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SOIL FUNGI ALTER INTERACTIONS BETWEEN THE INVADER *CENTAUREA MACULOSA* AND NORTH AMERICAN NATIVES

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Abstract. Soil microbes may affect the way exotic invasive plants interact with native neighbors. We investigated the effects of soil fungi on interactions between the invasive weed *Centaurea maculosa* (spotted knapweed) and six species native to the intermountain prairies of the northwestern United States. We also compared the effect of *C. maculosa* on the composition of the soil microbial community to that of the native species. In the field, fungicide (Benomyl) reduced AM mycorrhizal colonization of *C. maculosa* roots by >80%. Fungicide did not significantly reduce non-AM fungi. When grown alone, the biomass of *C. maculosa* was not affected by the fungicide application. However, depending on the combination of native competitor and fungicide, *C. maculosa* biomass varied from 10-fold decreases to 1.9-fold increases. In untreated soils, *C. maculosa* grew larger in the presence of *Festuca idahoensis* or *Koeleria cristata* than when alone. When fungicide was applied these positive effects of *Festuca* and *Koeleria* on *C. maculosa* did not occur. A third native grass, *Pseudoroegneria spicata*, had much stronger competitive effects on *C. maculosa* than *Festuca* or *Koeleria*, and fungicide reduced the competitive effects of *Pseudoroegneria*. Fungicide increased *Centaurea* biomass when competing with the forb *Gallardia aristata*. However, fungicide did not affect the way two other forbs; *Achillea millefolium* and *Linum lewisii*, interacted with *C. maculosa*. Rhizosphere microbial communities in the root zones of the three native bunchgrass species differed from that of *C. maculosa*. However, despite the strong effects of soil fungi in field interactions and differences in microbial community composition, soil biota from different plant rhizospheres did not affect the growth of *C. maculosa* in the absence of native competitors in greenhouse experiments. Our results suggest that successful invasions by exotic plant species can be affected by complex and often beneficial effects of local soil microbial communities. These effects were not manifest as simple direct effects, but become apparent only when native plants, invasive plants, and soil microbial communities were interacting at the same time.

Key words: Centaurea; *communities; competition; fungi; invasive exotics; mutualism; mycorrhizae; phospholipid fatty acids (PLFA); rhizosphere; soil microbes; spotted knapweed; weeds.*

INTRODUCTION

Soil microbes can have substantial effects on interactions among plants and the diversity and composition of plant communities (Bever 1994, West 1996, Van der Putten 1997, van der Heijden et al. 1998, Clay and Van der Putten 1999, Hooper et al. 2000, Packer and Clay 2000, Wardle 2002). For example, soil communities may alter competitive outcomes among plants by their pathogenic effects (Van der Putten and Peters 1997), by favoring obligate mycorrhizal species over non-mycorrhizal or facultative mycorrhizal species (Hetrick et al. 1989, Hartnett et al. 1993), or by the transfer of resources or fixed carbon between species (Chiarello et al. 1982, Francis and Read 1984, Grime et al. 1987, Moora and Zobel 1996, Walter et al. 1996, Watkins et al. 1996, Simard et al. 1997, Marler et al. 1999, but see Robinson and Fitter 1999). Plants can also affect soil microbes (Bever et al. 1996, Wardle and Nicholson 1996, Westover et al. 1997). Soil microbes can have strong effects on exotic, invasive plants (Allen and Allen 1990, Richardson et al. 2000), and plant–soil feedbacks may differ between some exotic and native species (Klironomos 2002, Van der Putten 2002, Callaway et al., *in press*). However, to our knowledge, the effects of the soil biota on interactions between invasive and native species have not been studied in manipulative field experiments.

Invasive *Centaurea* species (primarily knapweeds) appear to benefit from fungi present in the soil of newly occupied areas. When grown alone, the total biomass of *Centaurea melitensis* planted in native Californian soil was lower without fungicide than with fungicide. However, when *C. melitensis* was grown with the native bunchgrass *Nassella pulchra* the biomass of *C. melitensis* was greater with intact native fungal communities than when fungicide was added (Callaway et al. 2001). Marler et al. (1999) demonstrated that soil fungi enhanced the competitive effect of the noxious weed *Centaurea maculosa* on *Festuca idahoensis*, a bunchgrass native to the northern Rocky Mountains. Soil fungi had no effect on *C. maculosa* biomass when the

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PLATE 1. Intermountain grassland in the hills surrounding the Missoula Valley before and after invasion by *Centaurea maculosa*. Photographs by Sue Brown (left) and Dean Pearson (right).

weed was grown alone. However, when *C. maculosa* and *Festuca* were grown together, *Festuca* plants were larger in the absence of fungi than when soil fungi were present.

