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

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Abstract

Background and aims Understanding the impacts of ever more severe and widespread drought events has become a central focus of recent ecological research. Accordingly, the objective of this study is to investigate fundamental mechanisms that control drought effects on climate sensitive ecosystems by regulating soil-plant interactions.

Methods Field experiments were conducted in high altitudinal grasslands of the Tibetan Plateau. Based on historical records, we simulated extreme drought events, intercepting water inputs in early (spring), mid (summer), and late (autumn) periods of the plant-growing season (PGS). We measured vegetation responses to changes in soil physical, chemical, and biological properties, examining how the interplay of abiotic and biotic processes regulate the impacts of drought above and below ground.

Results Decreasing water input resulted in proportional increases in summer and autumn soil temperature, but reduced soil temperature during the spring drought. As a result, soil microbial biomass and available N and P concentrations remained stable during the early-PGS drought, while enzymatic activity, decomposition of organic materials, and nutrient release increased during the mid- and late-PGS. Concerted changes in microbial and plant activity determined seasonal fluctuations in carbon assimilation, microbial activity and nutrient dynamics, with varying degrees of resistance and resilience to drought stress observed at different PGS periods.

Conclusions Significant interactions were observed between plant productivity and microbial activity in response to moisture variability and associated changes in soil temperature, with the largest deleterious drought effects registered during the summer, when competition for limiting resources between plants and microorganisms was strongest.

Keywords Climate change · Drought stress · Nutrient limitation · Soil-plant interactions · Tibet
Introduction

Over the past few decades, the impact of climatic fluctuations on terrestrial ecosystems has emerged as a new field in ecology (Smith 2011). There is now mounting evidence indicating that both the frequency and intensity of regional and global extremes in temperature and moisture regimes have increased over the past few decades (Easterling et al. 2000; Jiang et al. 2012; Karl et al. 1995; Mastrandrea et al. 2011). Recent studies have demonstrated that many ecosystems are vulnerable to extreme climatic events that will likely lead to profound ecological and social impacts at local to global scales (e.g. IPCC 2007). Numerous studies have quantified the impacts of climate fluctuations through analyses of physiological responses, and vegetation distribution/displacement over the past century, in response to changes in atmospheric composition and climate (Silva et al. 2009, 2011; Leithead et al. 2010, 2012; Gomez-Guerrero et al. 2013; Silva and Anand 2013a, b; Silva and Horwath 2013). Despite evidence for a coherent process of global change presented by these studies, little is known about the underlying mechanisms controlling responses to climate variability.

Most experimental studies on drought effects focus on above-ground patterns including changes in plant productivity, phenology and gas-exchange (De Boeck et al. 2011; Godfree et al. 2011; Jentsch et al. 2011; Jimenez et al. 2011; Penuelas et al. 2007; Rich et al. 2008). Nevertheless, drought is expected to affect both vegetation and soil processes through changes in nutrient availability, resource use efficiency and ecophysiological performance of dominant plant species (Aanderud et al. 2010; Harper et al. 2005; Jensen et al. 2003; Zhou et al. 2009; Silva and Anand 2013a, b). Despite examples of soil-plant feedbacks determining tipping-points beyond which resilience is gained despite several disturbances (e.g. Silva and Anand 2013a, b), few experimental studies have dealt with interactions between soil and vegetation in response to extreme climatic events. Particularly, little is known about how microbial communities regulate or alter nutrient availability under extreme conditions (Bardgett et al. 2005), and new integrative studies are needed to assess the specific mechanisms involved in responses to drought stress.

The grasslands of 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 does not only yield relatively high vegetation growth rates with concentrated rainfall during the growing season, but also leads to very slow soil organic matter decomposition and nutrient release during dry cold winters (Kato et al. 2006; Zhao et al. 2006). As a result, strong seasonality is characterized by steep fluctuations in microbial and plant community structure, stability and productivity (Lipson et al. 1999, 2002). Typical alpine environments are dominated by drought-resistance grasses adapted to low resource availability, which can limit ecosystem resistance and resilience to disturbance or persistent stress (Körner 2003). Understanding coupled changes in plant and microbial communities would therefore bring valuable information about the resilience of these climate sensitive ecosystems, enhancing our ability to predict how terrestrial biomes will respond to climate change elsewhere.

