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Modification in Grassland Ecology under the Influence of Changing Climatic and Land Use Conditions

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Abstract

Grasslands are important terrestrial ecosystems in China, which are mainly distributed in arid and semiarid regions. Based on the multiyear field experiments in the semiarid grassland, the effects of land use practices on grassland above- and belowground community characteristics were investigated. In addition, how the annual climate factors regulate grassland productivity was also studied to detect critical periods for grass growth. Results showed that grazing exclusion increased grassland root biomass, root length density and root surface area with declining plant species richness. After grazing exclusion, with perennial bunchgrasses being predominant in root community all the time, proportion of perennial rhizome grasses increased and proportion of perennial forbs declined. Clipping significantly decreased the annual mean soil respiration and its components. The root respiration was more sensitive to clipping than microbial respiration. Temperature increments during the early stage of the growing season (April–May) were positively correlated with aboveground productivity. However, hot and dry summer (June–July) strongly inhibited aboveground productivity. Impacts of drought and heat in August on productivity were negligible. Increased temperature and precipitation during the senescence period (September–October) and a warmer dormancy phase (November–March) were negatively correlated with productivity in the following year, while precipitation during the dormancy period had no detectable effects.

Keywords: semiarid grassland, grazing exclusion, soil respiration, climate variation, biodiversity, productivity

1. Introduction

Grasslands are among the largest biomes in the world, accounting for nearly 25% of the land surface on earth [1, 2]. Grassland ecosystem plays a key role in balancing the concentrations of global atmospheric greenhouse gases through carbon storage and sequestration [3]. Grasslands

also significantly contribute to food security by providing food for ruminants, which are sources of meat and milk for human consumption. China has nearly 4 million km^2 of grasslands, accounting for 40% of China's total land area and 13% of the world's total grassland [4, 5]. Concurrent with population growth and socioeconomic development, however, China's grasslands have experienced rapid degradation over the last few decades due to climate change and unsound anthropogenic impacts [6, 7]. To combat the grassland degradation and restoration of the environment, the Chinese government has launched batches of national-scale conservation policies during the late 1990s and early 2000s. Two of them, the Grain for Green Program (GGP) and the Grazing Withdrawal Program (GWP) cover most of the grassland regions [8–10]. Restoring degraded grassland ecosystems is critical to the ecological and economical sustainability of these systems.

About 90% of grassland was degraded as a consequence of overgrazing by livestock in China [11]. Overgrazing induced considerable destructive effects on plant community and soil resources [12]. Grazing exclusion has been proven to be a successful practice to restore degraded grasslands throughout the world [13, 14]. Many studies pointed out significant enhancing effects of grazing exclusion on plant coverage, density and aboveground biomass in the early stage, which were diluted or even reversed as grazing exclusion time increased [11, 15]. Meanwhile, grazing exclusion not only significantly increased storage and availability of soil water and nutrients through more litter inputs [14, 16], but also played an important role in structuring community of soil eukaryotes [17]. Contrasted with numerous researches on aboveground responses to grazing exclusion, researches about root responses are largely limited by the studying difficulties and complexity of plant roots. Current studies on fenced grassland root mainly focused on root biomass and its distribution pattern in different types of grassland [12, 18]. Root morphology and/or physiology traits and plasticity have received considerable attentions due to their capability of foraging soil nutrients [19, 20]. There is a considerable difference in root traits and plasticity among different plant species, normally with greater ones in graminaceous species [21]. The hierarchy of root trait values and plasticity among species and plant functional groups in the vegetation could drive early-stage competition for water and nutrients, which ultimately made an effect on vegetative succession [22, 23]. However, major knowledge gaps still exist, concerning responses of plant root morphological traits and root community composition to grazing exclusion in long-term restored grassland.

Soil respiration plays an important role in regulating soil C pools and net C balance in terrestrial ecosystems [24]. The rate of soil respiration can be influenced by climate change (global warming, precipitation regimes, etc.), as well as anthropogenic activity (land use change and management practice), with consequent impacts on terrestrial C cycling and feedbacks to climate change [25, 26]. As one of the common land use practice, clipping or mowing of hay is regarded as a critical component of global change [27]. The effect of clipping on soil respiration had been investigated widely in different ecosystems; however, the results were various and inconsistent with each other [28, 29]. One reason for the variability of previous studies in clipping effect on soil respiration is that soil respiration is composed of two different components. One of the components is root respiration, which refers to the CO_2 emission from plant roots, mycorrhizal fungi and other associated microorganisms

(rhizosphere microorganisms) that depend on the contemporaneous [30]. Another component is microbial respiration, which is defined as the CO_2 emission from the decomposition of plant litter and soil organic matter by soil microorganisms [31]. Substrate sources of the two soil respiration components have different magnitudes, turnover rates and seasonal patterns, which make the two soil respiration components respond differently to climate change and land use practice [27, 32]. In addition, the contributions of root respiration to soil respiration are various in different ecosystems, which may also be responsible for the inconsistent results of the clipping effect on soil respiration [33]. Hence, quantifying the individual changes of root and microbial respiration in response to clipping is imperative for a comprehensive understanding of ecosystem carbon cycling.

Climate-driven variability in grassland productivity impacts the global carbon balance, ecosystem service delivery, and profitability of pastoral livelihoods. Aboveground net primary productivity (ANPP) of grasslands is highly temporally variable, as compared to other ecosystems, such as forest and cropland [34]. Much of the previous work considering the impacts of climate variability on ANPP has focused on annual precipitation and temperature [35, 36]. While the importance of these annual-scale metrics has often been confirmed in studies at regional scales, numerous site-specific reports have indicated that inter-annual variability in ANPP is poorly or even not at all correlated with annual climate conditions [37], with much of the temporal variation in ANPP left unexplained [36]. Changes in precipitation or temperature during certain parts of the year are more relevant drivers of ANPP than annual changes [38, 39], since vegetation production responds differently to climatic variation during different seasons [38, 40]. Future climates are likely to include more frequent extreme weather events and more pronounced seasonal variation in temperature and precipitation.

To provide a new perspective of biodiversity restoration and the basis for management of degraded grassland in semiarid areas, we firstly conducted with a space for time substitution method at Yunwushan National Nature Reserve, a typical steppe grassland on the Loess Plateau with different grazing exclusion timescales to determine effects of grazing exclusion on grassland root biomass, morphological traits and root community compositions in plant functional group level. Then, a clipping experiment was carried out to investigate the effect of clipping on root and microbial respiration. Finally, long-term productivity and weather records since 1982 were collected to examine the impacts of climate variability at different times of the year on grassland productivity.

