

# Nutrient availability does not explain invasion and dominance of a mixed grass prairie by the exotic forb *Centaurea diffusa* Lam.

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## Abstract

Diffuse knapweed (*Centaurea diffusa*) is a noxious non-native forb that is currently invading and dominating western North American grasslands. We established a series of fertilization, reverse fertilization, and removal experiments in grasslands east of the Colorado Front Range, USA, to examine the response of *C. diffusa* and the rest of the grassland community to changes in nutrient availability. We asked whether changes in the availability of nitrogen, phosphorus or both affect weedy non-native species, and *C. diffusa* in particular, differently from the native species; and whether *C. diffusa* appears to control resource availability to a greater degree than native species. Neither addition nor reduction of available nitrogen, phosphorus, or both caused consistent changes in *C. diffusa* seed germination, rosette or mature plant density, biomass, seed head production, or seed production. Removal of *C. diffusa* from the community resulted in no significant change in rates of nitrogen cycling or pools of available nitrogen or phosphorus. Removal of *C. diffusa* had little effect on the relative cover or species richness of the remaining community, but removal of the remaining community caused a large increase in *C. diffusa* biomass. The results suggest that *C. diffusa* requires relaxation or removal of competition to invade grasslands, that it is tolerant of a wide range of nutrient availability, and that once established, it functions similarly to the native prairie grasses. Heterogeneity in cover and competitive interactions, generally thought to enhance species coexistence and overall diversity, also provide increased opportunity for invasion.

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## 1. Introduction

One mechanism proposed to explain the problem of invasion and dominance by exotic plant species is that changes in resource availability have opened windows of opportunity for invading species that can exploit the unused resources (Jefferies and Maron, 1997; Dukes

and Mooney, 1999; Sher and Hyatt, 1999; Davis et al., 2000; LeJeune and Seastedt, 2001). Recent research suggests that enhanced resource availability favors the invasion of non-native species (Huenneke et al., 1990; Burke and Grime, 1996; Harrison, 1999; Smith and Knapp, 1999, 2001). Humans have enriched the earth with nitrogen, phosphorus, and carbon dioxide and altered disturbance type and frequency across entire landscapes and ecosystems (Vitousek et al., 1997; Jefferies and Maron, 1997; Dukes and Mooney, 1999). If invading species respond more rapidly to nutrient enrichment than do native species, by pre-empting

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resources and increasing in population size, then it seems plausible that environmental eutrophication could facilitate exotic species invasions at regional and continental scales.

Among the most wide-spread grassland invaders in western North America are the *Centaurea* (knapweed, Asteracea) species (Skinner et al., 2000; Rice, 2002). Species of the genus *Centaurea*, including star thistle (*C. solstitialis* L.), diffuse (*Centaurea diffusa* Lam.) spotted (*C. maculosa* Lam.), squarrose (*C. virgata* Lam.), and Russian knapweed (*C. repens* L.), and 10 other *Centaurea* species have invaded millions of hectares of North American grasslands previously dominated by C<sub>3</sub> and C<sub>4</sub> grasses (Sheley and Petroff, 1999; Rice, 2002). To investigate the role of nutrient availability and limitation in the invasion and dominance of Colorado Front Range prairies by *C. diffusa*, we measured the response of *C. diffusa* to alterations in nutrient availability across a range of life stages and soil types, in the field, under ambient environmental conditions. In addition, we removed *C. diffusa* from a subset of the test plots to partition the effects of *C. diffusa* on nutrient cycling and on the response of the community under varying nutrient availability. We asked the following questions: (1) does increased availability of nitrogen, phosphorus or both favor weedy non-native species, and *C. diffusa* in particular? (2) Does reduced availability of nitrogen, phosphorus, or both favor the native species over non-natives? and (3) Is the response of the native community to reductions in nutrient availability different in the presence versus absence of *C. diffusa*?

## 2. Methods

### 2.1. Research site

The Lastoka Prairie is located about 10 km east of the Colorado Front Range, near Superior, Colorado, USA (39°55'35"N, 105°11'8"W). We conducted nutrient manipulation experiments in adjacent fields at the site (North and South). The site is a degraded 65 ha parcel of mixed grass prairie, historically grazed, and heavily invaded by *C. diffusa*. Grazing ceased in early 1997. The soils are part of the Valmont series and are predominantly clayey over loamy-skeletal montmorillonitic, mesic Aridic Arigiustolls formed on old high terraces and benches in gravelly and cobbled loamy alluvium (USDA SCS, 1975). The surface layer (0–10 cm), which is the zone of greatest rooting density, is a dense, compacted, grayish-brown clay loam that contains varying amounts of cobble and gravel.

The vegetation of Lastoka is mixed grass prairie, but the composition is highly altered by exotic species.

Native grass species include *Agropyron smithii*, *Aristida longiseta* Steud., *Bouteloua gracilis* (H.B.K.) Lag. ex Steud., *Buchloe dactyloides* (Nutt.) Engelm., *Koeleria macrantha* (Ledeb.) Schult., *Poa compressa* L., and *Schedonnardus paniculatus* (Nutt.) Trel. Non-native grasses include *Bromus japonicus* Thunb. ex Murr., *Bromus tectorum* L., *Poa pratensis* L., and *Agropyron intermedium*. Native forbs include *Artemisia frigida* Willd., *Erigeron flagellaris* A. Gray, *E. divergens* Torr & A. Gray, *Sphaeralcea coccinea* (Pursh) Rydb., and *Gutierrezia sarothrae* (Pursh) Britt. & Rusby. Non-native forbs include *Alyssum minus* (L.) Rothm., *C. diffusa*, *Convolvulus arvensis* L., and *Hypericum perforatum* L. At the beginning of the studies, non-native cover averaged about 30%.

Average annual precipitation is 48.5 cm. Precipitation in April, May, and June averages 6.5 cm/month (Western Regional Climate Center, Reno, Nevada, USA). Summer precipitation (July, August, and September) is in the form of scattered thunderstorms, and averages 4.3 cm/month. Fall and winter are relatively dry. The average frost-free growing season length is 140–155 days (USDA SCS, 1975).

### 2.2. *C. diffusa*

*C. diffusa* is an exotic, typically biennial and forb (Watson and Renny, 1974). During the juvenile stage, *C. diffusa* forms a rosette with a central crown and tap root. At maturity, *C. diffusa* produces a single upright stem 0.3–1 m tall, with numerous spreading branches. Seed heads are solitary or born in clusters of 2–3 at the ends of branches. The larger senescent adults may break at the base and tumble, dispersing seeds. A single plant can produce up to 18,000 seeds (Watson and Renny, 1974; Schirman, 1981). Seed production by area can be as high as 11,200–48,000 seeds/m<sup>2</sup> but is affected by site conditions and water availability (Sheley et al., 1998). Seed germination rate typically exceeds 90%, and seeds can germinate at any time during the year when conditions are favorable.

