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ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Nitrogen limitation, ^{15}N tracer retention, and growth response in intact and *Bromus tectorum*-invaded *Artemisia tridentata* ssp. *wyomingensis* communities

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Abstract Annual grass invasion into shrub-dominated ecosystems is associated with changes in nutrient cycling that may alter nitrogen (N) limitation and retention. Carbon (C) applications that reduce plant-available N have been suggested to give native perennial vegetation a competitive advantage over exotic annual grasses, but plant community and N retention responses to C addition remain poorly understood in these ecosystems. The main objectives of this study were to (1) evaluate the degree of N limitation of plant biomass in intact versus *B. tectorum*-invaded sagebrush communities, (2) determine if plant N limitation patterns are reflected in the strength of tracer ^{15}N retention over two growing seasons, and (3) assess if the strength of plant N limitation predicts the efficacy of carbon additions intended to reduce soil N availability and plant growth. Labile C additions reduced biomass of exotic annual species; however, growth of native *A. tridentata* shrubs also declined. Exotic annual and native perennial plant communities had divergent responses to added N, with *B. tectorum* displaying greater ability to use added N to rapidly increase aboveground biomass, and native perennials increasing their tissue N concentration but showing little growth response. Few differences in N pools between the annual and native communities were detected. In contrast to expectations, however, more ^{15}N was retained over two growing seasons in the invaded annual grass than in the native shrub community. Our data suggest that N cycling in converted exotic annual grasslands of the northern Intermountain West, USA, may retain N more strongly than previously thought.

Keywords Cheatgrass · Exotic annual grass · Sagebrush steppe · Nutrient amendments · Carbon addition

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Introduction

The spread and dominance of nonnative plant species within ecosystems is a key component of global change. Exotic annual grasses are problematic in many shrub steppe ecosystems worldwide due to their role in altering fire cycles and displacing historic vegetation communities (D'Antonio and Vitousek 1992). In North America, invasion by *Bromus tectorum* L. (cheatgrass) is one of the most studied examples due in part to the large area of land affected. *Bromus tectorum* dominates over 2 million hectares or 7 % of land cover in the Great Basin (Bradley and Mustard 2005) and is especially prone to invading *Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle and Young (Wyoming big sagebrush) communities (Knapp 1996). *Bromus tectorum* prolifically produces seed that persists in the seed bank (Hulbert 1955; Humphrey and Schupp 2004;

Hempy-Mayer and Pyke 2008) and competes successfully with native perennial bunchgrasses and *A. tridentata* seedlings (Harris 1967; Young and Evans 1978). Periodic disturbance, especially by wildfire, helps *B. tectorum* establish areas that are nearly monocultures with little remaining native vegetation (Knapp 1996). Despite the large body of research on *B. tectorum*, little progress has been made on ways to prevent further invasion or to restore invaded lands.

Understanding how annual grass invasion alters ecosystem processes is critical to understanding potential long-term changes in these communities and will likely aid in understanding restoration of native plant communities. Increased nutrient availability after disturbance is one factor shown to favor invasion by exotic annual species. Increased nitrogen (N) facilitates their growth, while limited nutrient supply through carbon (C) addition can reduce productivity and may favor native plant dominance (McLendon and Redente 1991; Paschke et al. 2000).

Nitrogen is the nutrient that most often limits primary production in terrestrial ecosystems (LeBauer and Treseder 2008). *Artemisia tridentata* systems are thought to be efficient in N use; recycling of low available N is considered a characteristic of these communities (Norton et al. 2007). In contrast, *B. tectorum* has a superior competitive ability in systems with higher available soil N, which occurs following disturbance and is thought to be a factor that allows *B. tectorum* to quickly dominate *A. tridentata* systems (McLendon and Redente 1991). There is evidence from pot studies that *B. tectorum* growth responds more than native species to N fertilization (Monaco et al. 2003; Vasquez et al. 2008), but it is difficult to extrapolate these results to field conditions because soil sieving in pot studies can artificially increase N availability (Johnson et al. 1995), thus favoring *B. tectorum* over native species even at "low N" treatments.

Biogeochemical theory predicts that ecosystems retain and recycle essential growth-limiting nutrients more effectively than non-limiting nutrients (Vitousek and Reiners 1975). In addition, ecosystems that are more N-limited are thought to retain N more effectively than those where N limitations are less intense (Menge 2011). However, there are few direct experimental tests of the idea that N limitation intensity drives N retention efficiency, particularly in semi-arid ecosystems. Moreover, it has been suggested that plant-type conversion of perennial shrub steppe communities to annual *B. tectorum* communities may decrease ecosystem-level N retention by enhancing opportunities for dormant-season N losses prior to *B. tectorum* germination (Evans et al. 2001; Norton et al. 2007). If correct, enhanced N losses under annual-dominated communities could ultimately reduce site fertility and productivity, and alter future competitive relationships between nutrient-demanding invasive annuals and nutrient-conservative native perennials. However, this reduction in site productivity has not yet been documented.

Carbon addition has long been suggested as a technique to reduce soil N availability in restoration approaches where native species are adapted to low N conditions (McLendon and Redente 1991, 1992; Paschke et al. 2000; Blumenthal et al. 2003; Eschen et al. 2007). Carbon additions stimulate microbial demands for N, thus reducing soil N available for plant uptake (Zink and Allen 1998; Schaeffer et al. 2003; Eschen et al. 2007). Despite the short-term efficacy of such treatments in reducing available soil N, stoichiometric theory predicts that the addition of C-rich substrates could increase long-term N retention at the ecosystem-level, with unknown consequences for future N availability and plant competitive hierarchies. Yet despite abundant evidence that C reduces available inorganic N in the short term, it is unknown how C addition shapes overall ecosystem N balances. ^{15}N tracer approaches provide an opportunity to examine how C additions may shape small annual, yet cumulatively/decadally significant, changes in ecosystem N retention that are otherwise difficult to detect using traditional N-mass balance applied to large and variable soil N pools.

