Effects of *Stellera chamaejasme* removal on ~~the~~ plant community composition and nutrient stoichiometry of *S. chamaejasme*-dominated grasslands in the Qinghai-Tibetan Plateau

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Abstract

Background. Controlling *Stellera chamaejasme* is one of the main methods used to recover degraded grasslands in the Qinghai-Tibetan Plateau. ~~Limited~~ We know little about~~studies have been conducted on~~ the changes of plant ecological stoichiometry ~~of plants~~ during grassland~~this~~ recovery~~ period~~, especially in different ~~plant~~biological organization levels (species, functional group, and community levels).

Methods. ~~This study~~We investigated the effects of *S. chamaejasme* removal on ~~ecological stoichiometry by estimating the~~ C:N:P stoichiometry in different biological organization levels of the ecosystem. Factors influencing the ecological stoichiometry, such as biomass and soil nutrients, were also investigated.

Results. ~~Our results showed that f~~For the plants that became dominant after *S. chamaejasme* removal, N content decreased and their C:N increased. *S. chamaejasme* removal significantly affected the nutrient stoichiometry of different functional groups. In the *S. chamaejasme* removal sites (SR), ~~Specifically,~~ Gramineae ~~in the *S. chamaejasme* removal sites (SR) had~~ decreased N content and N:P, and increased C:N; ~~however,~~while forbs ~~had~~ increased N content, C:P, and N:P, and decreased P content and C:N~~, when compared to those at the control site (CK)~~. At the community level, the N content was lower and the C:N higher in SR communities compared to CK. ~~In addition, examination of plant performance showed~~There was an increase in biomass and decline in ~~community~~species diversity after *S. chamaejasme* removal. The N content ~~of the plant~~at community level ~~community~~ was positively correlated with soil total N content. Overall, ~~this study found that~~ *S. chamaejasme* removal increase~~s~~d the production and decrease~~s~~d ~~the~~ species diversity of alpine grassland, and ~~influences~~affected community composition and ~~the~~ plant stoichiometry~~ of grassland species, functional groups, the community, and~~ from species to community level~~-community composition~~. These results provide insight ~~into the role of~~for the restoration of *S. chamaejasme*-dominated grasslands~~ in ecological protection and conservation,~~. ~~and the conclusions~~Findings from this study will be ~~used to~~useful for developing effective and sustainable measures for *S. chamaejasme* control in the

Qinghai-Tibetan Plateau.

Keywords *Stellera chamaejasme*, ~~plant removal, nutrient~~ecological stoichiometry, Qinghai-Tibetan Plateau, toxic weed removal, community composition

Introduction

Ecological stoichiometry is used to examine the relationships between organisms and an ecosystem structure and function, and reflects the dynamic balance of multiple key elements in an ecological system (*Elser et al., 1996; Elser et al., 2000; Sterner & Elser, 2002*). In terrestrial ecosystems, carbon (C) is an essential part of the structural composition of plants (*Liu et al., 2011; Yang, Liu & An, 2018*); nitrogen (N) is a key component of the protein associated with plant production, photosynthesis, and litter decomposition (*Chen et al., 2016; Daufresne, 2004*). Phosphorus (P) is regarded as a limiting element that makes up DNA and RNA, and is responsible for cell membrane structure (*Bai et al., 2012; Naeem & Li, 1997; Yang, Liu & An, 2018*). ~~In any ecosystem,~~ C:N:P stoichiometry plays an ~~influential~~important role in ~~key~~regulating fundamental ecological processes, including ~~but not limited to plant-herbivore-predator relationships~~trophic interaction (*Kagata & Ohgushi, 2006; Tibbets & Molles, 2005*), community assembly~~ecosystem-specific composition and diversity~~ (*Güsewell et al., 2005; Olde Venterink et al., 2003*), ~~and the capacity of a system to~~ plant adaptation to environmental stress (*Sardans et al., 2008; Sardans, Rivas-Ubach & Peñuelas, 2012; Song et al., 2015*). The C:N and C:P ratios in the plant represent the capacity of plant to assimilate C while simultaneously capturing N and P, and the N:P ratio indirectly represents the dynamic balance between soil nutrients and plant nutrition requirements (*Güsewell, 2004; Koerselman & Meuleman, 1996; von Oheimb, et al. 2010*). For plants in grassland ecosystems, assimilation of atmospheric CO_2 via photosynthesis is the main C source, while N and P mainly come from soil and thus can be easily affected by the external environment. Therefore, plant N and P content are the main factors influencing C:N and C:P ratios (*Hedin, 2004; Jiang et al., 2012*). Plant C:N:P ratios ~~change significantly when the availability of one or more of these~~are sensitive to the changes of soil nutrient availability ~~elements change in soil~~ (*Sardans, Rivas-Ubach & Peñuelas, 2012*). While scholars have examined the stoichiometric traits of plants in alpine grasslands (*Chen et al., 2016; Fan, Harris & Zhong, 2016*), few have studied the stoichiometric response of plants to community composition change.

