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Drought accelerated recalcitrant carbon loss by changing soil aggregation and microbial communities in a subtropical forest

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ABSTRACT

Subtropical forests are considerable carbon (C) sinks in Asia, yet are facing the threat of drought with increased frequency and prolonged duration. Drought may directly and indirectly impact soil C cycling, potentially affecting the fate of the soil organic carbon (SOC) storage. In a subtropical evergreen broad-leaved forest of eastern China, five years of rainfall reduction experiment resulted in an average of 52.6% decrease in soil water content. In this study, the responses of SOC composition, soil aggregate stability, microbial extracellular enzymatic activities, fungal and bacterial community structures under long-term drought were assessed. Our results showed that drought resulted in loss of a third of large macroaggregates, and doubled the proportion of microaggregates. The non-hydrolyzed carbon (NHC) content decreased by over 50% in large macroaggregates, leading to increased sensitivity of SOC to decomposition. Compared with fungi, bacteria were more sensitive to drought. The majority of the affected taxa showed reduced abundances, while that of *Actinobacteria*, a group commonly associated with recalcitrant C degradation, significantly increased. Drought also increased the overall peroxidase activity typically involved in recalcitrant C turnover, although it reduced hydrolytic enzyme activities in macroaggregates. These findings revealed that drought not only decreased SOC stability through macroaggregate disintegration and changing its chemical characteristics, but also shifted microbial communities in both composition and activities toward enhanced abilities of recalcitrant C conversion. This study highlights the importance of understanding microbially-mediated C turnover processes to better predict the fate of SOC storage in response to long-term drought.

1. Introduction

Terrestrial ecosystems around the world are experiencing unprecedented extreme drought, with the frequency and intensity of drought projected to continually increase over the next 30–60 years (Dai, 2013). Drought may substantially influence a series of ecosystems functions, such as soil C storage and the associated C cycling processes (Bouskill et al., 2013). For example, in the summer of 2013, southern China experienced the most severe drought in the past one hundred years, resulting in a net reduction of 101.5 Tg C in C sequestration (Yuan et al., 2016). The effect of drought on C storage have been assessed in a range of field and modeling studies, primarily based on plant primary productivities and the intensities of soil respiration (Huang et al., 2016; Yuan et al., 2016; Wagg et al., 2017). Yet the response of the belowground SOC turnover processes to drought and how such responses were mediated by the inhabited microbial communities were less explored, particularly in forest ecosystems. Nonetheless, such investigations may provide mechanistic understandings crucial for better predictions of the response and fate of the forest SOC pool under the scenario of prolonged drought.

Physical protection of SOC through soil aggregation is one essential mechanism for long-term stability of the SOC pool (Oades and Waters,

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1991; Blanco-Canqui and Lal, 2004; Six et al., 2004). Fresh organic matter first enters large aggregates (Six et al., 1999), where efficient water and nutrient exchange owing to the porous structure favors penetration of fungal hyphae, promoting the initiation of litter decomposition. Residual organic substances circulate into microaggregates, which are in turn enriched in relatively recalcitrant C substrates. Compared to macroaggregates, microaggregates offer stronger physical and chemical protection over SOC due to lower porosity as well as higher mineral binding (e.g., sorption) and chelation (Kaiser and Guggenberger, 2003; King, 2011). As a result, the turnover rate of SOC is much slower in microaggregates, with estimated turnover time of up to 80 years (Carter, 2002). Hence, microaggregates comprise an important part of the stable soil C pool.

Due to the relative fragility of the large aggregates (>2 mm in diameter), influences of the changing climate or land management practices are typically first observed by loss of large aggregates, as well as release of microaggregates (<0.25 mm in diameter) and altered dynamics of SOC conversion (Vos et al., 2013). Previous studies mostly focused on how environmental or anthropogenic disturbances affected the turnover and SOC storage of large aggregates (Puget et al., 1995; Jastrow et al., 1998; Bailey et al., 2012; Cheng et al., 2015), yet effects on microaggregates were often neglected. Until recently, increasing evidence has suggested that compared to large aggregates, microaggregates support more diverse microbial communities and higher enzymatic activities (Bach and Hofmockel, 2014; Kumar et al., 2017; Bach et al., 2018). Importantly, microaggregates contain higher fractions of recalcitrant SOC and offer key contribution to the overall SOC stability (Verchot et al., 2011). Hence, we propose that more attention should be paid to changes in microaggregates, particularly those of the microbial communities and microbially mediated C turnover processes, to better predict the fate of SOC in the context of future climate change.

The effect of drought on microbially mediated C turnover processes could be through impacts on the structure and functions of soil microbial communities (Carney et al., 2007; Zhou et al., 2012a, b; Bouskill et al., 2013; Classen et al., 2015). Osmotic stress associated with drought could create selective pressure, which affects microbial physiology and favors growth of drought-resistant taxa (Bouskill et al., 2013). Under long-term drought, changes in plant primary productivity and biomass distribution may further impact microbial community composition and decomposing functions by changing substrate supply (Farooq et al., 2009). For instance, increased abundances of microbial taxa or functional genes specialized in decomposition of certain types of organic matter may in turn impact the structure of the SOC pool (Cheng et al., 2017). Drought could also affect the activities of microbially secreted extracellular enzymes, which is rate-limiting for SOC decomposition. Reduced enzymatic activities under conditions of drought were repeatedly observed (Sardans and Peñuelas, 2005). However, higher oxidase potential is typically found in arid soils, which could facilitate decomposition of chemically recalcitrant compounds and limit SOC accumulation (Stursova and Sinsabaugh, 2008; Sinsabaugh, 2010). Therefore, microbial responses to drought, particularly in those taxa or functions critical to SOC decomposition and cycling, may considerably influence the dynamics of the SOC storage.

Subtropical forests have higher average net ecosystem productivity (NEP) compared to tropical and temperate forests, accounting for 8% of global forest NEP (Yu et al., 2014). Due to the low atmospheric conditions controlled by the Western Pacific Subtropical High System, subtropical regions are typically influenced by seasonal droughts (Zhang et al., 2016). Prolonged duration of drought frequently occurs, especially over the hot season (Stocker et al., 2013), which may strongly impact primary productivity and biodiversity, and further change the regional C cycle. In order to assess the response and microbial mechanisms of SOC dynamics under prolonged drought as well as the implications on the fate of the SOC storage, field and laboratory experiments were conducted based on a rainfall reduction platform established in a subtropical evergreen forest in eastern China. Soil aggregation structure, the composition of microbial communities as well as extracellular enzymatic activities were investigated. Through integrative analyses of these results, we aim to answer the following questions: (1) how SOC storage and the C turnover processes respond to drought and (2) how such responses were mediated by the changes of soil microbial community structure and extracellular enzymatic activities.

2. Materials and methods

2.1. Study site

The experimental site is located in Tiantong National Forest Ecosystem Observation and Research Station (29°52′ N, 121°39′ E, 163 m above sea level) in Zhejiang Province, Eastern China. The region is characterized of subtropical monsoon climate, with a hot summer and a cold winter. Mean annual temperature and precipitation are 16.2 °C and 1374.7 mm, respectively. The soil type is Acrisol, with its texture being a clay loam with 6.8% sand, 55.5% silt, and 37.7% clay (Zhou et al., 2017). The dominant species include *Schima superba, Castanopsis fargesii* and *Lithocarpus glaber*.

