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

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.

BELOWGROUND MECHANISMS THAT AFFECT NUTRIENT UPTAKE AND RESPONSE TO HERBIVORY OF *CENTAUREA MACULOSA* AND NATIVE BUNCHGRASSES

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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.

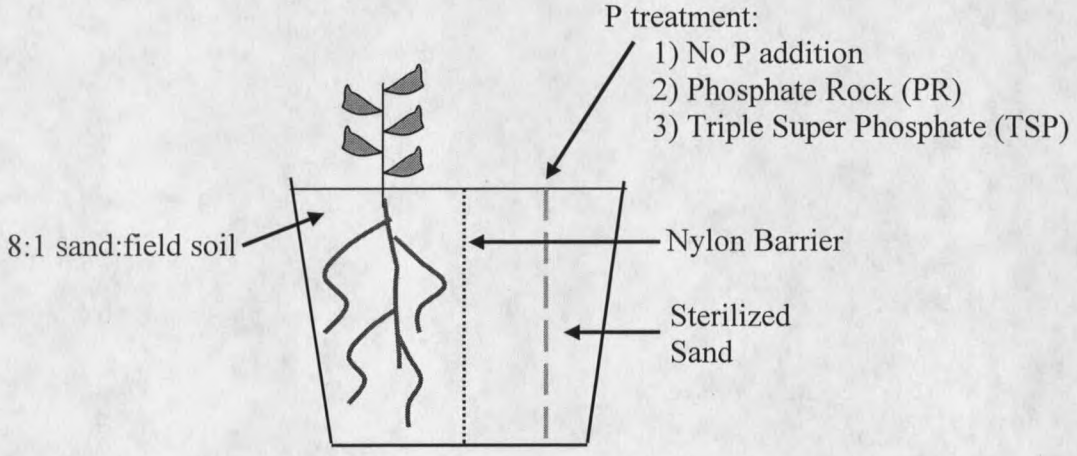

Figure 1. Diagram of pot construction, depicting PC and HC divisions with P treatment addition location.

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 $P \leq 0.05$.

	Centaurea maculosa			*Festuca idahoensis*		
P type	**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).

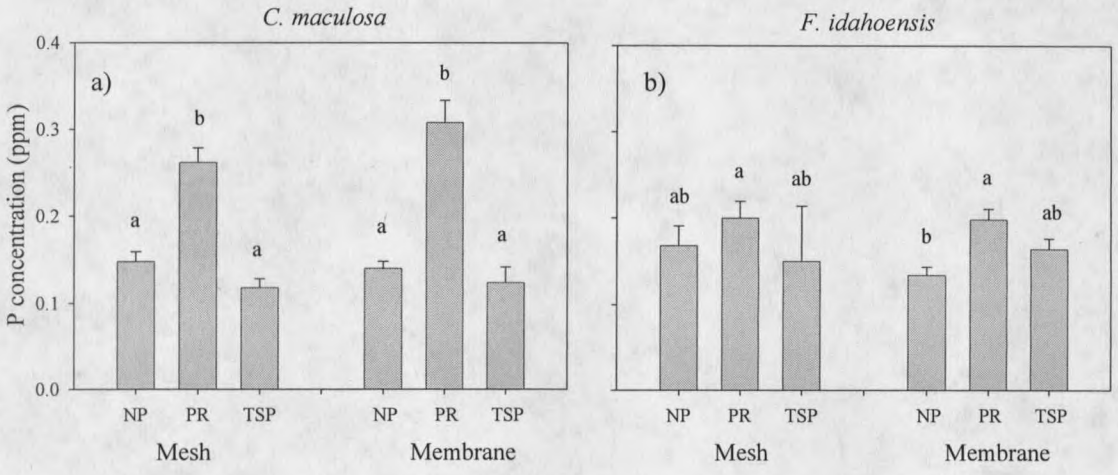

Figure 2a, b. Plant P concentration (%) for each species by barrier and P treatment; bars represent the standard error of the mean and letters denote significant differences at p<0.05.

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,

p=0.5), but differed with P treatment ($F_{2, 25}$=5.9, P=0.008). Plants amended with PR had lower percent colonization (22%) than TSP-amended plants (38%; Figure 3), and NP-amended plants had AM colonization levels intermediate of the other P treatments (33%).

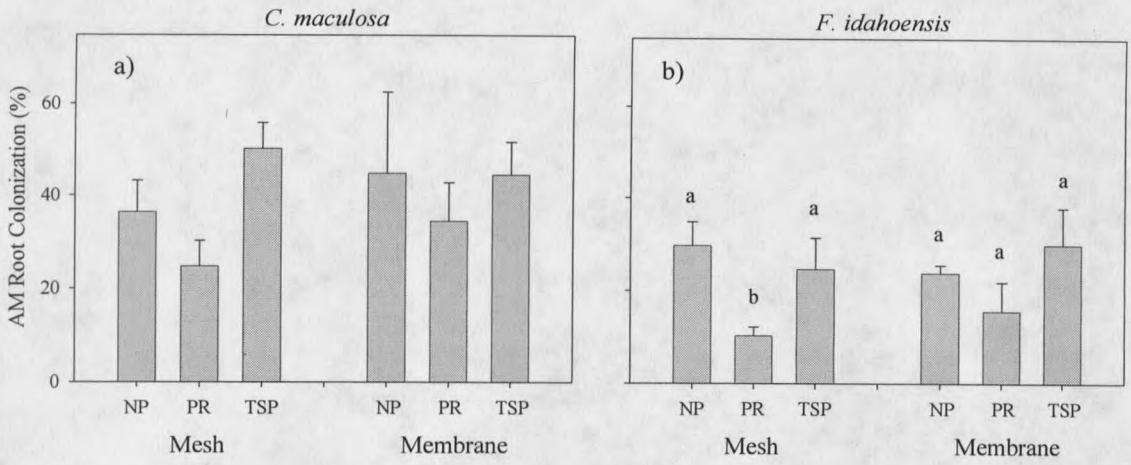

Figure 3a, b. Percent AM colonization for each plant species; bars represent the standard error of the mean and letters denote significant differences at P<0.05.

Roots of *C. maculosa* contained more vesicles than roots of *F. idahoensis* ($F_{1, 27}$=25.8, P<0.001); and both species contained more vesicles in their roots when amended with TSP than amended with NP or PR ($F_{2, 27}$=5.3, P=0.012; Table 2). The proportion of roots with vesicles was not affected by barrier types ($F_{1, 27}$=1.6, P=0.2). The proportion of plant roots with arbuscules did not differ by plant species ($F_{1, 25}$=1.8, P=0.19), but was affected by P treatment ($F_{1, 25}$=8.8, P=0.001), with PR-amended plants having less arbuscule formation than plants in TSP-amended pots (see Table 2). The proportion of roots with arbuscules was similar between barrier types ($F_{1, 25}$=0.5, P=0.5).

Mean ERH lengths were similar to those reported by Abbott and Robson (1985) and Abbott et al. (1984). *Centaurea maculosa* had more PC ERH than *F. idahoensis*

($F_{1, 36}$=13.6, P=0.001; Figure 4), and plants in membrane-divided pots had more PC ERH than plants in mesh-divided pots (33.2 and 28.4 m g^{-1} dry soil respectively; $F_{1, 36}$=3.1, P=0.09). Phosphorus treatment had an effect on PC ERH lengths for both species ($F_{2, 36}$=22.8, P<0.001). Pots amended with TSP had approximately 120% more PC ERH than NP- or PR-amended pots. Plants in mesh-divided pots had more PC ERH when amended with either NP or PR than plants in the membrane-divided pots exposed to the same P treatments (barrier x treatment interaction; $F_{2, 36}$=2.7, P=0.088).

Lengths of HC ERH were much greater with *C. maculosa* than with *F. idahoensis* (0.47 and 0.09 m g^{-1} dry soil respectively; $F_{1, 31}$=17.1, P<0.001). In mesh-divided pots, *C. maculosa* had nearly 3.5 times more HC ERH than *F. idahoensis* ($F_{1, 14}$=12.5, P=0.003; Figure 5). Hyphal compartment ERH lengths were not affected by P treatment in mesh-divided pots ($F_{2, 14}$=0.2, P=0.8).

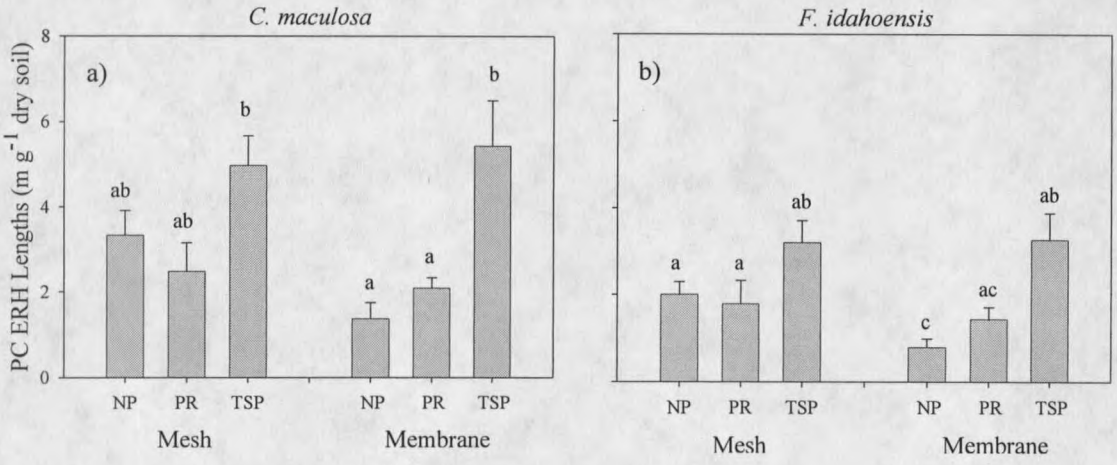

Figure 4 a, b. PC ERH lengths; bars represent the standard error of the mean and letters denote significant differences at P<0.05.

Membrane barriers effectively reduced HC ERH development with 0.07 and 0.52 m g^{-1} dry soil in membrane- versus mesh-divided pots respectively ($F_{1, 41}$=28.6, P<0.001). Extra radical hyphae were not expected in the HC of membrane-divided pots. The very low levels of ERH detected may be the result of contamination of soil samples during the coring or the extensive filtration process. Alternatively, hyphae of non-AM fungi may have been misidentified and counted as AM hyphae.

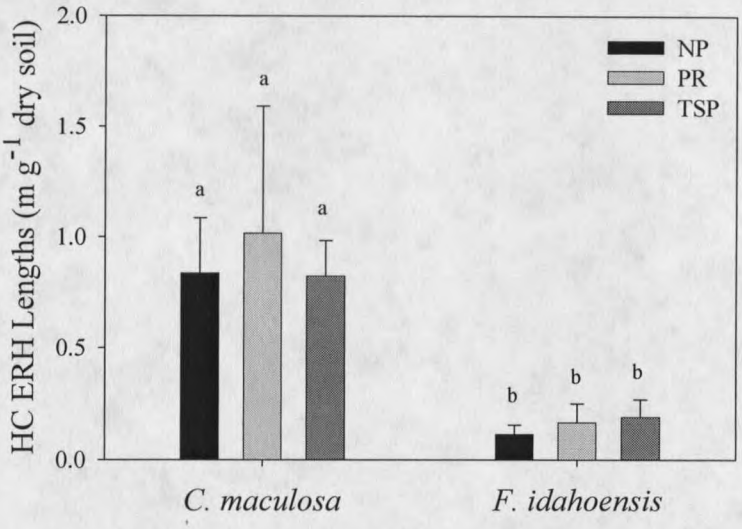

Figure 5. HC ERH lengths in mesh-divided pots; bars represent the standard error of the mean and letters denote significant differences at P<0.05.

Discussion

Defining mechanisms contributing to *C. maculosa*'s competitive success is necessary to reduce its negative impacts on North American grass- and rangeland ecosystems. LeJuene and Seastedt's (2001) speculation that *C. maculosa* may be better able to preempt P from neighboring plants was supported by Zabinski et al. (2002), who

report that AM increase *C. maculosa* P uptake to a greater degree than for native grasses. Identifying the mechanisms by which AM assist *C. maculosa* in P preemption remains critical to understanding the relationship between AM and this invasive forb.

P Sources

This experiment was designed to test the hypothesis that *C. maculosa* may access more P from a distant source through AM hyphae than *F. idahoensis*. We did not anticipate a high degree of P mobility in the sand, because the large pore sizes were expected to reduce water holding capacity and therefore any dissolved P. Additionally, PR is considered highly insoluble due to low available P content and lack of chemical or physical refinement (Waggaman 1952a). Because TSP is manufactured to be highly water-soluble (Waggaman 1952a), we expected this treatment to produce the largest amount of dissolved P, however this was not the case. In my soil system, the lack of sufficient organic matter and CEC sites (Table 1) likely caused the TSP to be less soluble than is normally observed in the field, although this logic could also be applied to the PR amendment (Chein and Menon 1995). However, the high P application rate (142 kg/ha) may have caused a higher percentage of PR to solubilize (N. Comerford, pers. comm.), and the P-deficient soil in the PC could have created a diffusion gradient strong enough to move soluble P through the large-pored growth medium. Due to the differential P movement between P treatments, we inadvertently created high- and low-P environments in the pots.

Plant P Utilization

Festuca idahoensis did not increase its above- or belowground biomass, total P content, or P concentration as P availability increased, indicating that plants were either limited by another nutrient, or were unable to take advantage of increased P availability. Plant species native to low-nutrient soils generally exhibit low nutrient demand, low relative growth rates, and low nutrient absorption rates (Aerts and Chapin 2000). Like many other native grassland species, *F. idahoensis* is adapted to the low nutrient environments characteristic of Montana grasslands and may not be able to take advantage of the pulse of P that occurred in PR-amended pots.

