Grazing limits natural biological controls of woody encroachment in Inner Mongolia Steppe

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Summary statement

Our study demonstrated that grazing could negatively affect herbivorous insects and parasitic plant on woody plants, and thus limit the effectiveness of their natural biological control effects on woody encroachment.

Abstract

Woody encroachment in grasslands has become increasingly problematic globally. Grazing by domestic animals can facilitate woody encroachment by reducing competition from herbaceous plants and fire frequency. Herbivorous insects and parasitic plants can each exert forces that result in the natural biological control of encroaching woody plants through reducing seeding of their host woody plants. Whereas, the interplay of grazing and dynamics of herbivorous insects or parasitic plants, and its effects on the potential biological control of woody encroachment in grasslands remain unclear. We investigated the flower and pod damage by herbivorous insects and the infection rates of a parasitic plant on the shrub *Caragana microphylla* that is currently encroaching in Inner Mongolia Steppe, under different grazing management treatments (33-year non-grazed, 7-year non-grazed, currently grazed). Our results showed that *Caragana* biomass was the highest at the currently grazed site, and the lowest at the 33-year non-grazed site. Herbaceous plant biomass followed the opposite pattern, suggesting that grazing is indeed facilitating the encroachment of *Caragana* plants in Inner Mongolia Steppe. Grazing also reduced the abundance of herbivorous insects per *Caragana* flower, numbers of flowers and pods damaged by insect herbivores, and the infection rates of the parasitic plant on *Caragana* plants. Our results suggest that grazing may facilitate woody encroachment in grasslands not only through canonical mechanisms (e.g., competitive release via feeding on grasses, reductions in fires, etc.), but also by limiting natural biological controls of woody plants (herbivorous insects and parasitic plants). Thus, management efforts must focus on preventing overgrazing to better protect grassland ecosystems from woody encroachment.
Introduction

The encroachment of woody plants into grasslands has become one of the most significant biological phenomena globally over the last century (Eldridge et al., 2011; Naito and Cairns, 2011). Woody encroachment can have important influences on grassland ecosystem structure and function (Eldridge et al., 2011; Ratajczak et al., 2012). A better understanding the mechanisms mediating woody encroachment is critical for better predictions of the potential alterations to plant community dynamics, and a better understanding of the consequences of such vegetation compositional shifts in grasslands (Van Auken, 2009).

Researchers have implicated many climatic and ecological factors mediating woody encroachment, such as changes in temperature, precipitation, CO$_2$ levels, fire regimes and overgrazing (Archer et al., 1995; Morgan et al., 2007; Coetzee et al., 2008; Gordijn et al., 2013; Kulmatiski and Beard, 2013; Matson and Bart, 2013). The effects of grazing by mammalian animals on woody encroachment across different rangeland ecosystems could be complex (Van Auken, 2009; Sankaran et al., 2013). For example, studies in African savannas showed that browsing and trampling by mammalian animals could reduce establishment of woody seedlings (Sankaran et al., 2008; Staver et al., 2009; Sankaran et al., 2013). While studies in North American semi-arid grasslands showed that high and constant levels of grass herbivory by domestic animals could facilitate woody encroachment (Scholes and Archer, 1997; Van Auken, 2000, 2009). Grazing by domestic animals can reduce biomass of herbaceous plants, which results in increased resource availability for the establishment of woody plants in grasslands (Coetzee et al., 2008). Grazing by domestic animals also leads to reduction in fire frequency and intensity by reducing biomass of herbaceous plants (Oba et al., 2000; Roques et al., 2001), favoring woody encroachment in grasslands.

