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Linking nitrogen partitioning and species abundance to invasion resistance in the Great Basin

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Abstract Resource partitioning has been suggested as an important mechanism of invasion resistance. The relative importance of resource partitioning for invasion resistance, however, may depend on how species abundance is distributed in the plant community. This study had two objectives. First, we quantified the degree to which one resource, nitrogen (N), is partitioned by time, depth and chemical form among coexisting species from different functional groups by injecting ^{15}N into soils around the study species three times during the growing season, at two soil depths and as two chemical forms. A watering treatment also was applied to evaluate the impact of soil water content on N partitioning. Second, we examined the degree to which native functional groups contributed to invasion resistance by seeding a non-native annual grass into plots where bunchgrasses, perennial forbs or annual forbs had been removed. Bunchgrasses and forbs differed in timing, depth and chemical form of N capture, and these patterns of N partitioning were not affected by soil water content. However, when we incorporated abundance (biomass) with these relative measures of N capture to determine N sequestration by the community there was no evidence suggesting that functional groups partitioned different soil N pools. Instead, dominant bunchgrasses acquired the most N from all soil N pools. Consistent with these findings we also found that bunchgrasses were the only functional group that inhibited annual grass establishment. At natural levels of species abundance, N partitioning may facilitate coexistence but may not necessarily contribute to N sequestration and invasion resistance by the plant community. This suggests that a general mechanism of invasion resistance may not be expected across systems. Instead, the key mechanism of invasion resistance within a system may depend on trait variation among coexisting species and on how species abundance is distributed in the system.

Keywords Cheatgrass · Great Basin · Medusahead · Niche · Nitrogen

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Introduction

Emerging theories of invasion resistance are linked to the ability of the native plant community to maintain low levels of limiting resources (Stohlgren et al. 1999; Davis et al. 2000). Several mechanisms may be important in reducing the amount of resources available to an invader. Theory and empirical evidence have widely identified a critical role for dominant species in maintaining low resources levels (Grime 1987, 1998; Hooper and Vitousek 1997; Crawley et al. 1999). Other research, however, has suggested that invasion resistance may be linked to species patterns of resource capture as opposed to species biomass per se. For example, resource partitioning among coexisting species or functional groups may allow more diverse communities to sequester more resources (Tilman et al. 1996; Tilman et al. 1997).

There is much evidence indicating that coexisting species can differ in the timing, soil depth or chemical

form in which they acquire a limiting resource (Veresog-lou and Fitter 1984; McKane et al. 1990; Miller and Bowman 2002). Moreover, several recent studies have demonstrated that resource partitioning among coexisting species may align with differences in productivity among species (McKane et al. 2002; Weigelt et al. 2005; Kahmen et al. 2006). Differences in patterns of resource capture among dominant and subordinate species may not only facilitate species coexistence but may also allow both species groups to contribute to invasion resistance (Naeem et al. 2000; Fargione and Tilman 2005). The relative importance of resource partitioning for invasion resistance, however, may depend on how species abundance is distributed in the plant community. For example, if species differ in pattern of resource capture and sequester large amounts of a limiting resource, then resource partitioning may contribute to species coexistence and invasion resistance. Alternatively, however, if species differ in patterns of resource capture but the absolute amount of resource captured by the plant community is largely driven by one or two species, then resource partitioning may facilitate species coexistence but may not contribute to invasion resistance.

Resource partitioning among coexisting species might be critical for invasion resistance when physiological or life history traits allow the invader to largely avoid interference from dominant species. For example, perennial bunchgrasses were historically the dominant herbaceous component in the Great Basin in the western USA, but many of these landscapes have been invaded by the exotic winter annual grasses cheatgrass (*Bromus tectorum* L.) and medusahead [*Taeniatherum caput-medusae* (L.) Nevski]. These annual grasses have higher rates of germination and root growth at lower soil temperatures, and produce thinner leaves and roots than bunchgrasses, allowing them to achieve higher relative growth rates and rates of root elongation than their perennial counterparts (Harris 1967; Harris and Wilson 1970; Arredondo et al. 1998). Combined, these traits enable annuals to capture a substantial portion of their resources when interference from dominant bunchgrasses is minimal. In this scenario, other less dominant functional groups, such as forbs, that may differ in patterns of resource capture, could be instrumental in minimizing the amount of resources available to these invaders.

