

Plant volatiles inhibit restoration of plant species communities in dry grassland

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Abstract

Benefits from livestock grazing have declined in regions where vegetation has been degraded by overgrazing. The vegetation can be restored by excluding livestock for a period, but it takes longer in drier regions. Here we propose a possible mechanism for delays in the recovery of poor vegetation for livestock grazing in dry grassland, introducing a case in Mongolia where steppe vegetation dominated by *Stipa krylovii*, a palatable grass, can become dominated by *Artemisia adamsii*, an unpalatable forb, when the grassland is overgrazed. Our long-term field experiment shows that the exclusion of livestock has not enhanced the recovery of palatable species in 6 years, indicating that *A. adamsii* is a strong competitor in the plant community. To understand why livestock exclusion is ineffective, we examined the ecological significance of volatile organic compounds (VOCs) released by *A. adamsii*. In *ex situ* experiments, the VOCs promoted photosynthesis of *S. krylovii* with enhanced stomatal conductance, and *S. krylovii* grew faster and consumed more water when exposed to the VOCs even with water deficiency. These findings imply that *S. krylovii* would be more likely to face severe drought before the next rain falls. We therefore conclude that plant volatiles may reduce the resilience of overgrazed vegetation in arid environments.

Zusammenfassung

Die Erträge der Viehbeweidung sind in Regionen zurückgegangen, in denen die Vegetation durch Überweidung geschädigt wurde. Die Vegetation kann sich erholen, wenn das Vieh für eine gewisse Zeit ausgeschlossen wird, was aber in trockenen Gebieten länger dauert. Wir schlagen hier einen möglichen Mechanismus vor, der für die Verzögerung bei der Erholung spärlicher Vegetation für die Viehbeweidung in trockenen Steppen verantwortlich ist, indem wir einen Fall in der Mongolei vorstellen, bei dem die Steppenvegetation, die durch das fressbare Gras *Stipa krylovii* dominiert wird, bei einer Überbeweidung des Graslandes durch *Artemisia adamsii* dominiert wird, das ein nicht fressbares Kraut ist. Unsere langfristigen Freilandexperimente zeigen, dass der Ausschluss des Viehs die Erholung der fressbaren Arten in 6 Jahren nicht gefördert hat, und weisen darauf hin, dass *A. adamsii* in der Pflanzengemeinschaft ein starker Konkurrent ist. Um zu verstehen, warum der Ausschluss des Viehs nicht effektiv ist, untersuchten wir die Bedeutung von flüchtigen organischen Substanzen (VOCs), die von *A. adamsii* freigesetzt werden. In Laborexperimenten förderten die VOCs die Photosynthese von *S. krylovii* über eine stärkere Durchlässigkeit der Stomata, und *S. krylovii* wuchs schneller und verbrauchte mehr Wasser, wenn es den VOCs ausgesetzt war, selbst wenn es Wassermangel

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gab. Diese Ergebnisse lassen vermuten, dass *S. krylovii* mit größerer Wahrscheinlichkeit vor dem nächsten Regen ernste Austrocknungserscheinungen zeigen würde. Wir schließen daraus, dass flüchtige Pflanzensubstanzen die Widerstandsfähigkeit einer überweideten Vegetation in trockenen Umgebungen reduzieren können.

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Introduction

Vegetation maintains an equilibrium with wild animals but can be disturbed by domestic livestock. Continuous overgrazing can debase vegetation, leading to the dominance of unpalatable species over palatable species. Further, overgrazing may result in a catastrophic shift of vegetation (Scheffer, Carpenter, Foley, Folke, & Walker, 2001; Van de Koppel, Rietkerk, & Weissing, 1997) and soil loss (Belnap, 1995). To reduce the risk of land degradation such as loss of soil organic matter, the best option is to reduce anthropogenic effects; the reduction or exclusion of livestock can allow palatable species to be restored in short periods in semi-arid and dry sub-humid regions (Harrison & Shackleton, 1999; Walker, Langridge, & McFarlane, 1997). Although the resilience of degraded land is relatively high in such regions, erratic rainfall causes primary production to vary greatly from year to year (Milchunas & Lauenroth, 1993; O'Connor, Haines, & Snyman, 2001). In arid regions, the resilience is much lower, so long-term exclusion of livestock will be required to return poor vegetation for livestock grazing to its original state (Lovich & Bainbridge, 1999).

The steppes of Mongolia and Inner Mongolia (China), which have a long history of livestock grazing, feature xerophytic plant communities that are adapted to severe cold winters. Distinct wet and dry seasons, corresponding to the growing season and the off-season, respectively, typify the climate. Yet dry spells often occur during the growing season; accordingly, plant growth is interrupted from time to time. *Stipa krylovii*, a palatable perennial grass, shares the community with small colonies of *Artemisia adamsii*, an unpalatable perennial forb. The grass generally predominates under low grazing pressure, but there is a clearly perceptible extreme of community structure across the steppe, typified by *A. adamsii*-dominated communities under high grazing pressure (Hilbig, 1995). *Artemisia frigida*, a palatable semi-shrub, also predominates under high grazing pressure in some cases (Cheng, Tsendeekhuu, Narantuya, & Nakamura, 2008).

