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Effects of *Bromus tectorum* invasion on microbial carbon and nitrogen cycling in two adjacent undisturbed arid grassland communities

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Abstract Soil nitrogen (N) is an important component in maintaining ecosystem stability, and the introduction of non-native plants can alter N cycling by changing litter quality and quantity, nutrient uptake patterns, and soil food webs. Our goal was to determine the effects of *Bromus tectorum* (C_3) invasion on soil microbial N cycling in adjacent non-invaded and invaded C_3 and C_4 native arid grasslands. We monitored resin-extractable N, plant and soil $\delta^{13}C$ and $\delta^{15}N$, gross rates of inorganic N mineralization and consumption, and the quantity and isotopic composition of microbial phospholipid biomarkers. In invaded C_3 communities, labile soil organic N and gross and net rates of soil N transformations increased, indicating an increase in overall microbial N cycling. In invaded C_4 communities labile soil N stayed constant, but gross N flux rates increased. The $\delta^{13}C$ of phospholipid biomarkers in invaded C_4 communities showed that some portion of the soil bacterial population preferentially decomposed invader C_3-derived litter over that from the native C_4 species. Invasion in C_4 grasslands also significantly decreased the proportion of fungal to bacterial phospholipid biomarkers. Different processes are occurring in response to *B. tectorum* invasion in each of these two native grasslands that: 1) alter the size of soil N pools, and/or 2) the activity of the microbial community. Both processes provide mechanisms for altering long-term N dynamics in these ecosystems and highlight how multiple mechanisms can lead to similar effects on ecosystem function, which may be important for the construction of future biogeochemical process models.

Keywords Global change · Exotic species invasion · Nitrogen cycling · Carbon cycling · Stable isotopes · Phospholipid fatty acids

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Introduction

Invasive species threaten the stability of ecosystems worldwide by altering resource availability, trophic structure, and biodiversity (Vitousek 1992; D'Antonio and Vitousek 1992). Soil nitrogen (N) is a key resource affected by the introduction of non-native plants, which change plant-associated organic inputs (Wolfe and Klironomos 2005; Evans et al. 2001), nutrient uptake patterns (Wedin and Tilman 1993; Ehrenfeld 2003; Sperry et al. 2006), and soil food webs (Belnap and Phillips 2001; van der Putten et al. 2007). Soil N dynamics are a function of the input of plant-associated organic material and the diversity and metabolism of the soil microbial community (Barrett and Burke 2000; van der Putten et al. 2007). The response of arid ecosystem N dynamics to invasion remains largely unknown as we have incomplete understanding of the belowground carbon (C) and N dynamics in these ecosystems.

Soil bacterial and fungal populations mediate soil organic matter (SOM) transformations, thereby influencing the composition of SOM and controlling dynamics of inorganic nutrients (NH_4^+ and NO_3^-) in the soil (Wolfe and Klironomos 2005). Since N mineralized from SOM by soil microbial activity is available for uptake by plants and microbes, increases in soil C availability could potentially alter overall microbial activity (Groffman 1999; Zak et al. 2000; Billings et al. 2002; Hooker and Stark 2008) and thus immobilization of available N (Schimel et al. 1989; Davidson et al. 1990; Schaeffer and Evans 2005). The positive effect of increased C availability could stimulate immobilization of N into microbial biomass, further acting as a negative feedback on plant growth (Rowe et al. 2009). In order to more fully understand soil C and N dynamics, there is a need to relate the sources of C utilized by heterotrophic microorganisms to decomposer community composition.

One of the most significant plant invasions in North America has been the establishment of the cleistogamus annual grass *Bromus tectorum* L. in arid regions of the Intermountain West (Mack 1981). Many habitats in western North America have been converted from bunchgrass/shrub communities to monospecific stands of *B. tectorum.* (Mack 1981). In semi-arid shrub ecosystems, invasion by *B. tectorum* can significantly alter microbial N cycling (Booth et al. 2003a) and SOM dynamics (Norton et al. 2004, 2008) demonstrating that the susceptibility of ecosystems to invasion is affected by differences in the resource use characteristics between native plant species and *B. tectorum* (Booth et al. 2003b). However, separating the ecosystem effects of invasion from other disturbances, such as grazing (Stark and Hart 1999), has been difficult because of the rarity of physically undisturbed, invaded communities (Evans and Belnap 1999; Evans et al. 2001; Rimer and Evans 2006; Sperry et al. 2006). Virginia Park is a grassland in southeastern Utah that has never been grazed by livestock and has long been closed to all human use. This area was invaded by *B. tectorum* in the fall of 1995, and has provided the opportunity to study the influence of *B. tectorum* invasion in the absence of physical disturbance.

Previous studies at this site addressed the immediate effect (<2 years post-invasion) of plant invasion on ecosystem N dynamics. These studies found that total soil N content at the soil surface increased, labile N concentrations in the top 10 cm of soil decreased, and soil net N mineralization rates decreased in native C_3 communities (Rimer and Evans 2006). Evans et al. (2001) observed that in addition to decreases in net N mineralization rates, litter fall in invaded but undisturbed C_3 and C_4 communities increased relative to non-invaded communities, and that overall aboveground litter quality (lignin:N) was lower. A follow-up study by Sperry et al. (2006) found that by 4 years post-invasion, *B. tectorum* utilized NO_3^- from subsurface soil layers, which led to greater surface soil N-availability, which then led to increased litter production by *B. tectorum* and NO_3^- leaching in a positive feedback loop. This positive feedback was stronger in wet years. *B. tectorum* invasion has also lowered soil food web diversity and increased the proportions of active bacterial component of the soil microbial community (Belnap and Phillips 2001; Belnap et al. 2005). Two important points from these studies are: (1) changes in N cycling can occur rapidly ($\sim$2 years) after *B. tectorum* establishment, and (2) these changes in N cycling may occur at the same time as changes in soil microbial community composition and substrate availability (i.e. addition of *B. tectorum*-derived organic materials).

Our goals for this project were to characterize the mechanisms by which invasion alters soil N cycling in these C_3 and C_4 native arid grassland communities and to determine whether *B. tectorum* invasion has affected the associated soil bacterial and fungal

components of the soil microbial community. First, we hypothesized that once *B. tectorum*-associated organic materials enter the SOM pool (increased soil C from greater productivity, and N from access to previously sequestered deep sources), these inputs would increase the rate of soil N cycling. Both the gross supply (mineralization) and demand (immobilization) of inorganic N by the microbial community would increase, possibly altering net N availability. Second, we hypothesized that changes in SOM composition due to *B. tectorum*-associated inputs would lead to shifts in the structure and activity levels of soil bacteria and fungi. The specific response of these components of the soil microbial community to *B. tectorum* inputs is difficult to predict a priori because the organic matter deposited may favor "opportunistic" decomposers (e.g., bacteria), or the composition of the organic matter may favor decomposers able to metabolize relatively complex substrates (e.g., fungi). To test these hypotheses, we monitored net inorganic N mineralization and plant and soil isotopic composition (Evans et al. 2001; Sperry et al. 2006; Rimer and Evans 2006), and made an independent assessment of soil bacterial and fungal abundance and activity following Belnap and Phillips (2001). To link ecosystem N availability to changes in microbial community dynamics, we measured soil C and N fluxes, gross rates of inorganic N mineralization/consumption, microbial phospholipid fatty acid (PLFA) biomarkers, and microbial C substrate utilization via $\delta^{13}C$ of microbial PLFA.

