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Clipping defoliation eliminates the stimulating effects of nitrogen enrichment on the aboveground productivity of an alpine meadow

NING ZONG^{1*}, PEILI SHI^{1,2}

¹Lhasa National Ecological Research Station, Key Laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, P.R. China

²College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, P.R. China

*Corresponding author: zongning@igsnr.ac.cn

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Abstract: To investigate how clipping (CL) regulates the effects of nutrient addition, an experiment, including CL and nitrogen (N) addition, was conducted in an alpine meadow. Nitrogen treatment increased community coverage (48–113% higher than the control) and aboveground biomass (29–117% higher than the control), which was mainly attributed to grass growth. Both N and N + CL treatments showed a tendency to reducing species richness, while significant reduction only occurred in 2016 and 2017 in CL treatment. Clipping showed a tendency to decrease community cover (3–37% lower than the control) and aboveground biomass (2–34% lower than the control), while N + CL treatment had no effect, indicating that clipping can eliminate the simulated effects of N addition. Nitrogen addition significantly increased soil inorganic N (SIN, 528–1230% higher than the control), while SIN in N + CL was 25–48% lower than N treatment. The decrease in stimulated effects in N + CL was attributed to SIN decrease, which resulted from the aboveground biomass removal by clipping. Our results show that clipping can take away aboveground biomass and cause soil nutrients to decrease, which slows down the degraded grassland recovery. This suggests that grazing exclusion may eliminate the effect of nitrogen deposition on aboveground production in alpine grasslands.

Keywords: compensatory growth; nutrient improvement; plant functional group; selective clipping; Tibetan Plateau

Nitrogen (N) is broadly considered as the major limiting nutrient that affects the structure of terrestrial ecosystems on a global scale (LeBauer and Treseder 2008, Fay et al. 2015). Ecosystem vegetation composition, diversity, and function are all modulated by this nutrient resource (Vitousek et al. 1997). Grassland is one of the types of ecosystems that are restricted by nutrients. Studies have shown that the degradation of the grassland community is accompanied by the depletion of soil nutrients (Foster and Gross 1998, Zong and Shi 2019), so the restoration of soil nutrients is an important process for the improvement of degraded grasslands. Therefore, fertilisation is an essential measure in degraded

grassland restoration that can significantly promote productivity. However, the effects of N enrichment depend on the effects of other interfering factors, such as grassland utilization measures.

Clipping is one of the most common land-use practices in grasslands and generally considered to be an important component of global change (Bahn et al. 2006). Selective clipping can cause changes in community structure due to the difference in clipping tolerance among plant species (Hernán et al. 2019). Studies have also shown that moderate clipping can promote compensatory growth, enhance accumulative grassland production, and compensate for greater growth under N input (Siddappaji et al. 2013). Factors

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affecting resource allocation include clipping intensities as well as growth stage, but nutrient conditions in which plants are located are also important factors.

Clipping can affect N distribution patterns, which in turn, regulates N addition effects. Studies have shown that after clipping, N distribution patterns in plants changed rapidly (Schmitt et al. 2013). Plants can increase the distribution of N to the aboveground parts, providing the material basis for aboveground regeneration under moderate clipping intensity (Schmitt et al. 2013). However, more studies also showed that regular clipping could periodically remove aboveground biomass, reduce litter return and photosynthetic products distributing to belowground, which can cause changes in soil physico-chemical properties (Liu et al. 2019). Especially in the process of degraded grasslands recovery, clipping can take away the aboveground part and synchronously take away nutrients from ecosystems, which may, in turn, affect the effects of nutrient addition on grassland restoration. However, there are few studies on the interactive effects of N addition and clipping on grassland restoration, especially in alpine grasslands intensively restricted by N availability.

Alpine grassland is the main ecosystem type on the Tibetan Plateau, and grazing is one of the main practices in grassland utilization (Lu et al. 2017). In addition, N deposition is also an environmental issue on a global scale, also on the Tibetan Plateau. Therefore, studies on how grazing regulates the effects of N deposition are of great significance under future climate change scenarios, and also important

for the improvement of degraded grasslands as well as animal husbandry development on the Tibetan Plateau. Our objective was to evaluate whether and how grazing affects the effects of N addition on alpine grasslands in this study. We conducted a long-term manipulative experiment including clipping and N addition in an alpine meadow ecosystem. We hypothesized that clipping defoliation could reduce soil nutrient content, due to the removal of aboveground biomass, which may impede the effects of N addition on the restoration of degraded grasslands. This research was aimed to provide a scientific basis for the prediction of alpine grasslands under future N deposition scenarios as well as the restoration and improvement of degraded grasslands.

