Nitrate uptake and water use of Centaurea maculosa (spotted knapweed) and native grasses
by Pamela Sue Blicker

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Resources and Environmental Sciences
Montana State University
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Abstract:

Centaurea maculosa (spotted knapweed), a perennial tap-rooted forb, was introduced to the Pacific Northwest in the late 1800s from Eurasia. It has no natural enemies in North America and has rapidly spread throughout native grassland systems in the western United States.

Semiarid grasslands are characterized by low nutrient and water availability. Generally, competition for soil resources in low-nutrient environments is intense. Centaurea 's success in semiarid grasslands may be attributed to its ability to preempt limiting resources, or alternatively, its ability to use these limiting resources more efficiently.

We hypothesized that as a weedy species, Centaurea has a greater ability than dominant native grasses to: 1) take advantage of pulses of nitrate availability, 2) preempt N under low and moderately high N levels, and 3) use water more efficiently.

Three studies were used to test these hypotheses. First, we conditioned Centaurea and two native grass species, Pseudoroegneria spicata and Pascopyrum smithii, to different durations (pulses) of nitrate availability. Plants were grown in mixed- and monoculture pots. 15N- nitrate was use to determine N uptake. Second, we grew Centaurea, Pseudoroegneria, and Pascopyrum in mixed- and monoculture, and supplied them with different rates of N. These N supply rates mimicked low and moderately high levels of N mineralization rates in semiarid grassland of Montana. Third, water use of Centaurea, Pseudoroegneria, Pascopyrum, and Festuca idahoensis was determined in a greenhouse and in the field. Total water use and water use efficiency (WUE) was evaluated in the greenhouse using the traditional method (biomass (g) / water used (kg)) and carbon isotope discrimination (A). Only A was used to determine WUE in the field.

Centaurea's responses to pulses of N availability and two levels of N depended on the identity of its neighbor. Centaurea faced much greater competition for N from Pascopyrum than from Pseudoroegneria in both N studies. In addition, Centaurea did not have the highest WUE, nor did it use the most water compared with Pseudoroegneria, Pascopyrum and Festuca. Finally, Centaurea appears to have traits that allow it to function as an early-seral species, yet also has the plasticity to function as a late-seral species depending on the plant community.

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

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ABSTRACT

Centaurea maculosa (spotted knapweed), a perennial tap-rooted forb, was introduced to the Pacific Northwest in the late 1800s from Eurasia. It has no natural enemies in North America and has rapidly spread throughout native grassland systems in the western United States.

Semiarid grasslands are characterized by low nutrient and water availability. Generally, competition for soil resources in low-nutrient environments is intense. *Centaurea's* success in semiarid grasslands may be attributed to its ability to preempt limiting resources, or alternatively, its ability to use these limiting resources more efficiently.

We hypothesized that as a weedy species, *Centaurea* has a greater ability than dominant native grasses to: 1) take advantage of pulses of nitrate availability, 2) preempt N under low and moderately high N levels, and 3) use water more efficiently.

Three studies were used to test these hypotheses. First, we conditioned *Centaurea* and two native grass species, *Pseudoroegneria spicata* and *Pascopyrum smithii*, to different durations (pulses) of nitrate availability. Plants were grown in mixed- and monoculture pots. ^{15}N-labeled nitrate was use to determine N uptake. Second, we grew *Centaurea, Pseudoroegneria*, and *Pascopyrum* in mixed- and monoculture, and supplied them with different rates of N. These N supply rates mimicked low and moderately high levels of N mineralization rates in semiarid grassland of Montana. Third, water use of *Centaurea, Pseudoroegneria, Pascopyrum*, and *Festuca idahoensis* was determined in a greenhouse and in the field. Total water use and water use efficiency (WUE) was evaluated in the greenhouse using the traditional method (biomass (g) / water used (kg)) and carbon isotope discrimination (Δ). Only Δ was used to determine WUE in the field.

Centaurea's responses to pulses of N availability and two levels of N depended on the identity of its neighbor. *Centaurea* faced much greater competition for N from *Pascopyrum* than from *Pseudoroegneria* in both N studies. In addition, *Centaurea* did not have the highest WUE, nor did it use the most water compared with *Pseudoroegneria, Pascopyrum* and *Festuca*. Finally, *Centaurea* appears to have traits that allow it to function as an early-seral species, yet also has the plasticity to function as a late-seral species depending on the plant community.

CHAPTER 1

INTRODUCTION

Centaurea maculosa Lam. (spotted knapweed), a perennial tap-rooted forb, was introduced to the Pacific Northwest in the late 1800s from Eurasia. It has no natural enemies in North America and has rapidly spread throughout native grassland systems in the western United States. Millions of hectares of semiarid grasslands have been invaded by *Centaurea* and millions of dollars are spent annually on costs associated with *Centaurea* infestations (Lacey *et al.* 1995). The shift of grasslands from native species to *Centaurea* may be the single greatest threat to native grassland systems in the western United States.

Semiarid grasslands are characterized by low nutrient and water availability (Eagles 1972). Low-nutrient environments are generally characterized by intense competition for soil resources (Tilman 1988; Wilson & Tilman 1991). *Centaurea's* success in semiarid grasslands may be attributed to its ability to preempt limiting resources, or alternatively, its ability to use these limiting resources more efficiently.

Nitrogen (N) is one of the most important limiting resources in grassland systems (Gleeson & Tilman 1990), and frequently limits plant productivity in rangeland ecosystems (Coyne, Trlica & Owensby 1995). Essential nutrients may be available to a plant for only short periods during the growing season (Gupta & Rorison 1995; Campbell & Grime 1989; Jonasson & Chapin 1991). Wetting events such as rainfall and snowmelt influence N concentrations in the soil (Campbell & Grime 1989; Jonasson & Chapin

1991; Cabrera 1993). A species' success may be attributed to its ability to preempt limiting nutrients during pulse events (Gupta & Rorison 1975; Jonasson & Chapin 1991).

A species' response to different concentrations of N in the soil is another attribute that may help explain its success in low-N environments. A plant with the plasticity to preempt N in low and high N conditions may be more successful. Alternatively, a plant that uses limited N more efficiently, particularly under low N conditions, may have an advantage in semiarid grassland systems.

Water is frequently a limiting resource in semiarid grasslands (Johnson *et al.* 1990). A species' ability to use limited water more efficiently may help its success, or alternatively, the species that preempts soil water, leaving less for its neighbors, may have an advantage in environments where water is a limiting resource.

Centaurea rapidly invades disturbed areas (Watson & Renney 1974); high nutrient availability and fewer competitors usually characterize disturbed areas (Picket & White 1985). Most weedy, or more ruderal species, have high nutrient uptake rates to take advantage of elevated levels of nutrients associated with disturbance (Burke & Grime 1996). These species are characterized by rapid growth and high nutrient uptake (Wedin & Tilman 1993). However, *Centaurea* is also known to invade pristine grasslands dominated by native grass species (Chicoine, Fay & Nielsen 1985). Plant species that dominate in late-seral, low-nutrient environments tend to have characteristics that allow them to compete for limiting resources. These characteristics include slow growth rates, highly developed root systems, and high resource use efficiency (Tilman & Wedin 1991). Apparently, *Centaurea* has the ability to take advantage of disturbed sites by rapidly acquiring nutrients, and to compete for limiting nutrients on grasslands dominated by native grass species.

Our research was based on three hypotheses, each related to attributes that may help explain *Centaurea's* success in semiarid grasslands. As a weedy species, we hypothesized that *Centaurea* has a greater ability than dominant native grasses to: 1) take advantage of pulses of nitrate availability, 2) preempt N under low and moderately high N levels, and 3) use water more efficiently.

Responses of *Centaurea* and two native grass species, *Pseudoroegneria spicata* [Scribn. and Smith] A. Love and *Pascopyrum smithii* [Rybd.] A. Love, to nitrate pulses were evaluated (Chapter 2). These species were grown in mixed- and monoculture pots and conditioned to different durations (pulses) of nitrate availability. These pulses were designed to mimic events of nutrient availability in semiarid grasslands (Cui & Caldwell 1997a). After 8 weeks, plants were exposed to ^{15}N-labeled nitrate for 8 hours and analyzed for ^{15}N uptake, nitrogen use efficiency, root to shoot ratios, and total biomass.

Plant responses to different levels of N were also evaluated (Chapter 3). *Centaurea, Pseudoroegneria,* and *Pascopyrum* were grown in mixed- and monoculture pots and conditioned to one of two N supply rates. These rates mimic low and moderately high levels of N mineralization rates in semiarid grassland of Montana (Neill 1995). After 8 weeks, plants were exposed to ^{15}N-labeled nitrate for 24 hours and analyzed for ^{15}N uptake, nitrogen use efficiency, root to shoot ratios, and total biomass.

Water use of *Centaurea* and three native grass species, *Pseudoroegneria, Pascopyrum*, and *Festuca idahoensis* Elmer was determined in a greenhouse and in the field (Chapter 4). In the greenhouse study, water use efficiency (WUE) was determined with the traditional method (biomass (g) / water used (kg)) and with carbon isotope discrimination (Δ). Total water use was also evaluated in the greenhouse study. Quantifying WUE using the traditional method requires measuring the amount of water used by the plant during the growing period and is difficult to do in the field, therefore only Δ was used to determine WUE in our field study.

A general overview of the results of these three studies and their implications are presented in Chapter 5.

CHAPTER 2

NITRATE PULSES

Introduction

Millions of hectares of semiarid grasslands are being invaded by aggressive, introduced forbs (Lacey *et al.* 1995). When successful, these introduced plant species can alter native ecosystem structure and function (Mooney & Drake 1986). *Centaurea maculosa* Lam. (spotted knapweed), a perennial forb was introduced to the Pacific Northwest in the late 1800s from Eurasia. It has no natural enemies in North America and has become a serious threat to native semiarid grasslands (Watson & Renney 1974; Strang, Lindsay & Price 1979). Invasive species may strongly influence ecosystem properties such as productivity, and nutrient and water cycles (Mooney & Drake 1986). The productivity of desirable forage plants decreases by up to 80% or more as density of *Centaurea* increases (Watson & Renney 1974). *Centaurea* also lowers biodiversity (Tyser & Key 1989) and may create near-monocultures (Watson & Renny 1974). To predict invasion and spread of weeds we need adequate information on species characteristics, interaction between species, and properties of the system being invaded (Mooney & Drake 1986).

Nitrogen (N) is one of the most important limiting resources in grassland systems (Gleeson & Tilman 1990), and frequently limits plant productivity in rangeland ecosystems (Coyne, Trlica & Owensby 1995). Essential nutrients may be available to a plant for only short periods during the growing season (Gupta & Rorison 1975; Campbell

& Grime 1989; Jonasson & Chapin 1991). Wetting events such as rainfall and snowmelt influence soil N concentrations (Campbell & Grime 1989; Jonasson & Chapin 1991; Cabrera 1993). For example, soil nitrate concentrations increased considerably for 24-hours following simulated rainfall (Cui & Caldwell 1997a). These pulses of nutrient availability are an important component of a plant's nutrient supply (Campbell & Grime 1989; Jonasson & Chapin 1991). Pulses of available soil nutrients following a wetting event may occur for a variety of reasons. Wetting of dry soil promotes the release of nutrients by increasing the turnover of microbial biomass and organic matter (Birch 1960). Some microorganisms die during drying periods; rewetting of soil enhances decomposition of dead microbial cells (Marumoto *et al.* 1977). Furthermore, microbial populations regenerate after rewetting, stimulating mineralization (Birch 1958; Soulides & Allison 1961). Although microorganisms may be the source of a nutrient pulse, they also compete for these nutrients, eventually immobilizing them. This immobilization generally constrains nutrient availability following a wetting event to less than one week (Cui & Caldwell 1997a).

A plant's success may be attributed to its ability to preempt limiting nutrients available during nutrient pulse events (Gupta & Rorison 1975; Jonasson & Chapin1991). Enhancing uptake capacity by existing roots is probably more important than growing new roots because of time constraints associated with nutrient availability during pulse events (Cui & Caldwell 1997a).

Alternatively, a plant's success may depend on its ability to use N more efficiently. Plants competing for N in N-limited environments tend to have low tissue N levels which indicates high nitrogen use efficiency (NUE; total biomass (g) / [N]) (Gleeson & Tilman 1990). Dominance by a perennial species may be determined by its ability to conserve mineral nutrients rather than maximize rate of capture (Aerts, Boots, & Van der Aart 1991). In low soil fertility conditions, high NUE is considered advantageous because it represents high biomass production per unit of nutrient taken up (Aerts & Chapin 2000).

Most ruderal or weedy species are thought to have high nutrient uptake rates to take advantage of elevated quantities of nutrient resources associated with disturbance (Burke & Grime 1996). We hypothesized that as a weedy species, *Centaurea* would have a greater ability to take advantage of pulses of nitrate availability than dominant native grasses. Our objectives were to: 1) determine if *Centaurea* and native grasses differ in their ability to take up nitrate during pulse events of different durations, 2) determine which species used N more efficiently, and 3) evaluate growth responses of each species. To accomplish our objectives, we conditioned *Centaurea* and two native grass species to different durations (pulses) of nitrate availability. These pulses were designed to mimic events of nutrient availability in semiarid grasslands (Cui & Caldwell 1997a). After 8 weeks, plants were exposed to ^{15}N-labeled nitrate for 8 hours and analyzed for %N root, %N shoot, %^{15}N acquired of applied, ^{15}N uptake by root (μmol g^{-1} h^{-1}), and NUE (total biomass (g) / total N (g)). Root to shoot ratios and total biomass were also determined.

Materials and methods

Plant species

Centaurea is an invasive, tap-rooted forb introduced from Eurasia. It currently infests over 1.8 million hectares in the northwestern United States (Lacey 1989), and has the potential to invade several million more hectares (Chicoine, Fay, & Nielsen 1978). *Pseudoroegneria spicata* [Scribn. & Smith] A. Love (bluebunch wheatgrass) is a native perennial bunchgrass. *Pascopyrum smithii* [Rybd.] A. Love (western wheatgrass) is a native perennial rhizomatous grass. These grasses frequently dominate semiarid grasslands in the northwestern United States.

Experimental design

Combinations of *Centaurea/Pseudoroegneria, Centaurea/Pascopyrum,* and monocultures of each species were grown in columns in a greenhouse and conditioned to one of three N pulse durations. Treatments were replicated six times and the experiment was arranged as a randomized complete block design. The columns were constructed of polyvinylchloride (PVC) pipe, 10 cm in diameter and 40 cm in height, with perforated end caps as bottoms. The columns were filled to within 2 cm of the top with pasteurized sand (< 2.0 mm in diameter). Seeds of each species were placed 1 cm into the sand in two groups of three per column. Seeds groups were spaced 6 cm apart and each group consisted of the same species. Mixed culture columns received three seeds of two species, monoculture pots received six seeds of the same species. Seeded pots were placed in a cold room for a two week vernalization period. The sand surface was misted with water three times per week during vernalization. Columns were then moved to a greenhouse where they were watered with a solution of commercial fertilizer (Miracle-Gro, Stern's Nurseries, Geneva, New York; 30:30:30 NPK) until seedlings were established. Established seedlings were thinned to two per pot, one from each group. Greenhouse temperatures averaged 20°C during the day and 15°C at night and plants were grown during summer, without augmented light.

Pulse treatments

An automated watering system delivered 150 mL of dilute (0.1x) N-free modified Hoagland solution (KH_2PO_4, K_2SO_4, $CaSO_4$, $MgSO_4$, and micronutrients; Cui & Caldwell, 1997b) twice daily. Nitrate was made available to plants once a week by substituting 150 mL of dilute (0.1x) N+ modified Hoagland solution (K_2PO_4, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, and micronutrients; Cui & Caldwell, 1997b) for the N-free nutrient solution. To mimic different durations of nitrate availability (pulses) pots were flushed with 3 L (2.5 pore volumes) of water at 8, 24, or 72 h following the nitrate treatments. Plants in the 24 h treatment continued to receive N+ during the next scheduled watering events while plants in the 72 h treatment received N+ during the first two scheduled watering events and then once daily during the next 48 hours.

^{15}N labeling

After 8 weeks of conditioning plants to different durations of nitrate availability, 35 mg per pot of ^{15}N-labeled nitrate as $K^{15}NO_3$ (99 atom %) in aqueous solution was substituted in the weekly N+ application. Pots were flushed with 3 L of water 8 hours after applying the ^{15}N-labeled nitrate, and harvested 24 hours later. Dried plant material was fine ground using a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colo. USA). Plant tissue N concentrations, atom % ^{15}N, and δ^{15}N were measured using Europa mass spectrometers (Europa Scientific, PDZ Europa Ltd., Cheshire, UK; U.C. Berkeley, Isotope Analysis Laboratory, Berkeley, Calif. USA, and U.C. Davis, Stable Isotope Facility, Davis, Calif. USA).