Herbivorous insects and parasitic plants, on the other hand, can exert important control on plant communities, including the spread of species that are woody encroachers. Insect herbivores can strongly affect the establishment and performance of plant species (McPherson, 1993; Prittinen et al., 2003), and thereby having important influence on plant community structure and dynamics (Brown, 1985; Crawley, 1989; Brown and Gange, 1992; Carson and Root, 2000; Borer et al., 2014). Insect herbivores can especially reduce seeding rates of their host plants directly by feeding on, laying on, or developing in seeds, or indirectly by damaging the reproductive parts (e.g. flowers) of the plants, and thereby
inhibiting seed formation (Hoffmann and Moran, 1998; Hoffmann et al., 2002; van Klinken et al., 2003; Impson et al., 2011). Through these mechanisms, insect herbivores feeding on flowering plants, including woody plants, can act as natural biological controls on the growth and expansion of these plants. Similar to herbivore effects (Pennings and Callaway, 2002; Pennings and Simpson, 2008), parasitic plants can also have strong impacts on plant community structure and dynamics by limiting the growth and development of their host plants (Pennings and Callaway, 1996; Callaway and Pennings, 1998; Bardgett et al., 2006). Thus, parasitic plants on woody host plants can also function as natural biological controls on woody encroachment. Many previous studies have shown that grazing by large herbivorous animals could affect insect diversity and abundance (Cagnolo et al., 2002; Kruess and Tscharntke, 2002; Hartley et al., 2003; Huntzinger et al., 2008; Zhu et al., 2012). Whereas, the interplay of grazing and dynamics of herbivorous insects or parasitic plants, and its effects on the potential biological control of woody encroachment in grasslands remain unclear.

We investigated whether natural biological controls of woody encroachment in grasslands, herbivorous insects and parasitic plants on woody plants, would be altered by different grazing management approaches. We hypothesized that grazing would negatively impact herbivorous insects and parasitic plants on woody plants, thereby limiting the roles that these natural biological controls play in limiting the expansion of woody plants under grazing, further facilitating woody encroachment in grasslands. To test this hypothesis, we examined how three different grazing management treatments affect the flower and pod damage by herbivorous insects and the infection rates of a parasitic plant on the encroaching legume shrub, Caragana microphylla (referred to as “Caragana” hereafter), in the semi-arid Inner Mongolia Steppe.

Results

Aboveground biomass

Caragana biomass was the highest at the currently grazed site, and the lowest at the 33-year non-grazed site ($F_{2, 27} = 13.10, P < 0.01$; Fig. 1A). In contrast, the aboveground biomass of herbaceous plants was the highest at the 33-year non-grazed site, and was lower at the 7-year non-grazed and currently grazed sites ($F_{2, 27} = 23.72, P < 0.01$; Fig. 1A). Caragana plants dominated plant communities in terms of biomass at the currently grazed site, but
made up a low proportion of the plant community at the 7-year non-grazed and 33-year non-grazed sites ($F_{2,27} = 42.12, P < 0.01$; Fig. 1B).

**Flower and pod damage by herbivorous insects on Caragana plants**

Insect abundance was the lowest at the currently grazed site and the highest at the 33-year non-grazed site ($F_{2,87} = 30.96, P < 0.01$; Fig. 2A). The percentages of flowers and pods damaged by herbivorous insects on Caragana plants followed a similar pattern: the damage percentages were the greatest at the 33-year non-grazed site, intermediate at the 7-year non-grazed site, and the lowest at the currently grazed site, respectively (flower damage: $F_{2,12} = 363.04, P < 0.01$, Fig. 2B; pod damage: $F_{2,12} = 49.78, P < 0.01$, Fig. 2C).

**Infection rates of the parasitic plant Cuscuta on Caragana plants**

The Caragana plants had the highest levels of Cuscuta infection at the 33-year non-grazed site, intermediate infection rates at the 7-year non-grazed site, and the lowest infection rates at the currently grazed site ($F_{2,12} = 27.00, P < 0.01$, Fig. 3).

**Discussion**

As has been commonly found in studies of woody encroachment and grazing by domestic animals in arid and semi-arid grasslands (Scholes and Archer, 1997; Van Auken, 2000, 2009), we found that continuous stocking of grazing domestic animals enhanced the growth of Caragana plants, which are currently encroaching in the semi-arid Inner Mongolia Steppe. The facilitative effects of grazing on Caragana encroachment could partially be due to the commonly recognized mechanisms of reduced competition from herbaceous plants (Coetzee et al., 2008) and reduction in fire frequency under grazing (Oba et al., 2000; Roques et al., 2001). Our results revealed that such facilitation on Caragana encroachment in Inner Mongolia Steppe could additionally be, in part, because of the decreased natural biological controls of herbivorous insects and parasitic plants on Caragana encroachment by grazing.