MATERIAL AND METHODS

Site description. This study was conducted at the Damxung grassland station (91°05'E, 30°29'N), Tibet Autonomous Region, China. The altitude is 4 333 m a.s.l., with a semiarid continental climate. The mean annual temperature during this study period was 2.8°C, and the mean annual precipitation was 466.5 mm (Figure 1). The soil is classified as meadow soil with sandy loam, with a depth of approximately 30–50 cm. Detailed soil properties can be found in Zong et al. (2014). The vegetation type is classified as the alpine meadow, with a community cover approximately 30–50%. The dominant species are *Kobresia pygmaea* C.B. Clarke var. *pygmaea*, *Carex montis-everestii*, and *Stipa capillacea* Keng.

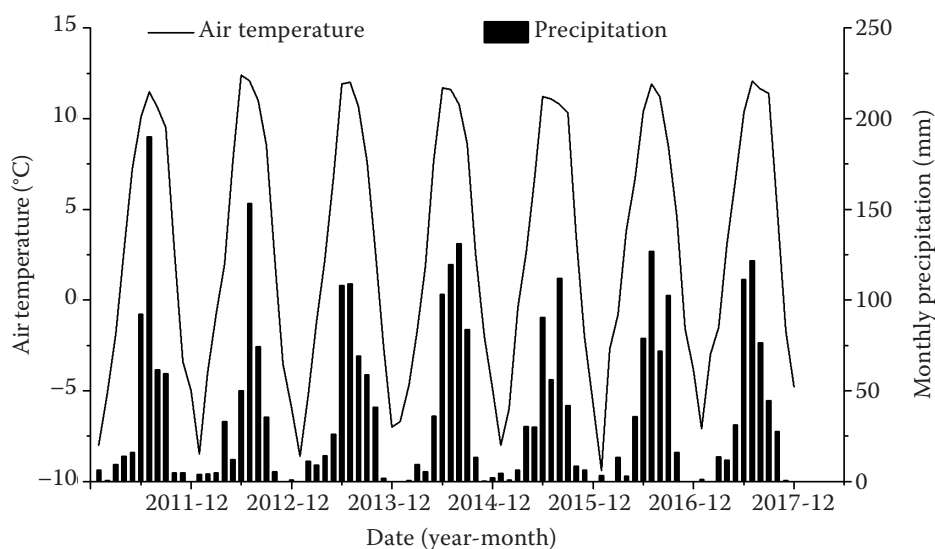


Figure 1. Inter-annual variation of mean annual temperature and monthly average precipitation during the study period in Damxung grassland station

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Experimental design. An area of 20 m × 20 m alpine meadow was selected for N fertilisation and clipping experiment since July 2010. A randomized block design was used for the experimental arrangement. Four blocks were established, and four 3 m × 3 m split plots were set up in each block, with 16 plots in total. Four treatments in each block were randomly assigned for the control (CK), N addition (N), clipping (CL), and N addition combined with clipping treatments (N + CL). The N addition rate was 40 kg N/ha/year, roughly equal to four times of the current N deposition rate (10 kg N/ha/year) (Zong et al. 2016), corresponding to the projected atmospheric deposition rate in this region till 2050 (Galloway et al. 2004). Two-meter aisles were left as buffering areas between the adjacent plots.

The CL and N + CL plots were clipped twice in early June and late July every year. Plants were manually clipped to an approximately 5-cm height above the ground, according to light grazing intensity outside the fence. In addition, only plant functional groups with palatable edibility for livestock were clipped, including grasses and sedges. The clipped aboveground materials were collected, oven-dried at 65 °C for over 48 h and weighed to determine the aboveground biomass removed by clipping. The clipped aboveground biomass in CL and N + CL treatments was 6.64–11.25 g/m² and 5.27–14.89 g/m² from 2011 to 2017, respectively. Nitrogen fertiliser was sprayed as aqueous NH₄NO₃ solution (18 L in each plot) in early June and early August each year. Water addition accompanied by N fertiliser represented a 6-mm precipitation increase, approximately 1.2% of the annual precipitation, which is well within the magnitude of inter-annual variations (Zong et al. 2014). The same amount of water was also added to the no N addition plots.