### 2.3. Fertilization experiments

The fertilization experiments consisted of randomized complete block designs with four replications in North and South Fields at Lastoka. The North Field experiment included four additional control (unmanipulated) plots.

In South Field (beginning in 1999) and in North Field (beginning in 2000), nitrogen (as ammonium nitrate), phosphate (as superphosphate), both, or neither were

added to replicate 2 m × 2 m plots twice during the growing season each year. Nitrogen was added at a rate of 10 g N/m<sup>2</sup> per application in 1999 and 2000, and 5 g N/m<sup>2</sup> per application in 2001. Phosphorus was added at a rate of 1 g P/m<sup>2</sup> per application. Above-ground biomass of *C. diffusa* rosettes and bolting plants and other grassland components was estimated by clipping a 0.1 m<sup>2</sup> quadrat in each plot in September each year. Clipped biomass was dried in an oven at 60 °C to constant biomass. Mature and senescing *C. diffusa* density was recorded and the aboveground biomass harvested in late August or early September each year and air-dried. Oven-drying of a subset confirmed that the air-dried plants were >99% dry. Dried plants were weighed, and in 2000 (North Field) and 2001 (both fields), the number of viable seed heads per plant was counted. In 2001, the seed production was estimated by dissecting six seed heads from a subset of the adult *C. diffusa* plants harvested from each fertilization plot (approximately 48,000 seeds were counted).

We analyzed the fertilization results separately by field (North and South) and year (1999, 2000, 2001) because the two fields had distinct land use histories and fertilization treatments were initiated 1 year apart. Using an ANOVA model with two factors (addition of nitrogen, addition of phosphorus), and two levels (with and without addition), we assessed the effect of fertilization on biomass of mature *C. diffusa* (g/plant), total biomass of *C. diffusa* (g/m<sup>2</sup>), seed heads per *C. diffusa* plant, seeds per seed head, and biomass of native and introduced grasses and forbs (g/m<sup>2</sup>). Biomass estimates of *C. diffusa* and other vegetation, and *C. diffusa* seed head and seed production counts were square-root transformed to meet parametric assumptions.

#### 2.4. Reverse fertilization and removal experiments

Reverse fertilization and removal treatments consisted of two factors (addition of gypsum to reduce phosphorus, addition of sugar to reduce nitrogen) with two levels (with and without addition), and one biomass removal factor (removal of *C. diffusa*) with two levels (with and without removal) in a randomized complete block design with seven replicate blocks, all in North Field. We reduced nitrogen availability by adding carbon (table sugar, or sucrose, C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) to stimulate growth of the microbial community, phosphorus availability by adding gypsum (CaSO<sub>4</sub>), and nitrogen and phosphorus availability by adding both. We added sugar at a rate of 84 g/m<sup>2</sup> C twice in the 2000 growing season and three times in the 2001 growing season. The quantity of carbon added was determined based on

previous experiments in nearby field sites (Seastedt et al., 1996; Reeve-Morghan and Seastedt, 1999). In 2001, we added a small amount of phosphorus (0.2 g/m<sup>2</sup>) to carbon treatments to replace phosphorus taken up by the microbial community. We added gypsum at a rate of 10 g Ca/m<sup>2</sup> twice during each growing season. Applications were timed to correspond with precipitation to reduce the confounding risk of osmotic stress.

We removed *C. diffusa* biomass in May 2000 by painting all individual plants in the 1 m × 1.5 m plot with Roundup (glyphosate), as described in Suding et al. (2004). To minimize the potential artifacts of removals, we delayed monitoring of the removal plots for a full growing season after the initial removals. Plots measured 1 m × 1.5 m. One square meter of each plot was reserved for cover and biomass measurements, and one-quarter square meter was reserved for soil sample collection. The remaining quarter square meter was reserved for individual plant competition studies (Suding et al., 2004).

We measured vegetation cover in June and August 2001 using a point-frame intercept with 36 intercepts. Plants intersected by the cross hairs were recorded to species level where possible. In August 2001, we harvested, air-dried, and weighed mature *C. diffusa* plants. For each plant, we counted the number of seed heads and removed six mature seed heads for dissection and enumeration of seeds. In September 2001 and April 2002, *C. diffusa* rosettes and seedlings with divided leaves in the square meter of the plot reserved for vegetation measurements were counted and classified by size as “large” (>5 cm) and “small” rosettes (1–5 cm).

In plots with no removal, simple correlations (Pearson's *r*) were used to assess relationships by nutrient treatment between cover, biomass, and fecundity of *C. diffusa* and cover of other species, grasses, and forbs. Using an ANOVA model with two factors (gypsum addition, sugar addition) and two levels (with and without addition), we assessed the effect of nutrient reduction on *C. diffusa* biomass (g/m<sup>2</sup>), density (plants/m<sup>2</sup>), rosette size, cover (%), seed heads per plant, and seeds per seed head, and on the biomass (g/m<sup>2</sup>) and cover (%) of other forbs and grasses. Rosette counts were rank transformed when necessary. The block term was not significant and there were no significant block by treatment interactions, so block was not included in the final model. Using an ANOVA model with the same two nutrient reduction factors and levels, plus removal of *C. diffusa* (with and without removal), we assessed the effect of *C. diffusa* on cover and biomass of other species and vegetation types. Again, block was excluded from the final model

because it was not a significant term and there were no significant block by treatment interactions.

To assess whether the effect of reverse fertilization on *C. diffusa* cover, total biomass, individual plant biomass, or fecundity, depended on species richness or the cover of the non-*C. diffusa* community, species richness, and square-root transformed biomass and cover of the non-*C. diffusa* vegetation were included as covariates in analyses of covariance.

For all analyses, post hoc tests to evaluate interaction terms were conducted on least-squares means using a Bonferroni adjustment for multiple comparisons. Initial protection levels were set at the 5% level. All analyses were performed in SAS V. 8.02 (SAS, 1999–2001).

### 2.5. Soil characteristics

Soil samples were collected from reverse fertilization plots in May, June, and September 2001 to assess the effectiveness of the nutrient manipulations. Samples were collected from the 0 to 10 cm depth using a 2.5 or 5 cm soil corer. Samples were analyzed for inorganic nitrogen (ammonium and nitrate), labile phosphorus (June and September only), and gravimetric moisture content. In situ nitrogen mineralization and nitrification were measured using the buried bag method (Eno, 1960; Westerman and Crothers, 1980; Aber et al., 1993). When each initial soil sample was collected, a second sample was retrieved, placed in a polyethylene bag, and returned to the collection hole for approximately 28 days. Total carbon and nitrogen, microbial biomass nitrogen, and pH were measured on a subset of the sampling dates.