The experimental platform was established in 2013 with three blocks of similar topography (slope and aspect), vegetation types and site properties (i.e., vegetation abundance and distribution, and the amount of rocks). Three plots were contained within each block, and were allocated randomly to the following three treatments: (1) Drought treatment (2) Disturbance control and (3) Control. The size of each plot is 25 m × 25 m, and the intervals among plots are at least 5 m. A buffer region of 2.5 m width was set around each plot. In order to simulate the extreme drought scenario, concave transparent polycarbonate boards were evenly fixed at a height of 1.5–3.5 m above ground in the Drought plots, and covered roughly 70% of the total area. To control for the effect of the shadows produced by these polycarbonate boards on the soil, a light interference treatment (Disturbance control) was set up by fixing the transparent polycarbonate boards convexly to allow the rainfall to pass through. The control plots were maintained in the natural condition without any disturbance. A 2 m-deep trench was dug around each plot, and polycarbonate isolation panels were placed around the plot to prevent lateral runoff of rainfall.

2.2. Soil sampling

Soil sampling was conducted in March 2018, a relatively wet season of the year. After removing litter and surface debris, the top 10 cm soil was sampled from each plot using soil corers (5 cm in diameter) with an inner bulldozer device to ensure the integrity of the soil core. Nine soil cores were collected in each plot following a grid-point sampling pattern, i.e., each plot was evenly divided into nine grids, and one soil sample was collected at the center of each grid. Intact soil cores were placed in sterile plastic bags, stored on ice and transported to the laboratory. The soil cores were gently broken along the natural points of weakness. Large roots and rocks were removed through an 8 mm sieve. The soils of each plot were combined, thoroughly mixed, and divided into two parts. One part was used for physical-chemical analyses of the unfractionated soils, the other part was used for soil aggregate fractionation.

2.3. Aggregate fractionation

Aggregate fractionation was performed with the 'optimal moisture' method, which exhibited less perturbation to soil microbial communities compared to regular dry sieving and wet sieving methods (Bach and Hofmockel, 2014; Schutter and Dick, 2002). Specifically, soil samples were dried to approximately 10% (0.10 g H_2O g^{-1} dry soil) at 4 °C, which took approximately five to six days depending on the original soil moisture. Although this approach had greatly decreased the soil water content, all soils were treated with the same procedures so that the

relative comparisons among treatments were still valid. Approximately 500 g of soil was passed through a stack of sieves, followed by shaking at 200–250 rpm for 2 min, generating four aggregate fractions: large macroaggregates (>2 mm, S1), medium macroaggregates (1–2 mm, S2), small macroaggregates (0.25–1 mm, S3), and microaggregates (<0.25 mm, S4). The aggregates were gently removed from the sieves. A part of the samples was stored at −80 °C for DNA extraction. The remaining soils were immediately used for soil physicochemical and enzymatic analyses.

The mean weight diameter (MWD, mm) and geometric mean diameter (GMD, mm) were used to assess soil aggregate stability (Kemper and Rosenau, 1986). The MWD (Eq. (1)) and GMD (Eq. (2)) were calculated by the following equations:

$$\mathrm{MWD} = \sum_{i=1}^{n} (Xi * Wi) \quad (1)$$

$$\mathrm{GMD} = \mathrm{EXP}\left[\left(\sum_{i=1}^{n} Wi * \lg(Xi)\right) \Big/ \left(\sum_{i=1}^{n} Wi\right)\right] \quad (2)$$

Where Xi is the mean diameter of the specific aggregate fraction (in mm), Wi is the mass proportion of soil aggregates in the i'th size fraction, and n is the number of size fractions. Hence, lower MWD and GMD indicate less stable soil aggregation.

2.4. Enzymatic assays

The activities of four hydrolases: cellobiohydrolase (CBH), β-1,4-glucosidase (βG), acid phosphatase (AP), β-1,4-N-acetylglucosaminidase (NAG), as well as two oxidases: polyphenol oxidase (PPO), peroxidase (PER) were determined according to the methods by Saiya-Cork et al. (2002). These enzymes are involved in the cycling of soil carbon, nitrogen and phosphorus. Hydrolase activities were measured by microplate fluorescence method and using substrates containing umbelliferone (MUB). Oxidoreductase activities were assessed in microplates based on light absorption using L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate. The functions of the six enzymes and the substrates used are shown in Table 1. Briefly, 1.5 g of soil was blended with 125 ml of 50 mM acetate buffer (pH 5) and homogenized with a magnetic stirrer. The hydrolase reactions last for 4 h at 26 °C, after which 0.2 mol L^{-1} NaOH was added to stop the reactions. The oxidoreductase reactions last for 20 h at 26 °C. After incubation, for the hydrolases, the fluorescence was measured using a multi-function microplate reader (SpectraMax M5, Molecular Devices, USA) with 365 nm excitation and 450 nm emission filters; whereas for the oxidases, absorbance was measured at 450 nm. The enzymatic activities were expressed in units of μmol h^{-1} dry soil g^{-1}.

Table 1
Summary of enzymes assessed in this study.

Enzyme	Abbreviation	EC	Substrate
Cellobiohydrolase	CBH	EC 3.2.1.91	4-MUB-β-D-cellobioside
β-glucosidase	βG	EC 3.2.1.21	4-MUB-β-D-glucopyranoside
Acid phosphatase	AP	EC 3.1.3.2	4-MUB-Phosphate
N-acetyl-glucosaminidase	NAG	EC 3.2.1.30	4-MUB-N-acetyl-β-D-glucosaminide
Peroxidase	PER	EC 1.11.1.7	L-dihydroxyphenylalanine
Phenol oxidase	PPO	EC 1.10.3.2	L-dihydroxyphenylalanine

2.5. Soil chemical analysis

Total carbon (TC), total nitrogen (TN) and non-hydrolyzed carbon (NHC) content of the soil aggregates were measured using an elemental analyzer (Elemental Analyzer, Vario EL III, Germany). NHC analysis was lightly modified according to Rovira and Vallejo's (Rovira and Vallejo, 2007). In brief, 1g of 100-mesh sieved air-dried sample was hydrolyzed with 20 ml of 6 mol L^{-1} HCl in a digestion tube, covered with a small funnel and heated at 105 °C for 18 h with manual interval oscillation. After cooling, the hydrolysate was discarded, and the non-hydrolysable residue was transferred to a 50 ml centrifuge tube and centrifuged at 4500 r min^{-1} for 20 min. The residue was washed twice with distilled water and centrifuging, which was then transferred a crucible and dried at 60 °C until weight was constant, then analyzed with an elemental analyzer (Elemental Analyzer, Vario EL III, Germany).

For the unfractionated soil, total carbon (Unf-TC) was analyzed using an elemental analyzer (Elementar Analysensysteme GmbH, Vario El, Germany). Total nitrogen (Unf-TN), total phosphorus (Unf-TP), nitrate (NO_3^--N) and ammonia (NH_4^+-N) concentrations were analyzed using a SMARTCHEM 200 automatic intermittent chemical analyzer (Zhou et al., 2012a, b). The pH was measured with a 1:2.5 dry soil/water ratio using a Sartorius pH electrode (Zhou et al., 2012a, b). Field volumetric soil water content (%V) was manually measured using Time Domain Reflectometry (TDR) (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) at 0–15 cm depth.

