**Enhancement of Precipitation Amount Stimulated Soil Respiratory Loss and Extracellular Hydrolases in a Switchgrass Experiment**

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***Abstract.***  Precipitation regimes are important controls on soil respiration but the underlying microbial mechanisms that likely mediate the effects remain rarely studied, particularly in a bioenergy cropland such as switchgrass (*Panicum virgatum* L*.*). Based on a three-year switchgrass mesocosm experiment under five manipulated precipitation regimes representing simulated mean annual precipitation amount (P0), two drought conditions (P-33 and P-50: 33% and 50% reduction relative to P0), and two wet conditions (P+33 and P+50: 33% and 50% enhancement relative to P0), soil samples (0-15 cm) were collected in February and March 2017. Soil organic carbon (SOC), total soil nitrogen (TN), SOC/TN (C: N), microbial biomass carbon (MBC), soil CO2 respiration rate (Rs), and extracellular enzymes activities (EEAs) were quantified. The proxy variables for hydrolytic C acquisition enzymes (*C-acq*), N acquisition (*N-acq*), and oxidative decomposition (*OX*) were calculated as the sum of α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), β-D-cellobiosidase (CBH) and β-1,4-xylosidase (BX); β-1,4-N-acetyl-glucosaminidase (NAG) and leucineamino peptidase (LAP); phenol oxidase (PHO) and peroxidase (PEO), respectively. The specific soil respiration rate (Rss), derived as the Rs per unit microbial biomass, was used to index microbial growth efficiency. Soil samples in a nearby switchgrass field plot were also collected and analyzed as a reference. Results showed insignificant differences in SOC and TN in all treatments. Relative to P0, Rs significantly increased by 121~312% and MBC little changed, leading to significantly higher Rss under P+33 (P < 0.05). The C-*acq* significantly increased by 102% but OX little changed under P+33. Rs and MBC slightly and insignificantly decreased, resulting insignificant changes in Rss in the drought conditions (P-33 and P-50). In addition, OX significantly decreased by 60% under P-33. These results suggest that the manipulative enhancement in precipitation amount stimulated soil respiratory C loss likely associated with both elevated hydrolases and reduced microbial growth efficiency. Whereas, microbial community responses to drought conditions are less pronounced in either hydrolases or microbial physiology in the switchgrass mesocosm experiment.

Key Words: Switchgrass, mesocosm experiment, manipulative precipitation, microbial activities, Soil carbon.

**Introduction**

Global climate models predict more extreme climatic events worldwide, such as intense drought and heavy rainfall ([Groisman and Knight 2008](#_ENREF_27), [Karl et al. 2008](#_ENREF_41), [Griffin-Nolan et al. 2018](#_ENREF_26), [Trugman et al. 2018](#_ENREF_76)). As a result, the global dry or wet land area resulted from precipitation extremes has been increased largely ([Stocker 2014](#_ENREF_73), [Dai et al. 2017](#_ENREF_18)). The changes in precipitation pattern directly affect soil moisture regimes and ecosystem functions ([Heisler and Weltzin 2006](#_ENREF_32)). While, availability of soil moisture can also be exacerbated with the elevated soil temperature due to climate warming ([Xu et al. 2013](#_ENREF_81)) and through accelerated evapotranspiration due to increased plant growth ([Wan et al. 2002](#_ENREF_77), [Xia et al. 2010](#_ENREF_79)). Altered soil moisture regimes impacted soil carbon decomposition rate and nutrients mineralization primarily due to shifting microbial activities ([Kreyling et al. 2008](#_ENREF_42), [Frank et al. 2015](#_ENREF_23), [Nielsen and Ball 2015](#_ENREF_61), [Ren et al. 2017](#_ENREF_63), [Nguyen et al. 2018](#_ENREF_60)). However, studies elucidating microbial mediation of soil carbon responses under altered precipitation regimes are limited ([Moyano et al. 2013](#_ENREF_58), [Huang et al. 2015](#_ENREF_37), [Zhao et al. 2016](#_ENREF_88), [de Nijs et al. 2018](#_ENREF_20)).

