NITROGEN DYNAMICS OF *CENTAUREA MACULOSA* AND

NATIVE SPECIES

by

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Elizabeth Anne D'Imperio

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ABSTRACT

Centaurea maculosa has invaded millions of hectares of disturbed and undisturbed semi-arid grasslands. Its success may be due to an ability to conserve nitrogen (N) or use N more efficiently than native species. It may also be able to acquire more N from early spring pulses than native vegetation.

Growth response, N acquisition, allocation, resorption and use efficiency of *C. maculosa* was compared with *Pseudoroegneria spicata, Pascopyrum smithii,* and *Rudbeckia hirta* by growing plants in high and low N supplies, which mimic disturbed and undisturbed sites. [15]Nitrogen acquired per unit of root mass was highest in *R. hirta*. *Centaurea maculosa* had the lowest C:N ratio. Resorption proficiency was lower in *C. maculosa* for Harvest 2 and 3, and similar among all species at Harvest 4. *Centaurea maculosa* had the highest mean residence time (MRT) and lowest N productivity. *Centaurea maculosa* had the highest nitrogen use efficiency (NUE) for plants receiving the high N supply whereas NUE was similar for all species receiving the low N supply. *Centaurea maculosa*'s success in disturbed sites may be due to a high NUE, which can be attributed to a high MRT.

Uptake and growth response of *C. maculosa* receiving an early spring, late spring or steady N treatment were determined in a glasshouse and a field study. *Centaurea maculosa*'s response was compared with *P. spicata, Festuca idahoensis* and *R. hirta* in the glasshouse, and *P. spicata, F. idahoensis* and *Balsamorhiza sagittata* in the field. In the glasshouse, with plants receiving the steady N treatment produced the most biomass whereas plants receiving the late N treatment produced the least. Grasses receiving the early N treatment had the highest root mass ratio (RMR), whereas RMRs of *C. maculosa* were similar among N treatments. Forbs acquired more N than grasses. In the field, biomass was similar among N treatments. Plants took up more N from the early treatments than the late or steady treatments. *Centaurea maculosa* may be able to acquire more N from pulses than grasses in disturbed sites when plants are small, but as the grasses grow, *C. maculosa* may lose its advantage in acquiring N.

CHAPTER 1

INTRODUCTION

Centaurea maculosa Lam. (spotted knapweed) is an invasive forb from Eurasia

first introduced into North America in the late 1800s. It has spread throughout the

Northwest invading semi-arid grasslands on disturbed and undisturbed sites. *Centaurea*

maculosa is considered an environmental and economic pest. It reduces forage for

wildlife and livestock and displaces native vegetation (Watson and Renny 1974). Some

of its success has been attributed to a lack of natural enemies and to allelopathic

chemicals.

In semi-arid grasslands, nitrogen (N) is considered the limiting nutrient (Gleason

and Tilman 1990; Loomis and Connor 1992). Nitrogen availability is linked to water

availability (Cui and Caldwell 1997; Hungate et al. 1997; Tsialtas et al. 2001) which is

also limiting in semi-arid grasslands. These resources are temporally variable and are

often available as pulses (Gupta and Rorson 1975; McKane et al. 1990). In western

Montana, most pulses occur in the spring in the form of snowmelt and spring rains;

approximately one third of the annual precipitation occurs in May and June (Western

Regional Climate Center). Nitrogen from pulses may only be available for a few days

(Cui and Caldwell 1997; Ivans et al. 2003). Some of *C. maculosa*'s success in grasslands

may be due to traits that allow it to use N efficiently, and to acquire and store N from

pulses.

Plants that grow well in grasslands have long-lived, low N tissue and large below

ground biomass (Crain et al. 2002). The long-lived tissue minimizes N loss whereas low

N concentration indicates that the plant has a low demand for N. A high root mass ratio

is also important in grassland systems (Casper and Jackson 1997), because large

belowground biomass increases a plant's ability to acquire limited resources.

Effects of N supply on nitrogen use efficiency (NUE) in plants have been studied,

most often in mesic systems (Berendse and Aerts 1987; Aerts 1990; Aerts and de Caluwe

1994; Vasquez de Aldana and Berendse 1997). Generally, plants have greater mean

residence time (MRT) for N or greater N productivity in low N soil. This is due to

physiological trade-offs that prevent plants from having a high MRT and high N

productivity simultaneously (Berendse and Aerts 1987; Aerts 1990). When N levels are

high, plants tend to produce more biomass and have higher turnover of tissue (Aerts

1990). When N levels are low, plants tend to produce low N tissue (Aerts 1990), and

increase N resorption (Aerts and de Caluwe 1994; Distel 2003).

Resorption is the movement of nutrients from senescing plant material. It is an

important part of N conservation in N limited soils (Killingbeck 1996). Nitrogen that is

resorbed can be reused to produce new biomass, and therefore reduce a plant's N uptake

requirements (Aerts 1996). Resorption may be affected by N supply (Aerts and de

Caluwe 1994; Distel 2003), although not all studies agree (Aerts 1996; Killingbeck

1996). In addition, resorption efficiency differs between grasses and forbs (Aerts 1996).

In grasslands, competition for belowground resources is more important for plant

survival than competition for aboveground resources (Wilson 1993). Because N is

temporally variable and may only be available for short periods of time, plants must be

able to take advantage of these pulses. Some of these adaptations may include a large root mass and high root density (BassiriRad et al. 1999; Fransen et al. 1999) or greater uptake capacity (BassiriRad and Caldwell 1992; Cui and Caldwell 1997; Fransen et al. 1999).

The timing of N pulses is important. The timing of a pulse relative to a plant's stage of growth may affect the plant's ability to acquire and utilize N (McKane et al. 1990; Miao and Bazzaz 1990; Bilbrough and Caldwell 1997; Gebauer and Ehleringer 2000). In semi-arid grasslands, most N pulses occur early in the spring when most plants are in their early growth phase.

Our research was based on the following hypotheses: 1) *Centaurea maculosa's* success in disturbed and undisturbed sites is due to a greater NUE than the native vegetation, and 2) *Centaurea maculosa* is able to acquire more N and produce more biomass from early spring pulses compared with native vegetation.

Nitrogen uptake, resorption and use efficiency of *C. maculosa*, *Rudbeckia, hirta* Lam., *Pseudoroegneria spicata* [Scribn. and Smith] A. Love, and *Pascopyrum smithii* [Rybd.] A. Love, were determined in a glasshouse study (Chapter 2). Plants were grown for 155 days in columns receiving one of two N supplies intended to mimic high or low N availability in the field (Neill 1995; Blicker et al. 2002). Plants received a ^{15}N label at the beginning of the study, and sets of plants were harvested at each of four times during the study. They were analyzed to determine N uptake, total biomass, root mass ratio, C:N ratios, resorption, MRT, N productivity and NUE.

Centaurea maculosa, *R. hirta*, *P. spicata* and *F. idahoensis* Elmer. were used to evaluate the response to different timing of N pulses (Chapter 3). In a glasshouse, plants

were grown in mixed- and mono-culture pots, and pulsed with an early or late treatment of ^{15}N intended to mimic the potential timing of N availability in Montana. Control plants received a steady supply of ^{15}N (early, mid- and late treatment). Plants were periodically measured during the study to determine growth rate. After 11 weeks when the plants began to senesce, they were harvested and separated. Biomass, root mass ratio, percent N, ^{15}N concentration, total ^{15}N, and N pool were determined.

In the field, response to pulses of N was measured, in a similar study, using *C. maculosa*, *Pseudoroegneria spicata*, *Festuca idahoensis* and *Balsamorhiza sagittata* (Pursh) Nutt (Chapter 3). At three sites in western Montana, plants received an early treatment, a late treatment, or a steady supply of ^{15}N (early, mid- and late), or they received only water. Plant material was collected seven days after the ^{15}N treatment. At the end of the growing season, all aboveground biomass was collected. ^{15}N and percent N data were used to determine from which treatments the plants acquired the most N. Above ground biomass, number of flowerheads, and percent N at the end of the growing season were determined to evaluate growth response to the pulses.

A general overview of these studies and their implications are presented in Chapter 4.

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CHAPTER 2

UPTAKE, ALLOCATION AND RESORPTION OF NITROGEN BY *CENTAUREA*

MACULOSA AND THREE NATIVE SPECIES.

Introduction

Centaurea maculosa Lam. (spotted knapweed) is an invasive Eurasian forb

introduced into North America in the late 1800s. It was first recorded in Montana in the

1920s and is now present in every county in Montana, Washington, Idaho and Wyoming.

Centaurea maculosa frequently invades disturbed sites such as roadsides and rangelands

(Watson and Renney 1974). These sites often have higher levels of available nitrogen

(N) and favor fast growing ruderals. *Centaurea maculosa* also grows in undisturbed sites

where nutrient availability is low. On these sites competition favors plants with low

nutrient requirements (Aerts 1990). *Centaurea maculosa's* success across such a range

of N availability may be linked to a greater physiologic and morphologic plasticity

allowing it to have higher nutrient use efficiency (NUE) than native vegetation in N-rich

and poor soils.

A plant's ability to utilize nutrients is measured as NUE, which is defined as a

ratio of outputs to inputs (Aerts and DeCaluwe 1994). Early studies measured NUE as

nutrient productivity, the biomass created per unit of nutrient, a figure inversely related to

the concentration of nutrients in the biomass (Chapin 1980; Shaver and Melillo 1984). A

modified approach to NUE incorporates mean residence time (MRT), the ratio between

the annual average of plant nutrient concentration and the annual nutrient loss (Aerts and

DeCaluwe 1994), in addition to nutrient productivity (Berendese and Aerts 1987; Aerts

1990).

Nutrient productivity and MRT are important components of NUE because they

incorporate different aspects of plant physiology. In nutrient rich soils, plants produce

large amounts of biomass with high turnover rates, which can result in high nutrient loss

(Aerts 1990). In contrast, a long MRT may benefit plants in nutrient poor soils (Aerts

1990). Plants with a long MRT often have slow growth rates, have lower turnover of

tissue, and retain nutrients for long periods of time. Because of these physiological trade-

offs, plant cannot have high productivity and long MRT simultaneously (Berendse and

Aerts 1987). However, NUE of plant species from high or low nutrient environments can

be similar with plants in high nutrient sites having high nutrient productivity but low

MRT, and with plants on low nutrient sites having low nutrient productivity but high

MRT (Aerts 1990).

A major determinant of MRT is resorption, the mobilization of nutrients, such as

N out of senescing plant tissue into living tissue where it can be stored for later use.

Resorption can be expressed as resorption efficiency, the percent of the nutrient removed

from the tissue, or resorption proficiency, the final concentration of nutrient in the plant

tissue (Killingbeck 1996). The process is considered biochemically complete for N when

concentrations decline to 0.70% in senesced plant material (Killingbeck 1996;

Killingbeck and Whitford 2001). Plants resorb approximately 50% of N from senescing

tissue (Aerts 1996), although the actual percent N varies among growth forms; resorption

efficiency averages 60% in graminoids and 40% in forbs (Aerts 1996).

Resorption is an important nutrient-conserving strategy of plants, especially those in nutrient-limiting environments, and affects competition, nutrient uptake and productivity (Killingbeck 1996). Resorbed N can be reused directly for new growth. It can also be stored for use during the next growing season, giving the plants access to N before it is readily available in cold soils. With resorbed N, the plant depends less on current uptake of N (Aerts 1996; Aerts 1997). High resorption also means lower N concentrations in the litter, perpetuating nutrient poor soils, and the dominance of plants adapted to low N soils (Monk 1966; Aerts 1995).

Resorption is greater at lower nutrient availability. For example, N resorption is greater in *Pinus thunbergii* trees along a decreasing N gradient (Enoki and Kawaguchi 1999). *Carex* spp. also has greater amounts of N in its litter when receiving high levels of N, indicating that it resorbs less N from senescing tissues (Aerts and DeCaluwe 1994). In contrast, N resorption is higher for two perennial herbs under high soil fertility (Boerner 1986). However, resorption and differences in N supply are not related in several plant species (Aerts 1996; del Arco et al. 1991; Cartaxana and Catarino 2002). For example, N availability is not related to resorption in the shrub, *Viburnum acerfolium*, at three sites with different soil types (Minoletti and Beorner 1994). In Alaska, the movement of phloem in birch trees affects resorption efficiency more than N availability (Chapin and Molanen 1991).

Based on these physiological processes, we hypothesized that *C. maculosa*'s success in soils with diverse N supplies reflects a greater NUE than native vegetation because of higher resorption, longer MRT, or greater N productivity. We predicted that *C. maculosa* uses nitrogen more efficiently (higher NUE) and resorbs more N from its

senescing tissue than native vegetation with a low N supply, while producing greater amounts of biomass per unit of N than native vegetation with a high N supply. We tested this hypothesis by growing *C. maculosa* and three native species under two different N supplies to determine N uptake, growth response, and N resorption when exposed to high and low nitrogen availability. Nitrogen concentration and growth response were used to determine NUE.

Methods

Experimental design

This glasshouse study involved four plant species, two N treatments, and four harvest dates with 8 replicates (n = 256) using a randomized block design. A nitrogen label (^{15}N) was added at the start of the study to determine ^{15}N uptake and allocation. Growth response was determined non-destructively during the study and from each harvest.

Plant materials

Centaurea maculosa Lam. is a tap-rooted, non-native perennial in the Asteraceae family (Watson and Renney, 1974). Three native perennial herbs were used to compare and contrast physiological responses among species. *Pseudoroegneria spicata* [Scribn. and Smith] A. Löve, a bunchgrass, and *Pascopyrum smithii* [Rybd.] A. Löve, a rhizomatous grass, are native grasses associated with semi-arid grasslands. *Rudbeckia hirta* Lam. is a short-lived, native perennial forb in the same family as *C. maculosa*.

Rudbeckia hirta grows in disturbed areas and has similar morphological characteristics to *C. maculosa,* including a taproot (Hitchcock and Cronquist 1973).

Growing conditions

Plants were grown from seed in a glasshouse in polyvinylchloride (PVC) columns, 10 cm in diameter and 40 cm in height. Columns were filled to within 4 cm of the top using an 8:1 mix of sand and soil collected from the Red Bluff Research Ranch, 60 km west of Bozeman, Montana. The sand was pasteurized to kill weed seeds, pathogens and insect eggs before mixing with the sieved soil. Soil organic matter was 4.31 % ; nutrient composition was 6.5 mg kg^{-1} of NO_3^-, 15.2 mg kg^{-1} of P, 448 mg kg^{-1} of K. To prevent phosphorus deficiency in the plants, 0.55 g of phosphorus in an aqueous solution was added to each pot before they were seeded and after the second harvest. Four seeds were originally planted into each pot. During the establishment phase, the plants were watered on alternate days and eventually thinned to one plant per pot. Supplemental lighting (GE Multi-vapor MVR1000/C/U, General Electric Company, Cleveland Ohio, USA) was used to maintain a constant 12 h day length starting on the first day of the study.