To characterize unmanipulated soils, samples were collected in North and South Fields at Lastoka Prairie in areas near the experimental plots, repeatedly during the 2000 and 2001 growing seasons. All samples were analyzed for inorganic nitrogen, in situ net nitrogen mineralization and nitrification, gravimetric moisture. On a subset of the sampling dates, labile phosphorus, total carbon, nitrogen and phosphorus, microbial biomass nitrogen, pH, texture, exchangeable calcium and magnesium, and extractable copper, iron, and zinc were measured as well.

Soil samples removed from the field were kept cool until they were extracted for nitrogen. Inorganic nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ) was extracted within 24 h of sample collection, from 10 g soil samples using 2 N KCl at a 5:1 KCl to soil ratio. Extracts were analyzed on a Lachat Automated Ion Analyzer or an Alpkem Autoanalyzer (Alpkem Corp. RFA Methodology No. A303-SO21). To determine gravimetric moisture content and for use in moisture correction

calculations, 5–10 g of each sample of field-moist soil was dried at 105 °C for 2 days.

Labile phosphorus was quantified by sequential extraction of the resin-extractable and bicarbonate-extractable inorganic and organic fractions (Tiessen and Moir, 1993). These extractions remove the inorganic phosphorus adsorbed on surfaces of crystalline phosphorus compounds, sesquioxides, and carbonates, and the more easily mineralizable organic phosphorus (Mattingly, 1975; Bowman and Cole, 1978; Tiessen and Moir, 1993), and are believed to represent the most labile and plant-available phosphorus fractions in soil (Hedley et al., 1982). Extractions were made on 0.5 g samples of air-dried soils. A subsample was oven-dried for moisture-correction. Extracts were analyzed on an Alpkem Autoanalyzer. Autoanalyzer matrix adjustments were made to match the normality of extracting solution of each fraction, to create an appropriate acidity at the detector (Townsend et al., 2002).

Total carbon and nitrogen were measured by Micro-Dumas combustion on an NA1500 C/H/N Analyzer (Carbo Erba Strumentazione, Milan). The nitrogen content in microbial biomass was estimated by chloroform-fumigation-extraction (Paul et al., 1999), using a 5 day fumigation period. The extracts were analyzed for nitrogen on a Lachat Automated Ion Analyzer. Determination of pH was made on field-moist samples using a 2:1 water slurry (Robertson et al., 1999). Texture was determined using the hydrometer method (Elliott et al., 1999). Exchangeable calcium and magnesium were extracted with 1 M ammonium acetate at pH 7.0 (Robertson et al., 1999) and measured by ICP. Copper, iron, and zinc were extracted with diethylenetriaminedipentaacetic acid (DTPA) and analyzed by ICP (Soil and Plant Analysis Council, 1992).

We analyzed the effect of nutrient reduction on ammonium, nitrate, net mineralization, net nitrification, resin-extractable phosphorus, and bicarbonate-extractable phosphorus. Block was again excluded from the final analysis for the reasons described previously. We assessed the effect of *C. diffusa* on soil nutrient characteristics using a model with two the nutrient reduction factors and the *C. diffusa* removal factor.

## 3. Results

### 3.1. Soil characteristics

South Field soils contained more iron, zinc, total carbon, total nitrogen, a greater C:N ratio than North Field soils, but pH, microbial biomass nitrogen, and

Table 1

Descriptive statistics for selected soil characteristics in North and Middle Fields and results of comparisons of means between the two fields

Measurement	Mean (S.E.)		d.f.	F	p
	North Field	South Field			
Sand (%)	39.2 (1.2)	46.0 (1.3)	34	15.2	0.0004
Silt (%)	26.9 (0.50)	26.8 (0.90)	34	0.01	ns
Clay (%)	33.9 (0.92)	27.2 (0.89)	34	27.6	<0.0001
Total C (%)	1.81 (0.047)	2.34 (0.83)	70	30.1	<0.0001
Total N (%)	0.162 (0.0034)	0.200 (0.0060)	70	29.7	<0.0001
C:N	11.1 (0.10)	11.6 (0.10)	70	10.3	0.001
pH (standard units)	5.22 (0.088)	5.10 (0.072)	30	1.18	ns
Microbial biomass N ( $\mu\text{g/g}$ )	12.4 (1.10)	13.4 (1.08)	30	0.408	ns
Exchangeable Ca ( $\mu\text{g/g}$ )	2650 (81)	2340 (97)	34	6.22	0.02
Exchangeable Mg ( $\mu\text{g/g}$ )	540 (24)	435 (20)	34	11.0	0.002
Extractable Cu ( $\mu\text{g/g}$ )	8.46 (1.9)	5.57 (0.68)	33	1.44	ns
Extractable Fe ( $\mu\text{g/g}$ )	27.3 (0.77)	32.2 (1.92)	33	5.79	0.02
Extractable Zn ( $\mu\text{g/g}$ )	2.09 (0.25)	2.95 (0.18)	33	10.5	0.003

S.E., standard error; d.f., degrees of freedom; F, F statistic; p, probability value; ns, not significant.

extractable copper did not differ between the two fields (Table 1). Most of the soils in both fields were clay loams or sandy clay loams.

Fig. 1 shows the temporal variability in concentrations of inorganic nitrogen pools and cycling rates in the North and South Fields in 2001. Resin-extractable phosphorus concentrations did not differ between the two fields, and bicarbonate-extractable organic and inorganic phosphorus concentrations were also similar on most sampling dates.

We did not measure nutrient availability in the fertilization plots. However, we noted a distinct greening response to nitrogen fertilization each year, and a distinct increase in total vegetation biomass and a shift to increased forb dominance in plots with added nitrogen and phosphorus.

Carbon addition reduced ammonium and nitrate concentrations ( $p < 0.001$ ) (Fig. 2). Rates of nitrification (May, June, and September) and mineralization (June and September) were also reduced in carbon-amended plots ( $p < 0.05$ ). In June, where both carbon and gypsum were added, labile phosphorus fractions were reduced. By September, gypsum addition reduced both resin-extractable phosphorus ( $F_{1,54} = 9.9$ ,  $p = 0.003$ ) and bicarbonate-extractable inorganic phosphorus ( $F_{1,54} = 5.32$ ,  $p = 0.03$ ).