The objectives of this study were to (1) evaluate the degree of N limitation of plant biomass in intact versus *B. tectorum*-invaded sagebrush communities, (2) determine if plant N limitation patterns are reflected in the strength of tracer ^{15}N retention over two growing seasons, and (3) assess if the strength of plant N limitation predicts the efficacy of carbon additions intended to reduce soil N availability and plant growth in native and invaded communities.

Materials and methods

Field sites and sampling

Six sites were selected in the northern Intermountain West, USA: three in eastern Oregon and three in southwestern Idaho. Each site contained a pair of native *A. tridentata* ssp. *wyomingensis* and exotic annual grass community plots located no further than 3 km apart. Pairs were carefully selected with similar soil types, precipitation, elevation, aspect, slope, and ecological sites (i.e., potential vegetation and production). All sites have deep loamy soils derived from alluvium and eolian deposits, receive between 203–305 mm of mean annual precipitation, and were historically dominated by *A. tridentata* ssp. *wyomingensis* (hereafter referred to as *A. tridentata*) and native perennial bunchgrasses. All of the annual grasslands had burned within the last 25 years. The date of the last fire at all

sagebrush sites was unknown but likely exceeded 50 years, the period of fire records in the area. Historically, all sites had been grazed by livestock but were fenced for the duration of the experiment. Additional details of each sampling location are provided in Witwicki (2005).

Native sagebrush communities had adult *A. tridentata* with varying cover of deep-rooted bunchgrasses [typically *Pseudoroegneria spicata* (Pursh) A. Löve (bluebunch wheatgrass), *Achnatherum thurberianum* (Piper) Barkworth (Thurber's needlegrass), and *Elymus elymoides* (Raf.) Swezey (squirreltail)] and biological soil crust with less than 15 % exotic annual grass cover. The exotic annual grass communities were dominated by *B. tectorum* but also had significant cover of *Poa secunda* J. Presl. (Sandberg bluegrass). *Taeniatherum caput-medusae* (L.) Nevski (medusahead), another exotic annual grass, was present at three of the annual grass sites. All annual grass sites had no remaining shrub component and less than 5 % native bunchgrass cover (excluding *P. secunda*). Hereafter, these plant communities will be referred to as "native" and "annual".

Each community plot contained three 5 × 5-m treatment plots to which N [ammonium nitrate (NH_4NO_3) at a rate of 100 kg N/ha], C (sucrose at a rate of 2,000 kg C/ha), or no treatment (control) was 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 the soil surface of all plots using a backpack sprayer to simulate a 0.5-mm rainfall event. On 2 × 2-m subplots located within each main treatment plot, 99 atom percent $^{15}NH_4^{15}NO_3$ was added to the water and applied at a rate of 0.19 g N m^{-2}. This low application rate allowed detection of ^{15}N to trace plant N retention but precluded fertilization effect.

Plant tissue for C, N, and ^{15}N analyses was collected 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 fully compare ^{15}N pools in native and annual communities, all six sites were sampled again on either 25–30 June 2005 or 12–13 July 2005 for plant biomass and cover, aboveground and belowground tissue, and soil C and N.

Because N should have a distinctly different spatial distribution between the two plant communities, and we wanted to accurately describe N pools at the community level, different numbers of soil samples were collected from native and annual plots. At each sampling interval, one composite mineral soil sample was collected in annual plots and three composite samples were collected in native plots (under sagebrush, under bunchgrasses, and in spaces between plants). Each composite sample consisted of five soil cores 2 cm in diameter and 10 cm deep. Samples were homogenized and passed through a 2-mm sieve before further analysis. Hereafter, the soil samples described above will be referred to as "bulk soil".

Soils collected on 20 October 2003 (pretreatment), 6 November 2003 (post-treatment), and 9 May 2004 (peak biomass) were 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, and total C. The amount of ^{15}N in total soluble N, microbial biomass N, and bulk soil was determined for the two later sampling dates. The amount of ^{15}N in soil organic nitrogen (SON) was determined by subtracting total soluble N and microbial biomass N from bulk soil. Soils and roots were collected in 2005, separated into fine roots (<2 mm), coarse roots (≥2 mm), and bulk soil, and each was analyzed for C, N, and ^{15}N.

To examine plant-available N over the entire growing season, ion-exchange resin capsules (Unibest, Bozeman, MT, USA) 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 between plants.

Plant cover, biomass, and tissue analysis

Plant cover was measured using the line-point intercept technique (Herrick et al. 2005) at plot establishment in autumn 2003 and at peak biomass in spring 2004. In 2004, live aboveground tissue of dominant plant groups (*A. tridentata*, bunchgrasses, and *B. tectorum*) was sampled for biomass and tissue analysis. *Bromus tectorum* biomass was estimated by clipping inside a 10 × 10-cm frame placed at regular intervals over 30 points within each plot. Bunchgrass biomass was estimated by clipping all live photosynthetic tissue in the plot except *P. secunda*, which is smaller and ubiquitous in both native and annual communities. For *A. tridentata*, only new shoot growth (green stems and leaves originating from them) was harvested as a representative measurement of current year's biomass; woody material and leaves found on woody stems (plant parts potentially produced before treatment application) were not used to estimate biomass. A 10 × 10-cm frame was randomly located at four points in an *A. tridentata* plant canopy, and all new shoot growth in the volume beneath the frame was clipped. Measurements were repeated on a second shrub within the plot, and cover data were used to scale biomass to the plot level.