Annual species (e.g., *Chenopodium album* and *Salsola collina*) are relatively abundant in both *S. krylovii*- and *A. adamsii*-dominated communities, but *S. krylovii* cannot coexist evenly with *A. adamsii*. *Artemisia* spp. are well known to emit large quantities of volatile organic compounds (VOCs). For example, *Artemisia californica* released $47 \mu\text{g}$ (monoterpenes) g^{-1} (dry leaf weight) h^{-1} (Arey, Crowley, Crowley, Resleto, & Lester, 1995). These species release constitutive and low-level VOCs when healthy, and large quantities of inducible VOCs when damaged (Holopainen, 2004). On the

steppe, *A. adamsii* is a major species that releases VOCs with a strong smell, so its effect is of interest.

To a greater or lesser degree, all plant species produce VOCs. A well-known paradigm is that plants emit VOCs to defend themselves against attack by insects and pathogens (Dudareva, Negre, Nagegowda, & Orlova, 2006; Shulaev, Silverman, & Raskin, 1997; Walling, 2000). Volatiles emitted by damaged plants can be detected by undamaged neighbors, which can then modify their own defenses for better protection against herbivores and pathogens (Baldwin, Halitschke, Paschold, von Dahl, & Preston, 2006; Farmer, 2001). However, the role of plant volatiles in ecosystems must be further elucidated.

Allelopathy is a function that can be additional to this paradigm (Kegge & Pierik, 2009). Plant volatiles have been shown to inhibit plant growth, and species that produce large quantities of VOCs can suppress coexisting species to some extent (Muller, Muller, & Haines, 1964; Rai, Gupta, & Singh, 2003; Tarayre, Thompson, Escarré, & Linhart, 1995). For example, *A. californica* emits several volatiles, such as camphor and 1,8-cineole, which inhibit seed germination of *Madia sativa*, a native forb of the west coast of North America (Halligan, 1975). VOCs in large amounts have been shown to cause inhibitory effects, but concentrations in nature are usually lower than those used in experiments (Cape, 2003). On the other hand, several studies have shown a prevalence of plant hormesis (or stimulatory allelopathy at low concentrations): a positive response to low levels of stressors (An, Johnson, & Lovett, 1993; Belz & Cedergreen, 2010; Calabrese & Blain, 2009; Stebbing, 1982; Vicherková & Polová, 1986).

In the process of vegetation degradation on the steppe, *S. krylovii* adjacent to *A. adamsii* is susceptible to grazing pressure as a result of the selective grazing habit of livestock, and the *Artemisia* colonies can proliferate. Although coexisting annual and perennial species play important roles in secondary succession from overgrazed vegetation, vigorous growth of *S. krylovii* after a cold and dry winter may be a prerequisite for restoration of the vegetation. This vigorous growth may also be susceptible to VOCs from the *Artemisia* plant residues remaining from the previous growing season, which might either stimulate or inhibit the growth of neighboring *S. krylovii* sprouts early in the growing season. In addition, older *A. adamsii* plants release more VOCs. An understanding of the survival strategy of unpalatable species that produce large quantities of VOCs is of importance to revealing their interaction with palatable species in the recovery of poor vegetation for livestock grazing. Here we show

the significance of the VOC-producing species in dry grassland, as exemplified by the *S. krylovii*–*A. adamsii* association. The specific objectives of this study were to investigate (i) the impact of livestock grazing on the plant community (*in situ* experiments) and (ii) hormetic responses of *S. krylovii* growth to VOCs released from *A. adamsii* plant residues (*ex situ* experiments). The response of *Stipa* to the live *Artemisia* VOCs is also discussed in relation to the results of *ex situ* experiments.

Materials and methods

Vegetation surveys

We conducted vegetation surveys at two sites in a typical Mongolian steppe during the period of peak vegetation cover (early September) in 2008 and 2009 (Cheng, Tsubo, Ito, Nishihara, & Shinoda, 2011). Site 1 (47°03'N, 105°57'E, 1198 m) was close to a settlement (or a water source) and regarded as a high-grazing-pressure field. Site 2 (47°01'N, 105°32'E, 1265 m) was away from such locality and hence under low grazing pressure. The vegetation survey followed the phytosociological method of Braun-Blanquet (1964); plant species composition of each plot of 10 m by 10 m was recorded on the basis of cover-abundance (plant coverage classes used in this study: +: <1% coverage; 1: 1–10%; 2: 11–25%; 3: 26–50%; 4: 51–75%; 5: 76–100%). To compare the species composition between the sites, we calculated Sørensen's similarity index as $2(C_1 \cap C_2)/(C_1 + C_2)$, where C_1 and C_2 are the number of species at Site 1 and Site 2, respectively (Magurran, 2004). Sheep and goats are mainly grazed at both sites.