The effects of soil fungi on plant interactions vary with resource availability (Hetrick et al. 1990, 1994, Johnson et al. 1997, Simard et al. 1997), the size of neighboring plants (Marler et al. 1999), and the composition of the fungal community (van der Heijden et al. 1998). The effects of soil fungal communities on the way plants interact also appear to vary for different plant species (Hartnett et al. 1993, Callaway et al. 2003). However, little is known about the effects of soil fungi on such species-specific plant interactions (see Francis and Read 1984, Grime et al. 1987, Simard et al. 1997), and even less about such interactions involving invasive plants. Furthermore, few studies have linked the mechanisms of fungi–plant interactions determined in the greenhouse to species interactions in the field.

While relatively rare in its native communities, *Centaurea maculosa* is among the most widespread and destructive grassland invaders in the Western United States and Canada (Griffith and Lucey 1991, Sheley and Jacobs 1997). The negative effects of *Centaurea* species on native plants are well documented (Muir and Majak 1983, Lesica and Shelly 1996) and *C. maculosa* may reduce the cover and diversity of native grassland species by more than 90% (Ridenour and Callaway 2001). *Centaurea maculosa* and the closely related *C. diffusa* appear to suppress natives via a number of different mechanisms including allelopathy (Muir and Majak 1983, Callaway and Aschehoug 2000, Ridenour and Callaway 2001, Bais et al. 2002) and competition for resources (Callaway and Aschehoug 2000, Le Jeune and Seastedt 2001). These studies have provided insight into the remarkable transmogrification of *C. maculosa* from native subordinate to invasive dominant, but the importance of soil microbes in this process remains uncertain for several reasons. First, there have been no field studies involving soil microbes, and second, studies of *Centaurea*-soil microbe interactions have been conducted with very few native species. The objective of this study was to measure interactions among native North American plant species and *C. maculosa* with and without manipulations of soil fungi in field conditions. Based on previous greenhouse experiments, our fundamental hypothesis was that soil fungi would enhance the competitive ability of *C. maculosa* against natives. We report on (1) a field experiment in which *C. maculosa* was grown alone and in competition with six different native North American species, and either with or without soil fungi reduced, (2) the composition of soil microbial communities occurring in the rhizospheres of a subset of the interacting species, and (3) a greenhouse experiment in which we measured the effects of sterilized and unsterilized soil from the field experiment on the growth of *C. maculosa*.

METHODS

Field experiment

We conducted a common garden experiment at The University of Montana Diettert Experimental Gardens in Missoula, Montana (see Plate 1). These gardens occupy land once covered by intermountain grassland, and are adjacent to natural intermountain grasslands that have been heavily invaded by *C. maculosa*. Approximately 300 m^2 in the garden were cleared of all plants and then covered with black geotextile fabric that allows water to pass but inhibits plant growth.

Holes, 20 cm in diameter, were cut into the mat where plants were established. In each hole, we planted either a native species with *C. maculosa* (original $n = 16$ per native species) or *C. maculosa* alone ($n = 12$). We chose this simple design to most clearly quantify the effects of native species on *C. maculosa*. This design does not allow us to compare the strength of intraspecific competition among *C. maculosa* plants to interspecific competition between *C. maculosa* and natives (Reynolds 1999). Competing plants were originally 5–10 cm apart. All native plants were established in the fall of 1998, and in the spring of 1999 we replaced native plants that didn't survive the winter and also transplanted *C. maculosa* seedlings next to them. The native species were the bunchgrasses *Pseudoroegneria spicata*, *Festuca idahoensis*, and *Koeleria cristata*, and the dicots *Linum lewisii*, *Gaillardia aristata*, and *Achillea millefolium*. In April 1999, we began to apply Benomyl (Bonide Products, Yorkville, New York, USA) in water at the concentration of 50 mg Benomyl/kg soil (Hetrick et al. 1989) to the soil around eight of the 16 pairs of *C. maculosa* and each native species, and continued to do so approximately every two weeks between April and September of 1999 and 2000. Six of the 12 solitary *C. maculosa* were also treated with Benomyl.

Benomyl has been shown to have minimal direct effects on plants in some studies (Paul et al. 1989), and is a recommended method for manipulating soil fungi in experiments with plants (Fitter and Nichols 1988, Smith et al. 2000), but Benomyl kills a wide range of other types of fungi as well as AM (arbuscular mycorrhizal) fungi (West et al. 1993, Newsham et al. 1994) and can have nontarget effects on other soil microorganisms (Van der Putten et al. 1990). To quantify the effects of Benomyl on *C. maculosa*, we subsampled dry fine roots from plants that had been randomly selected from the experimental array to include six *C. maculosa* plants treated with Benomyl and six that were not. We prepared these roots using modified methods of Phillips and Hayman (1970, also see Callaway et al. 2003) and checked the roots for fungal colonization under 100× magnification. We examined the roots for AM fungi and non-AM fungi using the ''magnified intersections'' method described in McGonigle et al. (1990) and Marler et al. (1999) to determine the percentage of colonized root length. AM fungi were distinguished from non-AM fungi by the presence of arbuscules, vesicles, hyphal coils, and nonseptate hyphae. Non-AM fungi included melanized hyphae and spores, septate hyphae, and nonseptate hyphae associated with non-AM fungi structures including oospores.