In contrast, *C. maculosa* increased its total P content considerably in the PR treatment. Although *C. maculosa*'s biomass increased slightly with soil P content (35% higher in PR-amended pots over TSP-amended pots), the increased plant P content (235% higher in PR versus TSP pots) suggests luxury consumption of P. Plants exhibiting luxury consumption may be able to store that nutrient for later use (van Iersel et al. 1998), although Burns (1992) found limited P redistribution in lettuce plants, with the vast majority of P stored in vacuoles, possibly for regulation of osmotic gradients. Alternatively, exploiting pulses of P may be a plant strategy to preempt the availability of P for its neighbors rather than for its own benefit (Hetrick et al. 1994a), although the usual result is increased biomass production in the plant (Aerts and Chapin 2000, Davis et al. 2000), which we did not observe in this study.

Luxury consumption of P may be a way for plants to increase seed quality and production (Stanley et al. 1993, Shumway and Koide 1995, Sanders and Koide 1994) and

offspring fitness (Heppell et al. 1998, Zhu and Smith 2001, LeJeune and Seastedt 2001, Allison 2002). Moreover, increased P uptake in mycorrhizal plants decreases the time to initiate flowering (Shumway and Koide 1994), increases the duration of flowering, and increases the number of flowers that produce fruit (Lu and Koide 1994). Considering the high rate of seed production exhibited in *C. maculosa* (Eddleman and Romo 1988), improving offspring size and competitive ability could be crucial for its early season germination and establishment.

AM Utilization

The role of AM in increasing host plant P acquisition has been clearly documented (Powell and Daniel 1978, Hetrick et al. 1990, Hetrick et al. 1994a, Jakobsen 2001). Because P moved across the barriers in the PR-amended pots, we were unable to quantify the role of AM for acquiring P from a distant source. However, the AM symbionts of *C. maculosa* produced 1.5 times more ERH in the rooting zone and 3.5 times more ERH at a distance of 5+ cm from the plant than the symbionts of *F. idahoensis*. This increase in ERH lengths represents increased soil volume explored and potential pool of nutrients for the host plant. This suggests that *C. maculosa* increases its competitive advantage over the native bunchgrass through its AM partnership, thereby enhancing its invasive potential.

The more effective *C. maculosa*-AM symbiosis may be the result of *C. maculosa* providing a larger portion of its photosynthetically-derived C to its fungal symbionts than the native *F. idahoensis*. For example, more vesicles, which are fungal carbon storage

structures, were produced in *C. maculosa* than *F. idahoensis* roots. For this and other reasons, the fungal symbiont may function differently with different host plants. Bever et al. (1996) found that sporulation rates of a single AM fungal isolate differed depending on the host plant. Alternatively, the hyphal architecture of AM fungi determines the distance from the root available for foraging of soil nutrients by the AM fungi (Dodd et al. 2000, Koide 2000). Arbuscular mycorrhizal fungal communities differ between individual plant species (Bever 2002), indicating that *C. maculosa* may form symbiotic relationships with different fungal species than does *F. idahoensis*, and may form AM with fungal species that extend further from the root surface.

Increases in plant-available P in the rooting zone diminish the role of AM hyphae in P acquisition (Hetrick et al. 1990, Hetrick et al. 1994). In my study, the increase in available P in the PC due to PR solubilization reduced the plant's reliance on its AM symbiont for P uptake. Arbuscular mycorrhizal colonization levels decreased 55% in *F. idahoensis* and 24% in *C. maculosa* between NP- and PR-amended treatments. Also, root arbuscule content, structures considered responsible for P transfer between the fungus and the plant, were 2 and 3 times higher in NP- and TSP-treated plants than in PR-treated plants, respectively.

Plant N

Plants growing in mesh-divided pots had lower N content and higher total ERH development than those in membrane-divided pots. Considering that 6% of fungal chitin is N (Leake and Read 1990), the reduction in plant N content could be the result of N

transfer to the fungus to support ERH development. However, because the N levels differed by only 0.1 mg plant^{-1} between barrier types, it is unlikely that this difference could have an effect on plant performance in the field or greenhouse, since wild plants are adapted to low N conditions and fluctuations in N availability are experienced often in natural settings (Aerts and Chapin 2000, Davis et al. 2000).

My research suggests several alternatives regarding AM function depending on host plant species. My study indicates that *C. maculosa* has greater P uptake, different patterns of AM root colonization, and greater ERH lengths and distribution than the native grass, all of which support my hypothesis that *C. maculosa*'s increased P acquisition is the result of its AM symbiosis. The ability of the host plant to more effectively exploit its fungal symbiont may contribute to its competitive success when invading North American grasslands.

DEFOLIATION, NITROGEN, AND PHOSPHORUS EFFECTS ON *CENTAUREA MACULOSA,* TWO NATIVE BUNCHGRASSES, AND ARBUSCULAR MYCORRHIZAE FUNGI

Introduction

Plants experiencing regular defoliation often respond through compensatory growth (Strauss and Agrawal 1999). Compensation is a measure of plant fitness following herbivory. However, because measuring plant fitness is complex, it is difficult to quantify compensatory growth (Maschinski and Whitham 1989). Instead, parameters such as plant biomass and/or seed production are used as surrogate measures of plant fitness (Chapin and McNaughton 1989), and compensation is evaluated by the amount of plant regrowth following herbivory.

A wide array of compensatory responses to herbivory have been documented depending on the plant species, grazing history, timing, duration, and intensity of herbivory, and nutrient availability (Paige 1992, Crawley 1997). Provided nutrients are not limiting, plants grazed early in the growing season have sufficient time before senescence for compensation to occur (Crawley 1997). Herbivory occurring late in the growing season often coincides with limited water and nutrient availability, which reduces the time available for plant regrowth before senescence, reducing the likelihood for compensatory growth.

Ungulate herbivory, in particular, can have detrimental effects on plant productivity and fitness due to the relatively large amount of aboveground biomass removed by grazing, and the negative correlation between the amount of tissue removed

and the amount of plant regrowth (Crawley 1997). Removal of shoot biomass decreases overall leaf area, resulting in a reduction of plant photosynthetic rates (Crawley 1997), and a decrease in the plant's ability to regenerate aboveground tissues. Often a reduction in root biomass occurs following herbivory (Trent et al. 1988), as plants reallocate nutrients (Bardgett et al. 1998) and carbon to the remaining shoot to facilitate regrowth (Dyer et al. 1991).

Herbivory can have potentially beneficial effects on plant productivity, including decreases in plant self-shading and increases in leaf number, tillering, and crown size (Wallace 1987). Plants adapted to herbivory often increase growth rates following herbivory through an increase in nitrogen accumulation (Grime 1979, Holland and Detling 1990). Also, rather than increasing chemical defenses, a reduction in concentration of plant secondary metabolites can occur following herbivory, allowing a greater amount of the plant's resources to go toward regrowth. Increases in fruit and seed production (Paige and Whitham 1987) and nutrient content (Wallace and Macko 1993) after herbivory have also been documented.

The amount of N and P available in the soil at the time of herbivory directly influences the amount of compensatory growth (Bergelson and Crawley 1992 a,b, Bergelson et al. 1996). In fertile systems, plant growth is not limited by nutrients, and increases in biomass compensation often occur (Crawley 1997). However, in unproductive systems, N and P are often limiting and reduce the ability for plants to compensate for tissue loss. In the same context, soil water content can govern plant available N and P (Cox and McEvoy 1983), thereby influencing compensatory growth.

Arbuscular mycorrhizae (AM) significantly influence plant nutrition (Smith and Read 1997) and could affect plant compensation after herbivory (Newingham et al. 2000). As obligate symbionts, AM fungi form a symbiotic relationship with plants and assist the host plant in P uptake (Smith and Read 1997). In return for assisting in nutrient uptake, the plant allocates a portion of its photosynthetically-derived carbon to the fungus.

In infertile environments, AM benefit the host plant by foraging for nutrients beyond the rhizosphere, often accessing nutrients in soils unexplored by the host plant's root system. In fertile systems the benefits of AM are often negated by the abundant nutrients available to the plant directly through their root systems, and by the carbon cost to the plant by the fungi. Because 85% of terrestrial plants form this symbiosis (Smith and Read 1997), many plants impacted by grazing are mycorrhizal. Increased soil nutrient acquisition inherent to the plant-fungal mutualism can assist in plant compensation for herbivore losses. However, when soil nutrient availability is high, the carbon drain by the fungus could reduce regrowth (Eom et al. 2001).

Simulated or natural herbivory may affect AM colonization levels if the host plant shifts carbon allocation away from or to the fungal symbiont. Gehring and Whitham (1994) found that in 23 of the 37 studies (60%) they reviewed, AM colonization levels decreased after simulated or natural herbivory events. The authors speculate that decreases in AM colonization may have profound effects on plant competitive interactions, reducing plant competitive abilities with reduced AM colonization.

Centaurea maculosa Lam. (spotted knapweed), a plant species native to Eurasia, was introduced to the United States in the early 1900s. Since its introduction, *C. maculosa* has invaded over 4 million hectares in the Rocky Mountain region of the United States (Boggs and Story 1987). *Centaurea maculosa* is characterized by its ability to outcompete neighboring plants for nutrient and water resources, forming dense monocultures in areas once dominated by native bunchgrasses (Boggs and Story 1987). The most common management techniques include herbicide application and manual removal (Sheley et al. 1999). With increased awareness of the short- and long-term effects of herbicide application, biological controls have been used to decrease *C. maculosa*'s abundance (Boggs and Story 1987). Grazing management to control *C. maculosa* infestations hinges on grazing effects being more detrimental to the invasive species than to native neighboring species (Olson 1999).

The objectives of my study were: 1) to examine compensatory growth of two native bunchgrasses, *Festuca idahoensis* and *Pseudoroegneria spicata*, and *C. maculosa* under high and low levels of N and P availability; 2) to evaluate the effect of AM on plant response to simulated herbivory; and 3) to assess the effects of simulated herbivory on AM structure and function. Specifically, I hypothesized that compensatory growth would be greatest in high nutrient conditions and that AM would increase plant N and P concentration and compensatory growth in low nutrient conditions, but decrease compensatory growth in high nutrient conditions. Regarding herbivory effects on AM, I hypothesized that AM colonization levels and ERH production would not be affected by herbivory.

Materials and Methods

Experimental Design

My experiment was a randomized, complete block design with 3 plant species, 2 mycorrhizal, 4 nutrient, and 2 herbivory treatments with 10 replicates of each treatment combination. Replicates were divided into blocks placed in a grid pattern to account for variations in temperature, light level, and humidity in the greenhouse. Plants were grown individually in 38 cm tall by 10 cm diameter PVC pots. An 8:1 mix of sand (masonry grade silica sand, 20:30 grit) and field soil was used. The field soil was taken from rangeland on Red Bluff Research Ranch in southwestern Montana (45.60°, 111.50°). Initial, ambient levels of N and P in the 8:1 sand:soil mix were 0.134 and 3.93 mg kg^{-1} soil, respectively.

Plant Species

Two native bunchgrasses, *Festuca idahoensis* Elmer (Idaho fescue) and *Pseudoroegneria spicata* [Scribn. & Smith] A. Love (bluebunch wheatgrass), and the invasive forb, *Centaurea maculosa* (spotted knapweed) were used in this study. Plant communities where these cool season grasses are common are often invaded by *C. maculosa* in the western United States (Tyser and Key 1988).

Planting

Four seeds of a single species were directly seeded into each pot, with ½ cm of sterilized sand applied on top of the seeds. *Festuca idahoensis* was planted seven days earlier than *P. spicata* and *C. maculosa* because it takes longer to germinate. Pots were watered twice daily for two weeks after seedling germination when watering dropped to once daily. A ⅛-strength modified Hoagland's solution was added twice at 50 ml per pot during seedling establishment to reduce potential nutrient stress. After a total of four weeks of growth, seedlings were large enough to initiate nutrient treatments.

Mycorrhizae

The field soil served as mycorrhizal inoculum for AM treatments. The soil mix for the non-AM treatment was pasteurized at 80 °C for 90 minutes to eliminate viable mycorrhizal inoculum sources and other soil biota (Thompson 1990). A microbial wash was added to pasteurized soils to reintroduce naturally occurring soil biota, by filtering the non-pasteurized field soil through a series of sieves, ending with an 11 μm sieve (modified method of Johnson 1993). The resultant slurry was applied at 50ml per non-AM treatment pot immediately after seeding.

Nutrient Treatments

Nutrient treatments included two levels of nitrogen (N) and phosphorus (P): low N/low P, low N/high P, high N/low P, and high N/high P. For P additions, triple super phosphate (TSP; available P_2O_5 46%; A.H. Hoffman, Inc. Lanchaster, PA, USA) was

ground to a fine powder and added to the pot approximately 4 cm from the soil surface prior to seeding. For pots receiving low P, 0.15 g TSP was added while pots receiving high P had 0.607 g TSP added. These P levels were based on rangeland soil P levels and fertilizer recommendations for sandy soils (MSU Extension Service, 1991), to attain 30 and 120 kg/ha respectively.

Nitrogen was added in solution through a drip irrigation system. The amounts of N added were based on field and lab nitrogen mineralization rates found by Holland and Detling (1990). Low N levels were applied at a rate of 0.0168 mmols N pot^{-1} day^{-1}, and high N pots received four times that amount, or 0.067 mmols N pot^{-1} day^{-1}, or a total for the study of 0.4704 and 1.876 mmols N pot^{-1} added for low and high N treatments respectively. Nitrogen was added as KNO_3 and $CaNO_3$ to a ⅛-strength modified Hoagland's solution. Nutrient solutions were administered every third day for four weeks and every other day for the remaining eight weeks of the experiment.

Herbivory Treatments

After 4 weeks of initial plant growth and 7 weeks of nutrient treatments (11 weeks total), half of all treatment combinations were clipped to simulate ungulate herbivory. Grasses were clipped to remove 75% of aboveground biomass (Lommasson and Jensen 1938; US Dept. of Interior 1984). For *C. maculosa*, 75% of its aboveground biomass was visually estimated and removed. Clipped biomass was dried at 60 °C for 48 hours, weighed, and recorded.