2. Materials and methods

2.1. Study area

This research was conducted in Yunwushan National Natural Grassland Protection Zone in Ningxia Hui Autonomous Region, China (36°10′-36°17′N, 106°21′-106°27′E, 1800–2100 m a.s.l.). Since 1982, the grassland has had been protected as a long-term monitoring sites for restoration of degraded grassland. The site is located at an elevation from 1800 to 2100 m and has a total area of 6660 ha. Mean annual temperature during 1982–2011 was 7°C with mean monthly

temperature extremes of $-22°C$ in January and $25°C$ in July. Annual precipitation averaged 425 mm. Annual evaporation is 1017–1739 mm, and the frost-free season averages 137 days. Soil type in the study area is montane gray-cinnamon soil. The vegetation community consists of 297 plant species and is dominated by *Stipa* plants (*Stipa bungeana*, *Spectrunculus grandis*, *Salvia przewalskii*), and main forbs include *Artemisia sacrorum* and *Thymus mongolicus*.

2.2. Experimental design and sampling

2.2.1. Grazing exclusion

Five experimental sites along a chrono-sequence of grassland restoration were selected in August 2012, when peak aboveground biomass occurred, with grazing exclusion for 30 years (GE30), 22 years (GE22), 9 years (GE09), 5 years (GE05) and continuous grazing at a medium density during the whole year (four sheep/ha) (GG), respectively. A transect of 300×100 m with representative vegetation was selected as the study area within each site, in which three pseudo-replicated plots (30×30 m) were established, and three subplots (2×2 m) were set up with a minimum interval of 15 m in each plot for field sampling.

2.2.2. Soil sampling

With aboveground plant parts being attached, a soil block of 50 cm long $\times$ 50 cm wide $\times$ 30 cm deep was excavated in each subplot and then was gently loosen by hand to get the intact root-soil mixtures with minimal breakage. Plant root-soil mixtures were soaked in water for twenty minutes and were gently shaken for several times to remove bulk soil.

2.2.3. Plant root sampling

Plant roots were carefully washed under flowing water to remove tightly attached organic matter and mineral soils and carefully identified roots in plant functional group level according to plant aboveground parts, root color, diameter, branches and texture. Five functional groups (PFGs) were categorized as perennial rhizome grass (PR), perennial bunchgrass (PB), PF perennial forbs (PF), shrubs and semishrubs (SS) and annuals and biennials (AB) [26, 41]. Functional group richness and species richness were the number of functional groups and plant root species appearing in one subplot, respectively.

After cutting down plant aboveground parts, roots in the same plant functional groups were spread on a transparent, plastic tray and scanned at a resolution of 300 dpi (Epson Scanner (10000XLPro, Canada). Root images were analyzed with WinRhizoPro software (V2012b, Regent Instruments, Canada) to measure root length (m), root surface area (cm^2). Thereafter, roots were oven-dried at $65°C$ for 48 h and then weighed to gain root mass. Root biomass, root length density, specific root length and specific root area are calculated in equations [42] as follows:

$$Root\ biomass\ (RB,\ g\ m^{-2}) = Root\ mass/Sampling\ area \qquad (1)$$

$$Root\ length\ density\ (RLD,\ mm^{-3}) = Root\ length/Sampling\ volume \qquad (2)$$

$$Specific\ root\ length\ (SRL,\ mg^{-1}) = Root\ length/Root\ mass \qquad (3)$$

$$Specific\ root\ area\ (SRA,\ cm^2\ g^{-1}) = Root\ surface\ area/Root\ mass \qquad (4)$$

2.2.4. Clipping management

The experiment was designed as a randomized block with five replicate blocks. Clipping was done once a year in the spring (June 20, 2014, and June 16, 2015). The trenching method was used in this study to separate soil respiration into root and microbial respiration [43]. In each plot, one root-free small plot (0.3 × 0.3 m) lined with nylon mesh (0.038 mm mesh size) in 0.5 m deep was randomly assigned. Soil respiration and its components were measured using an LI-6400 portable photosynthesis system attached to a soil CO_2 flux chamber (800 cm^3 in total volume; LI-COR 6400-09 TC, LI-COR Inc., Lincoln, NE, USA). The CO_2 efflux measured in the root-free plots reflects only microbial respiration, while CO_2 efflux measured in the whole-soil plots (roots are not removed) resulted from both microbial and root respiration. The difference between the CO_2 efflux values for root-free plots and whole-soil plots was used to indicate root respiration. However, we observed that the soil temperature and moisture in root-free plots were significantly higher than those in whole-soil plots. The actual root respiration would be underestimated if it is directly calculated from the difference of measured CO_2 flux between the whole-soil plot and root-free plot. To eliminate this error, we corrected the measured microbial respiration by using the linear Eq. (5), simulating the relationship between microbial respiration, soil temperature and soil moisture in root-free plots:

$$MR_{measured} = a \times T + b \times W + c \qquad (5)$$

where $MR_{measured}$, T and W are the microbial respiration (μmol CO_2 m^{-2} s^{-1}), soil temperature (°C) and volumetric soil water content (%) measured in the root-free plot, respectively. a, b and c are coefficients relevant to soil temperature and moisture.

Then, we determined the corrected microbial respiration ($MR_{corrected}$) using the soil temperature and moisture in the whole-soil plot. Root respiration (RR) calculated by the difference between the SR and the $MR_{corrected}$ is as follows:

$$RR = SR - MR_{corrected} \qquad (6)$$

Soil temperature at the depth of 5 cm was determined using a thermocouple probe connected to the LI-6400 adjacent to each PVC collar, and volumetric soil content in the 0–10 cm soil layers was measured using a TRIME TDR probe (IMKO, Ettlingen, Germany) adjacent to the same sites after soil temperature measurements. The root length production was measured using the minirhizotrons technique [44]. Peak aboveground biomass (AGB) was estimated by harvesting plant tissues above the soil surface from one 0.5 × 0.5 m quadrats at each plot in late September of both years. After aboveground plant residues cleaned, soil samples to depths of 10 cm were collected. Roots were collected from soil samples to determine belowground biomass (BGB). WSOC was measured using an automated total organic C analyzer

(TOC-Vcph, Shimadzu, Japan) [45]. SMBC was determined using the chloroform fumigation extraction method [46].

