AN ABSTRACT OF THE THESIS OF

Dana L. Witwicki for the degree of Master of Science in Environmental Sciences presented on June 3, 2005.

Title: Sugar Application and Nitrogen Pools in Wyoming Big Sagebrush Communities and Exotic Annual Grasslands.

Abstract approved:

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Paul S. Doescher David A. Pyke

Within *Artemisia tridentata* ssp. *wyomingensis* Nutt. (Wyoming big sagebrush) communities of the Great Basin, lands dominated by the exotic annual grass *Bromus tectorum* L. (cheatgrass) are increasing at an alarming rate. Carbon applications, which reduce plant-available soil N, have been suggested as a way to give native vegetation a competitive advantage over exotic annual grasses when reseeding after a fire. The main objectives of my study were to 1) compare N pools in intact *A. tridentata*- and *B. tectorum*-dominated communities to look for evidence of ecosystem changes associated with annual grass invasion, and 2) quantify and compare the effects of sugar and nitrogen additions on N pools in each of these communities. Research occurred at six sites in eastern Oregon and southwestern Idaho, each containing pairs of *A. tridentata* and exotic annual grass communities in close proximity. Pairs were carefully selected with similar soil types, precipitation, elevation, aspect, slope and ecological sites (i.e. potential vegetation and production). At the beginning of the cheatgrass growing season (late fall), three treatments (sugar, nitrogen or control) were applied. For soil pools, only one difference between untreated plots of annual and native communities was detected. In autumn, NO_3^- in the native community was about two-thirds the level in the annual community. The sugar treatment decreased inorganic N to near undetectable

levels one week after application, and levels remained low six months later (during peak *B. tectorum* biomass). Although the sugar treatment did not increase microbial biomass N from chloroform fumigation extraction, we found higher ^{15}N in microbial biomass and soil organic matter, suggesting that more N remained in the microbial pool over the growing season. The reduction of aboveground plant biomass by sugar and the increase of aboveground plant biomass from nitrogen addition were more pronounced for *B. tectorum* than for native plants. Plant responses indicated that treatment with labile carbon like sugar may be useful tools for restoration of native plants and for prevention of *B. tectorum* dominance, but additional research is necessary to quantify dose responses of *B. tectorum* to sugar.

Sugar Application and Nitrogen Pools in Wyoming Big Sagebrush Communities and Exotic Annual Grasslands

by

Dana L. Witwicki

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APPROVED:

Co-Major Professor, representing Environmental Sciences

Co-Major Professor, representing Environmental Sciences

Director of the Environmental Sciences Program

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Dana L. Witwicki, Author

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure Page

LIST OF TABLES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

Sugar Application and Nitrogen Pools in Wyoming Big Sagebrush Communities and Exotic Annual Grasslands

INTRODUCTION

Within *Artemisia tridentata ssp. wyomingensis* Nutt. (Wyoming big sagebrush) communities of the Great Basin, lands dominated by the exotic annual grass *Bromus tectorum* L. (cheatgrass) are increasing at an alarming rate. *B. tectorum* produces seed prolifically and competes successfully with native perennial bunchgrasses and *A. tridentata* seedlings. *B. tectorum* seedlings develop extensive fine root systems throughout the winter and early spring (Pyke and Novak 1994), allowing them to absorb water and accompanying nutrients more efficiently (Melgoza et al. 1990) and earlier in the growing season than native perennials. Periodic disturbance, especially by wildfire, helps *B. tectorum* establish near monocultures with little remaining native vegetation (Knapp 1996). This has raised concerns over the loss of native biodiversity and resulting changes in critical ecosystem processes (Billings 1992). Once established, these annual grass communities appear to cross an ecological threshold that prevents succession to sagebrush steppe, making restoration difficult (Laycock 1991). To successfully restore native plants on *B. tectorum*-degraded lands, it may be necessary to understand how this annual grass alters ecosystem processes such as nutrient cycling. Accordingly, this research focuses on potential changes in nitrogen pools associated with conversion of *A. tridentata* communities to *B. tectorum*-dominated communities.

When *B. tectorum* invades and outcompetes native vegetation, landscapes lose plant species diversity and heterogeneity (Whisenant 1990), which may additionally affect ecosystem processes. The structure of the plant community changes from biological soil crusts interspersed with *A. tridentata* and bunchgrasses to a near continuous cover of *B. tectorum* (Knapp 1996). Soil nutrient cycling can be influenced by plant species (Hobbie 1992) or plant functional group (Vinton and Burke 1995) through changes in litter quality and quantity, and these differences are most obvious in more labile soil C and N pools (Wedin and Pastor 1993, Vinton and Burke 1995). In *A.*

tridentata and other arid to semiarid communities, "islands of fertility" form on shrub-covered microsites (Charley and West 1975). When *A. tridentata* communities lose their shrub component due to mechanical removal or fire, nutrients are dispersed across the landscape (Burke et al. 1987, Halvorson et al. 1997). Conversion of *A. tridentata* communities to *B. tectorum*-dominated communities decreases spatial heterogeneity of soil nutrients (Bolton et al. 1990), increases decomposition rates in the upper layer of soil, and decreases stable carbon pools at deeper soil depths (Norton et al. 2004).

How *B. tectorum* invasion alters nitrogen cycling is a topic of considerable debate. Invasive species can increase soil N through N_2 fixation (Vitousek and Walker 1989) or decrease soil N through repeated loss from fires (D'Antonio and Vitousek 1992). Since *B. tectorum* increases fire frequencies in sagebrush steppe (Whisenant 1990), we might expect a decrease in total soil N over time. Even without fire, *B. tectorum* invasion decreases available N in the soil because its higher litter C:N than native grasses increases immobilization of N into soil microorganisms (Evans et al. 2001). There is also evidence that *B. tectorum* invasion leads to changes in the composition of soil microbial communities (Belnap and Phillips 2001), but how much this involves microorganisms that mediate soil N transformations is not known. However, comparisons at the landscape level showed little or no difference in N mineralization rates between areas dominated by *B. tectorum* and those dominated by native *A. tridentata* shrub steppe in the Columbia Basin, WA (Bolton et al. 1990, Svejcar and Sheley 2001). It is apparent that we do not have a clear understanding *B. tectorum's* effect on N cycling and that other factors could be critical, such as previous land use (including grazing and agriculture), fire history, seasonal timing of measurements, or nitrogen sampling method. In fact, none of these studies took place on land converted to *B. tectorum* by fire, the main mechanism for *B. tectorum* dominance in the Great Basin (Knapp 1996).

Better understanding of nitrogen cycling in these communities could lead to more successful restoration strategies in *B. tectorum*-invaded areas. Although past restoration efforts have successfully seeded degraded rangelands with introduced

perennial grasses such as *Agropyron desertorum* (Fisch.) Schult. (crested wheatgrass), high failure rates have accompanied attempts to seed native perennial vegetation (Young 1994). Past research suggests *B. tectorum* seedlings successfully compete with young *Chrysothamnus* spp., *A. tridentata*, *Stipa comata* Trin. and Rupr., and other native perennial bunchgrasses (Melgoza et al. 1990, Yoder and Caldwell 2002, Booth et al. 2003). Restoration failures may also be due to ecosystem changes in N cycling related to *B. tectorum* invasion and loss of the native shrub component. An alternative restoration approach uses carbon to manipulate N availability to plants. Carbon, applied as sugar, straw, or other organic material with a high C:N ratio, works by feeding carbon-limited soil microorganisms, allowing them to immobilize inorganic N normally available to plants (Zink and Allen 1998, Schaeffer et al. 2003). This favors slow-growing, low N-tolerant species over fast-growing species, such as *B. tectorum,* that need abundant N to thrive. Maintaining low soil inorganic N should result in an increase in relative abundance of native perennials and a decrease in relative abundance of invasive annuals (McLendon and Redente 1992, Paschke et al. 2000).

Carbon addition as a restoration treatment has gained considerable interest, but few studies have tested the assumed mechanism of N limitation along with plant response (Zink and Allen 1998, Morghan and Seastedt 1999, Alpert and Maron 2000). To use this tool appropriately, it is necessary to understand both ecosystem N dynamics and the effects of carbon treatment on N pools in target plant communities. The focus of this research was to 1) compare N pools in intact *A. tridentata* and *B. tectorum*-dominated communities to look for evidence of ecosystem changes associated with annual grass invasion, and 2) quantify and compare the effects of sugar and nitrogen additions on N pools in each of these communities, especially on biomass and tissue chemistry of dominant plants.

MATERIALS AND METHODS

Field sites and sampling

Six sites were selected in the northern Great Basin: three in eastern Oregon and three in southwestern Idaho. Each site contains a pair of native *A. tridentata* and exotic annual grass community plots located in close proximity (no further than 3 km apart). To account for confounding factors, pairs were carefully selected with similar soil types, precipitation, elevation, aspect, slope, and ecological sites (i.e. potential vegetation and production). All sites receive between 203-305 mm of mean annual precipitation and were historically dominated by *A. tridentata* ssp. *wyomingensis* (hereafter referred to as *A. tridentata*). All of the annual grasslands have burned within the last 25 years. The date of the last fire is unknown at all native sites, but likely exceeds 50 years, the period of fire records in the area. Historically, all sites have been grazed by livestock but were fenced for the duration of the experiment. Native communities have intact *A. tridentata*, varying bunchgrass and biological soil crust cover, and less than 15% exotic annual grass cover. The exotic annual grass communities are dominated by *B. tectorum* but also have a significant cover of *Poa secunda* Presl. (Sandberg's bluegrass). *Taeniatherum caput-medusae* (L.) Nevski (medusahead) is present at three of the annual grass sites. Additionally, all annual grass sites have no remaining shrub component and less than 5% native bunchgrass cover (excluding *P. secunda*). Hereafter, the plant communities described above will be referred to as "native" and "annual". Appendices A and B contain additional information on site characteristics. Appendices C and D contain additional information on field and laboratory techniques.

Each community plot contains three 5- x 5-m treatment plots to which nitrogen (ammonium nitrate [NH_4NO_3] at an application rate of 100 kg N/ha), sugar (sucrose at an application rate of 2000 kg C/ha), or no treatment (control) was randomly applied. Treatments were hand dispersed on 28-30 October 2003, before annual grass germination. To incorporate treatments into the soil, deionized water was applied to all

plots using a backpack sprayer to simulate a 0.5 mm rainfall event. On 2- x 2-m plots located within each main treatment plot, ninety-nine atom percent ^{15}N-labeled NH_4NO_3 was added to the water and applied at a rate of 0.525 g NH_4NO_3/m^2. This application rate did not significantly increase available N (fertilization effect), but allowed detection of ^{15}N in various pools to trace N cycling over the growing season. ^{15}N tracer was added only to smaller subplots due to high costs.

Because N should have a distinctly different spatial distribution between the two plant communities, and I wanted to accurately describe nitrogen pools at the community level, a different number of soil samples were collected in treatment plots: one soil sample in annual plots and three soil samples in native plots (under sagebrush, under bunchgrasses, and in interspace between plants). Each sample consisted of five 10-cm-depth soil cores that were homogenized and passed through a 2 mm sieve in the lab before further analysis.

