

## AN INVADER DIFFERENTIALLY AFFECTS LEAF PHYSIOLOGY OF TWO NATIVES ACROSS A GRADIENT IN DIVERSITY

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**Abstract.** Little is known about how exotics influence the ecophysiology of co-occurring native plants or how invader impact on plant physiology may be mediated by community diversity or resource levels. We measured the effect of the widespread invasive forb spotted knapweed (*Centaurea maculosa*) on leaf traits (leaf dry matter content, specific leaf area, leaf nitrogen percentage, leaf C:N ratios, and  $\delta^{13}\text{C}$  as a proxy for water use efficiency) of two co-occurring native perennial grassland species, *Monarda fistulosa* (bee balm) and *Koeleria macrantha* (Junegrass). The impact of spotted knapweed was assessed across plots that varied in functional diversity and that either experienced ambient rainfall or received supplemental water. Impact was determined by comparing leaf traits between identical knapweed-invaded and noninvaded assemblages. Virtually all *M. fistulosa* leaf traits were affected by spotted knapweed. Knapweed impact, however, did not scale with its abundance; the impact of knapweed on *M. fistulosa* was similar across heavily invaded low-diversity assemblages and lightly invaded high-diversity assemblages. In uninvaded assemblages, *M. fistulosa*  $\delta^{13}\text{C}$ , leaf nitrogen, and C:N ratios were unaffected by native functional group richness, whereas leaf dry matter content significantly increased and specific leaf area significantly decreased across the diversity gradient. The effects of spotted knapweed on *K. macrantha* were weak; instead native functional group richness strongly affected *K. macrantha* leaf C:N ratio,  $\delta^{13}\text{C}$ , and specific leaf area, but not leaf dry matter content. Leaf traits for both species changed in response to spotted knapweed or functional richness, and in a manner that may promote slower biomass accumulation and efficient conservation of resources. Taken together, our results show that an invader can alter native plant physiology, but that these effects are not a simple function of how many invaders exist in the community.

**Key words:** *Centaurea maculosa*; functional richness; invader impact; *Koeleria macrantha*; leaf traits; *Monarda fistulosa*; plant physiology; spotted knapweed; water use efficiency.

### INTRODUCTION

Exotic plants that occur at very high abundance, as virtual monocultures in recipient communities, are a growing problem worldwide. These “strong invaders” (sensu Ortega and Pearson 2005), by virtue of both their sheer abundance and novel functional attributes, can have broad effects on native ecosystems, from changing soil nutrient and water dynamics to altering fire frequency (reviewed by Levine et al. 2003). However, at smaller spatial scales, less is known about how exotics influence individual plants, particularly their physiology. Few studies have examined whether exotics alter the ecophysiology of co-occurring native plants or whether particular community characteristics mediate these effects. In isolation we know that, at small spatial scales, assemblages with increased native plant functional (Prieur-Richard et al. 2000, Symstad 2000, Fargione et al. 2003) or species diversity (Levine 2000,

Naeem et al. 2000, Dukes 2002, Kennedy et al. 2002, Zavaleta and Hulvey 2004, Maron and Marler 2007) are more resistant to invasion than are less diverse plant assemblages. Yet whether increasing diversity reduces the impact of an invader on co-occurring natives is wholly unanswered (Parker et al. 1999).

One mechanism responsible for the negative diversity–invasibility relationship is greater resource uptake and thus lower availability of soil resources in more diverse than in less diverse assemblages (Fargione et al. 2003, Maron and Marler 2007). Plant ecophysiological traits related to leaf morphology, nitrogen and carbon content, and water use efficiency (WUE) should respond to variation in resource availability; thus these traits are potentially sensitive to both surrounding plant diversity (Caldiera et al. 2001) and to the presence of a potent invader. Moreover, since physiological traits underlie variation in plant growth rates and seed production (Condon et al. 1987, Geber and Dawson 1990, Bazzaz and Grace 1997), they provide a metric to assess the impact of an exotic on a native, and explore how variation in native diversity and resource availability may mediate that impact.