Methods

The study site is located in the Needles District of Canyonlands National Park in Southeastern Utah, USA. It is a 100 ha arid grassland that has never been grazed by livestock. The climate is typical of the Colorado Plateau Desert with 215 mm precipitation annually. Soils are coarse–loamy, mixed, mesic Ustollic Camborthids (Begay series), and fall in the fine sandy loam texture class with 64% sand, 25% silt, and 13% clay from 0 to 0.3 m (Kleiner and Harper 1972, 1977). Full soil and vegetation descriptions are provided in Kleiner and Harper (1977). Two distinct vegetation associations are scattered throughout the site, and each community type can occupy up to 1 ha patches (Kleiner and Harper 1977). The first vegetation association is co-dominated by C_3 bunchgrasses: *Stipa hymenoides* R. & S. (Indian ricegrass), *Hesperostipa 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* (nomenclature follows Welsh et al. 1993). The second vegetation association is dominated by *Hilaria jamesii* (Torr.) Benth. (galleta grass), a C_4 warm season grass, and will be referred to as the *C_4 community*. *B. tectorum* cover in the site was $\sim$0.4% in 1994 until it increased to 20% in 1996 and 57% in 1998 (Kleiner and Harper 1972, 1977; Belnap and Phillips 2001). The invasion developed in a discontinuous fashion and did not represent a single invasion front. The invasion was much more severe into the C_4 community, with *B. tectorum* cover up to twice that found in the C_3 community during any given year. In 1995, 20 $\times$ 30 m permanent plots were established in distinct vegetation patches; three each in patches of the non-invaded and invaded C_3 and C_4 communities ($n = 12$). Plots were arranged in 3 blocks, roughly 500 m apart, with one replicate of each community type (non-invaded and invaded C_3 and C_4) in each block.

Plant and soil stable isotope composition

We collected foliage (2–3 leaves from three individuals in each plot) from the three grass species (*B. tectorum*, *S. hymenoides*, and *H. jamesii*) in 2001 in non-invaded and invaded C_3 and C_4 plots. Vegetation samples were oven-dried for 48 h at 60°C and ground to a fine powder. Soils were sampled from the top 10 cm using a 5 cm diameter soil-coring device. Soils were passed through a 2 mm mesh sieve, air dried, and ground to a fine powder. It was determined that washing three times with 3 N H_3PO_4 was sufficient to remove carbonates for isotopic analysis, yet not affect the $\delta^{13}C$ and $\delta^{15}N$ of the SOM via hydrolysis (data not shown). Both vegetation and soil samples were analyzed for %N, %C, $\delta^{13}C$ and $\delta^{15}N$ on a Carlo Erba Elemental Analyzer (NA1500 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan, Italy) coupled to a Finnigan Delta$^+$ mass spectrometer (Finnigan MAT, Bremen, Germany) via a Finnigan Conflo II Interface.

Resin-extractable nitrogen

An index of in situ net N mineralization rates is useful for tracking ecosystem-level N dynamics over long time periods (months to years). To accomplish this, we used 10 g of cation–anion exchange resin (MTO-Dowex MR-3, Supelco, Bellefonte, PA, USA) in nylon bags (Binkley and Matson 1983; Binkley 1984), and buried bags 5 cm from the soil surface. Three bags were installed on the perimeter of each of the 12 sampling plots and were replaced approximately every 8 weeks from September 2000 through December 2003. Each time bags were replaced, a different location was chosen. Upon removal from the soil, bags were transported to the laboratory, extracted in 50 ml 2 M KCl, placed on a shaker table for 1 h, and then gravity filtered (Whatman #4) to remove any resin beads or soil particles. Extracts were stored at 4°C until analyzed for NH_4^+ and NO_3^- concentrations using a colorimetric auto-analyzer (Alpkem FS3000, OI Analytical, College Station, TX, USA). Blanks (resin only) were measured, subtracted from field values, and net rates were calculated by dividing by the number of days the resin bag was in the soil.

Soil Organic N pools and C mineralization

We collected soil from non-invaded and invaded C_3 and C_4 grasslands for laboratory incubation (3 replicates for each grassland type and invasion status). Fifty grams dry weight of each soil type was placed in 5.3 cm diameter × 5.0 cm tall polyvinyl chloride cores, held by glass fiber filter paper taped to the bottom. We leached inorganic N from each soil sample by vacuum extraction with 75 ml N-free nutrient solution (Nadelhoffer 1990) at the beginning of the incubation and placed each soil sample in a 1 l gas-tight jar equipped with a gas sampling port. On days 7, 19, 42, 84, 228, 315, and 385 we extracted 9 ml of gas from each jar into a pre-evacuated, gas-tight glass vial. After each gas sampling, we leached inorganic N from each soil sample with N-free nutrient solution (75 ml), placed the samples back in the jars, and took four samples of ambient laboratory air before sealing the jars. Between sampling dates, samples were stored in the dark at 30°C. Leachate extracts were analyzed colorimetrically for ammonium (NH_4^+) and nitrate (NO_3^-) on an auto-analyzer. The difference in soil inorganic N concentrations between extraction dates was used to calculate net N mineralization rate. Gas samples were analyzed for CO_2 on a Shimadzu 14A gas chromatograph (GC14-A, Dallas, TX, USA) equipped with a thermal conductivity detector. Rates of CO_2–C evolution were calculated as the CO_2 concentration increase over time, accounting for jar headspace, soil mass, and initial concentrations in laboratory air.

We applied an exponential model of net N mineralization relative to total soil N (Bonde and Rosswall 1987; Wedin and Pastor 1993) to estimate pool sizes of labile N, rate constants for mineralization of labile pools, and mineralization rates of recalcitrant pools:

$$N_t = N_l(1 - e^{-h_l t}) + c_r t$$

where N_t is the amount of N mineralized at time t, N_l is the pool size of the labile pool of N relative to total soil N, h_l is the rate constant for the labile N pool, and c_r is the mineralization rate of the recalcitrant pool of N. The model assumes that mineralization of recalcitrant N is constant and does not decrease over time. We estimated parameter values using a non-linear curve fitting procedure (PROC NLIN, SAS 8.01) of net N mineralization data. This procedure finds the best fitting equation by minimizing the sum of squares of the residuals. We tested the robustness of parameter estimates by changing the starting values of the iterative procedure to values within the 95% confidence intervals; no change in parameter estimates resulted.

