

# Different mechanisms underlying divergent responses of autotrophic and heterotrophic respiration to long-term throughfall reduction in a warm-temperate oak forest

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## Research Article

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# Abstract

**Background:** There are many studies on disentangling the responses of autotrophic (AR) and heterotrophic (HR) respiration components of soil respiration (SR) to long-term drought, but few studies have focused on the mechanisms underlying its responses.

**Methods:** To explore the impact of prolonged drought on AR and HR, We conducted the 2-year measurements on soil CO<sub>2</sub> effluxes in the 7th and 8th year of manipulated throughfall reduction (TFR) in a warm-temperate oak forest.

**Results:** Our results showed long-term TFR decreased HR, which was positively related to bacterial richness. More importantly, some bacterial taxa such as *Novosphingobium* and *norank Acidimicrobiia*, and fungal *Leptobacillium* were identified as major drivers of HR. In contrast, long-term TFR increased AR due to the increased fine root biomass and production. The increased AR accompanied by decreased HR appeared to counteract each other, and subsequently resulted in the unchanged SR under the TFR.

**Conclusions:** Our study shows that HR and AR respond in the opposite directions to long-term TFR. Soil microorganisms and fine roots account for the respective mechanisms underlying the divergent responses of HR and AR to long-term TFR. This highlights the contrasting responses of AR and HR to prolonged drought should be taken into account when predicting soil CO<sub>2</sub> effluxes under future droughts.

## Background

Climate models predict widespread alterations in precipitation regimes, including longer, more intense droughts in the next decades (IPCC, 2013). The increased drought has a considerable effect on terrestrial carbon (C) cycling (Tian et al., 2000; Batson et al., 2015; Vidon et al., 2016), particular to soil respiration (SR), which is the largest CO<sub>2</sub> flux from terrestrial ecosystems back to the atmosphere (Janssens et al., 2001). Although multitudes of drought experiments have been conducted to explore the effect of drought on SR, the results of previous studies have been variable, including increase (Cleveland et al., 2010; Zhang et al., 2015), decrease (Schindlbacher et al., 2012; Selsted et al., 2012), and no change (Davidson et al., 2008; Lu et al., 2017). These inconsistent and often contradictory results constrain our understanding of feedbacks between soil C cycling and climate change.

Predicting the response of SR to drought is inherently difficult as SR is a combination of respiration associated with root activity (autotrophic respiration, AR) and soil organic matter (SOM) decomposition (heterotrophic respiration, HR) (Wang et al., 2014). Due to the difference in turnover times and control factors of plant and soil C pools, AR and HR often responded differently to drought (Borken et al., 2006; Huang et al., 2018; Sun et al., 2019). It has been shown that drought reduced AR, and thus SR, but had no effect on HR in a dry temperate forest (Hinko-Najera et al., 2015). Another study in a subtropical forest found drought decreased both AR and HR, and hence SR (Zhou et al., 2020). A previous study in a warm-temperate oak forest also suggested that drought increased AR but not HR, and therefore SR at a small scale (e.g., 4 m × 4 m roof) throughfall reduction (Liu et al., 2016). All of these indicate roots and soil

microbes have differential sensitivities to drought, and ultimately determine the direction of SR in response to drought in different ecosystems (Luo and Zhou, 2006). Nevertheless, the mechanisms underlying different responses of AR and HR to drought are far from clear, which limits our comprehension of whether soil acts as a C sink or C source in the scenario of increased droughts.

Although these past studies provide important insights into the effects of drought on SR and its components, they have almost conducted for short-term drought periods, rather than long-term consecutive drought. The long-term experiments are crucial to revealing not only the transient responses of AR and HR to drought but also the adaptive response. A throughfall reduction experiment in a tropical rainforest found that drought increased SR during the first 3 years (Zhang et al., 2015), but did not change SR after 6 years of continuous drought because of the increased HR and decreased AR (Zhou et al., 2019). Thus, the drought responses of AR and HR may shift with the duration of drought, with consequences for SR (Metcalf et al., 2007; da Costa et al., 2013).

Lu et al. (2017) reported that TFR did not affect HR during the first 4 years, and only increased AR as well as SR in the second year in this warm-temperate oak forest. In the present study, we explored if TFR still had a slight effect on SR after 6-year consecutive TFR treatment, and if AR and HR responded differently to long-term TFR. Soil microbial community attributes (e.g., diversity, abundance) as well as fine root properties (e.g., biomass, production) were studied to reveal the mechanisms underlying the responses of AR and HR to long-term TFR.

## Materials And Methods

### Study site

The research was conducted at the Baotianman Forest Ecosystem Research Station (111° 92' E, 33° 49' N), Henan province, central China. The study area has a continental monsoon climate and has four distinctive seasons, with humid and hot summer, and dry and cold winter. The annual average air temperature is 15.1°C, and the annual precipitation is 894 mm (1400 m a.s.l.) (Liu et al., 2016). The upland soil is dominated by Haplic luvisol and soil pH ranged from 4.4–5.1 (Luan et al., 2011; Lu et al., 2017). The soil has a sandy loam texture with 57–62% sand, 11–13% silt, and 27–30% clay (Luan et al., 2011). The dominant deciduous broadleaf tree species include *Quercus variabilis*, *Quercus aliena* var. *acuteserrata*, and *Fagus engleriana*, and coniferous tree species include *Pinus armandii*, *Pinus tabulaeformis*, and *Pinus massoniana*.

### Experimental design

In the spring of 2013, six plots (20 m × 20 m) were set up in a 60-year-old oak (*Q. aliena* var. *acuteserrata*) forest. Three plots with the ambient environment were designed as controls (“control”) and three plots were assigned to manipulated throughfall reduction (“TFR”). Detailed information for the TFR experiment refers to Lu et al., 2017. Briefly, about 160 shelter-panels (0.5 m × 3 m), covering 50% of the plot area, were installed in each TFR plot during the growing seasons (May–October) from 2013 to 2017. In the

spring of 2018, we adjusted the magnitude of TFR from 50% to 70%. We inserted plastic barriers to a depth of 0.7 m around each TFR plot to inhibit the subsurface flow of water, and extended plastic flashing 5 cm above the ground to prevent overland flow. A buffer zone of 2.5 m width is set off along the inner edge of each plot and no measurements were made in the buffer zone. Litter that fell on the panels was collected weekly and distributed evenly throughout the plot to avoid variations in litter input on the ground.

### **Measurements of SR and its components**

The HR was estimated using the trenching method as described by Lu et al. (2017). Briefly, five subplots (3 m × 3 m) were randomly assigned in each plot in March 2013 to measure HR. Trenches were dug about 1 m deep and placed plastic plates (5 mm thick) to inhibit root in-growth. In October of 2018, we dug the trenches again at the original position. To measure SR and HR, two PVC collars (19.6 cm inner diameter, 8 cm height) were installed 5 cm into the soil in each un-trenched and trenched subplot. We estimated AR as the difference between SR and HR.