C:N:P stoichiometry is species-specific and reflects the ability of a plant to capture resources under competition (*Berendse, 1998; Ehrenfeld, 2003; Vinton & Burke, 1995*). Plant stoichiometric traits can be ~~influenced~~affected by its neighboring species and the richness of the ecosystem (*Abbas et al., 2013; Borer et al., 2015; Guiz et al., 2018*). Plant communities can be described using a combination of the adaptation of a species to a specific environment and inter-species competition (*Yang, Liu & An, 2018*). Because there is a wide diversity in innate characteristics between plant species, such as life-history, physiology, and tissue chemistry, each has a unique influence on C, N, or P cycling and their stoichiometry in an ecosystem (*Ehrenfeld, 2003; Eviner, 2004; Scott, Saggar & McIntosh, 2001*). Aside from their innate characteristics, plants can influence C, N, and P cycling and stoichiometry by modifying the biomass, composition and/or activity of the soil microbial community (*Bezemer et al., 2006; Ehrenfeld, 2003; Groffman et al., 1996; Sun et al., 2009*). At the plant community ~~scale~~level, nutrient composition, and stoichiometry is influenced by ~~the diversity of plants and consumers~~species diversity and composition (*Abbas et al., 2013; Ebeling et al., 2014; Zhang, Han & Elser, 2011*). Stoichiometric homeostasis describes the capacity of an organism or ecosystem to maintain its internal elemental balance regardless of resource supply (*Borer et al., 2015; Sterner & Elser, 2002*); therefore, it is crucial to examine C:N:P stoichiometry at not only the species level but also at the community level. Previous studies in high altitude grasslands have found that net plant–plant interactions will shift from competitive to facilitative in response to environmental change (*Bret-Harte et al., 2004; Callaway et al., 2002*). While plants with different life strategies will compete for limited resources (water, light, and nutrient) they may also acquire facilitative shelter from their neighbors against severe climatic events such as solar radiation, strong winds, and low temperature in alpine grasslands *(Klanderud & Totland, 2005; Wang et al., 2008*). Although many studies have focused on the influence of species-species interactions on plant growth (*Callaway et al., 2002; Wang et al., 2008*), there has thus far been little attention paid to the potential impacts of species on the nutrient cycling processes in alpine grasslands at all levels from species to functional group and community.

The spread of weedy species is a serious problem in global grasslands ~~around the world~~ (*Baker, 1986; Shi et al., 2011*), and with their greater capacity to capture abiotic resources ~~such as nutrients, light, water, and CO_2, they are fierce competitors~~ (*Baker, 1974; Ziska, 2017*). ~~Their superior competitive ability and toxicity to livestock means that the spread of poisonous plants in a grassland ecosystem can lead to severe economic losses (*Jin et al., 2011; Li et al., 2014*).~~ *Stellera chamaejasme* is a toxic perennial weed ~~found~~ in the eastern alpine grassland of the Qinghai-Tibetan Plateau (QTP) of China~~.~~, which ~~*S. chamaejasme* has become a dominant species, especially in heavily-grazed grassland, and can~~ seriously threatens alpine grassland productivity and ecological sustainability (*Liu, Long & Yao, 2004; Xing & Song, 2002*). *S. chamaejasme*-dominated grasslands are spread widely across the plateau and are one of the main causes of degraded grassland found in the QTP (*Song et al., 2018*). ~~*S. chamaejasme* spreads for many reasons including toxicity to livestock preventing its consumption (*Liu, Long & Yao, 2004*), its allelopathic effects on forages (*Zhou, Huang & A, 1998*), and its association with creating fertility islands, which enable greater soil nutrient availability (*Guo & Wang, 2018; Sun et al., 2009*).~~ While many scholars have focused on *S. chamaejasme* exclusion and control for degraded grassland recovery (*Song et al., 2018; Wang et al., 2008; Wang et al., 2018*), some have stated that *S. chamaejasme* may play a positive role in alpine grasslands by providing protection for some species and helping to conserve plant diversity (*Cheng et al., 2014; Guo & Wang, 2018*). Most research on *S. chamaejasme* has focused on population spread dynamics (*Sun et al., 2009; Xing & Song, 2002*), allelopathic effects on forage plants (*Zhou, Huang & A, 1998*), and the use of its extracted flavonoids (*Jo et al., 2018; Kim et al., 2017*). Little is therefore known about the effects of *S. chamaejasme* removal on ecological stoichiometry, information that will help in understanding its role in ecological protection and conservation in alpine grasslands.

Because *S. chamaejasme* has become a dominant poisonous plant in degraded alpine grasslands, many herders utilize removal methods in order to maintain grassland productivity and ecological function. The relationship between poisonous plants and forage grass, and the nutrient dynamics of degraded grassland dominated by poisonous plants has, thus far, received little attention. Our study was conducted within this context in order to test the following hypotheses regarding *S. chamaejasme* removal: 1) it will affect stoichiometric traits in plant leaves; 2) it will elicit different stoichiometric responses at the species, functional group, and community levels; 3) it will influence the relationship between the stoichiometric traits of plants and surrounding environmental factors such as nutrient availability of soil and/or community composition.