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Here we ask how the widespread invasive forb spotted knapweed (*Centaurea maculosa*) affects the leaf physiology of two native co-occurring perennial grassland species, *Monarda fistulosa* (bee balm) and *Koeleria macrantha* (Junegrass). In particular, we explore how the impacts of knapweed on the physiology of these natives might change across an experimentally imposed gradient in native plant diversity that either is subject to resource (water) additions or is not. We examined the influence of spotted knapweed on leaf dry matter content (LDMC), specific leaf area (SLA), leaf nitrogen, leaf C:N ratios, and  $\delta^{13}\text{C}$  (water use efficiency [WUE]) because these traits can reveal patterns in resource uptake and use (Bazzaz and Grace 1997, Grime et al. 1997, Wilson et al. 1999, Garnier et al. 2001a, Roche et al. 2004) that might vary depending on (1) surrounding native plant diversity, (2) experimental water addition, and (3) levels of spotted knapweed invasion as mediated by diversity and resource availability. As well, *M. fistulosa* and *K. macrantha* differ in functional attributes. The forb *M. fistulosa* has a relatively shallow root system compared to the tap-rooted exotic forb spotted knapweed. The  $\text{C}_3$  grass *K. macrantha* flowers, sets seed, and goes dormant by early summer, before spotted knapweed attains a large size. In contrast, *M. fistulosa* flowers relatively late, and thus it has greater phenological overlap with spotted knapweed. We hypothesized that these differences in functional overlap between the two natives and spotted knapweed would influence the extent to which knapweed impacts the physiology of *M. fistulosa* and *K. macrantha*.

In previous work we found a strong negative relationship between native plant diversity and knapweed invasion (Maron and Marler 2007), with resource preemption contributing toward the ability of diverse assemblages to resist invasion. We also found that water additions increased invasibility, but this did not fundamentally change the slope of the negative diversity–invasibility relationship (Maron and Marler 2007). Here we are particularly interested in three issues. First, how might an invader such as knapweed affect the leaf physiology of surrounding natives? At high invader abundance natives might sacrifice rapid growth for efficient conservation of resources, which should depress SLA and leaf N and increase LDMC, C:N, and  $\delta^{13}\text{C}$ . Second, how might native diversity in the absence of an invader influence individual natives? Since increasing native diversity lowers resource availability, in the absence of an invader, LDMC, C:N, and  $\delta^{13}\text{C}$  might linearly increase across the diversity gradient, while leaf N and SLA should decrease. Third, how might the magnitude of invader impact scale with native diversity? If invader impact is solely a function of invader abundance, there should be a strong correspondence between invasibility and impact. This would then predict that effects of knapweed on native plant physiology would strongly decrease in more diverse assemblages.

Finally, since water addition increases “free” resources, belowground competition may be ameliorated, thus influencing the impacts of diversity on leaf traits in these assemblages. However, since water addition also leads to increased invasion (Maron and Marler 2007), we might expect greater knapweed impact in watered vs. unwatered assemblages.

## METHODS

### *Experimental design*

The experiment took place in a fallow field at Fort Missoula, Missoula, Montana, USA. In spring 2003 we created a total of 23 different native plant assemblages (12 mixed-species assemblages and 11 monocultures) containing one, two, three, four, or six plant functional groups and 1–16 species drawn from a pool of 16 native species. The 16 species naturally co-occur in grasslands of western Montana. Plants were classified into the following six functional groups based on their phenology and rooting architecture: dominant  $\text{C}_3$  grass species (*Festuca idahoensis*, *Poa sandbergii*, and *Koeleria macrantha*), extremely early-season forb species with a shallow tap root (*Dodecatheon pulchellum*, *Lewisia rediviva*), early-flowering forb species that branch by short rhizomes, stolons, or at the root crown (*Geum triflorum*, *Antennaria rosea*, and *Penstemon procerus*, respectively), mid-season forbs with woody root crowns (*Penstemon wilcoxii*, *Potentilla arguta*, and *Gaillardia aristata*), mid-season forbs with spreading rhizomes (*Arnica sorea*, *Achillea millefolium*, and *Monarda fistulosa*), and late-season forbs with a deep tap root (*Aster falcatus* and *Artemisia frigida*; nomenclature follows Dorn 1984). All forbs were  $\text{C}_3$ . Appendix A details the composition of each assemblage. Each diversity assemblage was assigned to an ambient rainfall or supplemental water treatment. Supplemental water was added to plots (starting in 2004) once per week in May and June. Each diversity  $\times$  water treatment was replicated across three blocks, each separated by 6 m, with plots within blocks separated by 3 m (23 diversity assemblages  $\times$  3 blocks  $\times$  2 water treatments = 138 plots total). See Maron and Marler (2007) for a complete description of the experimental setup.

