Competition between *Artemisia frigida* and *Cleistogenes squarrosa* under different clipping intensities in replacement series mixtures at different nitrogen levels

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Abstract

The relative competitive ability of *Artemisia frigida* Willd. (C3, semi-shrub) and *Cleistogenes squarrosa* (Trin.) Keng (C4, perennial bunchgrass), grown in a greenhouse, was studied under three nitrogen fertilizer levels, three defoliation intensities and five competitive replacement series in a factorial arrangement. Relative yield total and the respective crowding coefficients for each species were calculated with reference to above-ground, below-ground and total biomass. The index of aggressivity was also determined relative to total biomass.

*Cleistogenes squarrosa* was a consistently weak competitor at all nitrogen fertilizer and clipping levels compared with *A. frigida*. *Artemesia frigida* had a higher relative yield when grown in mixtures with *C. squarrosa* than in monocultures, whereas the yield of *C. squarrosa* was lower in mixtures with *A. frigida* than in monocultures. The relative competitive ability of *A. frigida* over *C. squarrosa* declined significantly at different clipping intensities with increasing nitrogen fertilizer. The interaction of nitrogen fertilizer with clipping was significant, although the clipping effect on aggressivity was not significant.

Keywords: plant competition, clipping treatment, steppe, nitrogen fertilizer, competitive ability, aggressivity

Introduction

Disturbance (herbivory) and stress (fertility) have profound effects on the competitive ability of plant species (Li and Watkinson, 2000; Hambäck and Beckerman, 2003). Herbivory may increase or decrease the competitive effect of one plant on another through its ability for plant regrowth (Whitham et al., 1991). The response of plants to grazing may differ between and within species, depending on nutrient availability, and on the intensity and frequency of herbivory (Wallace et al., 1984; Polley and Detling, 1988). Fertilizers can be used to change competitive relationships (Armstrong et al., 1993) leading to variations in the outcome of competition (Aerts, 1999). The persistence of plants in grassland ecosystems, therefore, largely depends on the ability of individuals to cope with complex and dynamic interactions between herbivory and the limitations of environmental resources.

Many studies have been conducted on successional dynamics of plant community in the Inner Mongolian steppe (Li, 1989; Wang, 1992; Wang et al., 1998). However, limited results on successional mechanisms are available, especially on competition among different species. *Stipa grandis* and *Leymus chinensis* are dominant species of the climax grassland communities in the Inner Mongolia steppe (Chen and Wang, 2000), but they will be replaced by *Cleistogenes squarrosa* at moderate grazing pressures and by *Artemisia frigida* at heavy grazing pressures on soils with a low fertility (Li, 1989; Wang et al., 1998; Chen et al., 2002).

*Artemesia frigida* Willd. is a C3, semi-shrub found in the steppes of the Eurasian Continent. The species has a taproot system and is a relatively high-quality forage in the autumn and winter. The *A. frigida* community, which has a low productivity, becomes the dominant community in the Inner Mongolian steppe, when the grassland is degraded. *Cleistogenes squarrosa* (Trin.) Keng., a short bunchgrass with characteristic...
C₄ anatomy, is the dominant species that accounts for proportionately 0.12 of the present whole grassland area on moderately degraded grassland in the Inner Mongolian steppe. *Cleistogenes squarrosa* has a fibrous root system and is a key species for sustainable grassland development. *Artemesia frigida* and *C. squarrosa* are excellent species for competition studies because they occur together naturally in preponderance in different successional stages (Wang et al., 2001).

The sandy chestnut soils of Inner Mongolia steppe are infertile, and pasture species have markedly different responses to nitrogen (N), for example *A. frigida* has a high requirement for soil N compared with *C. squarrosa*. The *A. frigida* community is approximately 0.20–0.25 of the total grassland area in the Inner Mongolia steppe (Wang et al., 2003). In particular, the degraded grassland area increases with population pressure and grazing intensity in the Inner Mongolia steppe. Usually, *C. squarrosa* is a subdominant species in the *A. frigida* community. Therefore, this study addresses the following question: by adjusting the grazing intensity and the amount of N fertilizer could the restoration of an *A. frigida* community be promoted or would *C. squarrosa* be the dominant species when no grazing or light grazing and N fertilizer is applied?

