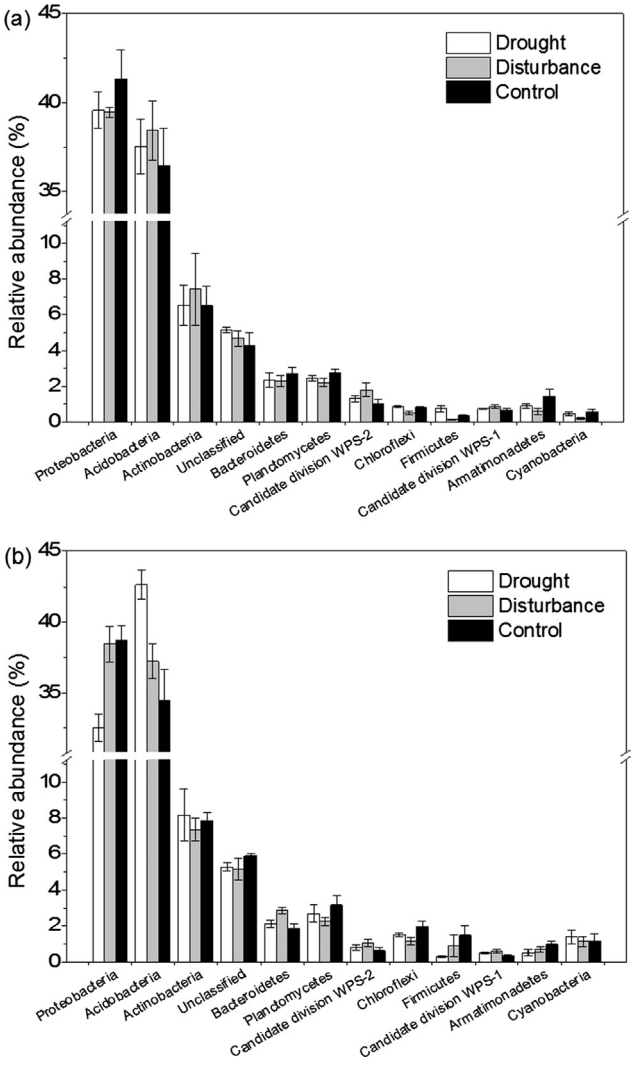

Fig. 4. The most abundant bacterial phyla under drought in a subtropical evergreen forest in August 2016 (a) and December 2016 (b). Data represent the means and standard errors (n = 3).

soil C and N cycling (Fig. 7), which provides a mechanistic understanding of soil C and N cycling in this region in the context of drought scenarios in the future.

4.1. Effects of drought on soil labile organic C and N contents

In this study, we found that drought decreased soil TC and TN contents by 4.5% and 6.3% respectively (Fig. S5 and Fig. 7), although there were no significant differences among the treatments (Table S1). The results were consistent with previous studies showing that soil organic C and total N contents were not sensitive indicators of changes in soil quality under different management practices (Haynes, 2005; Shahid et al., 2017).

Soil labile organic C and N pools play an important role in maintaining soil nutrient cycling in forest ecosystems (Yuan et al., 2017). These pools of organic matter are readily decomposed by microorganisms and act as a short-term nutrient reservoir for plants (Laik et al., 2009; Zhou et al., 2016). Some studies have used microbial biomass as an indicator of labile soil organic C and N pools (Degens and Sparling, 1996), whereas others considered the hot-water-extractable C and N pools (Cepáková et al., 2016).

In this study, no pronounced differences in soil labile organic C and N contents were found among the treatments in this subtropical

Table 3
F-values showing the effects of drought (D), sampling time (S) and their interactions on the dominant bacterial phyla in a subtropical evergreen forest during the period between August 2016 and December 2016.

	D	S	D × S
Proteobacteria	6.81*	15.16**	3.96*
Acidobacteria	3.79*	0.21	2.70
Actinobacteria	0.02	0.88	0.27
Unclassified	0.25	4.17	1.66
Bacteroidetes	0.79	0.50	3.01
Planctomycetes	2.46	0.85	0.16
Candidate Division WPS-2	3.08	7.37*	0.25
Chloroflexi	6.43*	40.75***	1.89
Firmicutes	1.63	5.67*	2.40
Candidate Division WPS-1	3.92*	17.07**	0.07
Armatimonadetes	4.32*	1.94	0.93
Cyanobacteria	0.50	14.32**	0.24

Significance level: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4
F-values showing the effects of drought (D), sampling time (S) and their interactions on the dominant bacterial genera in a subtropical evergreen forest during the period between August 2016 and December 2016.