Recent modeling and empirical work suggests that seasonal patterns of precipitation input and temperature are key factors determining regional variation in the spread of exotic annual grasses (Bradford and Lauenroth 2006; Chambers et al. 2007). In addition, other empirical and modeling work has suggested that, within a site, establishment of annual grasses is heavily influenced by year-to-year variation in precipitation timing and amounts

(Mack and Pyke 1983; Schwinning and Ehleringer 2001; Miller et al. 2006). While it is well known that water input exerts an overarching control on the timing and duration of biological activity in arid and semi-arid systems (Noy-Meir 1973), there is much evidence suggesting that dryland systems also are limited by nitrogen (N) (Hooper and Johnson 1999; Krueger-Mangold et al. 2004; Snyder et al. 2004) and that even small increases in N availability can facilitate the invasion of annual grasses (Paschke et al. 2000; Brooks 2003; Beckstead and Augspurger 2004; Chambers et al. 2007). Even with the potential advantage that annual grasses may have in terms of timing and rate of N capture relative to the historically dominant bunchgrasses, not all plant communities in the Great Basin are easily invaded (Booth et al. 2003; Beckstead and Augspurger 2004) and there is some evidence suggesting that N partitioning may be a critical mechanism for invasion resistance in these communities. For example, an earlier phenology and greater allocation of roots at depth exhibited by forbs compared to bunchgrasses may allow these species groups to partition N by time and soil depth, resulting in a more complete use of N by the native plant community (Blaisdell 1958; Sun et al. 1997). Likewise, greenhouse studies have shown a strong preference by bunchgrasses for NO_3^--N compared to NH_4^+-N (Monaco et al. 2003). While the chemical N preference of forbs has not been quantified, a stronger preference for NH_4^+ than NO_3^- may be another possible mechanism allowing forbs to minimize competition with bunchgrasses for N, increase N sequestration by the community and reduce invader establishment.

This study had two main objectives. First, we quantified the degree to which N is partitioned by time, depth and chemical form among coexisting species from different functional groups (non-native annual grasses, native perennial bunchgrasses and native perennial forbs, Table 1). A subset of plants was watered to examine whether observed patterns of N partitioning were influenced by soil water content. Second, we examined the degree to which native functional groups contributed to invasion resistance. We hypothesize that dominant bunchgrasses and subdominant perennial forbs differ in the timing, depth and form in which they acquire N. Specifically, we predict that, regardless of soil water content, bunchgrasses acquire relatively more N later in the growing season and from shallower soil layers than forbs, and that bunchgrasses acquire N as NO_3^- while forbs acquire N as NH_4^+. Based on expected differences in timing, depth and form of N capture between functional groups, we hypothesize that plots with all functional groups present will be less susceptible to invasion compared to plots where a functional group is removed.

Table 1 Functional group, codes and names of the seven species used in this study

Group	Code	Common name	Scientific name
Annual	BRTE	Cheatgrass	*Bromus tectorum* L.
Annual	TACA	Medusahead	*Taeniatherum caput-medusae* (L.) Nevski
Bunchgrass	PSSP	Bluebunch wheatgrass	*Pseudoroegneria spicata* (Pursh) A. Löve
Bunchgrass	ELEL	Bottlebrush squirreltail	*Elymus elymoides* (Raf.) Swezey
Bunchgrass	POSE	Sandberg bluegrass	*Poa secunda* J. Presl
Forb	LOTR	Nineleaf biscuitroot	*Lomatium triternatum* (Pursh) Coult. & Rose
Forb	CRIN	Grey hawksbeard	*Crepis intermedia* Gray

Species are arranged by functional group: invasive annual grass, native perennial bunchgrass and native perennial forb. Nomenclature and codes follow the USDA PLANTS database (http://plants.usda.gov/)

Materials and methods

Study site and species

This research was conducted in a sagebrush steppe community in eastern Oregon, USA (43°22′N, 118°22′W, 1,300 m elevation). Mean annual precipitation in Drewsey, OR, approximately 16 km north of the site, is 272 mm. Annual precipitation in 2006 was 274 mm. Soil at the site is a fine, montmorillonitic, mesic Xeric Haplargid. The herbaceous species selected for the experiment are representative of the steppe communities in the Great Basin (Tables 1 and 2). Bunchgrasses are the major herbaceous component followed by perennial forbs and annual forbs. Seasonal patterns of leaf biomass production were quantified for each species by clipping eight individual plants of the perennial species and eight plots (20 × 30 cm) of the annual grasses every two weeks. Green leaves were sorted, dried at 65 °C for 48 h and weighed.

