



Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology

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Abstract

Invasive exotic weeds pose one of the earth's most pressing environmental problems. Although many invaders completely eliminate native plant species from some communities, ecologists know little about the mechanisms by which these exotics competitively exclude other species. Mycorrhizal fungi radically alter competitive interactions between plants within natural communities, and a recent study has shown that arbuscular mycorrhizal (AM) fungi provide a substantial competitive advantage to spotted knapweed, *Centaurea maculosa*, a noxious perennial plant that has spread throughout much of the native prairie in the northwestern U.S. Here we present evidence that this advantage is potentially due to mycorrhizally mediated transfer of carbon from a native bunchgrass, *Festuca idahoensis*, to *Centaurea*. *Centaurea maculosa*, *Festuca idahoensis* (Idaho fescue, C₃), and *Bouteloua gracilis* (blue gramma, C₄) were grown in the greenhouse either alone or with *Centaurea* in an incomplete factorial design with and without AM fungi. *Centaurea* biomass was 87–168% greater in all treatments when mycorrhizae were present in the soil ($P < 0.0001$). However, *Centaurea* biomass was significantly higher in the treatment with both mycorrhizae and *Festuca* present together than in any other treatment combination ($P < 0.0001$). This high biomass was attained even though *Centaurea* photosynthetic rates were 14% lower when grown with *Festuca* and mycorrhizae together than when grown with *Festuca* without mycorrhizae. Neither biomass nor photosynthetic rates of *Centaurea* were affected by competition with the C₄ grass *Bouteloua* either with or without mycorrhizae. The stable isotope signature of *Centaurea* leaves grown with *Festuca* and mycorrhizae was more similar to that of *Festuca*, than when *Centaurea* was grown alone with mycorrhizae ($P = 0.06$), or with *Festuca* but without mycorrhizae ($P = 0.09$). This suggests that carbon was transferred from *Festuca* to the invasive weed. We estimated that carbon transferred from *Festuca* by mycorrhizae contributed up to 15% of the aboveground carbon in *Centaurea* plants. Our results indicate that carbon parasitism via AM soil fungi may be an important mechanism by which invasive plants out compete their neighbors, but that this interaction is highly species-specific.

Introduction

Mycorrhizal can modify or even reverse competitive interactions among plant species (Grime et al. 1987; Hetrick et al. 1989; Hartnett et al. 1993; Moora and Zobel 1996). The effects of mycorrhizae on the balance of competition is often attributed to direct and disproportional enhancement of species that rely on

mycorrhizal mutualisms, thereby improving their competitive ability against non-mycorrhizal species or those that have weak or facultative mutualisms (Caldwell et al. 1985; Allen and Allen 1990; Hartnett et al. 1993). Mycorrhizae may also alter competition by transferring nutrients and photosynthetically fixed carbon among plants (Chiariello et al. 1982; Grime et al. 1987; Hetrick et al. 1990; Francis and Read 1994;

Walter et al. 1996), but this process remains controversial (Robinson and Fitter 1999).

Little is known about the role of mycorrhizae in the intense competition that occurs among exotic, invasive weeds and native plants. Goodwin (1992) speculated that invasive species would be at a competitive disadvantage against natives if they required specific fungal mutualists to maximize their fitness. However, Marler et al. (1999a) demonstrated that arbuscular mycorrhizal (AM) fungi strongly enhanced the competitive effect of the noxious exotic, *Centaurea maculosa*, (spotted knapweed) on *Festuca idahoensis* (Idaho fescue), a bunchgrass common throughout the northwestern United States. They demonstrated that the direct positive effects of AM fungi on both *Centaurea* and *Festuca* were much weaker than the indirect negative effect of *Centaurea* on *Festuca* in the presence of AM fungi, and suggested that mycorrhizal transfer of carbon or resources would explain these results. Net transfer of carbon has been shown for ectomycorrhizae using stable and radioactive isotopes (Simard et al. 1997), but net transfer of carbon among plants by AM fungi remains a topic of debate (Robinson and Fitter 1999). Grime et al. (1987) established microcosms in which AM fungi were either present or eliminated and found that the growth of many competitive subordinates was many times higher in the presence of AM fungi. Simultaneously, the growth of *Festuca ovina*, the competitive dominant, was reduced. When a radioactive ^{14}C label was applied to *F. ovina*, ^{14}C was later detected in the shoots of the same subordinate species for which large increases in growth were detected, but only in microcosms with AM fungi.