Soil was collected on 20 October 2003 (pretreatment), 6 November 2003 (post-treatment), and 9 May 2004 (peak biomass) and analyzed for inorganic N (NH_4^+-N and NO_3^--N), total soluble N (inorganic N and dissolved organic N), potential net N mineralization, microbial biomass N, total N, total C, C:N, and water content. The amount of ^{15}N in total soluble N, microbial biomass N, and bulk soil was determined for the two later sampling dates. Plant tissue for C, $\delta^{13}C$, N, and ^{15}N analysis was collected within one week of the soil collection (2-4 May 2004). Aboveground plant biomass was harvested during peak biomass for each plant group (21-26 May 2004 for *B. tectorum*; 15-17 June 2004 for *A. tridentata* and bunchgrasses).

To examine plant-available N over the entire growing season, ion-exchange resin capsules (Unibest Inc., Bozeman, MT) were buried 10 cm beneath a column of undisturbed soil from October 2003 to May 2004. Two resin capsules were placed under *B. tectorum* in annual plots and under each of the following in native plots: *A. tridentata*, bunchgrasses, and interspace.

Soil nitrogen analysis

Within 24 hours of collection, resin capsules were extracted on a shaker table for 1 hour using 60 mL of 2 M KCl and poured through Whatman #42 paper filters. Extracts were analyzed colorimetrically for NH_4^+-N and NO_3^--N using an Alpkem RFA 300 at the Central Analytical Laboratory (CAL) at Oregon State University (Corvallis, OR).

Inorganic soil N (NH_4^+-N and NO_3^--N) and total soluble N were determined using 100 mL 0.5 M K_2SO_4 to extract 10 g soil for each sample. Samples were placed on a shaker table for one hour, filtered, and frozen until analysis. Microbial biomass N was determined using the chloroform fumigation extraction (CFE) method (Horwath and Paul 1994). Ten grams of soil were placed in glass beakers and fumigated for three days in a glass desiccator with ethanol-free chloroform ($CHCl_3$). Soil samples were then extracted with K_2SO_4 as outlined above. Potassium persulfate digests were performed to transform dissolved organic N to NO_3^- and allow for ^{15}N analysis. Extracts were digested in tightly sealed 50 mL test tubes using a 1:1 ratio of sample to oxidizing solution (Cabrera and Beare 1993), autoclaved at 120º C for 50 minutes, and diluted 10 times with deionized water. All soil extracts were analyzed on an Alpkem RFA 300 at CAL. Microbial biomass N was determined using equations by Horwath and Paul (1994), however, a correction factor was not used since we could not quantify the efficiency of extraction.

A 28-day laboratory incubation was performed to estimate potential net N mineralization (Hart et al. 1994). For each sample, ten grams of soil were placed in a plastic specimen cup, and moisture levels were maintained at 60% water-filled pore space at room temperature for the duration of the incubation. At the end of the incubation, soils were extracted with K_2SO_4, filtered, and analyzed using the same techniques detailed above. Net N mineralized was calculated using equations by Hart et al. (1994).

Gravimetric water content was determined by drying 10 g of each soil at 105° C for 48 hours. Before plots were treated (October 2003), total soil N, total soil C, and soil

C:N by mass were determined by combustion on a Costech Elemental Analyzer, Model 4010 (Costech Analytical Technologies, Inc., Valencia, CA). After plots were treated, bulk soil was analyzed additionally for ^{15}N on a Europa 20/20 SL Continuous Flow Isotope Ratio Mass Spectrometer (Europa Scientific, Crewe U.K.) at the Utah State University Stable Isotope Laboratory (Logan, UT).

To determine the amount of ^{15}N tracer in the total soluble N and microbial biomass N pools, digested extracts were diffused for 6 days onto acidified discs according to the procedure by Stark and Hart (1996). Discs were dried, wrapped in tin cups and sent to the Utah State University Stable Isotope Laboratory for analysis. The amount of ^{15}N tracer in the microbial biomass (mb) pool was determined using values for ^{15}N atom % and N concentration (mg N/kg soil) in digested K_2SO_4 extracts (initial) and digested chloroform-fumigated K_2SO_4 extracts (fum) in the following mixing model:

$$^{15}N_{mb} = [(mass_{fum} * {}^{15}N_{fum}) - (mass_{initial} * {}^{15}N_{initial})] / mass_{mb}.$$

Plant cover, biomass, and tissue analysis

Initially, plant cover for each site was measured by using the line-point intercept technique (Herrick et al. 2005) with six 25 m transects and points taken every meter. At peak biomass, plot cover was estimated using line-point transects to collect data at 100 points located at regular intervals across each plot.

Aboveground biomass for dominant plant groups was estimated differently depending on plant functional group. Dominant cover in annual communities was *B. tectorum*; dominant cover in native communities included both *A. tridentata* ssp. *wyomingensis* and different species of bunchgrasses depending on site. All aboveground photosynthetic tissue was harvested for both *B. tectorum* and bunchgrasses. *B. tectorum* biomass was estimated by clipping biomass inside a 10- x 10-cm frame placed at regular intervals over 30 points within the plot. Bunchgrass biomass was estimated by clipping all live biomass in the plot except *P. secunda*, which

is smaller and ubiquitous in both native and annual communities. For some extremely dense plots, smaller areas (1/2 or 1/4 plots) were clipped and used to estimate entire plot biomass. For *A. tridentata*, persistent leaves and woody material (plant parts potentially produced before treatment application) were not used to estimate biomass. Instead, I harvested only new shoot growth as a representative measurement of current year's biomass. This was estimated by randomly locating a 10- x 10-cm frame at 4 points in an *A. tridentata* plant and clipping all new shoot growth in the volume beneath the frame. I repeated the measurement on a second shrub within the plot and used cover data to scale up to plot level. All plant biomass was dried at 65° C for 48 hours before being weighed. Plant shoots for tissue analysis were collected from representative species, dried at 65° C for 48 hours, ground, and analyzed for total N, ^{15}N, C, and $\delta^{13}C$.

Statistical analysis

For all comparisons, ANOVA's were performed in SAS 8.2 using PROC MIXED for split plot design (SAS Institute, Cary, NC). The six sites acted as blocks with community (native or annual) as split plots within each site. To obtain an integrated "plot level" estimate of each soil pool (NH_4^+-N, NO_3^--N, total soluble N, microbial biomass N, total N, total C, soil C:N, net N mineralized, and % water) for native communities, percent plant cover was used to weight pool means from sagebrush, interspace, and bunchgrass. Data were log-transformed when necessary to improve normality and variance, assumptions of parametric statistics. All comparisons made between treatments and controls were adjusted using Dunnett's method. Bonferroni adjustment for multiple comparisons was used for all other contrasts. Due to the large number of comparisons in this study, I present data with 95% confidence intervals and estimates of differences rather than p-values from hypothesis tests (Johnson 1999). Confidence intervals incorporate multiple comparison adjustments and provide visual comparisons of differences between means.

RESULTS

Soil pools

Soil characteristics in native plots varied between plants and interspace and between different plant functional groups as exemplified by data from October (Figure 1). There were no differences among native groups for NH_4^+-N and NO_3^--N, although soil under cheatgrass tended to be higher than all native groups for NO_3^--N. Total N, total C, and soil C:N followed the trend of *A. tridentata* > bunchgrasses > interspace with *B. tectorum* most similar to bunchgrasses. There was little difference between groups for microbial biomass N and soil water content. There was less net N mineralized in soils under interspace than in soils under sagebrush, but there were no differences between any other groups. Since this was not the primary focus of my research and trends were generally similar across dates, data from November and May will not be presented. The main purpose of these measurements was to accurately account for spatial heterogeneity in soils of native communities.

When measurements from October and November (before *B. tectorum* germination) were scaled up to the community level by weighting the means of each native group based on its cover, a difference in only one soil pool was detected. NO_3^- in the native community was about two-thirds the level in the annual community in untreated plots (Figure 2; Appendix E). In May, when all plants were actively growing, inorganic N pools were lower, but similar for both communities. There were no differences between annual and native communities in untreated plots on any sampling dates for NH_4^+-N, microbial biomass N, total N, total C, soil C:N, net N mineralized, and water content, although treatments did not always have the same effect on both communities.

Application of sugar and nitrogen produced the intended effects on most soil N pools. The sugar treatment decreased inorganic N (mainly NO_3^-) in November and May, and when integrated over the entire time of the experiment in resin capsules;

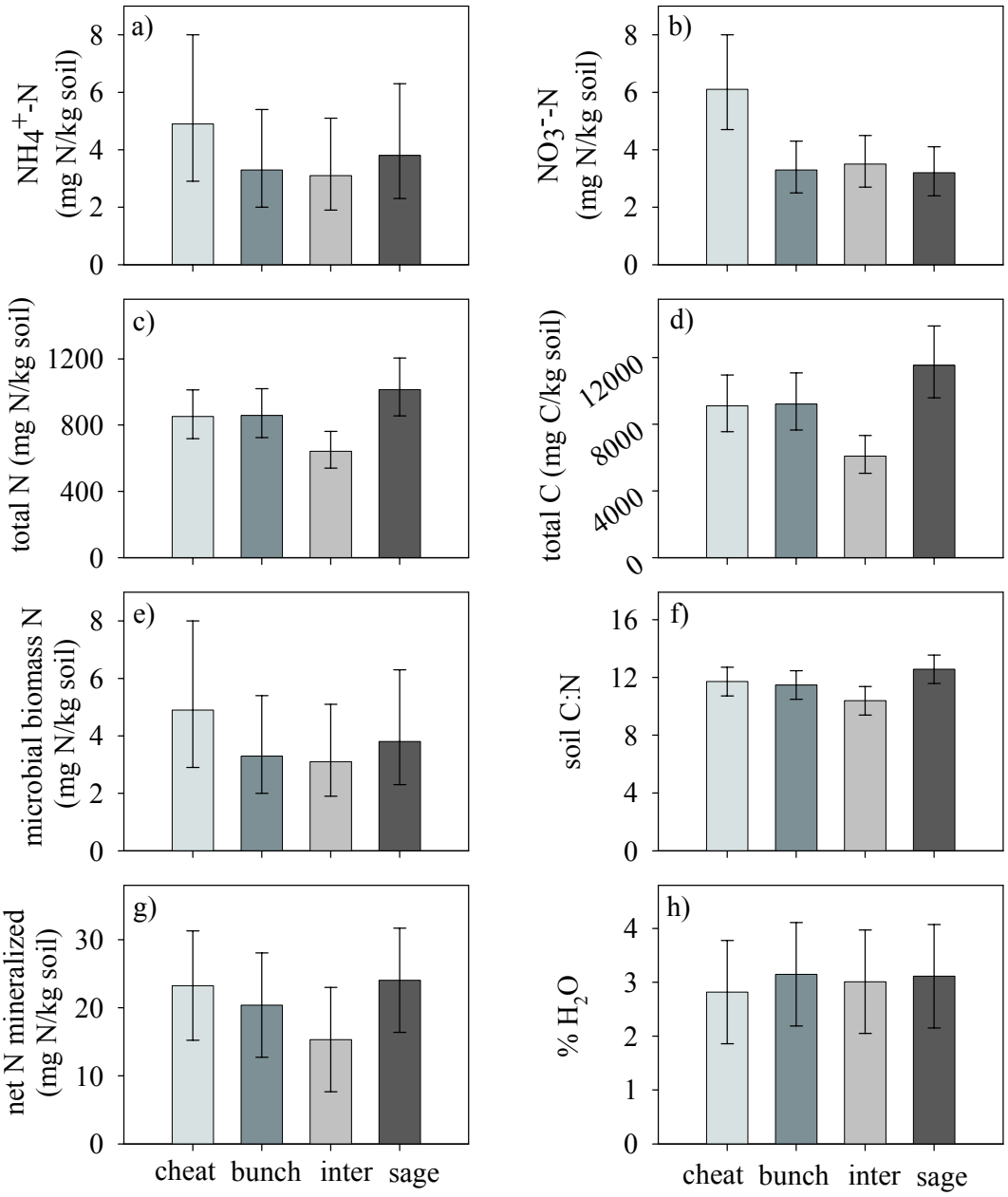

Figure 1. a) Back-transformed mean NH_4^+-N (mg N/ kg soil), b) back-transformed mean NO_3^--N (mg N/ kg soil), c) back-transformed mean total N (mg N/ kg soil), d) back-transformed mean total C (mg C/ kg soil), e) back-transformed mean microbial biomass N (mg N/ kg soil), f) mean soil C:N ratio, g) mean net N mineralized (mg N/ kg soil), and h) mean water content (% H_2O) in soil under *B. tectorum* (cheat) in annual communities and bunchgrasses (bunch), interspace (inter), and *A. tridentata* ssp. *wyomingensis* (sage) in native communities in October 2003. Bars represent 95% confidence intervals (α=0.025).