**Materials and methods**

**Soil and plant materials**

Degraded grassland soil (0–20 cm) was collected in August 2002 from the Inner Mongolia Grassland Ecosystem Research Station (IMGER), The Chinese Academy of Sciences, China, located at 43°26'–44°08'N, 116°04'–117°05'E, most of which is more than 1000 m a.s.l. The soil is a sandy chestnut soil (Chinese classification) or Calciustepts (US Soil Taxonomy classification) which is the main soil type of the Inner Mongolian grassland. Following air-drying at room temperature, the soil was screened through a 2-mm sieve. Soil pH (1:5 w:v soil suspension in 0.01 mol L⁻¹ CaCl₂ at 25°C) was 7.8, and soil phosphorus content (0.5 mol L⁻¹ NaHCO₃) was 1.6 mg kg⁻¹. Organic carbon and total N contents were 9.1 and 0.9 g kg⁻¹, respectively. Water holding capacity was 22.5%.

The seeds of two populations of *A. frigida* and *C. squarrosa* were collected from the IMGER in September 2002.

**Experimental procedures**

The experiment was performed in a greenhouse at the Institute of Botany, The Chinese Academy of Sciences, Beijing. One kilogram of soil (oven-dried at 110°C) was put into each of 180 plastic pots (15 cm deep). Each pot was lined with a plastic bag to prevent leaching of nutrients. All pots received an initial solution containing 0.8 mg FeEDTA, 104 mg KH₂PO₄, 50 mg MgSO₄·7H₂O, 0.8 mg MnSO₄·4H₂O, 2 mg CuSO₄·5H₂O, 2.2 mg ZnSO₄·7H₂O, 0.7 mg Na₂MoO₄·2H₂O and 0.8 mg H₂BO₃. Of the 180 pots, sixty pots received no nitrogen addition; sixty received 60 mg N kg⁻¹ per soil and sixty received 120 mg N kg⁻¹ in the form of ammonium nitrate. The soil moisture content was raised to 0.45 of water-holding capacity by adding deionized water and was maintained at approximately this level by frequently weighing and adding deionized water during the experiment.

A 3 × 3 × 5 factorial randomized complete block design was used with three nitrogen levels (N₀ = 0 N mg per pot, N₁ = 60 N mg per pot, N₂ = 120 N mg per pot), three defoliation intensities (heavy, light and no defoliation) and five competitive replacement series. The five planting proportions were monocultures of each species and mixtures at 0:75:0:25, 0:5:0:50 and 0:25:0:75. Each treatment was replicated four times. Approximately five seeds of the same species were sown in each of four holes in each pot. The populations were raised in a controlled greenhouse with a temperature range of 18–32°C and with additional illumination (sodium light) providing a 14-h day after sowing.

The plants were thinned to one per hole 14 d after seedling emergence so that individual plants were as homogenous in size as possible after thinning. This resulted in a planting density of four plants per pot. Plant density was equivalent to 977 seedlings m⁻², higher than the natural density in the field in order to shorten the period of time prior to competitive interaction occurring. The defoliation treatments were imposed at different stubble heights in each species because of their different sizes. Plant heights to the ligule of the uppermost fully expanded leaf were measured 2 d prior to defoliation, and the heavy defoliation intensity was set at 0:60 of these means: 31 mm stubble height above the ground for the *C. squarrosa* population and 25 mm for the *A. frigida* population. The stubble height for the light defoliation treatment was set at twice the height of the heavy treatment: 62 mm for *C. squarrosa* and 50 mm for *A. frigida*. The clipping was carried out at 2-week intervals. Overall, an average of 0.37 and 0.60 of total above-ground biomass produced by plants of both populations were clipped for light and heavy defoliation treatments over the 16-week duration of the experiment, respectively. Blocks were randomly assigned to a location within the greenhouse and pots were re-randomized within each block weekly.