Despite the demonstration of reciprocal increases and decreases in growth of receiver and donor species and the corresponding movement of ^{14}C to the shoots of the subordinate species, Bergelson and Crawly (1988) and Watkins et al. (1996), Robinson and Fitter (1999) have argued that the detection of the isotope in the receiver plant does not necessarily indicate net transfer since there may have been balancing movement to the donor, and that the ^{14}C experiments do not indicate the direction of movement. Furthermore, Grime et al. (1987) did not manipulate the presence and absence of the donor species. The debate has been fueled by reports that AM fungal connections only produce increases in either radioactive or stable (^{13}C) carbon labels in roots not in shoots, suggesting that the label may remain in the fungal tissue rather than being transferred to the plant

tissue (Waters and Borowicz 1995; Watkins et al. 1996; Graves et al. 1997; Fitter et al. 1998). Regardless of the direction of transfer, AM fungi have the potential to significantly alter competitive interactions within plant communities by providing a conduit for the transfer of carbon and nutrients from one plant to another. Unidirectional transfer of carbon via mycorrhizal connections would constitute parasitism and could promote species richness by permitting understory components or individuals in resource-poor microsites to persist (Grime et al. 1987; Simard et al. 1997). Alternatively these interactions could decrease species richness if they enhance the competitive ability of dominant species.

A limitation of many previous experiments is the use of experimental pulses of radioactive or stable isotope labels to detect carbon transfer, a process performed over very short time periods (hours to a few days). Radioactive isotopes decay and the application of labels must be performed in enclosed chambers that alter ambient CO_2 concentrations and leaf-to-air vapor gradients potentially confounding results. Therefore, the short application-to-harvest period of labels provides only a snapshot of the long-term interactions between the plants and does not provide enough time for large amounts of carbon to move through fungi or for carbon to move from fungal tissues into plants. Moreover, if labeled CO_2 is leaked from chambers or if chambers are left in place for longer periods of time, leaked label or labeled CO_2 that is fixed and then respired from the soil may be refixed by unenclosed vegetation leading to ambiguous results (Robinson and Fitter 1999). To avoid these potential problems others have tracked carbon transfer between plants by growing species that naturally differ in $^{12}\text{C}:^{13}\text{C}$ isotope ratios as a function of different photosynthetic pathways (Press et al. 1987; Watkins et al. 1996), hypothesizing that if mycorrhizae transfer carbon among plants, fungal connections among plants that differ in stable isotopic ratios would lead to an alteration of the isotopic ratios of the species receiving carbon. Plants using the C_3 and C_4 photosynthetic pathways differ greatly in their $^{12}\text{C}:^{13}\text{C}$ ratios reflecting differences in discrimination against the heavier isotope of carbon by their respective carboxylating enzymes ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPCase). As a result of greater discrimination by Rubisco C_3 plants have a signature that is more depleted in ^{13}C , as compared to the atmosphere, than the signature of C_4 plants; the

isotopic ratios of C₃ and C₄ species typically range between -20 and -35‰ and -7 and -15‰, respectively (Ehleringer and Osmond 1991). Furthermore, within C₃ plants individual species can be distinguished by their isotopic signatures, which reflect differences in time integrated internal CO₂ concentration and water-use efficiency. In this study we measured biomass, photosynthetic gas exchange, and ¹²C:¹³C ratios in the shoots of *Festuca idahoensis* (a C₃ species with high water-use efficiency), *Bouteloua gracilis* (a C₄ species), and *Centaurea maculosa* (a C₃ species with low water-use-efficiency) as these species interacted with and without AM soil fungi.

Methods

Members of the genus *Centaurea* are among the most economically destructive exotic plants in North America (Watson and Renney 1974; Griffith and Lucey 1991). *Centaurea maculosa* was introduced into North America from Eurasia early in the 20th century, and has invaded millions of hectares of natural Palouse prairie, decreasing the abundance and productivity of native species and reducing local plant diversity. *Centaurea* invasion is often characterized by the complete competitive exclusion of native plants and the development of dense monospecific patches. *Festuca idahoensis*, a native North American C₃ bunchgrass, is one of the dominant native plants in the Palouse and intermountain prairie of the north-west US. *Bouteloua gracilis* (blue gramma), a native North American C₄ bunchgrass, is a dominant species in mid- and short-grass prairie. All three plant species are typically well colonized by AM fungi (Allen et al. 1981; Hays et al. 1982; Marler et al. 1999a, 1999b).