Plant assemblages were created by planting seedlings in  $3 \times 3$  m plots divided into four subplots ( $1.3 \times 1.3$  m separated by a 0.2-m buffer strip). The four subplots within plots were planted with identical mixes and densities of native species. Assemblages were weeded throughout each growing season to maintain predetermined levels of species and functional richness. Subplots were invaded by one of three different exotic forb species, *Centaurea maculosa* (spotted knapweed), *Potentilla recta*, or *Linaria dalmatiana*, or remained as an uninvaded control. Here we focus on results collected from a subset of uninvaded and spotted knapweed-invaded subplots that contained the mid-season rhizomatous forb *Monarda fistulosa* and/or the co-occurring

early-season grass *Koeleria macrantha* (9 assemblages  $\times$  2 water treatments  $\times$  3 blocks = 54 plots  $\times$  invaded/uninvaded subplots = 108 for *M. fistulosa*; 10 assemblages  $\times$  2 water treatments  $\times$  3 blocks = 60 plots  $\times$  invaded/uninvaded subplots = 120 for *K. macrantha*). We chose *K. macrantha* because it is one of several dominant grass species both in terms of number and biomass in western intermontane grasslands; *M. fistulosa* was selected because it is a forb whose growing season partially overlaps with the forb spotted knapweed. We invaded subplots with spotted knapweed seed in September 2004 and again in 2005 (see Maron and Marler [2007] for details). We define invader impact as the difference between the values of a native plant physiological trait in knapweed-invaded and uninvaded assemblages.

In April 2005 the number of functional types (hereafter FR for functional richness) in both invaded and uninvaded subplots used in this study was highly correlated to the planted FR (Pearson  $r = 0.99$  and  $0.98$  for uninvaded and invaded subplots, respectively). In spring 2006 this was also the case (Pearson  $r = 0.97$  for both uninvaded and invaded subplots).

Over the course of three days in July 2005 and 2006, we collected 20 leaf samples from different *K. macrantha* and *M. fistulosa* at the same time of day (08:30 to 10:30 hours) in the 108 *M. fistulosa* and 120 *K. macrantha* subplots. We excised 10 pairs of leaves from plants in each subplot, with at least two leaves collected per genet. We sampled different individuals within subplots across years. We selected young, fully expanded and illuminated, undamaged leaves on each plant. Leaves were put into labeled plastic bags and stored in a cooler for transport back to the laboratory. In the laboratory, one leaf from each pair was rehydrated by placing a small amount of water in the bags and allowing the leaves to achieve turgor in a cool, dark environment (following Wilson et al. 1999 and Garnier et al. 2001b). We blotted dry the leaf samples and immediately weighed them to obtain saturated wet mass. Each sample was then pressed for no more than two days to facilitate measurement of leaf area to the nearest  $0.001 \text{ cm}^2$  on a calibrated LI-COR 3100 (LI-COR, Lincoln, Nebraska, USA). Leaf dry mass (g) was measured after these leaves were dried to a constant mass in an oven at  $45^\circ\text{C}$  for at least three days; leaf dry matter content (LDMC) was calculated as a ratio of leaf dry mass to fresh mass (mg/g). We calculated the average LDMC and specific leaf area (SLA;  $\text{m}^2/\text{g}$ ) values for the 10 samples collected per species per subplot.

For determination of leaf percentages of N and C, C:N, and  $\delta^{13}\text{C}$ , we used the second set of 10 leaves from plots where FR = 1, 3, 4, 6 ( $n = 60$  and  $n = 70$  subplots of *M. fistulosa* and *K. macrantha*, respectively), but not in subplots where FR = 2. The 10 harvested leaves were pooled together, air-dried for one week, dried for one day at  $60^\circ\text{C}$ , then immediately ground and sent to the

Colorado Plateau Stable Isotope Laboratory for analysis and calculation of leaf N, C, C:N, and  $\delta^{13}\text{C}$ .

#### Statistical methods

We used factor analysis to capture sets of variables that had unique variances and to group potentially correlated leaf traits so that we could perform subsequent analyses on uncorrelated factors. Factor analysis also was used as a data reduction technique, similar to what principal components analysis accomplishes. Factor analysis (JMP 2006) was performed on the five leaf traits measured in 2005 and 2006 to give 10 variables, and on an additional five variables created by averaging each leaf trait over the two-year period. Averages for the two years were calculated to determine if the averages described the data more efficiently than the two separate years. By including the average score, we devised an indirect test to detect if differences existed across the two years. If a strong correlation exists between the average scores and each year, then it is likely there were no differences between years, and the average can be used. We determined the factor loadings of these 15 variables using factor rotation, which transformed the principal components to a simple structure using an orthogonal rotation criterion. The rotation of the original variables revealed clustering of correlated variables in reduced dimensions. Each factor was completely uncorrelated with the other factors, and the factor loadings measured the correlation of each of the original variables to each of the factors. Leaf traits were considered as members of a factor when the correlation of each variable to a factor was  $\geq 0.82$ . The varimax method was employed to maximize variance of the squared elements of the factor matrix (JMP 2006). In this factor model, the new variables (reduced in number) are linear combinations of common factors and unique factors. A scree plot was used to determine the minimum number of factors required to explain the majority of the variation in the variable responses.

