Seasonality of annual plant establishment influences the interaction between the non-native annual grass *Bromus madritensis* ssp. *rubens* and Mojave Desert perennials

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Abstract

Competition between native and non-native species can change the composition and structure of plant communities, but in deserts the timing of non-native plant establishment can modulate their impacts to native species. In a field experiment, we varied densities of the non-native annual grass *Bromus madritensis* ssp. *rubens* around individuals of three native perennials– *Larrea tridentata*, *Achnatherum hymenoides*, and *Pleuraphis rigida* – in either winter or spring. Additional plots were prepared for the same perennial species and seasons, but with a mixture of native annual species. Relative growth rates of perennial shoots (RGRs) declined with increasing *Bromus* biomass when *Bromus* that was established in winter had 2-3 mo of growth and high water use before perennial growth began. However, this high water use did not significantly reduce water potentials for the perennials, suggesting *Bromus* that established earlier depleted other soil resources, such as N, otherwise used by perennial plants. Spring-established *Bromus* had low biomass even at higher densities and did not effectively reduce RGRs, resulting in an overall lower impact to perennials than when *Bromus* was established in winter. Similarly, growth and reproduction of perennials with mixed annuals as neighbors did not differ from those with *Bromus* neighbors of equivalent biomass, but densities of these annuals did not support the high biomass necessary to reduce perennial growth. Thus, impacts of native Mojave Desert annuals to perennials are expected to be lower than those of *Bromus* because seed dormancy and narrow requirements for seedling survivorship produce densities and biomass lower than those achieved by *Bromus*. In comparing the effects of *Bromus* among perennial species, the impact of increased *Bromus* biomass on RGR was lower for *Larrea* than for the two perennial grasses, probably because *Larrea* maintains low growth rates throughout the year, even after *Bromus* has completed its life cycle. This contrasts with the perennial grasses, whose phenology overlaps completely with (*Achnatherum*) or closely follows (*Pleuraphis*) that of *Bromus*.

Keywords: creosote bush, galleta grass, Indian ricegrass, invasive species, plant competition

Introduction

Non-native annual grasses introduced from the Mediterranean now dominate many of the annual floras of North American deserts (Billings 1990; Vitousek 1990, 1994; D'Antonio and Vitousek 1992). Of particular interest in the Mojave Desert is *Bromus madritensis* ssp. *rubens* (red brome), which was introduced into western North America a century ago (Hulbert 1955; Salo 2002). The density and biomass of *Bromus madritensis* ssp. *rubens* (hereafter referred to as *Bromus*) were initially low when it first became established in the early 20th century, but its abundance increased sharply in the 1970s, and it is now dominant across many landscapes in the Mojave Desert (Beatley 1966; Hunter 1991; Brooks 1999a). Most research on this annual grass has focused on increased frequency of wildfires associated with years of high shoot production (Beatley 1966, 1969; Brown and Minnich 1986; Brooks 1999a, 2002; Brooks and Esque 2002). Although the potential for invasive non-native annuals to reduce the performance of perennials is recognized (Harris 1967; Melgoza et al. 1990; Melgoza and Nowak 1991; Dyer and Rice 1999; Holzapfel and Mahall 1999; Brown and Rice 2000), few mechanistic studies have examined the interactions between *Bromus* and perennial species whose resources they share, and determined how the effects of *Bromus* on native species differ from those of ecologically similar winter annuals.

Similar to native Mojave Desert annuals, *Bromus* establishes best beneath the canopies of perennial shrubs (Samson 1986, Brooks 1999b) and shares the life history strategy of native winter annuals. For both *Bromus* and native annuals, early and rapid carbon gain occurs at the expense of great water loss, which may allow annual species (both native and non-native) to exploit soil water normally used by perennial shrubs and grasses. However, the net effect of the annual-perennial species interactions depends not only on resource depletion by annuals, but also on the ability of the perennial species to persist when resources are depleted (Goldberg 1990; Goldberg and Landa 1991). Thus, we hypothesized that growth and reproduction of three Mojave Desert perennials that represent contrasting life forms would respond differently to depletion of soil moisture by annual plant neighbors. We expected the evergreen shrub *Larrea tridentata* to be less sensitive to neighboring annuals because *Larrea* can photosynthesize and grow at soil water potentials well below those tolerated by winter annuals (Smith et al. 1997). Furthermore, root systems of annuals typically occur in shallow soil depths (Cable 1969; Forseth et al. 1984), and thus annuals should have little effect on *Larrea*, whose root system allows it to take advantage of soil moisture at a greater lateral and vertical extent than neighboring annual plants (Gile et al. 1998). In contrast, we expected annuals to reduce the performance of perennial grasses because of their overlapping root systems and phenology. For example, the phenology of the relatively slow-growing perennial grass *Achnatherum hymenoides* overlaps completely with annuals, and thus competition with annuals for the same available soil water will likely reduce the growth of *Achnatherum*. Optimal temperatures for growth of *Pleuraphis rigida* occur after the annuals have completed most of their development (Nobel 1980). In addition, *Pleuraphis* carbon uptake is dependent on favorable soil water potentials (Nobel 1980; Nobel and Zhang 1997). Thus, if annuals have extracted most of the available soil moisture before *Pleuraphis* is active, then little water will be available for *Pleuraphis* because of the pronounced late-spring/summer dry season.

The similar establishment and life history strategies of *Bromus* and native annuals suggest that these species may have similar effects on perennials. However, a recent glasshouse study showed that *Bromus* depletes soil resources such as water and N more rapidly than native winter annuals (DeFalco et al. 2003). Thus, neighboring plants comprised primarily of *Bromus* should reduce perennial plant performance more than if the neighboring plants are a mixture of native annual species.

The hypotheses above are assumed to be true when annuals are abundant. However, abundance of Mojave Desert annuals varies greatly from year-to-year (Hunter 1991). Germination and growth of winter annuals coincide with favorable growth temperatures and soil moisture recharge from winter precipitation falling between October and January (Beatley 1974). When winter precipitation is insufficient, germination of Mojave Desert annuals may correspond with early spring rainfall (February through March), although usually in lower densities and biomass. Thus, annuals that are established in the winter may have a greater impact on perennial plant performance than annuals established in the spring.