Plants were grown from seed from June through October in a greenhouse at the University of Montana under natural light and temperatures between 18–30 °C. Seeds of *Festuca idahoensis*, *Bouteloua gracilis*, and *Centaurea maculosa* were grown with or without AM fungi and with or without *Centaurea* competition for a total of 12 replicates per 10 treatments in an incomplete factorial arrangement of treatments within a randomized complete block design. The two treatments not included in the design were *Centaurea* grown in competition with itself, either with or without AM fungi. At the start of the experiment, seeds of the two grass species were sown into 7.5 L pots containing 20/30 grit silica sand. To provide mycorrhizal

inoculum, a 2-cm layer of field soil mixed with sand in the ratio 1:1 by volume was added two cm beneath the surface (Hartnett et al. 1993; Klironomos 2002). After germination, plants were thinned to one grass seedling per pot. The native grasses were given a head start because they grow more slowly than knapweed, knapweed invades already established grassland, and prior experiments (Marler et al. 1999a) indicated that knapweed benefited from parasitizing larger fescues. If the grasses were not established previously they would have been very small as compared to knapweed and therefore there would not have been as much carbon to parasitize. Nine weeks after the grasses were planted one half of the pots were treated with the fungicide Bonomyl (Dimethyl 4, 4'-o-phenylenebis, Bonide Products Inc., Yorkville, NY, USA), 185 mg per pot in 200 mL water, to establish the non-mycorrhizal treatments (Hetrick et al. 1989; Marler et al. 1999a). Following fungicide treatment *Centaurea* was seeded into the pots containing nine-week-old grasses and into pots by itself. One week after germination, *Centaurea* were thinned to one seedling per pot. Non-mycorrhizal pots were treated at three-week intervals with Bonomyl after planting *Centaurea*. Plants were watered daily and fertilized bi-weekly with 1/8 strength Hoagland's solution modified by the addition of phosphorus as inositol hexaphosphate. This form is not directly available to plants for uptake, and requires alteration in the soil by phosphatases produced by mycorrhizal fungi, soil microbes, or as root exudates (DeLucia et al. 1997). The final concentration of phosphorus in solution was 1.93 µg/ml, which is comparable to levels used by other researchers to investigate effects of mycorrhizal colonization in otherwise sterile substrates (Koide and Li 1990).

When the *Centaurea* plants were 6 weeks old and the grasses were 15 weeks old, we measured photosynthetic gas exchange on the most recent fully-developed foliage of each plant with a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). Measurements were made over the course of four days between the hours of 1100 and 1400. Plants were placed under halogen lamps at 900–1000 µmol m⁻² s⁻¹ PPFD for one half hour prior to measurement at 1000 µmol m⁻² s⁻¹ PPFD and 20 °C. Leaves were removed and the area of foliage used for gas exchange measurements was measured with a video camera and Mocha image analysis software (Jandel Scientific, Chicago, IL, USA). Photosynthetic rate, transpiration rate, and stomatal conductance

Table 1. Percent mycorrhizal colonization of *B. gracilis*, *F. idahoensis*, and *C. maculosa*, grown alone or with neighbors and with or without fungicide treatment to eliminate arbuscular mycorrhizal (AM) fungi.

Species	Mycorrhizal Colonization (%) ¹			
	+Fungicide + <i>Centaurea</i>	-Fungicide	+Fungicide - <i>Centaurea</i>	-Fungicide
<i>Bouteloua gracilis</i>	0.80a	12.50b	0.94a	19.33c
<i>Festuca idahoensis</i>	3.51a	19.90b	2.00a	13.88b
<i>Centaurea maculosa</i>	–	–	2.64a	32.63b
+ <i>Bouteloua</i>		+ <i>Festuca</i>		
<i>Centaurea maculosa</i>	8.47a	51.66b	4.86a	26.64c

¹ Within a row, letters indicate differences between means ($P < 0.05$, $n = 6$ plants per treatment).

were expressed per unit leaf area. Instantaneous water-use efficiency was calculated as μmols of CO_2 assimilated per mol of water transpired. The leaves used for gas-exchange measurements were dried and finely ground, and a subsample from plants of each treatment was analyzed for stable carbon isotope composition. Samples were analyzed at the Stable Isotope Laboratory of the Institute of Ecology (University of Georgia, Athens, GA, USA). Carbon isotope ratio was expressed relative to Pee Dee Belemnite.