In 2005, more detailed vegetation measurements were collected for plants grouped into six categories: (1) *A. tridentata*, (2) bunchgrass (excluding *P. secunda*), (3) *P. secunda*, (4) *B. tectorum*, (5) *T. caput-medusae*, and (6) other [*Vulpia octoflora* (Walter) Rydb. and annual forbs]. For all groups

except *A. tridentata*, ocular cover and biomass were collected in ¼ of each 5 × 5-m plot using six randomly located 1-m^2 quadrats. Aboveground biomass of two entire *A. tridentata* shrubs was collected at each native plot. Additional allometric measurements (height, greatest width, and greatest perpendicular width) were collected for all *A. tridentata* shrubs in each plot.

Plant biomass was dried at 65 °C for 48 h before being weighed or ground for tissue analysis. Plant shoots and roots were analyzed for total N, ^{15}N, and C using a Europa 20/20 SL continuous flow isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) at the Utah State University, Stable Isotope Laboratory (Logan, UT, USA).

Soil laboratory analyses

Within 24 h of collection, nutrients were extracted from resin capsules using 60 mL of 2 M KCl on a shaker table for 1 h 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 at Oregon State University (Corvallis, OR, USA). Inorganic soil N (NH_4^+–N and NO_3^-–N) and total soluble N were extracted from a 10-g soil sample for 1 h using 100 mL 0.5 M K_2SO_4 and filtered and analyzed as above. Microbial biomass N was determined by chloroform fumigation extraction (Horwath and Paul 1994) using 10 g of soil fumigated for 3 days with ethanol-free chloroform ($CHCl_3$) followed by extraction with 0.5 M K_2SO_4 and persulfate digestion (Cabrera and Beare 1993), with correction against unfumigated controls. No correction factors were applied for extraction efficiency. Gravimetric water content used in N calculations was determined by drying 10 g of each soil at 105 °C for 48 h.

Potential net N mineralization was estimated using a 28-day laboratory incubation (Hart et al. 1994) with 10 g of soil maintained at 60 % water-holding capacity at room temperature. At the end of the incubation, N was extracted with 0.5 M K_2SO_4, filtered, and analyzed as above, with net N mineralization corrected for initial N in samples.

Before plots were treated (October 2003), total soil C and N were determined by combustion on a Costech Elemental Analyzer, Model 4010 (Costech Analytical Technologies, Valencia, CA, USA). Post-treatment samples collected in 2003 and 2004 were analyzed for C, N, and ^{15}N using the same instrument detailed above at Utah State University. Soil samples collected in 2005 were analyzed on a Carlo Erba NC 2100 elemental analyzer interfaced with a delta v advantage isotope-ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Northern Arizona University, Colorado Plateau Stable Isotope Laboratory (Flagstaff, AZ, USA). A subset of samples run on instruments at both laboratories confirmed that results were comparable.

To determine ^{15}N in total soluble N and microbial biomass N pools, persulfate digested extracts were diffused for 6 days onto acidified discs following procedures 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 atom percent excess of ^{15}N tracer in the microbial biomass pool was determined using the following mixing model:

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

where $^{15}N_{mb}$ is the atom percent excess of microbial biomass, $mass_{fum}$ is the N concentration (mg N kg^{-1} soil) and $^{15}N_{fum}$ is the atom percent excess of digested chloroform-fumigated K_2SO_4 extracts, $mass_{initial}$ is the N concentration (mg N kg^{-1} soil) and $^{15}N_{initial}$ is the atom percent excess of digested K_2SO_4 extracts, and $mass_{mb}$ is the N concentration (mg N kg^{-1} soil) of microbial biomass. All tracer data were reported as ^{15}N atom percent excess, and a common estimate of natural abundance was used in all calculations. A maximum difference in ^{15}N natural abundance of 3.7 ‰ was found in similar Great Basin ecosystems (Hooker et al. 2008). In our study, this equates to a percent recovery of ^{15}N in *B. tectorum* 1 % lower than the values reported, and with the large amount of ^{15}N tracer added in our study, a potential bias of 1 % is well within sampling sources of error.

Statistical analysis

For all comparisons, data were analyzed as a randomized complete block with split-plot design in SAS 8.2 using PROC MIXED (SAS Institute, Cary, NC, USA). The six sites acted as blocks with community (native or annual) as the whole-plot factor and treatment (C, N, or control) as the split-plot factor. 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, soil C:N, and net N mineralized) for native communities in 2004, percent plant cover was used to weight pool means from sagebrush, interspace, and bunchgrass. In 2005, these same groups were used to weight pool means for bulk soil and root biomass, but the more detailed plant cover measurements were used to scale up aboveground biomass. Comparisons of the annual and native communities were made using the mean across all treatment types. Data were log-transformed when necessary to improve normality and homogeneity of 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, we present data with 95 % confidence intervals rather than p values for hypothesis tests (Johnson 1999). Confidence intervals incorporate multiple comparison adjustments and provide visual comparisons of differences among means.