In situ experiments

We have been monitoring changes in the species composition at Site 1 since 2003; the typical livestock density outside the enclosure is 1 sheep equivalent per 2.5 ha (Shinoda, Nachinshonhor, & Nemoto, 2010). The mean annual precipitation (1995–2008) is 156 mm (137 mm during the growing seasons, April–September), and the annual mean temperature is 0.3 °C with a minimum of –23.6 °C in January and a maximum of 20.7 °C in July. The soil is sandy: 1.3% clay (<0.002 mm), 0.6% silt (0.002–0.02 mm) and 98.1% sand (0.02–2 mm) in the top 10 cm. The field capacity and wilting point are 0.204 and 0.048 cm cm^{–1}, respectively.

We fenced off a field of 300 m by 300 m in June 2004 for a livestock exclusion experiment on grazing land in the Mongolian steppe (47°02'37"N, 105°57'05"E, 1200 m), where *A. adamsii* was dominant. It is noted that Site 1 is located within a few kilometers of our livestock exclusion field, and Site 2 is about 35 km away. In September 2003, plant species were recorded at two monitoring sites that had been established inside and outside the enclosure in June 2004. The

species composition has been monitored since every summer. To evaluate the similarity of the species composition between inside and outside, we calculated the Sørensen's similarity index. In addition, plant height, density and above-ground biomass of *A. adamsii* were measured in July 2007 in sample plots of 50 cm by 50 cm with three replications each. A two-tailed *t*-test was employed to examine the difference between communities of exclosures and grazing plots.

We further carried out a soil disturbance experiment within the enclosure. Three replicated randomized blocks were designed with no disturbance or with disturbance of the soil surface by stroking it with a broom a few times to simulate livestock trampling. This treatment could move buried seeds of annual species such as *C. album* to drier conditions at the soil surface and reduce germination of the seeds. The blocks were located within 100 m apart from each other and relatively uniform. The plot size was 4 m by 4 m. The treatment was applied in late April 2008 (4 years after enclosure). Plant species coverage was surveyed in early September 2008, following the phytosociological method of Braun-Blanquet (1964). A two-tailed *t*-test was used for comparison of total plant coverage between the treatments.

Ex situ experiments

We carried out *ex situ* experiments to test the possible hormetic effect of VOCs from *A. adamsii* on *S. krylovii*. We designed pot experiments to investigate the growth and photosynthesis of *S. krylovii* exposed to VOCs of *A. adamsii*. In all experiments, single seeds of *S. krylovii* were sown in individual plastic pots (top diameter of 10.5 cm, bottom diameter of 8 cm, height of 10.5 cm for Experiments 1 and 3; top area of 9 cm by 9 cm, bottom area of 7 cm by 7 cm, height of 7.5 cm for Experiment 2) filled with sandy soil (about 95% sand).

The *Stipa* plants were grown with adequate soil water and fertilizer until they reached a height of 20–25 cm and had 5–20 tillers. The plants were then grown under exposure to the VOCs for 24 days with either sufficient water (irrigated every 2 days to restore soil water content to around field capacity: Experiment 1, no-stress treatment in a glasshouse) or no additional water supply after an initial saturation (Experiment 2, stress treatment in a climate chamber). Meteorological environment for the *Stipa* plants grown in the experiments was as follows: a temperature of 16.0–25.1 °C, a relative humidity of 61–91% and a daylength of 0–11.1 h in Experiment 1, and 20–25 °C, 40–50% and 14 h with a maximum illumination of 80,000 lux in Experiment 2 (see Appendix A: Table 1). The soil surface of the pots was covered with a clear plastic sheet to minimize soil evaporation, and then naturally dried leaves of *A. adamsii* (<2 months after harvest at the end of summer) were placed on the plastic sheet: 1, 3 or 9 g of leaves in Experiment 1 ($n=4$) and 1, 2, 3 or 9 g in Experiment 2 ($n=3$). Experimental errors due to differences in plant height and the number of tillers between pots were minimized by grouping together pots with similar standard deviations of

growth. Wood-framed, top-closed plastic covers (20 cm by 20 cm wide by 40 cm high), the bottom part of which was opened for ventilation, were used to isolate the pots from each other. Aboveground dry matter was measured at the end of the experiments. In addition, plant water use was measured every 2 days in Experiment 1, and plant water use and the number of tillers were recorded weekly in Experiment 2. *Stipa* plants grown under the same environmental conditions without *Artemisia* leaves served as controls in both Experiments 1 ($n = 4$) and 2 ($n = 3$). The meteorological environment was different between the experiments, but this study compared treatments under the same condition in each experiment.

In Experiment 3, we used a photosynthesis meter (LI-6400, LI-COR, Lincoln, NE, USA) to measure leaf exchange rates of CO_2 and H_2O in *S. krylovii* with 30–40 tillers, grown with adequate water and fertilizer, in the climate chamber. The air inlet of the system was connected via a polyethylene tube to a closed container made of acrylic resin (20 cm by 15 cm wide by 30 cm high) containing naturally dried leaves of *A. adamsii* (1, 3 or 9 g). The ambient air was mixed with VOCs emitted from the leaves through the container, and the mixture was supplied to the leaf chamber, where CO_2 and H_2O exchange rates were measured at 25°C , a photosynthetic photon flux density of $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, and 400 ppm CO_2 at $700 \mu\text{mol s}^{-1}$. Three pots were assigned to each treatment. The measurements were taken first with a bunch of *S. krylovii* leaves (5–10 leaves) without the VOCs after the bunch had been acclimatized to the leaf chamber environment and then with the very same bunch after exposure to the VOCs for 10–15 min.