To test these predictions, we designed a field study that imposed two sets of treatments on three perennial species that differ in rooting depths and phenologies (*Larrea, Achnatherum*, and *Pleuraphis*). The first treatment focused on the composition of the annual neighbors associated with the perennial species (*Bromus* or a mixture of native annuals) while the second focused on when those annuals became established (winter or spring). The first treatment was imposed by establishing different densities of *Bromus* around target perennials in order to determine whether neighboring *Bromus* plants reduce the growth and reproduction of the perennial species. For comparison, a mixture of native annual species was also established around the same perennial species. The second treatment was designed to simulate the effects of timing of rainfall on annual plant establishment and production in the Mojave Desert. Thus, *Bromus* and native annual plants were established around perennials either in winter or in spring.

In particular, this study tested several specific hypotheses: (1) growth of perennials will decline as the abundance of annual neighbors increases, (2) annuals will have less effect on the growth of an evergreen shrub than on two perennial grass species; (3) annuals that germinate in the winter will have a more negative effect on the growth of associated perennials than will annuals that germinate in the spring; and (4) the invasive annual *Bromus* will have a more negative effect on the growth of associated perennial plants than will a mixture of native annual species.

Materials and methods

Study area, perennial species, and treatments

This study began in October 1999 and ended in June 2000 at Frenchman Flat, which is located at the Nevada Test Site (US Department of Energy) in southern Nevada, USA. Three native Mojave Desert perennial species were selected for study: creosote bush, *Larrea tridentata* (DC.) Cov.; Indian ricegrass, *Achnatherum hymenoides* (Roemer & Schultes) Barkworth; and galleta grass, *Pleuraphis rigida* Thurber. Each individual perennial plant plus the area 20 cm beyond the canopy edge of the perennial defined an individual study plot. Treatments were stratified among individual plots according to perennial plant volume, which was estimated as an inverted cone using each plant's canopy dimensions and height. Each individual perennial was randomly assigned a season for neighboring annual plant establishment (winter or spring) and a neighboring plant density (approximately 0, 200, 400, 600, 800 or 1000 individuals m^{-2}). To minimize competition from neighboring perennials, perennial vegetation within a 2 m radius of each *Larrea*, *Achnatherum*, and *Pleuraphis* individual was clipped to just below the crown.

For the winter establishment treatment, *Bromus* seeds previously collected from the area were hand broadcast on each plot on November 9 and 10, 1999 (i.e., beneath each individual perennial plant and out to an area 20 cm beyond the canopy edge). A light layer of straw mulch was placed on top of all plots (both the seeded winter establishment plots as well as the unseeded spring establishment plots) and secured in place with commercially available nylon bird netting. On February 9 and 10, 2000, straw and mesh were lifted from the spring establishment treatments, *Bromus* seed was sown as on winter treatment plots, and straw replaced. To ensure that the annuals established at the desired time of year, plots were irrigated in winter or in spring, as appropriate. The winter establishment treatment was irrigated with 25 mm water approximately once every 1 to 2 wks with a cyclone circular sprinkler, starting on November 11, 1999 and continuing until January 8, 2000. For the spring establishment treatment, irrigation occurred at the same rate as the winter treatment from February 11, 2000 until March 24, 2000. Straw from winter and spring plots was removed after irrigation ceased and annual seedlings were established. A total of 72 plots were established that had a single perennial plant with neighboring *Bromus* plants (3 perennial species × 2 seasons of establishment × 6 planting densities × 2 replicates per treatment combination).

Twenty-four additional plots were prepared that had a single perennial plant with neighboring annuals composed of a mixture of native species. For these native annual plots, seed trapped in litter beneath nearby *Larrea* canopies was collected and spread around the target perennial species and irrigated in a manner similar to the *Bromus* plots. Thus, the overall experimental design was similar to that of the *Bromus* plots (3 perennial species × 2 seasons of establishment), but only 4 replicates per treatment combination whose densities were not manipulated were allowed.

For both the *Bromus* and mixed native annual treatments, annuals were thinned up to 1 mo after germination in an attempt to maintain the density and composition of the neighboring annual plants. For perennials with *Bromus* neighbors, *Bromus* was thinned to approximate the target density by clipping plants at ground level, and any native annual species were similarly removed. For the perennials with neighboring plants of a mixture of native annual species, *Bromus* was removed but the native densities were not manipulated.

Shoots of neighboring annual plants were harvested when they senesced in late April and early May, sorted by species, and dried in a convection oven at 60°C to a constant mass. Plots with *Bromus* as the neighbor treatment had on average ≥85% *Bromus* by mass due to germination of native plants after thinning was completed. However, one plot each with *Pleuraphis* and *Larrea* had <85% of *Bromus* neighbors, and these plots were omitted from analyses. Biomass of native annuals for all the mixed species plots averaged 85%, with *Bromus* comprising the remaining biomass; thus, none of the mixed treatment plots were eliminated from analyses.

Soil water status

Volumetric soil water content (Θ_{vol}) was monitored for each plot throughout the study. An aluminum tube (41 mm outer diameter, 39 mm inner diameter) was placed vertically in the ground to a depth of 135 cm and at 10 cm from the canopy edge of each perennial. Θ_{vol} was measured monthly at 15, 35, 55, 75, 95, 115 and 135 cm depths using a calibrated neutron probe soil moisture device (Hydroprobe Moisture Depth Gauge, Campbell Pacific Nuclear, Martinez, California, USA). These measurements were used to calculate soil profile moisture (mm) according to Yoder and Nowak (1999) as in Anderson et al. (1993). We assumed deep drainage was negligible because Θ_{vol} did not change significantly below 75 cm throughout the duration of the study. We also assumed run-on and run-off were insignificant because the plots were situated far apart on a topographically flat area, and we observed no lateral surface movement of water when irrigation was applied. Thus, calculation of water use (mm) was based on calculation of evapotranspiration using a modified water balance equation: water use = precipitation + irrigation – Δ soil storage.

