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BROMUS TECTORUM INVASION ALTERS NITROGEN DYNAMICS IN AN UNDISTURBED ARID GRASSLAND ECOSYSTEM

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Abstract. The nonnative annual grass *Bromus tectorum* has successfully replaced native vegetation in many arid and semiarid ecosystems. Initial introductions accompanied grazing and agriculture, making it difficult to separate the effects of invasion from physical disturbance. This study examined N dynamics in two recently invaded, undisturbed vegetation associations (C_3 and C_4). The response of these communities was compared to an invaded/disturbed grassland. The invaded/disturbed communities had higher surface NH_4^+ input in spring, whereas there were no differences for surface input of NO_3^-. Soil inorganic N was dominated by NH_4^+, but invaded sites had greater subsurface soil NO_3^-. Invaded sites had greater total soil N at the surface four years post-invasion in undisturbed communities, but total N was lower in the invaded/disturbed communities. Soil $\delta^{15}N$ increased with depth in the noninvaded and recently invaded communities, whereas the invaded/disturbed communities exhibited the opposite pattern. Enriched foliar $\delta^{15}N$ values suggest that *Bromus* assimilated subsurface NO_3^-, whereas the native grasses were restricted to surface N. A Rayleigh distillation model accurately described decomposition patterns in the noninvaded communities where soil N loss is accompanied by increasing soil $\delta^{15}N$; however, the invaded/disturbed communities exhibited the opposite pattern, suggesting redistribution of N within the soil profile. This study suggests that invasion has altered the mechanisms driving nitrogen dynamics. *Bromus* litter decomposition and soil NO_3^- concentrations were greater in the invaded communities during periods of ample precipitation, and NO_3^- leached from the surface litter, where it was assimilated by *Bromus*. The primary source of N input in these communities is a biological soil crust that is removed with disturbance, and the lack of N input by the biological soil crust did not balance N loss, resulting in reduced total N in the invaded/disturbed communities. *Bromus* produced a positive feedback loop by leaching NO_3^- from decomposing *Bromus* litter to subsurface soil layers, accessing that deep-soil N pool with deep roots and returning that N to the surface as biomass and subsequent litter. Lack of new inputs combined with continued loss will result in lower total soil N, evidenced by the lower total soil N in the invaded/disturbed communities.

Key words: biological soil crust; Bromus tectorum; *cheatgrass; ecosystem nitrogen; mineralization; nitrate leaching; nitrogen isotope; plant invasion; soil nitrogen; surface disturbance.*

INTRODUCTION

Invasive plant species are a significant component of global change. They have substantial interactive effects on fire regimes, nutrient cycling, hydrology, and energy budgets and represent a threat to biodiversity, abundance, and survival of native species (Vitousek 1992, Chapin et al. 1996, Mack et al. 2000). Species diversity within an ecosystem can influence soil nutrient status because different functional groups utilize different resources in different seasons (Hooper and Vitousek 1998), and community composition can play an important role in regulating trace gas loss (Epstein et al. 1998). Invasive plant species can affect organic matter formation and decomposition, nutrient cycling, and soil development (Schlesinger et al. 1996) through changes in litter quality and quantity (Evans et al. 2001*b*), soil food webs (Belnap and Phillips 2001), and differential uptake of nutrients (Wedin and Tilman 1993).

Bromus tectorum L., a cleistogamous annual grass (hereafter referred to as *Bromus*), was introduced into the United States in the late 1800s (Mack 1981). Initial *Bromus* invasions often accompanied physical surface disturbance, as seeds were dispersed during cattle and agriculture expansion in the early 1900s (Mack 1981). Although introductions of *Bromus* into pristine ecosystems suggest that invasion may be enhanced by disturbance, it is not required (Evans et al. 2001*b*, Stohlgren et al. 2001). Natural revegetation into invaded areas is minimal due to greater competition for resources; *Bromus* sets seed in the spring, germinates in

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the fall, and continues root elongation throughout the winter (Harris 1967). The rapid establishment of an extensive root system by early spring renders resources unavailable to native plants that initiate growth at warmer temperatures (Harris 1967, Miller et al. 2001, Rafferty and Young 2002). *Bromus* is one of the most studied exotic plant species; however, the paucity of pristine communities that have been invaded by *Bromus* has confounded separating the effects of invasion and disturbance.

In arid and semiarid ecosystems, the soil surface is covered with a biological soil crust (Belnap and Lange 2001). These soil crusts are a matrix of cyanobacteria, mosses, lichens, and fungi that form a mat over the soil surface (Evans and Johansen 1999). Soil crusts are often found in areas where low water availability prevents continuous plant cover, allowing large areas to be exposed to direct sunlight. The biological soil crusts that develop under these conditions often represent an uninterrupted photosynthetic layer in plant interspaces (Lange 2001, Evans and Johansen 1999, and references cited within). In addition, lichens and cyanobacteria fix atmospheric N_2 (Evans and Lange 2001) and are important contributors to plant-available N (Evans and Ehleringer 1993). However, biological soil crusts are extremely fragile and susceptible to physical disturbance, thereby reducing rates of N_2 fixation and ecosystem N inputs (Evans and Belnap 1999, Belnap and Eldridge 2001, Evans et al. 2001*a*).

This two-year study examined how *Bromus* influenced N dynamics beginning four years after invasion in two pristine vegetation associations (C_3 and C_4). *Bromus* invaded in small distinct patches, creating adjacent noninvaded and invaded areas. Previous work at these sites shows an increase in total soil N, lowered labile N, and an increase in soil $\delta^{15}N$ in invaded communities 2–4 yr post-invasion (Rimer 1998, Evans et al. 2001*b*). In this study, these noninvaded and recently invaded communities were compared to a *Bromus*-dominated grassland that had been grazed by cattle until 1974, allowing for 24 years of natural recovery. We hypothesized that the presence of *Bromus* would (1) reduce N entering the soil from the surface by shading or disrupting the biological soil crust, reducing N_2 fixation, (2) reduce soil inorganic N by increasing uptake because an increased root biomass would occupy a greater soil volume, and (3) exploit subsurface N that was unavailable to the native grasses.

METHODS

Study site

This study was conducted in the Needles District of Canyonlands National Park in southeastern Utah, USA. The climate is typical of the Colorado Plateau Desert with 215 mm precipitation annually. The mean precipitation between 1965 through 1999 was 38, 52, 59, and 66 mm in winter, spring, summer, and fall, respectively. Winter storms originate in the Gulf of Alaska and move southeast onto the Colorado Plateau characterized by cool rain and snow. Canyonlands is located at the northern border of the Arizona monsoon system, resulting in unpredictable summer monsoons. Evaporation is greater than precipitation during the period from March to October, and precipitation rarely penetrates the surface, whereas deep soil moisture recharge occurs during winter months when temperatures are cooler.

Two locations within Canyonlands National Park were used in this study. The first is a 100-ha undisturbed arid grassland. The soils are Begay fine sandy loams, with an average of 64% sand, 25% silt, and 13% clay at 0–0.3 m and 1.33% organic matter in the top 0.05 m (Kleiner and Harper 1977*a*). Full soil and vegetation descriptions are provided in Kleiner and Harper (1977*a*, *b*). Two distinct vegetation associations are scattered throughout Virginia Park, and each community type can occupy up to 1 ha (Kleiner and Harper 1977*b*). The first vegetation association was co-dominated by C_3 bunchgrasses: *Achnatherum hymenoides* R. & S. Barkworth (Indian ricegrass), *Hesperstipa comata* T. & R. Barkworth (needle and thread grass), and *Sporobolus cryptandrus* (Torr.) Gray (sand dropseed) and will be referred to as the C_3 community. The second vegetation association was dominated by *Pleuraphis jamesii* Torr. (galleta grass), a warm season rhizomatous grass with C_4 photosynthesis (Kleiner and Harper 1977*b*), and will be referred to as the C_4 community. Nomenclature follows the USDA NRCS PLANTS database (*available online*).[6] *Bromus* cover was ~0.4% until 1994, when *Bromus* cover increased to 20% in 1996 and 57% in 1998 (Kleiner and Harper 1977*b*, Belnap and Phillips 2001). The invasion occurred into distinct patches of the communities and did not represent a single invasion front. The second study site was located 5.7 km northeast of the first. This area was heavily grazed by cattle until 1974. Soils are Ignacio-Leanto fine sandy loams, with 79% sand, 10% silt, and 11% clay, respectively. The second site was dominated by *Bromus* and will be referred to as the invaded/disturbed community. In 1995, 12 20 × 30 m permanent plots were established in the first study site in distinct patches; three in each in distinct patches of the noninvaded and recently invaded C_3 and C_4 communities. Three additional plots were established in the invaded/disturbed sites for a total of 15 plots. Because these plots are monitored in other studies, all destructive sampling occurred 1 m outside the perimeter of the plots.