Materials & Methods

Study site

The study was conducted in an alpine grassland at an elevation of 3,230 m in Haiyan County (N 37°04′, E 100°52′), approximately 125 km northwest of Xining, the capital city of the Qinghai Province, China. This area has a typical plateau continental climate, with a mean annual solar radiation of 2,580 h, mean annual temperature of 0.4 to 3.4 °C, and annual precipitation of 277.8 to 499.5 mm (most of which falls between May and September). Vegetation is typical of an alpine grassland with *Kobresia* and *Elymus* species being the dominant plants in our study area. Other companion species included *Festuca ovina*, *Poa pratensis*, *Melissitus ruthenica*, *Kobresia humilis*, *Carex atrofusca*, and *Lancea tibetica*. Local herders use the study site as a winter rangeland (grazing from September to May) with a heavy grazing intensity of about 7.94 sheep units per hm^2. Within the last few decades, *S. chamaejasme* has invaded the grassland and gradually become the dominant species in the study area resulting in the grassland facing serious degradation challenges. Field experiments were approved by the Haiyan County Grassland Station, Haibei, Qinghai (approval number: 2016-NK-136).

Experimental design

In May 2016, three quadrats measuring 40 m × 60 m were fenced off with each block being 60-80 m apart. In each quadrat, two treatments (Control, CK; *S. chamaejasme* removal, SR) were randomly established with four replicates (20 m × 7 m in size), eight plots in each quadrat, resulting in a total of 12 plots for both CK and SR. In SR, *S. chamaejasme* were artificially removed by pulling out in June 2016, and the soil which had been carried out was returned to the original site immediately. Plots were monitored weekly during the growing season to ensure there was no further *S. chamaejasme* growth.

Plant sampling and chemical analysis

To estimate the aboveground biomass and community diversity~~ index~~, three quadrats (0.5 m × 0.5 m) were randomly surveyed in each plot at the beginning of August 2017. Heights of all living plants were measured with a ruler, and plant coverage for each species were recorded using the step-point method (*Mueller-Dombois & Ellenberg, 1974*). All living~~ (aboveground)~~ plant biomass (aboveground) was harvested by ~~hand ~~clipping to ground level, sorted by species, oven dried at 85 °C for 24 h to a constant mass, and weighed. For the functional group level, the leaves of all species in each of the half plots~~,~~ were collected and sorted into four functional groups (Gramineae, sedges, legumes, and forbs)~~, were collected~~. For the community level, the green leaves of all species in each of the other half plots were collected and combined as one replication in each plot. For species level, *Elymus nutans*, *Poa crymophila*, *Koeleria litvinowii*, *Festuca ovina*, *Stipa aliena*, *Kobresia capillifolia*, *Kobresia humilis*, and *Carex atrofusca,* which together had ~~a ~~relative coverage of over 80%, were chosen to investigate the ~~influence~~effect of *S. chamaejasme* removal on ~~plant~~species-level C:N:P stoichiometry. After the survey of plant composition, in each plot, 30 consistent leaves of each species were collected and mixed as one sample for further analysis (*Lü & Han, 2010*). All leaves collected were oven dried and ground. Soil samples were collected with a soil auger at five random sites in each plot, then combined into one sample with a separation of 0–10 cm and soil depth of 10–20 cm soil. Soil samples were passed through a 1 mm sieve after air-drying to analyze nutrient content.

The total soil and plant organic C content was determined using the oil bath-K_2CrO_7 titration method - oxidization with dichromate in the presence of H_2SO_4, heated at 180 °C for 5 minutes and titration with $FeSO_4$ (*Bao, 1999*). The total N content of the soil and plant samples following a Kjeldahl digestion was assayed using a Nitrogen Analyzer System (Kjeltec 2300 Auto System II, Foss Tecator AB, Höganäs, Sweden); using H_2SO_4 for digestion, NH_3 was captured by H_3BO_3 and then titrated by HCl. Total P content was determined using the molybdate blue colorimetric method (*Bao, 1999*) using a spectrophotometer (SP-723; Shanghai, China) after digestion with H_2SO_4 and H_2O_2. The levels of NH_4^+-N and NO_3^- -N in the soil samples were measured using a FIAstar 5000 Analyzer FOSS TECATOR. The available P content of the soil was analyzed according to soil agricultural chemistry methods (*Bao, 1999*).

Diversity index calculation~~s~~

The following formulas were used:

(i) Species richness (*S*) = the number of species in the quadrat;

(ii) Shannon-Wiener index (*H*): $H = -\sum_{i=1}^{S} P_i \ln P_i$, in which P_i represents the relative important values (the average of relative biomass, relative cover, and relative height) of species i.

(iii) Simpson index (*D*): $D = 1 - \sum_{i=1}^{S} P_i^2$;

(iv) Pielou index (*E*): $E = -\frac{\sum_{i=1}^{S} P_i \ln P_i}{\ln(S)}$.