Greenhouse Environment

The greenhouse was maintained at 21 °C and 16 °C during the day and night respectively, with a photoperiod of 16 hours for the first 8 weeks of plant growth, after which time the natural day lengths reached approximately 16 hours. Lighting was supplemented with 10 GE Multi-Vapor MVR1000/C/U (GE Lighting, General Electric Company, Nela Park, Cleveland, OH 44112).

Harvesting

Plants were harvested after 16 weeks, separated into roots and shoots, and thoroughly washed of soil and debris. Plants were dried at 40 °C until a constant mass was reached. Soil cores (38 cm x 2.5 cm) for extra radical hyphae (ERH) quantification were taken from each pot before plants were removed. Bulk soil from each pot was thoroughly mixed after plants were removed and approximately 200 g was used for soil nutrient analysis.

After recording biomass, plant roots were re-hydrated for 60 seconds prior to removing small segments for determine AM colonization levels. Excised roots were cleared of pigments in 2.5% KOH, acidified using 3.0% HCl, and stained using 0.5% Trypan Blue. Root segments were mounted on microscope slides and measured for AM colonization using the magnified intersections method (McGonigle et al. 1990).

Nutrient analyses were conducted on 8 blocks of plant tissues after grinding in a UDY mill (Model #3010-030; Fort Collins, CO, USA) to 0.25 μm. Nitrogen content was analyzed on shoot and root tissues separately using mass spectrometry (University of

California, Davis; Stable Isotope Facility, Davis, CA, USA). Shoot and root tissues were combined for individuals and analyzed for P content, using Inductively Coupled Plasma Spectrometry (ICP; MDS Pharma Services, Lincoln, NE, USA). Soil N and P levels were determined using cadmium reduction and Olsen P methods, respectively (Klute 1986).

Extra radical hyphae (ERH) length (m g^{-1} dry soil) was quantified for 3 blocks of pots with a modified approach developed by Miller et al. (1995). Two 5-g soil subsamples were placed in 100 ml of 3.75% sodium hexametaphosphate for 12 hours to dissociate soil particles and hyphae. Each subsample was stirred for 5 minutes, and a 10 ml aliquot was added to 100 ml of distilled water and stirred for 30 seconds. A 20 ml aliquot was vortexed for 30 seconds, filtered through a 20 μm filter, and placed in a 20 ml centrifuge tube with 5 ml of 0.5% Trypan Blue. The filter and stain were vortexed for 30 seconds, allowed to sit for 5 minutes, and then vortexed again for 30 seconds. The solution was filtered through a 0.45 μm membrane filter, which was subsequently mounted on a microscope slide. Extra radical hyphae lengths were measured using the gridline intercept method (Newman 1966) at 200x magnification. Arbuscular mycorrhizal hyphae were distinguished from other soil fungi using criteria delineated by Nicolson (1959), Mosse (1959), and Sylvia (1992).

Total biomass was calculated as the sum of shoot and root tissues obtained at the time of harvest, and total yield was the sum of the total biomass at the time of harvest plus the amount of biomass removed four weeks earlier from clipped plants. Root mass ratio was calculated as root biomass divided by total biomass.

Mycorrhizal Effectiveness

Mycorrhizal effectiveness measures the benefit or detriment of being mycorrhizal in each treatment scenario. Mycorrhizal effectiveness (ME) was calculated for total biomass, total yield, plant N concentration and content, and plant P concentration and content according to the equation of Van der Heijden and Kuyper (2001) as:

$$ME = 1-(b/a)$$

where a is the value of the variable for the AM individual and b is the value of the variable of the non-AM individual of the same treatment combination in the same block.

Data Analysis

Each plant species was analyzed separately using 4-way analysis of variance (SPSS General Linear Model Univariate ANOVA, SPSS, Inc. Version 11.5). The model included 2- and 3-way interactions of AM, herbivory, and nutrient treatments, while blocking was solely a main effect to account for variance generated by the location of the pots in the greenhouse. Extra radical hyphae length was analyzed as a 1-way ANOVA for plant species, and as 3-way ANOVAs for plant species separately using the same model as described above. Relative biomass was analyzed as a 4-way ANOVA with species, AM, nutrient and clipping as main effects. Variables were transformed as necessary to pass assumptions of ANOVA. A series of Mann-Whitney non-parametric tests compared ME scores and colonization levels across plant species, because these data sets could not be transformed to pass homogeneity of variance tests for ANOVA.

Mycorrhizal effectiveness, AM colonization levels, and ERH lengths analyses included AM plants only for ANOVAs and non-parametric tests. All main effects and interactions with P-values less than 0.10 are reported. Post-hoc comparisons were conducted using Bonferroni comparisons with α=0.05. Soil nutrient levels (NO_3, NH_4, and Olsen P) were analyzed with student's t-tests.

Results

Soil Nutrients

Soil N (NO_3^- and NH_4^+) and P levels determined at the time of harvest are given in Table 3 below. Olsen P levels were significantly different between high and low P treatments. Soil NO_3^- and NH_4^+ levels were similar between high and low N treatments.

Table 3. Soil N and P analyses for high and low nutrient treatments: Mean ± (SE); different letters denote significant differences within a row at P≤0.05.

	High	Low	n
NO_3 (mg kg^{-1})	4.99 (0.23) a	5.02 (0.27) a	18
NH_4 (mg kg^{-1})	3.88 (0.19) a	4.14 (0.15) a	18
Olsen P (mg kg^{-1})	7.8 (1.15) a	2.3 (0.23) b	18

Total Biomass

Total biomass (g) of all three plant species was affected by AM, clipping and nutrient treatments (Table 4; Figure 6). In *C. maculosa,* non-AM plants had 39% more biomass than AM plants. Non-AM *F. idahoensis* and *P. spicata* plants were approximately twice as large as AM plants.

Table 4. Analysis of variance of total biomass and total yield for *C. maculosa, F. idahoensis,* and *P. spicata.*

	Total Biomass				Total Yield		
C. maculosa	d.f.[1]	MS	F	P	MS	F	P
AM	1	11.68	294.04	0.000	445.40	216.12	0.000
Nutrient	3	2.02	50.76	0.000	65.14	31.61	0.000
Clipping	1	9.93	250.11	0.000	154.72	75.07	0.000
Block	9	0.09	2.23	0.024	4.21	2.04	0.039
AM x Nutrient	3	0.81	20.48	0.000	15.45	7.50	0.000
AM x Clipping	1	0.21	5.28	0.023	0.04	0.02	0.897
Nutrient x Clipping	3	0.00	0.07	0.978	4.15	2.01	0.115
AM x Nutrient x Clipping	3	0.03	0.81	0.489	3.13	1.52	0.213
Error	134	0.04			2.06		
F. idahoensis							
AM	1	57.07	127.38	0.000	57.07	126.02	0.000
Nutrient	3	1.98	4.42	0.005	1.98	4.37	0.006
Clipping	1	29.94	66.82	0.000	28.85	63.69	0.000
Block	9	1.13	2.52	0.011	1.13	2.49	0.012
AM x Nutrient	3	0.87	1.95	0.125	0.87	1.93	0.128
AM x Clipping	1	0.03	0.07	0.789	0.08	0.17	0.678
Nutrient x Clipping	3	0.01	0.03	0.992	0.03	0.06	0.982
AM x Nutrient x Clipping	3	1.11	2.47	0.065	1.23	2.72	0.047
Error	133	0.45			0.45		
P. spicata							
AM	1	7.59	120.11	0.000	10.83	156.44	0.000
Nutrient	3	2.76	43.61	0.000	4.07	58.75	0.000
Clipping	1	18.89	298.81	0.000	10.43	150.64	0.000
Block	9	0.27	4.27	0.000	0.29	4.21	0.000
AM x Nutrient	3	0.32	5.09	0.002	0.39	5.59	0.001
AM x Clipping	1	0.27	4.34	0.039	0.13	1.81	0.181
Nutrient x Clipping	3	0.09	1.40	0.246	0.24	3.44	0.019
AM x Nutrient x Clipping	3	0.04	0.64	0.593	0.05	0.77	0.516
Error	134	0.06			0.07		

[1]degrees of freedom are consistent between plant N concentration and plant N content analyses for each species.

For all species, clipped plants had less total biomass than unclipped plants. In *C. maculosa,* clipping reduced total biomass by more than 40%, whereas clipped *F. idahoensis* and *P. spicata* plants were less than half the size of unclipped plants (Table 5). Total biomass was approximately 2 times greater in high N-treated versus low N-treated plants for all three species. Phosphorus level did not affect total biomass for any species.

Table 5. Total biomass, total yield, and RMR clipping by AM treatment means and standard error of the mean for *C. maculosa, F. idahoensis,* and *P. spicata.*

		C. maculosa	***F. idahoensis***	***P. spicata***
Total Biomass		Mean ± (SE)	Mean ± (SE)	Mean ± (SE)
AM	Unclipped	6.14 ± (0.34) a	1.09 ± (0.09) a	6.24 ± (0.35) a
	Clipped	3.48 ± (0.21) b	0.43 ± (0.04) b	2.90 ± (0.20) b
Non-AM	Unclipped	9.52 ± (0.34) c	3.37 ± (0.27) c	8.75 ± (0.40) c
	Clipped	6.23 ± (0.24) a	1.40 ± (0.13) a	4.82 ± (0.21) d
Total Yield				
AM	Unclipped	6.14 ± (0.34) a	1.09 ± (0.09) a	6.24 ± (0.35) a
	Clipped	4.18 ± (0.24) b	0.59 ± (0.05) b	3.71 ± (0.24) b
Non-AM	Unclipped	9.52 ± (0.34) c	3.36 ± (0.28) c	8.75 ± (0.40) c
	Clipped	7.52 ± (0.30) d	1.99 ± (0.17) d	6.24 ± (0.26) a
RMR				
AM	Unclipped	0.65 ± (0.01) a	0.39 ± (0.01) a	0.62 ± (0.01) a
	Clipped	0.68 ± (0.01) b	0.42 ± (0.01) a	0.62 ± (0.01) a
Non-AM	Unclipped	0.65 ± (0.02) a	0.49 ± (0.01) b	0.63 ± (0.01) a
	Clipped	0.76 ± (0.01) c	0.48 ± (0.01) b	0.69 ± (0.01) b

For *C. maculosa* and *P. spicata,* the AM by nutrient interaction was significant (Table 4; Figure 6a,e); in low N treatments there was a greater difference in biomass between non-AM plants and AM plants (54% and 44% for *C. maculosa* and *P. spicata* respectively), whereas in high N treatments, there was a smaller reduction in biomass

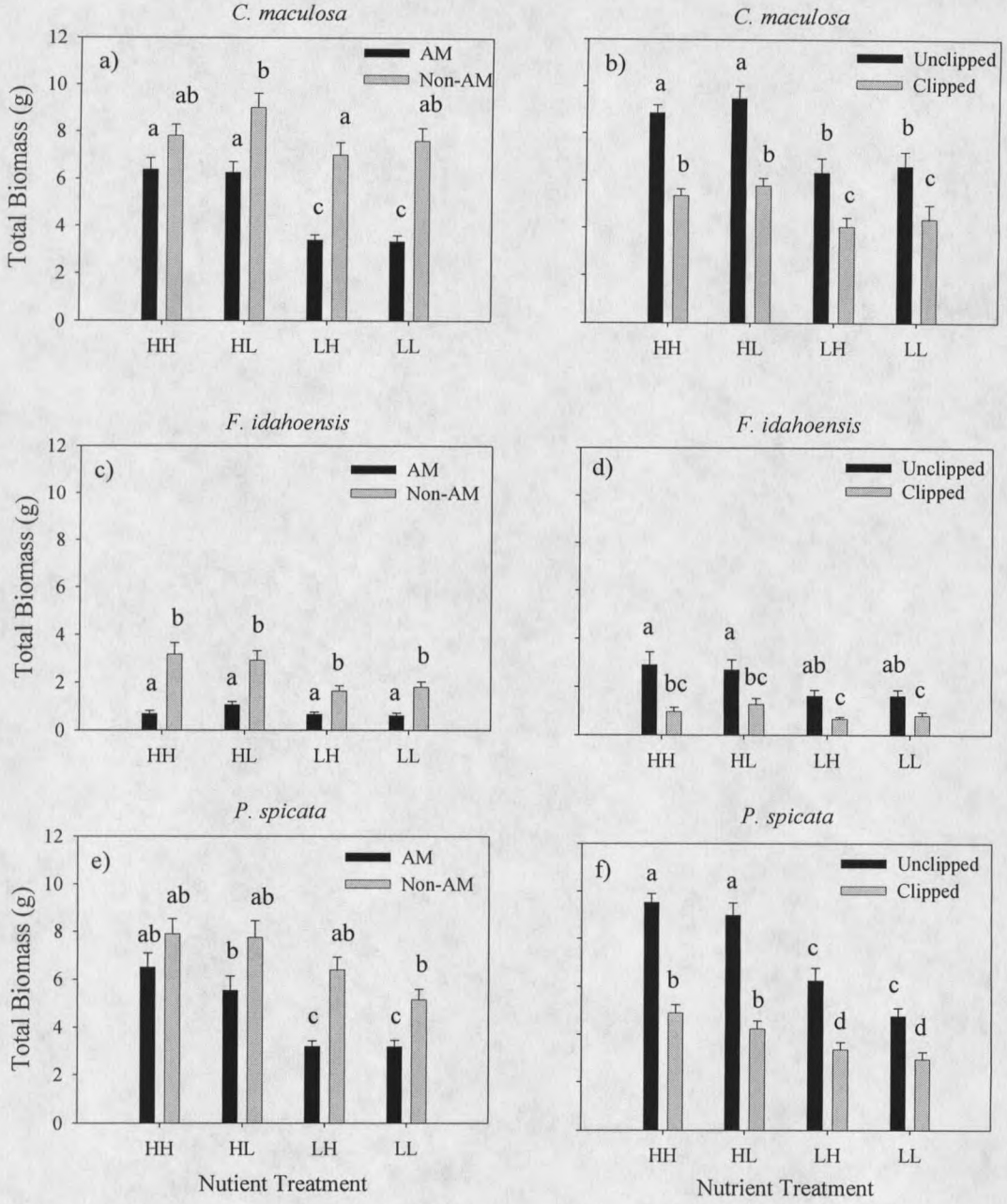

Figure 6. Total biomass (g) for each species between AM, clipping, and nutrient treatment interactions. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent standard error of the mean and different letters denote significant differences in a single species at $P<0.05$.

between non-AM and AM plants (24% for both species). An AM by clipping treatment interaction was detected in *C. maculosa* and *P. spicata*, with a larger reduction in total biomass in AM plants relative to non-AM plants for unclipped (43% and 53% reduction respectively) than clipped treatments (35% and 45% reduction respectively; Table 5).

