Belowground mechanisms that affect nutrient uptake and response to herbivory of Centaurea maculosa and native bunchgrasses
by Sara Theresa Zimmerley

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University
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Abstract:
Centaurea maculosa, an invasive forb from Eurasia, has come to dominate more than 4 million hectares of rangeland in the Rocky Mountain region (Boggs and Story 1987). Understanding mechanisms that increase C. maculosa's success are paramount to reducing its impacts on grassland communities. My research examined the effects of herbivory, arbuscular mycorrhizae (AM), and nutrient availability on C. maculosa and. native bunchgrasses. My first experiment examined the role of AM for phosphorus (P) acquisition from a distant source C. maculosa and Festuca idahoensis, a native bunchgrass. Plants were grown individually in pots divided by either a membrane barrier that excluded plant roots and AM hyphae from the opposite side of the pot, or a mesh barrier that excluded only plant roots. In the half of the pot without a plant, a layer of P , fertilizer was added. Centaurea maculosa was associated with greater quantities of extra radical hyphae (ERH) than F. idahoensis, suggesting that C. maculosa better utilizes its AM symbiont for P acquisition. The success of this exotic plant may be related to the fungal species that colonize the invader, with different fungal species accessing P from . different distances. Alternatively, C. maculosa may be providing more carbon for the AMF, resulting in greater ERH production, ERH soil exploration and soil nutrient pool exploitation.

My second experiment assessed the role of AM and nutrients on compensation for simulated herbivory of C. maculosa, F. idahoensis, and Pseudoroegneria spicata. Plants were grown in a combination of high and/or low nitrogen (N) and P for 11 weeks, with or without AM. After removing 75% of aboveground biomass from half of the plants, plants grew for an additional 4 weeks. All of the plants undercompensated for losses to simulated herbivory regardless of AM or nutrient levels. Centaurea maculosa regenerated biomass to a higher degree than either of the grasses. Clipping did not affect AM colonization levels or ERH production. Centaurea maculosa may be a better competitor in grassland systems through the use of its AM symbiosis and its greater ability to compensate for herbivory.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Catherine A. Zabinski   _Catherine A. Zabinski_   4-21-03
                                       (Signature)   Date

Approved for the Department of Land Resources and Environmental Sciences

Dr. Jeffery S. Jacobsen   _Jeffery S. Jacobsen_   4/21/03
                                  (Signature)   Date

Approved for the College of Graduate Studies

Dr. Bruce R. McLeod   _Bruce R. McLeod_   4-21-03
                                  (Signature)   Date

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ABSTRACT

Centaurea maculosa, an invasive forb from Eurasia, has come to dominate more than 4 million hectares of rangeland in the Rocky Mountain region (Boggs and Story 1987). Understanding mechanisms that increase *C. maculosa*'s success are paramount to reducing its impacts on grassland communities. My research examined the effects of herbivory, arbuscular mycorrhizae (AM), and nutrient availability on *C. maculosa* and native bunchgrasses. My first experiment examined the role of AM for phosphorus (P) acquisition from a distant source *C. maculosa* and *Festuca idahoensis,* a native bunchgrass. Plants were grown individually in pots divided by either a membrane barrier that excluded plant roots and AM hyphae from the opposite side of the pot, or a mesh barrier that excluded only plant roots. In the half of the pot without a plant, a layer of P fertilizer was added. *Centaurea maculosa* was associated with greater quantities of extra radical hyphae (ERH) than *F. idahoensis,* suggesting that *C. maculosa* better utilizes its AM symbiont for P acquisition. The success of this exotic plant may be related to the fungal species that colonize the invader, with different fungal species accessing P from different distances. Alternatively, *C. maculosa* may be providing more carbon for the AMF, resulting in greater ERH production, ERH soil exploration and soil nutrient pool exploitation.

My second experiment assessed the role of AM and nutrients on compensation for simulated herbivory of *C. maculosa, F. idahoensis,* and *Pseudoroegneria spicata.* Plants were grown in a combination of high and/or low nitrogen (N) and P for 11 weeks, with or without AM. After removing 75% of aboveground biomass from half of the plants, plants grew for an additional 4 weeks. All of the plants undercompensated for losses to simulated herbivory regardless of AM or nutrient levels. *Centaurea maculosa* regenerated biomass to a higher degree than either of the grasses. Clipping did not affect AM colonization levels or ERH production. *Centaurea maculosa* may be a better competitor in grassland systems through the use of its AM symbiosis and its greater ability to compensate for herbivory.

