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

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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A thesis submitted in partial fulfillment
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of

Master of Science

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Land Resources and Environmental Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 2000

APPROVAL

of a thesis submitted by

Pamela Sue Blicker

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

Dr. Bret E. Olson — Bret E. Olson (Signature) — 12/4/00 Date

Approved for the Department of Land Resources and Environmental Science

Dr. Jeffery S. Jacobsen — Jeff Jacobsen (Signature) — 12/4/00 Date

Approved for the College of Graduate Studies

Dr. Bruce R. McLeod — Bruce R. McLeod (Signature) — 12-4-00 Date

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ACKNOWLEDGEMENTS

I would like to express sincere appreciation to my committee members, Dr. Bret Olson, Dr. Rick Engel, and Dr. Jon Wraith. I would especially like to thank Dr. Olson for his patience and the tremendous amount of time he generously spent working with me. I would also like to thank Rosie Wallander for sharing her SAS expertise and for all of her help in the greenhouse.

Most of all, I am thankful to my best friend and husband, Brian. Without his unending support and encouragement this would not have been possible.

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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. Löve 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 $\delta^{15}N$ 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:

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

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].

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

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 (μmol 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.

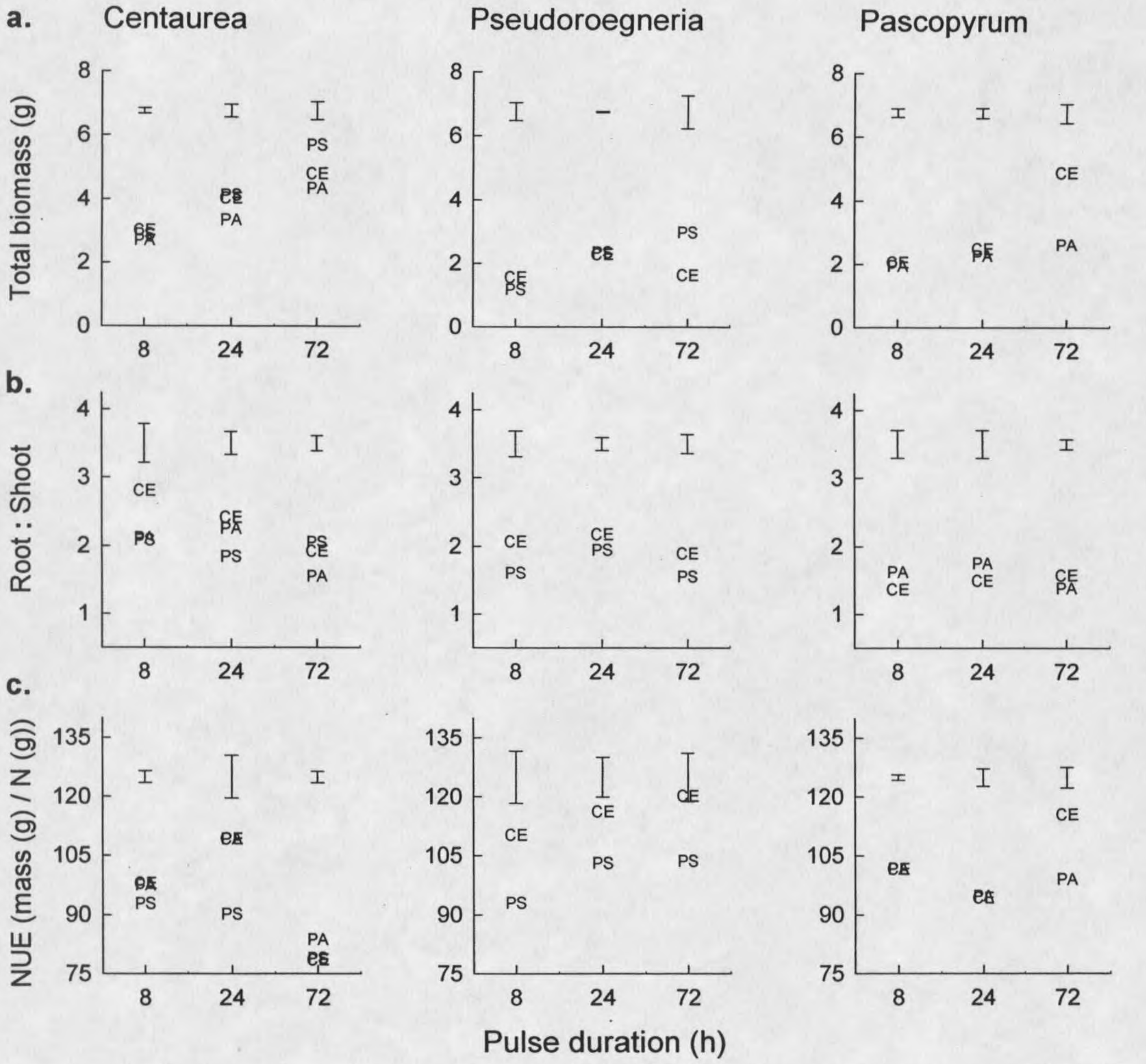

Figure 1. a. Total biomass (g), **b**. root : shoot, and **c**. nitrogen use efficiency (NUE; total biomass (g) / (total N (g)) of target species *Centaurea*, *Pseudoroegneria*, and *Pascopyrum* when growing with neighbors (*Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA) and conditioned 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.

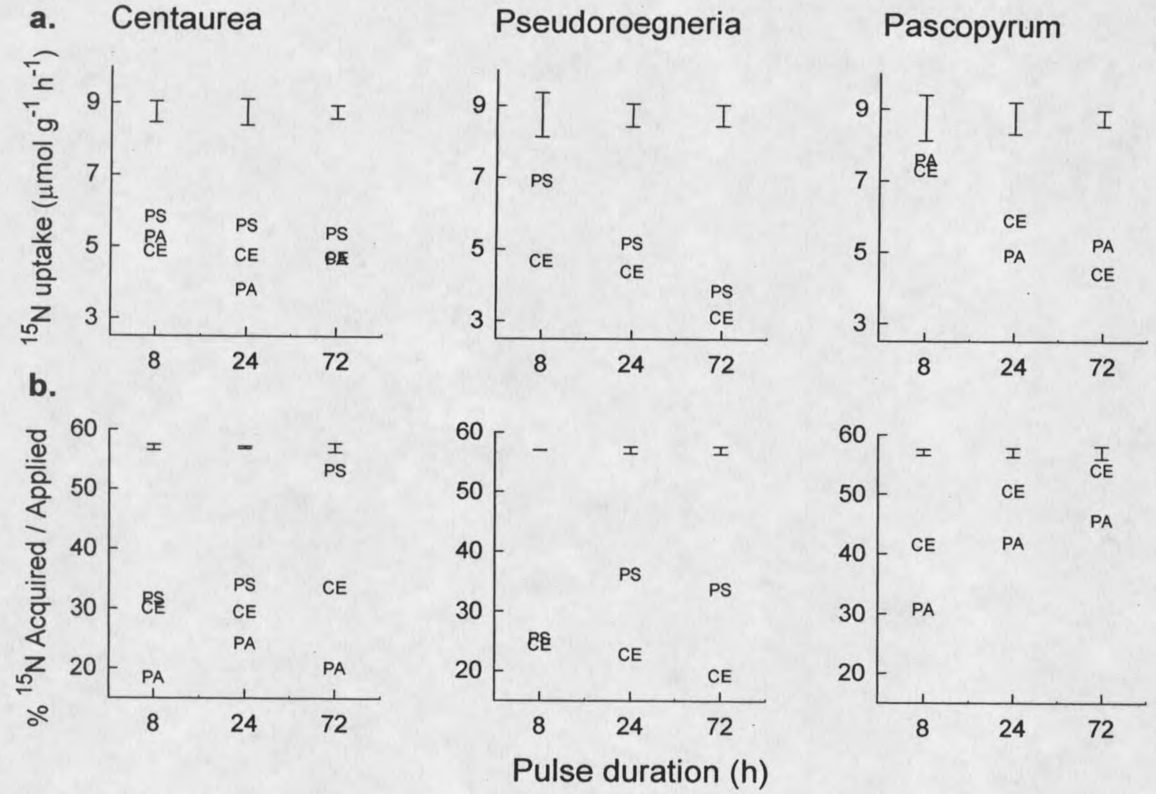

Figure 2. **a**. Root uptake of ^{15}N-labeled nitrate (μmol g^{-1} h^{-1}), **b**. percent ^{15}N-labeled nitrate acquired of applied of target species *Centaurea*, *Pseudoroegneria*, and *Pascopyrum* when growing with neighbors (*Centaurea* - CE, *Pseudoroegneria* - PS, *Pascopyrum* - PA) and conditioned 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 ($\mu mol\ g^{-1}\ h^{-1}$) 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