For each native species we performed a MANCOVA to determine significant effects of diversity, invasion treatment (spotted knapweed-invaded or uninvaded), water treatment, and block (including and up to the four-way interactions) on those factors identified in the factor analysis. We specified richness as an ordinal measure and invasion treatment (spotted knapweed-invaded or uninvaded), water treatment, and block as categorical variables. The ordinal measure controls for increasing plant diversity in the model. Because realized species and functional richness (FR) were highly correlated and because FR always explained more variation in the models than realized species richness, we only report results using FR as the ordinal covariate. To compare the effects across the factor component variables, the "mean" contrast was specified in the model; each response variable was contrasted with the other equally weighted response variables in the model.

TABLE 1. Results from ANCOVA testing effects of block, functional richness (FR), spotted knapweed (*Centaurea maculosa*), water treatment, and their interactions on bee balm (*Monarda fistulosa*) and Junegrass (*Koeleria macrantha*) leaf trait factors (factors identified by factor analysis).

Trait	Block	FR	Knapweed	Water	Knapweed × water	Knapweed × FR	Water × FR
<i>Monarda fistulosa</i>							
Factor 1: $\delta^{13}\text{C}$ , N, C:N	<b>5.93</b>	0.77	<b>6.09</b>	<b>5.76</b>	0.21	1.13	0.402
Factor 2: SLA	<b>2.66</b>	<b>4.97</b>	1.78	1.01	0.20	0.33	1.49
Factor 3: LDMC	<b>9.73</b>	<b>3.21</b>	<b>10.1</b>	3.23	0.02	1.79	1.24
<i>Koeleria macrantha</i>							
Factor 1: N, C:N	<b>17.9</b>	<b>19.2</b>	0.14	0.02	1.07	0.18	1.40
Factor 2: SLA	<b>18.8</b>	<b>3.23</b>	1.52	2.68	1.60	0.24	2.80
Factor 3: LDMC	<b>5.57</b>	1.46	1.37	<b>8.03</b>	0.73	0.28	0.36

Notes: *F* values are shown. Bold type denotes values that are significant at  $P < 0.05$ ;  $df = 17, 51$  for all reported tests. For *K. macrantha*,  $\delta^{13}\text{C}$  is spread equally between the first two factors.

Since none of the interactions were significant (see *Results*), we only report the main effects.

Since the MANCOVAs on the factors for each species were significant, we performed follow-up ANCOVAs on each factor to determine significant effects of diversity, invasion treatment (spotted knapweed-invaded or uninvaded), water treatment, and block including and up to the four-way interactions in the model (JMP 2006). Biologically relevant interaction terms test the following: (1) whether the effects of diversity on native plant physiology differed depending on water treatment; (2) whether the effects of diversity on native plant physiology differed depending on the presence or absence of spotted knapweed; and (3) the effects of spotted knapweed and water treatment on native plant diversity. If any of the interaction terms involving FR were significant, it would indicate that the effects of diversity on the response variable varied with the water or spotted knapweed-invasion treatment, and would cloud interpretation of main effects. However, these interaction terms were never significant.

## RESULTS

### *Monarda fistulosa*

A subset of the original 15 response variables loaded onto three factor components that accounted for 88% of the variation. Factor 1, which accounted for 49% of the total variation, showed high loading for average (i.e., the average of 2005 and 2006 values)  $\delta^{13}\text{C}$ , leaf N, and C:N ratio (loading factors = 0.82, 0.94, and -0.96, respectively). Factor 2, which accounted for 20% of the total variation, showed high loading, 0.98, for average specific leaf area (SLA). Factor 3, which accounted for 19% of the total variation, exhibited high loading for average leaf dry matter content (LDMC) at 0.98. Since trait averages across the two years had higher factor loading than in individual years, which suggests that there were no differences from one year to the next, we used the average rather than values for individual years. A MANCOVA using these three factors revealed significant main effects of spotted knapweed, water addition,

and functional richness (FR) but no significant interactions ( $F_{2,92} = 0.19$ ,  $P = 0.05$ ; Appendix B).