Altered precipitation regimes likely to influence the soil carbon and nitrogen dynamics. Globally, soil contains 1500-2500 Pg carbon; approximately three and four times the amount in atmosphere and in plant biomass, respectively ([Batjes 1996](#_ENREF_5), [Janzen 2004](#_ENREF_38), [Lal 2004](#_ENREF_44), [Batjes 2014](#_ENREF_6), [Ciais et al. 2014](#_ENREF_15)), and 133-140 Pg soil nitrogen in upper 100 cm ([Batjes 1996](#_ENREF_5), [2014](#_ENREF_6)). Soil moisture regulates the soil carbon cycle ([Austin and Sala 2002](#_ENREF_2)) through controlling soil accumulations of plant biomass and microbial decomposition rate ([Austin and Sala 2002](#_ENREF_2), [Moyano et al. 2012](#_ENREF_59), [Camenzind et al. 2018](#_ENREF_12), [Moinet et al. 2018](#_ENREF_57)). Likewise, soil nitrogen cycle is influenced by soil moisture through regulating soil nitrogen losses, and microbial mineralization and transformation ([Holtgrieve et al. 2006](#_ENREF_33), [Meyer et al. 2006](#_ENREF_56), [Borken and Matzner 2009](#_ENREF_10)). Elevated precipitation increased nitrogen mineralization from the organic matter ([Chen et al. 2017](#_ENREF_14)). At the same time, increased precipitation also enhanced the soil nitrogen leaching losses and denitrification rates ([Yahdjian and Sala 2010](#_ENREF_82), [Tiemann and Billings 2012](#_ENREF_75)). Thus, soil nitrogen pool is determined by the balance between soil nitrogen mineralization and losses ([Jongen et al. 2013](#_ENREF_40)). The drought intensity and length of drying and rewetting determines the magnitude of soil carbon efflux ([Borken and Matzner 2009](#_ENREF_10)). Soil respiration is an important determinant to predict the rate of carbon loss from the soil ([Hanson et al. 2000](#_ENREF_30), [Li et al. 2017](#_ENREF_47), [Yue et al. 2018](#_ENREF_85)). As a results, soils with fluctuating moisture regimes have more CO2 efflux into atmosphere ([Birch 1958](#_ENREF_8)) than those soils with constant moisture ([Borken and Matzner 2009](#_ENREF_10)). The inhibition of CO2 efflux was observed in drought ([Borken et al. 2006](#_ENREF_11), [Meier and Leuschner 2010](#_ENREF_55), [Darenova et al. 2017](#_ENREF_19)) and in anaerobic soil conditions ([Gaumont-Guay et al. 2006](#_ENREF_24)). This is due to the limited activities of organic matter decomposers ([Moyano et al. 2013](#_ENREF_58)) in dry soil and reduced production and transport of CO2 in anaerobic soils ([Yu et al. 2017b](#_ENREF_84)). Also, decreasing precipitation in permanently anaerobic soil enhanced soil respiratory loss of CO2 ([Couwenberg et al. 2010](#_ENREF_16), [Han et al. 2018](#_ENREF_29)) and reduced soil moisture in mesic or dry soil suppressed the respiratory loss ([Borken et al. 2006](#_ENREF_11)). A number of evidences supported that soil carbon loss enhanced with increasing precipitation ([Jongen et al. 2013](#_ENREF_40)).

