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RESEARCH ARTICLE

Soil fungi and fine root biomass mediate drought-induced reductions in soil respiration

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Abstract

1. Climate change has increased the frequency and intensity of droughts, with potential impacts on carbon (C) release from soil (i.e. soil respiration, *Rs*). Although numerous studies have investigated drought-induced changes in *Rs*, how roots and the soil microbial community regulate responses of *Rs* to drought remains unclear.
2. We conducted a 4-year field experiment (2014–2017) with three treatments (i.e. 70% rainfall reduction, control and ambient) in a subtropical forest to examine effects of drought on *Rs* and its components [i.e. autotrophic (*Ra*) and heterotrophic respiration (*Rh*)] and explore the mechanisms underlying these effects.
3. Drought significantly decreased *Rs* by 17% averaged over the 4 years, but it had no significant effect in the first experimental year. The decrease in *Rs* was mediated by soil fungi and fine root biomass. Fine root biomass was correlated negatively with *Ra* and *Rs* under drought, but positively in the control treatment. Furthermore, drought treatments increased physiological stress in the bacterial community. Structural equation model (SEM) analysis suggested that under drought conditions, microclimate affected *Rs* via its impact on fine root biomass and fungal biomass.
4. Our results highlight the complex interactions between microclimate, roots and soil microbes in regulating *Rs* under drought in subtropical forest ecosystems. Incorporating these interactions into land surface models may improve predictions of climate change impacts on forest ecosystems.

KEYWORDS

C-climate feedback, CO_2 emission, drought, fine root biomass, fungi community, physiological stress, structural equation model

1 | INTRODUCTION

Earth system models predict that global hydrological cycles will intensify in the next decades (IPCC, 2013), thereby altering the frequency and intensity of precipitation around the world (IPCC, 2013). Consequently, several regions will experience increased droughts, which threaten the biodiversity and stability of terrestrial ecosystems and alter ecosystem structure and function

(Carey et al., 2016; IPCC, 2013; Zhang et al., 2020). Actually, many regions are already affected by the consequences of increased droughts. For example, the drier regions of Northeast China are receiving progressively less summer precipitation since 1960, which has largely altered their carbon (C) balance (Piao et al., 2010). In addition, declines in summer precipitation in Europe significantly influenced plant photosynthesis, growth and productivity, which further modified ecosystem resilience to climate change (Beier et al., 2012). Drought-induced changes in terrestrial C cycling can create both positive and negative feedbacks to climate change, and could either amplify or diminish drought effects (Carey et al., 2016). Thus, mechanisms governing the response of ecosystem C dynamics to drought are crucial to understanding feedbacks between the C cycle and climate change.

Soil respiration (*Rs*) represents the CO_2 flux from the soil surface to the atmosphere and includes autotrophic respiration from roots and their microbial symbionts (*Ra*) and heterotrophic respiration from soil microbes decomposing litter and soil organic matter (*Rh*; Luo & Zhou, 2006; Zhou et al., 2017). *Rs* is the second-largest C flux (68–98 Pg C yr^{-1}) between terrestrial ecosystems and the atmosphere, approximately 10 times greater than the C flux associated with fossil fuel combustion (Bond-Lamberty et al., 2018; Zhou et al., 2017). Thus, minor changes in *Rs* could potentially affect atmospheric CO_2 concentrations (IPCC, 2013).

Model simulations and field experiments both indicate that drought decreases soil water availability (Ru et al., 2018) and influences *Rs* by modifying roots and soil microbial communities (Luo & Zhou, 2006). Drought generally decreases fine root biomass and soil microbial activity through decreasing input of photosynthates, thereby suppressing *Rs* and its components (Liu et al., 2016; Zhou et al., 2018). However, a 1-year study in a tropical rain forest of Costa Rica found that drought stimulated *Rs* by lowering soil moisture content and increasing oxygen availability for the growth of both roots and soil microbes (Cleveland et al., 2010). Thus, drought effects on *Rs* are ecosystem specific, and are determined by interactions with multiple environmental variables.

Predictions from regional and global *Rs* models differ both in magnitude and direction of root and microbial responses to drought (Bond-Lamberty et al., 2018; Yan et al., 2018). These differences partly reflect the uncertainties inherent to model parameterization of roots and microbes, especially their relative contribution, and their direct and indirect effects on *Rs* in response to drought. Complex interactions between microclimatic conditions, roots and soil microbes present a major uncertainty in predicting C-cycle dynamics (e.g. *Rs*, Zhou et al., 2012). Nonetheless, drought studies generally focus on only one of these three factors to investigate responses of *Rs* to drought, and do not quantitatively partition their direct and indirect effects (Bond-Lamberty et al., 2018).

Earth system models usually use empirical moisture functions to predict *Rs*. By neglecting the role of roots and microbes in regulating *Rs*, this approach causes large uncertainty in model projections (Yan et al., 2018). Model performance may be improved by incorporating drought responses of root and microbes from experimental studies into conceptual frameworks and biogeochemical models (Bond-Lamberty et al., 2018). Thus, accurate predictions of future climate-C feedbacks require manipulative experiments to quantify relationships between *Rs* and its driving factors under drought.