Virtually all *Monarda fistulosa* leaf trait factors were affected by both spotted knapweed and experimental water addition; only *M. fistulosa* SLA did not significantly change in response to spotted knapweed (Table 1). Regardless of the water treatment, average *M. fistulosa* leaf LDMC and C:N were significantly higher and average  $\delta^{13}\text{C}$  and leaf N percentage were significantly lower on plants inhabiting invaded vs. uninvaded subplots (Table 1, Fig. 1). Interestingly, spotted knapweed had substantial impacts on *M. fistulosa* leaf traits regardless of the functional richness (hence the number of spotted knapweed) in each assemblage (i.e., there was no significant FR × spotted knapweed interaction; Table 1). In other words, the difference in trait values between the control and the spotted knapweed-invaded subplots was similar across the FR gradient, despite the fact that spotted knapweed abundance was two to nine times greater in assemblages with lower vs. higher functional richness.

*M. fistulosa*  $\delta^{13}\text{C}$ , leaf nitrogen, and C:N ratios were not affected by FR (Table 1), whereas LDMC (Fig. 1) and SLA were significantly affected by FR (Table 1). Water addition resulted in higher leaf C:N and lower leaf N and  $\delta^{13}\text{C}$  (Table 1, Fig. 1). The impact of spotted knapweed or FR on leaf traits never changed in water addition plots compared to those that received ambient water (i.e., the spotted knapweed × water and FR × water interactions were not significant; Table 1).

### *Koeleria macrantha*

Eighty-six percent of the total variation in response variables was accounted for by three factor components. Factor 1 (accounting for 40% of the variation) was composed of average leaf N and average C:N (loading factors = 0.96 and -0.96, respectively). Factor 2 (accounting for 23% of the variation) was composed of average SLA with a loading factor of 0.93. Average  $\delta^{13}\text{C}$  also loaded equally across the first two factors. Factor 3

(accounting for 23% of the variation) was loaded with average LDMC at 0.97.

The MANCOVA using these three factors as independent variables and FR, spotted knapweed, and water addition as dependent variables was highly significant ( $F_{2,94} = 3.1$ ,  $P < 0.0001$ ; Appendix 1), however effects of spotted knapweed were not significant when tested with separate ANCOVAs on individual leaf traits (Table 1, Fig. 2), nor was the spotted knapweed  $\times$  FR interaction significant (Table 1). Native FR strongly affected *Koeleria macrantha* traits related to nutrient concentration,  $\delta^{13}\text{C}$ , and SLA. As the FR increased, *K. macrantha* foliar N significantly decreased and C:N increased, and *K. macrantha*  $\delta^{13}\text{C}$  became significantly more negative (Table 1, Fig. 2). SLA declined significantly as native species richness increased (Table 1), but LDMC was unaffected by native functional diversity (Table 1, Fig. 2). Water availability significantly reduced LDMC (Fig. 2), and there were no significant FR  $\times$  water addition or spotted knapweed  $\times$  water addition interactions (Table 1).

#### DISCUSSION

Our experiment produced several important results. First, spotted knapweed had strong impacts on the leaf physiology of the native forb *Monarda fistulosa*. This is one of the few studies to show that an invader can impact the physiology of a co-occurring native species. Second, the impact of spotted knapweed on *M. fistulosa* was not ameliorated in assemblages of increasing functional richness, nor did this impact change with the addition of water. These results are counter to our original predictions that invader impact would scale positively with invader abundance, as suggested by Parker et al. (1999) and Dukes (2004), and that water would increase the negative impact of spotted knapweed. Finally, contrary to the findings with the native forb *M. fistulosa*, the effects of spotted knapweed on the native grass *Koeleria macrantha* were relatively weak, especially relative to the effects of native plant diversity and experimental water additions.

Despite the fact that spotted knapweed abundance decreased significantly across assemblages with increasing functional richness (Maron and Marler 2007), spotted knapweed still had large impacts on *M. fistulosa* leaf traits, even in relatively invasion-resistant, high-diversity plots. Why might this be so? One possibility is that a few spotted knapweed plants in high-diversity plots may elicit the same physiologic responses in *M. fistulosa* as 50 or 125 spotted knapweed in the low-diversity plots because the ratio of *Monarda*:spotted knapweed across the functional richness (FR) gradient was relatively similar (approximately one *Monarda* for every three to four spotted knapweed; J. Maron and M. Marler, unpublished data). The density of both *Monarda* and spotted knapweed was highest in the low-diversity plots and steadily decreased with increasing functional