The amount of ^{15}N-labeled nitrate acquired during the final treatment was calculated using biomass (root and shoot), %N (root and shoot), and atom % (root and shoot). Atom % ^{15}N was determined by the following steps:

$$\delta\,^{15}\mathrm{N} = \left(\frac{R_{sample} - R_{std}}{R_{std}}\right) \qquad [1]$$

where R represents the absolute ratio (^{15}N/^{14}N) of the sample and standard gases. The absolute ratio for the standard gas (R_{std}) is 0.0036765. The absolute ratio of a sample (R_{sample}) can be determined by rearranging [1].

$$R_{sample} = \left[\frac{\delta^{15}\mathrm{N}}{1000} + 1\right] \times R_{std} \qquad [2]$$

R_{sample} is used to calculate F, the fractional abundance [^{15}N/(^{15}N + ^{14}N)]:

$$F = \left[\frac{R_{sample}}{R_{sample} + 1}\right] \qquad [3]$$

Atom % is used to express isotopic enrichment in samples highly enriched in ^{15}N:

$$\text{atom \%} = F \times 100 \qquad [4]$$

Harvest

Plants were harvested 17 weeks after planting which was 8 weeks after initiating treatments. Plant roots were washed free of sand and the plants were then separated by hand in a water bath. Shoot and root material was separated and then dried at 70°C for 72 h. Dried root and shoot material was weighed to determine total plant biomass, and root to shoot ratios. Before initiating treatments and immediately before harvest, the following information was collected on each plant. Number of tillers, height of total plant, and reproductive status were recorded for grass species. Number of leaves, length of longest leaf, and phenology (rosette, bolting, flowering) were recorded for *Centaurea.*

Data analysis

The experimental design included 3 pulse durations and 5 species combinations and was analyzed as a complete randomized block ($n = 6$). Total biomass, root to shoot ratios, %N root, %N shoot, %^{15}N acquired of applied, ^{15}N uptake by root ($\mu mol\ g^{-1}\ h^{-1}$), and NUE (total biomass (g) / total N (g)) were analyzed with ANOVA (SAS 1988). Main effects included pulse duration, species combination, and species. Pre-treatment plant height and number of tillers (grasses) or leaves (*Centaurea*) were used as covariates for total biomass and NUE. Planned contrasts between species combinations are listed in Table 1. Results of contrasts and *P*-values (Gill 1981) are located in Appendix A.

Species combination 1 vs species combination 2	Abbreviations
CENTAUREA/CENTAUREA vs *CENTAUREA/Pseudoroegneria*	CE CE vs CE ps
PSEUDOROEGNERIA/PSEUDOROEGNERIA vs *Centaurea/PSEUDOROEGNERIA*	PS PS vs ce PS
CENTAUREA/CENTAUREA vs *CENTAUREA/Pascopyrum*	CE CE vs CE pa
PASCOPYRUM/PASCOPYRUM vs *Centaurea/PASCOPYRUM*	PA PA vs ce PA
Centaurea/PASCOPYRUM vs *Centaurea/PSEUDOROEGNERIA*	ce PA vs ce PS
CENTAUREA/Pascopyrum vs *CENTAUREA/Pseudoroegneria*	CE pa vs CE ps

Table 1. Planned contrasts were used to compare plant responses to different pulses of N availability and different neighbors. For example, CE CE vs CE ps contrasted the response of *Centaurea* grown in monoculture (average of the two plants) with *Centaurea* when it was grown with *Pseudoroegneria.* Capital letters represent the species, or average of species, used in each contrast.

Results

Biomass

Biomass for all species was greater at the longer pulse durations except for *Pseudoroegneria* grown with *Centaurea* (Fig. 1a, Appendix A). The greatest difference in biomass between two species was at the 72 h pulse duration; *Pascopyrum* had considerably greater biomass than *Pseudoroegneria* when each were grown with *Centaurea.*

Root to shoot ratios

At the 8 h pulse duration, *Centaurea* in monoculture had higher root to shoot ratios than *Centaurea* when grown with either grass species (Fig. 1b, Appendix A). At the 24 h pulse duration, *Centaurea* in monoculture had higher root to shoot ratios than *Centaurea* grown with *Pseudoroegneria.* Conversely, *Pascopyrum* in monoculture had higher root to shoot ratios than *Pascopyrum* grown with *Centaurea. Pseudoroegneria* grown with *Centaurea* had higher root to shoot ratios than *Pascopyrum* grown with *Centaurea* at the 8 and 24 h pulse durations. At the 72 h pulse duration, *Centaurea* grown with *Pseudoroegneria* had higher root to shoot ratios than *Centaurea* grown with *Pascopyrum.*

Nitrogen use efficiency (NUE)

At the 8 h pulse duration, *Pseudoroegneria* in monoculture had lower NUE than *Pseudoroegneria* grown with *Centaurea* (Fig. 1c, Appendix A). At the 24 h pulse duration, *Centaurea* in monoculture had greater NUE than *Centaurea* grown with *Pseudoroegneria.* At the 24 h pulse duration, NUE was greater for *Pseudoroegneria* grown with *Centaurea* than *Pascopyrum* grown with *Centaurea.* Conversely, NUE was greater for *Centaurea* grown with *Pascopyrum* than *Centaurea* grown with *Pseudoroegneria.* At the 72 h pulse duration, *Pseudoroegneria* grown with *Centaurea* had greater NUE than *Pseudoroegneria* in monoculture.

^{15}N-labeled nitrate uptake (μmol g^{-1} h^{-1})

At the 8 h pulse duration, ^{15}N uptake was greater for *Pseudoroegneria* in monoculture than *Pseudoroegneria* grown with *Centaurea,* and also greater for *Pascopyrum* in monoculture than *Pascopyrum* grown with *Centaurea* (Fig. 2a, Appendix A). At the 24 h pulse duration, *Centaurea* grown with *Pseudoroegneria* had greater ^{15}N uptake than *Centaurea* grown with *Pascopyrum. Pascopyrum* grown with *Centaurea* acquired more ^{15}N than *Pseudoroegneria* grown with *Centaurea* at all pulse durations.

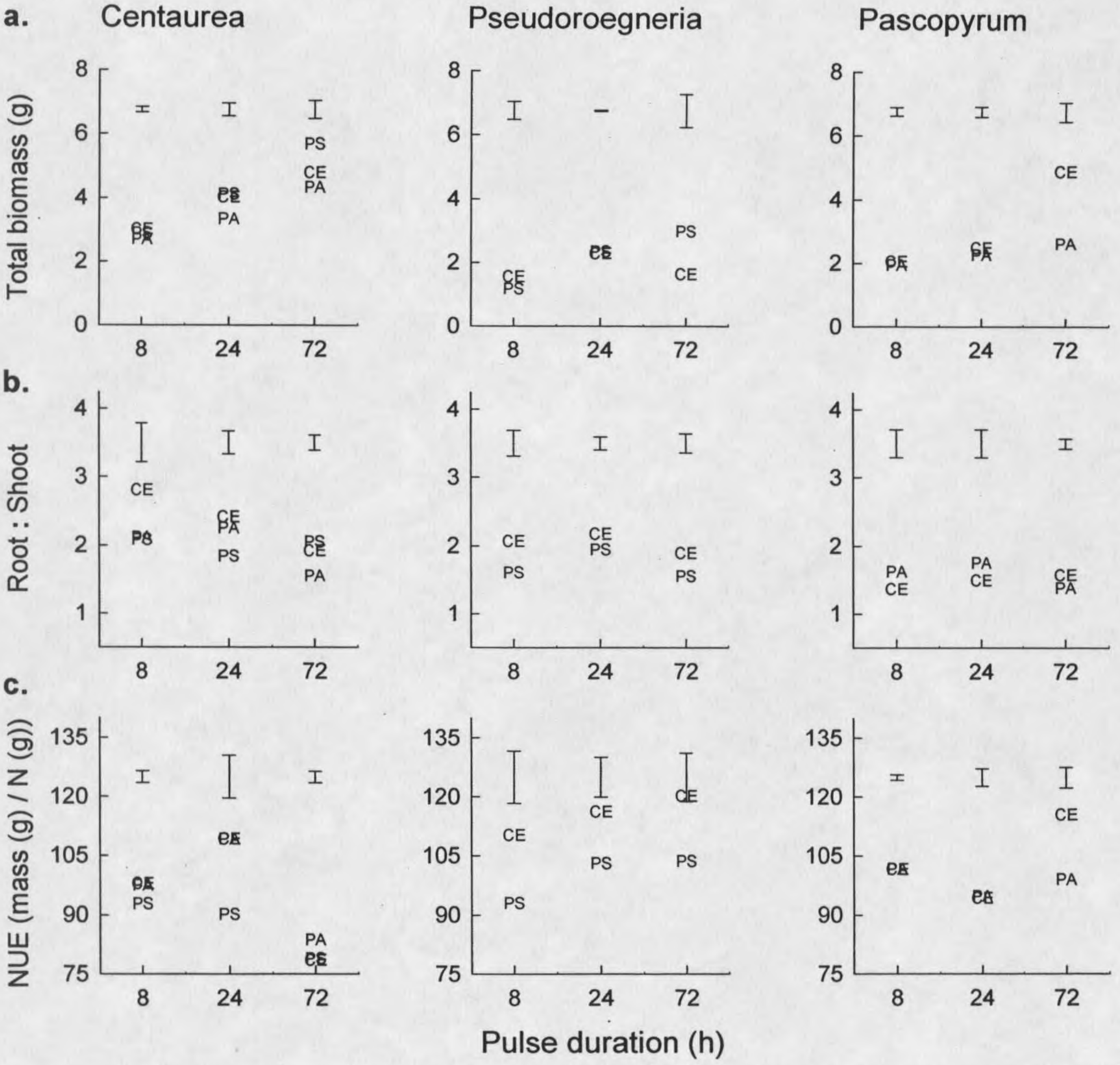

Figure 1. a. Total biomass (g), **b**. root : shoot, and **c**. nitrogen use efficiency (NUE; total biomass (g) / (total N (g)) of target species *Centaurea*, *Pseudoroegneria*, and *Pascopyrum* when growing with neighbors (*Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA) and conditioned to 8, 24, or 72 hours of nitrate availability each week. For example, Figure 1a. represents the biomass of *Centaurea* when grown with *Centaurea, Pseudoroegneria*, and *Pascopyrum*. Least square means standard error presented for each pulse duration.

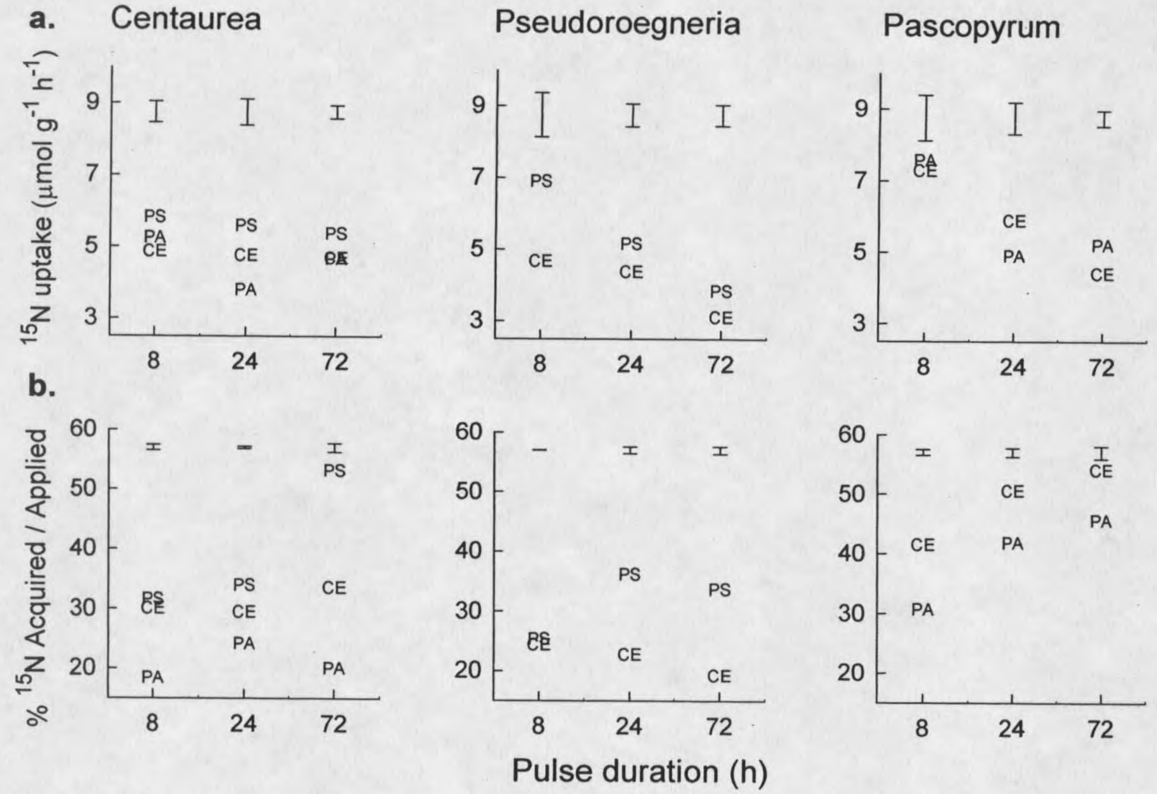

Figure 2. **a**. Root uptake of ^{15}N-labeled nitrate (µmol g^{-1} h^{-1}), **b**. percent ^{15}N-labeled nitrate acquired of applied of target species *Centaurea*, *Pseudoroegneria*, and *Pascopyrum* when growing with neighbors (*Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA) and conditioned to 8, 24, or 72 hours of nitrate availability each week. Least square standard error presented for each pulse duration

Percent ^{15}N acquired of applied

At the 8 h pulse duration *Centaurea* in monoculture acquired more ^{15}N than *Centaurea* grown with *Pascopyrum*. Conversely, *Pascopyrum* grown with *Centaurea* acquired more ^{15}N than *Pascopyrum* in monoculture (Fig. 2b, Appendix A). *Centaurea* grown with *Pascopyrum* acquired less ^{15}N than *Centaurea* grown with *Pseudoroegneria*. At the 24 h pulse duration, *Pseudoroegneria* in monoculture acquired more ^{15}N than

Pseudoroegneria grown with *Centaurea*, whereas *Pascopyrum* in monoculture acquired less ^{15}N than *Pascopyrum* grown with *Centaurea*.

At the 72 h pulse duration, *Centaurea* grown with *Pseudoroegneria* acquired more ^{15}N than *Centaurea* in monoculture, whereas *Pseudoroegneria* grown with *Centaurea* acquired less ^{15}N than *Pseudoroegneria* in monoculture. *Centaurea* in monoculture acquired more ^{15}N than *Centaurea* grown with *Pascopyrum*. Conversely, *Pascopyrum* in monoculture acquired less ^{15}N than *Pascopyrum* grown with *Centaurea*. The percent of ^{15}N acquired was much greater for *Pascopyrum* grown with *Centaurea* than for *Pseudoroegneria* grown with *Centaurea* at all pulse durations.

Discussion

Pulse Duration

Pulses of nutrient availability likely occur in soils as a result of accelerated mineralization and subsequent immobilization of nutrients by bacteria and fungi during drying and wetting, and freezing and thawing cycles (Soulides & Allison 1961; Marumoto *et al.* 1977). Soil microbes can quickly immobilize available nutrients, limiting the duration of pulse events to a few days to a week (Shield, Paul & Lowe 1973; Paul & Clark 1989; Cui & Caldwell 1997a). In our study, plants were conditioned to 8, 24, or 72 h pulses of N availability. Biomass, root to shoot ratios, and NUE reflect the long term response to these pulse events. After an 8 wk conditioning period, all plants were exposed to ^{15}N-labeled nitrate for 8 h and then harvested 24 h later. As a result of this labeling, ^{15}N uptake (μmol g^{-1} h^{-1}) represents relative root affinity for ^{15}N on a per gram basis. Percent ^{15}N acquired of applied reflects the amount of ^{15}N taken up during the labeling period, which is a function of affinity for N and total root mass.

Most plants had more biomass at the longer pulse durations. Plants from low-nutrient environments often respond positively to N pulse events (Cui and Caldwell 1997b). In a field study, plants produced more biomass when supplied with N in pulses than a continuous supply of N over a 10 wk period (Bilbrough & Caldwell 1997).

In our study, root to shoot ratios were generally greater at the shorter pulse durations. This indicates that most plants, whether grown in mixed- or monoculture, responded to lower N availability by allocating proportionally more resources to roots. Plants allocate relatively less biomass to leaves and more to roots when N is in short supply (Brouwer 1983), indicating that when N supply is limited, plants invest in the part of the plant that acquires N (roots), not the part that requires it most (shoots).