Shannon-Wiener index is commonly used in measuring the community species diversity, Simpson index is used in measuring the dominance status of community, and Pielou index means the evenness of community (*Magurran, 1994*).

Statistical analysis

Data analyses were performed using SPSS (version 17.0). Two-way ANOVA was used to determine the effects of either species and treatments, or functional groups and treatments on C, N, and P levels, on the ratios of C:N, C:P, and N:P, and transformed data was used, when necessary, to satisfy the assumptions of ANOVA. Independent t-tests were used to calculate significance of differences between CK and *S. chamaejasme* removal treatments in all parameters. Statistical significance was defined at the 95% confidence level. A principal component analysis (PCA) to assess the various effects of treatments on C, N, and P levels and the ratios of C:N, C:P, and N:P in different species or functional groups. A redundancy analysis (RDA) conducted in CANOCO 5.0 for Windows was utilized to assess variation ordination of community stoichiometry traits (contents of C, N, and P, ratios of C:N, C:P, and N:P) and soil nutrient levels (contents of Organic C, total N, total P, NH_4^+-N, NO_3^- -N, and available P in 10–20 cm deep soil).

Results

Species dominance, plant biomass, and diversity

~~In this study, t~~The dominance of *E. nutans* and *P. crymophila* ~~in~~were increased by SR ~~was significantly higher than that in the CK~~ ($P = 0.017$, $P = 0.042$, Table 1), while the dominance of ~~. The dominance of *K. litvinowii*, *F. ovina*, *S. aliena*, *K. capillifolia*, *K humilis*, and *C. atrofusca*~~ other species were ~~not different between CK and~~affected by SR ($P > 0.05$, Table 1). Plant aboveground biomass was significantly enhanced by~~in~~ SR~~ was significantly higher than that in the CK~~ ($P = 0.022$, Fig. 1). ~~Additionally, the a~~Aboveground biomass of Gramineae was also higher in the SR~~, when compared to~~ than that in the CK ($P = 0.023$, Fig. 1)~~.~~, whereas that of~~ However, for~~ forbs was~~, the aboveground biomass significantly~~ decreased ~~after *S. chamaejasme* removal~~by SR ($P = 0.049$, Fig. 1).~~, and~~ ~~no significant changes were found between SR and CK~~

~~for~~Aboveground biomass of sedges or legumes ~~(*P* = 0.586, *P* = 0.632, Fig. 1)~~ was not affected by SR (*P* = 0.586, *P* = 0.632, Fig. 1). In species level, the aboveground biomass of *P. crymophila* was significantly higher in the SR, when compared to CK (*P* = 0.023, Fig. 1). The biomass of *E. nutans, K. litvinowii*, *F. ovina*, *S. aliena, K. capillifolia, K humilis*, and *C. atrofusca* were not different between CK and SR (*P* > 0.05, Fig. 1). The diversity indices of plants in the SR treatment were significantly lower than that in CK (*P* = 0.040, *P* = 0.018 and *P* = 0.022 for species richness, Shannon-Wiener index and Simpson index, Table 2), indicating that *S. chamaejasme* removal had a vital impact on plant diversity. The Pielou index showed no significant difference between CK and SR (*P* = 0.074, Table 2).

Ecological stoichiometry

For species level, total C content of green leaves varied between species (F = 2.80, P = 0.012, Table 3), but no significant difference was found between SR and CK (F = 0.00, P = 0.985, Fig. 2a, Table 3). In total, the treatment and species both had significant impacts on N content (F = 22.17, *P* < 0.001 and F = 5.15, *P* < 0.001, respectively, Table 3), but only species significantly altered P content (F = 22.17, *P* < 0.001, Table 3). Specifically, significantly lower N contents were observed in the green leaves of *E. nutans*, *P. crymophila*, *K. litvinowii*, and *S. aliena* in SR than CK (*P* = 0.031, *P* = 0.002, *P* = 0.041 and *P* = 0.014, respectively, Fig. 2c). The total P content of *P. crymophila* was significantly higher in SR than CK (*P* = 0.004, Fig. 2e), but for *C. atrofusca*, the total P content was significantly lower in SR than CK (*P* = 0.016, Fig. 2e). No interaction between the treatment and species was found on the C, N, and P content (*P* = 0.596, *P* = 0.563 and *P* = 0.061, respectively, Table 3). Species and treatment both significantly affected the C:N, C:P, and N:P ratio in this study (Table 3), except the difference was not significant for treatment on the C:P ratio (F = 0.25, *P* = 0.618, Table 3). Species and treatment interacted, affecting the N:P ratio (F = 3.38, *P* = 0.003, Table 3). The C:N ratio was elevated in *P. crymophila*, *K. litvinowii*, *F. ovina*, and *S. aliena* leaves in SR compared to CK (*P* = 0.002, *P* = 0.033, *P* = 0.047, and *P* = 0.049, respectively), and no significant change was seen in the leaves of the other species (Fig. 2b). The C:P ratio significantly declined in *P. crymophila* and increased in *C. atrofusca* in SR compared to CK (*P* = 0.008 and *P* = 0.036, respectively), and no significant difference was seen in the other species (*P* > 0.05, Fig. 2d). The N:P ratio of *P. crymophila* and *K. litvinowii* significantly decreased in SR compared to CK (*P* = 0.000 and *P* = 0.004, respectively), but no difference was observed in the other species (*P* > 0.05, Fig. 2f).