Seasonal growth, reproduction, and water potentials of perennials

Shoot relative growth rates of the target perennials were determined by monitoring plant growth monthly beginning when plants were dormant in November and ending when plants senesced by June. For *Achnatherum* and *Pleuraphis*, two tillers were selected per plant and tagged at the base with a loose band of colored tape. The length of each leaf per tiller was measured at each sampling time. The relationship between leaf mass and leaf length was determined using leaves harvested from plants close to the experimental plants; this relationship was used to estimate the total leaf biomass on each tiller every time measurements were collected. For *Larrea*, four terminal twigs, each approximately 2 cm long, were tagged with tape. Twig length and the number of leaflet pairs were measured at each sampling time. Similar to the grasses, twig mass was estimated from twigs harvested from *Larrea* plants adjacent to the study plots. Because we wanted to examine the effect of neighboring annuals on growth of perennials, tiller and twig relative growth rates (RGR) were calculated from the time neighboring annuals were seeded (November for winter establishment treatments and February for spring) until perennials reached peak production (late April to late May). RGR was calculated according to Blackman (1919) and used the tiller or twig means of the $\log_e$-transformed masses to avoid bias in the estimates (Hoffmann and Poorter 2002). Growth of three *Larrea* individuals was difficult to measure because bagworms (*Thyridopteryx meadi*) altered the lengths of twigs and used leaflets for constructing cocoons; these *Larrea* individuals were omitted from analyses.

Flowers produced on the marked grass tillers and *Larrea* twigs were counted at each sampling time. Reproductive effort (RE) at peak flower production for each perennial was calculated as the ratio of the average number of flowers to the corresponding average production (g) among twigs or tillers.

Statistical analyses

All response variables were tested in analysis of variance (ANOVA) with qualitative and quantitative variables using SAS statistical software (SAS Institute, Cary, NC, version 8). Profile soil moisture was analyzed in separate fixed 2-way (perennial species × season of establishment) ANOVAs before irrigation, at the onset of perennial growth, and after *Bromus* senesced. The relationship between neighbor plant biomass and neighbor plant density was linearized by $\log_{10}$-transforming both variables and then tested in a 3-way ANOVA with neighbor identity (*Bromus* vs. mixed species) and season of establishment (winter vs. spring) as qualitative variables and neighbor density as a quantitative variable expressed through a linear term (Fernandez 2001).

Perennial responses (i.e., RGR, RE, water use, and water potentials) to the annual neighbor treatments (winter vs. spring establishment; *Bromus* vs. mixed species neighbors) were analyzed in two steps. First, a three-way ANOVA used only the *Bromus* neighbor treatment plots to determine if perennial responses were significantly affected by *Bromus* biomass (quantitative factor) and if the responses differed among perennial species and between the winter and spring establishment treatments (qualitative factors). Because sample sizes of the mixed species plots were smaller than those for *Bromus*, and because the range of biomass for the mixed annual plots was smaller and closer to zero than *Bromus*, we were unable to compare mixed annual and *Bromus* effects in ANOVA by simply adding neighbor identity as a qualitative factor. Instead we used outlier detection diagnostics to determine whether responses of perennials with mixed species neighbors deviated significantly from those with *Bromus* neighbors. This second step in the analysis tested perennial responses using 3-way ANOVAs for plots with both *Bromus* and mixed species neighbors: season and target species were qualitative factors, and biomass of the *Bromus* and mixed species plots was the quantitative factor. We expected mixed species neighbors to have less impact on perennials than *Bromus* neighbors. Therefore, we expected mixed species plots to be significant outliers, which were identified, then iteratively removed based on examination of studentized residuals, normal probability plots, and according to D'Agostino (1971). All statistical models tested linear and quadratic effects. Equal distribution of variances was determined from residual plots, and when violated (*i.e.*, RE), data were $\log_{10}$-transformed before analysis (Box and Cox 1964).

Results

The experiment occurred during a dry hydrological year (October 1999 through September 2000), which had 76% of the long-term mean annual rainfall. The total water added by irrigation and precipitation during the irrigation period was similar for both seasons of annual establishment (148 mm for winter and 147 mm for spring). Because rain events occurred after the irrigation period, total moisture input through May was 208 mm for the winter and 155 mm for the spring annual establishment treatments. Although direct comparisons between the amounts of sprinkler-applied water and natural precipitation are confounded by differences in intensity and duration of events as well as by evaporative conditions during and immediately after the event, the total moisture inputs for the annual establishment treatments were substantially greater than the average October through May precipitation for the area (90 mm), but were similar to the amount recorded during October 1997 through May 1998 (195 mm), which was a wet El Niño year.

Annuals germinated soon after water was applied (Fig. 1). Annuals that established in winter germinated, developed, and produced seed, and then senesced within 25 weeks whereas those established in spring completed their life cycle within 13 weeks. All annuals senesced in late April to early May, regardless of when establishment occurred. In contrast, perennial plant phenology was neither consistently induced by irrigation nor synchronized with the establishment of neighboring annuals (Fig. 1).

Because the different timing of irrigation confounds seasonal comparisons of soil water content on the same date, comparisons of soil moisture were made at three similar phenological stages. Prior to initial irrigation, soil moisture measured on November 10 and January 27 for winter and spring annual establishment treatments, respectively, was lower for *Larrea* compared with the perennial grasses (species effect, Table 1). As may be expected, soil moisture before irrigation began was lower in the spring establishment treatment compared with winter (season effect, Table 1) due to the 3-mo lag when irrigation began for the two seasons. At the onset of perennial growth (Fig. 1), soil moisture was higher in winter than in spring for *Achnatherum*, higher in spring than winter for *Larrea*, and the same in both seasons for *Pleuraphis* (species × season interaction, Table 1). By the time *Bromus* senesced in late April to early May, soil moisture again was lowest for *Larrea* plots compared with those of the perennial grasses, and all species had depleted soil moisture similarly for the two seasons of annual plant establishment (Table 1).

Seasonal influence on biomass of annual neighbors

The positive relationship between annual plant biomass and density ($\log_{10}$-$\log_{10}$ transformed) differed among the four different treatment combinations of annual species composition and season of annual establishment (density × species × season, Fig. 2, upper graph, Table 2). The slope of this relationship was lowest for the *Bromus*-winter and *Bromus*-spring treatments and greatest for the mixed annual-winter treatment (density × species). Nonetheless, mean biomass of *Bromus* was greater than that of the mixed annual species (species effect, Table 2), but greater densities of *Bromus* beneath perennials than mixed annual species also contributed to greater biomass for *Bromus* at both winter and spring establishment treatments compared with mixed annual species. Biomass of neighboring annuals was six times greater for plots established in winter than those in spring (season effect, Table 2) for both *Bromus* (45.1 ± 1.0 versus 7.2 ± 1.1 g m^{-2}) and mixed annual species (9.4 ± 1.2 versus 1.6 ± 1.1 g m^{-2}). Finally, a pronounced effect of self-thinning was observed for *Bromus* (*i.e.*, large negative slopes for the relationship between $\log_{10}$-$\log_{10}$ transformed plant size and annual density, Fig. 2, lower graph) compared with mixed species (density × species, Table 2). Analyzed separately, biomass of individual *Bromus* plants decreased as density increased in the winter ($\log_{10}$-$\log_{10}$ slope = -0.68, df = 1, 27, $P < 0.01$) and spring ($\log_{10}$-$\log_{10}$ slope = -0.48, df = 1, 27, $P < 0.01$) establishment treatments, but biomass of individual plants for the mixed annual treatment was not affected by crowding in either season (winter $\log_{10}$-$\log_{10}$ slope = 0.23, df = 1, 9, $P = 0.20$; spring = -0.40, df = 1, 11, $P = 0.18$).