To assess potential changes in C substrate assimilation and mineralization by microbes, jars were sampled on day 0 of the incubation for $\delta^{13}C$ of soil-respired CO_2 and the values compared to the $\delta^{13}C$ of PLFA biomarkers. Headspace CO_2 was sampled 24 h after the jars were sealed to allow time for mineralized C to accumulate. The $\delta^{13}CO_2$ was measured by collecting 20–100 µl of headspace air in a gastight syringe (Vici Precision Sampling, Baton Rouge, LA, USA) and injecting the air into a stable isotope mass spectrometer (Finnigan MAT, Bremen, Germany) interfaced with a GC column used to separate CO_2 and N_2O in air samples (Precon, Finnigan MAT). The volumes of gas analyzed varied, depending on sample CO_2 concentrations.

Gross N fluxes

A modified version of the ^{15}N isotope pool dilution method (Hart et al. 1994) was used to measure gross N flux rates. Five 100 g samples of soil were used for $^{15}NH_4^+$ and $^{15}NO_3^-$ addition along with two controls ($n = 12$). Each sample was placed in a plastic bag and labeled with 5 μg 99.9% ^{15}N as either $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ dissolved in 6 ml of deionized H_2O. Control soils received deionized water only. A 50 g subsample was extracted immediately in 100 ml of 2 M KCl. The remaining 50 g subsample was placed in an incubator (20°C) for 24 h and extracted at the end of that time period in 100 ml of 2 M KCl. After addition of KCl, extracts were shaken for 2 h and placed in a cold room (4°C) overnight. The next day, extracts were filtered (Whatman #4) and colorimetrically analyzed on an auto-analyzer for NH_4^+ and NO_3^- concentration. Remaining N in extracts was transferred onto acidified quartz filter disks using the diffusion procedure outlined in Hart et al. (1994) for stable isotope analysis. Changes in the inorganic N concentrations and the changes in the atom percent excess of soils at 0 and 24 h were used to calculate gross N flux rates.

Soil PLFA analysis

Total lipids were extracted from 50 g of freeze–dried, field-collected soil using a modified Bligh–Dyer procedure (Bligh and Dyer 1959; White and Ringelberg 1998; Pinkart et al. 2002). A silicic acid phase column (Bond Elut Mega BF-SI, 2 g, 12 ml, Varian Inc., Harbor City, CA, USA) on a vacuum manifold was used to separate the neutral lipids, glycolipids, and phospholipids following total lipid extraction (Dobbs and Findlay 1993; White and Ringelberg 1998). After mild alkaline hydrolysis with KOH and acidification with HCl, fatty acids in the phospholipid fraction were converted to their corresponding fatty acid methyl esters (FAME) using BF_3 in methanol (Dobbs and Findlay 1993). Each FAME was identified using a GC–mass spectrometer (GC–MS; Hewlett Packard HP 5890 series II plus, Palo Alto, CA, USA) with a 70% cyanopropyl polysilphenylene-siloxane column (SGE BPX-70, 50 m, 0.23 mm i.d.) with a temperature ramp from 60 to 280°C at 4°C min^{-1}, and interfaced with a mass-selective detector (HP 5970B). Identification of unknown FAMEs was accomplished by comparing retention times and mass spectra against a combination of known standards (Bacterial Fatty Acid Methyl Esters and 37 component FAME standards, Supelco Co., Rockford, IL, USA) and mass spectral libraries from the National Bureau of Standards. The concentrations of individual FAMEs extracted from each soil sample were determined by GC-flame ionization detection (GC-FID) using the same GC parameters for GC–MS analysis. Standard nomenclature for fatty acids was used.

The isotopic compositions of individual fatty acids were determined using an HP6890 GC coupled to a Finnigan Delta$^+$ via a Finnigan GC/CIII combustion interface. The same GC column, oven, and injector conditions used for the GC-FID and GC–MS analysis of FAMEs were employed in the isotopic analysis of the FAMEs. Each fatty acid was corrected for the addition of the methyl carbon from BF_3/methanol derivatization using mass balance of free methylated tridecanoic and tricosanoic acids added as internal standards (Abrajano et al. 1994).

Statistical analyses

For time-series data, a repeated measures, mixed effects analysis (PROC MIXED, SAS 8.01) was used to determine effect of invasion, date of sampling, plant community type, and their interaction on a given parameter. This test allowed us to model the covariance structure of the data set to account for unevenly spaced sampling dates (Littel et al. 1996). For all other normally distributed data, analysis of variance (PROC GLM, SAS 8.01) was used for determining effects of invasion status, plant community type, and their interaction. When necessary, data were log-transformed for normality. Pair-wise comparisons of means were made using Tukey's least significant difference. When data violated the assumption of normality, non-parametric methods were employed (PROC NPAR1-WAY, SAS 8.01) to determine the effect of invasion for each plant community type. All analyses were performed using SAS statistical software (Cary, NC, USA). Statistical significance was determined at $\alpha = 0.05$. Unless stated otherwise, all results reported as statistically significant occur at $P < 0.05$. Errors are presented as one standard error of the mean.

Results

Plant and soil stable isotope composition

Bulk soil data from the invaded sites showed no significant differences with *B. tectorum* invasion for mean soil organic $\delta^{13}C$, and $\delta^{15}N$ (Table 1). In C_3 communities, there was 23 ± 10% greater total soil N and 19 ± 7% greater soil organic C (SOC) in invaded compared to non-invaded communities when pair-wise comparisons were considered. These differences led to significantly lower soil C:N ratio when calculated as SOC:total N. There was no significant difference in SOC and total soil N among non-invaded and invaded C_4 communities.

The $\delta^{15}N$ of *Hilaria* (C_4) and *Stipa* (C_3) leaves (Table 2) were close to $\delta^{15}N$ values of the biological soil crust (~0‰), which is the dominate source of N input in undisturbed ecosystems (Evans and Ehleringer 1993). *B. tectorum* leaf $\delta^{15}N$ values were 1.5–2.8‰ greater than that of natives across all plots. The leaf $\delta^{13}C$ of *Hilaria* ranged from −14.2 ± 0.1 to −14.8 ± 0.7‰, and was not significantly different with invasion. Leaf $\delta^{13}C$ of *Stipa* ranged from −25.4 ± 0.2 to −25.5 ± 0.1‰ and did not differ with invasion.