Surface nitrogen input

Inorganic N input from soil crusts and litter was measured using ion-exchange resin bags at the soil crust/litter interface. Ten grams of mixed cation/anion resin (Dowex MR3) were placed into nylon bags and

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[6] ⟨http://plants.usda.gov/⟩

sealed with a string. After removing an approximately 0.10 m diameter plug of surface soil crust with a small trowel, an intact soil core directly beneath the removed crust was extracted with a 0.5 × 0.25 m clear plastic tube. One resin bag was placed on the top end of the tube, and the tube was reinserted into the soil. The previously removed soil crust was then placed over the top of the tube. Therefore N absorbed onto the upper resin bag represents input from the biological soil crust, litter, or both. The tubes remained in place until the next sampling date, at which time the resin bags were placed into individual plastic bags for transport to the University of Arkansas laboratory. The tube was reinserted using the above techniques with a fresh resin bag and crust into a different location on each sampling date. Three tubes were placed into each of the 15 plots. Sampling occurred during 1997 and 1998. Each resin bag was placed into 0.05-L 2 mol/L KCl for 1 h on a shaker table, allowed to settle at 4°C for 24 h, then gravity filtered using Whatman 40 filters. Each KCl extract was analyzed twice colorimetrically with an Alpkem 3000 Autoanalyzer (College Station, Texas, USA), and the mean was used in statistical analysis. Each value was divided by the number of days the bag was in the soil.

Soil moisture and inorganic N concentrations

A 0.08 m diameter sand soil auger was used to remove a 1-m soil core from all 15 plots from the five sites on the sampling dates described above, and the core was separated into the following increments: 0.00–0.05, >0.05–0.10, >0.10–0.25, >0.25–0.50, >0.50–0.75, and >0.75–1.00 m. Soil from each depth was sieved through a 2-mm soil sieve before subsampling. A 5-g subsample was removed from each depth, placed into 0.025-L 2 mol/L KCl, and extracted and analyzed colorimetrically according to the autoanalyzer methods outlined above. A second subsample (10 g) was weighed, oven-dried at 105°C for 24 h, and reweighed for soil moisture. Inorganic N concentrations were corrected for moisture present in the sample and are reported on a dry mass basis.

Soil isotopic composition and total N content

Soil isotopic composition and total soil N were determined on the soil cores collected in September 1999. Subsamples (10 g) from each depth were dried at 40°C for 48 h and ground on a rolling soil grinder to a fine powder. Approximately 50–100 mg from each subsample were placed into tin capsules and analyzed on a Carlo Erba Elemental Analyzer (NA1500 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan, Italy) in line with a Finnigan Delta$^+$ Mass Spectrometer (Finnigan MAT, Bremen, Germany) at the University of Arkansas Stable Isotope Laboratory. Isotope ratios are expressed in δ notation as parts per thousand (‰):

$$\delta^{15}\mathrm{N} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000 \qquad (1)$$

where R is the molar ratio of the heavier to the lighter isotope (^{15}N/^{14}N) for the standard or sample.

Soil δ^{15}N depends on the δ^{15}N of source N and subsequent fractionation. This typically results in a negative relationship between soil N concentration and δ^{15}N with depth and can be described by Rayleigh distillation kinetics (Evans and Ehleringer 1993):

$$\delta^{15}\mathrm{N}_{\mathrm{s},t} = k + \varepsilon \ln([\mathrm{N}]) \qquad (2)$$

where $\delta^{15}\mathrm{N}_{\mathrm{s},t}$ is the isotopic composition of the substrate at time t, k is a constant, ε is the slope of the linear relationship and is equal to the isotopic fractionation associated with decomposition, and ln([N]) is the natural log of total soil N (Evans and Ehleringer 1993). Plotting δ^{15}N against the natural log of total N results in a linear relationship with slope ε and intercept k.

Plant tissue analysis

Green leaf tissue was collected for foliar δ^{15}N on each sampling date through May 1999. Leaf tissue was collected from *Pleuraphis* in the C_4 communities, and all dominant species were collected in the C_3 communities and combined into one sample. *Bromus* was collected from all invaded communities. One leaf was collected from each of five randomly chosen plants and combined into one sample per plot for C_3, C_4, or *Bromus*. Samples were dried at 40°C for 48 h and ground to a fine powder using a mortar and pestle. Samples were analyzed using the methods outlined above for soil samples at the University of Arkansas Stable Isotope Laboratory.

Statistical analysis

Statistical analyses considered the five invasion/disturbance vegetation types as five independent communities. Each plot was located in a separate patch within each type. Surface input of NH_4^+ and NO_3^- were log transformed and analyzed using a two-way repeated measure with date and community as fixed effects. Soil moisture, NH_4^+, and NO_3^- were log transformed and analyzed as a three-way repeated measure with date, community, and depth as fixed effects. Pearson's coefficients were calculated to determine correlations between resin bag NH_4^+ and NO_3^- and soil moisture and inorganic soil N. Two-way ANOVAs with community and depth as fixed effects were used to test depth by soil δ^{15}N, total soil N, and ammonification and nitrification rates. Soil δ^{15}N was normally distributed, total N was natural log transformed, and ammonification and nitrification rates were ranked. Bonferroni adjustments were made on all post-hoc tests. Soil δ^{15}N and ln([N]) were fit to the Rayleigh distillation kinetics model using linear regression analysis, and the slopes were compared using ANCOVA. Statistics were performed with

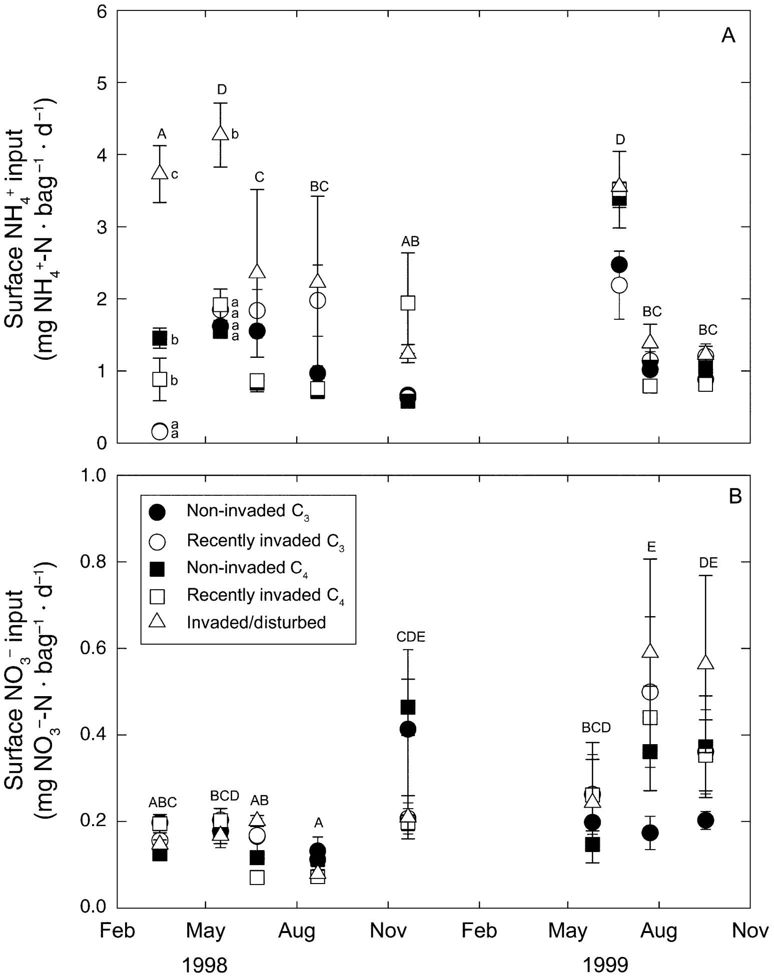

FIG. 1. NH$_4^+$ and NO$_3^-$ surface input (mean ± SE) for the five community types. Note the differences in scale for the *y*-axis between panels (A) and (B). Uppercase letters located above symbols signify the repeated-measures results for combined community means on that date, and lowercase letters adjacent to the community symbols represent differences between communities within that date. Different letters indicate differences at $P < 0.05$. The study was conducted in the Needles District of Canyonlands National Park in southeastern Utah, USA.