INTRODUCTION

The invasion of plant communities by exotics may impact ecosystem structure and function by altering community composition (Shea and Chesson 2002), making invasive plant species research a high priority for land managers and conservation biologists (D'Antonio and Kark 2002). *Centaurea maculosa* Lam. (spotted knapweed) is an invasive forb that dominates more than 4 million hectares of rangeland in the western United States, forming dense monocultures in areas once dominated by diverse grassland plant communities (Boggs and Story 1987). Native grasses, including *Festuca idahoensis* Elmer (Idaho fescue) and *Pseudoroegneria spicata* [Scribn. & Smith] A. Love (bluebunch wheatgrass), are often characterized as slow-growing species adapted to low-nutrient conditions. Fast-growing invaders can utilize nutrient pulses and outcompete these grasses. Understanding the mechanisms that allow exotic species to establish in native plant communities is a prerequisite to developing a comprehensive plan to reduce the success of invasive plants.

One mechanism that increases plant nutrient acquisition is the plant-fungal symbiosis, arbuscular mycorrhizae (AM). This symbiosis, ubiquitous among terrestrial plant species (Smith and Read 1997), involves an obligately symbiotic fungus that colonizes the host plant roots and extends via extra radical hyphae (ERH) into the surrounding soil to explore for nutrients, especially phosphorus (P). The fungus can acquire soil P from pore spaces that the much larger plant roots could not access. In exchange, the plant allocates up to 20% of its photosynthetically-derived carbon to its AM (Johnson et al. 1997).

While AM are generally mutualistic in low-nutrient soils, the symbiosis can become parasitic for the host plant when soil nutrients are abundant (Johnson 1993). The function of the symbiosis varies among host plants, fungal partners, and soil conditions (Bever et al. 1994, Johnson et al. 1997, Helgason 2002), and some plant species depend more on AM to acquire nutrients than others (Hetrick et al. 1986). In addition to colonizing plant roots, AM ERH extend into the soil, accessing nutrients or colonizing the roots of an adjacent plant (Friese and Allen 1991). Since AM are important in soil nutrient acquisition for the host plant, it is important to evaluate the contribution of AM in increasing invasive plant nutrient status, and subsequent effects on the success of the invader in establishing and dominating in native grassland assemblages. The role of ERH in host plant P acquisition and a comparison of *C. maculosa*'s and *F. idahoensis'* ability to exploit their AM for distant P source acquisition are investigated in Chapter 2.

Efforts to eradicate *C. maculosa* have mainly focused on herbicide application and, more recently, biological controls (Sheley et al. 1999). One form of biological control is grazing management to reduce *C. maculosa*'s seed production and population size (Olson 1999). The success of grazing management to reduce infestations hinge on grazing effects being more detrimental to the invasive species than to native neighbors.

Plants experiencing regular episodes of herbivory respond through compensatory growth (Strauss and Agrawal 1999). Compensatory growth is a measure of plant fitness following herbivory. Plant fitness, defined as the ability of an individual to contribute to subsequent generations, is difficult to measure, so parameters such as biomass and/or seed production are used as surrogate measures (Chapin and McNaughton 1989).

Additionally, under- and overcompensation often occur when the biomass of grazed plants is either lower (undercompensation) or higher (overcompensation) than the biomass of ungrazed individuals of the same species.

The effects of herbivory on plant growth vary due to the timing, duration, plant species, and soil nutrient availability (Crawley 1997, Paige 1992). Early-season herbivory events often result in complete or overcompensation, provided nutrients, water and light are not limiting, because sufficient time remains in the growing season to regenerate tissues lost to the herbivore (Crawley 1997). In contrast, late-season herbivory events often result in undercompensation since limited water and nutrient availability often coincide with late-season growing conditions and herbivory events.