For evaluation of the hormetic effect in Experiments 1 and 2, a mathematical model (An et al., 1993) was fitted to averaged data points of the *Stipa* plant response to the *Artemisia* VOCs as percentages of the control in dry matter (Y): $Y = 100 + [S_m \times X^q / (K_S^q + X^q)] - [I_m \times X^q / (K_I^q + X^q)]$, where X is the amount of *Artemisia* leaves, S_m is the potential response for maximum stimulation, I_m is the potential response for maximum inhibition, K_S is the leaf amount at which Y is half of S_m , K_I is the leaf amount at which Y is half of I_m , and q is a constant that determines the shape of the curve. In addition to the regression analysis, a one-tailed t -test was used to examine the null hypothesis (H_0) that the dry matter in each treatment is equal to that in the control, against the alternative hypothesis (H_1) that the former is greater than the latter. For Experiment 3, a paired one-tailed t -test was used to examine the difference between the control and each treatment (H_0 : treatment = control; H_1 : treatment > control).

Results

Effect of long-term grazing on species composition

The vegetation surveys in September of 2008 and 2009 (see Appendix A: Table 2) showed that *S. krylovii* and *Kochia*

prostrata, a shrub species, predominated in little-disturbed grazing land (Site 2), whereas *A. adamsii*, the annual forbs *C. album* and *Chenopodium aristatum*, and occasionally the perennial grass *Cleistogenes squarrosa* and the perennial sedge *Carex duriuscula* dominated severely overgrazed land (Site 1). *S. collina* was a major species observed at both sites. In 2008, we recorded 17 species at Site 1 and 20 species at Site 2, with 10 species in common between the sites. In 2009, we recorded 25 species at Site 1, 18 species at Site 2 and 11 species in common. The similarity index was 0.54 in 2008 and 0.51 in 2009, indicating differences in species compositions between sites under different grazing pressures for both years.

Effect of livestock exclusion on vegetation restoration

In September 2003, before the exclosure site was established, the species richness was 13 in each of the two plots. In June 2004, one of these two plots was subsequently positioned inside the exclosure and the other outside, respectively. Eleven of the 13 species were common between the plots. A total of 43 species were recorded from 2004 to 2010. Inside the exclosure, 32 species were recorded during that period, and four species (*A. adamsii*, *Caragana microphylla*, *C. squarrosa* and *S. krylovii*) were consistently present throughout the period. Outside the exclosure, the species richness was also 32, and three species (*A. adamsii*, *C. squarrosa* and *S. krylovii*) occurred throughout the period. The species richness varied between 9 and 18 from year to year outside the exclosure, and between 7 and 16 inside the exclosure. The similarity index varied between 0.69 and 0.96 during 2004–2010, with an average of 0.82, and there was no increasing or decreasing trend during the period. We surveyed the effect of livestock exclusion on the ecology of *A. adamsii*, and we measured its height, density and aboveground biomass in July 2007 (3 years after exclosure). Plant height inside the exclosure was more than twice that outside (Fig. 1A), but the density inside was about half that outside (Fig. 1B). The aboveground biomass inside was more than twice that outside, but the difference was not significant (Fig. 1C).

We also simulated the effect of livestock trampling on the plant community by disturbing the soil surface within the exclosure early in the growing season (April 2008), and investigated the coverage of every species late in the season (September). Eight species grew in all plots, and two grew in a few plots (Table 1). Soil disturbance decreased the total plant coverage ($n = 3$, $P < 0.001$). *C. album* dominated the undisturbed plots, and *A. adamsii* dominated the disturbed plots.

Hormetic effects of VOCs on plant growth

With adequate water (Experiment 1), growth of *S. krylovii* exposed to VOCs from *A. adamsii* leaves for

Table 1. The effect of simulated trampling by sheep and goats of the soil surface on plant coverage (+: <1%; 1: 1–10%; 2: 11–25%; 3: 26–50%; 4: 51–75%) inside our enclosure (47°02'37"N, 105°57'05"E, 1200 m).

	No disturbance			Simulated trampling		
	Block 1	Block 2	Block 3	Block 1	Block 2	Block 3
Total plant coverage (%) [*]	75	70	70	50	50	45
<i>Artemisia adamsii</i>	3	2	+	3	3	2
<i>Bassia dasyphylla</i>	+	+	1	+	+	+
<i>Caragana stenophylla</i>	+	+	1	+	1	+
<i>Carex duriuscula</i>	+	+		+	+	+
<i>Chenopodium acuminatum</i>	+	+	+	+	+	+
<i>Chenopodium album</i>	3	4	4	1	2	1
<i>Chenopodium aristatum</i>	2	+	2	2	1	2
<i>Cleistogenes squarrosa</i>						+
<i>Eragrostis pilosa</i>	1	+	+	+	+	+
<i>Salsola collina</i>	+	+	2	1	+	+
<i>Stipa krylovii</i>					+	

^{*} $P=0.00058$ based on a two-tailed *t*-test for comparison of total plant coverage between treatments.