SPSS (SPSS version 10.1, Chicago, Illinois, USA), and significance was determined at $\alpha = 0.05$.

RESULTS

Surface nitrogen input

Surface nitrogen input was NH$_4^+$ dominated ($T = 20.107$, $P < 0.0001$; Fig. 1A) for all communities ($P < 0.0001$) and all dates ($P < 0.05$). The two forms (NH$_4^+$ and NO$_3^-$) were not significantly correlated ($P = 0.576$). There was a significant interaction between community and date for surface nitrogen NH$_4^+$ input ($P < 0.0001$). NH$_4^+$ input changed significantly over time ($P < 0.0001$), with the highest NH$_4^+$ inputs in May 1998 and May 1999 (driven by the invaded plots) and the lowest in March 1998 and November 1998. The invaded/disturbed communities had the highest overall NH$_4^+$ input ($P < 0.001$), and all other communities were similar. There were significant differences between communities in March and May 1998 with more NH$_4^+$ input in the invaded/disturbed communities compared to the other four communities ($P < 0.05$). There was no interaction between community and date ($P = 0.197$; Fig. 1B), and no significant differences between communities ($P = 0.925$) for surface NO$_3^-$ input. The input of NO$_3^-$ changed significantly over time ($P < 0.0001$), with higher NO$_3^-$ input in July and September 1999.

Soil moisture and inorganic N concentrations

Soil moisture was higher for all communities during the winter months (January, March, and November 1998 and September 1999), compared to summer months (May, June, and August 1998 and May and July 1999) ($P < 0.0001$) (Fig. 2). Summer months had less soil moisture above 0.10 m (below 2%) compared to below 0.10 (3–4%) ($P < 0.0001$). There were sig-

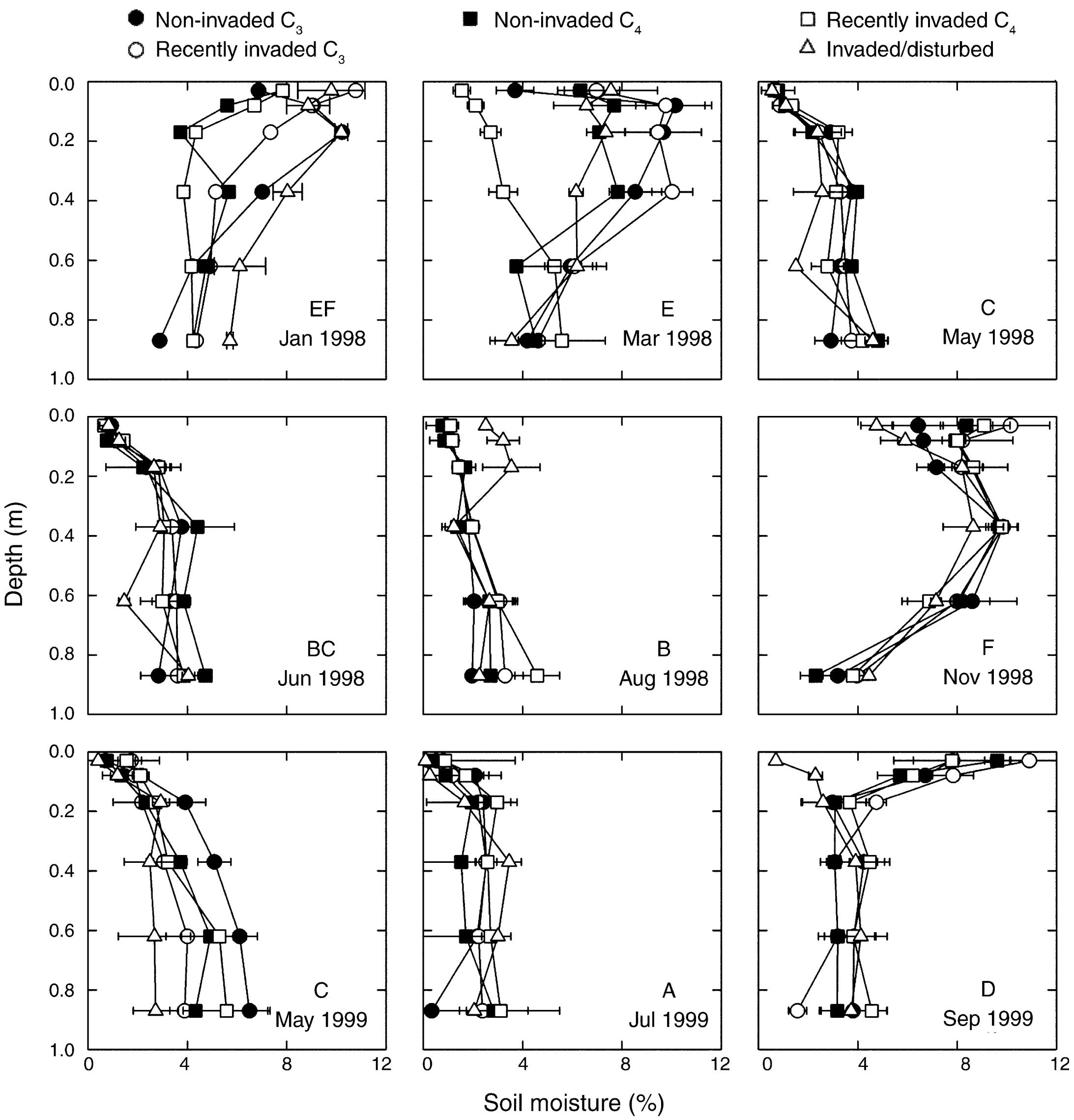

FIG. 2. Soil moisture (mean ± SE) collected from the five community types. Different letters above dates in the lower right corner of each graph indicate significant differences between dates for combined communities and depths at $P < 0.05$.

nificant differences between communities during March 1998 and September 1999; however, this was likely due to precipitation events that occurred during the sampling period ($P < 0.0001$).

Ammonium concentrations changed significantly over time ($P < 0.0001$, Fig. 3), with higher soil NH_4^+ for all communities in November 1998 and September 1999 compared to the other sampling dates. This was driven by high values at the soil surface. There was no interaction among community, depth, and date ($P = 0.998$) or community and depth ($P = 0.853$). There were significant interactions between date and community ($P < 0.0001$) and date and depth ($P < 0.05$). Soil NH_4^+ was higher in the recently invaded C_3 and invaded/disturbed communities compared to the non-invaded C_3 communities ($P < 0.0001$). This trend was driven by increased soil NH_4^+ in the recently invaded C_3 and invaded/disturbed communities in January 1998, March 1998, July 1999, and May 1999. Soil NH_4^+ was higher at the surface, decreasing with depth for all communities ($P < 0.0001$).