Research focusing on the effects of herbivory on AM has provided highly contradictory results. Some studies show a net decline in AM colonization following herbivory (Bethlenfalvey et al. 1988), while others cite increases or no changes (Trent et al. 1997) in AM colonization levels after herbivory (Wallace 1981, Wallace 1987). As AM can significantly affect plant nutrition (Smith and Read 1997), evaluation of the effects of AM on plant responses to herbivory and the effects of herbivory on AM are crucial to understanding the responses of *C. maculosa* and neighboring native grasses to herbivory.

Chapter 3 of this thesis addresses the role of AM in plant response to simulated herbivory under high or low nitrogen (N) and P levels, and simultaneously evaluates the effects of herbivory on AM in *C. maculosa, F. idahoensis,* and *P. spicata.* I hypothesized that compensatory growth would be greatest in high nutrient conditions, and that AM

would increase compensatory growth in low nutrient conditions, but decrease

compensatory growth in high nutrient conditions. Regarding herbivory effects on AM, I

hypothesized that AM colonization and ERH production would not be affected by

simulated herbivory.

DIFFERENCES IN PHOSPHORUS UPTAKE AND ARBUSCULAR MYCORRHIZAL EXTRA RADICAL HYPHAE DEVELOPMENT BETWEEN *CENTAUREA MACULOSA* AND *FESTUCA IDAHOENSIS*

Introduction

The invasion of native plant communities by exotics may impact ecosystem structure and function (Shea and Chesson 2002) by increasing soil erosion (Lacey et al. 1989), reducing soil organic matter and water infiltration rates (Lacey et al. 1989), depleting soil nutrient pools (Harvey and Nowierski 1989), and altering historic disturbance regimes (Whisenant 1990). Resource availability in invaded areas has been positively correlated with the ability of introduced exotics to establish and outcompete native residents (Burke and Grime 1996). Fungal mutualists and pathogens have also been linked to the success of exotic plants (Klironomos 2002), with fungal mutualists enhancing host plant resource acquisition. Understanding the mechanisms that allow exotic species to invade and establish in native plant communities is a prerequisite for developing a comprehensive plan to reduce the success of invasive plants.

Centaurea maculosa Lam. (spotted knapweed) is an invasive forb that dominates more than 4 million hectares of rangeland in the western United States, forming dense monocultures in areas once dominated by diverse grassland plant communities (Boggs and Story 1987). A tap-rooted, short-lived perennial that over-winters as a rosette, *C. maculosa* begins growth early in spring and flowers late in summer, while many neighboring native grasses and forbs are senescing (Sheley et al. 1999). This life strategy contributes to *C. maculosa*'s competitive success, because it can take advantage of soil

resources for a longer portion of the growing season. Many studies have attempted to identify factors that enhance *C. maculosa*'s invasibility in semi-arid rangeland systems; however, most focus on the role of aboveground ecological components in increasing the abundance and productivity of this exotic plant (Kennett et al. 1992, Maxwell et al. 1992, Olson et al. 1997, Sheley and Jacobs 1997).

Belowground-components of ecosystems are less studied, but an increasing amount of research shows that soil biota can affect plant community dynamics and succession (Wardle 2002). Arbuscular mycorrhizae (AM), for example, are symbiotic relationships between a plant and fungus, ubiquitous among terrestrial plant species (Read 2002), and an important influence on plant community structure (Hartnett and Wilson, 2002). The AM fungus assists the host plant in soil nutrient uptake, mainly phosphorus (George et al. 1995), via small (<10 μm diameter) extra radical hyphae (ERH) that explore the soil and access nutrients in spaces too small for plant roots to exploit. The plant allocates up to 20% of its photosynthetically-derived carbon (C) to the AM fungus (Johnson et al. 1997), which are obligate symbionts that require host plant C.

While AM are generally mutualistic in low-nutrient soils, the symbiosis can be antagonistic for the host plant when soil nutrients are abundant (Johnson 1993). Additionally, the function of the symbiosis varies among host plants and fungal partners (Bever et al. 1996, Johnson et al. 1997, Helgason 2002), and some plant species depend more on AM to acquire nutrients than others. Plants with coarsely-branched or tap-roots may depend more on AM hyphae to attain P than plants with fibrous root systems (Hetrick et al. 1986).

