Biotic and abiotic controls in determining exceedingly variable responses of ecosystem functions to extreme seasonal precipitation in a mesophytic alpine grassland

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Abstract

As a result of global climate change, frequent and intensive extreme climate events are observed and predicted. These extreme climate events can change ecosystem functions through driving plant growth and mortality, as well as ecological and evolutionary processes into different directions. We simulated a 1-in-100-year extreme precipitation event in different plant growth seasons (i.e. spring, summer, or autumn) to investigate the functional responses of a mesophytic alpine grassland ecosystem to climate change in the Tibetan plateau. The results demonstrated that the seasonal distribution of extreme precipitation is a critical factor in determining soil microbial- and plant-productivity. The response of vegetation net primary productivity (NPP) to extreme precipitation depends on the growth seasons and plant types. Total NPP in the treatments experienced extreme precipitation in the early (spring), mid- (summer) and late- (autumn) plant growth seasons were significant lower, higher, and no difference, respectively, compared with the control. Soil temperature and moisture were the key abiotic factors that affected ecosystem functions. For example, in the early plant growth season, no changes of soil moisture and the decreased temperature in response to the extreme precipitation resulted in a substantial decline in NPP. By contrast, in the mid-plant growth season, higher temperature and soil moisture elicited positive synergistic effects on plant growth and soil microbial processes. The increased sensitivity of above-ground NPP and the shift of dominant species from sedges to less palatable forbs might inevitably exaggerate the degradation of this grassland. Nevertheless, a high resilience index in the related ecological processes could potentially contribute to the grassland acclimation and stability, as most parameters returned to the similar levels as in the control after the extreme events ceased. Therefore, several synergistic or antagonistic mechanisms are hypothesized to operate in parallel or at different levels of organization and timescales. Further studies involving a range of different potential scenarios and longer periods are needed to predict the future climate change impacts on mesophytic grassland.

Key words: Extreme precipitation; Seasonality; Biomass accumulation and allocation; Resistance and resilience; Nutrient cycling; Mesophytic alpine grassland.

1. Introduction

As the importance of climate extremes in driving contemporary and future ecological dynamics has been recognized, there is an increasing need to understand their evolving directions and the functional changes of ecosystems in response to these extremes locally and globally (Jentsch et al., 2007; Gerten et al., 2008; Smith, 2011). For example, the intensity of precipitation events is projected to increase over most regions of the world, regardless of increase or decrease in total annual precipitation (Knapp et al., 2008; IPCC, 2012). The changes in precipitation regimes, such as increased intensification of the variability and stronger extremity, will be more spatially heterogeneous and less predictable than other climate changes, and will lead to altered precipitation inputs as more intense flooding or long-term droughts at local and regional scales (Beier et al., 2012; Brotherton and Joyce, 2015). Despite the prominent examples of the severe impacts of climate extremes, global climate change research has been dominated by the study on chronic environmental changes rather than on discrete climate extremes. Considering the sensitivity variance of ecosystem components, research oriented towards events (extreme) rather than trends (gradual) is urgently needed.

Ecological responses of system functions to climate extremes are exceedingly complicated, varying from minimal impacts to rapid mortality of populations or species, resulting in large-scale changes in ecosystem structures and functions, and even shifts in ecotone boundaries (Zeppel et al., 2014). Such variability in ecological resistance (capacity to withstand change) and resilience (capacity of functional recovery) are likely due to the differences in severities of climate extremes, sensitivities of various soil processes, vegetation types, and inherent regulatory mechanisms of plants and ecosystems (Zhang et al., 2013; Knapp et al., 2015). For example, k-strategists as the robust long-lived species may remain stable to climate extremes because of their long life span, or strong resistance abilities to the invading alien species and the changing environment (Beier et al., 2012; Reyser et al., 2012). Conversely, communities dominated by r-selected species may show better resilience after changes in the availability of water, i.e., a faster return to equilibrium (Combes, 2008; De Boeck et al., 2011). Furthermore, the changes of ecosystem functions also depend on whether extreme precipitation event leads to an increase or decrease in soil water content, contingent on the current precipitation and aridity index of the regional location (Knapp et al., 2008; Heisler-White et al., 2009). Studies to date have reported that extremely high precipitation can decrease the above-ground net primary productivity (ANPP) at mesic sites, but it increases ANPP at xeric sites (Krab et al., 2014; Zeppel et al., 2014). These results imply that plant-available water is a key factor in driving ecosystem responses to extreme precipitation. Specifically, Fay et al. (2008) reported a threshold of maximum soil water content at 600 mm mean annual precipitation in Kansas, U.S. Ultimately, information on climate extremes and the corresponding vulnerability of ecosystems is crucial to identify which species and regions, and thus which ecosystem services and functions, are most suffered by climate change (Gerten et al., 2008).

Since extreme climate can occur throughout the year and trigger the variability of plant responses, seasonality is

usually treated as a factor by considering precipitation simulation in spring, summer, and autumn. Moreover, seasonal changes in precipitation during warm or dry seasons may have more pronounced effects on ecosystems than changes during cool or wet seasons (Seneviratne et al., 2010; Zeppel et al., 2014). For example, in a limestone grassland in England, an increase of 20% in the summer precipitation significantly enhanced the plant growth, whereas increased precipitation in the autumn and winter had no effect (Morecroft et al., 2004). Similar results were observed in Oregon, U.S. (Bates et al., 2006). Accordingly, the response of ecosystem functions to the seasonal distribution precipitation involves a complex interplay among resultant influences on plant-available water, temperature fluctuation, and plant growth seasons (Fry et al., 2014; Hoover et al., 2014; Orsenigo et al., 2014). Therefore, studies across a range of seasons and plant functional types are critical for predicting vegetation responses to future climates.