Soil NO_3^- concentrations changed significantly over time ($P < 0.0001$, Fig. 4); January 1998, August 1998, and July 1999 had significantly higher NO_3^- concentrations than March 1998 and May 1999. The three-way interaction among community, date, and depth was not significant ($P = 0.352$). There were significant interactions between community and date ($P < 0.0001$), community and depth ($P < 0.0001$), and date and depth ($P < 0.0001$). Soil NO_3^- was higher in the top 0.10 m and below 0.75 m than between 0.10 m and 0.75 m ($P < 0.0001$) for all communities. There was more soil

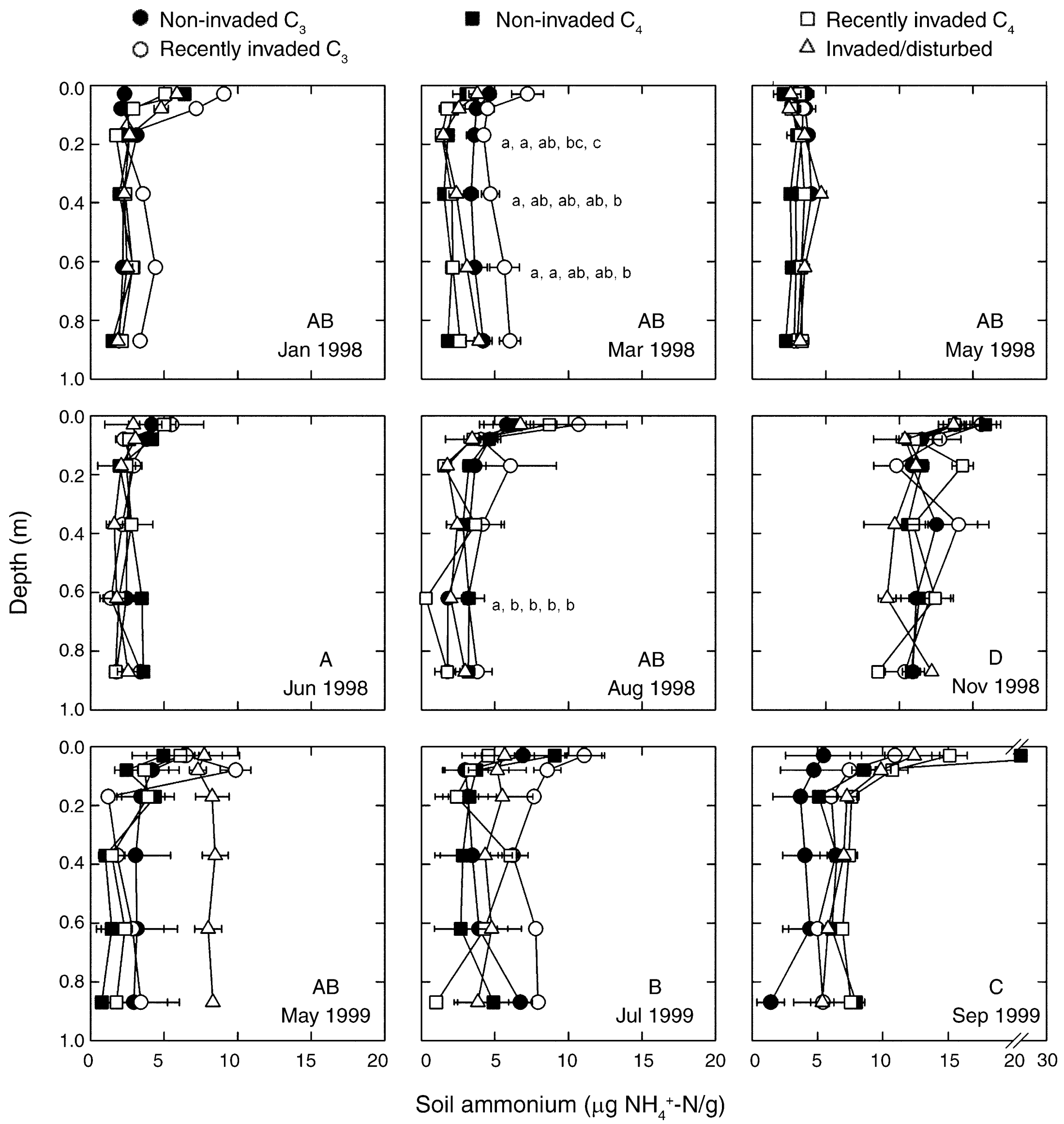

FIG. 3. Soil NH_4^+ concentrations (mean ± SE) collected from the five community types. Repeated-measures results are represented by uppercase letters above the date in the lower right corner of each graph; different letters signify differences at $P < 0.05$. Lowercase letters indicate differences between communities for a given depth on individual dates at $P < 0.05$.

NO_3^- in the invaded/disturbed compared to the other four communities ($P < 0.0001$), with higher subsurface NO_3^- in the invaded/disturbed communities in January 1998, August 1998, and May 1999. The January 1998 NO_3^- concentrations below 0.75 m in the invaded/disturbed communities were extremely high: 56.9 ± 22.4 μg N/g (mean ± 1 SE) compared to 0.65 ± 0.07 μg N/g for the other four communities.

Soil N isotopic composition and total N content

There was a significant interaction between community and depth for soil $\delta^{15}N$ ($P < 0.0001$, Fig. 5), and both main effects were significant (depth, $P < 0.05$; community, $P < 0.0001$). The invaded/disturbed communities had higher soil $\delta^{15}N$ at all depths compared to the other communities. There was a nonsignificant increase in soil $\delta^{15}N$ in the top 0.05 m in the recently invaded compared to the noninvaded C_3 and C_4 communities. Soil $\delta^{15}N$ increased with depth in the noninvaded and recently invaded C_3 and C_4 communities in the top 0.25 m and remained constant below 0.25 m ($P < 0.05$), whereas the invaded/disturbed communities exhibited the opposite pattern, with more positive soil $\delta^{15}N$ values in the surface 0.25 m and consistent soil $\delta^{15}N$ below 0.25 m ($P < 0.05$).

The two-way interaction for depth and community was not significant for total soil N ($P = 0.417$, Fig. 5). There were significant differences between communi-

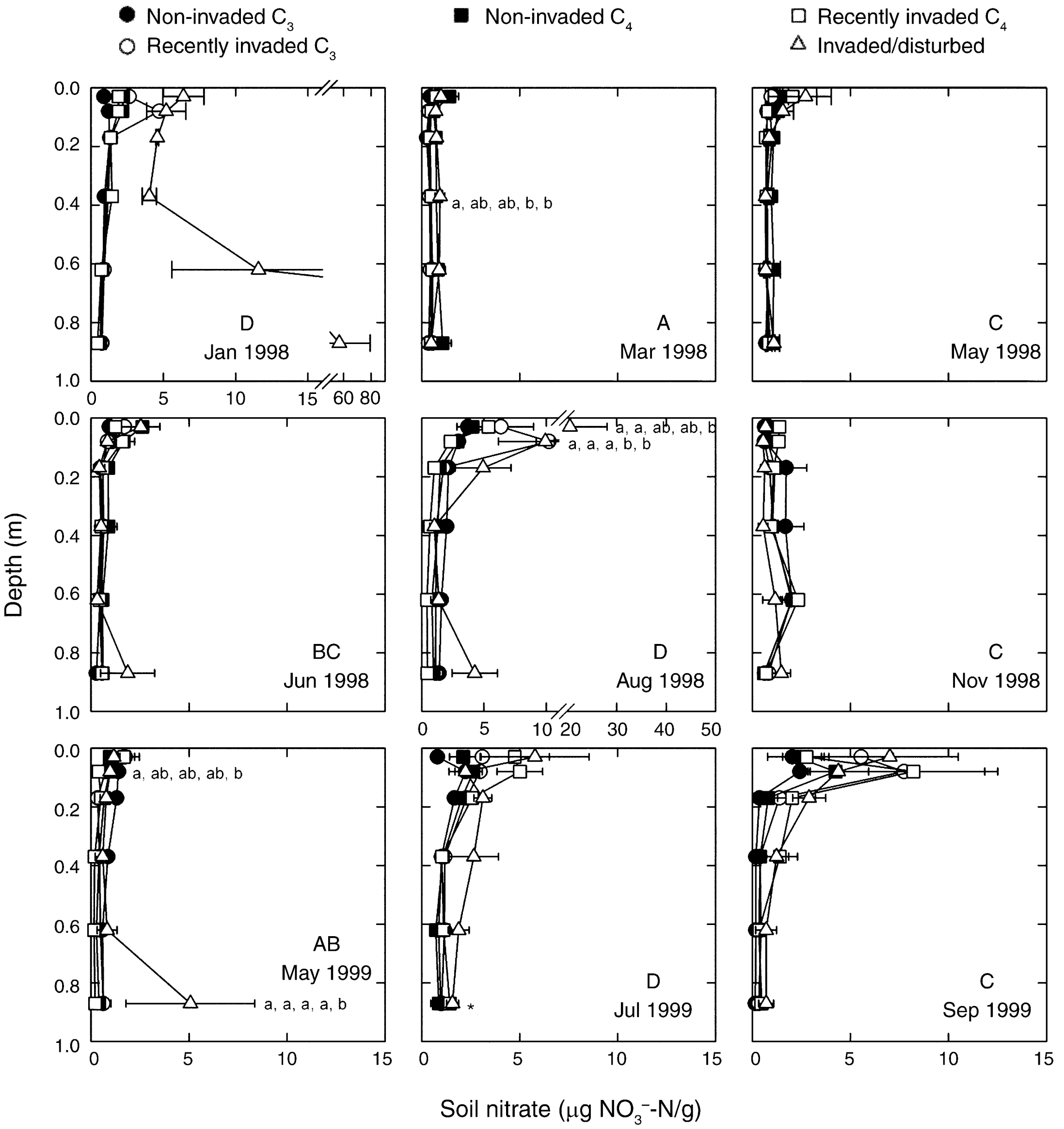

FIG. 4. Soil NO_3^- concentrations (mean ± SE) collected from the five community types. Repeated-measures results are represented by uppercase letters above the date in the lower right corner of each graph; different letters signify differences at $P < 0.05$. Lowercase letters indicate differences between communities for a given depth on individual dates at $P < 0.05$.