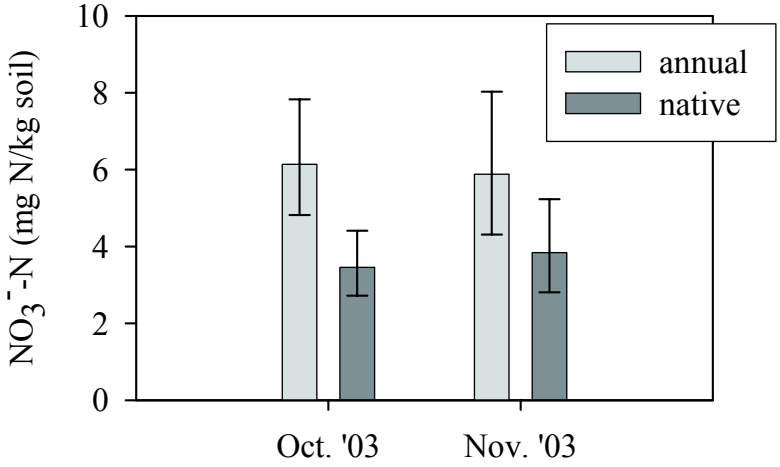

Figure 2. Back-transformed means of NO_3^--N (mg N/kg soil) in untreated plots of annual and native communities in October 2003 and November 2003. Note that only control plots were examined at the November sampling. Bars represent 95% confidence intervals.

nitrogen treatment had the opposite effect (Figure 3; Appendix E). Although treatments did not affect total soil N on either sampling date, soil C:N shifted somewhat in November in response to treatments, increasing from sugar treatment and decreasing from nitrogen treatment. These differences were negligible by May.

CFE resulted in no detectable increases in microbial biomass N from the sugar treatment (Figure 4; Appendix E). Instead, microbial biomass N decreased in nitrogen-treated native plots compared to the control in May. However, addition of sugar nearly doubled ^{15}N in microbial biomass and soil organic matter (SOM) compared to the control (Table 1). Similarly, the sugar treatment decreased net N mineralized in the November laboratory incubation (Figure 4), presumably the result of increased N immobilization in microbial biomass.

Soil pools additionally reflected plant community responses to treatments. For nitrogen-treated plots, plant-available N was consistently lower in annual communities than native communities, suggesting *B. tectorum's* superior ability to take advantage of higher available N. This was apparent for both NH_4^+-N and NO_3^--N in May (Figure 3;

Appendix E) and total soluble ^{15}N (Table 1). The sugar treatment limited access to N for plants in both communities, evidenced by more ^{15}N remaining in bulk soil (Table 1). More soil water remained in sugar-treated annual plots in both November and May, reflecting decreased water uptake by plants in this community (Figure 4; Appendix F).

^{15}N data collected for November were highly variable. This was only one week after treatment application, and apparently not enough time occurred for meaningful distribution of ^{15}N to various N pools. The only recognizable trend was higher ^{15}N in bulk soil of the native community. This does not necessarily indicate any important community difference because larger amounts of surface plant cover in annual plots probably delayed incorporation of ^{15}N into the soil. Although slightly more ^{15}N was recovered in May from control plots in the native community compared to the annual community (Table 1), this difference was not significant. Therefore, I will assume that similar amounts of ^{15}N had been incorporated into the soils of each community by May.

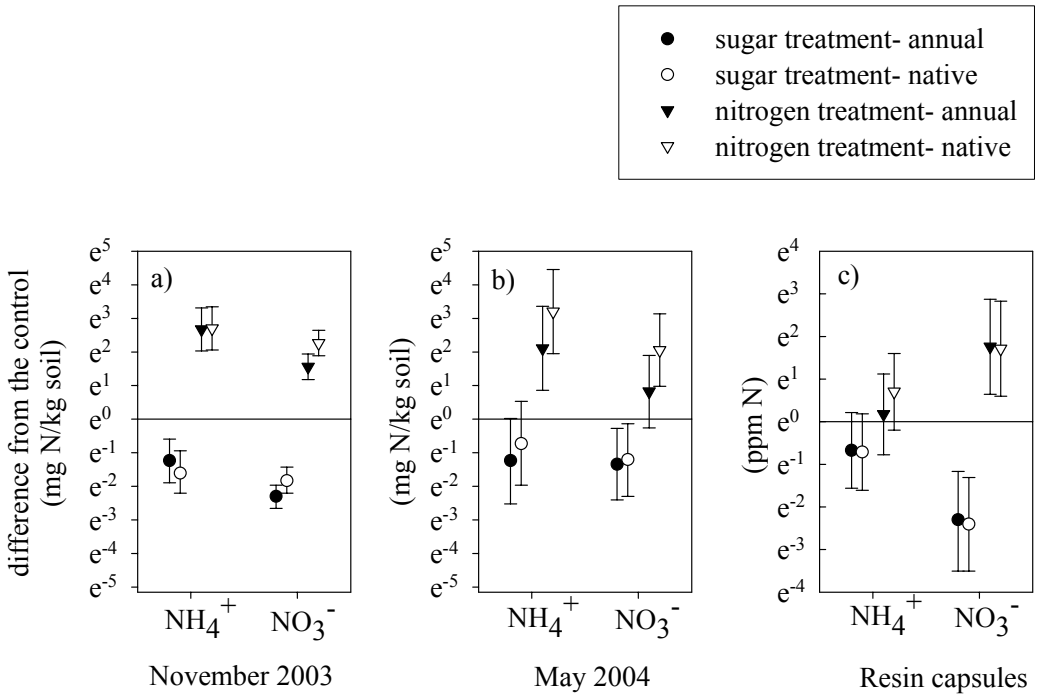

Figure 3. Differences of NH_4^+-N and NO_3^--N in sugar and nitrogen treatments compared to the control for a) November 2003 (mg N/kg soil), b) May 2004 (mg N/kg soil), and c) resin capsules (ppm N) in soil for the entire growing season (October 2003-May 2004). The reference line represents the control for each community. The y-axes are on the natural log scale. Bars represent 95% confidence intervals of the differences (α=0.025).

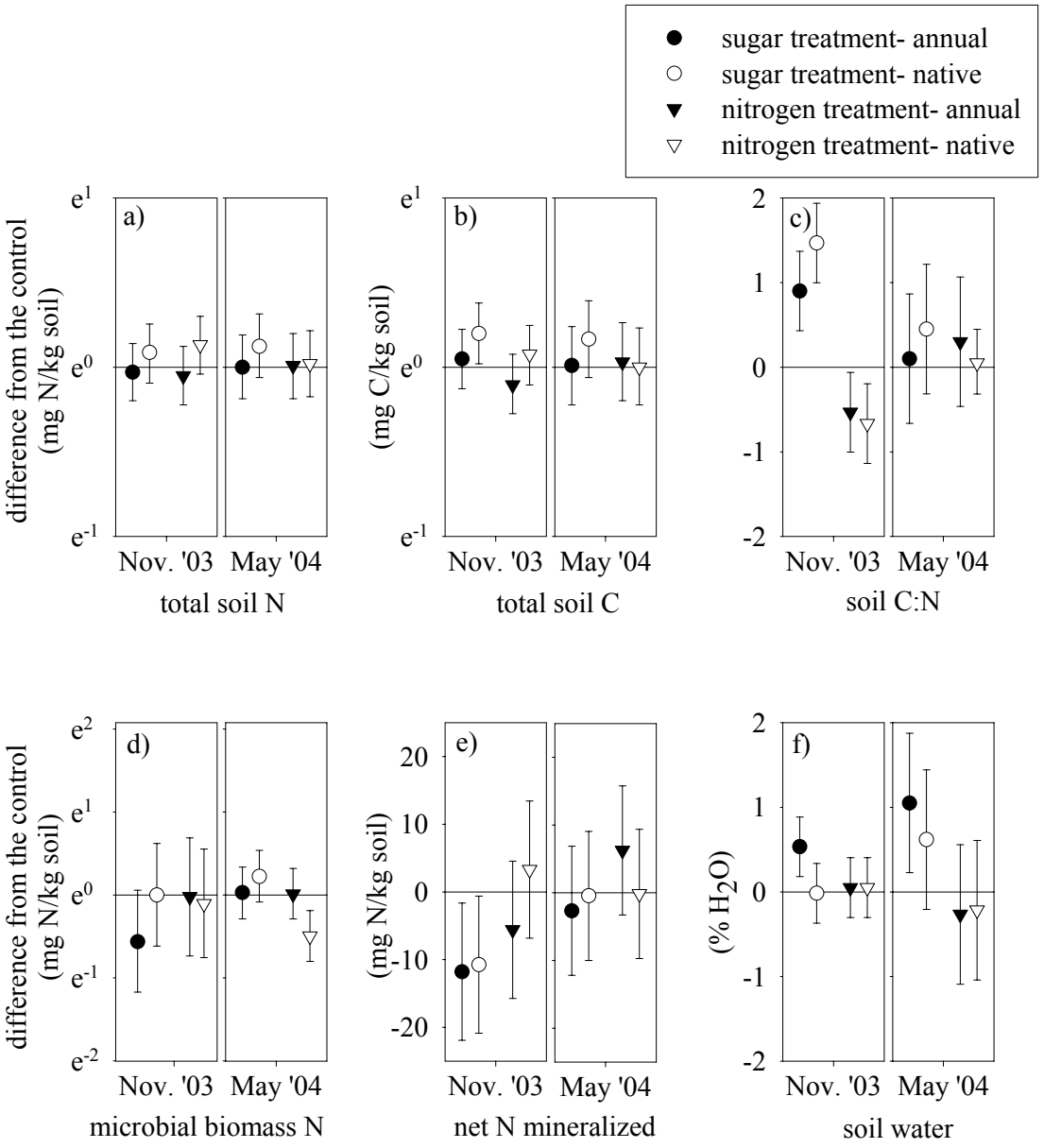

Figure 4. Differences of sugar and nitrogen treatments compared to the control for a) total soil N (mg N/kg soil), b) total soil C (mg C/kg soil), c) soil C:N, d) microbial biomass N (mg N/kg soil), e) net N mineralized (mg N/kg soil), and f) soil water (% H_2O). The y-axis for total soil N, total soil C, and microbial biomass N are presented on the natural log scale. The reference line (untransformed data = 0, natural log scale = 1) represents the control for each community. Bars represent 95% confidence intervals of the differences (α=0.025).

