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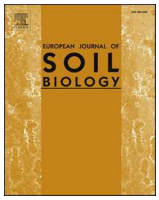

Original article

Effects of precipitation changes on soil heterotrophic respiration and microbial activities in a switchgrass mesocosm experiment

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ABSTRACT

Precipitation changes altered soil heterotrophic respiration, but the underlying microbial mechanisms remain rarely studied. This study conducted three-year switchgrass (*Panicum virgatum* L.) mesocosm experiment to investigate soil heterotrophic respiratory responses to altered precipitation. Five treatments were considered, including ambient precipitation (P0), two wet treatments (P+33 and P+50: 33% and 50% enhancement relative to P0), and two drought treatments (P-33 and P-50: 33% and 50% reduction relative to P0). The plant's aboveground biomass (AGB), soil organic carbon (SOC), total nitrogen (TN), microbial biomass carbon (MBC), heterotrophic respiration (R_s), biomass-specific respiration (R_{ss}: respiration per unit of microbial biomass as a reciprocal index of microbial growth efficiency), and extracellular enzymes activities (EEAs) were quantified in soil samples (0–15 cm). Despite significantly different soil moisture contents among treatments, results showed no impact of precipitation treatments on SOC and TN. Increasing precipitation had no effect, but decreasing precipitation significantly reduced plant AGB. Relative to P0, P+33 significantly increased R_s by more than 3-fold and caused no changes in MBC, leading to significantly higher R_{ss} ($P < 0.05$). P+33 also significantly increased hydrolytic enzyme activities associated with labile carbon acquisition (C_{acq}) by 115%. The only significant effect of drought treatments was the decreased β-D-cellobiosidase (*CBH*) and peroxidase (*PEO*) under P-33. Nonparametric analyses corroborated the strong influences of moisture and *CBH* on the enhanced precipitation, which stimulated soil respiratory carbon loss, likely driven by both elevated hydrolase activities and reduced microbial growth efficiency. However, the less sensitive drought effects suggested potential microbial tolerance to water deficiency despite depressed plant growth. This study informs the likely decoupled impacts of microbes and plants on soil heterotrophic respiration under changing precipitation in the switchgrass mesocosm experiment.

1. Introduction

Global climate models predict more extreme climatic events worldwide, such as intense drought and heavy rainfall [1,2]. As a result, the global dry or wet land areas from precipitation extremes have largely increased [2,3]. Changes in precipitation regimes affect soil moisture contents [4,5], which would affect microbial community activity, composition, and function [6,7], and consequently alter soil organic matter (SOM) decomposition and turnover [8]. In particular, the likely increased soil heterotrophic or microbial respiration rate under precipitation changes may lower global soil carbon storage and induce positive feedback to climate change [9,10]. It is thus imperative to discern the underlying mechanistic control of microbial communities that mediate soil respiratory losses. Switchgrass (*Panicum virgatum* L.) is a model bioenergy crop for global climate mitigation [11,12], and its biomass production is sensitive to altered precipitation [13–15] but soil respiration and microbial mechanisms in these systems are rarely investigated.

Soil heterotrophic respiration is highly responsive to the alteration in precipitation amount [16–18]. Reduced soil moisture caused by precipitation reduction can suppress microbial activities [17] due to reduced carbon accretion from plants [19,20] and limited extracellular enzymes and soluble carbon substrate diffusion [21–23]. On the other hand, increased plant carbon inputs and substrate diffusion following

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increased precipitation can stimulate soil microorganisms and their activities [24–26]. As such, increased precipitation is predicted to have a positive impact and decreased precipitation a negative impact on SOM mineralization and soil heterotrophic respiration [5,20,27] and microbial biomass carbon (MBC) [28–31]. However, increasing precipitation can increase, decrease, or have neutral effects on soil carbon storage depending on different ecosystems [32–34]. Nevertheless, insignificant responses of soil respiration [35,36] and MBC [37,38] under precipitation changes have also been reported.

Soil microbes and carbon-degrading extracellular enzymes activities (EEAs) are regarded as the rate-limiting steps in carbon decomposition and soil carbon efflux [39]. Soil microorganisms produce extracellular enzymes to mediate the decomposition of soil organic carbon (SOC) and plant litter, and contribute substantially to soil respiration [9,40]. Thereby, the EEAs are a good proxy of SOC decomposition [41–43]. The EEAs are sensitive to soil moisture [9,44], because soil moisture directly influences substrate availability and diffusion in the soil. Previous studies have explored an inconsistent pattern of precipitation effects on EEAs. A global meta-analysis study demonstrated that increased precipitation significantly stimulated oxidative enzymes (e.g., phenol oxidase and peroxidase) [28], while effects on hydrolytic carbon acquisition enzymes (e.g., α-1,4-Glucosidase, β-1,4-Glucosidase, β-D-Cellobiosidase, and β-1,4-Xylosidase) was insignificant. In another global meta-analysis study [45], increased precipitation stimulated the activities of oxidative enzymes and nitrogen acquisition enzymes (e.g., β-1,4-N-Acetyl-glucosaminidase and leucine amino peptidase) by 42.8% and 16.7%, respectively; and decreased precipitation reduced the oxidative enzyme and nitrogen acquisition enzyme by 47.2% and 17.6%, respectively; but neither increased nor decreased precipitation had impacts on carbon acquisition enzymes. However, the hydrolytic carbon acquisition enzyme was positively correlated with soil moisture in hardwood forests and corresponded with increased microbial biomass [46], while negatively linked in a mixed grass prairie [47]. As SOC is the result of an equilibrium between carbon inputs and outputs, the potential changes in EEAs can affect this linkage and consequently alter soil CO_2 respiration.

Altered precipitation also affects the soil respiration by controlling microbial physiology [48], such as microbial growth efficiency (MGE). MGE is described as the fraction of assimilated carbon partitioned for microbial growth, while the remaining carbon typically respired [49,50]. This microbial parameter is critical for simulating changes in soil carbon with precipitation changes [51,52]. In general, reduced precipitation is expected to reduce MGE, as low soil moisture limits microbial growth due to increased energy costs for survival mechanisms [26,53]. However, the opposite response has also been detected due to the accumulation of carbon rich osmolytes in their biomass [51]. Likewise, decomposer communities have shown variable MGE responses to increasing precipitation as well, such as MGE can be stimulated [52] or suppressed [54,55], likely due to different pathways of assimilated carbon allocation for growth and maintenance [22,52]. Despite the great importance of MGE on CO_2 respiration in soil, precipitation impacts on MGE and their consequences on the carbon loss through microbial respiration have been poorly studied.

Switchgrass (*Panicum virgatum* L.) is a model bioenergy crop [56], and past studies have mainly focused on switchgrass growth features to achieve high plant biomass under changing climate conditions [57–59]. Recent efforts have also promoted mechanistic understanding of switchgrass soil responses to climate warming and nitrogen fertilization as well as their interaction with soil, roots, and organic compounds' compositional change [60–62]. Studies also have implemented precipitation treatments in the field experiment [15,63], however very few have explored soil microbial community, activity, and their function in response to precipitation change. In a switchgrass mesocosm experiment that was implemented to discern the root and microbial respiration responses to precipitation change [64], the reliance upon a manually created root-free zone (for separation of root and microbial respiration) and no qualification of soil microbial features resulted in lack the capacity to study microbial mechanisms that may have regulated the respiratory loss under changing precipitation. Taking advantage of a switchgrass mesocosm experiment and sophisticated control of precipitation spanning a wide gradient of moisture regimes will thus allow one to test the plant growth and soil respiratory responses to precipitation changes. In such a mesocosm experiment, one can achieve a relatively consistent soil, plant, light, and other conditions except water input so that an accurate implementation of moisture or precipitation can be achieved. Using a switchgrass mesocosm experiment will provide a powerful way to discern the impact of precipitation and the underlying microbial processes that may contribute to respiratory loss from soils.