### 3.2. Vegetation description

In August 2001, *A. smithii*/*A. intermedium* and *E. flagellaris* dominated the Lastoka control plots (20.6 and 20.2% cover, respectively). Control plot cover

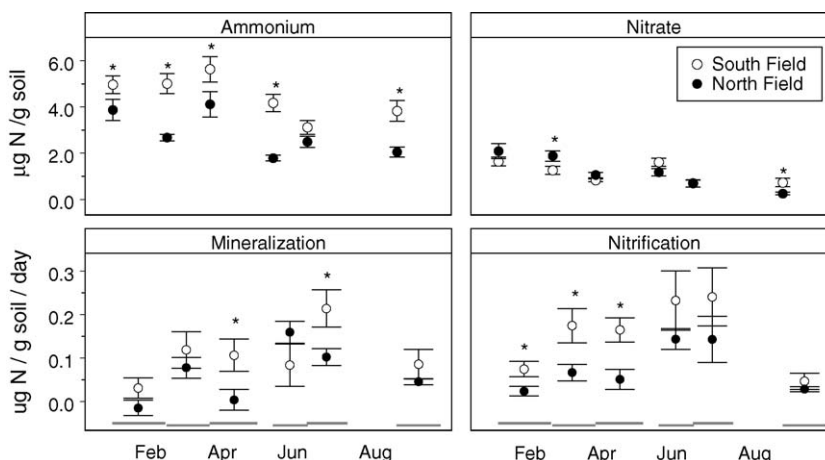


Fig. 1. Concentrations ( $\mu\text{g/g}$ ) of ammonium and nitrate and rates ( $\mu\text{g/g/day}$ ) of net nitrification and mineralization in North and South Field control soils, January–September 2001. Symbols represent mean and standard error of six samples. Horizontal lines on bottom panel represent duration of incubation period for buried bags for each sampling period. Asterisks indicate significant difference in fields for the sampling event ( $p < 0.05$ ).

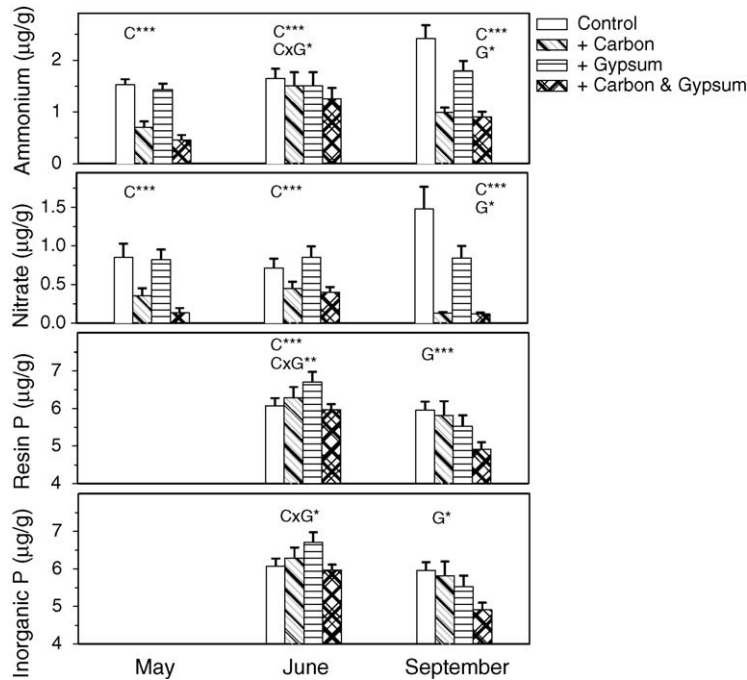


Fig. 2. Ammonium and nitrate concentrations (mean, standard error) in May, June, and September 2001, and resin-extractable and bicarbonate-extractable phosphorus in June and September 2001, in plots with added carbon, gypsum, both, or neither. C and G indicate significant single degree of freedom effects of carbon and gypsum addition on nitrogen concentrations and rates. C  $\times$  G indicates a significant carbon by gypsum interaction. \* $p < 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p < 0.0001$ .

averaged 53% native forbs and grasses and 47% non-native forbs and grasses. Of the non-natives, *C. diffusa* cover averaged 19.0%, *Poa pratensis* 15%, and *A. minus* 10%. The average stem density of *C. diffusa* in control plots declined in both fields from 1999 to 2001 (Table 2). North Field had a greater density of mature *C. diffusa* by 2001 ( $F_{1,9} = 4.9$ ,  $p = 0.03$ ), but individual plants were larger in South Field ( $F_{1,10} = 4.3$ ,  $p = 0.03$ ). The *C. diffusa* biomass was similar in the two fields in 2000 and 2001 ( $p > 0.05$ ). The larger plants in South Field produced more seed heads per plant in 2001 than the smaller plants in North Field ( $F_{1,10} = 4.8$ ,  $p = 0.03$ ), but not a significantly greater

number of seeds per seed head ( $p > 0.05$ ). Average seed production in 2001 was approximately 350 seeds/m<sup>2</sup> in North Field and 420 seeds/m<sup>2</sup> in South Field. *C. diffusa* rosette densities in North Field in March 2001 averaged  $7.63 \pm 1.70$  per m<sup>2</sup> and in South Field,  $6.94 \pm 1.73$  per m<sup>2</sup>. By August 2001, rosette density had changed little ( $7.96 \pm 1.50$  per m<sup>2</sup>).

### 3.3. Vegetation response to nutrient manipulation

#### 3.3.1. Lastoka, North Field

In 2000, the average biomass of mature *C. diffusa* (g/plant) increased by 45% with added nitrogen ( $F_{1,16}$

Table 2

*Centaurea diffusa* biomass, density, cover, and fecundity, and non-*C. diffusa* biomass in control plots in North and South Fields, August 1999–2001

Measurement	1999, Mean (S.E.)		2000, Mean (S.E.)		2001, Mean (S.E.)	
	North	South	North	South	North	South
<i>C. diffusa</i> (g/m <sup>2</sup> )	–	60 (20)	52 (17)	51 (16)	28 (14)	20 (7.9)
Non- <i>C. diffusa</i> (g/m <sup>2</sup> )	–	120 (19)	47 (6.6)	64 (11)	140 (42)	270 (26)
<i>C. diffusa</i> (g/plant)	–	5.5 (1.0)	0.9 (0.1)	2.5 (0.1)	1.3 (0.3)	2.8 (1.0)
<i>C. diffusa</i> (plants/m <sup>2</sup> )	18 (1.9)	10 (3.9)	20 (3.1)	20 (5.7)	13.6 (4.1)	4.3 (2.7)
% <i>C. diffusa</i>	–	32 (9.0)	49 (6.0)	41 (12)	17.4 (7.0)	6.3 (2.0)
Seed heads/plant	–	–	–	–	27 (2.0)	78 (11)
Seeds/seed head	–	–	–	–	0.89 (0.1)	1.26 (0.2)