Seasonal influence of neighbors on growth and reproduction of perennials

Bromus had an overall negative effect on shoot relative growth rate (RGR) when the model was fitted without the mixed annual species treatment (biomass effect, Table 3). RGRs were significantly different among the three perennial species, with the grasses having the highest RGRs (*Achnatherum*, 0.018 ± 0.003 d^{-1}; *Pleuraphis*, 0.018 ± 0.002 d^{-1}) and *Larrea* the lowest (0.006 ± 0.003 d^{-1}). The slopes of the relationship between perennial RGR and *Bromus* biomass were significantly different among species at $P = 0.06$ (df = 2, 53, Fig. 3, upper graphs), with the grasses having more negative slopes (*Achnatherum*, -0.00022 d$^{-1}\cdot$[g m^{-2}]$^{-1}$; *Pleuraphis,* -0.00019 d$^{-1}\cdot$[g m^{-2}]$^{-1}$) than *Larrea* (-0.00007 d$^{-1}\cdot$[g m^{-2}]$^{-1}$). Although growth reflects measurements made on two tillers per perennial grass, the reductions in RGR with increased *Bromus* biomass were not offset by increases in the total number of tillers per perennial grass (data not shown). The effects of *Bromus* biomass on perennial RGR were not significantly different between the winter and spring establishment treatments (biomass × season, Table 3). Nonetheless, stands of *Bromus* established in spring had lower biomass than those in winter, and consequently perennials with neighboring *Bromus* plants that were established in spring had higher overall RGRs compared to those established in the winter (0.018 ± 0.003 d^{-1} versus 0.010 ± 0.001 d^{-1}).

Reproductive effort (RE) for perennials with *Bromus* neighbors, measured as the number of flowers per g of production, did not vary with *Bromus* biomass or between seasons (Fig. 3, lower graphs). Averaged over neighbor biomass, RE was significantly lower for *Pleuraphis* with neighbors established in winter but was the same between seasons for both *Achnatherum* and *Larrea* (species × season, Table 3).

The relative effects of the *Bromus* and mixed species treatments on perennial responses such as RGR and RE could not be compared using traditional ANOVA because sample sizes for the mixed annuals were lower and the range of biomass for mixed annual treatments was smaller (and closer to zero) than those for *Bromus* (Fig. 3). Instead, we evaluated whether those perennials with the mixed species treatment were statistical outliers from the model fitted with both mixed species and *Bromus* treatments, which would suggest that perennials with neighbors of mixed annual species respond differently from those with neighbors of just *Bromus*. For perennial RGR, only two observations were outliers from the fitted model (|studentized residuals| > 2.5; D'Agostino Pearson Omnibus P-value < 0.01), and both observations were for *Pleuraphis* with the spring annual establishment treatment (Fig. 3). For these two outliers, one represented the *Bromus* neighbor treatment and the other represented the mixed annual species treatment. RE had no significant outliers. Thus, the effects of a mixture of annuals on perennial RGR and RE were quantitatively similar to those for *Bromus*.

Water relations

Total water use was greater for plants in the winter establishment treatment compared with that in the spring (Fig. 4). This greater water use was potentially influenced by the greater total water input as a result of rainfall that occurred after irrigation treatments were completed. Thus, to understand if the neighboring annual treatments affected water use for the three Mojave Desert perennials, total water use was analyzed separately for the winter and spring treatments. The slopes of the relationship between total water use and *Bromus* biomass varied among the three perennial species when *Bromus* was established in the winter (biomass × species, Table 4). Water use for plots with *Pleuraphis* and *Larrea* increased as neighboring *Bromus* biomass increased (slopes = 0.33 mm·$[\mathrm{g\ m}^{-2}]^{-1}$, df = 1, 10, $P < 0.01$ and 0.33 mm·$[\mathrm{g\ m}^{-2}]^{-1}$, df = 1, 6, $P = 0.04$ for *Pleuraphis* and *Larrea*, respectively), but water use for plots with *Achnatherum* did not change with *Bromus* biomass (df = 1, 9, $P = 0.88$). In other words, an additional 3.3 mm of water was used over the growth season for every increase of 10 g m^{-2} of neighbor *Bromus* biomass surrounding *Pleuraphis* and *Larrea* individuals. In contrast, total water use for the spring establishment treatment did not change with an increase in neighbor *Bromus* biomass, nor was it significantly different among perennial species (biomass × species and species effects; Table 4). To examine if total water use with mixed annuals was quantitatively different from that with just *Bromus* neighbors, we again examined the pooled model for statistical outliers. Only one plot, a *Larrea* with mixed annuals established in the spring, was a significant outlier.

Predawn (Ψ_{pre}) and midday (Ψ_{mid}) water potentials of the three perennial species were more negative for the spring annual establishment treatment than for the winter, but water potential did not vary with neighbor biomass (Table 4, Fig. 5). Among perennial species with *Bromus* neighbors only, Ψ_{pre} was significantly more negative for *Larrea* (-0.9 ± 0.1 MPa) than for either *Achnatherum* (-0.4 ± 0.1 MPa) or *Pleuraphis* (-0.5 ± 0.1 MPa) when neighboring *Bromus* plants were established in winter, but Ψ_{pre} was not significantly different among species when *Bromus* was established in spring. Ψ_{mid} was significantly lower for *Achnatherum* (-2.1 ± 0.1 MPa) and *Larrea* (-2.3 ± 0.1 MPa) than for *Pleuraphis* (-1.5 ± 0.1 MPa) when *Bromus* was established in winter, and was lower for *Larrea* (-3.2 ± 0.2 MPa) than for either *Achnatherum* (-2.7 ± 0.2 MPa) or *Pleuraphis* (-2.5 ± 0.1 MPa) when *Bromus* was established in spring. These differences in perennial plant water status between winter and spring establishment of *Bromus* should be interpreted carefully – measurements were made at analogous phenological stages (*i.e.* at peak perennial production, which corresponded to April 15, 2000 for winter establishment treatments and April 29, 2000 for spring), but water status was also affected at least in part by the additional soil drying that occurred during the 2 wk time difference between the measurements.