The grasslands in the Tibetan plateau have been described as a “bellwether of change”, due to their prompt response to climate variability (Wookey et al., 2009). The unique geography of this region not only yields relatively high vegetation growth rate with concentrated rainfall during the growing season, but also leads to a very low organic matter decomposition rate and nutrient release during the dry and cold winters (Zhou et al., 2013; Kato et al., 2006). As a result, strong seasonality has been characterized by steep fluctuations in microbial and plant community structure, stability and productivity (Lipson et al., 1999; Wang et al., 2015). Understanding the coupled changes in plant and microbial communities can offer valuable information regarding the resistance and resilience of these climate-sensitive ecosystems, which would facilitate the development of appropriate mitigation and adaptation strategies for the conservation of biodiversity, sustainable resource management and the protection of public safety (Zhou et al., 2013). In this study, we exposed a grassland in the Tibetan plateau to a simulated 1-in-100-year extreme precipitation scenario in different seasons to evaluate the abiotic factors (i.e. soil water content and soil temperature) that influence fundamental soil processes (i.e., soil microbial biomass, enzyme activities, and extractable nutrients) and net primary productivity (NPP) during different plant growth stages. Specifically, the following hypotheses were tested:

- 1) The response (positive, negative, or neutral) of NPP (above- and below-ground biomass) to extreme precipitation depends on the plant growth stage (spring, summer, or autumn) in this mesophytic ecosystem.
- 2) Extreme precipitation shifts the species’ composition and distribution, and eventually alters pasture quality and ecosystem functions due to the contrasting responses of different plant functional types to extreme precipitation.
- 3) As a result of a hierarchical response framework, a synergistic or antagonistic effect of soil moisture and temperature operates in parallel or at different levels of organization and timescales.
- 4) The relatively high recovery capability of the below-ground processes elicits a process of acclimation and adaptation, and eventually determines the resistance and resilience of ecosystem functions.

2. Materials and methods

2.1. Site description

The experimental site (32° 26' N, 102° 22' E) was located on a 1000 m² fenced area in an alpine meadow (elevation: 3570 m) in the eastern Tibetan plateau, Sichuan Province, China. The land management in this area is yak grazing (Wang et al., 2015). The annual mean precipitation is approximately 786 mm, and the annual mean temperature from 1962 to 2004 was 1.6 °C. The plant growth season begins with the bud break at the end of April or beginning of May and ends with the senescence of leaf in late September. Additionally, the aboveground biomass reaches its peak in August. The regional climate is characterized as typical plateau continental climate with high solar radiation, short-cool summer (mean July max/min temperature is 15/7.6 °C) and long-cold winter (mean January max/min temperature is -3.0/-17 °C). The short plant growth season is controlled by the southeastern monsoon, and the cold winter is dominated by the cold air from Siberia. In general, 77% of annual precipitation (607 mm) occurred during the plant growth season. The soil (0-10 cm depth) on the site is classified as alpine meadow soil with a pH of 5.3 ± 0.4 (soil/water ratio 1:2.5). The vegetation is dominated by *Kobresia humilis* (C. A. Mey) Serg., *Kobresia macrantha* Bocklr., *Festuca rubra*, *Agrostis hugoniana* Rendle, *Deschampsia caespitosa* (Linn.) Beauv., *Leontopodium nanum*, *Thalictrum alpinum*, *Potentilla discolor* Bunge., *Polygonum viviparum*, and *Oxytropis deflexa* (Pall) DC.

2.2. Experimental design

In the 2010 to 2011 growing seasons, four experimental treatments were set up as a randomized design with four replicates (total 16 plots). Each plot measured 1.5 m × 1.5 m and spaced 3 to 5 m apart from each other. Four experimental treatments were as follows (1) control, no simulated extreme precipitations were manipulated; (2) summer simulation, the extreme precipitations were performed in the mid- plant growth season in 2010 summer from August 1 to 13; (3) autumn simulation, the extreme precipitations were performed in the late-plant growth season in 2010 autumn from September 5 to 17; (4) spring simulation, the extreme precipitations were performed in the early-plant growth season in 2011 spring from May 13 to 25. The amount of simulated precipitation applied in each season were the same and was calculated based on the local historical rainfall data collected from 1962 to 2004 and intensified over 100-year extreme scale by following a Gumbel distribution (Gumbel, 1958). Here, extreme precipitation was defined as consecutive days with a daily precipitation of over 10 mm. Therefore, in each seasonal precipitation extreme event, water was applied at 16 mm per day for 13 consecutive days (a total of approximately 25% of the annual mean precipitation), according to the historical records of 167 mm of precipitation over 13 days in 1983.

Precipitation water was collected from a nearby river and filtered with 80- mesh screen before entering the manual sprayer. The filtered water was applied evenly on the plot surface at a rate of 36 L h⁻¹, an approximately natural intensity of

heavy rainfall. The precipitation background was measured in the control treatment using a tipping-bucket rain gage of the Onset HOBO weather station. Prior to treatment, all the plots were separated by inserting plastic films into 30 cm deep to prevent water penetration along the water potential gradients among the different treatments.

2.3. Soil moisture, temperature, soil sampling and analysis

Soil moisture and temperature were continuously monitored (every 30 min) by time- domain reflectometry device and Onset HOBO weather station, respectively, at a 10 cm depth in the soil of all the treatments. For soil analysis, composite soil samples were collected every 10 days after the extreme precipitation started in August 2010, September 2010, or May 2011 by homogenizing three auger borings from 0 to 10 cm soil depth. Over the course of the study, soil samples were taken on 5 sampling dates per treatment. The composite soil samples were sieved immediately to 2 mm and refrigerated (4°C) until analysis (<7 days).

Samples of 5 g composite soil were extracted with 20 mL of 0.5 M K₂SO₄ and the soil extracts were filtered using Pall A/E glass fiber filters and frozen until analysis. The ammonium (NH₄⁺), nitrate (NO₃⁻), and phosphate (PO₄³⁻) concentrations in the supernatant of the extracts were determined using a modified Berthelot reaction method, Griess reaction and the malachite green-based colorimetric micro-plate analysis, respectively, as described in our early study (Wang et al., 2015).