^{15}N labeling and N treatment

Eight weeks after seeding (d 0), 50 ml of a 0.06 g L^{-1} solution of K^{15}NO$_3^-$ (99 atom %; Isotec, Inc. Miamisburg Ohio, USA) was added to each pot (42 μg of ^{15}N per pot) as a N label. On d 5, the columns were thoroughly flushed to remove ^{15}N from the

soil. That same day, the first 25% of the plants were harvested to determine the initial amount of ^{15}N acquired by the plants.

On d 8, we began watering the plants with 150 ml of 2.5% modified Hoagland's solution (KH_2PO_4, KNO_3, $Ca(NO_2)_2$, MSO_4, micronutrients; Cui and Caldwell 1997) with either a high (0.067 mmols N per day) or low (0.0168 mmols N per day) N level to mimic high or low N availability in the field (Neill 1995; Blicker et al. 2002). Using drip emitters, the plants were watered every third day. Any water that drained from the columns was collected and poured back into the pot to ensure no N was lost. On d 101, the solution was diluted to half of the original concentration to reduce nutrient availability, mimicking reduced N availability in the field as soil water becomes limiting (Cui and Caldwell 1997; Hungate et al 1997; Tsialtas et al 2001). On d 119, the solution was diluted in half again. On d 137, plants were watered with only 75 ml of solution at the same concentration (0.0083 mmols N per day-high, 0.0021 mmols N per day-low) to induce senescence.

Harvest

Twenty-five percent of the plants were harvested on d 5, 43, 98, and 155 of the experiment, to sample plants across a large part of their life cycle. Shoots were removed with a razor, cleaned of debris, and separated from belowground material. Roots were washed in a tray to remove any organic and inorganic matter. Columns were stored at 4°C in a dark room after the shoots were removed, and before the roots were cleaned. For the final two harvests, dead roots were separated and collected. Dead shoot material was collected continually throughout the experiment.

Height, number of tillers or leaves, number of flowerheads, and stage of plant development (vegetative, bolting/stem elongation, flowering) were recorded for all remaining plants on the day before harvesting. All plant material was dried at 42° C for a minimum of 7 d, weighed, and then ground with a Udy mill cyclone grinder (Udy Corporation, Fort Collins, Colo.USA) for large samples, or with a mortar and pestle for small samples. All samples were analyzed for C (%), N (%) and ^{15}N content with an Isotope Ratio Mass Spectrometer (U.C. Davis Stable Isotope Facility, Davis, CA).

Growth response and N analyses

Growth response was determined by calculating total biomass (root mass plus shoot mass), root mass ratio (root mass/total mass) and dead biomass from Harvest 3. Harvest 3 was used to determine dead biomass because senescence was induced before Harvest 4, therefore dead biomass accumulated at an accelerated rate. ^{15}Nitrogen uptake per unit of root mass (μmol g^{-1}) was calculated using root mass, %N, and fractional abundance (F) from the first harvest to determine the initial uptake capacity of the plants when exposed to ^{15}N from d 0 to d 5. Percent acquired of ^{15}N applied was the sum of the product of root mass, % N, and F, and the product of shoot mass, percent N, and F. Fractional abundance and atom % ^{15}N were determined by the following steps (Boutton 1991; Shearer and Kohl 1993):

$$\delta\,^{15}N\,(‰) = [(R_{sample} - R_{std})/R_{std}] \times 10^3 \tag{1}$$

where $\delta\,^{15}N$ (‰) is ^{15}N discrimination, and R represents the absolute ratio (^{15}N/^{14}N) of the plant sample (R$_{sample}$) and standard gases (R$_{std}$). The absolute ratio for the standard

gas is 0.0036765. The absolute ratio of a sample (R_{sample}) can be determined by rearranging (1):

$$R_{sample} = {}^{15}N / {}^{14}N = [(\delta\ {}^{15}N / 1000) + 1] \times R_{std} \tag{2}$$

R_{sample} is used to calculate F, fractional abundance:

$$F = {}^{15}N / ({}^{15}N + {}^{14}N) = R_{sample} / (R_{sample} + 1) \tag{3}$$

For Harvests 2, 3 and 4, nitrogen resorption efficiency was the percentage of N resorbed from senesced aboveground material (Killingbeck and Whitford 2001). Nitrogen resorption proficiency was the %N remaining in senesced aboveground material (Killingbeck and Whitford 2001).

For Harvest 3, nitrogen productivity was the change in biomass from Harvest 1 to Harvest 3 divided by the average N pool (N concentration x biomass; Vazquez de Aldana and Berendse 1997). Because the same plant could not be used at Harvest 1 and 3, change in biomass and N pool were determined by comparing plants within blocks. Mean residence time (MRT) at Harvest 3 was the ratio of maximum N pool to annual N lost (Eckstein and Karlsson 2001).

$$MRT = Npool_{shoot} / [Npool_{shoot} - (biomass_{shoot} \times \%Ndead_{shoot})] / \text{ year} \tag{4}$$

where:

$Npool_{shoot}$ was the concentration of N in the shoot material x the biomass of the shoot material, $biomass_{shoot}$ was the biomass (g) of the shoot material and $\%Ndead_{shoot}$ was the g N in dead shoot material relative to g of biomass of dead shoot material. Only shoot material was used because the amount of N lost in roots could not be accurately determined. Nitrogen use efficiency was the product of N productivity and MRT at

Harvest 3 (Berendse and Aerts 1987). Harvest 3 was used to calculate N productivity, MRT, and NUE because at Harvests 1 and 2 many of the plants had not bolted and were still in early vegetative stages, and senescence had been induced between Harvests 3 and 4.

Data analysis

The experiment was a complete randomized block design. All variables were analyzed with ANOVA (R 1.8.1, 2003). Total biomass, RMR, ^{15}N acquired per unit root mass, and percent ^{15}N present of applied were transformed with square root. Carbon to nitrogen ratios for roots and shoots were transformed with $\lambda = 0.2$. Resorption proficiency and number of flowerheads per plants were transformed with natural log. Mean residence time, N productivity and NUE were transformed with the reciprocal. Non-transformed data and standard errors are presented in the figures. P-values less than 0.15 are presented (Gill 1981).

Results

^{15}N uptake

^{15}N uptake per unit of root mass at Harvest 1 was greater in *R. hirta* than *C. maculosa* and the two grasses (species, $P = 0.02$; Fig. 1). ^{15}N uptake per unit of root mass was only determined at Harvest 1, before we began treating the plants with the different N supplies.

The percent of ^{15}N present relative to applied in the plants differed by species

across harvests (species x harvest, $P < 0.001$; Fig. 2). At Harvest 1, *C. maculosa, R. hirta*
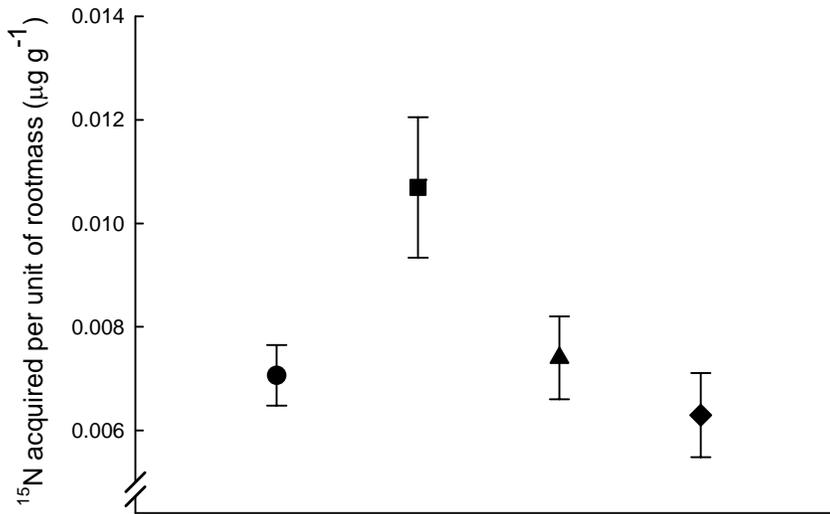
Figure 1. The amount of ^{15}N (µg) acquired per gram of root mass at Harvest 1 for *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦). Bars represent one standard error of the mean (S.E.M.).
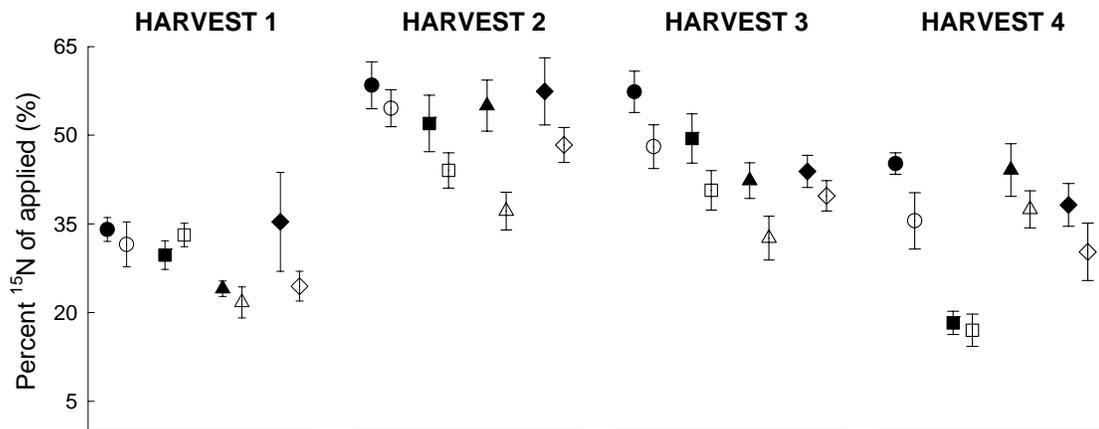
Figure 2. The percentage of ^{15}N present in the plant of the applied for *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦) for high (closed) and low (open) nitrogen supply at each harvest. Bars represent 1 S.E.M..

and *P. spicata* had similar amounts of ^{15}N. Overall, plants from Harvests 1 and 4 had a lower percent ^{15}N present than those from Harvests 2 and 3 (harvest, $P < 0.001$). Overall, the percent of ^{15}N present was greater in plants receiving the high N supply (N supply, $P < 0.001$).

Growth response

Total biomass differed by species across harvests (species x harvest, $P < 0.001$; Fig. 3a), and differed by N supply across harvests (N supply x harvest, $P < 0.001$). Total biomass was similar among all species and N supply in Harvests 1 and 2, whereas in Harvests 3 and 4, total biomass was greatest with the high N supply.

Root mass ratios differed by species among the four harvests (species x harvest, $P < 0.001$; Fig. 3b). Ratios were similar at all harvests for *R. hirta,* whereas they were greater in the later harvests for *C. maculosa* and the two grasses. Plants receiving the low N supply tended to have slightly greater RMRs than plants receiving the high N supply (N supply, $P = 0.06$).

Dead biomass at Harvest 3 was similar among N supplies for *C. maculosa* and *P. smithii*, but was greater for plants receiving the high N supply in *R. hirta* and *P. spicata* (species x N supply, $P = 0.06$, Fig. 4). Overall, *C. maculosa* had the least amount of dead biomass (species, $P = 0.02$).

Carbon to nitrogen ratios in live shoots differed for species across harvests (species x harvest, $P < 0.001$; Fig. 5a), and among N supply (species x N supply, $P = 0.03$). Carbon to nitrogen ratios were similar for all species at Harvest 1. At Harvest 4, *C. maculosa* had the lowest C:N, whereas *R. hirta* had the highest. Effects of N supply
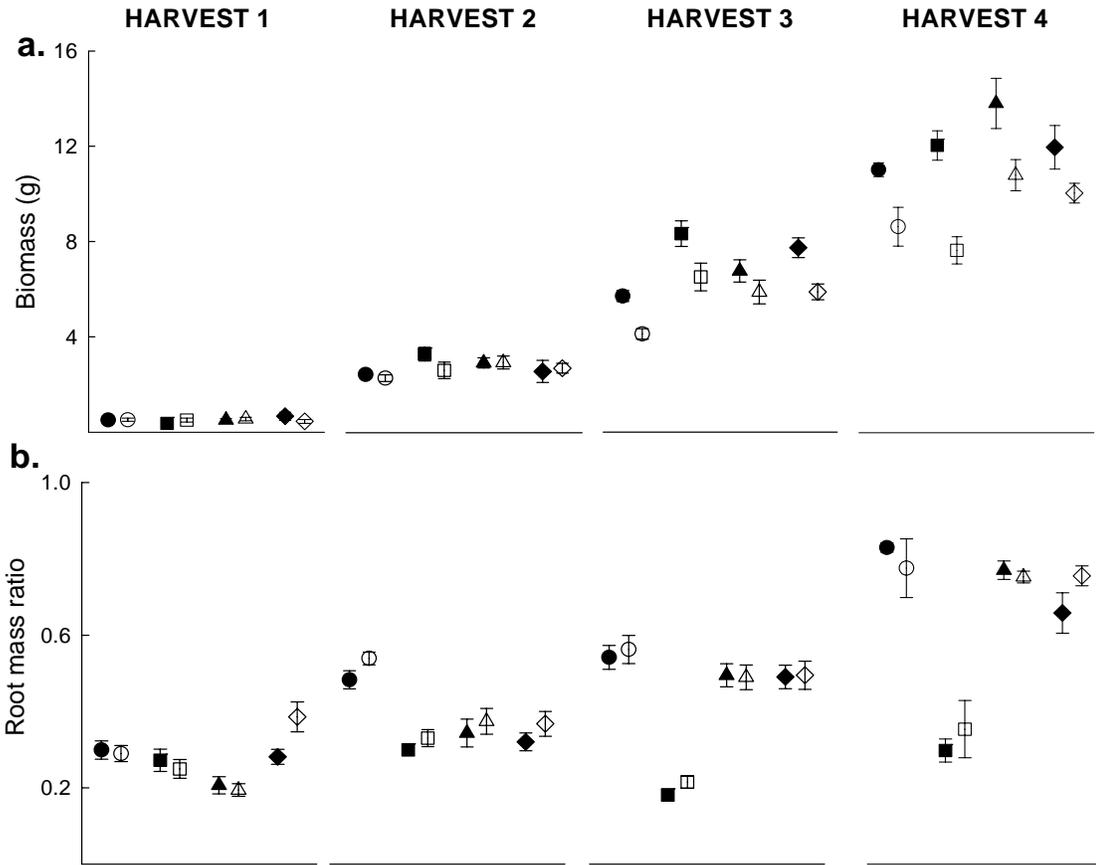
Figure 3. Total biomass in grams (a) and root mass ratio (b) for *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦) for high (closed) and low (open) nitrogen treatments at each harvest. Bars represent 1 S.E.M..
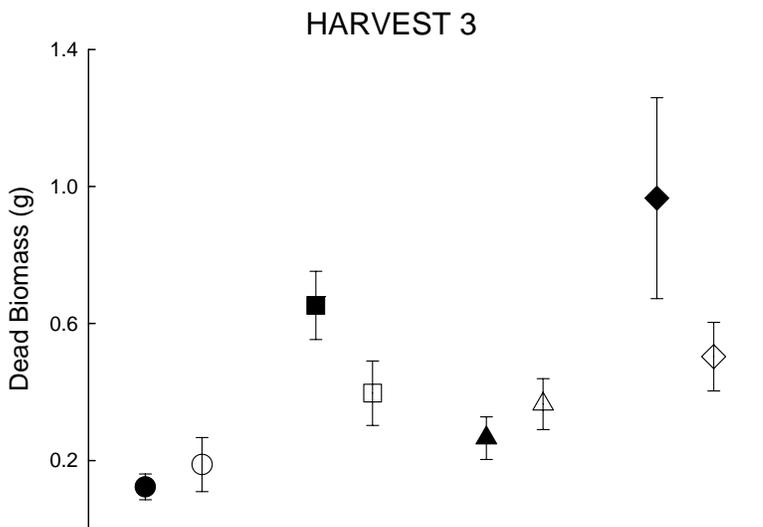
Figure 4. Total dead biomass (g) for *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦) for high (closed) and low (open) nitrogen treatments at Harvest 3. Bars represent 1 S.E.M..

also differed across harvests (N supply x harvest, P = 0.03). Carbon to nitrogen ratios were similar between N supplies at all harvests for *C. maculosa*, whereas C:N ratios for the two grasses were lower for those plants receiving the high N supply in Harvest 2, 3 and 4. Overall, *R. hirta* had the highest C:N ratios (species, P < 0.001); C:N ratios were greater with each harvest (harvest, P < 0.001).