Besides colonizing the root of a host plant, AM hyphae extend into the soil, accessing nutrients or colonizing the roots of adjacent plants (Freise and Allen 1991). Hyphal links between plants can form a belowground hyphal network, creating the possibility of resource transfer between species, potentially altering plant species interactions and community dynamics (Newman 1988). In *Festuca ovina* grasslands, carbon is transferred via AM hyphae from the dominant *F. ovina* to less common species, including *Centaurea nigra* (Grime et al. 1987).

Carbon transfer via hyphal linkages has been hypothesized to be a mechanism of plant competition in grassland systems. In the greenhouse when *C. maculosa* and *F. idahoensis* were grown together either with or without AM, *C. maculosa* was larger or *F. idahoensis* was smaller in AM-treated pots than non-AM pots, consistent with the hypothesis that C is transferred from *F. idahoensis* to *C. maculosa* (Marler et al. 1999). In a follow-up study, $^{13}CO_2$ was applied to one of two host plants grown in pots that divided the soil into halves that were or were not accessible by AM hyphae (Zabinski et al. 2002). Consistent with previous results and the hypothesis of C transfer, *C. maculosa* was larger when growing with a native grass in a pot with hyphal access to the opposite species. Those results could not be attributed to C transfer between species, as there was no evidence of ^{13}C transfer. However, tissue P concentration was higher in *C. maculosa* when growing with native grasses in pots with hyphal access to the grass, indicating that *C. maculosa* acquires P from soil on both sides of the pot. In contrast, native grasses did not have higher tissue P levels in pots with hyphal access to more soil. Therefore, the

invasive species may have exploited its AM symbiosis more efficiently than the native grasses to access a distant soil resource.

The objective of my experiment was to measure AM production and development in *C. maculosa* compared with *F. idahoensis*, by growing plants in a low nutrient soil with a distant P source that could only be accessed via AM. We hypothesized that the AM of *C. maculosa* would produce more ERH and at greater distances from the plant than the AM of *F. idahoensis*. As a result, the P concentration of *C. maculosa* would be greater than that of *F. idahoensis*.

Materials and Methods

Growth Environment

My experiment was a randomized complete factorial design with 2 plant species, 2 barrier types, 3 P treatments, and 10 replicates of each treatment combination. *Festuca idahoensis* and *C. maculosa* were grown singly in 2400 cm^3 (14 cm diameter x 15.25 cm height) pots in a greenhouse. The pots were divided in half with either a mesh barrier with 28 µm pores (Nitex™ nylon, Sefar America, Depew, NY, USA), or a membrane barrier with 0.45 µm pores (Magna™ nylon transfer membrane, Osmotics, Inc., Minnetonka, MN, USA), creating a plant compartment (PC) and hyphal compartment (HC). The mesh barrier excludes roots, but not hyphae from the hyphal compartment, whereas membrane barriers exclude roots and hyphae (Li et al. 1991), but allow water and solutes to cross (Zimmerley, unpublished data).

Each PC contained 8:1 sand (masonry grade silica sand, 20:30 grit) to field soil

mix. The field soil was obtained locally from a *Festuca idahoensis-Pseudoroegneria*

spicata habitat type (Mueggler and Stewart 1980) with *C. maculosa* present, and served

as AM inoculum. Each HC contained 100% silica sand with a 0.5 cm thick, vertical layer

of phosphate rock (PR) (available P_2O_5 3%, Pacific Calcium, Inc. Tonasket, WA), or

triple super phosphate (TSP) (available P_2O_5 46%, A.H. Hoffman, Inc. Lanchaster, PA),

added approximately 3.8 cm from the central barrier, in the center of the HC (Figure 1) or

silica sand with no phosphorus (NP). The two P fertilizers, differing in solubilization

rates and mineral composition, were selected to compare differential uptake of P by AM

hyphae. Each P type was added at a rate of 144 mg available P kg^{-1} soil (142 kg P ha^{-1}).

