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Drought changed soil organic carbon composition and bacterial carbon metabolizing patterns in a subtropical evergreen forest

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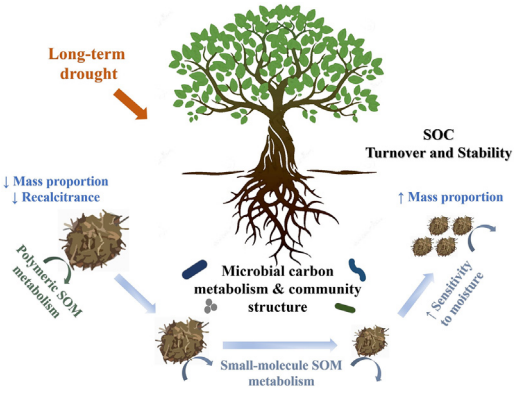
HIGHLIGHTS

- Drought reduced the proportion of large macroaggregates and the stability of the soil organic carbon pool.
- Effects of drought on microbial carbon metabolizing patterns varied with aggregate size fraction.
- Carbon metabolism in smaller aggregates had higher sensitivity to soil moisture.
- *Acidobacteria*, *Actinobacteria* and *Proteobacteria* were the most important mediators of soil carbon metabolism under drought.

GRAPHICAL ABSTRACT



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ABSTRACT

Subtropical forests are considerable carbon sinks in the northern hemisphere, yet are increasingly suffering from the impact of extreme drought. To better understand the dynamics and kinetics of forest soil carbon storage under long-term drought, a rainfall-reduction experiment was established in a subtropical evergreen forest of eastern China. Soil organic carbon (SOC) composition, microbial carbon metabolism and the interactions with soil microbial community structure were investigated across different soil aggregate size fractions. After five years' treatment of rainfall reduction, a significant loss of large macroaggregates, as well as an increase of microaggregates by over 100% was observed. Meanwhile, drought changed the composition of SOC, reducing the non-hydrolyzed carbon and humin contents in large- to medium-size macroaggregates. Microbial metabolizing capacity of polymeric compounds was also reduced especially in the above aggregate fractions, whereas the utilization of small-molecular compounds was more impacted in small macroaggregates and microaggregates. The changes in carbon metabolizing patterns were further associated with the abundance changes of specific

Abbreviations: SOC, soil organic carbon; SOM, soil organic matter; TC, total C; TN, total N; NHC, non-hydrolyzed C; HU, humus; HE, total alkali-extractable humus; HA, humic acid; FA, fulvic acid; HM, humin.

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Microbial communities

microbial taxa, revealing the microbially mediated mechanism of soil carbon metabolism under long-term drought. In addition, carbon metabolism in microaggregates was particularly sensitive to the changes of soil moisture, suggesting long-term drought may continually influence the functional resistance of the microbial communities. Taken together, our results provide insights into how biotic and abiotic processes together influence the SOC metabolizing processes, continued monitoring and investigation of which shall contribute to better understanding of the dynamics and kinetics of SOC storage under the impact of long-term drought.

1. Introduction

Currently, the majority of the Earth's terrestrial ecosystems are facing increased frequencies of extreme drought with greater intensity as well as prolonged duration, which may have marked effects on ecosystem functions such as carbon sequestration capacity (Dai, 2011; Stocker et al., 2013). In East Asia, subtropical evergreen broad-leaved forests are considerable carbon sinks that were vigorously influenced by extreme droughts over the past decades (Yu et al., 2014; Chevuturi et al., 2018). Both modeling and experimental studies have indicated that drought could reduce the net primary production, SOC storage and its partitioning across projected carbon pools of the impacted forest ecosystems (Zhao and Running, 2010; Hoover and Rogers, 2016). However, it is not yet fully understood how biotic and abiotic mechanisms together drive the progression of these changes, and such knowledge gaps may create great challenge for further understanding of the carbon-climate change feedback. Therefore, investigating the responses of SOC quantity and quality to drought and the microbial mechanisms may provide key insights for understanding the dynamics and fate of forest SOC pool under the long-term drought scenario.

Soil aggregation is one of the most important abiotic factors contributing to SOC storage and its stability through offering physical protection against degradation (Six et al., 2000). Soil aggregates are of different sizes, the distribution of which associates with a range of soil chemical characteristics and further influences the biotic processes. SOM heterogeneously distributes across different aggregate size fractions, with enrichment of SOM freshly derived from plant residues in macroaggregates, and accumulation of old and recalcitrant carbon mainly in microaggregates (Six et al., 2004; Davinic et al., 2012; Bach et al., 2018). In consequence, macroaggregates and microaggregates are markedly different in the kinetics of SOC turnover, which together substantiate the soil carbon cycling and the carbon sequestration capacity (Six et al., 2004; Lu et al., 2019a; Yılmaz et al., 2019). Consistently, uneven distributions of microbial community composition and metabolic activities across aggregate size fractions were repeatedly reported owing to preferential differences of the micro-environments (Fierer et al., 2007; Bach and Hofmockel, 2014; Chen et al., 2015). As a result, different aggregate size fractions, together with the inhabited microorganisms, play distinct roles in a range of biogeochemical processes in the belowground ecosystem (Six et al., 2000; Lagomarsino et al., 2012).

Both the quantity and quality of the SOC pool are important parameters of the stability of SOC storage. In studies of terrestrial carbon models, partitioning of SOC into sub-pools with different turnover kinetics, i.e., the fast- and slow- turnover pools, often yield better prediction of the C cycling dynamics (Gabarrón-Galeote et al., 2015; Zhang et al., 2019b). Under the drought scenario, disintegration of macroaggregates could contribute to the early change of the SOC storage (Zhang et al., 2019a). This change, on one hand, could expose the originally protected carbon to microbial decomposition, threatening the capacity of carbon fixation (Beare et al., 1994; Kushwaha et al., 2001); on the other hand, could shift mass distribution of macro- and microaggregates, leading to changes in the kinetics of carbon turnover processes. Nonetheless, the majority of current experimental studies on the response of SOC storage to climate change insufficiently evaluated SOC quality or its interactions with soil microorganisms (Canarini et al., 2016; Moreno et al., 2019). Further investigations on these aspects shall benefit understanding of how the SOC quality and the microbial drivers together affect the SOC turnover efficiency and kinetics under climate change.

Soil microbial communities are highly complex, and the effect of drought on soil microorganisms depend on both the duration and frequency of drought (Hoover and Rogers, 2016; Preece et al., 2019). During short-term droughts, the direct impact from osmotic stress and limited substrate diffusion on microbial activities and carbon degradation rates have been verified by a series of laboratory and field experiments (Uhlířová et al., 2005; Hueso et al., 2012; Preece et al., 2019). Whereas long-term drought may modify soil microbial community composition and indirectly alter the capacity and pattern of microbial carbon utilization. For example, drought-induced changes in both the quantity and quality of plant-derived SOM may influence the substrate preference and the composition of soil microbial communities (Fuchslueger et al., 2014; Preece and Peñuelas, 2016; Bastida et al., 2017). Particularly, these changes may have lasting and cascading effects, i.e., altered microbial communities could continually impact the belowground biogeochemical processes (De Vries et al., 2012; Bouskill et al., 2013), further changing and shaping the size and composition of the SOC pool (Wang et al., 2019a). For instance, elevated abundances of *Chloroflexi* and *Acidobacteria* in agriculture soils were associated with improved overall capacity of complex-organic-compound metabolism (Ai et al., 2015; Wang et al., 2019b); Increased *Actinomycetales* growth especially in the subsurface layer was associated with old carbon loss in thawing permafrost soils (Deng et al., 2015). Therefore, the mechanisms underlying the dynamics of SOC turnover over the short-term vs that over the long-term may be systematically different. Under the long-term drought scenario, the changes in microbial community composition and the associated C metabolizing functions are particularly worth attention for better understanding and prediction of the response and trajectory of the forest SOC storage.

In 2013, a rainfall reduction experimental platform was established in a subtropical evergreen forest of eastern China to simulate extreme drought scenario of this region. Long-term records have revealed significant reduction of soil respiration since 2015, and signs of changes in soil aggregation were observed no earlier than 2017 (Liu et al., 2018). In this study, we investigated the responses of SOC composition and microbial communities in 2018, after five years of extreme drought treatment. Across soil aggregates of different size fractions, SOC quantity and quality were analyzed; the microbial carbon metabolizing patterns were assessed by Biolog-Eco assays; the bacterial community composition characterized through high throughput sequencing was also incorporated. Through these attempts, this study was aimed to: (1) reveal the associations between biotic and abiotic processes involved in soil carbon metabolization, and (2) determine how these processes together impact and shape the response of forest soil carbon storage to long-term drought.