In roots, C:N ratios were lowest in Harvest 1 (harvest, P < 0.001; Fig. 5b). Overall, C:N ratios differed among species (species, P = 0.02). Plants receiving the low N supply tended to have higher C:N ratios than those receiving the high N supply (N supply, P = 0.10).
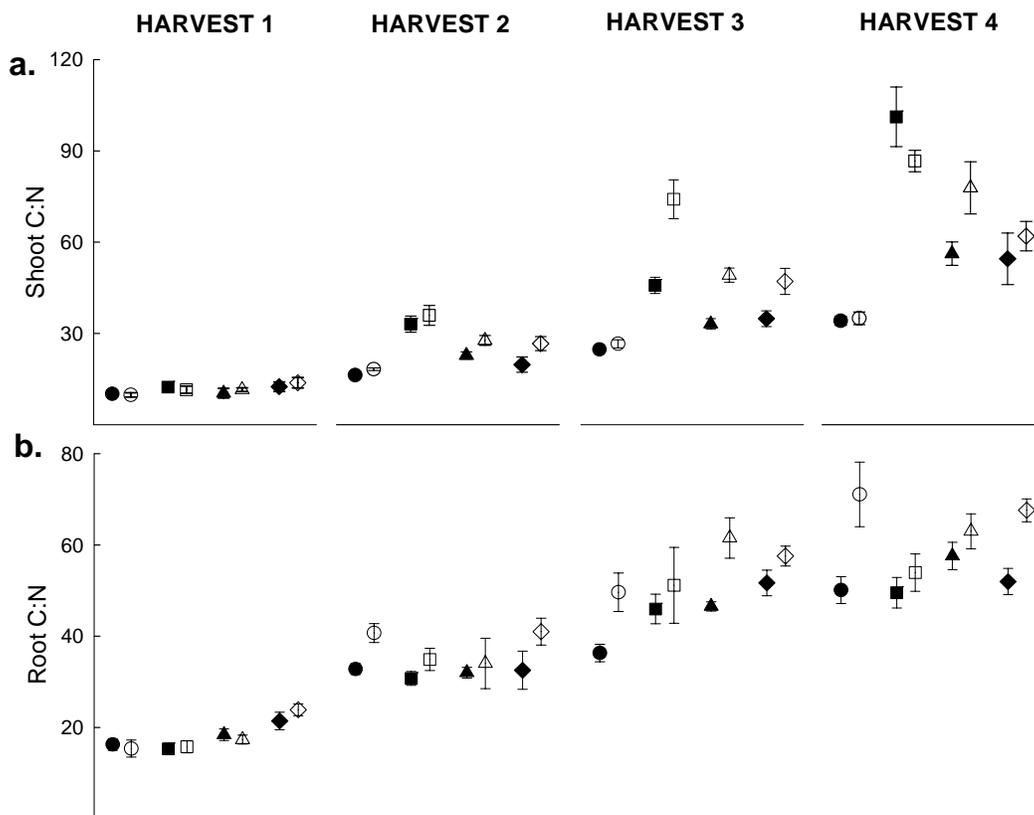
Figure 5. Carbon to nitrogen ratios (C:N) in the shoots (a) and roots (b) of *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦) for high (closed) and low (open) nitrogen supply at each harvest. Bars represent 1 S.E.M..

No plants flowered by Harvest 1, so Harvest 1 was not included in the statistical analysis. *Pascapyrum smithii* did not flower during the study so it was also not included. The number of flowerheads per plant at time of harvest differed among species by N supply (species x N supply, P = 0.07; Fig. 6) and across harvests (species x harvest, P < 0.001). *Centaurea maculosa* had more flowerheads with the high N supply than the low N supply, whereas *R. hirta* did not respond to N supply. *Pseudoroegneria spicata* had similar numbers of flowerheads at Harvests 2, 3 and 4, whereas the two forbs had greater numbers of flowerheads at the later harvests. Overall, *C. maculosa* and *R. hirta* had more flowerheads than *P. spicata* (species, P < 0.001).
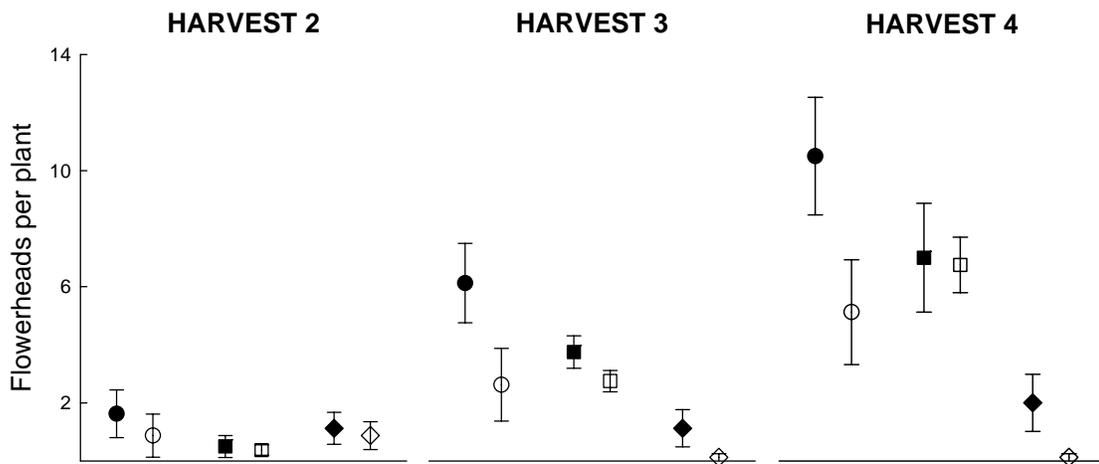
Figure 6. The number of flowerheads per plant at the time of harvest for *C. maculosa* (●), *R. hirta* (■) and *P. spicata* (♦) for high (closed) and low (open) nitrogen supply at Harvests 2, 3 and 4. Bars represent 1 S.E.M..

Resorption

Resorption efficiencies of species receiving high and low N differed among harvests, but there was no clear trend (species x N supply x harvest, P = 0.05; Fig 7a). Overall, *C. maculosa* had the lowest resorption efficiency (species, P < 0.001).

Resorption proficiency differed for species among harvests (species x harvest, P < 0.001, Fig 7b). *Centaurea maculosa* had the lowest resorption proficiency (highest concentration of N) in Harvest 2 and 3; by Harvest 4, resorption proficiency was similar to the other species. For all species, resorption proficiency tended to be lowest in Harvest 2 (Harvest, P < 0.001). Overall, resorption proficiencies were higher in plants receiving the low N supply than those receiving high N (N supply, P < 0.001).
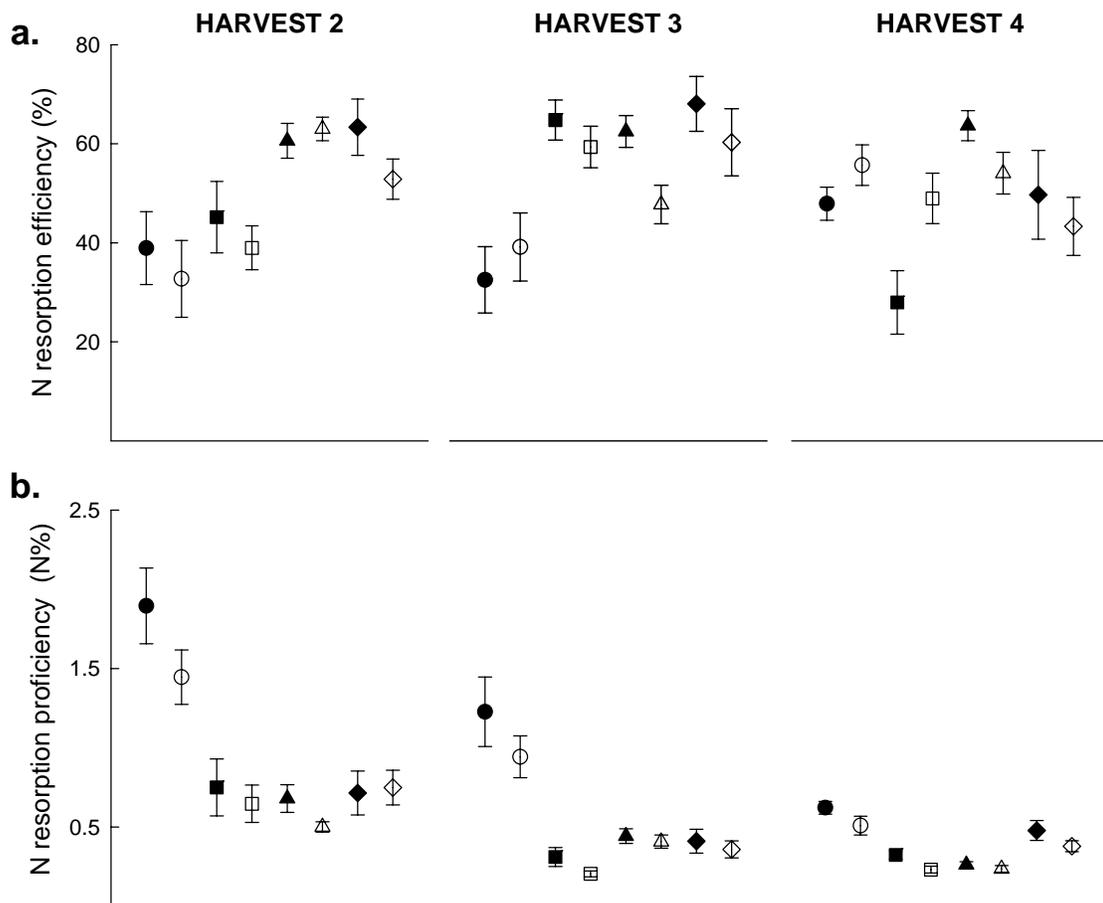
Figure 7. Nitrogen resorption efficiency (%) (a) and N resorption proficiency (% N in senesced tissure) (b) by harvest for for *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦)for high (closed) and low (open) nitrogen supply at Harvests 2, 3 and 4. Bars represent 1 S.E.M..

Nitrogen use efficiency

Mean residence time was greatest for *C. maculosa* (species, $P < 0.001$; Fig 8a). Overall, MRT tended to be greater in plants receiving the low N supply (N supply, $P = 0.05$). Nitrogen productivity was lowest in *C. maculosa* (species, $P < 0.001$; Fig 8b). Nitrogen productivity was greater in plants receiving the low N supply ($P = 0.001$).

Nitrogen use efficiency differed slightly among species by N supply (species x supply, $P= 0.14$; Fig 8c). Nitrogen use efficiency of high and low N supply plants of *C. maculosa* were similar, whereas NUEs of *R. hirta* and the grasses were greater with the low N supply.
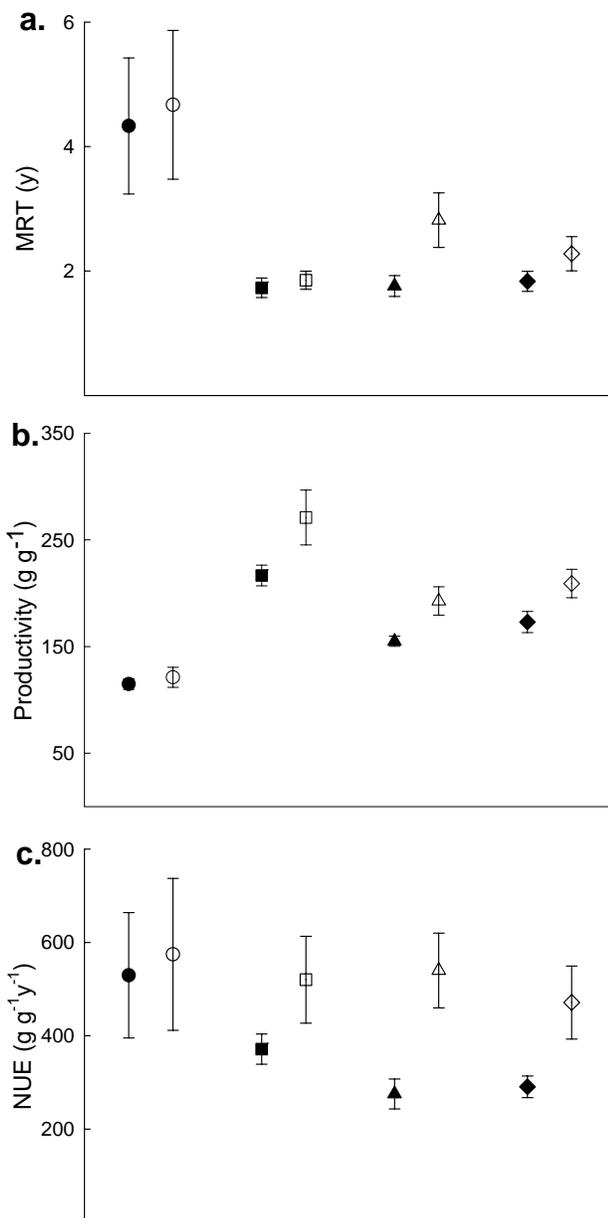
Figure 8. Mean residence time (a) N productivity (b) and NUE (c) at harvest 3 for for *C. maculosa* (●), *R. hirta* (■), *P. smithii* (▲) and *P. spicata* (♦) for high (closed) and low (open) nitrogen supply. Bars represent 1 S.E.M..