Resin-extractable nitrogen

Figure 1 presents resin-extractable N values for 1996–1998 from Rimer and Evans (2006) and Evans et al. (2001), and this study (2000–2003). There was significant seasonal variability in resin-extractable N with the greatest amount of N extracted during the wetter spring period compared to the dry summer. Compared to the invaded plots, resin-extractable N in non-invaded C_3 plots was higher at two of the 20 times sampled and not different 18 out of 20 times. Comparing non-invaded and invaded plots in the C_4 community, resin-extractable N was significantly higher in the non-invaded plots during spring in the early years after invasion (1996, 1998, 2000), higher in invaded plots in 2001, but not significantly different in 2002 and 2003.

Soil organic N pools and C mineralization

Long-term soil incubations of invaded and non-invaded soils show significant initial and long-term effects of *B. tectorum* invasion on soil N pools and microbial activity in C_3 communities but not in C_4 communities. The total net amount of N mineralized was 43 ± 2% greater in invaded C_3 communities compared to non-invaded communities (Table 3 and online resource 1). While mean cumulative net N mineralized was 83.17 ± 18.10 μg N g^{-1} dry soil in invaded C_4 communities, it was not significantly different from the 60.73 ± 13.00 μg N g^{-1} dry soil found in non-invaded C_4 communities. Application of the exponential model of mineralization of labile soil N pools revealed significant differences for non-invaded and invaded C_3 communities in labile N (N_l) pool size and the rate of mineralization of recalcitrant N (c_r). The labile soil N, c_r, and recalcitrant N pool size (calculated as the difference between total and labile N) were significantly higher in invaded compared to non-invaded C_3 communities (Table 3). The labile rate constant (h_l) and was not different between non-invaded and invaded plots, or between grassland communities (Table 3). In invaded C_3 communities, soils showed significantly greater mean total CO_2 evolution (2.30 ± 0.33 mg C g^{-1} dry soil) compared

Table 1 Properties of soils collected to a depth of 10 cm from adjacent plant community types (C_4 and C_3)

		SOC (mg C/g soil)	Total N (mg N/g soil)	SOC:total N	Soil $\delta^{13}C$ (‰)	Soil $\delta^{15}N$ (‰)
C_4	Non-invaded	4.39 (0.22)^{a}	0.46 (0.02)^{a,b}	9.5 (0.1)^{a}	−19.7 (0.8)	3.5 (0.7)
	Invaded	4.62 (0.33)^{a}	0.50 (0.04)^{a,c}	9.2 (0.1)^{a,b}	−20.1 (0.4)	4.1 (0.5)
C_3	Non-invaded	3.84 (0.16)^{b}	0.41 (0.02)^{b}	9.4 (0.1)^{a}	−22.0 (0.5)	3.5 (0.2)
	Invaded	4.73 (0.44)^{a}	0.53 (0.04)^{c}	9.0 (0.1)^{b}	−20.9 (0.5)	4.3 (0.3)

Shown are mean soil organic C (SOC), total soil N (Total N), the ratio of SOC to total N, and the isotopic composition of SOC ($\delta^{13}C$) and total N ($\delta^{15}N$). The standard errors of the means are in parentheses. Superscript letters (a,b) denote significantly different ($P < 0.05$) means between grassland communities and invasion status for a given parameter when pair-wise comparisons are considered

Table 2 Stable isotopic composition of plant leaves collected from the C_4 native grass (*H. jamesii*), C_3 native grass (*S. hymenoides*), and C_3 invasive grass (*B. tectorum*)

		H. jamesii		*S. hymenoides*		*B. tectorum*	
		Leaf $\delta^{13}C$ (‰)	Leaf $\delta^{15}N$ (‰)	Leaf $\delta^{13}C$ (‰)	Leaf $\delta^{15}N$ (‰)	Leaf $\delta^{13}C$ (‰)	Leaf $\delta^{15}N$ (‰)
C_4	Non-invaded	−14.2 (0.1)	0.2 (0.7)	–	–	–	–
	Invaded	−14.8 (0.7)	0.7 (0.3)	–	–	−26.9 (0.2)	2.7 (0.3)
C_3	Non-invaded	–	–	−25.5 (0.1)	0.9 (0.3)	–	–
	Invaded	–	–	−25.4 (0.2)	1.4 (0.7)	−26.6 (0.3)	2.9 (0.4)

Shown are mean $\delta^{13}C$ and $\delta^{15}N$ of bulk leaf material. The standard errors of the means are in parentheses

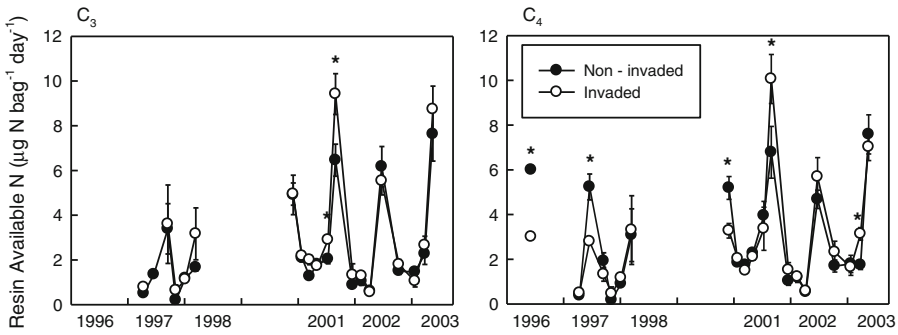

Fig. 1 Mean resin-available N in the top 5 cm of the soil in invaded/undisturbed sites from 1996 through 2003. *Data* shown for C_4 grasslands in 1996 comes from Rimer and Evans (2006), that for C_4 and C_3 from 1997 through 1998 comes from Evans et al. (2001), and 2000 through 2003 comes from this study. *Asterisks* (*) denote a significant difference ($P < 0.05$) between means of non-invaded and invaded communities. *Bars* represent the standard error of the mean

to non-invaded C_3 soils (1.90 ± 0.04 mg C g^{-1} dry soil) (Table 3). No such differences were evident for C_4 soils. The total CO_2–C evolved over 385 days was roughly 40–50% of the total organic C pool as measured by SOC analysis (Table 1).