Results

N limitation of plant biomass

Plant samples collected in May 2004 and June 2005 were used to examine the effects of N limitation on dominant vegetation (*A. tridentata*, native bunchgrass, *B. tectorum*) in each community. Dominant plants in each community varied in tissue N concentration, aboveground biomass, and how each responded to N treatment (Fig. 1). Nitrogen fertilization affected annual plants differently than native perennial plants. In May 2004, N increased tissue N but not biomass in *A. tridentata* and bunchgrass (Fig. 1a, b). In contrast, N increased biomass but not tissue N in *B. tectorum*. In June 2005, *B. tectorum* biomass in the N treatment was no longer significantly higher than controls, but *A. tridentata* and bunchgrasses maintained increased tissue N from the N treatment (Fig. 1b).

Plant samples collected in June 2005 were also used to make comparisons between the native and annual communities. Aboveground biomass and plant tissue N in native communities were greater by a factor of ten than in annual communities for all treatments, but no differences in root biomass or tissue N were detected between the communities (Fig. 2). Although increased plant tissue N persisted in *A. tridentata* in 2005 (Fig. 1b), there was no evidence that N application increased total biomass or tissue N in the native community (Fig. 2). The only difference detected at the community level from N treatment was increased aboveground plant tissue N in the annual community (Fig. 2b).

Soil samples confirmed that N fertilization substantially increased soil extractable inorganic N in November 2003 and May 2004 (Fig. 3a, b) and inorganic N detected in resin capsules (Fig. 4) in both communities. Soil samples collected on four sampling dates were also used to examine potential factors underlying plant species and community responses. In October and November 2003, soil NO_3^-–N in the untreated annual community was significantly higher than in the native community (Fig. 3b). In May 2004, when all plants were actively growing, inorganic soil N pools were lower but similar for both communities (Fig. 3a, b). In June 2005, the annual community had higher soil C:N than the native community (Fig. 5b). There were no differences between soils of annual and native communities

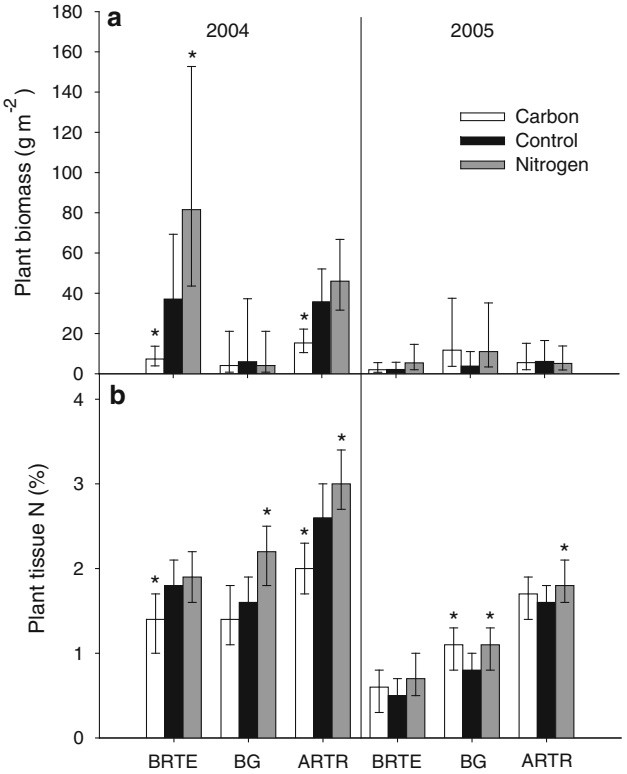

Fig. 1 Biomass (**a**) and % N (**b**) for current year's photosynthetic tissue of dominant plants in control and carbon- and nitrogen-treated annual [*Bromus tectorum* (*BRTE*)] and native [bunchgrass (*BG*) and *Artemisia tridentata* (*ARTR*)] communities collected in 2004 and 2005. Values are means of six replicates. *Bars* 95 % confidence intervals. *Asterisk* significant difference from the control ($\alpha = 0.025$)

for NH_4^+–N, net N mineralized, microbial biomass N, and total N on any sampling date (Figs. 3a, c, d, 5a).

Plant N limitation patterns in tracer ^{15}N retention

Tracer ^{15}N retention was compared in the bulk soils of native and annual communities over two growing seasons. In November 2003, one week after treatments were applied, less ^{15}N tracer was recovered in bulk soil of annual communities than native communities, but by May 2004, there was no difference between the communities (Fig. 6a). In June 2005, we recovered more ^{15}N tracer in bulk soil of the annual community than the native community (Fig. 6a). In addition, significantly more ^{15}N tracer was recovered from the aboveground and belowground pools in the annual community (Fig. 6b), and therefore tracer partitioning on this sampling date was examined relative to the total amount recovered per plot (Table 1).

Tracer ^{15}N retention was examined in dominant plants of native and annual communities in May 2004. Although numerous differences were detected in the way dominant