Soil respiration (Rs) and heterotrophic respiration (Rh) was measured once or twice monthly since January 2015 using an LI-8100 portable soil CO_2 flux system attached to the soil CO_2 flux chamber (LI-COR. Inc., Lincoln, NE, USA). In each plot, five PVC collars (20 cm diameter and 11 cm in height) were randomly insert into the soil (5–6 cm deep). Small living plants within each collar were removed manually at least 24 h prior to the measurements to eliminate respiration of the aboveground plants. Rs was measured three times between 9 a.m. and 2 p.m. For Rh measurement, three subplots of 0.65 m × 0.65 m were randomly set in each plot since October 2014. The trench was dug to a depth of 0.8 m (almost no fine roots at this depth). After PVC boards were placed around, the trench was refilled with the original soil to minimize interference. Autotrophic respiration (Ra) was obtained by subtracting Rh from Rs.

2.6. DNA extraction, 16S rDNA amplicon sequencing and sequence preprocessing

For each sample, 0.5 g of the soil was used for DNA extraction using the DNeasy Powersoil® DNA isolation kit (Qiagen) according to the manufacturer's manual. The obtained DNAs were checked with an UV–VIS spectrophotometer SMA 4000 (Merinton Instrument, Ann Arbor, MI, USA). The V4 region of the bacterial 16S rDNA gene was amplified with the primer set 515F/806R (Smets et al., 2016), and the fungal ITS2 region was amplified with the primer set ITS3/ITS4 (Zhang et al., 2019). Sequencing was performed on the Illumina HiSeq2500 platform at Guangdong Magigene Biotechnology Co., Ltd. Raw sequence data were processed and analyzed using USEARCH (Version 11.0.667) (Edgar, 2010). Barcodes were first removed and the sequences were assigned to individual samples. After merging paired-end reads and removing primers, sequences with average quality scores below 20 and lengths shorter than 100 bp were discarded to improve sequence quality. Chimeric sequence detection and elimination was through the UCHIME algorithm (Edgar, 2016), after which a total of 1,410,236 bacterial 16S rRNA gene sequences and 695678 fungal ITS sequences were obtained from the 36 soil aggregate samples. Operational taxonomic units (OTUs) were classified by UPARSE at 97% similarity level (Carini et al., 2017). Singletons were removed before downstream analyses. Taxonomic assignment was through the RDP classifier (Wang et al., 2007). The RDP 16S rRNA database and the UNITE Fungal ITS database (Nilsson et al., 2019) were used for bacterial and fungal classification, respectively,

with a 50% confidence threshold. All samples were rarefied to 30326 and 11866 sequences for the 16S rRNA gene and the ITS region, respectively. Sequence data were deposited in NODE database (https://www.biosino.org/node/) with the accession number OEP000677.

2.7. Statistical and data analyses

All statistical analyses were performed in RStudio (version 1.2.1335) (Team, 2015). The effect of treatments (TRT), soil aggregate fractions (AF) and their interactions (TRT × AF) on soil physical-chemical properties, enzymatic activities and microbial alpha diversity indices were tested by two-way ANOVA analysis followed by the least significant difference (LSD) test. For soil physical-chemical properties and enzymatic activities, the ANOVA analysis was based on rank order distributions to avoid extreme values. Only if the ANOVA analysis showed statistical significance, were the multiple comparisons from the LSD test statistics be considered valid. Variation partition analysis (VPA) was used to determine the effects of treatments, soil aggregate fractions and soil chemical properties on microbial community structures and soil enzymatic activities. Correlations between the activities of enzymes and TC, TN, NHC, TC/TN, as well as the abundances of the dominant microbial classes were explored using Pearson's correlation analysis. Linear regression analyses were conducted to reveal the effect of the treatment on fungi and bacteria within each aggregate size fractions; the proportion of the explained variations by the fitted function was evaluated according to the adjusted r-squared values. Most of the analyses were performed with the 'vegan' package for R (Oksanen et al., 2016). The least significant difference (LSD) test was performed with the 'agricolae' package (de Mendiburu and de Mendiburu, 2019).

3. Results

3.1. The effect of drought on soil respiration and soil physical-chemical properties

Throughout the year of 2018, the field measurement of soil water content decreased by an average of 52.6% in the drought plots compared to the control plots (Fig. 1a and b). At the time of sampling, soil moisture was also determined in the laboratory with the dry-weight-based approach, which showed a significant decrease under drought compared to the other two treatments, with a reduction of 19.7% compared to control (Fig. 1c). Meanwhile, significant reduction in the long-term averaged soil respiration, heterotrophic respiration, as well as autotrophic respiration by 24.2%, 24.0% and 35.8%, respectively, was observed, regardless of the non-significant differences at the time of sampling (Fig. S1).

Drought changed the size distribution of aggregate fractions, with decreased mass proportion of S1 by 32.4% and increased mass proportion of S4 by 119.0% (Fig. 2). MWD and GMD significantly decreased by 22.7% and 15.5%, respectively, under drought compared to control (Fig. S6). Across soil aggregate size fractions, most of the soil chemical properties were not significantly affected by drought. Nonetheless, a significant decrease in the NHC content was revealed in S1 ($P < 0.01$,