Few statistical outliers occurred for Ψ_{mid} when *Bromus* and mixed species treatments were considered together (Fig. 5). Only two of these outliers represented plots from the mixed species treatment, one established in spring for *Larrea* and the other established in winter for *Pleuraphis*.

Discussion

The non-native annual grass *Bromus madritensis* ssp. *rubens* reduced the growth of Mojave Desert perennials when *Bromus* individuals established during winter, which is also when native perennials were dormant, but had less effect in spring when germination and growth of *Bromus* were more synchronous with perennial plant activity. *Bromus* germinated shortly after seeds were sown and irrigated beneath the canopies of perennials in both winter and spring, but growth of *Bromus* plants that established in winter preceded perennial plant growth by 2 to 3 mo and resulted in greater *Bromus* biomass beneath canopies of the perennials and higher total water use. In contrast, germination in spring resulted in low biomass of *Bromus* around the perennials and had less effect on RGR of native perennials. Thus, the greater growth of *Bromus* when it establishes in winter likely depletes soil resources that would otherwise be used by perennial plants.

Despite the prominent increase in total water use when *Bromus* neighbors were established in winter, competition for water does not appear to be the major mechanism behind the observed differences in perennial responses to winter and spring establishment treatments. Other studies have shown that high water use by stands of non-native annual neighbors increases water stress and reduces performance of perennials in arid regions of western North America (Schultz et al. 1955; Eissenstat and Caldwell 1988; Gordon et al. 1989; Melgoza et al. 1990; Melgoza and Nowak 1991; Welker et al. 1991; Eliason and Allen 1997; Gordon and Rice 1993, 2000). However, in this study plant water potentials of perennials did not significantly decrease as a function of neighbor *Bromus* biomass. Furthermore, the pattern of plant water potential for the perennials was contrary to expectations if water use was a major competitive mechanism – greater total water use when *Bromus* establishes in winter is expected to result in greater stress for perennial plants, but the observed plant water potentials for the perennials with the winter establishment treatment indicated less water stress. Predawn plant water potentials imply that the perennial plants had access to ample soil water, even at peak shoot production. Indeed, the irrigation and natural rainfall that occurred during the annual plant establishment treatments exceeded average precipitation for the region, and evapotranspiration from the plots was commensurate with an above-average rainfall year (Yoder and Nowak 1999). Nonetheless, reduced perennial RGRs also may be a consequence of depletion of soil N by *Bromus*. Although we did not measure the status of soil and plant tissue N, *Bromus* has the potential to acquire soil N more rapidly than perennial species. For example, N-uptake by *Bromus* in a glasshouse experiment exceeded that of *Achnatherum* and *Pleuraphis*, especially with regard to *Bromus*' uptake of ammonium, which was more than two times that of the perennial grasses (Yoder et al. 2000). Clearly, further study of N-uptake by perennial species with and without *Bromus* neighbors and under conditions of contrasting establishment (*e.g.*, years with above- versus below-average precipitation) is necessary to further elucidate the mechanism of *Bromus* interference on perennial plants.

As predicted based on physiologically induced differences in phenology, declines in RGR associated with *Bromus* biomass were smaller for the evergreen shrub *Larrea* than for the grasses *Achnatherum* and *Pleuraphis*. *Larrea* is a drought-resistant perennial that maintains moderate to low rates of photosynthesis virtually all months of the year (Oechel et al. 1972; Smith et al. 1997), has high water use efficiencies through stomatal closure or increased photosynthetic capacity (Meinzer et al. 1988), and can withstand low soil water potentials (Oechel et al. 1972; Franco et al. 1994; Hamerlynck et al. 2000). In contrast, drought-evaders such as perennial grasses are sensitive to low soil water potentials, and their growing season is restricted to periods of high soil moisture (Smith et al. 1997). *Achnatherum* is an early-season C_3 species whose growth occurs above temperatures of 4°C (Pearson 1979) and has lower optimal leaf temperatures for photosynthesis compared to *Pleuraphis*, a late-season C_4 grass whose growth begins in late spring (Nobel 1980). Hence, growth of *Achnatherum* is synchronous with activity of winter annuals, thereby placing *Achnatherum* in direct competition with annuals, whereas reduction in *Pleuraphis* performance by neighbors may be pronounced because of the earlier growth and rapid soil moisture use of neighbors before *Pleuraphis* becomes active.

The effects of *Bromus* and mixed native annuals on perennial responses appear to be similar, based on the analysis detecting outliers from the fitted regression. Although this result is contrary to initial predictions that were based on an earlier study (DeFalco et al. 2003), the establishment of mixed species was markedly lower than *Bromus*, and thus the resulting native annual biomass was likely too low to significantly reduce perennial RGRs. Native annual species in the northern Mojave Desert germinate and grow at the same time, but typically in lower densities, than *Bromus* in any given year (Hunter 1991). This disparate establishment between the native and non-native desert annuals is due in part to a dormant seed fraction of native annuals that remains in the soil, which allows them to escape unfavorable germination conditions (Cohen 1966). Low densities of natives in the mixed species stands are consistent with the "bet-hedging" strategy found in annual species from unpredictable environments – in years of high seedling mortality, a viable portion of seed remains in the soil to germinate in subsequent years that have more favorable conditions for growth (Brown and Venable 1986; Philippi 1993; Clauss and Venable 2000). Natives may also have lower seedling survivorship than *Bromus,* whose less exacting soil moisture and temperature requirements and greater seed viability yields greater abundance than natives (Beatley 1966). In this study, the range of densities and resulting low biomasses for the mixed species neighbors falls within the range reported for native annuals censused in the area from 1963 through 1990 (Hunter 1991). Hence, the similar perennial responses between mixed annual and *Bromus* treatments reflect the same effect of these neighbors on perennials when their abundance is low. Native annuals do not often reach the high biomass achieved by their *Bromus* counterparts; thus negative effects of native annuals on perennials are likely infrequent.

