

SHORT COMMUNICATION

Soil fungal abundance and diversity: another victim of the invasive plant *Centaurea maculosa*

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Interactions between plants and soil microbes are important determinants of both above- and belowground community composition, and ultimately ecosystem function. As exotic plants continue to invade and modify native plant communities, there has been increasing interest in determining the influence of exotic invasives on native soil microbial communities. Here, using highly sensitive molecular techniques, we examine fungal abundance and diversity in the soil surrounding a particularly aggressive invasive plant species in North America, *Centaurea maculosa* Lam. In mixed stands, we show that this invasive weed can alter the native fungal community composition within its own rhizosphere and that of neighboring native plants. At higher densities, the effect of *C. maculosa* on native soil fungal communities was even greater. Our results demonstrate that this invasive weed can have significant effects not only on visible aboveground biodiversity but also on the native soil microbial community that extends beyond its rhizosphere.

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Plant–microbe interactions are an influential part of ecosystem functioning, and have recently gained attention in the context of invasion biology (Callaway *et al.*, 2004; van der Putten *et al.*, 2007). Previous research credits invasive weeds with decreasing the aboveground biodiversity of the invaded range by displacing or even eradicating native plants and disrupting the migration patterns of animals that are associated with those native plants (Hierro and Callaway, 2003). However, there is only limited information concerning the impact of invasive plants on the belowground biodiversity in soil microbial communities. Invasive plant species are thought to benefit from a lack of coevolved pathogens in their nonnative range (Keane and Crawley, 2002), yet few studies comprehensively examine soil microbial community dynamics and their response to novel plant influences. As below- and aboveground communities are linked through feedback interactions, assessing the impacts of invasive plants on soil microflora may provide new insight into the mechanism by which some

invaders are able to dominate landscapes and displace native plants (Klironomos, 2002; Wardle *et al.*, 2004; Wolfe and Klironomos, 2005).

To improve our understanding of the influence of nonnative plants on soil fungal communities, we collected soils from sites populated by the aggressive North American invasive weed *Centaurea maculosa* Lam. Fungal species diversity in high density (HD) stands of *C. maculosa* were compared to those found in adjacent low density (LD) stands at two sites. HD *C. maculosa* stands were near monocultures, consisting almost entirely of *C. maculosa* plants, whereas LD stands contained isolated *C. maculosa* (≥ 1 m spacing) along with a wide variety of native grasses and other plant species. DNA isolated from soil samples was analyzed by real-time PCR and length heterogeneity analysis (Manter and Vivanco, 2007).

Total fungal biomass (estimated by qPCR) and fungal phylotype richness varied between the two sites; however, at both sites the HD *C. maculosa* stand was associated with significant declines in fungal abundance and diversity. Bulk soil from HD stands of *C. maculosa* contained over 80% less fungal DNA, associated with the decline in abundance of six phylotypes, compared to LD stands (Figures 1a and b). In soils obtained from *C. maculosa* rhizospheres, fungal biomass was reduced nearly seven times in HD stands compared to LD stands (Figure 1a).