ties ($P < 0.0001$) with 104% and 53% more total N in the recently invaded compared to the noninvaded C_3 and C_4 communities, respectively, in the top 0.05 m. Total N was significantly lower in the invaded/disturbed communities compared to the recently invaded C_3 and C_4 communities in the top 0.10 m ($P < 0.05$). Total N decreased with depth ($P < 0.0001$) for all communities with greater total N at the surface 0.10 m compared to below 0.10 m. The invaded/disturbed communities had higher total soil N in the top 0.05 m and did not change with depth below 0.05 m ($P < 0.05$).

Total ecosystem soil nitrogen to 1.0 m depth was estimated by integrating the profile data for each plot. Total soil nitrogen was not significantly different for noninvaded and recently invaded communities ($P > 0.05$), and the mean value was 4200 ± 20 kg N/ha (Fig. 6). In contrast, total soil nitrogen in the invaded/disturbed community was 40% lower at 2500 ± 60 kg N/ha. The decrease in soil nitrogen corresponded with an increase in integrated soil $\delta^{15}N$ for the invaded/disturbed community (Fig. 6); mean values for this site were 5.1‰ greater than for the undisturbed communities.

Soil $\delta^{15}N$ was plotted against ln([N]) using the Rayleigh distillation model (Fig. 7). A comparison of the slopes from the noninvaded C_3 ($P = 0.900$) and C_4 ($P = 0.150$) communities indicates the slopes and inter-

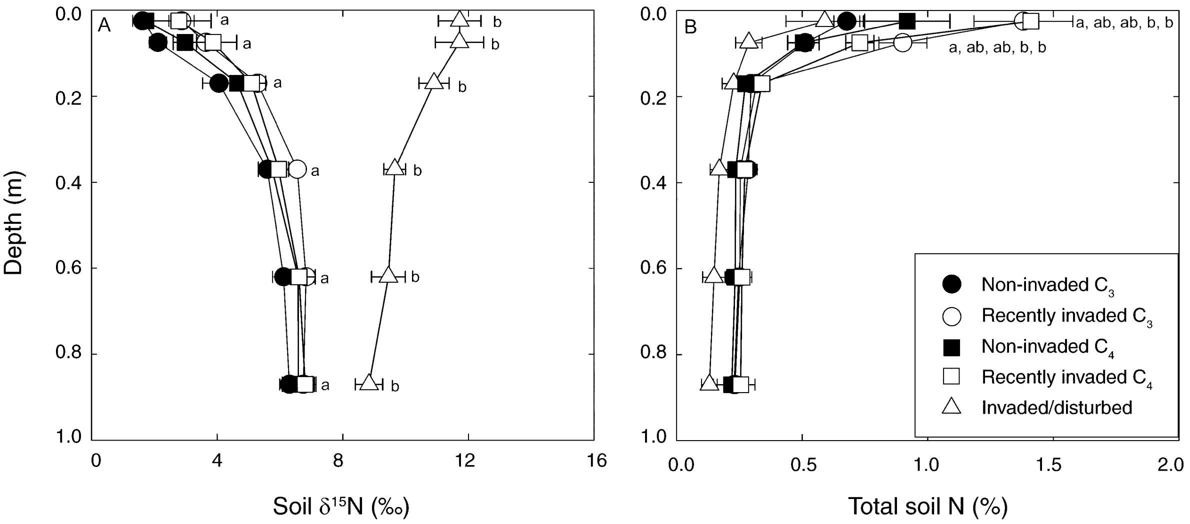

FIG. 5. Soil δ15N and total N (mean ± SE) for 1-m soil cores collected from the five community types. Different letters indicate differences between communities at $P < 0.05$; the noninvaded and invaded C_3 and C_4 communities were combined in panel (A) because there were no significant differences.

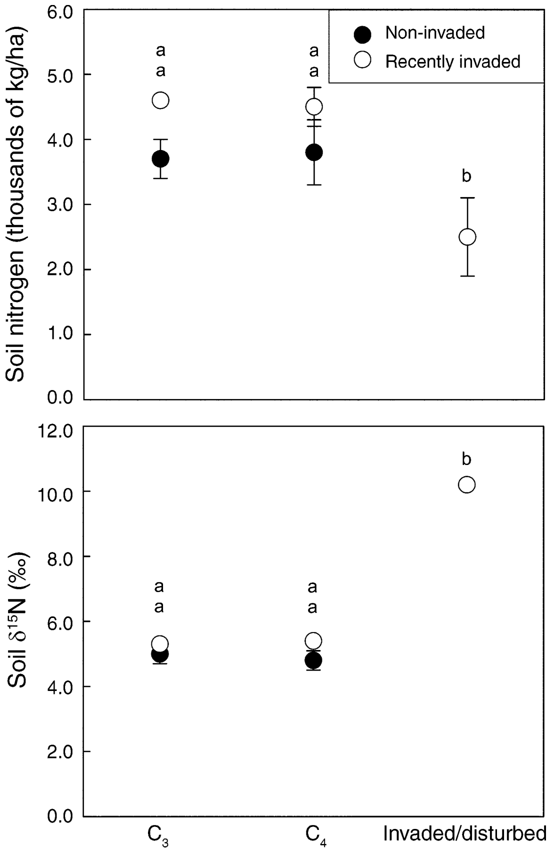

FIG. 6. Soil δ15N and total N (mean ± SE) calculated from soil profile data in Fig. 5 for the noninvaded C_3, recently invaded C_3, noninvaded C_4, recently invaded C_4, and the invaded/disturbed communities. Different letters indicate differences between communities at $P < 0.05$.

cepts are equal. Similarly, the slopes and intercepts from the recently invaded C_3 ($P = 0.828$) and C_4 ($P = 0.771$) communities are equal. Therefore, the two noninvaded communities were combined into a single value, as were the two recently invaded communities. The slopes of the communities with different invasion histories were significantly different ($P < 0.0001$). The linear model describing Rayleigh distillation kinetics was significant for all communities (noninvaded, $R^2 =$

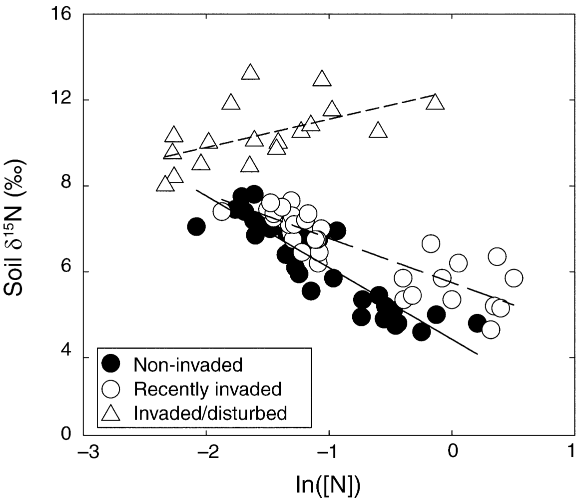

FIG. 7. The Rayleigh distillation model is represented by the relationship between soil δ15N and log-transformed N concentration from soils for the noninvaded, recently invaded, and invaded/disturbed communities. There was no significant difference between C_3 and C_4 communities, so they are grouped in this analysis. The slopes are significantly different for each community type (noninvaded, $\delta^{15}N = 0.83 - 0.90 \times \ln([N])$, solid line; recently invaded, $\delta^{15}N = 3.50 - 0.86 \times \ln([N])$, long dashed line; invaded/disturbed, $\delta^{15}N = 12.40 + 0.56 \times \ln([N])$, short dashed line).