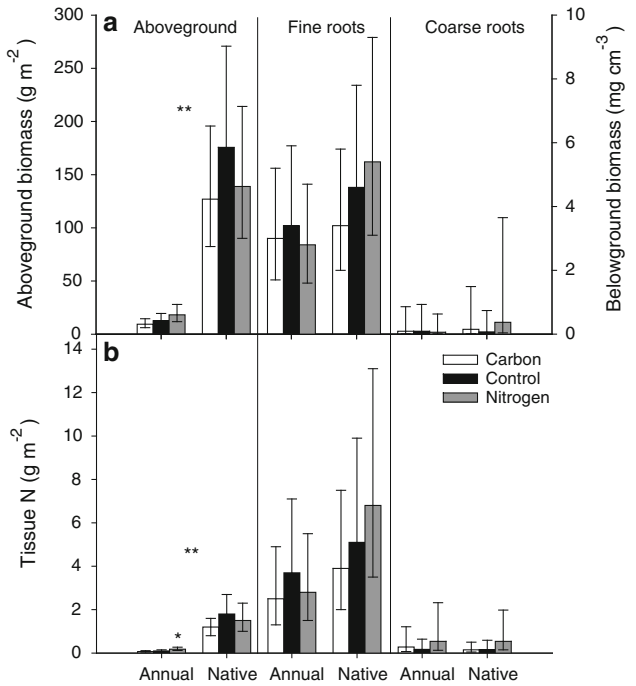

Fig. 2 Biomass (**a**) and tissue N (**b**) of aboveground vegetation, fine roots (<2 mm), and coarse roots (≥2 mm) collected in 2005 in annual and native communities treated with carbon, no treatment (control) or nitrogen. Values are means of six replicates. *Bars* 95 % confidence intervals. *Asterisk* significant difference from the control ($\alpha = 0.025$). *Two asterisks* significant difference between the annual and native communities ($\alpha = 0.05$)

plants in the communities responded to N fertilization (Fig. 1), patterns in tracer ^{15}N retention did not reflect any of these differences (Fig. 6c).

Tracer ^{15}N retention was examined in vegetation at the community level in June 2005. A higher relative percent of ^{15}N tracer was recovered in aboveground plant tissue in the native community compared to the annual community (Table 1), which reflected the pattern detected in aboveground biomass and tissue N (Fig. 2). There were no differences in relative tracer ^{15}N partitioning between N treatment and control for aboveground or belowground vegetation in either community (Table 1), even though we detected higher tissue N in aboveground biomass in the annual community with N treatment (Fig. 2b).

Tracer ^{15}N retention in soil pools highlighted some responses to N fertilization in the annual and native communities that were not revealed in the other soil sampling in May 2004. Less ^{15}N tracer was recovered in the soil organic N pool in the N treatment compared to the control for both communities (Fig. 6d). In addition, more ^{15}N tracer was recovered in the N treatment than the control in native communities for total soluble N (Fig. 6d). Similarly, N-treated plots in the native community had consistently higher soil extractable NH_4^+–N and NO_3^-–N than N-treated plots in the annual community (Fig. 3a, b), but this pattern was not tested for statistical significance. Although less ^{15}N tracer was recovered in bulk soil of the

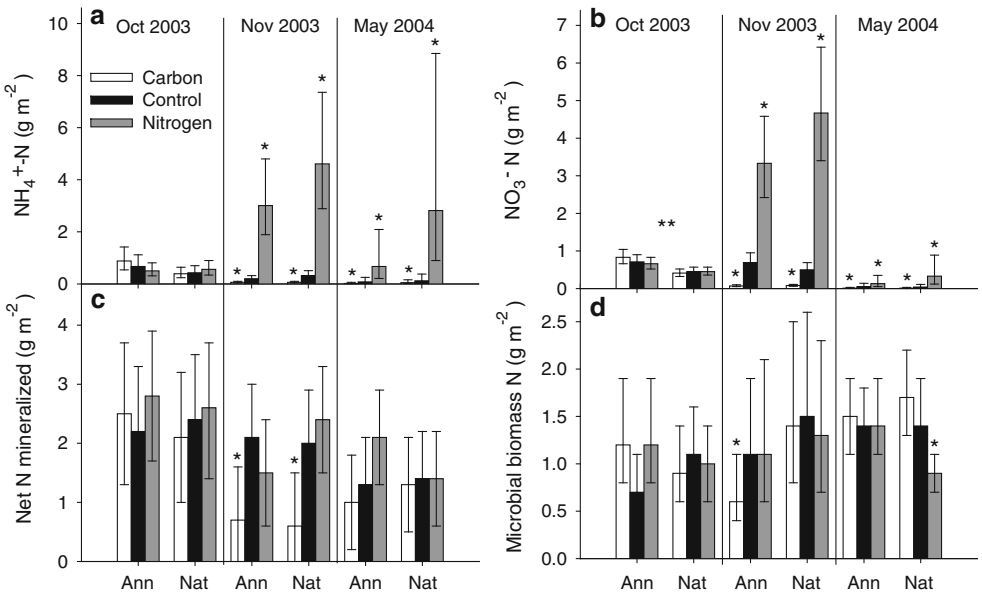

Fig. 3 Soil NH_4^+–N (**a**), NO_3^-–N (**b**), net N mineralized (**c**), and microbial biomass N (**d**) on three sampling dates for annual (*Ann*) and native (*Nat*) communities treated October 2003 with carbon, no treatment (control), or nitrogen. Values are means of six replicates. *Bars* 95 % confidence intervals. *Asterisk* significant difference from the control ($\alpha = 0.025$). *Two asterisks* significant difference between the annual and native communities ($\alpha = 0.05$)

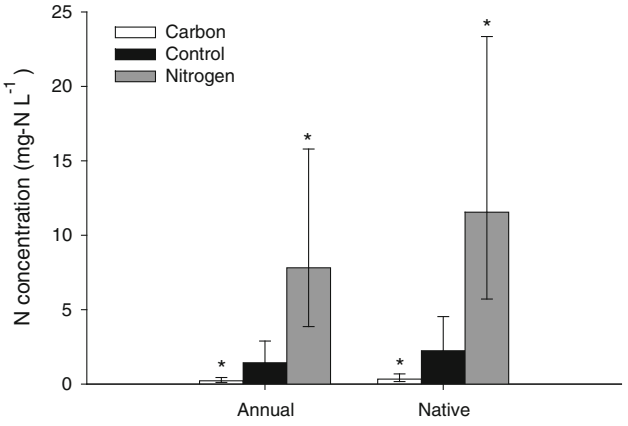

Fig. 4 Soil total inorganic N (NH_4^+–N and NO_3^-–N) concentration (mean ± 95 % CI, $n = 6$) in resin capsules for carbon, control, and nitrogen treatments for the entire growing season (October 2003–May 2004). *Asterisk* significant difference from the control ($\alpha = 0.025$)

native community in the N treatment compared to the control in June 2005 (Fig. 6a), this trend was not apparent in the relative ^{15}N tracer data (Table 1).