Plants competing for N in N-limited environments tend to have low tissue N levels, which may reflect high NUE (Gleeson & Tilman 1990). In a N pulse study, *Pseudoroegneria spicata* acquired less N but produced more biomass than *Agropyron desertorum*, indicating that *Pseudoroegneria* used N more efficiently than *Agropyron* (Cui & Caldwell 1997b). We expected that plants conditioned to the longer pulses of N would have lower NUE because the need to use N efficiently to produce biomass should decrease with greater N availability. However, NUE varied with the identity of a plant's neighbor. For example, grasses had higher NUE when grown with *Centaurea* than when grown in monoculture at the 72 h pulse duration. This suggests that a plant's ability to use N efficiently is plastic, and depends on the duration of N availability and the identity of its neighbor.

In general, plants conditioned to the shortest pulse of N availability had greater affinity for ^{15}N (μmol g^{-1} h^{-1}). Plants have a greater capacity to absorb N under N-limiting conditions (Lee 1982; Robinson 1996). Low N availability may signal a plant to synthesize additional N transport proteins, thereby increasing the plant's ability to acquire N (Lambers, Chapin & Pons 1998).

Plants conditioned to longer N pulse durations tended to acquire a greater percent of the applied ^{15}N during the 8 h labeling period. Presumably, this reflects the greater mass of plants conditioned to the 24 h and especially the 72 h pulse durations. However, within some mixed culture pots, one species took up a much greater percent of the applied ^{15}N, leaving less for its neighbor.

Centaurea vs. *Pseudoroegneria*

Overall, *Centaurea* had greater biomass than *Pseudoroegneria* at all pulse durations. Typically, weedy species that take advantage of disturbances have fast growth rates (Grime & Hunt 1975), whereas species that evolved in low-N environments tend to grow slow (Chapin 1980). However, at the 8 h pulse duration, *Centaurea* grown with *Pseudoroegneria* and *Centaurea* in monoculture had similar biomass, whereas *Pseudoroegneria* grown with *Centaurea* tended to have greater biomass than *Pseudoroegneria* in monoculture. This may reflect that *Pseudoroegneria* evolved to take advantage of short-term, episodic nutrient pulses when growing with different species in semiarid systems.

Differences in biomass of *Pseudoroegneria* and *Centaurea* at the 72 h pulse duration were opposite those of the 8 h pulse duration when grown together. *Centaurea* grown with *Pseudoroegneria* tended to have greater biomass than *Centaurea* in monoculture and *Pseudoroegneria* grown with *Centaurea* had lower biomass than *Pseudoroegneria* in monoculture. *Pseudoroegneria's* lower biomass at the 72 h pulse duration may reflect *Centaurea's* greater biomass. With a finite amount of N supplied at each pulse duration, *Centaurea* may have simply taken up more of the supplied N, especially at the longer pulse duration, leaving less for *Pseudoroegneria.* Alternatively, *Pseudoroegneria,* which evolved in low-nutrient environments, may not have been able to benefit from the longer durations of N availability. Nutrient uptake increases with increased nutrient supply up to some maximum uptake rate where a plateau is reached (Marschner 1995).

Pseudoroegneria grown with *Centaurea* had higher NUE (biomass / [N]) than *Centaurea* and *Pseudoroegneria* in monocultures, indicating that *Pseudoroegneria* uses limited resources more efficiently when growing with this invasive species. Slow growing plants that compete for nutrients in low-nutrient environments typically use limited resources efficiently (Aerts & Chapin 2000).

Pseudoroegneria in mixed- and monoculture pots had lower affinity for ^{15}N (μmol g^{-1} h^{-1}) at the longer pulse durations. Because the ^{15}N labeling period was only 8 h, *Pseudoroegneria* conditioned to the longer pulses of N availability (24 and 72 hours) may not have responded to N availability as rapidly as plants conditioned to shorter pulses of N availability. *Pseudoroegneria* in monoculture had greater affinity for ^{15}N than *Centaurea* and *Pseudoroegneria* in mixed- and monocultures at the 8 h pulse duration, indicating *Pseudoroegneria* had a greater ability to respond to short-term pulses of N. The ability to accumulate nutrients during short-term pulse events may enhance survival during periods of low nutrient availability (Campbell & Grime 1989).

Because *Centaurea* grown with *Pseudoroegneria* had a greater affinity for N than *Pseudoroegneria* grown with *Centaurea, Centaurea* was able to out-compete *Pseudoroegneria* for N when the two were grown as neighbors. This greater affinity for N combined with greater biomass enabled *Centaurea* to acquire a greater percent of the applied ^{15}N, especially at the longer pulse durations when N was most available. When grown with *Pseudoroegneria*, *Centaurea* appeared to have traits characteristic of ruderal or early-seral species in this greenhouse study. These traits include rapid growth rates and high nutrient uptake (Grime 1977; Wedin & Tilman 1993), which allow such plants to take advantage of elevated levels of nutrients associated with disturbance (Burke & Grime 1996).

Pseudoroegneria responded to pulses of N more effectively at the short pulse duration than at the longer pulse durations. Rapid response to short-term pulses of N availability may enhance *Pseudoroegneria's* survival between nutrient pulse events. Native grasses that evolved in low-nutrient environments with episodic events of nutrient availability may have traits which allow them to tolerate periods of low nutrient availability, but respond to short-term pulses of N availability. Such traits include low growth rates, high root to shoot ratios, and high NUE (Grime 1977; Chapin 1980; Brouwer 1983; Tilman & Wedin 1991; Aerts & Chapin 2000).

Overall, *Centaurea* had a greater ability than *Pseudoroegneria* to take advantage of pulses of N availability, especially at the 72 h pulse duration. *Centaurea* faced greater competition for N from neighboring *Centaurea* than from neighboring *Pseudoroegneria.*

Centaurea vs. Pascopyrum

The responses of *Centaurea* and its interaction with *Pascopyrum* were nearly opposite of *Centaurea's* response to *Pseudoroegneria.* For example, *Pascopyrum* tended to produce more biomass when grown with *Centaurea* than when grown in monoculture, whereas *Centaurea* had a tendency to produce more biomass when grown in monoculture than when grown with *Pascopyrum.* These results were consistent for all pulse durations. This was surprising because we expected the weedy species (*Centaurea*) to have a greater ability to take advantage of N pulse events than native grass species in general.

Root to shoot ratios were generally greater for *Centaurea* than *Pascopyrum,* especially when conditioned to the shorter pulse durations. Root to shoot ratios of *Centaurea* and *Pascopyrum* in mixed- and monoculture were similar when conditioned to 72 hours of nitrate availability. We expected that *Centaurea,* which has traits of a ruderal species, would have lower root to shoot ratios than the native *Pascopyrum.* Our observed higher root to shoot ratios for *Centaurea* may reflect greenhouse conditions, or that it had not bolted when this study ended. Usually, plants native to low-nutrient environments tend to have high root to shoot ratios (Chapin 1980) which increases their ability to compete for limited below ground resources (Boot & Mensink 1990).

Centaurea had lower NUE than *Pascopyrum* when conditioned to the 72 h pulse duration, indicating that N may have been in excess at this pulse duration, and thus

Centaurea did not need to use it efficiently. *Pascopyrum* had the greatest NUE when grown with *Centaurea* and conditioned to the 72 h pulse duration. This may reflect *Pascopyrum's* greater biomass accumulation at the longer pulse duration, and possibly an associated dilution effect. In contrast, *Centaurea* had higher NUE at the 24 h pulse duration than at the 8 or 72 h pulse durations, indicating that *Centaurea* was able to optimize growth with the amount of N available at this pulse duration. These results indicate biomass allocation and N tissue levels of *Pascopyrum* and *Centaurea* are plastic because NUE depended on the duration of nitrate pulses, and the identity of the plant's neighbor.

Affinity for ^{15}N (μmol g^{-1} h^{-1}) during the final 8 h labeling period was greatest for *Pascopyrum* grown in mixed- and monoculture at the 8 h pulse duration. This may reflect a more active root system, or that *Pascopyrum*, which evolved in a low-nutrient environment with episodic rainfall, can take advantage of brief periods of N availability following a wetting event.

Pascopyrum grown with *Centaurea* acquired a greater percent of the applied ^{15}N than *Pascopyrum* in monoculture and *Centaurea* in mixed- and monoculture. Conversely, *Centaurea* grown with *Pascopyrum* acquired less of the applied ^{15}N than *Centaurea* in monoculture. Because the affinity for N and root mass determines the ability to acquire N, *Pascopyrum* acquired more ^{15}N because of greater affinity, not greater root mass, at the shorter pulse durations because *Centaurea* had greater root mass. When these plants were conditioned to the 72 h pulse duration, affinity, biomass, and root to shoot ratios were similar for *Centaurea* and *Pascopyrum* when the two were grown together.

Pascopyrum's ability to take up a greater percent of ^{15}N at the final 8 h labeling period may reflect that it responded to the pulses more quickly, taking up a greater percent of applied ^{15}N, leaving less for *Centaurea.*

Unlike *Pseudoroegneria*, *Pascopyrum* had a greater ability than *Centaurea* to take advantage of pulses of N availability. Furthermore, *Centaurea* apparently faced greater competition for N from *Pascopyrum* than from neighboring *Centaurea* plants.

Conclusion

We expected that *Centaurea* would have a greater ability to take advantage of short-term N pulse events than native grasses, which may partly explain its success in invading semiarid grasslands dominated by native grasses. We found that plant species with the plasticity to respond to pulses of nutrient availability were able to out-compete their neighbors for N. Furthermore, *Centaurea's* responses to pulses of N availability differed depending on the identity of its neighbor. Finally, *Centaurea* did not consistently out-compete these two native grasses when exposed to pulses of N availability; *Pascopyrum* was more competitive for N than *Centaurea*, whereas *Centaurea* was more competitive for N than *Pseudoroegneria.*

CHAPTER 3

LOW AND HIGH NITROGEN

Introduction

Semi-arid grasslands in the northwestern United States are currently at risk of invasions by introduced, weedy plant species. For example, *Centaurea maculosa* (spotted knapweed) has invaded millions of hectares of semiarid grasslands (Lacey *et al.* 1995). *Centaurea*, a perennial, tap-rooted forb was introduced from Eurasia to the Pacific Northwest in the late 1800s. With no natural enemies in North America, it has continued to spread rapidly. *Centaurea*, which is not generally used as forage, decreases the productivity of desirable forage plants (Watson & Renney 1974) and reduces biodiversity (Tyser & Key 1989).

Past efforts to control invasions of *Centaurea* have generally focused on eradicating the weed using chemicals or reseeding. Predicting the outcome of eradication efforts is often unreliable and identifying reasons for success or failure is usually difficult. Treating the effects of an exotic weed invasion without understanding physiological factors that may contribute to the weed's success does not provide reliable, permanent control measures. To predict invasion and spread of weed populations we need adequate information on species characteristics, interaction between species, and properties of the system being invaded (Mooney & Drake 1986). More information on the biology and ecology of invasive plant species is essential (Wyse 1992; Burnside 1993).

Plant communities dominated by invasive species for long periods of time do not appear to fit Clement's (1916) model of plant succession. Instead, native plant communities that are replaced by exotic species appear to fit the multiple steady state, state and transition model (Westoby, Walker & Noy-Meir 1989). The "states" are relatively stable assemblages of species occupying a site. The "transition" between states is triggered either by natural events (e.g. fire, weather), management actions (e.g. grazing, destruction or introduction of plants), or a combination of the two. The term "suspended stages of succession" has also been used to describe plant communities that represent relatively unchanged assemblages of species over long periods of time (Laycock 1991). Laycock (1991) suggested that one of the reasons for suspended stages or different trajectories of succession may be dominance by highly competitive species or life forms. Some different trajectories of succession may not allow a disturbed ecosystem to return to its original state (Allen 1988). These concepts are closely related and describe ecosystems that do not fit the Clementsian approach. Plant communities dominated by *Centaurea* may be in suspended stages of succession.

Centaurea is known to rapidly invade disturbed areas (Watson & Renney 1974); distrubed areas are typically characterized by high nutrient availability and decreased competition (Picket & White 1985). Most ruderal, or weedy species are thought to have high nutrient uptake rates to take advantage of elevated levels of nutrients associated with disturbance (Burke & Grime 1996). These early-seral species are characterized by rapid growth and high nutrient uptake (Wedin & Tilman 1993). However, *Centaurea* is also known to invade pristine grasslands dominated by native grass species (Chicoine, Fay &

Nielsen 1985). Plant species that dominate in late-seral, low-nutrient environments tend to have characteristics that allow them to compete for limiting resources. These characteristics include slow growth rates, highly developed root systems, and high resource use efficiency (Tilman & Wedin 1991). Apparently, *Centaurea* has the ability to take advantage of disturbed sites by rapidly acquiring nutrients and to compete for limiting nutrients on grasslands dominated by native grass species.

Semiarid grasslands are characterized by low water and nutrient availability (Eagles 1972). Therefore low-nutrient environments should be characterized by intense competition for soil resources (Tilman 1988; Wilson & Tilman 1991). Plant species that use these resources most effectively may be superior competitors (Mamolos, Veresoglou & Barbayiannis 1995). On unproductive, nitrogen-depleted sandy soil, species associated with a superior ability to compete for limiting soil resources dominate over time (Tilman & Gleeson 1990).

Plants in low nutrient environments tend to grow slowly and have high root to shoot ratios (Chapin 1980; Wilson & Tilman 1991). These plants maintain low growth rates for two reasons. First, they have a lower nutritional demand for optimal growth. Second, they have lower plant tissue turnover rates and higher retention of nutrients (Crick & Grime 1987).

Plants from low-nutrient environments may benefit from high root to shoot ratios in several ways. High root to shoot ratios are in part, a phenotypic response to reduced nutrient availability (Chapin 1980). Plants from low-nutrient environments typically have greater root longevity, which partially accounts for greater root biomass. Chapin

(1980) suggested that because root absorption capacity generally declines with root age, increased root biomass does not necessarily imply high root absorption capacity. Many plants on infertile soils have evolved long-lived, extensive root systems that remain functional through the year but have relatively inflexible patterns of development (Grime 1977; Chapin 1980). Allocating more nitrogen to leaves maximizes growth rates, whereas allocating more nitrogen to roots may increase the ability to compete for nitrogen (Gleeson & Tilman 1990).

Nitrogen (N) is the most important limiting soil resource during plant succession (Tilman 1988). Nitrogen frequently limits plant productivity in rangeland ecosystems, enhancing the need to conserve, retain, and reuse N within the plant (Coyne, Trlica, & Owensby 1995); recycling internal, nitrogenous compounds allows perennial plants to maintain consistent levels of productivity. Maintaining these levels of productivity may not be possible if all N used in new growth was obtained from the soil (Coyne et al. 1995).

Nitrogen use efficiency (NUE) in plants is often expressed as the biomass produced per unit of plant N (Aerts 1997). Plants competing for N in N-limited environments tend to have low tissue N levels, which may indicate high NUE (Gleeson & Tilman 1990). Perennial plant species may dominate in low-nutrient environments because they conserve nutrients rather than maximize rate of uptake (Aerts, Boot & van der Aart 1991). Results from an experiment using pulses of N indicated that *Agropyron desertorum* acquired more N but produced less biomass than *Pseudoroegneria spicata*

(Cui & Caldwell 1997). These results indicate that *Pseudoroegneria* uses N more efficiently than *Agropyron.*

We hypothesized that *Centaurea* has a greater ability to preempt N than native grasses under low and moderately high N levels. Plants competing for limited resources are a major factor in determining successional dynamics (Tilman 1982; Walker & Chapin 1986). Alternatively, *Centaurea* may use N more efficiently than the native grasses. Greater NUE would concur with Aerts *et al.'s* (1991) suggestion that competition in low-nutrient environments is controlled by a plant's ability to conserve mineral nutrients.

Our objectives were to: 1) determine nitrate uptake of *Centaurea* and two native grasses at low and moderately high N levels, 2) determine which species used N more efficiently, and 3) evaluate growth responses of each species at each N level. To accomplish our objectives, we conditioned *Centaurea* and two native grass species to one of two N supply rates. These rates mimic low and moderately high levels of N mineralization rates in semiarid rangelands of Montana (Neill 1995). After 8 weeks, plants were exposed to ^{15}N-labeled nitrate for 24 hours and analyzed for %N root, %N shoot, %^{15}N acquired of applied, ^{15}N uptake by root (μmol g^{-1} h^{-1}), and NUE (total biomass (g) / total N (g)). Root to shoot ratios and total biomass were also determined.

Materials and methods

Plant species

Centaurea is a perennial, tap-rooted Eurasian forb introduced to the Pacific Northwest in the late 1800s. With no natural enemies in North American, *Centaurea* has spread rapidly throughout native grasslands in the western United States (Chicoine, Fay & Nielsen 1978). *Pascopyrum smithii* [Rybd.] A. Love (western wheatgrass) is a native, perennial rhizomatous grass. *Pseudoroegneria spicata* [Scribn. & Smith] A. Love (bluebunch wheatgrass) is a native, perennial bunchgrass. These native grasses are commonly associated with semiarid rangelands in the northwestern United States.