The SR and HR were measured once a month during the growing seasons from 2019 to 2020 using a Li-8100 soil CO<sub>2</sub> flux system (LI-COR Inc., Lincoln, NE, USA). In the meanwhile, soil temperature (ST) and soil moisture (SM) were manually measured by a portable temperature probe and soil moisture gauge (MPKit-BN, TZT Inc., Nantong, China) at three locations around each collar at 0–5 cm depth. Besides, in each plot, an EM50 data logger was installed with five 5TM soil temperature and moisture combined probes to continuously measure ST and SM at 30 min intervals. The precipitation data were obtained from the automatic weather station at Baotianman Forest Ecosystem Research Station, about 1 km away from the experimental plots.

### **Soil and fine root characteristics**

Soil samples were collected in August 2019 and August 2020 within the same day of SR measurements to examine the effects of TFR on soil microbial communities and fine root characteristics. Two soil samples (0–10 cm) from each subplot were collected using soil augers (inner diameter 3.8 cm). The fresh soil samples sieved by 2 mm mesh were used for chemical and microbial analyses. Fine root biomass (< 2 mm) was oven-dried to a constant weight and then weighted. The soil organic carbon (SOC) and total nitrogen (N), fine root C and N content were determined using an elemental analyzer. The soil total phosphorus (P) and fine root P contents were measured using the alkaline fusion molybdenum-antimony colorimetry (Bao, 2000). Fine root nonstructural carbohydrates (NSC): soluble sugar and starch were determined using the anthrone colorimetric method (Gao, 2006). Soil microbial biomass C (MBC) and nitrogen (MBN) were analyzed by chloroform fumigation extraction method with conversion factors of 0.45 (Wu et al., 1990) and 0.54 (Vance et al., 1987), respectively. Soil β-glucosidase, polyphenolase, peroxidase, and amylase activities were assayed by the colorimetric method according to Guan, 1986.

A modified ingrowth core method (Hertel and Leuschner, 2002) was conducted to determine fine root production. Five stainless steel cubes (20 × 20 × 20 cm) with a 2 mm mesh were installed in each plot,

and were refilled with rootless native soil in May 2019. The fine root in these in-growth blocks was collected at the end of October each year.

### Soil bacterial and fungal community analyses

Soil microbial communities were assessed using amplicon-sequencing technology. In brief, microbial DNA from each soil sample was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's manual. The bacterial 16S rRNA and fungal ITS genes were amplified using the primers 515F/907R (Yusoff et al., 2013) and the primer ITS1F/ITS2R (Adams et al., 2013), respectively. The PCR and high-throughput sequencing were conducted by the Majorbio Company (Shanghai, China) using the Illumina MiSeq PE300 platform. Operational taxonomic units (OTUs) were classified at 97% similarity level using UPARSE (version 7.1), and chimeric sequences identified by UCHIME were discarded (Edgar, 2013). The taxonomy of bacteria and fungi was assigned by RDP Classifier (Wang et al., 2007) against the Silva and Unite database, respectively, with a 70% confidence threshold. All samples were rarefied to the minimum sequence of the sample before the following analyses. We analyzed microbial composition and diversity on a platform ([www.i-sanger.com](http://www.i-sanger.com)) of Majorbio Company.

### Data analysis

The statistical analyses were carried out using SPSS version 24.0 for Windows (SPSS, Chicago, Illinois, USA). We used two-way ANOVA to test the effects of TFR and year on SR and its components, soil and root properties, microbial biomass, and enzymatic activities. We also used one-way ANOVA to assess the effects of TFR on fine root biomass and production, microbial community attributes (e.g., diversity, abundance).

Regression modeling was used to investigate the relationships between SR, HR, or AR and ST, as well as SM. The temperature sensitivity ( $Q_{10}$  value) was estimated by the following function (Lloyd and Taylor, 1994):

$$R = \alpha e^{\beta T}$$

$$\text{where, } Q_{10} = e^{10\beta};$$

$R$  represents SR, HR, and AR;  $\alpha$  and  $\beta$  are fitted parameters;  $T$  is the measured soil temperature.

Pearson correlation analyses were used to examine correlations between SR, HR, or AR and measured biotic and abiotic factors. Structure equation modeling (SEM) was conducted using AMOS 21.0 (SPSS Software, Chicago, Illinois, USA) to evaluate the significance of abiotic and biotic drivers in determining AR and HR (Wang et al., 2019). The SEM was fitted using the maximum likelihood estimation. The best model was selected using the lowest AIC value.

We used random forest analysis to identify the statistically significant bacterial and fungal predictors (genera) for HR (Trivedi et al., 2016). A total of 219 bacterial genera and 46 fungal genera were selected in the random forest modeling. After that, we used linear regressions to assess the relationships between the relative abundance of the selected predictive genera and HR.

## Results

### Soil physicochemical properties and soil enzymes

TFR decreased SM by 28% and 23% for un-trenched subplots and trenched subplots, respectively ( $P < 0.05$ ), but did not change ST during the study periods (Fig. 1 and Table 1).

TFR had no significant effects on MBC and MBN (Table S1), and had little effects on measured enzyme activities except for polyphenolase ( $P = 0.08$ ) (Table S1).

### Soil respiration and its components

SR showed no significant difference between TFR and control in 2019 ( $2.48 \pm 0.23$  vs.  $2.63 \pm 0.19$   $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ m}^{-1}$ ) and in 2020 ( $2.92 \pm 0.27$  vs.  $2.63 \pm 0.24$   $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ m}^{-1}$ ) ( $P > 0.05$ ; Fig. 2 and Table 1). TFR increased AR by 92% during the study periods ( $P < 0.05$ ; Table 1), with respective interannual variation of 96% in 2019 and 88% in 2020 ( $P < 0.05$ ; Fig. 2). TFR decreased HR by 23% during the study periods ( $P = 0.057$ ; Table 1), 29% in 2019 ( $P < 0.05$ ), and a slight decrease of 17% in 2020 ( $P = 0.09$ ) (Fig. 2). TFR decreased relative contribution of HR to SR from 79% to 60% during the study periods ( $P < 0.05$ ; Fig. 3).

**Table 1** Results ( $F$  and  $P$  values) of two-way ANOVA for the effects of throughfall reduction (TFR), year (Y) and their interactive effects (TFR $\times$ Y) on SR, HR, AR, soil moisture, and soil temperature in un-trenched subplots (SM-U and ST-U) and trenched subplots (SM-T and ST-T).