24 days was highest with 1 g of leaves (over 50% increase in aboveground biomass (Fig. 2A). The dry matter for the 1-g treatment, 1.077 ± 0.121 (mean \pm standard error) g plant⁻¹, was significantly greater than that for the control, 0.654 ± 0.085 g plant⁻¹ ($n=4$, $P=0.019$). The dry matter for the 3-g treatment (0.938 ± 0.184 g plant⁻¹) was about 40% higher than that for the control (Fig. 2A), but the difference was not significant ($n=4$, $P=0.106$). The dry matter for the 9-g treatment (0.610 ± 0.008 g plant⁻¹) was not different from that for the control ($n=4$, $P=0.368$). Water use by *S. krylovii* showed a positive association with growth throughout the experiment (Fig. 2B). Under water stress (Experiment 2), *S. krylovii* showed a similar response to the VOCs, but it peaked at a higher amount of *A. adamsii* leaves (Fig. 3A). The 3-g treatment increased the biomass (0.238 ± 0.050 g plant⁻¹, $n=3$, $P=0.152$) by about 50% of the control (0.160 ± 0.042 g plant⁻¹), and the 2- and 9-g treatments (0.203 ± 0.010 g plant⁻¹, $n=3$, $P=0.189$ and 0.199 ± 0.030 g plant⁻¹, $n=3$, $P=0.247$, respectively) by about 25%. The number of tillers reached the maximum on day 14 and then declined to the end of the experiment in all treatments above 1 g (Fig. 3B). The plant water use was large during the first 14 days, resulting in little available water in the soil for the latter days (Fig. 3C).

To understand the enhancement of *S. krylovii* growth by the VOCs, we measured the photosynthesis of *S. krylovii* exposed to VOCs under the well-watered condition (Experiment 3). The instantaneous net photosynthetic rates increased significantly by 2.2%, 4.0% and 1.2% with 1, 3 and 9 g, respectively, of *A. adamsii* leaves (Fig. 4A; $n=3$, $P=0.048$, 0.046, and 0.018, respectively). These enhancements corresponded to increases in stomatal conductance by 18.7%, 18.4% and 8.6% (Fig. 4B; $n=3$, $P=0.055$, 0.032, and 0.021, respectively). Thus, the physiological response clearly agreed with the growth response, particularly for the 1-g treatment in Experiment 1.

Discussion

Severe grazing pressure promoted the dominance of *A. adamsii*, as confirmed by the low values of the similarity index in association with relatively high cover-abundance of *A. adamsii* observed under high grazing pressure (see Appendix A: Table 2). The exclusion of herbivores generally tends to reduce the growth of *A. adamsii*, leading to an increase in the growth of *S. krylovii* (Hilbig, 1995). Our field monitoring in the enclosure, however, shows that the species composition did not change during the last 6 years. This suggests that it takes a long time to restore the poor vegetation naturally. In Mongolia, full livestock exclusion cannot be realized at the regional scale unless the current nomadic grazing system is changed. If livestock are not completely removed, the unpalatable *A. adamsii* will remain dominant as a result of trampling by livestock (Table 1), imposing a constraint on the recovery of the vegetation.

Studies from Inner Mongolia, China (Zhan, Li, & Cheng, 2007; Zhou, Sun, Huang, Gao, & Han, 2006), show potentially fast restoration of overgrazed *S. krylovii* steppes by the exclusion of livestock, with increased primary production and species richness. This restoration might have been successful because of the relatively wet environment (about 400 mm year⁻¹). In our study area, the species composition (the ratio of annual to other species types) is affected by rainfall variability (Cheng et al., 2011). Among annual species observed in the field survey (see Appendix A: Table 2), *S. collina* did not show a difference in abundance between the high- and low-grazing-pressure fields in 2008 (154 mm during the growing season), but was more abundant under high grazing pressure in 2009, an above-normal-rainfall year (184 mm). This indicates that increasing livestock grazing makes species composition more susceptible to rainfall variability. The studies from Inner Mongolia also show significant increases in the number of *S. collina* seeds buried in the soil and in species