Sample collection and analysis. From 2011 to 2017, community surveys were conducted during the peak growing season (mid-August) in every year. The subplots used for community surveys were 0.5 m × 0.5 m, evenly divided into twenty-five 0.1 m × 0.1 m grids by strings to determine the total community coverage and the coverage of each plant species. We manually counted the richness of each plant species. After that, we also manually cut the aboveground part of each plant species, placed them in page bags, and brought them back to the laboratory. We placed them in an oven at 65 °C for 48 h to constant weight and calculated as community aboveground biomass per square meter. We classified all species in the

community into three functional groups: grasses, sedges, and forbs. Grasses are mainly composed by *S. capillacea* Keng, *Stipa purpurea*, and *Poa crymophila* Keng, and sedges are mainly composed by *K. pygmaea* C.B. Clarke var. *pygmaea* and *C. montis-everestii*. Compositae, Rosaceae, Chenopodiaceae plants and other families were all classified as forbs. Five soil cores were collected in four corners and the center of the quadrat using an auger (3.8 cm in diameter, 0–15 cm in depth) after plant material collection in mid-August in 2011, 2013, 2015 and 2017. These cores were mixed as a composite sample and immediately passed through a 2-mm sieve to remove plant roots, gravel, and stones. Nitrate and ammonium N in soil samples were extracted using 2.0 mol/L potassium chloride (KCl) solution, filtered, and analysed in a continuous flow analyzer (AA3, SEAL Analytical, Norderstedt, Germany).

Statistical analysis. Statistical analyses were performed using the SPSS 16.0 software package (SPSS, Chicago, USA). A repeated two-factor analysis of variance (ANOVA) followed by Duncan's multiple comparisons (sampling year as a repeated factor) was used to detect the effects of N addition and clipping on community coverage, species richness, Shannon-Weiner index and aboveground biomass, the aboveground biomass of different functional groups and their proportions to total community. We also used Duncan's multiple comparisons to test the differences in the average aboveground biomass and their proportions. Multiple comparisons were also used to detect the differences in soil N content between treatments. Linear regression was used to analyse the relationships between soil inorganic N content and community coverage, aboveground biomass of community, grasses and forbs, as well as the proportion of grasses and forbs. Statistical significance was $P < 0.05$. All the figures were produced using Origin Pro 8.0 (OriginLab Corporation, Northampton, USA).

RESULTS AND DISCUSSION

Community structure and plant production. Nitrogen addition significantly increased community coverage (Figure 2, Table 1). CL treatment showed a tendency to reducing community coverage and reached significant levels in 2011, 2013 and 2016, which was consistent with the previous results on the Tibetan Plateau, indicating that grazing can decrease plant community aboveground biomass

and soil nutrient content (Fu and Shen 2017b; Lu et al. 2017). N + CL treatment increased community coverage only in 2012 and 2017 (Figure 2). Similarly, N treatment increased aboveground biomass in most sampling years (Figure 2, Table 1). This result is also consistent with the previous results of a meta-analysis on the Tibetan Plateau, indicating that N addition significantly increased plant community biomass by 29.7% (Fu and Shen 2016). N + CL treatment had no effect on aboveground biomass (Figure 2). These results are consistent with other studies (Bowman et al. 1993, Song et al. 2012) and suggest three conclusions. First, N was limiting in this alpine meadow, because N addition significantly stimulated ecosystem production. Second, we did not find strong evidence for N saturation, consistent with the N saturation threshold estimation (Zong et al. 2016, 50 kg N/ha/year), because the N addition level was 40 kg N/ha/year. So the results that N addition in this experiment did not inhibit ecosystem production were reasonable and interpretable. Third, the magnitude of aboveground biomass increase varied greatly among years, suggesting that the impacts of N addition on plant communities were modulated by other factors such as climatic conditions. These contrasting effects of clipping and nutrient addition on the plant were also observed at the individual and population levels in other studies (Wu et al. 2010), which indicates that clipping could modify the responsive patterns of plants to nutrient enrichment.

In the first few years, each treatment did not affect plant species richness, and significant changes occurred in the fifth year (2014). Only CL treatment significantly reduced plant species richness in 2016 and 2017 (Figure 2). Similar to plant species diversity, both N and N + CL treatments showed significant decreasing trends of the Shannon-Weiner index from the fifth year of treatment (2014), while CL treatment showed a significant downward trend in 2015 (Figure 2). Shannon-Weiner index under N treatment also showed a significant decreasing trend from the fifth year (2014), which is consistent with other studies (Hautier et al. 2009, Bai et al. 2010). The competition between plants from belowground for soil mineral resources converts to aboveground for light under fertilisation. Generally, competition for light between plant species reduces species diversity of plant communities either for aboveground or belowground light (Hautier et al. 2009, Ren et al. 2010). In addition, the changes in individual density after fertilisation can also induce species richness changes (Stevens and Carson 1999).

