Warming effects on plant regrowth after clipping are modified by repeated clipping in Mongolian pasture species

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Abstract

Global warming may pose a threat to the productivity of grazed grasslands. In this study, we investigated changes in the warming response of aboveground regrowth with the repetition of simulated grazing in two Mongolian pasture species, *Agropyron cristatum* and *Stipa krylovii*. Plants were grown under warming or non-warming conditions and subjected to repeated clipping of aboveground parts three times at 4-week intervals. Aboveground parts collected at each clipping and whole plants harvested at the end of the experiment were dried and weighed. In both species, warming had little effect on regrowth when clipping was repeated one or two times. In *A. cristatum*, however, warming significantly diminished regrowth when clipping was repeated three times. Belowground biomass decreased with clipping–regrowth cycles only in *A. cristatum*, and the decline was enhanced by warming, implying that the depletion of belowground reserves contributed to the reduction of regrowth in this species. Our results suggest that warming would likely have little effect on regrowth performance of grassland plants under lightly grazed conditions, but warming can decrease regrowth when grazing frequency is high, with the degree of decrease being species dependent.

Introduction

Grasslands are major ecosystems in arid and semi-arid regions, supporting pastoralism as an important industry in those regions. Grassland plants palatable to livestock experience repeated cycles of removal of aboveground parts by grazing and subsequent regrowth during the growing season (Oesterheld and McNaughton 1988, 1991; van Staalduinen et al. 2010). Therefore, the productivity of grazed grasslands depends largely on the regrowth ability of pasture species. During the regrowth of grasses, carbohydrates preserved in belowground parts generally support the early regrowth of aboveground parts after grazing, and then the photosynthetic products of new leaves begin to contribute to the regrowth (Chapin et al. 1990; Fulkerson and Donaghy 2001). However, the dependency on belowground reserves for regrowth and the photosynthetic capacity of juvenile leaves differ among species (Thornton et al. 1993; van Staalduinen and Anten 2005). Moreover, belowground reserves can be depleted by repeated grazing–regrowth cycles, such that regrowth performance may decline (X.L. Wang et al. 2012, 2013). For sustainable grassland use, it is necessary to understand the changes in pasture species' regrowth performance in response to repeated grazing.

Global warming can affect the regrowth performance of grassland species because plant physiological processes—including photosynthesis and respiration—are strongly temperature dependent (Luo 2007; Tjoelker and Zhou 2007). Although warming generally increases plant biomass production (Lin et al. 2010; S.P. Wang et al. 2012; Wang et al. 2019), there are reports of cases with no effect (Saleska et al. 2002) or with decreasing plant biomass in response to warming (Klein et al. 2007; Liu et al. 2021). Few studies, however, have focused on the effects of warming on plant regrowth after grazing, and the results of those limited studies were sometimes inconsistent. For example, experimental warming reduced the regrowth of grasses on the Canadian prairie (White et al. 2014), whereas warming did not affect the regrowth of aboveground biomass in an experiment conducted on the Tibetan Plateau (Li et al. 2020). To

predict the changes in grassland productivity due to global warming, it is necessary to study interspecific differences in the warming responses of regrowth after clipping in pasture plants.

The warming response of post-grazing regrowth can differ among species depending on the degree to which they rely on belowground resources for aboveground regrowth. Grass species can be categorized into two major growth forms, rhizomatous and caespitose, on the basis of the architecture and growth pattern of belowground parts. In addition to roots, rhizomatous species have a rhizome, a belowground storage organ that contains nonstructural carbohydrates such as starch and soluble sugar (Ottaviani et al. 2017; Steen and Larsson 1986). The regrowth of rhizomatous species seems to depend on belowground resources more strongly than that of caespitose species (van Staalduinen and Anten 2005). Probably because of those differences, regrowth after clipping is greater in rhizomatous than in caespitose species (van Staalduinen and Anten 2005; Zhang et al. 2018). Therefore, we would expect that the warming response of regrowth after grazing would be smaller in rhizomatous species, because their greater dependence on belowground storage allows for more stable regrowth performance than that of caespitose species.