2.2.5. Biomass data collection

Field harvest was conducted in mid or late August each year from 1982 to 2011, when the standing biomass reached its maximum. For each harvest in each year, 15 quadrats (1 × 1 m) were selected along a transect (300 × 100 m). Aboveground biomass was clipped and dried at 65°C to constant weight. Between 1982 and 1992, the degraded grassland recovered rapidly and biomass production increased almost linearly. It was mainly caused by the exclusion of human disturbance, particularly overgrazing. After 1992, grasslands assumed a relatively balanced state with lower variation in productivity and diversity. Further variation in productivity was likely caused primarily by climatic variation. We therefore used the peak aboveground biomass during 1992–2011 to evaluate the impacts of climate variability on grassland productivity. Mean daily temperature and precipitation during 1992–2011 were obtained from a weather station established in 1982, located only 0.9 km from the surveyed transect.

2.3. Data analyses

2.3.1. One-way analysis of variance

A one-way analysis of variance (ANOVA) followed by Tukey's HSD test was conducted to determine the effect of grazing exclusion time on grassland root traits (*RB, RLD, RSA*, plant functional group richness, plant species richness), the differences of root traits (*SRL, SRS*) and proportion in root community between plant functional groups, and the effects of clipping over time on soil respiration, microbial respiration, root respiration, soil temperature and soil moisture. Differences were considered significant for all statistical tests at $P < 0.05$. All the statistical analyses were conducted using IBM SPSS 18.0 (IBM, USA). Graphs were created with Sigma plot 12.5 (Systat Software, USA).

2.3.2. Partial least squares

Partial least squares (PLS) regression was used to analyze the responses of grassland productivity to variation in daily temperature and precipitation during all 365 days of the year based on data for 1992–2011. The two major outputs of PLS analysis are the variable importance in the projection (VIP) and standardized model coefficients. The VIP values reflect the importance of all independent variables for explaining variation in dependent variables. The VIP threshold for considering variables as important is often set to 0.8. The standardized model coefficients indicate the strength and direction of the impacts of each variable in the PLS model. The root-mean-square errors (RMSEs) of the regression analyses were calculated to determine the accuracy of the PLS model. In the PLS analyses, periods with VIP greater than 0.8 and high absolute values of model coefficients represent the relevant phases influencing grassland productivity. Positive model coefficients indicate that increasing temperature or precipitation during the respective period should increase ANPP, while negative model coefficients imply negative impacts on productivity.

3. Results and discussion

3.1. Effects of grazing exclusion on grassland root biomass and morphological traits

Results demonstrated that long-term grazing exclusion significantly increased grassland root biomass, root length density and root surface area ($P < 0.05$) (**Table 1**). The improved root biomass was mainly due to the increased aboveground productivity driven by the compensatory growth of dominant plant species after grazing removal [11, 18]. In the absence of herbivores, plants produced more roots to explore soil resource for aboveground growth, inducing increases in grassland total root length and surface area [47, 48]. Besides, our results indicated that the response of plant belowground richness to grazing exclusion followed a hump-like pattern, similar with responses of plant aboveground richness and diversity to grazing exclusion [11, 16], but with an earlier peak in the early-restoration stage (site GE05). Possibly long-term grazing exclusion caused a drastic decrease in bud bank size of forbs, followed with the decline or even disappearance of plant species relying on resprouting from bud bank after disturbance [49].

3.2. Root traits and proportional changes of five plant functional groups after grazing exclusion

Plant *SRL* and *SRS* showed significant differences between five plant functional groups ($P < 0.05$). Grasses had a much higher specific root length and specific root surface area than forbs. In detail, for *SRL*, PB had the highest value of 11.80 m g^{-1}, tripling that of SS (3.46 m g^{-1}), while PR and AB had similar *SRL* value, higher than that of PF (**Figure 1a**). For *SRS*, there were no marked variations among PR, PF and AB, and they were significantly higher and lower than those of SS and PB, respectively (**Figure 1b**). Our results indicated that plant functional groups differed significantly in their proportions ($P < 0.05$) (**Figure 2a–d**). As the predominant plant functional groups, PB and PF accounted for more than 50% in total. Based on root biomass, proportions of PR and PB significantly increased with a significant decrease in PF proportion after long-term grazing exclusion ($P < 0.05$), and SS and AB showed little change ($P > 0.05$) (**Figure 2a**). Based on root length density and root surface area, grazing exclusion significantly increased PR proportion and decreased PF proportion ($P < 0.05$), while PB and SS show little responses to grazing exclusion ($P > 0.05$) (**Figure 2b**, **c**). Interestingly, with the prolonged grazing exclusion years,

Site	Root biomass (g m^{-2})	*RLD* (10^3 m m^{-3})	*RSA* (10^4 cm^2 m^{-3})	PFG Richness	Plant species richness
GG	163.32 ± 11.27 c	1.76 ± 0.19 c	3.04 ± 0.32 b	3.55 ± 0.29	10.78 ± 0.74ab
GE05	172.22 ± 17.07 bc	2.10 ± 0.22 bc	3.37 ± 0.29 b	4.33 ± 0.17	13.00 ± 0.62 a
GE09	170.45 ± 12.35 bc	2.10 ± 0.32 bc	3.43 ± 0.30 b	4.00 ± 0.24	8.56 ± 0.76 bc
GE22	236.61 ± 21.52 ab	3.03 ± 0.29 ab	4.02 ± 0.24 b	3.44 ± 0.24	6.78 ± 0.74 c
GE30	244.41 ± 21.25 a	3.85 ± 0.25 a	5.21 ± 0.30 a	3.56 ± 0.24	7.44 ± 0.90 c

Different lowercase letters indicate significant differences ($P < 0.05$) between five study sites.