2. Materials and methods

2.1. Site description

The experimental site was located in Tiantong National Forest Park in Zhejiang Province of Eastern China (29°52′ N, 121°39′ E, 163 m above sea level). The region is a typical subtropical monsoon climate

zone with hot humid summer and cold winter. The mean annual temperature is 16.2 °C. The highest and lowest average monthly temperature occurs in July and January, respectively. Precipitation mainly occurs from May to August, with mean annual precipitation of 1374.7 mm (Bu et al., 2018). The average annual relative humidity is 82%. The zone contains representative vegetation types in the hilly area of eastern Zhejiang, with the dominant tree species *Schima superba*, *Castanopsis fargesii* and *Lithocarpus glaber* (Da et al., 2004).

2.2. Experimental design and treatments

In July of 2013, the rainfall-reduction experiment was established with nine 25 m × 25 m plots in a secondary forest with tree ages of 50–70 years. The plots had similar topography, including slope, vegetation type and abundance, as well as soil texture and the amount of rocks. Three treatments were set up, including drought, disturbance control (disturb) and control (Fig. S1), and three plots were randomly allocated to each treatment. In the drought treatment plots, V-shaped transparent polycarbonate plates were evenly fixed on steel frames at 1.5–3.5 m height aboveground to exclude rainwater, and covered roughly 70% of the plot area. The disturb plots were set up to control for light interference by the polycarbonate plates and the disturbance during construction of the plots. In the disturb plots, the same V-shaped transparent polycarbonate plates were placed in the downward-opening way to allow rainfall to pass through. In the control plots, natural conditions were maintained. Around each plot, a 2 m deep trench was dug and polycarbonate isolation sheets were fixed to prevent surface runoff of rainfall.

2.3. Soil sampling and aggregate sieving

Soil samples were collected in March 2018. In each plot, nine soil cores following a grid-point sampling pattern were taken from the top 10 cm soil using an inner bulldozer device (with inner diameter of 5 cm) to keep the integrity of soil core. Soil samples were transported to the laboratory on ice within 24 h. The nine soil cores from each plot were evenly combined into a composite sample. A sub-sample was immediately taken to determine soil moisture, ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) contents. Another part of the soil was used for aggregate sieving, with the remaining saved at −80 °C for further soil chemical analyses.

To minimize disturbance to microbial community composition and activities, the 'optimal moisture' sieving approach was adopted to separate soil aggregates of different sizes (Bach and Hofmockel, 2014). Briefly, soil samples were dried to optimal moisture (water content at approximately 10%) at 4 °C under sterile conditions. After removing the stones, animal and plant residues through 8-mm sieve (pre-cleaned with ethanol and uv irradiation), approximately 500 g of each soil sample was placed on a stack of sieves (2 mm, 1 mm and 0.25 mm), and shaken at 200–250 rpm for 2 min, through which the soil was separated into four size fractions: > 2 mm (S1, large macroaggregate), 1–2 mm (S2, medium macroaggregate), 0.25–1 mm (S3, small macroaggregate), and <0.25 mm (S4, microaggregate). All sieves were sterilized prior to aggregate sieving. A total of 36 fractionated soil aggregate samples were obtained. Each fraction was weighed to obtain the aggregate mass. A part of the aggregate samples was stored at 4 °C for Biolog assays, which were conducted within 48 h. The other part of the samples was stored at −80 °C for DNA extraction, and for analyses of total N (TN), total C (TC), non-hydrolyzed C (NHC), and humus contents.

2.4. Analyses of soil chemical properties

2.4.1. The unfractionated soil

Time Domain Reflectometry (TDR) (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) was used to measure field volumetric soil water content (%V) at the depth of 0–15 cm over the past five years. For the unfractionated soil, soil moisture was also measured with the gravimetric method by oven drying and calculated based on the dry weight of the soil; soil pH was determined with a glass electrode (soil: water = 1:2.5); NH_4^+-N and NO_3^--N were extracted with 1 M KCl (soil: KCl = 1:4); the total N (TN) and total P (TP) were digested by 18.4 M H_2SO_4. Then, the contents of NH_4^+-N, NO_3^--N, TN and TP were determined by a SMARTCHEM 200 automatic intermittent chemical analyzer (Germany, Clever Chem 200); total C (TC) of the unfractionated soil was analyzed by catalytic combustion at 850–1200 °C with a TOC analyzer (Vario TOC Elementar Analysensysteme, GmbH, Germany).

To determine fine root (<2 mm diameters) biomass, visible fine roots were first hand-picked; the attached residues (e.g. litter fractions) were carefully removed with a tweezer. Next, the rest of the soil was sieved with a 0.15 mm mesh, followed by gentle rinse with water to collect fine root parts. The obtained fine root biomass was dried at 70 °C until constant mass was reached.

2.4.2. The soil aggregates

TC, TN and NHC contents of soil aggregates were measured using an elemental analyzer (Elemental Analyzer Vario EL III, Germany), and the procedures were slightly modified from those by Rovira and Vallejo (Rovira and Vallejo, 2007). Briefly, 1 g of air-dried soil through 100-mesh was hydrolyzed with 20 mL of 6 M HCl in a digestion tube covered with a small funnel, and then heated at 105 °C for 18 h. 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, and then was transferred a crucible to be dried at 60 °C until weight was constant, which was analyzed with the elemental analyzer to determine the content of NHC.

Both the soluble humus (humic acid and fulvic acid) and the insoluble humus (humin) (van Zomeren and Comans, 2007) contents of aggregates were examined. Humus extraction was based on a modified procedure from Kumada et al (Kumada, 1988). First, humus-C (HU) was determined through oxidation with potassium dichromate followed by titration with ferrous sulphate (Walkley and Black, 1934). Specifically, 0.5 g of dried soil sample was passed through a 0.25 mm sieve and added into a hard glass test tube containing 10 mL of 0.4 M $K_2Cr_2O_7$-H_2SO_4 solution. The mixture was then heated in an oil bath at 180 °C for 5 min and transferred into a triangular flask followed by titration with ferrous sulphate solution. Total alkali-extractable humus (HE, humic acid and fulvic acid) was extracted with the sodium pyrophosphate-sodium hydroxide mixture (Kumada, 1988). HE was further separated into humic acid (HA) and fulvic acid (FA) through acidifying the extract to pH 2–3 using 0.5 M H_2SO_4, which was left to stand overnight. The precipitation was repeatedly washed with 0.05 M H_2SO_4 and filtered until the filtrate was colorless; the precipitated residue was the HA proportion. The rest of the components, i.e., FA and humin (HM), were calculated as follows:

$$FA = HE\text{-}HA;$$

$$HM = HU\text{-}HE.$$

The relationships among these humus contents were illustrated in Fig. S4.

2.5. Carbon metabolizing analysis through Biolog assays

Biolog-Eco Micro-Plates (Biolog Inc., Hayward, CA, USA) were adopted to assess microbial metabolic activities in soil aggregates. Specifically, the utilization of 31 carbon sources were assessed, which can be categorized into six groups: carbohydrates, amino acids, carboxylic acids, amines, phenolic compounds, and polymers. Soil samples (equivalent to 3 g of dry soil) were placed in sterile conical flasks and agitated with 27 mL of sterilized saline solution (0.85% NaCl, m/v) for 30 min at 200 rpm, followed by serial dilution to a final ratio of 1:1000. 150 μL of

the supernatant was transferred into each well of the Micro-Plate, which was then incubated at 25 °C in dark. The absorbance of each well was measured with an automated Micro-Plate reader at 590 nm following incubation after 0, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h. The absorbance values at 120 h of incubation showed approximation to maximal bacterial growth, and were therefore used for subsequent statistical analyses (Garland, 1996).

Average well color development (AWCD), which represents the overall carbon source utilization capacity of soil bacteria, was calculated according to (Kumar et al., 2017):

$$\mathrm{AWCD} = \sum(C_i - \mathrm{R})/\mathrm{n} \quad (1)$$

where C_i refers to the absorbance value in each Micro-Plate well. R refers to the absorbance value of the control well. The absorbance value was assigned 0 when $C_i - R < 0$. n is the number of carbon substrates.

Four diversity indices were calculated for the Biolog data: (1) the Shannon-Weaver index (H, the α-diversity index), (2) the Simpson index (D, the dominance of the functional diversity), (3) the McIntosh index (U, the evenness of the functional diversity) and (4) the substrate richness (S, the number of substrates effectively used, OD ≥ 0.25). Specifically, we used the following equation to calculate the biodiversity parameters (Manjunath et al., 2018; Sofo et al., 2018):

$$\mathrm{H} = -\sum P_i\,(\ln \mathrm{P}_i) \quad (2)$$

$$\mathrm{D} = 1 - \sum (P_i)^2 \quad (3)$$

$$\mathrm{U} = \sqrt{\sum (C_i - R)^2} \quad (4)$$

$$P_i = (C_i - \mathrm{R})/\sum(C_i - \mathrm{R}) \quad (5)$$

2.6. DNA extraction, 16S rDNA sequencing and data processing

For each sample, 0.5 g of soil was used to extract DNA with the DNeasy Powersoil® DNA isolation kit (Mo-Bio, Carlsbad, CA) according to the manufacturer's instructions. The concentration and quality of the extracted DNA was analyzed with an UV–VIS spectrophotometer SMA 4000 (Merinton Instrument, Ann Arbor, MI, USA). The primer pair 515F/806R was used to amplify the V4 hypervariable region of bacterial and archaeal 16S rRNA gene (Caporaso et al., 2012). Sequencing was performed on the Illumina HiSeq2500 platform at Guangdong Magigene Biotechnology Co., Ltd. The initial bioinformatic analysis of raw sequence data was through the USEARCH software (Edgar, 2013). After removing unique barcode, paired-end sequences of individual samples were merged and primers were removed. Reads with average quality scores below 20 and lengths shorter than 100 bp were discarded. The UCHIME algorithm was adopted to remove chimeras. A total of 1,410,236 bacterial 16S rRNA gene sequences were obtained from the 36 soil samples. Operational taxonomic units (OTUs) were clustered by the UPARSE program at the similarity level of 97%. For each OTU, one representative sequence was generated. Taxonomic annotation of the representative sequences was performed by the RDP classifier with a 50% confidence threshold (Wang et al., 2007). A randomly selected subset of 30,326 sequences per sample for bacteria was subjected to downstream analyses. All sequence data were deposited in the NODE database (https://www.biosino.org/node/) with the accession number OEP000677.