In this study, we tested the effect of warming on the regrowth performance after repeated shoot removal in two grass species, *Agropyron cristatum* (rhizomatous) and *Stipa krylovii* (caespitose), which are native to the Mongolian steppe and highly palatable to livestock (Undarmaa et al. 2018). Aboveground parts were clipped repeatedly during the experimental period to simulate periodical grazing, and regrowth after clipping was compared between the plants in warming and non-warming treatments. Nitrogen (N) content relates positively to the amount of crude protein and serves as an index of the nutritive value of pasture, and crude fiber (CF) content relates negatively to the digestibility of pasture (Schönbach et al. 2009; van Soest 1994); N and CF contents of leaves were measured to test the changes in nutritional value of pasture associated with regrowth and warming. We hypothesized that the regrowth performance of rhizomatous *A. cristatum* would be less sensitive to warming compared to that of caespitose *S. krylovii* because of the greater dependence of *A. cristatum* on belowground reserves.

Materials and Methods

Plant materials

In 2020, prior to the warming experiment, *A. cristatum* and *S. krylovii* plants were germinated from seed and then grown in 1.5-L plastic pots with one plant per pot in a greenhouse. During the 2020 growing season, aboveground parts of the plants were clipped with scissors at ground level four times at 3-week intervals to mimic periodic grazing (for details, see Hu et al. 2023). In April 2021, the plants were transplanted into larger pots (1/5000a Wagner pot) filled with washed dune sand, with one plant per pot to ease pot size restrictions on plant growth. Plants were watered with tap water as needed until the warming experiment.

Warming experiment

Beginning on 30 May 2021, 100 ml of 1/100 diluted commercial nutrient solution (Hyponex, N-P-K 6-10-5; Hyponex Japan, Osaka, Japan) was applied to each pot every week. During the experiment, plants received tap water every day except for rainy days.

For each of the two species, 24 individuals with aboveground parts of approximately the same size were selected. On 5 June 2021, six plants of each species were harvested to record the initial condition of plants before the warming treatment; the remaining plants were placed in six open-top chambers (OTCs; 2 × 2 × 2 m) at the Field Science Center, Tottori University, Japan (35°30.76'N, 134°10.30'E), with three plants per species in each OTC. The OTCs are four-walled enclosures made of plastic sheeting with metal pipe frames inside. Three OTCs were allocated to the non-warming treatment (control) and the other three to the warming treatment. To prevent warming, the lower 50-cm portion of the plastic sheeting of the control OTCs was kept open.

The temperature inside the OTCs was recorded by temperature loggers (RTR-52A, T&D, Nagano, Japan) at 15-min intervals. Daily mean temperature during the experimental period from 10 June to 30 August ranged from 20.9 to 30.3°C for control and 22.1 to 31.7°C for warming treatments (Fig. 1a, b). The degree of warming (i.e., difference in temperature between control and warming treatments) averaged over the experimental period was 0.96°C (mean daily temperature), 3.15°C (maximum), and − 0.04°C (minimum) (Fig. 1c).

Plant harvest and clipping

Plants collected at initial harvest (H_I) on 5 June were divided into leaves, stems, and belowground parts, and the leaf area was measured by a leaf area meter (Li-3100; Li-Cor, Lincoln, NE, USA). The harvested plant parts were oven dried at 70°C for 2 days and weighed. Immediately after H_I, aboveground parts of the remaining plants (9 plants per species for each treatment) were clipped with scissors at ground level to mimic grazing (first clipping, C_1). Clippings were collected, divided into leaves and stems, and leaf area was measured; clippings were dried as described above and weighed. Second clipping (C_2) was performed on 3 July, third clipping (C_3) on 31 July, and final harvest (H_F) on 28 August. Plants were divided into leaves, stems, and belowground parts, and leaf area and dry weight were determined as for H_I. The regrowth periods from H_I (or C_1) to C_2, C_2 to C_3, and C_3 to H_F were 28 days.

Leaf nitrogen and crude fiber contents

Leaves of three plants of each species within each OTC were combined into one sample to obtain enough material for the measurements. The samples were ground in a mill, and N content was measured with an NC analyzer (JM1000CN; J-Science Lab, Kyoto, Japan). CF was extracted by using a FibreBag System (FBS6; Gerhardt, Königswinter, Germany), oven-dried and weighed. The weighed CF extracts were then ashed at 600°C for 1 h to determine mineral content. CF content was calculated by subtracting the mineral content from the extract weight.