Gross N fluxes

All gross rates of soil N transformations, measured over 24 h, were significantly greater in *B. tectorum* invaded soils relative to non-invaded soils regardless of community type (Fig. 2). Gross N mineralization was significantly greater in invaded C_3 communities (2.7 ± 0.3 µg N g^{-1} dry soil d^{-1}) than in non-invaded C_3 communities (1.9 ± 0.2 µg N g^{-1} dry soil d^{-1}); this same pattern held for C_4 communities as well (3.3 ± 0.2 and 2.7 ± 0.1 µg N g^{-1} dry soil d^{-1} for invaded and non-invaded plots, respectively). Gross rates of NH_4^+–N consumption were significantly greater in *B. tectorum* invaded C_3 soils (−5.4 ± 0.4 µg N g^{-1} dry soil d^{-1}) compared to non-invaded C_3 soils (−3.1 ± 0.1 µg N g^{-1} dry soil d^{-1}), and in C_4 invaded soils (−5.5 ± 0.3 µg N g^{-1} dry soil d^{-1}) compared to C_4 non-invaded soils (−4.8 ± 0.2 µg N g^{-1} dry soil d^{-1}). Gross nitrification rates were also significantly greater in *B. tectorum* invaded soils in both C_3 and C_4 grassland communities. Roughly 0.5–2% of added $^{15}NO_3^-$ was recovered as NH_4^+ during the ^{15}N pool dilution experiment, indicating microbial uptake of labeled NO_3^- and its subsequent mineralization as NH_4^+. Gross NO_3^- consumption rates were relatively low and not significantly different across plots once remineralization was taken into account. A more robust estimate of N transformation rates is total gross mineralization (gross N mineralization + gross nitrification) which was significantly higher in both C_3 and C_4 invaded plots. Similarly, assuming that gaseous losses (NH_3 volatilization) was minimal, total gross N immobilization was calculated as: [(total gross N mineralization–total net N mineralization)–gross nitrification]. Gross immobilization was greater in invaded

Table 3 Parameter estimates for N mineralization model fitted to data and total N, N_2O–N, and CO_2–C mineralized from the laboratory soil incubation

		Labile N pool (N_l, μg g^{-1} soil)	Labile N constant (h_l)	Recalcitrant N mineralization rate (c_r, μg g^{-1} soil d^{-1})	Recalcitrant N pool (μg g^{-1} soil)	Total N mineralized (μg g^{-1} soil)	Total C mineralized (mg g^{-1} soil)
C_4	Non-invaded	40.93 (6.51)^{a}	0.04 (0.01)	0.05 (0.02)^{a}	420 (30)^{a}	60.73 (13.00)^{a}	2.20 (0.21)^{a,b}
	Invaded	46.89 (9.02)^{a}	0.05 (0.01)	0.09 (0.06)^{a,b}	460 (50)^{a}	83.17 (18.10)^{a,b}	2.22 (0.18)^{a,b}
C_3	Non-invaded	28.92 (1.07)^{b}	0.04 (0.01)	0.05 (0.01)^{a}	390 (30)^{b}	52.22 (3.87)^{a}	1.90 (0.04)^{b}
	Invaded	44.46 (7.28)^{a}	0.04 (0.01)	0.15 (0.03)^{b}	480 (10)^{a}	91.99 (5.07)^{b}	2.30 (0.33)^{a}

Mean labile N pool size (N_l), labile constant (h_l), and recalcitrant N mineralization rates (c_r) are direct parameter estimates while mean recalcitrant pool size was calculated as the difference between total N (Table 1) and N_l. Superscript letters (a,b) denote significantly different ($P < 0.05$) means between grassland communities and invasion status for a given parameter when pair-wise comparisons are considered. Numbers in parentheses denote the standard error of the mean

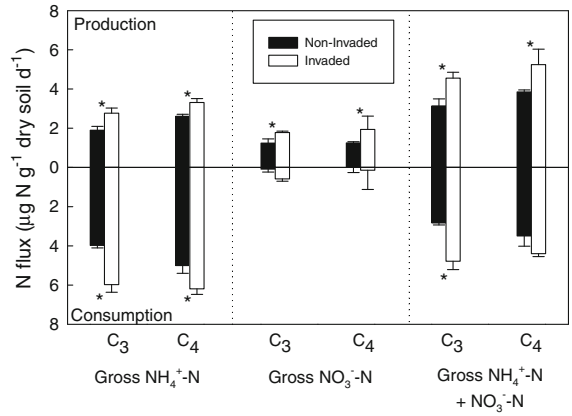

Fig. 2 Mean gross NH_4^+–N, NO_3^-–N, and total inorganic N (NH_4^+–N + NO_3^-–N) transformation rates (production and consumption) for soils from non-invaded and invaded C_3 and C_4 communities. Gross production rates are shown *above* the zero-reference line, and gross consumption rates *below*. *Asterisks* (*) denote a significant difference ($P < 0.05$) between means of non-invaded and invaded communities. *Bars* represent the standard error of the mean

C_3 soils (4.8 ± 0.4 μg N g^{-1} dry soil d^{-1}), compared to non-invaded C_3 soils (2.8 ± 0.1 μg N g^{-1} dry soil d^{-1}).

PLFA analyses

PLFAs were extracted from soil samples for determination of the relative abundances of bacterial and fungal biomarkers and substrate utilization patterns. In total, 13 PLFAs were identified for isotopic analysis. General biomarkers isolated were 16:0, 18:0, and 18:1ω9. The fatty acid 18:1ω9 has been used as a fungal biomarker in other studies, however, the enantiomers (*cis* and *trans*) of 18:1ω9 were not well separated chromatographically during isotopic analysis. Therefore, both 18:1ω9 enantiomers were combined and analyzed as one for the purposes of stable isotope composition, but not used as a fungal biomarker. A general bacterial biomarker (14:0) was isolated as well as biomarkers specific to $Gram^+$ (i15:0, a15:0, and i16:0) and $Gram^-$ bacteria (cy17:0 and cy19:0). The fungal biomarkers 18:3ω3 and 18:2ω6 were found, and an actinomycete biomarker (10me18:0) was identified, but sufficient amounts of 18:2ω6 and 10me18:0 were not present for accurate isotopic analysis. The total PLFA C content is not a measure of total microbial biomass C, but it can provide a useful comparison between treatments.

Mean total PLFA C was not significantly different between invaded (9.9 ± 0.3 μg C g^{-1} dry soil) and non-invaded C_3 soils (9.6 ± 1.6 μg C g^{-1} dry soil). The mean total PLFA C in soils from non-invaded C_4 soils was similar to that in C_3 soils (9.6 ± 0.8 μg C g^{-1} dry soil), but invaded C_4 communities had greater PLFA C by comparison (12.0 ± 0.8 μg C g^{-1} dry soil).

The proportions of bacterial, fungal, and actinomycete PLFA biomarkers varied with grassland type and invasion status (Fig. 3). In C_3 grassland soils, there were no significant differences in biomarker relative abundance between non-invaded and invaded plots (Fig. 3a). However, in soils from C_4 grasslands there were significant differences in fungal and bacterial biomarker abundance (Fig. 3b). The mean relative abundance of all fungal biomarkers measured as the percent of total PLFA C was significantly higher in the non-invaded community (7.1 ± 0.4%) compared to the invaded community (5.1 ± 0.3%). In contrast, the relative abundance of bacterial biomarkers (14:0, i15:0, a15:0, i16:0, and cy19:0) was significantly higher in the invaded community (45.7 ± 0.5%) when compared to the non-invaded community (43.1 ± 1.3%). The mean relative abundances of actinomycete and general biomarkers in C_4 grasslands showed no differences with *B. tectorum* invasion. A change in the ratio of the mean amount of PLFA C found in fungal versus bacterial biomarkers may be an indicator of overall changes in microbial community composition. Fungal:bacterial PLFA C (Fig. 3c) was significantly lower in invaded C_4 compared to non-invaded communities, while no such differences were observed in C_3 grasslands.