Seven weeks after *Centaurea* was planted all plants were harvested. Biomass was separated by roots and shoots, dried at 60°C for 48 hours, and weighed. Roots from six plants per treatment were cleared with 40% KOH for 48 hours, rinsed in 3% HCl for 24 hours, and stained with trypan blue before scoring for AM fungal infection according to McGonigle et al. (1990).

Because of the incomplete nature of the design, statistical analyses were performed by species. The effects of neighboring species and mycorrhizae on *Centaurea* growth and physiology were analyzed with a two-way analysis of variance with interaction. Effects of competition and mycorrhizae on *Festuca* and *Bouteloua* were also analyzed as a two-factor analysis with interaction. All data were analyzed using the general linear model procedure of SAS (Version 6.12, SAS Institute 1996). Error associated with blocking was significant, and therefore retained, only for analyses of physiology measurements.

Results

Bonomyl application reduced AM fungi in the roots of all species in the non-mycorrhizal treatments (Table 1). Bonomyl does not kill AM fungi selectively but is a systemic fungicide that eliminates or reduces all fungi in the soil.

The three species differed with respect to total mass ($P < 0.001$). *B. gracilis* had greater biomass than *F. idahoensis*, and both grasses were larger than the younger *Centaurea* plants (overall means by species: *Bouteloua* = 0.84 g, *Centaurea* = 0.11 g, *Festuca* = 0.25 g). No combination of *Centaurea* and AM fungi had a significant effect on the growth of the older *B. gracilis* or *F. idahoensis* plants. However, AM fungi improved *Centaurea* growth by an average of 125% across all treatments ($P < 0.001$, Figure 1, Table 2). Mycorrhizae enhanced *Centaurea* growth by 106% and 86% when grown alone and with *Bouteloua*, respectively. In contrast, *Centaurea* plants grown with *Festuca* and AM fungi together were 168% larger than *Centaurea* plants grown with *Festuca* and without AM fungi, and they were 75% larger than *Centaurea* plants grown alone with AM fungi. The combined effect of mycorrhizae and *Festuca* competition on *Centaurea* growth was much greater than the effect of AM fungi alone that was seen in the other treatment pairs ($P < 0.05$, Figure 1, Table 2). Both shoot mass and root mass responded to the treatment combinations in the same way as total mass and the results are not shown.

AM fungal colonization improved *Bouteloua* conductance, transpiration and photosynthetic rates ($P < 0.05$, Table 3). In contrast, conductance and transpiration of *Festuca* and *Centaurea* were not affected by

Table 2. Analysis of variance examining the effect of neighboring species (*Bouteloua gracilis* or *Festuca idahoensis*) and presence of arbuscular mycorrhizal (AM) fungi on total biomass of *Centaurea maculosa*.

Source of Variation	df	Mean Square	F	P
Species combination ¹	2	0.02388	8.75	0.004
AM fungi	1	0.11749	43.03	0.001
Species*AM fungi	2	0.01076	3.94	0.0233
Error	66	0.00273		

¹*Centaurea* with *Bouteloua*, with *Festuca*, or alone.

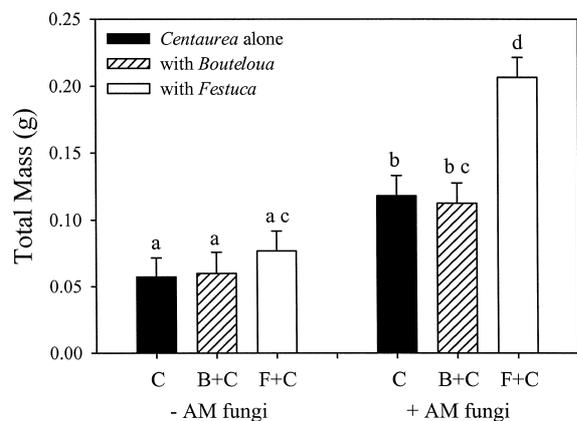


Figure 1. Total plant mass of *Centaurea maculosa* grown in competition with either *Bouteloua gracilis* or *Festuca idahoensis* and with or without arbuscular-mycorrhizal (AM) fungi. Different letters indicate differences between means ($P < 0.05$). Each bar is the mean (± 1 SE) of 12 plants.