This rate is higher than recommended for a field setting, and was chosen to create a

nearly contiguous layer of PR and TSP to increase the probability that AM hyphae would
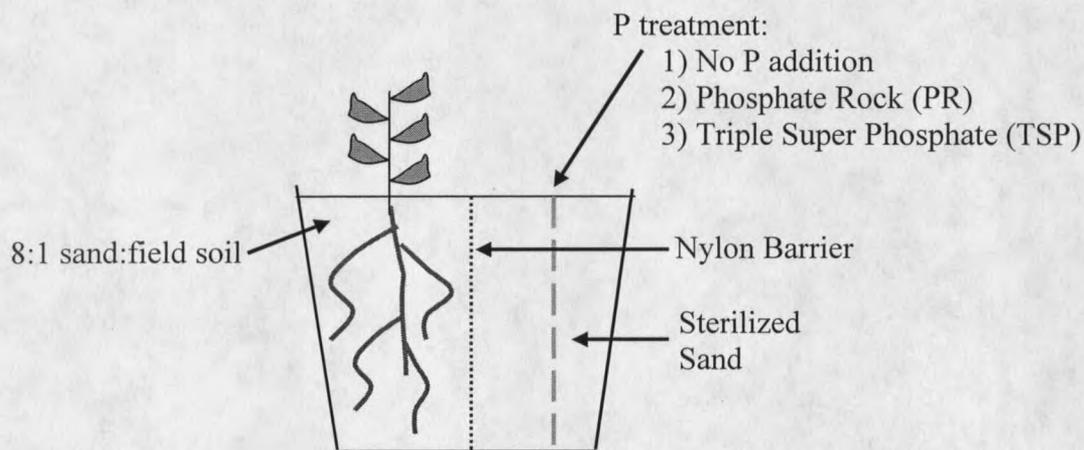
come into contact with the P fertilizers.

Figure 1. Diagram of pot construction, depicting PC and HC divisions with P treatment addition location.

Plants were grown in a greenhouse with a 16-hour day length, and an average day temperature of 22 °C and night temperature of 18 °C. Pots were watered daily and supplemented with 50 mL of ⅛-strength modified Hoagland's solution (minus P) every two weeks.

Plant Harvesting and Measurements

After 14 weeks, plants were harvested; roots and shoots were separated and thoroughly washed of soil and debris. Plants were dried for 48 hours at 60 °C and biomass was recorded. A subset of roots of each species from each treatment was cleared of pigments in 2.5% KOH, stained with 0.5% Trypan Blue (modified from Phillips and Hayman 1970), and analyzed for percent mycorrhizal colonization (McGonigle et al. 1990). Remaining plant tissues were finely ground and analyzed for nutrient content. Soil cores, 1.3 cm diameter, were taken from the PC and HC of each pot and analyzed for nutrient content and ERH length. Soil and plant nutrient concentrations were analyzed by MDS Pharma Services (Lincoln, NE). Soil cations (K, Mg, Ca, Na) were measured using ammonium extraction, soil P content was measured using the Olsen P method, NO_3 was analyzed using cadmium reduction and HCl extraction, and soil pH was measured with a 1:1 water: soil extraction (Klute 1986). Plant P and N concentrations were determined by wet-ash extraction and ICP analysis.

Extra radical hyphae were extracted from soils through the use of a modified soil filtration method (Miller et al. 1995) in which two 5 g soil subsamples were placed in 3.75% sodium hexametaphosphate solution for 12 hours to disperse soil particles and

hyphae. Each subsample was stirred for 5 minutes, a 10 ml aliquot was removed and added to 100 ml of distilled water, and this was stirred for 30 seconds. A 20 ml aliquot of that solution was vortexed for 30 seconds and poured through a 20 μm filter. The filter was placed into a centrifuge tube with 5 ml of 0.5% Trypan Blue, vortexed for 30 seconds, allowed to sit for 5 minutes, vortexed again for 30 seconds, and the solution was poured through a 0.45 μm filter. The filter was mounted on a microscope slide to quantify ERH lengths. Extra radical hyphal lengths were measured using the gridline intercept method (Reinhardt and Miller 1990) at 200x magnification. Arbuscular mycorrhizal hyphae were distinguished from other soil fungi using criteria delineated by Nicolson (1959), Mosse (1959), and Sylvia (1992).