Variables	TFR		Y		TFR $\times$ Y	
	$F$	$P$	$F$	$P$	$F$	$P$
SR	0.38	0.847	0.32	0.580	0.31	0.582
HR	4.10	0.057	0.05	0.834	0.38	0.544
AR	9.10	<b>0.007</b>	2.23	0.151	0.18	0.678
SM-U	12.41	<b>0.002</b>	0.01	0.931	0.24	0.630
SM-T	26.48	<b>&lt;0.001</b>	0.61	0.445	0.09	0.762
ST-U	0.01	0.943	0.01	0.775	0.001	0.970
ST-T	0.01	0.926	0.06	0.809	0.04	0.843

## Linking abiotic and biotic factors to soil CO<sub>2</sub> efflux

SR, HR, and AR were significantly related to soil temperature under both treatments (Fig. 4). TFR did not change the temperature sensitivity ( $Q_{10}$  value) of SR, while decreased the  $Q_{10}$  value of HR from 2.69 to 2.10 and increased the  $Q_{10}$  value of AR from 3.42 to 3.97 (Fig. 4). Soil moisture was significantly correlated with SR and HR ( $P < 0.05$ ) although at a lower coefficient of determination, but not AR (Fig. 4).

TFR increased fine root biomass and production during the study periods ( $P < 0.05$ ; Fig. 5), and was positively correlated with AR ( $P < 0.05$ ) (Fig. 5). The SEM indicated that AR was directly controlled by fine root biomass (Fig. 7).

TFR significantly decreased bacterial richness ( $P < 0.05$ ), and was marginally correlated with HR ( $P = 0.07$ ) (Fig. 6 and Table S2). The SEM also suggested that HR was directly driven by soil bacterial richness (Fig. 7). Our random forests modeling showed that many bacterial genera predictors of HR were distributed among the *Proteobacteria*, *Acidobacteriota*, *Chloroflexi*, and *Actinobacteriota*; fungal genera predictors of HR belong to the *Ascomycota* and *Mucoromycota* (Fig. S2). Some of these predictive genera were significantly related (linear regressions) to HR (Fig. 8). For example, TFR decreased the relative abundance of bacterial *Novosphingobium*, norank *11-24* and norank *Vicinamibacterales* ( $P < 0.05$ ) (Table S4), and were positively related to HR (Fig. 8). TFR increased the abundance of bacterial *1959-1* and norank *Acidimicrobiia* ( $P < 0.05$ ) (Table S4), and were negatively associated with HR (Fig. 8). In addition, TFR increased the relative abundance of fungal *Leptobacillium* ( $P < 0.05$ ; Table S4), and was negatively related to HR (Fig. 8).

## Discussion

### Effects of TFR on HR, AR and SR

Consistent with those of other studies (Borken et al., 2006; Risk et al., 2012), we also found long-term TFR decreased HR. TFR significantly decreased bacterial richness, which may lead to the decreased HR due to the positive correlation between bacterial diversity and soil CO<sub>2</sub> efflux (Liu et al., 2018; Delgado-Baquerizo et al., 2016). Generally, greater microbial species richness can allow for more metabolic activities, promoting the decomposition of organic matter (Naylor and Coleman-Derr, 2018). Thus, the decreased HR after 6-year consecutive TFR could arise from the decreased bacterial diversity (Hutchins et al., 2019; Singh et al., 2010). Another study in this oak forest has shown that short-term variation in soil moisture had no significant impact on bacterial diversity (Wei et al., 2018), which may explain the unchanged HR under TFR treatment during the first four years (Lu et al., 2017).

We subsequently identified major microbial taxa (genera) that predicted the changes in HR. We found the relative abundance of *Novosphingobium*, norank *11-24* and norank *Vicinamibacterales* belong to Gram-negative bacteria were positively associated with HR. In addition, the relative abundance of *1959-1* and

norank *Acidimicrobiia* belong to Gram-positive bacteria were negatively related to HR. Studies have shown that soils with a high abundance of oligotrophs or low abundance of copiotrophs may have low CO<sub>2</sub> emissions (Trivedi et al., 2013; Liu et al., 2018). It has also been shown that the Gram-negative bacteria have characteristics of copiotrophs, while Gram-positive bacteria contain characteristics of oligotrophs (Naylor and Coleman-Derr, 2018). Thus, the TFR-induced changes in the relative abundance of these bacterial taxa were responsible for the decreased HR. In addition, we found the relative abundance of fungal *Leptobacillium* belong to *Ascomycota* were negatively correlated to HR, despite a weak correlation between overall fungal community diversity with HR. We know little about the mechanism of the association between *Leptobacillium* and C emission, and postulated the taxa are tolerant to drought and may have high C use efficiency (Liu et al., 2018).

Previous studies have suggested that experimental drought often reduced AR due to the decreased fine root biomass or belowground C allocation among different ecosystems (Hinko-Najera et al., 2015; Huang et al., 2018; Zhou et al., 2019). However, we found long-term TFR increased AR, mainly because of higher fine root biomass and production under the TFR. According to the optimal partitioning theory (Bloom et al., 1985), plants should allocate more C to root growth from aboveground parts to reduce water limitation (Fuchslueger et al., 2014). However, many field throughfall exclusion experiments of forests have shown that fine root biomass did not always support this theory, including increase (Zhou et al., 2020), decrease (Moser et al., 2014), or little change during the first four years in this study (Lu et al., 2017), indicating that the responses of fine root to water deficit depends on intensity and duration of drought. In our system, we argued that mature trees increased belowground C allocation to adapt to the long-term drought, resulting in the higher AR.

Contrary to other studies (Sotta et al., 2007; Cleveland et al., 2010), we found long-term TFR had no significant effect on SR. Another study also showed that drought had no significant impact on soil CO<sub>2</sub> flux but did not mention AR and HR in a tropical forest (Davidson et al. 2008). This may mask the contrasting responses of AR and HR to long-term drought due to the different sensitivities of fine roots and microbes to water deficit (Zhou et al., 2019). Here, the present result showed AR and HR had opposite responses after 6-year consecutive TFR, leading to the unchanged SR.

### **Seasonal variability of soil CO<sub>2</sub> efflux**

The pronounced seasonal variations of SR, AR, and HR were explained by soil temperature, which was in agreement with previous studies (Vincent et al., 2006; Liu et al., 2016). Besides  $\theta$  soil moisture can also partly explained the seasonal patterns of SR and HR. These indicated that soil temperature and soil moisture can solely control soil respiration through influencing decomposition rates and microbial activity (Barthel et al., 2011). However, we found a weak relationship between soil moisture and AR along with the seasonal changes. This did not mean that soil moisture was not important to roots, but it may be the inherent growth rhythm of roots and the utilization of deep soil water that masked the effect of soil moisture on AR. TFR increased the Q<sub>10</sub> of AR while decreased it of HR. The changed Q<sub>10</sub> value may reflect

the shifts in the physiological status of plant roots and soil microbes (Zhang et al., 2014), which was potentially important for C-climate feedback models, and needs to be further evaluated.