Table 1. Repeated measure ANOVA analysis on the effects of the year (Y), nitrogen addition (N) and clipping (CL) on community coverage, species richness, Shannon-Weiner index, community aboveground biomass (AGB), AGB of grasses, sedges, other forbs, and the proportion of different functional groups to the total community

	Tests of between-subjects effects						Tests of within-subjects effects							
	N		CL		N × CL		Y		Y × N		Y × CL		Y × N × CL	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Coverage	206.318	< 0.001	150.884	< 0.001	42.454	< 0.001	23.760	< 0.001	5.524	< 0.001	4.620	0.003	4.094	0.001
Species richness	7.911	0.016	0.013	0.912	2.848	0.117	46.672	< 0.001	7.929	< 0.001	1.260	0.286	2.986	0.012
Shannon-Weiner index	11.853	0.005	1.979	0.185	0.524	0.483	15.758	< 0.001	3.972	0.002	4.037	0.002	2.398	0.070
Community AGB	52.276	< 0.001	66.409	< 0.001	36.309	< 0.001	13.854	< 0.001	5.598	0.004	3.474	0.005	1.626	0.152
grasses	89.367	< 0.001	64.738	< 0.001	42.416	< 0.001	37.788	< 0.001	13.897	< 0.001	4.530	0.013	3.119	0.009
sedges	0.786	0.393	20.771	0.001	2.654	0.129	13.140	< 0.001	2.856	0.052	1.097	0.363	2.371	0.088
other forbs	0.001	0.976	1.384	0.262	0.033	0.860	34.600	< 0.001	24.375	< 0.001	5.882	< 0.001	0.963	0.454
Proportion of to total community	105.903	< 0.001	56.888	< 0.001	19.619	0.001	62.281	< 0.001	16.692	< 0.001	9.854	< 0.001	0.979	0.446
sedges	3.767	0.076	8.400	0.013	7.276	0.019	10.643	< 0.001	2.029	0.073	1.999	0.077	3.958	0.018
forbs	62.503	< 0.001	61.142	< 0.001	6.404	0.026	49.286	< 0.001	16.446	< 0.001	15.045	< 0.001	2.302	0.105

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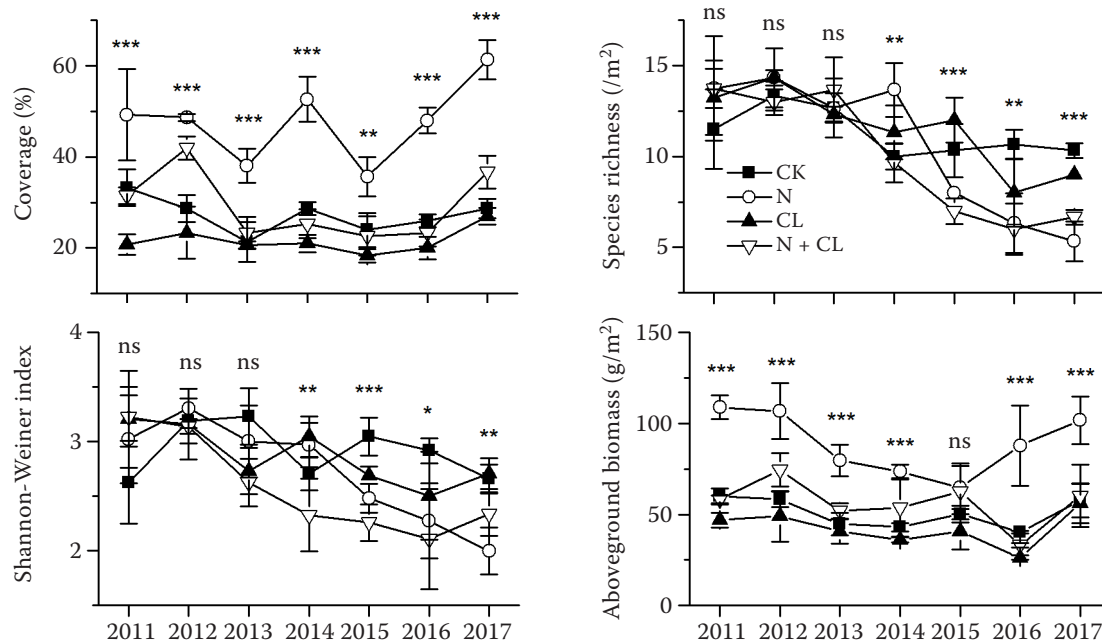


Figure 2. Effects of nitrogen addition (N) and clipping (CL) on community cover, species richness, Shannon-Weiner index, and aboveground biomass. CK – control; N – N addition; CL – clipping; N + CL – N addition combined with clipping treatment. The error bars represent the standard deviation (SD, $n = 4$). ns – not significant ($P \geq 0.05$); $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

Nitrogen addition can increase the productivity and size of individual plants (especially grasses) induced by soil available nutrient increase, which can result in the reduced density and cause rare plant species loss. This is verified by the stimulated growth of grasses under N addition. Meanwhile, the productivity increase caused by fertilisation can also increase litter amount. This can affect the germination of seed and the survival of seedlings caused by the decreased circulation of surface air and light reduction in the underlying plants (Li et al. 2009, Zhang et al. 2010). In addition, N + CL treatment showed a tendency to reducing species richness. Frequently selective clipping to certain kinds of plant species could reduce the photosynthesis products transferring from aboveground to plant roots and decrease the regeneration capacity (Wu et al. 2010), which would cause the clipped plant species to wither and decrease plant species richness.

