EFFECTS OF GRAZING INTENSITY ON SEXUAL AND CLONAL REPRODUCTION IN A CLONAL XEROPHYTIC SHRUB

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Abstract

Many plants can reproduce both sexually and clonally, with different consequences for the dispersal, distribution and long-term fitness of plant populations. Understanding the factors and underlying mechanisms that mediate the trade-offs between the two reproduction modes has long been of interest to biologists and ecologists. This knowledge is critical for better understanding the dispersal and sustainability of plant populations in changing environments. Herbivory affects plant growth, and yet how herbivory affects the reproduction mode in clonal flowering plants still remains unclear. In this study, we studied the influence of grazing on the reproduction mode and traits of a dominant xerophytic shrub *Caragana brachypoda* Pojark using field experiments that under three grazing intensities. Our results showed that both sexual and clonal reproduction of *C. brachypoda* decreased as grazing intensity increased. Thus, grazing disturbance will likely lead to an aging population structure. Moreover, the proportion of sexual reproduction versus clonal reproduction of this species significantly decreased as grazing intensity increased (with sexual reproduction decreasing to 0% under intensive grazing), which will likely decrease genetic diversity and reduce adaption potential to varying climate conditions over the long run. In addition, because grazing affected seeds and distal ramets more than proximate ramets, it is likely to create a more aggregated distribution pattern of plants, which in turn would enable livestock to graze on this shrub species even more easily due to reduction in travelling costs. Our results suggest that grazing can affect both sexual and clonal reproduction in clonal plants, and can affect mode of reproduction. The effects of grazing on plant reproduction mode are likely to ultimately affect population age structure, population genetic diversity and population distribution patterns, thereby influencing the long-term future of populations even when grazing appears to be sustainable based on plant density.

Key words: *Caragana brachypoda*; Sexual reproduction; Clonal reproduction; Reproduction strategy; Spatial distribution patterns; Grazing disturbance; Arid desert; Inner Mongolian Plateau.

Introduction

Many plants can reproduce both sexually and clonally, and both reproduction modes are ecologically important, because they influence the spread, distribution and long-term fitness of plant populations (Silvertown, 2008; Bailey *et al.*, 2009; Ma *et al.*, 2013; Liu *et al.*, 2014). Advantages of sexual reproduction include reducing deleterious mutation load (Hollister *et al.*, 2015), increasing offspring diversity (Wilk *et al.*, 2009), increasing dispersal potential (Silvertown, 2008; Wilk *et al.*, 2009), and increasing adaptive potential to environmental variation (Silvertown, 2008; Schulze *et al.*, 2012). Sexual reproduction, however, is often costly compared with clonal reproduction, as it requires more resources and time (Hoerandl, 2009; Bills *et al.*, 2015; Wang *et al.*, 2018). Clonal reproduction via resprouting avoids the costs of sexual reproduction (Wilk *et al.*, 2009; Lin *et al.*, 2016). Clonal reproduction, however, limits genetic variation and thus increases the probability of deleterious mutation accumulation, which may decrease fitness of the plant population in the long term (Lin *et al.*, 2016).

Understanding the factors and underlying mechanisms mediating the mode of reproduction has long been of interest to biologists and ecologists (Ceplitis, 2001a; He *et al.*, 2004; Karpaviciene, 2017; Wang *et al.*, 2018). In particular, this knowledge is critical for better understanding the spread and sustainability of plant populations in changing environments (Bengtsson & Ceplitis, 2000; Ceplitis, 2001b; Buchanan, 2015; Liu *et*

al., 2017), given anthropogenically caused changes in climate and land use. Previous studies have shown that plants tend to increase sexual reproduction under favorable environmental conditions (Levine & Feller, 2004; Ma *et al.*, 2013; Xie *et al.*, 2016), but to increase clonal reproduction under harsh environmental conditions (Wesche *et al.*, 2005; Ma *et al.*, 2013). Herbivory can affect plant reproduction directly through consumption of flowers, seeds and new clonal ramets, or indirectly by affecting plant biomass, but how herbivory influences mode of reproduction in clonal flowering plants has been rarely examined, and thus still remains unclear(Buchanan, 2015).

Xerophytic shrubs are the dominant vegetation in many arid desert regions, and a stable population of these shrubs is critical for maintaining the functions of desert ecosystems (Vetaas, 1992; Kidron,2010; Tracol *et al.*, 2011). *Caragana brachypoda* Pojark is a xerophytic shrub, dominant in the arid desert region of the Inner Mongolian Plateau (Zhou *et al.*, 2005), and it plays important roles in stabilizing sand, maintaining water resources, and providing food and refuge to animals. Grazing of livestock, especially sheep, can strongly affect plant success in this area (Xie *et al.*, 2018). *C. brachypoda* can reproduce both sexually, by producing seeds in pods, and clonally, by producing new ramets that grow from horizontal roots, but the extent to which grazing intensity affects its mode of reproduction has never been explicitly examined.