All plants were grown through the season of 2000. In the fall of 2000, all aboveground biomass of *C. maculosa* was harvested, dried at 60°C and weighed. The effects of neighbor species and fungicide on *C. maculosa* biomass were tested with two-way ANOVA with nontransformed variables. We also measured the diameter of the three bunchgrass species and the number of stems for each dicot species to evaluate the relative effect of *C. maculosa* on natives. Because the primary focus of this experiment was on the effect of soil fungi and different native species on the success of *C. maculosa*, native species were not planted alone as controls for the competitive effects of *C. maculosa*, therefore our comparisons are only among native species and do not include comparison of native species grown with versus without *C. maculosa*. Because different dependent variables were used for grass and forb growth, we conducted separate ANOVAs for each of these groups. In each ANOVA, we tested the effects of species and fungicide treatment.

Soil microbial communities

We conducted phospholipid fatty acid (PLFA) analyses to estimate the effect of Benomyl on soil microbial communities and to characterize the soil microbial communities that developed in the rhizospheres of different plant species (White and Ringelberg 1998). Broad taxonomic comparison of samples is possible because many fatty acids are specific to general taxa of microorganisms and can be used to determine the composition of the microbial community. We used the following criteria for fatty acid microbial indicators.

1) Actinomycete fungi. The 10 methyl branched fatty acids, especially 10me18:0, are thought to be produced almost exclusively by actinomycete fungi (Kroppenstedt 1985, Frostegård et al. 1993).

2) Bacteria. Terminally branched, cyclic, and monenoic fatty acids are thought to occur mainly in bacteria (Federle 1986). We used the PLFA markers i15:0, a15:0, 15:0, i16:0, 16:1ω 9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 as indicators of total bacterial populations (Frostegård and Bååth 1996).

3) Fungi to bacteria ratio. An estimate of the ratio of fungi to bacteria in soil samples is commonly made by dividing the amount of 18:2ω6, a fatty acid known to be produced mainly by fungi by the sum of the fatty acids known to be produced mainly by bacteria.

In the second week of June 2000, two weeks after Benomyl application, soil samples were collected (1–5 cm depth profile) from the root zones of grasses growing in competition with *C. maculosa* and from *C. maculosa* growing alone. All visible roots were hand picked from freeze-dried soil samples within 7 d of collection and lipids were extracted. PLFAs were extracted and analyzed according the method of White and Ringelberg (1998). Lipids were removed from samples into chloroform using a modified Bligh and Dyer (1956) extraction procedure. Phospholipids were separated from other lipids by silicic acid chromatography and derivatized to the corresponding fatty acid methyl esters (FAMEs) for analysis by gas chromatography. FAMEs were identified by gas chromatography–mass spectral analysis, relative retention times, coelution

with purchased standards, and comparison of samples between capillary columns of differing polarity (HP-5 and HP). FAMEs were quantified on a HP-225 column using a HP 6890 series GC system (Hewlett Packard, Wilmington, Delaware, USA) and protocol published by Frostegård et al. (1993). The classes of phospholipid fatty acids identified in *Centaurea* and bunchgrass rhizospheres were compared using detrended correspondence analysis in the PC-ORD software package (Hill 1979, McCune 1997).

To assess the effects of Benomyl on AM and general soil fungi, we collected soil from a subset of 19 plots chosen randomly from the *C. maculosa*-bunchgrass and *Centaurea*-alone treatments and tested the soil for the fungal biomarker 18:2ω6 using PLFA. Soil was collected from 0–10 cm in depth between five *C. maculosa*–grass pairs that had been treated with Benomyl, five other pairs that had not been treated, two solitary *C. maculosa* treated with Benomyl, two solitary *C. maculosa* that had not been treated, three grass rhizospheres treated with Benomyl, and two grass rhizosphere that were not treated.

Because plants can alter soil pH, and pH is a primary factor in the development of microbial communities (Saetre and Bååth 2000), we measured rhizosphere pH in the upper 10 cm of soil under each individual of nine *C. maculosa*–grass pairs (three for each species) and for five solitary *C. maculosa*. Additional samples were taken from the rhizosphere between each of the nine pairs of competing *C. maculosa* and grasses. pH was statistically analyzed with a two-way ANOVA in which the grass species of the pair and specific location in a plot (*C. maculosa*, grass, and intermediate) were used as effects. We used a one-way ANOVA and post-ANOVA students Tukey hsd test to compare pH in plots with competing species to plots with solitary *C. maculosa*.

