

# Carbon addition interacts with water availability to reduce invasive forb establishment in a semi-arid grassland

Dana Michael Blumenthal

Received: 7 January 2008 / Accepted: 25 July 2008 / Published online: 22 August 2008  
© US Government 2008

**Abstract** Increases in nitrogen (N) availability can favor fast-growing invasive species over slow-growing native species. One way to reduce N availability is to add labile carbon (C) to the soil, which can lead to microbial immobilization of plant available N. This method has been used, with widely varying degrees of success, to both study and control plant invasions. One reason that C addition might not work as expected is that N is not always the limiting resource for plant growth. For example, if plant growth is limited by water, changes in N availability might have little effect on invasion. Here I ask whether effects of C addition on N availability, resident plant biomass, and invasion depend on water availability in semi-arid mixedgrass prairie. Six invasive species were seeded into plots treated with a factorial combination of water (ambient or added) and N (+C, control or +N). Carbon addition reduced capture of mineral N by resin probes (by an average of 73%), and reduced biomass of resident species (from 336 g m<sup>-2</sup> to 203 g m<sup>-2</sup>), both with and without added water. In contrast, because there was little invasion in ambient-water plots, C addition reduced invasion only in added-water plots. Given

added water, C addition reduced biomass of *Centaurea diffusa* by 95%, and prevented invasion by *Gypsophila paniculata* and *Linaria dalmatica*. Mechanisms by which C addition reduced invasion varied by species, with added C reducing the growth of individual *C. diffusa* plants, but reducing numbers of *G. paniculata* and *L. dalmatica* individuals.

**Keywords** *Centaurea diffusa* (diffuse knapweed) · *Gypsophila paniculata* (baby's breath) · *Linaria dalmatica* (Dalmatian toadflax) · Mixedgrass prairie · Nitrogen limitation · Reverse fertilization · Sugar addition · Water · Weed invasion

## Introduction

Increases in nitrogen (N) availability have been linked to plant invasions worldwide (Bobbink et al. 1998; Smith et al. 1999; Daehler 2003). Such increases are thought to favor fast-growing weedy and invasive species over slower-growing native species (Chapin 1980; Davis et al. 2000; Blumenthal 2005). One possible solution to this problem is to reduce plant-available nitrogen by increasing the soil carbon (C):N ratio, and thereby increasing microbial N immobilization (McLendon and Redente 1992; Morgan 1994). For research, as well as some restoration applications, this has been accomplished through soil amendments of C, generally sugar or sawdust. Results of these

D. M. Blumenthal (✉)  
USDA—ARS Rangeland Resources Research Unit,  
Crops Research Laboratory, 1701 Center Ave,  
Fort Collins, CO 80526, USA  
e-mail: dana.blumenthal@ars.usda.gov

experiments have been mixed. Although there have been a number of experiments in which C addition has favored native over invasive species (McLendon and Redente 1992; Young et al. 1997; Zink and Allen 1998; Paschke et al. 2000; Alpert and Maron 2000; Baer 2003; Blumenthal et al. 2003; Perry et al. 2004; Averett et al. 2004; Prober et al. 2005; Eschen et al. 2007), there also have been many cases in which C addition had little effect on competition between native and invasive species (Wilson and Gerry 1995; Reeve Morghan and Seastedt 1999; LeJeune and Seastedt 2006; Corbin and D'Antonio 2004; Suding et al. 2004; Vinton and Goergen 2006; Seastedt and Suding 2007).

One reason that C addition might not work as expected is that N is not always the limiting resource for plant growth (Blumenthal et al. 2003). In particular, it is interesting to consider whether C addition should influence invasion in arid and semi-arid environments. If plant growth is limited by water availability, changes in N availability might have little effect on invasion. Conversely, if arid and semi-arid ecosystems are often co-limited by N and water (Hooper and Johnson 1999), C addition could strongly influence invasion. Among the C-addition studies that have been conducted in arid and semi-arid ecosystems, results have varied widely. Studies have found C addition to have little effect (Doescher et al. 1990; Miller et al. 1991; Huddleston and Young 2005), to reduce N or overall plant growth (Wilson and Gerry 1995; Horn and Redente 1998; Reeve Morghan and Seastedt 1999; LeJeune et al. 2006; Seastedt and Suding 2007), to inhibit invasive species (Young et al. 1998; Monaco et al. 2003; Beckstead and Augspurger 2004), accelerate succession (McLendon and Redente 1992; Paschke et al. 2000), or favor native over invasive species (Young et al. 1997). To date however, there have been no studies to explicitly test whether effects of C addition in arid or semi-arid environments depend on water availability.

The semi-arid mixedgrass prairie comprises 38% of the remaining grassland in North America. Although much of the mixedgrass prairie is relatively intact, invasive plants cause serious problems for both rural communities and biological diversity (Stohlgren et al. 1998; DiTomaso 2000). Many of the most problematic invaders are perennial forbs (LeJeune and Seastedt 2001). For example, *Euphorbia esula* reduces both species richness and productivity of native species

(Belcher and Wilson 1989). As a result, it costs an estimated \$129 million annually in direct management costs, reduced forage production, and reduced land value, in four states within the mixedgrass prairie region: Montana, North Dakota, South Dakota and Wyoming (Leitch et al. 1996). Similarly, invasive *Centaurea* species reduce forage for wildlife, and cost Montana livestock producers an estimated \$42 million annually (Trammell and Butler 1995). Increases in N availability, due in part to N deposition (Kochy and Wilson 2001), have been hypothesized to increase the success of such species in mixedgrass prairie (LeJeune and Seastedt 2001). Attempts to reduce invasibility by reducing N availability in mixedgrass prairie, however, have met with little success (Wilson and Gerry 1995; Suding et al. 2004; LeJeune et al. 2006; Seastedt and Suding 2007).