Relative Biomass

Relative biomass was quantified as the ratio of the clipped plant biomass to the unclipped plant biomass within the same treatment and block. Relative biomass measurements indicated that compensatory growth of each plant species was unaffected by AM or nutrient treatments (Table 5). Because my clipping treatment was a 75% removal, clipped plants were 25% the size of their unclipped counterparts at the point of clipping. At the time of harvest 28 days later, clipped *C. maculosa* plants were 62% of unclipped *C. maculosa* plants, clipped *F. idahoensis* plants were 41% of unclipped plants, and clipped *P. spicata* plants were 51% of unclipped plants, indicating an increase in plant growth rate after clipping (Figure 6).

Table 6. Analysis of variance of relative biomass for *C. maculosa, F. idahoensis,* and *P. spicata.*

	d.f.	MS	F	P
Species	2	0.51	7.83	0.001
AM	1	0.41	6.17	0.014
Nutrient	3	0.09	1.44	0.233
Spp * AM	2	0.03	0.39	0.681
Spp * Nutrient	6	0.01	0.16	0.988
AM * Nutrient	3	0.19	2.90	0.036
Spp * AM * Nutrient	6	0.07	1.042	0.399
Error	207	0.07		

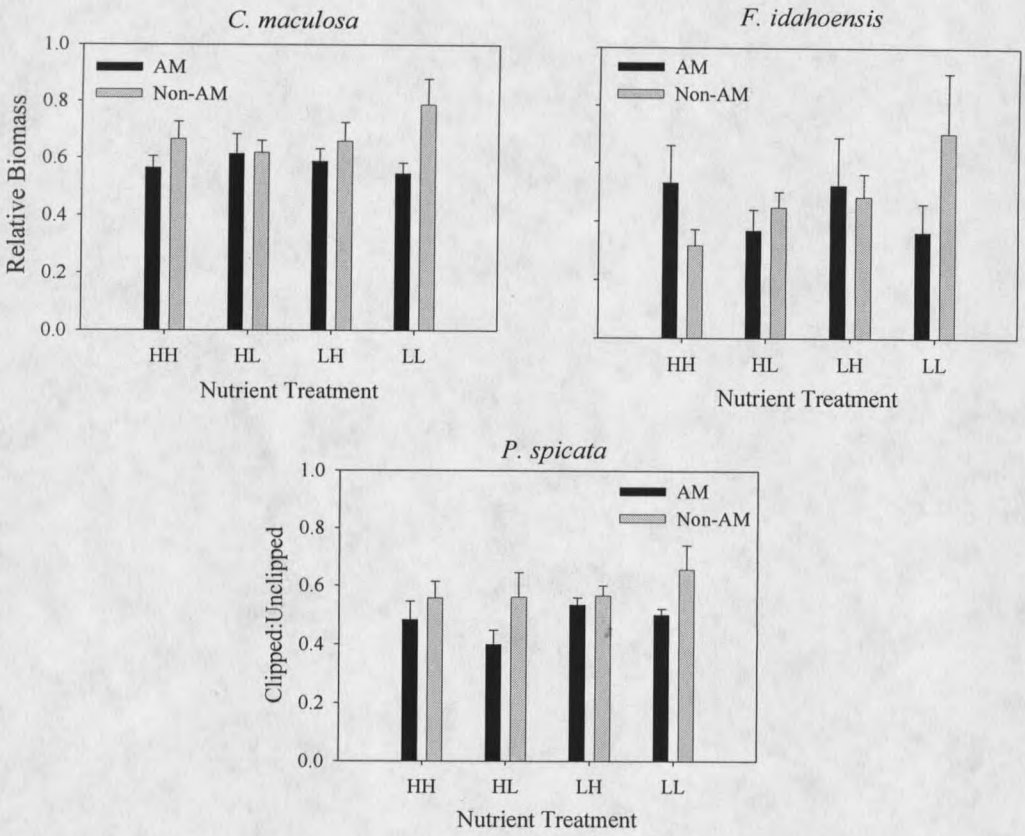

Figure 7. Relative biomass ratio of each species by AM and nutrient treatments. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent standard error of the mean and different letters denote significant differences in a single species at $P<0.05$.

Total Yield

Total yield for each plant species was affected by AM, clipping, and nutrient treatments (Table 5), and followed a pattern similar to total biomass. In *C. maculosa*, AM plants had 40% lower total yield than non-AM plants, and clipping reduced total yield by 25% (Figure 8a,b). Total yields of *P. spicata* and *F. idahoensis* were also lower with AM (34% and 68% respectively) and as expected total yields of clipped plants were

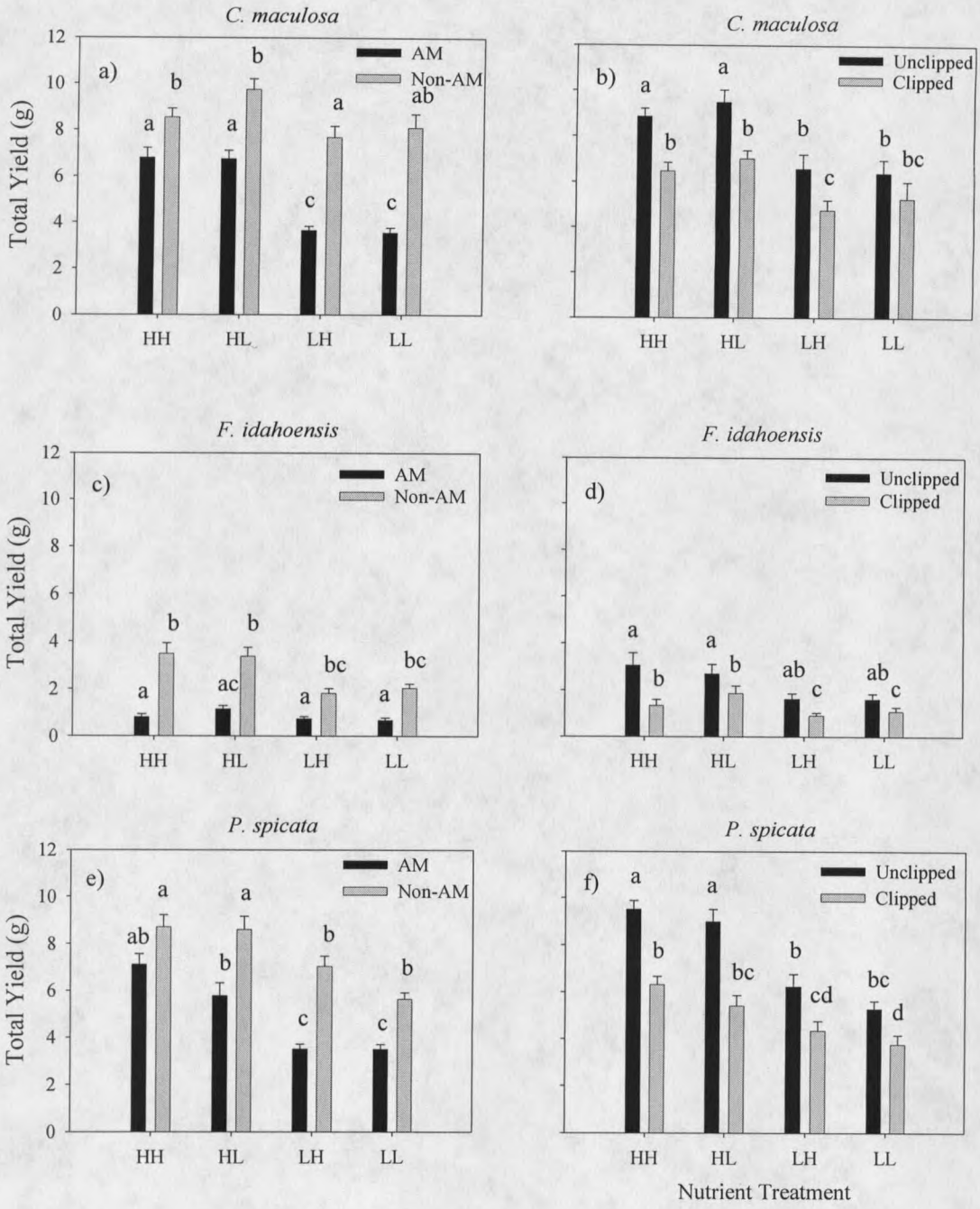

Figure 8. Total yield (g) for each species between AM, clipping, and nutrient treatment interactions. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent standard error of the mean and different letters denote significant differences in a single species at $P<0.05$.

lower (34% and 42% respectively; Table 6). All plant species showed greater total yield when grown in high N-treated than low N-treated pots.

For *C. maculosa* and *P. spicata*, an AM by nutrient treatment interaction was identified that was not evident with *F. idahoensis* (Table 5, Figure 8a,c,e). *Centaurea maculosa* and *P. spicata* AM plants grown in low N treatments were significantly smaller (54% and 45% respectively) than non-AM plants in low N treatments. The difference between the total yield of AM and non-AM plants grown in high N treatments was much less (25% for both species). *Pseudoroegneria spicata* was also affected by a clipping by nutrient treatment interaction (Table 5; Figure 8f); there was less of a difference (26%) in total yield between clipped and unclipped plants in low N versus the difference (31%) in high N-treated plants.

Root Mass Ratio

For all plant species, root mass ratio (RMR; root biomass/total biomass) was affected by AM, clipping, and nutrient treatments (Table 7; Figure 9). Non-AM plants had higher RMR than AM plants and clipped plants had consistently higher RMR than unclipped plants across all plant species. Plants grown in high N conditions had lower RMR than plants grown in low N pots. Root mass ratios were not affected by P levels. The clipping by AM interaction was significant for all three species; AM plants had similar RMR between clipping treatments, whereas clipped non-AM plants had larger RMR than unclipped non-AM plants (Table 6), indicating reallocation of root biomass in non-AM plants following clipping.

Table 7. Analysis of variance for root mass ratio of *C. maculosa, F. idahoensis,* and *P. spicata.*

	Root Mass Ratio			
C. maculosa	d.f.	MS	F	P
AM	1	0.14	7.34	0.008
Nutrient	3	0.12	6.28	0.001
Clipping	1	0.38	20.22	0.000
Block	9	0.05	2.55	0.01
AM x Nutrient	3	0.01	0.54	0.655
AM x Clipping	1	0.17	8.78	0.004
Nutrient x Clipping	3	0.00	0.09	0.966
AM x Nutrient x Clipping	3	0.01	0.38	0.766
Error	134	0.02		
F. idahoensis				
AM	1	0.24	71.38	0.000
Nutrient	3	0.05	13.97	0.000
Clipping	1	0.01	2.69	0.103
Block	9	0.01	1.44	0.179
AM x Nutrient	3	0.01	1.62	0.189
AM x Clipping	1	0.02	6.26	0.014
Nutrient x Clipping	3	0.00	1.30	0.278
AM x Nutrient x Clipping	3	0.10	2.22	0.089
Error	133	0.00		
P. spicata				
AM	1	0.17	19.94	0.000
Nutrient	3	0.14	16.14	0.000
Clipping	1	0.06	7.46	0.007
Block	9	0.02	2.68	0.007
AM x Nutrient	3	0.01	1.30	0.277
AM x Clipping	1	0.06	7.17	0.008
Nutrient x Clipping	3	0.01	1.20	0.314
AM x Nutrient x Clipping	3	0.01	0.93	0.431
Error	134	0.01		