Table 1. Partitioning of ^{15}N tracer in soil of exotic annual grass and native *A. tridentata* communities six months after tracer addition. Soils were collected May 2004 during peak *B. tectorum* biomass. Parentheses contain 95% confidence intervals.

		% of added ^{15}N recovered in:			
treatment	community	total soil N	total soluble N	microbial biomass N	soil organic matter N
sugar	annual	45.2* (34.4-59.5)	2.1 (1.2-3.8)	2.9* (1.9-4.5)	40.2* (27.6-58.5)
	native	47.2* (35.9-62.0)	3.3 (1.9-5.8)	4.2* (2.7-6.5)	39.9* (27.4-58.1)
control	annual	21.4 (16.3-28.1)	3.0 (1.8-4.9)	1.6 (1.0-2.4)	16.4 (11.3-23.9)
	native	26.3 (20.0-34.6)	3.1 (1.8-5.3)	2.1 (1.3-3.2)	19.5 (13.4-28.4)
nitrogen	annual	17.5 (13.3-23.0)	5.2 (3.2-8.7)	1.1 (0.7-1.6)	8.9* (6.1-12.9)
	native	24.1 (18.3-31.6)	14.7* (8.9-24.5)	0.6* (0.4-1.0)	8.5* (5.9-12.4)

*indicates significant difference from control for each community (α=0.025)

Vegetation

The dominant plants in each community varied in tissue N concentration, aboveground biomass, and how each responded to sugar and nitrogen treatments (Figure 5). *A. tridentata* had higher tissue N than both bunchgrasses and *B. tectorum*, however, tissue N was similar in both grass groups. Since entire plot biomass was not harvested, comparisons were limited to treatment responses within plant functional groups. For *B. tectorum*, sugar decreased both biomass and tissue N, while nitrogen increased only biomass. *A. tridentata* responded similarly to sugar as *B. tectorum*, but increased tissue N in response to nitrogen. In contrast, neither treatment affected bunchgrass biomass, although tissue N increased with nitrogen addition. Tracer partitioning data, an integrated measure of biomass and tissue chemistry over the growing season, showed significantly less ^{15}N tracer recovered from *B. tectorum* and *A. tridentata* in sugar-treated plots (Table 2).

Plant tissue C:N shifted in response to treatments, with a decrease of plant C:N in bunchgrasses from the nitrogen treatment as the most pronounced response (Figure 6; Appendix G). Both treatments negatively affected plant tissue C, with the exception of bunchgrasses in nitrogen-treated plots (Figure 6; Appendix G).

Evaluation of $\delta^{13}C$ indicated results were opposite of predictions. There was no evidence that sugar treatment limited plant growth through water stress because $\delta^{13}C$ in photosynthetic plant tissue was not different from the control. Unexpectedly, nitrogen treatment decreased $\delta^{13}C$ in *B. tectorum* (Figure 7).

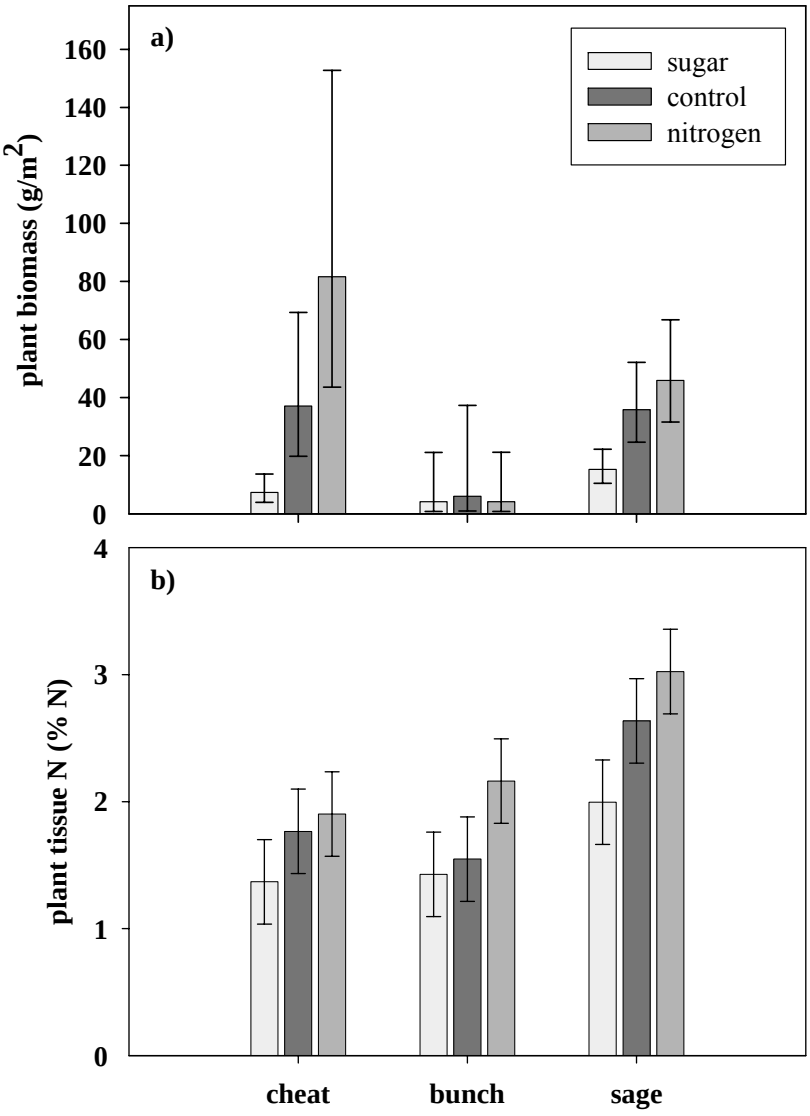

Figure 5. a) Back-transformed mean biomass (g/m^2), and b) mean tissue N (% N) for aboveground shoots of *B. tectorum* (cheat), bunchgrasses (bunch), and *A. tridentata* (sage). Bars represent 95% confidence intervals (α=0.025).

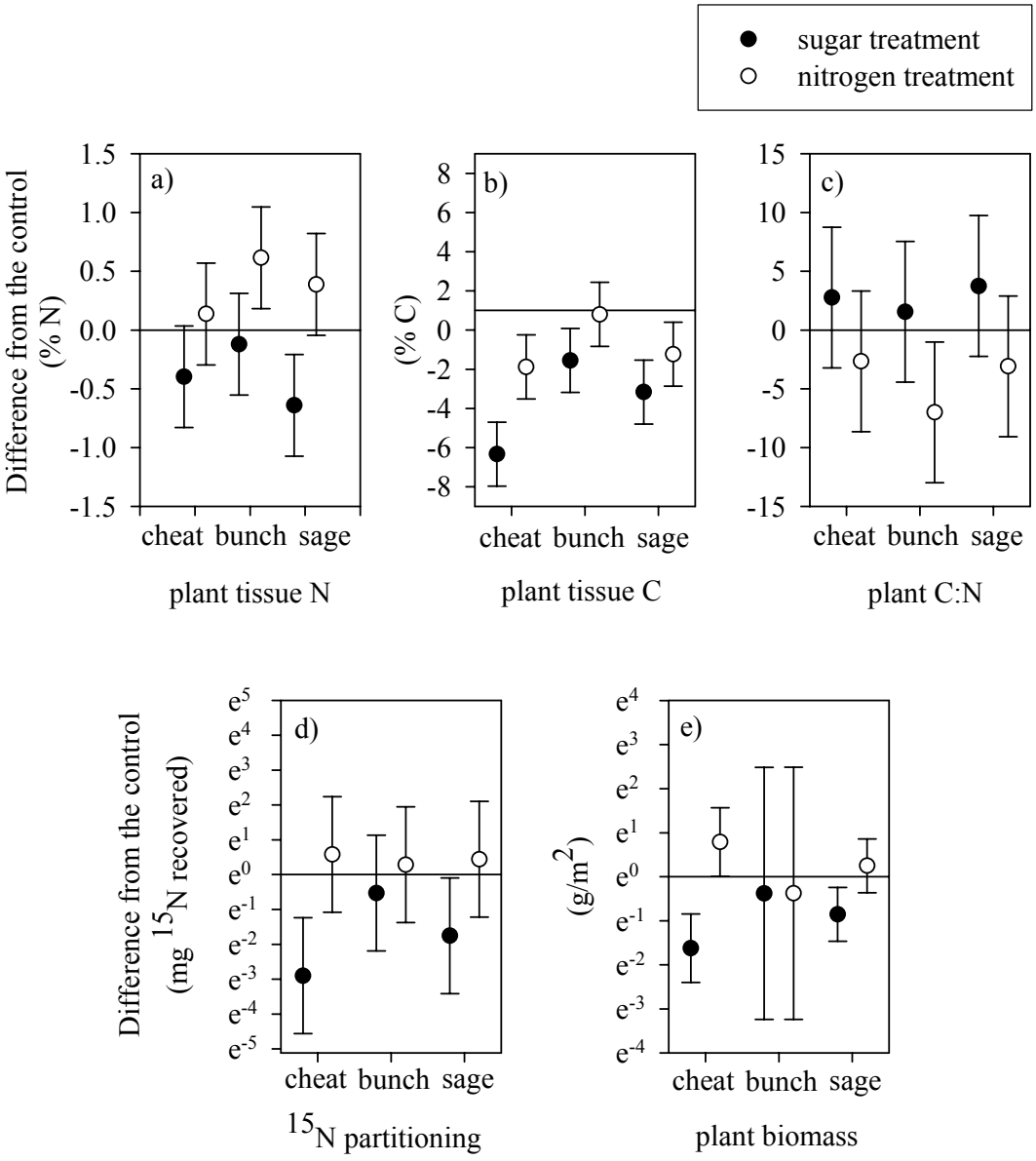

Figure 6. Differences of sugar and nitrogen treatments compared to the control for a) plant tissue N (% N), b) plant tissue C (% C), c) plant C:N, d) ^{15}N partitioning (mg ^{15}N recovered), and e) aboveground plant biomass (g/m^2) of *B. tectorum* (cheat), bunchgrasses (bunch), and *A. tridentata* (sage). The y-axis for ^{15}N partitioning and aboveground plant biomass are presented on the natural log scale. The reference line (untranformed data = 0, natural log scale = 1) represents the control for each community. Bars represent 95% confidence intervals of the differences (α=0.025).