This study aims to examine soil respiratory patterns and the underlying mechanisms in response to a wide range of precipitation treatments based on a 3-year switchgrass mesocosm experiment located in the Tennessee State University Agricultural Research and Education Center, Nashville, Tennessee, USA. Our main objectives were: (1) to explore the effects of sustained precipitation changes on SOC, soil heterotrophic respiration (R_s), and microbial activity; and (2) to elucidate the underlying microbial, enzymatic, and physiological controls on the responses of R_s to precipitation regimes in switchgrass cropland. Given the findings of global meta-analyses [29,32], we first hypothesized that the SOC content increases with increasing precipitation in the switchgrass mesocosm experiment. Considering that increased plant inputs and substrate diffusion following increased precipitation can stimulate soil microbial functions and activities, we set the second hypothesis that increased precipitation stimulated R_s. We expected that precipitation increased R_s was related to elevated plant biomass, reduced microbial growth efficiency, and stimulated hydrolase activities.

2. Materials and methods

2.1. Site description, soil sample collection and analysis

The switchgrass mesocosm experiment was carried out at the Tennessee State University Agricultural Research and Education Center (Latitude 36.12′N, Longitude 86.89′W, Elevation 127.6 m), Nashville, Tennessee, USA [15]. The mean annual temperature is 15.1 °C and mean annual precipitation is 1176 mm [65]. The greenhouse consisted of roof and wall to control inside air temperature and humidity. Roof and wall panels open automatically when the air temperature in the greenhouse is above 20 °C. Roof close automatically during rains. Plastic pots of 95 L. capacity (50 cm diameter and 50 cm height) were filled with an *Armour* silt loam soil collected from the nearby switchgrass field experiment in July 2013. The soil was slightly acidic (pH = 6.2) and low in phosphorus. Five tillers of two-year-old "Alamo" switchgrass collected from the switchgrass field experiment at the farm were transplanted to the experimental pots on July 13, 2013; one plant located at the center of the pot and the rest four surrounding it. No fertilizers were applied during the entire period of the three-year experiment. Water was applied during establishment of crop with an amount of water mimicking the long-term averaged ambient precipitation (see below). The aboveground plant biomass was harvested three times a year at the end of April, July, and October in 2017, and the dry weight of biomass (gram per pot) in April (close to March soil collection) was used in this analysis.

The mesocosm precipitation experiment was set up in a randomized complete block design with five precipitation amounts and five replications in each treatment. This experimental design was selected based on the balance of need of a high number replicates and the consideration of labor and analytical costs [66]. Five blocks were deployed at an increasing distance to the greenhouse door area, representing a varying degree of microclimate. Within each block, 5 large pots with a volume of 90 L. and 50 cm in diameter and 50 cm in height were randomly assigned to five treatments. Each pot was filled with soils and 5 tillers were planted in each pot with one tiller in the middle and four others circled around it about 25 cm away. This design thus avoided sidewall

impact on switchgrass growth. The total number of pots was 25 (5 levels × 5 replicates). In this experiment, the five precipitation amounts were implemented based on the relative change to the ambient condition. The ambient precipitation treatment (P0) represents the precipitation amount averaged over long-term records of annual precipitation from 1969 at Nashville, TN [64]. Two wet treatments (P+33 and P+50) were implemented with an enhanced precipitation amount of 33% and 50% relative to P0, respectively. Two drought treatments (P-33 and P-50) were implemented with a reduced precipitation amount of 33% and 50% relative to P0, respectively. Water was supplied three times in a day, ten days in a month in 2014 or two times in a day, ten days in a month in 2015 and 2016 with amount that summed to the set monthly total. Dry and wet treatments were maintained by increasing or decreasing individual watering duration. The precipitation level was controlled using an automatic irrigation system (RSC600i, Raindrip, Inc., Woodland Hills, CA) including a watering timer controller that automatically turns on and off the irrigation based on application duration for each treatment. The manipulation of precipitation treatments was initiated on February 1, 2014 and continued till present. Several pots in June and July 2015 received natural precipitation due to failure in roof control. We adjusted water in those pots by reducing the irrigation.

For soil sampling, two cores were collected midway between the plant base and the pot edge to minimize variability caused by edge effects in the pot. Soil samples (0–15 cm) were collected in March 2017 from the mesocosm experiment. Two cores were collected in each pot to derive a composited sample using a soil auger with a diameter of 5.0 cm. Soil samples were transported in a cooler filled with ice pack to the lab immediately after sampling. At the same day, soil samples were sieved using a 2 mm soil sieve after roots were removed. Gravimetric soil moisture content was determined by oven-drying a field moist subsample at 105 °C for 24–48 h.

2.2. Soil organic carbon and total nitrogen

Air-dried soil samples were ground to fine powder for soil organic carbon (SOC) and total nitrogen (TN) analysis. For each sample, about 50–100 mg equivalent of soil was weighed and packed in a tin capsule bag (4 × 6 mm). Packed soil samples were shipped to the University of North Carolina at Wilmington Center for Marine Science for analysis of organic carbon and TN using a Thermo Scientific HT Plus elemental analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA) interfaced with a Thermo Scientific Delta V Plus stable isotope mass spectrometer. The soil C/N ratio was calculated by dividing the SOC by TN.

2.3. Soil heterotrophic respiration and microbial biomass carbon

To quantify soil heterotrophic respiration (R_s), field moist soil subsamples (equivalent to 10.0 g dry weight) were weighted in a 7.5 cm tall PVC cores (5 cm diameter). The bottom side of each core was sealed with glass fiber paper. The PVC cores were placed in a mason jar (1 L. capacity) lined with glass balls at the bottom to prevent the cores resting in moisture. The mason jars were then connected to a Picarro G2131-*i* analyzer (Picarro Inc., Santa Clara, CA, USA) to measure total CO_2 concentration in the jars. The amount of CO_2 emitted over time was used to calculate the respiration rate based on dry soil weight [67]. Once soil samples were put in the mason jar and connected with the analyzer, the measurement started with CO_2 concentration immediately recorded. The respiration rate was estimated based on the average of CO_2 built up within a short time, saying 15–20 min. During the short measurement period, soil moisture was assumed to remain constant. Each soil sample was put into a different jar, which would connect with the analyzer for 15–20 min sequentially.