2.7. Statistical analyses

The effects of drought on soil chemical properties and the indices of microbial carbon source utilization patterns were analyzed by two- and one-way analysis of variance and the least significant difference test (LSD) with the 'agricolae' package (de Mendiburu and de Mendiburu, 2019). Principal component analysis (PCA) and pairwise PerMANOVA was used to evaluate the carbon metabolism differences among treatments and aggregate fractions. The contribution of carbon sources to the principal components was calculated with the 'factoextra' package (Alboukadel and Fabian, 2017). Variation partitioning analysis (VPA) was used to determine the effects of treatment, soil aggregate size, soil chemical properties as well as their interactions on microbial carbon metabolizing patterns. Correlations between TC, TN, NHC, TC/TN, NHC/TC, SOC composition, abundances of bacterial taxa and the utilization of individual carbon sources were analyzed based on Pearson's correlations. Canonical correlation analysis (CCA) was used to assess the contribution of soil chemical properties to carbon source utilization patterns. Most of the analyses were carried out with the 'vegan' package (Oksanen et al., 2016) in R (Version 3.5.1) (R Core Team, 2019).

3. Results

3.1. Effects of drought on chemical properties of the unfractionated soil and soil aggregates

During the five years of treatment, annual mean soil water content decreased under drought by 32.3% (Fig. 1). However, at the time of sampling, soil moisture did not show significant differences among treatments (Fig. S2g). Drought significantly increased fine root biomass by 34.2% compared to control condition (Fig. S2h). In the unfractionated soil, drought significantly decreased NH_4^+-N by 33.5%, while the concentrations of TN, TC or NO_3^--N did not vary significantly across treatments (Fig. S2).

Drought substantially affected the mass distribution of aggregate size fractions (Fig. 2a). Specifically, the proportion of large macroaggregates (S1) significantly reduced by 33.1%, while that of microaggregates (S4) significantly increased by 120.7%. The aggregate mass distribution did not differ significantly between disturb and control treatments. Across the four aggregate size fractions, TN, TC and TC/TN ratio shared a similar pattern with increasing trend toward smaller aggregate sizes, yet showing no significant differences across treatments (Fig. S3). Furthermore, NHC and fractions of humus contents were also analyzed (Fig. S4 and S5). In S1, the NHC content was significantly reduced under drought by 53.2% compared to control (Fig. S3d); In S2, the HM content was significantly reduced by 24.7% compared to the disturb (Fig. S4d), suggesting that drought could have changed the SOC composition.

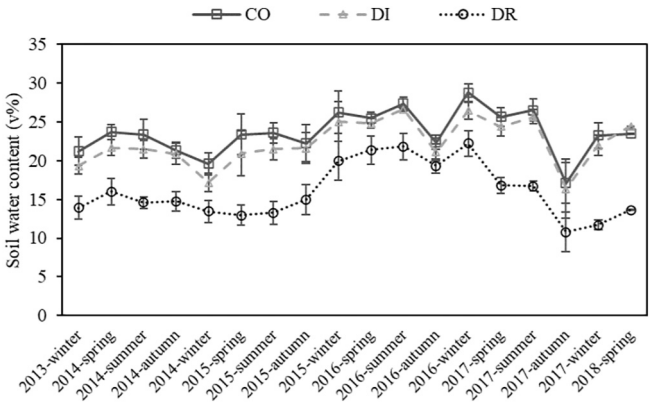

Fig. 1. Records of soil water content at the experimental site over the past five years. DR, DI and CO correspond to Drought, Disturb and Control treatment, respectively. Error bars represent standard deviations.

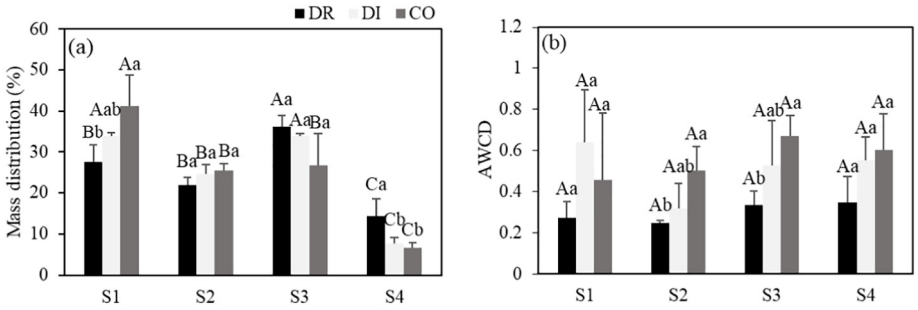

Fig. 2. Mass distribution (a) and AWCDs (b) of aggregate size fractions under three treatments. Different capital letters indicate significant differences ($P < 0.05$) among aggregate size fractions under the same treatment. Different lowercase letters indicate significant differences ($P < 0.05$) among treatments within the same aggregate size fraction. DR, DI and CO correspond to Drought, Disturb and Control treatment, respectively. Error bars represent the standard deviations.

3.2. Effects of drought on carbon utilization patterns across aggregate size fractions

Drought significantly affected AWCD, while aggregate size did not (Table S1). The average overall AWCDs under drought, disturb and control treatments were 0.30, 0.51 and 0.56, respectively, suggesting that carbon metabolism under drought was considerably suppressed. The effect of drought on carbon metabolizing patterns varied across aggregate size fractions. Particularly in S2 and S3, AWCD decreased by 50% and 49.2% under drought compared to control, respectively (Fig. 2b), and almost all four diversity indices of the carbon metabolizing patterns significantly decreased (Fig. S5).

Utilization of all six categories of carbon sources was in general negatively affected by long-term drought (Fig. 3). Overall, treatment significantly impacted the utilization of amino acids, polymers and carboxylic acids (Table S1). Drought specifically decreased utilization of polymers in larger aggregates (S1-S2), and that of carboxylic acids in smaller aggregates (S3-S4), with the former of which showing a decreasing trend toward aggregates of smaller sizes ($P < 0.05$). Drought also significantly decreased carbohydrates utilization in S2, and the utilization of amino acids and amines in S3.

The impact of treatment and aggregate size was further analyzed for individual substrates (Fig. 4, Table S2). In S1, the decrease of polymer utilization under drought was primarily due to the significant decrease of the utilization of C1 (tween 40) and D1 (tween 80); In S2, the reduction of carbohydrate utilization was mostly owing to the significant decrease in H2 (D, L-α-glycerol phosphate) utilization. In S3, the decrease in the utilization of C4 (L-phenylalanine) and A4 (L-arginine) together resulted in the reduction of amino acids utilization. In S4, the utilization of only H2 (D, L-α-glycerol phosphate) and H4 (putrescine) exhibited significant decrease under drought.