	D	S	D × S
Acidobacteria Subgroup 2	6.12*	4.73*	2.04
Acidobacteria Subgroup 1	0.57	5.85*	0.63
Gammaproteobacteria (unclassified)	6.37*	7.80*	2.48
Unclassified	0.25	4.17	1.66
Acidobacteria Subgroup 1 (unclassified)	2.49	1.47	1.28
Alphaproteobacteria Rhodospirillales (unclassified)	8.70**	175.39***	13.15**
Alphaproteobacteria Rhizobiales (unclassified)	1.46	1.15	0.84
Betaproteobacteria *Burkholderia*	2.52	6.62*	1.96
Gammaproteobacteria Sinobacteraceae (unclassified)	1.76	7.15*	0.67
Acidobacteria Candidatus Solibacter	0.70	7.23*	1.92
Alphaproteobacteria Rhodospirillaceae (unclassified)	0.27	3.30	2.61
Actinobacteria *Actinoallomurus*	0.22	0.36	0.30
Actinobacteria *Actinomycetales* (unclassified)	0.08	1.93	0.11
Alphaproteobacteria *Phenylobacterium*	4.98*	0.02	0.37
Alphaproteobacteria *Rhizomicrobium*	1.90	17.96**	0.85
Acidobacteria Subgroup 3	1.11	0.02	0.35

Significance level: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

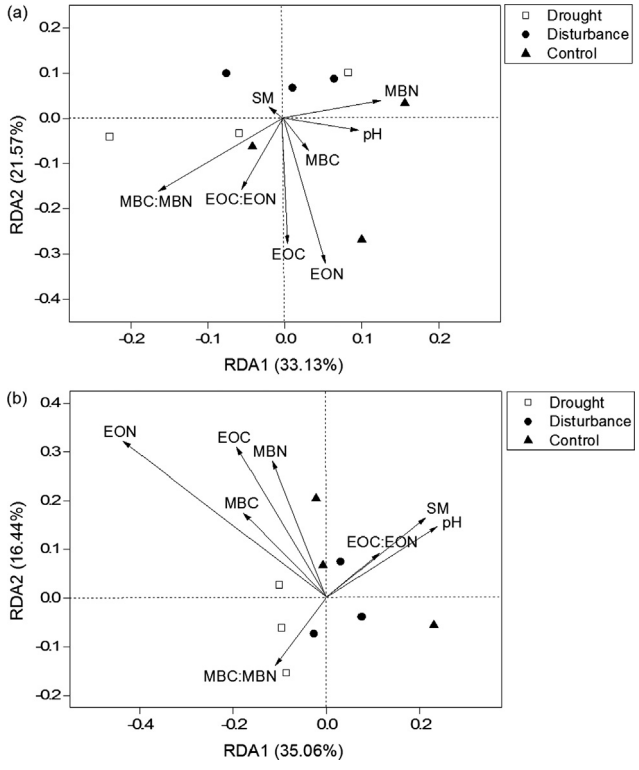

Fig. 5. Redundancy analysis (RDA) of the relationships between soil physicochemical properties and the patterns of microbial communities under drought in a subtropical evergreen forest in August 2016 (a) and December 2016 (b). SM, soil moisture; EOC, extractable organic C; EON, extractable organic N; MBC, microbial biomass C; MBN, microbial biomass N.

evergreen forest ecosystem (Table 1 and Table 2). The reason for this could be attributed to the large variations in the soil labile organic C and N pools, which may have masked the differences among the treatments across the sampling times. We also found that there were significant correlations between soil EOC and EON contents, and soil moisture content (EOC: $r = 0.654$, EON: $r = 0.645$, both $P < 0.001$), which was consistent with previous studies showing that changes in soil labile organic C and N contents were found to be closely associated with changes in temperature, soil moisture and substrate availability (Li et al., 2016; Murphy et al., 2007; Zhou et al., 2013). The large changes in soil moisture content seen in our study resulted in large variations in soil labile organic C and N contents across the sampling times (Fig. 2 and Fig. 3). It should be noted that the 70% rainfall reduction treatment was set up in the understory of the forest, implying that the forest canopy and tree trunks could have received rainfall. Given that forest ecosystems have some resilience to drought (Pickles and Simard, 2017), it may take longer to detect significant differences in soil labile organic C and N contents.