Discussion

### ^{15}N uptake

We expected that *C. maculosa* would acquire more ^{15}N per unit of root mass than the native grasses, which are slow growing species that rely on larger root mass rather than rapid uptake for N acquisition (Chapin 1980; Craine et al. 2002). However, uptake per unit of root mass was similar among *C. maculosa* and the grasses at Harvest 1. This differed from a previous study in which *C. maculosa* acquired more ^{15}N in a 24 hour period than *P. spicata* and *P. smithii* (Blicker et al. 2002), possibly because in our study the plants had five days to acquire ^{15}N and they did not have to compete with neighboring plants. *Rudbeckia hirta* had greater uptake per unit of root mass than the other species, allowing it to acquire a similar percentage of the applied ^{15}N as *C. maculosa* and *P. spicata* although it had less root mass. Further, the amount of ^{15}N acquired increased from Harvest 1 to Harvest 2 in all species, indicating that all the ^{15}N was not flushed from the pots after d 5.

Growth response

As expected, all species receiving the high N supply produced more biomass than those receiving the low N supply. Low N plants had higher root mass ratios. Plants often respond to low N availability by allocating more biomass belowground to acquire limiting resources (Poorter and Nagel 2000). In N-poor soils, like those in semi-arid grasslands, plants often have large, long-lived root systems, and high RMRs (Chapin 1980; Wilson 1993).

Root mass ratio did not increase over time in *R. hirta*; by Harvest 3, RMRs in *R. hirta* were much lower than the other species. Typically, RMRs increase over time (Aung 1974). Additionally, RMRs differed only slightly between *R. hirta* and *C. maculosa* in a previous study (Swan 2004). *Rudbeckia hirta*'s RMRs may not have changed over time because it had a high uptake per unit of root mass, which reduced the plant's need to increase root mass. Alternatively, the low RMR may have been an effect of the glasshouse, such as low light intensity, causing *R. hirta* to increase biomass allocation to shoots (Aung 1974).

Biomass and number of flowerheads differed among species as expected. Consistent with a previous glasshouse study, *C. maculosa*'s biomass was lower than the two native grasses (Blicker et al. 2003). *Pascopyrum smithii* did not produce any flowerheads. It does not produce flowers every year in the field so it is not surprising that this species did not flower under glasshouse conditions.

Centaurea maculosa produced the most flowerheads of the four species. High seed production may play a role in its success in the field (Watson and Renney 1974). High seed production may be of particular importance in disturbed sites. Centaurea maculosa produced significantly more flowerheads per plant in the high N supply, increasing its reproductive potential.

Resorption

Nitrogen resorption efficiency (percent resorbed) differed by species as expected. *Centaurea maculosa* had the lowest resorption efficiency, resorbing approximately 42% of the N, whereas both grasses resorbed slightly less than 60%. These means are close to

the averages for their respective growth forms (Aerts, 1996). *Rudbeckia hirta*'s

resorption efficiency (49%) was slightly higher than the average for forbs.

Effects of N supply on resorption efficiency and proficiency were not consistent.

Resorption efficiency was greater for plants receiving the high N supply, whereas

resorption proficiency was lower. Dissimilar responses are not uncommon (Killingbeck

1996; Wright and Westoby 2003). Nitrogen resorption efficiency was similar among two

bunchgrasses growing in their natural habitats of high or low N availability in Argentina,

but resorption proficiency is greater in species from low N soils (Distel et al. 2003).

Resorption efficiency is affected by N levels in green leaves and N withdrawal

(Killingbeck 1996). In our study, N concentrations in green leaves appeared to have

more of an effect on resorption efficiency than resorption proficiency, i.e. the

concentration of N in senesced tissue. Plants grown with high N had higher levels of N

in live biomass and therefore more N to resorb. Although N supply affected N

concentrations in senesced tissue, the difference was not enough to offset the effect that

N supply had on N levels in green tissue.

Resorption proficiency is greater in plants receiving the low N supply, which

agrees with similar studies (Aerts and de Caluwe 1994; Distel 2003). Increasing

resorption proficiency is an important N conserving strategy when N supplies are

limiting. Resorbed N can be reused for new biomass during the growing season, and

stored in remaining live tissue to be used the following growing season (Aerts 1996;

Bausenwein et al. 2001; Santos et al. 2002). In semi-arid grasslands, resorption may be

particularly important because N availability is temporally variable (Gupta and Rorson

1975; McKane et al. 1990). Plants can resorb N acquired from spring pulses and reuse it when N is no longer available in the soil.

Centaurea maculosa had the greatest difference in resorption proficiency between high and low N supply. Additionally, *C. maculosa* resorbed the least amount of N from senescing tissue at Harvest 2 and 3; by Harvest 4, when N supply was reduced to induce senescence, resorption efficiency and proficiency were similar to the other species. By having greater resorption with the low N supply, *C. maculosa* had greater NUE and MRT (Aerts 1987; Aerts 1990).

Nitrogen use efficiency

Nitrogen use efficiency is the product of MRT and N productivity. Our values for MRT were higher than those in a similar study (Aerts and de Caluwe 1994). These differences could reflect differences in plant species or growing environment. Our results with semi-arid species were similar to those from subarctic graminoids and herbs (Eckstein and Karlsson 2001). Both of these environments are nutrient limited and species may have evolved similar characteristics such as slow growth rates and high N retention.

Mean residence time was greater in low N plants indicating they were able to limit N losses by resorbing more N. *Centaurea maculosa* and *P. smithii* also had less loss of N with low N supply by reducing turnover of biomass, as indicated by the lower amount of dead biomass from those plants. Mean residence time is not always affected by N supply (Aerts and de Caluwe 1994; Vasquez de Aldana and Berendse 1997), but

others have had similar results as ours (Aerts 1989; Aerts and de Caluwe 1989; Eckstein
and Karlsson 2001).

Centaurea maculosa had the greatest MRT with both N supplies, possibly
because it had the least amount of dead biomass and a high resorption efficiency. This
was surprising because *C. maculosa* is a weedy species, which are known to have fast
growth rates. Fast growing plants often have high turnover and low MRT.

We expected that the native species would also have a longer MRT. Plants
adapted to low N soils often have high MRTs (Berendse and Aerts 1987; Vazques de
Aldana and Berendse 1997). Minimizing N loss in nutrient poor systems is an important
N conserving strategy (Monk 1966). In North American grasslands, plants with large
amounts of long-lived, low N tissue, similar to those in our study, grow well in low N
soils (Crain et al 2002). However, in our study, the species adapted to low N soils
seemed to conserve N by producing low N tissue more so than by producing long-lived
tissue.

Centaurea maculosa had the lowest N productivity. In addition, N productivity
differed only slightly among the high and low N supplies for *C. maculosa* compared with
the native species. Nitrogen productivity often decreases with increasing N availability
(Aerts and de Caluwe 1994; Vasquez de Aldana and Berendse 1997). Our values for N
productivity were similar to those from other studies (Aerts and de Caluwe 1994;
Vasquez de Aldana and Berendse 1997).

With the low N supply, all species had similar NUEs. Our values for NUE were
greater than those from similar studies (Aerts and de Caluwe 1994; Vasquez de Aldana
and Berendse 1997). Plants in those studies had lower MRTs.

Centaurea maculosa was able to have a high NUE because it had a high MRT, whereas the grasses and *R. hirta* had a high NUE because of higher N productivity. Plants have traits that result in a high MRT or N productivity, but they cannot have both (Berendse and Aerts 1987; Aerts 1990). Similar NUEs among *C. maculosa* and the native species in the low N supply may partly explain why *C. maculosa* can grow in late successional sites with other plants but often not dominate these sites.

With the high N supply, *C. maculosa* had the highest NUE. *Centaurea maculosa* had a high MRT with the high and low N supply, whereas the grasses and *R. hirta* had lower N productivity and MRT with the high N supply. As a result, *C. maculosa* was able to maintain a higher overall NUE. *Centaurea maculosa*'s success in high N soils, like disturbed sites, may be in part due to its higher NUE.

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CHAPTER 3

UPTAKE AND RESPONSE OF CENTAUREA MACULOSA AND NATIVE SPECIES

TO DIFFERENT TIMINGS OF NITROGEN PULSES

Introduction

Nitrogen (N) is one of the most limiting nutrients in grasslands (Gleeson and

Tilman 1990; Loomis and Connor 1992). Its availability, which is spatially and

temporally variable (Gupta and Rorson 1975; McKane et al. 1990), is linked to soil water

and wetting events (Cui and Caldwell 1997a; Hungate et al. 1997; Tsialtas et al. 2001).

Soil water increases N availability by increasing diffusion of N and the turnover of soil

microbes (Ivans et al. 2003). However, this increase in N availability may only last a few

days (Cui and Caldwell 1997a; Ivans et al. 2003).

To take advantage of these short pulses of N, plants must respond quickly or have

appropriate belowground morphology. Fast growing plants such as ruderals can respond

to pulses of available N by creating new roots (Robinson and VanVuuren 1998;

BassiriRad et al. 1999). Increased uptake capacity may also allow the plant to utilize

pulses of N (BassiriRad and Caldwell 1992; Cui and Caldwell 1997a; Fransen et al.

1999). Furthermore, plants can take advantage of increased N availability by having high

specific root length and root density. In two desert shrub species, differences in rooting

densities may account for the differences in N and water uptake after wetting events

(BassiriRad et al. 1999).

Certain plant species can benefit from pulses of N. Plants receiving pulses of nutrients have greater biomass and fruit production than plants receiving a continuous supply of nutrients (Benner and Bazzaz 1987; Bilbrough and Caldwell 1997). Leaf N concentrations were higher for six Great Basin, U. S. A. species receiving a pulsed supply of N compared with those receiving a steady supply (Bilbrough and Caldwell 1997). Nitrogen uptake is also greater in plants receiving pulsed supplies of N compared with steady supplies (Fransen et al. 1999; Bowman and Bilbrough 2001).

A plant's growth form can affect how it responds to irregular N availability (Bilbrough and Caldwell 1997; Robinson and Van Vuuren 1998; Bowman and Bilbrough 2001). In general, forbs and other fast-growing species are more plastic and can respond quicker to non-uniform supplies of N than slower growing species like grasses (Robinson and Van Vuuren 1998). In a study of four grass species, fast- and slow-growing species did not differ in root foraging characteristic and were able to acquire similar amounts of N from pulses, but the species differed in uptake capacity and root biomass (Fransen et al. 1999).

The timing of a N pulse affects how well a plant can utilize the pulse and how the plant will respond (Benner and Bazzaz 1987; Bilbrough and Caldwell 1997). Six old-field species differed in their temporal uptake patterns (McKane et al. 1990) and five arctic species differed in their seasonal maximum N uptake (McKane et al. 2002).

A plant's response to a pulse can be related to when the pulse occurs during its stage of growth (McKane et al. 1990; Miao and Bazzaz 1990; Bilbrough and Caldwell 1997; Gebauer and Ehleringer 2000). Six springtime ephemerals had higher growth rates

and greater biomass with early spring pulses (Bilbrough and Caldwell 1997), which mimic natural rain events and coincide with the early vegetative growth phase of plants.

Neighbor species may have a greater effect on uptake rates and the ability of a plant to acquire N from pulses. *Centaurea maculosa's* ability to acquire ^{15}N from pulses in glasshouse was influenced by its neighbor species (Olson and Blicker 2003). Nitrogen uptake, total biomass and root mass ratios differed for two grasses exposed to N pulses depending on whether they were growing in mixed- or mono-cultures (Cui and Caldwell 1997b).

In semi-arid grasslands such as those in Montana, most pulses occur in the spring in the form of snowmelt and spring rains. Approximately one third of the annual precipitation occurs in May and June (Western Regional Climate Center, Appendix C). Much of the vegetation is dominated by cool-season grasses and forbs. Our objective was to determine whether *Centaurea maculosa,* an invasive forb, is able to acquire more N and produce more biomass than native vegetation from early season pulses of N, compared with late season pulses or a steady supply, in the glasshouse and in the field.

Methods

Plant material

Centaurea maculosa Lam. is a tap-rooted, non-native perennial in the Asteraceae family (Watson and Renney 1974). It invades disturbed and undisturbed semi-arid grasslands throughout northwestern United States. *Festuca idahodensis* Elmer. and *Pseudoroegneria spicata* [Scribn. and Smith] A. Love are native, cool-season,

bunchgrasses (Moser et al. 1996; Stubbendieck et al. 2003). They begin growing in early

spring (Stubbendieck et al. 2003), and are common in areas where *C. maculosa* invades.

Balsamorhiza sagittata (Pursh) Nutt. is a native, cool season forb. Like *C. maculosa*, it

has a taproot and is in the Asteraceae family (Stubbendieck et al. 2003). *Balsamorhiza*

sagittata grows in well-drained soils along hillsides and plains, and flowers from May to

August (Stubbendieck et al. 2003). These four species were used in our field study.

     Centaurea maculosa and the same two grasses were used in our glasshouse

experiment but we used a different forb. *Rudbeckia hirta* Lam. is a short-lived, native

perennial in the same family as *C. maculosa*. It grows in disturbed areas, has a taproot,

and is similar in size to *C. maculosa* (Hitchcock and Cronquist 1973). *Rudbeckia hirta*

was used as the native forb in the glasshouse experiment because *B. sagittata* does not

establish easily in the glasshouse, and *R. hirta*'s growth form is more similar to *C.*

maculosa than *B. sagittata*.

Glasshouse study

     Centaurea maculosa, *R. hirta*, *F. idahoensis* and *P. spicata* were grown two

plants per pot in one of seven species combinations. *Centaurea maculosa* was grown

with each species or itself, as was *R. hirta*. The two grasses were not grown with

themselves or each other. The 8.8 L pots were 17 x 17 cm at the top, 37 cm tall, and

filled to within 4 cm of the top with a 10:1 mix of pasteurized sand and un-pasteurized

field soil from the Red Bluff Research Ranch 60 km west of Bozeman, Montana. The

sand was pasteurized to kill weed seeds, pathogens and insect eggs while the soil was left

unpasturized to maintain a natural soil biota. The nutrient composition of the soil

mixture was 0.7 mg kg^{-1} of NO_3^-, 0.6 mg kg^{-1} of P, 80 mg kg^{-1} of K, and less than 0.05%

(mass basis) organic matter. H_3PO_4 was added to each pot to raise P levels to 20 g kg^{-1},

which are similar to phosphorus levels in field soils.