To increase our understanding of the relationship between herbivory and reproduction modes in flowering clonal plants, we experimentally examined the influence of grazing intensity on the mode of reproduction in *C. brachypoda*. Our aims were to understand how grazing intensity affects sexual and clonal reproduction in *C. brachypoda*, to identify the mechanisms through which grazing affected its mode of reproduction, and to determine the consequences of these influences for long-term population stability of this dominant xerophytic shrub species.

Materials and Methods

Study sites and experimental design: Field experiments were conducted in the Alashanzuo area in the arid desert region of the Inner Mongolian Plateau (38.84º N, 105.66º E; altitude~1550 m). In the study area, mean annual temperature is 7.8ºC, mean annual precipitation is 110 mm, mean annual total sunshine time is 3200 h, and mean daily solar radiation intensity is 1.71 kJ cm^{-2} d^{-1}. Vegetation cover in the study area is low (< 20%), and the vegetation mainly consists of xerophytic shrubs. Relatively about 30% of the total plant biomass is represented by the dominant xerophytic shrub *C. brachypoda* (unpublished data) in the study area. The other plant species in the area include shrub species: *Caragana tibetica, Caragana roborovoskyi, Nitraria tangutorum, Artemisia desertorum, Atraphaxis frutescens, Helianthemum songaricum, Oxytropis aciphylla,* and herbaceous species: *Stipa glareosa, Asparagus cochinchinensis, Salsola ruthenica, Cynanchum thesioides，Stragalus membranaceus.* The soil in the study area is typical grey-brown desert soil.

In 2008, we located four study sites at least 50 km apart from each other, and within each site we established three plots with different grazing intensities: no grazing, mild grazing (0.4 sheep per hectare), and intensive grazing (0.8 sheep per hectare), for a total of 12 plots. At each site, the size of the no grazing plot was about 10 hectares, the size of the mild grazing plot was about 50 hectares, and the size of the intensive grazing plot was about 50 hectares. Local villagers grazed sheep, according to the grazing intensity settings, at the study sites from 2008 through to the period 2012-2013 when this study was conducted. No other management measures were applied to these study sites. A vegetation survey in 2012 showed that the average cover of *C. brachypoda* was 3.95% in the no grazing plots, 3.62% in the mild grazing plots, and 3.23% in the intensive grazing plots; the average crown size of *C. brachypoda* was 1.51 m^2 in the no grazing plots, 1.41 m^2 in the mild grazing plots, and 1.29 m^2 in the intensive grazing plots.

Sexual reproduction: We examined plant sexual preproduction in all the plots under three grazing intensities, including seed production, seed vigor, sapling establishment, annual sexual recruitment.

Seed production and vigor: We surveyed seed production twice respectively in 2012 and 2013. In each plot in each year, we established a 100 m long transect, and selected 10 individuals of *C. brachypoda* shrub at

intervals of 10 m along the transect, for a total of 20 shrubs sampled per plot over the two years. For each shrub, we counted the total number of flowers during each flowering season (we recorded the number of new flowers every 3 days during each flowering season, April 20 to May 23), and the total number of pods during each fruiting season (we recorded the production of new pods every 3 days during each fruiting season, May 15 to June 25). After all surviving pods matured, we harvested all mature pods to count seed number. We calculated fruit set for each shrub according to the formula:

$$Fruit\ set = mature\ pod\ number\ /\ flower\ number$$

In each plot, we randomly collected 50 mature pods from at least 20 shrubs, and counted the number of seeds within each pod. We determined the mass of one hundred seeds from each plot by collecting seeds from at least 20 shrubs per plot (we collected a lot of seeds, pooled them, and then randomly picked out a sub-sample of 100 seeds), air drying them for 7 days, and weighing them on an electronic balance.

In June 2012 and 2013, we collected and air-dried healthy *C. brachypoda* seeds from each plot. In the following March 2013 and 2014, we measured seed vigor on a haphazard sub-sample of 100 of these seeds. We germinated seeds on wet filter paper in the dark in an incubator set at 25℃ during the day and 15℃ during the night. Data from the two years were pooled, and germination rate and germination index values calculated using the following formulas:

$$Germination\ rate\ (\%) = (number\ of\ germinated\ seeds\ /\ total\ number\ of\ seeds) \times 100$$
$$Germination\ index = \sum Gt\ /\ Dt,$$

where Gt represents the number of germinated seeds on day t, and Dt represents the number of elapsed days.