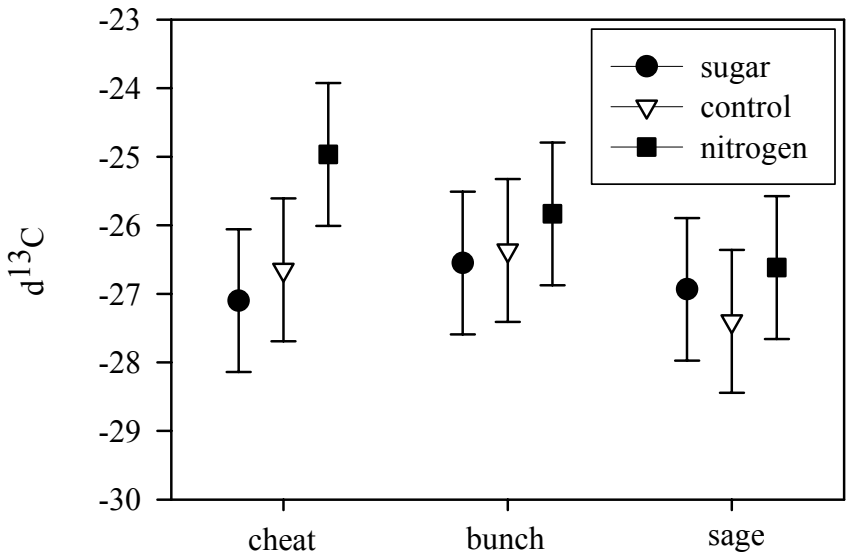

Figure 7. Mean $\delta^{13}C$ for current year's photosynthetic tissue in *B. tectorum* (cheat), bunchgrasses (bunch), and *A. tridentata* (sage). Error bars represent 95% confidence intervals (α=0.025).

Table 2. Partitioning of ^{15}N tracer in aboveground plant tissue of *B.tectorum* in exotic annual grass communities and bunchgrasses and *A. tridentata* in native communities at peak *B. tectorum* biomass. Parentheses contain 95% confidence intervals.

	% of added ^{15}N recovered in:		
	annual	native	
treatment	*B. tectorum*	bunchgrasses	*A. tridentata*
sugar	0.26* (0.07-0.95)	0.12 (0.03-0.45)	0.66* (0.18-2.42)
control	4.72 (1.30-17.19)	0.21 (0.06-0.75)	3.84 (1.06-14.00)
nitrogen	8.41 (2.31-30.62)	0.28 (0.08-1.00)	5.98 (1.64-21.79)

* significant difference from control (α=0.025)

DISCUSSION

We found little evidence of differences between soil N pools in the native and annual communities. The only difference in soil pools between communities was higher NO_3^--N in the annual community in October and November. At that time of year, native plants are mainly dormant but still have basic metabolic requirements. Cheatgrass, on the other hand, had not germinated yet, and therefore, was not taking up N from the soil. Seasonal increases in labile forms of N, mainly while cheatgrass lies in seed dormancy, could lead to a net loss of N from annual grass communities over time, however, we found no evidence of differences in total soil N or ^{15}N in annual grass communities converted 3 to 23 years ago. This period between annual grass senescence and the following year's seed germination is usually very dry (most rainfall occurs from late autumn through spring) so there is little opportunity for leaching. In fact, autumn rains typically stimulate cheatgrass germination (Mack and Pyke 1983), and N mobilized at this time is likely taken up by seedlings. However, we did not measure losses to system through leaching or denitrification, and potential losses during this transition from dry to wet could be important over longer time scales (Davidson et al. 1993).

Unlike soil pools, we saw clear differences in tissue chemistry between dominant plant groups in each community. Plant species affect nutrient cycling through differences in nutrient acquisition and loss, litter quality, and association with microbes (Hobbie 1992), and we would expect these differences between the native and annual communities to create an accompanying shift in N pools after *B. tectorum* invasion. This shift occurs spatially (Burke et al. 1987, Halvorson et al. 1997) and temporally, evidenced by greater NO_3^--N in annual communities during late summer and autumn (Svejcar and Sheley 2001). In a controlled laboratory experiment, *A. tridentata* and *A. desertorum* caused subtle but significant changes in soil C and N cycling (Chen and Stark 2000). We saw differences in soil C and N pools under bunchgrasses, interspace, and *A. tridentata* in native plots, but when scaled up to plot level, there were no

differences between native and annual communities. This is consistent with the results of other comparisons of *A. tridentata* and nearby *B. tectorum*-converted communities in the Columbia Basin (Bolton et al. 1990, Svejcar and Sheley 2001). Although few differences in soil N pools were detected in these studies, soil N is highly variable, and a greater sample size would be necessary to detect small changes in N pools between communities (n=1-6 in these studies). It is likely that the differences in plant tissue chemistry and biomass create feedbacks influencing soil N and C cycling (Wedin and Tilman 1990, Chen and Stark 2000), promoting the alternative stable state of *B. tectorum* dominance. This has been evidenced outside of the laboratory by Evans et al. (2001), who found greater N immobilization and decreased plant-available N with *B. tectorum* invasion on the Colorado plateau.

Soil pools generally responded to sugar and nitrogen treatments as predicted, but unexpectedly, sugar treatment did not increase microbial biomass N in either November or May. November sampling occurred only one week after treatment application, yet nearly all of the sugar had visibly been incorporated into the soil from water applied with treatments and precipitation during that period. Soil inorganic N in sugar-treated plots decreased to extremely low levels in November and May and, presumably, had shifted to the microbial pool. In the lab incubation performed in November, less net N was mineralized in soils from sugar-treated plots, indicating inorganic N immobilization in microbial biomass. Additionally, we recovered more ^{15}N from microbial biomass and SOM in sugar-treated plots than in control plots, demonstrating that, over the course of the growing season, N remained in organic forms unavailable to plants. Although inorganic N can be immobilized abiotically into SOM (Johnson et al. 2000), higher ^{15}N recovered in both SOM and microbial biomass suggest that this was mainly a biological process in our system. Because our community experiences seasonal wet/dry stress, instantaneous measurements of the microbial pool using CFE are probably insufficient to accurately characterize this pool. Others have experienced problems getting accurate estimates of microbial biomass N in drier soils using CFE (Sparling and West 1989,

Gallardo and Schlesinger 1992), and ^{15}N provides a more reliable estimate of the microbial pool integrated over the entire growing season.

Additional investigation of sugar's mechanism of N reduction using $\delta^{13}C$ natural abundance in photosynthetic plant tissue showed no evidence that sugar alters osmotic properties of soil water. If sugar decreased soil water potentials, thereby decreasing availability of soil water to plants, we would expect less negative $\delta^{13}C$ in plants from sugar-treated plots than control plots. Less negative $\delta^{13}C$ would indicate that plants discriminated less against ^{13}C because of decreased stomatal aperture from water stress (Farquhar et al. 1982). Yet, no differences in $\delta^{13}C$ between plants from the sugar treatment and the control were observed. Nitrogen treatment decreased $\delta^{13}C$ in *B. tectorum* instead. Nitrogen fertilization may increase drought stress because of larger plant size and higher transpiration rates (Harvey and Van Den Driessche 1999). Additionally, rates of photosynthesis may increase with N fertilization, decreasing carbon isotope discrimination regardless of stomatal aperture (Field and Mooney 1986). Both likely explain the decreased $\delta^{13}C$ in *B. tectorum*, however, the first explanation is consistent with my findings that *B. tectorum* increased biomass but not tissue N.

We saw clear differences in the way dominant plant groups in each community responded to sugar and nitrogen treatments. Nitrogen addition increased *B. tectorum* biomass more than native plant biomass, supporting the theory that dominance by annuals in sagebrush steppe is related to high nutrient availability (McLendon and Redente 1991, 1992). Alternatively, nitrogen addition increased tissue N in *A. tridentata* and bunchgrasses but not in *B. tectorum*. Similar results were found by Redente et al. (1992), including more dramatic decreases in biomass of early seral species with soil N reduction. Although sugar treatment decreased aboveground biomass and tissue N in both *B. tectorum* and *A. tridentata*, cheatgrass tended to have a greater reduction of biomass while *A. tridentata* had a greater reduction of tissue N. Additionally, sugar reduced ^{15}N recovered in *B. tectorum* more dramatically than ^{15}N recovered in *A. tridentata*.

In this study at the community level, sugar treatment did not affect bunchgrass tissue N or biomass. Without competition and under low N availability from straw additions, root and shoot growth of *B. tectorum* was equal to or greater than that of perennial bunchgrasses (Monaco et al. 2003). This does not correspond with my results, however, I question the accuracy of my bunchgrass biomass sampling method. Bunchgrass biomass within plots was highly variable due to its patchy distribution across the landscape, and we were unable to detect any changes resulting from treatments. In future studies evaluating treatment responses, I recommend comparing pre- and post-treatment measurements on individual bunchgrasses or greatly increasing plot size.

Since I was not able to destructively harvest entire plots or collect root biomass, inferences of plant community response were limited, especially for ^{15}N tracer partitioning. Individual plant groups responded as expected, although uncertainty in bunchgrass biomass results weakens our ^{15}N findings for this group. Roots in *A. tridentata* communities compose a significant portion of biomass, and Monaco et al. (2003) found that perennial grasses generally had higher root N while annual grasses generally had higher shoot N. Therefore, it is likely that we underestimated ^{15}N taken up by native plants.

Differential plant responses indicate that sugar has potential as a restoration treatment. Adding water after sugar application allows microorganisms to rapidly absorb this simple carbon compound, thus ensuring treatments stay in target areas. We also wanted to limit plant-available N the entire time that *B. tectorum* could potentially germinate, which could be as early as November or as late as May depending on climatic conditions. A single large dose of sugar was logistically practical as a restoration treatment and had potential for long term limitation of plant-available N. Although the cost of our treatment was extremely high ($4000/ha), a carbon source that remains in target areas and provides long term N limitation is highly desirable. Therefore, cost-effective alternatives should be investigated. Additionally, little work

has been done on responses of invasive annual grasses in the Great Basin to different doses of sugar, and lower doses of sugar could potentially provide similar results.

Most restoration treatments in the Great Basin involving sugar have used it in conjunction with native plant seeding on recently burned *B. tectorum*-dominated land. Our study is unique in that we applied sugar to both annual and native communities. Much of the remaining sagebrush in close proximity to annual grasslands has a dense understory of *B. tectorum* and, therefore, a high risk of losing the shrub component with repeated burns. Although not the main focus of this research, cover data from native sites suggest that sugar application has potential to inhibit further *B. tectorum* invasion and restore greater relative native plant cover in these areas. Sugar dramatically reduced *B. tectorum* biomass within native plots (personal observation), however, more research is needed to understand the degree and length of this reduction and whether perennials would retain a relevant competitive advantage over time.

CONCLUSION

Although soils in native and annual communities have similar N pools at the landscape level, plants within these communities responded differently to sugar and nitrogen treatments. Large doses of sugar decrease plant-available N for extended periods of time and highly reduce aboveground biomass of *B. tectorum*. Evidence that sugar reduces biomass more in *B. tectorum* than native plants suggests that this could be a useful tool for restoration of native plants and prevention of *B. tectorum* dominance. Additional research is necessary to assess the long term success of sugar additions. Also, investigating responses of native and exotic annual species to different doses of sugar would provide useful information for potentially interested land managers. Moreover, research on ecosystem level changes (such as soil microbial community structure) caused by cheatgrass invasion would be useful for directing successful restoration strategies.