Table 1. Root biomass, root length density (*RLD*), root surface areas (*RSA*), plant functional group (PFG) richness and plant species richness in study sites.
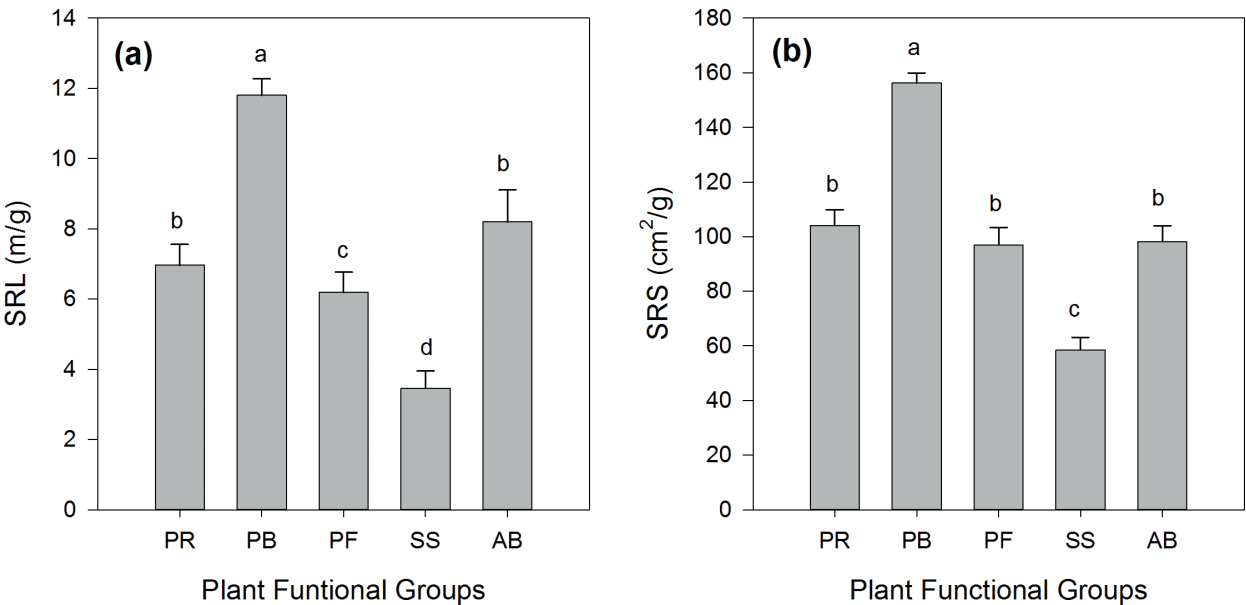
Figure 1. *SRL* traits (a) and *SRS* traits (b) of five plant functional groups. Different lowercase letters indicate significant differences ($P < 0.05$) between plant functional groups.

proportions of PR and PB in plant species richness significantly increased ($P < 0.05$), and those of PF and AB significantly decreased ($P < 0.05$), while SS showed little fluctuation ($P > 0.05$) (**Figure 2d**).

As the guerrilla plant species, PR had advantages in spatial propagation and exploration of adjacent nutrient patches by increasing rhizome and root length after grazing exclusion [50]. Additionally, dispersal by rhizomes allowed temporal release of PR plants from their natural enemies (i.e., root herbivores and pathogens), which stimulated plant growth in return [51]. The compositional changes of plant functional groups mainly resulted from their different responses to improved soil resources after grazing exclusion [52]. Compared with forbs, grasses had a stronger correlation with soil N [16], and grasses' higher *SRL* and *SRS* consolidated their superiority in acquiring soil resources [20]. Given that nitrogen deposition often occurs with accompanying rainfall events, which forms water and nutrient pulses [53], plants with larger root systems (i.e., grasses) gained more benefit than smaller plants at the start of the nutrient pulse [54]. Therefore, our study indicated that the hierarchy of root system size and root traits among five plant functional groups determined grassland root pattern in semiarid grassland after long-term grazing exclusion.

3.3. Effect of clipping on soil respiration

Clipping significantly reduced the mean soil respiration by 14.7% ($P < 0.001$) and 11.4% ($P < 0.05$) in 2014 and 2015, respectively (**Table 2**, **Figure 3a**). Previous research has reported that clipping could decrease the soil respiration in grassland ecosystems, which was most likely due to the restriction of translocation of photosynthate from aboveground plant tissues to roots and rhizosphere microorganisms [31]. In addition, clipping increased soil temperature by 0.6°C ($P > 0.05$)
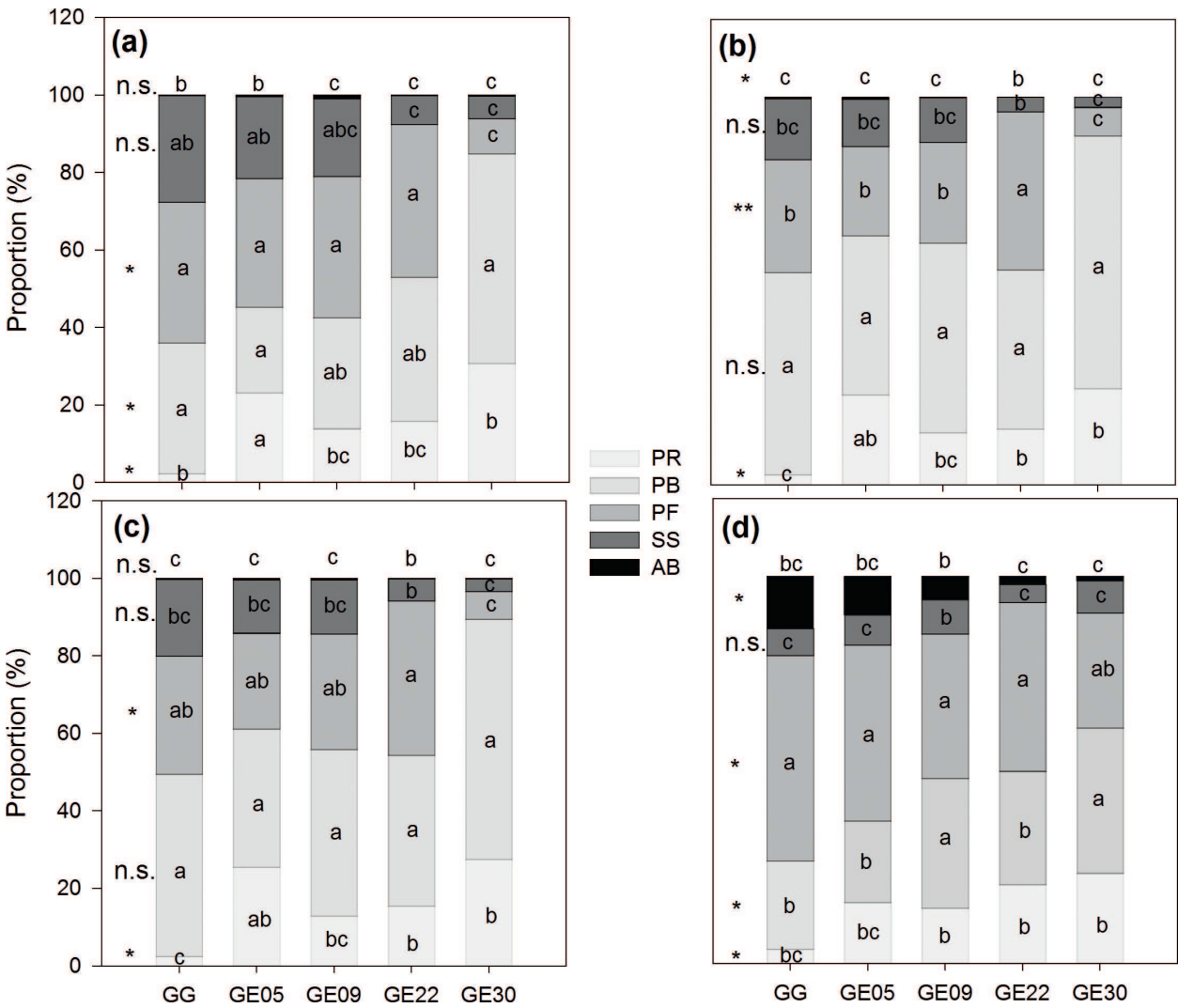
Figure 2. Distribution proportions of five plant functional groups in root biomass (a), root length density (b), root surface area (c) and plant species richness (d) in grazing grassland (GG), grassland with grazing exclusion for 5 years (GE05), 9 years (GE09), 22 years (GE22) and 30 years (GE30), respectively. Different lowercase letters indicate significant differences ($P < 0.05$) between five plant functional groups for each grassland type, and n.s. indicates no significant difference ($P > 0.05$) for each plant functional group between five grassland types; * and ** indicate significant differences for each plant functional groups between five grasslands in $P < 0.05$ level and $P < 0.01$ level, respectively.