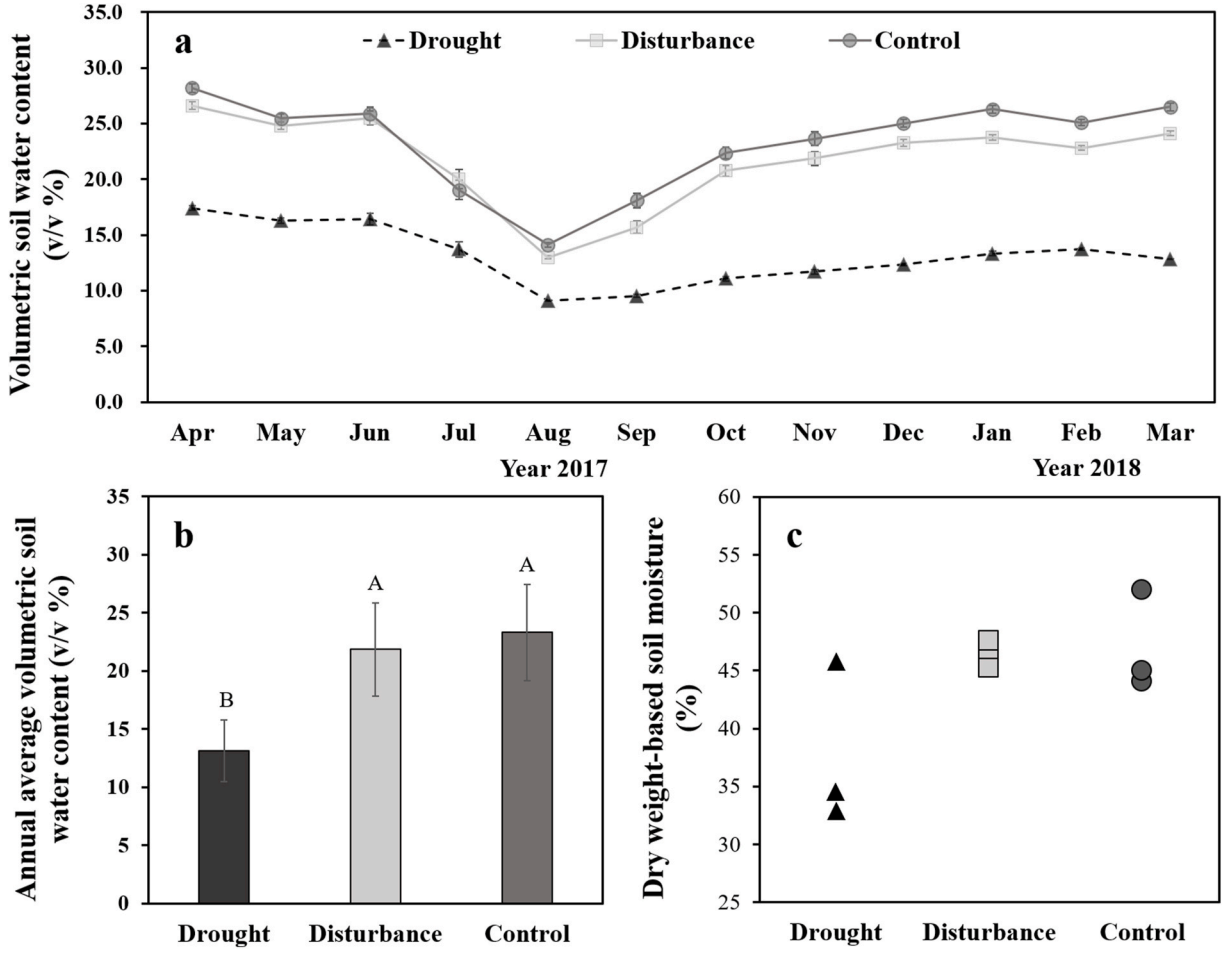

Fig. 1. The effect of treatment on soil moisture. **(a)** Field measurement of volumetric soil moisture to a depth of 5 cm (v/v%) from April 2017 to March 2018; error bars represent standard errors. **(b)** Annual averages of volumetric soil moisture (v/v%) under the three treatments; error bars represent standard deviations. **(c)** Dry-weight-based soil moisture of the soils from the nine plots collected at the day of sampling.

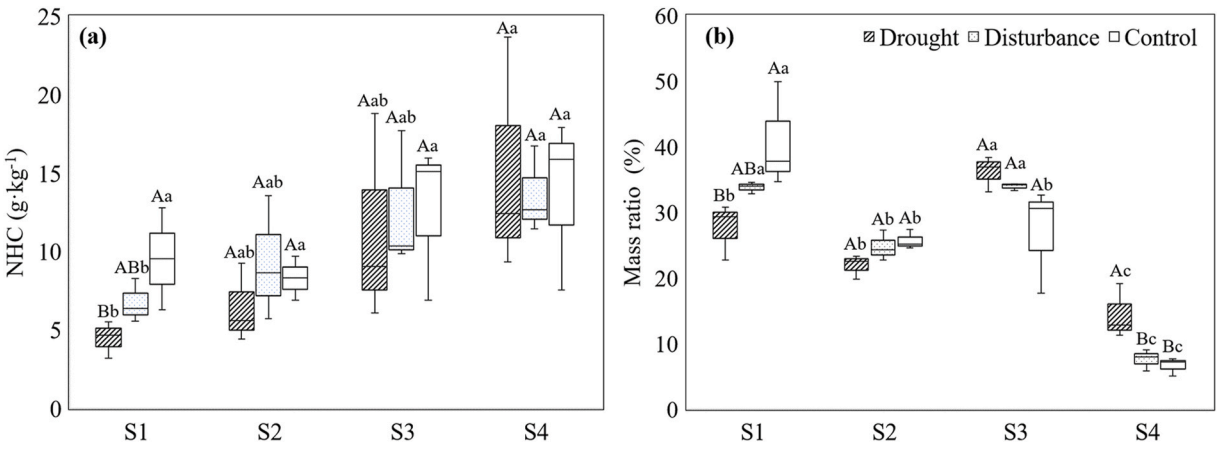

Fig. 2. Content of soil NHC **(a)** and the mass proportion of aggregates **(b)** within four aggregate fractions under the three treatments. Upper-case letters indicate statistically significant differences at $P < 0.05$ by LSD tests across treatments of the same aggregate fraction; different lower-case letters indicate statistically significant differences at $P < 0.05$ across aggregate fractions of the same treatment.

Fig. 2). The contents of TN, TC and NHC significantly varied across soil aggregate fractions ($P < 0.05$), and showed an increasing trend toward smaller aggregates under all three treatments (Fig. 2 and Fig. S5).

3.2. The effect of drought on soil enzymatic activities across aggregate size fractions

Drought reduced hydrolase activities especially in larger aggregates (S1 and S2, in Fig. 3). Specifically, the average activities of hydrolases CBH, βG, AP and NAG decreased by 47.8%, 67.8%, 25.4%, 42.2% in S1 and 59.8%, 58.5%, 24.0%, 57.3% in S2, respectively, compared to those under the control condition. After summarizing the soil enzymatic activities across each aggregate size fraction (Fig. S2), drought significantly reduced CBH activity in S1 and S2, but significantly increased βG and PER activities in S4. Correlation analyses further showed that most of the soil enzymatic activities were significantly correlated with soil TC, TN and NHC contents (Table 2); while NAG and AP activities were significantly correlated with TC/TN ratio ($P < 0.05$).

3.3. The effect of drought on microbial community structure and composition across aggregate size fractions

Both treatment and aggregate size significantly impacted the α-diversity of bacterial communities (Chao1, Shannon and Evenness indices), while fungal communities were less affected (Table 3). Bacterial diversity increased from S1 to S3, followed by a slight decrease in S4, while the impact of drought on bacterial diversity was the most significant in S1 (Fig. S3). The interaction between treatment and aggregate size had no significant effect on the bacterial or fungal diversity (Table 3).

Across all soil samples, the dominant bacterial phyla were *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*, which accounted for 43.8%, 24.6%, 6.1% and 5.9% of total abundance, respectively. Drought significantly increased the abundance of *Actinobacteria* by 114.7%. The fungal communities were mainly consisted of *Ascomycota*, *Basidiomycota* and *Mortierellomycota*, accounting for 45.9%, 30.4%, 19.3% of total fungi, respectively.

For bacteria, the impact of drought was found across all aggregate size fractions, and *Actinobacteria*, *Proteobacteria*, and *Acidobacteria* were the most affected phyla. In S2–S4, drought specifically increased *Actinobacteria* abundances, including its orders *Actinomycetales* and *Solirubrobacterales*, both of which also showed an increasing tendency toward smaller aggregate sizes (Fig. S7). Drought also significantly affected the phylum *Proteobacteria*, mostly by decreasing the abundances of the associated taxa. Specifically, *Caulobacterales* (*Alphaproteobacteria*) and *Legionellales* (*Gammaproteobacteria*) abundances decreased significantly in S1 and S3; while *Deltaproteobacteria* and *Myxococcales* (*Deltaproteobacteria*) abundances decreased across all aggregate size fractions (Fig. 4).

As for fungal communities, the effect of drought was mostly restricted within larger aggregates (Fig. 4). Specially, drought reduced the abundances of *Boletales*, *Mortierellales*, *GS11* and *Chaetosphaeriales* in S1, as well as that of *Mucoromycotina* in S2. Particularly, both *Boletales* and *Mucoromycotina* showed decreasing abundances towards smaller aggregate sizes (Fig. S7), whereas *Mortierellales* and *Chaetosphaeriales* showed the opposite distribution pattern (Fig. 4).

3.4. Factors affecting the composition and functions of microbial communities

To quantify the contribution of treatment, aggregate fraction, soil physical-chemical properties and their interactions to enzymatic activities and the structures of the bacterial and fungal communities, variance partitioning analysis (VPA) was performed (Fig. 5). Specifically, soil physical-chemical properties had the largest explanatory power compared to treatment and aggregate size, contributing 30.3%, 28.7% and 34.2% of the variance in soil bacterial, fungal communities and enzymatic activities, respectively (Fig. 5). The independent impact from treatment or aggregate fraction was comparable across the three comparisons. In addition, the interactions between the three variable categories explained 7.3%–10.9% of total variance across the comparisons.