Statistical analysis

Differences in dry weight, N content, and CF content among harvest and clipping occasions were tested by Tukey's multiple comparison test ($P < 0.05$). A *t*-test was used to assess the effect of temperature treatments on variables within each harvest and clipping occasion. To evaluate the contribution of root reserves to aboveground regrowth, linear regression was applied to the relationship between the aboveground and belowground biomass at the final harvest. Differences in the slopes of the regression lines between temperature treatments was tested by an analysis of variance (ANOVA). When no difference in slope was detected, we further tested the differences in the *y*-intercepts of regression lines between temperature treatments with an analysis of covariance (ANCOVA) using belowground biomass as a covariate. We used GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA) to perform the Tukey's multiple comparison test and *t*-test. We performed ANOVA and ANCOVA using R version 4.0.3 (R Foundation, Vienna, Austria).

Results

Aboveground biomass

Aboveground biomass at first clipping (C_1) was 2.23 g and 2.18 g in *A. cristatum* and 1.25 g and 1.02 g in *S. krylovii* in the control and warming treatments, respectively (Fig. 2). In *A. cristatum*, aboveground biomass decreased with repeated clipping; the decrease was significant from C_2 to C_3 in both temperature treatments (Fig. 2a). Aboveground biomass of *A. cristatum* did not significantly differ between temperature treatments at C_1, at C_2, or at C_3, but it was significantly smaller in warming than control treatments at H_F. In *S. krylovii*, aboveground biomass increased from C_1 to C_2 and decreased from C_2 to C_3, such that the biomass at C_3 and H_F was similar to that at C_1 (Fig. 2b). No significant difference was found in aboveground biomass of *S. krylovii* between treatments at any of the clipping or harvest occasions.

Belowground biomass

Belowground biomass at H_I was 10.91 g for *A. cristatum* and 4.28 g for *S. krylovii* (Fig. 3). In *A. cristatum*, the belowground biomass significantly decreased from H_I to H_F due to clipping repetitions; the decrease was significantly larger in warming than in control treatments (Fig. 3a). In *S. krylovii*, however, belowground biomass increased from H_I to H_F in both treatments (Fig. 3b).

Contribution of belowground biomass to aboveground biomass at final harvest

Linear regression was applied to the relationship between above- and belowground biomass at H_F (Fig. 4). Regression coefficients were significant and positive for *A. cristatum* in both treatments, but they were not significant for *S. krylovii*. The slopes of the *A. cristatum* linear regressions did not differ

between treatments ($P > 0.05$, ANOVA), but the *y*-intercepts differed significantly between them ($P < 0.05$, ANCOVA).

Morphology and nitrogen and crude fiber contents of leaves

In *A. cristatum*, specific leaf area (SLA) tended to increase with successive clippings; the increase was statistically significant from C_3 to H_F in both treatments (Fig. 5a). In *S. krylovii*, SLA increased from C_1 to C_2, decreased from C_2 to C_3, and then increased from C_3 to H_F in both treatments (Fig. 5b). For both species, no significant difference was found in SLA between treatments at any clipping or harvest occasion.

The leaf N content of *A. cristatum* tended to increase with repeated clipping and was significantly higher at C_3 and H_F than that at C_1 and C_2 (Fig. 5c), although no significant difference due to warming was found at any clipping or harvest occasion. In *S. krylovii*, leaf N content differed little among clipping and harvest occasions, with a temporary decrease at C_2 (Fig. 5d), and no significant differences were found between temperature treatments.

In *A. cristatum*, leaf CF content did not change with repeated clipping in both treatments, and no difference due to warming was found at any clipping or harvest occasion (Fig. 5e). Leaf CF content in *S. krylovii* changed little with clipping repetition (Fig. 5f), and a significant decrease due to warming was found only at C_3.

Discussion

Regrowth of aboveground biomass in the 4-week regrowth period following clipping tended to decrease with repeated clipping in *A. cristatum*, whereas the regrowth of *S. krylovii* decreased little with repeated clipping (Fig. 2). The observed decrease in the regrowth of *A. cristatum* was inconsistent with our previous study (Hu et al. 2023), which showed that aboveground biomass in both species consistently recovered to the same level as before clipping even after three clippings. This discrepancy might be attributed to the difference in the number of tillers and the amount of belowground reserves between the current-year plants used in our previous study and 1-year-old plants used in this one. Current-year plants have far fewer tillers than 1-year-old plants; therefore, greater belowground reserves may have enabled current-year plants to maintain abundant regrowth even after repeated clipping. Christiansen and Svejcar (1988) reported that the concentration of root carbohydrate reserves can decrease with root growth, which might have caused the decrease in regrowth with repeated clipping in 1-year-old plants of *A. cristatum*. Thus, the observed decrease in belowground biomass in response to repeated clipping (Fig. 3a) may reflect the depletion of belowground reserves for regrowth.