Source of variation	SR		MR		RR		ST		SM	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Treatment	<0.001	0.034	0.005	0.008	<0.001	0.092	0.110	0.023	0.305	0.544
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment × time	<0.001	0.011	0.746	0.134	<0.001	0.078	0.289	0.059	0.742	0.286

Table 2. *P*-values of repeated measures ANOVA of total soil respiration (*SR*), microbial respiration (*MR*), root respiration (*RR*), soil temperature (ST) and soil moisture (SM) in a temperate grassland of Loess Plateau.
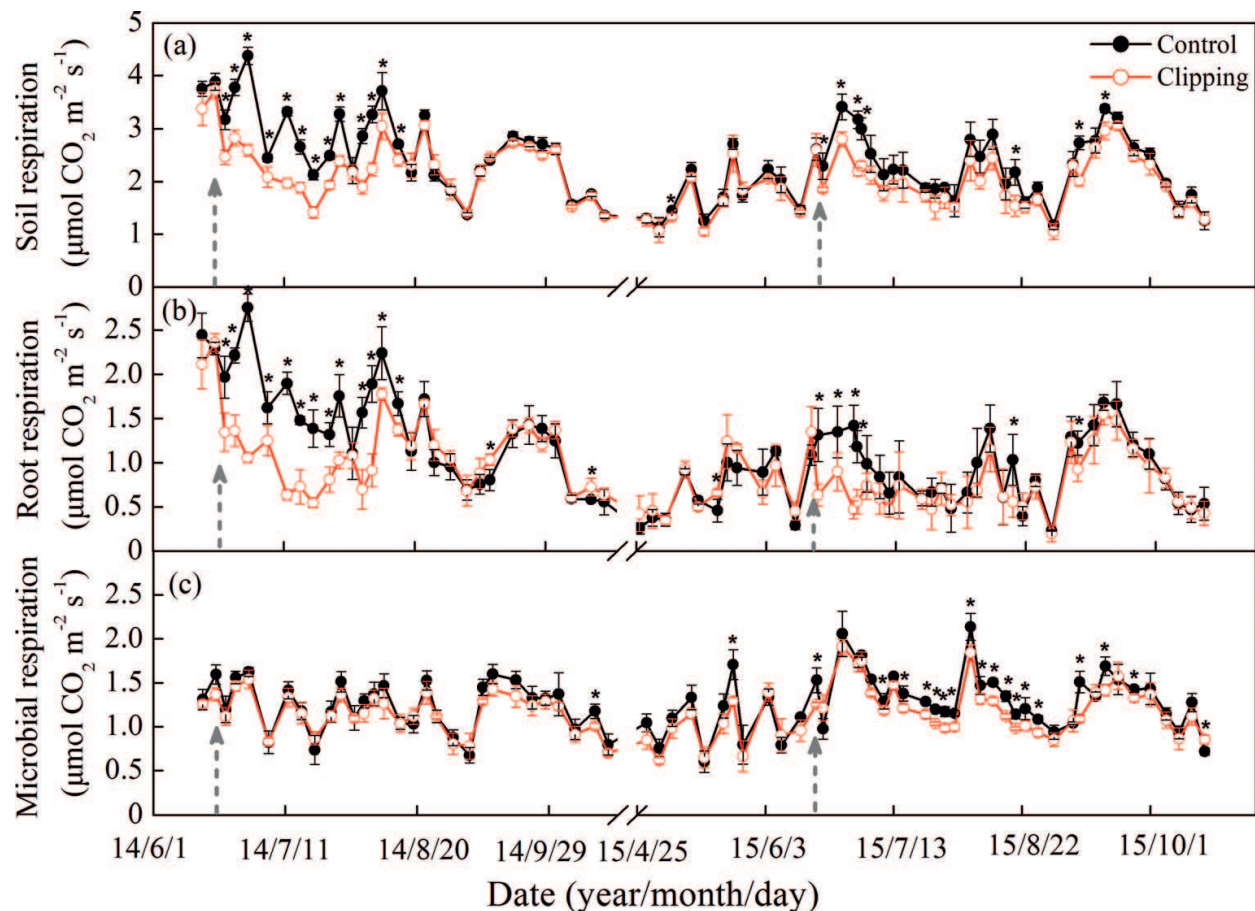
Figure 3. Seasonal variations of soil respiration and its components in the control and clipping treatments. Values are means, standard deviations (*n* = 5). Asterisks denote significant difference (*P* < 0.05) between treatments. Arrows indicate clipping dates.

in 2014 and 1.3°C (*P* < 0.05) in 2015 in our study (**Figure 4**). We speculated that there was a potential increase in soil respiration driven by soil temperature, because higher soil temperature has been reported to stimulate the activities of plant roots and soil microbes [29]. However, the increase of soil respiration due to elevated soil temperature may not compensate for decrease in soil respiration caused by reduced photosynthesis, leading to the decrease in soil respiration after clipping.