Precipitation regimes are important controls on microbial biomass and enzymes kinetics but their responses to altered precipitation regimes remained poorly understood. For example, the microbial biomass can be increased, decreased or no change with the changes in soil moisture regimes ([Lai et al. 2013](#_ENREF_43), [Bell et al. 2014](#_ENREF_7), [Nielsen and Ball 2015](#_ENREF_61), [Homyak et al. 2018](#_ENREF_34), [Zhang et al. 2018](#_ENREF_87)). Enhancement in precipitation amount in dry ecosystems significantly increased microbial biomass ([Huang et al. 2015](#_ENREF_37), [Huang et al. 2018](#_ENREF_36)), but in mesic ecosystems, enhancement in precipitation inhibits the microbial growth due to reduced oxygen levels ([Manzoni et al. 2014](#_ENREF_51), [Sierra et al. 2017](#_ENREF_69)). Microbial biomass was more sensitive to increased precipitations in dry soil than in mesic soils but more sensitive to decreased precipitation in mesic soils than in dry soils ([Zhou et al. 2018](#_ENREF_91)). In contrast, microbial biomass can also be increased in dry soil ([Boot et al. 2013](#_ENREF_9), [Schaeffer et al. 2017](#_ENREF_67), [Homyak et al. 2018](#_ENREF_34)) through their ability to tolerate drought stress ([Barnard et al. 2013](#_ENREF_3)), since they can grow in slow rate even in stress condition ([Manzoni and Katul 2014](#_ENREF_50)). However, microbial biomass also depends on resources available, season or ecosystem. For instant, soil microbial biomass reduced in forestland, but no responses in shrubs and grassland was observed in reduced precipitation regimes ([Ren et al. 2017](#_ENREF_63), [Ren et al. 2018](#_ENREF_62)). Soil microorganisms synthesizes extracellular enzymes, which are key for the metabolism of carbon, nitrogen and phosphorus in soil organic matter([Wang et al. 2015](#_ENREF_78)). However, the effects of precipitation regimes on EEAs are rarely understood ([Ren et al. 2017](#_ENREF_63)). Responses were inconsistent, including EEA activities decreased in drought ([Sardans and Peñuelas 2005](#_ENREF_66)), decreased in water addition ([Gutknecht et al. 2010](#_ENREF_28)), or no effect of increased precipitation ([McDaniel et al. 2013](#_ENREF_54), [Ren et al. 2017](#_ENREF_63)). Drought suppressed oxidase activities but no effect of precipitation change on the rest of the enzymes activities ([Xiao et al. 2018](#_ENREF_80)), whereas increased precipitation stimulate N-acquisition activities ([Zhou et al. 2013](#_ENREF_90), [Xiao et al. 2018](#_ENREF_80)).

The growth of microorganisms and their activities largely depend on the available soil moisture, since it is the medium to acquire resources. Moisture content in the soil regulates the rate of substrate and gas diffusion, and microbial dispersal to the available resources ([Manzoni et al. 2012](#_ENREF_52), [Manzoni et al. 2014](#_ENREF_51), [Tecon and Or 2017](#_ENREF_74)). In drought conditions, water filled pores in the soil disconnected leading to reduced substrate diffusion, thereby limiting microbial activities and growth ([Stark and Firestone 1995](#_ENREF_72), [Manzoni et al. 2014](#_ENREF_51)). In other hand, the supply of above-ground plant biomass to the soil, which is the source of nutrients for microbes, can also be reduced in the limited soil moisture regimes ([Hoover and Rogers 2016](#_ENREF_35), [Jin et al. 2018](#_ENREF_39)). In the limited soil moisture condition, microbes may invest to maintain osmotic equilibrium with the surroundings through accumulating solutes such as amino compounds in their cell ([Harris 1981](#_ENREF_31), [Schimel et al. 2007](#_ENREF_68)). Different degree of microbial responses to altered precipitation regimes were observed at community levels ([Zhao et al. 2017](#_ENREF_89)). For example, fungi are benefited with the hypha, a specialized structure in their body, and resistant cell walls ([Singh et al. 2010](#_ENREF_70)) and Gram positive bacteria with strong cell wall and improved osmoregulation strategy to cope with drought conditions ([Landesman and Dighton 2010](#_ENREF_45)). As a result, fungi can exploit resources from extremely dry soil even when solute diffusion is very low ([Lennon et al. 2012](#_ENREF_46), [Manzoni et al. 2012](#_ENREF_52), [Zeglin et al. 2013](#_ENREF_86)). Fungi are considered more resistant to soil drying and bacteria are more resilient to rewetting ([Canarini et al. 2017](#_ENREF_13)).