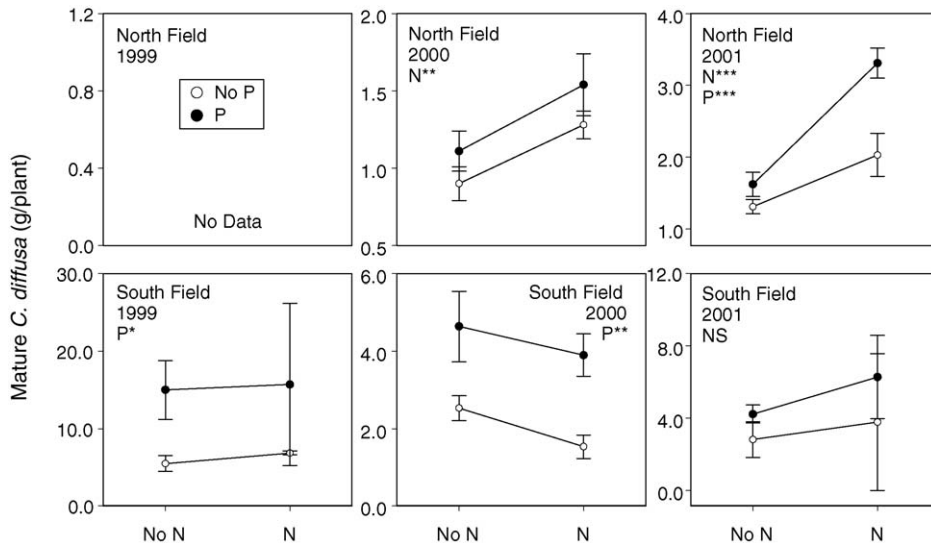


Fig. 3. Average mature *Centaurea diffusa* biomass, August 1999, 2000, and 2001 in North and South Field fertilization plots. N, nitrogen and P, phosphorus. Plots with added phosphorus supported the largest *C. diffusa* plants, although the effect of phosphorus was not significant in both fields in all years. \*  $p < 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p < 0.0001$ .

= 8.4,  $p = 0.01$ ) (Fig. 3). In 2001, the average biomass of mature *C. diffusa* increased with added nitrogen ( $F_{1,16} = 37.6$ ,  $p < 0.0001$ ) and phosphorus ( $F_{1,16} = 15.32$ ,  $p = 0.001$ ). Post hoc comparisons indicated that *C. diffusa* biomass in plots treated with both nitrogen and phosphorus was greater than in other treatments, and mature *C. diffusa* plants were approximately 150% larger than control plants. The total biomass of *C. diffusa* ( $\text{g/m}^2$ ) increased with nitrogen addition ( $F_{1,16} = 10.7$ ,  $p = 0.005$ ), as did the average number of seeds per seed head ( $F_{1,16} = 6.34$ ,  $p = 0.02$ ) from 1.1 in control plots to 1.8 in nitrogen plots. The average number of seed heads per *C. diffusa* plant increased by 95% (from 27 to 53) with nitrogen and phosphorus addition ( $F_{1,16} = 6.06$ ,  $p = 0.03$ ).

Phosphorus plots supported the greatest relative biomass of *C. diffusa* (30%), compared to 17% in control plots, 26% in nitrogen plots, and 19% in nitrogen plus phosphorus plots; these differences were not significant. Rosette biomass also was unrelated to fertilization treatment.

In plots fertilized with nitrogen and phosphorus, the relative biomass of *A. minus* was 39%, compared with 6% in control plots, 14% in phosphorus plots and 15% in nitrogen plots (Fig. 4). Biomass of forbs other than *A. minus* and *C. diffusa* increased with nitrogen addition ( $F_{1,16} = 8.8$ ,  $p = 0.009$ ). Grass biomass did not respond to nutrient addition.

Carbon addition reduced total vegetation cover, grass cover, native grass cover, forb cover, native forb cover, and species richness (Table 3). Carbon addition reduced the cover of *A. minus*, *E. flagellaris*, a native forb, and

*Schedonnardus paniculatus*, a native  $C_4$  grass. Gypsum addition (phosphorus reduction) reduced the cover of *A. minus* ( $F_{1,45} = 4.2$ ,  $p = 0.046$ ).

Controlling for non-*C. diffusa* biomass, carbon addition reduced the biomass ( $\text{g/m}^2$ ) of *C. diffusa* ( $F_{1,23} = 15.01$ ,  $p = 0.0008$ ). There were no other significant effects of carbon or gypsum addition on *C. diffusa* cover, biomass, density of mature plants or rosettes, or seed head or seed production.

### 3.3.2. Lastoka, South Field

Phosphorus addition caused an increase in the average biomass of mature *C. diffusa* plants by 150% in August 1999 ( $F_{1,11} = 4.87$ ,  $p = 0.0495$ ) and by 110% in August 2000 ( $F_{1,10} = 12.56$ ,  $p = 0.005$ ) (Fig. 3). Nitrogen (only) addition eliminated *C. diffusa* from the plots over the course of the study (Fig. 4). There were no other significant responses by *C. diffusa*, the non-*C. diffusa* community, or the total vegetation community to nitrogen, phosphorus, or both. In August 2001, there was a continued trend of larger *C. diffusa* in plots with added phosphorus (average increase of 60%  $\text{g/plant}$ ), but the difference was not significant. Overall biomass of *C. diffusa* (rosettes plus mature plants) increased significantly with phosphorus addition in 2001 ( $F_{1,12} = 7.37$ ,  $p = 0.02$ ). Seed heads per *C. diffusa* plant and seeds per seed head were unchanged by nutrient addition.

The relative percent biomass of *C. diffusa* was greater in phosphorus-addition plots than in all other plots. In 1999, the relative percent biomass of *C. diffusa* on all plot types ranged from 25 to 47%. By 2002, the