Although grazing by livestock at low intensity and frequency may have little effect on grassland plant communities, high levels of grazing by livestock can lead to reductions in biomass of herbaceous plants and changes in species composition of grasslands, facilitating the transition from grasslands to shrublands or woodlands (Coetzee et al., 2008; Knapp et al., 2008; Good et al., 2013). Caragana plants are widely distributed in the semi-arid Inner Mongolia Steppe, and they are relatively resistant to grazing disturbance (Zhang et al., 2006).
High levels of grazing by livestock during the recent decades in the semi-arid Inner Mongolia Steppe has significantly reduced the cover of herbaceous plants (Li et al., 2013; Peng et al., 2013), which favored the establishment and growth of the relatively grazing-resistant *Caragana* plants, thereby promoting the encroachment of this shrub species in the semi-arid Inner Mongolia Steppe. Additionally, our results showed that the herbivorous insect abundance per flower, and flower and pod damage by herbivorous insects on *Caragana* plants decreased from the 33-year non-grazed site to the 7-year non-grazed site, and to the currently grazed site, suggesting that grazing had negative impact on the herbivorous insects on *Caragana* plants, and thus limiting natural biological controls of herbivorous insects on the establishment and expansion of *Caragana* plants in Inner Mongolia Steppe.

Many studies have shown that herbivorous insects can reduce seeding of woody host plants by damaging seeds and reproductive parts, or by inhibiting seed formation through debilitating the host plants, which limits the establishment and spread of woody host plants. For example, Hoffmann and Moran (1998) found that two insect species, a bud-feeder, *Trichapion lativentre*, and a seed-feeder, *Rhyssomatus marginatus*, together caused substantial reductions in seeding by the invasive South American tree *Sesbania punicea* in South Africa. Meiners *et al.* (2000) found that insect herbivory had strong negative effects on the establishment of four tree species in New Jersey, United States. Hoffmann *et al.* (2002) reported that gall-forming wasp *Trichilogaster* sp. could effectively reduce seed production of the invasive Australian tree *Acacia pycnantha* in South Africa. Chaneton *et al.* (2010) reported that herbivory by tenebrionid beetles was the main factor limiting the establishment of the woody plant *Austrocedrus chilensis* in a steppe-woodland ecotone in Argentina. Similarly, at the 33-year and 7-year non-grazed sites in our study, the insect herbivores targeting *Caragana* plants reduced the fecundity of *Caragana* plants more strongly by damaging more flowers and pods than at the currently grazed site, indicating relatively strong biological controls of the insect herbivores on the establishment and expansion of *Caragana* plants at the non-grazed sites versus the currently grazed site.

In contrast, the herbivorous insects at the currently grazed site decreased in abundance under the impact of grazing disturbance. Studies in other ecosystems have shown that disturbances can cause local reductions in numbers of insects (Anderson, 1992; Dennis *et al.*, 2008; Schowalter, 2012); and in our case, insects may simply escape from the currently grazed site to avoid the disturbances from grazing by domestic animals and associated anthropogenic activities (Braschler *et al.*, 2009; Schowalter, 2012; Ben-Ari and Inbar, 2013).
Thus, with lower abundance of herbivorous insects on *Caragana* plants under grazing disturbance, biological control effects of herbivorous insects on the encroachment of *Caragana* plants decreased at the currently grazed site.