While most research has focused on *Bromus*' dramatic impact on plant community composition and structure by providing fuel for wildfire, this study highlights the potential for the decline of perennial species in undisturbed habitat through direct interaction with *Bromus*. As *Bromus* continues to integrate into the native flora and to dominate the landscape in the Mojave Desert, the composition of species within these communities, especially the shorter-lived perennial grasses, are subject to change even in the absence of wildfire. Future mitigation of the effects of *Bromus* in the Mojave Desert will require an understanding of the environmental cues that drive its establishment and dominance while simultaneously appreciating the interannual variation controlling these factors.

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Table 1. Profile soil water (mm, mean ± SE) to a depth of 1.35 m around three Mojave Desert perennial species measured at three different phenological stages. Factors in the 2-way ANOVA were the three perennial species (Species) and the two seasons when *Bromus* was established (Season).

	Before irrigation			Source	df	*P*
	Achnatherum	*Pleuraphis*	*Larrea*	Species	2, 55	0.02
Winter	118 ± 1	119 ± 2	114 ± 2	Season	1, 55	< 0.01
Spring	110 ± 1	110 ± 1	107 ± 1	Species × Season	2, 55	0.77
	Onset of perennial growth					
	Achnatherum	*Pleuraphis*	*Larrea*	Species	2, 58	< 0.01
Winter	138 ± 3	156 ± 4	141 ± 7	Season	1, 58	0.28
Spring	110 ± 1	153 ± 4	161 ± 5	Species × Season	2, 58	< 0.01
	After *Bromus* senescence					
	Achnatherum	*Pleuraphis*	*Larrea*	Species	2, 60	0.03
Winter	129 ± 5	131 ± 4	119 ± 3	Season	1, 60	0.34
Spring	124 ± 4	128 ± 5	118 ± 3	Species × Season	2, 60	0.92

Table 2. Statistics for the 3-way qualitative (neighbor species), qualitative (season), and quantitative (annual density) ANOVAs for annual biomass and plant size. Annual biomass and density were $\log_{10}$-$\log_{10}$ transformed before analysis.

		$\log_{10}$ [Biomass (g m^{-2})]	$\log_{10}$ [Size (g ind^{-1})]
	df	*P*	*P*
Density	1, 74	< 0.01	< 0.01
Species	1, 74	< 0.01	< 0.01
Season	1, 74	< 0.01	< 0.01
Species × Season	1, 74	0.70	0.74
Density × Species	1, 74	< 0.01	< 0.01
Density × Season	1, 74	0.81	0.74
Density × Species × Season	1, 74	0.01	< 0.01

Table 3. Statistics for the 3-way qualitative (perennial species), qualitative (season), and quantitative (*Bromus* biomass) ANOVAs for perennial relative growth rate (RGR) and reproductive effort (RE).

		RGR (d^{-1})	RE (flowers g^{-1})
	df	*P*	*P*
Biomass	1, 53	< 0.01	0.72
Species	2, 53	< 0.01	< 0.01
Season	1, 53	< 0.01	0.29
Species × Season	2, 53	0.02	< 0.01
Biomass × Species	2, 53	0.06	0.29
Biomass × Season	1, 53	0.13	0.77
Biomass × Species × Season	2, 53	0.22	0.44

Table 4. Statistics for separate seasonal 2-way qualitative (perennial species) and quantitative (*Bromus* biomass) ANOVAs for perennial water use, and predawn (Ψ_{pre}) and midday (Ψ_{mid}) water potentials.

		Water use (mm)	Ψ_{pre} (MPa)	Ψ_{mid} (MPa)
Winter treatment	df	*P*	*P*	*P*
Biomass	1, 26	< 0.01	0.15	0.61
Species	2, 26	0.16	< 0.01	< 0.01
Biomass × Species	2, 26	< 0.01	0.35	0.35

		Water use (mm)	Ψ_{pre} (MPa)	Ψ_{mid} (MPa)
Spring treatment	df	*P*	*P*	*P*
Biomass	1, 26	0.64	0.85	0.41
Species	2, 26	0.26	0.48	0.05
Biomass × Species	2, 26	0.68	0.21	0.38

FIGURE LEGENDS

Figure 1. Phenological stages of annual neighbors (*Bromus* and mixed annual species) and native perennials (*Achnatherum, Pleuraphis* and *Larrea*) where the annuals were established in the winter (top set of diagrams) or in the spring (bottom set of diagrams). Periods when plots were irrigated are also indicated by light diagonal shading.

Figure 2. Relationship of $\log_{10}$ transformed annual biomass (top) and of $\log_{10}$ transformed plant size (bottom) to $\log_{10}$ transformed plant density for the annual grass *Bromus* (circles) and for mixed annual species (triangles) that established beneath the canopies of perennials. The annual plants were established in winter (filled symbols) or spring (clear symbols). Regression lines emphasize different slopes for each neighbor and for winter (solid) and spring (dotted) establishment treatments.

Figure 3. Relationship of relative growth rate (RGR; top) and of reproductive effort (RE; bottom) for perennial species *Achnatherum* (left), *Pleuraphis* (center), and *Larrea* (right) to biomass of the annual grass *Bromus* (circles) or of mixed annual species (triangles) that established beneath canopies of the perennials. Annual plants were established in winter (filled symbols) or spring (clear symbols). Regression lines represent a significant relationship between perennial RGR and *Bromus* biomass over both the winter and spring annual establishment treatments. Arrows point to statistical outliers.

Figure 4. Relationship of total water use for the perennial species *Achnatherum* (left), *Pleuraphis* (center), and *Larrea* (right) to biomass of the annual grass *Bromus* (circles) or of mixed annual species (triangles) that established beneath canopies of the perennials. The annual plants were established in winter (filled symbols) or spring (clear symbols). Regression lines represent a significant relationship between total water use and *Bromus* biomass over both the winter and spring annual establishment treatments. Arrow points to statistical outlier.

Figure 5. Relationship of predawn (top graphs) and of midday (bottom graphs) water potentials for the perennial species *Achnatherum* (left), *Pleuraphis* (center), and *Larrea* (right) to biomass of the annual grass *Bromus* (circles) or of mixed annual species (triangles) that established beneath canopies of the perennials. Measurements were made on perennials at peak perennial production for winter (filled symbols) and spring (clear symbols) annual establishment treatments. At this time, annuals were senescing beneath canopies of the perennials. Arrows point to statistical outliers.