Because the $\delta^{13}C$ of PLFA C could reflect recent C inputs into microbial biomass, C input may be partitioned for communities that contain both C_3 and C_4 derived organic matter. This is possible only in invaded C_4 communities because the biomass $\delta^{13}C$ of *H. jamesii* (C_4) and *B. tectorum* (C_3) differs by 11–12‰ (Table 2). In contrast, $\delta^{13}C$ values of *S. hymenoides* (C_3) and *B. tectorum* biomass are indistinguishable from one another. There were significant differences in two biomarkers isolated between non-invaded and invaded C_4 communities: a 1.5 ± 0.3‰ greater mean $\delta^{13}C$ for the $Gram^+$ bacterial biomarker a15:0, and 2.4 ± 0.7‰ greater mean $\delta^{13}C$ for the $Gram^-$ bacterial biomarker cy19:0 in non-invaded relative to invaded C_4 communities, respectively,

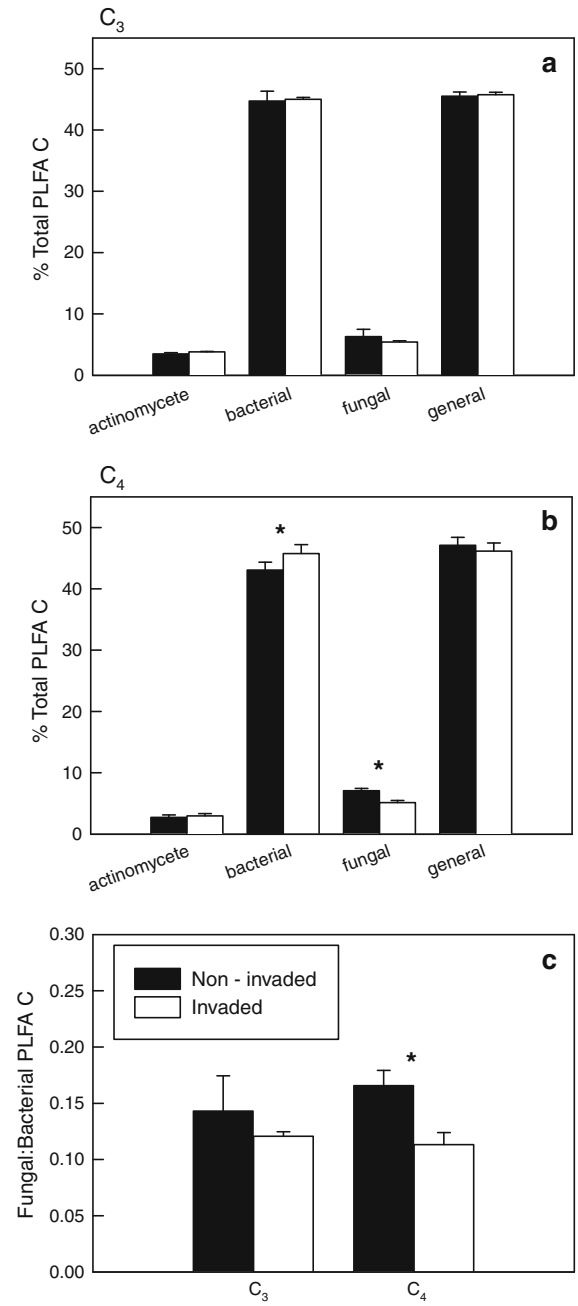

Fig. 3 Mean microbial community composition data for soils from non-invaded and invaded C_4 and C_3 communities. Mean % total PLFA C for C_4 (**a**) and C_3 (**b**) grasslands are shown for actinomycete, $Gram^+$ and $Gram^-$ bacteria, fungal, and general microbial PLFA biomarkers. The ratio of fungal to bacterial ($Gram^+$ and $Gram^-$) % total PLFA C is shown in (**c**). *Asterisks* (*) denote a significant difference ($P < 0.05$) between means of non-invaded and invaded communities. *Bars* represent the standard error of the mean

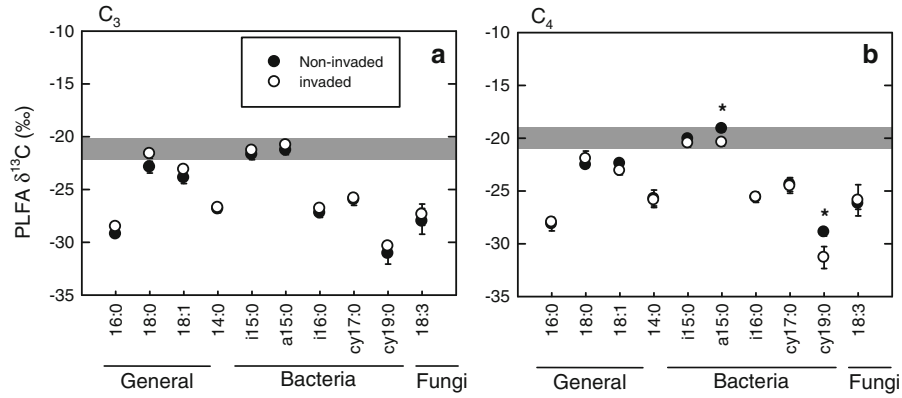

Fig. 4 Mean $\delta^{13}C$ of PLFA C biomarkers for soils from C_3 (**a**) and C_4 (**b**) communities. Biomarkers were isolated for general microbial, bacterial, and fungal biomass. *Shaded regions* show the range in $\delta^{13}C$ (mean with 2 standard errors) that was measured for bulk soil organic C in each community-type (Table 1). *Asterisks* (*) denote a significant difference ($P < 0.05$) between means of non-invaded and invaded communities. *Bars* represent the standard error of the mean

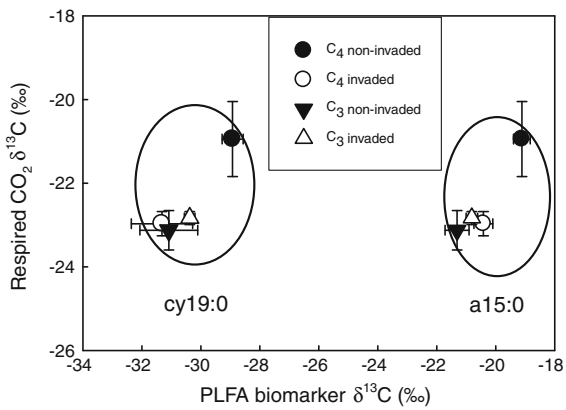

Fig. 5 Comparison of the mean isotopic composition of $\delta^{13}C$ respired (as CO_2) from the entire microbial community to the $\delta^{13}C$ of specific $Gram^+$ (a15:0) and $Gram^-$ (cy19:0) bacterial biomarkers. *Bars* represent the standard error of the mean

(Fig. 4b). For any given PLFA biomarker, there was no significant difference in the mean $\delta^{13}C$ between non-invaded and invaded C_3 communities. Phospholipid biomarkers were in general more depleted (more negative $\delta^{13}C$) in ^{13}C relative to the bulk SOM in non-invaded and invaded communities (shaded portion of Fig. 4).