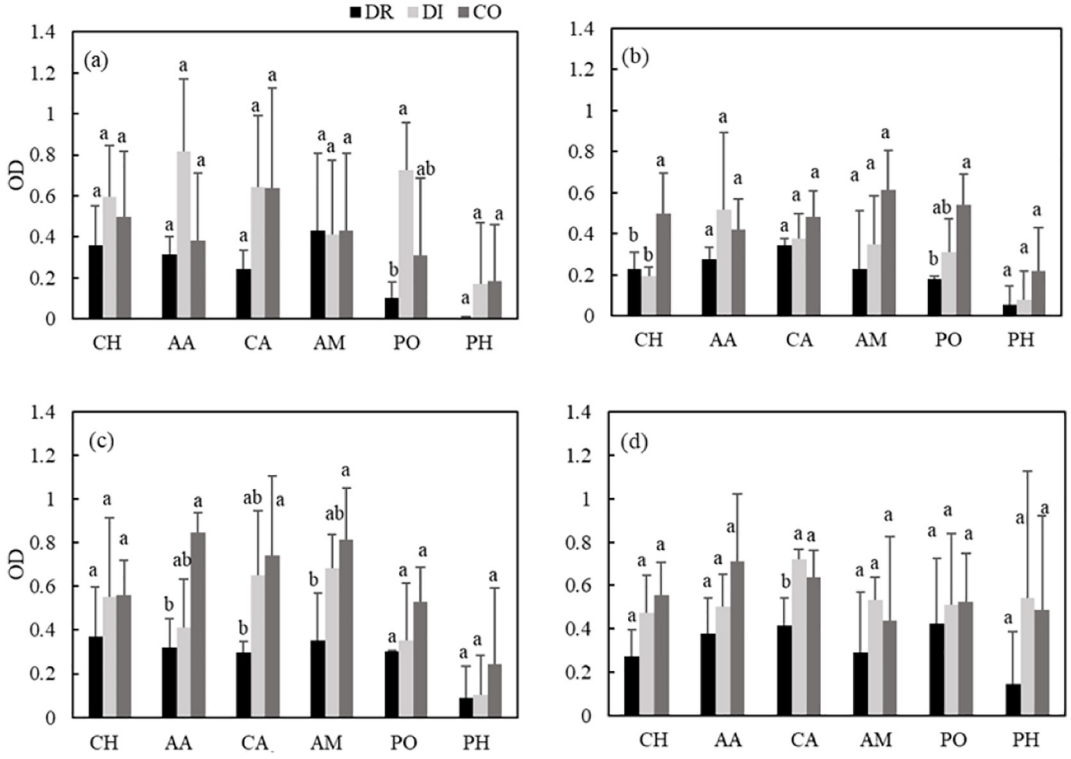

Fig. 3. The effect of treatment on utilization of six carbon source categories in (a) S1, (b) S2, (c) S3 and (d) S4. Different lowercase letters indicate significant differences ($P < 0.05$) among treatments within the same aggregate size fraction. Abbreviations are as follows: CH (carbohydrates), AA (amino acids), CA (carboxylic acids), AM (amines), PO (polymers), PH (phenolic compounds); DR, DI and CO correspond to Drought, Disturb and Control treatment, respectively. Error bars represent the standard deviations.

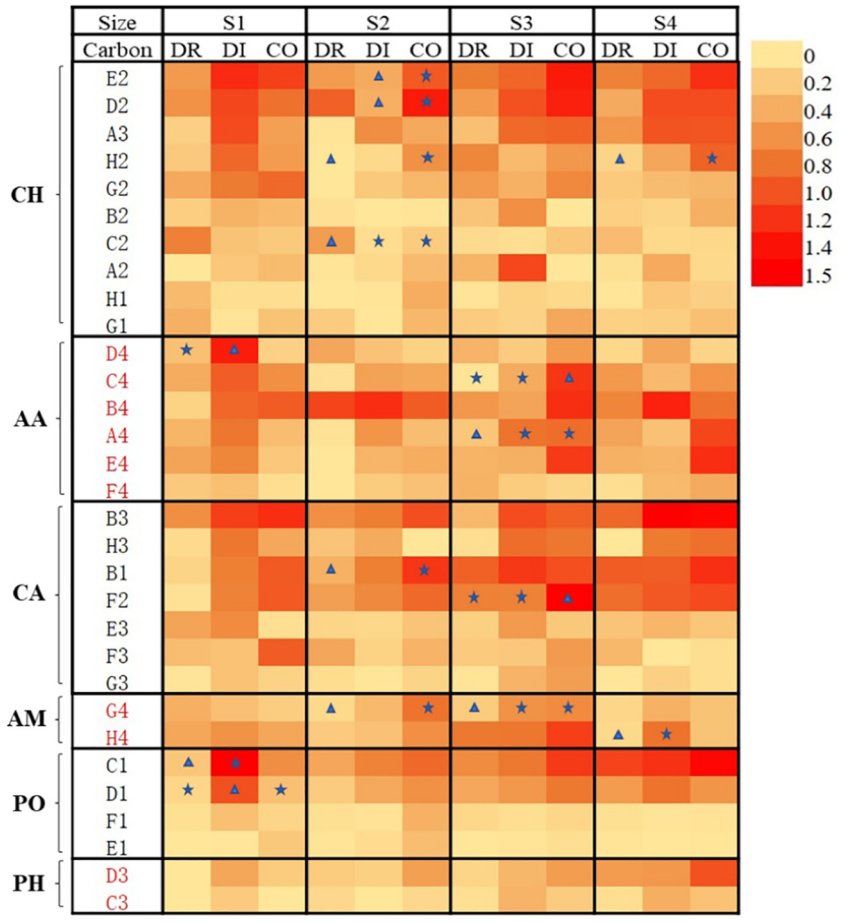

Fig. 4. The effect of treatment on utilization of 31 carbon sources across aggregate size fractions. Triangles and stars indicate the presence of significant differences ($P < 0.05$) in utilization capability between the two for the specific carbon source. Abbreviations are as follows: CH (carbohydrates), AA (amino acids), CA (carboxylic acids), AM (amines), PO (polymers), PH (phenolic compounds); DR, DI and CO correspond to Drought, Disturb and Control treatment, respectively; Colors indicate the average OD in the Biolog wells.

3.3. Principal component analysis of carbon source utilization patterns

The two-dimensional principle component analysis (PCA) explained 41.2% of the total variance of the carbon utilization patterns (Fig. 5a), with PC1 accounting for 31.0% and of considerable distinguishing power among treatments ($P < 0.01$). Specifically, drought samples mainly clustered at the negative side of PC1, while disturb and control samples primarily occupied the positive side. Note that treatment effect was primarily driven by the differences between samples from the drought treatment and those from the disturb and control treatments (Pairwise PERMANOVA, $R^2 = 0.085$ and 0.092, respectively; $P < 0.05$), with no significant differences between the latter two, suggesting that drought provided the main source of variation. Among the top ten carbon sources that contributed the most to the principle components,

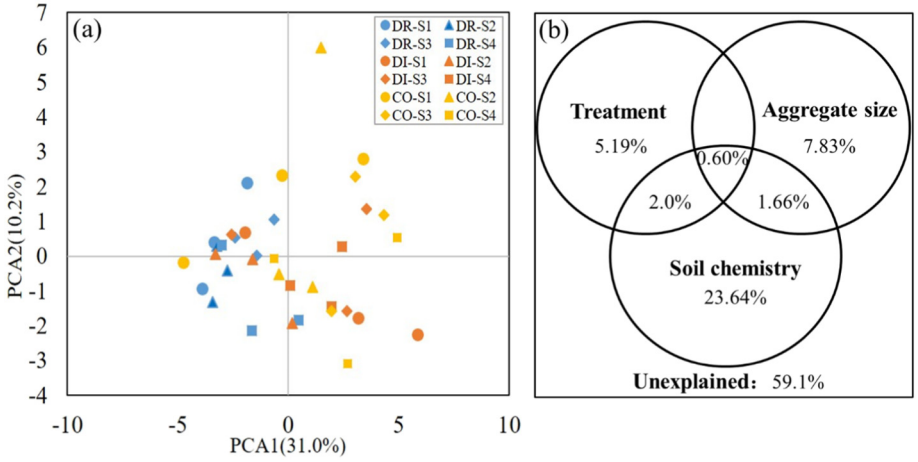

Fig. 5. (a) PCA of microbial carbon metabolizing patterns; (b) VPA of the effects of soil chemical properties, treatment and aggregate size on carbon metabolizing patterns. DR, DI and CO correspond to Drought, Disturb and Control treatment, respectively.

three belonged to carbohydrates, three were polymers, two were carboxylic acids, with the other two belonging to amino acids and phenolic compounds (Fig. S6). Hence, differential utilization of these carbon sources were important contributors to the overall metabolic differences across treatments.

3.4. Factors affecting carbon metabolizing functions

Variation partitioning analysis (VPA) was used to quantitatively assess the contribution from soil chemical properties, treatment, aggregate size and their interactions to carbon source metabolizing functions (Fig. 5b). In total, 40.9% of the carbon metabolic variation was explained. Soil chemical properties interpreted the greatest variation partition, by 23.64%, with treatment and aggregate size explaining 5.19% and 7.83%, respectively. There was little interaction among the variable categories.

Next, the associations between soil chemical properties and the overall carbon metabolizing patterns were analyzed across aggregate sizes (Table S3). None of the Biolog functional diversity indices was significantly correlated with TN, TC, NHC contents, or TC/TN and NHC/TC ratios. However, within and only in the smaller aggregate fractions S3 and S4, most of the diversity indices showed significant or extremely significant correlations with soil moisture. Canonical Correlation Analysis (CCA) also confirmed that the effect of soil moisture on carbon metabolizing patterns further intensified toward smaller aggregates (Table S3).

Correlations between soil chemical properties and the utilization of individual carbon sources were further analyzed (Fig. 6). Significant correlations were found between both the quantity and quality of soil nutrients and the utilization of specific carbon sources, and these associations varied across aggregate size fractions. Interestingly, more significant correlations between soil humus composition and carbon metabolism were found in S1 and S2. Particularly in S1, HA content was negatively associated with the utilization of a number of carbon sources, which likely contributed to the negative impact of HA on the overall carbon metabolizing capacity in this aggregate fraction (Table S3). Meanwhile, more correlations were found between TN, TC, NHC and carbon source utilization in S2 and S4; whereas in S3, the fewest significant correlations were revealed (Fig. 6).