Festuca idahoensis was seeded first because it takes longer to establish. Thirty

five days later the other species were seeded. After all plants were seeded, pots were

arranged in a randomized grid. The pots were rearranged twice before the study began to

ensure that placement in the greenhouse would not affect initial size of plants. Plants

were not re-randomized after the N treatments began. While plants were being

established they were watered every other day with 200 ml of water. Commercial

fertilizer (0.84 g; Miracle-Gro, Stern's Nurseries, Geneva, NY, USA: 30:30:30 NPK) was

added to each pot. Pots were thinned to two plants per pot. Supplemental lighting (GE

Multi-vapor MVR1000/C/U, General Electric Company, Cleveland Ohio, USA) was used

to maintain a constant 12 h day-length.

Pairs of plants were treated with one of three ^{15}N treatments: an early treatment

when plants were in a vegetative stage, a late treatment when most plants were bolting or

flowering, and a steady treatment. All pots received 0.433 g of $^{15}NH_4^{15}NO_3$ 10 atom %

(0.13 g ^{15}N) as follows.

On d 1 of the study, 0.433 g of $^{15}NH_4^{15}NO_3$ in 200 ml of H_2O was added to the

early treatment pots, 0.144 g $^{15}NH_4^{15}NO_3$ in 200 ml of H_2O was added to the steady

treatment pots, and 200 ml of H_2O was added to the late treatment pots. At this time

nearly all plants were in the early vegetative stage, although some of the *R. hirta* had

begun to bolt. On d 13, the second treatment of 0.144 g $^{15}NH_4^{15}NO_3$ in 200 ml of H_2O

was added to the steady treatment pots; all other pots received water. On d 25, 0.433 g of

$^{15}NH_4{}^{15}NO_3$ in 200 ml of H_2O was added to the late treatment pots, the last 0.144 g

$^{15}NH_4{}^{15}NO_3$ in 200 ml of H_20 was added to the steady treatment pots, and 200 ml of

water was added to the early treatment pots. Plants were watered with drip emitters every

other day. Seven days after each treatment, columns were watered beyond saturation to

remove any remaining ^{15}N from the soil.

Plant height, stage of growth, number of tillers, and number of flowers or buds

were recorded within 24 h of each treatment and at the time of harvest. Plants were

harvested on d 78 when most plants were finishing their reproductive stage and beginning

to senesce. Plants were harvested by removing all aboveground material and separating

live from dead material. Plant material was placed in drying ovens immediately

following harvest. Pots were stored at 4°C in a dark room after the shoots were removed,

and before the roots were cleaned. Roots were cleaned to remove any organic and

inorganic matter, and plants were separated from each other in a water bath.

Field study

Three sites were established in western Montana where *Centaurea maculosa*

grows with native vegetation. The Winston Site is a railroad right-of-way 120 km

northwest of Bozeman, Montana (45° 37' N, 111° 46' W). The soil is a Martinsdale

loam, a deep well-drained loamy soil (Olson et al. 1977). The average annual

precipitation at the site is 272 mm, and average precipitation for the months of May and

June is 95 mm (Western Regional Climate Center, 2004, Appendix C). The Hyalite Site

is on private property 12 km southwest of Bozeman, Montana (45° 37' N, 111° 07' W)

near Hyalite Creek. The soils are loamy, mixed frigid Typic Argiustolls (Brooker et al.

2002). The average annual precipitation is 490 mm, and average precipitation for May and June is 150 mm (Western Regional Climate Center, Appendix C). The Theide Site is 60 km west of Bozeman, Montana (45° 30' N, 111° 37' W) near the Red Bluff Research Ranch. The soils are loamy-skeletal mixed Pachic and Typic Cryoborolls (Boast and Shelito 1989). The average annual precipitation is 429 mm and the average precipitation for May and June is 95 mm (Western Regional Climate Center, Appendix C). All three sites have well-drained soils and are currently not grazed by livestock.

Eight clusters of plants were established at each site. Each cluster contained four target plants of each species. *Centaurea maculosa* was matched with *P. spicata* at the Winston Site, with *P. spicata* and *F. idahoensis* at the Theide Site, and with *F. idahoensis* and *B. sagittata* at the Hyalite Site.

Each site had three treatments and a control. The three treatments were: 1) an early spring treatment of ^{15}N (Day 1), 2) a late spring treatment of ^{15}N applied four weeks after the early spring treatment (Day 29), and 3) a steady treatment of ^{15}N which was one third the strength of the early and late spring pulses, applied three times at 14 d intervals (Day 1, 15 and 29). The control plants received water.

On d one of the study, 1 L of 14 mmol L^{-1} $^{15}NH_4$$^{15}NO_3$ 10 atom % was poured on the soil surface in a 15 cm radius around each early spring treatment plant, one L of 4.66 mmol L^{-1} $^{15}NH_4$$^{15}NO_3$ was applied to the steady treatment plants and one L of water was applied to late spring and control plants. On d 15, 1 L of 4.66 mmol L^{-1} $^{15}NH_4$$^{15}NO_3$ 10 atom % was applied to the steady treatment plants and water was applied to the early and late treatment plants and the control plants. On d 29, 1 L of 14 mmol L^{-1} $^{15}NH_4$$^{15}NO_3$ 10 atom % was applied to the late spring treatment plants, one L of 4.66 mmol L^{-1} $^{15}NH_4$^{15}

NO_3 was applied to the steady treatment plants, and one L of water was applied to the early treatment and control plants. In 2003, treatments began on 2 May at the Winston Site, 9 May at the Theide Site, and 16 May at the Hyalite Site. These treatments were intended to simulate typical spring rain events in western Montana.

Plant heights, and basal and canopy area were recorded for each target plant within 24 h of applying the first nutrient treatment to determine initial aboveground plant volume. Volumes were calculated as follows:

$$(3.1415h/3) \times [(cmax \times cmin) + 0.5(cmax + cmin) + (bmax \times bmin) + 0.5(bmax + bmin)] \qquad (1)$$

where h is height, cmax is maximum canopy width, cmin is minimum canopy width, bmax is maximum base width, and bmin is minimum base width (Johnson et al. 1988). Vegetative stage and the number of flowerheads were also recorded for each plant at the end of the growing season. One to three leaves from each target plant were collected 7 d after labeling, and aboveground biomass was collected at the end of the growing season to determine ^{15}N enrichment and percent N. Leaves from control plants were collected to determine percent N and background ^{15}N in the plants.

Growth response and N analyses

Leaves and plants were oven-dried at 42° for at least one week. Live and dead shoots and roots of the glasshouse plants were weighed to determine biomass and root mass ratios. All samples were ground in a Udy mill cyclone grinder (Udy Corporation, Fort Collins, Colo.USA). Samples were analyzed for C (%), N (%), and ^{15}N content with an Isotope Ratio Mass Spectrometer (U.C. Davis Stable Isotope Facility, Davis, CA).

For the glasshouse plants, growth response was determined by total biomass (root mass plus shoot mass), root mass ratio (root mass/total mass), and an index of relative growth rate (RGR). Relative growth rate was based on non-destructive measures of aboveground biomass only:

$$1/d \, [\ln (h_2 \times leaves_2) - \ln(h_1 \times leaves_1)] \tag{2}$$

where h is the height of the plant, leaves is the number of live leaves or tillers on the plants and d is the number of days between measurements. Relative growth rate was calculated for three time periods: Period 1 (d 0 - one day before the early treatment to d 12 - one day before applying the second steady treatment), Period 2 (d 12 to d 24 - one day before the late N treatment), and Period 3 (d 24 to d 39 - 14 days after the late treatment).

The total amount of ^{15}N acquired was the sum of the product of root mass, percent N, and fractional abundance (F), and the product of shoot mass, percent N, and F. ^{15}Nitrogen concentration was calculated as the total amount of ^{15}N acquired divided by total biomass. Nitrogen pool was calculated as the sum of the product of root mass and percent N and the product of shoot mass and percent N. Percent N in roots and shoots was also measured.

For the field plants, growth response was determined by the final aboveground biomass. ^{15}Nitrogen concentrations in the leaves 7 d after N treatments were applied the product of percent N and F. Percent N 7 d after N treatments and at the end of the growing season were determined.

Fractional abundance and atom % ^{15}N were determined by the following steps (Boutton 1991; Shearer and Kohl 1993):

$$\delta \ ^{15}N \ (\text{‰}) = [(R_{sample} - R_{std})/R_{std}] \ x \ 10^3 \tag{3}$$

where $\delta \ ^{15}N$ (‰) is ^{15}N discrimination, and R represents the absolute ratio ($^{15}N/^{14}N$) of

the plant sample (R_{sample}) and standard gases (R_{std}). The absolute ratio for the standard

gas is $3.6765 \ x \ 10^{-3}$. The absolute ratio of a sample (R_{sample}) can be determined by

rearranging (3):

$$R_{sample} = \ ^{15}N \ / \ ^{14}N = [(\delta \ ^{15}N \ / \ 1000) + 1] \ x \ R_{std} \tag{4}$$

R_{sample} is used to calculate F, fractional abundance:

$$F = \ ^{15}N \ / \ (^{15}N + \ ^{14}N) = R_{sample} \ / \ (R_{sample} + 1) \tag{5}$$

Statistical analyses

The glasshouse study was a complete randomized design with four species, seven

neighbor combinations, and three treatments. Data were fit using a general least squares

model. To account for correlation within pot, a general correlation structure was defined

with pot as the grouping factor (R 1.8.1 2003). Because of the large differences in

variances between species, variances were weighted to allow the data to be analyzed

using ANOVA. Total biomass, total number of flowerheads, total ^{15}N acquired, and ^{15}N

concentration were transformed with square root. Nitrogen pool and percent N in the

roots and shoots were transformed with natural log. Root mass ratios were not

transformed. Relative growth rate was analyzed using repeated measures with the

number of treatments as the between factor effect and the three time periods as the within

factor effects (R 1.8.1 2003). Each species was analyzed separately because of the large

differences in variances among species. Relative growth rate data were not transformed.

Each of the three field sites was a complete randomized block design with eight replicates. Pre-treatment plant volume was used as a covariate for all of the variables. Percent N and ^{15}N concentration were analyzed for leaves collected seven days after the early- and late N treatments, and seven days after the final steady treatment was applied. Shoot biomass, percent N, and ^{15}N present at the end of the growing season were analyzed. We included leaves from control plants collected at the same time as the steady treatment. All data were transformed with natural log and analyzed using ANCOVA (R 1.8.1 2003). Each *Centaurea*-neighbor combination was analyzed separately because these *Centaurea*-neighbor treatment combinations were at different sites, and thus treatment combinations were not interspersed.

Figures present non-transformed data. P-values less than 0.10 are presented (Gill 1981).

Results

Glasshouse growth response

Total biomass was greatest in *R. hirta* (species, $P < 0.001$, Fig 1a). Overall, plants receiving the steady N treatment tended to have the most biomass, whereas plants receiving the late N treatment had the least biomass (treatment, $P < 0.001$). Plants paired with *C. maculosa* or *R. hirta* had less biomass than those paired with the grasses (neighbor, $P < 0.001$).

Root mass ratios differed among species by N treatment (species x treatment, $P = 0.007$; Fig 1b). Root mass ratios were similar for all three N treatments in *C. maculosa*.

Figure 1. Total biomass (a) and root mass ratio (b) of glasshouse plants for *C. maculosa* (CEMA), *R. hirta* (RUHI), *F. idahoensis* (FEID) and *P. spicata* (PSSP) when receiving the early (●), late (■) and steady (▲) N treatments and growing with *C. maculosa* (C), *R. hirta* (R), *F. idahoensis* (F) and *P. spicata* (P). Bars represent one standard error of the mean (S.E.M.).

Root mass ratios for *F. idahoensis* were highest in plants receiving the early treatment, and similar for plants receiving the late and steady treatment. Root mass ratios in *P. spicata* were higher for plants receiving the steady treatment than those receiving the late treatment. Overall, RMRs was lowest for *R. hirta* (species, P < 0.001). Overall, RMRs were highest for plants receiving the early N treatment (treatment, P = 0.01).

Overall, RGRs were highest in *R. hirta*. Relative growth rates differed among treatments by time period (treatment x time; P < 0.001 for *C. maculosa*, *R. hirta*, and *F. idahoensis*, P = 0.02 for *P. spicata*, Fig. 2). For all species, RGRs for Periods 1 and 2
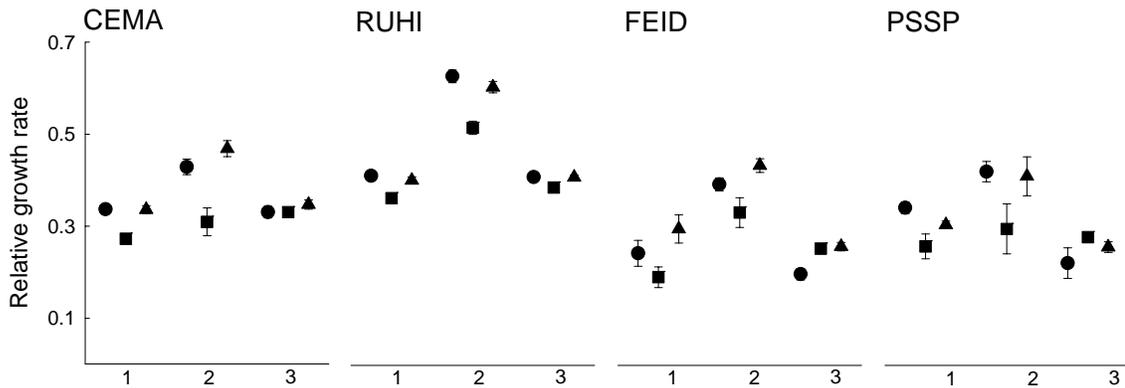
Figure 2. Index of relative growth rate for *C. maculosa* (CEMA), *R. hirta* (RUHI), *F. idahoensis* (FEID) and *P. spicata* (PSSP) when receiving the early (●), late (■) and steady (▲) N treatments at time period 1 (one day before the first N treatment was applied to one day before the second N treatment was applied), 2 (one day before the second N treatment was applied to one day before the last N application was applied), and 3 (one day before the last N application was applied to 14 days after the application was applied). Bars represent 1 S.E.M..

were lowest in plants receiving the late treatment, whereas for Period 3, RGRs were

similar among treatments. For all species RGRs across treatments were highest for

Period 2 (time, P < 0.001 for all species). Overall, RGRs were lowest for all species in

plants receiving the late treatment (treatment; P < 0.001 for *C. maculosa*, *R. hirta* and, *F.*

idahoensis, P = 0.06 for *P. spicata*).

Overall, plants receiving the late treatment had the greatest percent N (treatment,

P < 0.001, Fig. 3a). Percent N in the shoots was lowest for *R. hirta* (species, P < 0.001).

Percent N was highest in plants paired with *C. maculosa,* and lowest when paired with *R.*

hirta (neighbor, P = 0.05).