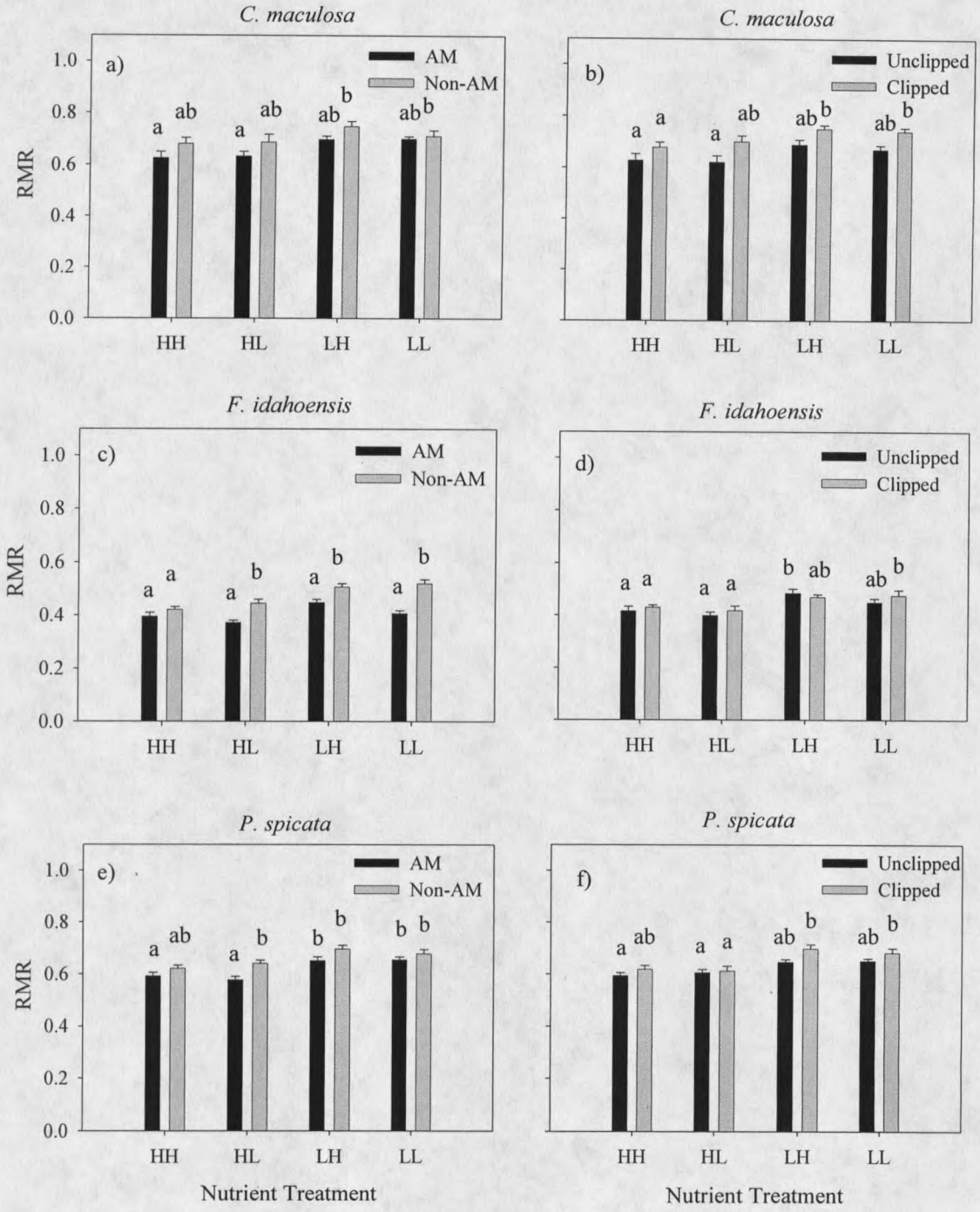

Figure 9. Root mass ratio for each species between AM, clipping, and nutrient treatment interactions. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent standard error of the mean and different letters denote significant differences in a single species at $P \leq 0.05$.

Plant Nitrogen

Plant N concentration (%) was affected by AM, clipping, and nutrient treatments for all three plant species (Table 8; Figure 10). Mycorrhizal plants had 25%, 20% and 22% higher N concentration than non-AM plants of *C. maculosa, F. idahoensis,* and *P. spicata* respectively. Clipped plants had higher N concentration than unclipped plants for all plant species. Plants grown in high N treatments had higher N concentration than plants grown in low N conditions regardless of plant species.

An AM by nutrient interaction occurred with *C. maculosa* and *F. idahoensis* (Figure 10a,c); non-AM plants grown in low N treatments had 34% and 27% lower N concentration than AM plants grown in the same nutrient treatments. In high N treatments, non-AM plants had 17% and 14% lower N concentration than AM plants for *C. maculosa* and *F. idahoensis* respectively.

The AM by clipping treatment interaction was significant for *C. maculosa* and *P. spicata* (Table 9); non-AM *C. maculosa* plants had similar N concentration between clipping treatments, whereas clipped AM plants had higher plant N concentration than unclipped AM plants. For *P. spicata,* the difference in plant N concentration was greater between AM clipped and unclipped plants than was the difference between non-AM clipped and unclipped plants. A nutrient by clipping interaction was significant for *C. maculosa,* with clipped plants grown in high N/high P having higher N concentration than clipped, low N-treated plants and any unclipped plants (Figure 10b).

Table 8. Analysis of variance of plant N concentration (%) and content (g plant^{-1}) for *C. maculosa, F. idahoensis,* and *P. spicata.*

	Plant N Concentration				Plant N Content		
C. maculosa	d.f.[1]	MS	F	P	MS	F	P
AM	1	0.75	96.38	0.000	0.03	66.45	0.000
Nutrient	3	0.27	35.11	0.000	0.07	182.83	0.000
Clipping	1	0.23	28.93	0.000	0.06	145.53	0.000
Block	7	0.02	2.42	0.025	0.00	0.71	0.665
AM x Nutrient	3	0.03	4.26	0.007	0.00	3.46	0.019
AM x Clipping	1	0.12	15.30	0.000	0.00	1.81	0.182
Nutrient x Clipping	3	0.02	2.37	0.075	0.00	0.26	0.858
AM x Nutrient x Clipping	3	0.01	0.87	0.457	0.00	0.77	0.514
Error	104	0.01			0.00		
F. idahoensis							
AM	1	4.61	69.63	0.000	24.96	86.84	0.000
Nutrient	3	0.97	14.74	0.000	3.86	13.42	0.000
Clipping	1	1.21	18.30	0.000	21.26	73.97	0.000
Block	7	0.19	2.99	0.007	0.94	3.26	0.004
AM x Nutrient	3	0.22	3.29	0.024	0.55	1.90	0.134
AM x Clipping	1	0.01	0.13	0.718	0.57	1.97	0.163
Nutrient x Clipping	3	0.07	1.01	0.390	0.11	0.37	0.777
AM x Nutrient x Clipping	3	0.007		0.953	0.40	1.38	0.253
Error	103	0.07			0.29		
P. spicata							
AM	1	2.10	91.88	0.000	0.85	21.45	0.000
Nutrient	3	0.61	26.79	0.000	5.12	130.39	0.000
Clipping	1	2.10	91.88	0.000	6.65	168.58	0.000
Block	7	0.09	3.88	0.001	0.04	0.90	0.507
AM x Nutrient	3	0.02	0.74	0.530	0.16	3.94	0.011
AM x Clipping	1	0.15	6.49	0.012	0.00	0.11	0.739
Nutrient x Clipping	3	0.04	1.85	0.142	0.04	1.01	0.391
AM x Nutrient x Clipping	3	0.00	0.16	0.925	0.04	1.00	0.398
Error	104	0.02			0.04		

[1]degrees of freedom are consistent between plant N concentration and plant N content analyses for each species.

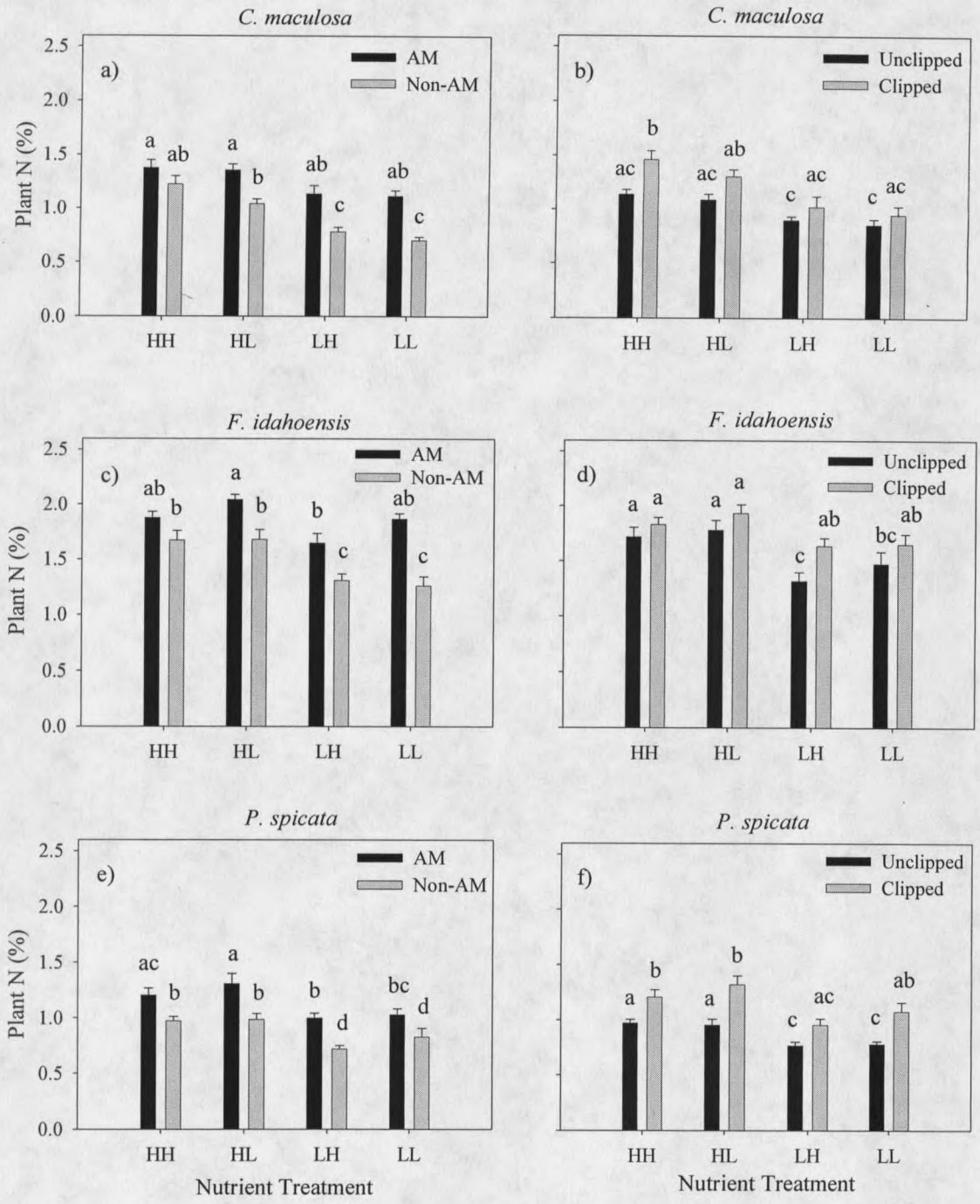

Figure 10. Plant N concentration (%) of each species between AM, clipping, and nutrient treatments. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent the standard error of the mean and different letters denote significant differences in a single species at $P \leq 0.05$.

Table 9. Plant N concentration (%) and content (g plant^{-1}) means and standard error of the mean for *C. maculosa, F. idahoensis,* and *P. spicata* by AM and clipping treatments. Different letters denote significant differences in means for a single species at $P \leq 0.05$.

		C. maculosa	***F. idahoensis***	***P. spicata***
Plant N (%)		Mean ± (SE)	Mean ± (SE)	Mean ± (SE)
AM	Unclipped	1.09 ± (0.03) a	1.77 ± (0.05) a	0.94 ± (0.03) a
	Clipped	1.41 ± (0.05) b	1.95 ± (0.05) b	1.30 ± (0.05) b
Non-AM	Unclipped	0.90 ± (0.04) c	1.37 ± (0.07) c	0.79 ± (0.03) c
	Clipped	0.97 ± (0.06) ac	1.59 ± (0.05) d	0.96 ± (0.05) a
Plant N (g plant^{-1})				
AM	Unclipped	0.07 ± (0.005) a	0.02 ± (0.002) a	0.06 ± (0.005) a
	Clipped	0.05 ± (0.004) b	0.01 ± (0.001) b	0.04 ± (0.003) b
Non-AM	Unclipped	0.08 ± (0.005) c	0.04 ± (0.005) c	0.07 ± (0.004) a
	Clipped	0.06 ± (0.004) ab	0.02 ± (0.002) a	0.04 ± (0.002) b

Plant N content (g plant^{-1}) for all plant species was affected by AM, clipping, and nutrient treatments (Table 8; Figure 11). Plant N content (g plant^{-1}) was 20%, 57%, and 12% higher in *C. maculosa, F. idahoensis* and *P. spicata* non-AM plants than AM plants. Unclipped *C. maculosa* plants had 30% more plant N than unclipped plants. Unclipped *F. idahoensis* plants had 55% more plant N than clipped plants, and unclipped *P. spicata* plants had 37% more plant N than clipped plants. *Centaurea maculosa* and *F. idahoensis* plants grown in high N-treated pots had higher plant N than low N-treated plants and were not affected by P level, whereas *P. spicata* plants grown in high N/high P had higher plant N content than any other nutrient treatment.

In *C. maculosa* and *P. spicata,* AM by nutrient treatment interactions were significant, with AM plants having lower plant N content than non-AM plants under low N conditions (0.035 and 0.053 g plant^{-1} respectively). With high N treatments, AM and non-AM *C. maculosa* plants had comparable plant N content (Figure 11a,e).

