

Postprint: Effects of Genotypic Diversity of Different Neighbor Species on *Artemisia frigida* Growth

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Abstract

Plant species diversity and genotypic diversity play important ecological roles in community structure and function. In recent years, the influence of plant genotypic diversity on plant-plant interactions has become an important scientific question of interest to researchers. This experiment selected the degraded grassland dominant species *Artemisia frigida* as the target plant, and the stable community constructive species *Leymus chinensis* and community companion species *Koeleria cristata* as neighbor plants, to investigate the effects of neighbor plants with different genotypic diversity on the growth performance (plant height, aboveground biomass, belowground biomass, and total biomass) of *Artemisia frigida*, and to further explore the underlying mechanisms of neighbor plant genotypic diversity effects on the target plant by measuring relative competition intensity and neighbor plant trait variation. The results showed that: (1) When the neighbor species was *Leymus chinensis*, genotypic diversity had significant effects on the growth performance of *Artemisia frigida*. When neighbors were of 6 genotypes, the plant height, aboveground biomass, and total biomass of *Artemisia frigida* were significantly lower than those under monoculture and 3-genotype treatments ($P < 0.05$), and the relative competition intensity was higher than the other two treatments. In contrast, when the neighbor species was *Koeleria cristata*, genotypic diversity had no significant effects on all observed indicators of *Artemisia frigida* or on relative competition intensity ($P > 0.05$). (2) Using principal component analysis to analyze the effects of genotypic diversity on its own trait variation revealed that when the neighbor species was *Leymus chinensis*, genotypic diversity had significant effects on trait variation responses, mainly manifested as the plant height, total biomass, and aboveground biomass of *Leymus chinensis* populations being significantly higher under the 3-genotype treatment than under the monoculture treatment ($P < 0.05$). When the neighbor species was *Koeleria cristata*,

genotypic diversity had no significant effect on trait variation ($P > 0.05$). (3) When the neighbor species was *Leymus chinensis*, the total biomass and specific leaf area of *Leymus chinensis* were significantly negatively correlated with the aboveground biomass and total biomass of *Artemisia frigida* ($P < 0.05$). When the neighbor species was *Koeleria cristata*, there were no significant correlations between the traits of *Koeleria cristata* and the traits of *Artemisia frigida* ($P > 0.05$). The experimental results reveal that the effects of genotypic diversity on target plant growth are influenced by neighbor plant species, and high-genotype combinations of the stable community constructive species *Leymus chinensis* can significantly suppress the growth of *Artemisia frigida*, which may be related to the large trait variation in *Leymus chinensis* populations with high genotypic diversity and their high relative competition intensity against *Artemisia frigida*. These results provide experimental evidence that constructive species genotypic diversity influences interspecific interactions, and offer theoretical guidance for the rational utilization and conservation of grasslands.

Full Text

Effects of Genotypic Diversity of Neighboring Species on the Growth of *Artemisia frigida* Plants

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Abstract

Plant species diversity and genotypic diversity play crucial ecological roles in community structure and function. In recent years, the influence of plant genotypic diversity on plant-plant interactions has become an important research focus. This study selected *Artemisia frigida*, a dominant species in degraded grasslands, as the target plant, and used *Leymus chinensis*, a climax community constructive species, and *Koeleria cristata*, a companion species, as neighboring plants to investigate how neighboring plants with different genotypic diversities affect the growth performance of *A. frigida*. We measured plant relative competition intensity and neighbor plant trait variation to further explore the underlying mechanisms.

The experiment revealed three key findings: (1) When *L. chinensis* served as the neighbor species, genotypic diversity significantly affected *A. frigida* growth. Plant height, aboveground biomass, and total biomass of *A. frigida* were significantly lower under high genotypic diversity (G6) compared to monoculture (G1) ($P < 0.05$), and relative competition intensity was significantly higher. However, when *K. cristata* was the neighbor, genotypic diversity showed no significant effects on any measured indicators or relative competition intensity of *A. frigida*. (2) Principal component analysis of neighbor trait variation indicated that *L. chinensis* trait variation was significantly affected by genotypic diversity, with

aboveground biomass significantly higher in high-diversity treatments, while *K. cristata* showed no significant trait variation responses. (3) Kendall rank correlation analysis revealed significant negative correlations between *L. chinensis* total biomass and specific leaf area (SLA) and *A. frigida* aboveground and total biomass, whereas no significant correlations existed between *K. cristata* traits and *A. frigida* traits.