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APPENDIX

Appendix A. Site Descriptions

Table 1. Description of Idaho field sites

site	plant community	elevation (m)	latitude/ longitude	ecological site description	soil depth (cm)*	last known fire(s)	current dominant vegetation
Bowns Creek	native	1010	43°20'00" 115°56'00"	loamy 10-12" *A. tridentata* / *A. spicatum*	42	unknown (>50 years)	*Artemisia tridentata ssp. wyomingensis, Agropyron spicatum, Poa secunda*
	annual	1010	43°20"00" 115°55'00"		39	1982	*Bromus tectorum, T. caput- medusa, P. secunda*
Mayfield	native	1095	43°22'00" 115°51'00"	loamy 10-12" *A. tridentata* / *A. spicatum*	25	unknown (>50 years)	*A. tridentata, Elymus elymoides, P. secunda*
	annual	1065	43°22'00" 115°50'00"		29	1985	*B. tectorum, Taniantherum caput-medusa, P. secunda*
Nicholson Road	native	855	43°24'00" 116°29'00"	loamy 8-10" *A. tridentata* / *A. spicatum*	30	unknown (>50 years)	*A. tridentata, P. secunda*
	annual	860	43°24'00" 116°29'00"		55	1987	*B. tectorum, P. secunda*

* depth to clay or hardpan

Table 2. Description of Oregon field sites

site	plant community	elevation (m)	latitude/ longitude	soil texture*	soil depth (cm)**	last known fire(s)	current dominant vegetation
Shell Rock Butte	native	1056	43°45'45" 117°15'55"	silt loam	45	unknown (>50 years)	*Artemisia tridentata ssp. wyomingensis, Agropyron spicatum, Poa secunda*
	annual	1059	43°45"46" 117°15'50"		46	1996	*Bromus tectorum, P. secunda*
Two Forks	native	953	43°48'47" 117°18'01"	loam	94+	unknown (>50 years)	*A. tridentata, A. spicatum, Stipa thurberii, P. secunda*
	annual	970	43°48'32" 117°18'15"		94+	1996, 2002	*B. tectorum, P. secunda*
Lincoln Bench	native	865	43°53'20" 117°08'13"	silt loam	36	unknown (>50 years)	*A. tridentata, A. spicatum, S. thurberii, Elymus elymoides, P. secunda*
	annual	866	43°53'33" 117°08'15"		40	1999	*B. tectorum, Taniantherum caput-medusa, P. secunda*

*Ecological site descriptions were not available for Oregon sites so soil for each site was characterized (see Appendix B)
** depth to clay or hardpan

Appendix B. Oregon Soil Descriptions

Shell Rock Butte Experimental Site- Annual Community

General site description: south-facing, 6% slope angle with no curvature, 5% surface stoniness.

A: 0-7cm; pale brown (10YR6/3) silt loam; brown (10YR4/3) moist; moderate, medium granular structure; many, very fine roots; all pores filled with roots; slightly sticky and slightly plastic; no effervescence.

B_t: 7-36cm; pale brown (10YR6/3) clay silt; brown (10YR4/3) moist; moderate, subangular blocky structure; common, very fine roots; many, very fine, dendritic tubular and vesicular pores; moderately sticky and slightly plastic; no effervescence.

B: 36-46cm; yellow brown (10YR5/4) loam; dark yellow brown (10YR3/4) moist; weak subangular blocky structure; moderately few, fine roots; common, fine and medium, irregular and dendritic tubular pores; slightly sticky and moderately plastic; no effervescence.

C or B_m: below 46cm.

Comments: This boundary between the Bt and B horizon is classified as “broken”, because the B horizon is discontinuous pockets that vary in depth. We believe these pockets are silt-filled old sagebrush root pores because a few dead roots were observed in these pockets.

Shell Rock Butte Experimental Site- Native Community

General site description: south-facing, 8% slope angle with no curvature, 1% surface stoniness.

A: 0-8cm; pale brown (10YR6/3) silt loam; dark brown (10YR3/3) moist; very fine, granular structure; many, very fine roots; all pores filled with roots; non-sticky and moderately plastic; no effervescence.

B_t: 8-31cm; brown (10YR5/3) clay loam; dark brown (10YR3/3) moist; moderate, subangular blocky structure; few, very fine roots; common, very fine, dendritic tubular pores; slightly sticky and very plastic; no effervescence.

B: 31-45cm; pale brown (10YR6/3) silt loam; brown (10YR4/3) moist; weak, very fine, subangular blocky structure; very few, fine roots; common, fine, dendritic tubular pores; slightly sticky and slightly plastic; no effervescence.

C: below 45cm; >75% rock with silt and small rocks.

Two Forks Experimental Site- Annual Community

General site description: northwest aspect, 8% slope angle with concave curvature, located on crest of slope, 2% surface stoniness.

A: 0-20cm; brown (10YR5/3) loam; dark yellow brown (10YR4/3) moist; fine, weak granular structure; many, very fine roots; common, very fine, dendritic tubular pores; non-sticky and moderately plastic; no effervescence.

B_t: 20-37cm; pale brown (10YR5/3) sandy clay loam; dark brown (10YR4/3) moist; moderate, medium, angular blocky structure; moderately few, very fine roots; many, very fine, dendritic tubular pores; moderately sticky and moderately plastic; no effervescence.

B_2: 37-94cm; very pale brown (10YR6/3) silt loam; dark brown (10YR4/3) moist; weak, medium, massive to granular structure; very few, very fine roots; very few, very fine, dendritic tubular pores; non-sticky and moderately plastic; no effervescence.

Two Forks Experimental Site- Native Community

General site description: northwest aspect, 5% slope angle with slightly concave curvature, located on crest of slope, 5% surface stoniness.

A: 0-17cm; light brown gray (10YR6/2) loam; dark brown gray (10YR4/2) moist; weak, fine granular, platy structure; many, very fine roots; many, very fine, dendritic tubular pores; non-sticky and moderately plastic; no effervescence.

B_t: 17-40cm; brown (10YR5/3) clay loam; dark brown (10YR3/3) moist; moderate, medium, subangular blocky structure; few, very fine roots; many, very fine, dendritic tubular pores; slightly sticky and very plastic; no effervescence.

B_2: 40-94cm; pale brown (10YR6/3) silt loam; dark brown (10YR3/3) moist; very weak, very fine, massive granular structure; very few, very fine roots; very few, very fine, dendritic tubular pores; non-sticky and moderately plastic; no effervescence.

Lincoln Bench Experimental Site- Annual Community

General site description: east facing, 15% slope angle with concave curvature, located on backslope, 0% surface stoniness.

A_1: 0-12cm; pale brown (10YR6/3) silt loam; brown (10YR4/3) moist; granular structure; many, very fine roots; many, very fine, dendritic tubular pores; slightly sticky and slightly plastic; no effervescence.

A_2: 12-26cm; pale brown (10YR6/3) silt loam; dark brown (10YR3/3) moist; angular blocky structure; moderately few, very fine roots; common, very fine, dendritic tubular pores; slightly sticky and moderately plastic; no effervescence.

AB: 26-40cm; pale brown (10YR6/3) silt clay; brown (10YR6/3) moist; angular blocky structure; common, very fine roots; very few, common, dendritic tubular pores; slightly sticky and very plastic; no effervescence.

B_t: below 40cm; brown (10YR5/3) clay; brown (10YR4/3) moist; subangular blocky structure; very few, very fine roots; very fine, dendritic tubular pores; moderately sticky and very plastic; no effervescence.

Lincoln Bench Experimental Site- Native Community

General site description: north-facing, 8% slope angle with convex curvature, located on backslope, 2% surface stoniness.

A: 0-18cm; pale brown (10YR6/3) silt loam; dark brown (10YR3/3) moist; weak, fine granular structure; moderately few, very fine roots; many, very fine, dendritic tubular pores; non-sticky and slightly plastic; no effervescence.

AB: 18-36cm; pale brown (10YR6/3) silty clay; brown (10YR4/3) moist; moderate, fine, angular blocky structure; moderately few, very fine roots; many, very fine, dendritic tubular pores; very sticky and very plastic; no effervescence.

B_t: below 36cm; pale brown (10YR6/3) clay; brown (10YR4/3) moist; medium, moderate, angular blocky structure; moderately few, very fine roots; common, very fine, dendritic tubular pores; very sticky and very plastic; no effervescence.

Appendix C. Field Methods

Soil Collection and Processing Protocol

Materials needed:
2 small diameter soil corers
ziplock bags/Fisher bags
portable hanging balance
large cooler
cool packs
sharpie
2mm sieve

Procedure:

1) For cheatgrass, collect 5 soil cores from each plot using a ¾ inch inner diameter soil corer. This should be about 80g of soil; a portable hanging balance can be used to double check this amount. Collect soils from 0-10cm depth. Combine soils in a ziplock bag.

2) For native plots, collect 5 soil cores from interspace to specifications above. Soil should be collected at least 40cm from bunchgrasses and sagebrush.

3) Repeat soil collection under sagebrush. Collect soils within 15cm from base of sagebrush.

4) Repeat soil collection under bunchgrasses. Collect soils within 10cm from base of bunchgrass.

5) Chill soils in a cooler or refrigerator until they can be processed in the lab. Do not place directly on ice! Soils should be processed within 24 hours.

6) Mix soils thoroughly within bag.

7) Pass soils through a 2mm sieve.

Resin Insertion Protocol

Materials needed:
Unibest resin capsules
plastic tweezers
latex gloves
spade
hory hory knife
flags with plastic stems
ruler

Procedures:
Use spade to remove a plug from the soil surface. Use the hory hory knife to deepen the hole to just over 10cm and create a clean wall on one side. At 10 cm, use the hory hory knife to make a small pocket underneath an undisturbed column of soil. Try to make the hole just big enough to fit the resin capsule. Insert the resin capsule with plastic tweezers and cover the hole with soil. Mark the resin capsule location with a plastic-stemmed flag. It is also a good idea to draw a map of resin placement within the plot in case a flag is blown out of place. For native plots, I marked S=sagebrush, B=bunchgrass and I=interspace on flags.

Treatment Protocol

Materials needed:
2- 5 gallon field king backpack sprayers
adjustable wrench (to attach pumping lever)
water carboys (total of 100 gallon volume)
1000mL graduated cylinders
paper lunch bags
portable balance for weighing sugar
tube for siphoning

Chemicals needed:
Ammonium nitrate (NH_4NO_3) (Application rate = 2000 kg C/ha/yr)
Cane sugar (sucrose) (Application rate = 100 kg N/ha/yr)
^{15}N double-labeled ammonium nitrate

^{15}N preparation:
Weigh 2.10g double-labeled ^{15}N enriched ammonium nitrate. Place in 20mL scintillation vial. Add 15mL deionized water to vial. Seal tightly. Make 36 vials.

Ammonium nitrate preparation:
Weigh 714.17g ammonium nitrate and place in a paper bag. This is the amount to be applied to each plot. Weigh 12 bags.

Sugar preparation:
An application rate of 2000 kg C/ha/yr means that 11.88kg (26.2lb) of sugar will be spread on each plot. This equals one 25 lb bag of sugar plus 544.3g (1.2lb) sugar. This volume will be determined and will be applied in addition the 25lb bag.

Procedure:
1) Hand spread one 25lb bag and one 1.2lb scoop of sugar in each carbon treatment plot.
2) For the nitrogen plots, hand spread one bag of ammonium nitrate.
3) Add 1.66 liter (0.44 gallon) deionized water to the enriched backpack sprayer. Then add the vial of ^{15}N. Mix thoroughly and spray on $4m^2$ isotope plot. Repeat at other two plots at the site.
4) Add 10.52 liters (2.78 gallons) deionized water to the "water only" sprayer. (This volume is marked on the DI only backpack sprayer). Use a hose to siphon water from the heavy jugs. Spray water evenly over the non-isotope enriched plot area. Repeat at other two sites at plot.