To quantify microbial biomass carbon (MBC), field moist soil subsample (5.0 g) was weighted in 50 ml centrifuge tube and fumigated with 3 ml ethanol-free chloroform for 24 h in the fume hood. Another equivalent weight subsample was weighted but kept unfumigated. Both the fumigated and unfumigated subsamples were added with 25 ml of 0.5 M K_2SO_4 and shaken on a mechanical shaker for 30 min. The extracts were filtered through Whatman #4 filter paper using vacuum pump. Soil extract (5 ml) and persulfate reagent (5 ml) were added in culture tubes for both fumigated and unfumigated and placed into the drying oven set at 85–90 °C for 18 h [68]. The tubes were removed from oven and cooled to room temperature before analyzing. Extractable organic carbon was measured using total organic carbon and nitrogen analyzer (Shimadzu Corp., Kyoto, Japan). The MBC was determined by subtracting extractable organic carbon in the unfumigated samples from that in the fumigated samples. An extraction coefficient of 0.45 was used [69]. Microbial biomass-specific respiration rate (R_{ss}) was determined by the ratio derived from the R_s rate over MBC.

2.4. Extracellular enzyme activity assay

Field moist soil samples (1.0 g) were used to quantify the extracellular enzymes activities (EEAs) following established protocols [70,71]. The fluorometric method was used to assess β-1,4-glucosidase (*BG*), β-D-cellobiosidase (*CBH*), leucineamino peptidase (*LAP*), β-1,4-N-acetyl-glucosaminidase (*NAG*), and acid phosphatase (*AP*) [72] and colorimetric method was used to assess phenol oxidase (*PHO*) and peroxidase (*PEO*) activities [73]. In this study, the proxy variable for hydrolytic carbon acquisition enzymes (C_{acq}) was calculated as the summation of *BG* and *CBH*; the proxy variable for hydrolytic nitrogen acquisition enzymes (N_{acq}) was calculated as the summation of *LAP* and *NAG*.

To quantify EEAs, 1 g of fresh soil sample was first homogenized with 125 ml of 50 mM sodium acetate buffer (pH = 5.0) using a hand blender to make a soil slurry. Hydrolyzes were assessed using 96-well fluorometric (black) plates. Sample wells received 200 μl soil slurry and 50 μl sodium acetate buffer. Quench control wells received 200 μl soil slurry and 50 μl 4-Methylumbelliferone (MUB) standard. For *LAP*, 7-Amino-4-methyl coumarin (AMC) standard was used instead of MUB. Assay wells received 200 μl soil slurry and 50 μl fluorescence-labeled substrate. Standard reference wells received 200 μl sodium acetate buffer and 50 μl MUB or AMC standard. Substrate control wells received 200 μl sodium acetate buffer and 50 μl substrate. Eight replicate wells were used for each. Fluorometric plates were incubated for 18 h at 20 °C. All fluorometric plates received 10 μL (0.5 M) NaOH before measurement to increase the MUB to make it detectable. For the *PEO* assay, L-3,4-dihydroxyphenylalanine (DOPA) substrate and 96-well colorimetric (clear) plates were used. Sixteen wells were used for the assay, eight additional wells were used for samples and another eight for the substrate. Assay wells received 200 μL soil slurry and 50 μL of DOPA substrate. The sample wells received 200 μL soil slurry and 50 μL of sodium acetate buffer. Substrate wells received 200 μL sodium acetate buffer and 50 μL DOPA substrate. For the *PEO* plates, an additional 10 μL 0.3% H_2O_2 solution was added to every well. Oxidase plates were incubated for 18 h at 20 °C. A multi-model microplate reader (Molecular Devices, Sunnyvale, CA, USA) was used to quantify enzyme activities. The fluorometric plates were quantified at an excitation wavelength of 365 nm and an emission wavelength of 460 nm, while colorimetric plates were measured at 460 nm for absorbance.

2.5. Statistical analysis

All data were analyzed using SPSS version 22.0 (IBM Corporation, Chicago, USA), R version 3.6.2 [74], and Origin 2023 (Origin Lab Corporation, Northampton, MA, USA). Before analysis, data were log transformed if they violated the assumptions of normal distribution and equal variance. One-way blocked analysis of variance (ANOVA) was used to test the effect of precipitation on plant aboveground biomass (AGB), soil moisture, SOC, TN, R_s, R_{ss}, MBC, and EEAs, and TUKEY *post hoc* test was performed to compare the means when significant differences were observed. The significance level was set at $P < 0.05$. The

principal component analysis (PCA) was conducted to explore the relationship between principal components (i.e., associated with the precipitation effect) and R_s and corresponding soil parameters (e.g., AGB, soil moisture, C/N, MBC, and EEAs). SOC, and TN were not included in the PCA because of their positive relationships. Linear regression models were utilized to determine the relationships of R_s to AGB, soil moisture, SOC, TN, C/N, MBC, and EEAs. The random forest (RF) analysis was used to determine the most important and credible predictors of AGB, soil moisture, SOC, TN, C/N, MBC, and EEAs for R_s. As derived from R_s, R_{ss} was not included in the PCA and RF analyses. The RF mean predictor importance (i.e., per cent increase in mean square error (MSE)) was applied to identify the main predictor variable.

3. Results

There were no significant effects of block on all variables ($P > 0.05$; Table S1), only the effects of precipitation were thus presented.

3.1. Soil moisture, AGB, SOC, and TN

There was a significant precipitation effect on gravimetric soil moisture content ($P < 0.05$; Fig. 1a). In addition to an ascending soil moisture content of 8.17%, 12.38%, 17.48%, 23.66%, and 24.40% with the increasing precipitation gradient (e.g., P-50, P-33, P0, P+33, and P+50), post hoc tests showed significant differences between all treatments except the two wet treatments (Fig. 1a). There were no significant treatment effects on AGB between high precipitation and control treatments, but there was significantly higher AGB under control than drought treatments (Fig. 1b). There were no significant precipitation effects on the SOC content (12.81–14.76 g kg^{-1}) and TN content (0.92–1.05 g kg^{-1}) across the treatments ($P > 0.05$; Fig. 1c and d).

3.2. R_s

There was a significant precipitation effect on R_s ($P < 0.05$; Fig. 2a). The highest R_s was observed under P+33 (2.24 μg C g_{soil}^{-1} h^{-1}), and post hoc tests showed that relative to P0, R_s significantly increased by more than 3-fold with a higher precipitation treatment (P+33) and by more than 2-fold with the highest precipitation treatment (P+50), but insignificantly changed with the lower precipitation treatments (P-33 and P-50). R_s was also significantly higher under two wet treatments (P+50 and P+33) than those under two dry treatments (P-33 and P-50).

3.3. Soil MBC and R_{ss}

There was no significant precipitation effect on MBC ($P > 0.05$; Fig. 2b), and there was a significant precipitation effect on R_{ss} ($P < 0.05$; Fig. 2c). The highest R_{ss} was observed under P+33 (0.06 μg CO_2–C mg_{MBC}^{-1} h^{-1}), and post hoc tests showed that relative to P0, R_{ss} was significantly increased by more than 4-fold under P+33 but insignificantly changed under other precipitation treatments (Fig. 2c). Moreover, no significant difference was observed for R_{ss} among the P+50, P-33, and P-50 treatments (Fig. 2c).