The number of tillers of *C. squarrosa* and branches of *A. frigida* were monitored at each harvest and any losses...
were recorded. After herbage was removed at the final harvest (16 weeks), stems, roots, green leaves, reproductive stems and vegetative stems were separated from the potting mix, washed, and sorted into their component parts for each species, dried (48 h at 80°C), and weighed. Material removed at each defoliation was sorted in the same way and summed to estimate total production.

Data analysis

Parameters of the competition model of de Wit (1960) for the dry weight of *A. frigida* and *C. squarrosa* at different clipping intensities along the N gradient were estimated using a maximum likelihood ratio statistic (Machin and Sanderson, 1977). The procedures are described in detail by Hill and Gleeson (1988), Robinson and Whalley (1991) and Armstrong et al. (1993).

The essential features of de Wit’s model are as follows: the yields of *A. frigida* (y) and *C. squarrosa* (z) are given by Equations 1 and 2 respectively:

\[ y = \frac{M_i k_{ij} t}{(k_j - 1) t + 1} \]  
\[ z = \frac{M_i k_{ij} v}{(1 - k_j) t + k_j} \]  

where y and z are the respective yields per pot of *A. frigida* and *C. squarrosa* in the proportions \( i : j \); the notation \( i \) was used for *A. frigida* and \( j \) for *C. squarrosa*; \( M_i \) and \( M_j \) are the respective monoculture yields per pot of *A. frigida* and *C. squarrosa*, and \( k_{ij} \) and \( k_{ji} \) are the respective relative crowding coefficients for one species towards the other, and \( u \) and \( v \) are the respective proportions of seedlings of *A. frigida* and *C. squarrosa* in the mixture (proportions vary from 0 to 1).

The relative yield total (RYT), i.e. the sum of the relative yields of species \( i \) and \( j \), is given by:

\[ (\text{RYT})_{ij} = \frac{y}{M_i} + \frac{z}{M_j} = k_{ij} \times k_{ji}. \]

The de Wit relative crowding coefficient, \( k \), provides a measure of the aggressiveness of each species \( i \) grown with species \( j \) in the mixture (Machin and Sanderson, 1977). Relative yield total is the product of the respective crowding coefficients \( (k_{ij} \times k_{ji}) \) for each species (Harper, 1977; Hill and Gleeson, 1991). An RYT value =1.0 indicates that the two species are making the same demands on the same limiting resource (Harper, 1977). An RYT value >1.0 implies that species either make different demands on resources, avoid competition with each other or show a symbiotic relationship, and an RYT <1.0 suggests mutual antagonism.

The procedure of Machin and Sanderson (1977) also provides a test for the adequacy of fit of four submodels \((k \neq t, k = t, k = t = 1\) and the default \(k = t \neq 1\), where \(k = k_j\) and \(t = 1/k_j\)). The biological interpretations of each submodel are given in Harper (1977).

In order to assess the competitive abilities of the two species in mixtures effectively, another index of aggressivity \( (A) \) (McGilchrist and Trenbath, 1971) was used. For the mixtures of equal numbers of *A. frigida* and *C. squarrosa*, so the aggressivity of *A. frigida* against *C. squarrosa* \((A_{AC})\) was calculated by the modified formula:

\[ A_{AC} = \frac{W_{AM}}{W_{AP}} - \frac{W_{CM}}{W_{CP}} \]

where \(W_{AP}\) and \(W_{CP}\) are total dry weights per pot of *A. frigida* and *C. squarrosa* in their respective monocultures, and \(W_{AM}\) and \(W_{CM}\) are those of *A. frigida* and *C. squarrosa* in their mixtures, respectively. When \(A_{AC} < 0\), \(A_{AC} = 0\) or \(A_{AC} > 0\), *A. frigida* is less competitive than, as competitive as or more competitive than *C. squarrosa* respectively.

