# Effects of Soil Nitrogen Reduction on Nonnative Plants in Restored Grasslands

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### Abstract

**We studied the cumulative effects of 3 years of carbon amendments on previously disturbed mixed-grass prairie sites near Boulder, Colorado. Analysis of soil inorganic nitrogen during the third field season indicated statistically significant but short-term nitrogen reduction in response to addition of a combination of sugar and sawdust treatments. Plant foliage production was significantly reduced by these carbon amendments and averaged 377 g/m$^2$/year on control plots versus 219 g/m$^2$/year on treated plots. Undesirable species such as *Centaurea diffusa* (diffuse knapweed) exhibited a similar biomass response. But after three years of treatment there is little evidence to suggest a relative increase in desirable, reseeded species such as *Agropyron smithii* (western wheatgrass). We suggest that the carbon amendment treatment alone is an inadequate remediation technique in areas exposed to extensive seed rain by exotic species.**

### Introduction

One of the greatest problems facing land management and land restoration agencies is the invasion of ecosystems by nonnative species (Westman 1990; Morgan 1994; Hobbs & Humphries 1995). To find a way to protect areas against invasion, we must first understand the processes that makes an area susceptible to invasion. Two processes that contribute to such susceptibility are disturbance and nutrient additions (Hobbs 1989; Huenneke et al. 1990; Westman 1990; Hobbs & Huenneke 1992; Cowie & Werner 1993; Lodge 1993; Hobbs & Humphries 1995).

While invasive species do not all share the same characteristics, many of them are colonizing species capable of living in highly disturbed habitats (Hobbs & Huenneke 1992). Many of the characteristics found commonly in these colonizing invasive species are similar to characteristics found commonly in early seral species: high population growth rates, short generation times, abundant seed production, and very effective seed dispersal strategies (Westman 1990; Lodge 1993; Rejmánek & Richardson 1996). Similarly, the factors that favor native species over these invasive species are the same factors that favor late seral species over early seral species, such as the ability to persist at low nutrient levels and the capacity to out-compete other species under such conditions (Wedin & Tilman 1990; Redente et al. 1992).

One technique that can be used to lower soil nitrogen levels and favor later seral species is addition of carbon amendments to the soil. Carbon amendment treatment involves the addition of organic matter, such as sugar, sawdust, straw, or grain hulls, that is high in carbon and low in nitrogen to the soil of an experimental site (Morgan 1994). The addition of carbon stimulates soil microbe growth, and the soil microbes accumulate soil nitrogen in their biomass, making it unavailable to plants (Vitousek 1982; Hunt et al. 1988; McLendon & Redente 1992; Morgan 1994). Because native plants and late seral species are more competitive than invasive species and early seral species in the resulting low-nitrogen environment (Wedin & Tilman 1990; Redente et al. 1992), the nitrogen-lowering effects of carbon amendment treatment can help speed the rate of succession in disturbed areas by countering the nutrient increases due to disturbance and by favoring late seral species.

Published results (Huenneke et al. 1990; Mountford et al. 1996) are consistent in showing that nitrogen additions reverse succession and/or allow for invasion by nondominant late-successional species. But the hypothesis that nitrogen removal or nitrogen reduction can speed succession and/or maintain dominants has not been tested in a variety of habitats. Here, we tested the hypothesis that a native perennial grass, such as the C3 species *Agropyron smithii* (western wheatgrass), can persist under reduced nitrogen conditions, while annual or semelparous perennial species, such as *Alyssum minus* (alyssum) and *Centaurea diffusa* (diffuse knapweed), cannot.

### Methods

#### Plots and Treatment

Eighteen experimental plots were located in Boulder Open Space land, south of the city of Boulder, Colo-

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rado. These plots measure 3 × 1.5 m and were randomly divided into a control half and a treatment half (Fig. 1). Twelve of these plots were on the site of a 1991 disturbance, and six were on the site of a 1993 disturbance. Burial of utility cables was responsible for both disturbances. The sites were seeded in 1991 and 1993 with a mixture of grasses consisting of western wheatgrass, *Bouteloua gracilis* (blue grama), *Andropogon gerardii* (big bluestem), *Andropogon scoparium* (little bluestem), and *Bouteloua curtipendula* (side oats grama), but only the western wheatgrass was still present at the time of the study. The site was fenced to exclude cattle, but cattle entered the plots for limited periods of time each year. All plots had large populations of invasive species and potentially elevated nitrogen levels due to disturbance, atmospheric enrichment, and feedback effects from the decomposition of weedy species. These plots were also used in carbon amendment studies conducted in 1994 and 1995, as reported by Seastedt et al. (1996). Carbon amendments were applied by hand to the soil surface in the center square meter of the treatment plots. The total amount of carbon added in 1996 was 1000 g of sucrose and 650 g of air-dried sawdust. About 200 g of sucrose were applied each month from February to June, and 325 g of sawdust were applied in March and May only.