Plant functional groups. The most conspicuous effects of N addition were the shifts in different plant functional groups, with grasses becoming increasingly dominant in plant community, while forbs showed opposite trends (Figures 3–5, Table 1), which was also consistent with the previous meta-analysis on the response of alpine plants to N addition on the Tibetan Plateau (Fu and Shen 2016). N + CL treatment only showed significant effects in 2017 and

had no effect on the average aboveground biomass. CL treatment had no effect on both the annual and the average aboveground biomass and proportion (Figures 3 and 4). Although significant effects of forbs occurred in some sampling years, the average showed no difference (Figure 3). However, CL treatment increased the proportion of forbs to the total community (Figure 4). As a nitrophilous species, rhizomatous grasses are better adapted to N addition and have acquisitive resource-use strategies (Bai et al. 2010). As a faster-growing and tall species, grasses occupy the upper part of the community canopy and outcompete other species for light (Hautier et al. 2009). Furthermore, the fibrous root systems of grasses have greater abilities to compete for soil water and nutrient resources (Wang et al. 2012). As a kind of palatable forages, the increasing growth in grasses is beneficial to the recovery of degraded rangelands and the development of livestock husbandry.

However, sedges were not so responsive to N addition. The significant effects occurred from the fifth year of the treatments. Both CL and N + CL showed a tendency to decreasing sedge aboveground biomass, and this also can be seen from the average aboveground biomass, while N treatment had no effect (Figures 3 and 5). However, all the treatments had negative effects on the proportion

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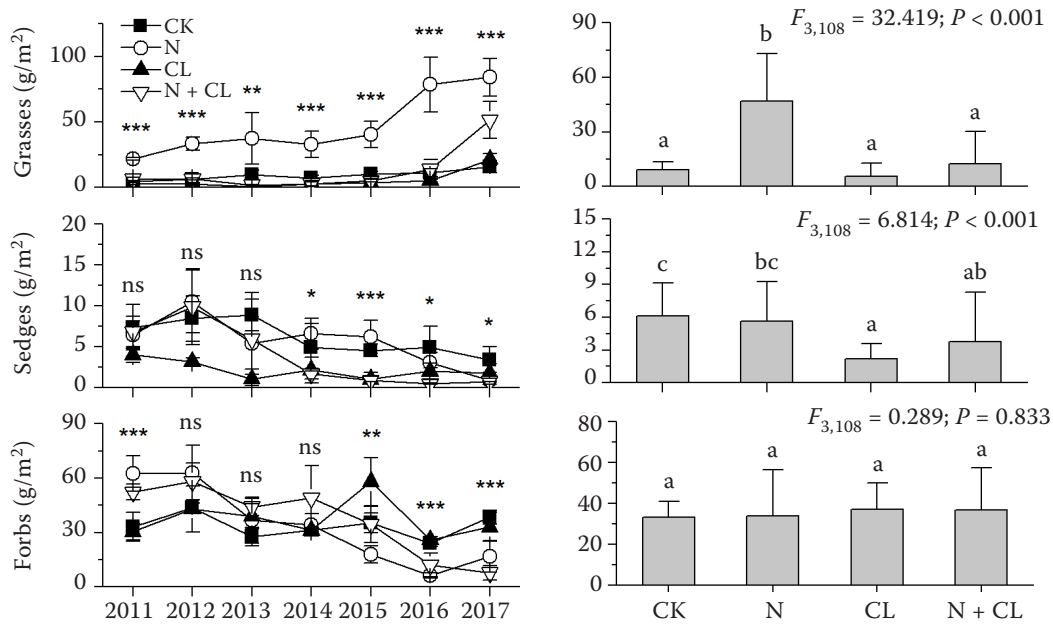


Figure 3. Effects of nitrogen addition (N) and clipping (CL) on the aboveground biomass of grasses, sedges, and forbs in different sampling years, as well as the average aboveground biomass from 2011 to 2017. Different lowercase letters on the bars represent significant differences among different treatments ($P < 0.05$). CK – control; N – N addition; CL – clipping; N + CL – N addition combined with clipping treatment; ns – not significant ($P \geq 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