Results

Respective crowding coefficients and relative yield total

The relative crowding coefficients, based on above-ground biomass, suggested that *A. frigida* was generally more dominant than *C. squarrosa* as indicated by a higher crowding coefficient. Under-yielding (no or moderate N addition) or over-yielding (high N addition) occurred in the no clipping treatment. Under-yielding \((k \neq t \neq 1, \text{RYT} < 1.0\) occurred under light clipping intensity regardless of N addition (Figure 1a–c). However, when heavy clipping was imposed on the two species, over-yielding (no N applied) or competition (moderate or high N addition) occurred (Figure 1a–c). These results indicated that the dry-matter (DM) weight of *C. squarrosa* was strongly inhibited by the presence of *A. frigida* in the no- and light-defoliation treatments (*A. frigida* adversely affects the DM weight of *C. squarrosa*), whereas the two species were competing for the same environmental resources (N) (in the moderate and high N addition) when heavy defoliation occurred. Relative yield total and the respective crowding coefficients based on total biomass (sum of above-ground and below-ground biomass per pot) showed similar trends to that of above-ground biomass (Figure 1; Table 1). When heavy clipping was imposed on the two species, over-yielding occurred (Table 1) no matter what N fertilizer treatment was applied, indicating some niche differentiation between species under heavy clipping.
Replacement diagrams for below-ground biomass from mixtures of *A. frigida* and *C. squarrosa* under different N additions were given in Figure 2. The pattern of competition using below-ground biomass was similar to that using total biomass and above-ground biomass except under light clipping at the low N addition (RYT = 1·55). *Artemesia frigida* was generally more dominant than *C. squarrosa* (as indicated by higher crowding coefficients). When the two species were subjected to heavy clipping, over-yielding occurred (Figure 2) at all N additions, which indicated that *A. frigida* and *C. squarrosa* were not competing for the same environmental resources and so they escaped some measure of competition with each other.

**Competitive ability**

In order to clarify the relative competitive abilities of the two species, the competitive abilities of the two species in mixtures at 0·5:0·5 were assessed by the index

\[
\text{Relative Crowding Coefficient} = \frac{Y_{A.frigida} - Y_{C.squarrosa}}{Y_{C.squarrosa} - Y_{A.frigida}}
\]

where \(Y_{A.frigida}\) and \(Y_{C.squarrosa}\) are the yields of *A. frigida* and *C. squarrosa*, respectively.

*Figure 1* Replacement diagrams of *C. squarrosa* and *A. frigida* based on above-ground biomass per pot under different clipping intensities. Relative crowding coefficients, measuring the competitive power of *A. frigida* relative to *C. squarrosa*, are on the left-hand side of the diagram, and for *C. squarrosa* relative to *A. frigida* on the right-hand side. The relative yield totals are shown at the top of the diagram. Actual total yields are plotted as ▼. Mean data points for *C. squarrosa* and *A. frigida* are indicated by ● and ○ respectively. Lines were fitted to all data. (a) No nitrogen addition, (b) moderate nitrogen addition and (c) high nitrogen addition.

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of aggressivity based on total biomass. Both N addition and the interaction between N addition and clipping significantly affected the relative competitive ability of *A. frigida* and *C. squarrosa*, but clipping intensity has no significant effect on the relative competitive ability (Table 2). With increase in N addition, there was a reduction in the relative competitive ability of *A. frigida* against *C. squarrosa* at the different clipping intensities with the exception of the high N addition in the no-clipping treatment (Figure 3a). The change in the relative competitive ability with clipping intensity did not show a consistent pattern. The relative competitive ability of *A. frigida* against *C. squarrosa* declined in the no-N addition treatment with increase in clipping intensity, while there was an increase in the moderate N treatment and a decrease in the high N treatment compared with no clipping (Figure 3b).

**Discussion**

Results from using replacement series and diallel designs have been prominent in the search for competitive hierarchies in which a species will out-compete (in the sense of outcome of competition) all species ranked below it in the hierarchy and be out-competed by those above it (Shipley, 1993) although there are critics of the method (Jolliffe *et al.*, 1984; Connolly, 1988, 1997; Snaydon, 1991, 1994). Increasing the complexity, both temporally and spatially, of experimental designs increases the logistical problems of carrying out the experiment. Simple experimental designs are preferable when they can validly address the question of interest without an unacceptable sacrifice of realism (Gibson *et al.*, 1999). Indeed, substitutive experiments, in which overall density is held constant while the frequency of component species is varied, have provided an elegant and useful method for the study of plant interactions involving two species (Watkinson, 1981).