To determine how roots and soil microbes regulate *Rs* and its components (*Ra* and *Rh*) under drought, we conducted a 4-year field experiment in a subtropical forest in Eastern China. We then applied structure equation model (SEM) techniques to explore the direct and indirect effects of microclimate, roots and soil microbes on *Rs* under control and drought conditions. We aimed to answer the following questions: (a) How does drought affect roots and soil microbes? (b) What is the relative importance of microclimate, roots and soil microbes in determining the response of *Rs* to drought? (c) How do microclimate, roots and microbes interact to determine *Rs* under control and drought conditions?

2 | MATERIALS AND METHODS

2.1 | Site description

The experiment was carried out at the Tiantong National Field Observation Station for Forest Ecosystems (29°48′N, 121°47′E), Zhejiang Province, China (Figure S1). The study region has a subtropical monsoon climate with humid, hot summers and dry cold winters. Mean annual temperature is 16.2°C, ranging from 4.2°C in January to 28.2°C in July. The mean annual precipitation is 1,374 mm, which mainly occurs from May to August (1978–2016, China Climatological Survey in Tiantong, Zhejiang). The growing season of forest ecosystems in this region lasts from May to the end of October. The experimental site was located in a natural forest without human disturbance, and the average forest age ranged from 50 to 55 years (Liu, Zhou, Wang, et al., 2019). The study region is water-limited based on the global relationship between precipitation and net primary productivity (NPP; Taylor et al., 2017). The soil at our site is an Acrisol, with a pH ranging from 4.4 to 5.1 (Liu, Zhou, Bai, et al., 2019). The soil has a clay loam texture with 6.8% sand, 55.5% silt and 37.7% clay (Liu, Zhou, Bai, et al., 2019; Yan et al., 2006). Dominant tree species include *Schima superba*, *Castanopsis fargesii* and *Lithocarpus glaber*.

2.2 | Experimental design and treatments

Our throughfall exclusion experiment had a randomised complete block design, with three replications and three treatments: drought, control and ambient conditions. Three blocks with similar topography (i.e. slope and aspect), vegetation species and site properties (i.e. the amount of vegetation and rock) were established in July 2013. Three 25 m × 25 m plots were established in each block, and each treatment was randomly allocated to one of the three plots. Each plot was enclosed with PVC board (2.5 mm in thickness), which was inserted into the soil to a depth of 2 m to prevent lateral water movement into the plots and to prevent

it from escaping the plots. The individual plots were at least 5 m apart. Buffer zones (2.5 m wide) were marked along the inside edge of each plot. To minimize artefacts related to plot establishment, no measurements were taken inside the buffer zone (Gao et al., 2015; Su, Su, Yang, et al., 2020).

In each drought plot, transparent V-shaped polycarbonate plates were uniformly fixed at 2.5 m height above the ground to evenly reduce the rainfall by *c*. 70% (Figure S1). In the control plot, we also uniformly fixed transparent V-shaped polycarbonate plates with the same size and shape as in the drought treatment. In the control plots, the plates were turned upside down; this design allowed rainfall to reach the forest floor but provided the same shading conditions as the drought plots induced by polycarbonate plates. The ambient plots did not include any polycarbonate plates. We inferred drought effects from comparisons between drought plots and control plots; ambient plots were used to test whether shading by the polycarbonate plates affected the responses of any measured variables to drought.

2.3 | Soil respiration measurements

To measure *Rs*, PVC collars (20 cm in inner diameter and 11 cm in height) were permanently inserted into the soil at 5–6 cm depth at the centre of each plot, and in each quarter of each plot. Thus, each plot contained five PVC collars for *Rs* measurements. Each PVC collar had twenty-four 8-mm holes distributed evenly in the pipe wall below the ground level to allow root growth. Small living plants inside the collars were removed manually without soil disturbance 24 hr prior to *Rs* measurement to eliminate above-ground plant respiration. *Rs* was measured once or twice a month between 9:00 a.m. and 14:00 pm, using an LI-8100 portable soil CO_2 flux system attached to soil CO_2 flux chamber (LI-COR. Inc.) between July 2013 and December 2017.

We estimated *Ra* and *Rh* using the trench method as described by (Zhou et al., 2007). In short, three subplots with an area of 0.65 m × 0.65 m were randomly established in each plot in October 2014 to measure *Rh*. A trench was dug to a depth of 0.8 m (with little fine root distribution below this depth) and PVC plates (2.5 mm thick) were placed against the trench walls. Each PVC plate had 120 holes with 5 mm diameter distributed evenly to allow water movement, and each plate was covered with a nylon net with 400 mesh to prevent outside roots growing inside the subplots. We then refilled the trench according to its original soil profile to minimise the disturbance of trenching. The same size PVC collars used to measure *Rs* were inserted into the centre of each subplot to measure *Rh*. We calculated *Ra* as the difference between *Rs* and *Rh*. After a 3-month recovery period, *Rh* was measured once or twice a month from January 2015. At the same time as the *Rh* measurements, soil temperature (15 cm depth) was recorded adjacent to each PVC collar using a thermocouple probe connected to the LI-COR 8100. Soil temperature and moisture data were logged by the device with 5s intervals. Both air temperature and daily precipitation were recorded by the automatic weather station of Tiantong National Field Observation Station for Forest Ecosystems.