3.4. Effect of clipping on root respiration

In the present study, clipping reduced the mean root respiration by 22.1% (*P* < 0.001) and 13.3% (*P* > 0.05) in 2014 and 2015, respectively (**Table 2**, **Figure 3b**). We found a prompt response in root respiration in the first measurements after two days of clipping treatment, following the sharp reduction of 49.2 and 26.4% within two weeks after treatment in 2014 and 2015, respectively (**Figure 3b**). We also found that the sharp decrease in root respiration was consistent with the sudden reduction of root production in the same periods (**Figures 3b** and **5a**). Considering the significant correlation between the root production and root respiration (**Figure 5a**), we attributed the decrease of root respiration after clipping to the limited supply
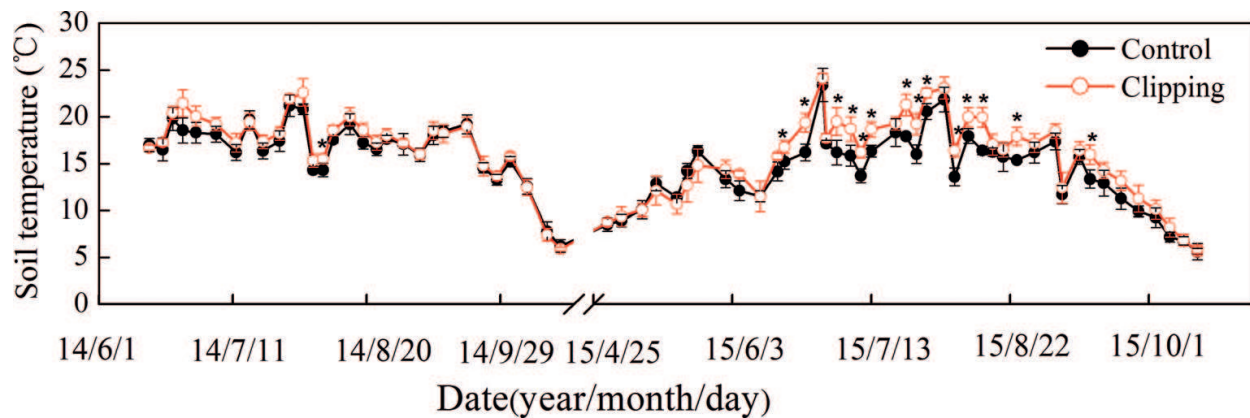
Figure 4. Seasonal variations of soil temperature in the control and clipping treatments. Values are means, standard deviations ($n = 5$).

of substrate for root growth and production. However, in September–October in 2014 and April–May in 2015, a higher root respiration was observed in the clipping plots (**Figure 3b**). Previous studies by Wan et al. [55] and Zhou et al. [33] reported that clipping could stimulate root respiration by promoting plant regrowth and root biomass. In our study, the higher root production observed in clipping plots in September–October in 2014 and April–May in 2015 might be responsible for the higher root respiration in the same periods (**Figures 3c** and **5a**).

3.5. Effect of clipping on microbial respiration

Microbial respiration exhibited relatively constant lower values in clipping plots almost through-out the study period in our study. Clipping significantly reduced microbial respiration by 6.0% ($P < 0.05$) and 9.9% ($P < 0.05$) in 2014 and 2015, respectively (**Table 2**, **Figure 3c**). The main explanation of this result was the reduced supply of labile C for mineralization by soil microor-ganism after clipping [56]. In the present study, clipping reduced the WSOC by 20.6% ($P > 0.05$) and 27.1% ($P < 0.05$) in 2014 and 2015, respectively (**Figure 5b**). The decrease of WSOC might be responsible for the reduction of SMBC in clipping plots in our study (**Figure 5c**), because WSOC was one of the main labile C substrates for soil microorganism. In addition, SMBC was reported to be significantly related to microbial respiration in previous research [57], which was similar to our results (R^2=0.88, $P < 0.05$). Hence, we attributed the decrease of microbial respiration after clipping to the reduction of available C supply for microbial mineralization.

3.6. Response of grassland productivity to variation in daily temperature

Between 1992 and 2011, the average harvest date of peak aboveground biomass for grassland at Yunwushan National Nature Reserve was 15th of August. The 365 daily temperature values between the previous September and August of the year of harvest were used as independent variables in the PLS regression. A low root-mean-square error (RMSE) of 8.13 g m^{-2} for the resulting PLS model indicated that the model was a good fit for the data. Based on the VIP and standardized model coefficients of the PLS analysis, we found that warming during different periods had varied impacts on grassland productivity (**Figure 6**).
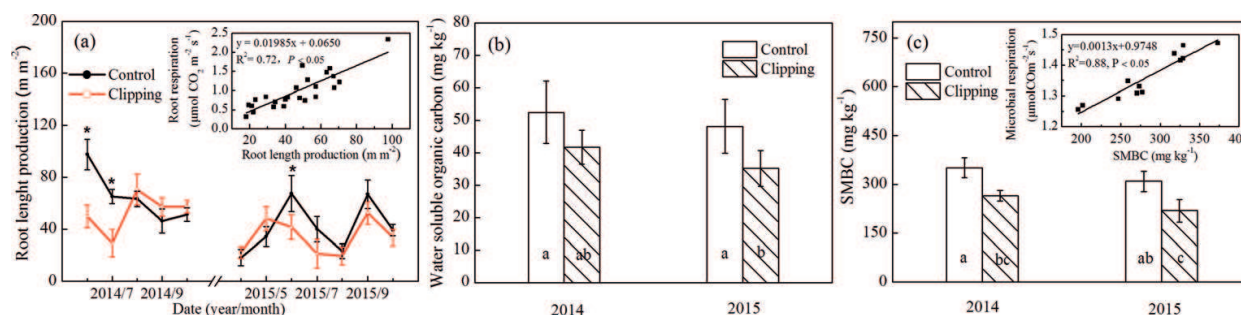
Figure 5. Comparison of root length production (a), water-soluble organic carbon in trenched plots (b) and soil microbial biomass C in trenched plots (c) among treatments. Asterisks and different letters denote significant difference ($P < 0.05$) between treatments. Arrows indicate clipping dates.