Our objectives in this study were (1) to test whether effects of C addition on N availability, plant growth, and invasion depend on water availability in a semi-arid environment, and (2) to determine how C and N addition influence invasion by several invasive plant species in intact mixedgrass prairie.

## Materials and methods

### Study site

We conducted the study at the USDA-ARS High Plains Grassland Research Station (HPGRS), west of Cheyenne, Wyoming. The HPGRS is at the southern end of the Northern Mixedgrass Prairie (41°N, 104°W; Schuman et al. 1999; LeCain et al. 2000). Elevation at the HPGRS is 1,930 m, mean annual precipitation is 38 cm, and there are an average of 127 frost-free days per year. Average temperatures in summer and winter are 18°C and -2.5°C, respectively. The resident plant community is dominated by native species, including cool-season graminoids (C<sub>3</sub>) such as *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass), *Stipa comata* (needle-and-thread) Trin and Rupr, and *Carex duriuscula* C.A. Mey., a warm-season (C<sub>4</sub>) grass *Bouteloua gracilis* (H.B.K.) Lab. Ex Steud (blue grama), and a diverse array of forbs and subshrubs. The soils are mixed, mesic, Aridic Argiustolls; the soil series is an Ascalon sandy loam (Schuman et al. 1999). Although the site was not grazed during the study, it had been

grazed at a moderate stocking rate (6.29 ha cow-calf pair<sup>-1</sup>) for 8 years prior to the study.

### Experimental design and treatment applications

To examine the interactive effects of water and N availability on invasion, we seeded six invasive species into intact, resident communities, and manipulated water and N or C. Treatments included two water treatments (ambient, added) crossed with three N treatments (+C, control, +N). Treatments were arranged in a blocked, split-plot design, with three blocks, each containing one replication of all treatments. N treatments were randomly assigned to subplots within water-manipulation whole plots. Subplots measured 2.95 m × 1.00 m and were separated by undisturbed buffers zones at least 0.35 m in width. Each subplot was comprised of two 1.3 m × 1.0 m treated areas, separated by a 0.35 m walkway, each of which received seed of all six invasive species.

We added water during both the winter, using snow fences, and the summer, using irrigation. Snow was increased with three lath and wire snow fences. Each fence was 1 m high by 25 m long and was oriented perpendicular to the prevailing winter winds. Snow accumulation behind the fences averaged 50 cm. Water-manipulation plots were 2.95 × 6.40 m. Added-water plots were located in the zone of maximum snow accumulation, 1 m to the leeward side of the fence. Plots receiving ambient snowfall were located 20 m to the windward side of each snow fence. During the growing season, fences were removed to equalize air movement between ambient-water and added-water plots. We increased summer precipitation in added-water plots by adding 50% of the 30-year average monthly precipitation in weekly increments from mid May through mid August. During the first month of watering in 2005, we mistakenly applied only 25% of the 30-year average. Water was applied with a watering wand powered by a battery powered pump.

Nitrogen treatments were applied to 2.95 m by 1.00 m subplots within the larger water-manipulation plots. Carbon-addition plots received a total 2,948 g m<sup>-2</sup> of dextrose (1,167 g m<sup>-2</sup>°C) over the course of the experiment. This quantity of C is intermediate among the C-addition quantities used in previous studies finding strong effects on vegetation (e.g., Baer 2003; Blumenthal et al. 2003; Prober et al.

2005; Eschen et al. 2007; Seastedt and Suding 2007). I used dextrose as a C source because it is produced from corn, and can be less expensive than the more commonly used sucrose. Preliminary experiments have shown that dextrose and sucrose amendments have similar effects on N availability in rangeland soils under laboratory conditions (Mark Paschke, unpublished data). We applied dextrose in seven increments, on 1 April, 20 May, 12 August, 21 October, and 19 November in 2004, and on 5 May and 5 July in 2005. At each date, we applied 416 g m<sup>-2</sup> of powdered dextrose (Sigma, St. Louis, MO) by hand. To wash the dextrose off above-ground plant tissue, we then sprayed the plots with 0.5 l of water. The same amount of water was sprayed onto plots not treated with C. N-addition plots received 2.63 g m<sup>-2</sup> of N annually, a rate similar to the highest rates of N deposition measured in a survey of the Canadian Mixedgrass Prairie (Kochy and Wilson 2001). We divided fertilizer into two applications each year. In April 2004, August 2004, May 2005, and July 2005 we applied 3.9 g m<sup>-2</sup> of 33.5-0-0 ammonium nitrate fertilizer to N-addition plots. For each plot, fertilizer was dissolved in 0.5 l of water and sprayed on using a handheld sprayer. The same amount of water was sprayed onto plots not treated with N.

### Study species and seed addition

We seeded six invasive species into all plots: *Bromus tectorum* L. (cheatgrass), *Centaurea diffusa* Lam. (diffuse knapweed), *Cirsium arvense* (L.) Scop. (Canada thistle), *Euphorbia esula* L. (leafy spurge), *Gypsophila paniculata* L. (baby's breath), and *Linaria dalmatica* (L.) P. Mill. (Dalmatian toadflax). These species all invade intact mixedgrass prairie, and were all present at the HPGRS prior to this study. *C. diffusa*, *C. arvense*, *E. esula*, and *L. dalmatica* are listed as noxious weeds in Wyoming (<http://plants.usda.gov>). *Bromus tectorum*, while most problematic in the Intermountain West, is also common in Wyoming. *Gypsophila paniculata* is on the noxious weed lists for both California and Washington State. It was planted as an ornamental at the HPGRS and has been spreading into adjacent mixedgrass prairie.