Our results also indicated that grazing also had negative impacts on the parasitic plant *Cuscuta*, and thus decreasing the biological control effects of *Cuscuta* on the encroachment of *Caragana* plants in Inner Mongolia Steppe. Previous studies have shown that parasitic plants can have strong effects on the structure and dynamics of plant communities by selectively suppressing the growth of infected plants (Pennings and Callaway, 1996; Callaway and Pennings, 1998; Bardgett et al., 2006). Additionally, parasitic plants often prefer legumes as host plants because of their relatively high nitrogen content (Marvier, 1998; Pennings and Callaway, 2002). At our study sites, the parasitic plant *Cuscuta* mainly attacked *Caragana* plants (leguminous shrub species) and had negative effects on the growth of *Caragana* plants. As the infection rates of *Cuscuta* on *Caragana* plants were higher at the non-grazed sites than the currently grazed site, we expect that growth-suppressing effects of the parasitic plant *Cuscuta* on *Caragana* plants would be stronger at the two non-grazed sites versus the currently grazed site. *Cuscuta* infection might contribute to the lower aboveground biomass of *Caragana* plants at the non-grazed sites versus the currently grazed site. Thus, at the non-grazed sites, the parasitic plant *Cuscuta* could exert a relatively strong biological control on the encroachment of *Caragana* plants, as it suppresses the growth, and thereby limiting the reproduction of *Caragana* plants. At the currently grazed site, the infection rate of *Cuscuta* on *Caragana* plants was lower, probably due to grazing by domestic animals on *Cuscuta* plants or seeds (Nicol et al., 2007). This indicated that the parasitic plant *Cuscuta* was negatively affected by grazing disturbance, and thereby having limited biological control effects on the encroachment of *Caragana* plants at the currently grazed site.

**Conclusions**

Overall, our results demonstrated that grazing could negatively affect the herbivorous insects and the parasitic plant *Cuscuta* on *Caragana* plants, and thus limit the effectiveness of their natural biological control effects on the establishment and expansion of *Caragana* plants in the semi-arid Inner Mongolia Steppe. Grazing, then, would release *Caragana* plants from the pressure of the herbivorous insects and the parasitic plant *Cuscuta* to some extent, which in turn would favor the further encroachment of *Caragana* plants in the semi-arid Inner Mongolia Steppe. These indicated that overgrazing would facilitate woody encroachment at a
faster rate than previously expected. Also, the relatively weaker biological control effects of the herbivorous insects and the parasitic plant *Cuscuta* on the expansion of *Caragana* plants at the 7-year non-grazed site versus the 33-year non-grazed site indicated that it might need a long period of time for grassland ecosystems to recover their functioning after overgrazing disturbance. Our results revealed that grazing facilitates woody encroachment in grasslands not only through the canonical mechanisms commonly known, but also by limiting natural biological controls (herbivorous insects and parasitic plants). Thus, management efforts should focus on preventing overgrazing to better protect grassland ecosystems from woody encroachment.

**Materials and methods**

**Study sites**

Field work was conducted at the Inner Mongolia Grassland Ecosystem Research Station, Chinese Academy of Sciences (IMGERS), which is located in Xilinhaote area on the Inner Mongolia Plateau (43.95 °N, 116.07 °E). The mean annual temperature and precipitation of Xilinhaote area is ~2.3 °C and ~286 mm, respectively. The mean annual sunshine time and mean daily solar radiation intensity of Xilinhaote area is ~2970 h and ~1.59 kJ cm\(^{-2}\) d\(^{-1}\), respectively. The vegetation in Xilinhaote area is typical semi-arid Inner Mongolia Steppe, and mainly consists of herbaceous plants, such as the grasses *Leymus chinensis* and *Stipa grandis*. The woody plant, *Caragana microphylla* (referred to as “*Caragana*” hereafter), is encroaching into these grasslands. *Caragana* plants are legume shrubs, which are relatively resistant to grazing animals (Zhang et al., 2006). In the study area, the herbivorous insects on *Caragana* plants were mainly the beetle species *Mylabris speciosa*, *Mylabris sibirica*, *Epicauda gorhami*, *Epicauda erythrocephala*, and *Labidostomis bipunctata*, which mainly prefer to feed on the flowers or pods of *Caragana* plants (Guo and Ma personal observation), and thereby having strong negative effect on seeding of *Caragana* plants. In the study area, the parasitic plant on *Caragana* plants was *Cuscuta chinensis* (referred to as “*Cuscuta*” hereafter), and it generally prefers legume plants as hosts (Marvier, 1998, Guo and Ma personal observation; Pennings and Callaway, 2002), and thereby debilitating *Caragana* plants.