The pools of soil labile C and N were mainly derived from the mineralization of litter and soil organic matter in soils (Zhou et al., 2012) and soil microorganisms are critical in these processes. Overall, drought decreased soil EOC, EON, MBC and MBN contents by 13.4%, 5.7%, 11.1%, and 15.2% respectively at the study site (Fig. 7). Two possible reasons could explain this. First, drought may have reduced soil C inputs from plants, as we found less litter biomass in the drought plots (593.96 g/m^2 vs 654.89 g/m^2 in the control plots) in 2016. Second, drought inhibited soil microbial activities (Fig. 6b) and altered the microbial community structure (see Fig. 4 and Fig. S4).

4.2. Effects of drought on the soil microbial community structure

Overall, the soil microbial community in this subtropical evergreen forest was dominated by Proteobacteria, Acidobacteria and Actinobacteria (Fig. 4), which have been extensively reported to be the most abundant phyla in many other forest ecosystems by high-throughput sequencing analysis (Chodak et al., 2015; Zhou et al., 2017). We found that the effects of drought on soil microbial community composition mainly occurred through its influence at the bacterial genus level. For example, unclassified taxa of *Sinobacteraceae* (Gammaproteobacteria) and *Rhizomicrobium* (Alphaproteobacteria) were mainly found in August 2016 (Fig. S4b), but *Actinoallomurus* (Actinobacteria) and *Actinomycetales* (Actinobacteria) were mainly found in December 2016 (Fig. S4d).

Different bacterial phyla have different responses to drought stress depending on their desiccation-related survival strategies (Barnard et al., 2013), which results in a considerable shift in the bacterial community structure (Chodak et al., 2015). Gram-positive bacteria such as Actinobacteria and Firmicutes are tolerant to desiccation stress, as they have rigid cell walls and are able to form spores (Chodak et al., 2015; Uhlířová et al., 2005). In our study, however, drought had no effect on the relative abundance of Actinobacteria, although drought increased the relative abundance of Actinobacteria by 2.3% compared with the control treatments (Fig. 7). In addition, we found a decrease in the relative abundance of Firmicutes in the drought plots (Fig. 7). The different responses of these two Gram-positive bacteria to drought maybe further suggest that it may take a longer time to detect significant differences in microbial communities in our rainfall reduction manipulation plots.

Gram-negative bacteria such as Proteobacteria have been reported to be vulnerable to drought stress (Chodak et al., 2015) and the abundance of Betaproteobacteria has been found to be positively correlated with the net C mineralization rate (an index of C availability) (Fierer et al., 2007). In this study, we found that drought significantly decreased the relative abundance of Proteobacteria (Table 3 and Fig. 4). The reason for this could be attributed to the lower soil EOC contents in the drought plots, as there is a close relationship between Proteobacteria and soil labile organic C availability (Singh et al., 2007).

4.3. Relative contributions of direct and indirect drought effects on the patterns of soil microbial communities

It is known that soil microbial community composition and diversity vary greatly among different ecosystems, and this variation has been thought to be correlated with changes in many soil factors such as soil pH (Jones et al., 2009), soil organic C and TN contents (Chodak et al.,