The possible future path of precipitation pattern is highly unpredictable, so the effects on soil ecosystems. However, earlier studies on the roles of microbes in soil carbon dynamics under climate change scenario were primarily focused on experimental warming ([DeAngelis et al. 2015](#_ENREF_21), [Romero-Olivares et al. 2017](#_ENREF_64)). Precipitation manipulation studies were mostly concentrated on the aboveground parameters ([Nielsen and Ball 2015](#_ENREF_61), [Grant et al. 2017](#_ENREF_25), [Basto et al. 2018](#_ENREF_4), [Cowles et al. 2018](#_ENREF_17)), but our understanding on the consequences of altered precipitation on soil ecosystems is incomplete ([Huang et al. 2015](#_ENREF_37), [Canarini et al. 2017](#_ENREF_13), [Ren et al. 2017](#_ENREF_63), [Nguyen et al. 2018](#_ENREF_60)). In other hand, below-ground studies were mostly carried out in natural grasslands either in increased ([Lai et al. 2013](#_ENREF_43), [Bell et al. 2014](#_ENREF_7), [Huang et al. 2015](#_ENREF_37)), or decreased precipitation regimes ([Ren et al. 2018](#_ENREF_62)), and the results are highly contrasting in directions and magnitudes. This may be due to the confounding effects in natural grasslands, including temperature, plant diversity and nutrients contents in the soil. Therefore, we aim to carryout mesocosm experiment to observe the soil microbial respiration loss and their determinants under manipulated precipitation using bioenergy switchgrass crop in temperate climatic regions with the three hypotheses. First, we hypothesized that microbial respiratory C loss from soil ecosystems increases with the enhancement of precipitation amount due to activated microbial activities and enzyme kinetics. Second, in contrast, we hypothesized that microbial respiration C loss is less sensitive to reductions in precipitation amount due to microbial resistance in switchgrass experiment. Third, enhancement of precipitation amount activates the soil enzymatic decompositions and less sensitive to precipitation reductions.

**Materials and Methods**

**Site description, soil sample collection and analysis**

The switchgrass mesocosm experiment was carried out at the Tennessee State University Agricultural Research and Education Center, Nashville, Tennessee, USA. The mean annual temperature (MAT) is 15.1 °C and mean annual precipitation is 1200 mm ([Deng et al. 2015](#_ENREF_22)). The greenhouse consisted of roof and wall to control inside air temperature and humidity. Roof and wall panels opened automatically when the air temperature in the greenhouse is above 20C. Roof closed automatically during rains. Plastic pots of 95 L capacity (50 cm diameter and 50 cm height) were filled with soil collected from the nearby switchgrass field experiment in July 2013. The Talbott silt clay loam soil (Fine, mixed, semi-active, thermic Typic Hapludalfs; 25% sand, 55% silt, and 20% clay) is slightly acidic (pH=5.97) and low in carbon (2.37 g kg-1) and nitrogen (0.14 g kg-1) ([Deng et al. 2015](#_ENREF_22)). 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. The aboveground plant biomass was harvested three times a year at the end of April, July, and October.

The mesocosm experiment was set up in a randomized complete block design (RCBD) with five precipitation treatments and five replications in each treatment. The total number of pots were 25 (5 level × 5 replicate). Five precipitation amounts were implemented in the experiment. The ambient precipitation condition (P0) represents the precipitation amount averaged over long-term records of annual precipitation from 1969 at Nashville, TN ([Yu et al. 2017a](#_ENREF_83)). Two wet conditions (P+33 and P+50) were implemented with an enhanced precipitation amount of 33% and 50% relative to P0, respectively. Two drought conditions (P-33 and P-50) were implemented with a reduced precipitation amount of 33% and 50% relative to P0, respectively. Soil samples collected from a nearby switchgrass field plot exposed to natural precipitation also taken as a basis. 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.

Soil samples (0-15 cm) were collected in February and March 2017 in all 25 pots. The 25 samples were collected from the experiment and three samples collected from the nearby switchgrass field plot as a basis. All 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 subsamples at 1050C for 24 hours. Oven-dried soil subsamples were used to analyze C and N contents using total organic carbon and nitrogen analyzer (Shimadzu Corp., Kyoto, Japan).

**Heterotrophic soil respiration and microbial biomass carbon**

Heterotrophic soil respiration rates were measured in soil samples collected in both February and March 2017. 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 CO2 concentration in the jars. The amount of CO2 emitted over time was used to calculate the respiration rate based on dry soil weight ([Li et al. 2018](#_ENREF_49)).