Percent N in the roots differed among N treatments depending on neighbor

(neighbor x treatment, P = 0.09; Fig. 3b). Overall, percent N in roots differed among

species (species, P < 0.001). Percent N in roots was highest in plants receiving the late

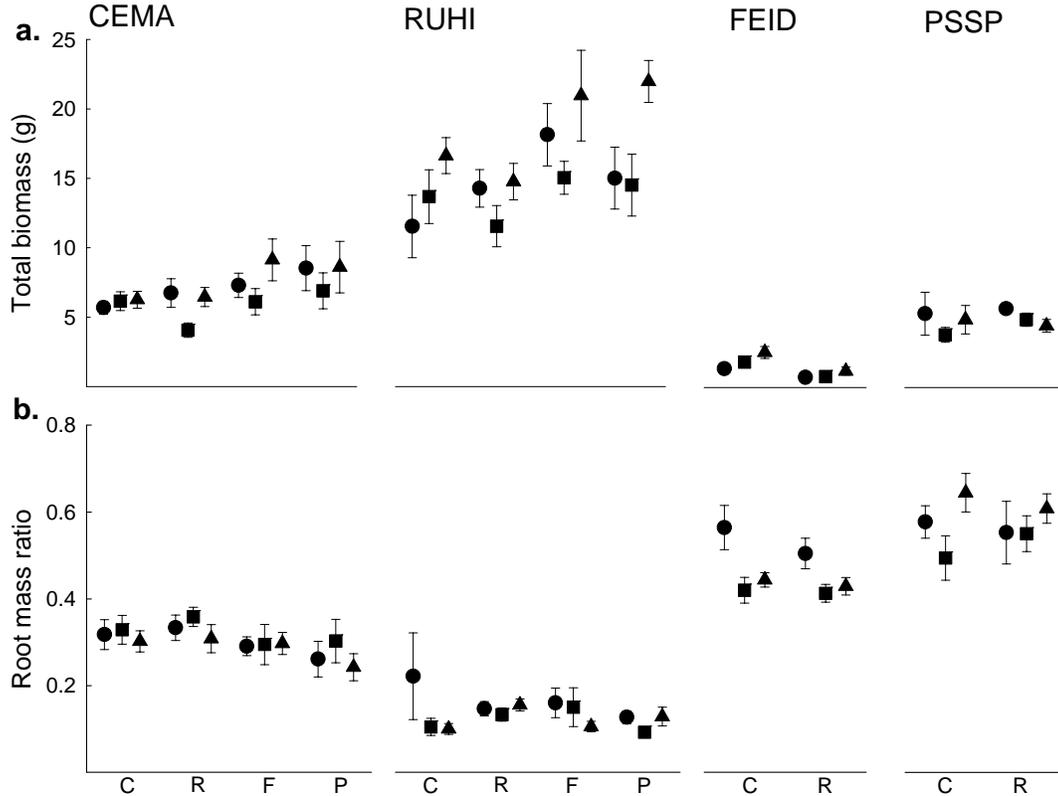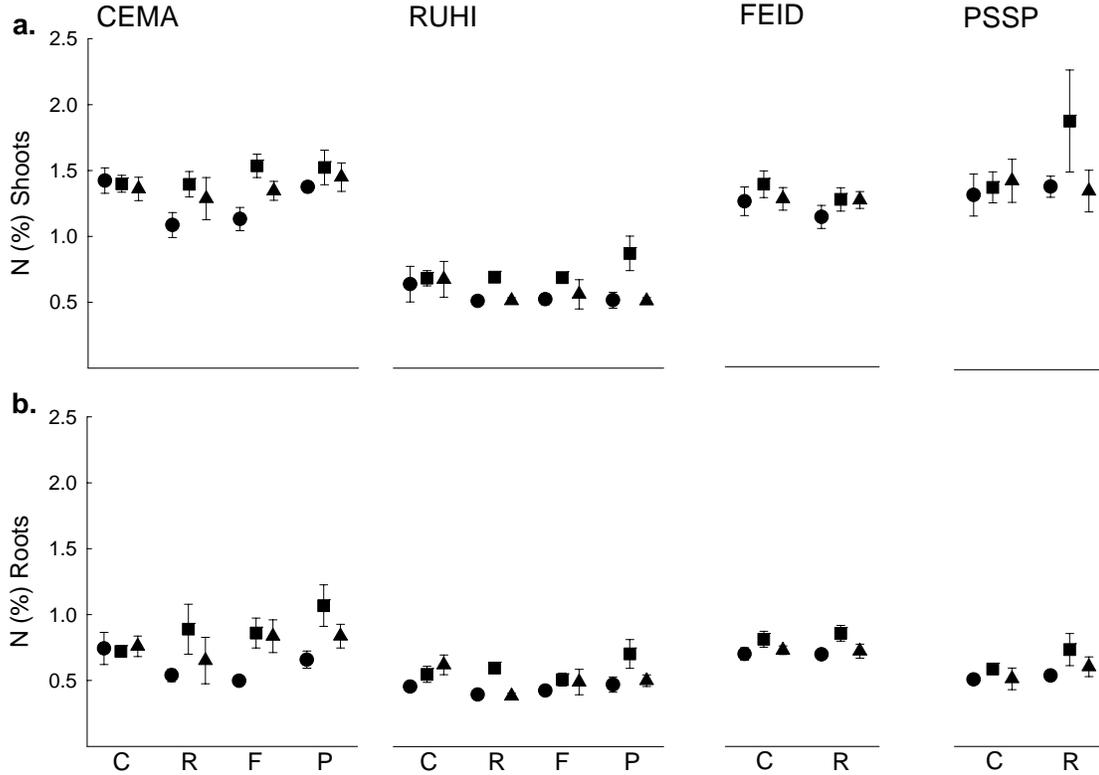Figure 3. Percent N in shoots (a) and roots (b) of glasshouse plants for *C. maculosa* (CEMA), *R. hirta* (RUHI), *F. idahoensis* (FEID) and *P. spicata* (PSSP) when receiving the early (●), late (■) and steady (▲) N treatments and growing with *C. maculosa* (C), *R. hirta* (R), *F. idahoensis* (F) and *P. spicata* (P). Bars represent 1 S.E.M..

treatment (treatment, $P < 0.001$). Percent N in the roots tended to be highest in plants

paired with *P. spicata*, and lowest for those paired with *R. hirta* and *F. idahoensis*

(neighbor, $P = 0.04$).

At the time of harvest, only *C. maculosa* and *R. hirta* produced enough

flowerheads for statistical analysis. Only one *P. spicata* plant flowered; none of the *F.*

idahoensis flowered. Of the two forbs, *C. maculosa* had the most flowerheads per plant

(species, $P < 0.001$). The number of flowerheads per plant did not differ between N

treatments for *C. maculosa* or *R. hirta*. Plants paired with *R. hirta* had the lowest number

of flowerheads (neighbor, $P < 0.001$).

Glasshouse ^{15}N uptake

Total ^{15}N uptake by species, ranging from highest in the forbs, lowest in the grasses (species, P < 0.001; Fig 4a). Overall, plants receiving a steady treatment of N had the highest ^{15}N, whereas those receiving the early treatment had the lowest ^{15}N (treatment, P = 0.001). Total ^{15}N was lowest for those paired with *R. hirta* (neighbor, P = 0.001).

^{15}N concentration (^{15}N per unit of biomass) of species differed among N treatments (species x treatment, P = 0.02; Fig 4b). For *C. maculosa* and *F. idahoensis,* plants receiving the early treatment had the lowest concentration of ^{15}N, whereas *R. hirta* plants receiving the late treatment plants had the highest. Overall, concentration of ^{15}N was highest in *C. maculosa* (species, P < 0.001). Plants receiving the late treatment had the greatest concentration of ^{15}N, whereas plants receiving the early treatment had the lowest (treatment, P < 0.001). Overall, plants paired with *C. maculosa* had the highest concentration of ^{15}N of biomass, whereas those paired with *R. hirta* had the lowest (neighbor, P = 0.06).

Total N (N pool) was highest in the forbs and lowest in the grasses (species, P < 0.001; Fig. 5). Overall, total N was lowest in plants receiving the early treatment (treatment, P < 0.001). Plants paired with *P. spicata* had the highest total N, whereas plants paired with *C. maculosa* and *R. hirta* had the lowest (neighbor, P < 0.001).

Field growth response

Aboveground biomass did not differ among treatments at any site. At the Hyalite Site, *B. sagittata* had the greatest biomass, whereas *F. idahoensis* had the lowest (species,
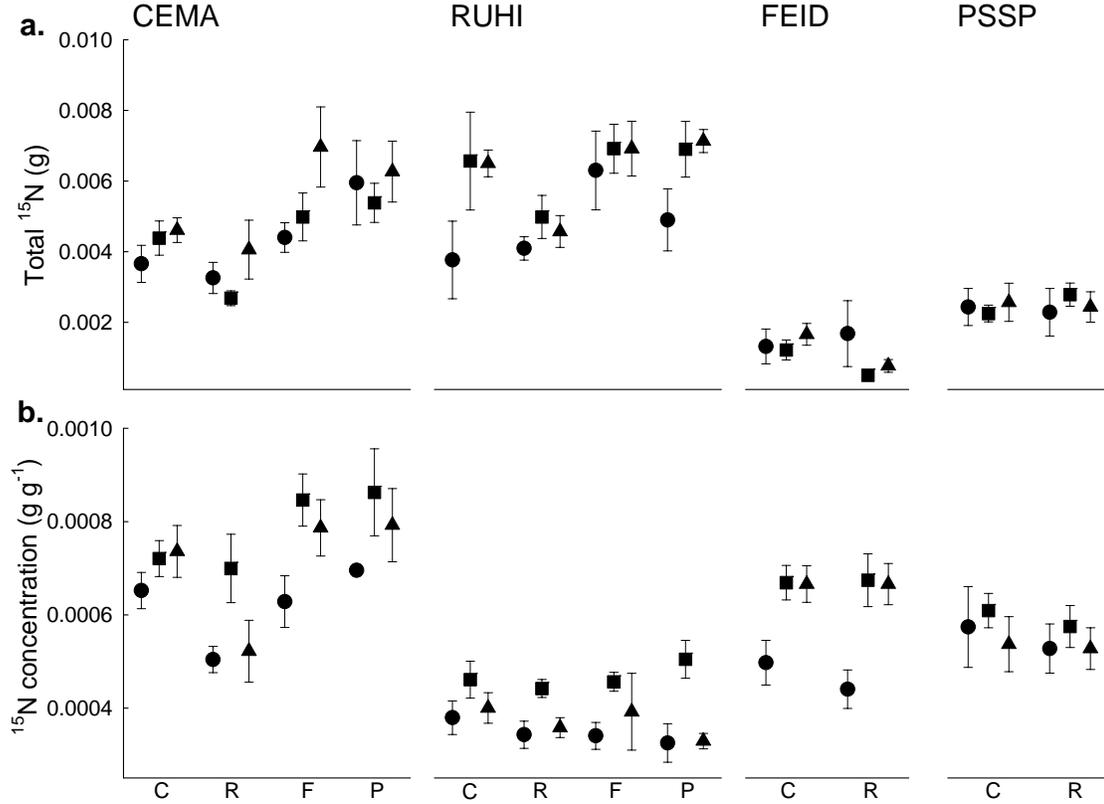
Figure 4. Total ^{15}N (g) present in the plant for above- and belowground biomass (a) and the concentration of ^{15}N (b) for glasshouse plants in *C. maculosa* (CEMA), *R. hirta* (RUHI), *F. idahoensis* (FEID) and *P. spicata* (PSSP) when receiving the early (●), late (■) and steady (▲) N treatments and growing with *C. maculosa* (C), *R. hirta* (R), *F. idahoensis* (F) and *P. spicata* (P). Bars represent 1 S.E.M..
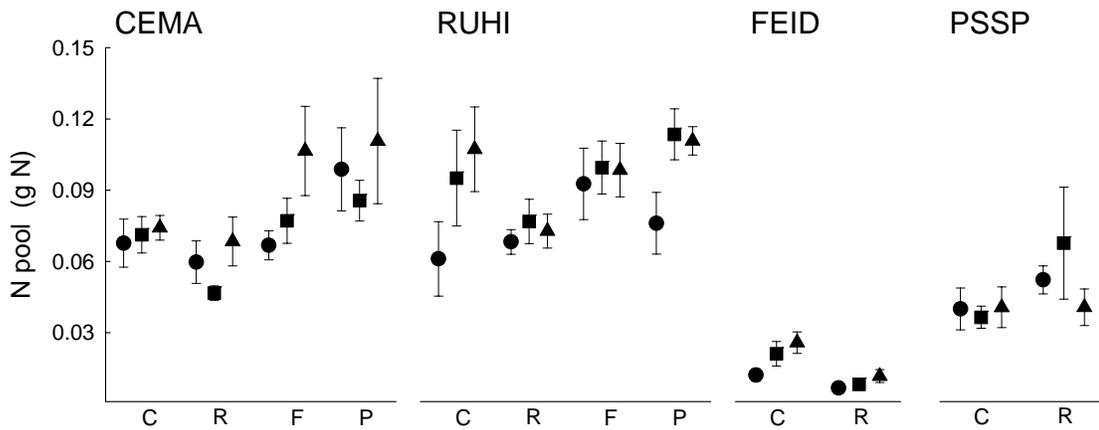
Figure 5. N pool (total g of N present) in *C. maculosa* (CEMA), *R. hirta* (RUHI), *F. idahoensis* (FEID) and *P. spicata* (PSSP) when receiving the early (●), late (■) and steady (▲) N treatments and growing with *C. maculosa* (C), *R. hirta* (R), *F. idahoensis* (F) and *P. spicata* (P). Bars represent 1 S.E.M..

P < 0.001; Fig. 6). At the Theide Site, *F. idahoensis* had the lowest biomass (species, P <

0.001). Biomass was similar for *C. maculosa* and *P. spicata* at the Winston Site (species,

P = 0.6). The number of flowerheads did not differ by treatment at any site; *C. maculosa*

had the most flowerheads at all sites.

Figure 6. Biomass at the final harvest for *C. maculosa* (CEMA), *F. idahoensis* (FEID), *P. spicat*a (PSSP) and *B. sagittata* (BASA) for the early (●), late (■), and steady (▲) N treatments, and the control (♦) at their respective sites. Bars represent 1 S.E.M.. Sample sizes at the Hyalite Site ranged from 2 to 6.

At the Hyalite Site, percent N in the leaves seven day after the treatments differed

among species across treatments (species x treatment, P < 0.001; Fig 7a). Percent N was

lowest in the control for *C. maculosa* and *F. idahoensis*. For *B. sagittata,* percent N was

highest in the early N treatment, and similar among the late and steady treatments and the

control. Overall, percent N was highest in plants receiving the early treatment (treatment,

P < 0.001). *Festuca idahoensis* had the lowest percent N (species, P < 0.001).

At the Theide and Winston Sites, percent N in leaves seven days after the early

treatments was greatest in plants receiving the early treatment, and lowest in those

receiving the steady treatment (treatment, P < 0.001). Percent N also differed among

species at the Theide Site; *C. maculosa* had the highest percent N and *F. idahoensis* had

the lowest (species, P < 0.001). At the Winston Site, *C. maculosa* had the highest percent

N (species, P = 0.01).