Data Analysis

Total plant biomass, plant N concentration (%) and content (mg nutrient plant^{-1}), plant P concentration (%) and content (mg nutrient plant^{-1}), AM colonization, arbuscule and vesicle density, PC ERH, and PC and HC soil P data were analyzed with full factorial, 3-way ANOVAs with plant species, barrier type, and P treatment as the main effects (SPSS General Linear Model Univariate ANOVA, SPSS, Inc., Version 11.5). We analyzed HC ERH length as 2-way ANOVAs with barrier and P treatment as the main effects, and species differences in HC ERH were compared with student's t-tests. Data were transformed as needed to pass assumptions of ANOVA. All main and interaction effects with P-values less than 0.10 are reported. Post-hoc comparisons were made using the Bonferroni comparison with α=0.05.

Results

Phosphorus availability in the PC of each pot was highest in the PR treatment, the result of net movement of P across the barriers (Table 1); TSP movement was limited, yet also increased soil P in the PC. The quantity of P in the soil was not affected by plant species ($F_{1, 48}$=0.80, P=0.38) or barrier type ($F_{1, 48}$=1.5, P=0.23; Table 1).

Table 1. Soil data; mean (± SE). Different letters denote significant differences within columns at P≤0.05.

	P (ppm)	OM (%)	pH	CEC
Hyphal Compartment				
NP	1.75 (0.16) a	0.19 (0.01) a	8.74 (0.07) ab	0.52 (0.05) a
PR	8.35 (0.37) b	0.11 (0.01) b	8.67 (0.10) a	0.64 (0.03) a
TSP	3.40 (0.15) c	0.15 (0.01) c	8.99 (0.04) b	0.61 (0.05) a
Plant Compartment				
NP	2.25 (0.16) a	0.35 (0.01) a	8.30 (0.04) a	1.57 (0.06) a
PR	9.65 (1.17) b	0.22 (0.01) b	8.05 (0.04) b	1.67 (0.06) a
TSP	3.95 (0.15) c	0.24 (0.02) b	8.45 (0.04) c	1.57 (0.07) a

Plant Response

Centaurea maculosa plants had twice as much biomass as *F. idahoensis* plants ($F_{1, 112}$=100.7, P<0.001; Table 2). Barrier type did not affect total biomass for either plant species ($F_{1, 112}$= 0.1, P=0.7), whereas P treatment did affect biomass ($F_{2, 112}$=5.4, P=0.006). *Centaurea maculosa* plants amended with PR had more biomass than those amended with TSP or NP. This pattern was similar for *F. idahoensis*, but the differences

Table 2. Total biomass, total P, P concentration, total N, percent AM colonization, percent of roots with vesicles, and percent of roots with arbuscules for *Centaurea maculosa* and *Festuca idahoensis* across P treatments. Mean (±SE); different letters denote significant differences across rows for each species across rows at P≤0.05.

P type	*Centaurea maculosa*			*Festuca idahoensis*		
	NP	PR	TSP	NP	PR	TSP
Total Biomass (g)	1.45 (0.03) a	1.63 (0.06) b	1.21 (0.08) a	0.71 (0.06) c	0.76 (0.12) c	0.60 (0.14) c
Total P (mg plant^{-1})	0.20 (0.01) a	0.49 (0.03) b	0.15 (0.01) a	0.11 (0.02) ab	0.14 (0.01) b	0.07 (0.01) a
N concentration (%)	1.20 (0.05) a	1.00 (0.04) b	1.25 (0.06) a	1.18 (0.03) a	1.14 (0.04) a	1.33 (0.15) b
Total N (mg plant^{-1})	1.64 (0.05) a	1.70 (0.07) a	1.50 (0.03) b	0.93 (0.09) c	0.90 (0.06) c	0.82 (0.08) c
Vesicles (%)	4.8 (1.1) a	4.7 (1.3) a	11.0 (1.5) b	1.5 (1.1) ab	0.5 (0.5) a	3.2 (1.6) b
Arbuscules (%)	6.0 (2.0) a	3.4 (1.4) a	12.6 (2.3) b	10.8 (0.7) a	4.8 (2.2) b	12.8 (2.8) a

were not statistically significant.