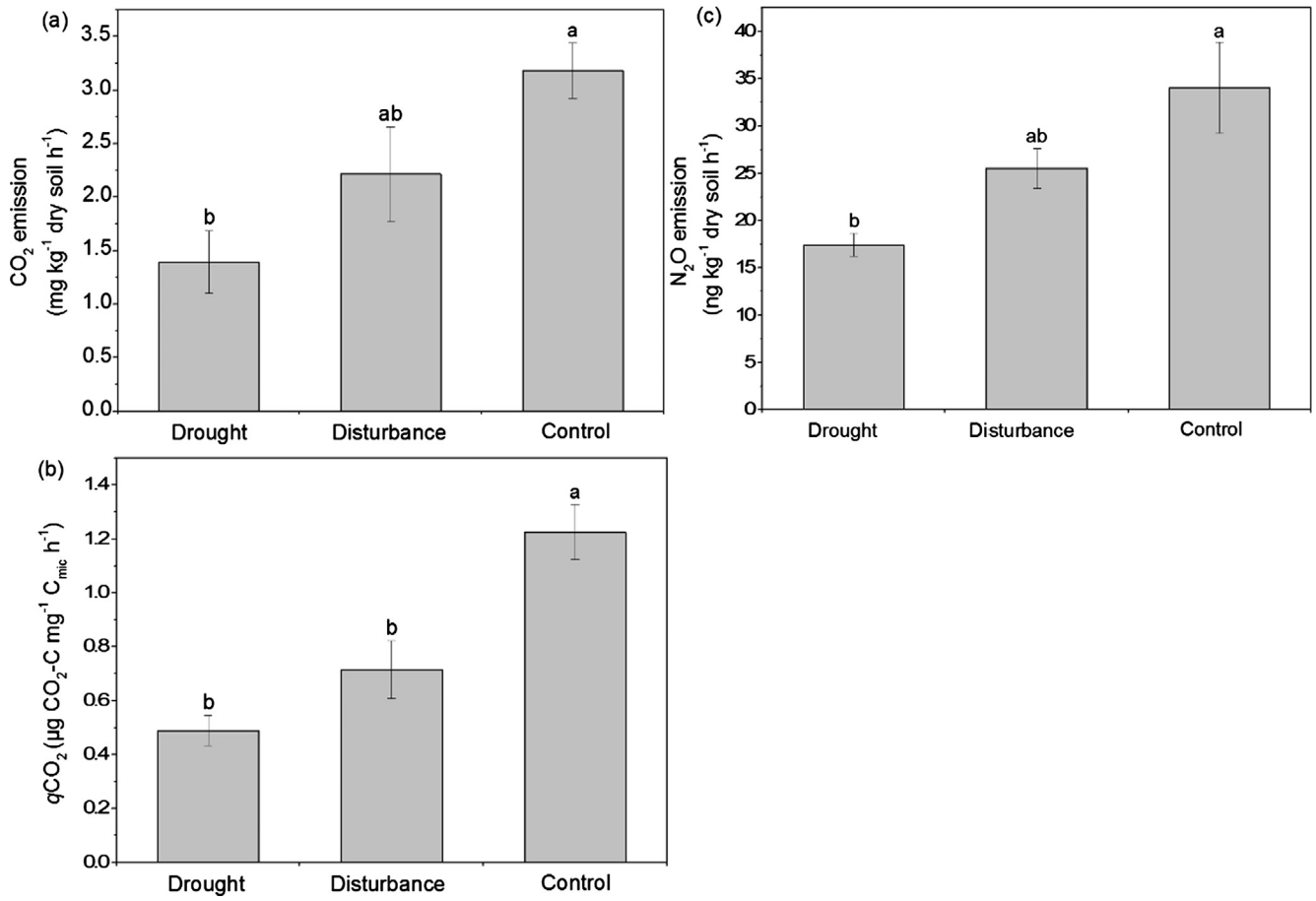

Fig. 6. Variations in soil potential CO_2 emissions (a), microbial quotient (qCO_2) (b) and soil potential N_2O emissions (c) under drought in a subtropical evergreen forest in December 2016. Data represent the means and standard errors (n = 3).