mycorrhizae. *Festuca* photosynthetic rates were the same for both levels of AM fungi infection, but *Centaurea* photosynthetic rates were higher in Bonymyl treated plants (Figure 2A, Tables 3 and 4). Photosynthetic rates of *Centaurea* were lowest in the two treatments in which it was grown with a neighbor and AM fungi together (Figure 2B, Table 3). The only significant difference between mycorrhizal and non-mycorrhizal treatment pairs was seen in *Centaurea* grown with *Festuca*. *Centaurea* grown with *Festuca* and AM fungi had the second lowest rates of photosynthesis per unit leaf area (Figure 2B), but attained the greatest biomass (Figure 1); in this treatment, *Centaurea* photosynthetic rates were lower than in the *Centaurea* alone plus mycorrhizae treatment (13.95 ± 0.84 , SE vs 15.08 ± 0.84 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively), and significantly lower than *Centaurea* grown with *Festuca* but without mycorrhizae (13.95 ± 0.84 vs 16.29 ± 0.84 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $P < 0.05$). There was no effect of AM fungi on water-use efficiency in any species. Competition with *Bouteloua* decreased water-use ef-

iciency of *Centaurea* (2.10 ± 0.11 , SE $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ with neighbor versus 2.43 ± 0.11 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$ without neighbor, $P < 0.05$). There were no other effects of competition on leaf-level gas exchange for any species (Table 3). Instantaneous water-use efficiency was greatest in *Bouteloua* (8.4 ± 0.2 , SE $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$), followed by *Festuca* (4.8 ± 0.2 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$) and *Centaurea* (2.3 ± 0.2 $\mu\text{mol CO}_2/\text{mol H}_2\text{O}$).

Stable carbon isotope composition of *Bouteloua*, *Centaurea*, and *Festuca* differed as expected and reflected the photosynthetic pathways and water-use efficiencies of the three species (Figure 3, $P < 0.0001$). *Bouteloua* had an isotopic signature that is typical for a C_4 grass, and *Centaurea* had lower stable isotope contents than the more water-use efficient *Festuca* (overall means by species: *Bouteloua* = -14.61‰ , *Centaurea* = -31.61‰ , *Festuca* = -28.87‰). Neither AM fungi nor *Centaurea* neighbors affected stable carbon isotope discrimination of either *Festuca* or *Bouteloua* (data not shown). Therefore, stable carbon isotope composition was averaged across treatments for *Festuca* and *Bouteloua* (Figure 3). Stable isotope composition of *Centaurea* foliage was significantly different in treatments with AM fungi and grass neighbors compared with *Centaurea* grown alone without AM fungi. However, the direction of the response differed when *Centaurea* was grown with *Festuca* versus with *Bouteloua* or by itself, resulting in a species by mycorrhizae interaction (Table 5). When *Centaurea* plants were grown with AM fungi, either alone or with a *Bouteloua* neighbor, their stable carbon isotope content decreased in comparison to non-mycorrhizal treatments (Figure 3, $P < 0.05$). In contrast, sharing soil with a *Festuca* neighbor and AM fungi had the opposite effect, i.e., stable carbon isotope content of *Centaurea* increased in comparison to the *Festuca* minus AM fungi treatments and became more similar to the signature of *Festuca* (Figure 3). The isotopic signature of *Centaurea* grown with *Festuca* and AM fungi (-31.31‰) was greater than that of *Centaurea* grown alone with AM fungi (-31.73‰ , $P < 0.06$) or *Centaurea* grown with *Festuca* but without AM fungi (-31.78‰ , $P < 0.09$). The stable carbon isotope composition of *Festuca*, *Centaurea* grown with *Festuca*, and *Centaurea* grown alone, all in mycorrhizal treatments, was -29.01‰ , -31.31‰ , and -31.73‰ , respectively. There were no concomitant effects on instantaneous water-use efficiency in either *Festuca* or *Centaurea* suggesting that increased ^{13}C content is from carbon transfer from *Festuca* to

Table 3. Mean values of conductance, photosynthesis, transpiration and water-use efficiency for all treatments. Standard errors are given in parentheses. Different letters within a species for a given physiological measurement indicate significant differences among means ($P < 0.05$).