2.4 | Soil microbial analyses

Soil samples were collected eight times during 2014–2017 (January, May, August and November 2014, January, May and August 2015, August 2016, February 2017 and August 2017) within the same day of *Rs* measurements. Three soil cores were randomly collected from each plot using a soil corer (inner diameter 5 cm) at 0–10 cm and 10–20 cm depths. For each plot, the three soil cores were pooled by depth and stored at −20°C before further analyses. All samples were taken outside the trenched subplots.

We assessed the microbial community composition in soils (e.g. bacteria, fungi, actinobacteria and glomeromycota) using phospholipid fatty acids (PLFA) analyses (Hackl et al., 2005; Xu et al., 2015). In brief, 1.5 g soil was used for BligheDyer lipid extraction. A stream of N_2 was used for drying the different phases. All samples were dried and analysed by GC following trans-esterification for quantitative analysis relative to an internal standard (Bligh & Dyer, 1959; Canarini et al., 2016). The gas chromatography conditions were set by the MIDI Sherlock program (MIDI, Inc.). The resulting peaks were identified using bacterial fatty acid standards and the software SHERLOCK version 6.2.

The areas measured by GC-FID were used to calculate the abundance of PLFA markers (in nmol PLFA g^{-1} dry soil). Total lipid abundance was calculated as the sum of lipids with chain length from C10 to C20; this value was used as an indicator of total microbial biomass (e.g. Huang et al., 2013). Gram-positive bacteria were represented by all iso- and anteiso branch chain fatty acids (Landesman & Dighton, 2010), whereas Gram-negative bacteria were represented by monounsaturated and cyclopropane fatty acids (Ushio et al., 2008). PLFAs 18:2ω6,9 and C18:1ω9 were used as an indicators of fungi (Hu et al., 2017; Zeglin et al., 2013), while C16:1ω5*c* was used to indicate arbuscular mycorrhizal fungi (Swallow et al., 2009). PLFAs 10 Me16:0, 10 Me17:0 and 10 Me18:0 were used as indicators for actinomycetes (Hu et al., 2017). The abundance of individual PLFAs was calculated as the absolute amount of C (nmol PLFA-C g^{-1} soil) and then converted to mole percentage of PLFA-C (e.g. Huang et al., 2013). The ratio of the sum of cyclopropyl PLFAs to the sum of their monoenoic precursors ((cy17:0 + cy19:0)/(16:1ω7 + 18:1ω7), 'cy/pre' for short) was used as an indicator of physiological/nutritional stress in bacterial communities (Kieft et al., 1997).

2.5 | Fine root biomass measurements

Considering the sensitivity of mature trees to drought, we sampled soil to measure fine root biomass (<2 mm diameters) once a year (Valverde-Barrantes et al., 2015). Specifically, fine root biomass was

determined using the soil core method on June 16, 2016 and August 20, 2017 when *Rs* was also measured. Soil cores ($n = 10$) were randomly collected in each plot, approximately 1.5 m apart from the nearest tree using a soil corer (inner diameter 9 cm) at depths of 0–10 cm and 10–20 cm. The fine root biomass was calculated as the total biomass from 0 to 20 cm. Visible fine roots were hand-picked and all attached residues (e.g. soil, dead roots, stem materials and litter fractions) were carefully removed with tweezers. The remaining soil was sieved through a 0.15-mm mesh sieve and was gently rinsed to collect the remaining fine root segments. Fine root biomass was oven dried at 70°C for 48 hr to reach a constant mass.

2.6 | Statistical and data analyses

One-way ANOVA was used to evaluate treatment effects on *Rs*, *Ra*, *Rh*, microbial biomass, fine root biomass and physiological or nutritional stress of bacterial communities. The average of all replicates within a plot was treated as one data point. Means were compared using least significant difference and Duncan tests. Repeated measures ANOVAs was used to examine the effects of drought treatment (*D*), Sampling time (*T*), and their interaction ($D \times T$) on all variables (i.e. *Rs*, *Rh*, *Ra*, microbial community composition, soil temperature and moisture). In all analyses, the probability level used to determine significance was $p < 0.05$.

The effects of treatment, root biomass and microbial abundance on *Rs* were assessed through linear regression analysis in R using the CAR package. For the subset of data from days on which we simultaneously measured *Rs*, soil microbial biomass, root biomass and soil microclimate, we evaluated the relation between these factors and *Rs*. Variation partition analysis was used to determine the effects of microclimate, roots and microbes on *Rs* under both control and drought conditions (Su, Su, Zhou, et al., 2020).

We then used SEM to assess the relative importance of soil abiotic environment and microbial biomass in determining *Rs*. Prior to SEM analysis, we examined the normality and heteroscedasticity for data as well as all bivariate relationships for signs of nonlinearities. Mardia's test was used to ensure the skewness or kurtosis was appropriate for assuming multivariate normality. We chose the best model to present their multivariate effects of concerned variables using the lowest AIC values among different models. SEM analyses were conducted using the PIECEWISESEM package in R.