Sapling establishment and annual sexual recruitment: We measured sapling establishment twice respectively during 2013-2014 and 2014-2015 seasons. We established two 90 m long transects in April 2013 and April 2014 in each plot, and placed nine 1m × 1m quadrats at intervals of 10 m along each transect, thus got 18 quadrats per plot over the two years. The vegetation in quadrats were representative for each of the plots. We sowed 100 seeds of *C. brachypoda* in each quadrat. Our previous survey showed that the ambient density of *C. brachypoda* seeds in the soil was only about 0.5 seeds/m^2 (unpublished data), and thus the number of pre-existing seeds in the soil was negligible. After 18 months (at the end of the two growing seasons), we recorded the number of surviving saplings in each quadrat.

We quantified the annual sexual recruitment of *C. brachypoda* population for each plot using the following formula:

$$Annual\ sexual\ recruitment\ (saplings\ per100\ shrubs) = sapling\ establishment\ ratio\ (saplings/seeds) \times seed\ number\ per\ shrub \times 100\ shrubs$$

Clonal reproduction: We investigated plant clonal reproduction by counting the size of the bud bank, and examining the clonal architecture, and annual ramet recruitment. We surveyed the bud bank and inter-ramet distance in April 2013 and 2014. In each year, we randomly sampled 10 individuals of *C. brachypoda* shrubs in each plot. For each shrub sampled, we carefully removed the sand accumulated around the base of the shrub, and then dug down 30-40 cm from the surface to expose the belowground biomass. *C. brachypoda* produces new buds along horizontal roots extending out from the taproots of the mother plant or adult ramets. Live buds belowground represent the "bud bank". Buds that produce aboveground shoots are defined as "clonal ramets", and the length of horizontal root between clonal ramets along a single horizontal root is the "inter-ramet distance". We recorded the size of the bud bank for each shrub, the number of ramets in each clone (not including the parent shrub), and the inter-ramet distance for all the ramets. We surveyed the annual ramet establishment in the autumn each year, i.e., in September 2014 and 2015. We randomly sampled 100 individuals of *C. brachypoda* shrubs in each plot. For each shrub sampled, we recorded the number of ramets from the previous year's crop that had successfully established. Ramets from the previous year's crop could be distinguished from older ramets based on their size. We calculated the annual ramet recruitment of *C. brachypoda* population for each plot using the following formula:

Annual ramet recruitment = annual number of ramets established per 100 shrubs

Annual population mortality and growth: We also documented annual population mortality to evaluate annual population growth. We surveyed the annual population mortality in September 2014 and 2015, respectively. In each year, we randomly sampled 100 *C. brachypoda* shrubs in each plot. For each shrub sampled, we recorded the number of *C. brachypoda* plants that had died. We calculated the annual population mortality for each plot using the following formula:

Annual population mortality = annual number of plants died per100 shrubs

Based on annual sexual recruitment, annual ramet recruitment and annual population mortality, we quantified annual population growth for each plot using the following formula:

Annual population growth = (annual sexual recruitment + annual ramet recruitment) - annual population mortality

Proportion of sexual reproduction: We calculated proportion of sexual reproduction during population recruitment for each plot using the following formula:

Proportion of sexual reproduction (%) =annual sexual recruitment / (annual sexual recruitment + annual ramet recruitment) × 100

Data analysis

We analyzed data using Generalized Linear Mixed Models (GLMMs) with grazing intensity as a fixed factor.

When we examined the differences in flower number per shrub, juvenile pod number per shrub, mature pod number per shrub, fruit set, seed number per shrub, bud bank and inter-ramet distance of *C. brachypoda* among the three grazing intensities, we treated sites under each grazing intensity and individual shrubs within each site (individual shrubs were nested within sites) as random factors. When we examined the differences in sapling establishment percentage of *C. brachypoda* among the three grazing intensities, we treated sites under each grazing intensity and quadrats within each site (quadrats were nested within sites) as random factors. When we examined the differences in seed number per pod, seed weight, seed vigor, annual sexual recruitment, annual ramet recruitment, annual population mortality, annual population growth, and proportion of sexual reproduction during population recruitment of *C. brachypoda* among the three grazing intensities, we treated sites under each grazing intensity as a random factor. All analyses were performed using SPSS 17.0.

Results

Seed production: As grazing intensity increased, the number of flowers, juvenile pods and mature pods per shrub sharply decreased (Fig. 1a, b, c). The strength of the differences gradually increased from the flower phase to the juvenile pod phase and then to the mature pod phase. Similarly, fruit set sharply decreased as grazing intensity increased (Fig. 1d). The number of seeds per pod slightly decreased as grazing intensity increased (Fig. 1e). The sharp decrease in the number of mature pods per shrub coupled with the slight decrease in the number of seeds per pod led to a sharp decrease in the number of seeds produced per shrub as grazing intensity increased (Fig. 1f), with the number of seeds per shrub in the no grazing treatment being roughly 4 times of that in the mild grazing treatment and being about 45 times of that in the intensive grazing treatment. Seed weight of *C. brachypoda* did not vary with grazing intensity (Fig. 2a), but seed germination rate and the germination index decreased with increasing grazing intensity (Fig. 2b).