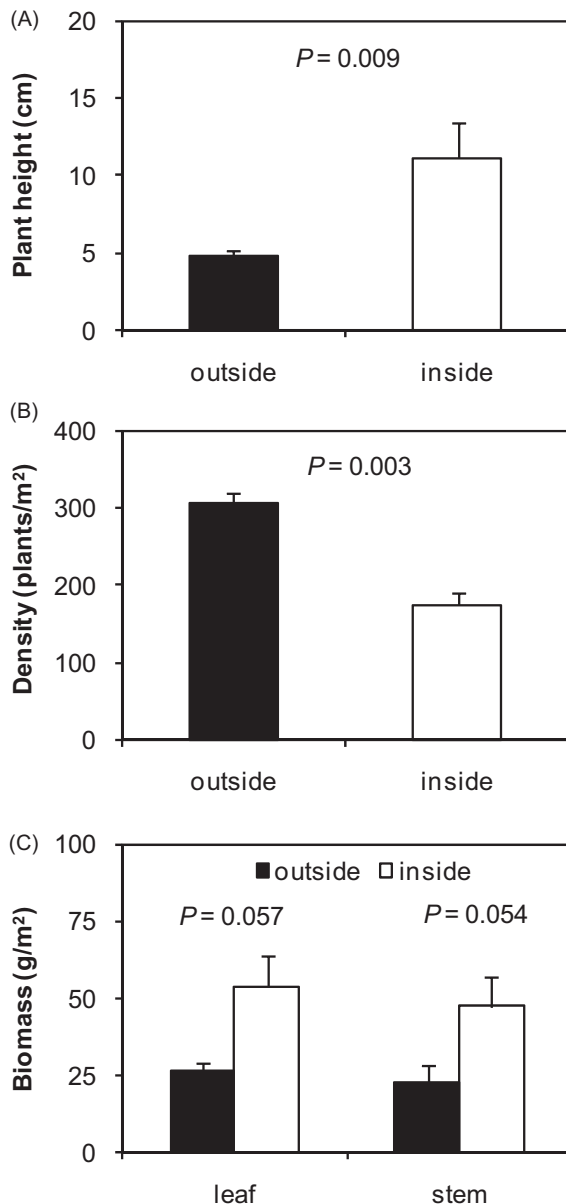


Fig. 1. Mean + standard error of (A) plant height, (B) density and (C) aboveground biomass of *A. adamsii*, which were measured inside and outside our enclosure in July 2007. The P -values are based on a two-tailed t -test to examine the difference between the outside and inside.

presence above the soil surface in the enclosure (Zhan et al., 2007; Zhou et al., 2006). Therefore, in association with perennial species (e.g., *C. squarrosa* and *C. duriuscula*) that coexist with *A. adamsii* (see Appendix A: Table 2), *S. collina* would be one of the key species in the restoration of overgrazed vegetation on the steppe.

To understand the resilience of vegetation in the study area, we have investigated hormesis by VOCs, on which very little has been reported (An et al., 1993; Belz & Cedergreen, 2010; Calabrese & Blain, 2009; Stebbing, 1982; Vicherková & Polová, 1986). Our experiments confirmed this hormetic

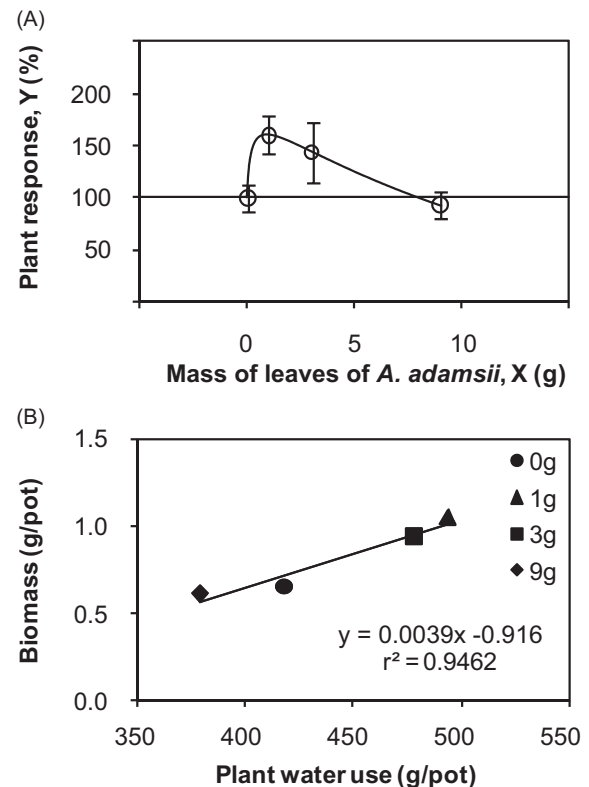


Fig. 2. Hormetic effects of volatile organic compounds (VOCs) from *A. adamsii* leaves on *S. krylovii* under well-watered condition. (A) Aboveground biomass of *S. krylovii* exposed to the VOCs; values (mean + standard error) are shown as percentages of the control, and the model (An et al. 1993) is fitted as $Y = 100 + [S_m \times X^q / (K_s^q + X^q)] - [I_m \times X^q / (K_l^q + X^q)]$ where $S_m = 90$, $I_m = 310$, $K_s = 0.2$, $K_l = 20$ and $q = 1$ ($r^2 = 0.9998$). (B) Relationship between aboveground biomass and total water use by the plant.