In our experiment, regrowth after repeated clipping decreased with warming only in *A. cristatum* at H_F, whereas warming did not affect regrowth of *S. krylovii* (Fig. 2). This implies that experiments applying a single clipping treatment may overlook the negative effect of warming on plant regrowth performance, thus underestimating the interspecific difference in the warming response of plant regrowth. The

response of species' belowground biomass to repeated clipping and warming may be the driving factor here. Belowground biomass was decreased by three clipping repetitions and the decrease was enhanced by warming in *A. cristatum*, whereas belowground biomass tended to increase after repeated clipping in *S. krylovii* (Fig. 3). In addition, a significant positive regression between aboveground and belowground biomass was found only in *A. cristatum* (Fig. 4). These results suggest that carbohydrate reserves made a greater contribution to aboveground regrowth in *A. cristatum*, a rhizomatous species, than in *S. krylovii*, a caespitose species (van Staalduinen and Anten 2005). The positive regression slopes for *A. cristatum* (Fig. 4) indicate that the decrease in belowground carbohydrate reserves may have contributed to the reduction in regrowth of this species at H_F in response to warming. Increased carbon loss due to enhanced root respiration by warming (Lawrence and Oechel 1983; Rachmilevitch et al. 2006) may have decreased belowground biomass. The regression line for *A. cristatum* was shifted downward by warming, indicating that a factor other than the decrease in belowground reserves also contributed to reduced regrowth. What that additional factor is remains unclear, but the suppression of leaf photosynthesis due to warming might have contributed (Bauweraerts et al. 2013; Wertin et al. 2011).

SLA did not change with warming but changed temporally with repeated clipping in both species (Fig. 5). This result was in line with the findings of Jessen et al. (2020) that mammalian herbivory has a greater effect on SLA of grassland species than that of warming. SLA represents leaf morphological features and can reflect leaf-level plant strategies against grazing; leaves with greater SLA are softer and exhibit rapid compensatory growth through larger light-harvesting area, whereas leaves with lower SLA are tougher and minimize damage by grazing (Cingolani et al. 2005; Díaz et al. 2001). Thus, warming would have little effect on leaf-level plant strategies against grazing in our two Mongolian pasture species. However, it should be noted that the response of SLA to warming varies between studies; warming either had no effect on SLA (Rodgers et al. 2018) or increased SLA in some cases (Hartman and Nippert 2013; Xu and Zhou 2006).

In our study, warming had little effect on leaf N (Fig. 5c, d) and CF contents (Fig. 5e, f) in both species, even after three clipping repetitions. Because leaf N relates positively to forage protein content and leaf CF relates negatively to digestibility of forage (Waghorn and Clark 2004), our results imply that warming will generally not affect the nutritive value of these two Mongolian pasture species under grazing use. These observations are consistent with a meta-analysis conducted by Dumont et al. (2015) in which warming did not clearly affect N and CF contents of forage species. However, a meta-analysis performed by Moyo and Nsahlai (2021) revealed an increase in CF content and decrease in N content due to warming. Such variations in the responses of CF and N contents to warming might reflect the modification of warming effects by water availability. Warming effects on leaf CF and N contents are reportedly more obvious under drier conditions (Li et al. 2018; White et al. 2014). In grassland species in Mongolia, the increase in CF content by warming was mitigated by adding water (Yoshihara et al. 2022). In our study, plants were watered daily and thus were not suffering from water deficit. Presumably, our experimental design resulted in no response of CF and N contents to warming.

Conclusion

In our experiment, warming had little effect on regrowth after clipping in two Mongolian pasture species, *A. cristatum* and *S. krylovii*, but warming diminished the regrowth of *A. cristatum* when clipping was repeated three times. These results suggest that warming would generally have little influence on the regrowth of grassland plants after grazing, although warming can decrease regrowth when grazing frequency is high. The degree of that decrease can differ among grassland species. Thus, experiments applying a single clipping treatment may overlook the negative effect of warming on plant regrowth. To better predict the effect of global warming on grasslands under grazing use, more research on interspecific differences in the warming response of plant regrowth after repeated clipping is warranted. Our findings suggest that Mongolian grasslands dominated by *S. krylovii* would be more robust against livestock grazing under global warming than those dominated by *A. cristatum*.

Declarations

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Competing interests

The authors have no competing interests to declare.