Experimental design

Combinations of *Centaurea/ Pseudoroegneria*, *Centaurea/Pascopyrum,* and monocultures of each species were grown in columns in a greenhouse and conditioned to low or moderately high N levels. Treatments were replicated nine times and the experiment was arranged as a randomized complete block design. The columns were constructed of polyvinylchloride (PVC) pipe, 10 cm in diameter and 40 cm in height, with perforated end caps as bottoms. The columns were filled to within 2 cm of the top with pasteurized sand (< 2.0 mm in diameter). Seeds of each species were sterilized in a 2% NaClO solution for 2 min. and placed 1 cm into the sand in two groups of three per column. Seeds groups were spaced 6 cm apart and each group consisted of the same species. Mixed culture columns received three seeds of two species, monoculture pots received six seeds of the same species. Seeded pots were placed in a cold room for an 8 wk vernalization period. The sand surface was misted with water three times per week during vernalization. Columns were then moved to a greenhouse where they were watered with a solution of commercial fertilizer (Miracle-Gro, Stern's Nurseries, Geneva, New York; 30:30:30 NPK) until seedlings were established. Established seedlings were thinned to two per pot, one from each group. Greenhouse temperatures averaged 20°C during the day and 15°C at night. Grow lights were on between 0700 and 2100 each day during the experiment.

Low / High N treatments

During the 8 wk treatment period, plants were watered daily with a nutrient solution using an automated watering system. Plants in the low N treatment (low N) received 200 mL of dilute (0.1x) modified Hoagland solution (K_2PO_4, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, and micronutrients; Cui & Caldwell, 1997b) containing 7.5 mM N. Plants in the moderately high N treatment (high N) received 200 mL of dilute (0.1x) modified Hoagland solution containing 15 mM N. These N supply rates mimic low and moderately high levels of N mineralization rates in semiarid rangelands of Montana (Neill, 1995).

^{15}N labeling and uptake

At the end of the treatment period, ^{15}N-labeled nitrate as $K^{15}NO_3$ (99 atom%; Isotec, Inc. Miamisburg, Ohio, USA) in an aqueous solution (150 mL per column) was substituted for the daily watering of nutrient solution. Each column received the same amount of N, in the form of ^{15}N, that had been supplied daily during the treatment period. Plants were harvested 24 h later. Dried plant material was fine ground using a Cyclone

Sample Mill (UDY Corporation, Fort Collins, Colo. USA). Plant tissue N concentrations and $\delta^{15}N$ were measured using a Europa mass spectrometer (Europa Scientific, PDZ Europa Ltd., Cheshire, UK, U.C. Davis, Stable Isotope Facility, Davis, Calif. USA).

The amount of ^{15}N-labeled nitrate acquired during the final treatment was calculated using biomass (root and shoot), %N (root and shoot), and atom % (root and shoot). Atom % ^{15}N was determined by the following steps:

$$\delta\,^{15}N = \left(\frac{R_{sample} - R_{std}}{R_{std}}\right) \qquad [1]$$

where R represents the absolute ratio ($^{15}N/^{14}N$) of the sample and standard gases. The absolute ratio for the standard gas (R_{std}) is .0036765. The absolute ratio of a sample (R_{sample}) can be determined by rearranging [1].

$$R_{sample} = \left[\frac{\delta^{15}N}{1000} + 1\right] \text{x } R_{std} \qquad [2]$$

R_{sample} is used to calculate F, the fractional abundance [$^{15}N/(^{15}N + ^{14}N)$]:

$$F = \left[\frac{R_{sample}}{R_{sample} + 1}\right] \qquad [3]$$

Atom % is used to express isotopic enrichment in samples highly enriched in ^{15}N:

$$\text{atom \%} = F \times 100 \qquad [4]$$

Harvest

Before initiating treatments and immediately before harvest, the following information was collected on each plant. Number of tillers, height of total plant, and reproductive status for grasses and number of leaves, length of longest leaf, and phenology (rosette, bolting, flowering) for *Centaurea*. Plants were harvested 17 weeks after the vernalization period which was 8 weeks after initiating treatments. Shoots were removed from the roots and placed in a drying oven at 70° C for 72 h. Roots were washed free of sand and separated by hand in a water bath, then placed in a drying oven at 70° C for 72 h. These plant materials were weighed to determine total plant biomass, and root to shoot ratios.

Data analysis

The experimental design included 2 N treatments and 5 species combinations and was analyzed as a completely randomized block (n = 9). Total biomass, root to shoot ratios, %N root, %N shoot, %^{15}N acquired of applied, ^{15}N uptake by root (μmol g^{-1} h^{-1}), and NUE (total biomass (g) / total N (g) total biomass $(g)^{-1}$) were analyzed with ANOVA (SAS 1988). Main effects included N treatment, species combination, and species. Pre-treatment plant height and number of tillers (grasses) or leaves (*Centaurea*) were used as covariates for total biomass and NUE. Planned contrasts were used to compare plant responses to the two N treatments and neighbor effects (Table 2). Results of contrasts and *P*-values (Gill 1981) are in Appendix B.

Species combination 1 vs species combination 2	Abbreviations
CENTAUREA/CENTAUREA vs *CENTAUREA/Pseudoroegneria*	CE CE vs CE ps
PSEUDOROEGNERIA/PSEUDOROEGNERIA vs *Centaurea/PSEUDOROEGNERIA*	PS PS vs ce PS
CENTAUREA/CENTAUREA vs *CENTAUREA/Pascopyrum*	CE CE vs CE pa
PASCOPYRUM/PASCOPYRUM vs *Centaurea/PASCOPYRUM*	PA PA vs ce PA
Centaurea/PASCOPYRUM vs *Centaurea/PSEUDOROEGNERIA*	ce PA vs ce PS
CENTAUREA/Pascopyrum vs *CENTAUREA/Pseudoroegneria*	CE pa vs CE ps

Table 2. Planned contrasts were used to compare plant responses to different N supply rates and different neighbors. For example, CE CE vs CE ps contrasted the response of *Centaurea* grown in monoculture (average of the two plants) with *Centaurea* when it was grown with *Pseudoroegneria.* Capital letters represent the species, or average of species, used in each contrast.

Results

Biomass

Biomass for all species was consistently greater at high N than at low N. At both N treatments, biomass was greater for *Centaurea* grown with *Pseudoroegneria* than *Centaurea* in monoculture, but lower for *Centaurea* grown with *Pascopyrum* than *Centaurea* in monoculture (Fig. 1a, Appendix B). Biomass was greater for *Pseudoroegneria* in monoculture than *Pseudoroegneria* grown with *Centaurea* at low N but was similar at high N. At both N treatments, *Pascopyrum* in monoculture had lower biomass than when grown with *Centaurea.* Biomass was much greater at both N treatments for *Pascopyrum* grown with *Centaurea* than *Pseudoroegneria* grown with *Centaurea*, whereas *Centaurea* grown with *Pseudoroegneria* consistently had greater biomass than *Centaurea* grown with *Pascopyrum.*

Root to shoot ratios

At both N treatments, *Centaurea* in monoculture had higher root to shoot ratios than *Centaurea* grown with *Pascopyrum* (Fig. 1b, Appendix B). Root to shoot ratios were consistently greater for *Pascopyrum* grown with *Centaurea* than *Pascopyrum* in monoculture. At high N, *Pascopyrum* grown with *Centaurea* had higher root to shoot ratios than *Pseudoroegneria* grown with *Centaurea.* At both N treatments, root to shoot ratios were greater for *Centaurea* grown with *Pseudoroegneria* than *Centaurea* grown with *Pascopyrum.*

Nitrogen use efficiency (NUE)

At low N, NUE was greater for *Centaurea* when it was grown with the grasses (mixed culture pots) then when it was grown in monoculture. *Pascopyrum* in monoculture had greater NUE than *Pascopyrum* grown with *Centaurea* (Fig. 1c, Appendix B). Nitrogen use efficiency was greater at both N treatments for *Pseudoroegneria* grown with *Centaurea* than *Pseudoroegneria* in monoculture.

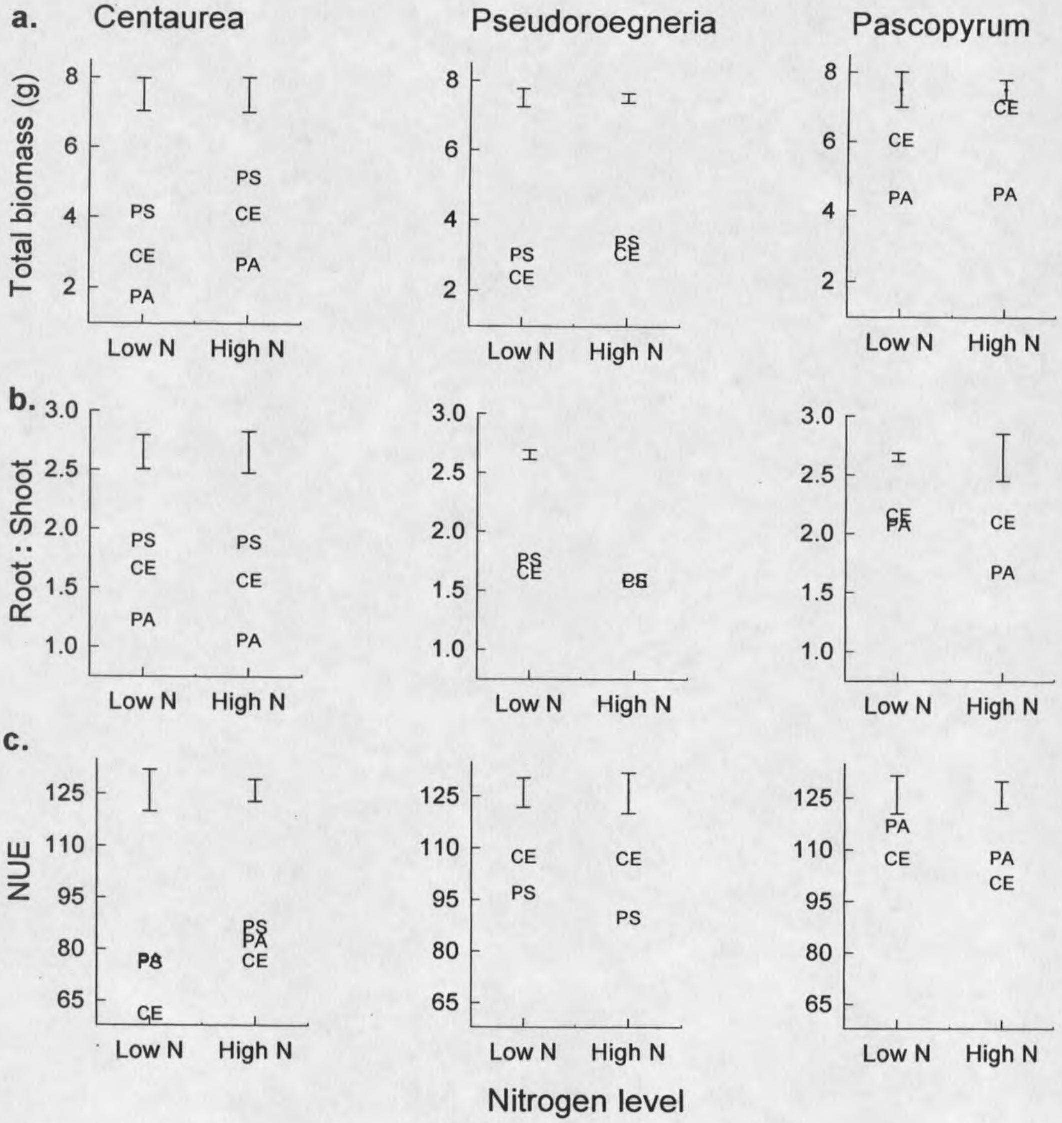

Figure 3. **a**. Biomass (g), **b**. root : shoot ratios, and **c**. nitrogen use efficiency (total biomass (g) / total N (g)) of target species *Centaurea*, *Pseudoroegneria*, and *Pascopyrum* when growing with neighbors (*Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA) and conditioned to low N levels or high N levels during the treatment period. For example, Figure 1a. represents biomass of *Centaurea* when grown with *Centaurea*, *Pseudoroegneria*, and *Pascopyrum*.

^{15}N-labeled nitrate uptake (μmol g^{-1} h^{-1})

At both N treatments, *Centaurea* in monoculture had greater ^{15}N uptake than when grown with either grass species (Figure 2a and Appendix B). At low N, *Pascopyrum* grown with *Centaurea* had slightly greater ^{15}N uptake than *Pascopyrum* in monoculture, whereas *Pascopyrum* grown with *Centaurea* had lower ^{15}N uptake than *Pascopyrum* in monoculture at high N.

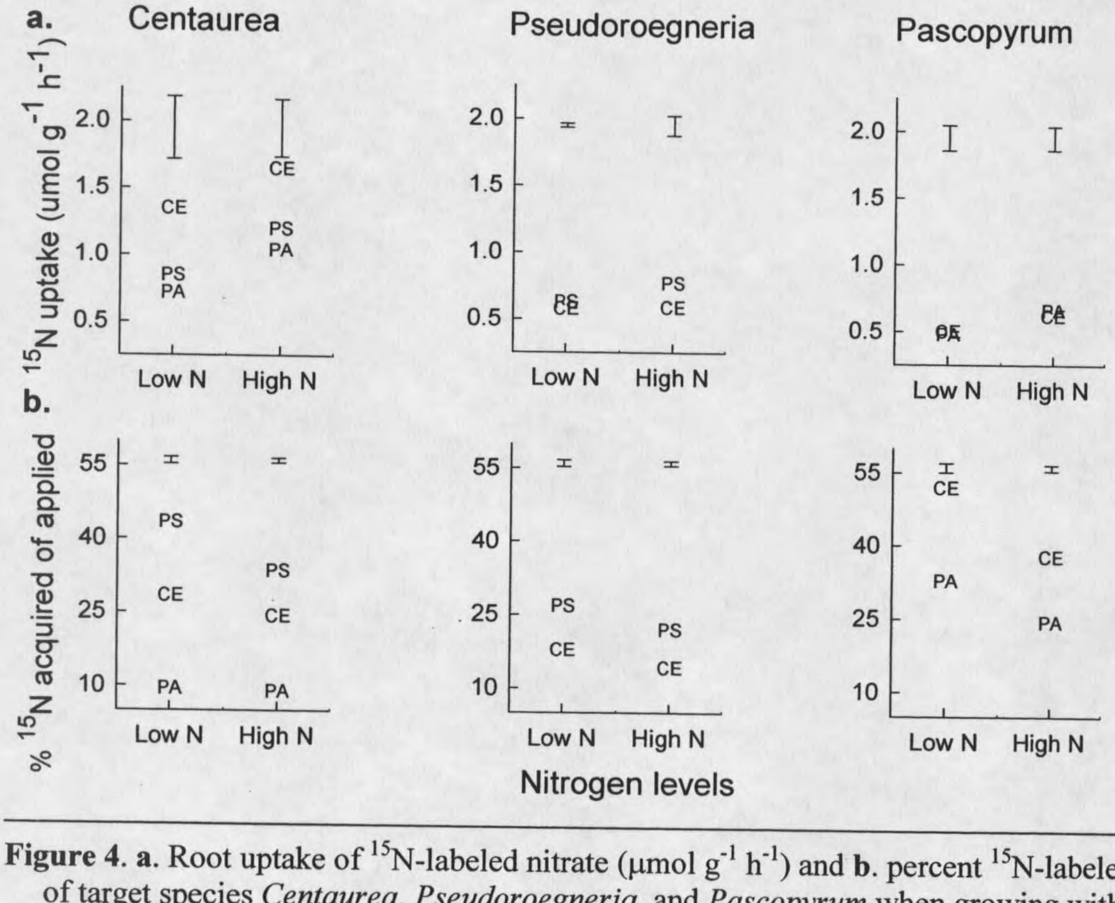

Figure 4. **a**. Root uptake of ^{15}N-labeled nitrate (μmol g^{-1} h^{-1}) and **b**. percent ^{15}N-labeled of target species *Centaurea*, *Pseudoroegneria*, and *Pascopyrum* when growing with neighbors (*Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA) and conditioned to low N levels or high N levels during the treatment period.