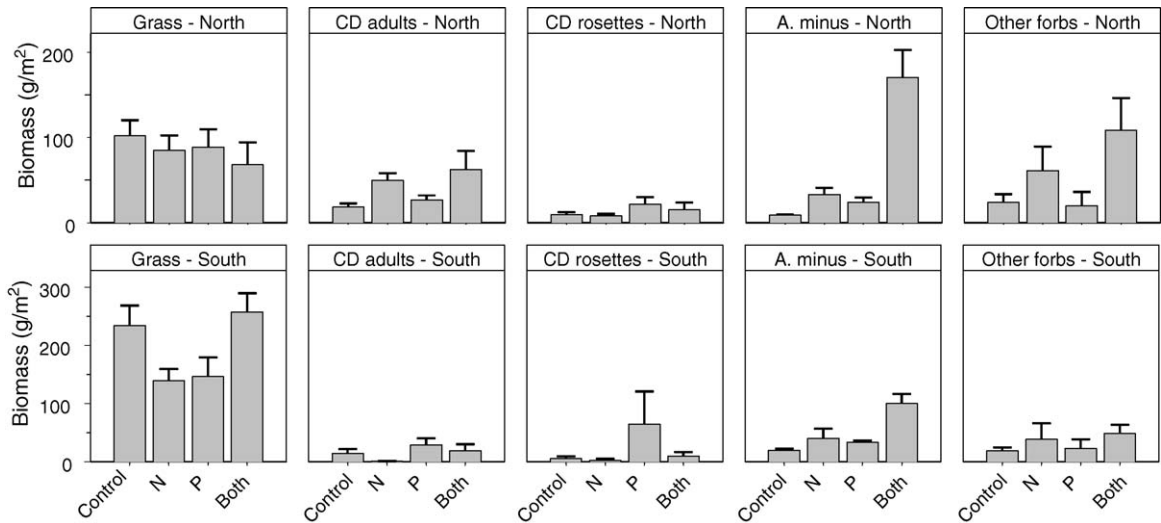


Fig. 4. Aboveground biomass ( $\text{g/m}^2$ ), August 2001 in North and South Field fertilization plots. Mean and standard error by treatment and field. CD, *Centaurea diffusa*.

relative biomass of *C. diffusa* ranged from 2% in nitrogen addition plots to 7% in control and nitrogen plus phosphorus addition plots, but in phosphorus plots, *C. diffusa* biomass remained 30% of the whole vegetation biomass (significantly greater at  $p = 0.0516$ ).

The biomass of *A. minus* increased with addition of nitrogen ( $F_{1,12} = 11.4$ ,  $p = 0.006$ ) and phosphorus ( $F_{1,12} = 10.4$ ,  $p = 0.007$ ) (Fig. 4). Grass biomass and forb biomass other than *C. diffusa* and *A. minus* in South Field did not respond to nutrient additions. The non-*C. diffusa* biomass was co-limited by nitrogen and phosphorus (significant  $N \times P$  interaction,  $F_{1,12} = 11.0$ ,  $p = 0.006$ ). The non-*C. diffusa* biomass was dominated by *A. minus*. The biomass of *A. minus* was not recorded separately in 1999 and 2000, but by 2001,

it comprised 24% of the biomass in nitrogen plus phosphorus plots, compared with 7% in control plots, 11% in phosphorus plots and 19% in nitrogen plots.

### 3.4. Effect of *C. diffusa* on nutrient cycling and availability

The biomass of *C. diffusa* removed in May 2000 averaged  $21.6 \pm 1.5 \text{ g/m}^2$  and comprised  $25.7 \pm 2.8\%$  of the total vegetation biomass. Elimination of *C. diffusa* from the community had no significant effect on ammonium or nitrate concentrations, rates of nitrogen cycling, or on phosphorus concentrations in 2001 (Fig. 5). Elimination of *C. diffusa* from the community also had no effect on microbial biomass nitrogen, soil

Table 3

Species and vegetation components that decreased in response to carbon addition. Mean and standard error,  $F$ , and  $p$  statistics for plots with no added carbon vs. added carbon

Cover (%)	$n$	Mean (S.E.)		ANOVA results	
		No carbon added	Carbon added	$F_{(1,45)}^a$	$p$
<i>A. minus</i>	28	9.3 (1.1)	0.10 (0.10)	298	<0.0001
<i>E. flagellaris</i>	28	12 (3.1)	4.3 (1.5)	8.53	0.005
<i>S. paniculatus</i>	28	7.3 (2.4)	1.2 (0.8)	10.9	0.002
<i>C. diffusa</i>	14	19 (3.3)	16 (2.8)	ns	ns
Total vegetation	28	102 (5.1)	69 (2.5)	36.2	<0.0001
Grasses	28	54 (4.1)	38 (2.8)	10.8	0.002
Native grasses	28	39 (3.2)	29 (2.7)	5.3	0.03
Forbs	28	48 (4.7)	31 (2.3)	10.5	0.002
Native forbs	28	20 (3.5)	11 (2.4)	7.6	0.008
Richness (#)	28	10 (0.33)	9.0 (0.34)	7.8	0.008

<sup>a</sup> *C. diffusa* was removed from half of the plots.  $F_{(1,19)}$  for *C. diffusa*.

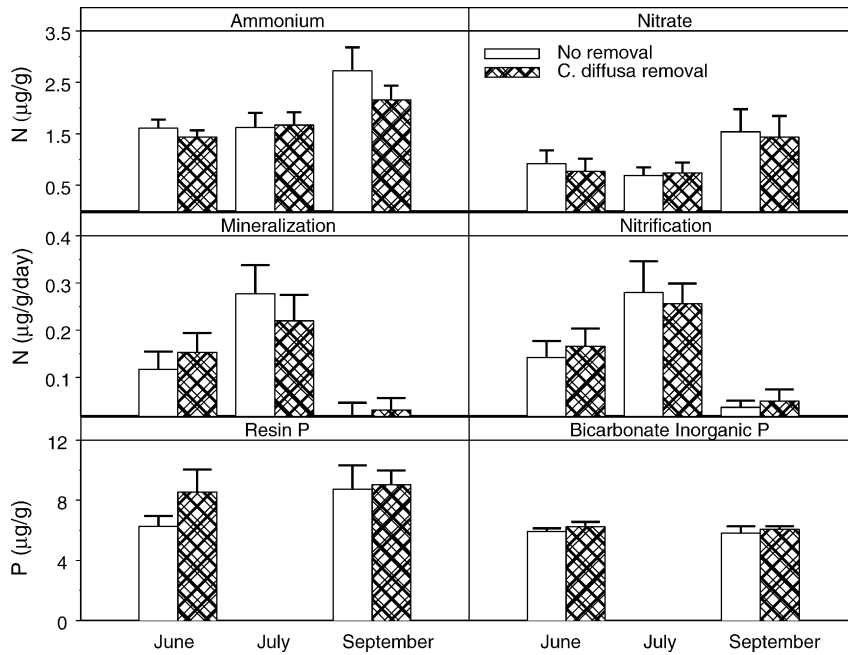


Fig. 5. Concentrations of nitrogen and phosphorus ( $\mu\text{g/g}$  soil) and in situ rates of net nitrogen transformation ( $\mu\text{g N/g soil/day}$ ) in soils from plots containing *Centaurea diffusa* and plots from which *C. diffusa* was removed 1 year previous (mean, standard error). Removal of *C. diffusa* resulted in no significant differences in soil nutrient availability.

pH, total carbon, total nitrogen, or the C:N ratio (Table 4).