Two 0.7 × 1.0 m seeded areas were located within each subplot, surrounded by 0.15 m buffer areas that received the same water and N treatment as seeded areas. To make it possible to repeatedly find

and measure seedlings of individual species, we seeded invasive species in rows oriented perpendicular to the snow fence. Each species was sown into two randomly located 11.66 cm × 100 cm rows within each subplot (one in each seeded area). To increase safe sites for germination, we lightly disturbed half of each row (5.83 cm × 100 cm) to a depth of <3 cm using a pointed hoe. We added seed by hand in late November 2003.

#### Measurement of soil water, mineral N, plant density and plant biomass

We measured the effects of water addition on volumetric soil water content with 30 cm deep CS616 time-domain reflectometry probes attached to CR10X data loggers (Campbell Scientific, Logan Utah, USA). We placed probes in control and added-water plots within two of the three blocks, for a total of four probes. Probes were installed outside of areas planted with weeds or treated with N or C. We calibrated the probes using gravimetric water content data collected over the course of the study.

We measured available  $\text{NO}_3^-$  and  $\text{NH}_4^+$  with Plant Root Simulator (PRS)<sup>TM</sup> resin probes (Western Ag Innovations, Saskatoon, SK, Canada). Four cation and four anion probes were installed in each subplot. Probes were located in the buffer area that was subjected to experimental treatments but not seeded with invasive species. The 17.5 cm<sup>2</sup> resin membranes of the probes were placed vertically, between 2 and 7.6 cm below the soil surface, the zone of maximum root activity (Schuman et al. 1999; LeCain et al. 2006). Due to initial concerns that the probes would become saturated, we left them in the ground for only 1 month the first year, inserting them May 27, and removing them June 23. Because 2004 probes were not close to being saturated, we extended this period in 2005, inserting them May 17 and removing them August 4. Probes were cleaned with deionized water immediately after being removed from the soil and shipped to Western Ag Innovations for analysis. At Western Ag, probes were eluted with 17.5 ml of 0.5 M HCl for 1 h, and inorganic N ( $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) was determined colorimetrically, using a Technicon Autoanalyzer II (Hangs et al. 2004).

We counted all seedlings of each invasive species in early April, early May, late June and late September in 2004 and in late July 2005. Seedlings

were counted separately in disturbed and undisturbed sides of the row during 2004. By 2005 it was no longer possible to assign seedlings to disturbed or undisturbed areas. Invasive species were harvested immediately after the final count. A resident population of *L. dalmatica* in one of the whole plots made it difficult to be sure that plants observed in September 2004 and July 2005 had been added as seed. For this species, therefore, analyses of data from these dates included only two whole plots. We harvested resident species within seeded areas following the invasive species harvest, in early August 2005. We separately clipped all remaining monocots and dicots in one randomly located 50 × 37.5 cm frame within each planted area. All plants were dried at 60°C for 3 days prior to weighing.

#### Data analysis

We used JMP version 5 (SAS Institute 2002) for all analyses. When necessary, data were transformed to meet model assumptions, using either  $\log_{10}$  or square root transformations. Mineral N captured by resin probes, and resident plant biomass data, were analyzed using models including Block, Water (ambient, added), N (+C, control, +N), and appropriate interactions, with Block and Block × Water as random effects. Because resin probe burial durations differed between 2004 and 2005, mineral N data from each year were analyzed separately. Volumetric water content was analyzed with a repeated measures model including Block, Water, Date, and interactions, with Block and Block × Water as random effects.

Because added invasive species were rarely observed within plots without added water, resulting in a large number of zeros, we tested for effects of Water on invasive species biomass using a Welch's nonparametric *t*-test. Effects of N on invader biomass within added-water plots were then tested using one-way ANOVAs including Block and N. Effects of N on invader numbers within added-water plots were tested using repeated measures models that included Block, N, Date, and interactions, with Block and Block × N as random effects. To examine effects of disturbance (only measured in 2004) on invader numbers, separate models were used that included Block, N, Disturbance, Date, and interactions, with Block, Block × N, and Block × N × Disturbance as random effects. In all ANOVAs, appropriate error

terms (see results for degrees of freedom) were used to test for whole-plot and sub-plot effects. Where significant effects of N were found, Tukey HSD means separation tests were used to test for significant differences among N treatments.

## Results

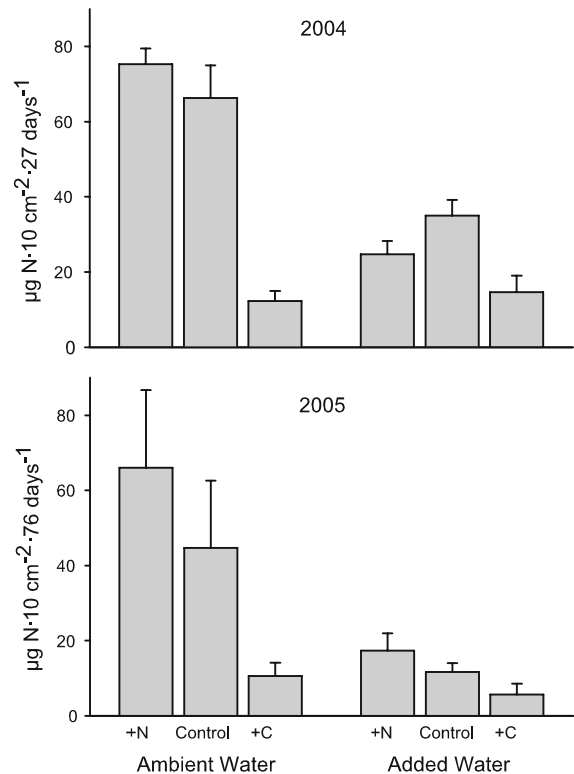
### Precipitation and mineral N

Precipitation was 84% of the long-term average for the HPGRS over the course of the experiment, from November 2003 to August 2005. The winter of 2003–2004, immediately following invasive species seeding, was quite dry, with November–April precipitation being only 60% of average. November 2004–April 2005 precipitation was 83% of average. May–October precipitation in 2004 was 87% of average, and May–August precipitation in 2005 was 98% of average. Water addition increased average volumetric soil water content from 0.12 to 0.17 over the course of the study ( $F_{1,2} = 558, P = 0.027$ ).