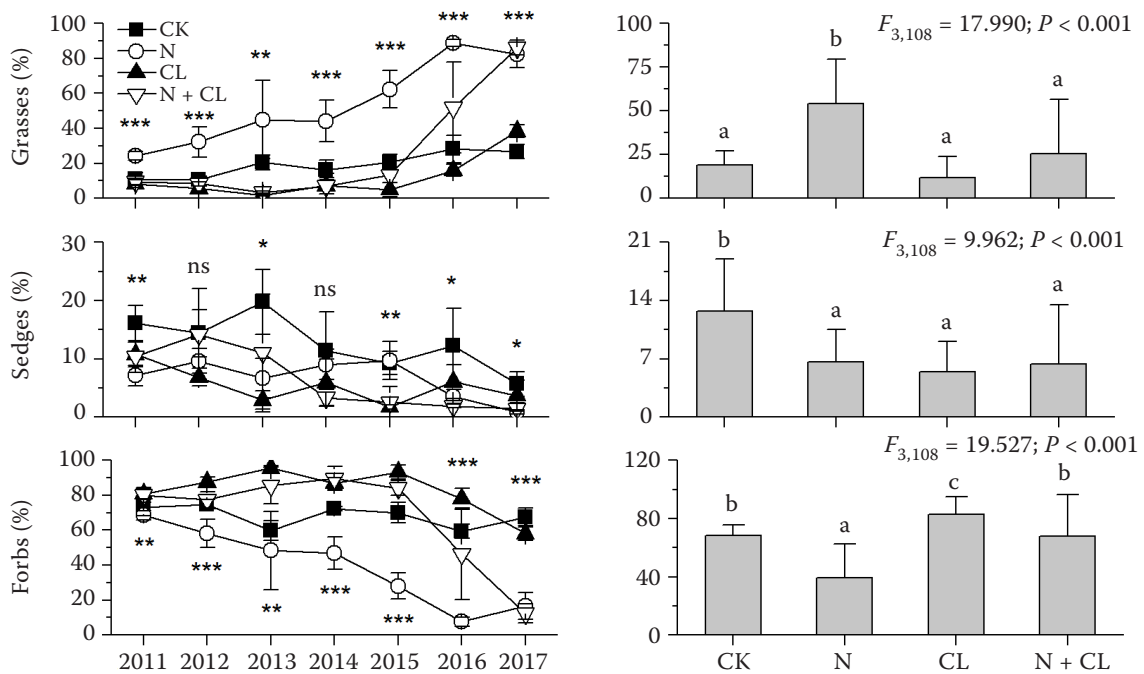


Figure 4. Effects of nitrogen addition (N) and clipping (CL) on the proportion of grasses, sedges, and forbs to the total community in different sampling years, as well as the average proportion during 2011 to 2017. Different lowercase letters on the bars represent significant differences among different treatments ($P < 0.05$). CK – control; N – N addition; CL – clipping; N + CL – N addition combined with clipping treatment; ns – not significant ($P \geq 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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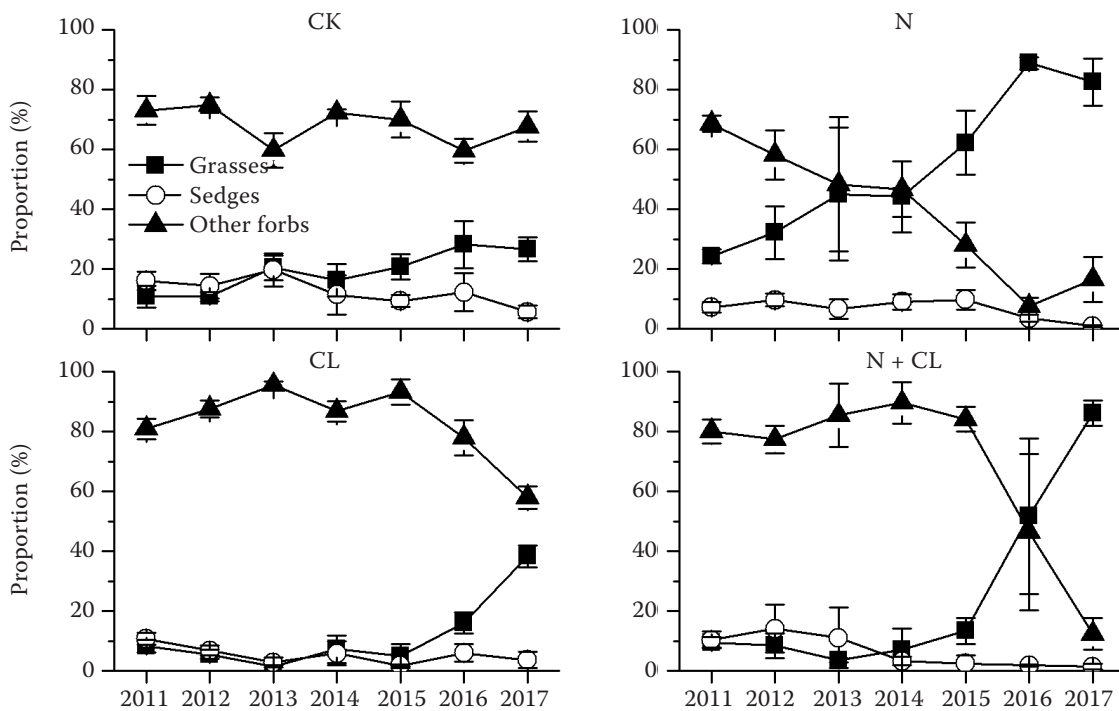