3.5. Interaction between carbon metabolizing patterns and bacterial community compositions

Significant correlations between AWCD and the Shannon and Chao1 diversities of bacterial communities suggested positive interactions between microbial community composition and function (Fig. S7). Mantel test also revealed significant associations between β-diversities of the overall carbon source utilization patterns and bacterial community structure (Mantel test, $r = 0.21$, $P < 0.05$). In order to further analyze the effect of bacterial community composition on carbon utilization patterns, RDA was performed (Fig. S8). Bacterial community structure could explain 38.8% of the total variation in microbial carbon metabolism, with RDA1 explaining 30.4% of the variation. Utilization patterns of carbohydrates, carboxylic acids, amino acids, polymers as well as amines oriented along RDA1, while utilization of phenolic compounds oriented along RDA2. *Actinobacteria* and *Deltaproteobacteria* were the two taxa most related to microbial carbon metabolism, with the former showing increased abundance toward drought samples and negatively associated with utilization of majority of the carbon source categories, and the latter showing decreased abundance toward drought samples and positively associated with the carbon source categories.

Pairwise correlation analyses further revealed significant correlations between carbon source utilization and the abundances of major bacterial classes and orders across aggregate size fractions (Fig. 7). Significant associations were observed for 16 bacterial classes and 14 orders, and were mostly within smaller aggregates S2–S4. In some cases, utilization of one substrate was associated with the abundances of a number of bacterial taxa. For instance, amine utilization was correlated with the abundances of *Acidobacteria_Gp3*, *Planctomycetia* and *Betaproteobacteria* in S2, and with those of *Acidobacteria_Gp1*, *Actinobacteria*, *Subdivision3* and *Deltaproteobacteria* in S3. Meanwhile, the abundance of one bacterial taxon could also associate with the utilization of several carbon substrates. For instance, in S2, the abundance of *Betaproteobacteria* was correlated with utilization patterns of carbohydrates, carboxylic acids, amines as well as phenolic compounds, suggesting a key role of this group in carbon metabolism within this aggregate fraction.

Among the bacterial taxa significantly correlated with carbon metabolism, we found that their abundances were also affected by drought

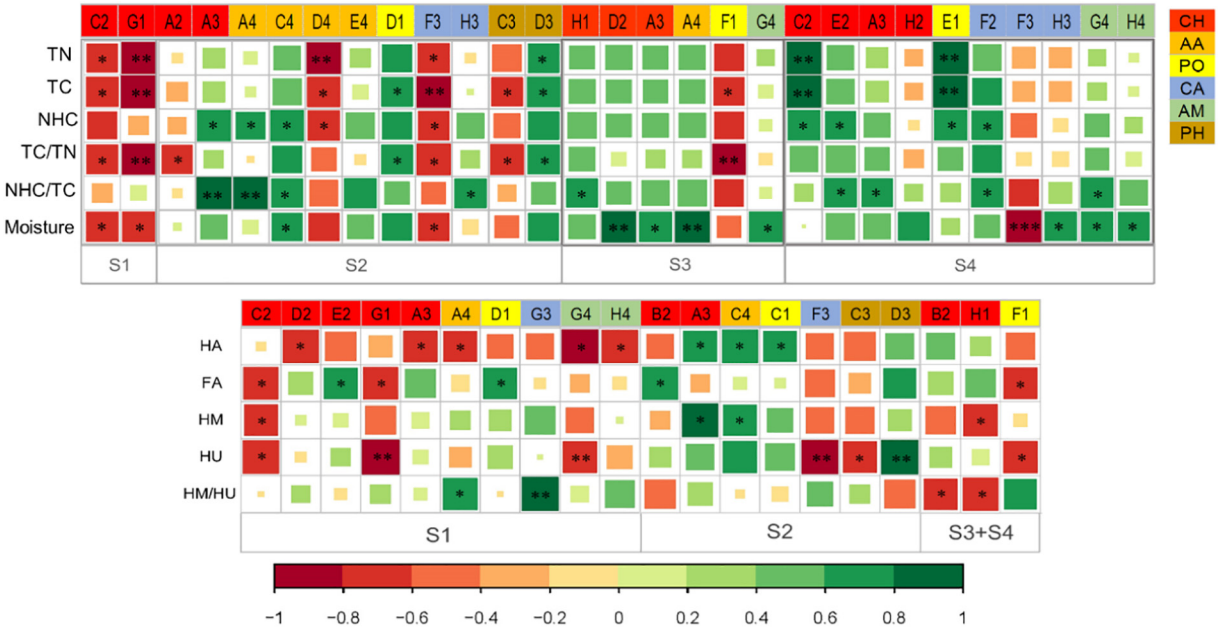

Fig. 6. The correlations between soil chemical properties and carbon source utilization across aggregate size fractions. Abbreviations are as follows: CH (carbohydrates), AA (amino acids), CA (carboxylic acids), AM (amines), PO (polymers), PH (phenolic compounds); DR, DI and CO correspond to Drought, Disturb and Control treatment, respectively; the color and size of the squares indicate the corresponding correlation coefficients; ***, **, * indicate significant correlations at $P < 0.001$, $P < 0.01$ and $P < 0.05$ levels, respectively.

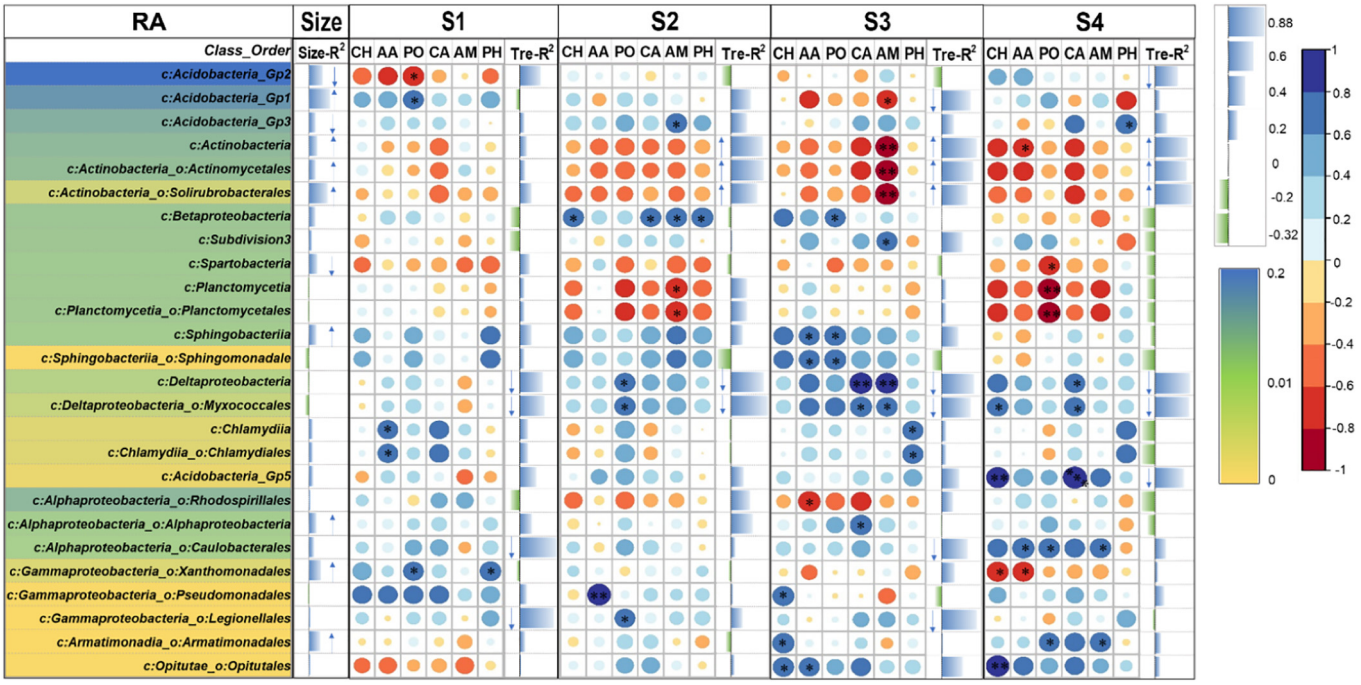

Fig. 7. The correlations between carbon source utilization and the abundances of bacterial classes and orders. The bacterial taxa were ordered based on the relative abundances of the classes. The color and size of the circles indicate the corresponding correlation coefficients; ***, **, * indicate significant correlations with $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively. The bars under Size-R^2 indicate the R^2 values based on ANOVA of the effect of aggregate size fraction; The bars under Tre-R^2 indicate the R^2 values based on ANOVA for the effect of treatment. ↑ and ↓ indicate significant increase or decrease with aggregate size or under drought treatment. Background colors of bacterial taxon names correspond to the average relative abundance of the specific class or order.

and aggregate size to varying degrees (Fig. 7). For instance, *Acidobacteria-Gp2* showed decreased abundances with the decrease of aggregate sizes, whereas *Acidobacteria-Gp1* showed the opposite pattern. The abundances of *Deltaproteobacteria* and *Myxococcales* were significantly reduced across all aggregate size fractions under drought, while that of *Actinobacteria* were significantly increased across S2 to S4. Altogether, these observations revealed a complex network of the impact of drought on bacterial community compositions and its relationships with carbon source utilization patterns.