3.5. Associations between soil enzymatic activities and microbial community composition

Soil enzymatic activities are important indicators of soil microbial functions and are influenced by changes in microbial community structure and composition. In order to assess the impact of bacterial and fungal community structure on the patterns of enzymatic activities, the redundancy analysis (RDA) was performed (Fig. S4). Here, bacterial and fungal community structure could explain 40.6% and 39.1%, respectively, of the total variation in soil enzymatic activities. For both bacterial and fungal communities, PER and AP oriented along RDA1, whereas the hydrolases primarily oriented along RDA2. For the bacterial communities, RDA1 explained 32.8% of the variation, with *Gammaproteobacteria* and *Acidobacteria_Gp2* related the most to RDA1, positively and negatively. RDA2 accounted for 7.8% of the variation, along which *Actinobacteria* showed an increased abundance toward the drought samples. For the fungal communities, *Agaricomycetes*, *Mortierellomycetes* and *Leotiomycetes* were among those most closely related to RDA1,

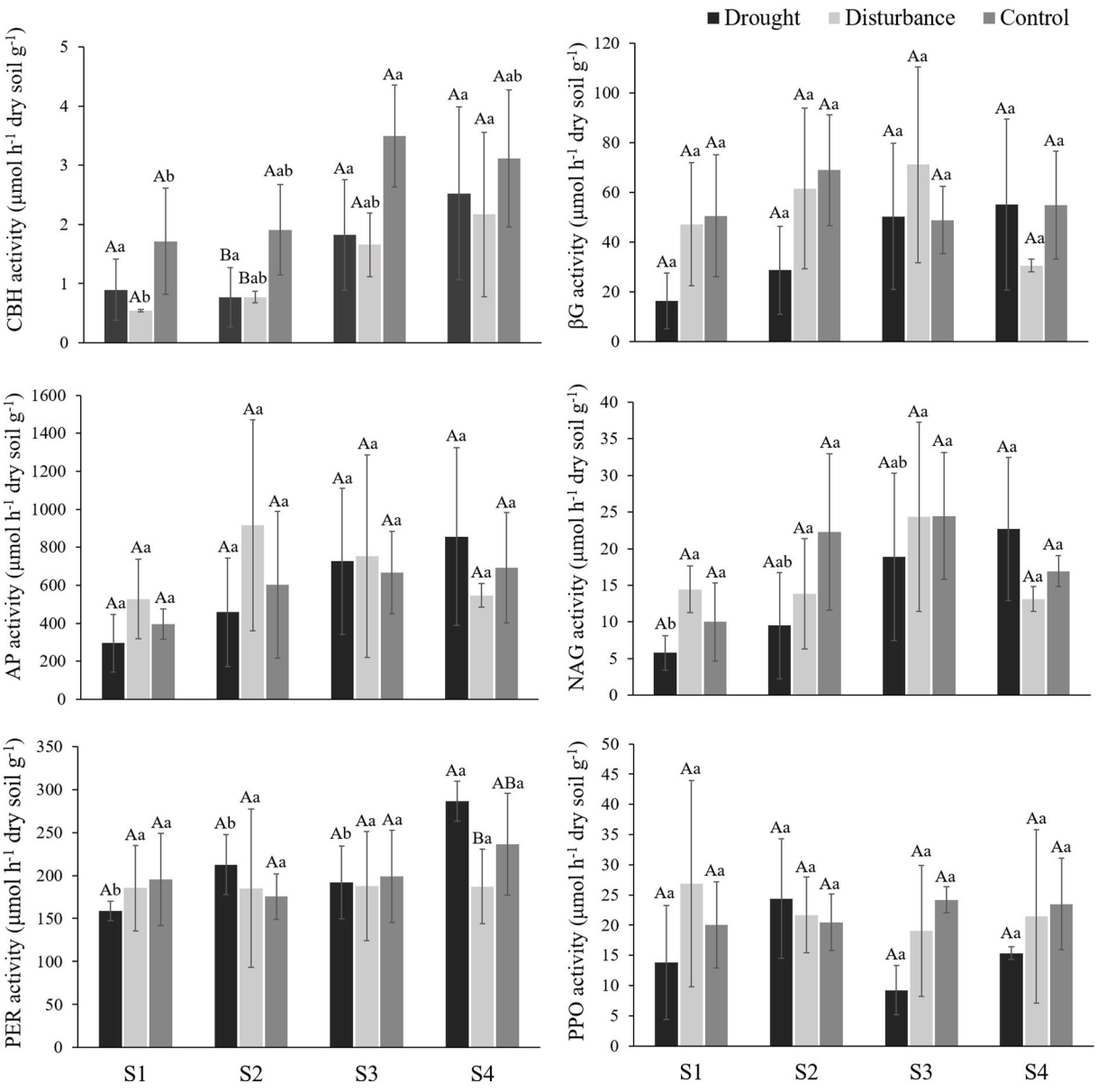

Fig. 3. Soil enzymatic activities within the four aggregate fractions under different treatments. Different upper-case letters indicate statistically significant differences at $P < 0.05$ across treatments of the same aggregate fraction; different lower-case letters indicate statistically significant differences at $P < 0.05$ across aggregate fractions of the same treatment.

Table 2
Correlation matrix of enzyme activities with soil chemical properties.

	CBH	βG	AP	NAG	PPO	PER
TN	0.614**	0.422*	0.652**	0.494**	0.103	0.439**
TC	0.58**	0.412*	0.696**	0.486**	0.099	0.446**
NHC	0.594**	0.444**	0.725**	0.513**	0.079	0.471**
TC/TN	0.278	0.291	0.733**	0.362*	0.127	0.314

Values indicate correlation coefficients; *$P < 0.05$; **$P < 0.01$.

Table 3
Effects of treatment, soil aggregate fractions and their interaction on the alpha-diversity of the bacterial and fungal communities.

	Bacteria			Fungal		
	Chao 1	Shannon	Evenness	Chao 1	Shannon	Evenness
TRT	24.313***	12.04***	3.081	3.286	0.859	0.849
AF	3.877*	10.13***	11.055***	1.902	3.043*	2.831
TRT × AF	1.094	0.78	0.951	1.795	1.563	1.464

F-values show the effects of treatment (TRT), soil aggregate fraction (AF) and their interaction (TRT × AF) on the alpha-diversity of the bacterial and fungal communities. (Significance level: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

which explained 31.7% of the variation. RDA2 accounted for only 7.4% of the variation, along which *Dothideomycetes* abundance increased toward the drought samples.