Greenhouse experiment

To see if soil treatment effects could be observed in the absence of interacting plants, in May 2000, we collected ~100 g of soil from the upper 10 cm of the rhizosphere between each pair of competing *C. maculosa* and native species and each the rhizosphere of solitary *C. maculosa* plants. Soil from each rhizosphere sample was kept separate for the experiment, but each individual sample was thoroughly mixed before adding 50 cm^3 of the sample each of two replicate 525-cm^3 rocket pots in which *C. maculosa* was grown from seed. The seed source was from local populations in the Missoula valley in western Montana. This inoculum was added to 20-grit silica sand. *Centaurea maculosa* plants were grown for 11 wk in greenhouse conditions during the summer (natural light, daily low temperatures ~10°C, daily high temperatures ~23°C), and then harvested, dried at 60°C, and weighed. The mean biomass of the two *C. maculosa* replicates was used to represent the effect of the soil from a single field microsite. Species effects and Benomyl effects were tested with two-way ANOVA (SPSS 1999).

Results

The concentration of the fungal biomarker 18:2ω6 was 3.13 ± 0.30 (mean ± 1 SE) molar percent in untreated soils vs. 2.40 ± 0.28 molar percent in soil with fungicide ($t = 1.767$, $P = 0.095$). Our fungal biomarker provides only a crude estimate of total fungi, but the application of Benomyl at the rates used here commonly reduces AM without altering plant growth (Fitter and Nichols 1988, Paul et al. 1989, West et al. 1993, Smith et al. 2000, Callaway et al. 2003; but see Pedersen and Sylvia 1997). AM fungi and non-AM fungi were present in the roots of all *C. maculosa* roots that we analyzed, but the percent colonization of AM fungal hyphae was 4.7 ± 3.6% in fungicide treatments vs. 44.2 ± 7.9% in no-fungicide treatments (*t* test, $P < 0.001$). Non-AM fungi did not decrease significantly with Benomyl treatments in the roots (2.7 ± 1.9% in fungicide treatments vs. 7.2 ± 3.7%, $P = 0.304$), and the trend in the proportional decrease was much less than for AM fungi. Other studies have shown that Benomyl reduces the AM component more than non-AM fungi (Smith et al. 2000, Callaway et al. 2001, 2003). The study by Smith et al. (2000) suggests that Benomyl does not have strong effects on the soil microbial community as a whole.

Field experiment

Planted alone in the field, *C. maculosa* grew to an average aboveground biomass of 4.9 ± 1.1 g per plant, and growth was not affected by the application of fungicide (Fig. 1; *t* test; df = 1, 11; $P = 0.891$). However, depending on the combination of plant competitor and fungicide, *C. maculosa* biomass varied from decreases of >10-fold to increases of 1.9-fold. As found in several previous greenhouse experiments (Marler et al. 1999), *C. maculosa* biomass increased (*t* test, df = 1, 11; $P = 0.021$) when planted with *Festuca* without fungicide applied to the soil, but decreased when planted with *Festuca* in soil with fungicide (Fig. 1; *t* test; df = 1, 11; $P = 0.033$). This pattern was almost identical when *C. maculosa* was planted with *Koeleria*. In contrast, *C. maculosa* biomass decreased from 5.15 ± 1.1 g per plant when planted alone without fungicide to 0.21 ± 0.09 g when planted with *Pseudoroegneria* without fungicide (*t* test, df = 1, 11; $P < 0.001$). When fungicide was applied where *Pseudoroegneria* and *C. maculosa* were grown together *C. maculosa* biomass increased significantly (*t* test, df = 1, 11; $P < 0.001$).

Overall, dicots were much stronger competitors against *C. maculosa* than grasses (Fig. 1). The biomass of *C. maculosa* was lower when grown with *Gallardia* and *Linum* than when grown alone, but *Achillea* did not reduce *C. maculosa* growth. The species-specific effects of dicots on *C. maculosa* were much less variable than those of bunchgrasses. As for *Pseudoroeg-*