Sapling establishment and annual sexual recruitment: Sapling establishment sharply decreased with increasing grazing intensity (Fig. 3a). The sapling establishment percentage in the mild grazing treatment was 60% of that in the no grazing treatment, and the sapling establishment percentage in the intensive grazing treatment was 0%. Annual sexual recruitment of *C. brachypoda* sharply decreased as grazing intensity increased (Fig. 3b). Annual sexual recruitment in the no grazing treatment was about 7 times of that in the mild grazing treatment. There was no sexual recruitment in the intensive grazing treatment.

Bud bank, clonal architecture, and annual ramet recruitment: The size of the bud bank of *C. brachypoda* decreased as grazing intensity increased (Fig. 4a). The bud bank in the no grazing treatment was 1.2 times of that in the mild grazing treatment, and 1.5 times of that in the intensive grazing treatment.
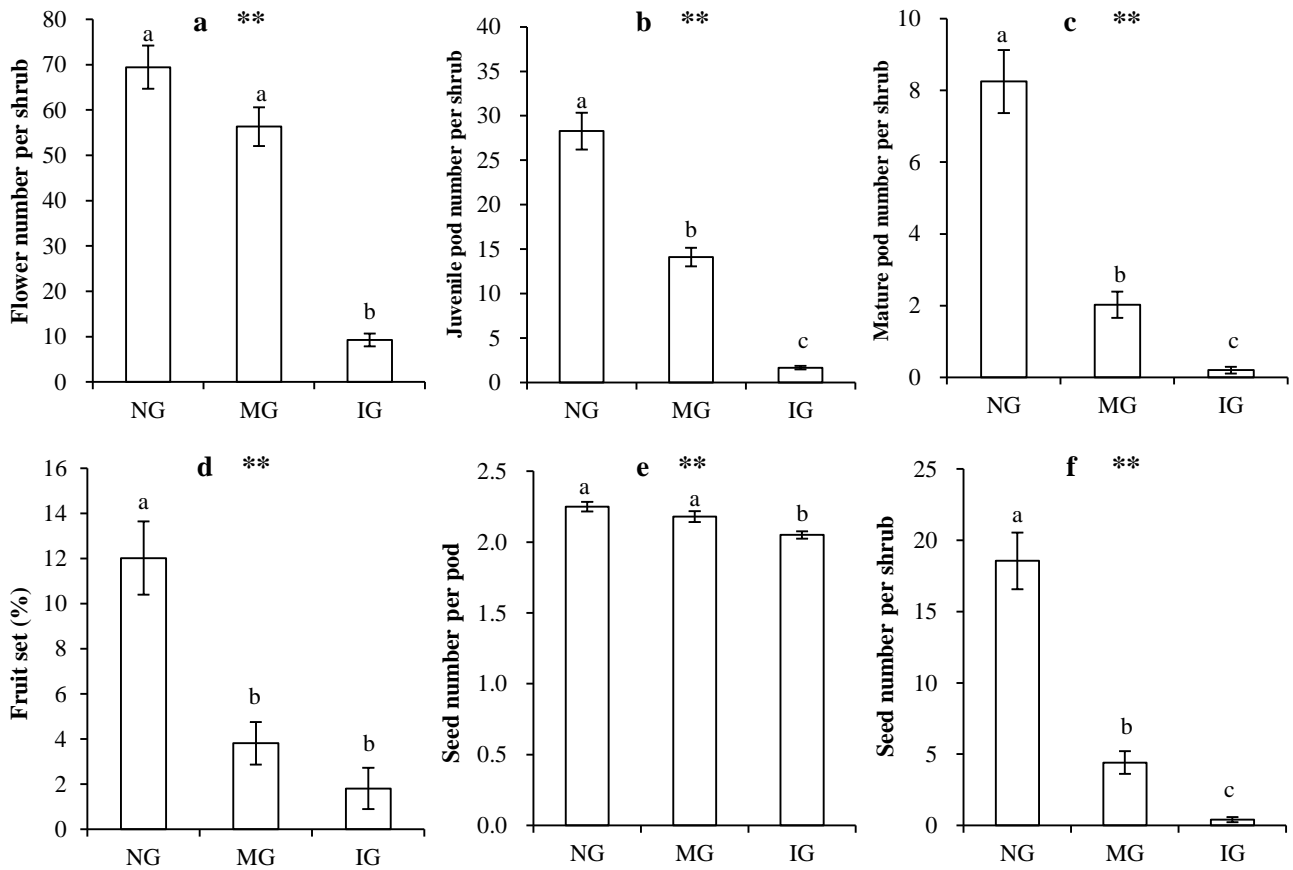
Fig. 1. Flower number per shrub (a), juvenile pod number per shrub (b), mature pod number per shrub (c), fruit set (d), seed number per pod (e) and seed number per shrub (f) of *C. brachypoda* under different grazing intensities. Data are means ± SE. Abbreviations: NG, no grazing; MG, mild grazing; IG, intensive grazing. ** indicates $p<0.01$ in GLMMs. Different letters indicate significant differences between grazing intensities ($p<0.05$).
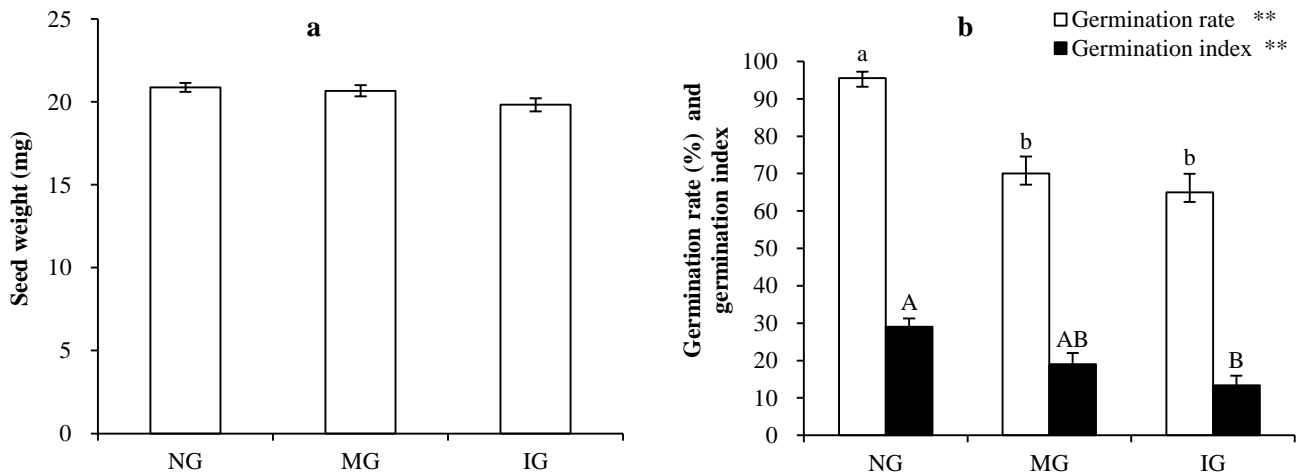