Percent N at the end of the growing season was similar among treatments at the

Hyalite Site. Percent N at the site was lowest for *B. sagittata* and highest for *F.*

idahoensis (species, P < 0.001; Fig. 7b).
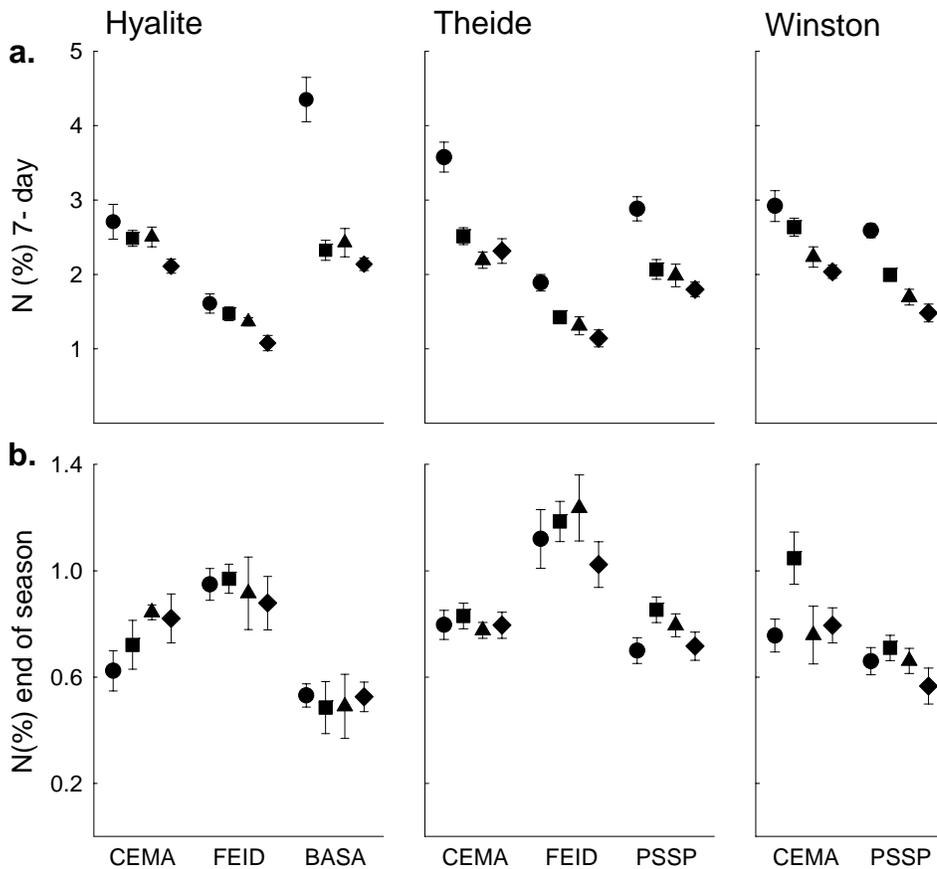
Figure 7. Percent N from the leaves seven days after the early and late treatment, and seven days after the final application for the steady treatment (a) and percent N in the total aboveground biomass at the end of the growing season after senescence (b) for *C. maculosa* (CEMA), *F. idahoensis* (FEID), *P. spicata* (PSSP) and *B. sagittata* (BASA) for the early (●), late (■) and steady N treatments (▲), and the control (♦) at their respective sites. Bars represent 1 S.E.M. Samples sizes at the Hyalite site range from 2 to 6 for end of season data.

At the Theide Site, percent N at the end of the growing season differed among treatments (treatment, P = 0.05). Percent N for *C. maculosa* and *P. spicata* was similar, and higher than percent N in *F. idahoensis* (species, P < 0.001).

At the Winston Site, percent N at the end of the growing season was highest in plants receiving the late treatment and lowest in the control plants (treatment, P = 0.01). *Pseudoroegneria spicata* had lower percent N in aboveground biomass than *C. maculosa* at the end of the growing season (species, P= 0.05).

Field ^{15}N uptake

The concentration of ^{15}N (^{15}N per unit of biomass) in leaves seven days after the early and late N treatments, and seven days after the final steady treatment of N differed by treatment at all three sites (treatment, P < 0.001; P < 0.001; P = 0.003, Winston, Theide, Hyalite respectively; Fig. 8). At the Theide and Hyalite Sites, ^{15}N concentrations were highest in plants receiving the early treatment, and were similar among plants receiving the late and steady treatments. At the Winston Site, ^{15}N concentrations were similar among plants receiving the early and late treatments; ^{15}N concentrations were lowest in plants in the steady treatment. ^{15}N concentrations were similar among species except at the Hyalite Site, where the ^{15}N concentration of *B. sagittata* was the lowest (species, P < 0.001).
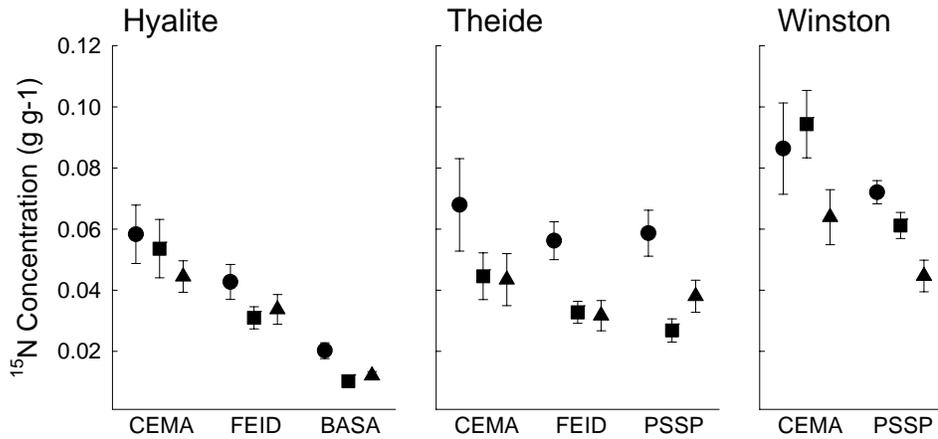
Figure 8. ^{15}N concentration from the leaves seven days after the early and late treatments and seven days after the final application of the steady t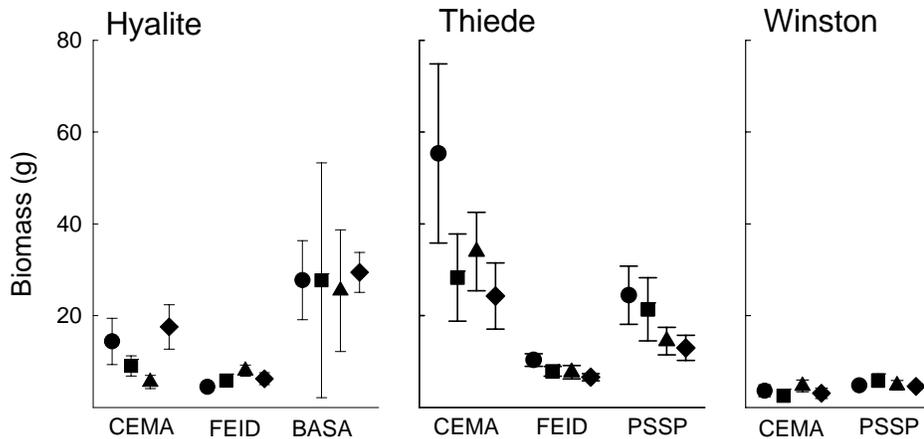reatment for *C. maculosa* (CEMA), *F. idahoensis* (FEID), *P. spicata* (PSSP) and *B. sagittata* (BASA) for the early N pulse (●), late N pulse (■), and steady N treatment (▲)and the control (♦) at their respective sites. Bars represent 1 S.E.M..

Discussion

Glasshouse study

     The timing of a nutrient pulse in relation to a plant's stage of growth may

determine how well a plant will be able to use the nutrients (McKane et al. 1990;

Bilbrough and Caldwell 1997; Gebauer and Ehleringer 2000). Cool-season adapted

plants, such as the ones used in this study, had greater biomass and higher growth rates

with early spring pulses that coincided with the early growth phase of the plants

compared with mid- and late spring pulses (Bilbrough and Caldwell 1997). Similarly, in

our glasshouse study, plants receiving the late treatment had the lowest total biomass and

lowest overall RGRs. Percent N was also highest in the late treatment plants, indicating

that these plants had the lowest N productivity (biomass per unit of N). The late

treatment was applied after most of the plants in the glasshouse, except for *F. idahoensis*,

had bolted or flowered. Apparently, it was too late in the growth cycle for the plants to use the late N treatment to produce additional biomass.

Overall, plants receiving the steady N treatment had the greatest biomass and highest RGR. We had predicted that plants receiving the early treatment would have greater biomass than those receiving the steady N treatment, similar to Bilbrough and Caldwell (1997). Perhaps the presence of neighbor species inhibited a growth response. In a previous study of *C. maculosa* and two native grasses, neighbor species had a strong influence on biomass (Olson and Blicker 2003).

Nitrogen may also have been stored for later use rather than being converted into biomass. Luxury consumption is common among slow-growing species; excess nutrients are acquired during pulses and stored in tissue until nutrients in the soil are no longer available (Chapin 1980). Our glasshouse plants may have acquired and stored excess N, but plant material was only harvested at the end of the study; therefore it is unknown if N levels in the plants were elevated within a few days of N treatments.

Although the plants receiving the early N treatment did not have the greatest biomass, RMRs were highest in plants receiving the early treatment and similar among plants receiving the late and steady treatments. All species receiving the early N treatment allocated more biomass belowground; apparently this was in response to low N levels in the soil which presumably developed later during the growing period (Aung 1974; Poorter and Nagel 2000). High RMRs may be advantageous in semiarid grasslands where water and nutrients often limit growth. In grasslands belowground resources are more important to plant growth and survival than aboveground resources, therefore plants with large root systems are better competitors (Wilson 1993; Casper and Jackson 1997).

Root mass ratios in *C. maculosa* were lower than the grasses and did not differ among the three treatments, indicating that it may have another mechanism for acquiring N in a nutrient-limited system. *Centaurea maculosa* may be able to respond to changes in soil N by increasing its uptake kinetics (BassiriRad and Caldwell 1992; Cui and Caldwell 1997a; Fransen et al. 1999), or by reducing root growth of its neighbors through allelopathic compounds, as shown in sand culture or hydroponics (Ridenour and Callaway 2001; Bais et al. 2003). However, *C. maculosa* simply may be more physiologically plastic than the native grasses. *Centaurea maculosa* plants receiving the early treatment had lower percent N in roots and shoots compared with *C. maculosa* plants receiving the late and steady treatments, indicating that it was able to respond to low levels of N later in the growth cycle by increasing its N productivity (biomass per unit of N) rather than by increasing its root system. Although the timing of the pulse affected percent N for all species, the effect was most pronounced in *C. maculosa*.

Total ^{15}N may be a better indicator than ^{15}N concentration as to how much N was acquired during the treatments because additional biomass was created after the first treatment, reducing the concentration of ^{15}N. As expected, the forbs had more total ^{15}N, possibly because the forbs had more biomass, thus this does not necessarily indicate a greater uptake capacity or uptake per unit of root mass

Plants receiving the early treatment had the lowest total ^{15}N, possibly because these plants were relatively small at the time of the early N treatment. Although the plants may have been able to respond by taking up a large amount of ^{15}N per unit of biomass, the plants had little biomass, therefore the total amount of ^{15}N was still low.

Thus ^{15}N concentration from the glasshouse study may not be a good indicator as to which pulse elicited the greatest uptake response.

Field study

In the field, biomass did not differ by N treatment at any site, possibly because precipitation at all three field sites in 2003 was below average and it was the fifth year of a drought (Heim 2004; WRCC 2004). Nitrogen is often the limiting factor in semiarid grasslands and N additions have increased aboveground biomass in some of the species used in this study (Gleeson and Tillman 1990; Loomis and Connor 1992; Krueger-Mangold et al. 2003). However, in grassland communities N is only limiting when water is freely available (Dodd and Lauenroth 1979). Therefore, low precipitation may have offset any potential growth response that the plants had from the N treatments.

The lack of a treatment response may be in part due to luxury consumption. Percent N in leaves collected seven days after the treatment was highest in plants receiving the early treatment and lowest in control plants, indicating that early N treatment plants were able to take up more N than they needed (luxury consumption). Excess N taken up in these pulses could be stored until later in the growing season when N in the soil is not as available (Chapin 1980).

The early treatment plants also had a higher percent N and ^{15}N concentration seven days after the treatment than the late and steady treatments. This supports our hypothesis that plants were able to take up more N from the early treatment that coincides with normal soil moisture and N availability, than from late or steady supplies of N. This

is similar to the results comparing the response of spring ephemerals to early, mid-, and late pulses of N (Bilbrough and Caldwell 1997).

We predicted that *C. maculosa* would have a greater concentration of ^{15}N from the early treatment than the native vegetation and except for *B. sagittata*, this did not happen. Although *C. maculosa* acquired more ^{15}N than the grasses in the glasshouse study, this may have been caused by size differences among species. In the field, the grasses and *C. maculosa* may have been able to obtain similar amounts of nutrients through different mechanisms (Fransen et al. 1999). The grasses may have been able to take up N by maintaining a greater root biomass than *C. maculosa* as indicated by the RMRs from the glasshouse study, whereas *C. maculosa* may have had a higher uptake rate than the grasses.

Percent N in the aboveground biomass at the end of the study indicates resorption proficiency, the final concentration of N in senesced tissue, because plant material was collected after the plants had senesced. Plants with higher percent N remaining in senesced tissue have lower resorption proficiency (Killingbeck 1996). At the end of the study, *F. idahoensis* had the highest percent N, *C. maculosa* and *P. spicata* were intermediate, and *B. sagittata* had the lowest. This indicates that *F. idahoensis* had the lowest resorption proficiency and *B. sagittata* had the highest. This may affect how plants respond to pulses because resorbed N can be reused the following growing season. Plants that have high resorption efficiency may depend less on uptake of nutrients (Killingbeck 1996). This may explain why *B. sagittata* had the highest percent N in the leaves after the early N treatment but had the lowest concentration of ^{15}N.

Neighbor effect

Neighbors have an important affect on uptake and growth response of individual plants in response to pulses of N (Olson and Blicker 2003; Cui and Caldwell 1997a). Uptake rates of *C. maculosa* and *P. spicata* after N pulses were affected by neighbor species in a previous glasshouse study (Olson and Blicker 2003). Similarly, neighbor species influenced growth response and N uptake in this study. Plants paired with *C. maculosa* and *R. hirta* had less biomass than those paired with the two grass species. Grasses also had less ^{15}N and a lower N pool when paired with the forbs. As stated earlier, some of these effects may be due to the larger size of the forbs than the grasses.