Pairwise correlation analyses further confirmed the associations between the abundances of individual microbial taxa and soil enzymatic activities, and between those and soil chemical properties. For the bacterial communities, hydrolase activities (CBH, BG, AP and NAG) were positively correlated with the abundances of *Proteobacteria* classes

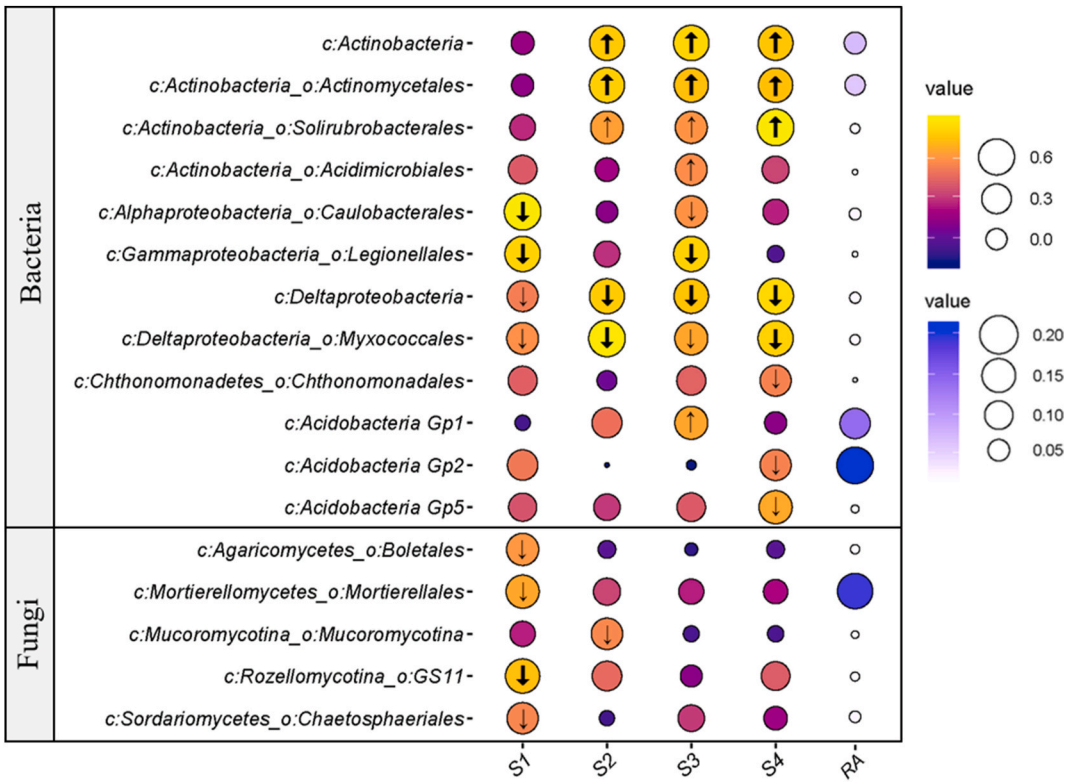

Fig. 4. Effect of Drought treatment on the relative abundances of fungal and bacterial taxa within four aggregate size fractions compared to the control treatment. The size and color of the circle indicates the degree of drought impact. The symbols '↑' and '↓' indicate significant increase/decrease by Drought. Significance level: thin arrow: $p < 0.05$, thick arrow: $p < 0.01$. RA refers to the relative abundance of the corresponding class/order. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

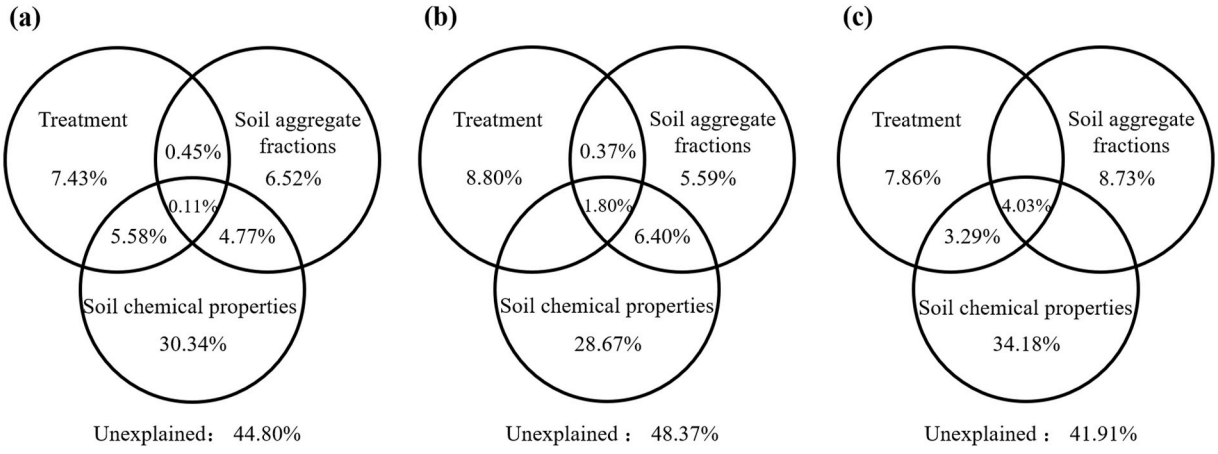

Fig. 5. Variation partitioning analysis (VPA) to determine the effects of treatment, soil aggregate fraction and soil chemical characteristics and the interactions between these variable categories on **(a)** bacterial and **(b)** fungal community structures, as well as **(c)** enzymatic activities.

and *Sphingobacteria*, yet were negatively correlated with those of some *Acidobacteria* groups (Fig. 6). Amongst fungi, enzymatic activities (CBH, BG, NAG and PPO) were positively associated with *Sordariomycetes* and *Mortierellomycetes*, and were negatively associated with *Dothideomycetes*. These patterns were in general consistent with those observed based on RDA. In addition, due to the tight associations between enzymatic activities and soil chemical properties (Table 2), the correlations between microbial taxa abundances and enzymatic activities were also largely consistent with those between microbial taxa abundances and soil chemical properties (Fig. 6).

4. Discussion

Soil aggregation is a vital regulator of soil C storage and microbially mediated SOC decomposition (Six et al., 2006). Based on the results from this study, a schematic diagram was made depicting how long-term drought altered the SOC distribution, the diversity, composition as well as the activities of the microbial communities across aggregate size fractions (Fig. S8). Notably, drought not only changed the structure of soil aggregation and resulted in re-distribution of SOC, but also differentially impacted the microbial communities and microbially mediated SOC decomposition across aggregate size fractions. The combination of