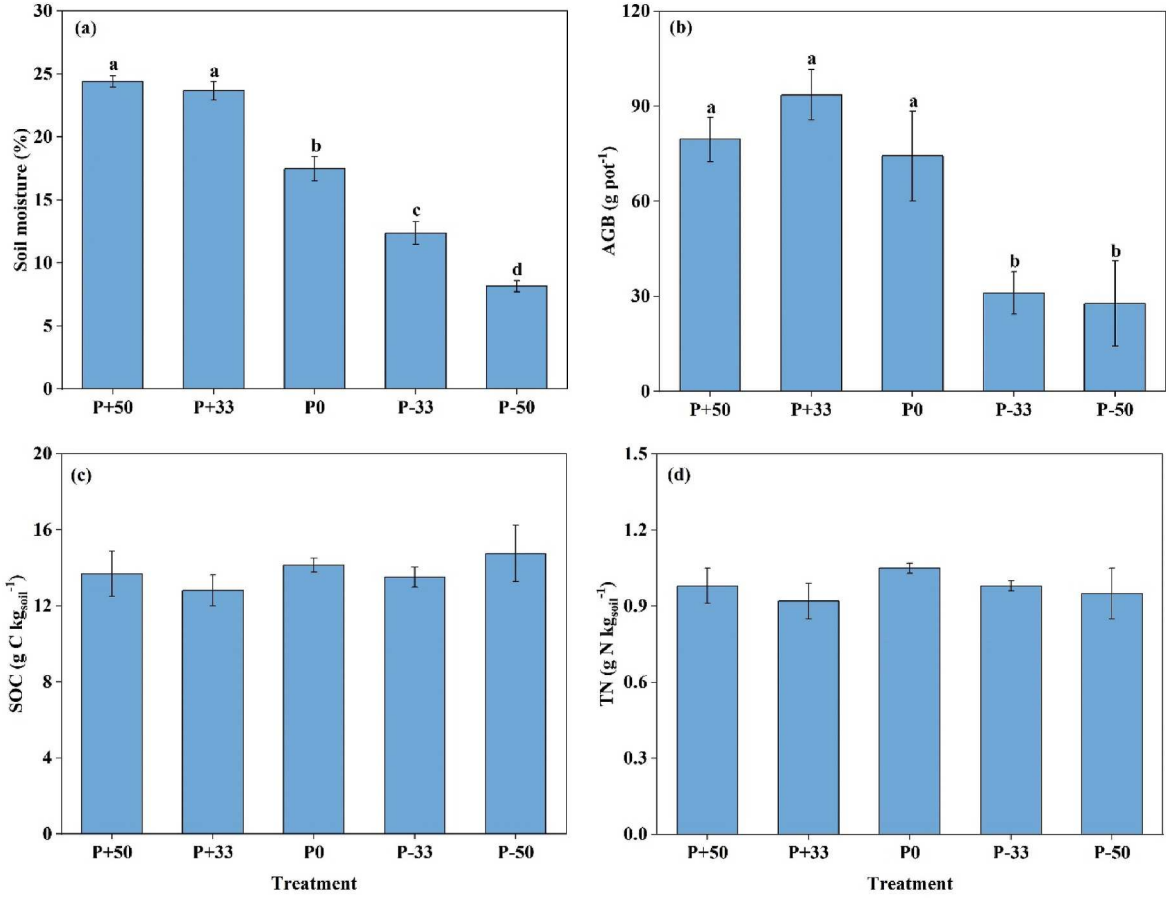

Fig. 1. Mean (±SE) soil moisture, AGB, SOC, and TN (a–d) under five precipitation treatments in the three-year switchgrass (*Panicum virgatum* L.) mesocosm experiment. Different lowercase letters indicate significant differences between treatments ($P < 0.05$). No letters indicate insignificant differences between treatments ($n = 5$). P+50, P+33, P-33, and P-50 represent the enhanced precipitation by 50%, 33% and reduced precipitation by 33% and 50% relative to P0, respectively. P0: ambient precipitation. AGB: plant aboveground biomass; SOC: soil organic carbon; TN: total nitrogen.

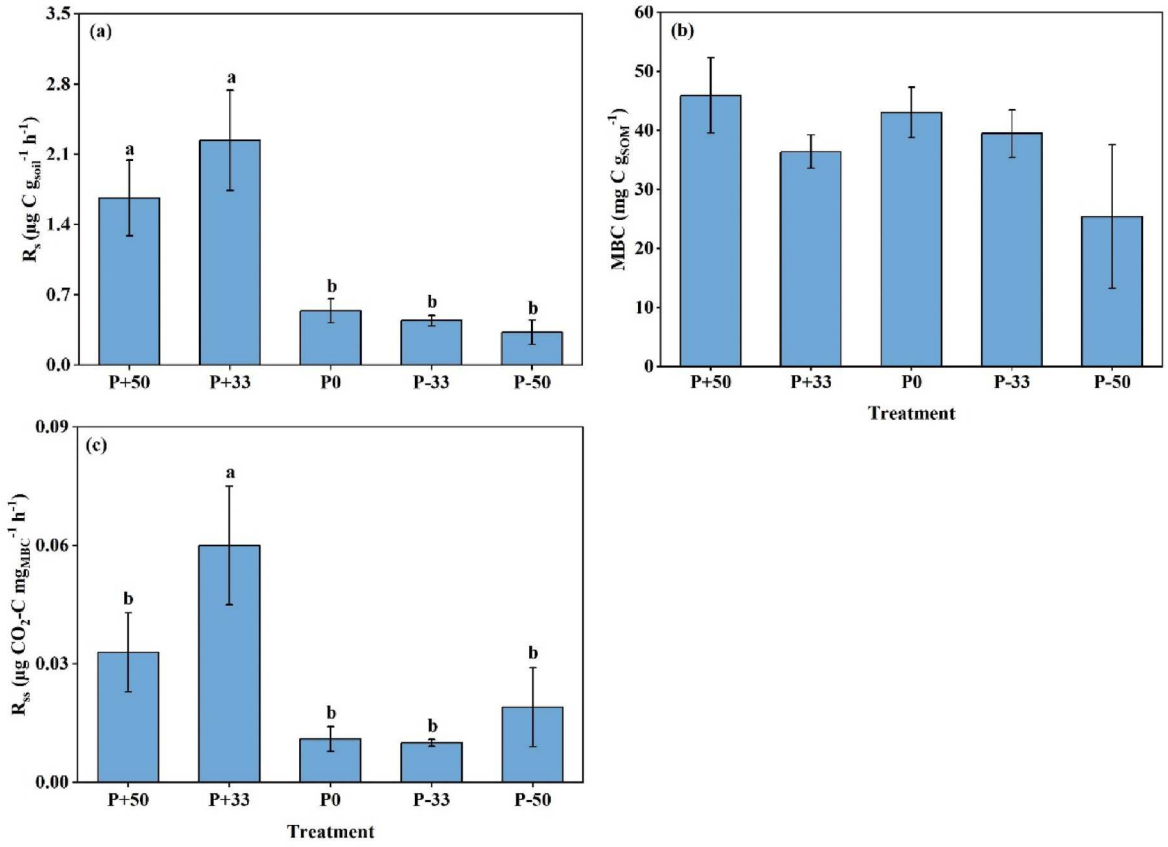

Fig. 2. Mean (±SE) R_s, MBC, and R_{ss} (a–c) under five precipitation treatments (see Fig. 1 for five precipitation treatments) in the three-year switchgrass (*Panicum virgatum* L.) mesocosm experiment. Different lowercase letters indicate significant differences between treatments ($P < 0.05$). No letters indicate insignificant differences between treatments ($n = 5$). R_s: soil heterotrophic respiration. MBC: microbial biomass carbon; R_{ss}: microbial biomass-specific respiration.