Appendix D. Laboratory Methods

Soil Processing Protocol

Material needed:
sieved soil
balance
scoopula
soil tins
drying oven
237mL sample cups with lids
120mL sample cups with lids
folded Whatman 42 filter paper (12.5cm circles)
6.5cm funnels
funnel holder
20mL scintillation vials
larger Nalgene bottles
squirt bottle with K_2SO_4
100mL bottle pump
large glass bottle with K_2SO_4
shaker table
bungee cords
boxes (for shaker table)
vacuum desiccators
vacuum pump
vacuum grease
fume hood
30mL beakers
50mL Erlenmeyer flasks
boiling chips
dark-colored garbage bags
incubator
latex gloves

Chemicals needed:
0.5M K_2SO_4
ethanol-free chloroform

Gravimetric Soil Moisture

Material needed:
sieved soil
balance
scoopula
soil tins
drying oven

Procedure:
1) Weigh 10 g soil into a numbered soil tin. Record weight of tin and soil. Place tin in drying over at 105C for 48 hours. Record mass of dry soil and tin. Determine percent moisture.

Inorganic N & Total Soluble N

Material needed:
sieved soil
balance
scoopula
237mL sample cups with lids
folded Whatman 42 filter paper (12.5cm circles)
6.5cm funnels
funnel holder
20mL scintillation vials
computer labels
squirt bottle with K_2SO_4
100mL bottle pump
large glass bottle with K_2SO_4
shaker table
bungee cords
boxes (for shaker table)

Chemicals needed:
0.5M K_2SO_4

Procedure:
1) Weigh 20 g soil for initial K_2SO_4 extraction. Place in 237mL sample cup. Add 200mL 0.5M K_2SO_4. Shake for 1 hour on shaker table. (Shaker speed should be set at 180.) Allow to settle 24 hours.

2) Place folded Whatman 42 paper filters in 6.5cm diameter funnels. Rinse filters with 10-20mL K_2SO_4 and allow to sit for 5 minutes. Pour samples in funnel and collect filtrate in labeled 257mL sample cups. Place 20mL of solution in a scintillation vial for initial inorganic nitrogen analysis. Place 20mL in another scintillation vial for persulfate digest. Keep the rest of the soil in the large sample cup for inorganic nitrogen diffusions.

Chloroform Fumigation Extraction

Material needed:
sieved soil
balance
scoopula
120mL sample cups with lids
folded Whatman 42 filter paper (12.5cm circles)
6.5cm funnels
funnel holder
20mL scintillation vials
computer labels
squirt bottle with K_2SO_4
100mL bottle pump
large glass bottle with K_2SO_4
shaker table
bungee cords
boxes (for shaker table)
vacuum desiccators
vacuum pump
vacuum grease
fume hood
30mL beakers
50mL Erlenmeyer flasks
boiling chips
dark-colored garbage bags
incubators

Chemicals needed:
0.5M K_2SO_4
ethanol-free chloroform

Procedure:
1) Weigh 10 g soil into a 30mL beaker for chloroform fumigation. Place samples in a vacuum desiccator. Add 50mL ethanol-free chloroform and boiling chips to a 50mL Erlenmeyer flask; place in vacuum desiccator. Seal desiccator and place in fume hood; apply vacuum pump until chloroform boils. Release seal; evacuate for a minute; release seal again. Repeat 2 more times. Evacuate 3 minutes and keep sealed in fume hood. Place dark-colored garbage bag over desiccators. Incubate for 3 days.

2) Transfer soil to labeled 120mL sample cups. Add 100mL 0.5M K_2SO_4 to each cup. Shake for 1 hour on shaker table. (Set shaker speed at 180.) Allow to settle for 24 hours.

3) Place folded Whatman 42 paper filters in 6.5cm diameter funnels. Rinse filters with 10-20mL K_2SO_4 and allow to sit for 5 minutes. Pour samples in funnel and collect filtrate in labeled scintillation vials.

Persulfate Digest Protocol

Persulfate digestion oxidizes organic N to NO_3^- under alkaline pH (with NaOH), with measurement on autoanalyzer as NO_3^-.

** Always digest standards (3 reps) and matrix sufficient to supply the autoanalyzer.

Materials needed:
Pyrex screw top test tubes (25mm x 150mm)
Nalgene caps (28mm wide mouth)
pipetter (0.5mL)
pipette tips
Glycine N standards
1000mL volumetric flask
100mL volumetric flasks (5)
1L bottle
large Erlenmeyer flask to digest matrix
1.5 L container to hold matrix solution
DI water autopipetter (7.5 mL)

autoclavable baking dishes (to hold water)
autoclavable test tube racks
autoclave
scintillation vials

Chemicals needed:
low nitrogen $K_2S_2O_8$ (potassium persulfate)
10N NaOH

Procedure:

Glycine N Standards:
Glycine (NH_2CH_2COOH) FW=75.07
To make 100mgN/L stock solution: $\frac{100}{14} \times 75.07 \div 1000 \, {}^{mL}\!/_{L} = 0.5362 \, {}^{g}\!/_{L}$
Add 0.536 g glycine to a 1000 mL volumetric flask. Bring to volume using DI water, store in 1 L container and refrigerate.
To make standards, pipette (0.125, 0.25, 0.685, 1.25, 1.875) mL stock solution into 250 mL volumetric flasks. Record actual mass of stock solution added. Bring to volume using K_2SO_4. Label and pour into small beakers for easier pipetting.

Matrix:
Add (1:1 ratio) 200 mL K_2SO_4 and 200 mL OX to a large Erlenmeyer flask. Cover the top with foil and fasten securely with labeling tape. Place in autoclave with samples.

Persulfate Digest Oxidizing Solution (OX):
Make OX fresh in an Erlenmeyer flask, gently heated and stirred:
10 g $K_2S_2O_8$, 200 mL DI water, 7.64 mL 10 N NaOH

Without ^{15}N diffusion:
- Pipet 0.5 mL OX and 0.5 mL sample into clean test tube
- Cap immediately
- Place in autoclavable baking dish with water to sample level
- Autoclave for 50 minutes at 120 C
- Remove samples and allow to cool 30 minutes
- Dilute with 4.5mL DI water, cap, ready for nitrate analysis

With ^{15}N diffusion:
- Pipet appropriate amount of sample and OX into 150 mL test tube (5-80 mL; you may have to use 2 test tubes)
- Cap immediately
- Place in autoclavable container with water to sample level

- Autoclave for 50 minutes at 120 C
- Remove samples and allow to cool 30 minutes
- Pour digest into specimen container for ^{15}N diffusion
- Pipet 0.5 mL of digest into clean test tube, dilute with 4.5 mL DI water, cap, ready for nitrate analysis
- Rinse digested test-tube with 3 mL DI water and transfer to diffusion cup

Don't forget to do some duplicate samples!

^{15}N Diffusion Protocol

Materials needed:
latex gloves
craft paper
12.5mm wide PTFE (teflon tape)
paper punch (with 7mm holes)
#1 Whatman filter paper
0.5 microliter pipetter
0.5 microliter pipette tips
stainless steel wire
11 mm glass culture tube
microforceps
Devarda's alloy scoop
MgO scoop
NaCl scoop
glass beads
5 by 8 mm tin cups

Chemicals needed:
K_2SO_4
concentrated H_2SO_4 (sulfuric acid)
Devarda's alloy
MgO
NaCl

2.5M $KHSO_4$ Preparation:
Add 7mL concentrated H_2SO_4 and 22g C to 50mL DI water. Bring up to 100mL volume and stir until dissolved. Store refrigerated in small container. Use the same 2.5M $KHSO_4$ for all discs throughout research.

Prepare ahead of time:
Make 7mm discs by punching holes in Whatman #1 filter paper. Rinse 3 times with deionized water and dry in an oven at 35C. Store discs in a sealed bag. Acid wash glass beads, dry and store. Wash stainless steel wires with K_2SO_4 then rinse 3 times with DI. Dry and store in a clean container.

Procedure:
Cover lab surface with craft paper. Cut PTFE tape into 7 cm length strips. Pipette 5 microliters of 2.5 M $KHSO_4$ onto each disc. Place 2 discs about 4 mm apart on one half of the strip of PTFE tape (3.5 cm). Fold the other half of the PTFE strip over to cover the discs and gently smooth it out to remove wrinkles. Use the open end of an 11 mm glass culture tube to seal the PTFE around each disc. Prepare all of the acid traps before setting up diffusion cups.

Add solution to a specimen cup (amount will depend on the ^{15}N and total N in the solution.)

5-40mL for digested initial samples
5-30mL for digested fumigated samples

Add a few clean glass beads and NaCl (increases saltiness so that water will not collect around discs) to each specimen cup. (1.5g NaCl per 30mL K_2SO_4 effectively eliminates water accumulation around discs) I used a minimum of 2 scoops and added 1 scoop for every extra 10 mL of sample. Complete this step for all specimen cups before proceeding.

Place one acid trap on the surface of the solution and add 1 scoop of MgO (increases pH so ammonium will volatilize to ammonia) and 1 scoop of Devarda's alloy. Cap and swirl. Include blanks and standards.

Incubate solution at 22C for 6 days. Invert containers 2 or 3 times every three days to eliminate droplets that form on the sides of the containers. MgO will settle and form a crust. Tap container and use beads to break apart the crust.

At the end of the incubation, remove the acid traps with forceps, dip briefly in deionized water, and place on Kimwipe. Dry carefully so that no fluid touches the disc. Peal away PTFE with forceps and impale each disc with a stainless steel wire. Placing a few layers of paper on lab surface makes it easier to impale the disc. Use a clean paper clip to push the disc onto the wire. Impale both discs from an acid trap on the same wire. Place wire on drying rack in desiccator containing concentrated H_2SO_4 and dry for at least 4 hours. Have an Excel sheet ready with location of discs on the drying rack.

Wrap dry disks in 5 by 8 mm tin capsules. Wrap both discs together unless you suspect that one is not good. Analyze for total N and ^{15}N enrichment on a continuous-flow direct combustion mass spectrometer. Make appropriate blanks and standards.

Standard solution:
Make 10gN/L stock solution by adding 180mg 99 atom % NH_4Cl and 3.6g NH_4Cl (natural abundance = 0.3663 atom %) to a 100mL volumetric flask. Store in tightly sealed clean Nalgene container.

Diffused standards:
Pipette 5µL standard in K_2SO_4 (20, 60, 100 µL K_2SO_4). Make 3 replicates.
Add MgO and Devarda's alloy and diffuse with other samples.

Non-diffused standards:
Pipette 5µL ^{15}N NO_3 standard directly on 1 disc. Make 3 replicates. Complete this step when you remove discs from pillows on day 6, then place on drying rack with samples.

Potential Nitrogen Mineralization

Material needed:
sieved soil
balance
scoopula
237mL sample cups with lids
folded Whatman 42 filter paper (12.5cm circles)
6.5cm funnels
funnel holder
20mL scintillation vials
computer labels
squirt bottle with K_2SO_4
100mL bottle pump
large glass bottle with K_2SO_4
shaker table
bungee cords
boxes (for shaker table)
incubator

Chemicals needed:
0.5M K_2SO_4

Procedure:
1) Record mass of labeled 118mL sample cup and lid. Place 10 g of soil into sample cup and screw the lid on loosely.