At the functional group level, total C content of green leaves varied between groups (F = 3.36, *P* = 0.046, Table 4), but no significant difference was found between SR and CK (F = 0.75, *P* = 0.401, Fig. 3a, Table 4). There was no significant interaction between groups and treatments in C content (F = 0.38, *P* = 0.767, Table 4). Groups had significant impacts on N and P content (F = 66.79, *P* < 0.001 and F = 22.01, *P* < 0.001, respectively, Table 4), but only P content was significantly affected by the treatment (F = 4.09, *P* = 0.040, Table 4). The interaction of groups and treatments was significant for N and P content (F = 7.61, *P* = 0.002 and F = 4.00, *P* = 0.026, respectively, Table 4). Legumes had the highest N content compared to Gramineae, sedges, and forbs, and SR treatment did not significantly affect the N content of legumes (*P* = 0.124, Fig. 3c). Total N content declined significantly in Gramineae but increased significantly in forbs in SR compared to CK (*P* = 0.049 and *P* = 0.014 for Gramineae and forbs, Fig. 3c). Additionally, forbs had a significantly lower P content in SR compared to CK (*P* = 0.013, Fig. 3e), while no significant difference was seen in Gramineae, sedges, or legumes (*P* = 0.732, *P* = 0.356 and *P* = 0.848, respectively, Fig. 3e). Groups had significant impacts on C:N and C:P ratios (F = 43.58, *P* < 0.001 and F = 28.14, *P* < 0.001, respectively, Table 4), but treatments had no significant impact on C:N, C:P and N:P (F = 0.10, *P* = 0.757 for C:N, F = 2.31, *P* = 0.147 for C:P and F = 1.99, *P* = 0.178 for N:P, respectively, Table 4). The interaction of groups and treatments was significant for C:N, C:P and N:P (F = 5.36, *P* = 0.010 for C:N, F = 3.31, *P* = 0.047 for C:P and F = 5.80, *P* = 0.007 for N:P, respectively, Table 4). In Gramineae, there was no change in the C:P ratio, but the C:N significantly increased and the N:P significantly declined in SR compared to CK (*P* =

0.649 for C:P, $P = 0.048$ for C:N and $P = 0.049$ for N:P, respectively, Fig. 3b, d, f). In the leaves of forbs, the C:N ratio was significantly lower but the C:P and N:P ratios were significantly higher in SR compared to CK ($P = 0.032$ for C:N, $P = 0.010$ for C:P and $P = 0.004$ for N:P, respectively, Fig. 3b, d, f). There was no significant difference in the C:N, C:P, and N:P ratios in leaves of sedges or legumes between SR and CK ($P > 0.05$, Fig. 3b, d, f).

At the community level, plant leaves had significantly lower total N content and higher C:N ratio in SR compared to CK ($P = 0.047$ for N content and $P = 0.049$ for C:N, Fig. 4b, c). There was no significant difference in the other parameters at the community level between SR and CK ($P > 0.05$, Fig. 4a, d, e, f).

Soil nutrient availability

There was no difference in the organic C content of 0–10 cm deep soil between SR and CK ($P = 0.546$); however, C content in 10-20 cm depth soil was significantly lower in SR compared to CK ($P = 0.049$, Fig. 5a). There was no difference in the total N and ammonium N (NH_4^+ - N) content in 0–10 cm deep soil between SR and CK ($P = 0.498$ for total N and $P = 0.244$ for NH_4^+ - N, Fig. 5b, c), while in the 10–20 cm deep soil the total N content was lower and the NH_4^+ -N content higher in SR compared to CK ($P = 0.043$ for total N and $P = 0.016$ for NH_4^+ - N, Fig. 5b, c) and there was no significant difference in soil nitrate N (NO_3^- -N) content at any depth ($P = 0.659$ for 0-10 cm and $P = 0.240$ for 10-20 cm, Fig. 5d). Total and available P content of 10–20 cm deep soil were lower in SR compared to CK ($P = 0.049$ for total P and $P = 0.046$ for available P, Fig. 5 e, f), but no significant difference was seen in total P content and available P content at 0–10 cm soil depth ($P = 0.647$ for total P and $P = 0.246$ for available P, Fig. 5e, f).

Driving factors of plant stoichiometry traits

The PCA analysis showed that the different species and functional groups all showed varying degrees of changes in their leaf C, N, and P levels, and C:N, C:P, and N:P ratios between SR and CK (Fig. 6, 7). The first two axes of the PCA account for over

80% of the variation in species traits across the sites for all eight species with *P. crymophila* and *K. litvinowii* showing significant differentiation in the first axis (Fig. 6a-h). At the functional group level, all groups besides legumes showed significant differentiation, with *S. chamaejasme* removal responsible for over 75% variations for all four functional groups (Fig. 7a-d).