Between 30 March and 30 May, model coefficients for temperature analysis (**Figure 6**) were always positive and VIP values mostly exceeded 0.8 (the threshold for variable importance), indicating that warming in April and May increases grassland productivity. During 31 May–1 August, model coefficients were consistently negative and VIP values were mostly important, implying that temperature increase in summer (June–July) depressed productivity, forming a striking contrast with the impacts of spring warming. It was of interest that the relevant periods influencing productivity, as identified by PLS regression, were almost the same as the phases of plant growth (i.e., the early and middle stages of the growing season) at our study area. No obvious impacts of temperature variation in August on grassland productivity were apparent. During September–October (the senescence period for vegetation), most model coefficients were negative, indicating that high temperature at that time was unfavorable for productivity of the following year. During 1 November–29 March, the dormancy period, model coefficients were mostly negative, although this phase also included some short intervals with positive coefficients. This variation might indicate that dormancy for grassland is a complex physiological and ecological process. Moreover, it seems possible that the strength of temperature impacts varies throughout the dormancy period. Taking a broader view at model coefficients and aiming at consistency with established phonological phases, we interpreted the entire period (November–March) as another relevant period during which temperature increases appeared to reduce grassland productivity.

3.7. Response of grassland productivity to variation in daily precipitation

The 365 daily precipitation values between the previous September and August were also used as independent variables in the PLS analysis. The resulting model still proved to be a good fit for the data, with an RMSE of 6.53 g m^{-2}. In contrast to the positive effects of higher precipitation in June and July, increasing rainfall during the senescence period (September–October) and the early growing season (April–May) was correlated with low productivity (**Figure 6**). Similar to temperature effects in August, no significant relationship was found between grassland ANPP and precipitation in August. During the dormancy period, there was no consistent correlation between precipitation and productivity. Positive impacts were almost offset by negative ones.
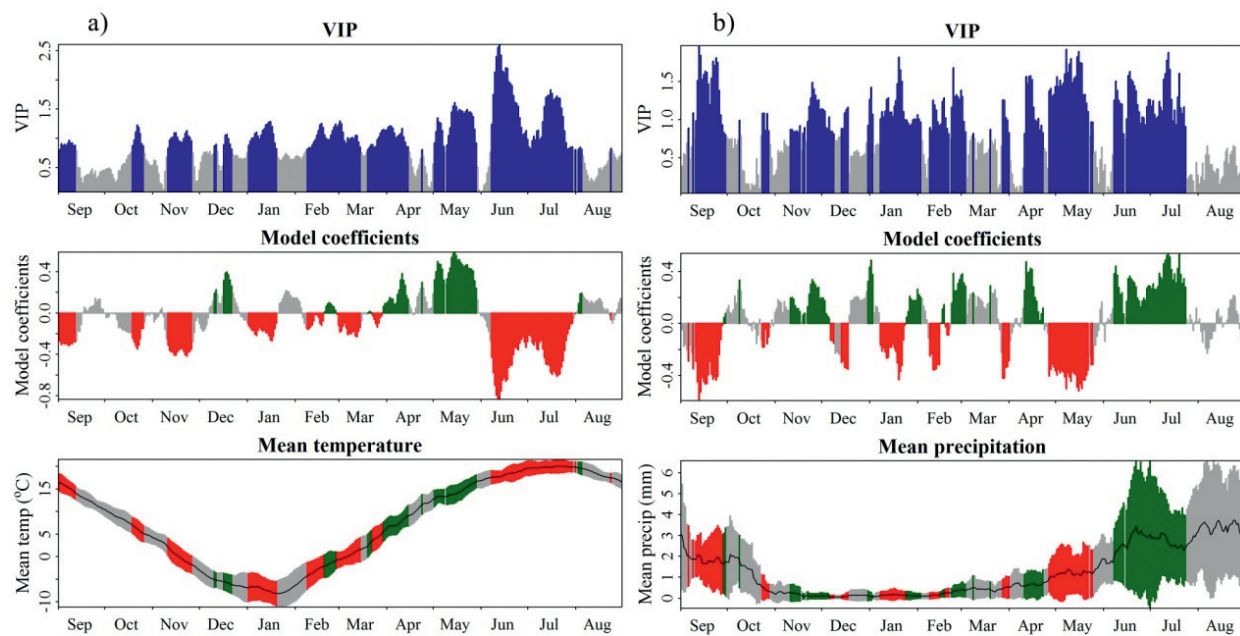
Figure 6. Results of partial least squares (PLS) regression correlating grassland productivity at Yunwushan during 1992–2011 with 15-day running means of (a) daily mean temperature and (b) daily precipitation from previous September to August. Blue bars in the top row indicate that VIP values are greater than 0.8, the threshold for variable importance. In the middle row, red color means model coefficients are negative and important, while green color indicates important positive relationships between grassland productivity and climate variables. The black lines in the bottom panel stand for daily mean temperature and precipitation, while gray, green and red areas represent the standard deviation of daily climate variables.

The increased temperature with reduced precipitation in spring (April–May) could improve grassland productivity. Biomass produced in spring is often believed to be limited by cold temperatures at mid or high latitude [58]. Temperature increases early in the growing season may stimulate plant growth directly by raising leaf temperatures or indirectly by increasing water absorption and N mineralization (**Figure 7**) [40]. Additionally, warmer springs also likely accelerate snowmelt and advance spring greening [59], which might lengthen the growing season and result in increased photosynthesis and carbon acquisition [60]. In contrast to some studies reporting that more precipitation during April–May promoted grassland productivity [39], we found a negative relationship between these variables. To some extent, this discrepancy can be explained by the site hydrology. Frequent winter snow (lasting from November to March) in our study area provides sufficient soil water for plant growth in early spring. The sporadic precipitation during April–May (with an average of 59.5 mm during these two months between 1992 and 2011) may not have important direct impacts on productivity. In contrast, low air and soil temperature, as well as limited solar radiation caused by frequent rain events in May, might partially explain the negative correlations between spring rainfall and grassland productivity.