Efficacy of carbon addition in reducing soil N and plant growth

Carbon addition decreased soil extractable inorganic N in November 2003 and May 2004 (Fig. 3a, b) and inorganic N detected in resin capsules (Fig. 4) in both the native and annual communities. Soil C:N decreased modestly in November 2003 in response to the C treatment, but this difference was negligible by May 2004 (Fig. 5b). In May 2004 and June 2005, more ^{15}N remained in bulk soil of C-treated plots compared to control plots in both annual and native communities (Fig. 6a). In both communities in June 2005, carbon treatment also increased the total amount of tracer recovered compared to the control (Fig. 6b), largely due to the high amount of ^{15}N recovered in bulk soil in the C treatment (Fig. 6a).

Carbon treatment reduced plant-available N through immobilization of N in microbial biomass (Fig. 6d). By May 2004, addition of C treatment had nearly doubled ^{15}N in microbial biomass and SON (Fig. 6d). Similarly, carbon treatment significantly decreased net N mineralized in the November 2003 laboratory incubation by more than one-half in both communities (Fig. 3c).

A. tridentata and *B. tectorum* responded similarly to C treatment in 2004, with considerable decreases in both biomass and tissue N relative to the control (Fig. 1a, b). The reduction in *B. tectorum* biomass from C treatment was more extreme than the reduction in *A. tridentata* biomass (80 vs. 57 % reduction, respectively). In addition, significantly less ^{15}N tracer was recovered from aboveground tissue of *B. tectorum* and *A. tridentata* in C-treated

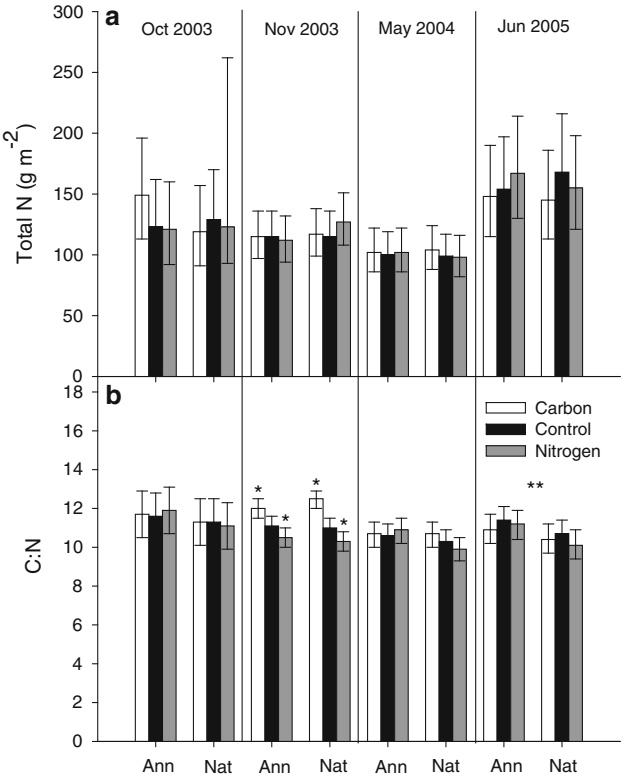

Fig. 5 Soil N (**a**) and C:N ratios (**b**) on four sampling dates for annual (*Ann*) and native (*Nat*) communities treated 28–30 October 2003 with carbon, no treatment (control), or nitrogen. Values are means of six replicates. *Bars* 95 % confidence intervals. *Asterisk* significant difference from the control ($\alpha = 0.025$). *Two asterisks* significant difference between the annual and native communities ($\alpha = 0.05$)

plots (Fig. 6c) than controls. Carbon treatment did not affect tissue N for bunchgrasses (Fig. 1b). We did not detect a decrease in aboveground bunchgrass biomass from C treatment, although bunchgrass cover was low and extremely variable in native plots (around 1 % at half the sites and 15–21 % at the other half). By June 2005, we no longer detected any decreases in biomass or tissue N from C treatment in any plant group (Fig. 1a, b).

In the community-level comparisons made in 2005, annual and native communities responded similarly to C treatment. Less ^{15}N was incorporated into aboveground plant tissue and fine roots in the C treatment than the control for both communities, over a year and a half after C application (Table 1). However, there were no differences between the C treatment and the control for above and belowground biomass or tissue N for either community (Fig. 2).