2) Place in incubator at 25 degrees Celsius for 28 days.

3) Determine the gravimetric water content of the samples. Adjust water in sample cup to 60% water-filled pore space, and be sure that the water adjusted soils are well mixed. Reweigh samples.

3) Periodically check weigh of samples. Add deionized water as necessary to bring samples back to correct percent moisture.

4) On day 28, add 100mL 0.5M K_2SO_4 to each cup. Shake for 1 hour on shaker table. (Set shaker speed at 180.) Allow to settle 24 hours.

5) Place folded Whatman 42 paper filters in 6.5cm diameter funnels. Rinse filters with 10-20mL K_2SO_4 and allow drip. Pour samples in funnel and collect filtrate in labeled scintillation vials.

Total Soil N & C Protocol:

Materials needed:
drying oven
mortar and pestle
wig-L-bug grinder
timer
20mL scintillation vials
tins cups
microbalance
microbalance tweezers, scoop, brush
96-well sample tray

Procedure:
Place soils in drying oven for 48 hours at 105ºC. Grind sample with mortar and pestle and place in clean dry scintillation vial labeled "coarsely ground" in addition to other identifying information.

Place a fraction of the sample in the wig-l-bug grinder with metal ball. Make sure not to fill capsule more than 1/3 with sample. Grind for 60 seconds. Place in new scintillation vial labeled "finely ground" in addition to other identifying information. Repeat grinding 2 more capsules of the sample. Repeat for all samples.

Calculate amount of sample necessary to have 80-120 µg N/sample or at least 50 µg N if you have extremely low N samples. Do not place more than 80 mg of soil into the tin cup. Check any other specifications for the lab that will run your samples.

Dry all samples for 24 hours at 105ºC before weighing. Weigh appropriate amount of sample and wrap in tin cup. Place sample in 96-well sample tray and identify samples on a data sheet for the mass spec lab. Keep samples is dessicator until they are mailed to mass spec lab.

Resin Processing Protocol

Materials needed:
resin capsules
plastic tweezers
120mL sample cups
folded Whatman 42 filter paper (12.5cm circles)
6.5cm funnels
funnel holder
20mL scintillation vials
larger Nalgene bottles
squirt bottle with 2M KCl
100mL bottle pump
large bottle with 2M KCl
shaker table
bungee cords
boxes (for shaker table)

Chemicals needed:
2M KCl

Procedure:
1) Place resin capsule in 120mL sample cup using plastic tweezers. Add 60 mL 2M KCl. Shake for 1 hour on shaker table. (Set shaker speed at 180.) Allow to settle overnight.

2) Place folded Whatman 42 paper filters in 6.5cm diameter funnels. Rinse filters with 10-20mL 2M KCl and allow to drain completely. Pour samples in funnel and collect filtrate in 2 or 3 labeled scintillation vials.

Plant Tissue Processing Protocol:

Materials needed:
drying oven
wig-L-bug grinder
mortar and pestle
dry ice chips
timer
20mL scintillation vials
tins cups
microbalance
microbalance tweezers, scoop, brush
96-well sample tray

Procedure:
Place plant material in drying oven for 48 hours at 65ºC. Grind sample with mortar and pestle and dry ice chips, and place in clean dry scintillation vial labeled "coarsely ground" in addition to other identifying information. Place scintillation vial with lid removed in drying oven for 24 hours at 65ºC (make sure not to mix up sample lids).

Place a fraction of the sample in the wig-l-bug grinder with metal ball. Make sure not to fill capsule more than 1/3 with sample. Grind for 60 seconds. Place in new scintillation vial labeled "finely ground" in addition to other identifying information. Repeat grinding 2 more capsules of the sample. Repeat for all samples.

Dry all samples for 24 hours at 65ºC before weighing. Weigh appropriate amount of sample and wrap in tin cup. Place sample in 96-well sample tray and identify samples on a data sheet for the mass spec lab. Keep samples is dessicator until they are mailed to mass spec lab.

	amount of plant tissue in sample (mg)		
treatment	sage	bunchgrass	cheat
sugar	4	4	5
control	4	4	5
nitrogen	3	3	4

Appendix E. Soils N Pools

Table 3. Back-transformed mean soil N pools and C:N ratios on 3 sampling dates for native *A. tridentata* and exotic annual grass communities treated Oct. 28-30, 2003 with sugar, no treatment (control), or nitrogen. Parentheses contain 95% confidence intervals.

		Soil N pool (mg N/kg soil)				
Date/ treatment	Community	NH_4^+-N	NO_3^--N*	Microbial biomass N	Total N	C:N ratio
Oct. 20, 2003						
sugar	annual	7.3 (3.9-13.5)	6.9 (5.3-9.0)	6.5 (3.5-12.2)	1239 (938-1635)	11.7 (10.5-12.9)
	native	3.2 (1.7-5.9)	3.4 (2.6-4.4)	7.7 (4.3-13.8)	977 (740-1290)	11.3 (10.1-12.5)
control	annual	3.8 (2.0-7.0)	6.1 (4.7-7.9)	6.2 (3.5-11.0)	1046 (792-1391)	11.6 (10.4-12.8)
	native	3.3 (1.8-6.1)	3.5 (2.7-4.5)	8.1 (4.6-14.5)	988 (748-1305)	11.3 (10.1-12.5)
nitrogen	annual	4.1 (2.2-7.7)	5.5 (4.3-7.2)	10.5 (5.9-18.6)	1013 (767-1337)	11.9 (10.7-13.1)
	native	4.4 (2.4-8.2)	3.5 (2.7-4.6)	7.6 (4.3-13.5)	970 (735-1281)	11.1 (9.9-12.3)

* indicates significant difference between annual and native communities (α=0.05)

Table 3. (Continued) Back-transformed mean soil N pools and C:N ratios on 3 sampling dates for native *A. tridentata* and exotic annual grass communities treated Oct. 28-30, 2003 with sugar, no treatment (control), or nitrogen. Parentheses contain 95% confidence intervals.

		Soil N pool (mg N/kg soil)				
Date/ treatment	Community	NH_4^+-N	NO_3^--N	Microbial biomass N	Total N	C:N ratio
Nov. 6, 2003						
sugar	annual	0.5* (0.3-0.8)	0.6* (0.4-0.8)	5.2* (3.0-9.0)	953 (788-1154)	12.0* (11.5-12.5)
	native	0.5* (0.3-0.8)	0.6* (0.5-0.9)	11.4 (6.6-19.6)	956 (790-1157)	12.5* (12.0-12.9)
control	annual	1.7 (1.1-2.7)	5.9** (4.3-8.0)	9.2 (5.4-15.8)	982 (811-1189)	11.1 (10.6-11.6)
	native	2.4 (1.6-3.9)	3.8 (2.8-5.2)	11.4 (6.6-19.6)	881 (727-1066)	11.0 (10.5-11.5)
nitrogen	annual	25.1* (16.0-39.5)	27.9* (20.4-38.0)	9.0 (4.8-17.1)	933 (771-1130)	10.5* (10.0-11.0)
	native	36.4* (23.1-57.3)	36.9* (27.0-50.3)	10.3 (5.7-18.5)	1003 (829-1215)	10.3* (9.8-10.8)

*indicates significant difference from control for each community (α=0.025)
****** indicates significant difference between annual and native community for NO_3^--N in control (α=0.025)

Table 3. (Continued) Back-transformed mean soil N pools and C:N ratios on 3 sampling dates for native *A. tridentata* and exotic annual grass communities treated Oct. 28-30, 2003 with sugar, no treatment (control), or nitrogen. Parentheses contain 95% confidence intervals.

		Soil N pool (mg N/kg soil)				
Date/ treatment	Community	NH_4^+-N	NO_3^--N	Microbial biomass N	Total N	C:N ratio
May 9, 2004						
sugar	annual	0.2 (0.1-0.6)	0.1* (0.0-0.3)	12.1 (9.3-15.9)	850 (700-1033)	10.7 (10.0-11.3)
	native	0.4* (0.1-0.4)	0.1* (0.0-0.3)	13.9 (10.6-18.1)	855 (704-1039)	10.7 (10.0-11.3)
control	annual	0.7 (0.2-2.1)	0.5 (0.2-1.2)	11.8 (9.0-15.4)	851 (700-1033)	10.6 (9.9-11.2)
	native	0.9 (0.3-2.8)	0.3 (0.1-0.9)	11.1 (8.5-14.4)	754 (621-916)	10.3 (9.6-10.9)
nitrogen	annual	5.7* (1.8-17.7)	1.0 (0.4-2.8)	12.0 (9.2-15.7)	856 (705-1040)	10.9 (10.2-11.5)
	native	22.3* (7.1-69.6)	2.6* (1.0-7.8)	6.8* (5.2-8.8)	770 (634-936)	9.9 (9.3-10.5)

*indicates significant difference from control for each community (α=0.025)

Appendix F. Soil Water

Table 4. Gravimetric soil water content (% H_2O) of annual and native communities for 3 sampling dates. Parentheses indicate 95% confidence intervals.

		% H_2O	
date	treatment	annual	native
October 20, 2003	sugar	2.9 (2.0-3.8)	3.1 (2.1-4.0)
	control	2.9 (2.0-3.9)	3.1 (2.2-4.0)
	nitrogen	2.6 (1.7-3.5)	3.0 (2.0-3.9)
November 6, 2003	sugar	3.9* (2.7-5.0)	3.6 (2.5-4.7)
	control	3.3 (2.2-4.4)	3.7 (2.5-4.8)
	nitrogen	3.4 (2.3-4.5)	3.7 (2.6-4.8)
May 9, 2004	sugar	6.7* (4.8-8.5)	6.1 (4.2-8.0)
	control	5.6 (3.7-7.5)	5.5 (3.6-7.3)
	nitrogen	5.3 (3.5-7.2)	5.3 (3.4-7.1)

* significant difference from control (α=0.025)

Appendix G. Plant C and N Pools

Table 5. C:N, % N, and % C for current year's photosynthetic tissue of *B. tectorum* in annual grass communities and bunchgrasses and *A. tridentata* in native communities. Parentheses indicate 95% confidence intervals.

plant	treatment	plant C:N	plant tissue N (% N)	plant tissue C (% C)
B. tectorum	sugar	27.3 (22.6-31.9)	1.4* (1.0-1.7)	35.9* (34.7-37.2)
	control	24.5 (19.9-29.1)	1.8 (1.4-2.1)	42.3 (41.0-43.5)
	nitrogen	21.8 (17.2-26.4)	1.9 (1.6-2.2)	40.4* (39.2-41.6)
bunchgrasses	sugar	27.9 (23.3-32.5)	1.4 (1.1-1.8)	38.4* (37.2-39.7)
	control	26.3 (21.7-31.0)	1.6 (1.2-1.9)	40.0 (38.8-41.2)
	nitrogen	21.8* (14.7-24.0)	2.2* (1.8-2.5)	40.8 (39.6-42.0)
A. tridentata	sugar	21.7 (17.0-26.3)	2.0* (1.7-2.3)	42.8* (41.6-44.0)
	control	14.8 (10.2-19.4)	2.6 (2.3-3.0)	46.0 (44.7-47.2)
	nitrogen	17.9 (13.3-22.5)	3.0* (2.7-3.4)	44.7 (43.5-45.9)

* significant difference from control (α=0.025)