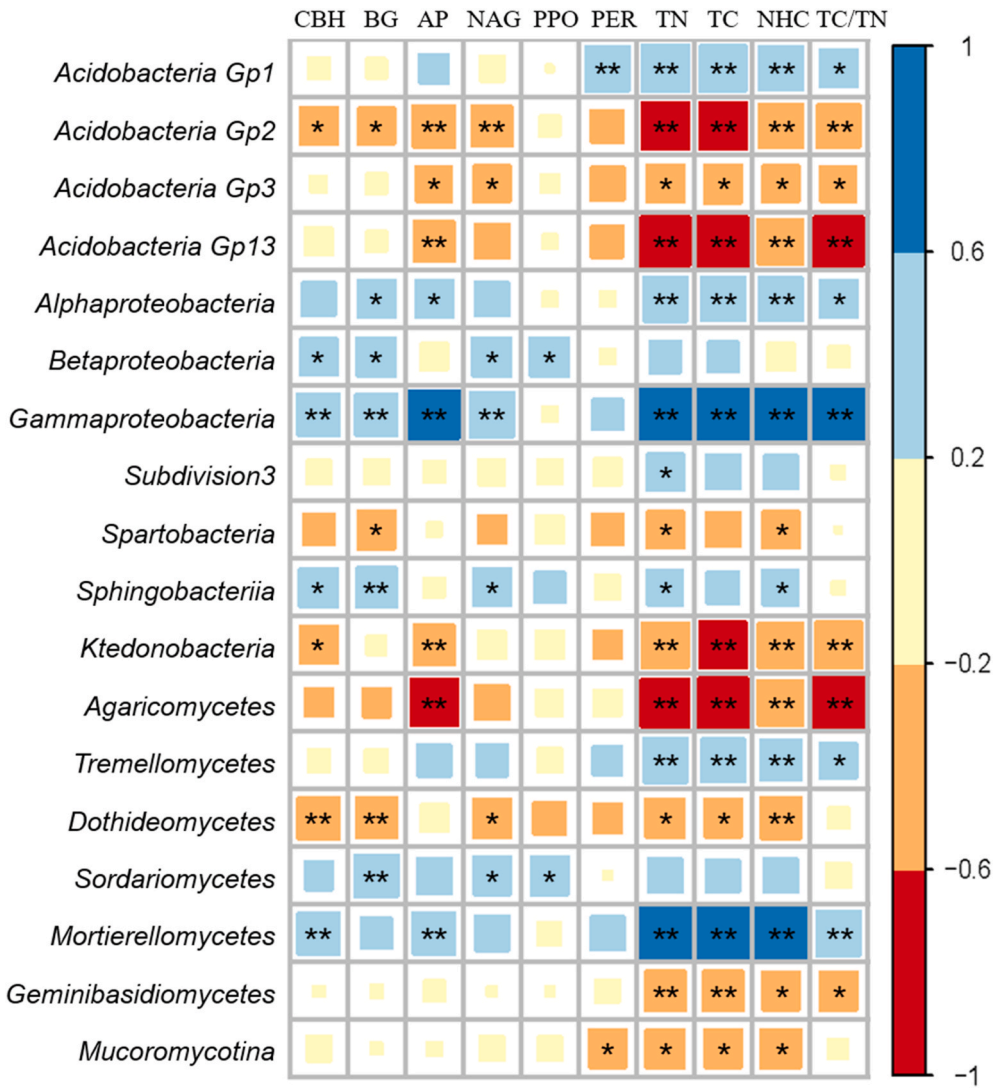

Fig. 6. Correlations between dominant bacterial and fungal classes and environmental variables. The numerical values in the table indicate the correlation coefficient between the dominant classes and environmental variables. Only the most abundant classes with relative abundances >0.1% are shown. Significance level: $^{*}P < 0.05$ and $^{**}P < 0.01$.

these effects could potentially impact the SOC turnover processes, which, over the long term, would mediate the response of the soil C pool under prolonged drought scenario.

4.1. Effect of drought on soil aggregation and SOC quality

Firstly, drought changed SOC distribution by reducing the proportion of large macroaggregates (S1), evidenced by decreased MWD and GMD by 22.7% and 15.5%, respectively (Fig. S6). Disintegration of large macroaggregates resulted in release of microaggregates and exposure of the originally protected SOC to microbial decomposition, which may further alter the SOC turnover processes. Previous studies have revealed that reduction in plant-derived soil organic matter input and microbial polysaccharide secretion could both contribute to loss of large aggregates (Tisdall and Oades, 1982; Six et al., 1999; Fonte et al., 2009). In this experimental site, reduced litter biomass by 9.3% under drought was reported (Bu et al., 2018); in addition, the fungal and bacterial biomass showed significant decrease in the unfractionated soil based on analysis of phospholipid fatty acids (PLFAs) (unpublished data). These observations together support that drought had a suppressing effect on the formation of large aggregates and the associated C sequestration capacity.

Drought also impacted SOC quality, particularly with a significant decrease in NHC content in S1. The NHC reduction could result from weakened physical protection of SOC due to decreased stability of large macroaggregates under the impact of drought. Both modeling and experimental studies have revealed that the dynamics of the recalcitrant C pool determined the long-term SOC storage in soils rather than that of the labile C pool (Belay-Tedla et al., 2009). Turnover of recalcitrant C was also found to be more sensitive compared to labile C under the global warming scenario (Knorr et al., 2005; Hartley and Ineson, 2008; Xu et al., 2010). Kinetic investigation revealed that the labile C pool could rapidly approach new steady state conditions during simulated warming, i.e., within five years, while over the longer run, the recalcitrant C pool was of greater contribution to the total soil respiration and determined soil C losses (Hartley and Ineson, 2008). Hence, long-term tracking of the dynamics of the recalcitrant C pool coupled with mechanistic understanding of its kinetics is particularly important to better evaluate the stability and fate of the SOC pool under prolonged drought.

4.2. Soil enzymatic activities were regulated by and feed back to SOC dynamics

Soil enzymes participate in SOM decomposition, and contribute a

key step to nutrient mineralization and sustaining of soil fertility (Nannipieri et al., 2012). Substrate availability regulates soil enzymatic activities not only through providing necessary reactants but also through impacting the growth of microorganisms from which soil enzymes are produced (Tabatabai et al., 2002; Tiemann et al., 2015; Trivedi et al., 2017). In this study, higher enzymatic activities were observed in microaggregates, consistent with higher levels of TC and TN (Fig. 3 and Fig. S5). This pattern was also supported by a number of other studies that found that decomposing enzyme activities were associated with SOC contents (Qin et al., 2010; Lagomarsino et al., 2012). In addition, microaggregates are of larger surface area, which is also crucial for supporting the colonization of more abundant microorganisms (Bach et al., 2018).

Results from this study also showed that the effect of drought on soil enzymatic activities varied across aggregate size fractions. Soil enzymatic activities in larger aggregates (S1, S2) were more susceptible to drought, particularly for the hydrolases (Fig. 3). Specifically, the decrease in the activities of CBH was the most significant, whose substrate was cellulose primarily derived from plant residues (Deng and Tabatabai, 1994; Xu et al., 2017). Previous work had revealed a significant decrease of litter material under drought in this experimental site (Bu et al., 2018), which might have contributed to lowered hydrolase activities through substrate limitation. Soil moisture could be an additional important regulator of soil enzymatic activities (Waldrop and Firestone, 2006; Bach and Hofmockel, 2016), due to its influence on nutrient exchange, as well as the destabilizing effect of desiccation on hydrolases' physical structures (Li and Sarah, 2003). Hence, the changes in soil enzymatic activities could result from those in both substrate availability and soil moisture.

Notably, when unit enzymatic activities were combined with aggregate mass fractions, the gross activities of some enzymes (βG, PER) significantly increased in S4, by nearly or even over 100% under drought. With continuously increasing proportions of microaggregates, the microbial activities in this fraction is particularly worth attention. Microaggregates contain greater fractions of protected C, a key part of soil recalcitrant C pool (Fansler et al., 2005). This SOC fraction may become susceptible to microbial decomposition upon disruption of the soil aggregate structure. In particular, the PER enzyme mediates key processes in recalcitrant C turnover including lignin degradation (Orth and Tien, 1995). Hence, the increased PER activity in S4 may facilitate the transformation of recalcitrant C, potentially contributing to decreased stability of the SOC pool.

4.3. Soil physical-chemical conditions drove microbial community distributions across aggregate size fractions

Spatial heterogeneity of nutrient availability across aggregate size fractions strongly selects for microbial lifestyles and influences the distribution of microbial communities, which in turn shapes the processes and dynamics of SOC turnover (Mummey and Stahl, 2004). In this study, soil physical-chemical properties explained the most variation in both bacterial and fungal community compositions. For bacterial communities, smaller aggregates (S3, S4) hosted the highest diversity, consistent with higher concentrations of TC and TN, similar to the patterns observed by Bach et al. (2018). Oligotrophic bacteria including *Acidobacteria* and *Chloroflexi* showed higher abundances in larger aggregates (Delgado-Baquerizo et al., 2017). On the contrary, the abundances of *Proteobacteria* and *Actinobacteria* increased toward smaller aggregates (Fig. S7), both of which in general favor environments rich in organic materials (Fierer et al., 2007; Wang et al., 2019). Many groups of *Proteobacteria* are copiotrophic microorganisms preferably utilizing labile substances; whereas *Actinobacteria* are capable of breaking down a wide range of organic matter in soils including cellulose and chitin, hence playing an important role in SOC decomposition (Ramirez et al., 2010; Fierer et al., 2012; Sun et al., 2016). Increased abundances of both of the above taxa were possibly due to the presence of rich and diverse organic substrates in smaller aggregates.