Author contributions

RH and TK designed and performed the experiments. RH analyzed the data, RH and TK wrote the manuscript. BG helped with the collection of plant seeds in 2014. YY provided editorial advice.

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Figures

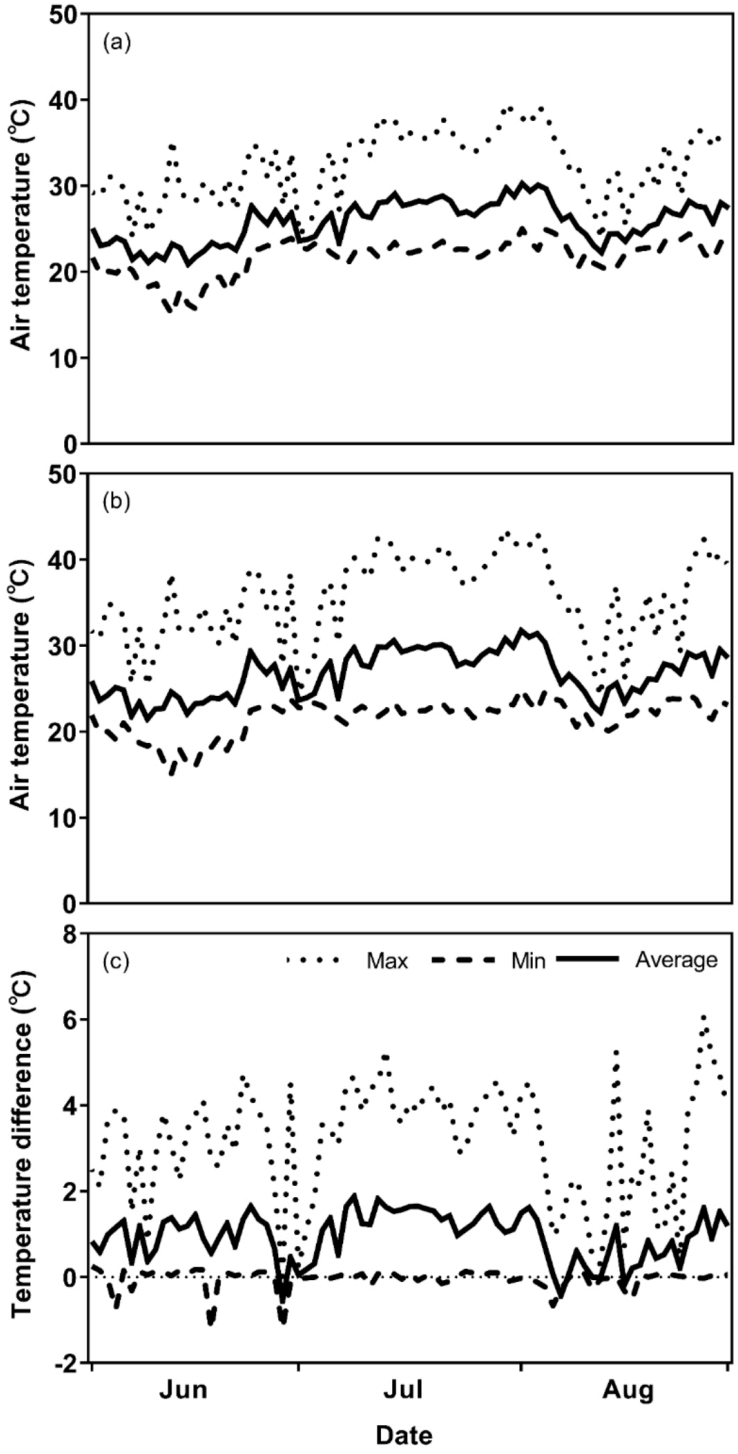

Figure 1

Daily maximum, minimum, and average temperature inside open-top chambers for (a) control and (b) warming treatments during the experimental period. (c) The effect of the warming treatment on daily maximum, minimum, and average temperature.