3 | RESULTS

3.1 | Effects of drought on microclimate

Drought slightly increased soil temperature at the depth of 15 cm compared with ambient and control treatments ($p < 0.001$, Table 1; Figure S2). Averaged across the study period, drought decreased volumetric soil moisture content at the depth of 0–15 cm by 30% relative to the control ($p < 0.05$, Table 1; Figure S2). Soil temperature and moisture did not differ significantly between the ambient and control treatments (Figure S2). The interaction between drought treatments and sampling time was significant for soil moisture, but not for soil temperature during the whole period (Table 1).

3.2 | Drought-induced change in soil respiration and its components

Averaged across the 4 years, drought decreased *Rs* by 16.5% (Table 1; Figure 1). Differences in *Rs* and its components between the control and ambient plots were negligible (Figure S3). Drought significantly decreased *Rs* by 12% in 2015, by 18% in 2016 and by 21% in 2017, but it did not affect *Rs* in 2014 (Figure 1). There was a significant interaction between drought and sampling time for *Rs*, *Ra* and *Rh* (Table 1). Drought decreased *Ra* and *Rh* by 27% and 21% across the last 3 years of the experiment (2015–2017), respectively (Figure 1). Drought reduced *Rs*, *Ra* and *Rh* during the growing season

TABLE 1 Results (*F* and *p* values) of repeated measurements analysis of variance: effects of drought (*D*), sampling time (*T*) and their interactive effects ($D \times T$) on *Rs*, *Ra*, *Rh*, soil temperature, soil moisture content, fine root biomass and biomass of bacteria, fungi and Cy/Pre

	D		*T*		$D \times T$	
Variables	***F***	***p***	***F***	***p***	***F***	***p***
Rs	53.41	**0.002**	151	**<0.001**	7.72	**<0.001**
Ra	22.12	**0.009**	21.5	**<0.001**	2.56	**0.007**
Rh	105.1	**0.001**	22	**<0.001**	2.94	**<0.001**
Soil temperature	45,221	**<0.001**	2,335	**<0.001**	1.21	0.289
Soil moisture	960.9	**<0.001**	25.8	**<0.002**	2.68	**0.005**
Fine root biomass	111.2	**<0.001**	1.08	0.36	3.38	0.14
Bacteria	377.6	**<0.001**	3.52	0.13	1.49	0.29
Fungi	226.7	**<0.001**	2.2	0.09	0.75	0.59
Cy/Pre	125.14	**<0.001**	1.56	0.08	1.13	0.58

Abbreviations: Cy/Pre, physiological/nutritional stress; *Ra*, autotrophic respiration; *Rh*, heterotrophic respiration; *Rs*, soil respiration.

The bold values represent the treatment effect is significant.

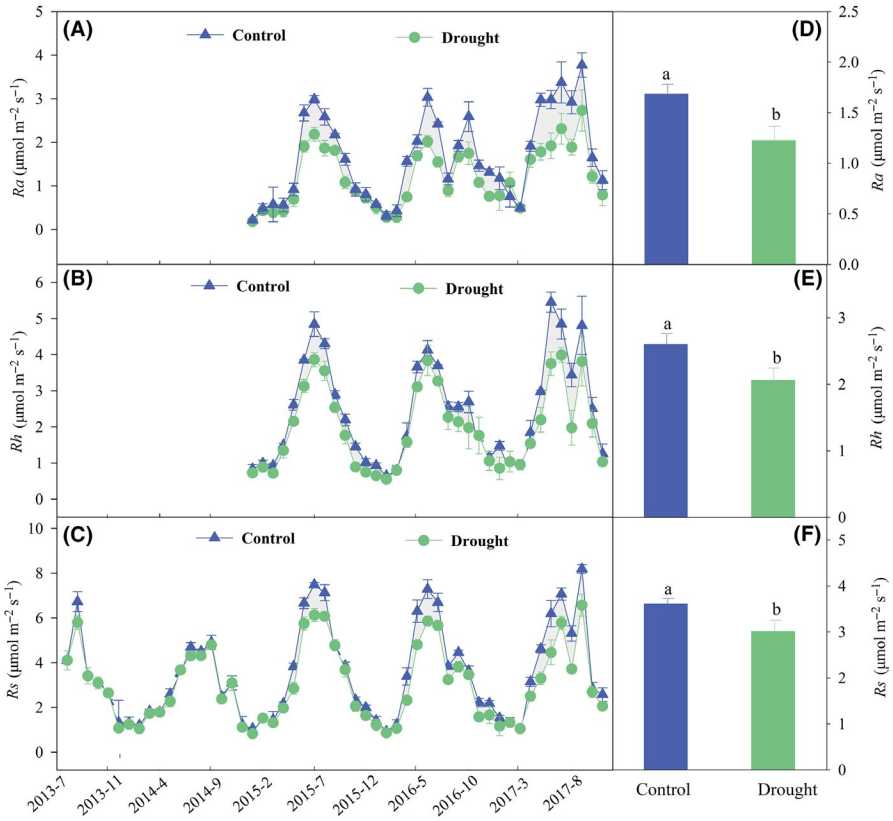

FIGURE 1 Effects of drought on seasonal variability and average value of autotrophic respiration (*Ra*, A, D), heterotrophic respiration (*Rh*, B, E) and soil respiration (*Rs*, C, F) from 2013 to 2017. Error bars represent the mean standard error. Grey fill indicates significant differences in *Ra*, *Rh* and *Rs* between control and drought treatments during the growing seasons. Different lower case letters identify significant differences between control and drought for each respiration variable at $p < 0.05$

by 16%, 27% and 21%, respectively, but it did not affect these fluxes in the non-growing seasons (Figure 1; Figure S4).