3.4. Soil EEAs

There was a significant precipitation effect on *BG*, *CBH*, and C_{acq} ($P < 0.05$; Fig. 3a, b, and c), and there was no significant precipitation effect on *LAP*, *NAG*, and N_{acq} ($P > 0.05$; Fig. 3d, e, and f). The highest *BG*, *CBH*, C_{acq}, and *LAP* (1.98, 0.29, 2.28, and 0.15 μmol g_{SOM}^{-1} h^{-1}, respectively) were observed under P+33. Post hoc tests showed that relative to P0, *BG*, *CBH*, and C_{acq} significantly increased by 117.58%, 93.33%, and 115.09% under P+33, respectively, and little changed under other precipitation treatments (Fig. 3a, b, and c). *BG* and C_{acq} were significantly higher under P+33 than under P-33 (Fig. 3a and c). *CBH* was significantly higher under two wet treatments (P+50 and P+33) than those under two dry treatments (P-33 and P-50) (Fig. 3b).

There was a significant precipitation effect on *AP* and *PEO* ($P < 0.05$; Fig. 3g and h). Post hoc tests showed that relative to P0, *AP* significantly increased by more than 1.5-fold under P+50 and little changed under other precipitation treatments (Fig. 3g). *AP* was also significantly higher under P+50 than those under P-50 and P-33 (Fig. 3g). Relative to P0, *PEO* significantly decreased by 64.1% under P-33 and was generally lower under P-33 and P-50 than other treatments (Fig. 3h). Being generally high under P+50, P+33 and P0, *PEO* was significantly higher under P+33 than those under two dry treatments (P-33 and P-50), and also significantly higher under P+50 than under P-33 (Fig. 3h).

3.5. Relationship of R_S and corresponding soil parameters

The PCA showed that the total explanations for PC1 and PC2 were 29.90% and 20.30%, respectively, and these parameters greatly differed among different treatments (Fig. 4). The PCA exhibited that *BG*, C_{acq}, *CBH,* R_s, soil moisture, AGB, *PEO*, and *AP* were mainly correlated with the two wet treatments, especially greater effects of P+33 (dark diamond).

The result of linear regression showed that R_s was significantly related to soil moisture ($R^2 = 0.43$, $P < 0.001$; Fig. 5a), R_{ss} ($R^2 = 0.85$, $P < 0.001$; Fig. 5e), AGB ($R^2 = 0.21$, $P < 0.05$; Fig. 5f), and *CBH* ($R^2 = 0.46$, $P < 0.001$; Fig. 5h). There was no significant correlation between R_s and SOC, TN, MBC, *BG*, C_{acq}, *LAP*, *NAG*, N_{acq}, *AP*, *PHO*, *PEO*, and C/N ($P > 0.05$; Fig. 5b, c, d, g, i, j, k, l, m, n, o, and p).

The RF analysis showed that soil moisture, and *CBH* were the most dominant drivers for R_s ($P < 0.01$; Fig. 6). It was also observed that moisture (increase in MSE = 14.55%) was more important than other driving factors for R_s. The RF model explained 33.13% of the variance in R_s.

4. Discussion

4.1. Altered precipitation had no significant impacts on SOC

Contrary to our first hypothesis, we found that neither increased nor decreased precipitation, based on mesocosm conditions, led to significant changes in SOC at 0–15 cm soil depth in the switchgrass cropland (Fig. 1). This is in line with the conclusion of no precipitation impact on SOC as reported in a previous meta-analysis study [75] and other studies in temperate grassland of southern Great Plains [33] and in desert grasslands [37]. The enhanced decomposition and respiration losses of soil carbon under increased precipitation were likely compensated by the increased aboveground carbon inputs to soils through litterfall and rhizodeposition, and thus resulting in constant soil carbon storage [33]. Moreover, reduced precipitation might have limited microbial decomposition by retarded substrate diffusion [76,77]. It was unknown how