Species	Stomatal conductance mol H ₂ O m ⁻² s ⁻¹		Photosynthesis μmol CO ₂ m ⁻² s ⁻¹		Transpiration mol H ₂ O m ⁻² s ⁻¹		Water-use efficiency μmol CO ₂ /mol H ₂ O	
	+AM	-AM	+AM	-AM	+AM	-AM	+AM	-AM
	<i>Bouteloua gracilis</i>							
grown alone	0.09 (.005) ^a	0.07 (.005) ^b	13.37 (.73) ^a	10.48 (.73) ^b	1.59 (.08) ^a	1.22 (.08) ^b	8.44 (.30) ^a	8.61 (.30) ^a
grown with <i>Centaurea</i>	0.09 (.005) ^a	0.07 (.005) ^b	11.73 (.77) ^{ab}	10.90 (.77) ^b	1.51 (.09) ^a	1.24 (.09) ^b	7.97 (.31) ^a	8.76 (.31) ^a
<i>Centaurea maculosa</i>								
grown alone	0.51 (.05) ^a	0.48 (.05) ^a	15.08 (.87) ^{ab}	15.70 (.84) ^{ab}	6.48 (.40) ^a	6.65 (.41) ^a	2.44 (.13) ^a	2.43 (.14) ^a
grown with <i>Bouteloua</i>	0.50 (.05) ^a	0.57 (.05) ^a	13.51 (.87) ^a	15.05 (.84) ^{ab}	6.52 (.40) ^a	7.27 (.41) ^a	2.10 (.13) ^a	2.09 (.14) ^a
grown with <i>Festuca</i>	0.54 (.05) ^a	0.53 (.05) ^a	13.95 (.84) ^a	16.29 (.84) ^b	6.32 (.40) ^a	7.00 (.40) ^a	2.21 (.13) ^a	2.34 (.13) ^a
<i>Festuca idahoensis</i>								
grown alone	0.19 (.03) ^a	0.17 (.03) ^a	15.99 (2.1) ^a	9.90 (2.0) ^b	3.14 (.38) ^a	2.71 (.38) ^a	5.09 (.64) ^a	3.51 (.61) ^b
grown with <i>Centaurea</i>	0.21 (.03) ^a	0.23 (.03) ^a	15.74 (2.0) ^a	16.25 (2.0) ^a	3.29 (.38) ^a	3.60 (.38) ^a	5.71 (.61) ^a	4.88 (.61) ^{ab}

Table 4. Analysis of variance examining the effect of neighboring species (*Bouteloua gracilis* or *Festuca idahoensis*) and presence of arbuscular mycorrhizal (AM) fungi on photosynthetic rates of *Centaurea maculosa*.

Source of Variation	df	Mean Square	F	P
Species combination ¹	2	7.67	0.91	0.409
AM fungi	1	39.31	4.66	0.035
Species*AM fungi	2	4.36	0.52	0.599
Error	64	8.44		

¹*Centaurea* with *Bouteloua*, with *Festuca*, or alone.

Centaurea rather than from changes in integrated water-use efficiency as measured by ¹³C content.

Discussion

Our results suggest that AM fungi enhance the growth of the invasive weed, *Centaurea maculosa*, both directly as is typical of many mycorrhizal mutualisms (Allen and Allen 1990), and indirectly by providing a link for carbon transfer from neighboring *F. idahoensis* to *C. maculosa*. Carbon transfer is supported by the following evidence: 1) in the treatment with *Festuca* and AM fungi together *Centaurea* was significantly larger than in any other treatment (as also shown by Marler et al. (1999a)), 2) the carbon isotope ratio of *Centaurea* shoots was significantly more like that of *Festuca* only when AM fungi were present, and 3) the increase in *Centaurea* biomass corresponded with a significant decrease in photosynthetic rate of the same *Centaurea* plants. While any of these observations considered alone might not sug-