Table 2 Leaf biomass (g m^{-2}) of the seven study species, other perennial forbs (consisting of two species) as well as the total leaf biomass of each functional group during each of the three ^{15}N injections periods (mean (SE), $n = 18$ per injection period)

Group	Species	April	May	June
Annual grasses	BRTE	5.0 (0.4)	7.3 (0.9)	0.0
	TACA	1.9 (0.2)	1.9 (0.1)	2.3 (0.2)
	Total	6.9 (0.4)	9.2 (0.9)	2.3 (0.2)
Bunchgrasses	PSSP	8.7 (1.0)	37.4 (5.4)	48.8 (8.5)
	ELEL	6.1 (0.8)	12.6 (1.4)	10.0 (1.6)
	POSE	7.1 (0.6)	10.9 (1.4)	0.0
	Total	21.9 (1.5)	60.9 (5.8)	58.8 (9.4)
Perennial forbs	LOTR	1.6 (0.1)	3.3 (0.3)	2.7 (0.4)
	CROC	1.8 (0.4)	3.8 (0.4)	4.9 (0.5)
	Other forbs	1.4 (0.4)	1.8 (0.5)	1.0 (0.3)
	Total	4.8 (0.4)	8.4 (0.5)	8.6 (0.5)
Annual forbs		11.6 (4.1)	4.2 (1.01)	4.3 (0.3)

Soil inorganic N concentration and gross N transformation rates

Soil inorganic N concentration and gross N transformation rates were quantified during the April, May and June ^{15}N injections. Gross N-mineralization and NH_4^+ consumption were determined by $^{15}NH_4^+$ isotope dilution, and gross nitrification and NO_3^- consumption were determined by $^{15}NO_3^-$ isotope dilution following procedures outlined by Hart et al. (1994). In the field, 6 ml of 2 mM K^{15}NO$_3$ or (^{15}NH$_4$)$_2$SO$_4$ (99 atom %) was injected into soil cores (ca.180 g soil core^{-1}, 5 cm diam × 7.5 cm) which increased inorganic N by ca. 1 µg N g^{-1} soil. The ^{15}N solutions were delivered using a syringe and a needle with four holes drilled above a sealed tip. The solution was evenly divided across five injection points in each core and was delivered over a 1–6.5 cm depth, creating an even application zone through the core. Immediately following injection, one pair of cores was extracted with 2 M KCl to quantify initial recovery of $^{15}NH_4^+$ or $^{15}NO_3^-$, while another set of cores were extracted four days later to measure isotope dilution. Enrichment of soil extracts were determined by diffusion (Stark and Hart 1996) and continuous flow-direct combustion mass spectrometry at University of California Davis Stable Isotope Facility (UCDSIF, Europa Integra, London). Gross rates of mineralization, nitrification and consumption were calculated based on changes in NH_4^+ or NO_3^- concentration and atom percent excess of ^{15}N during the incubation, following Stark (2000). Inorganic N concentrations were determined colorimetrically following Forster (1995) for NH_4^+ and Miranda et al. (2001) for NO_3^-.

Nitrogen partitioning by time, depth and chemical form

To quantify temporal, spatial and chemical patterns of plant N capture, we injected ^{15}N compounds into the soil around naturally established target plants of the seven

study species three times during the growing season (25 April, 23 May and 20 June 2006), at two different depths (2–7 and 17–22 cm) and in two chemical forms (NH_4^+ and NO_3^-). Each treatment combination was replicated five times in a randomized complete block design. Different plants were used for each treatment replicate. To examine how soil water content may influence the pattern of N capture by the study species, a second set of plants were watered with a simulated 25 mm rain event two days prior to the N injections in May and June. The water treatment was not applied in April, since soils were close to field capacity at this time.