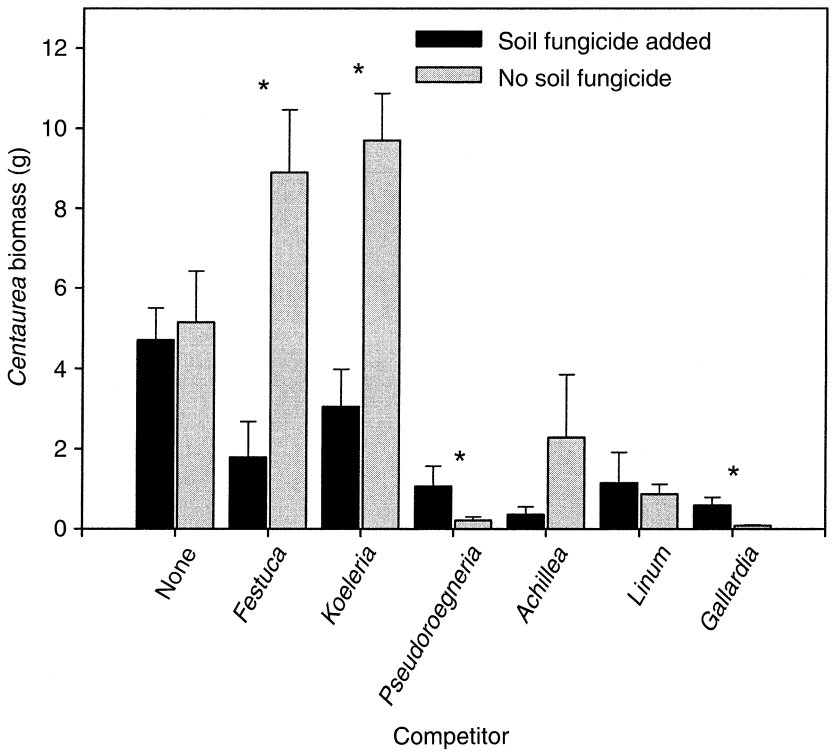

FIG. 1. Aboveground biomass of *Centaurea maculosa* either grown alone or with six different native species, and either with or without the fungicide Benomyl added to the soil. Error bars represent +1 SE, and asterisks denote a significant effect of fungicide on an individual species. Two-way ANOVA: effect of treatment, $F_{1, 119} = 8.503$, $P = 0.004$; effect of species, $F_{6, 119} = 5.548$, $P < 0.001$; effect of treatment × species, $F_{6, 119} = 3.829$, $P = 0.002$. Important pairwise comparisons are presented in *Results*.

neria, soil fungicide increased *Centaurea* biomass when the weed was competing with *Gallardia*. Soil fungicide did not affect the way either *Achillea* or *Linum* interacted with *C. maculosa*.

In general, the effects of most native species and fungicide on *C. maculosa* were reflected in the effects of *C. maculosa* and fungicide on native species (Fig. 2). For all grass species combined, soil fungicide did not significantly affect their final size (two-way ANOVA, effect of fungicide, $F_{1,59} = 1.162$, $P = 0.188$; effect of species, $F_{2,59} = 3.118$, $P = 0.011$). However, even though natives were planted in advance of *C. maculosa*, fungicide significantly increased the diameters of *Festuca* and *Koeleria* growing with *C. maculosa* (effect of species × fungicide, $F_{2,59} = 4.008$, $P = 0.008$). Fungicide did not change the effects of *C. maculosa* on *Pseudoroegneria*. For all forb species combined, soil fungicide had significant effects on their growth (two-way ANOVA, effect of fungicide, $F_{1,60} = 3.220$, $P = 0.004$; effect of species, $F_{5,60} = 8.118$, $P < 0.001$), but this was due to the strong response of *Gallardia* to Benomyl (Fig. 2). In contrast to *Festuca* and *Koeleria*, but mirroring *Gallardia's* effect on *Centaurea*, *Gallardia* stem number was highly suppressed by *C. maculosa* when fungicide was added to the soil.

Soil microbial communities

Multivariate analyses of PLFAs indicated that microbial communities in the rhizospheres of *C. maculosa* differed from those in the rhizospheres of the grass species (Fig. 3). These differences corresponded strongly with differences in PLFA markers for fungi and bacteria. The fungal fatty acid biomarker, 18:2ω6, was higher in native grass rhizospheres than in *C. maculosa* rhizospheres (1.844 ± 0.108 molar percent vs. 1.263 ± 0.157, $t = 3.10$, df = 1, 23, $P = 0.005$) as was the fungus to bacteria ratio estimated from PLFAs (0.142 ± 0.009 vs. 0.094 ± 0.012, $t = 3.12$, df = 1, 23, $P = 0.005$). The 16:1ω5 marker, which has been tentatively linked to AM fungi (Olsson et al. 1995, Olsson 1999), did not differ between native and *C. maculosa* rhizospheres (1.80 ± 0.35 vs. 1.79 ± 0.41, $t = 0.31$, df = 1, 23, $P = 0.760$).