Percent ^{15}N acquired of applied

At both N treatments, all of the following contrasts were significant. *Centaurea* grown with *Pseudoroegneria* acquired more ^{15}N than *Centaurea* in monoculture, whereas *Centaurea* grown with *Pascopyrum* acquired less ^{15}N than *Centaurea* in monoculture (Figure 2b and Appendix B). *Pseudoroegneria* in monoculture acquired more ^{15}N than *Pseudoroegneria* grown with *Centaurea,* whereas *Pascopyrum* in monoculture acquired less ^{15}N than *Pascopyrum* grown with *Centaurea. Pascopyrum* grown with *Centaurea* consistently acquired much more ^{15}N than *Pseudoroegneria* grown with *Centaurea,* whereas *Centaurea* grown with *Pascopyrum* acquired much less ^{15}N than *Centaurea* grown with *Pseudoroegneria.*

Discussion

Nitrogen treatments

The N treatments in this study were designed to mimic low and moderately high N mineralization rates of semiarid grasslands in Montana (Neill 1995). Biomass, root to shoot ratios, and NUE reflect the long term exposure to the N treatments. After an 8 wk treatment period, all plants were exposed to ^{15}N-labeled nitrate for 24 h and then harvested. The amount of ^{15}N supplied to plants corresponded to their respective treatment supply rates. As a result of this labeling, ^{15}N uptake (μmol g^{-1} h^{-1}) represents relative root affinity for N on a per gram basis. Percent ^{15}N acquired of applied reflects the amount of ^{15}N taken up in 24 h, which is a function of affinity for N and total root mass.

Biomass for all species was consistently greater at high N than at low N. In a field study *Centaurea* and associated vegetation had greater biomass with greater N supply rates (Story, Boggs & Graham 1989). Root to shoot ratios tended to be slightly lower at high N. Root to shoot ratios generally decrease with increased N availability (Fichtner & Schultz 1992; Reynolds & D'Antonio 1996; Robinson 1996). Plants typically allocate more biomass to roots and less to leaves when N is in short supply because investing resources in the part of the plant that acquires limited resources (roots) is more important than investing in the part that requires most of the nutrient (shoot) (Brouwer 1983).

Nitrogen use efficiency was generally greater for the grasses at low N than at high N, although the grasses had greater NUE than *Centaurea* at both N treatments. Plant species that evolved in low-nutrient environments tend to have low tissue N concentrations, which may reflect high NUE (Gleeson & Tilman 1990). In contrast to the grasses, *Centaurea* grown in mixed- and monoculture had slightly greater NUE at high N than at low N. We expected to see greater NUE at low N than at high N for both species because the need to use N efficiently to produce biomass should decrease with greater N availability. A plant's ability to produce more biomass per unit of N is considered advantageous in low-nutrient environments (Aerts *et al.* 1991).

Affinity for ^{15}N (μmol g^{-1} h^{-1}) was greater at high N for nearly all species. This may simply reflect that twice as much ^{15}N was available to plants in the high N treatment than at the low N treatment. In contrast, the percent of applied ^{15}N-labeled nitrate acquired during the final labeling period was generally greater for plants at low N. This may reflect that plants conditioned to low N had a greater capacity to absorb N under N-limiting conditions (Lee 1982; Robinson 1996). Low N availability may signal a plant to synthesize additional N transport proteins, thereby increasing the plant's ability to take up N (Lambers, *et al.* 1998).

Centaurea vs. *Pseudoroegneria*

Centaurea grown with *Pseudoroegneria* had greater biomass than *Centaurea* in monoculture at both N treatments. Conversely, *Pseudoroegneria* grown with *Centaurea* tended to have lower biomass than *Centaurea* in monoculture at both N treatments. This indicates that *Centaurea* grew faster with *Pseudoroegneria* as a neighbor than in monoculture. Plants that grow fast are able to rapidly occupy space, which is advantageous when competing for limited resources (Lambers *et al.* 1998). Although not tested, it appears that *Centaurea* in monoculture tended to have greater biomass than *Pseudoroegneria* in monoculture at high N, whereas *Centaurea* in monoculture and *Pseudoroegneria* in monoculture tended to have similar biomass at low N. In low N environments, the growth rate of potentially fast-growing species is generally reduced more than slow-growing species (Fichtner & Shulze 1992). In a greenhouse study comparing response to a high and low N nutrient solution, *Holcus lanatus*, a fast-growing species, had greater differences in growth rates between low and high N treatments than *Deschampsia flexuosa*, an inherently slow-growing species (Poorter *et al.* 1995).

Root to shoot ratios were similar for *Centaurea* and *Pseudoroegneria* in mixed- and monocultures at both N treatments. Apparently, *Centaurea* and *Pseudoroegneria* were not very plastic when allocating biomass to roots and shoots based on the slight responses to N supply rate and identity of its neighbor. Plasticity in root to shoot ratios of species from low-nutrient and high-nutrient environments is often similar (Reynolds & D'Antonio 1996). In contrast, Aerts et al (1991) found that *Erica tetralix* and *Calluna vulgaris,* species from nutrient-poor soils, and *Molinia caerulea*, a species from nutrient-rich soils, allocated less biomass to roots at high N than at low N. In addition, *Molinia* allocated about 2 - 3 times more biomass to roots than *Erica* and *Calluna.*

Pseudoroegneria in mixed-and monoculture had greater NUE than *Centaurea* in mixed- and monoculture, especially at low N. Overall, *Pseudoroegneria* grown with *Centaurea* had greater NUE than *Pseudoroegneria* in monoculture at both N treatments. Presumably, *Pseudoroegneria* had to use N efficiently because it was competing with a larger *Centaurea* plant for limited N. *Centaurea* grown in mixed- and monoculture had greater NUE at high N than at low N, which may partially explain why *Centaurea* readily invades high-nutrient environments, such as disturbed areas. At low N, *Centaurea* may not have been able to produce as much biomass per unit N because the low N treatment may have limited growth of this weedy species.

Centaurea in mixed- and monoculture had a greater affinity for ^{15}N (µmol g^{-1} h^{-1}) than *Pseudoroegneria* in mixed- and monoculture at both N treatments, particularly at high N. *Pseudoroegneria*, indigenous to low N, semiarid grasslands, may have evolved a root system that does not have as great an affinity for N as *Centaurea*, a weedy species. This may help explain *Centaurea's* ability to take advantage of disturbed areas where N mineralization rates are typically higher than undisturbed areas (Burke & Grime 1996).

Centaurea grown with *Pseudoroegneria* acquired a greater percent of the applied ^{15}N-labeled nitrate than *Centaurea* in monoculture at low and high N. Conversely, *Pseudoroegneria* grown with *Centaurea* acquired a lower percent of ^{15}N than *Pseudoroegneria* in monoculture at both N treatments. This response may reflect the greater root mass of *Centaurea* which allowed it to acquire more of the applied ^{15}N, leaving less for *Pseudoroegneria* when the two were grown as neighbors.

Centaurea vs. Pascopyrum

The responses of *Centaurea* to *Pascopyrum* were nearly opposite *Centaurea's* responses to *Pseudoroegneria*. We expected that *Centaurea,* as a weedy species, would have a greater ability to compete for N at the high and low N than the two native grasses in this study.

Pascopyrum in mixed- and monoculture had more biomass than *Centaurea* in mixed- and monoculture at both N treatments, particularly at low N. We expected that *Centaurea* would have greater biomass than *Pascopyrum* because weedy species are generally presumed to grow faster than native rangeland grasses. Species that have the potential to grow fast typically have similar or higher growth rates under nutrient-limited conditions than potentially slow-growing species (Poorter *et al.* 1995).

Plants from low-nutrient environments tend to have high root to shoot ratios (Chapin 1980) which increases their ability to compete for limited below-ground resources (Boot & Mensink 1990). *Pascopyrum* grown with *Centaurea* had greater root to shoot ratios than *Pascopyrum* in monoculture, whereas *Centaurea* grown with *Pascopyrum* had lower root to shoot ratios than *Centaurea* in monoculture. This may indicate that *Centaurea* plants competing with *Pascopyrum* allocated proportionally more resources to above ground growth to more rapidly complete its life cycle. Often, faster growing ruderal species have the capacity to grow quickly, which facilitates rapid completion of the life cycle and maximizes seed production (Grime 1977). Alternatively, *Centaurea* may have allocated more resources to above ground growth (lower root to shoot ratio) because it was in the rosette stage, and therefore competing for light with the taller *Pascopyrum*. These results indicate that *Centaurea* and *Pascopyrum* are plastic in allocating biomass to roots and shoots, depending on the identity of their neighbor. *Pascopyrum* may have evolved to allocate more resources to below ground biomass when growing with different species, whereas *Centaurea* may have evolved to allocate more resources to above ground biomass when growing with different species.

Like *Pseudoroegneria, Pascopyrum* in mixed-and monoculture had greater NUE than *Centaurea* in mixed- and monoculture at both N treatments. *Pascopyrum* in monoculture had greater NUE than *Pascopyrum* grown with *Centaurea* at low N than at high N. Greater NUE under low N conditions was expected, because plants native to low-nutrient environments tend to use nutrients efficiently (Gleeson & Tilman 1990). In mixed- and monoculture *Centaurea* had slightly greater NUE at the high N treatment than at the low N treatment, which again may reflect that as a weedy species, *Centaurea* simply had greater NUE when N levels more closely resembled those associated with disturbance.

Centaurea in mixed- and monoculture had a much greater affinity for ^{15}N (μmol g^{-1} h^{-1}) than *Pascopyrum* in mixed- and monoculture at both N treatments. *Pascopyrum* had similar affinity for ^{15}N in mixed- and monoculture at both N treatments. *Pascopyrum's* lower affinity may reflect that it evolved in a low-nutrient environment. Plants from such environments rely on extensive root systems to capture nutrients in limited supply (Aerts *et al.* 1991). However, greater root mass does not necessarily increase root absorption capacity. As root systems become more extensive, absorption capacity generally declines in plants from low-nutrient environments (Chapin 1980).

Pascopyrum grown with *Centaurea* acquired a greater percent of the applied ^{15}N-labeled nitrate than all other plants at low and high N, whereas *Centaurea* grown with *Pascopyrum* acquired a lower percent of ^{15}N than all other plants at both N treatments. This response may reflect the greater root mass of *Pascopyrum,* which offset its lower affinity for ^{15}N compared with *Centaurea.* This allowed *Pascopyrum* to acquire a greater percent of the applied ^{15}N, leaving less for *Centaurea* when the two were grown as neighbors.

Conclusion

We expected that *Centaurea* would have a greater ability than native grasses to preempt N under low and moderately high N levels, which may partly explain its success in invading semiarid grasslands dominated by native grasses. We found that *Centaurea* was able to out-compete *Pseudoroegneria* for N at both N treatments, although it was not able to out-compete *Pascopyrum* for N at either N treatment. This indicates *Centaurea's* ability to compete for N depended on the identity of its neighbor.

Overall, *Centaurea* had a greater ability to compete for N at the higher N level, which may help explain its ability to take advantage of disturbance. Furthermore, *Centaurea* had greater affinity for N than *Pseudoroegneria* and *Pascopyrum* at both N treatments. This may be one of *Centaurea's* traits that enhances its ability to invade undisturbed semiarid grasslands dominated by native grass species. *Centaurea* appears to have traits that allow it to function as an early-seral species, yet also has the plasticity to function as a late-seral species, depending on the plant community.

CHAPTER 4

WATER USE

Introduction

Centaurea maculosa Lam. (spotted knapweed), a perennial tap-rooted forb, was introduced to the Pacific Northwest in the late 1800s from Eurasia. It has no natural enemies in North America and has spread rapidly throughout native grassland systems in the western United States. This rapid spread of persistent, invasive species may alter plant communities and ecosystem function (Mooney & Drake 1986). Millions of hectares of semiarid grasslands have been invaded by *Centaurea,* and millions of dollars are spent annually on costs associated with *Centaurea* infestations (Lacey *et al.* 1995). The shift of grasslands from native species to *Centaurea* may be the single greatest threat to native grassland systems in the western United States.

The expansion of persistent weedy species increases the need to better understand the mechanisms associated with these species' ability to displace native species. Plant communities dominated by invasive species for long periods of time do not appear to fit Clement's (1916) model of plant succession. Instead, native plant communities that are replaced by exotic species appear to fit the multiple steady state, state and transition model (Westoby, Walker & Noy-Meir 1989). Laycock (1991) used the term "suspended stages of succession" to describe plant communities that represent relatively unchanged assemblages of species over long periods of time. These concepts are closely related and describe ecosystems that do not fit the Clementsian approach, possibly because invasive species preempt limiting resources or use limiting resources more efficiently, therefore inhibiting establishment, survival, and reproduction of native species.

In low-nutrient environments, plant species compete mainly for nutrients and water (Wilson & Tilman 1991). Plant species that use these resources most effectively may be more competitive (Mamolos, Veresoglour & Barbayiannis 1995). Nutrient-poor habitats support lower growth rates and higher root to shoot ratios (Wilson & Tilman 1991). Below ground competition usually affects the balance between competing species more than aboveground competition (Wilson 1988). Competitive ability has been positively correlated with high root to shoot ratio, or high root mass (Baan Hofman & Ennik 1980; Aerts *et al.* 1991). Therefore, nutrient-poor habitats may be characterized by intense competition for soil resources (Tilman 1988).

Water is frequently a limiting resource in semiarid grasslands (Johnson *et al.* 1990). A species' success may be related to its ability to use water efficiently in water-limited environments. Alternatively, a species' success could simply reflect its ability to extract more water from the soil, potentially leaving less for its neighbors.

Water use efficiency (WUE) is generally measured using one of three methods. First, the traditional method requires measuring dry matter produced and the amount of water used by a plant during a growing period (WUE = D/W, where D = mass of dry matter, and W = mass of water used). Second, water use efficiency can be measured by assessing the carbon dioxide (CO_2) and water vapor exchange of individual leaves. This gas exchange efficiency is the ratio of the mass of CO_2 gained to the mass of water lost (A/g), where A refers to assimilation and g refers to stomatal conductance. This method assesses instantaneous water use efficiency but may be a poor indicator of long-term water use efficiency (Kramer & Boyer 1995). A third technique, carbon isotope discrimination (Δ), is negatively correlated with long-term water use efficiency in C_3 plants (Farquhar *et al.* 1988; Ehleringer *et al.* 1990). The carbon isotopic composition ($\delta^{13}C$) of plant tissues has been correlated with long- and short-term estimates of the ratio of dry matter production to water, providing a useful tool to evaluate correlation between *A* and *g* (Farquhar, O'Leary & Berry 1982; Farquhar & Richards 1989).

Atmospheric CO_2 contains 98.9% ^{12}C and 1.1% ^{13}C; they are identical in chemical properties but ^{13}C has greater mass. Plants discriminate against the heavier isotope, with discrimination being particularly high in plants that fix C by the C_3 pathway (Farquhar *et al.* 1982). Some discrimination occurs because $^{12}CO_2$ diffuses faster toward the carboxylation site than $^{13}CO_2$, but most of the discrimination against ^{13}C occurs during carboxylation catalyzed by ribulose bisphophate carboxylase (rubisco) (Kramer & Boyer 1995). Because of this discrimination, plant tissue accumulates relatively less ^{13}C than ^{12}C.

Carbon isotope composition is a result of the ^{13}C:^{12}C ratio of the CO_2 in the intercellular air spaces of leaves (c_i) compared with the ^{13}C:^{12}C ratio of atmospheric CO_2 (c_a) (Farquhar *et al.* 1982, Farquhar *et al.* 1989). Carbon isotope discrimination (Δ) is calculated from $\delta^{13}C$ using a carbon isotope ratio of -8‰ for C_a. Carbon isotope discrimination values provide a long-term estimate of the ratio of c_i/c_a, which is related to A/g. .

One link between WUE and carbon isotope composition ($\delta^{13}C$) is through stomatal conductance (Farquhar, Ehleringer & Hubick 1989). When stomatal conductance is high, leaf intercellular c_i concentrations rise, increasing the opportunity for rubisco to discriminate against ^{13}C. This decreases the $^{13}C:^{12}C$ ratio in plant material (Taiz & Zeiger 1998), resulting in a higher Δ. When stomatal conductance is low, c_i decreases; with lower c_i, rubisco discriminates less against ^{13}C, resulting in a higher $^{13}C:^{12}C$, and thus a lower Δ in plant material (Taiz & Zeiger 1998). A lower Δ value in plant material correlates with higher WUE (Farquhar *et al.* 1982); conversely, a plant with a higher Δ value denotes lower WUE because open stomata facilitate greater water loss. Because C is continually assimilated, Δ values integrate *A/g* over the period of tissue development. This results in a long-term indicator of WUE for the tissue of interest (Johnson *et al.* 1990).

We hypothesized that the aggressive, successful *Centaurea* uses water more efficiently than dominant native grass species. Alternatively, *Centaurea* may simply use more water than native grasses. Our objectives were to determine: 1) WUE for *Centaurea* and three dominant native grasses using the traditional method (biomass (g) / water used (kg)) and carbon isotope discrimination (Δ) in a greenhouse, 2) total water use for *Centaurea* and three native grasses in a greenhouse, and 3) WUE based on Δ of *Centaurea* and three native grasses in the field over two growing seasons.