In 2004, the quantity of mineral N ( $\text{NH}_4^+\text{-N}$  plus  $\text{NO}_3^-\text{-N}$ ) captured by resin probes was significantly affected by water addition ( $F_{1,2} = 60, P = 0.016$ ), N treatment ( $F_{2,8} = 33, P = 0.0001$ ) and the interaction between water addition and N treatment ( $F_{2,8} = 9.2, P = 0.0085$ ; Fig. 1). Post-hoc analyses revealed that mineral N was influenced by N treatment only without water addition ( $F_{2,4} = 56, P = 0.0012$ ), and was lower in C-addition plots without added water than in control plots. In 2005, mineral N was significantly affected by water addition ( $F_{1,2} = 19, P = 0.05$ ) and N treatment ( $F_{2,8} = 27, P = 0.0003$ ) but not their interaction (Fig. 1). Means comparisons showed that C addition significantly reduced probe-N, but that N-addition had no significant effect on probe-N (Fig. 1).

### Resident species

Total above-ground biomass of resident species increased with N addition and decreased with C addition ( $F_{2,8} = 54, P < 0.0001$ ; Fig. 2). Resident biomass also increased with water addition ( $F_{2,8} = 30, P < 0.032$ ). Carbon addition reduced biomass of resident monocots ( $F_{2,8} = 41, P < 0.0001$ ), comprised primarily of perennial grasses and *Carex*

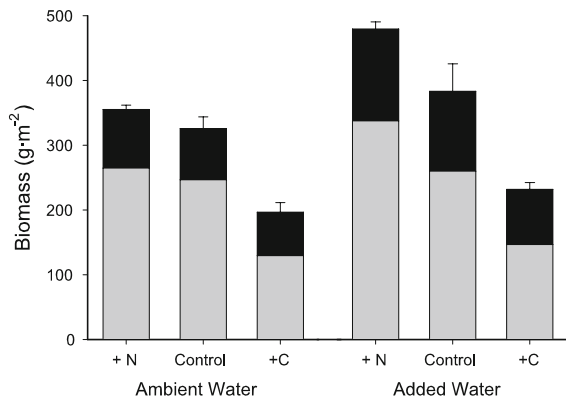


**Fig. 1** Mineral nitrogen ( $\text{NH}_4^+\text{-N}$  plus  $\text{NO}_3^-\text{-N}$ ) extracted from resin probes as a function of water and nitrogen treatment: added nitrogen (+N), no amendment (Control), or added sugar (+C). Error bars are +1 SE. Note the different y-axes for 2004 and 2005, which reflect different burial durations. Water addition significantly reduced mineral nitrogen in both years. Nitrogen treatment significantly affected mineral nitrogen with ambient water in 2004, and with ambient and added water in 2005. In each case, +C plots had significantly lower mineral nitrogen than +N and Control plots, based on Tukey HSD ( $P < 0.05$ )

*duriuscula* (Fig. 2). Although N treatment also influenced resident dicots ( $F_{2,8} = 5.4, P = 0.032$ ), comprised primarily of perennial forbs and subshrubs, dicot biomass in C-addition plots differed only from that in N-addition plots and not from that in control plots. Water addition had no effect on monocot or dicot biomass, either alone or in conjunction with C or N addition.

### Invasive species

Only three of the six added invasive species successfully invaded the experiment: *Centaurea diffusa*, *Gypsophila paniculata*, and *Linaria dalmatica*. Results therefore will be limited to these species.



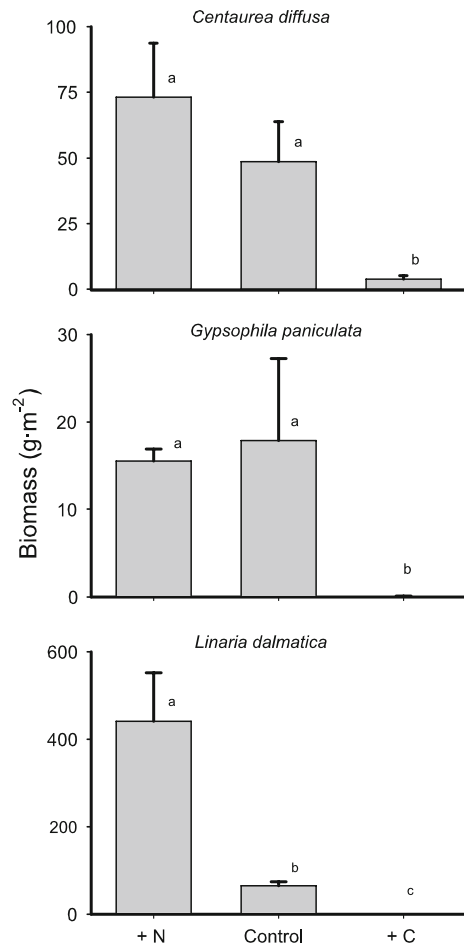
**Fig. 2** Biomass of resident species, including monocots (gray bars) and dicots (black bars) as a function of water and nitrogen treatment: added nitrogen (+N), no amendment (Control), or added sugar (+C). Error bars are +1 SE of total resident biomass. Effects of nitrogen treatment were significant for total resident biomass, resident dicot biomass, and resident monocot biomass. In pair-wise comparisons, biomass in +C plots was lower than that in Control and +N plots for total resident biomass and monocot biomass, and lower than that in +N plots for dicots. Biomass in +N plots was higher than that in Control plots only for total resident biomass. Effects of water were significant only for total resident biomass

Populations of these three invasive species persisted 3 and 4 years after the initial seeding, reestablishing after the harvest via resprouting or germination.

