

Short communication

The invasive forb, *Centaurea maculosa*, increases phosphorus availability in Montana grasslands

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Abstract

Centaurea maculosa Lam. (Asteraceae, spotted knapweed) was introduced to the United States from Eurasia in the late 1800s and now covers over 3 million ha in Washington, Idaho, Montana, and Wyoming. Several recent studies have suggested that the success of *C. maculosa* may be partly due to its ability to outcompete native species for phosphorus (P), through high root mass and/or association with arbuscular mycorrhizal fungi. We used a combination of field and greenhouse studies to explore the P efficiency of *C. maculosa* and its effects on soil P levels. *C. maculosa* was P efficient in both the field study and greenhouse experiment. In the field, P concentration in *C. maculosa* was more than twice that of three native species (*Pseudorogneria spicata*, *Festuca idahoensis*, and *Lupinus sericeous*). In the greenhouse experiment, even at extremely low levels of soil P availability, uptake of P by *C. maculosa* was six times greater than that by the native, *Lupinus argenteus*. However, soil P levels were elevated in sites invaded by *C. maculosa*, which is the opposite of what would be expected if root or mycorrhizal uptake were responsible for the higher P efficiency. These results indicate that the success of *C. maculosa* may be due to its greater ability to acquire P than native species, but do not indicate that *C. maculosa* is actually outcompeting natives for the P that it acquires. In contrast, *C. maculosa* appears to have the ability to increase the availability of P in some soils.

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1. Introduction

Invasion by exotic plant species provides an opportunity to demonstrate how the properties of individual species may affect ecosystem-level processes, such as nutrient cycling (Vitousek, 1990; Ehrenfeld et al., 2001). *Centaurea maculosa* Lam. (Asteraceae) is one of the most widespread invaders in grasslands of the United States and Canada and has greatly decreased diversity in invaded systems (Tyser and Key, 1988; Ridenour and

Callaway, 2001). Recent studies have suggested that the success of *C. maculosa* may be at least partially attributed to its greater competitive ability for phosphorus (P) compared to native species (LeJeune and Seastedt, 2001; Zabinski et al., 2002). Although competition was not directly tested, soil P has been shown to be up to 88% lower in sites dominated by *C. maculosa* than in sites dominated by native grasses (Harvey and Nowierski, 1989 in Olson, 1999), suggesting higher uptake by *C. maculosa*. Higher P uptake by *C. maculosa* may be facilitated by its deep, extensive root development and/or colonization by arbuscular mycorrhizal fungi (AMF; Marler et al., 1999; Zabinski et al., 2002). This paper presents the results of several experiments wherein we tested soil P levels in invaded grasslands and the P efficiency of *C. maculosa* in the field and the greenhouse.

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2. Methods

2.1. Soil P

We studied the effect of *C. maculosa* on soil P levels using a paired comparison of plots sprayed with herbicide to eliminate *C. maculosa* and unsprayed plots located in western Montana, USA. Soils at all three sites are moderately alkaline soils classified as Calciothidic or Typic Haploxerolls. Site 1 was sprayed with Picloram (4-amino-3,5,6-trichloropicolinic acid) in 1999. Sites 2 and 3 were sprayed with 2,4-dichlorophenoxy acetic acid (2,4 D) in 1997 and 1998, respectively. Native species composition and percent bare ground were similar between sprayed and unsprayed plots. In each plot, six samples from 0 to 15 cm depth were taken. Samples were dried for 48 h at 100 °C and then sieved through 2 mm mesh. Plant available P was estimated by extraction in 1.0 M NaHCO₃ (Kuo, 1996). Briefly, 10 g of oven-dried soil were shaken in 50 ml of 1.0 M NaHCO₃ for 30 min and then filtered through Whatman no. 42 filter papers. The P concentration in the extracts was determined calorimetrically using the ascorbic acid method (Kuo, 1996). The effects of site and treatment tested using an analysis of variance (SPSS 10.0).

2.2. Phosphorus efficiency, field study

To determine the relative uptake of P in field conditions, leaves of *C. maculosa*, *Pseudoroegneria spicata* (Pursh) A. Löve, *Festuca idahoensis* Elmer, and *Lupinus sericeus* Pursh were collected from three paired native and invaded sites in western Montana. The native site at the National Bison Range (Moiese) has not yet been invaded. The native sites at the University of Montana Bandy Ranch (Ovando) and Mount Sentinel (Missoula) have been maintained by spraying with either 2,4 D or Picloram. *C. maculosa* cover at the invaded sites was approximately 60–80%.

At each site, 10 plants of each species were randomly selected. Leaves were collected in June 2002, when all species were actively growing. Leaves were dried for 3 days at 60 °C then ground through a 1 mm mesh using a Wiley Mill. Determination of PO₄-P was done by the Oregon State University Central Analytical Lab. A two-way ANOVA was used to test for the effect of species and community type (invaded versus uninvaded) on P concentration (SPSS 10.0). Differences between species were determined by a Bonferroni test for multiple comparisons.