Yet for fungal communities, higher diversity was found in S2 and S3 (Fig. S3). In general, soil fungi preferably inhabit low density soils with higher porosity (Harris et al., 2003). Hence, the distribution patterns of fungal communities may result from the compromise between nutrient availability and soil structural conditions. In addition, although TC and TN were the highest in microaggregates, plant litter residues, which enter macroaggregates in the first place and favorably utilized by fungi, provide important substrates for the growth of saprophytic fungi (Clemmensen et al., 2013; Santalahti, 2018). Here, greater abundances of *Ascomycota*, *Basidiomycota* and *Mucoromycota* were found in S1 and S2 (Fig. S7). The dominant members of *Ascomycota* included *Eurotiales* and *Helotiales*, both of which were capable of producing extracellular enzymes and decomposing lignin and cellulose (Vandenkoornhuyse et al., 2002; Osono and Takeda, 2006; Yelle et al., 2008; Žifčáková et al., 2011). Similar characteristics were also reported for the orders of *Basidiomycota* including *Russulales* and *Agaricales*. *Mucoromycota* was shown to be capable of decomposing cellulose and monosaccharides (Osono, 2007). All of these above-mentioned groups exhibited greater abundances in larger aggregates. In contrast, the abundance of the fast-growing saprophytic fungi *Mortierellomycota* (primarily the order *Mortierellales*), which mainly uses simple soluble substrates, showed increasing abundances toward smaller aggregates (Liao et al., 2018). Taken together, the combined effects of nutrient availability and soil structure could together determine soil fungi distribution across aggregate fractions.

4.4. Drought differentially impacted the bacterial and fungal community compositions

In this study, we found that fungal communities were more resistant to drought than bacterial communities (Table 3 and Fig. 4). Not only that fewer fungal taxa were affected, but also that the impact of drought was primarily within larger aggregates. It is generally acknowledged that fungi are better adapted to drought conditions, owing to stronger cell wall structures (Bowman and Free, 2006), as well as the abilities to form spores, cysts or other resistant cell types (Augé, 2001; Crowther et al., 2014; Meisner et al., 2018). Extension of mycelial networks in filamentous fungi also enables efficient transportation of water and nutrients over longer distances (Allen, 2007; Bapiri et al., 2010; Herrera et al., 2011). Consistently, the abundance of *Boletales*, an order of large ectomycorrhizal fungi lacking hydrophilic hyphae, showed significant decrease in S1, possibly due to its weaker ability of water acquisition (Ekblad et al., 2013). The impact of drought on fungi may also be associated with changes in substrate availability, e.g., reduced litter biomass may drive the changes in fungal taxa specialized in litter decomposition (Fuchslueger et al., 2014). A notable example was significant decrease of *Mortierellales* in S2, members of which belong to saprophytic fungi and are usually involved in soil cellulose and lignin degradation (Větrovský and Baldrian, 2013). Altogether, drought may affect fungal communities either through direct effect of water reduction, or through indirect changing of SOM by its effect on plant primary production.

In contrast, bacterial communities were more susceptible to drought, particularly within smaller-size aggregates. The changes in bacterial community composition were dominated by increased abundance of *Actinobacteria* including the orders of *Actinomycetales*, *Acidimicrobiales* and *Solirubrobacterales*, and decreased abudance of *Proteobacteria* across S2 to S4. Many members of *Proteobacteria* are r-strategists, whose growth heavily depends on nutrient availability and are in general sensitive to environmental perturbations (Bouskill et al., 2013). On the contrary, the gram-positive *Actinobacteria* are known for their competitive advantages under stressed conditions including drought (Bachar et al., 2010; Bouskill et al., 2013). Morphology of this group resembles that of filamentous fungi with hyphae structure and spore formation capability (Williams et al., 1972; Okoro et al., 2009). Furthermore, *Actinobacteria*

are known to be involved in decomposition of resistant organic materials such as soil humus (Goodfellow and Williams, 1983; Mackelprang et al., 2011; Deng et al., 2015). Hence, changes in *Actinobacteria* abundance may couple recalcitrant C turnover, which is particularly worth attention under long-term drought scenario. Drought also changed the abundance distribution of *Acidobacteria*. Most of its groups showed decreased abundances under drought except for *Gp1*, possibly due in part to its ability of extracellular mucus production to cope with drought stress and to utilize a broad range of C substrates (Lee and Cho, 2009; Sul et al., 2013). In summary, the changes in microbial community composition and functions reflected selective power of long-term drought; More importantly, these changes have the potential to continuously influence the soil biogeochemical cycling processes, and eventually may impact the SOC storage of the forest ecosystem.

4.5. SOC turnover in microaggregates could drive long-term SOC response to drought

Based on our findings and those by others, microaggregates exhibited a paradox in terms of microbial processes and SOC stability. It is widely acknowledged that, compared to macroaggregates, microaggregates offer greater protection over SOC, both physically and chemically (Cambardella and Elliott, 1994; Kaiser and Guggenberger, 2003; Denef et al., 2004; Lehmann et al., 2007; Stewart et al., 2009; Dungait et al., 2012). However, microaggregates hosted the most diverse bacterial communities, and showed the highest enzymatic activities (Bailey et al., 2012; Bach et al., 2018). Hence, we postulate that microaggregates were characterized by relatively stable inner cores, with highly active outer surface, which provided important hotspots for microbial activities. Under long-term regimes of climate change or anthropogenic perturbation, macroaggregate disintegration may contribute a first stage in soil degradation, leading to relative quick loss of soil labile C; While during the later stage of prolonged perturbation, the highly active microbial communities on the outer surface may continuously be selected and interfere with the C storage within the microaggregates, eventually leading to loss of the stable C pool. In our experimental site, results from this study showed clear evidences from the first stage and emerging clear evidences from the later stage. Continued monitoring is yet undergoing to further track the changes in soil structure, SOC quantity and quality, microbial activities as well as community compositions to better understand how the interplay of biotic and abiotic processes together mediate the response of the soil C transformation processes and SOC storage under prolonged drought condition.

5. Conclusion

In this subtropical evergreen broad-leaved forest site, five years of drought treatment substantially reduced macroaggregates' stability and changed the distribution of aggregate size fractions. Drought also changed the activities and composition of soil microbial communities, which may further impact the soil C cycling processes. In large aggregates (S1, S2), microbial responses to drought primarily suggested reduced SOC decomposition due to decreased microbial biomass, diversity and hydrolase (CBH) activities. Whereas in microaggregates, increased abundance of *Actinobacteria* as well as the PER activity was revealed, both of which were associated with decomposition of complex C compounds. Therefore, despite of the insignificant change in soil TC, our results suggest increased potential of recalcitrant C decomposition as the result of both the changes in soil aggregation and in microbial community structure and functions. This study highlights the need for continued monitoring especially on the dynamics of the recalcitrant C pool under prolonged drought. In addition, our results indicated the SOC turnover processes in macro- and microaggregates may be regulated by different drivers, proper consideration of which may benefit terrestrial C models for better prediction of the trajectories of the soil C storage.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107898.

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