Differences in the $\delta^{13}C$ of respired CO_2 were observed during the first 24 h of incubation between non-invaded and invaded C_4 grassland soils. The $\delta^{13}C$ for the bacterial biomarkers a15:0 and cy19:0 are plotted with the $\delta^{13}C$ of the CO_2 respired from the soil microbial community during the first 24 h of incubation (Fig. 5). The mean $\delta^{13}C$ of CO_2 respired from the soil in the first 24 h of incubation was 2.0 ± 0.5‰ lower in invaded compared to non-invaded C_4 communities. Comparable 1.5–2.0‰ differences in mean $\delta^{13}C$ were only observed for a15:0 and cy19:0 in the invaded versus the non-invaded C_4 communities. There were no significant differences in mean $\delta^{13}C$ of microbial respiration observed for any soils from C_3 communities.

Discussion

We utilized a combination of field and laboratory approaches to determine the effects of *B. tectorum* invasion on ecosystem C and N cycling in two adjacent native grassland communities. We found that, depending upon the composition of the native plant community, *B. tectorum* invasion led to changes in the composition of soil N pools, or altered the structure and function of the microbial community. Both processes ultimately feed back to changes in ecosystem-level C and N cycling. In the C_3 community, *B. tectorum* invasion appears to have: 1) increased surface-soil pools of SOC, total N, and labile organic N, 2) increased rates of gross inorganic N cycling and microbial immobilization of N, but 3) not altered the microbial biomass or PLFA biomarker composition. In the invaded C_4 community, gross inorganic N cycling rates were also greater, but there was no measured effect of *B. tectorum* invasion on the size of the soil organic N pool or microbial activity. However, the $\delta^{13}C$ values of different microbial

functional groups in the invaded C_4 community suggest that *B. tectorum* invasion alters the substrate use of at least a portion of the soil bacterial community, which preferentially decomposes *B. tectorum*-derived C_3 organic inputs rather than that from C_4 *H. jamesii*. The relative abundance of bacterial PLFA biomarkers, and the ratio of bacterial, relative to fungal, biomarkers also increased in soils in the C_4 community. This suggests that *B. tectorum* invasion significantly alters soil microbial community structure, which may then lead to changes in substrate utilization and increased rates of soil N cycling.

Seasonal and interannual variations in N dynamics

In the 8 years since invasion of *B. tectorum* into these grassland communities, there have been seasonal and inter-annual variations in the dynamics of soil inorganic N as measured by resin-extractable N. Resin-extractable N was seasonally lower in the invaded C_4 community from 1996 to 2000, but not significantly different in the invaded C_4 community from 2001 to 2003. The decrease in surface inorganic N immediately following invasion likely results from the increased deposition of *Bromus* leaf litter with high C:N and lignin:N ratios (Evans et al. 2001) promoting microbial immobilization in surface soil layers. Over time however, total labile soil N in invaded communities appears to increase, possibly due to further SOM deposition and utilization of deep soil NO_3^- sources by *B. tectorum* (Sperry et al. 2006). Indeed, neighboring sites that have a longer history of invasion (Sperry et al. 2006) along with studies in other ecosystem types, show that invasion by *B. tectorum* can increase soil N and net N mineralization in the long-term (Brooks 2004; Booth et al. 2003a; Norton et al. 2004).

Large temporal variation in inorganic N could also be related to soil water availability. On an intra-annual scale, net N mineralization rates are highest in the spring, when soils are wettest, and differences between invaded and non-invaded soils are evident. Mean annual precipitation may also be driving differences in net N mineralization on an inter-annual scale. The years of our study encompass a severe regional drought, while the years 1997–1998 were significantly wetter. This pattern in precipitation may explain why there were no significant effects of invasion on net N mineralization in the springs of 2002 and 2003 (see online resource 1).

An increase in available soil C accompanying invasion may increase the rate of soil N cycling through organic and inorganic pools (with commensurate gaseous losses), leading to a progressive enrichment in the $\delta^{15}N$ of total soil N in these ecosystems (Billings et al. 2002). Sperry et al. (2006) found alterations in N dynamics along a chronosequence since *B. tectorum* invasion (including the site used in this study) using a Rayleigh decomposition model of soil $\delta^{15}N$ to determine alterations in ecosystem N inputs and cycling (Evans and Ehleringer 1993; Billings et al. 2003). We found differences in soil $\delta^{15}N$ verging on statistical significance, so perhaps the time since *B. tectorum* invasion has not been long enough to change the $\delta^{15}N$ of the bulk soil. In our case, changes in leaf $\delta^{15}N$ may be more indicative of changes in N dynamics (Billings et al. 2002; Sperry et al. 2006). Sperry et al. (2006) used $\delta^{15}N$ of plants and depth profiles of soil NO_3^- to show that changes in leaf $\delta^{15}N$ were due to utilization of leached NO_3^- by *B. tectorum*. Our results are similar to those of Sperry et al. 2006 with respect to *B. tectorum* (i.e., leaf $\delta^{15}N$ of *B. tectorum* was greater than that of the native grasses). Additionally, we observed slight increases (albeit not statistically significant) in leaf $\delta^{15}N$ of C_3 and C_4 grasses in invaded relative to non-invaded plots suggesting that these plots could indeed be on the trajectory hypothesized by Sperry et al. (2006).