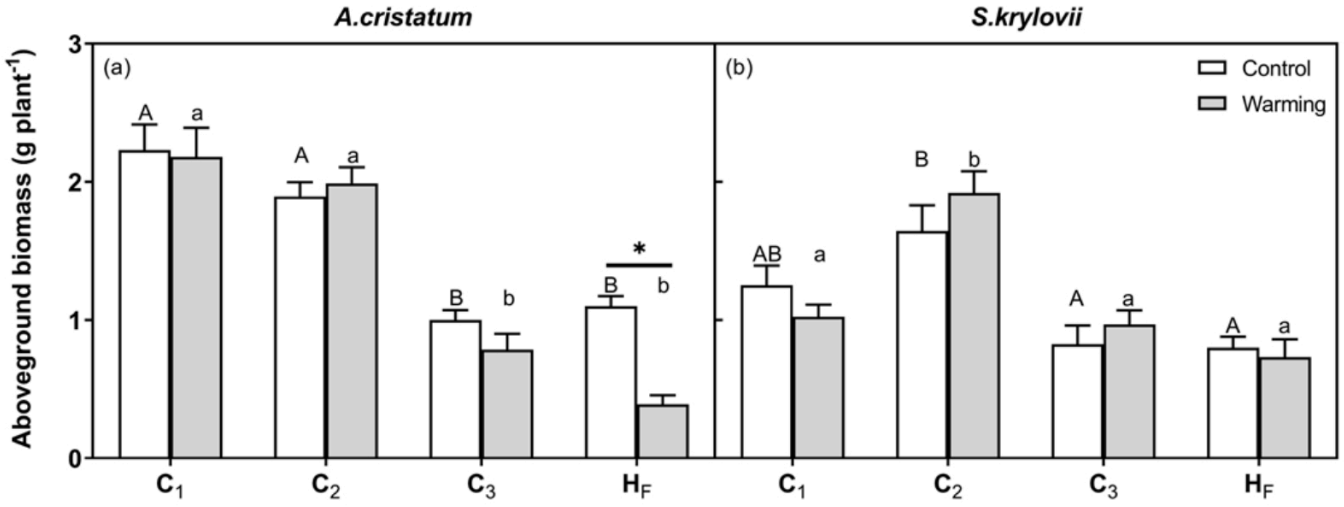

Figure 2

Aboveground biomass of (a) *Agropyron cristatum* and (b) *Stipa krylovii* growing under control and warming treatments. C_1, C_2, C_3, and H_F indicate first, second, and third clipping and final harvest. Different uppercase or lowercase letters above the columns indicate statistically significant differences among clipping or harvest occasions within each treatment ($P < 0.05$, Tukey's multiple comparison test). An asterisk above columns indicates a significant difference between temperature treatments ($P < 0.05$, *t*-test). Error bars indicate +1SE ($n = 9$).