The invasive species attained little or no biomass in plots without water addition. Average above-ground biomass of *C. diffusa*, *G. paniculata*, and *L. dalmatica* in ambient-water plots was 0% ( $P = 0.0021$ ), 0.83% ( $P = 0.0017$ ), and 1.0% ( $P = 0.018$ ) of that in added-water plots, respectively.

Given added water, and therefore invasive species, N treatment significantly affected above-ground biomass of *C. diffusa* ( $F_{2,9} = 13$ ,  $P = 0.018$ ), *G. paniculata* ( $F_{2,9} = 9.2$ ,  $P = 0.032$ ), and *L. dalmatica* ( $F_{2,6} = 243$ ,  $P = 0.0041$ ). Specifically, C addition dramatically reduced biomass of all three invasive species, and N addition increased biomass of *L. dalmatica* more than fivefold (Fig. 3).

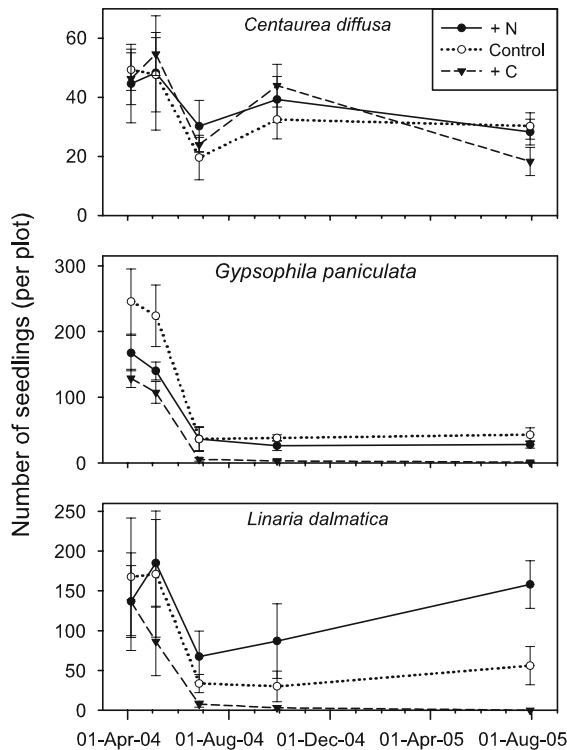
Results for plant numbers in added-water plots were less consistent among invasive species (Fig. 4). N treatment had no effect on *C. diffusa* numbers. In contrast, N treatment did influence *G. paniculata* numbers ( $F_{2,4} = 12$ ,  $P = 0.020$ ), with many fewer plants in C-addition than control plots. For *L. dalmatica* numbers, both the main effect of N ( $F_{2,4} = 7.5$ ,  $P = 0.045$ ) and its interaction with Date ( $F_{2,18} = 6.5$ ,  $P = 0.0005$ ) were significant. In



**Fig. 3** Biomass of seeded invasive species in added-water plots as a function of N treatment: added N (+N), no amendment (Control), or added sugar (+C). Bars with different letters are significantly different based on Tukey HSD ( $P < 0.05$ ). To obtain biomass/m<sup>2</sup>, per-plot biomass was divided by the area within which each species was planted rather than the entire area of the plot, and is therefore not directly comparable to the resident species biomass values in Fig. 2

post-hoc, within-date analyses, N influenced *L. dalmatica* numbers in both September 2004 ( $F_{2,2} = 107$ ,  $P = 0.0092$ ), and August 2005 ( $F_{2,2} = 208$ ,  $P = 0.0048$ ), with fewer plants in C-addition than in control plots. N addition did not influence plant numbers of any of the invasive species.

Effects of C addition on the size of individual plants could be examined only for *C. diffusa*, because it was the only species to survive in sufficient numbers in all N treatments. The average size of individual *C. diffusa* plants was significantly affected by N treatment ( $F_{2,4} = 58$ ,  $P = 0.0011$ ); plant size was lower in



**Fig. 4** Invasive species numbers ( $\pm 1$  SE) in added-water plots as a function of Date and N treatment: added N (+N), no amendment (Control), or added sugar (+C). Only Date significantly influenced *C. diffusa* density. Both Date and N treatment influenced *G. paniculata* density, with lower density in +C than in Control plots. N treatment and Date  $\times$  N treatment influenced *L. dalmatica* density, with lower density in +C than in Control plots on the last two sampling dates

C-addition plots ( $0.10 \text{ g m}^{-2}$ ) than in Control ( $0.76 \text{ g m}^{-2}$ ) or N-addition ( $1.3 \text{ g m}^{-2}$ ) plots.

Disturbance increased the numbers of all three species in added-water plots ( $P < 0.001$  in all cases), but did not interact with either Date or N treatment. All three species were able to establish without disturbance in added-water plots, however. In September 2004, surviving seedling numbers per plot in disturbed and undisturbed areas, respectively, were 11 and 29 for *C. diffusa*, 4 and 19 for *G. paniculata*, and 5 and 35 for *L. dalmatica*.