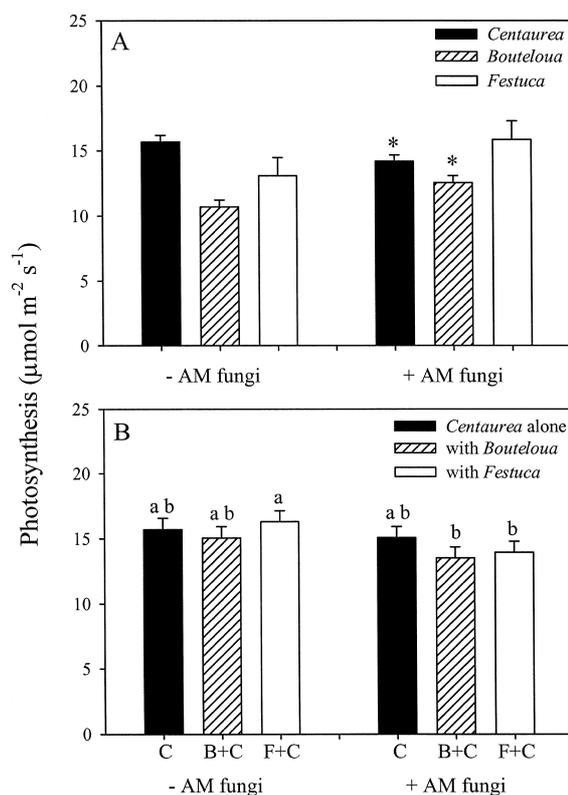


Figure 2. Photosynthetic rates of A) *Centaurea maculosa*, *Bouteloua gracilis*, and *Festuca idahoensis* grown with or without arbuscular-mycorrhizal (AM) fungi. Asterisks indicate significant differences between mycorrhizal treatments ($P < 0.05$). B) *Centaurea maculosa* grown in competition with either *Bouteloua gracilis* or *Festuca idahoensis* and with or without arbuscular-mycorrhizal (AM) fungi. Different letters indicate differences between means ($P < 0.05$). In A, each bar represents the mean (\pm 1 SE) of 24 plants for *Centaurea* and 48 plants for *Bouteloua* and *Festuca*. In B, each bar is the mean (\pm 1 SE) of 12 plants.

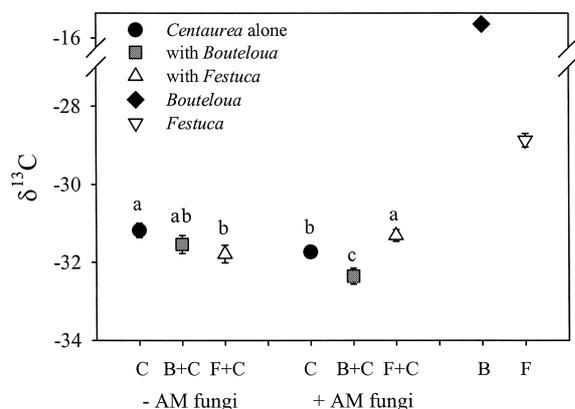


Figure 3. Stable carbon isotope composition of *Centaurea maculosa* grown in competition with either *Bouteloua gracilis* or *Festuca idahoensis* and with or without arbuscular-mycorrhizal (AM) fungi. Different letters indicate differences between means ($P < 0.10$). Values for *B. gracilis* and *F. idahoensis* are plotted for reference. Each symbol is the mean (\pm 1 SE) of between 4 and 9 plants.

Table 5. Analysis of variance examining the effect of neighboring species (*Bouteloua gracilis* or *Festuca idahoensis*) and presence of arbuscular mycorrhizal (AM) fungi on stable carbon isotope content of *Centaurea maculosa*.

Source of Variation	df	Mean Square	F	P
Species combination ¹	2	0.686	3.33	0.049
AM fungi	1	0.720	3.49	0.071
Species*AM fungi	2	1.230	5.97	0.006
Error	31	0.206		

¹ *Centaurea* with *Bouteloua*, with *Festuca*, or alone.

gest C transfer, when taken together they provide strong evidence in support of transfer.

Altered carbon isotope ratios in the shoots of *Centaurea* indicate that carbon was not simply shifted around belowground in the fungal tissue. In contrast to the *Festuca* plus *Centaurea* treatments, the stable carbon isotope composition for the mycorrhizal treatments of *Centaurea* with *Bouteloua* and *Centaurea* by itself shifted in the reverse direction. Our data suggest that carbon transfer was species-specific as *Bouteloua gracilis* did not enhance the growth of *Centaurea* in the presence of AM fungi, and the change in isotopic composition of *Centaurea* plants grown with *Bouteloua* and AM fungi, as compared to plants grown with *Bouteloua* but without AM fungi, was opposite the signature of the C_4 grass. Although these data suggest carbon transfer, differences in stable isotope content may simply reflect differences in water-use efficiency. There were no differences among the six *Centaurea* treatments with respect to

instantaneous water-use efficiency, measured when plants were well watered, however, variations in stable carbon isotope composition may be due to differences in long-term water-use efficiency. When *Centaurea* was grown either alone or with *Bouteloua*, AM fungi treatments had lower stable isotope compositions indicative of decreased long term water-use efficiency in these mycorrhizal treatments. In contrast, when grown with *Festuca*, ^{13}C data suggest increased water-use efficiency in the mycorrhizal treatment. We believe that carbon transfer better explains the large enhancement of growth of *Centaurea* grown with *Festuca* and AM fungi than does increased water-use efficiency.