Within a species, similar sized plants were selected for the ^{15}N injections. Because the annual grasses tended to grow in localized dense stands in the community, ^{15}N injections were applied to 20 × 30 cm plots within these stands rather than to individual plants. All experimental plants were spaced at least 1 m away from other plants receiving injections. The ^{15}N label was injected as either 11.9 mM $^{15}NH_4Cl$ or 11.9 mM $K^{15}NO_3$ (80 atom%) using a syringe and a 30-cm-long needle. The label for each treatment was applied in 12 × 5 ml injection points, resulting in a total N addition of 10 mg. For the perennial plants, the 12 injection points were distributed evenly around the plant 10 cm from the plant base. For the annual grass plots, the 12 injection points were distributed evenly over the plot using a 10 × 10 cm grid pattern. We used a fine-grain injection pattern because widely spaced injections points favor species with larger lateral rooting distance (Dukes and Caldwell 2001). The injection distances correspond to distances where the study species had demonstrated high root length densities (J. James, personal observation) suggesting that the effects of injection spacing on species absolute N capture would be minimal.

Plants were harvested three days after injecting the labeled solution. Aboveground biomass was clipped and green biomass was dried at 65°C for 48 h and weighed. This material was then coarsely chopped and a subsample was finely ground to pass through a 600 μm mesh screen. Tissue N concentration and ^{15}N enrichment were measured by continuous flow direct combustion and mass spectrometry at the UCDSIF (Europa Integra, London). A mass balance approach following Nadelhoffer and Fry (1994) was used to quantify plant ^{15}N capture, allowing comparisons to be made among species with different biomass or leaf N concentration. Here, plant N capture (mg N plant^{-1}) = $m_f \times [(N_f - N_i)/(N_{lab} - N_i)]$ where m_f is the mass of the N pool (mg), N_f and N_i are the final and initial atom% ^{15}N of the sample and N_{lab} is the atom % ^{15}N of the labeled solution. Spatial, temporal and chemical patterns of N capture by a species were normalized by expressing N capture by a species in a particular treatment as a percentage of the total amount of N capture by a species in all

treatments (McKane et al. 2002; Kahmen et al. 2006). Percentages were calculated individually for each block. Also, we estimated the absolute amount of N per species captured in the plant community in the different treatments. During each of the injections, eighteen 1 × 1 m frames were distributed randomly along three transects within a 2 ha area in the community. Aboveground green biomass was clipped in these frames and sorted by species. The average biomass of a species (g m^{-2}) during each of the three injection times was used to scale plant N capture to an area basis. One limitation of using N content in aboveground biomass as a measure of absolute N capture is that it ignores N stored in roots. Large differences in N storage patterns among species can confound comparisons of species abilities to sequester N. Studies on individual plants show that forbs and bunchgrasses allocate about 55% and 65%, respectively, of newly acquired N to shoots during periods of active growth (J. James, unpublished). This suggests that quantitative comparisons of N sequestration among natives are feasible but should be done with some caution, recognizing that a portion of the difference observed in total N capture between these species groups is due to differences in N storage patterns. Invasive annuals, however, allocate up to 90% of newly acquired N to shoots, limiting our ability to quantitatively compare N sequestration by natives and invasives.

Functional group removal plots

Removal plots were established at two sites in the community to evaluate the degree to which the three most common functional groups in this system resist invasion by the annual grass *T. caput-medusae*. Four removal treatments were applied at each site including: (1) nothing removed; (2) annual forbs removed; (3) perennial forbs removed; (4) bunchgrasses removed. Logistical constraints of large sample size prevented the quantification of N capture by annual forbs. Nevertheless, we included this functional group in the removal plot portion of this study because their relatively high biomass production early in the growing season (Table 1) may be important in inhibiting the establishment of annual grasses. Treatments were replicated four times at each site in a randomized block design. Removal plots were 2 × 2 m. Functional groups were removed in spring 2004 by brushing a 6% glyphosate solution on all species within the functional group targeted for removal. Plots were monitored through the experiment and additional plants were removed with glyphosate as needed. We choose plant removals over plant additions because removal plots are particularly valuable to understanding the effects of nonrandom variation in species composition and natural levels of species abundance on invasion resistance (Diaz et al. 2003; Zavaleta and Hulvey 2006). A disadvantage of this approach is that below

ground biomass cannot be removed, so plots are subject to nutrient turnover from decomposing roots. We expect that this flush, however, would dampen over time with microbial immobilization. *Taeniatherum caput-medusae* was seeded in the fall of 2005 at 3,000 seeds m^{-2}. The density of *T. caput-medusae* in the entire 2 × 2 m plot was measured in June 2006 and 2007.