3.5. Effect of *C. diffusa* on the vegetation community

*C. diffusa* density was negatively correlated with species richness (Pearson's  $r = -0.84$ ,  $p = 0.02$ ) and total grass cover (Pearson's  $r = -0.84$ ,  $p = 0.02$ ). *C. diffusa* cover was negatively correlated with cover of non-*C. diffusa* vegetation (Pearson's  $r = -0.83$ ,  $p = 0.02$ ) (Fig. 6). Total vegetation cover decreased

Table 4

Soil characteristics in plots in which *Centaurea diffusa* was removed from the community ( $n = 7$ ) and plots in which *C. diffusa* was present ( $n = 7$ )

	No removal	<i>C. diffusa</i> removal
Total C (%)	1.59 (0.068)	1.14 (0.051)
Total N (%)	0.153 (0.0043)	0.154 (0.0046)
C:N	10.4 (0.17)	10.7 (0.073)
pH	5.03 (0.040)	5.24 (0.088)
Microbial N ( $\mu\text{g/g}$ )	10.4 (2.9)	9.76 (1.1)

Mean (standard error). There were no significant differences in soil characteristics between the two.

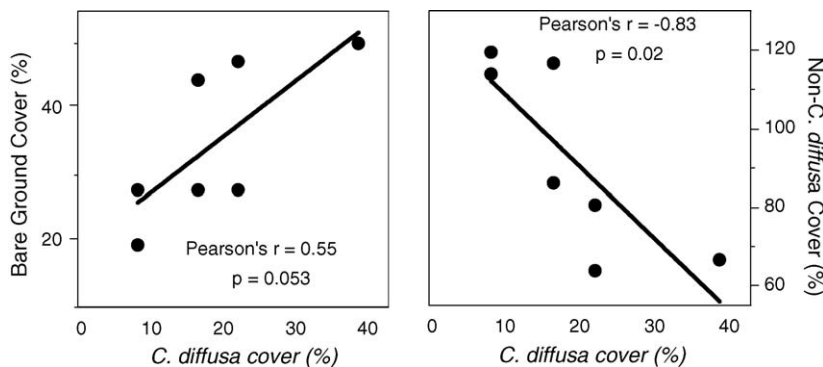


Fig. 6. Simple correlations between *Centaurea diffusa* cover (%) and cover of bare ground and non-*C. diffusa* vegetation in control plots, August 2001. Data were square-root transformed for analysis but are presented in the original scale.

and amount of bare ground increased with increasing *C. diffusa* cover and mature plant density ( $p < 0.053$ ).

One year after the removal of *C. diffusa*, cover of the native species *Buchloe dactyloides* (C<sub>4</sub> grass) and *Gutierrezia sarothrae* (native forb) increased. The effect for *G. sarothrae* was significant in June 2001 ( $F_{1,45} = 11.3$ ,  $p = 0.002$ ) and August 2001 ( $F_{1,45} = 6.21$ ,  $p = 0.02$ ). For *Buchloe dactyloides*, the effect was only significant in June 2001 ( $F_{1,45} = 6.5$ ,  $p = 0.01$ ). Cover of *Bromus japonicus* (a non-native C<sub>3</sub> grass) increased in the absence of *C. diffusa* in June 2001 ( $F_{1,45} = 5.6$ ,  $p = 0.02$ ), and cover of *Convolvulus arvensis* (a non-native forb) increased in the absence of *C. diffusa* in August 2001 ( $F_{1,45} = 6.1$ ,  $p = 0.02$ ).

*C. diffusa* removal had no significant effect on the non-*C. diffusa* vegetation cover, the cover of grasses, native grasses, forbs, native forbs, species richness, or cover of bare ground ( $p > 0.05$ ). There was no difference in the response to *C. diffusa* removal in plots with added carbon, gypsum, or both, compared to the response in the control plots ( $p > 0.05$ ).

#### 4. Discussion

The hypothesis that elevated nitrogen levels might be causing a shift in resource ratios, favoring species that respond maximally to nitrogen availability, or species that are good competitors for the next most limiting resource does not appear to explain *C. diffusa* invasion and dominance. We did not see a strong or consistent growth response by *C. diffusa*, other forbs, or by the whole vegetation biomass to nitrogen addition in any year of the fertilization experiments. Instead, the strongest response was by *A. minus*, to addition of both nitrogen and phosphorus, but not to nitrogen alone. Addition of both nutrients caused a shift in composition from dominance by grasses to co-dominance by grasses and *A. minus*.

Addition of phosphorus increased the aboveground biomass of individual mature *C. diffusa* in South Field but not in North Field. South Field is more nitrogen-rich (Fig. 1) and phosphorus may have been initially more limiting in South Field than in North Field. Inorganic nitrogen to labile phosphorus ratios averaged 0.24 in South Field versus 0.13 in North Field, which might explain the co-limitation of *C. diffusa* by nitrogen and phosphorus in North Field.

The lack of *C. diffusa* biomass response to lowering of phosphorus availability was unexpected. The reduction of nitrogen and phosphorus availability also did not produce the opposite effect that increasing nitrogen and phosphorus availability produced. Gyp-

sum addition did not significantly reduce labile phosphorus concentrations until mid-2001, so the effect of phosphorus reduction on a biennial species might be delayed. All soil samples were collected from the 0 to 10 cm depth, so it is possible that *C. diffusa* primarily accesses nitrogen, phosphorus, and other resources at greater depths. Tap roots of mature *C. diffusa* at the site extend to approximately 20–30 cm below ground surface, but roots of *C. diffusa* rosettes are typically less than 15 cm. The gypsum and carbon treatments reduced labile phosphorus and inorganic nitrogen in at least the 0–10 cm depth. Results of several microcosm competition trials suggest resource partitioning by *C. diffusa* and other *Centaurea* species (*C. maculosa*, *C. solstitialis*) as mechanism of the wide-spread success of *Centaurea* spp. (Sheley and Larson, 1994, 1996, 1997; Jacobs and Sheley, 1999; Dukes, 2002). Access to deeper resources, at least by bolting and mature plants, could possibly explain the lack of response of *C. diffusa* to the treatments, and the lack of response in nutrient pools and rates of cycling following *C. diffusa* removal. Hook et al. (2004) were unable to demonstrate consistent effects on soil carbon and mineral nitrogen pools by *C. maculosa*.