RDA analysis showed that approximately 80% of the variations had been explained and that *S. chamaejasme* removal had a significant influence on the plant N content and C:N ratio (Fig. 8). The content of organic C, total N, and available P in soil were positively correlated with the N content of leaves, but negatively correlated with the C:N ratio of the community. Total P content in the soil was positively correlated with the C:P and N:P ratios of the community and negative correlation with leaf P content.

Discussion

Elemental stoichiometry ratios are used as proxies for elemental C, N, and/or P use efficiencies (*Castellanos et al., 2018*). Our results showed that *S. chamaejasme* removal could ~~influence~~affect the ecological stoichiometry of plants at all levels, from species level to functional group and community level (Fig. 2, 3, 4 and Table 3, 4). These changes were closely related to species domina~~tion~~nce, biomass, and community diversity, and soil nutrient availability (Fig. 1, 5 and Table 1, 2).

Species level response

S. chamaejasme removal from degraded grasslands distinctly changed plant dominance. Besides *K. humilis* and *C. atrofusca*, all importance values of the other studied species increased by varying amounts after *S. chamaejasme* removal, and there was a significant difference between the values of *E. nutans* and *P. crymophila* (Table 1). In addition, the aboveground biomass of *P. crymophila* increased in SR compared to CK (Fig. 1). *S. chamaejasme* removal may significantly enhance the competitive potential of these species via increasing available space, which allows them to inhibit the growth of their competitors (*Niinemets, Valladares & Ceulemans, 2003*). The variation in plant–plant interaction responses to neighbor removal results from resource reallocation induced by different nutrient utilization strategies (*Wang et al., 2016*). In a terrestrial ecosystem, nutrient availability is one of the most limiting factors of plant growth, and thus nutrient use strategies will help determine plant distribution and dominance (*Güsewell et al., 2005*). The nutrient contents of green tissues could reflect the efficiency of nutrient utilization. Low nutrient concentrations in green tissues are considered to be an efficient mechanism of nutrient conservation and utilization (*Carrera, Sain & Bertiller, 2000*). Sistla and Schimel (*2012*) showed that a high C content in green tissues led to higher nutrient use efficiency. In our study, nutrient contents and C levels were species-specific (Fig. 2a). The N content of four species, *E. nutans*, *P. crymophila*, *K. litvinowii*, and *S. aliena*, decreased and the C:N ratio increased following *S. chamaejasme* removal (Fig. 2b, c). This result may help explain the increased dominance of these six species and also suggests that they may have developed a N storage strategy in response to neighbor removal in which more N is transported to the reproductive organs during the reproductive growth process or to the roots before the wilt period begins. Therefore, the leaf N content was maintained at a low level in August (*Rong et al., 2015*).

Tilman (*1982*) speculated that at the resource competition scale, species with low nutrient element concentrations were more suitable for growing in nutrient poor environments. In our study, the N:P ratio of most species (except *P. crymophila* in CK and *C. atrofusca* in SR) at both the CK and SR sites were lower than the threshold of 10:1 (*Güsewell, 2004*), suggesting that N is limited, rather than P, in this alpine grassland (Fig. 2f). In a N poor environment, enhancing the efficiency of N utilization is an important strategy to increase species dominance, and species with lower N concentrations should have a competitive advantage over other species in N-restricted environments (*Fan, Harris & Zhong, 2016; Tilman, 1997*). The N:P ratio of *P. crymophila* in CK and *C. atrofusca* in SR were between 10 and 20, which means that there was N and P co-limitation. This could partly explain the increase in the P content of *P. crymophila* and the decrease of P content in *C. atrofusca* after *S. chamaejasme* removal. Overall, these results show that plants could develop a nutrient utilization strategy in response to *S. chamaejasme* removal.

Functional group level response

Following *S. chamaejasme* removal, we saw that the biomass of Gramineae plants rapidly increased, but the biomass of forbs decreased (Fig.1). Previous studies have found that both competition and facilitation exist between individual plants due to the various microclimatic impacts induced by neighboring vegetation (*Chu et al., 2009; Klanderud & Totland, 2005; Wang et al., 2008*). Our study shows that the interaction between Gramineae and *S. chamaejasme* was competitive, yet between forbs and *S. chamaejasme* it was facilitative. *S. chamaejasme* may provide protection for neighboring forbs against severe climatic conditions such as high solar radiation, strong winds, or low temperatures (*Klanderud & Totland, 2005*) and when it is removed, the growth of forbs may be inhibited by Gramineae plants because of their greater competitive ability (*Wang et al., 2016*).