2.4. Soil enzymatic activity

Six enzymes involved in C, nitrogen (N), and phosphorus (P) cycles, were analyzed and used to evaluate the microbial functions influenced by extreme precipitation. The enzyme activities investigated include acid phosphatase (AP), which hydrolyzes organic bound phosphates, cellobiohydrolase (CBH) and β -glucosidase (BG), which participate the decomposition of organic carbon, Polyphenol oxidase (PPO) and peroxidase (POD), which are involved in the degradation of lignin, and N-releasing enzyme N-acetyl-glucosaminidase (NAG). These extracellular soil enzymatic activities were measured using the method reported by Sinsabaugh et al. (1993). Briefly, samples of approximately 1 g fresh composite soil were extracted with 60 mL of 50 mM sodium acetate buffer at pH 5.0. The soil extraction was homogenized using Polytron homogenizer for 1 minute and centrifuged at 8,000g at 4 °C for 20 minutes. Meanwhile, enzyme substrate solution was prepared by dissolving five substrates in 5 mL of 50 mM acetate buffer (pH 5.0). After that, 0.75 mL of the supernatant was mixed with 0.75 mL of enzyme substrate solution in a 2 mL centrifuge tube. Three analytical replicates were developed for each sample. The tubes with the mixture of supernatant and enzyme substrate were placed in racks on a shaker and shaken for 1 to 6 h at 25 °C. The blanks were prepared in the same manner as the samples except acetate buffer was added instead of enzyme substrate solution. For POD assay, 75 μ L 0.3% H₂O₂ and 75 μ L 1.0 N NaOH were used, and for AP, CBH, BG,

and NAG assay, 75 μ L 1.0 N NaOH was used, to terminate the reaction and develop the color. The absorbance of the reaction products was measured by the Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, Inc.). Enzymatic activities were expressed in μ mol of the substrate hydrolyzed per h per g of dry soil.

2.5. Soil microbial biomass

Soil microbial biomass C, N, and P content were determined by using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, samples of 10 g fresh soil were fumigated with chloroform for 24 h in vacuum desiccators, and another 10 g soil samples were not fumigated to serve as control. Carbon and N in the fumigated or non-fumigated soils were extracted with 50 mL of 0.5 M K_2SO_4 for 30 minutes. The soil extracts were filtered using Pall A/E glass fiber filters and frozen at $-20^\circ C$. The total dissolved C and N in the filtered soil extracts were determined using a total organic C and N analyzer (Multi NC 2100S, Analytik Jena, Jena, Germany). Phosphorus in the fumigated or non-fumigated soils was extracted with 50 ml of 0.025 N HCl and 0.03 N NH_4F for 30 minutes. The soil extracts were then processed using the same procedure as mentioned above and the total P in the extracts were measured according to Bray and Kurtz (1945). Microbial biomass C, N, and P were calculated by subtraction of the extractable C, N or P of the unfumigated soils from that of the fumigated soils and then divided by an efficiency factor of 0.45 (Vance et al., 1987).

2.6. Plant biomass

Plants in the autumn and summer simulation treatments were harvested by hand at the end of experiment on September 25, 2010 and those for the spring simulation treatment on September 25, 2011. Firstly, the whole plants were collected by dig a 30 $\times$ 30 $\times$ 30 cm hole and all the plants were sorted into sedges, grasses, and forbs. Then, shoots were separated from roots, and all the samples were oven dried at 70 $^\circ C$ and weighted. The NPP of each species was calculated as the sum of aboveground and belowground biomass.

2.7. Statistical analysis

Statistical analysis was performed with SAS (version 9.1, SAS Institute, Cary, NC, USA). The responses of microbial biomass, enzyme activities and soil nutrients (NH_4^+ , NO_3^- , and available P) concentrations to the simulated extreme precipitations in different seasons of the plant growth were analyzed separately. The differences between means of control and treatments were evaluated by using an independent-sample *t*-test. Differences of NPP in sedges, grasses, forbs, and total species among different plant growth seasons were assessed by one-way ANOVA. Prior to statistical analysis, the normality of the data was tested and natural log (ln)-transformation was carried out if the transformation improved the normality. In any case, if the normality was not reached by transformation approach, distribution-free nonparametric tests (i.e. the

Kruskal-Wallis H test and Nemenyi test) were used.

The observed environmental factors were used for redundancy discriminatory analysis with CANOCO 4.5 software (ter Braak and Smilauer, 2002). Detrended correspondence analysis indicated that if axis lengths were less than 3, redundancy discriminatory analysis was an appropriate method. Structural equation modeling (SEM) was adopted to test the casual relationships among NPP, abiotic (soil moisture and temperature), and biotic (microbial biomass, enzyme activities, and nutrient concentrations) controls. The AMOS 18.0 software package (SmallWaters, Chicago, IL, USA) was used to design the model and calculate the path coefficients. In contrast to other approaches such as multiple regression analysis, SEM techniques not only provide a higher degree of support for the question of causation, but also deal with the interdependence of variables by dividing the total effects into the direct and indirect effects (Grace, 2006; Lienin and Kleyer, 2012). Moreover, SEM techniques can conduct the comparison of competing models through several different measures. The most frequently used indicator in SEM is the likelihood of chi-square value χ^2 ; the occurrence of non-significant χ^2 indicates that the pattern of covariance predicted by the hypothesis is not different from the observed data, and therefore the model can be accepted. However, it is generally accepted that the chi-square test should be interpreted with caution and supplemented with other goodness of fit indices when data depart from multivariate normality and sample sizes are small (Matías et al., 2012). Under this circumstance, the Bentler–Bonnet normed fit index (NFI) and the goodness-of-fit index (GFI) were used, and the values (ranging between 0 and 1) greater than 0.9 indicate an acceptable fit of the model to the data.