Materials and Methods

Plant species.

Centaurea is an introduced perennial, tap-rooted forb that has the potential to invade over 15 million hectares in Montana (Chicoine, Fay & Neilsen 1985). *Pseudoroegneria* *spicata* [Scribn. and Smith] A. Love (bluebunch wheatgrass), a perennial bunchgrass, *Pascopyrum smithii* [Rybd.] A. Love (western wheatgrass), a perennial rhizomatous grass, and *Festuca idahoensis* Elmer (Idaho fescue), a perennial bunchgrass, are native species. These grasses frequently dominate semiarid grasslands typified by our study sites.

Greenhouse study

Centaurea, Pseudoroegneria, Pascopyrum, and *Festuca* plants were grown at low, medium, or high soil water status in a greenhouse to determine levels of $\delta^{13}C$ and WUE for each species. Empty weight of each column was recorded. Two parts silt loam and one part sand were mixed, pasteurized, and dried to a constant weight, the columns were then filled with the soil mixture and weighed again. Columns were constructed of polyvinylchloride (PVC) pipe, 10 cm in diameter and 40 cm in height, with perforated end caps as bottoms. Seeds were sterilized in 1.5% NaClO solution. The soil was moistened and three seeds of each grass species were planted in 15 columns, one species per column. Five seeds of *Centaurea* were planted in 15 columns. Seeded columns were placed in a cold room for a three week vernalization period, then moved to a greenhouse. Greenhouse temperatures averaged 20°C during the day and 15°C at night. Grow lights were used from 0700 to 2100 for the first 10 weeks, ambient summer light conditions were used for the remainder of the experiment. The soil was kept moist during seedling emergence. Once established, seedlings were thinned to one per column.

Treatments began six weeks after columns were removed from the cold room. Before initiating treatments and immediately before harvest, plant height and number of tillers (grasses) or leaves (*Centaurea*) were recorded for each plant. Most plants were at a 4-leaf stage.

A soil water retention curve was determined for the soil using pressure membrane and pressure plate extractors (Soilmoisture Equipment, Santa Barbara, Calif. USA). The measured data were then fitted to the van Genuchten (1980) parametric water retention model. Soil water retention describes the functional relationship between soil water content and soil matric potential under equilibrium conditions (Or & Wraith 1999). Mass water content (kg/kg) corresponding to three predetermined soil matric potentials was determined from the soil water retention relationship. Soil matric potentials -0.01, -0.1, and -1.0 MPa were used as the high, medium, and low soil water status (treatments), respectively. Soil matric potential represents a consistent, repeatable, and biologically meaningful water treatment. Each soil matric potential (3) - species (4) combination was replicated 5 times (n = 60). Three columns, one for each matric potential, were added to the study to estimate the amount of water loss due to evaporation from bare soil. These columns were filled with soil, but were not seeded.

All columns were weighed three times per week to determine mass water content. Soils were then brought up to target mass water content by adding the amount of water required to attain the desired soil matric potential. The target water status thus represents the maximum soil wetness that the plants experienced. Frequent watering minimized the range in soil matric potential experienced by the planted columns. Plants were fertilized biweekly by substituting 50 mL of 0.25x solution of commercial fertilizer (Miracle-Gro, Stern's Nurseries, Geneva, New York; 30:30:30 NPK) for the first 50 mL of water required to restore target soil matric potential in each column.

Following an 8 wk treatment period, plants were harvested. Shoot and root material was separated and then placed in a drying oven at 70° C for 72 h. Total biomass (g), root mass ratio (root mass (g) / total biomass (g)), total water use (kg), water use efficiency (g/kg), and carbon isotope discrimination (Δ) were determined for all plants at each matric potential. Dried root and shoot material was weighed to determine total plant biomass, and root mass ratio (RMR). Total water used by plants represents the total amount of water added to pots to maintain the desired soil water status over the treatment period. Water use efficiency (g/kg) is the total biomass (g) produced per unit of water used (kg). To determine Δ, shoot material was fine ground and sent to a laboratory for analysis (see carbon isotope discrimination, this section).

Field study

Three sites representing semiarid grasslands in Montana were used for this study. All sites were infested with *Centaurea*. The Helena Site was 130 km northwest of Bozeman, Montana, USA. Elevation is 1280 m with an average annual precipitation of 305 mm. Soils are coarse loamy, mixed frigid Aridic Argiustolls (USDA 1999). *Pseudoroegneria* and *Pascopyrum* are the dominant native grasses; several species of native forbs are also common. The site is between an active rail line and a cultivated field, about 100 m from a state highway. The Belgrade Site is 24 km west of Bozeman, Montana. Elevation is 1350 m with an annual average precipitation of 364 mm. Soils are coarse loamy, mixed frigid Aridic Ustifluvents (USDA 1999). *Pascopyrum* is the dominant native grass. This site is in a horse pasture in a low-density, rural residential subdivision. The Hyalite Site is 12 km southwest of Bozeman, Montana at an elevation of 1615 m. The average annual precipitation is 466 mm and soils are fine loamy, mixed frigid Typic Argiustolls, tending toward mixed frigid Udic Argiustolls (USDA 1999). The dominant native grass is *Festuca*. *Lupinus sericeus* Pursh (lupine) and other native forbs are common at the site. This site borders a rural residential subdivision.

Total aboveground biomass of the dominant native grass species and *Centaurea* was collected from three locations at each field site in early June, July, and August 1999 and mid-May, June, July, and August 2000. Three samples from each location within a study site were composited. Samples representing different vegetative stages of *Centaurea* were collected when possible, including rosette, bolting, and flowering stages. The samples were placed in a drying oven within 4 h of harvest and dried for 72 h at 70°C.

Carbon isotope discrimination (Δ)

Carbon isotope discrimination values are integrated over the period of tissue development, thus they are a long-term indicator of intercellular CO_2 concentrations, (Johnson & Rumbaugh 1995). Total above ground biomass was collected to determine Δ over the period of plant tissue development. Selecting only a part of the plant (i.e. youngest leaf) would have represented only the period of tissue development for that plant part.

Dried shoot material from the greenhouse and field study were fine ground using a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado USA) and sent to the

Isotope Analysis Laboratory (Ecosystem Sciences; U.C. Berkeley, Berkeley, Calif. USA) to determine carbon isotope composition. Carbon isotope composition ($\delta^{13}C$) is calculated by comparing the ^{13}C to ^{12}C composition of a sample (R_{sample}) relative to the Pee Dee Belemnite (PDB) standard (R_{PDB}):

$$\delta\ ^{13}C_{sample} = \left[\frac{R_{sample}}{R_{PDB}} - 1\right] \text{x } 1000 \qquad [1]$$

These $\delta^{13}C$ values were used to calculate isotopic discrimination (Δ), as described by Farquhar & Richards (1984):

$$\Delta = \frac{\delta\ ^{13}C_{air} - \delta\ ^{13}C_{sample}}{1 + \left[\frac{^{13}C_{sample}}{1000}\right]} \qquad [2]$$

where the C isotope composition of air ($\delta^{13}C_{air}$) was assumed to be -8 $^{0}/_{00}$ (Farquhar *et al.* 1989).

Data analysis

Greenhouse study. The research design was a 4 (species) by 3 (soil matric potential) factorial with 5 replications (n=60). Water use efficiency can be expressed by biomass per unit of water used and by Δ. Both were analyzed with ANOVA in a completely randomized block design (SAS 1988). Species and matric potential main effects and their interactions were determined. Biomass (total and shoot) was correlated with Δ to determine if there was an association between these two variables.

Field study. The research design included species, which varied by site, and growth stage (*Centaurea*: rosette, bolting, mature), which varied by month, as main effects. Carbon isotope discrimination was analyzed for *Centaurea* and grasses separately by site and by month because there were different native grasses at each site, and different growth stages by month. ANOVA (SAS 1988) was used for all analysis.

Results

Greenhouse study

Total biomass differed among species depending on matric potential (treatment by species, P = 0.01, Fig. 1a). Across matric potentials, total biomass differed among species (species, P < 0.0001). *Pascopyrum, Pseudoroegneria,* and *Centaurea* had similar biomass at the lowest matric potential. At the -0.10 MPa matric potential, biomass was greatest for *Pascopyrum* and intermediate for *Pseudoroegneria* and *Centaurea.* At the -0.01 MPa matric potential, *Pascopyrum* and *Pseudoroegneria* had greater biomass than *Centaurea*, which was intermediate. *Festuca* had considerably less biomass than the other species at each matric potential. For each species, biomass was greater at the highest matric potentials (treatment, P < 0.0001).

Root mass ratio (RMR) differed among species across matric potentials (species, P = 0.06, Fig. 1b); RMR was highest for *Pascopyrum*, slightly lower for *Pseudoroegneria*, intermediate for *Centaurea*, and lowest for *Festuca.* Root mass ratio was relatively similar across matric potentials for each species (treatment, P = 0.56).

Total water use differed among species depending on matric potential (treatment by species, P = 0.02, Fig. 2a). Overall, total water use was greatest for *Pascopyrum*, intermediate for *Centaurea* and *Pseudoroegneria*, and lowest for *Festuca* (species, P < 0.0001). For each species, water use was greater at the higher matric potentials (treatment, P < 0.0001).

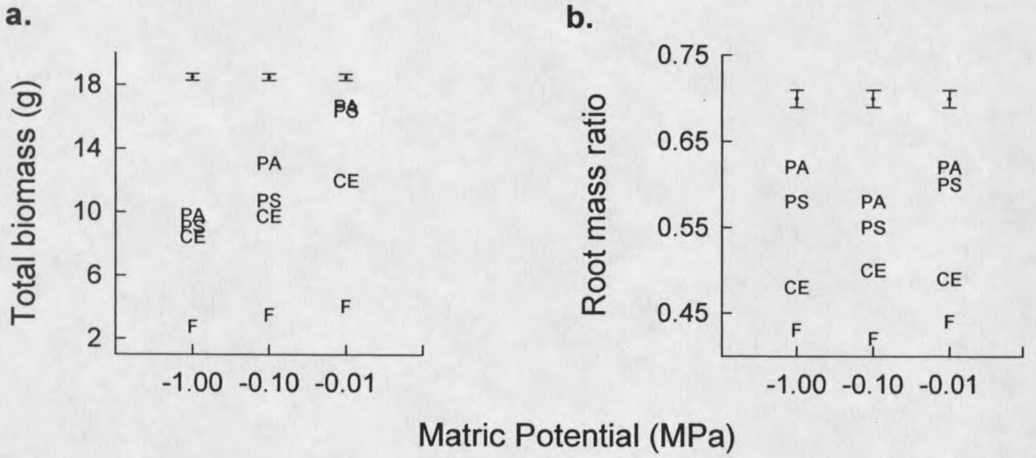

Figure 5. **a**. Total biomass (g) and **b**. root mass ratio (root (g) / total biomass (g)) at low (-1.00), moderate, (-0.10), and high (-0.01) matric potentials (MPa)which represent low, medium, and high soil water status, respectively. Species: *Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA, and *Festuca* - F. Least square means standard error presented for each matric potential.

Water use efficiency (WUE; biomass (g) / water used (kg)) differed among species (species, P < 0.0001, Fig. 2b). WUE was greatest for *Pascopyrum* and *Pseudoroegneria*, intermediate for *Centaurea*, and lowest for *Festuca*. Water use efficiency was relatively consistent for each species across the three matric potentials (treatment, P = 0.85).

Carbon isotope discrimination (Δ) differed among species across matric potentials (species, P < 0.0001, Fig. 2c). Overall, Δ was greatest for *Pascopyrum*, intermediate for *Centaurea* and *Festuca*, and lowest for *Pseudoroegneria.* Carbon isotope discrimination differed across the matric potentials (treatment, P = 0.001). For each species, Δ was highest at the highest matric potential. Carbon isotope discrimination and biomass (total and shoot) were not correlated.

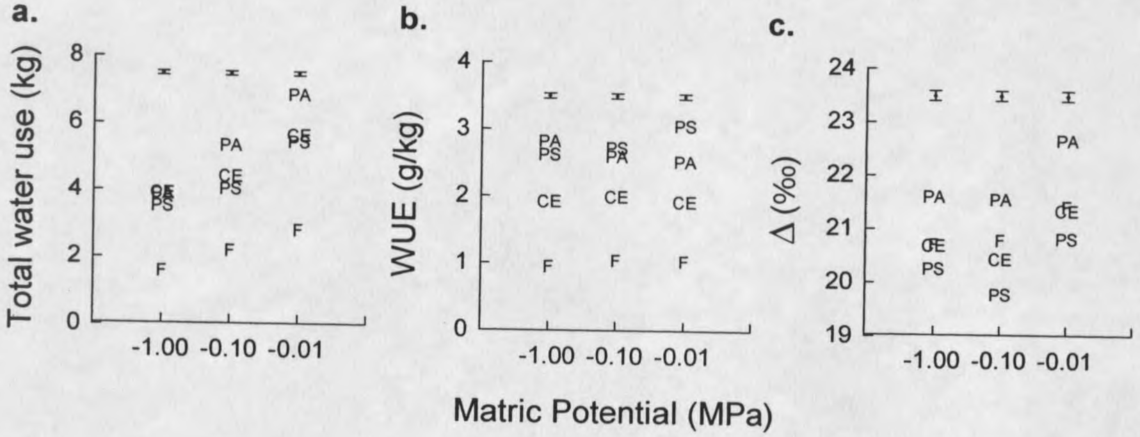

Figure 6. **a**. Total water use (kg), **b**. water use efficiency (biomass (g) / water used (kg)), **c**. carbon isotope discrimination (Δ) at low (-1.00), moderate (-0.10), and high (-0.01) matric potentials (MPa) which represent low, medium, and high soil water status, respectively. Species: *Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA, and *Festuca* – F. Least square means standard error presented for each matric potential.

Field study

Precipitation. In 1999, precipitation was generally in the normal range during the first half of the growing season (Fig. 3). July was particularly dry, whereas August had several rainfall events, which is not normal for the month of August at these field sites. In 2000, May was drier than usual in Helena, whereas the other two sites had consistent

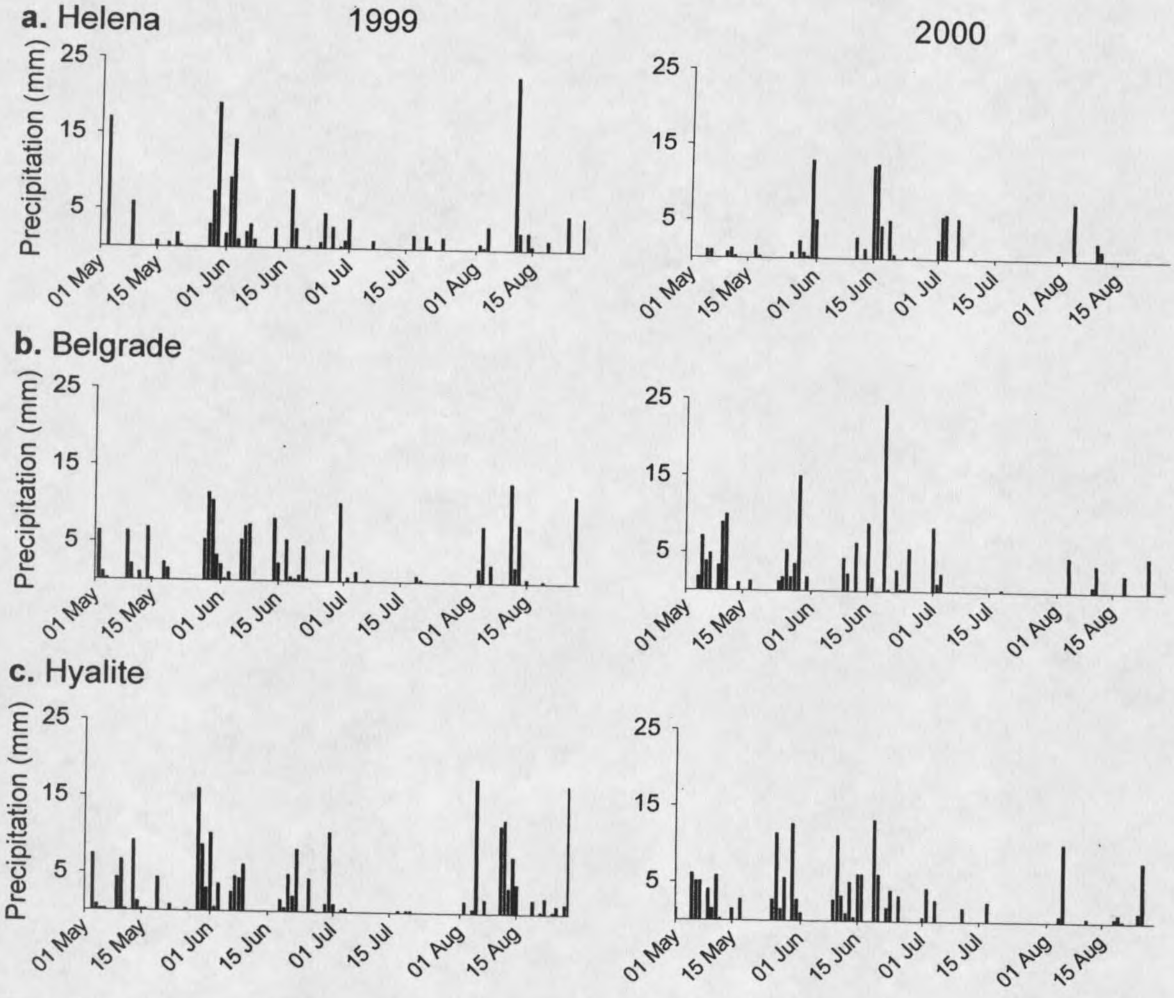

Figure 7. Daily precipitation (mm) for May, June, July, and August, 1999 and 2000 for the **a**. Helena, **b**. Belgrade, and **c**. Hyalite field sites.