Figure 5. The proportion of grasses, sedges, and forbs to the total community under different treatments. CK – control; N – nitrogen (N) addition; CL – clipping; N + CL – N addition combined with clipping treatment

of sedges to the community (Figure 4). With dense cluster roots, sedges generally respond differently to N addition compared with other plant species. Studies in Northwestern Caucasus reported that sedge plants responded intensively to N addition (Onipchenko et al. 2012), while studies in Ellesmere Island showed that sedges only responded positively to N combined with phosphorus fertiliser (Henry et al. 1986). These divergent responses may be attributed to the differences in other factors because nutrient additions had different effects in physiognomically similar communities under different water conditions (Bowman et al. 1993). In addition, our study also indicates that sedge growth is not only limited by N availability and long-term N addition may have inhibiting effects in these semi-arid alpine grasslands. These results support the functional-based hypothesis that N enrichment benefits species with acquisitive resource-use strategies and excludes those with conservative resource-use strategies (Suding et al. 2005).

Correlation analysis between soil nutrient and community change. Nitrogen addition significantly increased soil inorganic N (SIN), while CL treatment had no significant effect, which was also consistent with the previous meta-analysis on the response of alpine soil to N addition and grazing on the Tibetan

Plateau (Fu and Shen 2017a, Lu et al. 2017). N + CL treatment also significantly increased SIN, but SIN in N + CL treatment was 25–48% lower than N addition (Figure 6), which suggests that clipping decreased SIN under N addition. Other studies also showed that nutrient concentration in plant aboveground parts and soil were decreased by clipping treatment (Fu et al. 2012, 2015, Liu et al. 2019), and clipping could also alter the response of biomass production

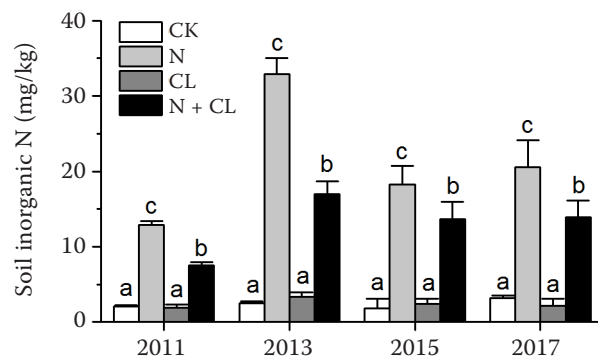


Figure 6. Soil inorganic nitrogen (N) content under different treatments in 2011, 2013, 2015 and 2017. Different lowercase letters on the bars represent significant differences among different treatments ($P < 0.05$). CK – control; N – N addition; CL – clipping; N + CL – N addition combined with clipping treatment