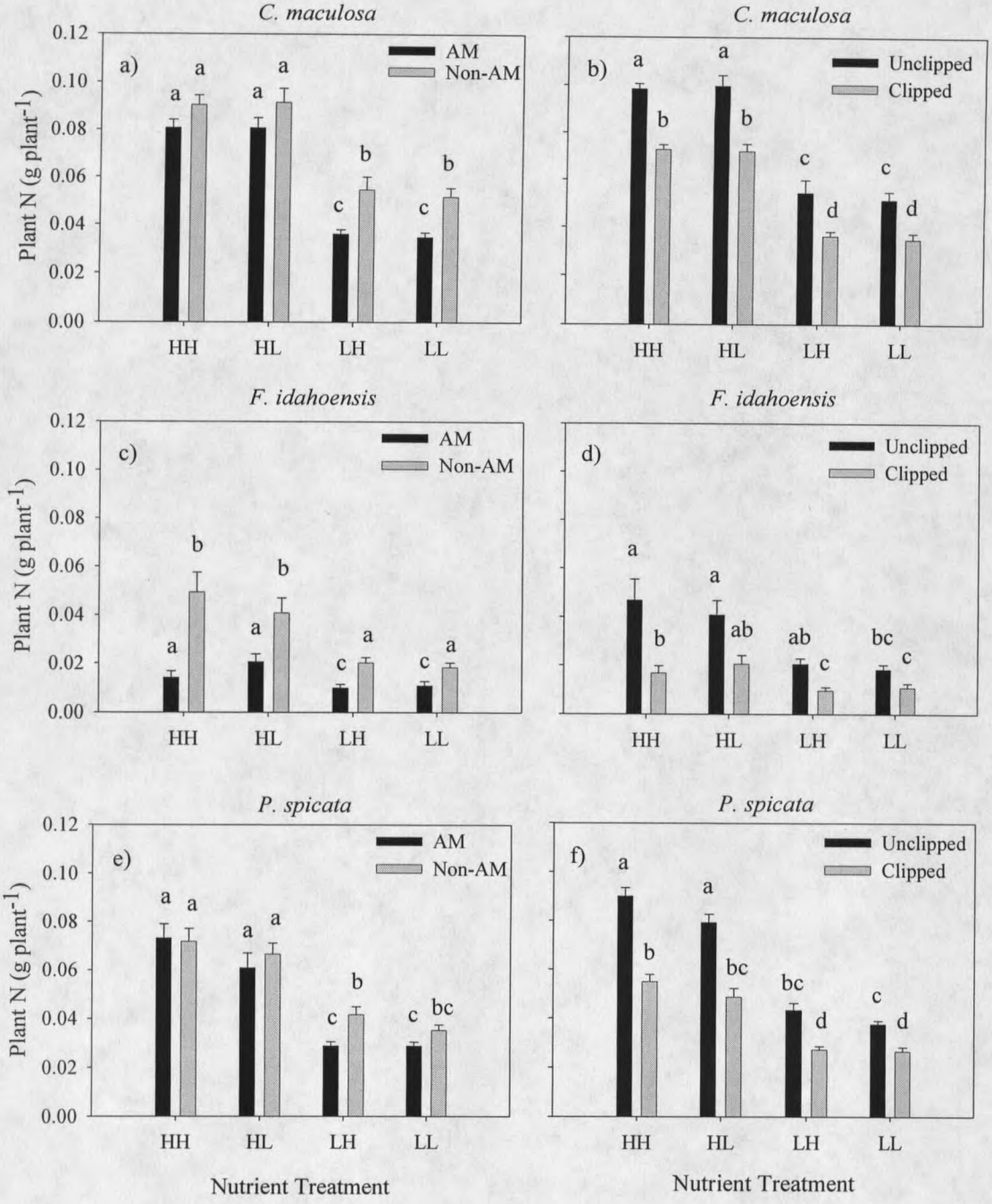

Figure 11. Plant N content (g plant^{-1}) of each species between AM, clipping, and nutrient treatments. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent the standard error of the mean and different letters denote significant differences at $P \leq 0.05$ for each species.

Plant Phosphorus

Plant P concentration (%) of *C. maculosa* and *P. spicata* was affected by AM, clipping, and nutrient treatments (Table 10). In *C. maculosa* and *P. spicata,* AM plants had approximately 15% higher P concentration than non-AM plants. Clipped plants had 30% and 25% higher P concentrations than unclipped *C. maculosa* and *P. spicata* plants respectively. *Centaurea maculosa* grown in high P treatments had higher P concentration than plants grown in low P conditions. Nutrient treatment affected *P. spicata* plant P concentration with plants grown in low N/high P having lower plant P concentration than plants grown in any other nutrient treatment.

Table 10. Plant P concentration (%) and content (g plant^{-1}) means and standard error of the mean for *C. maculosa, F. idahoensis,* and *P. spicata* by AM and clipping treatments. Different letters denote significant differences in means for a single species at P≤0.05.

		C. maculosa	***F. idahoensis***	***P. spicata***
Plant P (%)		Me ± (SE)	Mean ± (SE)	Mean ± (SE)
AM	Unclipped	0.22 ± (0.01) a	0.24 ± (0.01) a	0.15 ± (0.005) a
	Clipped	0.30 ± (0.02) b	0.21 ± (0.01) b	0.21 ± (0.009) b
Non-AM	Unclipped	0.20 ± (0.01) a	0.21 ± (0.01) b	0.14 ± (0.011) a
	Clipped	0.24 ± (0.02) ab	0.25 ± (0.01) a	0.19 ± (0.009) a
Plant P (g plant^{-1})				
AM	Unclipped	0.013 ± (0.0008) a	0.003 ± (0.002) a	0.009 ± (0.0008) a
	Clipped	0.010 ± (0.0007) b	0.001 ± (0.001) b	0.006 ± (0.0005) b
Non-AM	Unclipped	0.018 ± (0.0011) b	0.007 ± (0.006) c	0.011 ± (0.0013) a
	Clipped	0.015 ± (0.0007) a	0.003 ± (0.003) a	0.007 ± (0.0011) b

Table 11. Analysis of variance of plant P concentration (%) and P content (g plant^{-1}) for *C. maculosa, F. idahoensis,* and *P. spicata.*

	Plant P Concentration				**Plant P Content**		
C. maculosa	d.f.[1]	MS	F	P	MS	F	P
AM	1	0.77	8.68	0.004	5.08	48.39	0.000
Nutrient	3	0.84	9.48	0.000	1.35	12.86	0.000
Clipping	1	1.71	19.24	0.000	2.23	21.25	0.000
Block	7	0.25	2.79	0.011	0.22	2.10	0.050
AM x Nutrient	3	0.19	2.12	0.102	0.16	1.56	0.204
AM x Clipping	1	0.05	0.52	0.471	0.10	0.93	0.336
Nutrient x Clipping	3	0.08	0.93	0.428	0.07	0.67	0.574
AM x Nutrient x Clipping	3	0.03	0.28	0.840	0.05	0.51	0.674
Error	104	0.09			0.11		
F. idahoensis							
AM	1	0.001	0.30	0.585	31.53	120.20	0.000
Nutrient	3	0.003	1.33	0.269	1.48	5.63	0.001
Clipping	1	0.003	0.72	0.398	23.50	89.59	0.000
Block	7	0.015	5.60	0.000	0.59	2.26	0.036
AM x Nutrient	3	0.004	1.36	0.261	0.62	2.35	0.078
AM x Clipping	1	0.022	8.53	0.004	0.38	1.46	0.231
Nutrient x Clipping	3	0.002	0.70	0.557	0.11	0.42	0.743
AM x Nutrient x Clipping	3	0.008	2.96	0.036	0.43	1.65	0.183
Error	95	0.003			0.26		
P. spicata							
AM	1	1.40	18.65	0.000	1.29	20.44	0.000
Nutrient	3	3.89	51.71	0.000	0.21	3.33	0.022
Clipping	1	6.22	82.67	0.000	2.27	35.93	0.000
Block	7	0.38	5.07	0.000	0.15	2.39	0.026
AM x Nutrient	3	0.20	2.61	0.055	0.05	0.75	0.526
AM x Clipping	1	0.01	0.15	0.704	0.14	2.24	0.138
Nutrient x Clipping	3	0.17	2.20	0.092	0.02	0.38	0.767
AM x Nutrient x Clipping	3	0.06	0.81	0.493	0.01	0.10	0.958
Error	101	0.08			0.06		

[1]degrees of freedom are consistent between plant P concentration and plant P content analyses for each species.

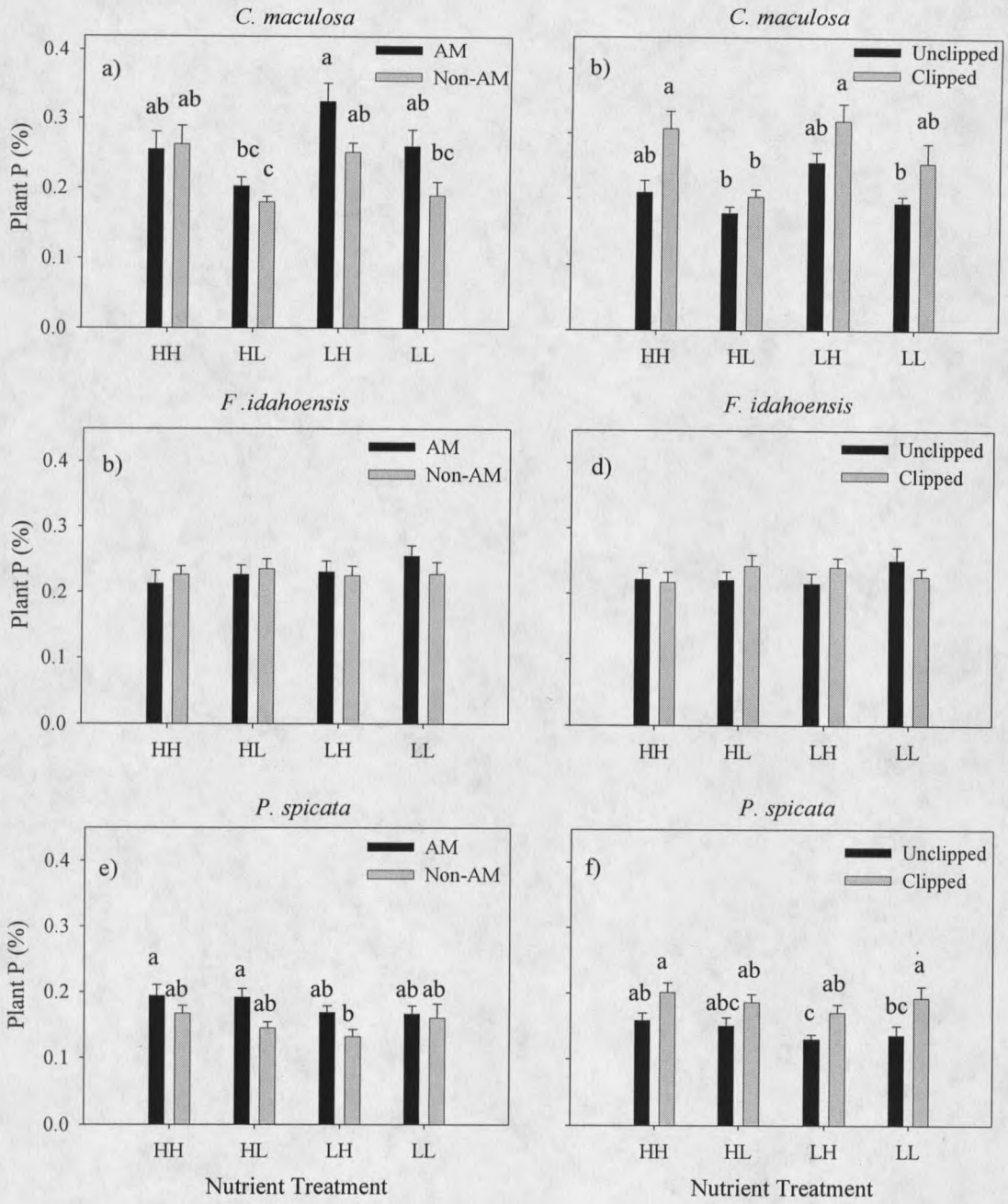

Figure 12. Plant P concentration (%) of each species between AM, clipping, and nutrient treatments. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent the standard error of the mean and different letters denote significant differences at $P \leq 0.05$ for each species.

Although *F. idahoensis* plant P concentration was not affected by AM, clipping, or nutrient treatments as main effects (Table 11), an AM by clipping interaction was identified (Table 10). For AM plants, P concentration decreased with clipping, whereas in non-AM plants, P concentration increased with clipping.

Plant P content (g plant^{-1}) of all three plant species was affected by AM, clipping, and nutrient treatments (Table 11). Non-AM *C. maculosa* plants had 30% greater P content than AM plants. Non-AM *F. idahoensis* and *P. spicata* plants had 60% and 18% more P in their tissues than AM plants for each plant species respectively. Clipping reduced plant P by approximately 20%, 55%, and 37% compared with unclipped plants for *C. maculosa, F. idahoensis,* and *P. spicata* respectively. *Centaurea maculosa* plants grown in low N/low P had lower P content than plants grown in any other nutrient combination. For *F. idahoensis* and *P. spicata*, high N-treated plants had higher P content than low N-treated plants, and plants had similar P content between P treatments.

The nutrient by AM interaction was significant for *F. idahoensis,* with AM plants having similar P content across nutrient treatments, whereas non-AM plants had lower P content in low N treatments; no trend was apparent across P treatments (Figure 13c). An AM by nutrient treatment interaction was significant in *P. spicata*, with AM plants grown in low N treatments having lower plant N content than non-AM plants grown in the same nutrient treatments (Figure 12e). Nutrient by clipping treatments were also significant for *P. spicata*, with unclipped plants grown in high N-treated pots having higher P content than clipped plants grown in the same nutrient treatments (Figure 12f). Low N treated plants had similar P contents between clipping treatments.