Competitive hierarchies may be the rule along gradients of resource quantity, or where one environmental factor alone is of overwhelming importance (Keddy, 1990). In this experiment, the disturbance (clipping) and stress (fertility) did change the competition intensity between the two species, but they did not change the position in the hierarchy (*A. frigida* out-competed *C. squarrosa* under all conditions). *Artemesia frigida* performed better (i.e. was more competitive) than *C. squarrosa* under both N application and clipping treatments (*kji* > *kjji* and *AC > 0*). Under most situations, no matter what the above-ground biomass, below-ground biomass and total biomass were, pairwise asymmetric competition can be said to exist because *kji* > 1 and *kjji* < 1. That is, *A. frigida* grows better in mixtures with *C. squarrosa* but *C. squarrosa* grows worse in mixtures with *A. frigida*. Therefore, *A. frigida* should eventually exclude *C. squarrosa*.

Some experiments have shown that stress (fertility) and disturbance (herbivory) have profound effects on the competitive ability of species (Li *et al.*, 1999; Li and Watkinson, 2000; Hambäck and Beckerman, 2003). Li and Watkinson (2000) found a reversal of outcomes in competition at intermediate levels of nutrients when they studied two species (*Daucas carota* L. and *Chenopodium album* L.) in a greenhouse experiment along a nutrient gradient. In this study, with increased N addition, the relative competitive ability of *A. frigida* against *C. squarrosa* significantly declined at different clipping intensities, but there was no reversal in relative competitive ability. It is tentatively proposed that there are three possible reasons: (i) the perennial grass (*C. squarrosa*) has a higher relative growth rate than the semi-shrub (*A. frigida*) with increased N addition; (ii) clipping has a more adverse effect on *A. frigida* than

![Table 1](https://example.com/figure1.png)

**Table 1** The fitted model, *kji*, relative yield total (RYT) values, and the biological interpretation for *Artemisia frigida* and *Cleistogenes squarrosa* competition at different clipping intensities based on total biomass under different nitrogen levels.

<table>
<thead>
<tr>
<th></th>
<th>Fitted model</th>
<th><em>kji</em></th>
<th><em>kjji</em></th>
<th><strong>RYT</strong></th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No nitrogen addition</td>
<td>No clipping</td>
<td>3·35</td>
<td>0·27, <em>t</em> = 3·70</td>
<td>0·90</td>
<td>Under-yielding</td>
</tr>
<tr>
<td></td>
<td>Light clipping</td>
<td>1·87</td>
<td>0·35, <em>t</em> = 2·86</td>
<td>0·65</td>
<td>Under-yielding</td>
</tr>
<tr>
<td></td>
<td>Heavy clipping</td>
<td>4·10</td>
<td>0·37, <em>t</em> = 2·70</td>
<td>1·52</td>
<td>Over-yielding</td>
</tr>
<tr>
<td>Moderate nitrogen addition</td>
<td>No clipping</td>
<td>1·10</td>
<td>0·27, <em>t</em> = 3·70</td>
<td>0·30</td>
<td>Under-yielding</td>
</tr>
<tr>
<td></td>
<td>Light clipping</td>
<td>2·88</td>
<td>0·35</td>
<td>1·00</td>
<td>Competition</td>
</tr>
<tr>
<td></td>
<td>Heavy clipping</td>
<td>2·13</td>
<td>0·52, <em>t</em> = 1·92</td>
<td>1·11</td>
<td>Over-yielding</td>
</tr>
<tr>
<td>High nitrogen addition</td>
<td>No clipping</td>
<td>6·81</td>
<td>0·45, <em>t</em> = 0·15</td>
<td>3·06</td>
<td>Over-yielding</td>
</tr>
<tr>
<td></td>
<td>Light clipping</td>
<td>1·35</td>
<td>0·63, <em>t</em> = 1·59</td>
<td>0·85</td>
<td>Under-yielding</td>
</tr>
<tr>
<td></td>
<td>Heavy clipping</td>
<td>1·96</td>
<td>0·62, <em>t</em> = 1·61</td>
<td>1·22</td>
<td>Over-yielding</td>
</tr>
</tbody>
</table>

C. squarrosa, because C. squarrosa growth over-compensates for herbage removal; and (iii) the extent to which nitrogen addition changed the competitive relationship was not sufficient to reverse the outcome of competition between the two species.