3.3 | Drought-induced change in fine root biomass and soil microbes

On average, drought significantly increased fine root biomass by 20% across the experimental period ($p < 0.05$, Figure 2A). Drought also decreased bacterial biomass (−21%) and fungal biomass (−24%; Figure 3A). Furthermore, drought significantly increased physiological/nutritional stress in the bacterial community by 19% (Figure 3B).

3.4 | Linking microclimate, roots and microbes to soil respiration

Both abiotic and biotic factors influenced *Rs* under the drought and control treatments. Fine root biomass was correlated negatively with *Ra* and *Rs* under drought, but showed a positive correlation in control treatments (Figure 2B,C). *Ra* and *Rs* were positively correlated under both drought and control treatments (Figure 2D). *Rs* and its components (*Ra* and *Rh*) were correlated positively with fungal PLFAs, but were negatively correlated with bacterial PLFAs under both control and drought treatments ($p < 0.01$, Figure 4). Soil temperature accounted for 75%, 61% and 65% of temporal variation in *Rs*, *Rh* and *Ra* in the control treatment, respectively, and for 79% (*Rs*), 74% (*Rh*) and 66% (*Ra*) in the drought treatment (Figure 5A–C). Weak correlations with volumetric soil moisture content were found for *Ra*, *Rh*, and *Rs* in both control and drought treatments (Figure 5D–F).

Our SEM analysis showed that the influence of throughfall on *Rs* was mediated through soil moisture, fungi and root biomass, and its effects differ between control and drought conditions (Figure 6; Figure S6). Specifically, changes in fine root biomass caused by seasonal variation of soil moisture were positively correlated with *Rs* in the control treatment, but were negatively correlated under drought conditions. Fungi were positively correlated with *Rs* under drought conditions, but no correlation was observed in the control treatment (Figure S6). In contrast, bacteria were positively correlated with *Rs* in the control treatment, but showed no correlation with *Rs* under drought conditions. These results suggest that fungi play a more important role in regulating *Rs* than bacteria under drought condition. In addition, seasonal soil temperature was positively correlated with *Rs* under both control and drought conditions.

Taken together, our results suggest that both soil fungi and fine root biomass largely mediate reductions in soil respiration with drought (Figure S7). Specifically, drought-induced increases in fine root biomass were negatively correlated with *Ra* and *Rs*. Drought-induced decreases in fungi were positively correlated with *Rs* and its components. Microclimate, root biomass and microbial biomass jointly explained 65% of the variance in *Rs* for drought treatments. Furthermore, drought-induced changes in soil microclimate affected *Rs* indirectly via changes in fine root and fungi biomass.

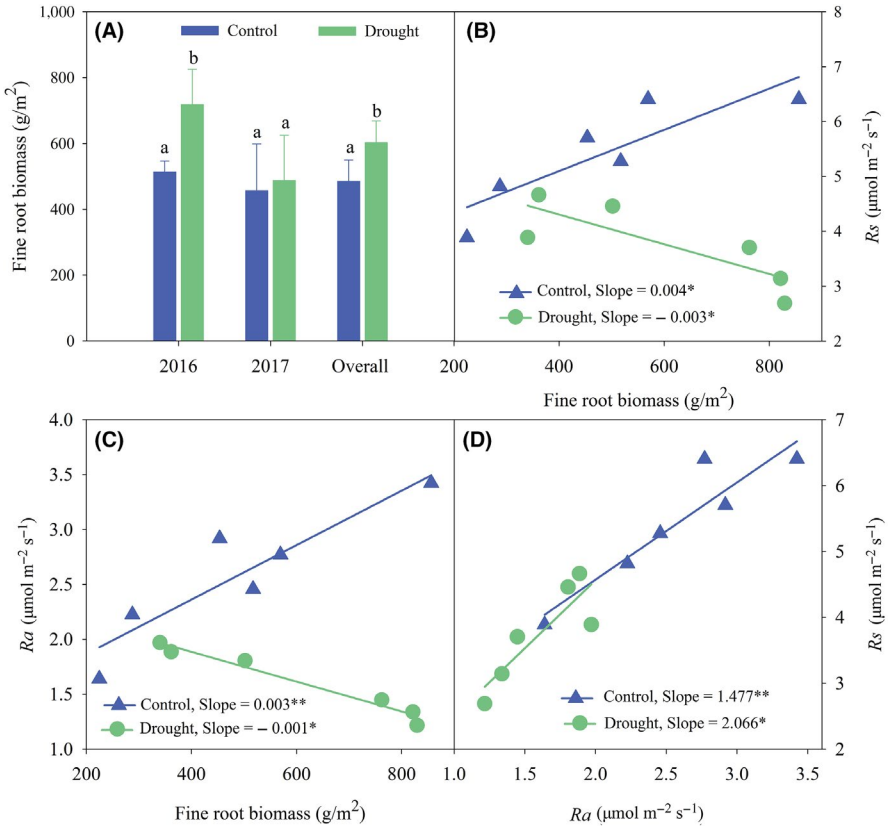

FIGURE 2 Effects of drought on fine root biomass (A), and relationships between fine root biomass and soil respiration (*Rs*, B) as well as autotrophic respiration (*Ra*, C), between *Ra* and *Rs* (D). Error bars represent the mean standard errors. Different lower case letters identify significant differences between control and drought for each respiration variable at $p < 0.05$. Each point was the average value through time. $^{*}p < 0.05$, $^{**}p < 0.01$