Previous studies have emphasized that the impacts of global climate change can be evaluated seasonally rather than simply described based on (more commonly assessed) annual trends (Orlowsky and Seneviratne 2012). Accordingly, here we evaluate fundamental soil processes (i.e. enzyme activities, soil respiration, nitrogen mineralization and soil microbial biomass) as they are affected by drought during different period of the plant growing season (PGS). We describe key factors (soil water content and soil temperature) influencing biogeochemical linkages that would affect above- and below-ground responses in any terrestrial ecosystem, testing the hypothesis that the magnitude and duration of impacts depend on seasonal variations in SOM decomposition and productivity of microbial communities. We anticipate that the net outcome of biotic and abiotic interactions will alter nutrient dynamics and enzymatic degradation of organic materials in a predictable fashion, reflecting how extreme drought events would impact the ecosystem by altering soil-plant interactions.

Materials and methods

The experimental site (32°26′N, 102°22′E) was established within a 100 m² area of alpine meadow at 3570 m a.s.l. in eastern Tibet-Plateau, i.e. western part of the Sichuan Province, PR China. Originally, the land used to be employed for raising yaks, but our study site
has not been grazed since 2007. The mean annual precipitation is approximately 786 mm, and the mean annual temperature is 1.6 °C based on data from 1961 to 2004 collected at the Hongyuan meteorological station. The growing season begins at the end of April or the beginning of May when the earliest plant species sprout, and ends in late September when most species are withered. The regional climate is characterized as typical plateau continental climate with high solar radiation, short-cool summer (mean July max/min 14.6/7.6 °C) and long-cold winter (mean January max/min −3.0/−17.2 °C). The short season for vegetation growing is controlled by the southeastern monsoon, and the cold winter is dominated by cold from Siberia. Approximately 77.2 % of the annual precipitation (~605 mm) is distributed during the growing season. The soil is classified as a typical alpine Mat Cry-gelic Cambisols (Chinese Soil Taxonomy Research Group 1995), which consists of 19 % clay, 66 % silt and 15 % sand, pH=5.0–6.0 (5.3±0.4, 0–10 cm, 1:2.5 soil: water v:v ratio). The average of soil organic matter content in our experiment site is 118±2.87 mg/kg (dry soil) from our analysis. The vegetation is representative of alpine communities which are typical of Tibetan grasslands, dominated by Kobresia humilis, Kobresia macrantha, Festuca rubra, Agrostis hugoniana, Deschampsia caespitosa, Leontopodium nanum, Thalictrum alpinum, Potentilla discolor, Polygonum viviparum, Oxytropis deflexa. (Sichuan Vegetation Research Group 1980).

Experimental design

Drought simulations in the field were performed at the plot level in randomized distribution during the 2010–2011 growing season. The experiment consisted of 16 plots (1.5 m×1.5 m) with four replicates each, following treatments: control (ambient conditions) and simulated drought events in three different periods of the plant growing season (PGS), i.e., the mid-PGS (mean temperature 10.3 °C) in 2010, the late-PGS (mean temperature 6.8 °C) in 2010 and the early-PGS (mean temperature 3.8 °C) in 2011. Extremeness of drought events here was determined by statistical extremity with respect to a historical reference period (extreme value theory) independent of its effects on organisms. Drought was defined as the consecutive time of days with precipitation of less than 0.1 mm in the study area (Gumbel 1958). Thus, a drought period of 20 days was applied by intercepting precipitation during mid-PGS 2010 (from 29 July to 16 August), late-PGS in 2010 (1 September to 20 September early-PGS in 2011 (10 May to 29 May) in the present study.

Drought was applied by rainout shelter, which can transmit 90 % of photo-synthetically active radiation. The shelters were built with a steel frame and covered with a 2 mm polymethylmethacrylate sheet (light transmittance >85 %, SuQianHuanYu Plastic Products Co., Ltd, China). The distance between the roof and land was 80 cm to allow natural air recirculation and prevent greenhouse effects. The experimental plots were 3–5 m apart and contained similar vegetation structure and composition. Ambient rainfall of control was measured by the tipping-bucket rain gauge of HOBO weather station. A 30 cm depth impermeable trench was used to separate all plots and prevent water penetration by potential gradient.

Soil sampling

The water content and temperature of each plot were monitored at interval of 30 mins at 5 cm depth using HOBO weather station. Top soil (0–10 cm below surface) samples were collected 3 times during the treatment period and after simulation we sampled 2 times. At each collection date, composite soil samples comprised of three sub-samples were collected for each plot. All samples were sieved through 2 mm screen and stored at 4°C until further analysis.