Specifically, we found HR showed little difference between control and TFR in May and October 2020 (Fig. 2). A similar pattern was also found in AR in both 2019 and 2020 (Fig. 2). This may be attributed to the lower activities of both roots and soil microorganisms in May and October, and hence lower water requirement (Chapin et al., 2002). It is worth noting that the maximum AR in July 2019 and 2020 can determine the response of SR to drought, and thus the monitoring frequency of SR should be increased to accurately assess the response of SR to drought.

### **Relative contribution of AR and HR to SR**

Although partitioning AR and HR of SR and evaluating the responses of AR and HR to drought is vital to understanding whether and how drought facilitates soil C sequestration, there was limited research on this crucial issue in the warm-temperate forests of China (Luan et al., 2012; Liu et al., 2016; Lu et al., 2017). We used the trenching method to distinguish AR and HR, which was widely applied in forest ecosystems (Liu et al., 2016; Huang et al., 2018). This method may underestimate HR due to the elimination of roots and associated root exudations, which are respired by soil microbes and often lead to a priming effect resulting from SOC decomposition (Hanson et al., 2000). It may also overestimate HR due to the elevated soil moisture in the trenched subplots (Yan et al., 2010). Moreover, the AR in our study may be overestimated as the estimation of AR includes both root respiration and rhizosphere respiration (e.g., mycorrhizal respiration) (Hopkins et al., 2013).

Nevertheless, our study provides empirical evidence that long-term TFR decreased the relative contribution of HR to SR, indicating that the proportion of CO<sub>2</sub> released from microbial SOM decomposition was lower than that from root activities under TFR treatment. As is known to all, HR is an important indicator of SOC decomposition and also plays a crucial role in the stability of SOC (Janssens et al., 2010). Therefore, the decreased relative contribution of HR to SR may lead to more soil C sink (Wang et al., 2019). On the other hand, soil C storage is also largely dependent on the C input (Riggs et al., 2015), thus the higher fine root biomass and production under TFR treatment may also facilitate soil C sequestration. However, recent studies have demonstrated that plant roots or belowground C allocation can drive the soil SOM decomposition (Moore et al., 2020; Street et al., 2020), indicating that the fine roots had a dual role in regulating soil C storage (Dijkstra et al., 2020). Our results suggest that how prolonged drought will ultimately influence SR, and therefore soil C storage, will depend not only on soil microorganisms but also on plant belowground C allocation.

## **Conclusions**

In this warm-temperate oak forest, long-term TFR decreased HR and was positively associated with bacterial richness. More importantly, some bacterial taxa such as *Novosphingobium* and *norank Acidimicrobia*, and fungal *Leptobacillum* were identified as the key drivers of HR. However, TFR

significantly increased AR, which was attributed to the increased fine root biomass and production. The increase in AR offset the decrease in HR, resulting in unaltered SR under the TFR treatment. Our findings highlight the different response mechanisms of AR and HR to prolonged drought should be considered when predicting soil CO<sub>2</sub> emissions under future droughts.

## Abbreviations

SR: soil respiration; AR: autotrophic respiration; HR: heterotrophic respiration; TFR: throughfall reduction; SOM: soil organic decomposition; SM: soil moisture; ST: soil temperature; SOC: soil organic carbon; C: carbon; N: nitrogen; P: phosphorus; NSC: nonstructural carbohydrates; MBC: microbial biomass C; MBN: microbial biomass nitrogen; SEM: structure equation modeling.