These results demonstrate that the effects of neighbor genotypic diversity on target plant growth depend on neighbor species identity. High genotypic diversity combinations of the constructive species *L. chinensis* significantly inhibited *A. frigida* growth, likely due to greater trait variation and higher relative competition intensity in high-diversity *L. chinensis* populations. This study provides experimental evidence that constructive species genotypic diversity influences interspecific interactions and offers theoretical guidance for rational grassland utilization and conservation.

Keywords: neighboring plant; target plant; plant-plant interactions; genotypic diversity; competition; traits

1. Materials and Methods

1.1 Experimental Materials

Artemisia frigida target plants were obtained from a single genotype through cutting propagation. *Leymus chinensis* plants were collected from the field, and *Koeleria cristata* genets were germinated from seeds. After molecular marker identification of different genets, tillers of the same genotype were propagated vegetatively. Since *K. cristata* reproduces through wind-pollinated outcrossing, the probability of different seeds being the same genotype is extremely low, so molecular marker identification was not performed for this species.

1.2 Experimental Population Construction

We established microcosms using either *L. chinensis* or *K. cristata* as neighbor species at three genotypic diversity levels: monoculture (G1), three-genotype combination (G3), and six-genotype combination (G6). Each 19 cm diameter plastic pot contained 2500 g of fresh topsoil collected from native *L. chinensis* grassland in the typical steppe region. A single *A. frigida* ramet of the same genotype was transplanted into the center of each pot. For neighbor plant establishment, nine tillers of *L. chinensis* (for G1, G3, G6) or 12-15 tillers of *K. cristata* (for G1, G3, G6) were planted 10 cm from the central *A. frigida* plant, with tillers labeled according to their genet origin. Rhizomes were removed from *L. chinensis* tillers, and all neighbor tillers were trimmed to 10 cm aboveground height and 10 cm root length. To calculate relative competition intensity, we also established monoculture controls of *A. frigida* alone, *L. chinensis* alone, and *K. cristata* alone. Dead tillers were replanted within 15 weeks.

The experiment lasted 130 days. All pots were randomly arranged and repositioned weekly to avoid location effects. Soil water content was maintained at $(10\pm 2)\%$, weeds were removed regularly, and no high-temperature or shading stress was applied.

1.3 Index Measurement

At the experimental conclusion, we measured plant height and harvested aboveground and belowground biomass for each species per pot. For specific leaf area (SLA) determination, the second fully expanded sunlit leaf from the inside out was selected from each genet, scanned at 400 dpi (Epson, Long Beach, USA) to measure leaf area, then oven-dried at 105°C for 2 hours and 80°C to constant weight. SLA was calculated as leaf area divided by dry weight. Root length was measured by scanning neighbor plant root systems using an EPSON 1680 scanner and analyzing with WinRHIZO software.

1.4 Data Analysis

Relative competition intensity (RCI) was calculated at the pot level as: $RCI = (P_{\text{monoculture}} - P_{\text{mixture}}) / P_{\text{monoculture}}$, where P represents performance (aboveground biomass, belowground biomass, or total biomass). RCI reflects competitive ability between individuals, with $RCI > 0$ indicating competition, $RCI = 0$ indicating no competition, and $RCI < 0$ indicating facilitation.

We used one-way ANOVA to test effects of neighbor genotypic number on *A. frigida* height, aboveground biomass, belowground biomass, and total biomass, followed by Duncan's test for multiple comparisons. t-tests were used to compare RCI values against zero. Principal component analysis (PCA) quantified trait variation in neighbors at different genotypic diversity levels. Kendall rank correlation analysis examined relationships between neighbor traits (*L. chinensis* or *K. cristata*) and target species traits. Data were transformed to meet normality and homogeneity assumptions before statistical testing. All analyses were conducted using SPSS 21.0 and Canoco 4.5.

2. Results

2.1 Effects of Neighbor Genotypic Diversity on *A. frigida* Growth

Leymus chinensis genotypic diversity significantly affected *A. frigida* growth. Plant height, aboveground biomass, and total biomass of *A. frigida* decreased with increasing *L. chinensis* genotypic diversity. Specifically, when *L. chinensis* had six genotypes (G6), *A. frigida* aboveground biomass and total biomass were significantly lower than when *L. chinensis* had one genotype (G1) ($P < 0.05$). In contrast, *K. cristata* genotypic diversity showed no significant effects on any growth variables of *A. frigida*.