## Discussion

This study shows that C addition can reduce N availability, resident plant biomass, and invasibility of northern mixedgrass prairie. Carbon addition

reduced N availability both with water addition (in 2004) and without water addition (in both years; Fig. 1), suggesting that microbes were C-limited even with ambient, and somewhat lower than average, levels of precipitation. These results are in accord with those of several other studies in which addition of sucrose (Wilson and Gerry 1995; Suding et al. 2004; LeJeune et al. 2006) or sucrose and gypsum (Seastedt and Suding 2007) reduced N availability in mixedgrass prairie. Other studies have found that C addition can reduce N availability in even more arid ecosystems (McLendon and Redente 1992; Young et al. 1997; Paschke et al. 2000). In this study, water addition also reduced N availability, but the mechanism behind this effect is not clear. Added water may have stimulated N uptake by plants or microbes, or have leached mineral N below the 7.6 cm probe depth prior to probe insertion in late May.

Like N availability, above-ground biomass of resident plants was reduced by C addition both with and without water addition (Fig. 2). Thus, even with somewhat lower than average precipitation in this semi-arid grassland, C addition still had strong effects on plant growth. It seems likely that effects of C addition were mediated by reduced N availability, but it is also possible that reduced availability of other essential nutrients was involved. Both N addition and water addition increased resident plant biomass, results that are qualitatively similar to those observed by Lauenroth et al. (1978) in the nearby shortgrass steppe, and in accord with the suggestion that N and water may co-limit productivity in many semi-arid ecosystems (Hooper and Johnson 1999).

C addition had stronger effects on invasive species biomass than on resident plant biomass, reducing biomass of *C. diffusa* by 95%, and preventing invasion by *G. paniculata* and *L. dalmatica* (Fig. 3). These results were observed only with added water, suggesting that low water availability can limit the influence of C addition on invasion (the direct effects of summer and winter water addition on invasion are explored in detail in a separate paper; Blumenthal et al. 2008). Because invasive species were largely absent in ambient-water plots, however, it was not possible to fully examine the interaction between water addition and C addition. Furthermore, the focus on invasive species recruitment, which was very sensitive to water, may have accentuated the importance of water, and therefore the interaction between water and C addition

in this study. Effects of C addition on competition between established invasive and native species might be less sensitive to water availability.

Multiple factors might explain the relatively strong effects of C addition on invasive species in added-water plots. First, the invasive species may have had higher N requirements for growth than the resident species. Carbon addition had large effects on *C. diffusa* growth, reducing per-plant biomass by 87%. Second, unlike most resident species, invasive species had to establish from seed during the course of the experiment. Establishing seedlings may have been particularly sensitive to low N availability. Although most invasive species germination occurred shortly before the first C was added, seedling mortality was quite high, and C addition greatly reduced the number of surviving seedlings of both *G. paniculata* and *L. dalmatica* (Fig. 4). Furthermore, C addition reduced seedling numbers in both disturbed and undisturbed areas, indicating that even with disturbance, lower N availability can influence recruitment. These results are in accord with several other studies that have found C addition to reduce invasive species recruitment (Young et al. 1998; Beckstead and Augspurger 2004; Seastedt and Suding 2007). Such reductions may be particularly problematic for the short-lived invasive forbs in this experiment, which rely on frequent recruitment.

The present study is one of several that have examined effects of C addition on *C. diffusa* in mixedgrass prairie. In experiments involving resident populations of *C. diffusa*, sucrose addition did not affect *C. diffusa* biomass (LeJeune et al. 2006), and sucrose plus sawdust addition reduced *C. diffusa* biomass by 40%, but affected other resident species similarly (Reever Morghan and Seastedt 1999). A study of *C. diffusa* transplants into mixedgrass prairie found that sucrose addition reduced *C. diffusa* biomass by 75%, and had similarly large effects on transplanted native species (Suding et al. 2004). Finally, an experiment in which *C. diffusa* was established from seed found that sucrose addition reduced *C. diffusa* biomass by 81% but only when resident grass competitors were removed (Seastedt and Suding 2007). Among the three invasive species I examined, *C. diffusa* was the least affected by C addition, but its biomass was still reduced by 95%. The present study is the first to find that C addition reduced biomass of *C. diffusa* more than that of native species,

but is in other ways quite similar to the study by Seastedt and Suding (2007). Both studies involved addition of *C. diffusa* seed and relatively high levels of C to mixedgrass prairie. Further, both studies found that C addition influenced *C. diffusa* only when conditions were particularly favorable for *C. diffusa* growth, due to the removal of competitors or the addition of water. Together, these results suggest that although *C. diffusa* can survive under conditions of low N availability, limited N can reduce its ability to thrive in environments high in other resources.

In sum, this study suggests that reducing N availability can inhibit invasion even in semi-arid ecosystems, and that effects of N are most likely to be observed during wet periods. It also suggests that the degree to which effects of C addition interact with water availability may depend on the sensitivity of different processes to water limitation. Given sufficient water for invasion, C addition inhibited or precluded invasion. Without added water, however, invasive species recruitment was strongly water-limited, which reduced the effect of C addition on invasion. In contrast, neither N availability nor resident species biomass were strongly limited by water, and water did not influence their responses to C addition.

**Acknowledgements** Many thanks to Kayla Aspen, Sanjay Advani, Ingrid Burke, Rod Chimmer, Justin Derner, Erika East, Cara Ferrier, Erik Hardy, Dan LeCain, Jack Morgan, Matt Mortenson, Valerie O'Neill, Mark Paschke, Jean Reeder, Gerald Schuman, David Smith, Jeffrey Welker, and Mark West for contributions to experimental design, execution, and interpretation; and Ruth Hufbauer, Aníbal Pauchard, Laura Perry, Timothy Seastedt, and two anonymous reviewers for helpful comments on earlier versions of this manuscript.