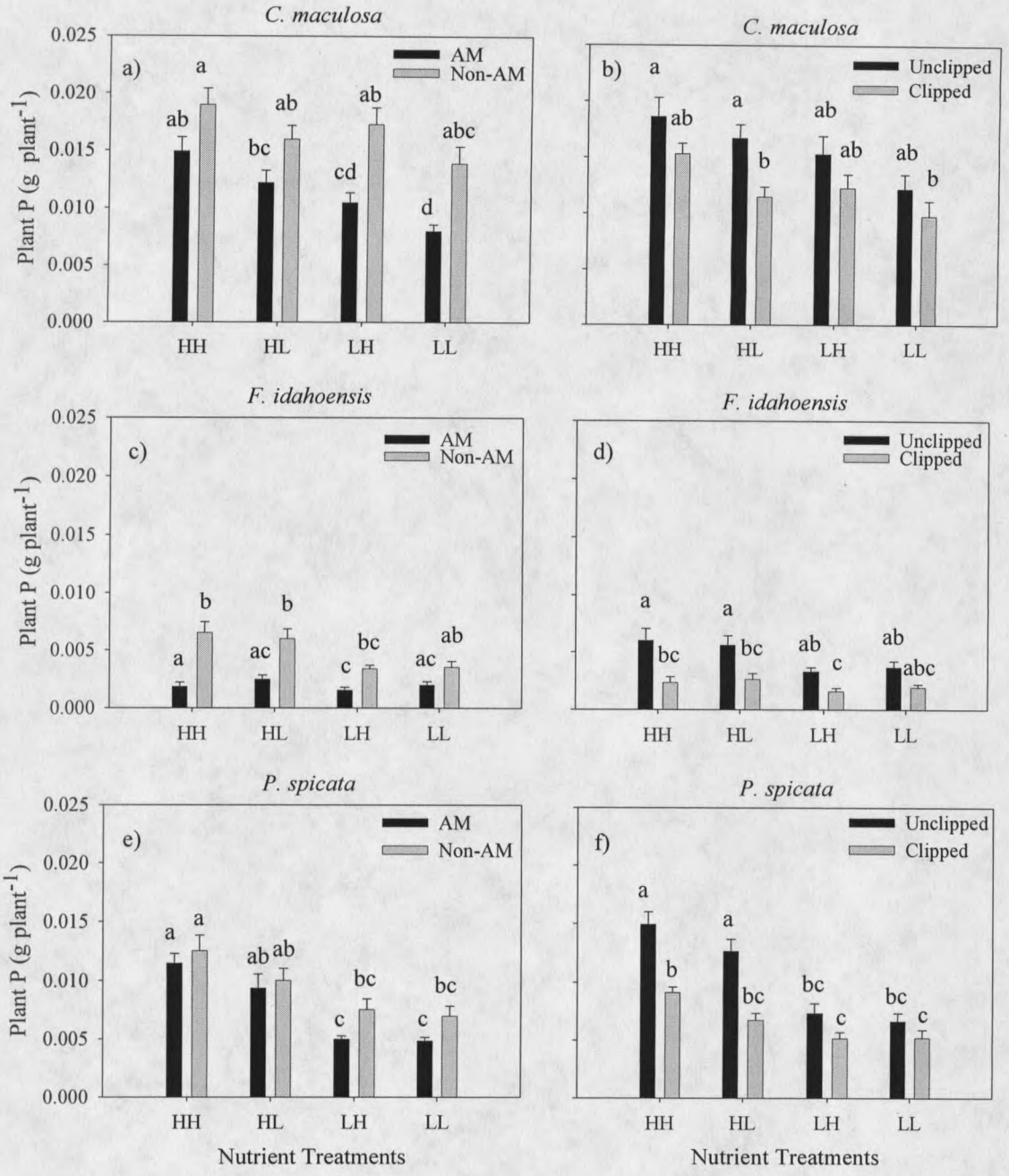

Figure 13. Plant P content (g plant^{-1}) of *C. maculosa, F. idahoensis,* and *P. spicata* among AM, clipping, and nutrient treatments. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent the standard error of the mean and different letters denote significant differences at $P \leq 0.05$ for each species.

Mycorrhizae

Mycorrhizal structures were not found in non-AM plant root samples (0% colonization, n=42). Colonization levels of AM plants compared across species using Mann-Whitney non-parametric tests showed plant species differed in colonization levels, with 25%, 18%, and 1.3% for *C. maculosa, P. spicata,* and *F. idahoensis* respectively (Table 12; Figure 14). Considering each species separately, colonization of *C. maculosa* and *F. idahoensis* roots by AM fungi was not affected by clipping, whereas clipping decreased AM colonization levels by 37% in *P. spicata* compared with unclipped plants (Table 13). Nutrient treatments did not affect AM colonization of *C. maculosa,* although AM colonization levels of *F. idahoensis* roots in low P treatments were higher than colonization levels in high P treatments. Colonization levels in *P. spicata* were also affected by nutrient treatments; high N/high P-treated plants had lower AM colonization than low N/low P-treated plants. The remaining two nutrient treatments had colonization levels intermediate of, and not significantly different from, the two extremes. The clipping by nutrient treatment interaction was significant for *P. spicata* and *C. maculosa,* with clipped high N/low P plants having higher colonization levels than any other nutrient treatment combination (Figure 14).

Table 12. Mann-Whitney non-parametric test statistics for AM colonization between each species combination.

	z	P
C. maculosa vs. *F. idahoensis*	-8.74	0.000
C. maculosa vs. *P. spicata*	-2.72	0.006
F. idahoensis vs. *P. spicata*	-8.32	0.000

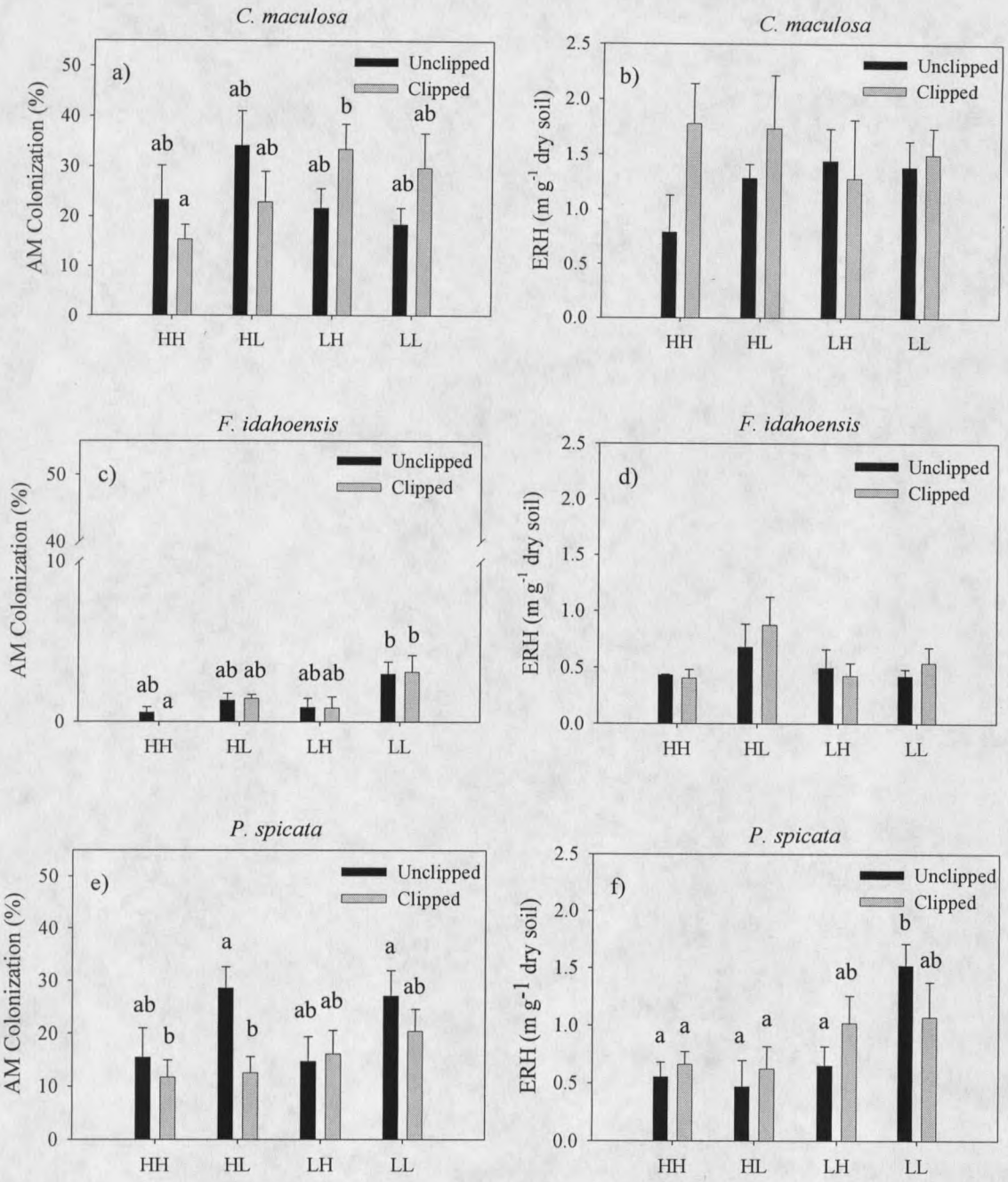

Figure 14. AM colonization levels and ERH lengths for each species across nutrient and clipping treatments. HH=high N/high P; HL=high N/low P; LH=low N/high P; LL=low N/low P. Bars represent the standard error of the mean and different letters denote significant differences at $P \leq 0.05$ for each plant species.

Table 13. Analysis of variance of AM colonization levels of *C. maculosa, F. idahoensis,* and *P. spicata.*

	AM Colonization (%)				ERH (m g-1 dry soil)			
C. maculosa	d.f.	MS	F	P	d.f.	MS	F	P
Nutrient	3	2.52	2.32	0.090	3	0.06	0.17	0.912
Clipping	1	0.35	0.32	0.575	1	0.54	1.68	0.219
Block	7	7.08	6.54	0.000	2	0.29	0.90	0.434
Nutrient x Clipping	3	2.66	2.45	0.078	3	0.34	1.10	0.389
Error	38	1.08			12	0.32		
F. idahoensis								
Nutrient	3	10.57	13.09	0.000	3	0.04	1.08	0.393
Clipping	1	0.14	0.17	0.638	1	0.00	0.01	0.913
Block	9	1.34	1.66	0.144	2	0.20	5.25	0.021
Nutrient x Clipping	3	0.50	0.61	0.402	3	0.04	0.96	0.440
Error	32	0.81			13	0.04		
P. spicata								
Nutrient	3	316.36	3.78	0.017	3	0.63	9.38	0.001
Clipping	1	772.04	9.23	0.004	1	0.00	0.06	0.815
Block	8	445.52	5.32	0.000	2	0.45	6.80	0.010
Nutrient x Clipping	3	330.22	3.95	0.014	3	0.18	2.64	0.093
Error	43	83.68			13	0.07		

Extra radical hyphae length was affected by plant species (1-way ANOVA; $F_{2,75}$=2.3, P=0.104) with 1.4, 0.83, and 0.49 m g^{-1} dry soil for *C. maculosa, P. spicata,* and *F. idahoensis,* respectively. Analyzing plant species separately showed that clipping did not affect ERH lengths for any plant species, and ERH lengths associated with *C. maculosa* and *F. idahoensis* were not affected by nutrient treatments (Table 13; Figure 14). *Pseudoroegneria spicata* ERH lengths (m g^{-1} dry soil) were affected by nutrient treatments, with plants grown in low N/low P associated with more ERH than plants

grown in either the high N/high P or high N/low P conditions (Figure 14). Plants grown in low N/high P had ERH lengths similar to all other nutrient treatments.

Mycorrhizal Effectiveness

Mycorrhizal effectiveness (ME) measures the benefit to the plant of having the symbiotic relationship. A ME score > 0 shows enhanced activity in the AM plant relative to the non-AM plant within the same treatment and block. All ME scores are shown in Table 14 and Mann-Whitney non-parametric test statistics are shown in Table 15.

Table 14. Mycorrhizal effectiveness scores for *C. maculosa, F. idahoensis,* and *P. spicata* for total biomass, total yield, plant N concentration and content, and plant P concentration and content. Mean ± (SE). Different letters denote significant difference between species at $P \leq 0.05$.

	C. maculosa	*F. idahoensis*	*P. spicata*
$ME_{biomass}$	- 0.86 ± (0.08) a	-3.17 ± (0.43) b	-0.66 ± (0.07) a
ME_{yield}	-0.82 ± (0.09) a	-3.34 ± (0.48) b	-0.59 ± (0.06) a
$ME_{N\ concentration}$	0.17 ± (0.05) a	0.10 ± (0.04) a	0.17 ± (0.03) a
$ME_{N\ content}$	-0.44 ± (0.08) a	-2.45 ± (0.39) b	-0.31 ± (0.06) a
$ME_{P\ concentration}$	0.04 ± (0.06) a	-0.04 ± (0.05) b	0.11 ± (0.05) a
$ME_{P\ content}$	-0.70 ± (0.15) a	-2.98 ± (0.66) b	-0.31 ± (0.09) a

For *F. idahoensis,* $ME_{biomass}$ and ME_{yield} were considerably lower than those for *P. spicata* and *C. maculosa,* which had similar ME scores, although all plant species had ME scores lower than zero. The same trends were apparent in $ME_{N\ content}$, with all species having scores less than zero and *F. idahoensis* being the most detrimentally

affected by the AM symbiosis. *Centaurea maculosa* and *P. spicata* had similar $ME_{N\ content}$ scores. In contrast, $ME_{N\ concentration}$ scores for all three plants were slightly above zero for plant N concentration, indicating a net benefit of AM on plant N concentration. All three species had $ME_{P\ content}$ scores less than zero, but *F. idahoensis* had the lowest $ME_{P\ content}$ score. For $ME_{P\ concentration}$, *C. maculosa* and *P. spicata* had positive scores, although for *F. idahoensis* $ME_{P\ concentration}$ was negative.

Table 15. Mann-Whitney non-parametric test statistics for ME scores for each species combination.

	C. maculosa vs. *F. idahoensis*		*C. maculosa* vs. *P. spicata*		*F. idahoensis* vs. *P. spicata*	
	z	P	z	P	z	P
$ME_{biomass}$	-5.77	0.000	-1.52	0.128	-6.57	0.000
ME_{yield}	-6.00	0.000	-1.83	0.067	-6.94	0.000
$ME_{N\ concentration}$	-1.82	0.069	-1.05	0.295	-0.76	0.450
$ME_{N\ content}$	-5.38	0.000	-0.65	0.515	-5.76	0.000
$ME_{P\ concentration}$	-1.97	0.048	-0.55	0.583	-2.81	0.005
$ME_{P\ content}$	-4.50	0.000	-2.72	0.006	-5.68	0.000

Discussion

Compensatory growth following herbivory has significant effects on plant fitness (Maschinski and Whitham 1989). Replacement of photosynthetic tissues is necessary for increasing carbon acquisition to produce viable seed and promote offspring success. For invasive species, compensatory growth is no less important than for the neighboring

native species impacted by frequent herbivory events. Exotic species are often freed from the specialized herbivores they experienced in their areas of origin (Keane and Crawley 2002), although predation often shifts from less specialist and more generalist herbivores, as is the case for ungulate grazers. Therefore, the ability of invasive species to compensate for herbivory in their new environments is imperative for their successful establishment and persistence.