Warming in summer coinciding with drought can generate physiological stress for plant growth (**Figure 7**) [61], which can explain the reduced productivity in our study area. Moreover, increases in summer temperature can also lower ANPP, perhaps by reducing soil moisture through increased evapotranspiration. Decrease in precipitation amounts and lengthening of intervals between precipitations events during the past 20 years further reduced soil water
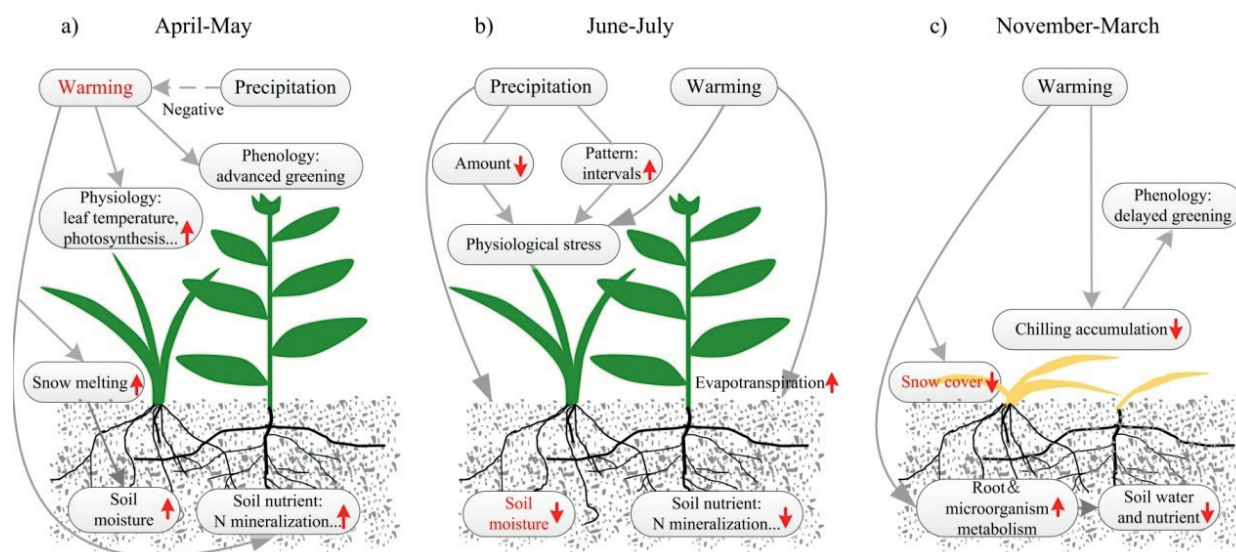
Figure 7. Potential relationships between grassland productivity and climate variability during (a) April–May, (b) June–July and (c) November–March at Yunwushan.

availability in our study region. This is in line with the hypothesis that impacts of climate variation and change on plant productivity might occur via variability in soil moisture [36]. Continuous warming and drought in summer could also affect N mineralization negatively and limit soil resource availability, thereby reducing productivity.

PLS regression did not detect a response of grassland productivity to climatic variation in August. Compared to climate variation during June–July, August shows more variable temperature and precipitation in our study region, although August is cooler on average than July. For instance, the coefficient of variation (CV) of precipitation in August between 1992 and 2011 was 53.3%, while it was only 33.5% for June–July. It is also worth noting, however, that in our study biomass was mostly harvested around the 15th of August, so that the vegetation was only exposed to half a month of August conditions.

Increases in temperature and precipitation during September–October in the previous year were negatively correlated with productivity in the current year, which can be partially explained by the widely reported delays of senescence caused by warming and wetting later in the year [62]. Delay in the senescence period may be related to some extent to increased soil nutrient and water depletion. This would imply that fewer resources may have been available for biomass production in the following year.

While some studies reported that weather during the dormancy period had limited impacts on grassland productivity [38], such effects may become more important, as temperature in winter further increases. Our results indicated that high temperatures during the dormancy period were negatively correlated with productivity. This is consistent with warming experiments in two limestone grasslands in the UK, which showed that winter heating combined with drought reduced the biomass of both communities [63]. Warmer winter can lead to some unanticipated consequences (**Figure 7**). The most direct impacts have been a shortening of the

snow season and a reduction in snow cover, which have been observed in our study area. Declines in the area and depth of snow cover may expose the land surface to more frequent freezing events, exerting negative effects on plant growth. This is supported by observations in northern Scandinavia where extensive areas of vegetation died due to loss of snow cover after extreme winter warming in December 2007 [64]. Increased demands of soil nutrients and water due to accelerated root and microorganism metabolism caused by winter warming might also contribute to the productivity reduction. Finally, variation in spring phenology can also help explain this phenomenon. The timing of spring phenology in most temperate plants results from the interplay of winter cold and spring heat. Plants that evolved in temperate climates fall dormant in autumn to protect themselves from winter freezes and will only resume growth in spring when they have been exposed sufficiently to cold conditions [65]. Temperature increases in spring can advance spring phenology (e.g., greening for grassland), but warming in winter may delay the fulfillment of chilling requirements and thus lead to a slowdown in the advance of spring events or even later onset of spring phenology [65, 66]. The advancing trend in spring greening still dominates climate change responses of plants in our study region so far, since chilling requirements for vegetation are easily satisfied in all winters under the present cold climate with a mean temperature of $-2.6°C$ for the dormancy period. As global warming progresses, especially when rates and effects of warming in winter exceed those in spring, advances in greening might be slowed or even turn into delays. We therefore recommend increased scientific attention to impacts of winter warming on grassland productivity and the timing of spring phenology events.

4. Conclusion

Based on the results of the long-term experiments highlighted in this chapter, grassland root biomass and root morphological traits significantly increased after long-term grazing exclusion, accompanying with significantly declined plant species richness. The higher *SRL* and *SRS* may determine the increased proportion of grasses. The root respiration and microbial respiration exhibited different response patterns to the spring clipping. Compared with the relatively constant lower values in clipping plots almost throughout the study period for microbial respiration, root respiration fluctuated greatly in response to clipping treatment. In addition, soil water content could affect the response of soil respiration and its components to clipping in aspect of magnitudes and resilience in the semiarid grassland ecosystem. PLS regression between ANPP and daily climate variables during the past 20 years successfully delineated how timing of temperature and precipitation variability affected grassland productivity on the Loess Plateau in China. The analysis of productivity responses should account not only for the magnitude of climate variation but also for its timing.

5. Future perspective

The land use practices have substantially improved the above- and belowground ecophysiological processes in the degraded semiarid grasslands. At the site level, plant roots and soil

organisms are two important parts in the plant-soil feedback system, which affect the growth of plants and formation of interspecific competition relationship and vegetation pattern. Therefore, future studies will focus on evaluating the rules of plant-soil feedback during the plant growth process. At the macro level, has climate variation contributed to grassland restoration, and how the LUCC and human activities have affected grasslands in the ecological projects implemented regions? These are still open questions that need to be addressed. These studies will provide insights in effective management measurements for the ecological restoration projects and may serve as guidelines for government and policy makers in adjusting future ecological policies and managing grassland production in the western China for adapting to climate conditions.

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