Statistical analyses

Analysis of variance (ANOVA) was used to evaluate changes in soil inorganic N pools and gross N transformation over time (SAS 1999). Likewise, the effect of ^{15}N injection time, depth and chemical form on the relative and absolute amount of N captured by a species was analyzed with ANOVA (SAS 1999). Because two of the species, *P. secunda* and *B. tectorum*, had senesced by the June injection, only the April and May injection data were used to compare the effects of time, depth and form on species N capture. Normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests, respectively. The relative N capture data, expressed as a percentage, were arcsine-transformed. The homogeneity of variance of the absolute N capture did not improve with transformation, so these data were weighted by the inverse of the variance in the ANOVA model (Neter et al. 1990). Contrasts were used to test hypotheses about differences in N capture between species from different functional groups. When these comparisons were not orthogonal, sequential Bonferroni corrections were made to maintain an experiment-wise error rate of $\alpha = 0.05$ (Rice 1989).

ANOVA was also used to evaluate the effect of removal treatment, site and year on the density of *T. caput-medusae*. However, since plots were repeatedly sampled, the ANOVA was conducted as a split–split plot in time using Proc Mixed with site and time as the split factors (SAS 1999). In this case, replicate(site) was used as the error term for site. Removal treatment × replicate(site) was used as the error term for removal treatment within site. Year × replicate(site) was used as the error term for testing year and year × site.

Results

Leaf biomass production

The bunchgrasses as a group were the largest contributor to leaf biomass in the community (Table 2). Forbs and annual grasses had higher rates of leaf production earlier in the growing season than bunchgrasses (Fig. 1). The bunchgrasses *P. spicta* and *E. elymoides* maintained vegetative growth later in the growing season than the bunchgrass
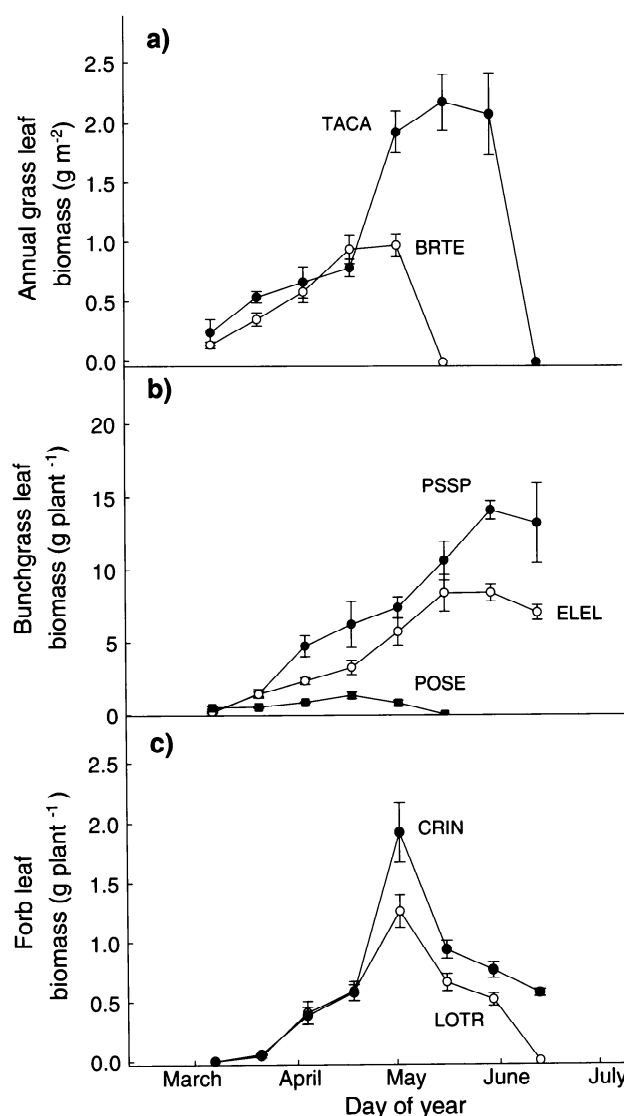

Fig. 1 Leaf biomass over the course of the growing season for the seven study species. For grasses, tillers excluding seed stalks were considered leaves (mean ± SE, $n = 8$ for each harvest)

P. secunda. The annual grass, *T. caput-medusae*, maintained vegetative growth later in the growing season than the other annual grass, *B. tectorum*.