Figure 1

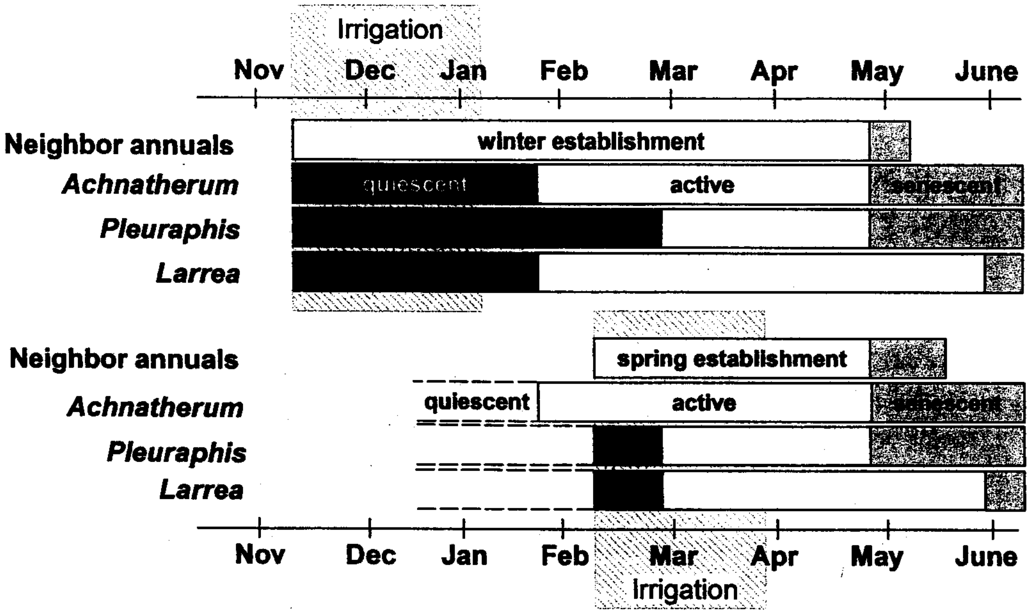

Irrigation
Nov
Dec
Jan
Feb
Mar
Apr
May
June
Neighbor annuals
winter establishment
Achnatherum
quiescent
active
senescent
Pleuraphis
Larrea
Neighbor annuals
spring establishment
Achnatherum
quiescent
active
senescent
Pleuraphis
Larrea
Nov
Dec
Jan
Feb
Mar
Apr
May
June
Irrigation

Figure 2

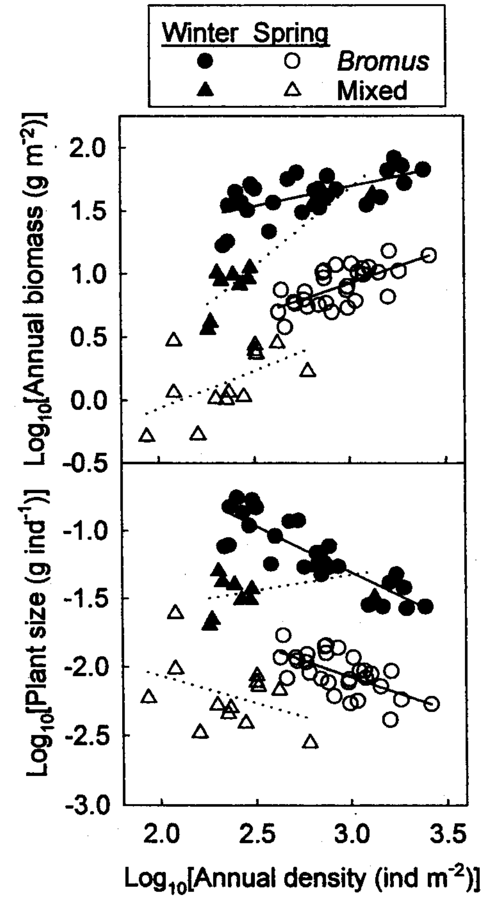

Winter Spring
● ○ *Bromus*
▲ △ Mixed
Log$_{10}$[Annual biomass (g m^{-2})]
2.0
1.5
1.0
0.5
0.0
-0.5
Log$_{10}$[Plant size (g ind^{-1})]
-1.0
-1.5
-2.0
-2.5
-3.0
2.0
2.5
3.0
3.5
Log$_{10}$[Annual density (ind m^{-2})]