2.3. Phosphorus efficiency, greenhouse study

To investigate the effects of soil P availability on plant tissue P content, *C. maculosa* and *L. argenteus* Pursh were grown in soils with three levels of available P. This experiment was conducted in a greenhouse at the University of Montana that was kept on a 12-h light:12-h dark cycle. Six species/treatment combinations were replicated 14 times for a total of 84 pots. Plants were seeded into 500 g of soil in 450 ml pots. The soil was a calcareous, native mineral soil from the Missoula valley footslopes which was depleted of available P by mixing two parts mineral soil with one part sand and growing *Lolium perenne* for 8 weeks prior to use.

Four weeks after *C. maculosa* began to germinate; *L. argenteus* seeds that had been scarified and aerated in water overnight were planted. Each pot was thinned to one plant per pot. Pots were watered every two to three days. Pots were fertilized with 50 ml of fertilizer solution at planting, weeks 2, 6, and 10. P applications were graduated for P treatment totals of 0, 20, and 100 ppm. Phosphorus levels were based on P efficiency studies by Johnson et al. (1994).

Plants were harvested after 16 weeks. Plant roots and shoots were separated, washed, dried at 60 °C for 24 h, and weighed for biomass. Plant P was analyzed using a combination of methods from Braum and Helmke (1995) and Kuo (1996). Fifty milligrams of *C. maculosa* and 20 mg of *L. argenteus* tissue were ground through a no. 4 mesh screen using a Wiley Mill. Samples were then ball milled to pass through 200 mesh, ashed at 550 °C for 2 h, acidified with 1N H₂SO₄, brought to a 25 ml volume, and shaken for 16 h. P analysis was performed using the ascorbic acid method (Kuo, 1996).

A two-way ANOVA was used to test for differences among all treatment means for tissue P (SPSS 10.0). Differences between treatments and contrasts were determined using a Bonferroni test for multiple comparisons.

3. Results

3.1. Soil P

C. maculosa rhizospheres contained greater soluble P at two of three sites (site* treatment $P = 0.028$; Fig. 1). Across all sites combined, sites that were not sprayed had twice the soluble P concentrations than sites that had been sprayed (10.09 ± 2.86 and 4.98 ± 1.27 S.E. $\mu\text{g P g}^{-1}$ soil, respectively; treatment $P = 0.015$).

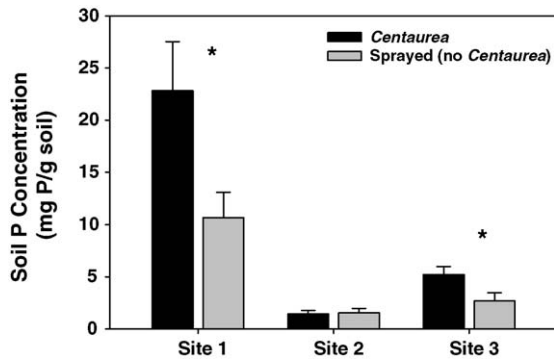


Fig. 1. Soil P concentration ($\mu\text{g P/g soil}$) was higher at two *C. maculosa* (unsprayed) sites compared to sites sprayed to eliminate *C. maculosa* (site \times treatment $P = 0.028$). Significant differences within sites are indicated by asterisk (*). Bars are means + 1 S.E.

3.2. Phosphorus efficiency, field study

While no species showed differences in tissue P concentration in invaded versus uninvaded communities ($P = 0.058$), there were significant differences between species ($P < 0.0005$). *C. maculosa* tissues contained at least twice the P (3185 ± 76 S.E. $\mu\text{g mg}^{-1}$) than the native species tested. There was a significant difference between the nitrogen fixing *L. sericeus* and the shallow-rooted grass *F. idahoensis* (1536 ± 72 and 1180 ± 56 S.E. $\mu\text{g mg}^{-1}$, respectively). The leaf P concentration of the deeply rooted grass, *P. spicata* was intermediate to the other native species (1407 ± 68 S.E. $\mu\text{g mg}^{-1}$).

3.3. Phosphorus efficiency, greenhouse experiment

Compared to *L. argenteus*, *C. maculosa* acquired greater P ($P = 0.001$) and biomass ($P < 0.005$); even in the most extremely P limited soil (Fig. 2). However, *C. maculosa* concentrated less P than *L. argenteus* ($P < 0.02$). As fertilizer levels increased, *L. argenteus* plant P content increased, but there was no change in biomass ($P = 0.2$). This led to three times the concentration of P in the high versus the low fertilizer treatments ($P < 0.005$). In comparison, as fertilizer levels increased, *C. maculosa* increased both plant P content and biomass, leading to an increase in P concentrations of only 1.5 times ($P < 0.02$).