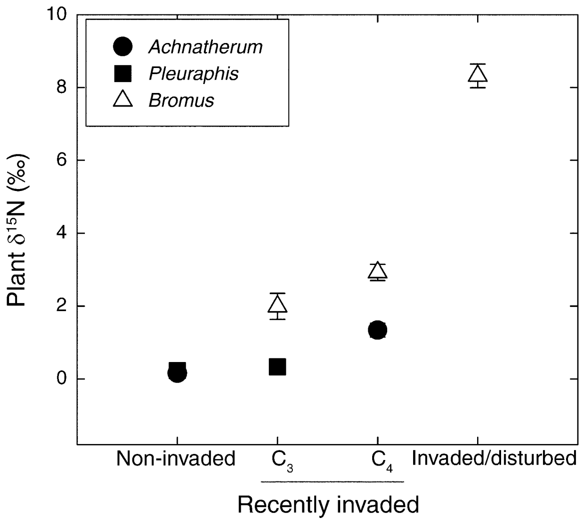

FIG. 8. Plant $\delta^{15}N$ (mean ± SE) for *Achnatherum* spp., *Pleuraphis jamesii*, and *Bromus*. *Achnathrum* spp. and *P. jamesii* were collected in the noninvaded and recently invaded C_3 and C_4 communities, and *Bromus* was collected from the recently invaded C_3 and C_4 and invaded/disturbed communities.

0.80, $P < 0.0001$; recently invaded, $R^2 = 0.74$, $P < 0.0001$; invaded/disturbed, $R^2 = 0.32$, $P < 0.05$, Fig. 7). Invasion significantly increased the slope from -0.90 in the noninvaded communities to -0.86 in the recently invaded communities; the slope in the invaded/disturbed communities changed direction to a positive 0.56 ($P < 0.0001$).

Plant tissue analysis

There was an increase in leaf $\delta^{15}N$ for *Bromus* in March 1998 collected from the invaded C_4 communities ($P = 0.001$). However, because this was the only isotope value that changed over the course of this study, all dates were combined within each population to compare means. There were significant differences between populations for mean foliar $\delta^{15}N$ ($P < 0.0001$; Fig. 8). Plant $\delta^{15}N$ was similar in the noninvaded C_3 and C_4 communities with a mean of 0.2‰. Invaded sites had greater plant $\delta^{15}N$ in the C_3 communities by 1.2‰, whereas the C_4 communities were not affected. *Bromus* $\delta^{15}N$ was 1.6‰ and 1.7‰ more positive than the C_3 and C_4 grasses collected in the recently invaded communities, and *Bromus* collected from the invaded/disturbed communities was 5.4‰ and 6.3‰ more positive than *Bromus* collected in the invaded C_3 and C_4 communities, respectively.

DISCUSSION

Bromus invasion into the arid grasslands of the Colorado Plateau has altered the mechanisms controlling N input and loss, resulting in changes in both the short and the long term. Previous studies in these communities have shown that *Bromus* invasion increased total N content and decreased labile N at the soil surface two years following the initial invasion (Rimer 1998). Three and four years after invasion, Evans et al. (2001*b*) found that increased litter quantity in invaded communities also sequestered N in the litter layer. Additionally, the presence of *Bromus* decreased N_2 fixation rates and altered soil food web composition, biomass, and activity (Belnap and Phillips 2001). This study examined how these changes in N input and loss have altered plant-available N both spatially and temporally beginning four years after the invasion and the effects of those changes on uptake and assimilation by both native grasses and *Bromus*.

Input of surface N appeared to be controlled by N_2 fixation in the noninvaded communities and *Bromus* litter decomposition in the invaded communities. The changes in surface input of N seen in this study were minimal, suggesting inorganic N input from the biological soil crust were similar to the inorganic N released from decomposing *Bromus* litter. However, below 0.5 m, there was an increase in inorganic NO_3^- in invaded communities. Soil and plant isotopes suggest deep soil N was taken up by *Bromus* roots, assimilated into green leaf tissue, and redeposited at the soil surface as litter. In the short term, this has led to an increase in surface N, as evidenced by the increase in total N content in the top 10 cm found in both this study and Rimer's (1998). However, over time, physical destruction of the litter biomass from UV, high temperatures, and wind has resulted in mass loss due to wind and water erosion decreasing total N from the surface.

Both the noninvaded C_3 and C_4 communities have well-developed biological soil crusts containing N_2-fixing lichens and cyanobacteria (Belnap and Phillips 2001). Nitrogen fixation is highest during cool wet conditions (Belnap 2002), and N leaks from these organisms, primarily in the form of NO_3^- (Garcia-Pichel and Belnap 2001). The organisms that fix atmospheric N_2 in the soil crust obtain their energy through photosynthesis. Litter was 95% greater in recently invaded communities (Evans et al. 2001*b*), and the subsequent shading of the soil crust reduces photosynthetic rates (Lange et al. 1998). However, the reduction in N_2 fixation rates due to *Bromus* shading did not decrease N input as expected. This is likely due to decomposition of *Bromus* litter. Decomposition of plant litter is comprised of two phases: N immobilization followed by N mineralization (Melillo et al. 1989). Nitrogen immobilization early in decomposition is accompanied by increased fungal biomass as labile C is consumed, increasing N requirements for growth and development (Parker et al. 1984), and soil fungi were more abundant in both the C_3 and C_4 communities three years following invasion (Belnap and Phillips 2001). In addition, there was a reduction in labile N at these study sites two years post-invasion during the initial immobilization phase (Rimer 1998, Evans et al. 2001*b*). As the easily decomposable C compounds are consumed and

C becomes limiting, the associated N requirements decrease, resulting in increased N mineralization rates (Melillo et al. 1989) and increased surface N input from decomposing *Bromus* litter.

Total soil N in the upper 10 cm was greater in recently invaded communities, but lower in the invaded/disturbed communities, presumably due to N loss rates exceeding new N inputs due to the lack of a functional soil crust with N_2-fixing organisms (Peterjohn and Schlesinger 1991). Interestingly, there is a lack of *Bromus* litter buildup in the invaded/disturbed communities, suggesting considerable mass loss. Buried litter and root decomposition can be predicted by the effects of soil environmental conditions on microbial processes (Moorhead and Reynolds 1991, Silver and Miya 2001), whereas surface litter is often not exposed to soil organisms, yet exhibits higher rates of mass loss (Schaefer et al. 1985, Harrison 2003). Abiotic factors, such as UV breakdown, wind, and water erosion, were attributed to the higher decomposition rates of surface litter in the summer in Chihuahuan (Schaefer et al. 1985, Moorhead and Reynolds 1991, Gehrke et al. 1995) and Great Basin Deserts (Harrison 2003). The sites used in this study were exposed to high temperatures, intense sunlight, and high winds that may have contributed to mass loss and subsequent N loss.

Subsurface inorganic N was higher in the invaded/disturbed communities. Soil NO_3^- concentrations were greater after invasion, with high surface soil NO_3^- in August 1998, July 1999, and September 1999 and was similar to *Bromus*-invaded communities in the Great Basin following *Bromus* senescence (Svejcar and Sheley 2001, Booth et al. 2003). Subsurface NO_3^- in the invaded/disturbed communities was highest in January 1998; NO_3^- concentrations were two orders of magnitude higher than all other communities. Three mechanisms could be responsible for this increase in subsurface NO_3^-: N_2 fixation by symbiotic root bacteria, mineralization from plant roots, or leaching. Although nitrogenase activity has been detected in some species in the C_3 communities (Wullstein et al. 1979), the soil $\delta^{15}N$ profile suggests N_2 fixation was not a substantial form of N input in the invaded/disturbed communities (Kohl and Shearer 1980). Root decomposition and associated N mineralization could elevate deep-soil N concentrations (Parker et al. 1984). However, subsurface mineralization rates are minimal (Evans and Ehleringer 1994), and bacterial biomass decreased with depth at these study sites, reducing the potential for root decomposition (Kuske et al. 2002). Leaching is the most probable mechanism influencing elevated subsurface NO_3^- concentrations. Subsurface soil moisture remained constant throughout the year, suggesting substantial moisture is percolating through the profile, balancing moisture recharge and root uptake at these depths (Kremer and Running 1996). Positively charged NH_4^+ molecules adhere to negatively charged soil particles, restricting movement, and NH_4^+ concentration decreased with depth. However, NO_3^- is mobile and can easily move down through the soil profile (Ylaranta et al. 1993).