Microbial communities in the rhizospheres of the different grass species showed general species-specific patterns in the DCA (Fig. 3). However, there were no significant differences in the axis scores, nor were there any differences in single PLFA markers among native grass species suggesting that the DCA patterns were determined by broad differences in relative abundances

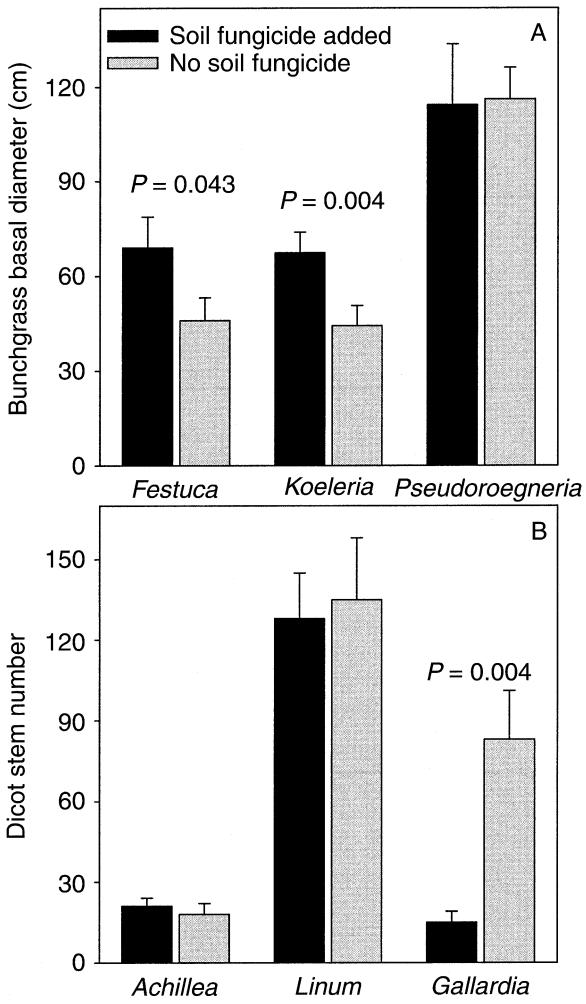

FIG. 2. (A) Diameters of bunchgrass species and (B) stem number of dicots grown with *Centaurea maculosa*, either with or without the fungicide Benomyl added to the soil. Error bars represent +1 SE, and asterisks denote a significant effect of fungicide on an individual species.

of different microbial taxa rather than the presence or absence of taxa.

The rhizosphere pH of each grass species was significantly different than that of each other grass species (ANOVA, $P = 0.002$) and soil pH was lower in *C. maculosa* rhizospheres when it was grown next to *Festuca* and *Pseudoroegneria* than when it was grown alone (Fig. 4). *Festuca* rhizospheres, when alone, were more acidic than any other, but soil between neighboring *Festuca* and *C. maculosa* and soil in the *C. maculosa* rhizospheres were higher in pH than that of *Festuca* neighbors ($P = 0.011$ and $P = 0.052$, respectively), but almost identical to the rhizospheres of solitary *C. maculosa*. Rhizospheres of *Pseudoroegneria* were less acidic than for any other species, but soil pH between competitors and under adjacent *C. maculosa* was significantly more acidic.

Greenhouse experiment

Unlike the field experiment and despite the differences in microbial community composition and pH among rhizospheres, we found no effect of soils from the microsites that were occupied by the six different native species paired with *C. maculosa*, or solitary *C. maculosa*, on the growth of *C. maculosa* in the greenhouse (Fig. 5). The effect of Benomyl was not significant for any microsite type considered alone, but *C. maculosa* growth was significantly lower in soils across all species combinations that had been treated with Benomyl in the field (two-way ANOVA, effect of fungicide, $F_{1, 113} = 7.181$, $P = 0.009$).

DISCUSSION

Our results from manipulative field experiments contribute to the growing body of evidence for the crucial role of soil biota in plant interactions and community function (Van der Putten et al. 1993, Bever 1994, van der Heijden et al. 1998, Clay and Van der Putten 1999, Hooper et al. 2000, Packer and Clay 2000, Klironomos 2002, Wardle 2002). Our results suggest that native soil biota can have large effects on the success of exotic invaders, but these effects are most evident within the context of interacting plant species. In our field experiments, soil fungicide reduced AM fungi and dramatically altered the competitive ability of *C. maculosa* and these effects were highly specific to the native species with which *C. maculosa* interacted. These specific effects of native-species–fungicide combinations varied by orders of magnitude in scale and were often reversed. For example, *C. maculosa* increased in aboveground biomass when grown with *Festuca* (see Marler et al. 1999) and *Koeleria* without fungicide. However, soil fungicide eliminated the beneficial effects of *Festuca* and *Koeleria* on *C. maculosa*. This pattern was reversed for *Pseudoroegneria*, which suppressed *C. maculosa* when soil fungi were not reduced, but *Pseudoroegneria* lost much of this competitive advantage when fungicide was added. Overall, forbs were much more competitive than grasses with *C. maculosa*. However, *C. maculosa*–forb–microbe interactions were also species specific.