Nutrient availability

Nitrogen in the forms of soil ammonium (NH₄⁺), nitrate (NO₃⁻) and phosphorus in the form of phosphate (PO₄³⁻) were extracted with 0.5 M K₂SO₄ and soil extracts were 4 filtered by Pall A/E glass fiber filters and frozen until analysis. Ammonium was measured using a modified method of Berthelot reaction (Rhine et al. 1998). Nitrate was measured using a modified Griess reaction (Doane and Horwath 2003).
Phosphate was measured by using the malachite green-based colorimetric micro-plate analysis (D’Angelo et al. 2001). All nutrients were expressed as μg g⁻¹ of dry soil.

Net N mineralization was measured as the total change in soil inorganic N (NO₃⁻ + NH₄⁺) concentration before and after simulated drought, both in treated and control plots, by using in situ incubation of soils in buried bags (Eno 1960), with incubation periods of 20 days. Net N mineralization rates were expressed as μg g⁻¹ of dry soil day⁻¹.

Soil enzyme activity

We targeted 6 enzymes (Table 1) involved in carbon, nitrogen and phosphorus cycling, to evaluate changes in microbial activity and function as affected by extreme-drought treatments.

The determination of enzyme activities involved acid phosphatase that mineralizes organic phosphate, cellobiohydrolase and β-glucosidase related to the decomposition of organic carbon and N-releasing enzyme N-acetyl-glucosaminidase (NAG). Polyphenol oxidase (PPO) and peroxidase were involved in the degradation of lignin. We measured these extracellular enzyme activities in soil with assay techniques modified from those proposed by Sinsabaugh (1993). We prepared two fractions of fresh soil (~1 g), weighed one fraction and dried at 70 °C to constant dry mass, and then determined water content. To the other fraction we added 60 ml of 50 mM sodium acetate buffer, pH 5, followed by mixing with an ultrasonic (Polytron) homogenizer for 1 min. The homogenate was centrifuged at 8,000 r/min at 4 °C for 20 min. The substrates with a concentration of 50 mM at pH 5 in acetate buffer were mixed with 0.75 ml homogenate in 2 ml centrifuge tube, using 3 analytical replicates per sample.

We prepared blank controls and placed them together with samples on a shaker (Multi-Therm Thermal Mixer, Benchmark Scientific, Inc.) for 1–6 h (Table 1) at 200 r/min at 25 °C. Controls were prepared in duplicate. To terminate the reaction and develop the color, we added 75 μl 0.3 % H₂O₂, and 0.075 ml 1.0 N NaOH to samples and controls. After centrifuging, we measured the absorbance of reaction products in the supernatant on a UV-vis spectrophotometer (Thermo Scientific Varioskan Flash, Thermo Fisher Scientific, Inc.). The reaction products were determined for AP, CBH, BG and NAG assays at 410 nm. The reaction products for polyphenol oxidase and peroxidase were measured at 460 nm. The enzyme activity was expressed in μmol of substrate hydrolyzed per hour per g of dry soil, except polyphenol oxidase and peroxidase, which were expressed as μmol substrate converted per hour per g of dry soil. The concentrations were calculated based on solutions of known concentrations of pNP compounds in buffer. To determine polyphenol oxidase activity, we used the absorbance at 460 nm of a reaction mixture of a known amount of pyrogallic with prepared polyphenol oxidase (Sigma-Aldrich, Inc.). The peroxidase activity was expressed as the difference in activity between the PPO and the peroxidase assay samples.

Microbial C, N, and P

Soil microbial C (MBC), N (MBN) and P (MBP), were determined using a chloroform fumigation-extraction procedure through the difference in DOC, DON and available P between fumigated and non-fumigated soil subsamples (Brookes et al. 1985; Vance et al. 1987).