Subsurface NO_3^- has been found in other arid ecosystems (Walvoord et al. 2003), often located directly beneath shrubs (Hunter et al. 1982, Schlesinger et al. 1996). Nitrification increases during periods of adequate moisture under trees and shrubs as litter decomposition ensues, and *Bromus* litter enhanced nitrification rates in warm wet months (Burke 1989, Gallardo and Schlesinger 1992, Schlesinger et al. 1996, Burke et al. 1998, Chen and Stark 2000). Water easily infiltrates sandy soils such as those found on the Colorado Plateau (Ylaranta et al. 1993, Kao and Hunt 1996) and can move the NO_3^- down through the soil profile. Because *Bromus* is inactive during summer the increased precipitation from summer and fall monsoons can leach NO_3^- through the soil profile (Clothier and Green 1997). During summer months, soil temperatures rise, increasing surface evaporation rates, and subsequently soil water vaporizes and moves up the soil column. However, NO_3^- does not move up the soil column and remains at subsurface layers. The downward movement of NO_3^- during winter recharge and the concentration of subsurface NO_3^- during periods of water vapor loss are evidenced by the elevated NO_3^- concentrations in the invaded communities below 0.5 m. Mean monthly precipitation in September 1997 was 142% above average, and the high subsurface NO_3^- concentrations in the invaded/disturbed communities the following January may be the result of soil water carrying NO_3^- down the soil profile.

The subsurface NO_3^- pools created by *Bromus* invasion as litter decomposes and the NO_3^- leaches down through the soil profile may create a N source for the following year's cohort of *Bromus*. The ability of *Bromus* to access and assimilate subsurface N is suggested by more positive *Bromus* foliar $\delta^{15}N$ compared to the shorter rooted native species. Foliar $\delta^{15}N$ did not change over time for either the C_3 or C_4 perennial grasses, suggesting the natives were not reallocating stored N (Kolb and Evans 2002) but instead were dependent on surface N throughout the year. Fractionation with uptake can occur above 0.3 mol N/m^3 (Kolb and Evans 2003), and inorganic N concentrations in this study ranged from 0.002 to 4.13 mol N/m^3. Therefore, foliar $\delta^{15}N$ values may have been influenced by physiological factors associated with uptake and assimilation or mycorrhizal influences (Yoneyama et al. 1991, Evans and Ehleringer 1994, Evans et al. 1996, Hobbie and Colpaert 2003). The differences between the native and *Bromus* $\delta^{15}N$ are large, likely beyond fractionation effects, and suggest *Bromus* is accessing subsurface N. Moreover, the native grasses appear to use more surface N than *Bromus*, and their foliar $\delta^{15}N$ reflect N produced by N_2-fixing processes. Invasion has altered spatial acquisition of plant-available N from surface N controlled by input from the biological soil crust to subsurface N leaching through the soil profile. The contrasting patterns of resource acquisition will have important implications for native species survival in invaded communities.

Decomposing *Bromus* litter was incorporated into surface soils, increasing surface soil $\delta^{15}N$. Surface soil $\delta^{15}N$ in noninvaded communities reflect N_2 fixation from the biological soil crust; isotopic fractionation associated with N_2 fixation is $<2‰$, and the organisms that fix N, as well as the surrounding soil, will have low $\delta^{15}N$ values (Kohl and Shearer 1980, Turner et al. 1983). *Bromus* $\delta^{15}N$ was 8.3 ± 0.3‰, and surface soil $\delta^{15}N$ was 11.7 ± 0.7‰ in the invaded/disturbed communities, suggesting N mineralization and trace gas loss were additional factors decreasing surface soil $\delta^{15}N$. Subsurface soil $\delta^{15}N$ was greater in the noninvaded communities as NO_3^- moved down through the soil profile. The uptake and assimilation of subsurface NO_3^- with a more positive $\delta^{15}N$ was evident in *Bromus* with more positive foliar $\delta^{15}N$ than the native grasses. In addition to the incorporation of leachate from the *Bromus* litter with high $\delta^{15}N$ values, continuous fractionating processes, such as mineralization and trace gas loss, increased surface soil $\delta^{15}N$ (Nadelhoffer and Fry 1988). These data suggest N was recycled in the system by *Bromus*: subsurface N was assimilated, redistributed to the surface in biomass, and released during litter decomposition, where it was leached to subsurface layers, becoming available for *Bromus* uptake once again.

The negative slope of the Rayleigh distillation kinetics model describes loss of N during decomposition, and the accompanying increase in soil $\delta^{15}N$ (Evans and Ehleringer 1993). The slope for the noninvaded communities was similar to those found in arid ecosystems (Evans and Ehleringer 1993), where N loss is due to decomposition and mineralization as N moves down through the soil profile. The slope in the invaded/disturbed community was positive, suggesting the mechanisms controlling N in the soil profile had been altered. The Rayleigh Distillation model assumes mass loss follows typical $\delta^{15}N$ enrichment patterns, and this was clearly not the case in the invaded/disturbed communities, where lower total N included physical mass loss as well as biotic loss. Moreover, the uptake of subsurface NO_3^- with high $\delta^{15}N$ maintained the pattern of more positive soil $\delta^{15}N$ at the surface as *Bromus* litter was exposed to fractionating processes. This pattern of soil $\delta^{15}N$ with depth acts as a clock tracing the changes in N dynamics with invasion over time.

The invasion of *Bromus* has greatly impacted N dynamics in both undisturbed and disturbed arid grasslands on the Colorado Plateau. Surface NO_3^- appeared to have leached downward, resulting in elevated subsurface soil NO_3^- pools. This subsurface NO_3^- may have supplied *Bromus* with a N source unavailable to native grasses, as evidenced by the greater *Bromus* $\delta^{15}N$ values. Total N was greater four years post-invasion in the top 0.10 m, possibly due to leachate from the additional *Bromus* litter and increased N immobilization rates, whereas the reduced total N in the invaded/disturbed communities was likely due to high temperatures and UV that increased decomposition rates of surface *Bromus* litter. These data suggest *Bromus*-dominated grasslands generate a positive feedback loop by acquiring and assimilating subsurface N, depositing it at the surface as litter, where decomposition and leaching return that N to subsurface layers that are again available for *Bromus* uptake. *Bromus* has altered the general pattern of N cycling from input and loss controlled by the biological soil crust to recycling within the system, with subsurface N uptake and assimilation, depositing that N at the surface as litter, and leaching the N to subsurface levels as *Bromus* litter decomposes.

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LITERATURE CITED

Belnap, J. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biology and Fertility of Soils **35**:128–135.

Belnap, J., and D. J. Eldridge. 2001. Disturbance and recovery of biological soil crusts. Pages 363–384 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function and management. Ecological Studies Series. Volume 150. Springer Verlag, New York, New York, USA.

Belnap, J., and O. L. Lange. 2001. Biological soil crusts: structure, function and management. Ecological Studies Series. Volume 150. Springer Verlag, New York, New York, USA.

Belnap, J., and S. L. Phillips. 2001. Soil biota in an ungrazed grassland: response to annual grass (*Bromus tectorum*) invasion. Ecological Applications **11**:1261–1275.

Booth, M. S., J. M. Stark, and M. M. Caldwell. 2003. Inorganic N turnover and availability in annual- and perennial-dominated soils in a northern Utah shrub-steppe ecosystem. Biogeochemistry **66**:311–330.

Burke, I. C. 1989. Control of nitrogen mineralization in a sagebrush steppe landscape. Ecology **70**:1115–1126.

Burke, I. C., W. K. Lauenroth, M. A. Vinton, P. B. Hook, R. H. Kelly, H. E. Epstein, M. R. Aguiar, M. D. Robles, M. O. Aguilera, K. L. Murphy, and R. A. Gill. 1998. Plant–soil interactions in temperate grasslands. Biogeochemistry **42**:121–143.

Chapin, III, F. S., M. S. Torn, and M. Tateno. 1996. Principles of ecosystem sustainability. American Naturalist **148**: 1016–1037.

Chen, J., and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology and Biochemistry **32**:47–57.