Fig. 2. Seed weight (a) and seed vigor (b) of *C. brachypoda* under different grazing intensities. Data are means ± SE. Abbreviations: NG, no grazing; MG, mild grazing; IG, intensive grazing. ** indicates $p<0.01$ in GLMMs. For the same index, different letters indicate significant differences between grazing intensities ($p<0.05$).

The inter-ramet distance of *C. brachypoda* decreased as grazing intensity increased (Fig. 4b). Compared with that in the no grazing treatment, inter-ramet distance decreased 26% in the mild grazing treatment, and 45% in the intensive grazing treatment.

Annual ramet recruitment of *C. brachypoda* sharply decreased as grazing intensity increased (Fig. 4c). Annual ramet recruitment in the mild grazing and intensive grazing treatments decreased by 23% and 50%, respectively, compared with that in the no grazing treatment

Annual population mortality and annual population growth: Annual population mortality of *C. brachypoda* increased as grazing intensity increased (Fig. 5a). Annual population mortality increased 1.3 times in the mild grazing treatment and times in the intensive grazing treatment, compared with that in the no grazing treatment.
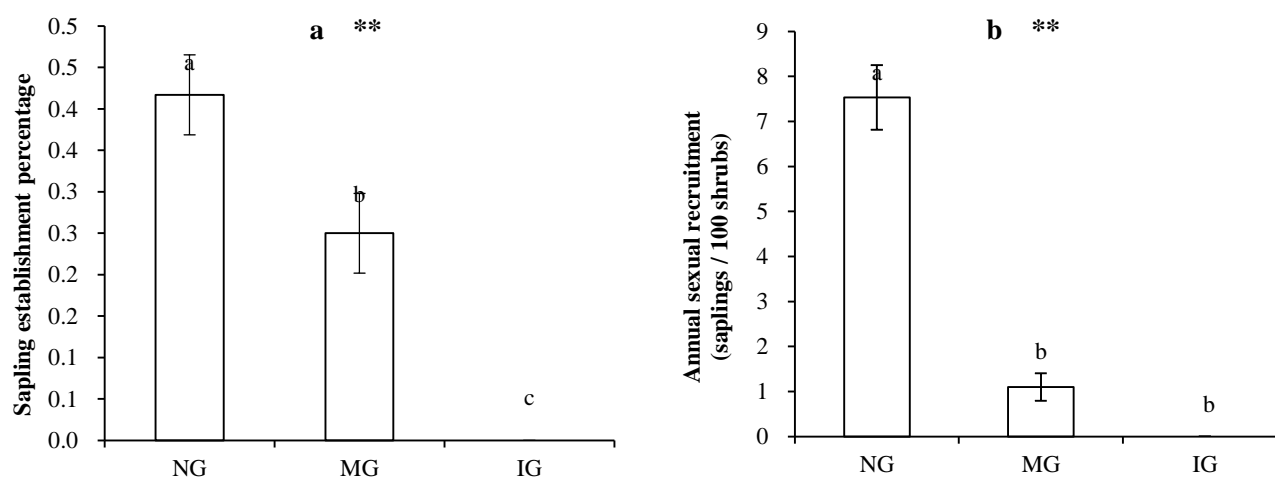
Fig. 3. Sapling establishment percentage (a), annual sexual recruitment (b) of *C. brachypoda* under different grazing intensities. Data are means ± SE. Abbreviations: NG, no grazing; MG, mild grazing; IG, intensive grazing. ** indicates *p*<0.01 in GLMMs. Different letters indicate significant differences between grazing intensities (*p*<0.05).