Positive effects of AM fungi on plant growth have been reported in a wide variety of species, including those of western grasslands (Hetrick et al. 1989, 1990), and it has been suggested that mycorrhizae may enhance the competitive ability of native bunchgrasses and thus play a role in combating plant invasions (Goodwin 1992). However, our results indicate that mycorrhizae may have the opposite effect and ultimately facilitate the establishment of invasive weeds such as *Centaurea*. This is consistent with previous experiments with *Centaurea*, except that the direct effects of AM fungi on *Centaurea* were not significant in these prior works (Marler et al. 1999a).

Although the total biomass of *Centaurea* was greater in the presence of AM fungi and *Festuca*, there was no corresponding decrease in the biomass of *Festuca* in this treatment. This was probably due to fact that we provided the grasses with a nine-week head start before planting *Centaurea* seeds. Therefore, any differences in total biomass of the grasses that developed during the later half of the experiment arising in response to elimination of mycorrhizae or introduction of *Centaurea* were likely too small to detect. Similarly, because *Festuca* and *Bouteloua* plants were larger than *Centaurea*, bi-directional transport, if it occurred, may have been swamped by the large size of the grasses. In similar experiments in which *Centaurea* and *Festuca* plants were germinated at the same time, leading to smaller *Festuca* plants relative to *Centaurea*, Marler et al. (1999a) reported significant decreases in *Festuca* biomass in the presence of both *Centaurea* and AM fungi together in comparison to the presence of either alone.

The difference between the average isotopic signature of *Festuca* (28.87‰) and the signature of *Centaurea* when grown alone with mycorrhizae (31.73‰) was 2.86‰. The shift of 0.42‰ in the signature of

Centaurea foliage towards the signature of *Festuca* foliage in the *Festuca* + AM treatments (31.31%, Figure 3), accounts for approximately 15% of this difference and suggests that in this treatment *Centaurea* may have acquired up to 15% of its aboveground carbon from *Festuca*. This would explain why above and belowground biomass of *Centaurea* were greater in the *Festuca* plus AM fungi treatment than in the *Festuca* minus AM fungi treatment, even though photosynthetic rates were 14% lower in the mycorrhizal treatment.

Bouteloua photosynthetic rates were lower in non-mycorrhizal treatments, but *Festuca* rates were unaffected by AM fungi. Higher rates of photosynthesis and conductance, with no concomitant increases in growth, in response to mycorrhizal infection have been shown previously for *Bouteloua* (Allen et al. 1981; Allen and Allen 1984). Stable carbon isotope data suggest that *Centaurea* was less water-use efficient over the long term when grown with *Bouteloua* or in pots by itself with mycorrhizae (Figure 3); however, these trends were not reflected in the instantaneous water-use efficiency data (Table 3).

Our results indicate that transfer of carbon from the native *Festuca idahoensis* to the invasive weed *Centaurea maculosa* may be an important mechanism by which this exotic competitively excludes some of its new North American neighbors. However, evidence of carbon transfer was species-specific and did not operate in the interaction between *Centaurea* and *Bouteloua gracilis*. The current study and a previous study by Grime et al. (1987) that demonstrated transfer of ^{14}C among neighboring species only when AM fungi were present in the soil had two genera in common, *Festuca* and *Centaurea*. In the Grime et al. experiment, the carbon donor was *Festuca ovina* and one of the recipients was *Centaurea nigra*, raising the possibility that particular taxonomic groups may function differently than species chosen from communities at random. The lack of evidence in support of carbon transfer to aboveground organs in other studies (Fitter et al. 1998; Robinson and Fitter 1999) may be due to species choice. Although only certain families have been identified as having developed the potential to function as myco-heterotrophs, i.e., parasitic plants that acquire carbon from other plant species through fungal intermediaries (Leake 1994), our results suggest that we should broaden our view of myco-heterotrophy. Grime et al. (1987) argued that common mycorrhizal networks have the potential to maintain high levels of community diversity; but our

results suggest the opposite may also be true. In communities invaded by *Centaurea maculosa*, or similar weeds, common mycorrhizal networks may ultimately contribute to the development of exotic monocultures.

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