4. Discussion

4.1. Drought decreased soil aggregate stability

Soil aggregate size distribution is a vital indicator of the stability of the soil's physical structure, and is the joined result from both processes of soil aggregation and degradation (Zhang et al., 2018a). Our results found that drought significantly decreased the large macroaggregate (S1) proportion while increased the microaggregate (S4) proportion (Fig. 2a). A range of factors may influence soil aggregate stability, including litter input, soil moisture, plant root extension, soil microbial activities, etc. (Algayer et al., 2014; Demenois et al., 2018). In this long-term experiment, our previous work revealed drought significantly reduced primary productivity and decreased litter biomass from an average of 654.89 g/m^2 in control plots to 593.96 g/m^2 in drought plots (Bu et al., 2018). We postulate that reduced litter biomass could be an important contributor to the loss of large macroaggregates, as this aggregate fraction is typically enriched in fresh litter-derived organic matters. Meanwhile, reduced water content may also weaken the effects of adhesive agents in large macroaggregates, which are structurally relatively loosely assembled compared to microaggregates (Chen et al., 2019). However, our study also revealed that drought significantly increased fine root biomass compared to that under control condition (Fig. S2h). Increased root growth, on one hand, could facilitate more efficient water and nutrient uptake by the plants (Brunner et al., 2015); while on the other, shall benefit soil aggregation through enmeshing fine particles and releasing polyvalent cations (Demenois et al., 2018). Yet increased fine root biomass, though mitigated, fail to offset the negative effect of long-term drought on macroaggregate stability, indicating that other forces were more strongly impacting the structural stability of soil macroaggregates.

4.2. Effects of drought on soil chemical properties

Five years of rainfall reduction significantly reduced soil water content, by 32.3% in annual mean soil moisture throughout the year of sampling (Fig. 1). In this study, however, sampling was conducted in a season with relatively frequent rainfall. Although the average soil moisture was lower in drought samples, differences from that in disturb and control plots were not statistically significant. Therefore, the observed differences in carbon metabolizing patterns and microbial community compositions among treatments, although partly associated with short-term fluctuations of soil moisture, may to a greater extent result from the cumulative changes over the long-term treatment (Preece et al., 2019).

Aside from soil moisture, no significant differences in the content of TC or TN were found among treatments (Fig. S2). Despite of the decrease in plant primary productivity, the overall rate of SOM decomposition was also decreased, evidenced by 21.3% annual mean reduction of soil respiration over the past four years (unpublished data). Hence, the TC and TN contents appeared to be a balanced net outcome of both the decrease in SOM input and in microbial decomposition activities. To a certain extent, this balance may provide an indicator for the resistance of the forest SOC storage under the influence of drought (Moreno et al., 2019). Nonetheless, the soil TC remains a rather coarse evaluation of the forest SOC storage function.

Within TC, the recalcitrant C fraction provides key contribution to long-term C storage owing to its longer residence time (McLauchlan and Hobbie, 2004). Compared to TC, NHC content is typically enriched in lignin, resin, suberin, etc., and is considered to represent the

recalcitrant portion of SOC (Rovira and Vallejo, 2002). Here, although soil TC has not changed, the recalcitrant carbon components showed decreasing trends. The NHC content significantly decreased by over 50% in S1 under drought, and the HM content in S2 also showed some decrease (Fig. S3 and S4). This indicates a change in the chemical stability of the SOC, which could associate with the C sequestrating potential of the forest ecosystem. We consider that the reduced plant litter input, especially the lignin and phenol components, could result in insufficient replenishment of vegetal carbon particularly into macroaggregates and may to some extent be responsible for the decline in recalcitrant C contents. In comparison, no significant difference in NHC content was observed in microaggregates. It is known that microaggregates in general contain greater fractions of recalcitrant compounds and have slower C turnover rates, i.e., by over a hundred years compared to weeks or months in macroaggregates (Tisdall and Oades, 2012). Hence, we postulate that it may require a longer time for the SOC composition within microaggregates to respond to drought (Tisdall and Oades, 2012; Grunwald et al., 2016). Therefore, it remains necessary to continually track the changes in SOC composition and other soil chemical characteristics over prolonged duration of drought for better understanding of the long-term dynamics and kinetics of the forest SOC pool.

4.3. Soil physical-chemical properties drove microbial carbon metabolizing patterns

Soil aggregates of varied sizes create distinct niches for microorganisms due to the differences in soil physical-chemical characteristics, therefore promoting the colonization of distinct microbial assemblages (Davinic et al., 2012; Tiemann et al., 2015). The soil microbial metabolic patterns are impacted by the joined effects of stratified soil physical-chemical conditions and the heterogeneity in microbial community composition (Chen et al., 2015). This study identified a series of significant associations between carbon substrate utilization abilities and soil chemical properties, suggesting that both the quantity and quality of SOC are critical factors influencing soil microbial activities (Fig. 6). Nonetheless, a substantial proportion of variation remained unexplained, which may be associated with additional environmental factors that was not investigated in the present study (Fierer and Jackson, 2006; Stein et al., 2014; Tian et al., 2015).

Soil moisture is one of the most important factors influencing the functions and activities of soil microorganisms (Alster et al., 2013; Bu et al., 2018; Wang et al., 2019a). In this study, we found that the carbon metabolizing functions in microaggregates are particularly sensitive to changes in soil moisture (Fig. 6 and Table S3). This phenomenon may in part be due to the structural differences among aggregates of different sizes (Zhou et al., 2008). Macroaggregates are penetrated with fungal hyphae, plant root tissues, and are characterized of higher porosity (Mangalassery et al., 2013; Yang et al., 2019), which is beneficial for retaining of soil water (Regelink et al., 2015; Lu et al., 2019b). In contrast, microaggregates contain smaller-size pores with microorganisms mostly inhabiting the surface area, resulting in higher sensitivity of the microbial metabolic processes to moisture change of the outer environment (Utomo and Dexter, 1981; Upton et al., 2019). Therefore, resistance of microbial processes to drought may be partially mediated by soil's physical structure. More importantly, with the growing proportion of microaggregates under the influence of long-term drought, the sensitivity of microbial metabolism to soil moisture may continually increase, which could further decrease the functional resistance of the microbial communities.

4.4. Effects of drought on microbial carbon metabolizing capacities

The color development within the Biolog plates reflects the potential of bacterial growth on the provided substrate (Braun et al., 2010; Kumar et al., 2017). In this study, long-term drought has not only weakened the overall carbon utilization abilities of soil microorganisms, but also changed the carbon metabolic patterns across aggregate sizes (Figs. 2b and 3). Specifically, the utilization of small-molecule compounds, including amino acids, carboxylic acids and amines, reduced significantly under drought and particularly in smaller aggregates (S3–S4), which implicates decreased availability of these carbon sources. This may to some extent associate with reduced circulation of partially decomposed carbon sources into smaller aggregates due to decreased plant litter biomass (Liu et al., 2018). In addition, small-molecule compounds in the soil including amino acids and carboxylic acids, could derive from plant root exudates (Barnard et al., 2015; Preece and Peñuelas, 2016), while diffusion of these compounds from fine root-enriched large macroaggregates to microaggregates could be restricted in dried soil (Schimel et al., 2007). However, although drought may reduce root exudation (Brunner et al., 2015), it is not yet known how total root exudation could change with increased fine root biomass in this experimental site (Bu et al., 2018). Hence, our observations also suggest further experimental quantification of root exudates may facilitate better understanding of the belowground SOC balance and the associated decomposing activities.

4.5. Impacts of drought on carbon metabolizing patterns through its effects on soil microbial community composition

Our results showed that the composition of soil bacterial communities was an important driver of the carbon source utilization patterns. Firstly, the overall carbon utilization capacity was significantly correlated with bacterial α-diversity ($P < 0.05$, Fig. S7), suggesting greater microbial taxonomic diversity sustained greater functional capacity and diversity. This phenomenon was also supported by many other studies using unfractionated soils (Cesarano et al., 2017; Wang et al., 2019b). Here, loss of bacterial diversity under long-term drought resulted in not only reduced growth on the tested carbon sources, but also decreased number of carbon sources that could be consumed. Hence, both the intensity and the versatility of the belowground microbial carbon metabolism were changed. Specifically, the effect of drought on carbon utilization patterns was most significant in S2 and S3, consistent with our finding that the impact of drought on bacterial community structure was also more significant in smaller aggregates (S2-S4). In contrast, fungal communities were more impacted in large macroaggregates (S1) (unpublished data by a parallel study). Moreover, more significant correlations were present between consumption of carbon substrates and the abundances of specific bacterial taxa in smaller aggregates (Fig. 7), further supporting that the inhabited microorganisms in these aggregate fractions were responsible for utilization of the related organic compounds (Bach et al., 2018).