Plant P concentrations (%) differed between plant species ($F_{1, 46}$=8.047, P=0.007) and P treatment ($F_{2, 54}$=22.9, P<0.001), but not between barrier types ($F_{1, 54}$=0.1, P=0.75; Figure 2). *Centaurea maculosa* had higher P concentrations than *F. idahoensis,* and PR-amended plants had 135% higher P concentrations than NP- or TSP-amended plants. A species by P treatment interaction was identified ($F_{2, 46}$=10.4, P<0.001). *Centaurea maculosa* contained higher concentrations of P in the PR treatment, whereas *F. idahoensis* did not increase its P concentration when amended with PR. A barrier by P treatment interaction also occurred ($F_{2, 46}$=4.1, P=0.023), with a greater difference in P concentrations between P treatments in membrane-divided pots than in mesh-divided pots (Figure 2).
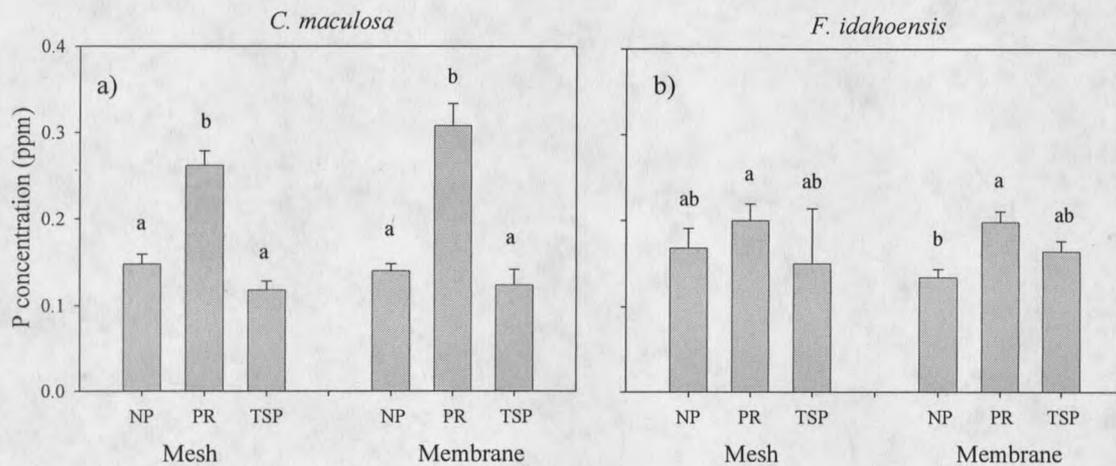

Figure 2a, b. Plant P concentration (%) for each species by barrier and P treatment; bars represent the standard error of the mean and letters denote significant differences at p<0.05.

Total P content (mg P plant^{-1}) was higher for *C. maculosa* than *F. idahoensis* ($F_{1, 54}$=58.0, P<0.001; Table 2), was not affected by barrier type ($F_{1, 54}$=0.3, P=0.58), but was affected by P treatment ($F_{2, 54}$=32.2, P<0.001; Table 2), with total P highest in plants from PR-amended pots. Additionally, a plant species by P treatment interaction was identified ($F_{2, 49}$=43.5, P<0.001) with *C. maculosa* having more total P when amended with PR, whereas *F. idahoensis* total P was similar across P treatments (Table 2).

Tissue N concentration was similar across plant species ($F_{1, 43}$=2.1, P=0.2), and varied among barrier ($F_{1, 43}$=10.6, P=0.002) and P treatments ($F_{2, 43}$=7.4, P=0.002; Table 2). Plants in membrane-divided pots had higher N concentrations than plants in mesh-divided pots, and PR-amended plants had lower N concentrations than plants in either the NP- or TSP-amended pots.

Total N content differed between plant species ($F_{1, 43}$=193.5, P<0.001), barrier types ($F_{1, 43}$=5.3, P= 0.03) and P treatments ($F_{2, 43}$= 4.2, P=0.02; Table 2). *Centaurea maculosa* had higher total N content than *F. idahoensis*. Plants in membrane-divided pots had higher N content than plants in mesh-divided pots, although *F. idahoensis* did not show this trend (plant species by barrier interaction, $F_{2, 43}$=6.0, P=0.02). Plants in PR-amended pots contained more N than plants in NP- or TSP-amended pots, although this trend was only observed for *C. maculosa* and not *F. idahoensis*.

Mycorrhizae

Centaurea maculosa had more roots colonized by AM than *F. idahoensis* ($F_{1, 25}$=17.3, P<0.001). Colonization levels were similar between barrier types ($F_{2, 25}$=0.5,