The interactive effect between herbivory and plant competition may arise through two major routes: first, rates of herbivory may increase or decrease when the abundance of plant neighbours, or some factor correlated with plant abundance, is altered (Louda et al., 1990; Trenbath, 1993; Holt and Lawton, 1994); second, herbivory may increase or decrease the competitive effects on the ability to regrow (Whitham et al., 1991). In this experiment, although the main clipping effect is

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Figure 2 Replacement diagrams of C. squarrosa and A. frigida based on below-ground biomass per pot under different clipping intensities. Conventions and symbols are as for Figure 1. The de Wit model did not fit the data for light clipping under the high nitrogen addition treatment.
not significant in the analysis of variance ($P > 0.05$) on aggressivity, calculated from individual masses, the interaction between N addition and clipping was significant ($P < 0.05$). This shows that the relative competitive ability of *A. frigida* against *C. squarrosa* with additional N is different at different clipping intensities (Figure 3b). At both low and high N additions, the relative competitive ability of *A. frigida* against *C. squarrosa* tended to decrease with increasing clipping intensity, while at intermediate levels of N, their relative competitive ability tended to increase with clipping intensity. This indicates that clipping has a complicating effect on the relative competitive ability of the two species along a nutrient gradient, probably because *A. frigida* and *C. squarrosa* have different regrowth abilities when clipping is imposed.

In conclusion, *A. frigida* is a superior competitor to *C. squarrosa* under most circumstances. Although the competitive ability of seedlings of the two species was only studied for about 4 months, the result is almost the same trends as restoration succession in the field where there has not been grazing since 2000. That is, *C. squarrosa* is still not yet a dominant species after a restoration period of 4 years in an *A. frigida* community (Y.Z. Gao, unpubl. data). Christensen *et al.* (2003) assessed the sustainability and resilience of a grazing ecosystem in the Inner Mongolia steppe of China by determining thresholds and stable states with an ecosystem simulation model. They found that a grazing intensity ($1 \times u$; $g =$ biomass in grazed area, $u =$ biomass in ungrazed area) higher than $0.49$, in combination with low precipitation events, resulted in decreased herbaceous net primary production and root biomass, and increased shrub net primary production and root biomass. Consequently, the system shifted to a stable shrub-dominant state that could

### Table 2 Two-way analysis of variance on the relative competitive ability of *A. frigida* against *C. squarrosa* ($A_{AC}$).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F-value</th>
<th>P-level</th>
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</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td>2</td>
<td>0.19</td>
<td>0.095</td>
<td>71.97</td>
<td>***</td>
</tr>
<tr>
<td>Clipping (C)</td>
<td>2</td>
<td>0.007</td>
<td>0.004</td>
<td>2.69</td>
<td>NS</td>
</tr>
<tr>
<td>N × C</td>
<td>4</td>
<td>0.116</td>
<td>0.029</td>
<td>21.97</td>
<td>***</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>0.024</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant effects are indicated in bold. The significance levels are given by: NS, not significant ($P > 0.05$); *, $P < 0.05$; ***, $P < 0.001$.

![Figure 3](image-url) **Figure 3** The effects of nitrogen addition (a) and clipping (b) on the relative competitive ability of *A. frigida* against *C. squarrosa*. N0, no nitrogen addition; N1, moderate nitrogen addition; N2, high nitrogen addition; NC, no clipping; LC, light clipping; HC, heavy clipping.
not return its original composition even without further grazing. Therefore, a long-term field experiment would be justified.

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