Secondly, responses of carbon metabolism functions to drought were associated with abundance changes of specific bacterial taxa across aggregate size fractions (Fig. 7 and S5). In S1, drought particularly reduced the utilization of polymers, which was positively correlated with the abundance of *Acidobacteria_Gp1*, while negatively correlated with that of *Acidobacteria_Gp2*. Although the abundance of neither class varied significantly with treatment, the former showed increased abundances toward microaggregates; whereas the latter exhibited the opposite pattern. Such observations suggest groups of *Acidobacteria* may have different preference of carbon sources, hence their response to changing climate may differentially impact the processes of SOC cycling.

In S2, utilization of both polymers and carbohydrates was specifically reduced under drought. Decreased polymer utilization was significantly associated with decreased abundance of *Deltaproteobacteria* and especially its order *Myxococcales*, the latter of which was known for its capabilities of secreting extracellular hydrolytic enzymes to facilitate decomposition of complex carbon sources (Brenner, 2006). In the meanwhile, the abundance of *Betaproteobacteria* was positively correlated not only with the utilization of carbohydrates, but also with that

of carboxylic acids, amines as well as phenolic compounds. Many members of *Betaproteobacteria* are copiotrophic bacteria and able to rapidly utilize bioavailable SOM and prosper in nutrient-rich environments (Cesarano et al., 2017). Our results suggest this group may be heavily involved in metabolism of a range of carbon sources in medium-size macroaggregates.

In smaller aggregates S3 and S4, drought significantly decreased the abundances of *Deltaproteobacteria* and *Myxococcales*, by over 50% for both taxa. Abundances of the two taxa were positively correlated with the utilization of carboxylic acids in both aggregate fractions, and with that of amines in S3. It is known that *Myxococcales* typically favor organic-rich environments, and are metabolically versatile in SOC utilization (Wang et al., 2020). Hence, substantially decreased abundance of *Myxococcales* could indicate lack of bioavailable carbon sources, especially small-molecule compounds in smaller aggregates. In S3, reduced amine utilization was also associated with increased abundances of *Acidobacteria_Gp1* and *Actinobacteria* under drought, both taxa of which likely preferentially utilize more complex carbon sources rather than small-molecule compounds (Kumar et al., 2017; Zhang et al., 2018b). In S4, utilization of a range of carbon sources including amino acids, polymers and amines was associated with the abundances of *Caulobacterales* and *Acidobacteria_Gp5*. Altogether, these observations revealed that members of *Proteobacteria*, *Acidobacteria* and *Actinobacteria* played major regulatory roles in soil carbon cycling; in addition, the effect of long-term drought on soil carbon metabolization was closely associated with its impact on the abundance changes of these taxa.

5. Conclusions

Understanding the effects of drought on soil microbial carbon metabolization is crucial for better prediction of the changes in soil carbon cycling and storage under future's inevitable extreme climate. In this study, after five years of rainfall-reduction experiment, drought substantially reduced the proportion of large macroaggregates and the quality of SOC contained. Carbon metabolism was impacted in all aggregate size fractions, which was associated with the changes in both soil chemical properties and the structure of the microbial communities. Therefore, biotic and abiotic forces together impacted and shaped the responses of carbon metabolizing processes and the SOC storage to drought. This study also highlights that more attention shall be paid on changes in environmental sensitivity of microbial communities for better understanding of the resistance and resilience of ecosystem functions under the long-term drought scenario.

CRediT authorship contribution statement

Xin Su: Formal analysis, Investigation, Writing - original draft, Software, Visualization. **Xueling Su:** Formal analysis, Investigation, Visualization, Data curation. **Songchen Yang:** Formal analysis, Conceptualization, Methodology. **Guiyao Zhou:** Resources, Writing - review & editing. **Mengying Ni:** Formal analysis, Investigation. **Chao Wang:** Formal analysis, Investigation. **Hua Qin:** Conceptualization, Methodology. **Xuhui Zhou:** Resources. **Jie Deng:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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

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References

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., He, X., 2015. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biol. Biochem. 80, 70–78.
Alboukadel, K., Fabian, M., 2017. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R Package Version 1.0.5.
Algayer, B., Le Bissonnais, Y., Darboux, F., 2014. Short-Term Dynamics of Soil Aggregate Stability in the Field. 78 pp. 1168–1176.
Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol. Biochem. 64, 68–79.
Bach, E.M., Hofmockel, K.S., 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biol. Biochem. 69, 54–62.
Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F., Hofmockel, K.S., 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biol. Biochem. 118, 217–226.
Barnard, R.L., Osborne, C.A., Firestone, M.K., 2015. Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. ISME J. 9, 946.
Bastida, F., Torres, I.F., Andrés-Abellán, M., Baldrian, P., López-Mondéjar, R., Větrovský, T., Richnow, H.H., Starke, R., Ondoño, S., García, C., López-Serrano, F.R., Jehmlich, N., 2017. Differential sensitivity of total and active soil microbial communities to drought and forest management. Glob. Chang. Biol. 23, 4185–4203.
Beare, M., Hendrix, P., Coleman, D., 1994. Water-stable aggregates and organic matter fractions in conventional-and no-tillage soils. Soil Sci. Soc. Am. J. 58, 777–786.
Bouskill, N.J., Lim, H.C., Borglin, S., Salve, R., Wood, T.E., Silver, W.L., Brodie, E.L., 2013. Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. ISME J. 7, 384–394.
Braun, B., Böckelmann, U., Grohmann, E., Szewzyk, U., 2010. Bacterial soil communities affected by water-repellency. Geoderma 158, 343–351.
Brenner, D.J., 2006. Bergey's Manual® of Systematic Bacteriology Volume Two the Proteobacteria Part C the Alpha-, Beta-, Delta-, and Epsilonproteobacteria. Bergey's Manual Trust, Boston, MA.
Brunner, I., Herzog, C., Dawes, M.A., Arend, M., Sperisen, C., 2015. How tree roots respond to drought. Front. Plant Sci. 6, 547.
Bu, X., Gu, X., Zhou, X., Zhang, M., Guo, Z., Zhang, J., Zhou, X., Chen, X., Wang, X., 2018. Extreme drought slightly decreased soil labile organic C and N contents and altered microbial community structure in a subtropical evergreen forest. For. Ecol. Manag. 429, 18–27.
Canarini, A., Carrillo, Y., Mariotte, P., Ingram, L., Dijkstra, F.A., 2016. Soil microbial community resistance to drought and links to C stabilization in an Australian grassland. Soil Biol. Biochem. 103, 171–180.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621.
Cesarano, G., De Filippis, F., La Storia, A., Scala, F., Bonanomi, G., 2017. Organic amendment type and application frequency affect crop yields, soil fertility and microbiome composition. Appl. Soil Ecol. 120, 254–264.
Chen, X., Li, Z., Liu, M., Jiang, C., Che, Y., 2015. Microbial community and functional diversity associated with different aggregate fractions of a paddy soil fertilized with organic manure and/or NPK fertilizer for 20 years. J. Soils Sediments 15, 292–301.
Chen, Z., Zhou, X., Geng, S., Miao, Y., Cao, Y., Chen, Z., Zhang, J., Han, S., 2019. Interactive effect of nitrogen addition and throughfall reduction decreases soil aggregate stability through reducing biological binding agents. For. Ecol. Manag. 445, 13–19.
Chevuturi, A., Klingaman, N.P., Turner, A.G., Hannah, S., 2018. Projected changes in the Asian-Australian monsoon region in 1.5° C and 2.0° C global-warming scenarios. Earth's Future 6, 339–358.
Da, L., Yang, Y., Song, Y., 2004. Population structure and regeneration types of dominant species in an evergreen broadleaved forest in Tiantong National Forest Park, Zhejiang province, eastern China. J. Plant Ecol. 376–384.
Dai, A., 2011. Drought under global warming: a review. Wiley Interdiscip. Rev. Clim. Chang. 2, 45–65.
Davinic, M., Fultz, L.M., Acosta-Martinez, V., Calderón, F.J., Cox, S.B., Dowd, S.E., Allen, V.G., Zak, J.C., Moore-Kucera, J., 2012. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biol. Biochem. 46, 63–72.
de Mendiburu, F., de Mendiburu, M.F., 2019. Package 'Agricolae.' R Package, Version 1–2.
De Vries, F.T., Liiri, M.E., Bjørnlund, L., Setälä, H.M., Christensen, S., Bardgett, R.D., 2012. Legacy effects of drought on plant growth and the soil food web. Oecologia 170, 821–833.
Demenois, J., Carriconde, F., Bonaventure, P., Maeght, J.-L., Stokes, A., Rey, F., 2018. Impact of plant root functional traits and associated mycorrhizas on the aggregate stability of a tropical Ferralsol. Geoderma 312, 6–16.