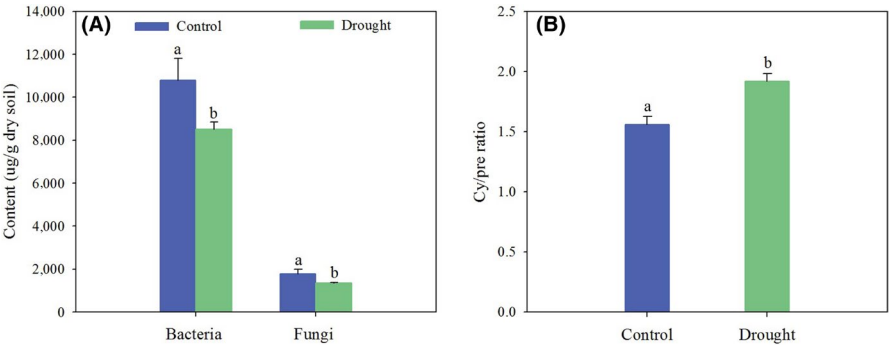

FIGURE 3 Effect of drought on the content of bacteria, fungi (A) and physiological/nutritional stress of bacterial communities (B). Error bars represent the standard error of the mean. Different lower case letters identify significant differences between control and drought for each respiration variable at $p < 0.05$

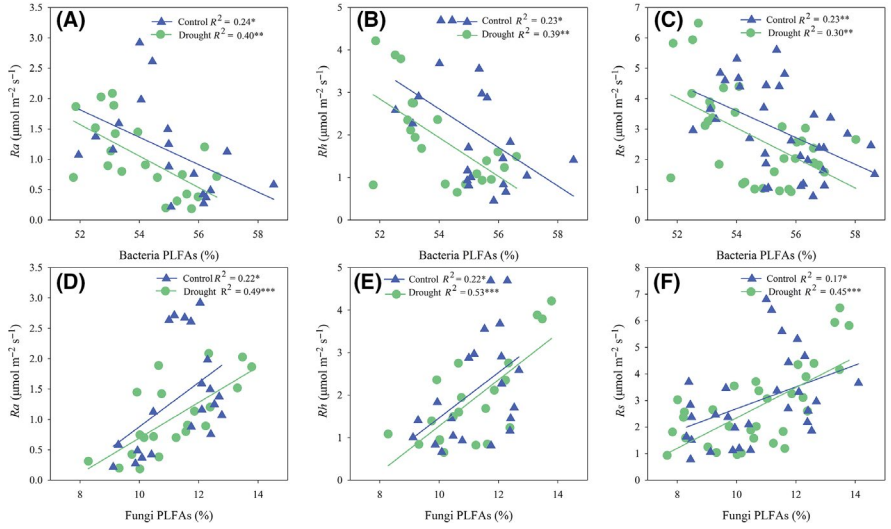

FIGURE 4 Effects of drought on the relationship between soil respiration and its component with the phospholipid fatty acids (PLFA) of bacteria (A–C), and fungi (C & D). *Ra*, autotrophic respiration; *Rh*, heterotrophic respiration; *Rs*, soil respiration. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

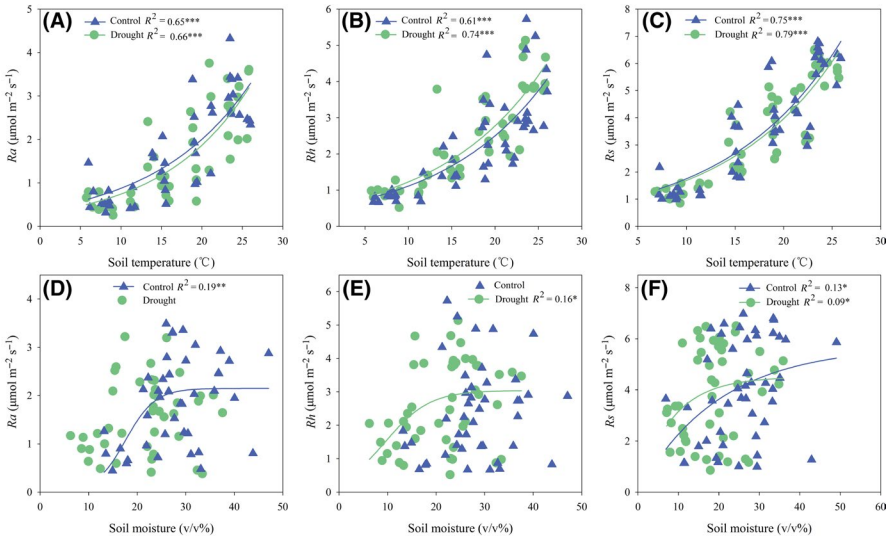

FIGURE 5 Effects of drought on relationships between soil temperature and autotrophic respiration (*Ra*, A), heterotrophic respiration (*Rh*, B), and soil respiration (*Rs*, C), relationships between volumetric soil moisture and *Ra* (D), *Rh* (E) and *Rs* (F). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