#### Soil Analyses

Soil was analyzed for extractable ammonium and nitrate on three dates—during treatment, 1 month after, and 2 months after—to evaluate the duration of the immobilization potential of the amendments. All plots were sampled in May 1996 during the carbon amendment addition period. Twelve of the paired plots were measured in July, 1 month after we had stopped adding carbon amendments to the plots. This sample of 12 plots consisted of six paired plots from the 1991 disturbance site and six paired plots from the 1993 disturbance site. The remaining six paired plots, all from the 1991 disturbance site, were measured in August. A sample consisted of four 10-cm soil cores, two from each half of the paired plots. The two soil cores from one-half of the plot were bulked together and sifted through a 2-mm mesh sieve. A subsample was weighed, dried at 60°C, and re-weighed to measure moisture content. We prepared each soil sample by adding 10 g of soil to 50 ml of 2N KCl, shaking the sample for 1 hour and filtering it the following day. The solution was frozen until it was analyzed on a Lachet analyzer for nitrate ($NO_3^-$) and ammonium ($NH_4^+$).

#### Plant Census and Foliage Production

We identified all the native and invasive plants in the plots in midsummer. We counted the total number of alyssum and western wheat within the southwest 0.1-$m^2$ quadrat and the total number of knapweed within the full 1-$m^2$ plot. The first biomass harvest occurred in mid-July at a time of peak foliage biomass. All biomass except flowering knapweed was clipped at the soil surface in the northwest 0.1-$m^2$ quadrat of each plot. The previous season's standing dead material and litter were not collected. This harvest underestimates annual

Figure 1. A view of one of the paired plots with the control plot in the background (inside the quadrat) and the carbon-amended plot in the foreground. The areas to the left and right of the plots were untreated. The control plot contains predominantly the invasive grasses *Bromus tectorum* (cheat-grass) and *Bromus japonicus* (Japanese brome), while the carbon-amended plot contains *A. smithii* (western wheatgrass) with cheat-grass along the right edge. A drift of the previous year's *C. diffusa* (knapweed) can be seen at the upper left corner.

productivity because many of the early-season plants had already senesced, but we assumed that this bias would not affect treatment comparisons. All biomass was dried at 60°C to constant weight. Flowering knapweed was left in the field until its seed matured. The second harvest occurred in late August and consisted of all the mature knapweed found in 1 $m^2$ of all 36 plots. All knapweed was air-dried to constant weight. We estimated knapweed seed production by harvesting and counting the number of seeds per 10 random seed heads from the knapweed plants in each plot.

All data were analyzed with a standard paired-plot $t$ test (SAS Institute 1988), except for mature knapweed density. Knapweed was not found in all plots, and both densities and biomass exhibited no correlations within the paired plots. A one-way analysis of variance employing a log-transformation to homogenize the variance was used to test for knapweed differences.

## Results

### Soil Analyses

Soil inorganic nitrogen levels differed significantly between the treated and control plots both during treatment and 1 month after treatment, but the differences were not statistically significant 2 months after treatment (paired $t$ test, $p < 0.05$, except in last collection; Fig. 2). Ammonium levels were initially substantially lower in the treated plots than in the control plots; these differences diminished after carbon additions ceased. Nitrate levels were also initially lower in the treatment plots than in the control plots, but the difference again became less pronounced after carbon additions were stopped. In addition to the soil nitrogen decrease, the soil analyses showed a significant increase in soil moisture in the treated plots during the first soil collection ($p < 0.05$), which was not observed during the subsequent soil analyses.

### Plant Census and Biomass

The plant census showed that these plots had a high rate of invasion. We compared our species list to a collection of exotic species listed by Mack (1989) and found that 62% of the species in the plots were nonnative species.

The census data on the native species, western wheat, and the two invasive species, alyssum and knapweed, showed no significant treatment effect (Table 1). There was no significant change in native species density between the control and the treatment plots ($p = 0.68$). In addition, the density of the two invasive species did not change significantly between the control and the treatment plots ($p = 0.34$ for knapweed; $p = 0.44$ for alyssum).