### Table 1 Extracellular enzymes and their substrates

| Enzyme                          | Substrate                      | Shaking time |
|---------------------------------|--------------------------------|--------------|
| Acid phosphatase (AP)           | 5 mM pNP-phosphate             | 1 h          |
| cellobiohydrolase (CBH)         | 2 mM pNP-β-D-cellobioside      | 6 h          |
| β-glucosidase (BG)              | 5 mM pNP-β-D-glucopyranoside   | 1 h          |
| β-N-acetyl-glucosaminidase (NAG)| 2 mM pNP-acetyl-β-D-glucosaminid| 3 h          |
| polyphenol oxidase (PPO)        | 5 mM L-DOPA                    | 2 h          |
| peroxidase (POD)                | 5 mM L-DOPA                    | 2 h          |

*a pNP = p-nitrophenyl

b L-DOPA = L-3,4-dihydroxyphenylalanine
Briefly, 10 g soil sample were fumigated with chloroform for 24 h in a vacuum desiccator and another 10 g served as non-fumigated controls. Carbon and N were extracted with 50 ml of 0.5 M K$_2$SO$_4$ for 30 min, from fumigated and non-fumigated samples, and the extracts were filtered and frozen at $-20^\circ$C before analyzing by a Total Dissolved Organic Carbon and Nitrogen Analyzer - multi NC 2100S (Analytik Jena AG, Analytik Jena Co., Jena, Germany). Phosphorus was extracted with 50 ml of 0.025 N HCl +0.03 N NH$_4$F and treated the same way as C and N as described above. Available P of extracts was determined by the Bray Kurtz method (Bray and Kurtz 1945), and MBP was also calculated by the difference of available P between the fumigated and the non-fumigated extracts. There were no correction factors (ie.: Kc, Kn and Kp) employed in the calculation, and the three microbial nutrients were expressed as $\mu$g g$^{-1}$ of dry soil.

Soil respiration

Soil respiration rates were measured by an infrared gas analyzer (Licor LS6400, Licor Biosciences, Inc.) fitted with a soil respiration chamber (Glen Spectra Ltd). Patches of vegetation (diameter 10 cm) were clipped to ground surface to provide a plant-free patch. The chambers were placed directly onto the soil surface to a depth of 1 cm. Mean flux rates were calculated through duplicate measurements at each plot and all treatments. Specific areas for measurement were selected randomly, but the measuring time was always between 10 am and 1 pm.

Stability to perturbation

For stability analysis, we used the approach of McNaughton (1993). All measurements were done four times: Day 10, Day 20, Day 30 and Day 40, since the onset of drought simulation treatments. Measurements of stability during the simulated drought periods were taken as the measurements of resistance, and after the end of the experiment, these measurements were taken as a proxy for resilience. Due to absolute differences in the investigated parameters (respiration rate, enzyme activity, microbial biomass) between soils and sampling dates, Resistance = % change from control, i.e.: Resistance = (treatment-control/control)$\times$100 %, while resilience was measured as change in resistance over time. Water deficit = (treatment-control)/control$\times$100 %, as graphically shown in Fig. 2.

Statistical analysis

Statistical analysis was performed with SAS (version 9.1, SAS Institute Inc., Cary, NC, USA). Responses to the simulated extreme-drought in different periods of the plant growing season, including mid-, late- and early-PGS were analyzed separately. The samples collected from controls and treatments in the same time were paired with each other. Differences between means were tested by Paired t-test. All collected data were tested for normality using Shapiro-Wilkinson’s test and log-transformed if necessary. Statistical significance was considered at $p$-values<0.05.

Results

Soil moisture and temperature

The simulated extremes significantly reduced the soil water content (SWC) in all the three drought treatments. In average, the SWC decreased by 33, 41 and 56 % during the periods of treatments in summer 2010, autumn 2010 and spring 2011, respectively. The result showed rapid decreases of SWC immediately took place after the drought-treatments, and a subsequent narrowing of the SWC differences with the control plots occurred after the treatments (Fig. 1). Additionally, the fluctuations of SWC in the controlled plots were obviously correlated to the precipitation events during the simulated droughts, while those in the drought treatment plots were not, as also justified the validity of our experiment treatment.

The simulated drought progressively reduced SWC through the different period of PGS. The water deficit in relation to control plots was up to 54 % during the summer, 46 % during the autumn, and 45 % during the spring. These differences were very large with rapid decreases in SWC (shown as increases in stress curves; Fig. 2) after the simulated drought started in all periods of PGS.

Drought treatment also affected soil temperature, as shown by three different indexes; namely, minimum (Min-), maximum (Max-) and average (Ave-)
temperature values (Fig. 3). During the summer and autumn (mid- and late-PGS), temperatures were always higher in the drought treatment in relation to the control, reflecting an association between SWC and the specific heat capacity of soils. The temperature curves of the early-PGS showed different trends, with control plots showing higher temperature records than treated plots.