Figure 3

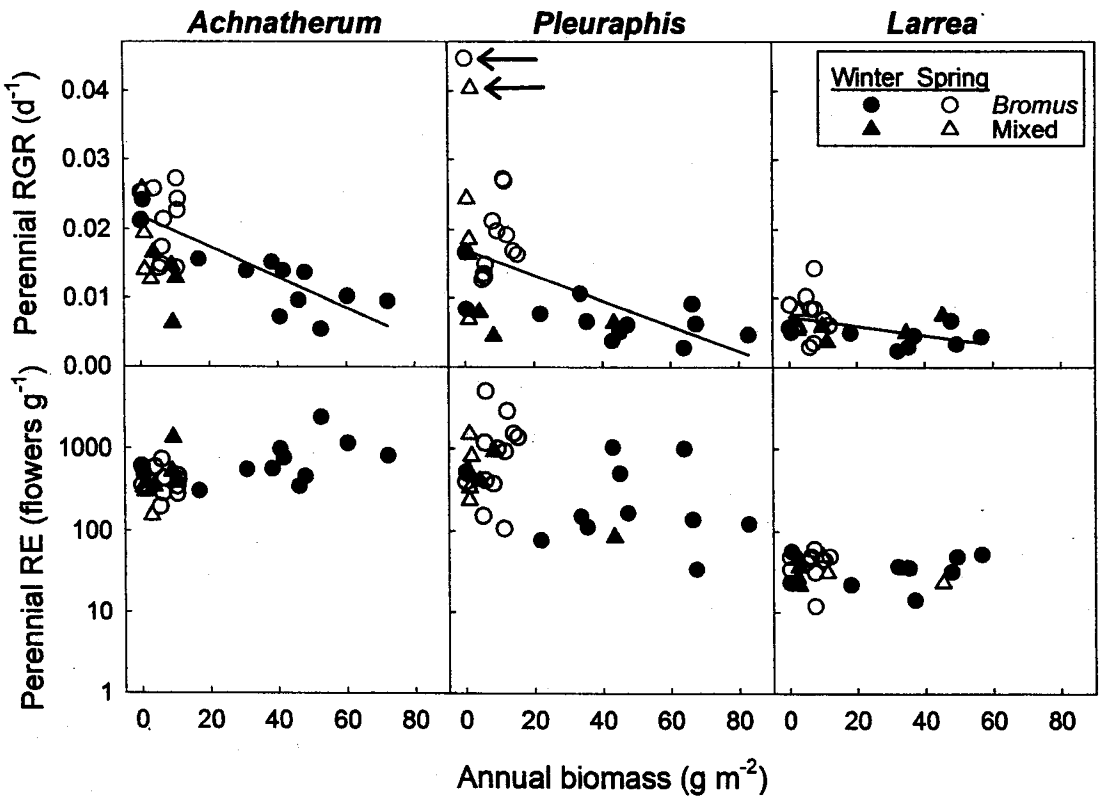

Achnatherum
Pleuraphis
Larrea
Winter Spring
● ○ Bromus
▲ △ Mixed
Perennial RGR (d^{-1})
0.04
0.03
0.02
0.01
0.00
Perennial RE (flowers g^{-1})
1000
100
10
1
0
20
40
60
80
Annual biomass (g m^{-2})

Figure 4

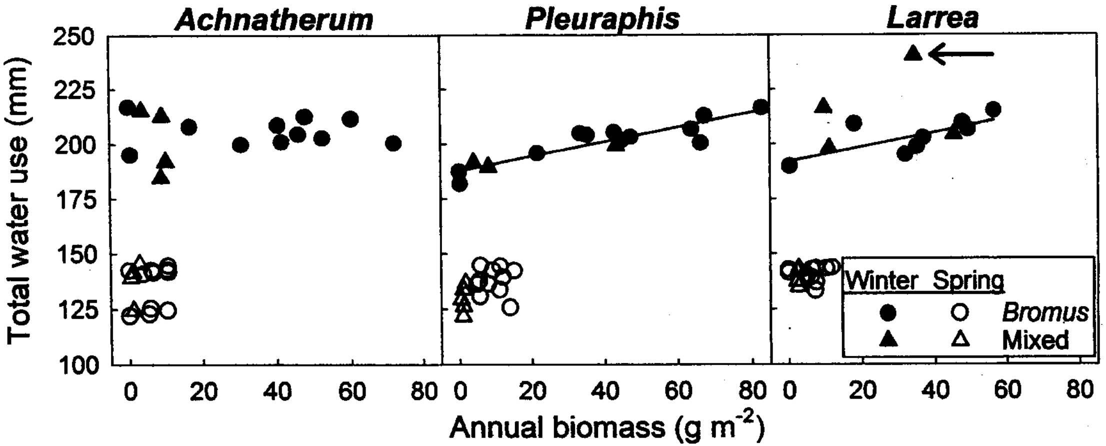
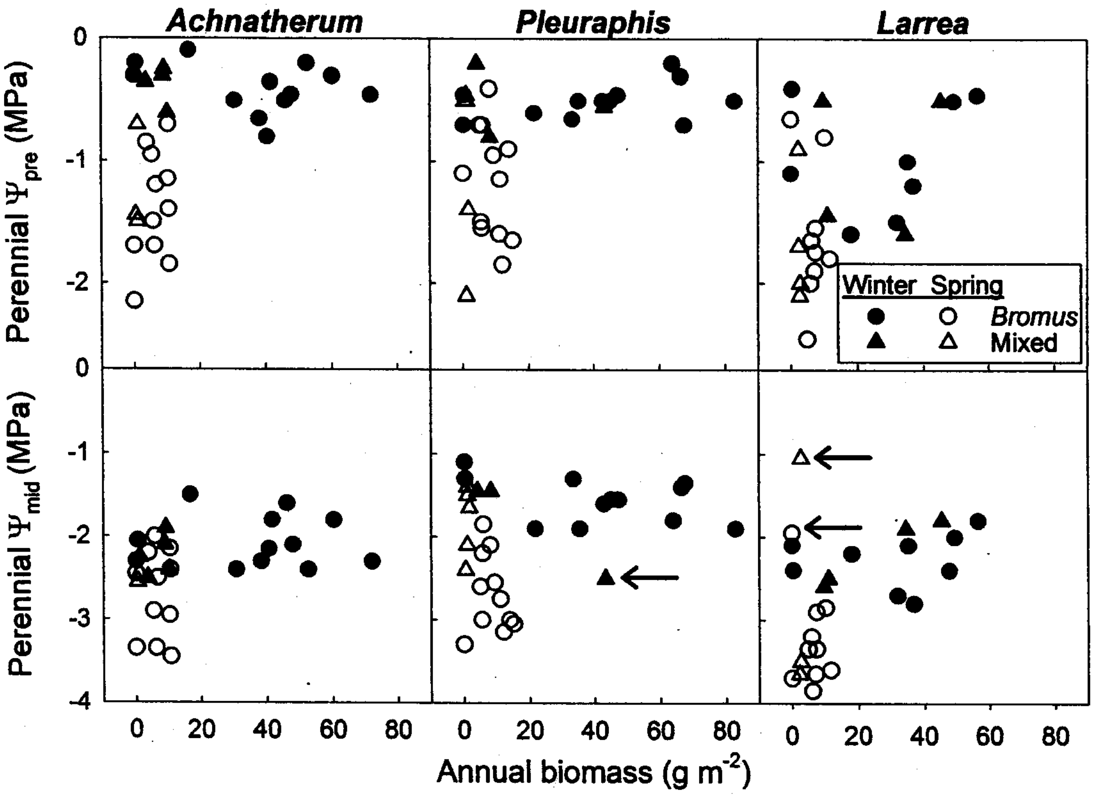

Achnatherum
Pleuraphis
Larrea
Total water use (mm)
250
225
200
175
150
125
100
0
20
40
60
80
Annual biomass (g m-2)
Winter Spring
● ○ Bromus
▲ △ Mixed

Figure 5


Achnatherum
Pleuraphis
Larrea
Perennial Ψ_{pre} (MPa)
Perennial Ψ_{mid} (MPa)
0
-1
-2
-3
-4
0
20
40
60
80
Annual biomass (g m^{-2})
Winter Spring
Bromus
Mixed