## Declarations

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### Authors' contributions

Conceived and design the experiment: Shirong Liu, Jinglei Zhang. Obtained data and samples in the field: Jinglei Zhang, Cuiju Liu, Xiaojing Liu. Processed samples in the lab: Jinglei Zhang, Xingwei Guo, Baoliang Niu. Analyzed the data and wrote the manuscript: Jinglei Zhang, Shirong Liu, Junwei Luan, Hui Wang, Cuiju Liu. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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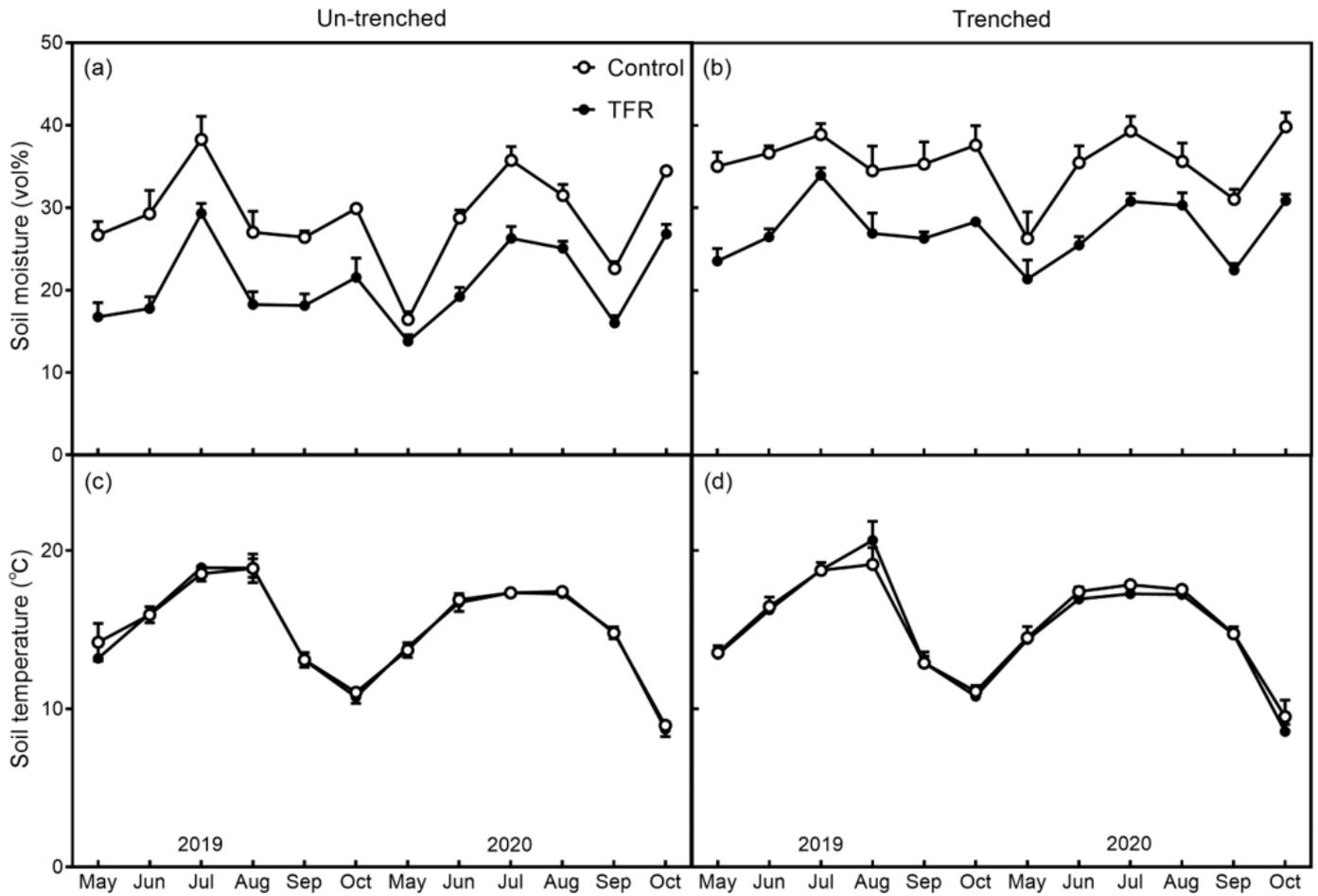
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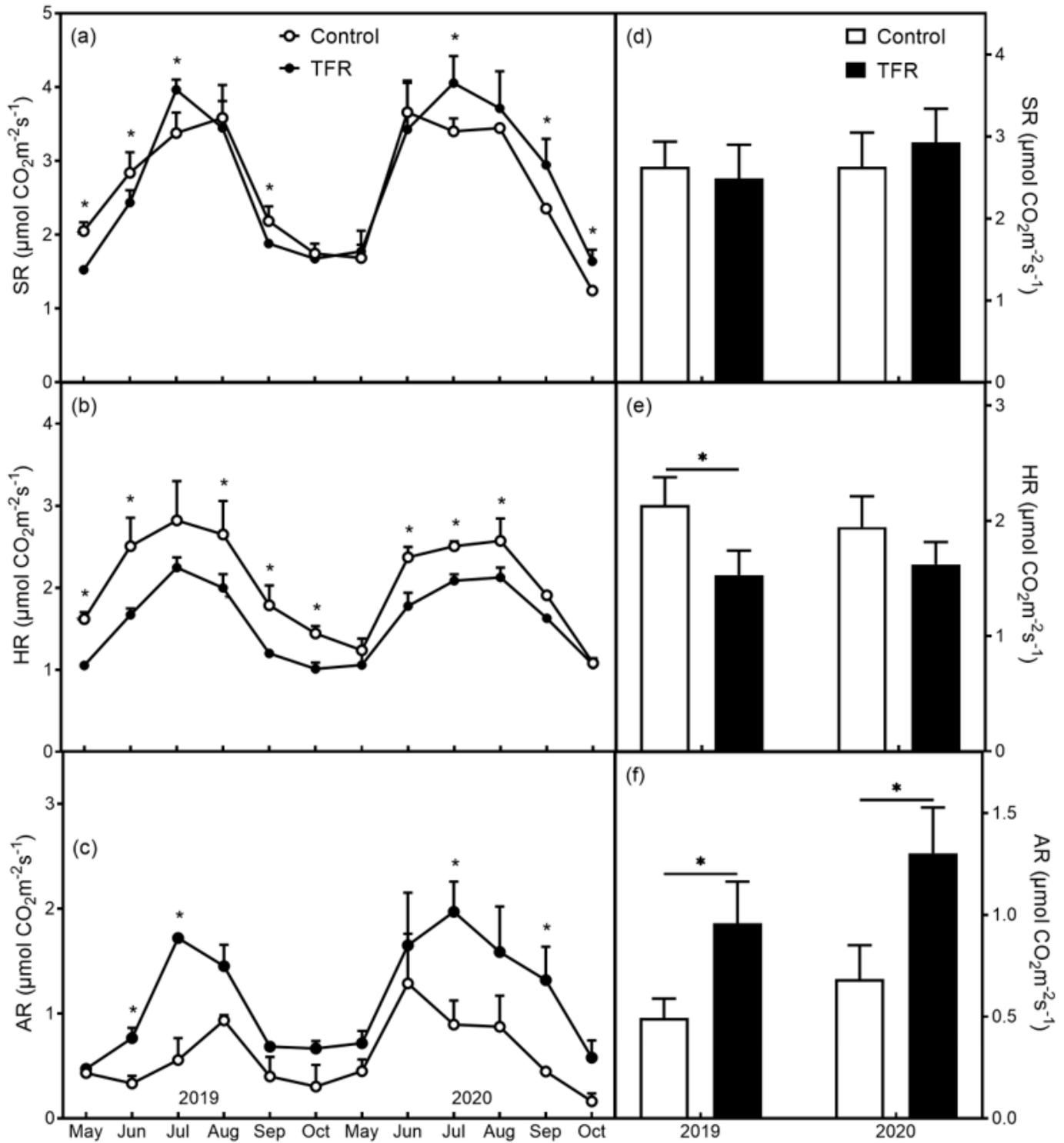
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## Figures



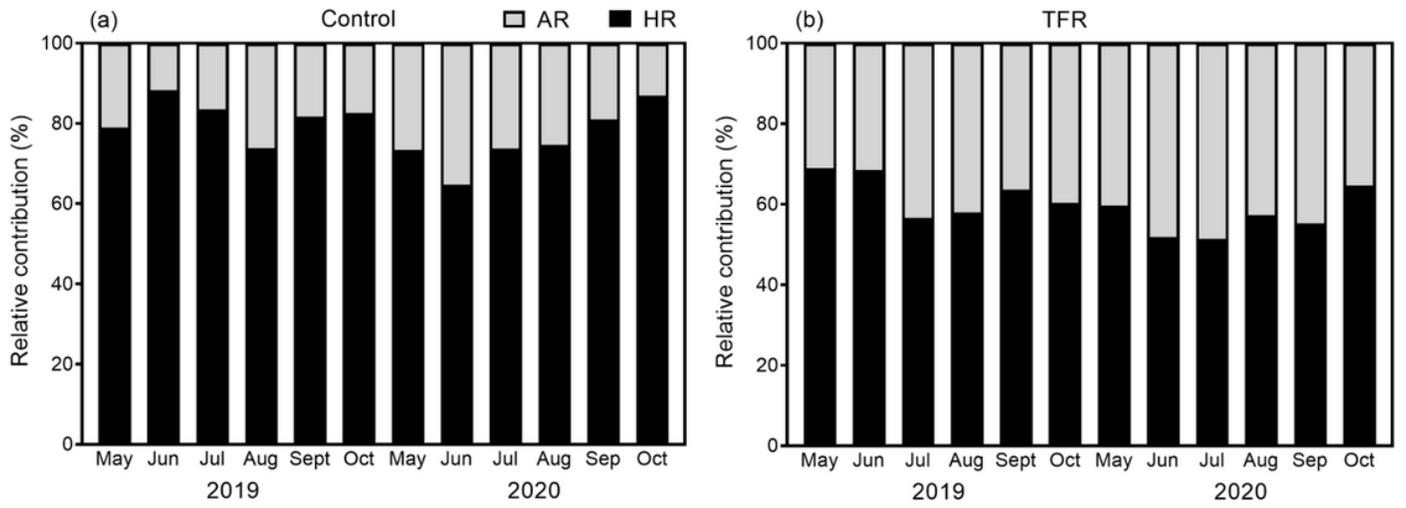
**Figure 1**

Effects of TFR on seasonal variability of soil temperature (ST; a and b) and soil moisture (SM; c and d) for the un-trenched and trenched subplots from 2019 to 2020. Error bars represent the mean standard error.