The lack of growth, seed head, and seed production response of *C. diffusa* to added nitrogen in South Field, and the fact that nitrogen addition ultimately eliminated *C. diffusa*, contrasted with the strong growth, seedhead, and seed production response to nitrogen and phosphorus addition in North Field, indicate that *C. diffusa* does not always function like the weedy species that are typically thought to dominate after natural disturbances in western grasslands (Seastedt et al., 1991; McLendon and Redente, 1991). Lowe et al. (2002) similarly demonstrated that nitrogen availability has inconsistent effects on a range of western grassland exotic invaders including *Centaurea repens* (Russian knapweed). The response of native grasses to added ammonium nitrate in greenhouse trials was as strong or stronger than the response of *C. repens*. However, Lowe et al. (2002) found a significant positive linear relationship between *C. repens* biomass and inorganic nitrogen. Story et al. (1989) also observed a significant increase in the biomass of *C. maculosa* in a field study of inorganic nitrogen (as ammonium nitrate) effects. That *C. diffusa* in this study did not respond consistently to nitrogen addition may reflect species-level differences in resource needs, the influence of competitors, or differences in pre-existing nitrogen concentrations and C:N ratios in the soil (Table 1). Both mineral and total nitrogen concentrations were greater in South Field, and South Field plants were larger. However, over the course of the study, nitrogen addition in South Field

eliminated *C. diffusa* from the plots, suggesting that *C. diffusa* is not able to convert additional nitrogen to biomass (or seeds), or it is not able to effectively compete at high nitrogen levels, or both. The small but statistically significant differences in micro-nutrient concentrations (Ca, Mg, Fe, Zn, Table 1) and texture seem unlikely to explain the field level differences in response by *C. diffusa*.

Other vegetation types in the community exhibited more consistent and expected responses. The relative cover of native grasses increased with carbon addition, from 38 to 42%, which indicates that they are relatively good competitors under low-nutrient conditions. *Alyssum minus* exhibited the strongest responses to nutrient additions, and was virtually eliminated from the community by nutrient reduction treatments. For certain invading exotic species like *A. minus*, reverse fertilization has promise as control strategy (e.g., Morgan, 1994; Pegtel et al., 1996; Tilman et al., 1999).

The negative correlations between *C. diffusa* cover and density and non-*C. diffusa* vegetation and positive correlation between *C. diffusa* and bare ground suggest either that *C. diffusa* eliminates other vegetation through competitive exclusion, or that *C. diffusa* is more successful where neighbors are absent. The results of our removal experiments suggest that *C. diffusa* does not eliminate or preclude native vegetation growth: we saw little indication that removal of *C. diffusa* from the community resulted in significant change in native plant diversity or cover. Moreover, removal of *C. diffusa* did not correspond with a decrease in bare ground. Our results suggests that *C. diffusa* has little competitive effect on other species, just as it appears to have little effect on soil nutrient availability.

*Centaurea* species have been reported to be successful competitors for resources (Callaway and Aschehoug, 2000; Marler et al., 1999; Zabinski et al., 2002). Suding et al. (2004) found that *C. diffusa* can tolerate competition from neighbors better than other species, but that it does not have strong effects on neighbors. If *C. diffusa* were strongly affecting resource availability, through either input processes (such as root exudates, root turnover, or aboveground litter chemistry), or through uptake processes (such as resource pre-emption), its removal should alleviate resource limitations on the rest of the community, or result in changes in the quantity, ratio, or timing of resource availability. However, we found that removal did not change the way that the rest of the community responded to the nutrient reduction treatments. Either *C. diffusa* partitions resource use so that it does not compete directly with other species (e.g., Sheley and

Larson, 1994, 1996, 1997; Jacobs and Sheley, 1999; Dukes, 2002), or its effects on resources are insignificant relative to the reduction imposed by our nutrient reduction treatments.

LeJeune (2002) found that patterns of inorganic nitrogen, labile phosphorus, and water availability beneath *C. diffusa* and native grasses were similar throughout the growing season and did not indicate temporal pre-emption of resources. This further supporting evidence that *C. diffusa* has little effect on resource availability suggests that *C. diffusa* does not acquire resources more efficiently or reduce available resources to a lower level than the native species. If resource acquisition patterns and litter quality inputs are similar, then invasive species may function very similarly to the native community, or as a “twin invader” (after Hoopes and Hall, 2002).

The strongest biomass response by *C. diffusa* that we observed was an artifact of complete removal plots in the reverse fertilization experiments (Suding et al., 2004). On the edges of plots in which we removed all vegetation, *C. diffusa* biomass increased by approximately 40-fold, and the relative cover of *C. diffusa* increased from approximately 40 to nearly 80% (LeJeune, 2002). The strong response of *C. diffusa* to removal of competition is consistent with the interpretation that *C. diffusa* has little effect on existing communities, but is most successful where disturbance has eliminated competitors. These results are surprising for such a successful invader, and one that reportedly can invade well managed rangelands (Sheley et al., 1998). However, the results are consistent with observations of *C. diffusa* growing along roadsides and in disturbed fields in the Colorado Front Range. These results are not consistent with the theory that *Centaurea* species dominate by mycorrhizal subsidy at the expense of neighbors (Marler et al., 1999; Carey and Callaway, 1999; Zabinski et al., 2002).

Our results suggest that lack of competition, possibly for water or light, is more influential than nutrient availability in the persistence and dominance of mature *C. diffusa*. Survival during the seedling stage, at least in drought years, appears to be limited by moisture and largely unrelated to competition and nutrient availability (LeJeune, 2002). Protection from desiccation by “nurse plants” may negate or even override competition for soil resources. As seedlings age, competition probably becomes more important. Invasion of disturbed areas most likely occurs in wet years, when cover is less crucial to seedling establishment.

The results of the nutrient manipulation experiments do not suggest a biogeochemical or resource pre-emption

explanation for *C. diffusa* dominance, nor do they suggest a biogeochemical restoration angle at this site. The results are inconsistent with the hypothesis that nutrient-rich soils are more susceptible to invasion by *C. diffusa* and other exotic species (LeJeune and Seastedt, 2001; Stohlgren et al., 1999, 2002), and that nutrient-poor soils are more resistant to invasion (Huenneke et al., 1990; Hoopes and Hall, 2002). Instead, *C. diffusa* appears to be unresponsive to soil nutrient status, and fairly tolerant of a wide range of nutrient availability, like the native grasses.

The wide range of tolerance of *C. diffusa* to resource availability, its ability to function like the native grasses, its prolific seed production and germination rate (Watson and Renny, 1974; Schirman, 1981; Sheley et al., 1998; LeJeune, 2002) at a range of levels of nutrient availability, and its escape from the species-specific herbivory (Seastedt et al., 2003) may explain its ability to invade and dominate western North American rangelands.

Experiments involving plant competition (Suding et al., 2004), soil and vegetation community relationships (this study) and response to insect herbivory (Seastedt et al., 2003; LeJeune et al., 2005) by *Centaurea* species in Colorado Front Range prairies all appear to produce finding substantially different from studies of *Centaurea* in more northerly environments. Whether these differences are due to soils, regional climatic differences, or genetic differences in the invasive species itself remains unknown. Such differences do, however, point out the need for 'site-specific' management approaches to widespread invaders.

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