Soil inorganic concentration and transformation rates

The NH_4^+ concentration in upper soil layers was lower in May than in April or June, while the NO_3^- concentration remained relatively constant across the three injection periods (Table 3). Inorganic N concentrations on average were about 1.7-fold lower in the 17–22 cm soil layer compared to the 2–7 cm layer. Soil water content declined through the season, with lower soil layers remaining wetter than upper soil layers. Gross N-mineralization rates declined from April to May and remained low in June,

Table 3 Soil N concentration, water content and N flux rates at each of the three injection times (mean (SE), $n = 8$)

	Soil depth (cm)	April	May	June
Soil N concentration and water content				
NH_4^+ pool (mg N kg^{-1})	2–7	0.48 (0.10) a	0.16 (0.05) b	0.41 (0.11) a
NH_4^+ pool (mg N kg^{-1})	17–22	0.17 (0.05) b	0.22 (0.12) ab	0.35 (0.11) a
NO_3^- pool (mg N kg^{-1})	2–7	0.33 (0.07) a	0.31 (0.09) a	0.31 (0.13) a
NO_3^- pool (mg N kg^{-1})	17–22	0.14 (0.01) b	0.40 (.039) a	0.46 (0.16) a
Water content (g H$_2$O g^{-1})	2–7	0.41 (0.11) a	0.11 (0.01) b	0.12 (0.01) b
Water content (g H$_2$O g^{-1})	17–22	0.28 (0.01) a	0.16 (0.01) b	0.13 (0.01) c
N flux rates (mg N kg^{-1} day^{-1})				
Gross N-mineralization	2–7	0.53 (0.06) a	0.22 (0.05) b	0.32 (0.06) b
Gross NH_4^+ consumption	2–7	0.49 (0.03) b	0.35 (0.04) b	0.84 (0.13) a
Gross nitrification	2–7	0.26 (0.05) a	0.54 (0.07) b	0.76·(0.10) b
Gross NO_3^- consumption	2–7	0.22 (0.03) c	0.58 (0.08) b	0.85 (0.06) a

Soil N concentration and water content were sampled in the 2–7 and 17–22 cm soil layers. N flux rates were measured in the 2–7 cm soil layer

Different letters indicate significant differences across labeling times, as determined with LS means ($P < 0.05$)

while gross nitrification increased three-fold between April and June over these three months. Consumption of NO_3^- by microbes increased almost four-fold over the three months and paralleled changes in gross nitrification. In contrast, the consumption of NH_4^+ was higher in June than April and May, and consumption of NH_4^+ in June was higher than gross N-mineralization ($P < 0.001$, data not shown).

Nitrogen partitioning by time, depth and chemical form

Water addition prior to ^{15}N labeling increased N capture by the study species about 1.3-fold ($P = 0.021$, data not shown), but did not differentially affect the timing, depth or form of N capture of the study species ($P > 0.05$). The timing and depth of N capture, however, differed significantly among species ($P < 0.001$ and $P < 0.001$ for species × time and species × depth; Fig. 2). Forbs acquired a greater proportion of N in April compared to May, while bunchgrasses acquired a greater proportion of N in May compared to April ($P < 0.001$ and $P < 0.001$, respectively). Annual grasses captured the majority of N in April compared to May ($P < 0.001$). Forbs acquired a greater proportion of N from the 17–22 cm soil layer than bunchgrasses ($P = 0.006$). Annuals captured more N from shallow soil layers compared to deep soil layers ($P < 0.001$).