3. Results

3.1. Soil moisture and temperature

The response of soil moisture during the treatment and recovery stages to the simulated extreme precipitation depends on the plant growth seasons (Fig. 1). In the summer and autumn simulation treatments, soil moisture increased significantly after 3 days of precipitation. In the spring simulation treatment, however, no changes of soil moisture were detected until day 8 of the precipitation. At the end of the simulation, soil moisture was increased by 10, 34, and 47% in the spring, summer, and autumn simulation treatments, respectively, compared to the control. Soil moisture also showed different patterns during the recovery stage in different seasonal treatments. In the spring and summer simulation treatments, soil moisture dropped to the similar level as in the control at days 3 and 15, respectively, after the simulated precipitation ceased. In the autumn simulation treatment, however, it remained higher than in the control over the course of the study (Fig. 1). Extreme precipitation increased soil temperature in the summer and autumn simulation treatments, but reduced it in the spring simulation treatment (Fig. 2). Furthermore, more pronounced temperature fluctuations between control and treatment were observed in spring than during other seasons (Fig. 2). Unlike soil moisture, soil temperature in all the growth season

treatments did not return to the levels as in the control after the extreme precipitation ceased (Fig. 2).

3.2. Microbial biomass and enzyme activities

Extreme precipitation in the summer simulation treatment caused a more significant impact on microbial biomass than in the spring and autumn simulation treatments (Fig. 3). For example, microbial biomass increased by 209% after 13 days of consecutive precipitation in the summer simulation treatment. By contrast, the simulated precipitation did not change the microbial biomass in the spring simulation treatment throughout the study course. Moreover, simulated precipitation significantly decreased the microbial C: N ratio in the summer simulation treatment compared to the control, while it hardly affected the microbial C: N ratio in the autumn simulation treatment (Fig. 3).

The activities of six enzymes displayed exceedingly divergent patterns (Fig. 4). In the spring simulation treatment, all the enzymes were maintained at a steady level throughout the study, indicating a strong resistance to the precipitation extremes happened in this season. Compared to the control, the activities of four enzymes, i.e. β -glucosidase, cellobiohydrolase, *N*-acetyl-glucosaminidase, and phosphatase, in the summer simulation treatment were significantly reduced after 14 days of consecutive precipitation. No changes of peroxidase and polyphenol oxidase were found between the control and summer precipitation treatments during the precipitation period, while peroxidase were significant decreased in the summer precipitation treatment after the precipitation ceased for 7 days (Fig. 4). In the autumn simulation treatment, significant differences were only observed after 7 days of recovery, i.e., polyphenol oxidase was enhanced and the other five enzymes were reduced by the previous extreme precipitation compared to the control. All enzyme activities returned to the level as in the control at the end of recovery stage, regardless of the precipitation seasons (Fig. 4).

3.3. Soil nutrients availabilities

Extreme precipitation simulated in the summer had a more profound influence on soil nutrient availabilities compared to it simulated in the spring and autumn (Fig. 5). The soil NH_4^+ concentration was increased by 86% and 70% by the summer simulated precipitation applied for 7 and 14 days, respectively, and maintained at a significantly higher level compared to the control during the recovery stage. The NO_3^- and PO_4^{3-} concentrations in the summer simulation treatment also increased significantly after the start of the precipitation, but returned to levels similar to those of the control when precipitation ceased (Fig. 5). The concentrations of PO_4^{3-} and NO_3^- were unresponsive during the whole study period (include precipitation and recovery stages) in the spring and autumn simulation treatments, but the NH_4^+ concentration were reduced by the extreme precipitation in the spring (Fig. 5).

3.4. Net primary productivity

The effect of precipitation extremes on NPP depends on precipitation season, plant functional type, and above- or below-ground organs (Fig. 6). Compared to the control, the total NPP was significantly decreased by the extreme precipitation simulated in the spring, while it was significantly increased by the summer simulation. No influence of the extreme precipitation on the total NPP was found in the autumn simulation treatment. Specifically, the total biomass of forbs showed little response to the precipitation extremes that happened in all the plant growth seasons, except in the summer simulation treatment, a significant increase in the below-ground accumulation was found (Table S1). For sedges, significant decreases were observed in both aboveground and belowground biomass in the spring simulation treatment compared to the control, but levels remained stable in both summer and autumn (Fig. 6). For sensitive grasses, the precipitation extremes in the spring and autumn significantly decreased the biomass by 78.1% and 32.8%, respectively, compared to the control.

3.5. Correlations between NPP and biotic and abiotic factors

Redundancy discriminatory analyses 1 and 2 explained total 73.8% of the variations that caused by the abiotic and biotic factors in the control and seasonal precipitation treatments ($P = 0.002$) (Fig. 7). A great difference was observed between the control and simulation treatment in spring, which was related to microbial biomass C and PO_4^{3-} . Small differences were found in the summer and autumn treatments, mainly due to the small differentiations in soil temperature, and enzyme activities of peroxidase and phosphatase (Fig. 7).

According to the non-significant χ^2 values ($P > 0.05$) and the goodness-of-fit indices (NFI and GFI > 0.9), the proposed path analysis model provided an adequate fit for data collected during different seasonal treatments (Fig. 8). The model implied that in the spring simulation treatment, soil temperature played a pivotal role in determining NPP (total effect = 0.72), and in the summer and autumn simulation treatments, soil temperature and moisture exerted similar impacts (Table S2). Biotic factors, i.e. microbial biomass, enzyme activities, and nutrients concentrations, had positive and direct effects on NPP (Fig. 8).

4. Discussion

In this study, we demonstrated that the timing of extreme precipitation events is a critical factor in predicting soil microbial and plant productivities, as well as ecosystem resilience. We confirmed that the interactions between biotic and abiotic processes govern the seasonal nutrient dynamics, and the enzymatic decomposition of organic matter in Tibetan grasslands. Furthermore, a suite of acclimation and adaptation mechanisms pointed to a high resilience in many ecological processes that potentially contribute to the grassland acclimation and stability.