2.2 Relative Competition Intensity (RCI) Between *A. frigida* and Neighbors

2.2.1 *A. frigida*-*L. chinensis* Competition When mixed with *L. chinensis*, *A. frigida* showed RCI values ranging from -0.42 to 0.73. For G1, RCI values were not significantly different from zero, indicating no significant competition. However, for G3 and G6, RCI values for aboveground, belowground, and total biomass were significantly greater than zero ($P < 0.05$), indicating that *L. chinensis* significantly inhibited *A. frigida* growth. Moreover, genotypic diversity significantly affected competition intensity: RCI values for G6 were significantly greater than for G1 ($P < 0.05$), showing stronger competitive suppression at higher genotypic diversity .

2.2.2 *A. frigida*-*K. cristata* Competition When mixed with *K. cristata*, *A. frigida* RCI values ranged from 0.57 to 0.75, all significantly greater than zero ($P < 0.05$), indicating significant competitive effects. However, *K. cristata* genotypic diversity did not significantly affect competition intensity. *K. cristata* itself showed RCI values close to zero, indicating minimal impact from *A. frigida* .

2.3 Effects of Genotypic Diversity on Neighbor Trait Variation

PCA of *L. chinensis* traits revealed that trait variation increased with genotypic diversity. For G1, individual traits were mainly distributed along the first axis from -1.0 to 1.0. For G3, distribution expanded to -1.0 to 1.5 on the first axis and -1.0 to 1.0 on the second axis. For G6, distribution further expanded to -0.8 to 1.0 on the first axis and -0.8 to 0.8 on the second axis, indicating greater trait variation at higher genotypic diversity [Figure 2: see original paper]. In contrast, *K. cristata* trait variation showed no significant differences across genotypic diversity levels.

2.4 Trait Correlations Between Neighbors and Target Species

Kendall rank correlation analysis revealed significant negative correlations between *L. chinensis* total biomass and SLA and *A. frigida* aboveground biomass and total biomass ($P < 0.05$). No significant correlations were detected between *K. cristata* traits and *A. frigida* traits .

3. Discussion

Our experiment demonstrates that whether increased neighbor genotypic diversity enhances inhibition of target plants depends on neighbor species identity. When *L. chinensis* served as neighbor, high genotypic diversity significantly suppressed *A. frigida* growth, supporting our hypothesis. However, *K. cristata* genotypic diversity showed no significant effects. This species-specific pattern

aligns with previous studies showing inconsistent effects of neighbor diversity on target plants.

The mechanisms underlying these effects likely involve changes in neighbor trait variation. Our PCA results show that *L. chinensis* trait variation increased substantially with genotypic diversity, particularly for total biomass and SLA. These traits showed significant negative correlations with *A. frigida* biomass, suggesting that genotypic diversity alters competitive outcomes by modifying neighbor functional traits. Similar results have been reported in biodiversity experiments, where increased diversity enhances trait variation, broadens niche space, and improves resource acquisition efficiency. For example, increased specific leaf area variation reflects enhanced light use efficiency, while greater root length variation improves water and nutrient uptake.

The lack of significant effects from *K. cristata* may reflect its different ecological role. As a companion species co-occurring with *A. frigida* in degraded communities, *K. cristata* may have less competitive impact regardless of its genotypic diversity. In contrast, *L. chinensis* as a constructive species may exert stronger competitive effects that are amplified by genotypic diversity, similar to how species diversity functions in communities.

These findings have important implications for grassland management. Maintaining high genotypic diversity in constructive species like *L. chinensis* can enhance their competitive suppression of undesirable species such as *A. frigida*, thereby slowing grassland degradation. This provides experimental evidence that intraspecific genetic variation in dominant species can influence interspecific interactions and community dynamics, complementing the well-documented effects of species diversity.

Future studies should combine population genetic surveys with trait-based ecology approaches to better quantify how intraspecific genetic variation impacts plant-plant interactions under global change scenarios, facilitating improved ecological restoration strategies for the typical steppe ecosystems of northern China.

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