Differences in nutrient uptake and conservation strategies across growth forms and functional groups have also been previously observed (*Aerts, 1996; Yuan & Chen, 2009*). The nutrient element contents in plant leaves are continually affected by the plant's structural features and growth regulation (*Baldwin et al., 2006*). Our results were consistent with those observations. Legumes had the highest N content and lowest C:N ratio compared to Gramineae, sedges, and forbs, which had no significant change after *S. chamaejasme* removal (Fig. 3b, c). In Gramineae, the N content decreased and the C:N ratio increased after *S. chamaejasme* removal. This suggests that the increase in the biomass of Gramineae may be due to their higher utilization efficiency of N and is in accordance with the "dilution theory," where nutrient element concentration may be diluted in plant bodies when there is a rapid increase in plant biomass (*Rong et al., 2015; Sardans & Peñuelas, 2008*). The light:nutrient hypothesis states that the C:N ratios of plants are higher in bright environments because of the increased gains in photosynthetic C at any N concentration (*Sterner et al., 1997; Sterner & Elser, 2002*). Following *S. chamaejasme* removal, environmental light levels may increase, therefore, species, such as those in Gramineae, will rapidly increase in biomass and have a lower C:N ratio. After *S. chamaejasme* removal, the N content increased and the C:N ratio declined in forbs (Fig. 3b, c), which could be explained by the utilization efficiency theory that states that a lower efficiency of N usage results in less biomass. This may also be related to the increase of soil extractable inorganic N content (NH_4^+-N, for example, Fig. 5b) after *S. chamaejasme* removal (*Ehrenfeld, 2003*) and it also suggests that the ability of forbs to absorb N, in order to maintain growth and adapt to a more severe environment, has been enhanced.

The P level of an organism is partly driven by the allocation of P to ribosomal RNA, which is related to the increase in its growth rate (*Hessen et al., 2007; Song et al., 2015; Vrede et al., 2004*). In our study, the total P content of forbs decreased after *S. chamaejasme* removal (Fig. 3e), which may be partly explained by the measured decrease of the biomass of the forbs. After *S. chamaejasme* removal, the P content decreased and the C:P ratio increased in forbs (Fig. 3d) and the growth rate decreased. This agrees with the Growth Rate Hypothesis (GRH) that a higher plant growth rate is usually accompanied by lower C:N or C:P ratios (*Elser et al., 1996; Hessen et al., 2007; Vrede et al., 2004*). Previous studies in natural ecosystems have confirmed that plant biomass growth is limited by leaf N:P ratios (*Das, Dang & Shivananda, 2006; Van Duren & Pegtel, 2000*). After *S. chamaejasme* removal, the N:P ratio of Gramineae declined while that of the forbs increased (Fig. 3f). The variation of autotrophs in the C:N:P composition ratio has interspecific and intraspecific components. Some analyses of the percentage of N and P of photosynthetic biomass showed that the P increased faster than N in a rapidly growing organism (*Elser et al., 2000; Nielsen et al., 1996*). This theory was reflected in the Gramineae, which had a faster growth rate and lower N:P ratio after *S. chamaejasme* removal. As for the forbs, the increased N:P ratio may be related to the increased availability of N in the soil following *S. chamaejasme* removal (Fig. 5b). Some studies have shown that N availability increased the N:P ratio of plants (*Güsewell et al., 2005*), which may explain the decline of the biomass of forbs after *S. chamaejasme* removal.

Community level response

In most terrestrial ecosystems, N and P are the main elements that control plant growth (*Aerts & Chapin, 1999*). The stoichiometric ratios of C:N:P in plant leaves and litter in many ecosystems have been widely used as indicators to estimate nutrient limitations on plant growth, primary productivity, and litter decomposition (*Gusewell, 2005; Tessier & Raynal, 2003; Zhan et al., 2017*). Our results showed that the N:P ratio in the alpine grassland community did not change after *S. chamaejasme* removal (Fig. 4f), which indicates that in a short time *S. chamaejasme* removal has little influence on nutrient limitation in this ecosystem. The average N:P ratio was 7.88 at CK and 6.98 at SR. These values are both < 10 and therefore suggest that N was the limiting nutrient in this alpine grassland. The N content of the community declined and the C:N ratio increased after *S. chamaejasme* removal (Fig. 4b, c). The aboveground biomass at SR was significantly higher than in CK, indicating that the plants in this community have adapted to *S. chamaejasme* removal by increasing their N utilization efficiency. A previous study has shown that improving the ability to use an element in the environment where the element is limiting is important for plant growth in nutrient poor soils (*Tilman, 1997*). The decreases in the N utilization efficiency of forbs resulted in a decrease in biomass (Fig. 1), which helps to explain the plant community composition changes after *S. chamaejasme* removal. In this study, plant diversity was shown to significantly decrease after *S. chamaejasme* removal (Table 2). Some studies have shown that N availability increases the body N:P ratio and reduces the species diversity of communities (*Güsewell et al., 2005; Roem & Berendse, 2000; Seastedt & Vaccaro, 2001*). Our results showed that there was no significant change in the N:P ratio of the community between CK and SR; however, the available N (NH_4^+-N) content of the soil increased and the N:P ratio increased significantly after *S. chamaejasme* removal. The decline of community diversity seen in this study may therefore be attributed mainly to the reduction of species richness in the forbs group.