4.1. Changes in NPP with extreme precipitation seasons, plant functional groups, and plant biomass allocation

Net primary productivity showed exceedingly variable responses to the extreme precipitation seasons, plant functional types, and above- or below-ground biomass (Figs. 6, 7). Extreme precipitation happened in the summer caused a significant increase in the total NPP, with 14.6% enhancement in the below-ground biomass of forbs, and some slight increase in the above- and below-ground biomass of sedges and grasses. On the contrary, the extreme precipitation happened in the early plant growth season (spring) elicited a significant decrease in the total NPP, probably due to the substantial reductions in above- and below-ground biomass of sedges and grasses. Nevertheless, the extreme precipitation simulated in the late plant growth season (autumn) only caused a slight decrease in grasses and simultaneous increase in forbs, resulted in a neutral net effect on the total NPP (Figs. 6 and 7).

Above-ground biomass was found to be more sensitive to the extreme precipitation events than the below-ground, as indicated by an increase of 23% (vs. 17%), decrease of 10% (vs. 6.0%) and 57% (vs. 40%) in the summer, autumn, and spring simulation treatments, respectively, compared to the control. These results were supported by the low shoot/root ratio observed in other studies (Gao et al., 2007; Ma et al., 2010), and ascertained that the species in the alpine meadow allocated a large fraction of biomass below ground, in order to acclimate and mitigate the impact of the harsh environment in this region. Furthermore, extreme precipitation not only changed the biomass accumulation and allocation, but also changed the composition of community vegetation. For example, the proportion of forbs to the entire plant community increased from 30.5% in the control treatment to a domination of 63.4% in the spring simulation treatment, with concurrent reductions in sedges, from 43.2 to 26.2%, and grasses from 26.3 to 10.4% (Fig. 6; Table S1). Thus, extreme precipitation in the early plant growth season not only reduced ecosystem productivity, but also affected pasture quality through the shift from perennial graminoids (grasses and sedges) to less palatable annual forbs. An increase in forbs cover is usually considered as an early symptom of alpine meadow degradation in the Tibetan grassland (Kato et al., 2006; Wang et al., 2015). Zhou et al. (2011, 2014) also found that the community of monsoon evergreen broad-leaved forest in South China is undergoing a transition from a cohort of fewer and larger individuals to a forest of great numbers of smaller individuals, with different species composition, indicated that the subtropical forests are threatened by the long-term climate change due to the lack of ecosystem resilience.

4.2. Abiotic factors affecting ecosystem functions

Among all the treatments, soil moistures were never exceeded 21.5%, suggesting that this value was the maximum threshold for the water held by the soil in this region (Fig. 1). Soil moisture were not significantly increased by the extreme precipitation in the spring, likely due to the high background soil moisture that caused by the snow melting from the preceding winter (Fig. 1). Soil temperatures were significantly decreased by the extreme precipitation simulated in the

spring, which indicated that the variations in soil processes and plant productivity were mainly caused by soil temperature. The results from redundancy discriminatory and path analysis also supported this conclusion (Figs. 7 and 8).

The combination of high temperature and precipitation can have drastic synergistic impacts on activating microbial populations, accelerating enzymatic nutrient mineralization, and subsequently favoring vegetation growth in the peak growing season (Buckeridge and Grogan, 2008). The path analysis also reinforced the importance of abiotic factors (soil temperature and moisture) for determining the microbial and plant productivities in the summer simulation treatment (Fig. 8). Compared to the perennial sedges and grasses, more pronounced stimulation was observed in forbs, the shallow-rooted annual (Fig. 6). This is probably because at the maximum growth season, small pulses of water addition were either intercepted by the canopy or evaporated quickly from the soil surface, leaving deeper-rooted plants without advantage. In the autumn, the extreme precipitations did not change the plant biomass; likely because the plants in this season passed their growth peak and were lack of incentive to exploit additional available water due to the costs outweigh any potential returns in C gain (Schwinning and Sala, 2004). The lack of response in the late plant growth season (autumn) was also probably resulted from the relative short term between the beginning of the extreme precipitation and the final harvest, as the duration was only 20 days in this season, compared with 132 and 55 days in the early (spring) and mid- (summer) plant growth seasons, respectively.

4.3. Biotic factors affecting ecosystem responses

The high microbial biomass caused by the extreme precipitation in the mid-plant growth season (summer) stimulated an increase in the soil nutrients availability in the mid-plant growth season, thus enhanced the plant growth in this season (Figs. 3, 5 and 6). The differences of the microbial C: N ratio between the control and summer simulation treatments suggested that the extreme precipitation changed not only microbial productivity, but also microbial composition and possible functions. Moreover, the microbial C: N ratio of 6 - 8 in the mid-plant growth season under the simulated precipitation showed a dominance of bacteria that high microbial activities could be induced. This was supported by the high concentration of nutrients in this season (Fig. 3). In addition to microbial activities, the increased nutrient availability in the summer simulation treatment might also be attributed to the microbial lysis, due to physical disturbance from soil drying and wetting cycles caused a pulse of nutrient releasing from microbial biomass, in a process known as “slaking” (Bunemann et al., 2013). Ren et al. (2015) highlighted a “fertilization effect” of water addition. High water supply promoted soil microbial activity and stimulated N and P mineralization, thus achieved the same plant growth rate as the N fertilizer application in semi-arid grassland. Furthermore, in the same region, Wang et al. (2015) observed the largest deleterious drought effects for plant productivity and microbial activity during the summer, in which competitions for limiting resources between plants and microorganisms were strongest.

In the early plant growth season, the lack of responses in extracellular enzymes that involved in the breakdown of organic matter to the extreme precipitation caused no changes in the soil nutrient concentrations except the NH_4^+ concentration, which reduced by the extreme precipitation and contributed to the significant decreases in NPP in this season (Figs. 4, 5 and 6). In the late plant growth season, the relatively small vegetation changes could be owed to the lack of treatment effects on soil enzyme activities and extractable nutrients. Labile C released through root exudation has been suggested to trigger positive plant-soil feedback via rhizosphere priming, which is an important mechanism that accounts for sustained forest growth during the experimental warming and CO_2 enrichment (Phillips et al., 2013; Yin et al., 2014). Thus, the low temperature and plant growth activity in the late plant growth season might substantially reduce the rhizodeposition, thereby hindered soil organic matter decomposition and subsequent nutrient cycling for further feedback on growth stimulation.