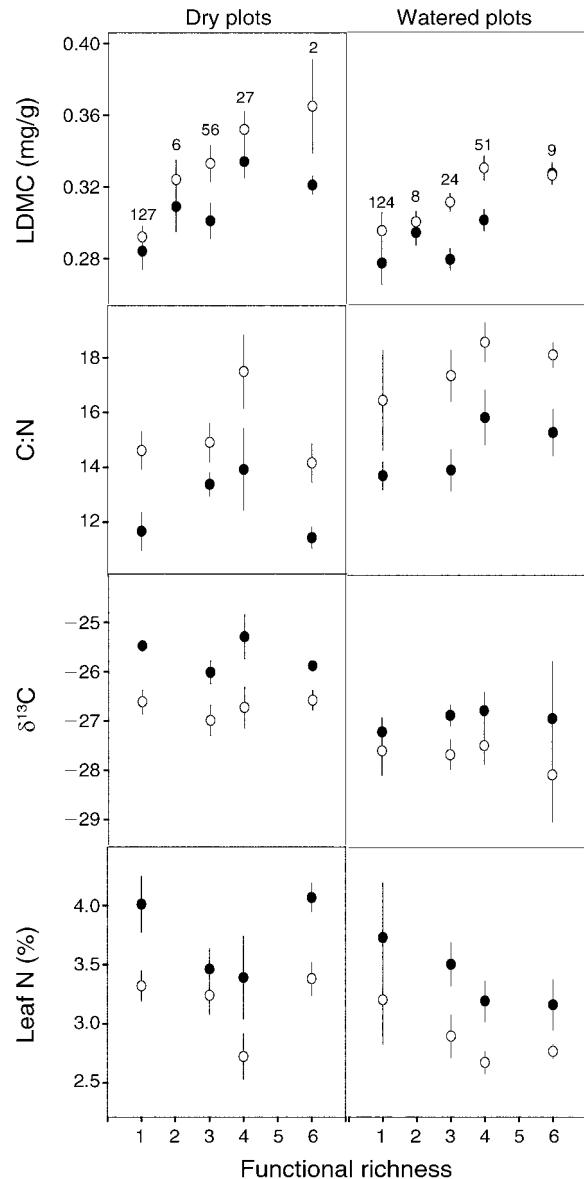


FIG. 1. Effect of spotted knapweed (*Centaurea maculosa*) and native functional richness on leaf dry matter content (LDMC), C:N values,  $\delta^{13}\text{C}$ , and leaf N values of bee balm (*Monarda fistulosa*) under ambient rainfall (dry plots) and supplemental watering (data are shown as means  $\pm$  SE, pooled for 2005 and 2006). Values above the points are the average number of flowering spotted knapweed plants in invaded plots in 2006. Solid circles are the uninvaded control, while open circles denote spotted knapweed-invaded subplots. The numbers of knapweed plants given in the upper panels apply to all panels. Sixteen naturally co-occurring grassland species were classified into six functional groups based on phenology and rooting architecture. Assemblages were planted to yield a functional richness of 1, 2, 3, 4, or 6 (see *Methods: Experimental design*).