	Helena			Belgrade			Hyalite		
	1999	2000	30 - yr	1999	2000	30 - yr	1999	2000	30 - yr
May	55.6	23.1	45.2	58.7	72.4	62.5	64.0	75.7	80.7
Jun	54.6	44.2	47.5	50.0	57.2	62.2	64.7	64.7	72.6
Jul	10.4	20.3	27.9	3.0	12.2	28.5	2.5	11.9	34.3
Aug	48.8	11.0	32.8	50.5	15.7	32.3	87.1	22.4	38.1

Table 3. Monthly total precipitation (mm) and 30-year precipitation (mm) averages for Helena, Belgrade, and Hyalite field sites in 1999 and 2000.

rainfall in early May. Mid-May through the end of June had sporadic rainfall events at all sites. Conditions were unusually dry at each site in July and through the end of the growing season.

Carbon isotope discrimination 1999. At the Helena site in June, Δ was higher (lower WUE) for *Centaurea*-rosettes and *Centaurea*-bolting than for *Pascopyrum* and *Pseudoroegneria* (species, P = 0.0002; Fig. 4a). In July, Δ was highest for *Centaurea*-rosettes, intermediate for *Centaurea*-bolting and *Pascopyrum*, and lowest for *Pseudoroegneria* (species, P < 0.0001). In August, Δ was again highest for *Centaurea*-rosettes followed by *Pascopyrum.* Carbon isotope discrimination of *Centaurea*-mature and *Pseudoroegneria* were considerably lower than *Centaurea* rosettes and *Pascopyrum* (species, P = 0.008). Overall, Δ of *Centaurea* declined from June to August, whereas Δ of the grasses was similar in July and August.

At the Belgrade site in June, Δ was highest for *Centaurea* rosettes; and slightly lower for *Centaurea*-bolting and *Pascopyrum* (species, P = 0.07). In July, Δ of *Centaurea*-rosettes was highest and *Centaurea*-bolting and *Pascopyrum* were similar (species, P = 0.03). In August, Δ of *Centaurea*-rosettes, *Centaurea*-mature and *Pascopyrum* was similar, although *Centaurea*-rosettes had slightly higher Δ than *Centaurea* mature (species, P = 0.12). Overall, Δ declined for all species from June to August.

At the Hyalite site in June, Δ was highest for *Centaurea*-rosettes and *Centaurea*-bolting and lowest for *Festuca* (species, P = 0.04). In July, Δ was highest for *Centaurea*-rosettes, intermediate for *Centaurea*-mature, and lowest for *Festuca* (species, P <

0.0001). In August, Δ was again highest for *Centaurea*-rosettes and lower for *Centaurea*-mature and *Festuca* (species P = 0.04). Overall, Δ of *Centaurea*-rosettes and *Centaurea*-mature was lower in August than July but remained relatively constant for *Festuca* from June to August.

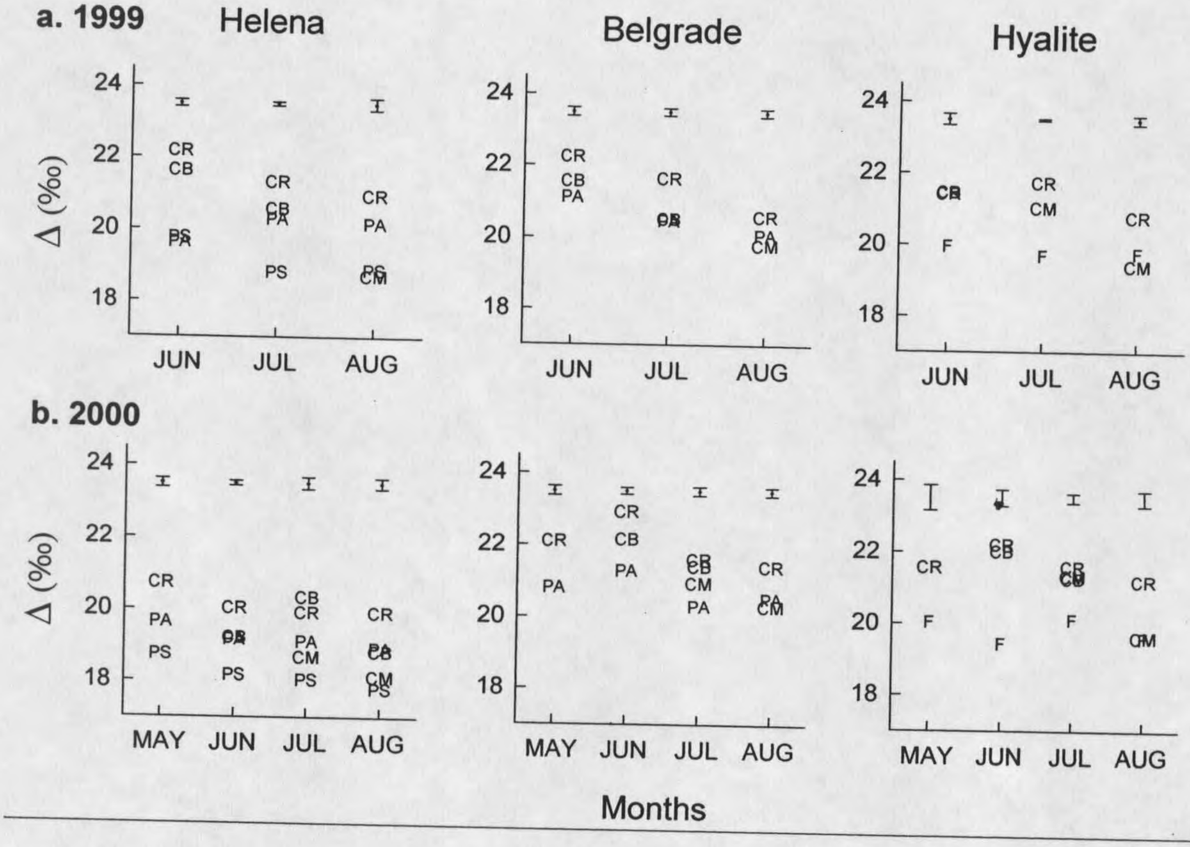

Figure 8. Carbon isotope discrimination (Δ) in **a**. 1999 and **b**. 2000 of *Centaurea* and native grasses at Helena, Belgrade, and Hyalite field sites. Species: *Centaurea*-rosettes (CR), *Centaurea*-bolting (CB), *Centaurea*-mature (CM), *Pseudoroegneria* (PS), *Pascopyrum* (PA), and *Festuca* (F). Least square means standard error presented for each month.

Carbon isotope discrimination 2000. At the Helena site in May, Δ was highest for *Centaurea*-rosettes, intermediate for *Pascopyrum*, and lowest for *Pseudoroegneria* (species, P = 0.01; Fig. 4b). In June, Δ was highest for *Centaurea*-rosettes, intermediate for *Centaurea*-bolting and *Pascopyrum*, and lowest for *Pseudoroegneria* (species, P = 0.0004). In July, Δ was highest for *Centaurea*-rosettes and *Centaurea*-bolting, intermediate for *Pascopyrum* and *Centaurea*-mature, and lowest for *Pseudoroegneria* (species, P = 0.007). In August, Δ was highest for *Centaurea*-rosettes, intermediate for *Centaurea*-bolting and *Pascopyrum*, and lowest for *Centaurea*-mature and *Pseudoroegneria* (species, P = 0.007). Overall, Δ was lower in June than May for all species, but remained relatively constant for *Centaurea*-rosettes, *Pascopyrum*, and *Pseudoroegneria* from June to August. Carbon isotope discrimination was lower in August than July for *Centaurea*-bolting and *Centaurea*-mature.

At the Belgrade site in May, Δ was higher for *Centaurea*-rosettes and lower for *Pascopyrum* (species, P = 0.07). In June, Δ was highest for *Centaurea*-rosettes, intermediate for *Centaurea*-bolting, and lowest for *Pascopyrum* (species, P = 0.008). In July, Δ was highest for *Centaurea*-rosettes and *Centaurea*-bolting, intermediate for *Centaurea*-mature, and lowest for *Pascopyrum* (species, P = 0.03). In August, Δ was highest for *Centaurea*-rosettes, and lowest for *Centaurea*-mature and *Pascopyrum* (species, P = 0.06). Overall, Δ increased from May to June, and decreased from June to July. From July to August, Δ of *Centaurea*-rosettes and *Pascopyrum* remained relatively constant, whereas Δ of *Centaurea*-mature was lower in August than in July.

At the Hyalite site in May, Δ was similar for *Centaurea*-rosettes and *Festuca* (species, P = 0.26). In June, Δ was highest for *Centaurea*-rosettes and *Centaurea*-bolting and lowest for *Festuca* (species, P = 0.02). In July, Δ was similar for *Centaurea*-rosettes, *Centaurea*-bolting, and *Centaurea*-mature but lower for *Festuca* (species, P = 0.03). In August, Δ was highest for *Centaurea*-rosettes and slightly lower for *Centaurea*-mature and *Festuca* (species, P = 0.09). Overall, Δ was inconsistent across the months for *Centaurea*-rosettes and *Festuca*. Carbon isotope discrimination for *Centaurea*-bolting was higher in June than in July and higher in July than in August *Centaurea*-mature.

Discussion

Greenhouse study

Total biomass was greater for all species at the higher soil matric potentials because of greater water availability. *Centaurea* had similar or lower biomass than *Pascopyrum* and *Pseudoroegneria* at all matric potentials, indicating that *Centaurea* did not grow faster than these two grasses. Weedy, or early-seral species, are often characterized by rapid growth rates (Wedin & Tilman 1993). *Centaurea* was in a rosette stage and had not yet bolted at the time of harvest, which may partially explain its lower biomass. *Centaurea* consistently had greater biomass than *Festuca*, which grew much more slowly than all other species. The lower biomass of *Festuca* was not surprising because many plants that evolved in low-nutrient environments grow slowly (Chapin 1980, Wilson & Tilman 1991), however, this does not explain why *Pseudoroegneria* and *Pascopyrum* produced greater biomass than *Centaurea*.

Root mass ratio (RMR: root mass (g) / total biomass (g)) is a measure of how total biomass is partitioned. *Pascopyrum* and *Pseudoroegneria* tended to have a greater RMR than *Centaurea.* Plants from environments limited by water and nutrients generally have high RMR, which increases their ability to compete for limited below ground resources (Boot & Mensink 1990). Overall, RMR of each species remained relatively constant across matric potentials. However, when we were harvesting the plants, we noticed that water did not infiltrate the entire length of the planted columns, which may have limited growth of the plants' root system. This reflects that water contents corresponding to the -0.1 and -1.0 matric potentials were lower than column capacity, and thus the added water was held in the upper portions of the columns.

Total water use followed a pattern similar to total biomass. Plants used more water at the higher matric potential, and plants with greater biomass used more water. *Centaurea* did not use the most water nor was it the largest plant, which may reflect its phenological stage when harvested. *Festuca* used considerably less water than all other plants at all matric potentials, which presumably is related to the much smaller size of these plants.

Water use efficiency (WUE; total biomass (g) / total water used (kg)) was greatest for *Pascopyrum* and *Pseudoroegneria* at all matric potentials. We hypothesized that *Centaurea* would use water more efficiently than the native grasses, which, if true, may help explain its success in semiarid grasslands. In low-nutrient environments, plants compete mainly for water and nutrients (Wilson & Tilman 1991) and plants that use these resources most efficiently may be more competitive (Mamolos *et al.* 1995).

Inconsistent responses of WUE have been observed in response to water deficits. Water use efficiency of *Agropyron desertorum* and *Leymus angustus* increased in response to water deficits (Johnson *et al.* 1990). Severe drought decreased WUE in winter wheat, whereas mild drought had an inconsistent affect on WUE (Heitholt 1989). Furthermore, the relationship between WUE and drought stress was inconsistent for four cool-season grasses (Johnson & Bassett 1991). In our study, WUE of the different species was relatively constant between the matric potentials, indicating that at the different soil matric potentials, the plants were consistent in how they used water to produce biomass.

Festuca had the lowest WUE based on the traditional measure of biomass produced per unit of water used (g/kg). *Festuca* grew much slower than the other species, leaving a greater amount of bare soil exposed in *Festuca* columns than columns of the other species. Plants lose water via the evaporative process of transpiration. In addition, water is also lost directly from the soil surface by evaporation. We included blank columns (no plant) in this study to estimate evaporation loss from the soil surface at each matric potential. Because *Festuca* plants were small and these columns had a considerable amount of bare soil, a similar amount of water was required to attain the desired soil matric potential in *Festuca* columns as the blank columns. *Festuca's* apparent low WUE may reflect high water evaporation from the bare soil more than actual water use by *Festuca.*

Water use efficiency (*A/g*) based on Δ differed among species. In a cold desert plant community, Δ varied among the species, indicating differences in WUE (Gebauer &

Ehleringer 2000). In addition, for four cool season grasses in a field study, *Agropyron desertorum* had higher Δ than *Dactylis glomerata*, *Festuca arundinacea*, and *Lolium perenne*, indicating *Agropyron* had lower WUE than the other species (Johnson & Bassett 1991). In our study, Δ was greatest at the highest soil matric potential, indicating that plants could afford to be less efficient when water was most available. Carbon isotope discrimination was highest for 22 plant species on the wettest microsites along a soil moisture gradient within a Sonoran Desert community (Ehleringer & Cooper 1988). In our study, Δ was relatively constant for each species between the moderate and low soil matric potentials. Because drier conditions are generally more common in semiarid grasslands, the similar Δ at the two lowest matric potentials may simply indicate that these Δ values represent the normal range of Δ for these plants.

Carbon isotope discrimination was greatest for *Pascopyrum*, which is generally located in swales and bottomlands where water is more readily available. This may indicate that *Pascopyrum* did not evolve to use water as efficiently as *Pseudoroegneria*, a species generally located on dry, coarse-textured upland soils (USDA Range Plant Handbook 1937). *Pseudoroegneria* had the lowest Δ which indicates it used water more efficiently than the other species. *Centaurea* did not have the lowest Δ nor the highest Δ which was surprising because we hypothesized that *Centaurea* would either use water more efficiently than native grasses, or alternatively, use more water to produce biomass.

Biomass and Δ were not correlated in this study. Relationships between biomass and Δ have varied among several studies and may depend on the particular species and experimental conditions (Farquhar & Richards 1989; Hubick *et al.* 1986). Carbon isotope discrimination was positively correlated with biomass and yield in field-grown wheat (Condon, Richards & Farquhar 1987); conversely, Δ was negatively correlated with biomass in field-grown peanuts (Wright, Hubick, & Farquhar 1988). Overall, our results do not support our hypothesis that *Centaurea* would have greater WUE than native grasses.

Field study

Precipitation. Rainfall for each site was below the 30-year average in May, June, July, and August 1999 and 2000, except for August 1999. The growing season was drier in 2000 than in 1999. Because of the extensive dry period from early July through August 2000, the native grasses appeared to have become dormant earlier in 2000 than 1999.

1999. Carbon isotope discrimination was generally lower for all species at each site by the end of the growing season. Conditions were very dry at each site in July and although the sites received August rainfall, Δ values are integrated throughout the growing season and represent conditions during tissue development. *Centaurea*-rosettes generally had the highest Δ which indicates they used water less efficiently than the other species. Grasses tended to have higher Δ in June than in July and August. For the grasses, similar Δ in July and August indicates the grasses were quiescent, or no longer physiologically active, during these drier months. At the Helena site in July and August, *Pseudoroegneria* had lower Δ than *Pascopyrum*, which agrees with results from the greenhouse study. *Centaurea*-mature consistently had lower Δ than *Centaurea*-rosette, which may be a function of tissue age; for example, older tissue of *Centaurea*-mature may be less physiologically active than the younger tissue of *Centaurea*-rosette. Nitrogen levels and photosynthetic rates generally decrease as plant tissues age (Mooney & Winner 1991).