4.4. High resilience of ecosystem functions

Ecological resilience is the ability of an ecosystem to return to its original state after a perturbation, thereby maintaining its essential characteristic taxonomic composition, structures, ecosystem functions, and rates of key processes (Sun et al., 2014). The studied grassland appeared to be fairly resilient, in short term, to a more extreme precipitation changes than that was predicted for the region, as most of the below-ground parameters returned to the control levels once the extreme precipitation ceased. This increased the intriguing possibility that resilience in function, rather than in resistance, might be expected for ecosystems with a history of frequent climate extremes. At individual level, if the climate extremes repeatedly occur, such as the harsh conditions in the Tibetan plateau, a plant can develop a so-called stress memory, by which it can remembers the experienced stresses and changes the present or future response to the environmental stresses (Reichmann et al., 2013; Walter et al., 2013). Ecological memory has recently received attention as an important variable in relation to the ecosystem dynamics and resilience, which consists of retrospective memory and prospective memory (Niu et al., 2014). Retrospective memory can “remember” the past ecological events and situation, which helps the disturbed ecosystem return to its previous stable stage. Prospective memory takes charge of “remembering” the direction of succession and ensures that the ecosystem development along a natural succession trajectory (Sun et al., 2014). In this way, climate extremes directly influence plant physiology and soil processes, triggering a suite of acclimation and adaptation mechanisms to explain the high resilience in this mesophytic grassland.

5. Conclusions

Understanding the response of plant functional groups, above- and below-ground biomass, as well as soil microbial- and plant- productivities to the extreme precipitation events that happen in different seasons is crucial knowledge for

ecologist to predict the effect of future climate change on ecological and evolutionary processes in mesophytic grassland. Our results demonstrate that significant interactions occurred between soil moisture and temperature, with the strongest synergistic effects observed during the spring and summer. Furthermore, the increased sensitivity of the above-ground NPP and the shift of dominant species from sedges to less palatable forbs under the extreme spring precipitation, would inevitably exaggerate the degradation of this grassland. These results indicate that an integrative framework including biotic and abiotic drivers and responses to decipher the past and predict future climate change impacts on terrestrial ecosystems is needed. Given the truth of hitherto poorly studied biomes and ambient climatic conditions in the alpine meadow ecosystem, the thorough investigation of the responses of ecosystem functions to relevant complex scenarios, i.e. precipitation frequency and amplitude, seasonality, extremity, and interactions with other global climate change drivers, warrants further in-depth research.

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References

- Bates, J.D., Svejcar, T., Miller, R.F., Angell, R.A., 2006. The effects of precipitation timing on sagebrush steppe vegetation. *J. Arid Environ.* 64, 670–697.
- Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., de Peck, B., Christensen, J.H., Leuzinger, S., Janssens, I.A., Hansen, K., 2012. Precipitation manipulation experiments – challenges and recommendations for the future. *Ecol. Lett.* 15, 899–911.
- Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 59, 39–45.
- Brotherton, S.J., Joyce, C., 2015. Extreme climate events and wet grasslands: plant traits for ecological resilience. *Hydrobiologia* 750, 229–243.
- Buckeridge, K.M., Grogan, P., 2008. Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Appl. Soil Ecol.* 39, 210–222.
- Bunemann, E.K., Keller, B., Hoop, D., Jud, K., Boivin, P., Frossard, E., 2013. Increased availability of phosphorus after drying and rewetting of a grassland soil: processes and plant use. *Plant Soil* 370, 511–526.

432 Combes, C., 2008. The role of extreme events in evolution. *CR Geoscience* 340, 591–594.

433 De Boeck, H.J., Dreesen, F.E., Janssens, I.A., Nijs, I., 2011. Whole system responses of experimental plant communities to
434 climate extremes imposed in different seasons. *New Phytol.* 189, 806–817.

435 Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W., 2008. Changes in grassland ecosystem function due
436 to extreme rainfall events: implications for responses to climate change. *Glob. Change Biol.* 14, 1600–1608.

437 Fry, E.L., Manning, P., Power, S.A., 2014. Ecosystem functions are resistant to extreme changes to rainfall regimes in a
438 mesotrophic grassland. *Plant Soil* 381, 351–365.

439 Gao, Y.H., Luo, P., Wu, N., Chen, H., Wang, G.X., 2007. Grazing intensity impacts on carbon sequestration in an alpine
440 meadow on the eastern Tibetan Plateau. *J. Agr. Biol. Sci.* 3, 642–647.

441 Gerten, D., Luo, Y., Le Maire, G., Parton, W.J., Keough, C., Weng, E., Beier, C., Ciais, P., Cramer, W., Dukes, J.S.,
442 Hanson, P.J., Knapp, A.K., Linder, S., Nepstad, D., Rustad, L., Sowerby, A., 2008. Modelled effects of precipitation on
443 ecosystem carbon and water dynamics in different climatic zones. *Glob. Change Biol.* 14, 2365–2379.

444 Grace, J.B., 2006. Structural equation modeling and natural systems. Cambridge: Cambridge University Press.

445 Gumbel, E., 1958. Statistics of Extreme. Columbia University Press: New York.

446 Heisler-White, J.L., Blair, J.M., Kelly, E.F., Harmony, K., Knapp, A.K., 2009. Contingent productivity responses to more
447 extreme rainfall regimes across a grassland biome. *Glob. Change Biol.* 15, 2894–2904.

448 Hoover, D.L., Knapp, A.K., Simth, M.D., 2014. Resistance and resilience of a grassland ecosystem to climate extremes.
449 *Ecology* 95, 2646–2656.

450 IPCC., 2012. Managing the risks of extreme events and disasters to advance climate change adaptation. in C. Field et al.,
451 editors. A special report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge
452 University Press, Cambridge, UK.

453 Jentsch, A., Kreyling, J., Beierkuhnlein, C., 2007. A new generation of climate change experiments: events, not trends.
454 *Front. Ecol. Environ.* 5, 315–324.