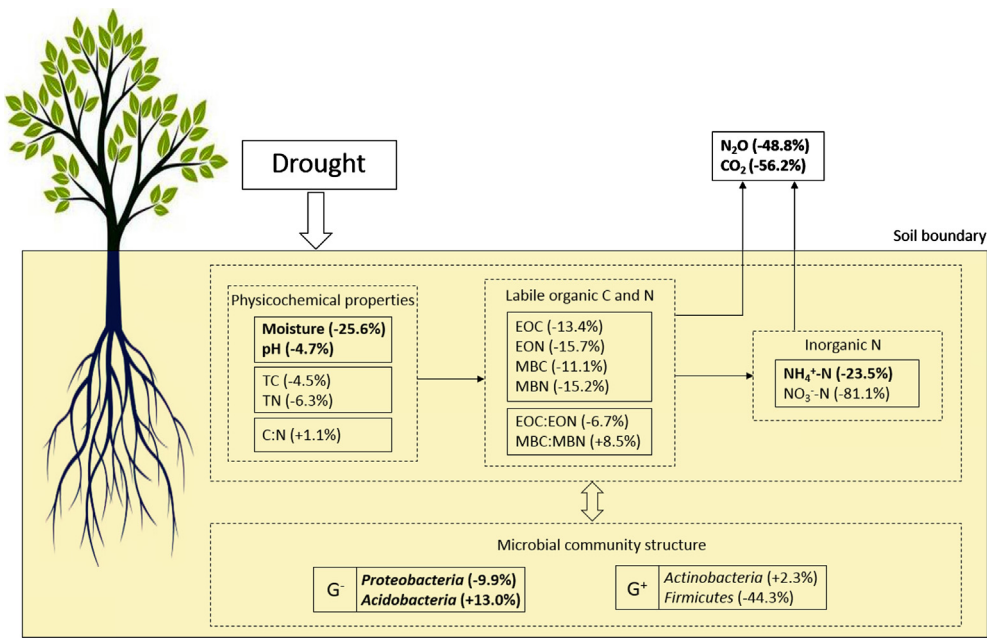

Fig. 7. The responses of soil labile organic C and N and microbial communities to drought in a subtropical evergreen forest, where EOC is extractable organic C, EON is extractable organic N, MBC is microbial biomass C and MBN is microbial biomass N. The response of each parameter to drought is shown in parentheses. The percentages followed by "+" and "−" indicate the extent to which drought increases and decreases the parameter on average. The parameters in bold were significantly affected by drought.

2015), net soil C mineralization rate (Fierer et al., 2007) and soil nutrient quality (Zhou et al., 2017). In this study, we speculated that drought might influence soil microbial communities via direct and indirect pathways. We found that the indirect drought-induced effects of soil EOC and EON contents played a weightier role in influencing the patterns of soil microbial communities than the direct drought-induced effects of soil moisture among the treatments (Fig. 5). Previously, soil pH has often been reported to be the overarching factor driving the patterns of soil microbial communities in many ecosystems (Chodak et al., 2015; Fierer, 2017; Jones et al., 2009; Zhou et al., 2017). It is

interesting to note that soil pH correlated better with the control plots for both sampling times than the other soil properties (Fig. 5), highlighting the importance of soil pH in shaping soil microbial communities at the ambient site. On the other hand, we found that drought significantly decreased soil pH (Table 1 and Fig. 1b), which was supported by the higher relative abundance of Acidobacteria in the drought plots (Table 3 and Fig. 4). Previous studies have stated that Acidobacteria are more abundant at lower pH in soils, and can indicate changes in soil pH (Jones et al., 2009). Fierer (2017) showed that soil pH was the best predictor of bacterial community composition at the landscape scale (from pH 4 to pH > 8). Overall, our results indicated that apart from drought-induced changes in soil labile organic C and N contents, soil pH also played an important role in driving soil microbial communities among the treatments.

4.4. Effects of drought on soil potential N_2O emissions

N_2O is an important greenhouse gas involved in global warming, with a global warming potential of about 298 times that of CO_2. N_2O also contributes to stratospheric ozone depletion (Nakicenovic and Swart, 2000; Ravishankara et al., 2009). Generally, dissolved organic N first converts to NH_4^+ through ammonification, then to NO_3^- by nitrification and finally to N gas via denitrification (Shen et al., 2014). It has been reported that denitrification was the main source of N_2O emissions in subtropical forests (Zhang et al., 2011). In this study, we found that drought significantly decreased soil potential N_2O emissions (Fig. 6c), which resulted from the increase in soil aeration under drought, thus inhibiting the N_2O emissions from denitrification. Further studies are needed to clarify the contributions of nitrification and denitrification to total N_2O production under drought in this subtropical evergreen forest.