## References

- Alpert P, Maron JL (2000) Carbon addition as a countermeasure against biological invasion by plants. *Biol Invasions* 2:33–40. doi:10.1023/A:1010063611473
- Averett JM, Klips RA, Nave LE et al (2004) Effects of soil carbon amendment on nitrogen availability and plant growth in an experimental tallgrass prairie restoration. *Restor Ecol* 12:568–574. doi:10.1111/j.1061-2971.2004.00284.x
- Baer SG (2003) Soil resources regulate productivity and diversity in newly established tallgrass prairie. *Ecology* 84:724–735. doi:10.1890/0012-9658(2003)084[0724:SRR PAD]2.0.CO;2
- Beckstead J, Augspurger CK (2004) An experimental test of resistance to cheatgrass invasion: limiting resources at different life stages. *Biol Invasions* 6:417–432. doi:10.1023/B:BINV.0000041557.92285.43



- Belcher JW, Wilson SD (1989) Leafy spurge and the species composition of a mixed-grass prairie. *J Range Manage* 42:172–175. doi:[10.2307/3899318](https://doi.org/10.2307/3899318)
- Blumenthal DM (2005) Interrelated causes of plant invasion. *Science* 310:243–244. doi:[10.1126/science.1114851](https://doi.org/10.1126/science.1114851)
- Blumenthal DM, Jordan NR, Russelle MP (2003) Soil carbon addition controls weeds and facilitates prairie restoration. *Ecol Appl* 13:605–615. doi:[10.1890/1051-0761\(2003\)013\[0605:SCACWA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2003)013[0605:SCACWA]2.0.CO;2)
- Blumenthal DM, Chimner RA, Welker JM et al (2008) Increased snow facilitates plant invasion in mixedgrass prairie. *New Phytol*. doi:[10.1111/j.1469-8137.2008.02475.x](https://doi.org/10.1111/j.1469-8137.2008.02475.x)
- Bobbink R, Hornung M, Roelofs JG (1998) The effects of airborne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *J Ecol* 86:717–738. doi:[10.1046/j.1365-2745.1998.8650717.x](https://doi.org/10.1046/j.1365-2745.1998.8650717.x)
- Chapin FSI (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst* 11:233–260. doi:[10.1146/annurev.es.11.110180.001313](https://doi.org/10.1146/annurev.es.11.110180.001313)
- Corbin JD, D'Antonio CM (2004) Can carbon addition increase competitiveness of native grasses? A case study from California. *Restor Ecol* 12:36–43. doi:[10.1111/j.1061-2971.2004.00299.x](https://doi.org/10.1111/j.1061-2971.2004.00299.x)
- Daehler CC (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annu Rev Ecol Syst* 34:183–211. doi:[10.1146/annurev.ecolsys.34.011802.132403](https://doi.org/10.1146/annurev.ecolsys.34.011802.132403)
- Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. *J Ecol* 88:528–534. doi:[10.1046/j.1365-2745.2000.00473.x](https://doi.org/10.1046/j.1365-2745.2000.00473.x)
- DiTomaso JM (2000) Invasive weeds in rangelands: species, impacts, and management. *Weed Sci* 48:255–265. doi:[10.1614/0043-1745\(2000\)048\[0255:IWIRSI\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2000)048[0255:IWIRSI]2.0.CO;2)
- Doescher PS, Miller RF, Wang JG et al (1990) Effects of nitrogen availability on growth and photosynthesis of *Artemisia-Tridentata* ssp *Wyomingensis*. *Great Basin Nat* 50:9–19
- Eschen R, Mortimer SR, Lawson CS et al (2007) Carbon addition alters vegetation composition on ex-arable fields. *J Appl Ecol* 44:95–104. doi:[10.1111/j.1365-2664.2006.01240.x](https://doi.org/10.1111/j.1365-2664.2006.01240.x)
- Hangs RD, Greer KJ, Sulewski CA (2004) The effect of interspecific competition on conifer seedling growth and nitrogen availability measured using ion-exchange membranes. *Can J For Res* 34:754–761. doi:[10.1139/x03-229](https://doi.org/10.1139/x03-229)
- Hooper DU, Johnson L (1999) Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. *Biogeochemistry* 46:247–293
- Horn BE, Redente EF (1998) Soil nitrogen and plant cover of an old-field on the shortgrass steppe in southeastern Colorado. *Arid Soil Res Rehabil* 12:193–206
- Huddleston RT, Young TP (2005) Weed control and soil amendment effects on restoration plantings in an Oregon grassland. *West N Am Nat* 65:507–515
- Kochy M, Wilson SD (2001) Nitrogen deposition and forest expansion in the northern Great Plains. *J Ecol* 89:807–817. doi:[10.1046/j.0022-0477.2001.00600.x](https://doi.org/10.1046/j.0022-0477.2001.00600.x)
- Lauenroth WK, Dodd JL, Sims PL (1978) The effects of water- and nitrogen-induced stresses on plant community structure in a semi-arid grassland. *Oecologia* 36:211–222. doi:[10.1007/BF00349810](https://doi.org/10.1007/BF00349810)
- Leitch JA, Leistriz FL, Bangsund DA (1996) Economic effect of leafy spurge in the upper great plains: methods, models, and results. *Impact Assess* 14:419–433
- LeCain DR, Morgan JA, Schuman GE et al (2000) Carbon exchange rates in grazed and ungrazed pastures of Wyoming. *J Range Manage* 53:199–206. doi:[10.2307/4003283](https://doi.org/10.2307/4003283)
- LeCain DR, Morgan JA, Milchunas DG et al (2006) Root biomass of individual species, and root size characteristics after five years of CO<sub>2</sub> enrichment on native shortgrass steppe. *Plant Soil* 279:219–228. doi:[10.1007/s11104-005-2301-9](https://doi.org/10.1007/s11104-005-2301-9)
- LeJeune KD, Seastedt TR (2001) *Centaurea* species: the forb that won the west. *Conserv Biol* 15:1568–1574. doi:[10.1046/j.1523-1739.2001.00242.x](https://doi.org/10.1046/j.1523-1739.2001.00242.x)
- LeJeune KD, Suding KN, Seastedt TR (2006) Nutrient availability does not explain invasion and dominance of a mixed grass prairie by the exotic forb *Centaurea diffusa* Lam. *Appl Soil Ecol* 32:98–110. doi:[10.1016/j.apsoil.2005.01.009](https://doi.org/10.1016/j.apsoil.2005.01.009)
- McLendon T, Redente EF (1992) Effects of nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. *Oecologia* 91:312–317. doi:[10.1007/BF00317618](https://doi.org/10.1007/BF00317618)
- Miller RF, Doescher PS, Wang JG (1991) Response of *Artemisia-Tridentata* ssp *Wyomingensis* and *Stipa-Thurberiana* to nitrogen amendments. *Am Midl Nat* 125:104–113. doi:[10.2307/2426373](https://doi.org/10.2307/2426373)
- Monaco TA, Johnson DA, Norton JM et al (2003) Contrasting responses of Intermountain West grasses to soil nitrogen. *J Range Manage* 56:282–290. doi:[10.2307/4003820](https://doi.org/10.2307/4003820)
- Morgan JP (1994) Soil impoverishment a little-known technique holds potential for establishing prairie. *Restor Manage Notes* 12:55–56
- Paschke MW, McLendon T, Redente EF (2000) Nitrogen availability and old-field succession in a shortgrass steppe. *Ecosystems* (NY, Print) 3:144–158. doi:[10.1007/s100210000016](https://doi.org/10.1007/s100210000016)
- Perry LG, Galatowitsch SM, Rosen CJ (2004) Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinaceae* in carbon-enriched soil. *J Appl Ecol* 41:151–162. doi:[10.1111/j.1365-2664.2004.00871.x](https://doi.org/10.1111/j.1365-2664.2004.00871.x)
- Prober SM, Thiele KR, Lunt ID et al (2005) Restoring ecological function in temperate grassy woodlands: manipulating soil nutrients, exotic annuals and native perennial grasses through carbon supplements and spring burns. *J Appl Ecol* 42:1073–1085. doi:[10.1111/j.1365-2664.2005.01095.x](https://doi.org/10.1111/j.1365-2664.2005.01095.x)
- Reever Morghan KJ, Seastedt TR (1999) Effects of soil nitrogen reproduction on nonnative plants in restored grasslands. *Restor Ecol* 7:51–55. doi:[10.1046/j.1526-100X.1999.07106.x](https://doi.org/10.1046/j.1526-100X.1999.07106.x)
- Schuman GE, Reeder JD, Manley JT, Hart RH, Manley WA (1999) Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. *Ecol Appl* 9:65–71. doi:[10.1890/1051-0761\(1999\)009\[0065:IOGMO\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1999)009[0065:IOGMO]2.0.CO;2)
- Seastedt TR, Suding KN (2007) Biotic constraints on the invasion of diffuse knapweed (*Centaurea diffusa*) in North American grasslands. *Oecologia* 151:626–636. doi:[10.1007/s00442-006-0620-z](https://doi.org/10.1007/s00442-006-0620-z)

- Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ Pollut* 100:179–196. doi:[10.1016/S0269-7491\(99\)00091-3](https://doi.org/10.1016/S0269-7491(99)00091-3)
- Stohlgren TJ, Bull KA, Otsuki Y et al (1998) Riparian zones as havens for exotic plant species in the central grasslands. *Plant Ecol* 138:113–125. doi:[10.1023/A:1009764909413](https://doi.org/10.1023/A:1009764909413)
- Suding KN, LeJeune KD, Seastedt TR (2004) Competitive impacts and responses of an invasive weed: dependencies on nitrogen and phosphorus availability. *Oecologia* 141:526–535. doi:[10.1007/s00442-004-1678-0](https://doi.org/10.1007/s00442-004-1678-0)
- Trammell MA, Butler JL (1995) Effects of exotic plants on native ungulate use of habitat. *J Wildl Manage* 59:808–816. doi:[10.2307/3801961](https://doi.org/10.2307/3801961)
- Vinton MA, Goergen EM (2006) Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. *Ecosystems* (NY, Print) 9:967–976. doi:[10.1007/s10021-005-0107-5](https://doi.org/10.1007/s10021-005-0107-5)
- Wilson SD, Gerry AK (1995) Strategies for mixed-grass prairie restoration: herbicide, tilling, and nitrogen manipulation. *Restor Ecol* 3:290–298. doi:[10.1111/j.1526-100X.1995.tb00096.x](https://doi.org/10.1111/j.1526-100X.1995.tb00096.x)
- Young JA, Clements CD, Blank RR (1997) Influence of nitrogen on antelope bitterbrush seedling establishment. *J Range Manage* 50:536–540. doi:[10.2307/4003710](https://doi.org/10.2307/4003710)
- Young JA, Trent JD, Blank RR et al (1998) Nitrogen interactions with medusahead (*Taeniatherum caput-medusae* ssp. *asperum*) seedbanks. *Weed Sci* 46:191–195
- Zink TA, Allen MF (1998) The effects of organic amendments on the restoration of a disturbed coastal sage scrub habitat. *Restor Ecol* 6:52–58. doi:[10.1046/j.1526-100x.1998.00617.x](https://doi.org/10.1046/j.1526-100x.1998.00617.x)