Summary

The early treatment seemed to benefit the plants more than the late treatment. In the glasshouse, *C. maculosa*, *R. hirta* and *P. spicata* plants receiving the early N treatment all had greater biomass than those receiving the late treatment. In the field, all species acquired more ^{15}N from the early treatment than from the late and steady treatments.

Centaurea maculosa's response compared with the native vegetation differed some between the glasshouse and field studies. In the glasshouse *C. maculosa* acquired more ^{15}N than the native grasses and was able to reduce the size and uptake of its neighbors. In the field, all plants were able to respond similarly to the pulses. In the glasshouse, plants were young and the slow growing grasses were relatively small. In the field, grasses had well-established root systems and therefore may have an advantage in acquiring resources that the young glasshouse plants did not.

Centaurea maculosa's response to pulses compared with the grasses in the glasshouse may not confer a competitive advantage in mature grasslands, where species such as the bunchgrasses used in this study are already established. Grasses with different growth rates acquire similar amounts of N through different mechanisms (Fransen et al. 1999). Such differences may partly explain the dynamics between *C. maculosa* and the native grasses in mature grasslands. However, in disturbed sites which are more similar to our glasshouse study, *C. maculosa* may have an advantage because the grasses have not had the time to establish an extensive root system.

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CHAPTER 4

SUMMARY

We hypothesized that: 1) *C. maculosa* has a greater nitrogen use efficiency (NUE) than native vegetation with high and low N supplies because of greater resorption, longer mean residence time (MRT), or higher N productivity, and 2) *C. maculosa* acquires more N and produces more biomass from an early pulse of N, than from a late pulse or a steady supply, and that it can acquire more N than the native vegetation.

Centaurea maculosa had a higher NUE than the native vegetation with the high N supply but NUEs were similar among *C. maculosa* and the native species with the low N supply. *Centaurea maculosa* had the longest MRT with the high and low N supplies. This is likely due to a low turnover of biomass and high resorption efficiency. *Centaurea maculosa* had a higher NUE than the other species in the high N supply because of its high MRT. *Centaurea maculosa* may have an advantage in disturbed sites where N availability is high because it has a higher NUE than the native species.

Nitrogen use efficiency was similar among all species with low N supply although they achieved it through different mechanisms. For the grasses, N productivity was greater with the low N supply than with the high N supply, whereas, N productivity was similar and MRT was high at both N supplies for *C. maculosa*. Similar NUEs among *C. maculosa* and the native grasses may explain *C. maculosa*'s ability to grow and persist, but not dominate, in mature grasslands where N availability is low.

In the field, *C. maculosa* and the grasses acquired more N from the early N treatment. This was expected because the early N treatment mimicked early spring wetting events in western Montana. These early spring pulses of N coincide with the early growth phase of plants, when they are beginning to bolt.

In the glasshouse, plants may not have acquired more N from the early N treatment because they were small. However, the glasshouse plants had the lowest biomass from the late N supply, indicating that the late treatment was applied too late in the growth cycle of the plant.

Centaurea maculosa and *R. hirta* acquired more N than the grasses in the glasshouse study. These plants were larger than the grass species and it is likely that they acquired more N because of their size rather than having greater uptake kinetics. Plants paired with *C. maculosa* and *R. hirta* however had less N and biomass indicating that the forbs were more competitive in the glasshouse setting.

In the field, the grasses seemed to acquire as much N as *C. maculosa* from the early treatment. Root mass ratios may help explain the discrepancy between the glasshouse and field study. Semi-arid grasslands plants often rely on large, long-lived root systems to acquire limited resources. In our glasshouse study, the grasses were still young and had not yet developed extensive root systems and therefore were not able to acquire as much N as the forbs. In the field, the grasses were mature, and we presume had high root mass ratios. This may explain why *C. maculosa* is able to invade disturbed sites. *Centaurea maculosa* can acquire more N from early spring pulses when plants are young. Grasses on disturbed sites have not yet established an extensive root system and therefore may not be as competitive as *C. maculosa*.

The results of our studies may partly explain why *C. maculosa* is able to invade disturbed sites. *Centaurea maculosa* is able to use N more efficiently than the native vegetation and can acquire more N from early spring pulses. Our research does not explain why *C. maculosa* is able to invade undisturbed sites, but it partly explains why *C. maculosa* is able to persist on these sites.

APPENDICES

APPENDIX A

ANOVA TABLES REFERENCED IN CHAPTER 2

ANOVA tables for data using square root, $\lambda = 0.2$ or natural log transformations.

^{15}N uptake per unit of root mass

Factor	df	p-value
Block	1	0.4366
Species	3	0.0130
Residuals	59	

Percent ^{15}N aquired of applied

Factor	df	p-value
Block	1	0.0072
Species	3	<0.0001
Treatment	1	<0.0001
Harvest	3	<0.0001
Species * Treatment	3	0.4086
Species * Harvest	9	<0.0001
Treatment * Harvest	3	0.5254
Species * Treatment * Harvest	9	0.6250
Residuals	223	

Total biomass

Factor	df	p-value
Block	1	<0.0001
Species	3	<0.0001
Treatment	1	<0.0001
Harvest	2	0.0086
Species * Treatment	3	0.3386
Species * Harvest	9	<0.0001
Treatment * Harvest	3	<0.0001
Species * Treatment * Harvest	9	0.3716
Residuals	224	

Dead biomas at Harvest 3

Factor	df	p-value
Block	1	<0.0001
Species	2	0.0240
Treatment	1	0.2049
Species * Treatment	3	0.0649
Residuals	56	

Root mass ratio

Factor	df	p-value
Block	1	<0.0001
Species	3	<0.0001
Treatment	1	0.0644
Harvest	2	<0.0001
Species * Treatment	3	0.2280
Species * Harvest	9	<0.0001
Treatment * Harvest	3	0.8009
Species * Treatment * Harvest	9	0.6372
Residuals	224	

Shoot C:N ratios

Factor	df	p-value
Block	1	0.0293
Species	3	<0.0001
Treatment	1	<0.0001
Harvest	3	<0.0001
Species * Treatment	3	0.0256
Species * Harvest	9	<0.0001
Treatment * Harvest	3	0.0328
Species * Treatment * Harvest	9	0.3470
Residuals	223	

Root C:N ratios

Factor	df	p-value
Block	1	0.1364
Species	3	0.0220
Treatment	1	0.0970
Harvest	3	<0.0001
Species * Treatment	3	0.2775
Species * Harvest	9	0.3615
Treatment * Harvest	3	0.6196
Species * Treatment * Harvest	9	0.5415
Residuals	223	

Number of flowers per plant

Factor	df	p-value
Block	1	0.1226
Species	2	<0.0001
Treatment	1	0.0046
Harvest	2	<0.0001
Species * Treatment	2	0.0645
Species * Harvest	4	0.0001
Treatment * Harvest	2	0.4151
Species * Treatment * Harvest	4	0.6957
Residuals	125	

Resorption efficiency

Factor	df	p-value
Block	1	0.3434
Species	3	<0.0001
Treatment	1	0.0431
Harvest	2	0.0851
Species * Treatment	3	0.0727
Species * Harvest	6	<0.0001
Treatment * Harvest	2	0.4032
Species * Treatment * Harvest	6	0.0541
Residuals	152	

Resorption proficiency

Factor	df	p-value
Block	1	0.9631
Species	3	<0.0001
Treatment	1	0.0008
Harvest	2	<0.0001
Species * Treatment	3	0.5220
Species * Harvest	6	<0.0001
Treatment * Harvest	2	0.8388
Species * Treatment * Harvest	6	0.7609
Residuals	159	

Mean residence time

Factor	df	p-value
Block	1	0.1772
Species	3	0.0001
Treatment	1	0.0461
Species * Treatment	3	0.2303
Residuals	49	

Nitrogen productivity

Factor	df	p-value
Block	1	0.8429
Species	3	<0.0001
Treatment	1	0.0308
Species * Treatment	3	0.6793
Residuals	54	

Nitrogen use efficiency

Factor	df	p-value
Block	1	0.3625
Species	3	0.4582
Treatment	1	0.0085
Species * Treatment	3	0.0862
Residuals	49	

APPENDIX B

ANOVA TABLES REFERENCED IN CHAPTER 3

ANOVA tables for data from glasshouse study, using weighted variances, and natural log, square root or no transformations.

Total biomass

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	<.0001
Treatment	2	<.0001
Species* Treatment	6	0.185
Neighbor* Treatment	6	0.4887

Root mass ratio

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	0.1084
Treatment	2	0.0094
Species* Treatment	6	0.0068
Neighbor* Treatment	6	0.6256

%N shoot

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	0.0486
Treatment	2	<.0001
Species* Treatment	6	0.1457
Neighbor* Treatment	6	0.3503

%N root

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	0.0409
Treatment	2	<.0001
Species* Treatment	6	0.5999
Neighbor* Treatment	6	0.0931

Flowerheads at harvest

Factor	df	p-value
(Intercept)	1	<.0001
Species	1	<.0001
Neighbor	3	0.0009
Treatment	2	0.2258
Species* Treatment	2	0.3223
Neighbor* Treatment	6	0.2623

Total ^{15}N

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	<.0001
Treatment	2	<.0001
Species* Treatment	6	0.2907
Neighbor* Treatment	6	0.2871

^{15}N Concentration

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	0.066
Treatment	2	<.0001
Species* Treatment	6	0.0184
Neighbor* Treatment	6	0.6675

N Pool

Factor	df	p-value
(Intercept)	1	<.0001
Species	3	<.0001
Neighbor	3	<.0001
Treatment	2	0.0002
Species* Treatment	6	0.1197
Neighbor* Treatment	6	0.7214

Relative growth rate

Cetaurea maculosa

Factor	df	p-value
treatment	2	<.0001
time	2	<.0001
plant	85	0.0001
treatment * time	4	<.0001
Residuals	170	

Rudbeckia hirta

Factor	df	p-value
treatment	2	<.0001
time	2	<.0001
plant	85	<.0001
treatment * time	4	<.0001
Residuals	170	

Festuca idahoensis

Factor	df	p-value
treatment	2	<.0001
time	2	<.0001
plant	33	0.0028
treatment * time	4	0.0061
Residuals	66	

Pseudoroegneria spicata

Factor	df	p-value
treatment	2	0.0637
time	2	<.0001
plant	33	0.4812
treatment * time	4	0.0242
Residuals	66	

ANOVA tables of field variables by field site transformed with natural log.

Final Biomass

Winston

Factor	df	p-value
(Intercept)	1	<0 .0001
Species	1	0.5971
Treatment	3	0.4714
Block	7	0.1617
Starting volume	1	0.0002
Species * treatment	3	0.9505
Residuals	48	

Theide

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	<0 .0001
Treatment	3	0.2541
Block	7	0.0018
Starting volume	1	<0 .0001
Species * treatment	6	0.9425
Residuals	75	

Hyalite

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	0.0004
Treatment	3	0.3735
Block	7	0.1791
Starting volume	1	0.0700
Species * treatment	6	0.7016
Residuals	29	

Number of flowers

Winston

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	1	<0 .0001
Treatment	3	0.7709
Block	7	0.0841
Starting volume	1	0.0007
Species * treatment	3	0.3199
Residuals	48	

Theide

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	<0 .0001
Treatment	3	0.4313
Block	7	0.0928
Starting volume	1	0.0856
Species * treatment	6	0.5637
Residuals	75	

Hyalite

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	<0 .0001
Treatment	3	0.1681
Block	7	0.4144
Starting volume	1	0.1535
Species * treatment	6	0.6359
Residuals	29	

%N seven days after treatment

Winston

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	1	0.0001
Treatment	3	<0 .0001
Block	7	0.4096
Starting volume	1	0.9708
Species * treatment	3	0.2598
Residuals	48	

Theide

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	<0 .0001
Treatment	2	<0 .0001
Block	7	0.2419
Starting volume	1	0.5269
Species * treatment	4	0.8638
Residuals	55	

Hyalite

	Df	p-value
(Intercept)	1	<0 .0001
Species	2	<0 .0001
Treatment	3	<0 .0001
Block	7	0.1467
Starting volume	1	0.3766
Species * treatment	6	0.0001
Residuals	74	

%N at the end of the growing season

Winston

Factor	Df	p-value
(Intercept)	1	0.0001
Species	1	0.0519
Treatment	3	0.0206
Block	7	0.5453
Starting volume	1	0.2584
Species * treatment	3	0.3306
Residuals	48	

Theide

Factor	Df	p-value
(Intercept)	1	0.0022
Species	2	<0 .0001
Treatment	3	0.0817
Block	7	0.0146
Starting volume	1	0.6866
Species * treatment	6	0.6173
Residuals	75	

Hyalite

Factor	Df	p-value
(Intercept)	1	5.0196
Species	2	2.7520
Treatment	3	0.7343
Block	7	0.3464
Starting volume	1	0.9860
Species * treatment	6	0.4265
Residuals	63	

^{15}N Concentration seven days after treatment

Winston

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	1	0.2252
Treatment	2	0.0007
Block	7	0.1456
Starting volume	1	0.1489
Species * treatment	2	0.2682
Residuals	34	

Theide

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	0.5195
Treatment	2	<0 .0001
Block	7	0.3459
Starting volume	1	0.0262
Species * treatment	4	0.6680
Residuals	55	

Hyalite

Factor	Df	p-value
(Intercept)	1	<0 .0001
Species	2	<0 .0001
Treatment	2	0.0029
Block	7	0.9517
Starting volume	1	0.1814
Species * treatment	4	0.2853
Residuals	55	

APPENDIX C

PRECIPITATION DATA FOR FIELD SITES IN CHAPTER 3

The total monthly and annual precipitation in mm collected by the Western Region Climate Center at weather stations nearest study sites. The average precipitation collected from 1971 to 2000.

	Percipitation (mm)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Hyalite Site (Bozeman MSU Station)													
Average (1971-2000)	20.57	20.07	35.05	53.09	76.71	74.17	38.10	37.59	45.97	39.88	27.94	22.10	490.98
2003	37.34	61.98	42.67	91.19	66.55	71.88	9.14	10.16	3.81	27.94	18.54	50.04	491.24
Theide Site (Norris Madison Pump House Station)													
Average (1971-2000)	13.18	13.91	28.30	43.68	73.93	70.27	39.77	37.82	39.53	34.65	9.27	14.88	429.20
2003	18.54	28.30	10.74	78.08	64.66	50.75	13.66	46.12	3.66	16.59	0.00	27.57	367.95
Winston Site (Townsend, Montana Station)													
Average (1971-2000)	8.89	8.13	13.46	18.29	42.16	52.83	34.29	33.27	26.67	15.49	10.16	8.89	272.54
2003	7.87	14.48	11.43	51.05	34.54	36.83	11.43	21.34	10.92	2.54	3.05	8.89	214.38

(Western Region Climate Center, 2004)