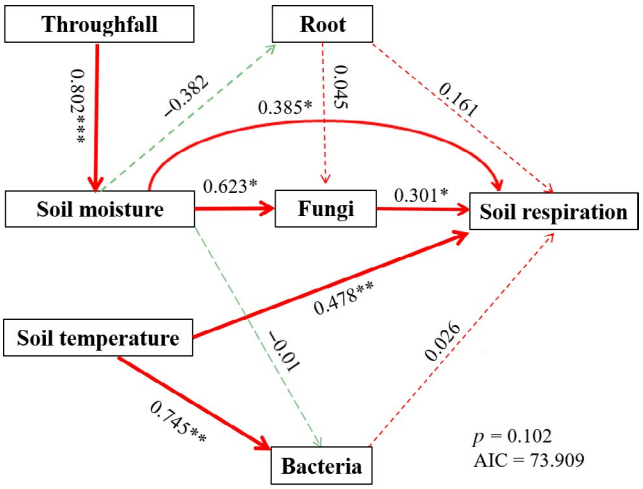

FIGURE 6 Structure equation models (SEM) outlining the influence of microclimate, root biomass, soil microbes and their interactions on soil respiration. Single-headed arrows indicate positive and negative relationships respectively. Arrow width is proportional to the strength of the relationship. The numbers adjacent to arrows are standardized path coefficients, which reflect the effect size of the relationship. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

4 | DISCUSSION

4.1 | Root biomass and microbial community regulate the responses of *Rs* to drought

Predicting ecosystem responses to drought and assessing climate–C cycle feedbacks requires understanding of drought-induced effects on soil respiration (*Rs*) and its components (Bond-Lamberty et al., 2018; Huang et al., 2018). Our results show that drought significantly decreased *Rs* and its components (Figure 1). The decrease in *Rs* was attributed to reductions in both *Rh* and *Ra* (Figure 3). Drought-induced reductions in *Rh* resulted from decreased soil microbial activity and biomass (Figure 3A), probably due to the decrease in substrate availability under drought in the organic layer and the top mineral horizon (Fuchslueger et al., 2014). These results are consistent with drought-induced reductions in fungal and bacterial biomass, and the increased physiological/nutritional stress in the bacterial community.

In contrast, we found that drought significantly increased fine root biomass across the experimental period (Figure 2A). The increased fine root biomass likely reflects the increased C allocation to fine roots from above-ground parts to sustain water uptake and photosynthesis in response to drought stress (Fuchslueger et al., 2014). However, the increased root biomass did not stimulate *Ra* and then *Rs* as we expected. These results can probably be explained by drought-induced changes in root phenology, causing plants to shift C to root growth rather than respiration. Such shifts can decrease specific root respiration (Hinko-Najera et al., 2015; Zhou et al., 2018). Furthermore, drought may decrease mycorrhizal respiration, an important component of root respiration that is often neglected when distinguishing the components of *Rs* (Nottingham et al., 2010).

Our results indicate that in the drought treatment induced change in *Rs* was positively correlated with fungi biomass, but negatively correlated with bacterial biomass (Figure 4). Decreases in soil moisture caused relative increases in fungal abundance, both in the control and drought treatment. These results probably reflect that fungi are more tolerant to drought stress than bacteria, due to their higher metabolic potential to obtain resources for survival (Fuchslueger et al., 2014). Thus, under drought condition, fungi may have a stronger ability to decompose SOM and then stimulate CO_2 emission than bacteria (Fanin et al., 2019; Phillips et al., 2012).

Our results further indicate that drought decreased *Rs* and its components in the growing seasons, but not in the non-growing seasons. Both roots and microbes have a greater water demand in the growing season than the non-growing season (Chapin et al., 2002). Lower soil water contents in the growing season may impede canopy photosynthesis and then suppress root activity (e.g. water and nutrient uptake, Ru et al., 2018). Decreased transport of assimilated C towards the rhizosphere could also suppress soil microbial biomass

and activity, thereby reducing the decomposition of soil organic matter and then *Rh* (Fuchslueger et al., 2014). A recent study at our site found that even in the absence of roots, the direct negative effect of drought on microbial carbon metabolism during the growing season was greater than in the non-growing season (Su, Su, Yang, et al., 2020). This decrease in microbial carbon metabolism would further suppress soil organic matter decomposition and soil respiration.

Although drought had no effect on *Rs* in the first year, drought effects on *Rs* gradually increased in later years (Figure 1; Figure S4). These results suggest that continuous drought progressively decreased root activity and microbial biomass, and that short-term drought experiments may underestimate drought effects on *Rs*. Nonetheless, drought affected soil moisture content similarly in all years (Figure S2), and drought effects on root biomass did not increase over time. Thus, the mechanisms underlying this trend of progressive drought effects on *Rs* remain unclear, and require further research.