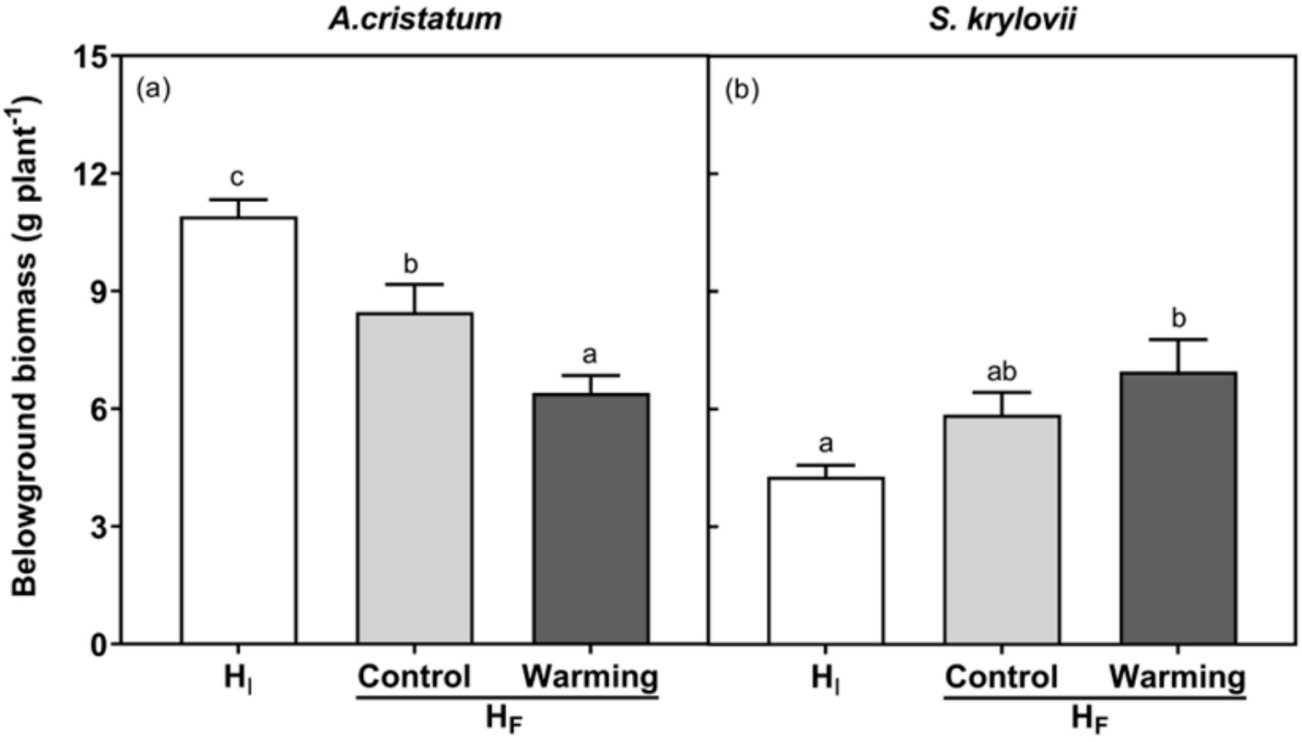

Figure 3

Belowground biomass of (a) *Agropyron cristatum* and (b) *Stipa krylovii* at the initial (H_I) and final harvest (H_F). Different lowercase letters above the columns indicate a significant difference within species ($P < 0.05$, Tukey's multiple comparison test). Error bars indicate +1SE (n = 6 for H_I, 9 for control at H_F, and 9 for warming treatment at H_F).

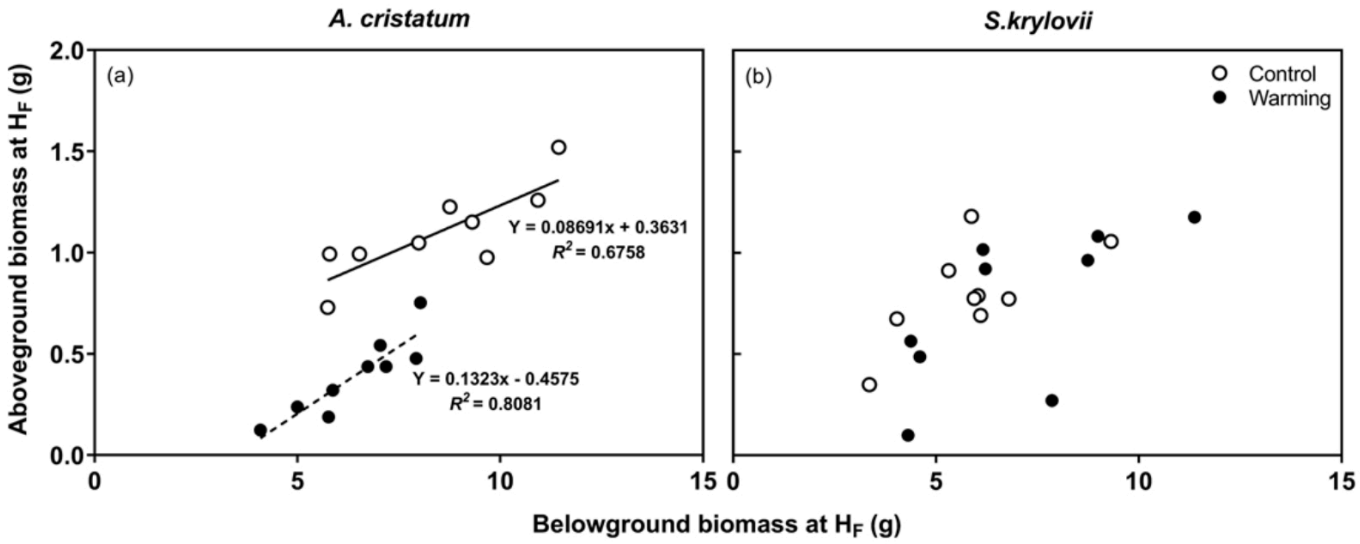

Figure 4

Relationships between above- and belowground biomass at final harvest (H_F) in (a) *Agropyron cristatum* and (b) *Stipa krylovii* growing under control and warming treatments. Solid and broken lines represent linear regression for control and warming treatments, respectively. Regression lines are not shown for *S. krylovii* because regression coefficients were not statistically significant in either temperature treatment.