2000. From early July through the remainder of the growing season, conditions at all field sites were unusually dry. Carbon isotope discrimination was similar for grasses during the last two months of the growing season, again indicating that these plants were quiescent during this period. Carbon isotope discrimination was consistently highest for *Centaurea*-rosette and *Centaurea*-bolting and consistently lower for the grasses and *Centaurea*-mature. This indicates that *A/g* was lower for *Centaurea*-rosette and *Centaurea*-bolting, suggesting that these plants were less efficient at using water than the grasses and mature *Centaurea* plants. Because the plant tissue of *Centaurea*-rosettes and *Centaurea*-bolting are younger than the plant tissue of *Centaurea*-mature, it is not surprising that they had higher Δ.

At the Helena site, *Pseudoroegneria* had lower Δ than *Pascopyrum*, which agrees with results from the greenhouse study. Carbon isotope discrimination was similar for most plants early in the growing season at Helena. In addition, Δ was considerably lower in June 2000 than June 1999, which most likely reflects early dry conditions during the 2000 growing season.

At the Belgrade site, Δ was higher in June than May, which may reflect the late May precipitation. At the higher elevation Hyalite site, Δ remained relatively constant for *Centaurea*-rosettes, *Centaurea*-bolting, and *Festuca* over the growing season, although Δ for *Centaurea*-mature was considerably lower in August than July. The lower Δ for *Centaurea*-mature later in the growing season may reflect tissue age, and presence of reproductive structures. *Centaurea* flower buds had Δ values averaging 16.7‰ (unpublished data).

Conclusion

In the greenhouse, *Centaurea* did not use water more efficiently than the native grasses nor did it use the most water. We hypothesized that *Centaurea* would have greater WUE, because this would help explain its success in semiarid grasslands. Alternatively, we suggested that *Centaurea* may simply use more soil water, leaving less for its neighbors.

In the field, Δ was lower later in the growing season indicating that plants were using water more efficiently when it was more limited. The ranking of Δ for the different species and physiological stages was generally consistent across sites and between years. The results of this field study indicate that *Centaurea*-rosettes have higher Δ (lower *A/g*, lower WUE) than native grasses and *Centaurea*-mature. Low Δ of mature *Centaurea* may reflect that the majority of biomass was in the form of stems. In alfalfa plants, the stem portion of the plant had the lowest Δ, and the youngest leaves had the highest Δ (Johnson & Rumbaugh 1995). High stomatal conductance, low photosynthetic rates, or both can lead to low *A/g*, (or higher Δ), which results in lower water use efficiency (Gebauer & Ehleringer 2000).

Centaurea had the lowest WUE (highest Δ) in the rosette stage and the greatest WUE when the plants were mature. *Centaurea* plants in the mature (flowering) stage may prolong their growing season by greatly increasing WUE during mid- and late summer when neighbor semiarid grasses are quiescent. In general, the native grasses had greater WUE than *Centaurea*-rosettes, and similar WUE to *Centaurea*-mature.

Carbon isotope discrimination was lowest (greater WUE) when water was most limiting. These results agree with theoretical predictions that Δ is negatively related to WUE (Farquhar *et al.* 1982; Farquhar *et al.* 1989; Ehleringer *et al.* 1990; Johnson *et al.* 1990).

CHAPTER 5

SUMMARY

We hypothesized that *Centaurea,* as a weedy species, has a greater ability than dominant native grasses to: 1) take advantage of pulses of nitrate availability, 2) preempt N under both low and moderately high N levels, and 3) use water more efficiently. Our results did not support these hypotheses; *Pascopyrum* was more competitive for N than *Centaurea,* whereas *Centaurea* was more competitive for N than *Pseudoroegneria* in both N studies. In addition, *Centaurea* did not use water the most efficiently, nor did it use more water than *Pseudoroegneria, Pascopyrum,* and *Festuca* in general.

Centaurea had a greater ability to take advantage of pulses of N availability than *Pseudoroegneria.* For *Centaurea,* competition for N was apparently greater from neighboring *Centaurea* than from neighboring *Pseudoroegneria.* The responses of *Centaurea* and its interaction with *Pascopyrum* were nearly opposite of *Centaurea's* responses to *Pseudoroegneria. Pascopyrum* had a greater ability to take advantage of pulses of N availability than *Centaurea.* Furthermore, for *Centaurea,* competition was apparently greater from *Pascopyrum* than from neighboring *Centaurea* plants. *Centaurea's* responses to pulses of N availability differed depending on the identity of its neighbor.

The low and high N study results were similar to the results of the nitrate pulse study. For *Centaurea,* competition for N was apparently greater from neighboring *Centaurea* than from neighboring *Pseudoroegneria,* and greater from *Pascopyrum* than from neighboring *Centaurea* plants. *Centaurea* had greater biomass, greater affinity for ^{15}N, and acquired a greater percent of the applied ^{15}N than *Pseudoroegneria* at both N treatments. Conversely, *Pascopyrum* had greater biomass and acquired more ^{15}N than *Centaurea* at both N treatments. This again indicates that *Centaurea's* response to N depended on the identity of its neighbor.

Overall, *Centaurea* had a greater ability to compete for N at the higher N level, which may help explain its ability to respond to disturbances. Although *Centaurea* did not use N more efficiently than *Pseudoroegneria* and *Pascopyrum* at either N treatment, it had greater affinity for ^{15}N than the native grasses at both N levels. The higher affinity for ^{15}N may help explain its ability to invade undisturbed semiarid grasslands dominated by native grasses. Apparently, *Centaurea* has traits that allow it function as an early-seral species, yet also has the plasticity to function as a later-seral species depending on the plant community.

Results from these N studies may not answer the question of *Centaurea's* success at invading semiarid grasslands dominated by native grass species. But they do give us insight into *Centaurea's* responses to its neighbor. Identifying *Centaurea's* ability to out-compete one species of native grass for N, but not the other, may help us identify appropriate weed control strategies. In addition, these results may encourage additional studies on the physiological traits of *Centaurea* that may enhance its ability to invade undisturbed semiarid grasslands.

In the greenhouse water use study, *Centaurea* did not use water more efficiently than the native grasses in general, nor did it use the most water. Furthermore, *Centaurea* did not have the greatest water use efficiency in the field. Water use efficiency was lowest (highest carbon isotope discrimination; Δ) for *Centaurea* in the rosette stage and greatest when the plants were mature. *Centaurea* plants in the mature (flowering) stage may prolong their growing season by greatly increasing water use efficiency during mid- and late summer when neighboring grasses are quiescent. By being more active longer into the summer than native grasses, *Centaurea* may deplete soil water, and possibly affect water or nutrient availability in subsequent seasons.

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APPENDICES

APPENDIX A

NITRATE PULSE CONTRAST TABLES

Table 4a. Total Biomass (g) Contrasts. Plants were grown in mixed- or monoculture pots and exposed to 8, 24, or 72 h of nitrate per week. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Total Biomass (g)									
Contrasts	8 h		*P*-value	24 h		*P*-value	72 h		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		1	2		
CE CE vs CE ps	3.03	2.91	0.71	4.04	4.14	0.83	4.84	5.70	0.21	0.33
PS PS vs ce PS	1.18	1.57	0.23	2.35	2.30	0.91	3.00	1.64	0.07	0.53
CE CE vs CE pa	3.03	2.72	0.37	4.04	3.35	0.11	4.84	4.35	0.49	0.13
PA PA vs ce PA	1.95	2.06	0.32	2.27	2.49	0.34	2.62	4.90	0.28	0.01
ce PA vs ce PS	2.06	1.57	0.24	2.49	2.30	0.77	4.90	1.61	0.00	0.09
CE pa vs CE ps	2.72	2.91	0.63	3.35	4.14	0.11	4.35	5.70	0.11	0.03

Table 4b. Root : Shoot Contrasts. Plants were grown in mixed- or monoculture pots and exposed to 8, 24, or 72 h of nitrate per week. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Root : Shoot Ratio									
Contrasts	8 h		*P*-value	24 h		*P*-value	72 h		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		1	2		
CE CE vs CE ps	2.81	2.09	0.06	2.42	1.85	0.10	1.93	2.06	0.63	0.05
PS PS vs ce PS	1.61	2.07	0.22	1.95	2.18	0.50	1.56	1.90	0.23	0.07
CE CE vs CE pa	2.81	2.12	0.07	2.42	2.26	0.64	1.93	1.56	0.19	0.04
PA PA vs ce PA	1.63	1.37	0.18	1.76	1.50	0.14	1.39	1.58	0.52	0.04
ce PA vs ce PS	1.37	2.07	0.11	1.50	2.18	0.09	1.58	1.90	0.32	0.01
CE pa vs CE ps	2.12	2.09	0.94	2.26	1.85	0.29	1.56	2.06	0.13	0.94

Table 4c. Nitrogen Use Efficiency (NUE; total biomass (g) / total N (g)) Contrasts. Plants were grown in mixed- or monoculture pots and exposed to 8, 24, or 72 h of nitrate per week. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Nitrogen Use Efficiency (NUE; total biomass (g) / total N (g))									
Contrasts	8 h		*P*-value	24 h		*P*-value	72 h		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		1	2		
CE CE vs CE ps	98.1	93.0	0.53	109.3	90.3	0.04	78.6	79.0	0.95	0.11
PS PS vs ce PS	93.2	110.3	0.05	103.2	116.3	0.17	103.7	120.3	0.06	0.04
CE CE vs CE pa	98.1	97.7	0.96	109.3	109.3	0.99	78.6	83.8	0.52	0.47
PA PA vs ce PA	101.8	101.7	0.84	94.9	94.5	0.55	99.2	115.6	0.41	0.40
ce PA vs ce PS	101.7	110.3	0.42	94.5	116.3	0.13	115.6	120.3	0.64	0.07
CE pa vs CE ps	97.7	93.0	0.62	109.3	90.3	0.07	83.8	79.0	0.62	0.44

Table 4d. Nitrate Uptake of Roots (umol g^{-1} h^{-1}) Contrasts. Plants were grown in mixed- or monoculture pots and exposed to 8, 24, or 72 h of nitrate per week. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Nitrate Uptake of Roots (umol g^{-1} h^{-1})									
Contrasts	8 h		*P*-value	24 h		*P*-value	72 h		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		1	2		
CE CE vs CE ps	4.88	5.83	0.36	4.77	5.60	0.32	4.70	5.39	0.26	0.05
PS PS vs ce PS	6.91	4.68	0.04	5.17	4.39	0.35	3.85	3.12	0.23	0.01
CE CE vs CE pa	4.88	5.26	0.71	4.77	3.81	0.25	4.70	4.68	0.97	0.63
PA PA vs ce PA	7.57	7.29	0.03	4.91	5.90	0.19	5.22	4.41	0.37	0.00
ce PA vs ce PS	7.29	4.68	0.04	5.90	4.39	0.12	4.41	3.12	0.07	0.00
CE pa vs CE ps	5.26	5.83	0.63	3.81	5.60	0.07	4.68	5.39	0.31	0.04

Table 4e. Percent Nitrate Acquired of Applied Contrasts. Plants were grown in mixed- or monoculture pots and exposed to 8, 24, or 72 h of nitrate per week. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Percent Nitrate Acquired of Applied									
Contrasts	8 h		*P*-value	24 h		*P*-value	72 h		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		1	2		
CE CE vs CE ps	30.17	31.63	0.79	29.59	34.00	0.55	33.63	53.22	0.04	0.11
PS PS vs ce PS	25.43	24.48	0.86	36.22	22.80	0.08	33.75	19.30	0.12	0.07
CE CE vs CE pa	30.17	18.45	0.04	29.59	24.27	0.48	33.63	20.12	0.15	0.06
PA PA vs ce PA	30.74	41.53	0.03	41.85	50.58	0.02	45.66	54.11	0.01	0.00
ce PA vs ce PS	41.53	24.48	0.01	50.58	22.80	0.00	54.12	19.30	0.00	0.00
CE pa vs CE ps	18.45	31.63	0.04	24.27	34.00	0.26	20.12	53.22	0.00	0.00

APPENDIX B

LOW AND HIGH NITROGEN CONTRAST TABLES

Table 5a. Total Biomass (g) Contrasts. Plants were grown in mixed- or monoculture pots and conditioned to low or moderately high N supply rates. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

Contrasts	Total Biomass (g)						
	Low N		*P*-value	High N		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		
CE CE vs CE ps	2.86	4.11	0.01	4.09	5.11	0.04	0.01
PS PS vs ce PS	2.99	2.35	0.15	3.35	3.02	0.49	0.14
CE CE vs CE pa	2.86	1.72	0.01	4.09	2.64	0.00	0.00
PA PA vs ce PA	4.36	6.02	0.01	4.49	6.99	0.15	0.02
ce PA vs ce PS	6.02	2.35	0.00	6.99	3.02	0.00	0.00
CE pa vs CE ps	1.72	4.11	0.00	2.64	5.11	0.00	0.00

Table 5b. Root : Shoot Contrasts. Plants were grown in mixed- or monoculture pots and conditioned to low or moderately high N supply rates. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Root to Shoot Ratio						
Contrasts	Low N		*P*-value	High N		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		
CE CE vs CE ps	1.66	1.89	0.42	1.56	1.88	0.19	0.06
PS PS vs ce PS	1.75	1.65	0.73	1.58	1.58	0.99	0.70
CE CE vs CE pa	1.66	1.22	0.12	1.56	1.05	0.04	0.01
PA PA vs ce PA	2.07	2.15	0.00	1.67	2.10	0.01	0.00
ce PA vs ce PS	2.15	1.65	0.13	2.10	1.58	0.07	0.00
CE pa vs CE ps	1.22	1.89	0.04	1.05	1.88	0.00	0.00

Table 5c. Nitrogen Use Efficiency (NUE; total biomass (g) / total N (g)) Contrasts. Plants were grown in mixed- or monoculture pots and conditioned to low or moderately high N supply rates. CE (ce): (*Centaurea maculosa*), PA (pa): (*Pascopyron smithii*), PS (ps): (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

Contrasts	Nitrogen Use Efficiency (NUE; total biomass (g) / total N (g))						
	Low N		*P*-value	High N		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		
CE CE vs CE ps	61.14	75.94	0.04	76.64	86.34	0.19	0.00
PS PS vs ce PS	96.94	107.39	0.13	89.69	106.89	0.02	0.00
CE CE vs CE pa	61.14	76.54	0.03	76.64	82.40	0.43	0.01
PA PA vs ce PA	116.78	107.34	0.01	107.66	100.43	0.19	0.00
ce PA vs ce PS	107.34	107.39	0.99	100.43	106.89	0.48	0.83
CE pa vs CE ps	76.54	75.94	0.94	82.40	86.34	0.65	0.52

Table 5d. Nitrate Uptake of Roots (umol g^{-1} h^{-1}) Contrasts. Plants were grown in mixed- or monoculture pots and conditioned to low or moderately high N supply rates. CE (ce); (*Centaurea maculosa*), PA (pa); (*Pascopyron smithii*), PS (ps); (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

Contrasts	Nitrate Uptake of Roots (umol g^{-1} h^{-1})						
	Low N		*P*-value	High N		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		
CE CE vs CE ps	1.35	0.85	0.00	1.65	1.20	0.03	0.00
PS PS vs ce PS	0.63	0.58	0.80	0.77	0.59	0.39	0.26
CE CE vs CE pa	1.35	0.72	0.00	1.65	1.04	0.00	0.00
PA PA vs ce PA	0.48	0.50	0.13	0.66	0.62	0.07	0.04
ce PA vs ce PS	0.50	0.58	0.64	0.62	0.59	0.88	0.53
CE pa vs CE ps	0.72	0.85	0.46	1.04	1.20	0.51	0.05

Table 5e. Percent Nitrate Acquired of Applied Contrasts. Plants were grown in mixed- or monoculture pots and conditioned to low or moderately high N supply rates. CE (ce); (*Centaurea maculosa*), PA (pa); (*Pascopyron smithii*), PS (ps); (*Pseudoroegneria spicata*). Only species in capital letters were used in contrasts, values were averaged for species grown in monoculture.

	Percent Nitrate Acquired of Applied						
Contrasts	Low N		*P*-value	High N		*P*-value	*P*-value across trmts
1 vs 2	1	2		1	2		
CE CE vs CE ps	28.47	43.43	0.00	24.41	33.57	0.01	0.01
PS PS vs ce PS	26.87	17.95	0.02	22.08	14.25	0.02	0.00
CE CE vs CE pa	28.47	9.55	0.00	24.41	9.00	0.00	0.00
PA PA vs ce PA	32.80	51.96	0.00	24.45	37.81	0.00	0.61
ce PA vs ce PS	51.96	17.95	0.00	37.81	14.25	0.00	0.00
CE pa vs CE ps	9.55	43.43	0.00	9.00	33.35	0.00	0.00

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