Plant Compensation

Both the invasive forb and native grasses in this study increased their overall growth rate following simulated herbivory, although not enough to completely compensate for defoliation. *Centaurea maculosa* had the most post-clipping tissue regeneration, indicating a potential competitive advantage over the native grasses post-herbivory. Had my study continued beyond the 28 days of growth post-clipping, we may have seen full compensation in one or more of the species.

Studies examining the response of *C. maculosa* to herbivory have had contrasting results, with most documenting a reduction in *C. maculosa* productivity following defoliation (Muller-Scharer 1991, Kennett et al. 1992). In my study, although *C. maculosa* did not fully compensate, it was the least affected of the three species by defoliation.

In the field, *F. idahoensis* recovers well from heavy defoliation (Olson and Wallander 1997). In my experiment, *F. idahoensis* plants had relatively low total biomass, as is common for slow-growing species. The field studies cited examined

responses of much larger plants, and this size discrepancy may account for its undercompensation following defoliation in my study.

My results show *P. spicata* increased growth following herbivory, yet *P. spicata* shows poor recovery from defoliation in other studies (Caldwell et al. 1981). My control of growth factors in the greenhouse may have resulted in increased compensatory growth in *P. spicata,* because water and nutrient availability and competition limit plant productivity in field settings.

Nutrient Effects

Plant response to herbivory is contingent upon the amount of resources available for regrowth (Maschinski and Whitham 1989). Invasibility is also linked to nutrient availability as changes in resources pools affect competition and promote invasion by plants that can better compete in those conditions (Davis and Pelsor 2001). We expected to see greater compensation with higher nutrient availability, as resources such as N and P would not limit plant regrowth.

In my study, N had the greatest effect on plant productivity. Plants were consistently larger in high N treatments, regardless of plant species, and plant N content was consistently higher in high N treatments. Soil available N levels at the end of the experiment were the same between high and low soil N conditions, indicating that N was taken up by plants or soil microbes in the quantities provided by the treatments. Wallace and Macko (1993) speculate that grazed plants that exhibit increased N content and/or concentration following herbivory could be superior competitors under grazed conditions.

Increased photosynthetic rates with increased N content and the preemption and storage of soil N for growth during times of low N availability may result in a competitive advantage for grazed plants.

Increases in N concentration may be the plant's first step to regenerate biomass (Nowak and Caldwell 1984, Aerts and Chapin 2000) following tissue removal by grazers, indicating the potential for increased plant growth rates and biomass production. Plant N concentration (%) was always higher in AM and clipped plants than non-AM and unclipped plants. If we had allowed the experiment to continue for a longer period of time following clipping, both clipped and AM plants may have shown less of a size differential relative to unclipped and non-AM plants. Alternatively, increased N concentration could be explained by increases in N-based herbivory defenses (Findlay et al. 1996; Vrieling et al. 1996). Either way, clipped plants had less biomass per unit nitrogen than unclipped plants, suggesting that unclipped plants use N more efficiently than clipped plants.

Phosphorus availability as a factor in compensatory response was expected to be especially important for the regrowth of *C. maculosa,* which exhibits high levels of P consumption in the grasslands it invades (LeJeune and Seastedt 2001). In my system P treatment did not affect biomass or yield, suggesting that N, not P, is the limiting nutrient (Chapin et al. 1986). *Centaurea maculosa* and *P. spicata* increased P uptake in high P conditions, whereas *F. idahoensis* did not. Increased P uptake in high P conditions may indicate better competitive ability, since storage of P for use during times of low P

availability (van Iersel et al. 1998) and may result in higher seed quality and quantity (Sanders and Koide 1994).

Mycorrhizal Effectiveness

The well-documented ability of AM to increase plant nutrient acquisition suggests that AM may have a significant effect on plant compensatory responses (Newingham et al. 2000), especially in low nutrient conditions. We hypothesized that AM plants would have greater compensation in low nutrient conditions, although this did not occur. Instead, AM showed inconsistent effects on plant responses to herbivory. Based on ME scores, AM had a beneficial or neutral affect on N concentration all plant species. Mycorrhizae benefited *C. maculosa* and *P. spicata* for increasing P concentration, but not *F. idahoensis*. For *F. idahoensis,* an AM symbiosis was highly detrimental in regard to increasing biomass production and overall nutrient contents, indicating a lower competitive ability for small *F. idahoensis* plants in the field where AM are ubiquitous among the majority of rangeland plants, including invasive species. However, ME scores for *C. maculosa* and *P. spicata* were only slightly negative for most variables (ME scores ranged from -0.86 to -0.31), indicating that AM has a nearly neutral effect on these species in my study. The transition from mutualistic to parasitic interactions with AM is widespread (Johnson et al. 1997) and understanding the dependency of plants on AM is paramount to understanding belowground mechanisms that affect plant productivity.

Assessment of AM benefits on plant productivity for *F. idahoensis* are difficult due to the uncharacteristically low AM colonization levels detected in this study

compared with those found in other AM studies using *F. idahoensis* (20-40%; see Chapter 2). The low colonization levels could be attributed to the relatively small root mass, which may diminish the amount of roots available for AM colonization. Alternatively, the low colonization levels may be due to the placement of P fertilizer in the top 4-cm of the pots. The small root masses of *F. idahoensis* plants were contained in the top 5-10 cm of the pots, also the area where the P fertilizer was applied. The higher P levels in that zone may have limited AM colonization levels.

Although *F. idahoensis* AM colonization levels were low, ERH lengths were comparable to those quantified for *P. spicata,* which had colonization levels similar to those quantified in other studies using this species. Similar ERH lengths suggest a similar carbon costs to AM for *F. idahoensis* and *P. spicata,* although lower occurrences of internal structures, especially arbuscules, were observed in *F. idahoensis* roots. Because arbuscules are considered the sites for P exchange between the plant and fungi, the AM symbiont of *F. idahoensis* may be transporting lower amounts of P from the soil to the plant, thereby reducing the AM benefit to *F. idahoensis.*

Although there was a significant AM affect on several plant variables in *F. idahoensis*, other soil components including fungal pathogens could have affected biomass production and nutrient uptake for *F. idahoensis.* The addition of microbial wash to non-AM pots reintroduces bacteria and small-spored fungi. Non-AM *F. idahoensis* roots had little non-AM fungal hyphae or fruiting bodies associations, while AM roots had considerable non-AM associated fungal structures. This suggests that fungal pathogens may have been present in the original field soil that was absent from the

microbial wash, thereby reducing the productivity of AM *F. idahoensis* plants. Alternatively, colonization levels may have continuously declined through the course of the experiment to the quantity detected at harvest in response to nutrient levels, indicating tight plant control of its AM, as was the case for *F. idahoensis* in a previous experiment (see Chapter 2).

Herbivory Effects on Mycorrhizae

If plants can control the degree of AM colonization and if AM benefit the host plant in low-nutrient environments, then I would expect greater colonization in the clipped treatment and reduced colonization in high P treatments. That AM plants in this experiment have less biomass than non-AM plants suggests that the carbon cost of the plant for retaining its AM symbiont is greater than the benefit of possible increased nutrient acquisition. Clipping did not have a consistent affect on AM colonization levels. Mycorrhizal colonization levels were similar between clipped and unclipped *C. maculosa* and *F. idahoensis* plants, although AM colonization levels in *P. spicata* were lower in clipped than unclipped plants.

Effect of nutrient levels on AM root colonization varied with plant species. *Centaurea maculosa* exhibited no changes in root colonization levels across nutrient treatment. *Pseudoroegneria spicata* and *F. idahoensis* experienced lower AM colonization rates when growing in high N/high P soils than when in low N/low P conditions. Increased AM colonization has often been linked to increased nutrient acquisition for the host plant and subsequent increases in plant growth (Smith and Read

1997). *Festuca idahoensis* had higher AM colonization levels in the low N/low P treatment than the high N/high P treatment; evidence of its ability to control AM colonization of its roots. A plant-induced reduction in AM colonization was documented in Chapter 2 for *F. idahoensis* roots in the presence of relatively high soil P levels, and may therefore explain the extremely low AM colonization levels documented in this study.

Lengths of ERH were not affected by clipping for any of the plant species and nutrient treatment effects on ERH varied with plant species. *Centaurea maculosa* had ERH lengths significantly greater than those of either *F. idahoensis* or *P. spicata,* although ERH lengths detected in this experiment were lower than those found in my previous experiment (see Chapter 2), ERH lengths documented here are similar to those found by Abbott and Robson (1985). As discussed in Chapter 2, higher levels of ERH production may increase *C. maculosa*'s competitive radius in the soil through increased soil volume available for nutrient extraction, increased nutrient acquisition (Jakobsen et al. 1992), and therefore increased competitive ability (Wallace and Macko 1993).

Pseudoroegneria spicata was the only species in my study to have higher ERH production in the low N/low P treatment than in either the high N/high P or high N/low P soils. Control over AM colonization and ERH development assist in reducing the parasitic effects of AM on the host plant by reducing the carbon costs to the plant in times of high nutrient availability (Smith and Read 1997). Interestingly, P did not seem to be the only cause of changes in ERH development with *P. spicata;* low N and P levels resulted in ERH length to increase. Because the main component in fungal cell walls is

chitin, a molecule composed of up to 6% N (Leake and Read 1990), the N and P demands of the fungi and host plant respectively could have increased ERH length to augment soil resource exploration.

In conclusion, my study provides no evidence that increased nutrient availability or AM increase plant compensatory growth following defoliation. Additionally, AM do not always confer an advantage to the host plant, and may actually reduce the plant's competitive abilities under certain conditions. Clipping had variable effects on AM structure depending on the host plant, and some species showed greater control over their AM than others. Nitrogen, not P, was the limiting nutrient for plant growth in this experiment, suggesting its importance in competitive interactions between *C. maculosa* and neighboring native grasses.

CONCLUSIONS

For my research, I hypothesized: 1) that the invasive forb, *C. maculosa,* would produce more ERH and at greater distances from the plant when compared to the AM of *F. idahoensis*; 2) that compensatory growth would be greatest in high nutrient conditions; 3) that AM would increase compensatory growth in low nutrient conditions, but decrease compensatory growth in high nutrient conditions; and 4) that AM colonization levels and ERH production would not be affected by herbivory.

Centaurea maculosa produced 1.5 times more ERH in the rooting zone and 3.5 times more ERH at a distance of 5+ cm from the plant than the symbionts of *F. idahoensis.* This increase in ERH length represents increased soil volume explored and potential pool of nutrients for the host plant. This suggests that *C. maculosa* increases its competitive advantage over the native bunchgrass through its AM partnership, thereby enhancing its invasive potential.

In my second experiment, both the invasive forb and native grasses increased their overall growth rate following simulated herbivory, although not enough to completely compensate for defoliation. *Centaurea maculosa* had the most post-clipping tissue regeneration, indicating a potential competitive advantage over the native grasses post-herbivory. Had my study continued beyond the 28 days of growth post-clipping, I might have seen full compensation in one or more of the species.

I hypothesized that AM plants would have greater compensation in low nutrient conditions, although this did not occur. Instead, AM showed inconsistent effects on plant responses to herbivory. For *F. idahoensis,* AM symbiosis was highly detrimental in

regard to increasing biomass production and nutrient content, indicating a lower competitive ability for small *F. idahoensis* plants in the field where AM are ubiquitous among the majority of grassland plants, including invasive species. In contrast to *F. idahoensis,* the roots of *C. maculosa* and *P. spicatum* occupied the entirety of the pots, which may indicate a reduced reliance on AM in my study, because nutrients may have been easily attainable by the plant without the use of AM. The transition from mutualistic to parasitic interactions with AM is widespread (Johnson et al. 1997) and understanding the dependency of plants on AM is paramount to understanding the ways belowground mechanisms affect plant productivity.

Mycorrhizal colonization levels were similar between clipped and unclipped *C. maculosa* and *F. idahoensis* plants, although AM colonization levels in *P. spicatum* were lower in clipped than unclipped plants. Lengths of ERH were not affected by clipping for any of the plant species, although *C. maculosa* was associated with higher ERH lengths than either of the two grasses, indicating a consistent ERH response between experiments.

My research identifies several interesting trends regarding plant nutrient dynamics. In my first experiment, *C. maculosa* increased its total P content considerably in the PR treatment. Although *C. maculosa*'s biomass increased slightly with soil P content, the increased P content suggests luxury consumption of P. Plants exhibiting luxury consumption may be able to store that nutrient for later use (van Iersel et al. 1998), or luxury consumption may be a way for plants to increase seed quality and production (Stanley et al. 1993, Shumway and Koide 1995, Sanders and Koide 1994) and offspring

fitness (Heppell et al. 1998, Zhu and Smith 2001, LeJeune and Seastedt 2001, Allison 2002). Moreover, increased P uptake in mycorrhizal plants decreases the time to initiate flowering (Shumway and Koide 1994), increases the duration of flowering, and increases the number of flowers that produce fruit (Lu and Koide 1994). Considering the high rate of seed production exhibited in *C. maculosa* (Eddleman and Romo 1988), improving offspring size and competitive ability could be crucial for its early season germination and establishment.

In my second study, N had the greatest effect on plant productivity. Plants were consistently larger in high N treatments, regardless of plant species, and plant N content was consistently higher in high N treatments. Wallace and Macko (1993) speculate that grazed plants that exhibit greater N content and/or concentration following herbivory could be superior competitors under grazed conditions. Increased photosynthetic rates with increased N content and the preemption and storage of soil N for growth during times of low N availability may result in a competitive advantage for grazed plants.

Plant N concentrations (%) were always higher in AM and clipped plants than non-AM and unclipped plants. Increases in N concentrations may be the plant's first step to regenerate biomass (Nowak and Caldwell 1984, Aerts and Chapin 2000) following tissue removal by grazers, indicating the potential for increased plant growth rates and biomass production. Had I continued the experiment for a longer period of time following clipping, clipped plants may have completely compensated for herbivory losses. Clipped plants had less biomass per unit nitrogen than unclipped plants, suggesting that unclipped plants use N more efficiently than clipped plants.

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