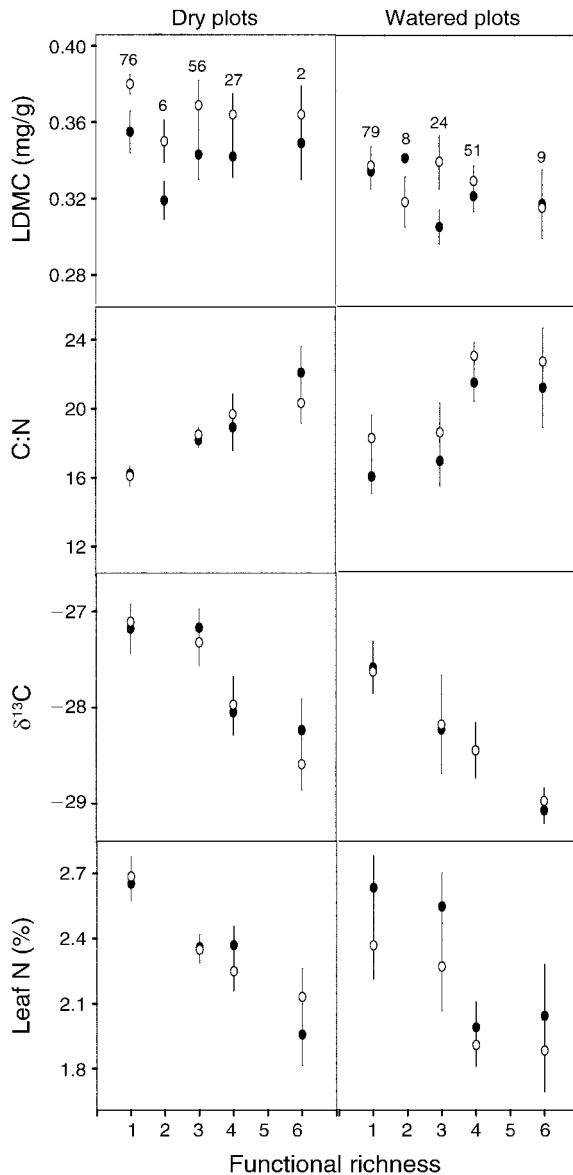


FIG. 2. Effect of spotted knapweed and native functional richness on leaf dry matter content (LDMC), C:N,  $\delta^{13}\text{C}$ , and leaf N values of Junegrass (*Koeleria macrantha*) (data are shown as means  $\pm$  SE, pooled for 2005 and 2006). Values above the points are the average number of flowering spotted knapweed stems in invaded plots in 2006. Solid circles are the uninverted control, while open circles denote spotted knapweed-invaded subplots.

diversity of the assemblage, leaving the ratio of relative abundance of the two interacting species similar.

*M. fistulosa* leaf dry matter and C:N increased, and leaf percent N and  $\delta^{13}\text{C}$  (water use efficiency [WUE]) decreased in response to the presence of spotted knapweed. This plastic response to spotted knapweed may represent a strategy for growing in poorer nutrient environments and is typical of plants conserving resources in stressful conditions (Grime et al. 1997,

Garnier et al. 2001a). The spotted knapweed-associated decline in *M. fistulosa*  $\delta^{13}\text{C}$  may have been caused by lower photosynthetic capacity due to the lower leaf N concentrations. However, our experiment was not designed to determine if stomatal conductance was higher, which could also cause declines in  $\delta^{13}\text{C}$ . It seems reasonable to assume that the effect of spotted knapweed on *M. fistulosa* leaf traits was likely mediated through an alteration of soil resource levels, since we found that plant-available nitrogen and soil moisture levels were significantly reduced in invaded vs. uninvaded assemblages; in contrast, light levels were the same across uninvaded and invaded subplots (J. Maron and M. Marler, unpublished data). Other studies have also shown that exotics can have important effects on resource availability experienced by co-occurring native plants (Dunbar and Facelli 1999, Dyer and Rice 1999, Hamilton et al. 1999). Alternatively, allelopathic compounds exuded by spotted knapweed could affect the physiology of natives in the field, as has been demonstrated in greenhouse experiments (Callaway and Aschehoug 2000, Bais et al. 2003, Callaway et al. 2005).

In contrast to its strong and specific effects on *M. fistulosa*, we only detected effects of spotted knapweed on *K. macrantha* leaf traits when all traits were tested together. Since statistically significant effects of spotted knapweed disappeared when traits were analyzed separately, it appears that spotted knapweed has rather diffuse effects on specific *K. macrantha* leaf traits. These effects are congruent with our prediction that spotted knapweed would have a bigger impact on the forb relative to the grass. The marginal impact of spotted knapweed on *K. macrantha* traits related to nitrogen and water use may be due to a lack of functional overlap between the native grass and the invader. *K. macrantha* actively grows and accumulates most of its biomass in the spring and early in the summer, at a time when spotted knapweed is still a small rosette and has yet to bolt and flower. Spotted knapweed also extracts water from deeper in the soil relative to native grasses, and it photosynthesizes from midsummer until late fall (Hill et al. 2006). Due to offset phenologies and rooting depth, *K. macrantha* and spotted knapweed probably compete less than do spotted knapweed and *M. fistulosa*. Both spotted knapweed and *M. fistulosa* are active mid-to late-season forbs. Even though separate traits of *K. macrantha* were not affected early in the invasion by spotted knapweed, it is plausible that spotted knapweed could reduce absolute levels of soil resources over successive seasons, thereby exerting stronger specific effects on *K. macrantha* over time.

The experimental addition of water, a limiting resource in the dry grasslands of the intermountain west (Sheley et al. 1999), had substantial effects on some leaf traits of both native species, but not on others. On average, *K. macrantha* LDMC and  $\delta^{13}\text{C}$  (WUE) declined in watered plots, and *M. fistulosa* had lower  $\delta^{13}\text{C}$  (WUE) and higher leaf C:N in wet vs. dry plots

(Figs. 1, 2). For water-limited species,  $\delta^{13}\text{C}$  (WUE) may decrease in watered plots because the plants may not regulate water as conservatively when soil moisture is greater. Plants in watered plots may keep their stomata open longer relative to plants in dry plots. For the grasses, LDMC may decline in watered plots because leaf architecture may be maintained via turgor pressure rather than relying more on structural carbohydrates. Contrary to initial predictions, impacts of spotted knapweed (or FR) on leaf traits did not change across the water treatment.