Deng, J., Gu, Y., Zhang, J., Xue, K., Qin, Y., Yuan, M., Yin, H., He, Z., Wu, L., Schuur, E.A.G., Tiedje, J.M., Zhou, J., 2015. Shifts of tundra bacterial and archaeal communities along a permafrost thaw gradient in Alaska. Mol. Ecol. 24, 222–234.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. 103, 626–631.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecol. Lett. 88, 1354–1364.
Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., Richter, A., 2014. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytol. 201, 916–927.
Gabarrón-Galeote, M.A., Trigalet, S., van Wesemael, B., 2015. Effect of land abandonment on soil organic carbon fractions along a Mediterranean precipitation gradient. Geoderma 249-250, 69–78.
Garland, J.L., 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem. 28, 213–221.
Grunwald, D., Kaiser, M., Ludwig, B., 2016. Effect of biochar and organic fertilizers on C mineralization and macro-aggregate dynamics under different incubation temperatures. Soil Tillage Res. 164, 11–17.
Hoover, D.L., Rogers, B.M., 2016. Not all droughts are created equal: the impacts of interannual drought pattern and magnitude on grassland carbon cycling. Glob. Chang. Biol. 22, 1809–1820.
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.
Kumada, K., 1988. Chemistry of Soil Organic Matter. Elsevier.
Kumar, U., Shahid, M., Tripathi, R., Mohanty, S., Kumar, A., Bhattacharyya, P., Lal, B., Gautam, P., Raja, R., Panda, B.B., 2017. Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. Ecol. Indic. 73, 536–543.
Kushwaha, C.P., Tripathi, S.K., Singh, K.P., 2001. Soil organic matter and water-stable aggregates under different tillage and residue conditions in a tropical dryland agroecosystem. Appl. Soil Ecol. 16, 229–241.
Lagomarsino, A., Grego, S., Kandeler, E., 2012. Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. Pedobiologia 55, 101–110.
Liu, Y., Cai, R., Liu, Y., Ge, G., 2018. Dynamic response of wetland soil microbial carbon source metabolic activity to different water conditions – a case study of Poyang lake. Soils 50, 705–711.
Lu, M., Yang, M., Yang, Y., Wang, D., Sheng, L., 2019a. Soil carbon and nutrient sequestration linking to soil aggregate in a temperate fen in Northeast China. Ecol. Indic. 98, 869–878.
Lu, S., Yu, X., Zong, Y., 2019b. Nano-microscale porosity and pore size distribution in aggregates of paddy soil as affected by long-term mineral and organic fertilization under rice-wheat cropping system. Soil Tillage Res. 186, 191–199.
Mangalassery, S., Sjögersten, S., Sparkes, D.L., Sturrock, C.J., Mooney, S.J., 2013. The effect of soil aggregate size on pore structure and its consequence on emission of greenhouse gases. Soil Tillage Res. 132, 39–46.
Manjunath, M., Kumar, U., Yadava, R.B., Rai, A.B., Singh, B., 2018. Influence of organic and inorganic sources of nutrients on the functional diversity of microbial communities in the vegetable cropping system of the Indo-Gangetic plains. Comptes Rendus Biol. 341, 349–357.
McLauchlan, K.K., Hobbie, S.E., 2004. Comparison of labile soil organic matter fractionation techniques. Soil Sci. Soc. Am. J. 68, 1616–1625.
Moreno, J.L., Torres, I.F., García, C., López-Mondéjar, R., Bastida, F., 2019. Land use shapes the resistance of the soil microbial community and the C cycling response to drought in a semi-arid area. Sci. Total Environ. 648, 1018–1030.
Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., O'Hara, R., 2016. Vegan: Community Ecology Package. R Package 2.3–3.
Preece, C., Peñuelas, J., 2016. Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. Plant Soil 409, 1–17.
Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Peñuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biol. Biochem. 131, 28–39.
Regelink, I.C., Stoof, C.R., Rousseva, S., Weng, L., Lair, G.J., Kram, P., Nikolaidis, N.P., Kercheva, M., Banwart, S., Comans, R.N.J., 2015. Linkages between aggregate formation, porosity and soil chemical properties. Geoderma 247-248, 24–37.
Rovira, P., Vallejo, V.R., 2002. Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. Geoderma 107 (1), 109–141.
Rovira, P., Vallejo, V.R., 2007. Labile, recalcitrant, and inert organic matter in Mediterranean forest soils. Soil Biol. Biochem. 39, 202–215.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecol. Lett. 88, 1386–1394.
Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103.
Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79, 7–31.
Sofo, A., Elshafie, H.S., Scopa, A., Mang, S.M., Camele, I., 2018. Impact of airborne zinc pollution on the antimicrobial activity of olive oil and the microbial metabolic profiles of Zn-contaminated soils in an Italian olive orchard. J. Trace Elem. Med. Biol. 49, 276–284.
Stein, A., Gerstner, K., Kreft, H., 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecol. Lett. 17, 866–880.
Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., 2013. Climate Change 2013: The Physical Science Basis. Cambridge University Press Cambridge.
Team, R.C., 2019. R-A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, p. 2019.
Tian, J., McCormack, L., Wang, J., Guo, D., Wang, Q., Zhang, X., Yu, G., Blagodatskaya, E., Kuzyakov, Y., 2015. Linkages between the soil organic matter fractions and the microbial metabolic functional diversity within a broad-leaved Korean pine forest. Eur. J. Soil Biol. 66, 57–64.
Tiemann, L., Grandy, A., Atkinson, E., Marin-Spiotta, E., McDaniel, M., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecol. Lett. 18, 761–771.
Tisdall, J., Oades, J., 2012. Landmark papers: no. 1. Organic matter and water-stable aggregates in soils. Eur. J. Soil Sci. 63, 141–163.
Uhlířová, E., Elhottová, D., Tříska, J., Šantrůčková, H., 2005. Physiology and microbial community structure in soil at extreme water content. Folia Microbiol. 50, 161.
Upton, R.N., Bach, E.M., Hofmockel, K.S., 2019. Spatio-temporal microbial community dynamics within soil aggregates. Soil Biol. Biochem. 132, 58–68.
Utomo, W., Dexter, A., 1981. Soil friability. J. Soil Sci. 32, 203–213.
van Zomeren, A., Comans, R.N., 2007. Measurement of humic and fulvic acid concentrations and dissolution properties by a rapid batch procedure. Environ. Sci. Technol. 41, 6755–6761.
Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261.
Wang, H., Wang, S., Wang, R., Zhang, Y., Wang, X., Li, J., 2019a. Direct and indirect linkages between soil aggregates and soil bacterial communities under tillage methods. Geoderma 354, 113879.
Wang, S., Li, T., Zheng, Z., Chen, H.Y., 2019b. Soil aggregate-associated bacterial metabolic activity and community structure in different aged tea plantations. Sci. Total Environ. 654, 1023–1032.
Wang, W., Luo, X., Ye, X., Chen, Y., Wang, H., Wang, L., Wang, Y., Yang, Y., Li, Z., Cao, H., Cui, Z., 2020. Predatory *Myxococcales* are widely distributed in and closely correlated with the bacterial community structure of agricultural land. Appl. Soil Ecol. 146, 103365.
Yang, C., Liu, N., Zhang, Y., 2019. Soil aggregates regulate the impact of soil bacterial and fungal communities on soil respiration. Geoderma 337, 444–452.
Yılmaz, E., Çanakcı, M., Topakcı, M., Sönmez, S., Ağsaran, B., Alagöz, Z., Çıtak, S., Uras, D.S., 2019. Effect of vineyard pruning residue application on soil aggregate formation, aggregate stability and carbon content in different aggregate sizes. CATENA 183, 104219.
Yu, G., Chen, Z., Piao, S., Peng, C., Ciais, P., Wang, Q., Li, X., Zhu, X., 2014. High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon region. Proc. Natl. Acad. Sci. 111, 4910–4915.
Zhang, Q., Shao, M.a., Jia, X., Zhang, C., 2018a. Understory vegetation and drought effects on soil aggregate stability and aggregate-associated carbon on the Loess Plateau in China. Soil Sci. Soc. Am. J. 82, 106–114.
Zhang, X., Liu, S., Huang, Y., Fu, S., Wang, J., Ming, A., Li, X., Yao, M., Li, H., 2018b. Tree species mixture inhibits soil organic carbon mineralization accompanied by decreased r-selected bacteria. Plant Soil 431, 203–216.
Zhang, Q., Shao, M., Jia, X., Wei, X., 2019a. Changes in soil physical and chemical properties after short drought stress in semi-humid forests. Geoderma 338, 170–177.
Zhang, Y., Li, X., Gregorich, E.G., McLaughlin, N.B., Zhang, X., Guo, Y., Gao, Y., Liang, A., 2019b. Evaluating storage and pool size of soil organic carbon in degraded soils: tillage effects when crop residue is returned. Soil Tillage Res. 192, 215–221.
Zhao, M., Running, S.W., 2010. Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. Science 329, 940–943.
Zhou, X., Lin, H.S., White, E.A., 2008. Surface soil hydraulic properties in four soil series under different land uses and their temporal changes. CATENA 73, 180–188.