From the population level to the functional group level, and then on to the community level, the variation of N and P concentrations and C:N:P ratios gradually decreased and stabilized. This may relate to grassland ecosystem homeostasis, where the differences among species are balanced out with a greater number of species in the higher vegetation levels (*Fan, Harris & Zhong, 2016*). This implies that the influence of *S. chamaejasme* removal on ecosystem level stoichiometry may be inhibited by inertia effects.

Factors driving leaf nutrient stoichiometry

It is well known that plants and soil are interdependent (*Silva & Batalha, 2008*) and there are an increasing number of reports that show that the nutrient traits of plant cannot be separated from the dynamics of soil nutrient availability (*Eskelinen, Stark & Mannisto, 2009; Li et al., 2014)*. The occurrence of weeds is highly related to soil properties (*Korres et al., 2017; Walter, Chritensen & Simmelsgaard, 2002*). In *S. chamaejasme*-dominated grasslands, the organic C content in the soil decreased significantly after *S. chamaejasme* removal (Fig. 5a). This result was consistent with previous work. For example, *S. chamaejasme* increases the organic C content of soil because of the greater plant production and litter input or the higher microbial biomass (*Sun et al., 2009*). ~~The results of this study showed that the organic C content in soil without *S. chamaejasme* was lower than that with a *S. chamaejasme* community.~~ After *S. chamaejasme* removal, the total N, P, and available P content of the soil decreased (Fig. 5c, e, f). This may be due to the fast growth rate of the grasses, which requires greater N and P uptake in the absence of competition from *S. chamaejasme*. The content of NH_4^+-N in soil increased significantly after *S. chamaejasme* removal (Fig. 5b). *S. chamaejasme* is widely distributed throughout alpine grasslands creating islands of fertility, as determined by greater soil nutrient availability (*Sun et al., 2009*). Therefore, when *S. chamaejasme* has been removed, the soil nutrients gathered by the plants may be released from these “fertility islands,” which was reflected by the change of NH_4^+-N content observed in the results (Fig. 5b). Because of the longer and more widely distributed root system of *S. chamaejasme* (*Guo & Wang, 2018; Sun et al., 2009*), the significant changes seen in soil nutrient levels resulting from *S. chamaejasme* removal were mostly at the 10–20 cm depth rather than surface soil. As most of the changes in soil nutrient levels after *S. chamaejasme* removal appeared at the 10–20 cm soil depth the nutrient traits of this depth were used in the RDA.

The leaf trait–environment relationship is used to explain and predict the underlying mechanisms of leaf nutrient trait variation, environmental change, and to identify the nutrient limitations in an ecosystem (*Güsewell, 2004; Kerkhoff et al., 2005; Zhang et al., 2019*). Our results showed that *S. chamaejasme* removal could positively influence the community soil NH_4^+-N levels and C:N ratio and negatively influence the soil organic C, total N, available P, and leaf N contents (Fig. 8). It is well known that plant nutrient concentrations will often reflect soil nutrient conditions (*Han et al., 2011; Ordoñez et al., 2009*). Consistent with this, in *S. chamaejasme* removal sites, plant N content decreased and plant C:N increased with decreasing soil total N. Our results showed there was a negative relationship between soil NH_4^+-N and plant N, and a positive relationship between soil NH_4^+-N and plant C:N (Fig. 8). This may be due to different forms of available N (NH_4^+-N or NO_3^--N) being used by different plants.

Conclusions

This study was the first to investigate the effect of *S. chamaejasme* removal on the stoichiometric traits of plants in alpine grasslands. Different species were shown to have different nutritional and stoichiometric responses to *S. chamaejasme* removal~~ as seen in their C, N, and P levels and C:N, C:P, and N:P ratios~~. Firstly, the species that showed an increased dominance after *S. chamaejasme* removal had a lower N content and higher C:N ratio. Secondly, at the functional level, Gramineae had a lower N content and N:P ratio, and a higher C:N ratio in SR compared to CK. In forbs, the N content, C:P ratio, and N:P ratio increased, and P content and C:N ratio decreased after *S. chamaejasme* removal. These results were related to the increase in the biomass of Gramineae and the decrease in the biomass of the forbs. Lastly, at the community level, the N content was lower and the C:N ratio higher in SR than in CK, which may account for the observed biomass increase and decline in community diversity. Plant N content was positively correlated with the soil total N content and negatively with the NH_4^+-N content. Overall, this study has shown that *S. chamaejasme* removal ~~influences~~affects the stoichiometric traits of species, functional groups, and the community, and will also affect the dominance of species and community composition. This study presents the analysis of results obtained from one year of data collection. More systematic studies need to be carried out in alpine *S. chamaejasme*-dominated grasslands in order to reveal the influence of longer time periods and other factors, such as microorganisms, climate, and grazing, or the impact of other alternative measures such as fertilization and grazing prohibition on the recovery of *S. chamaejasme*-dominated degraded grassland.

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