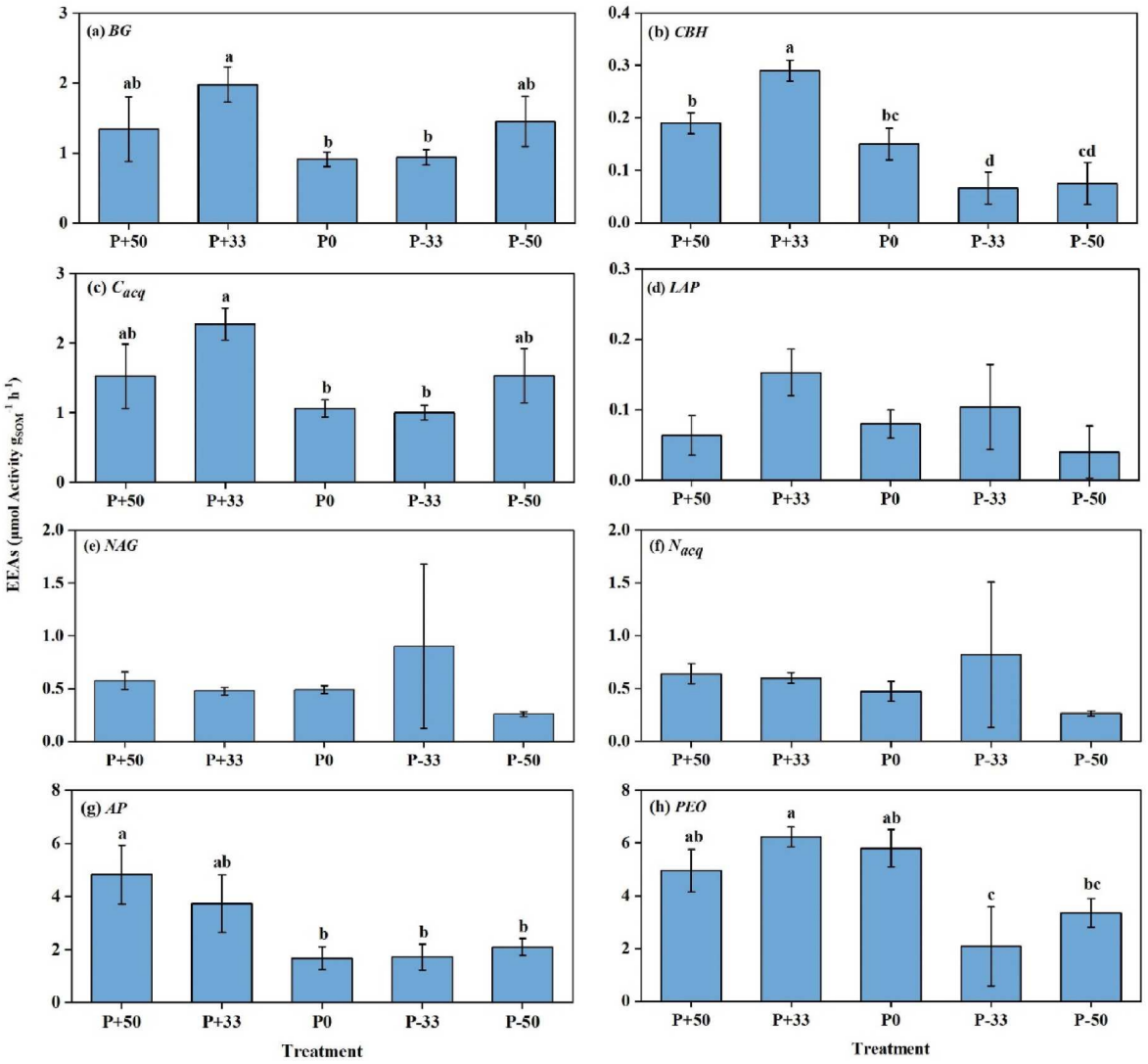

Fig. 3. Mean (±SE) *BG*, *CBH*, C_{acq}, *LAP*, *NAG*, N_{acq}, *AP*, and *PEO* (a–h) under five precipitation treatments (see Fig. 1 for five precipitation treatments) in the three-year switchgrass (*Panicum virgatum* L.) mesocosm experiment. Different lowercase letters indicate significant differences between treatments ($P < 0.05$). No letters indicate insignificant differences between treatments ($n = 5$). *BG*: *β*-1,4-glucosidase; *CBH*: *β*-D-cellobiosidase; C_{acq}: carbon acquisition enzyme; *LAP*: leucineamino peptidase; *NAG*: *β*-1,4-N-acetyl-glucosaminidase; N_{acq}: nitrogen acquisition enzyme; *AP*: acid phosphatase; *PEO*: peroxidase.

belowground plant biomass contributed to soil carbon storage because we did not perform destructive root biomass measurements. If decreased precipitation stimulates the root/shoot ratio in grasslands [78], the abovementioned suppressed decomposition and increased belowground carbon inputs possibly play important roles in maintaining soil carbon storage under reduced precipitation [33,37]. This result was consistent with the global meta-analysis study of 179 published papers that showed SOC remained constant under decreased precipitation, attributed to higher root/shoot ratio and reduced decomposition [79]. In this experiment, lack of N and P fertilizations also likely created a nutrient imbalance and deficient condition, which likely limited plant and microbial growth and consequently SOC accrual. Nevertheless, the insignificant effects of precipitation on SOC imparted the necessity to explore whether distinct SOC fractions (e.g., labile vs. recalcitrant C) may respond to the enhanced or reduced precipitation.

4.2. Increased precipitation stimulated R_s

In support of the second hypothesis, R_s was significantly increased by more than 3-fold under P+33 and increased by more than 2-fold under P+50, while reduced precipitation (P-33 and P-50) did not significantly affect R_s (Fig. 2). Accordingly, soil moisture had a significant positive effect on R_s (Fig. 6). In consistent with previous studies, enhanced precipitation stimulated R_s in switchgrass croplands either in field or mesocosm conditions [63,64], and on the global scale [5,20]. As precipitation are closely related to soil moisture, these patterns of precipitation effects could be explained by soil moisture which affects R_s by favoring or limiting substrate diffusion and carbon inputs from plant production [16,24,63,64,80].

In addition, the response of R_s to increased precipitation in our study was consistent with studies in grasslands [16,27,81], but the magnitude of increase in respiration in our study was much higher. For example, R_s was enhanced by 107.7% when precipitation was increased by 30% in a precipitation experiment conducted on a temperate desert plant [82]. Likewise, a 30% increase in precipitation caused a 31% increase in R_s in arid and semiarid grasslands [16]. Most of the precipitation manipulation experiments are conducted in natural ecosystems [83], where part of precipitation may be lost due to surface runoff with increasing precipitation amount [84], possibly causing a lower increment of soil moisture and thus the smaller increase in R_s. In our experiment, the absence of runoff (i.e., loss of water) could have supported faster microbial decomposition and respiration. Evapotranspiration loss of soil

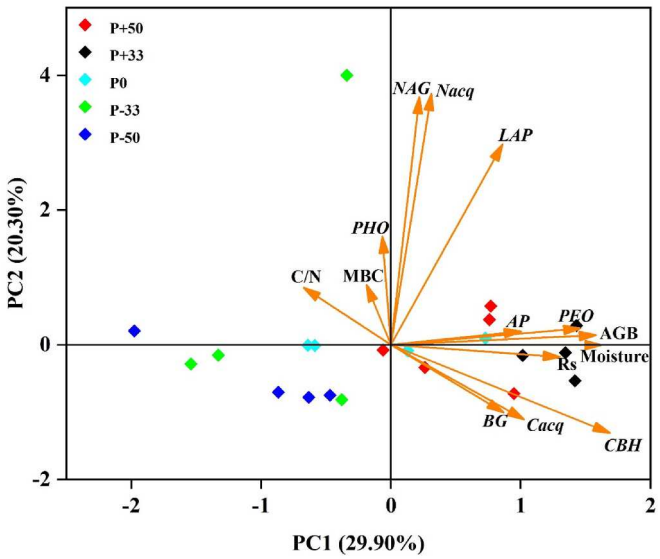

Fig. 4. Principal component analysis (PCA) for all soil parameters by five precipitation treatments (see Fig. 1 for five precipitation treatments) in the three-year switchgrass (*Panicum virgatum* L.) mesocosm experiment. R_s: soil heterotrophic respiration; MBC: microbial biomass carbon; R_{ss}: microbial biomass-specific respiration; AGB: plant aboveground biomass; *BG*: *β*-1,4-glucosidase; *CBH*: *β*-D-cellobiosidase; C_{acq}: carbon acquisition enzyme; *LAP*: leucineamino peptidase; *NAG*: *β*-1,4-N-acetyl-glucosaminidase; N_{acq}: nitrogen acquisition enzyme; *AP*: acid phosphatase; *PEO*: peroxidase.

moisture was likely minimal in our experiment due to low sunlight and wind as well as the relatively high humidity. Nevertheless, switchgrass demonstrates a relatively larger volume of root mass [85,86] and possibly add more carbon for microbial decomposition and respiration [87].

At the microbial level, P+33 stimulated C_{acq} (Fig. 3), which could have accelerated the decomposition of SOC and therefore increased R_s [9,25,88]. Also, P+33 enhanced R_{ss} as microbes allocated more assimilated carbon for maintenance than for biomass production [54,89]. Another explanation is the altered composition of decomposer communities such as an increase in the relative abundance of bacteroidetes in a semi-arid grassland [37]. +50 induced increase in R_s was less pronounced than under P+33, which was consistent with the proportional decrease of the elevated soil C_{acq} (Fig. 3). The explanation may lie in the fact that high moisture inhibited oxygen diffusion, reduced microbial activities, and thus suppressed decomposition and R_s [26,90]. A previous study highlighted that R_s dropped when soil moisture exceeded 26% in a mesic ecosystem with mean annual precipitation of 1063 mm [91], and similarly, R_s dropped at soil moisture content of 24.4% in our study (Fig. 1).

Decreased precipitation did not significantly reduce R_s compared to the control (Fig. 2). Despite its consistence with some previous studies [35,36], other studies demonstrated decreased precipitation suppressed R_s due to reduced carbon allocation, substrate diffusion and microbial activities [26,63,92–94]. Given that the relatively high mean annual precipitation in our study (1176 mm per year), we speculated that the reduction of precipitation in the drought treatment of our experiment was insufficient to induce significant changes in substrate diffusion to microbes and thus little alteration in microbial activities. Microbes could also have physiologically adapted to the reduced precipitation and showed resistance to drought treatment [95]. Under the two reduced precipitation treatments, the insignificant responses of microbial biomass, microbial growth efficiency and hydrolytic carbon acquisition activities were evident. Another explanation lies in the size and timing of precipitation on R_s [36,96], as maneuvering three times per day in 2014 and two times per day in 2015 and 2016 throughout the year could maintain a moisture condition in favor for microbial activities.