4.2 | Linking microclimate, root biomass and soil microbes to *Rs* response to drought

Since soil respiration (*Rs*) is regulated by both abiotic (e.g. soil temperature and moisture) and biotic factors (e.g. fine root biomass and fungi biomass), understanding their multivariate effects on *Rs* is important to predict future forest C cycling (Luo & Zhou, 2006). For instance, drought-induced decreases in soil moisture could trigger a water limitation of the microbial community, thereby lowering microbial decomposition and *Rs* (Zhou et al., 2018). In addition, drought stimulated accumulation of fine root biomass in our experiment, which may suppress root activity and decrease C input to fungi, leading to a decrease in the size of the fungal community (Chapin et al., 2002). Indeed, our SEM analysis suggests that changes in fine root biomass by drought were negatively correlated with fungal biomass (Figure S6), probably because fungal growth is largely determined by the chemical composition and quantity of plant inputs (Brant et al., 2006). Therefore, the altered root–fungi relationship by drought would further modify *Rs* pattern compared with those in the control.

Furthermore, we found that microclimate played a more important role in regulating *Rs* than root biomass and soil microbes under both drought and control conditions (Figures S5 and S6). The dominant role of microclimate likely reflects that soil temperature affect most aspects of respiration processes, especially root and microbial activity along the seasonal changes (Fuchslueger et al., 2014; Luo & Zhou, 2006). For example, soil temperature can affect C turnover (both input and output) through altering soil biochemistry and microbial physiology, which in turn affects *Ra* and *Rh* (Ohashi et al., 2015). Our results showed that drought slightly increased soil temperatures relative to control treatments, and drought-induced changes in soil moisture were positively correlated with soil temperature (Table 1; Figure S6). We speculate that drought decreased ground cover due to the increased herb mortality, thereby increasing the absorption of sunlight by the shrub floor (Guadagno et al., 2017), which could slightly increase the soil temperature. In addition, the lower plant cover caused by drought would also increase evaporation and lower soil moisture, resulting in increased fine root biomass and decreased fungi biomass (Chapin et al., 2002; Luo & Zhou, 2006). Through this mechanism, drought-induced changes in soil microclimate regulate *Rs* via its effect on root biomass and soil microbes in subtropical forests.

4.3 | Implications for terrestrial C modelling and future experiments

Understanding the effects of drought on *Rs* and its components may improve predictions of ecosystem C dynamics under future climate climatic conditions (Carey et al., 2016; Fuchslueger et al., 2014). Our experiment provided several important insights into the mechanisms underlying drought effects on *Rs* (Figure S7). Based on these results, we offer some suggestions for the design of manipulative experiments and the improvement of land surface models.

First, our results indicate that microclimate, root and soil microbes all played vital roles in regulating *Rs* in response to drought in a subtropical forest. Effects of these abiotic and biotic factors on *Rs* were frequently observed in diverse climate and ecosystems types. However, it is still unclear how microclimate, roots and soil microbes interact to affect responses of *Rs* to drought in different ecosystems (e.g. agricultural and grassland ecosystems). Future experiments should focus on quantifying these interactions, because they largely determine *Rs*. Meanwhile, we used the trenched method to estimate *Rh* and *Ra*, which may overestimate or underestimate microbial respiration due to dead root decomposition or that the additional carbon input from dead root biomass would trigger priming effects (Kuzyakov & Bol, 2006). Therefore, these factors should be considered in the future experiments to better examine the effects of drought on *Rs* in forest ecosystems.

Second, drought duration may be crucial in evaluating the responses of *Rs* to drought, since the drought-induced effects on ecosystem processes will largely change over time (Luo & Zhou, 2006). We found that drought had no effect on *Rs* in the first year, but gradually decreased *Rs* in years 2–4 (Figure 1). Moreover, *Rs* and its components were more responsive to drought in the growing season than in the non-growing seasons. The multivariate effects of microclimate, root and microbes on *Rs* at longer time scale also remain unknown. Temporal changes in the responses of *Rs* as well as its driving factors to drought should thus be considered in model predictions in the future.

Third, microclimate, roots and soil microbes are crucial in regulating responses of *Rs* to drought. However, current land-surface models usually do not consider the effects of these three factors, especially microbial community composition under drought. This creates a great challenge to realistically predict the climate–C cycle feedback (Lal, 2004). Future ecosystem and global model parameters would thus need to consider these three factors to develop more precise process-based models for predicting effects of future climate change on C cycling in terrestrial ecosystems.

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AUTHORS' CONTRIBUTIONS

X.Z. and G.Z. designed, and oversaw the research; G.Z. synthesised data, conducted experiment and wrote the manuscript; R.L., Z.D., L.Z., S.L., H.L., J.W., M.Z. conducted the field experiment; J.S. and S.B. discussed and revised the manuscript together.

DATA AVAILABILITY STATEMENT

Data are available via the Dryad Digital Repository https://doi.org/10.5061/dryad.83bk3j9pk (Zhou et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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