Potential mechanisms driving long-term changes in N dynamics

Laboratory incubations reveal two potential mechanisms that could drive long-term changes in soil N cycling in these two grassland communities. First, our results show that *B. tectorum* invasion is significantly altering SOC and N availability in relatively resource poor C_3 grassland soils. Previously, Rimer and Evans (2006) found lower amounts surface soil N in invaded C_3 communities 2 years after *B. tectorum* invasion. However, 7 years after invasion, we found that soil from those same invaded C_3 communities showed significantly greater amounts of labile N, and greater rates of net N mineralization, compared to non-invaded communities. In a related study, Sperry et al. (2006) showed that *B. tectorum* utilized soil NO_3^- that had leached below the rooting zone of the native grasses. We suggest that, over time, this deep N is being accessed by *B. tectorum* and then deposited on

the soil surface as litter, increasing soil N availability. In addition, changes in N dynamics caused by invasion may be more evident in C_3 soils because differences in atmospheric dust deposition (Reynolds et al. 2001) lead to soils with lower resource availability relative to C_4 soils. Higher nutrient availability is known to facilitate *B. tectorum* invasion (Miller et al. 2006; Rowe et al. 2009), and this may explain why the invasion is more swift and severe in C_4 communities compared to C_3 communities. It also suggests that there is a positive feedback effect of *B. tectorum* invasion in C_3 communities that may facilitate the establishment and persistence of the invasive grass species.

If soil microbial N cycling were stimulated by increased organic inputs from *B. tectorum* invasion, then we would expect to see increased microbial activity, and increased gross N cycling rates. Microbial activity as measured by CO_2 efflux from soil incubations was greater in the invaded C_3 grassland, but there appeared to be no consistent differences in overall microbial biomass. In addition, SOC content and gross N fluxes were greater in the invaded C_3 community when compared to those not invaded by *B. tectorum*. Increases in gross N fluxes in *B. tectorum*-dominated communities can be related to the input of highly labile organic matter (Saetre and Stark 2005). Similar patterns of gross fluxes of N have been observed with invasion in other semi-arid ecosystems (Stark et al. 2002; Booth et al. 2003a). This is consistent with our hypothesis that increased litter inputs with *B. tectorum* invasion (Evans et al. 2001) lead to increased microbial activity and increased rates of soil N cycling (Sperry et al. 2006).

The second potential mechanism is evident in the C_4 grassland soils where the effect of invasion appears to be a shift toward a more bacterially dominated community where soil bacteria are decomposing *B. tectorum*-derived organic material. Ecosystem-scale perturbations that lead to changes in soil C availability have been shown to favor the activity of one or more classes of soil microorganisms (Zak et al. 1993; Rillig et al. 1997; Waldrop and Firestone 2004). Previous studies show increases in litter deposition (Evans et al. 2001) and decomposition rates (Norton et al. 2004) with *B. tectorum* invasion, possibly due to increased inputs of organic matter into soils via increased root density and/or exudation. Increases in soil C availability can lead to greater turnover of soil bacteria and fungi (Hungate et al. 2000) or enhanced utilization of substrates (Rillig et al. 1997; Hungate et al. 2000; Phillips et al. 2002). While our results show no consistent differences in overall microbial biomass N (data not shown) or PLFA C with invasion, there were important differences in the abundance and isotopic composition of PLFAs that can be used to link microbial substrate utilization to soil N dynamics. The observed differences in the $\delta^{13}C$ of $Gram^-$ and $Gram^+$ PLFA biomarkers, coupled with depletion of respired $\delta^{13}CO_2$, is consistent with the hypothesis that a significant fraction of respiration in invaded C_4 soils is coming from microbial populations that are preferentially utilizing *B. tectorum*-derived organic material. This shift in substrate utilization by bacteria in response to *B. tectorum* invasion is accompanied by a significant shift in the fungal:bacterial ratio in C_4 communities invaded by *B. tectorum*. Together these data suggest that *B. tectorum* invasion may stimulate the activity and/or abundance of bacterial populations (Belnap and Phillips 2001; Kuske et al. 2002; Belnap et al. 2005), while adversely affecting the abundance of fungal populations in C_4 communities (Hawkes et al. 2006).

The stoichiometric relationship between decomposers and their substrates may also explain our observations. Bacteria possess lower C:N ratios ($\sim$5:1) than fungi ($\sim$15:1), and this stoichiometry may drive large differences in substrate demand between these two groups when soil C availability changes (Phillips et al. 2002). Coupled with the large change in litter composition caused by *B. tectorum* invasion (Evans et al. 2001; Saetre and Stark 2005), this appears to favor bacteria over fungi. Isotopic partitioning of C substrates was not possible in C_3 grassland due to the fact that both natives and the non-native *B. tectorum* share C_3 physiology. Interestingly, no differences in bacterial and fungal abundance were observed between non-invaded and invaded soils in C_3 grasslands even though they showed the greatest number of N transformation differences (gross and net fluxes, labile N, and recalcitrant N mineralization rate).

Conclusions

Different processes appear to be occurring in these C_3 and C_4 communities that are leading to similar effects

on ecosystem N dynamics as measured over 8 years since the introduction of *B. tectorum*. In invaded C_3 communities, there is an increase in bulk soil organic C and total N, the amount of labile soil organic N, recalcitrant N mineralization, gross N transformation rates, and C mineralization. It is possible that changes in the activity of the microbial community, rather than its structure, are controlling changes in soil N dynamics in C_3 grasslands (Belnap et al. 2005). In invaded C_4 grasslands, we observed that *B. tectorum* invasion leads to greater gross N transformation rates and an increase in the relative abundance of soil bacteria, which preferentially decompose more *B. tectorum*-derived (C_3) organic matter than that from *H. jamesii* (C_4). These changes in microbial community composition and substrate utilization may be important controls on soil N cycling in C_4 grassland soils. However, it is important to note that our stable isotopic approach was unable to resolve C substrate utilization in C_3 grasslands, and so does not preclude the possibility that the bacterial portion of these soil microbial communities is also preferentially decomposing *B. tectorum*-derived organic matter.

When this study is taken in context with previous work at the site, it demonstrates the succession of changes in ecosystem function that occur 2–10 years post-invasion. Past studies showed that invasion increased the amount and decreased the quality of litter deposited (Evans et al. 2001). At 2 years post-invasion, total N at the soil surface increased while N present in labile and inorganic pools decreased as more N was sequestered in *B. tectorum* litter (Rimer and Evans 2006) and microbial activity was suppressed. Then, at 4 years post-invasion, surface soil N increased due to utilization of deep NO_3^- by *B. tectorum* and its cumulative deposition on the surface as plant litter (Sperry et al. 2006). Now, 8 years post-invasion, the activity and/or composition of the bacterial and fungal components of the soil microbial community has changed to the point that this formerly sequestered N may be decomposed providing the substrate to fuel increased N transformation rates. However, these changes are likely to be seasonal and influenced by soil moisture levels as driven by precipitation. It is evident that by altering soil C substrate quantity and composition in invaded ecosystems, *B. tectorum* invasion may significantly alter either the activity, or the proportion of bacterial to fungal components, of the soil microbial community. These shifts in microbial dynamics will lead to shifts in ecosystem C and N dynamics, such as altering the amounts of plant available N and rates of soil organic N deposition, in these grassland ecosystems.

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