The published work [64] and the current study were based on the same experiment, and both found out increased precipitation increased soil respiration or microbial respiration; However, significant drought effects on microbial respiration were found in the previous work [64] but not in the current study. This might be caused due to the different research focus and the resulting measurement approaches. In the published work [64], the investigators conducted microbial and root respiration measurements by inserting collars and removing roots from some collar area in the experimental pot, however, microbial respiration measured by removing roots [64] may not exclusively eliminate the influence of root respiration due to root growth in the artificial root-free zone. While in the current study, soil samples were collected by excluding roots and then respirations were measured in the laboratory, thus microbial respiration was distinctly quantified without the influence of roots; Besides different collection dates, the experimental duration is longer in the current study.

4.3. Precipitation impacts on soil MBC, R_{ss}, and EEAs are mediated by microbial physiology and enzyme kinetics

MBC remained unaffected by increased precipitation amount in this study likely because moisture is no longer a limiting factor for microbial growth in both control and treatment plots, for instance, in the site where the mean annual precipitation is > 600 mm [28]. The annual ambient precipitation is high up to 1176 mm per year in our site. Likewise, we observed no significant effect of drought treatments on MBC, suggesting that soil microbes grown under limited soil moisture conditions showed inherent and ubiquitous resistance to soil moisture stress [17,22,97] based on a global synthesis in grasslands [98].

By accounting for both R_s and microbial biomass, R_{ss} was significantly increased under the P+33 treatment (Fig. 2), which indicated a decrease in microbial growth efficiency (MGE). This suggested that decomposer communities allocate a higher fraction of assimilated carbon for maintenance than for growth under moist conditions. This was consistent with a reduced MGE with increasing precipitation in a semiarid temperate steppe in northern China, where the leaching loss of dissolved organic carbon may serve as a cause for the reduced MGE [54]. It appears feasible that the decreased nutrients availability, caused by enhanced nutrients uptake by plant growth, may also contribute to a decline in MGE [52].

Enhanced precipitation also tended to stimulate various EEAs, particularly C_{acq}, *BG*, *CBH*, and *AP* (Figs. 4 and 5), while reduced precipitation generally caused no changes in nearly all studied EEAs except for *PEO*, which was suppressed under P-33. The general pattern of increasing EEAs under enhanced precipitation is consistent with our expectation that wetter soils promote enzyme activities as compared to drier soils [9,99]. We likened the little impacts of reduced precipitation on most of the EEAs to that of microbial biomass given their close association [46,100,101]. The positive impacts of increased precipitation on C_{acq} were previously reported in different precipitation manipulation studies [46,101,102]. Significant increases in C_{acq} under P+33 indicated that there was greater microbial carbon demand [103]. This might be due to soil nutrient depletion, and the depletion of the simple form of nutrients under increased precipitation could have promoted the production of C_{acq} for the decomposition of complex substrates [104]. As such, higher C_{acq} can promote the microbial breakdown of SOM and thus stimulate soil carbon turnover and carbon losses through R_s [9,105]. C_{acq} enzyme activities in our study suggested that higher precipitation might have positive feedback on soil carbon loss, consistent with an earlier study in native tallgrass prairie [48]. Among the EEAs, *CBH* was the most stimulated microbial extracellular enzymes involved in carbon cycling under increased precipitation (Fig. 3), which hints to higher root turnover [106]. Because soil microbes produce *CBH* for the hydrolysis of cellulose, the increased root turnover can provide more cellulolytic organic matter for decomposition, and thus promoting *CBH* activities