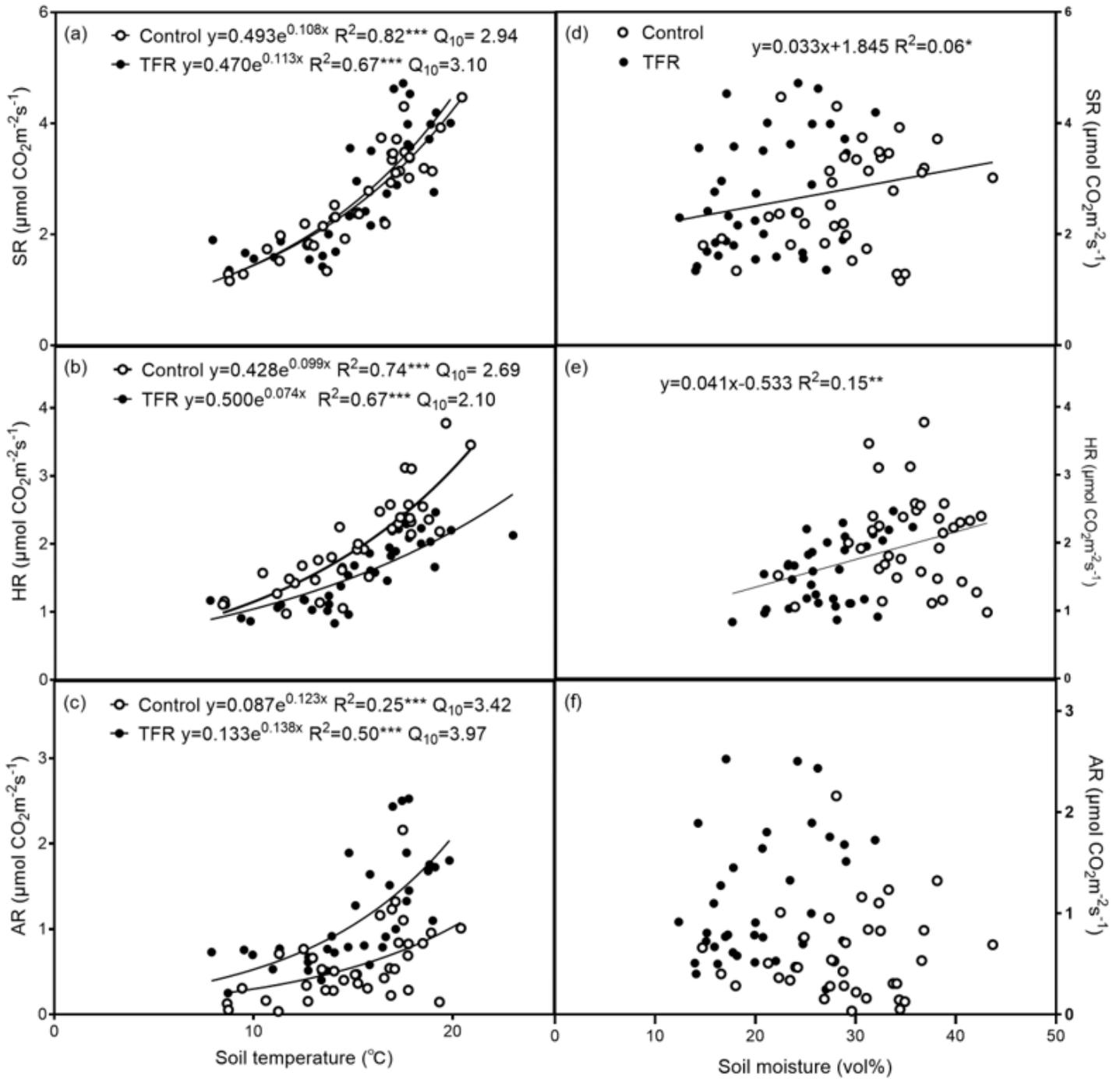
**Figure 2**

Seasonal variation and average value of SR (a, d), HR (b, e) and AR (c, f) from 2019 to 2020. \*P < 0.05.