5. Conclusions

Through analyzing soil labile organic C and N contents, the microbial community structure and its ecosystem function under extreme drought (i.e., a 70% rainfall reduction) in a subtropical evergreen forest ecosystem, we found that drought slightly decreased soil EOC, EON, MBC and MBN contents by 13.4%, 5.7%, 11.1% and 15.2% respectively in general, though there were no significant differences between the drought plots and the control plots. The dominant bacterial phyla across all soils were Proteobacteria, Acidobacteria and Actinobacteria. Different bacterial phyla had different responses to the drought stress: (1) drought had no effects on Actinobacteria and Firmicutes, which indicated that it may take a longer time to detect significant differences in these two Gram-positive bacteria under drought; (2) drought significantly increased the relative abundance of Acidobacteria, which was supported by the lower soil pH in the drought plots; (3) drought produced a significantly lower relative abundance of Proteobacteria, which was supported by the lower soil labile organic C and N contents under drought. Soil microbial communities might, to some extent, be more sensitive to drought stress at the genus level in this subtropical evergreen forest. We found that the indirect drought-induced effects of soil EOC and EON contents had more influence on the patterns of soil microbial communities than the direct drought-induced effects of soil moisture. Additionally, drought had significantly lower soil potential CO_2 and N_2O emissions, further confirming that drought inhibited soil microbial activity. Overall, drought had a cascading effect on soil physicochemical properties, labile organic C and N contents and soil microbial communities (Fig. 7). Given how soil labile organic C and N contents act as a nutrient reservoir for plant growth, our results imply that drought may decrease forest ecosystem productivity in this subtropical evergreen forest in the future.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foreco.2018.06.036.

References

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J. 7, 2229–2241.
Brookes, P.C., Kragt, J.F., Powlson, D.S., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biol. Biochem. 17, 831–835.
Canarini, A., Dijkstra, F.A., 2015. Dry–rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling. Soil Biol. Biochem. 81, 195–203.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Cepáková, Š., Tošner, Z., Frouz, J., 2016. The effect of tree species on seasonal fluctuations in water-soluble and hot water-extractable organic matter at post-mining sites. Geoderma 275, 19–27.
Chen, L., Luo, Y., Xu, J.M., Yu, Z.Y., Zhang, K.L., Brookes, P.C., 2016. Assessment of bacterial communities and predictive functional profiling in soils subjected to short-term fumigation–incubation. Microb. Ecol. 72, 240–251.
Chodak, M., Gołębiewski, M., Morawska-Płoskonka, J., Kuduk, K., Niklińska, M., 2015. Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. Ann. Microbiol. 65, 1627–1637.
Degens, B., Sparling, G., 1996. Changes in aggregation do not correspond with changes in labile organic C fractions in soil amended with ^{14}C-glucose. Soil Biol. Biochem. 28, 453–462.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.
Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive Earth's biogeochemical cycles. Science 320, 1034–1039.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590.
Gao, Q., Hasselquist, N.J., Palmroth, S., Zheng, Z.M., You, W.H., 2014. Short-term response of soil respiration to nitrogen fertilization in a subtropical evergreen forest. Soil Biol. Biochem. 76, 297–300.
Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Adv. Agron. 85, 221–268.
Hueso, S., García, C., Hernández, T., 2012. Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol. Biochem. 50, 167–173.
Huse, S., Huber, J., Morrison, H., Sogin, M., Welch, D., 2007. Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biol. 8, R143.
IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J. 3, 442–453.
Kaisermann, A., Roguet, A., Nunan, N., Maron, P.-A., Ostle, N., Lata, J.-C., 2013. Agricultural management affects the response of soil bacterial community structure and respiration to water-stress. Soil Biol. Biochem. 66, 69–77.
Kwon, M.J., Haraguchi, A., Kang, H., 2013. Long-term water regime differentiates changes in decomposition and microbial properties in tropical peat soils exposed to the short-term drought. Soil Biol. Biochem. 60, 33–44.
Laik, R., Kumar, K., Das, D.K., Chaturvedi, O.P., 2009. Labile soil organic matter pools in a calciorthent after 18 years of afforestation by different plantations. Appl. Soil Ecol. 42, 71–78.
Li, Z.Q., Zhao, B.Z., Zhang, J., 2016. Effects of maize residue quality and soil water content on soil labile organic carbon fractions and microbial properties. Pedosphere 26, 829–838.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Murphy, D.V., Stockdale, E.A., Poulton, P.R., Willison, T.W., Goulding, K.W.T., 2007. Seasonal dynamics of carbon and nitrogen pools and fluxes under continuous arable and ley-arable rotations in a temperate environment. Eur. J. Soil Sci. 58, 1410–1424.
Nakicenovic, N., Swart, R., 2000. Special report on emissions scenarios: a special report of