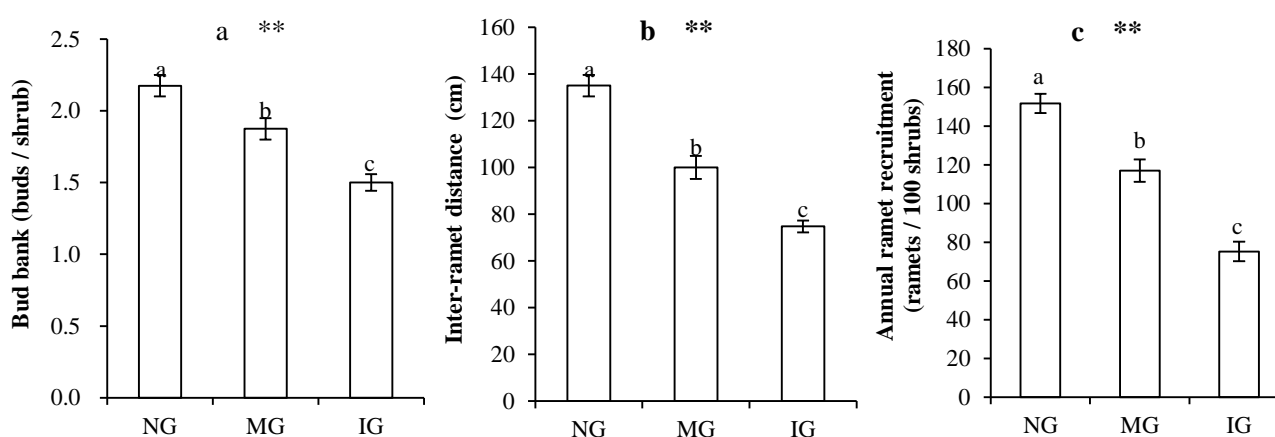Fig. 4. Bud bank (a), inter-ramet distance (b), annual ramet recruitment (c) of *C. brachypoda* under different grazing intensities. Data are means ± SE. Abbreviations: NG, no grazing; MG, mild grazing; IG, intensive grazing. ** indicates *p*<0.01 in GLMMs. Different letters indicate significant differences between grazing intensities (*p*<0.05).

Annual population growth of *C. brachypoda* sharply decreased as grazing intensity increased (Fig. 5b). In the no grazing and mild grazing treatments, annual population recruitment (sum of annual sexual recruitment and annual ramet recruitment) of *C. brachypoda* was greater than annual population mortality, and thus annual population growth was positive. In contrast, in the intensive grazing treatment, annual population recruitment was less than annual population mortality, and thus annual population growth was negative.

Proportion of sexual reproduction during population recruitment: In all treatments, most of the population growth was due to clonal production of new ramets rather than sexual production. This was especially true as grazing increased. The proportion of sexual reproduction during population recruitment sharply decreased with the increase of grazing intensity, as it was 4.7% in the no grazing treatment, 0.9% in the mild grazing treatment, and 0% in the intensive grazing treatment (Fig. 6).