Microbial nutrients

Microbial N, P, and C content decreased significantly under simulated drought during the summer (mid-PGS), whereas C and P levels were inconsistently variable over all growing seasons (Fig. 4), but contrasting with the summer response, drought plots generally showed higher N, P, and C levels than control plots during the autumn (late-PGS), when drought induced increases in microbial N, P, and C. At the end of the experiment during the late-PGS, N values decreased significantly (84 %; \( p < 0.001 \)), while C showed no statistical differences, and P remained consistently higher (59 %; \( p = 0.047 \)) in relation to controls.

Soil respiration and enzyme activity

The drought had a strong (41 %; \( p < 0.05 \)) negative effect on soil respiration after approximately 8 days of drought during the summer (mid-PGS; Fig. 5). This effect was smaller with no significance (9 %; \( p = 0.47 \)) during the autumn, while different responses were observed during the spring, possibly as a result of early root development. Although spring drought increased soil respiration in at least two occasions following the treatment (Fig. 5), soil respiration rates in drought and control plots eventually converged. These results indicate varying resistance and resilience of soil microbiota to drought stress at different seasons. This is corroborated by the extracellular activity of six soil enzymes (Fig. 6).
that showed strong seasonal patterns, with large differences between drought (high values) and control (low values) plots observed during the summer and autumn, and small differences during the spring. In most cases, enzymatic activity returned to basal levels after the end of the experiment.

Soil available nutrients

Analyzing each season/PGS-phase (Fig. 7), we have observed that drought significantly decreased soil nitrate concentration by 83 % ($p<0.001$) during the summer, 36 % ($p=0.05$) during the autumn and 41 % ($p=0.015$) during the early-PGS (2011 spring).
during the spring. However, nitrate levels tended to converge after the end of the experiment, with no significant difference observed except in the late-PGS (autumn). Drought also had a negative effect on ammonium concentrations during the summer and autumn, with no significant effects during the spring, and decreased the availability of P during the summer, with no significant differences observed during other PGS-phases.

N mineralization

Drought had a lasting negative effect on net N mineralization both during the treatment period and after simulations. Net N mineralization was stable under drought during the autumn (late-PGS), compared with a dramatic decline observed during the summer and spring. Net nitrification showed the same responses as N mineralization except that it stayed stable during and after drought in the late-PGS. Additional data are given in Online Resource 5.

Discussion

Confirming our central hypothesis, our results show that the impact of drought stress on below-ground processes depends on the phase of plant growth. Drought treatments invariably resulted in large reductions of soil water availability of similar magnitude throughout the year (relative to control plots). Nevertheless, the impacts of reductions in water availability also depended on the interplay of biotic and abiotic processes.

Drought greatly increased soil temperatures during the summer and autumn, but significantly reduced soil temperatures during spring. Despite the intrinsic relationship between soil water content and specific heat capacity (Petrone et al. 2004), establishment and initial growth of plants after cold winters (early-PGS) determines reductions in albedo (retaining heat), enhancing evapotranspiration, activating microbial populations, and accelerating enzymatic nutrient mineralization, which further favors vegetation growth (Buckeridge and Grogan 2008; Larsen et al. 2007). This positive feedback loop is dependent on sufficient water supply, which was attained in control plots but not in drought treatments during spring. Later in the growing season (summer and autumn), the positive effect of initial plant growth on heat fluxes is not as important as in the cold spring, which helps explain the divergent temperature patterns between PGS periods.

Previous experimental studies concluded that drought-induced increases in temperature improve above- and below-ground productivity (De Boeck et al. 2011). Here, temperature increases happening concurrently with water deficit, caused significant reductions in plant productivity (up to 24 % above and ~17 % below ground, see Online Resource 6), particularly, in the late growing season. Climate fluctuations, as well as seasonal variations in water regime, and drought stress, have been shown to alter plant productivity, phenological cycles, reproductive traits, and patterns of resource use across biomes (Gomez-Guerrero et al. 2013; Hueso et al. 2011; Jentsch et al. 2009; Kreyling et al. 2008; Silva and Anand 2013a, b; van Ruijven and Berendse 2010). Our results show that in climate...
sensitive Tibetan grasslands, these plant-soil interactions represent a major factor determining responses to drought through alteration in C and nutrient dynamics.