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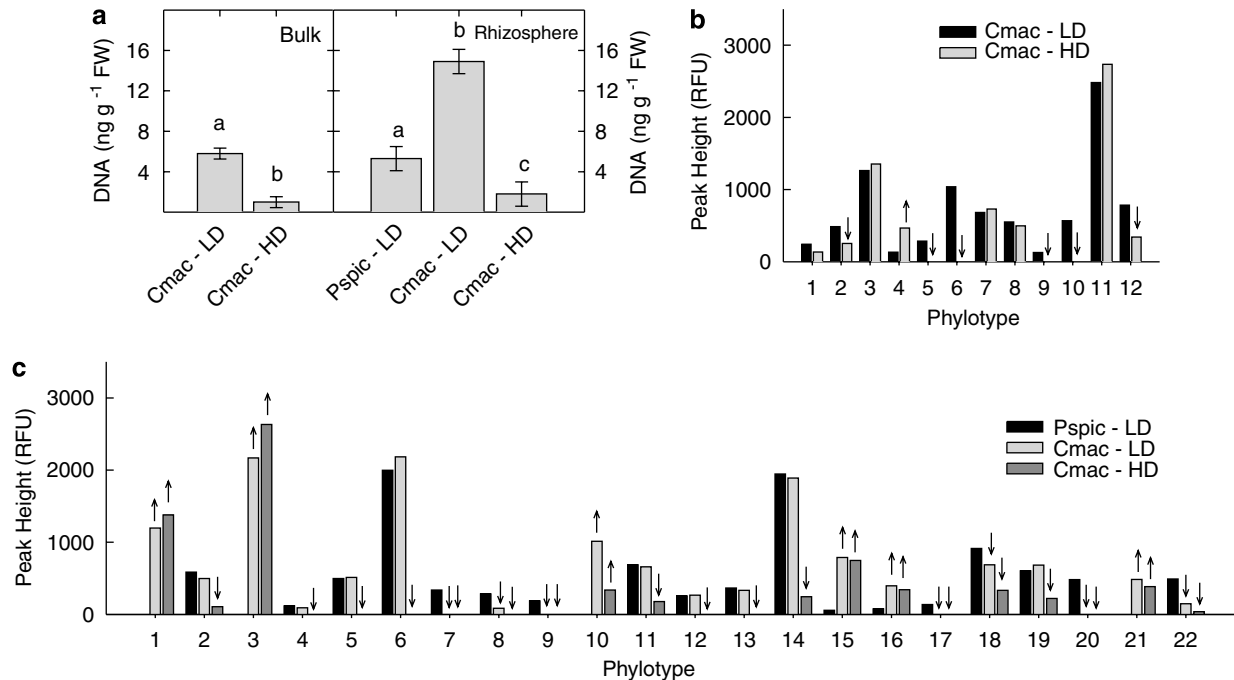


Figure 1 Total fungal DNA (a) and individual phylotype abundance (peak height) from bulk (b) and rhizosphere soil (c) from two sites in Montana that contained adjacent high- and low-density stands of *C. maculosa*. Pspic-LD = *Pseudoroegneria spicata* in the low-density stands, Cmac-LD = *Centaurea maculosa* in the low-density stands, Cmac-HD = *C. maculosa* in the high density stands. Bars are LSmeans and s.e., means with different letters are significantly different ($P < 0.05$, panel a). Bars are LSmeans and s.e., arrows indicate significantly increasing or decreasing phylotype abundance between high-and-low density stands ($P < 0.05$, panel b). Bars are LSmeans and s.e.s, arrows indicate significantly increasing or decreasing phylotype abundance relative to the *P. spicata* rhizosphere ($P < 0.05$, panel c).

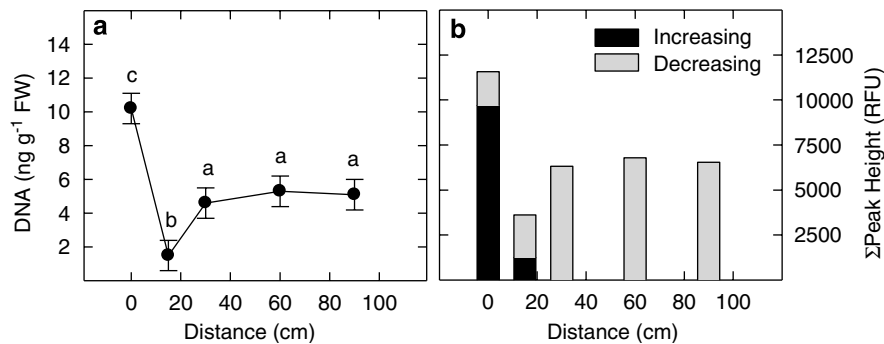


Figure 2 Total fungal DNA (a) and phylotype abundance (b) from the rhizosphere of *Poa secunda* plants at various distances from a *Centaurea maculosa* plant. Points are LSmeans and pooled s.e.s, means with different letters are significantly different from the control, *P. secunda* at 90 cm ($P < 0.05$, panel a). Bars are the total abundance (Σ of peak heights) of significantly increasing or decreasing phylotypes. At each sample point, the observed phylotypes were assigned to the above categories if they were significantly different between the *P. secunda* rhizosphere at 0 and 90 cm⁻¹ ($P < 0.05$, panel b).