In the field, soil microbial communities in *C. maculosa* rhizospheres were different than those in grass rhizospheres, corroborating a number of studies that have demonstrated species-specific plant effects on soil microbes (Bever et al. 1996, Wardle and Nicholson 1996, Westover et al. 1997, Eom et al. 2000, Saetre and Bååth 2000). *Centaurea maculosa* also affected soil pH, shifting pH in the soil between the competitors towards the pH of the rhizospheres of *C. maculosa* when grown alone (Fig. 5). However, soils from different rhizospheres did not affect the growth of *C. maculosa* in the absence of native competitors in greenhouse experiments. This suggests that the controlled greenhouse conditions and small pots changed micro-

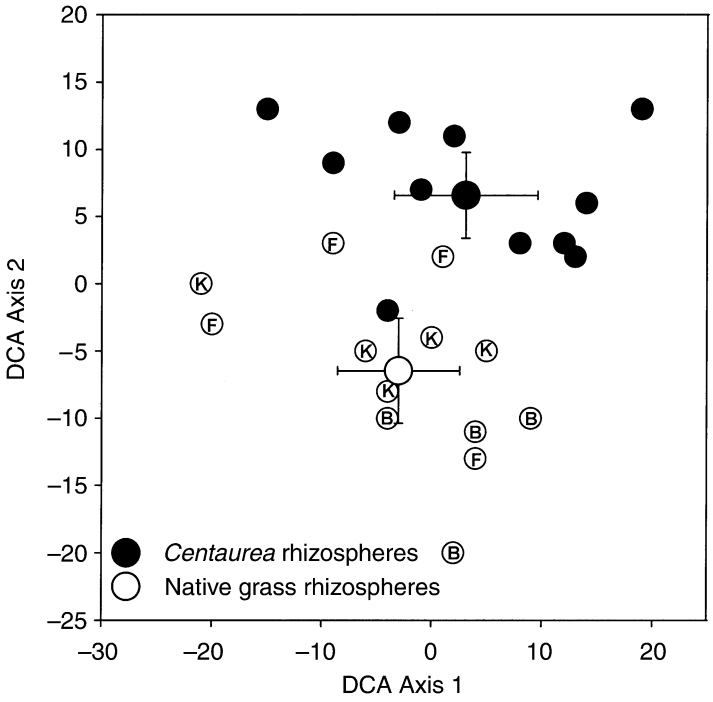

FIG. 3. Detrended correspondence analysis (DCA) of soil microbial communities associated with *Centaurea maculosa* or native grass rhizospheres in the field. Closed circles represent *Centaurea* rhizospheres, and open circles represent grass rhizospheres. Symbols represent the mean for each group on the *x*- and *y*-axes and are shown with 95% confidence limits in each direction. Eigenvalues were 0.29 for the *x*-axis and 0.20 for the *y*-axis. Letters inside open circles denote the native grass species under which the soil was collected: B, *Pseudoroegneria spicata* (bluebunch wheatgrass); F, *Festuca idahoensis*; K, *Koeleria cristata*.

bial communities, or that the field effects were dependent on the presence of all interacting parties—the native, the invader, and the soil microbes. Other experiments with soil microbial communities and grass competitors conducted with similar protocols in our greenhouse have demonstrated effects on *C. maculosa* growth and *C. maculosa*-–soil-microbe feedbacks (Marler et al. 1999; Callaway et al., *in press*). Therefore the absence of microbial effects on *C. maculosa* in the greenhouse in the experiment may be due to the absence of grasses and the elimination of complex three-way interactions.

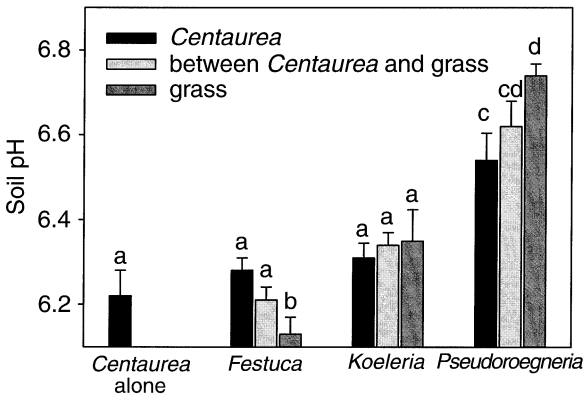

FIG. 4. Soil pH associated with *Centaurea maculosa* or native grass rhizospheres in the field. For each native–*Centaurea* pair, soil was collected from the rhizosphere of each species and between the competing species. Error bars represent +1 SE, and different letters denote significant differences within a species in post-ANOVA Tukey hsd tests with a values adjusted for multiple comparisons.