Experimentally imposed variation in functional richness influenced some native plant physiological traits but not others. As functional diversity increased, *M. fistulosa* LDMC increased (Fig. 1) and SLA decreased, while correlated traits related to nutrient concentration and water use efficiency remained relatively stable (Table 1). Since leaf N was positively correlated with  $\delta^{13}\text{C}$ , in order for  $\delta^{13}\text{C}$  to remain relatively constant at higher levels of FR, it is likely that *M. fistulosa* closed their stomata during the day. As functional richness increased, plants shifted toward more conservative leaf construction (i.e., higher LDMC and reduced SLA). In contrast to *M. fistulosa*, the effect of FR on *K. macrantha* nutrient concentrations,  $\delta^{13}\text{C}$ , and SLA was much greater than the effect of spotted knapweed (Table 1). Leaf percent N,  $\delta^{13}\text{C}$ , and SLA were strongly depressed while C:N increased with increasing native FR, but LDMC was not affected at all by native functional richness. The negative relationship between *K. macrantha*  $\delta^{13}\text{C}$  and FR could be a result of the lower leaf nitrogen. Lower leaf N could lead to reduction in photosynthetic capacity and may be why  $\delta^{13}\text{C}$  is strongly depressed, but higher stomatal conductance can also partly explain depressed  $\delta^{13}\text{C}$  in higher FR plots. In a removal experiment, Suding et al. (2004) found that diffuse knapweed, *Centaurea diffusa*, had no impact on the performance of target natives; instead, neighboring competitors had a stronger effect on the biomass of the target bunchgrasses. In our experiment the leaf traits of this shallow, fibrous rooted grass were more strongly affected by native richness.

If the presence of spotted knapweed in assemblages was equivalent to adding another native functional group to an assemblage, then we would expect these two factors to have relatively similar effects on the leaf traits of natives. However, this was not the case. Spotted knapweed impacts on *M. fistulosa* traits were of greater magnitude than were the effects of increasing native FR by one. This suggests that spotted knapweed has unique effects on *M. fistulosa* that are not simply tied to the fact that spotted knapweed-invaded assemblages contain an added functional type.

Our results show that in the presence of spotted knapweed, or with increased native FR, *M. fistulosa* and *K. macrantha* shifted leaf traits associated with a strategy that may include slower accumulation of biomass and efficient conservation of resources (Poorter and Garnier

1999). Lower leaf N,  $\delta^{13}\text{C}$  (WUE), and higher investments in LDMC and C:N may lead to smaller plants (Grime et al. 1997). Individuals may also retain leaves longer. Plastic responses to spotted knapweed or native FR may represent a trade-off for perennial species; plants with a "persistence" strategy (higher LDMC, lower individual biomass) may sacrifice biomass accumulation for prolonged reproductive bouts and a longer life (McGinley et al. 1987, Grime et al. 1997). Whether effects of spotted knapweed on functional attributes of *M. fistulosa* leaf traits ultimately compromise whole-plant biomass and abundance remains to be seen. Although our study was not designed to examine how invader impact on native physiological traits affects native demographics, we will quantify the impact of spotted knapweed on native biomass, which should shed light on how average physiological response maps to biomass.

This study represents the only test, to our knowledge, of how native FR, water addition, and a potent exotic individually and interactively influence the leaf physiology of native co-occurring plants. Our findings suggest that native diversity and invasibility do not scale identically to native diversity and invader impact. While spotted knapweed numbers and biomass can be negatively affected by different components of native diversity (Pokorny et al. 2005, Maron and Marler 2007), the impact of this invader on native physiological traits is similar across the diversity gradient. Furthermore, the overall impact of spotted knapweed on natives can vary with the identity and functional attributes of those natives, with variation in invader impact likely due to the extent to which particular natives overlap phenologically with an invader. These findings point to the importance of quantifying both the overall impact of exotics on natives, as well as quantifying how variation in native functional type, resource availability, and community composition may mediate these impacts.

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#### APPENDIX A

Composition of species richness and functional richness used in experimental assemblages (*Ecological Archives* E089-081-A1).

#### APPENDIX B

MANCOVAs for (A) *Monarda fistulosa* and (B) *Koeleria macrantha* on the three leaf trait factors (*Ecological Archives* E089-081-A2).