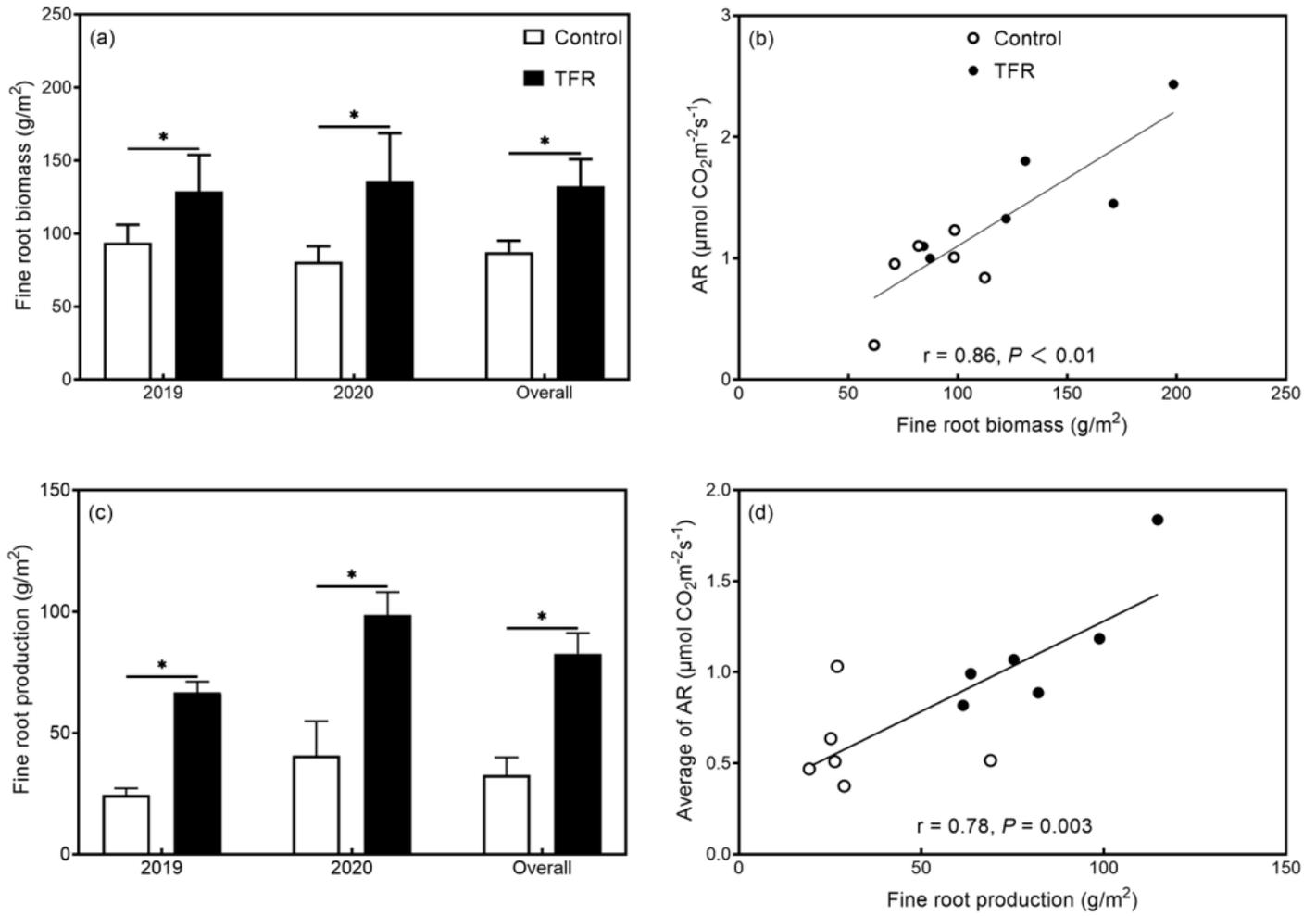
**Figure 3**

Seasonal variability in the relative contribution of AR and HR to SR under control (a) and TFR treatment (b).



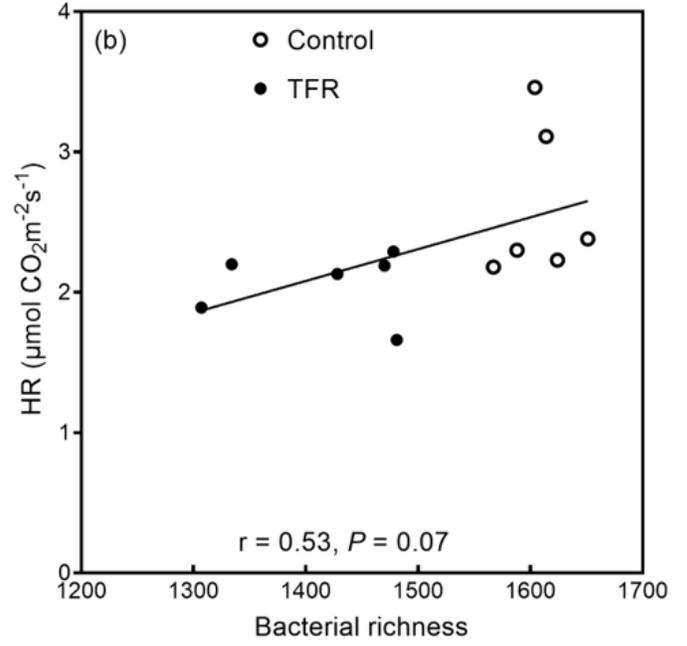
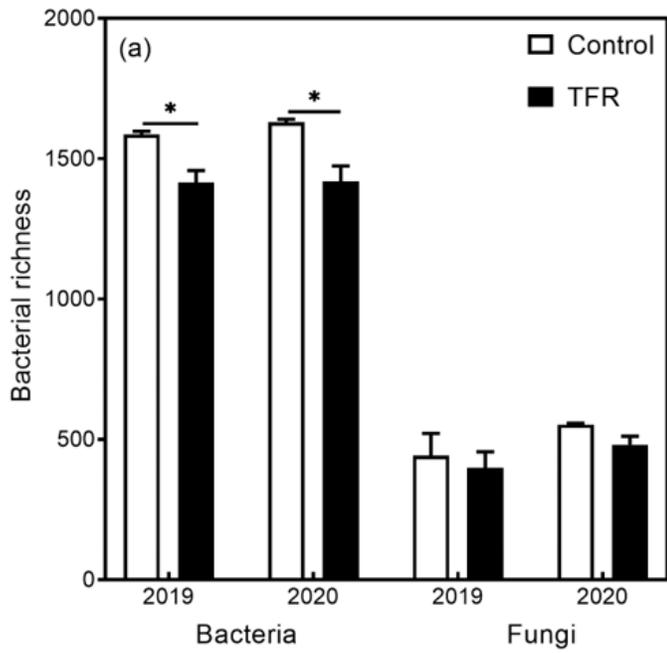
**Figure 4**

Relationships between soil temperature and SR (a), HR (b), and AR (c), and relationships between soil moisture and SR (d), HR (e) and AR (f) under control and TFR treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