Total fungal biomass in the rhizosphere of *Pseudoroegneria spicata*, a native grass present in the LD stand, was significantly lower than that of the *C. maculosa* rhizosphere, but was greater than that of the *C. maculosa* rhizosphere of the HD stand. A comparison of the individual abundance of each phylotype present in the various rhizospheres showed that seven phylotypes were significantly reduced in the *C. maculosa* LD and sixteen in the *C. maculosa* HD when compared to *P. spicata* (Figure 1c). Conversely, six phylotypes were increased in abundance in both the HD and LD

C. maculosa rhizosphere as compared to the rhizosphere of *P. spicata*. Though previous work revealed that *C. maculosa* disrupts the arbuscular mycorrhizal fungal community of native and naturalized grasses (Mummey *et al.*, 2005; Mummey and Rillig, 2006), our results present the first analysis of the effects of *C. maculosa* on the broader soil fungal community.

It is possible that the reduction in plant diversity in HD *C. maculosa* stands leads to the observed reduction in fungal diversity. However, recent reports suggest that soil characteristics, such as

resource availability (Carney *et al.*, 2004; Waldrop *et al.*, 2006) and pH (Fierer and Jackson, 2006), are better predictors of microbial community diversity than the existing plant community. Soils from HD and LD stands had similar C/N ratios and similar amounts of humic acids (data not shown) however, soil characteristics that were not tested may also play a role in the modification of fungal communities between HD and LD *C. maculosa* stands.

To examine if and at what distance *C. maculosa* could affect the fungal community present in the soil rhizosphere of native grasses, we collected soils from another LD stand of *C. maculosa* (>5 m spacing), but focused on a native grass species (*Poa secunda*) growing at various distances from *C. maculosa* adults. Rhizosphere soils collected from *P. secunda* growing directly adjacent to *C. maculosa* had significantly higher fungal biomass than those collected from distances further away (Figure 2a). This could be an additive effect, as the rhizospheric zone of both plant species overlapped at this distance. Interestingly, *P. secunda* growing 15 cm from *C. maculosa* had the lowest amount of microbial biomass out of all distances tested. A more detailed analysis of the individual phylotype abundances suggests that this pattern of biomass is the culmination of two different effects of *C. maculosa* on fungi in the rhizosphere (Figure 2b). For example, total abundance (Σ peak heights) of some phylotypes decreased within 15 cm of a *C. maculosa* plant; whereas, other phylotypes show a dramatic increase within the *C. maculosa* rhizospheric zone. When the resultant total abundance of all phylotypes is determined (Figure 2b), this pattern is consistent with our observed estimates of total fungal biomass. It should also be noted that *C. maculosa* roots were not apparent in grass rhizosphere samples at a distance of 15 cm or more from the *C. maculosa* plant, suggesting that diffusible root exudates may be partially responsible for the observed decrease in microbial biomass at this distance.

Soil microbes can have a profound influence on molecular and biochemical processes in individual plants, plant communities and ultimately the ecosystem (Klironomos, 2002; Callaway *et al.*, 2004; Stinson *et al.*, 2006), but the consequences of modification of microbial biodiversity in the rhizospheric and nonrhizospheric soil are not well understood. Disruption of the balance between native plant and microbial communities has been shown to have a negative effect on native plants but a positive effect on invasive plants (Klironomos, 2002; Stinson *et al.*, 2006), and it is possible that such a disruption could cause an imbalance to arise between plant species, causing some to flourish at the expense of others. Here, we show that the aggressive invasive weed *C. maculosa* alters soil fungal communities in its own rhizosphere and bulk soil when it is found in HD versus LD stands, and provide evidence that these microbial community

alterations extend to neighboring native grass species. In addition, we show that *C. maculosa* favored phylotypes that were rare, and often undetectable, in the native soil microbial community. A better understanding of the interactions between invasive plants, native plants and soil microbial communities is essential for developing appropriate land management and restoration strategies.

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