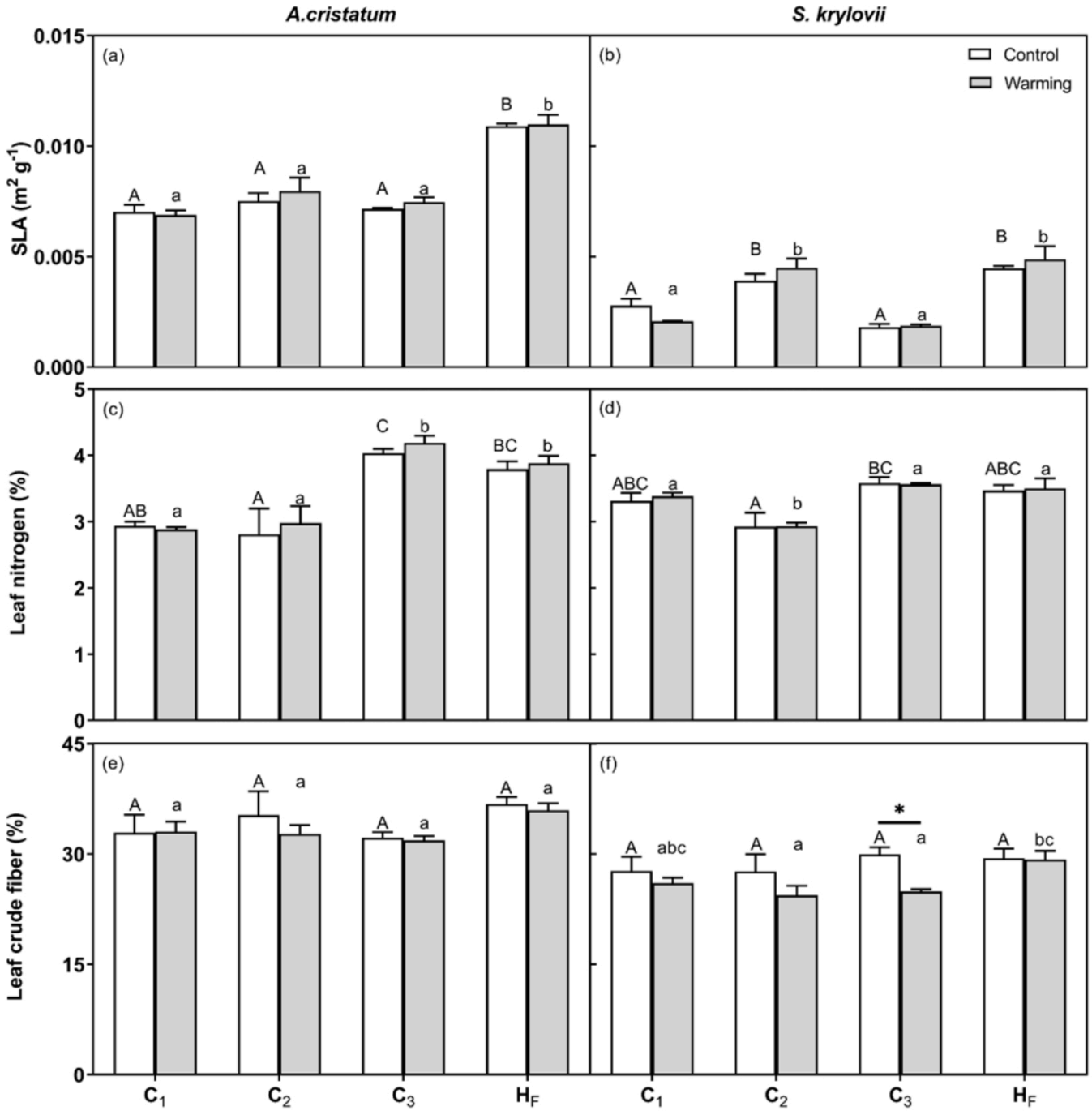

Figure 5

Specific leaf area (SLA), leaf nitrogen content, leaf crude fiber content in *Agropyron cristatum* (a, c, e) and *Stipa krylovii* (b, d, f) growing under control and warming treatments. C_1, C_2, C_3, and H_F indicate first, second, and third clipping and final harvest. Different uppercase or lowercase letters above the columns indicate significant differences among clipping and harvest occasions within treatments ($P < 0.05$, Tukey's multiple comparison test). An asterisk above columns indicates a significant difference between temperature treatments ($P < 0.05$, *t*-test). Error bars indicate +1SE ($n = 3$).