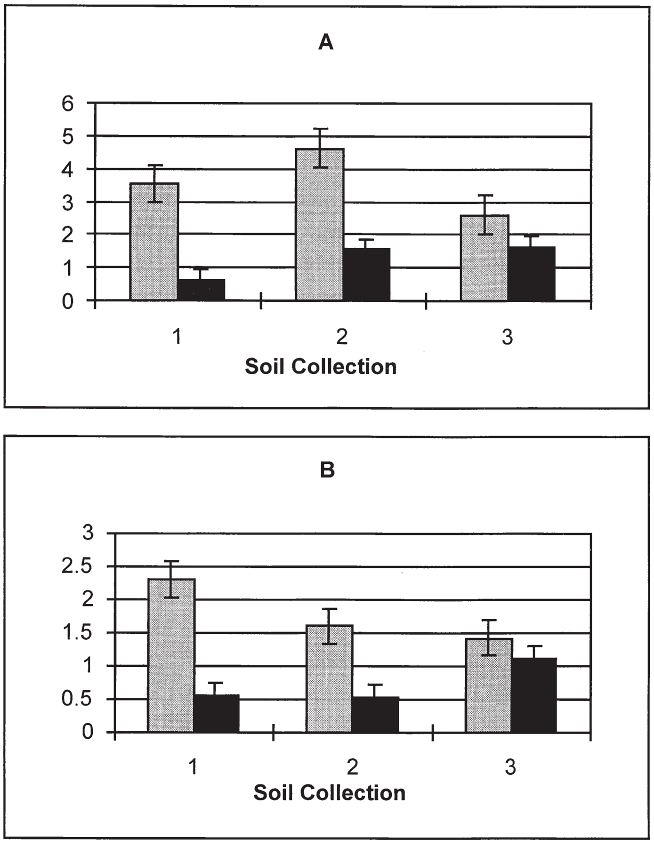


Figure 2. Ammonium (A) and Nitrate (B) in carbon-amended plots (black bars) and control plots (gray bars). Values are means with standard-error bars ($n = 18$, 12, and 6 per treatment, respectively).

Both the non-knapweed biomass and the mature knapweed biomass were significantly lower in the treated plots than in the control plots ($p < 0.05$ for both; Table 2). Knapweed was present in nine of the treatment plots and five of the control plots; it weighed an average of 81.72 g/$m^2$ in each of the nine treatments plots, compared with an average of 236.26 g/$m^2$ in each

**Table 1.** Comparisons of average density of invasive (*A. minus* and *C. diffusa*) and native (*A. smithii*) species in the control and treatment plots.*

| | *Plant Densities (number/m²)* | |
|---|---|---|
| *Species* | *Control (mean ± SE)* | *Treated (mean ± SE)* |
| *Alyssum minus* | 326.1 ± 109.6 | 226.7 ± 72.0 |
| *Centaurea diffusa* | 2.5 ± 0.8 | 7.3 ± 4.5 |
| *Agropyron smithii* | 120.6 ± 23.7 | 123.9 ± 27.7 |

*$n = 18$. None is significantly different ($p > 0.05$).

**Table 2.** Comparison of average biomass amounts for control and treatment plots.*

| | Biomass (g/m$^2$) | |
|---|---|---|
| *Variable* | *Control (mean ± SE)* | *Treated (mean ± SE)* |
| Non-Knapweed | 311.8 ± 53.8 | 178.4 ± 18.1 |
| Knapweed | 65.6 ± 34.4 | 40.9 ± 19.1 |
| Total | 377.5 ± 70.3 | 219.2 ± 27.6 |

*Biomass is broken down into three categories: non-knapweed biomass, mature knapweed biomass, and total biomass. The amounts listed reflect the average biomass of all 18 treatment or control plots. The biomass of *C. diffusa* (knapweed) in the plots containing knapweed (nine treatment plots and five control plots) was averaged over all 18 plots. All are significantly different ($p < 0.05$).

of the five control plots. When the zero values for all other plots are included in the analysis, average biomass of knapweed in the treated plots was about two-thirds that of knapweed in the control plots. The total biomass of all plants was also significantly lower in the treatment plots than in the control plots ($p < 0.05$).

The knapweed seed count showed no significant difference in seed production per gram of tissue between the knapweed in the control plot (8.2 ± 1.6) and that in the treatment plots (6.2 ± 2.2; $p > 0.05$).