effect: the biomass of *S. krylovii* was greater with than without exposure to VOCs from dried leaves of *A. adamsii* under well-watered condition (Fig. 2). Further, the *A. adamsii* VOCs stimulated photosynthesis by *S. krylovii* (Fig. 4). This result implies that *Stipa* plants that neighbor the *Artemisia* species producing large quantities of VOCs photosynthesize more than distant *Stipa* plants. The enhanced photosynthesis can be interpreted as the increased intake of CO₂ as a result of stomatal widening induced by plant volatiles (Fig. 4). In addition, the *Stipa* plants tended to consume more soil water with exposure of the VOCs, as the photosynthetic rate increased, until soil water deficit occurred (Fig. 3). Our findings show that the grass *S. krylovii* grows faster and consumes more water when it grows near the forb *A. adamsii*. This would expose the grass to increased water stress before the next rain. In the study area, where drought often occurs, soil moisture that increases from the wilting point even after reasonable rainfall (e.g., 10 mm) quickly returns to the wilting point within about 2 weeks (Shinoda et al., 2010). Therefore, *A. adamsii* can promote *S. krylovii* growth during short wet spells after rain events but may inhibit it over the long term during the recovery of the poor vegetation under erratic, low rainfall.

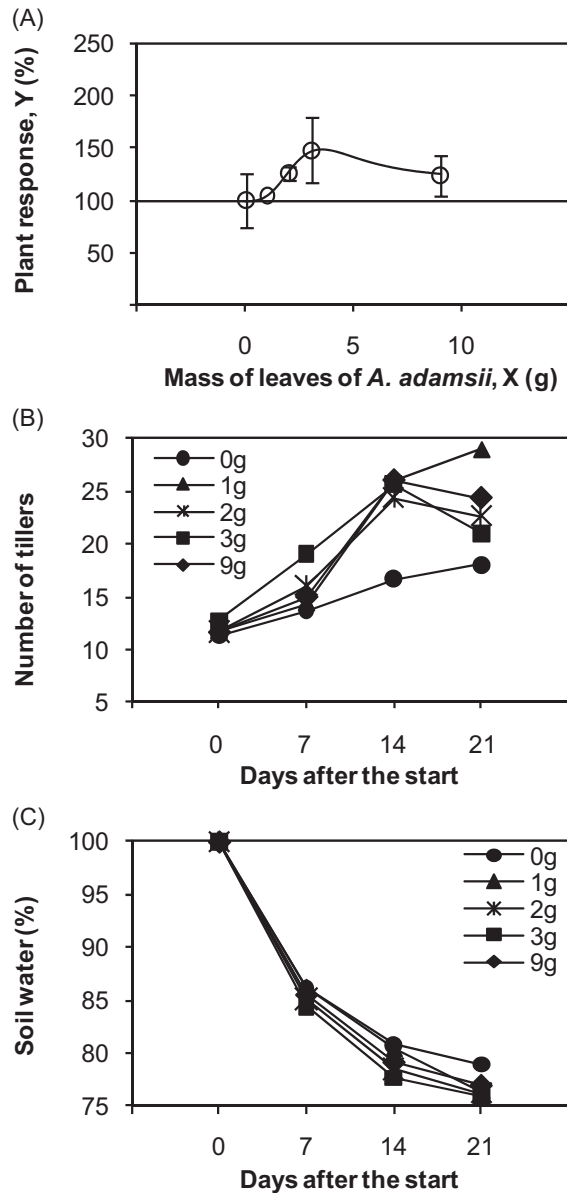


Fig. 3. Hormetic effects of volatile organic compounds (VOCs) from *A. adamsii* leaves on *S. krylovii* under water stressed condition. (A) Aboveground biomass of *S. krylovii* exposed to the VOCs; values (mean + standard error) are shown as percentages of the control, and the model (An et al. 1993) is fitted as $Y = 100 + [S_m \times X^q / (K_S^q + X^q)] - [I_m \times X^q / (K_I^q + X^q)]$ where $S_m = 340$, $I_m = 320$, $K_S = 3$, $K_I = 3.5$ and $q = 3$ ($r^2 = 0.9957$). (B) The mean number of tillers of *S. krylovii* exposed to the VOCs; only green (live) tillers were counted. (C) Mean water use by *S. krylovii* exposed to the VOCs; the percentage signifies the decrease in soil water content from the initial value, weight of the pot filled with the soil at field capacity.

Unlike the hormetic effect, the inhibitory effect of VOCs on plant growth has been widely reported (Muller et al., 1964; Rai et al., 2003; Tarayre et al., 1995). We thought this allelopathic effect could be detected with the large amount (9 g) of leaves of *A. adamsii*, which was about four times the