Working Group III of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
Pickles, B.J., Simard, S.W., 2017. Mycorrhizal networks and forest resilience to drought. In: Johnson, N.C., Gehring, C., Jansa, J. (Eds.), Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. Elsevier Academic Press, Amsterdam, pp. 319–339.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.
Core Team, R., 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326, 123–125.
Schaeffer, S.M., Homyak, P.M., Boot, C.M., Roux-Michollet, D., Schimel, J.P., 2017. Soil carbon and nitrogen dynamics throughout the summer drought in a California annual grassland. Soil Biol. Biochem. 115, 54–62.
Schlesinger, W.H., Dietze, M.C., Jackson, R.B., Phillips, R.P., Rhoades, C.C., Rustad, L.E., Vose, J.M., 2016. Forest biogeochemistry in response to drought. Glob. Chang Biol. 22, 2318–2328.
Shahid, M., Nayak, A.K., Puree, C., Tripathi, R., Lal, B., Gautam, P., Bhattacharyya, P., Mohanty, S., Kumar, A., Panda, B.B., Kumar, U., Shukla, A.K., 2017. Carbon and nitrogen fractions and stocks under 41 years of chemical and organic fertilization in a sub-humid tropical rice soil. Soil Tillage Res. 170, 136–146.
Shen, J.P., Xu, Z.H., He, J.Z., 2014. Frontiers in the microbial processes of ammonia oxidation in soils and sediments. J. Soils Sediments 14, 1023–1029.
Singh, B.K., Munro, S., Potts, J.M., Millard, P., 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl. Soil Ecol. 36, 147–155.
Song, Y.C., Da, L.J., 2016. Evergreen broad-leaved forest of East Asia. In: Box, E.O. (Ed.), Vegetation Structure and Function at Multiple Spatial, Temporal and Conceptual Scales. Springer International Publishing, Cham, pp. 101–128.
Uhlířová, E., Elhottová, D., Tříska, J., Šantrůčková, H., 2005. Physiology and microbial community structure in soil at extreme water content. Folia Microbiol. 50, 161–166.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.
Wang, X.H., Kent, M., Fang, X.F., 2007. Evergreen broad-leaved forest in Eastern China: its ecology and conservation and the importance of resprouting in forest restoration. For. Ecol. Manag. 245, 76–87.
Wang, Y.H., Yan, D.H., Wang, J.F., Ding, Y., Song, X.S., 2017. Effects of elevated CO_2 and drought on plant physiology, soil carbon and soil enzyme activities. Pedosphere 27, 846–855.
Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol. Biochem. 27, 1601–1610.
Xiao, Y., Huang, Z.G., Lu, X.G., 2015. Changes of soil labile organic carbon fractions and their relation to soil microbial characteristics in four typical wetlands of Sanjiang Plain, Northeast China. Ecol. Eng. 82, 381–389.
Yuan, Y., Zhao, Z.Q., Li, X.Z., Wang, Y.Y., Bai, Z.K., 2017. Characteristics of labile organic carbon fractions in reclaimed mine soils: evidence from three reclaimed forests in the Pingshuo opencast coal mine, China. Sci. Total Environ. 613–614, 1196–1206.
Zhang, J.B., Cai, Z.C., Zhu, T.B., 2011. N_2O production pathways in the subtropical acid forest soils in China. Environ. Res. 111, 643–649.
Zhou, X.Q., Chen, C.R., Wu, H.W., Xu, Z.H., 2012. Dynamics of soil extractable carbon and nitrogen under different cover crop residues. J. Soils Sediments 12, 844–853.
Zhou, X.Q., Wu, H.W., Li, G.D., Chen, C.R., 2016. Short-term contributions of cover crop surface residue return to soil carbon and nitrogen contents in temperate Australia. Environ. Sci. Pollut. Res. 23, 23175–23183.
Zhou, X.Q., Guo, Z.Y., Chen, C.R., Jia, Z.J., 2017. Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. Biogeosciences 14, 2101–2111.
Zhou, X.Q., Wang, J.Z., Hao, Y.B., Wang, Y.F., 2010. Intermediate grazing intensities by sheep increase soil bacterial diversities in an Inner Mongolian steppe. Biol. Fert. Soils 46, 817–824.
Zhou, X.Q., Chen, C.R., Wang, Y.F., Xu, Z.H., Duan, J.C., Hao, Y.B., Smaill, S., 2013. Soil extractable carbon and nitrogen, microbial biomass and microbial metabolic activity in response to warming and increased precipitation in a semiarid Inner Mongolian grassland. Geoderma 206, 24–31.