455 Kato, T., Tang, Y.H., Gu, S., Hirota, M., Du, M.Y., Li, Y.N., Zhao, X.Q., 2006. Temperature and biomass influences on
456 interannual changes in CO₂ exchange in an alpine meadow on the Qinghai-Tibetan Plateau. *Glob. Chang Biol.* 12,
457 1285–1298.

458 Knapp, A.K., Beier, C., Briske, D.D., Classen, A.T., Luo, Y.Q., Reichstein, M., Smith, M.D., Smith, S.D., Bell, J.E., Fay,
459 P.A., Heisler, J.L., Leavitt, S.W., Sherry, R., Smith, B., Weng, E., 2008. Consequences of more extreme precipitation
460 regimes for terrestrial ecosystems. *Bioscience* 58, 811–821.

461 Knapp, A.K., Hoover, D.L., Wilcox, K.R., Avolio, M.L., Koerner, S.E., La Pierre, K.J., Loik, M.E., Luo, Y.Q., Sala, O.E.,
462 Smith, M.D., 2015. Characterizing differences in precipitation regimes of extreme wet and dry years: implications for

climate change experiments. *Glob. Change Biol.* 21, 2624–2633.

Krab, E.J., Aerts, R., Berg, M.P., van Hal, J., Keuper, F., 2014. Northern peatland Collembola communities unaffected by three summers of simulated extreme precipitation. *Appl. Soil Ecol.* 79, 70–76.

Lienin, P., Kleyer, M., 2012. Plant trait responses to the environment and effects on ecosystem properties. *Basic Appl. Ecol.* 13, 301–311.

Lipson, D.A., Schmidt, S.K., Monson, R.K., 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* 80, 1623–1631.

Ma, W.L., Shi, P.L., Li, W.H., He, Y.T., Zhang, X.Z., Shen, Z.X., Chai, S.Y. 2010. Changes in individual plant traits and biomass allocation in alpine meadow with elevation variation on the Qinghai -Tibetan Plateau. *Sci. China – Life Sci.* 53, 1142–1151.

Matías, L., Catro, J., Zamora, R., 2012. Effect of simulated climate change on soil respiration in a Mediterranean-type ecosystem: rainfall and habitat type are more important than temperature or the soil carbon pool. *Ecosystems* 15, 299–310.

Morecroft, M.D., Masters, G.J., Brown, V.K., Clarke, I.P., Taylor, M.E., Whitehouse, A.T., 2004. Changing precipitation patterns alter plant community dynamics and succession in an ex-arable grassland. *Funct. Ecol.* 18, 648–655.

Niu, S.L., Luo, Y.Q., Li, D.J., Cao, S.H., Xia, J.Y., Li, J.W., Smith, M.D., 2014. Plant growth and mortality under climatic extremes: an overview. *Environ. Exp. Bot.* 98, 13–19.

Orsenigo, S., Mondoni, A., Rossi, G., Abeli, T., 2014. Some like it hot and some like it cold, but not too much: plant responses to climate extremes. *Plant Ecol.* 215, 677–688.

Phillips, R.P., Midgley, M.G., Brozstek, E., 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in forests. *New Phytol.* 199, 41–51.

Reichmann, L., Sala, O., Peters, D., 2013. Precipitation legacies in desert grassland primary production occur through previous-year tiller density. *Ecology* 94, 435–443.

Ren, H.Y., Xu, Z.W., Huang, J.H., Lü, X.T., Zeng, D.H., Yuan, Z.Y., Han, X.G., Fang, Y.T., 2015. Increased precipitation induces a positive plant-soil feedback in a semi-arid grassland. *Plant Soil* 389, 211–223.

Reyer, C., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R.P., Bonfante, A., de Lorenzi, F., Dury, M., Gloning, P., Abou Jaoudé, R., Klein, T., Kuster, T.M., Martins, M., Niedrist, G., Riccardi, M., Wohlfahrt, G., de Angelis, P., de Dato, G., François, L., Menzel, A., Pereira, M., 2012. A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. *Glob. Change Biol.* 19, 75–89.

Schwinning, S., Sala, O.E., 2004. Hierarchy of response to resource pulses in arid and semi-arid ecosystems. *Oecologia* 141, 211–220.

Seneviratne, S.I., Corti, T., Davin, E.L., Hirschi, M., Jaeger, E.B., Lehner, I., Orlowsky, B., Teuling, A.J., 2010.

Investigating soil moisture-climate interactions in a changing climate: a review. *Earth Sci. Rev.* 99, 125–161.

Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., Mcclaugherty, C.A., Rayburn, L., Repert, D., Weiland, T., 1993. Wood decomposition- nitrogen and phosphorus dynamics in relation to extracellular enzyme-activity. *Ecology* 74, 1586–1593.

Smith, M.D., 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *J. Ecol.* 99, 656–663.

Sun, Z.Y., Ren, H., Schaefer, V., Guo, Q.F., Wang, J. 2014. Using ecological memory as an indicator to monitor the ecological restoration of four forest plantations in subtropical China. *Environ. Monit. Assess.* 186, 8229–8247.

ter Braak, C.J.F., Smilauer, P., 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5), Biometris, Wageningen

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass-C. *Soil Biol. Biochem.* 19, 703–707.

Walter, J., Jentsch, A., Beierkuhnlein, C., Kreyling, J., 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environ. Exp. Bot.* 94, 3–8.

Wang, Z.Y., Silva, L.C.R., Sun, G., Luo, P., Mou, C.X., Horwath, W.R., 2015. Quantifying the impact of drought on soil-plant interactions: a seasonal analysis of biotic and abiotic controls of carbon and nutrient dynamics in high-altitudinal grasslands. *Plant Soil* 389, 59–71.

Wookey, P.A., Aerts, R., Bardgett, R.D., Baptist, F., Brathen, K.A., Cornelissen, J.H.C., Gough, L., Hartley, I.P., Hopkins, D.W., Lavorel, S., Shaver, G.R., 2009. Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. *Glob. Change Biol.* 15, 1153–1172.