## Discussion

The carbon amendment treatment lowered nitrogen levels in our treated plots substantially below the levels found in control plots. This nitrogen level decrease in response to carbon amendments was also seen by other researchers (McLendon & Redente 1992; Wilson & Gerry 1995). The soil nitrogen–lowering effects did not last long after the treatment (Fig. 2). To continuously lower soil nitrogen levels, labile carbon amendments must be added at regular intervals. Morgan (1994) added carbon amendments to his study plots once and noted no decrease in the effectiveness of carbon amendments over time in the plots where carbon amendments successfully stopped weed growth. He did not, however, measure soil nitrogen during the growing season.

Our carbon treatment had no significant effect on weed density. There was no significant increase in native species density in the treated plots and no significant decrease in the density of invasive species (Table 1). The reduction in knapweed and non-knapweed biomass on treated sites indicated that nitrogen limitation in the carbon-amended treatments affected plant growth (Table 2). The majority of the biomass in the plots was made up of invasive species. Many of the species were early seral species, and Redente et al. (1992) found that early seral species tend to be more dependent on nitrogen than later seral species. The most prevalent native grass species was a C3 species, western wheat, which has also been shown to increase in growth with increases in nitrogen level (Hunt et al. 1988). Native forb biomass in these plots was too small to give insight into whether the native forbs decreased in biomass in conjunction with reduced nitrogen, or whether they increased in biomass as nitrogen level and competition decreased, as seen by Huenneke et al. (1990). The overall reduction in biomass found in our study compares with the findings of Willems and van Nieuwstadt (1996).

The practicality of carbon amendment treatment for a particular site must take into account three considerations: the length of effectiveness, the amount of labor involved in application, and the cost and amount of carbon amendments. First, our results indicate that the length of effectiveness depends on regular application and on the characteristics of the carbon source added. While we knew that the sugar would disappear rapidly, we hoped that the sawdust would generate a long-term effect. This was obviously not the case.

Second, the amount of labor involved in application can affect the practicality of carbon amendment treatment. The use of carbon amendments is made more practical by surface application than by Morgan's (1994) technique of raking the carbon sources into the soil. The labor investment, however, appears linearly related to the number of carbon applications.

Finally, the amount and cost of carbon amendments needed affects the large-scale practicality of this treatment. The amount of carbon needed to create the desired effect varies widely with the site and initial soil nitrogen levels. We achieved a significant nitrogen reduction with 1000 g/m$^2$ of sucrose and 640 g/m$^2$ of sawdust per year. Wilson and Gerry (1995) noted a decrease in available soil nitrogen with 400 g/m$^2$ of sawdust per year and no sucrose. McLendon and Redente (1992) achieved a significant soil nitrogen reduction using no sawdust and only 160 g/m$^2$ of sucrose per year. Hunt et al. (1988) noted that 150 g/m$^2$ of sucrose per year controlled invasive species in his sucrose-added plots. Seastedt et al. (1996) noted a significant decrease in density of one invasive species with 300 g/m$^2$ in 1994 and 200 g/m$^2$ in 1995. Morgan (1994) used a substantially larger amount of carbon than any of the other researchers, 2000 g/m$^2$ of sucrose and 16 liters of sawdust per square meter (about 15 times the amount of sawdust used here) to achieve the desired control in one of his sites. Sucrose has proven its effectiveness in all studies, but it is the most expensive of the carbon sources. Waste carbon sources such as sawdust or (in eastern Colorado) sugar beet pulp may prove far more practical for large-scale treatments. Further studies will have to be conducted before guidelines can be set up for effective use of carbon amendments in different types of sites.

The limitations of this experiment include the small size of the plots in relation to sources of seed rain and our inability to keep cows out of the plots. These plots are surrounded by invasive species. While the treatment may have reduced seed sources of annual and biennial weeds within the treatment plots, seeds from adjacent areas undoubtedly reseeded the plots. Reducing available nitrogen to all plants in an area that is subject to high seed rain can potentially open up space for invasive species to gain or regain a foothold. The experiment was also potentially affected by cattle activities during 3 days in mid-June. While our soil analyses indicate that we were successful in reducing inorganic nitrogen availability during spring, the subsequent activities of cattle may have minimized the treatment effects. Our results may therefore represent a conservative assessment of the effects of nitrogen limitation.

In conclusion, our study confirms that carbon amendment treatment is an effective treatment for decreasing plant biomass and temporarily lowering soil nitrogen levels. But seed rain into our small plots likely negated any possible restoration benefits of the amendments. Better controls on external influences to plots are needed if this procedure is to become a practical tool for land restoration.

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