Despite increasing temperature, which in the study region was expected to stimulate soil microbiota (Davidson and Janssens 2006), drought stress resulted in reductions in net microbial respiration during the summer and autumn. This effect was observed in both microbial biomass (expressed as C) and microbial-derived nutrients (N and P), which were substantially reduced under drought during the summer, but increased during the spring. These results illustrate the fact that in the early-PGS drought suppressed both above- and below-ground productivity, favoring accumulation of soil nutrients. Conversely, relatively higher microbial immobilization (and vegetation growth) helps explain the depletion in soil nutrient pools later in the growing season as a result of increased competition (Michelsen et al. 1999).

Changes in soil respiration relative to control showed that the microbial community was more resistant to drought stress in the late-PGS, while significantly lower
resistance was observed in the early- and mid-PGS as shown in the figure (Online Resource 7). Following the end of the simulated drought, the microbial activity returned to its basal state, showing significantly higher resilience in the mid-PGS and late-PGS than in the early-PGS (Godfree et al. 2011). Similarly, the soil extracellular enzymes activity (see the figure in Online Resource 8) during the period of simulated extreme drought was consistent with changes in soil respiration and microbial nutrients.

From the change of soil enzyme activities, it can be inferred that low decomposition rates (in fact the lowest) induced nutrient limitation in drought treatments during the spring. Following the interruption of treatments, however, both soil respiration and enzyme activities showed a quick recovery during all growing seasons, indicating that the microbial community at the study site is resilient to short seasonal drought events (Godfree et al. 2011). Nevertheless, it is still uncertain what would be the response to more severe or persistent drought.

Extreme droughts may occur naturally throughout the year. Previous studies of plant communities’ response to drought, suggest that the timing of drought is a critical factor determining its impact on vegetation (De Boeck et al. 2011). Our data shows that the timing of drought is also a critical factor controlling belowground responses. Furthermore, we show how different biotic processes control nutrient cycling seasonally, which should exacerbate those previously observed aboveground responses (Burke et al. 2011; Pfeifer-Meister and Bridgham 2007). During the summer, soil water availability is typically high in the region due to frequent monsoonal rain events. By contrast, during spring and autumn less water input from precipitation occurs. For this reason, the resistance of soil microbial activity during the summer (mid-PGS) was significantly lower than that during early and late-PGS (Online resource 8). This shows that the combination of higher temperatures and lower precipitation would have drastic synergistic impacts.
on climate sensitive ecosystem by limiting the recovery ability of microbial communities.

Under drought stress, different ecosystems may have different levels of resistance and resilience. Most existing evidence suggests that temperate grasslands and forests respond to drought similarly, with reductions in productivity, reproductive success and, when the stress is persistent, alteration in structure and species composition (Breda et al. 2006; Gomez-Guerrero et al. 2013; Kahmen et al. 2005; Leithead et al. 2010; Lewis et al. 2004). Mirroring responses expected for vegetation, microbial productivity (MBC) declined in drought treatments. Curiously, the impact of drought on MBC showed higher recovery in summer than in the autumn and spring after treatment interruption (Online Resource 9). The MBC: N response shows that the simulated drought has not only altered microbial activity, but also composition and possible function. Higher ratio of MBC: N indicated the dominance of fungi, which showed significantly higher resilience than other communities (Online Resource 9). The resistance of soil respiration showed a lower stability with positive-effect in early-PGS and negative-effect in mid-PGS, which was consistent with the change of soil microbial biomass.

In conclusion, varying degrees of resistance and resilience of soil microbiota to drought stress were observed at different PGS periods. Significant interactions occurred between moisture and temperature, with the strongest effects observed during the summer, when competition for nutrients is strongest. Reduction of soil water can reduce soil carbon storage and affect CO₂ fluxes seasonally (Joos et al. 2010). Other simulated-drought experiments during warm seasons have shown not only changes in microbial N mineralization but also P mineralization (van Meeteren et al. 2008), and in nutrient-rich soils, such simulations have induced soil acidification (VanHaesebroeck et al. 1997). Here we confirmed that interactions between biotic and abiotic processes govern seasonal C and nutrient dynamics, total soil CO₂ efflux, and enzymatic decomposition of organic matter in Tibetan grasslands. Furthermore, we demonstrated that the timing of drought events is a critical factor in predicting both soil microbial- and plant-productivities, and ecosystem resilience and stability. Our results show the need for a new integrative framework, including biotic and abiotic drivers and responses, to decipher the past and predict the future climate change impacts on terrestrial ecosystems.

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