Field moist soil subsample (5.0 g) was weighted in 50 ml centrifuge tube and fumigated with 3 ml ethanol-free chloroform for 24 hours in the fume hood. Another equivalent weight subsample was weighted and kept unfumigated. Both fumigated and unfumigated tube were labeled as ‘fumigated’ and ‘unfumigated’. All the fumigated and unfumigated subsamples were added with 25 ml of 0.5 M K2So4 and shaken in a mechanical shaker for 30 minutes. 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 ºC for 18 hours. 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 microbial biomass carbon was determined by subtracting extractable organic carbon in the unfumigated samples from that in the fumigated samples. Microbial biomass-specific respiration rate was determined by the ratio derived from soil heterotrophic respiration rate over microbial biomass carbon.

**Extracellular Enzyme Activity (EEA) Assay**

Soil samples for each treatments were assessed to determine the potential extracellular enzymes activities using protocols ([Sinsabaugh et al. 2000](#_ENREF_71), [Allison et al. 2008](#_ENREF_1), [Li et al. 2012](#_ENREF_48)) in the units of µmol activity gsom-1 h-1. Fluorometric techniques were used to assess α-1,4-gulcosidase (AG), β-1,4-glucosidase (BG), β-D-cellobiosidase (CBH), β-1,4-xylosidase (BX), leucineamino peptidase (LAP), β-1,4-N-acetyl-glucosaminidase (NAG) and acid phosphatase (AP) ([Marx et al. 2001](#_ENREF_53)) and colorimetric method was used to assess phenol oxidase (PHO) and peroxidase (PEO) activities ([Saiya-Cork et al. 2002](#_ENREF_65)). In this study, the proxy variables for hydrolytic C acquisition enzymes (*C-acq*), N acquisition (*N-acq*), and oxidative decomposition (*OX*) were calculated as the sum of α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), β-D-cellobiosidase (CBH) and β-1,4-xylosidase (BX); β-1,4-N-acetyl-glucosaminidase (NAG) and leucineamino peptidase (LAP); phenol oxidase (PHO) and peroxidase (PEO), respectively.

One gram of fresh soil sample and 125 ml of 50 mM Na acetate buffer (pH 5.0) were placed in a 500 ml container and homogenized at a highest speed for 1 minute to make a slurry using hand blender. For the PHO and PEO assay, L-3,4-dihydroxyphenylalanine (DOPA) substrate and 96-well clear plates were used. Sixteen wells were used for each soil samples (assays), eight additional wells were used for control and another eight for negative control. Essay wells consisted of 200 µL soil slurry and 50 µL of DOPA substrate. The control wells contained 200 µL soil slurry and 50 µL of sodium acetate buffer. Negative control wells comprised of 200 µL sodium acetate buffer and 50 µL DOPA substrate. For PER plates, additional 10 µL 0.3% H2O2 solution was added to every well. Molecular Devices (spectrophotometrically) were used to quantify PEO and PHO at 460 nm. Black microtiter plate containing 96-wells were used for hydrolase assays. In addition to above assays design, eight replicate wells were used for quench coefficient and additional eight wells were used for standard references. Quench coefficient wells received 200 µL soil slurry and 50 µL of standard (MUB). Standard reference wells contained 200 µL of buffer and 50 µL MUB. All plates received 10 µL (0.5 M) NaOH to increase the MUB to make it detectable. Florescence was quantified using a microliter plate fluorometer with emission and excitation wavelength set to 460 nm and 365 nm respectively. Spectrophotometer; molecular device, was used to measured spectrophotometric activity.

**Statistical Analysis**

Two-way ANOVA was used to examine the main and interactive effects of date and precipitation on soil respiration rate using PROC GLM in SAS software 9.4 (SAS Inc., Cary, NC, USA). One-way ANOVA was used to examine the effect of precipitation regime on SOC, TN, C/N ratio, Rs, MBC, and EEAs. When significant differences were observed, *post hoc* test (Tukey) was performed for the multiple comparisons. The significance level was set at P<0.05.