Yin, H.J., Wheeler, E., Phillips, R.P., 2014. Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biol. Biochem.* 78, 213–221.

Zeppel, M.J.B., Wilks, J.V., Lewis, J.D., 2014. Impacts of extreme precipitation and seasonal changes in precipitation on plants. *Biogeosciences* 11, 3083–3093.

Zhang, Y., Susan, M.M., Nearing, M.A., Ponce, C.G.E., Huete, A.R., Buda, A.R., Bosch, D.D., Gunter, S.A., Kitchen, S.G., Henry, M.W., Morgan, J.A., McClaran, M.P., Montoya, D.S., Peters, D.P.C., Starks, P.J., 2013. Extreme precipitation patterns and reductions of terrestrial ecosystem production across biomes. *J. Geophys. Res.* 118, 148–157.

Zhou, G.Y., Houlton, B.Z., Wang, W.T., Huang, W.J., Xiao, Y., Zhang, Q.M., Liu, S.Z., Cao, M., Wang, X.H., Wang, S.L., Zhang, Y.P., Yan, J.H., Tang, X.L., Zhang, D.Q., 2014. Substantial reorganization of China's tropical and subtropical forests: based on the permanent plots. *Glob. Change Biol.* 20, 240–250.

Zhou, G.Y., Peng, C.H., Li, Y.L., Liu, S.Z., Zhang, Q.M., Tang, X.L., Liu, J.X., Yan, J.H., Zhang, D.Q., Chu, G.W., 2013. A climate change-induced threat to the ecological resilience of a subtropical monsoon evergreen broadleaved forest in south

525 China. Glob. Change Biol. 19, 1197–1210.

526 Zhou, G.Y., Wei, X.H., Wu, Y.P., Liu, S.G., Huang, Y.H., Yan, J.H., Zhang, D.Q., Zhang, Q.M., Liu, J.X., Meng, Z., Wang,

527 C.L., Chu, G.W., Liu, S.Z., Tang, X.L., Liu, X.D., 2011. Quantifying the hydrological responses to climate change in an

528 intact forested small watershed in Southern China. Glob. Change Biol. 17, 3736–3746.

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Figure captions

Figure 1. Daily rainfall and soil moisture (0–10 cm) during (gray boxes) and after (white boxes) the simulated extreme precipitation in different plant growth seasons (spring 2011, summer 2010, and autumn 2010). Gray and black lines indicate moisture fluctuations in the control and extreme precipitation treatments, respectively.

Figure 2. Maximum, average, and minimum temperatures measured daily in soil surface layer (0–10 cm) during (gray boxes) and after (white boxes) the simulated extreme precipitation in different plant growth seasons (spring 2011, summer 2010, and autumn 2010). Gray and black lines indicate moisture fluctuations in the control and extreme precipitation treatments, respectively.

Figure 3. Soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP), and microbial C: N ratio during (gray boxes) and after (white boxes) the simulated extreme precipitation in different plant growth seasons (spring 2011, summer 2010, and autumn 2010). Differences between the control and extreme treatments are compared at each sampling date and indicated by significance at $p < 0.05$ (*); 0.01 (**); and 0.001 (***) according to *t*-tests. Standard errors were shown as line bars. $n=4$.

Figure 4. Effects of simulated extreme precipitation on the activities of β -glucosidase (BG), cellobiohydrolase (CBH), N-acetylglucosaminidase (NAG), peroxidase (POD), polyphenol oxidase (PPO) and phosphatase (PS) during (gray boxes) and after (white boxes) the simulated extreme precipitation in different plant growth seasons (spring 2011, summer 2010, and autumn 2010). Differences between the control and extreme treatments are compared at each sampling date and indicated by significance at $p < 0.05$ (*); 0.01 (**); and 0.001 (***) according to *t*-tests. Standard errors were shown as line bars. $n=4$.

Figure 5. Mean soil available nutrients $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, PO_4^{3-} during (gray boxes) and after (white boxes) the simulated extreme precipitation in different plant growth seasons (spring 2011, summer 2010, and autumn 2010). Differences between the control and extreme treatments are compared at each sampling date and indicated by significance at $p < 0.05$ (*); 0.01 (**); and 0.001 (***) according to paired *t*-tests. Standard errors were shown as line bars. $n=4$.

Figure 6. Net primary productivity responses to the extreme precipitation events during different plant growth season in sedges, grasses, forbs, and total species. Different lowercase letters indicate significant differences ($P < 0.05$) in above- or

below- ground biomass among different seasonal treatments, whereas different upper letters indicate significant differences in the total NPP (above- plus below- ground biomass). Standard errors were shown as line bars. n=4. Blank: control; fine hatched bars: SPRING; coarse hatched bars: SUMMER; black: AUTUMN.

Figure 7. Redundancy discriminatory analysis of the abiotic and biotic factors in the control and extreme treatments during different growth seasons (spring 2011, summer 2010, and autumn 2010) with the indices of soil available nutrients, microbial biomass, and enzyme activities at the end of extreme precipitation treatments. BG: β -glucosidase; MBC: microbial biomass of carbon; MBN: microbial biomass of nitrogen; MBP: microbial biomass of phosphorus; NAG: *N*-acetyl-glucosaminidase; POD: peroxidase; PPO: polyphenol oxidase; PS: phosphatase; ST: soil temperature; SWC: soil moisture.

Figure 8. Path model depicting causal relationships between abiotic and biotic factors in determining net primary productivity (NPP) in SPRING (A), SUMMER (B) and AUTUMN (C). Arrows represent positive (solid) or negative (dashed) path coefficients. Double arrows indicate correlations between soil water content and soil temperature. Traits and paths represent unmeasured residual variations were not included for simplicity. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

$\chi^2 = 15.28$, $P = 0.182$, NFI = 0.890, GFI = 0.918

$\chi^2 = 25.28$, $P = 0.282$, NFI = 0.922, GFI = 0.969

$\chi^2 = 10.28$, $P = 0.102$, NFI = 0.849, GFI = 0.868

Figure 8.