In 2012, we conducted field work at three adjacent sites with different grazing management treatments within the IMGERS: the first site (~3 hectares in size) was a mature grassland community that has been fenced to exclude grazing from large herbivorous animals.
for 33 years (since 1979), following long-term grazing by local livestock (grazing intensity: ~2 sheep unit ha\(^{-1}\); this site is referred to as “33-year non-grazed site” hereafter); the second site (~3 hectares in size) has been fenced to exclude grazing from large animals for seven years (since 2005), following a long-term grazing by local livestock (grazing intensity: ~2 sheep unit ha\(^{-1}\); this site is referred to as “7-year non-grazed site” hereafter); and the third site has been grazed by local livestock all the time (grazing intensity: ~2 sheep unit ha\(^{-1}\); this site is referred to as “currently grazed site” hereafter). The three study sites are geographically close to each other and have relatively uniform environmental conditions and plant species compositions, and experienced similar relatively high level grazing by domestic animals before applying the grazing management treatments (Bai et al., 2010; Xie et al., 2016). No other management measures were applied to these study sites.

**Aboveground biomass**

At each study site, we surveyed the aboveground biomass of the herbaceous plants and *Caragana* plants, respectively. In August 2012, we randomly located 10 1×1m sampling plots within each study site, with distance of at least 20m between plots. In each plot, we harvested the aboveground biomass of the herbaceous plants and *Caragana* plants, respectively. Biomass was dried at 65°C to a constant weight and weighed.

**Flower and pod damage by herbivorous insects on Caragana plants**

In the flowering season, April 2012, we set up five parallel transects (120m each) with a distance of at least 20m between transects at each study site, and we randomly chose six branches of *Caragana* plants (from six *Caragana* plants) along each transect (in total 30 branches for each site) to count the number of herbivorous insects on each flower in the field, and then calculated the average number of herbivorous insects per flower for each branch (30 averages per study site). We also randomly sampled 100 flowers from *Caragana* plants (approximately 30 *Caragana* plants) along each of the transects to count the number of flowers with damage by herbivorous insects, and then calculated the percentage of flowers damaged by herbivorous insects for each transect (five replicates per study site). We visited the study sites again in August 2012, when the pods of *Caragana* plants had matured. At each study site, we set up five parallel transects (120m each) with a distance of at least 20m between transects, and we randomly sampled 100 pods from *Caragana* plants (approximately 30 *Caragana* plants) along each transect to count the number of pods with damage by
herbivorous insects, and then calculated the percentage of pods damaged by herbivorous insects for each transect (five replicates per study site).

**Infection rates of the parasitic plant Cuscuta on Caragana plants**

At each study site, we studied the infection rates of the parasitic plant *Cuscuta* on *Caragana* plants. In August 2012, we set up five parallel transects (100m each) within each study site, with a distance of at least 20m between transects. Along each transect, we sampled *Caragana* plants approximately every 10m (10 *Caragana* plants sampled per transect), and counted the number of *Caragana* plants infected by *Cuscuta*, and then we calculated the infection rate of *Cuscuta* plants on *Caragana* plants for each transect (five replicates per study site).

**Data analysis**

Data analyses were performed with JMP9 statistical software (SAS Institute, 2010). We used ANOVAs to test the differences of means among the grazing management treatments for all variables (all data met the assumptions of normality and homogeneity of variances), and then we performed post-hoc Tukey’s HSD tests to explicitly compare the means between the grazing management treatments for all the variables.

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**Competing interests**

The authors declare no competing or financial interests.

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Fig. 1 Aboveground biomass of *Caragana* and herbaceous plants (A), and proportional abundance of *Caragana* (% of total plant biomass) (B) under different grazing management treatments. Data are means±s.e.m.; n=10. Within each panel, shared letters (upper-case or lower-case) indicate means that are not significantly different from each other (Tukey’s HSD tests, significance level of 0.05).
Fig. 2 Abundance of insect herbivores (number per Caragana flower) (A), and percent flower damage (B) and percent pod damage (C) by insect herbivores on Caragana plants under different grazing management treatments. Data are means+s.e.m.; n=30 in panel A, n=5 in panel B, and n=5 in panel C. Within each panel, shared letters indicate means that are not significantly different from each other (Tukey’s HSD tests, significance level of 0.05).
Fig. 3 Infection rates of the parasitic plant *Cuscuta* on *Caragana* plants under different grazing management treatments. Data are means±s.e.m.; n=5. Within each panel, shared letters indicate means that are not significantly different from each other (Tukey’s HSD tests, significance level of 0.05).