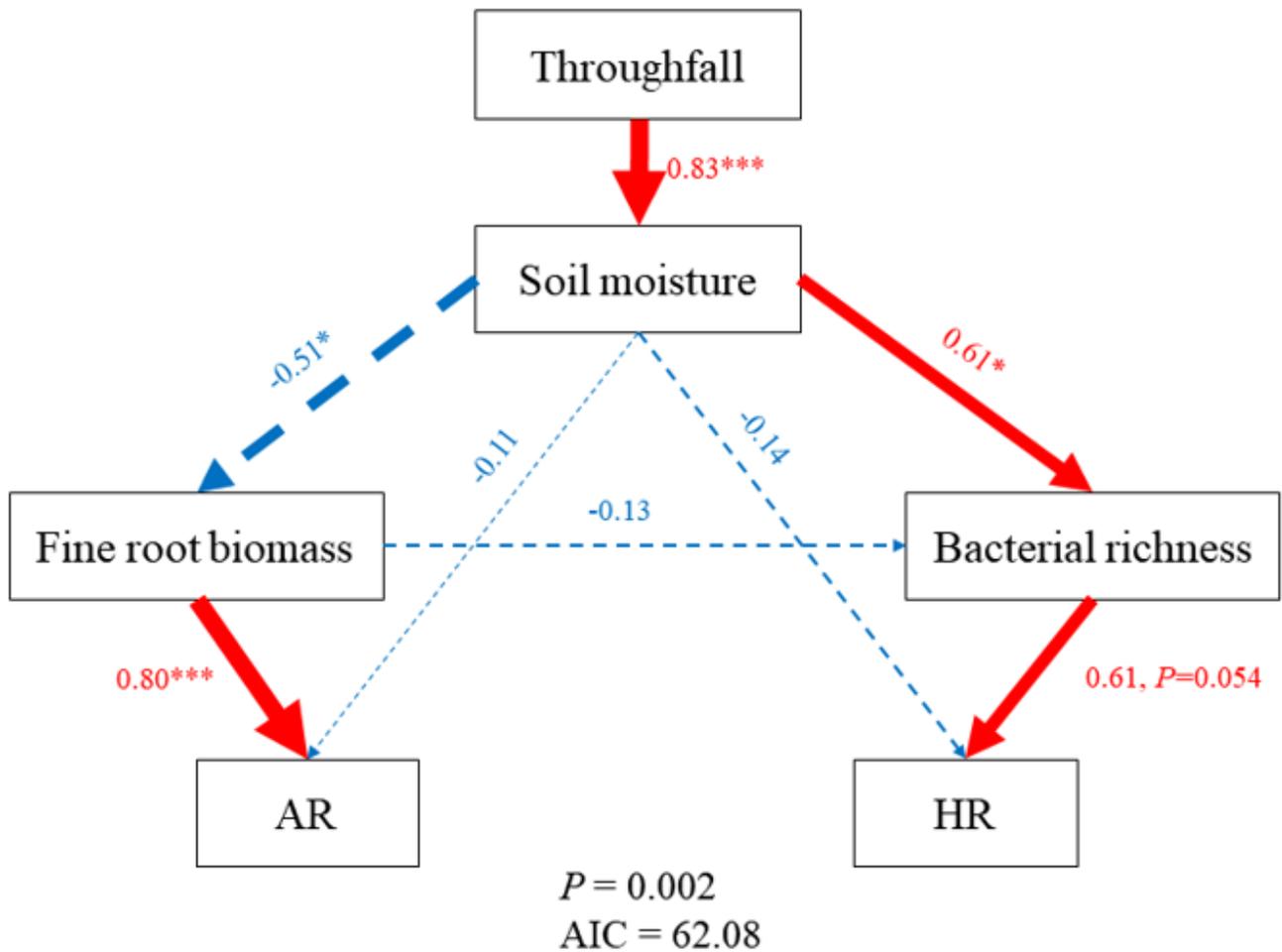
**Figure 5**

Fine root biomass (a) and production (c) under control and TFR treatment, and relationships between fine root biomass and AR (b), between fine root production and the average of AR (d). \*P < 0.05.



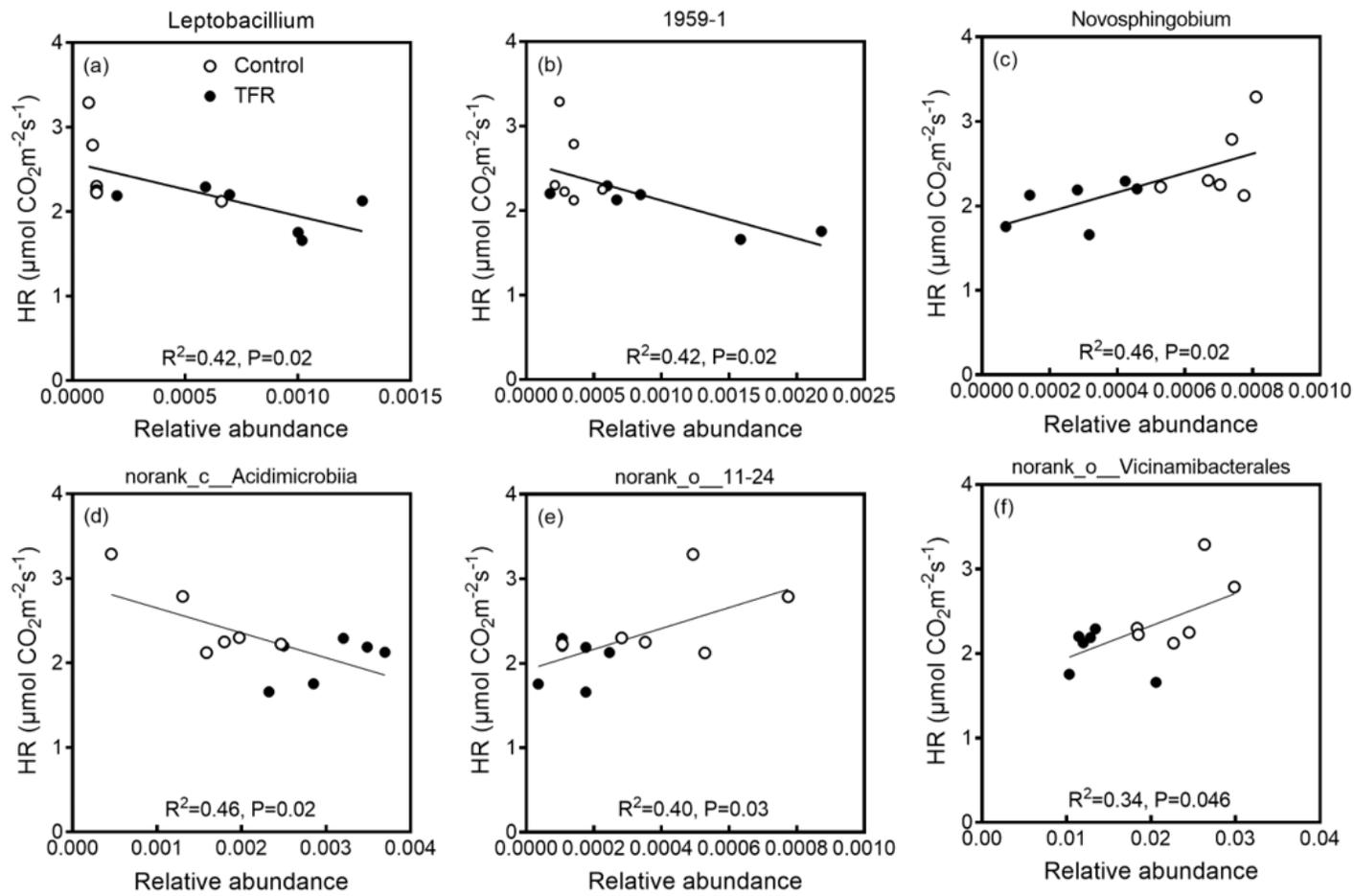
**Figure 6**

Effects of TFR on bacterial and fungal richness (Sobs index) (a), and relationships between bacterial richness and AR (b). \*P < 0.05.



**Figure 7**

Structure equation modeling (SEM) assessing the direct and indirect influences of driving factors on AR and HR. Blue and red arrows indicate negative and positive relationships, respectively. The numbers on arrows are standardized path coefficients, and arrow width is proportional to the strength of the relationship. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**Figure 8**

Linear regressions between the relative abundances of selected microbial genera and HR across different treatment.

## Supplementary Files

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