Discussion

Information on ecosystem nutrient limitation provides a basis for understanding fundamental controls on net

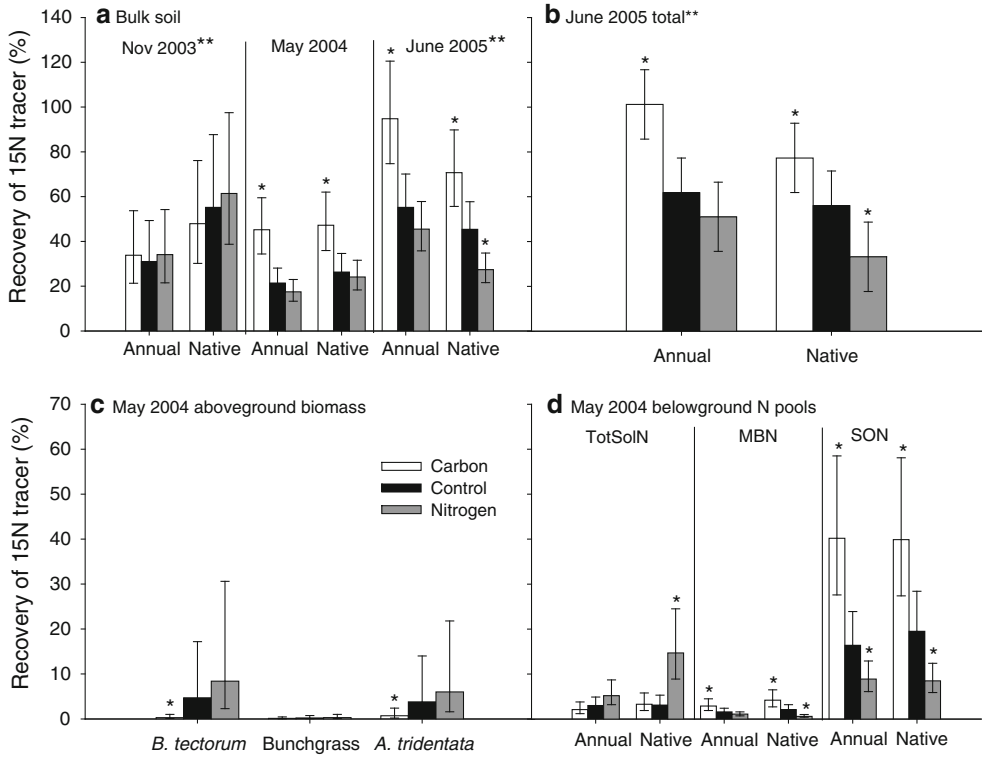

Fig. 6 Percent recovery of ^{15}N tracer in bulk soil (**a**), total aboveground and belowground pools (**b**), dominant plants (**c**), and other soil pools (**d**) in annual and native communities on each sampling date. Other soil pools include total soluble N (*Tot Sol N*), microbial biomass N (*MBN*), and soil organic N (*SON*). Values are means of six replicates. *Bars* 95 % confidence intervals. *Asterisk* significant difference from the control ($\alpha = 0.025$). *Two asterisks* significant difference between the annual and native communities ($\alpha = 0.05$)

Table 1 Relative partitioning (%) of recovered ^{15}N tracer in aboveground plant tissue, belowground root tissue, and bulk soil in control and C- and N-treated annual and native plant communities, June 2005

	Carbon		Control		Nitrogen	
	Annual	Native	Annual	Native	Annual	Native
Aboveground						
Total aboveground**	0.1* (0.1–0.2)	1.1* (0.6–2.0)	0.4 (0.2–0.8)	3.2 (1.7–5.9)	0.8 (0.4–1.5)	5.5 (3.0–10.1)
Belowground						
Fine roots	3.3* (1.9–5.7)	4.5* (2.6–7.7)	8.4 (4.9–14.0)	9.4 (5.5–15.6)	6.4 (3.7–10.9)	9.7 (5.7–16.1)
Coarse roots	0.1 (0.02–0.4)	0.1 (0.01–0.3)	0.2 (0.05–1.2)	0.1 (0.02–0.4)	0.1 (0.03–0.6)	0.3 (0.1–1.7)
Bulk soil**	96.4* (94.3–97.8)	94.0* (90.5–96.3)	90.7 (85.6–94.2)	85.2 (77.8–90.5)	92.2 (87.7–95.1)	83.8 (75.8–89.5)

Parentheses contain 95 % confidence intervals

* Significant difference from control for each community ($\alpha = 0.025$)

** Significant difference between the annual and native community ($\alpha = 0.05$)

primary production and for interpreting the efficacy of management treatments designed to restore native vegetation through nutrient manipulation. In our fertilization experiment, we found that N limits aboveground productivity in both annual grass and sagebrush communities, with greater N limitation of annual grass than sagebrush communities. The greater response of annual grass, especially *B. tectorum*, to N addition supports the idea that dominance by annuals in sagebrush steppe is related to high nutrient availability (McLendon and Redente 1991, 1992). Although N fertilization did not increase tissue N concentrations in *B. tectorum* (Fig. 1), we found that ^{15}N tracer recovery in aboveground biomass nearly doubled in N-fertilized *B. tectorum* plots relative to controls (Fig. 3b), indicating that fertilizer N uptake was used to support added biomass growth. In contrast, N fertilization

increased tissue N in *A. tridentata* and bunchgrasses without substantially increasing biomass. Overall, these results illustrate divergent responses of annual versus perennial plant communities to added N, with the exotic annual *B. tectorum* displaying a greater ability to use added N to rapidly increase aboveground biomass, followed by *A. tridentata* and then bunchgrasses.

The dominant plants in our study, each representing a different life form (shrub, perennial grass, and annual grass), responded uniquely to the treatments, with *A. tridentata* and bunchgrasses functioning more similarly than *B. tectorum* with N fertilization, and *A. tridentata* and *B. tectorum* functioning more similarly than bunchgrasses with C additions. This contrasts previous research in pots that found higher tissue N in aboveground *B. tectorum* biomass with N fertilization and similar reductions of aboveground biomass and tissue N in *B. tectorum* and perennial bunchgrasses from C additions (Monaco et al. 2003). Our data suggest that there are important differences in how tissue N and biomass of the dominant life forms in our study are affected by high and low soil N availability.