The beneficial effects of *Festuca* and *Koeleria* on *C. maculosa* in the absence of fungicide were like those reported by Marler et al. (1999) from greenhouse experiments. They found that soil fungi enhanced the negative effect of *C. maculosa* on *Festuca*, but soil

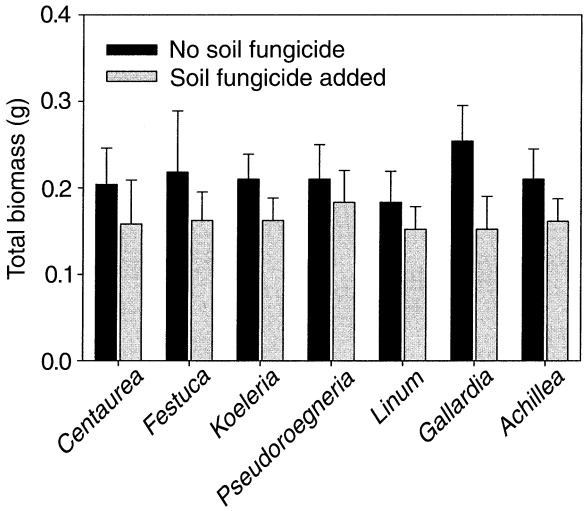

FIG. 5. Total biomass of *Centaurea maculosa* grown in soil collected from microsites occupied by either a native Montana species and competing *Centaurea* or a solitary *Centaurea*, and with soil that had been treated with Benomyl fungicide in the field or left untreated. No fungicide was added during the greenhouse phase of the experiment. Error bars represent +1 SE. Two-way ANOVA: effect of fungicide, $F_{1, 113} = 7.181$, $P = 0.009$; effect of species, $F_{6, 113} = 0.182$, $P = 0.981$; effect of fungicide × species, $F_{6, 113} = 0.212$, $P = 0.972$.

fungi had no direct effect on either species when they were grown alone. When the species were grown together, *Festuca* plants were 170% larger in the absence of soil fungi (sterilization and addition of microbial slurry protocol) than when soil fungi were present. In contrast, *C. maculosa* plants were 66% larger in the presence of soil fungi (Benomyl protocol) than in their absence. Although the phenomenon has been consistent in different experiments with *F. idahoensis*, we do not know the mechanism by which *C. maculosa* increases in growth with some natives and soil fungi. Research with other *Centaurea* species (Grime et al. 1987) and *C. maculosa* (Marler et al. 1999; E. Carey and R. M. Callaway, *unpublished data*) suggests that *C. maculosa* may benefit from soil fungi-mediated parasitism in which fixed carbon or other resources are transferred from the grasses to *C. maculosa* via a common network of AM fungi. However, one experiment using ^{13}C labels found no evidence for carbon transfer from *F. idahoensis* to *C. maculosa* (Zabinski et al. 2002). They found that AM fungi appear to enhance the ability of *C. maculosa* to acquire more soil phosphorus than native grasses. Alternatively, different combinations of plant species may change the composition of the microbial community, by shifts in the composition of the total fungal community (see Bever 1994, Bever et al. 1997), or the AM fungal community (Johnson et al. 1997, Egerton-Warburton and Allen 2000). A third possibility is that some native species have strong positive effects on the growth of soil fungi, but the positive feedback of soil fungi to the natives is less than the positive feedback to *C. maculosa*. The results of the field experiment reported here, however, suggest that previous greenhouse experiments are far too simplistic in terms of understanding the role of soil microbes on *C. maculosa* success. For some species, such as *Festuca*, *Koeleria*, and *Gallardia*, the soil microbes existing in native soils appear to contribute to their demise. For other species, such as *Pseudoroegneria*, soil microbes appear to confer resistance to the competitive effects of *C. maculosa*.

Our results suggest that successful invasions by exotic plant species are not determined only by competitive, consumer-driven, and demographic processes, but by complex and often beneficial effects of the local soil microbial communities. Mutualisms have been associated with many successful invasions (Richardson et al. 2000), including those occurring between mycorrhizal fungi and exotic plants (Schmidt and Scow 1986, Koske 1992, Richardson et al. 1994), perhaps because of associations between invaders and relatively non-specific mycorrhizal fungi. Invasions may be enhanced by escaping relatively more species-specific soil pathogens. Understanding mutualisms and antagonisms involving invasive plants and soil microbes have the potential to increase our understanding of the devastating increase in noxious exotics around the globe. Better understanding of the role of soil microbes in plant invasions will require focused experiments on plant–soil feedbacks (Bever 1994, Bever et al. 1997), plant-specific effects on the diversity and function of mycorrhizal and pathogenic fungi (Bever et al. 1996, van der Heijden et al. 1998), defense responses of plants to microbial infection, and the effects of soil microbes on plant interactions. Although microbes had dramatic effects on interactions among different plant species, soil cultures from the rhizospheres of different species did not. Our experiments were limited to pairwise interactions among plants species. Many studies have demonstrated that pairwise interactions do not accurately represent the complex indirect interactions that occur among plant species (Miller 1994, Levine 1999, Callaway and Pennings 2000). Our results suggest that some natural functions in plant-soil communities depend on the community as a whole, and not on isolated components.

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