**Results**

**Soil moisture among precipitation gradient**

Soil moisture content (SM; %) slightly differed with month (Fig 1). SM significantly enhanced with the increased in precipitation levels and declined with the decreased in precipitation levels.



Fig. 1 Mean soil moisture content (%) in each precipitation levels measured on February and March 2017.

Higher SM was observed in wet treatments (P+50 and P+33) as compared to drought treatments (P-50 and P-33).

**Soil organic carbon, total nitrogen and C:N** **ratio**

ANOVA results showed insignificant differences in SOC and TN in all precipitation treatments (Fig 2a,b; P<0.05). The Drought (P-50) condition led to significantly higher C:N (Fig 2c; P<0.05), but P-33 did not show effects. Field condition significantly reduced C:N. The C:N was slightly and insignificantly changes in wet treatments over the control.



Fig. 2 Mean (±SE) soil organic carbon (a), total nitrogen (b) and C:N (c) measured for each precipitation levels in March 2017. Different letters indicate significant difference.

**Soil respiration**

The precipitation effects on Rs was significant (Fig 3a,b; P<0.05). Rs increased with the enhancement in precipitation levels, peaked at P+33 and declined with the further increased in precipitation amount to P+50. Relative to P0, Rs significantly increased by 121% and 312% in February and March respectively under P+33. Whereas, P+50 insignificantly increased Rs by 52% and 209% in February and March respectively, but the effect was significant with P-50 in February. Drought treatments had marginal effects. P-50 and P-33 reduced Rs by 41% and 33%; and 39% and 18% in February and March respectively. The differences in Rs were insignificant between wet and between drought treatments. Slightly higher Rs was observed in March over February but the effects were not significant.



Fig. 3 Mean (±SE) soil respiration rate measured for each precipitation levels in February and March 2017. Different letters indicate significant difference.

**Soil microbial biomass carbon and specific soil respiration**

MBC was marginally affected (Fig 4a; P<0.05), but Rss significantly affected by the precipitation treatments (Fig 4b; P<0.05). Rss significantly increased from 0.012 µg CO2-C mgmbc-1 h-1 under P0 to 0.065 µg CO2-C mgmbc-1 h-1 under P+33, but insignificantly increased to 0.036 µg CO2-C mgmbc-1 h-1 under P+50. No significant effects of drought on Rss were detected.



Fig. 4 Mean (±SE) microbial biomass carbon (a) and biomass specific soil respiration (b) measured for each precipitation levels. Different letters indicate significant difference.

**Soil extracellular enzyme activities**

Precipitation treatments showed significant effects on C-acq, AP and OX but had little effect on N-acq (Fig 5a,b,c,d; P<0.05). C-acq enhanced significantly by 192% under field condition and by 102% under P+33. The effects of P+50 and drought conditions over the control was insignificant. Among C\_acq enzymes, CBH differed significantly, but AG, BG and BX changed little with precipitation regimes (Fig 6; P<0.05). CBH increased significantly under field condition. Also, P+33 measured significantly higher over drought treatments but drought treatments has little effects over control. Precipitation treatments has insignificant effects on NAG and LAP (Fig 7; P<0.05). AP increased gradually with the increased in precipitation amounts, which was observed significantly higher by 189% under P+50. The drought treatments had little effects on AP. OX demonstrated the significant reductions under field condition and P-33 by 61% and 60% respectively but reduced insignificantly under P-50 by 49%. OX was slightly influenced by the wet treatments. OX increased by 12% under P+33 but reduced by 17% under P+50. PEO decreased significantly under P-33 and field condition but increased precipitation amount has little effects (Fig 7; P<0.05). The differences on the effects in C-acq, AP and OX was insignificant between wet and between drought treatments.



Fig. 5 Mean (±SE) C-acq (a), N-acq (b), AP (c) and OX (d) enzyme activities measured for each precipitation levels. Different letters indicate significant difference.



Fig. 6 Mean (±SE) CBH (a), AG (b), BG (c) and BX (d) activities measured for each precipitation levels. Different letters indicate significant difference.



Fig. 7 Mean (±SE) LAP (a) and NAG (b) activities measured for each precipitation levels. Different letters indicate significant difference.

**Discussions**

**Conclusion**

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