Based on nutrient retention theory (Vitousek and Reiners 1975), we expected stronger N limitation of annual grass than sagebrush communities would be reflected in ^{15}N tracer retention. Our results followed these expectations and demonstrated stronger N retention in shallow (0–10 cm) soils of annual communities. Although it is possible that our sampling to 10 cm did not capture N leached and retained in deeper mineral soil, other studies have found that most belowground ^{15}N tracer recovered in grasslands and shrublands was in the top 10 cm (Schimel et al. 1986; Curtis et al. 2005; Hungate et al. 2006) and $<$10 % of ^{15}N tracers are lost via leaching (Templer et al. 2012). It is likely that annual grass communities took up and recycled ^{15}N to soil through growing and senescent biomass, which is consistent with Hooker et al.'s (2008) findings that *B. tectorum* invasion leads to shallow soil C and N enrichment. Although we did not measure deeper soil, it is also possible that *B. tectorum* may have also displayed weaker N retention at depth, because *B. tectorum* is associated with loss of deep SOM (Norton et al. 2004) that can influence grassland N retention (Epstein et al. 2001). However, other evidence suggests the potential for high N loss in *A. tridentata* communities. Gaseous pathways dominate N losses from aridlands (Peterjohn and Schlesinger 1990), and interspace areas of *A. tridentata* shrub communities can display particularly high rates of N gas loss, up to 75 % of applied ^{15}N tracers (Klubek and Skujins 1981). In contrast, the higher soil C:N that we observed under annual grass is associated with lower rates of N gas loss from aridland soils (Stark et al. 2002), and high soil C:N promotes strong ecosystem ^{15}N retention across a range of terrestrial ecosystems worldwide (Templer et al. 2012). More detailed process studies and sampling at depth are needed to determine possible fine-scale differences in the fate of ^{15}N between annual grass- and perennial plant-dominated communities.

Previous comparisons of native and invaded communities indicate that *B. tectorum* invasion leads to net N losses, but the mechanisms of this N loss are not well resolved (Evans et al. 2001; Booth et al. 2003; Hooker et al. 2008). It is widely thought that annual grass communities are inherently more N "leaky" than native perennial communities due to faster N cycling and pulse N losses during dormant season storms and at the beginning of growing seasons before grasses germinate (Norton et al. 2007). However, our data showing stronger short-term ^{15}N retention in shallow soils of invaded annual communities raises the possibility that other mechanisms may explain prior observed differences in ecosystem N capital between invaded and native communities. To reconcile these observations, we suggest that increased wildfire frequency with *B. tectorum* invasion, rather than a more leaky annual N cycle of invaded grasslands, may promote ecosystem N loss with *B. tectorum* invasion. Accelerated fire frequencies are often observed on sites invaded by *B. tectorum* (Miller et al. 2011); however, our invaded grassland sites were likely free of fires for nearly 25 years. If dominance by exotic annual grass alone leads to leaky N cycling, then we should have detected this loss at our sites.

Differences in N limitation between annual grass and native perennial communities can also explain the differential impact of sugar on community plant growth in our restoration treatment study. Although sugar treatment reduced aboveground biomass in both communities, it had a greater impact on biomass in the annual community. The stronger negative effect of sugar on annual plant growth mirrors our finding that N was more limiting to annual grass than shrub communities. Indeed, sugar treatment greatly reduced soil inorganic N to extremely low levels and increased microbial ^{15}N immobilization relative to control plots. Keeping soil inorganic N low for extended periods of time is essential for continued response by vegetation in the communities. We demonstrated that the effects of C addition can last longer than 1 year with a very high application rate of sugar (Table 1); however, reduction in annual grass biomass was not sustained (Figs. 1a, 2a). Repeat sugar applications or use of a longer lasting C source, such as straw, is needed to achieve a longer term reduction in soil N availability (Zink and Allen 1998; Blumenthal et al. 2003; Eschen et al. 2007).

Timing of C addition may have affected how the dominant plants in our study responded. Seasonal growth of native bunchgrasses is delayed relative to *A. tridentata* and *B. tectorum* such that both the shrub and annual grass were actively growing when C applications occurred, but

the native bunchgrasses were not. Root competition between adult *A. tridentata* and seedlings for nutrients during winter months appears important for seedling growth and survival (Reichenberger and Pyke 1990), thus early winter C applications may impact those plants that can photosynthesize and grow with cold soil temperatures such as *A. tridentata* and *B. tectorum* (Pearson 1975 cited in McArthur and Welch 1982; Harris 1977; Aguirre and Johnson 1991). The timing of C addition may also allow other plants, such as native C3 perennial bunchgrasses that delay growth until warmer temperatures return, to escape this period of N limitation. More detailed temporal studies of N availability are needed during winter to better understand these relationships.

Our results are consistent with others who have found that C addition decreases biomass of early seral species compared to late seral species (Redente et al. 1992) and annuals compared to perennials (Eschen et al. 2006). Importantly, our data build on previous work by linking community response to C additions to concurrent observations of community N limitation to explain differences in response to C addition. Overall, our data suggest that information on patterns of N limitation in annual grass versus native perennial communities can help inform patterns of N retention and of community response restoration treatments targeted at reducing soil N availability.

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