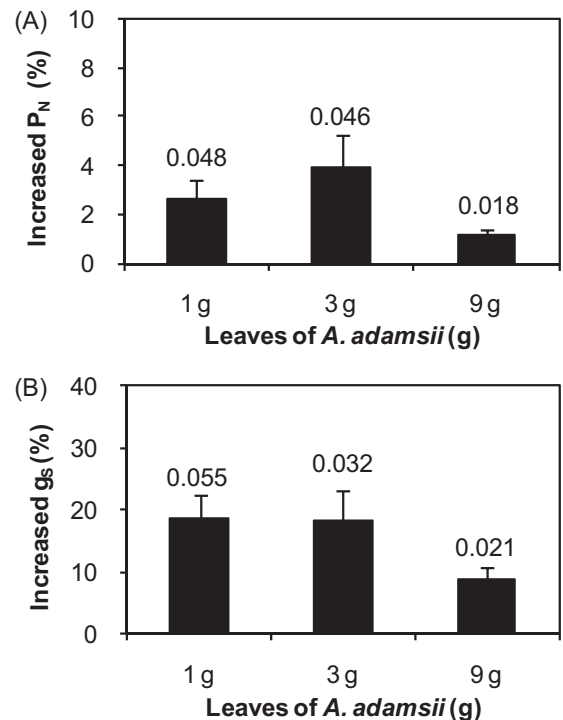


Fig. 4. Hormetic effects of VOCs from *A. adamsii* leaves on (A) leaf photosynthesis (P_N) and (B) stomatal conductance (g_s) of *S. krylovii* under well-watered condition. Values (mean + standard error) are shown as percentage increase relative to the control. For the 1-g, 3-g and 9-g treatments, the control had mean P_N values of 21.1, 15.0 and 16.9 $\mu\text{mol bunch}^{-1} \text{s}^{-1}$, respectively, and mean g_s values of 0.72, 0.35 and 0.44 $\text{mol bunch}^{-1} \text{s}^{-1}$. The numbers above each bar are P -values based on a paired one-tailed t -test to examine the difference between the control and each treatment (H_0 : treatment = control; H_1 : treatment > control; $n = 3$).

estimated 54 g dry leaf m^{-2} at our study site in July 2007 (Fig. 1). However, our experiments indicate that this large amount might still promote the growth of *S. krylovii* under water stress (Fig. 3). Small amounts of plant volatiles enhance stomatal conductance and photosynthesis, as shown here, but large amounts can cause stomatal closure (Rai et al., 2003). Thus, VOCs emitted from *A. adamsii* can both stimulate and inhibit *S. krylovii*. However, the large leaf biomass of *A. adamsii* that we used could be the maximum available *in situ*, so perhaps there is no inhibitory effect of *A. adamsii* on *S. krylovii* even in higher-rainfall areas with higher biomass production.

We recently identified 1,8-cineole and camphor as major VOCs in naturally dried leaves of *A. adamsii* (Tsubo, Nishihara, Nakamatsu, Cheng, & Shinoda, 2011). On the other hand, Shatar, Dung, and Karahawa (2003) detected α -thujone and 1,8-cineole as major compounds, but not camphor, in fresh aboveground parts of *A. adamsii* collected near Ulaanbaatar (wetter and colder than our study site). The difference between the studies might be due to how the plant samples were dried or to geographical variation in the

environmental conditions of the sampling sites (Lokar, Maurich, Mellerio, Moneghini, & Poldini, 1987; Oyedeji, Afolayan, & Hutchings, 2009). Our recent study shows that the growth of *S. krylovii* under water stress tends to be promoted by a small amount of volatile cineole (Tsubo et al., 2011), which is a major compound in both dead and live *Artemisia* plants. This implies a potential hormetic effect of live *A. adamsii* VOCs on *S. krylovii* throughout the growing season.

On the basis of our results, we propose that when dry spells are long, *S. krylovii* ceases to grow earlier. This is due to water deficit arising as a result of greater water use by the grass on exposure to VOCs from *A. adamsii*, while *A. adamsii*, a xerophytic plant, may reduce photosynthesis and emit VOCs (Sharkey & Loreto, 1993; Velikova, 2008) and then maintain its own vigor under water stress. This therefore implies that the forb may not be easily replaced by the grass. As a consequence, a longer time might be required to restore the overgrazed vegetation than expected. In addition, as plant species producing large quantities of VOCs are adapted to heat stress as a result of the emission of plant volatiles (Sharkey & Singsaas, 1995; Tyson, Dement, & Mooney, 1974; Yuan, Himanen, Holopainen, Chen, & Stewart, 2009), future climate change might further delay the recovery of the vegetation.

In conclusion, we have shown only one of the possible mechanisms underlying the combined effects of biotic stimulants or inhibitors and abiotic stresses on plant communities in dry grassland. Another possible mechanism is the physical effect of livestock grazing on the emission of plant volatiles. The quantity of VOCs (e.g. 1,8-cineole) released from a plant (e.g., *A. frigida*) can increase when the plant is damaged (Zuo et al., 2010). Our recent study shows an inhibitory effect of large amounts of volatile cineole on the growth of *S. krylovii* (Tsubo et al., 2011), indicating that *S. krylovii* may be inhibited when live *A. adamsii* is damaged by livestock trampling and thus produces more VOCs. Thus, the VOC-related mechanism involved in the *S. krylovii*–*A. adamsii* association is complex throughout the growing season. Further investigation will be necessary to understand the ecological role of plant volatiles in the restoration of vegetation debased by overgrazing on the steppe.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.baae.2011.11.005.

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