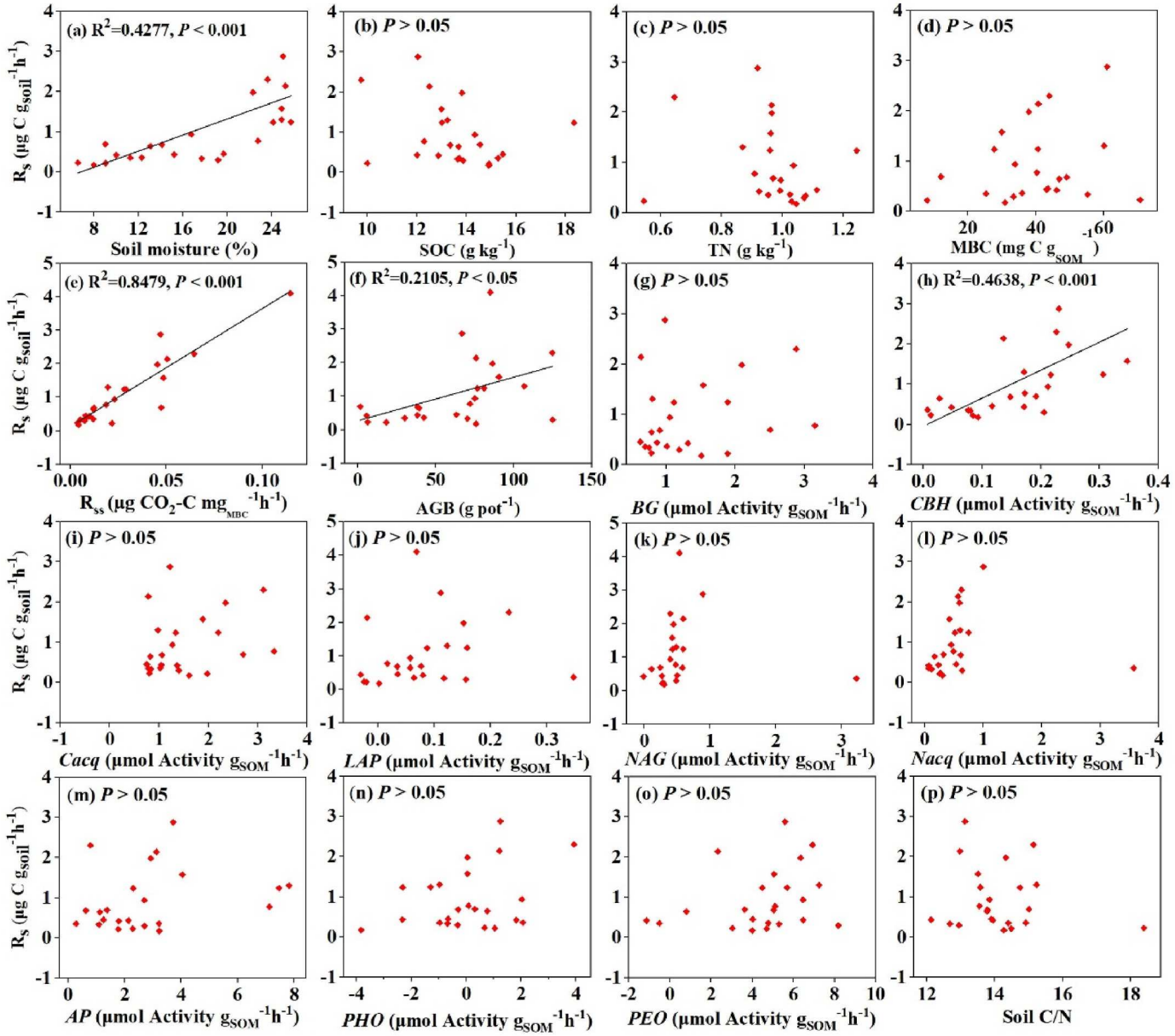

Fig. 5. Relationships between R_s and (a) soil moisture, (b) SOC, (c) TN, (d) MBC, (e) R_{ss}, (f) AGB, (g) *BG*, (h) *CBH*, (i) C_{acq}, (j) *LAP*, (k) *NAG*, (l) N_{acq}, (m) *AP*, (n) *PHO*, (o) *PEO*, and (p) soil C/N ratio in the three-year switchgrass (*Panicum virgatum* L.) mesocosm experiment. R_s: soil heterotrophic respiration; SOC: soil organic carbon; TN: total nitrogen; MBC: microbial biomass carbon; R_{ss}: microbial biomass-specific respiration; AGB: plant aboveground biomass; *BG*: *β*-1,4-glucosidase; *CBH*: *β*-D-cellobiosidase; C_{acq}: carbon acquisition enzyme; *LAP*: leucineamino peptidase; *NAG*: *β*-1,4-N-acetyl-glucosaminidase; N_{acq}: nitrogen acquisition enzyme; *AP*: acid phosphatase; *PEO*: peroxidase.

[107]. N_{acq} was relatively less sensitive to precipitation changes in the switchgrass grassland (Fig. 3), likely because the highly limited N supply in both control and precipitation treatments may induce large responses but mask the changes of these enzyme activities associated with N acquisitions.

In our experiment, soil was low in phosphate content [15], and soil microbes may have moderated this limitation through stimulating *AP* activities under increased precipitation [7]. Increased precipitation had no significant impact on *PEO* (Fig. 3) likely due to low soil pH (pH = 6.2), as *PEO* expression has higher soil pH requirements of 8 ± 1 [42]. At the global scale, *PEO* activity was significantly promoted under increased precipitation [28], As oxidase facilitates the decomposition of recalcitrant fraction of SOM, the unchanged *PEO* activities are potentially favorable for SOC stabilization [108]. The depressed *PEO* activities under reduced precipitation can thus preserve aromatic compounds and the lignin fraction of SOM from decomposition [42]. Also, many EEAs remained unaffected under reduced precipitation, indicating the complex and high order of interaction of multiple factors in controlling of enzymatic activities such as the insufficient reduction of precipitation triggering detectable soil microbial responses and the microbial community's physiological adaptation to decreased precipitation [48,106].

4.4. Implications for future studies

Based on the switchgrass mesocosm experiment, this study revealed little response of soil heterotrophic respiration to drought but larger positive response to increasing precipitation. This implied that as to the respiratory loss, switchgrass may be resilient to warming and drought conditions but subject to a large enhancement under extreme high precipitation in future scenarios. This study also enabled a better

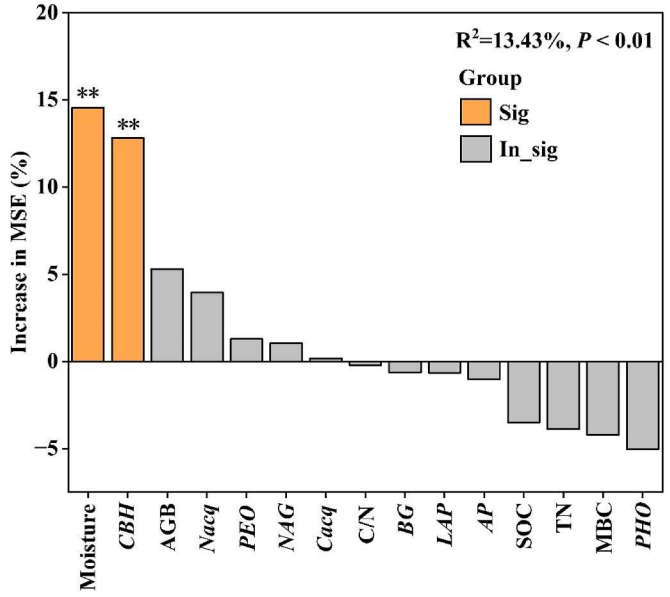

Fig. 6. Important predictors of R_s. The figure displays the random forest mean predictor importance (% of increase in mean square error (MSE)) of soil parameters drivers of R_s. Statistical significance levels expressed as $^*P < 0.05$ and $^{**}P < 0.01$. R_s: soil heterotrophic respiration; SOC: soil organic carbon; TN: total nitrogen; MBC: microbial biomass carbon; R_{ss}: microbial biomass-specific respiration; AGB: plant aboveground biomass; *BG*: β-1,4-glucosidase; *CBH*: β-D-cellobiosidase; C_{acq}: carbon acquisition enzyme; *LAP*: leucineamino peptidase; *NAG*: β-1,4-N-acetyl-glucosaminidase; N_{acq}: nitrogen acquisition enzyme; *AP*: acid phosphatase; *PEO*: peroxidase.

understanding of the underlying microbial mechanisms. Although microbial biomass tended to resist to change at the community level, the extracellular enzymatic activities respond suggesting a key control of microbial communities' physiology and likely community composition, rather than overall community abundance. Interestingly, the increasing precipitation little impacts aboveground biomass but significantly changed soil microbial features, whereas, decreasing precipitation reduced aboveground biomass but little changed soil microbial function and activities. It implied a clear decoupling of plant and microbial responses to changing precipitations and thus a different role in regulating soil heterotrophic respiratory loss. As a sophisticated and well-controlled system, the switchgrass mesocosm experiment enabled a better understanding of plant, soil, and microbial responses and processes thus contributing to future projection of soil respiratory losses under more extreme precipitation scenarios. Nevertheless, future studies are expected to acquire the knowledge of plant roots and their interaction with microbes so that a mechanistic understanding of switchgrass soil respiration in response to changing precipitation can be achieved. To maintain a sophisticated mesocosm experiment over long-term duration (e.g., a decade or longer) should be prioritized.

5. Conclusion

This study demonstrated that despite no significant changes in SOC or plant aboveground biomass, the increasing precipitation stimulated soil respiratory carbon losses consistent with elevated hydrolases' activities and decreased microbial growth efficiency. Despite significant reduction in plant aboveground biomass, drought conditions had no significant impacts on respiratory carbon losses likely due to insignificant changes in the substrate diffusion, enzyme kinetics, or microbial physiology, the latter suggesting microbial tolerance to drought conditions in the mesocosm environment. This study informs the key role of microbe on soil respiratory loss under changing precipitation, and this has important implications for understanding soil carbon responses in bioenergy croplands under future climate change. Given the more extreme precipitation distribution globally [109], our study can be upscaled to project even large respiratory carbon losses from soils in the future after accounting for the aggregated responses under drought and wetter conditions.

CRediT authorship contribution statement

Wei Dai: Data curation, Formal analysis, Software, Validation, Visualization, Writing – review & editing. **Madhav Parajuli:** Data curation, Formal analysis, Software, Validation. **Siyang Jian:** Data curation, Formal analysis, Resources, Software, Validation, Visualization, Writing – review & editing. **Dafeng Hui:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Philip Fay:** Conceptualization, Investigation, Writing – review & editing. **Jianwei Li:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – original draft, 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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