4. Discussion

The results of our studies suggest that *C. maculosa* is more P efficient than the native species tested and it has the ability to alter soil P cycling. Soil P was elevated in

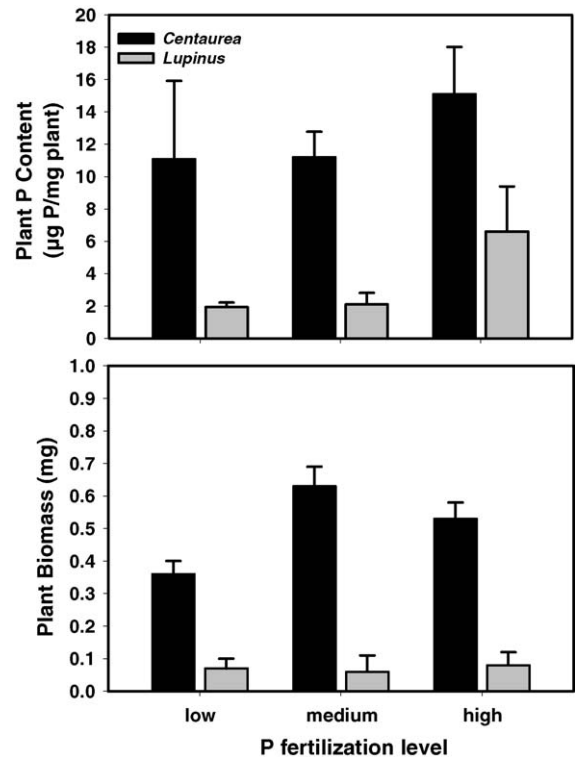


Fig. 2. Tissue P content and biomass differences between *L. argenteus* and *C. maculosa*. *Lupinus argenteus* concentrated tissue P, while *C. maculosa* increased biomass with increasing added P in soil. Bars are means + 1 S.E.

two of the sites invaded (unsprayed) by *C. maculosa* (Fig. 1). The lack of detectable difference at site 2 may be due to unmeasured soil characteristics at that site. Localized soil properties may have a strong influence on the ability of plant species to impact soil P (Chen et al., 2003).

Both the field and greenhouse studies demonstrated high P efficiency for *C. maculosa*. In the field, above-ground biomass of *C. maculosa* contained approximately twice the P as the three native species studied. Although there was no difference in P concentrations in *C. maculosa* and *L. argenteus* in the greenhouse experiment at each of the fertilizer levels, there was a difference in the magnitude of response to fertilization (Fig. 2). As fertilization increased, *L. argenteus* concentrated P, whereas *C. maculosa* appeared to utilize the P for increased growth.

Previous studies have attributed the effective P uptake capacity of *C. maculosa* to association with AMF (Zabinski et al., 2002). If AMF were the mechanism for P efficiency, it would be expected that soil P levels would be lower in invaded communities

due to the formation of depletion zones around colonized roots and AMF hyphae (Smith and Read, 1997), as was found by Harvey and Nowierski (1989 in Olson, 1999). However, we found higher levels of soil P in soils invaded by *C. maculosa*. This result is more consistent with increases in soluble P found in the rhizospheres of plants that exude phosphatases (Grierson and Adams, 2000) or chelating compounds (Grierson, 1992; Stevenson and Cole, 1999).

C. maculosa produces many root exudates, including the polyphenol, (\pm)-catechin (Bais et al., 2002, 2003). (+)-Catechin is frequently found in the extracts of the leaves of forest trees and can be important in the complexation of Fe, Al, and Ca (Stevenson and Cole, 1999; Kidd et al., 2001). Soils in western Montana grasslands and foothills invaded by *C. maculosa* tend to be calcareous (Montagne et al., 1982), which limits P availability through the precipitation of Ca–P compounds. Thus, higher levels of P in rhizospheres of *C. maculosa* may be due to chelation of Ca by (\pm)-catechin (see Watt and Evans, 1999). Alternatively, the herbicide treatment itself may have caused the differences observed. However, this is unlikely due to the persistence in the difference 3 years post-spraying and that other than the presence of live or dead *C. maculosa*, the native species composition and percent bare ground were similar between treated and untreated sites.

It has been suggested that the success of *C. maculosa* may be due to its ability to outcompete native species for soil P (Harvey and Nowierski, 1989 in Olson, 1999; LeJeune and Seastedt, 2001), although this has not been tested directly. Our results support the hypothesis that *C. maculosa* has the ability to acquire more P than native species, potentially enhancing its competitive success. However, greater acquisition of P was not related to depletion of the resource, a requisite for competition. We suggest that there may be multiple mechanisms responsible for the P efficiency of *C. maculosa* and that these may be dependent on local soil conditions. In some invaded soils, *C. maculosa* may actually have the ability to increase P availability. The evidence that an invader can directly alter soil nutrient cycling has important implications for the management and restoration of invaded communities.

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