Clothier, B. E., and S. R. Green. 1997. Roots: the big movers of water and chemical in soil. Soil Science **162**:534–543.

Epstein, H. E., I. C. Burke, A. R. Mosier, and G. L. Hutchinson. 1998. Plant functional type effects on trace gas fluxes in the shortgrass steppe. Biogeochemistry **42**:145–168.

Evans, R. D., and J. Belnap. 1999. Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology **80**:150–160.

Evans, R. D., J. Belnap, F. Garcia-Pichel, and S. Phillips. 2001*a*. Global change and the future of biological soil crusts. Pages 417–429 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function and management. Ecological Studies Series. Volume 150. Springer Verlag, New York, New York, USA.

Evans, R. D., A. J. Bloom, S. S. Sukrapanna, and J. R. Ehleringer. 1996. Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. Plant Cell and Environment **19**:1317–1323.

Evans, R. D., and J. R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from ^{15}N of soils. Oecologia **94**:314–317.

Evans, R. D., and J. R. Ehleringer. 1994. Water and nitrogen dynamics in an arid woodland. Oecologia **99**:233–242.

Evans, R. D., and J. R. Johansen. 1999. Microbiotic crusts and ecosystem processes. Critical Reviews in Plant Sciences **18**:183–225.

Evans, R. D., and O. L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. Pages 263–279 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function and management. Ecological Studies Series. Volume 150. Springer Verlag, New York, New York, USA.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001*b*. Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecological Applications **11**:1301–1310.

Gallardo, A., and W. H. Schlesinger. 1992. Carbon and nitrogen limitations of soil microbial biomass in desert ecosystems. Biogeochemistry **18**:1–17.

Garcia-Pichel, F., and J. Belnap. 2001. Small-scale environments and distribution of biological soil crusts. Pages 193–201 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function and management. Ecological Studies Series. Volume 150. Springer Verlag, New York, New York, USA.

Gehrke, C., U. Johanson, T. V. Callaghan, D. Chadwick, and C. H. Robinson. 1995. The impact of enhanced ultraviolet-B radiation on litter quality and decomposition processes in Vaccinium leaves from the Sub-Arctic. Oikos **72**:213–222.

Harris, G. A. 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. Ecological Monographs **37**:89–111.

Harrison, K. S. 2003. Litter decay processes and soil nitrogen availability in native and cheatgrass-dominated arid rangelands. Thesis. Oregon State University, Corvallis, Oregon, USA.

Hobbie, E. A., and J. V. Colpaert. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytologist **157**:115–126.

Hooper, D. U., and P. M. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs **68**:121–149.

Hunter, R. B., E. M. Romney, and A. Wallace. 1982. Nitrate distribution in Mojave Desert soils. Soil Science **134**:22–30.

Kao, C. S., and J. R. Hunt. 1996. Prediction of wetting front movement during one-dimensional infiltration into soils. Water Resources Research **32**:55–64.

Kleiner, E. F., and K. T. Harper. 1977*a*. Occurrence of four major perennial grasses in relation to edaphic factors in a pristine community. Journal of Range Management **30**:286–289.

Kleiner, E. F., and K. T. Harper. 1977*b*. Soil properties in relation to cryptogamic groundcover in Canyonlands National Park. Journal of Range Management **30**:202–205.

Kohl, D. H., and G. Shearer. 1980. Isotopic fractionation associated with symbiotic N_2 fixation and uptake of NO_3^- by plants. Plant Physiology **66**:51–56.

Kolb, K. J., and R. D. Evans. 2002. Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. New Phytologist **156**:57–64.

Kolb, K. J., and R. D. Evans. 2003. Influence of nitrogen source and concentration on nitrogen isotopic discrimination in two barley genotypes (*Hordeum vulgare* L.). Plant Cell and Environment **26**:1431–1440.

Kremer, R. G., and S. W. Running. 1996. Simulating seasonal soil water balance in contrasting semi-arid vegetation communities. Ecological Modelling **84**:151–162.

Kuske, C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology **68**:1854–1863.

Lange, O. L. 2001. Photosynthesis of soil-crust biota as dependent on environmental factors. Pages 217–240 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function and management. Ecological Studies Series. Volume 150. Springer Verlag, New York, New York, USA.

Lange, O. L., J. Belnap, and H. Reichenberger. 1998. Photosynthesis of the cyanobacterial soil-crust lichen *Collema tenax* from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO_2 exchange. Functional Ecology **12**:195–202.

Mack, R. N. 1981. Invasion of *Bromus tectorum* L. into western North America: an ecological chronicle. Agro-Ecosystems **7**:145–165.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications **10**:689–710.

Melillo, J. M., J. D. Aber, A. E. Linkins, A. Ricca, B. Fry, and K. J. Nadelhoffer. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant and Soil **115**:189–198.

Miller, M., J. Belnap, S. Beatty, and B. Webb. 2001. Components of spatial and temporal soil variation at Canyonlands National Park: implications for P dynamics and cheatgrass (*Bromus* tectorum) performance. Pages 154–162 *in* USDA Forest Service Proceedings RMRS-P-21. USDA Forest Service, Washington, D.C., USA.

Moorhead, D. L., and J. F. Reynolds. 1991. A general-model of litter decomposition in the northern Chihuahuan Desert. Ecological Modelling **56**:197–219.

Nadelhoffer, K. J., and B. Fry. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Science Society of America Journal **52**:1633–1640.

Parker, L. W., P. F. Santos, J. Phillips, and W. G. Whitford. 1984. Carbon and nitrogen dynamics during the decomposition of litter and roots of a Chihuahuan desert annual, *Lepidium lasiocarpum*. Ecological Monographs **54**:339–360.

Peterjohn, W. T., and W. H. Schlesinger. 1991. Factors controlling denitrification in a Chihuahuan Desert ecosystem. Soil Science Society of America Journal **55**:1694–1701.

Rafferty, D. L., and J. A. Young. 2002. Cheatgrass competition and establishment of desert needlegrass seedlings. Journal of Range Management **55**:70–72.

Rimer, R. L. 1998. The influence of plant invasion and surface disturbance on nitrogen cycling in a cold desert ecosystem. Thesis. University of Arkansas, Fayetteville, Arkansas, USA.

Schaefer, D., Y. Steinberger, and W. G. Whitford. 1985. The failure of nitrogen and lignin control of decomposition in a North American desert. Oecologia **65**:382–386.

Schlesinger, W. H., J. A. Raikes, A. E. Hartley, and A. F. Cross. 1996. On the spatial pattern of soil nutrients in desert ecosystems. Ecology **77**:364–374.

Silver, W. L., and R. K. Miya. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia **129**:407–419.

Stohlgren, T. J., Y. Otsuki, C. A. Villa, M. Lee, and J. Belnap. 2001. Patterns of plant invasions: a case example in native species hotspots and rare habitats. Biological Invasions **3**: 37–50.

Svejcar, T., and R. Sheley. 2001. Nitrogen dynamics in perennial- and annual-dominated arid rangeland. Journal of Arid Environments **47**:33–46.

Turner, G. L., F. J. Bergersen, and H. Tantala. 1983. Natural enrichment of ^{15}N during decomposition of plant material in soil. Soil Biology and Biochemistry **15**:495–497.

Vitousek, P. M. 1992. Global environmental change: an introduction. Annual Review of Ecology and Systematics **23**: 1–14.

Walvoord, M. A., F. M. Phillips, D. A. Stonestrom, R. D. Evans, P. C. Hartsough, B. D. Newman, and R. G. Striegl. 2003. A reservoir of nitrate beneath desert soils. Science **302**:1021–1024.

Wedin, D., and D. Tilman. 1993. Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. Ecological Monographs **63**:199–229.

Wullstein, L. H., M. L. Bruening, and W. B. Bollen. 1979. Nitrogen fixation associated with sand grain root sheaths (rhizosheaths) of certain xeric grasses. Physiologia Plantarum **46**:1–4.

Ylaranta, T., J. Uusikamppa, and A. Jaakkola. 1993. Leaching of nitrogen in barley, grass ley and fallow lysimeters. Agricultural Science in Finland **2**:281–291.

Yoneyama, T., T. Omata, S. Nakata, and J. Yazaki. 1991. Fractionation of nitrogen isotopes during the uptake and assimilation of ammonia by plants. Plant Cell Physiology **32**:1211–1217.