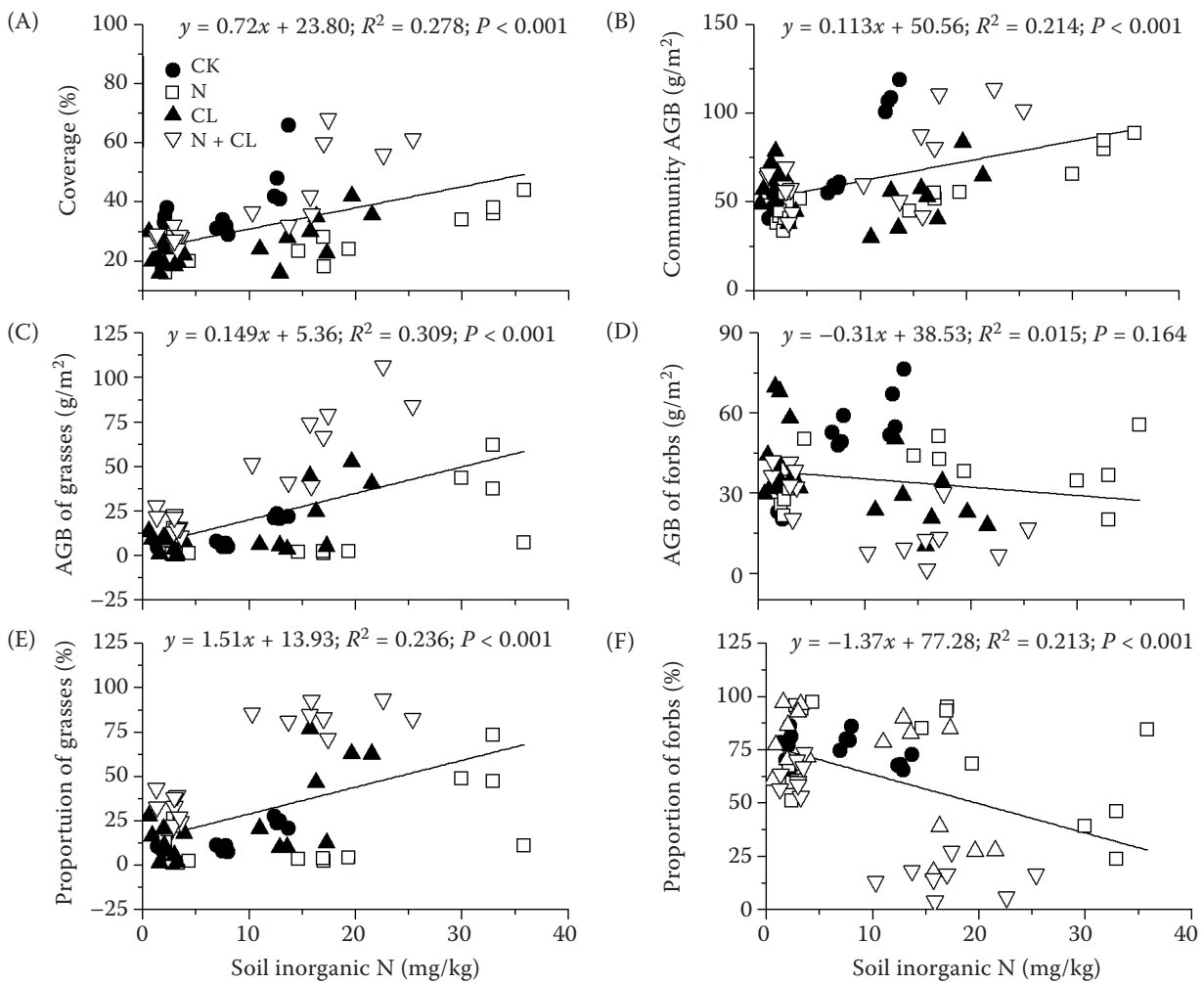
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Figure 7. Correlation analysis between soil inorganic nitrogen (N) content and community coverage, aboveground biomass (AGB) of community, grasses, and forbs as well as the proportion of grasses and forbs. CK – control; N – N addition; CL – clipping; N + CL – N addition combined with clipping treatment

to experimental warming by reducing soil N availability (Fu et al. 2015). Correlation analysis showed that there were positive correlations between SIN and community coverage as well as aboveground biomass. Meanwhile, the biomass and the proportion of grasses showed significant increasing trends with SIN. Although there was no significant correlation between forb biomass and SIN, the proportion of forbs decreased significantly with SIN increase (Figure 7). We also analysed the correlation between SIN change and aboveground biomass change (compared with CK) under N and N + CL treatments, which showed that they were positively correlated with each other (Figure 8). These results indicate that the difference in the stimulating effects in N + CL treatment compared with N treatment was attributed to SIN decrease, which was caused by biomass removal under

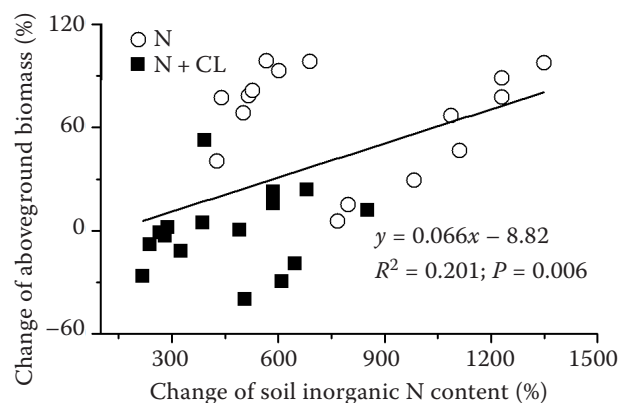


Figure 8. Correlation analysis between the changes of soil inorganic nitrogen (N) content and the aboveground biomass under N and N + clipping (CL) treatments compared with those in the control

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clipping. This verified in the correlations between the changes of SIN and aboveground biomass under N and N + CL treatments (Figure 8). This suggests that grazing exclusion is an initial necessity when fertilisation is used to restore degraded alpine grasslands. We also can infer that grazing exclusion may eliminate the effect of nitrogen deposition in this alpine grassland under future global change scenarios on the Tibetan Plateau.

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