Discussion

In this study, we found that, grazing significantly decreased both sexual and ramet recruitment of *C. brachypoda*, and increased its population mortality. These results were probably due to the following reasons. First, grazing sheep directly consumed flowers, pods, seeds and new sprouting ramets, and may also have destroyed new sprouting ramets by trampling. Similar negative effects of livestock have been seen in other arid land shrubs (Zhang *et al.*, 2006; Foroughbakhch *et al.*, 2012). Second, consumption of foliage by sheep led to reduced photosynthesis per shrub, and thus a deficiency of organic matter accumulation, resulting in decreased fertility and increased population mortality. This would also lead to smaller shrub size and lower density of reproductive organs on shoots. Third, grazing also likely increased soil compaction, as this has been found in other systems (Pulido *et al.*, 2017; Pulido *et al.*, 2018), which would limit successful seed germination of this species, resulting in decreased sexual recruitment.
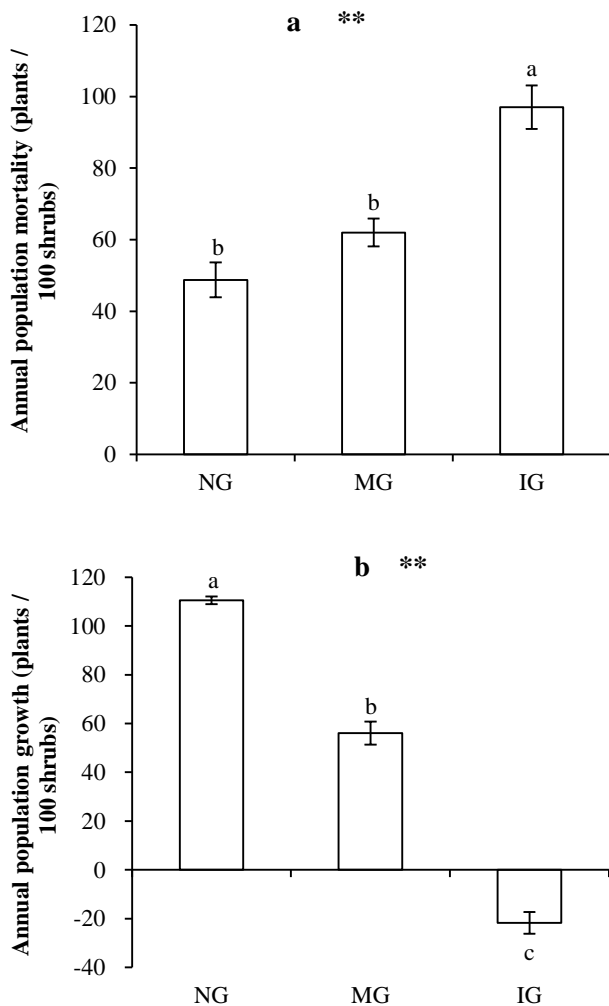
Fig. 5. Annual population mortality (a) and annual population growth (b) of *C. brachypoda* under different grazing intensities. Data are means ± SE. Abbreviations: NG, no grazing; MG, mild grazing; IG, intensive grazing. ** indicates *p<0.01* in GLMMs. Different letters indicate significant differences between grazing intensities (*p<0.05*).
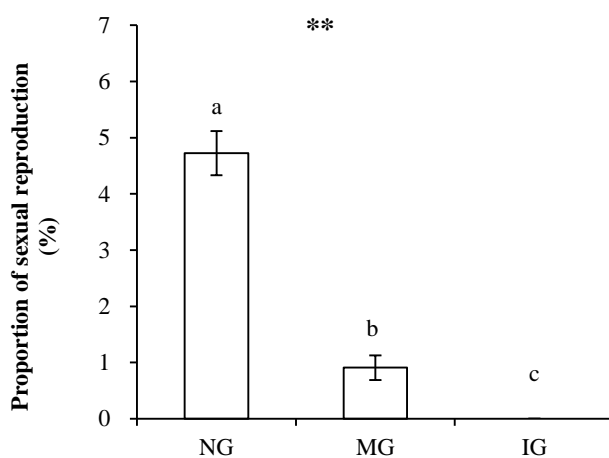

Fig. 6. Proportion of sexual reproduction during *C. brachypoda* population recruitment under different grazing intensities. Data are means ± SE. Abbreviations: NG, no grazing; MG, mild grazing; IG, intensive grazing. ** indicates *p<0.01* in GLMMs. Different letters indicate significant differences between grazing intensities (*p<0.05*).

The negative effects of herbivory on plant traits which decrease the ability of plants to reproduce sexually or clonally can also be found in other plant species. For example, Elle & Hare (2002) studied floral traits in *Datura wrightii*, and found that plants attacked by herbivores had fewer open flowers, which negatively affected sexual reproduction. Steets & Ashman (2004) investigated effects of herbivory on the mating system of *Impatiens capensis*, and showed that leaf damage indirectly affected reproduction by reducing chasmogamous flower size, leading to a reduction in pollinator visitation. Xie *et al.*, (2018) showed that the sapling establishment percentage of *Caragana stenophylla* was negatively affected by livestock grazing, leading to decreased sexual recruitment. Brathen & Junttila (2006) studied the tolerance of the clonal sedge *Carex bigelowii* to damage in genets, and found that ramet weight decreased under damage treatments, indicating that herbivory reduced clonal reproduction. Our results were consistent with these previous studies and indicated that grazing disturbance could exert strong limiting effects on sexual and clonal reproduction of plants simultaneously.

The negative effects of grazing on both sexual and clonal recruitment of *C. brachypoda* not only resulted in a negative population trend, they also resulted in an aging population age structure, as the proportion of young individuals decreased under grazing disturbance. Although little is known about senescence in *C. brachypoda*, it is likely that an older population will be less vigorous, further increasing the negative effects of grazing on the population growth rate.

Our results revealed that the proportion of sexual reproduction versus clonal reproduction of *C. brachypoda* decreased as grazing intensity increased, to the point that there was no sexual recruitment under intensive grazing. These suggested that population growth of this species by sexual reproduction was more sensitive than by clonal reproduction to grazing disturbance. This happened because sexual reproduction in *C. brachypoda* occurs mainly aboveground, while clonal propagation occurs mainly belowground, and the influence of grazing by livestock is generally stronger aboveground than belowground (Sun *et al.*, 2014; Larreguy *et al.*, 2017; Sun *et al.*, 2018). Thus, grazing would have a stronger effect on sexual reproduction than clonal propagation for this species. Moreover, because sexual reproduction is generally a costly strategy (Wang *et al.*, 2018), it is possible that *C. brachypoda* plants experiencing heavy grazing reduced their energetic investment into sexual reproduction.

The high proportion of clonal reproduction and low proportion or even absence of sexual reproduction under grazing disturbance may reduce the adaptation potential of this shrub species to varying climate conditions in the long run, as sexual reproduction is very important for maintaining genetic diversity and promoting overall fitness (Stocklin & Winkler, 2004; Wang *et al.*, 2004). These indicated that populations with high versus low levels of grazing will not only experience changes in abundance, but also over time will have different genetic structures and evolutionary potentials.

Because seeds can travel farther from plants than rhizomes, sexual reproduction endows plants with a greater dispersal potential, and thus allows plants to be widely distributed within a habitat (Xie *et al.*, 2015). In contrast, clonal propagation is spatially restricted, and leads plants to form an aggregated distribution (Cristobal *et al.*, 2014). In this study, grazing disturbance decreased the proportion of sexual reproduction and increased the proportion of clonal propagation of *C. brachypoda*. Over time, this should decrease the chance of individual dispersal and thus promote an aggregated distribution of *C. brachypoda* populations. In addition, other studies have shown that grazing causes a high mortality of ramets that are relatively far away from and thus less protected by "nurse effects" of the mother shrub plants (Xie *et al.*, 2015; Saixiyala *et al.*, 2017), which would make the distribution of *C. brachypoda* populations even more aggregated. Finally, grazing decreased the inter-ramet distance of *C. brachypoda* ramets. Two mechanisms might lead to the decrease of inter-ramet distance. First, new ramets with a shorter spacer could receive more protection from the mother shrub against animal browsing. Second, grazing led to poor performance of *C. brachypoda* shrubs, and a consequent lack of resources may have restricted spacer growth. The negative effects of grazing on inter-ramet distance were also found in other clonal plant species, such as *Trientalis europaea* (Piqueras, 1999), and *Carex moorcrofii* (Sun *et al.*, 2014). Shortened inter-ramet distance of *C. brachypoda* ramets under grazing disturbance would also promote aggregated distribution of *C. brachypoda* populations.

Thus, the decreased proportion of sexual reproduction, increased relative importance of population clonal propagation, and morphological responses of *C. brachypoda* to grazing should lead over time to a more aggregated distribution pattern of this shrub species, which is consistent with the trend of distribution pattern of *C. brachypoda* observed in the plots with different grazing intensities (authors' personal observation). An aggregated distribution of plants may facilitate further livestock grazing on the species due to reduction in travelling costs (Bailey *et al.*, 1998; Matsumura *et al.*, 2010). If so, this would represent an adverse feedback that would further negatively affect the sustainability of *C. brachypoda* populations.

Conclusions

In summary, grazing decreases both sexual and clonal reproduction of the shrub *C. brachypoda*, and the decrease to sexual reproduction is greater than that to clonal reproduction. These effects likely negatively influence population age structure, genetic diversity and distribution patterns of *C. brachypoda*, leading to population degradation of this species as grazing intensity increases. Our study suggests that grazing can influence the mode of plant reproduction and thereby population dynamics through multiple mechanisms. One important consequence is that the age structure and genetic structure of the population may be affected even if the population density is stable, suggesting that by affecting plant reproduction modes, grazing may have important negative effects on plant populations even when in the short term it appears to be at sustainable levels.

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