The chemical form of N captured by different species depended on the time and depth from which N was acquired (species × time × form, $P = 0.007$ Fig. 2a,b; species × depth × form, $P < 0.001$ Fig. 2c,d). The forbs

Fig. 2a–d ^{15}Nitrogen capture by the seven study species, as influenced by the time, depth and form of tracer addition. Panels **a** and **b** show the effects of time and N form on species N capture, while panels **c** and **d** show the simple effects of depth and N form on species N capture. Plant N capture data are expressed as a percentage of each species' total ^{15}N capture. Percentages were calculated individually for each block (mean + SE, $n = 10$)
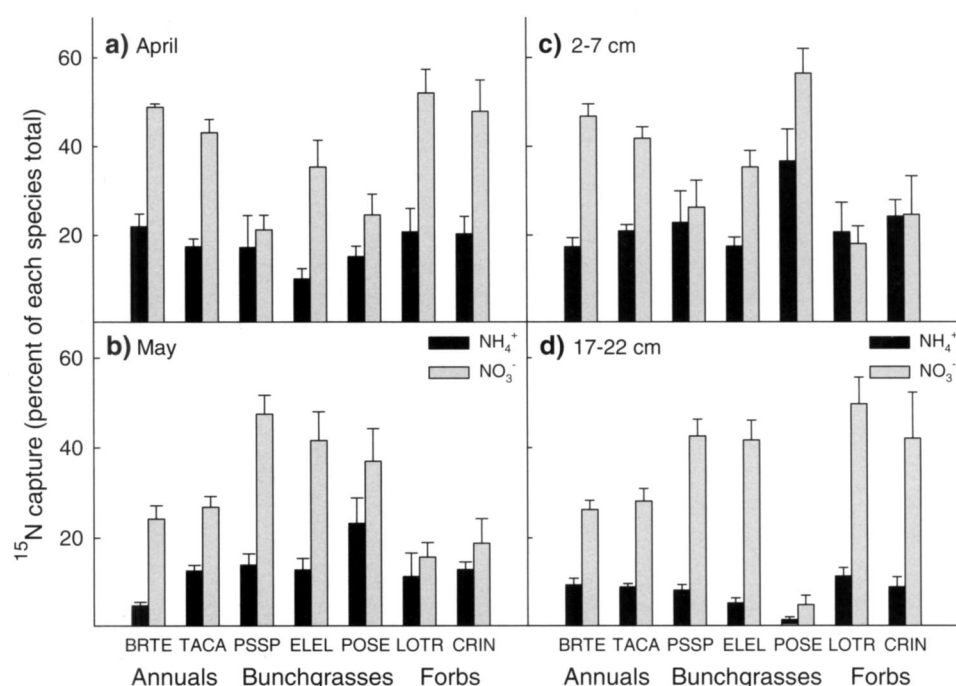
acquired the majority of N as NO_3^- in April ($P < 0.001$), but comparable amounts of N as NO_3^- and NH_4^+ in May. The bunchgrass *P. spicata* captured similar amounts of N as NO_3^- and NH_4^+ in April, but in May *P. spicata* captured over twofold more N as NO_3^- compared to NH_4^+. The other two bunchgrasses, E. *elymoides*, and *P. secunda*, displayed trends similar to annuals grasses, capturing the majority of N as NO_3^- in both April and May ($P < 0.05$). All species acquired N mainly as NO_3^- in the 17–22 cm soil layer.

Nitrogen capture per unit biomass and total N capture by each species declined during the growing season (Figs. 3, 4, $P < 0.001$ and $P < 0.001$, respectively). Bunchgrasses on average captured more N per unit leaf biomass than forbs ($P = 0.008$), but this appeared to be largely driven by the high uptake per unit biomass of *P. secunda*. The total amount of N captured following ^{15}N injection at different times, depths and chemical forms differed significantly among native species (species × time, species × depth, species × form, $P < 0.001$; Fig. 4). In all treatments, however, bunchgrasses acquired more N than forbs ($P < 0.001$).

Functional group removal plots

There was a significant main effect of removal treatment on *T. caput-medusae* density ($P = 0.009$; Fig. 5). Bunchgrass removal was the only treatment that significantly increased *T. caput-medusae* density compared to the intact control plots. There was no significant effect of site or interaction between removal treatment and site on *T. caput-medusae* density ($P = 0.671$ and $P = 0.501$). Density of *T. caput-medusae* varied across years ($P = 0.052$), but there was no removal treatment by year interaction on *T. caput-medusae* density ($P = 0.155$).
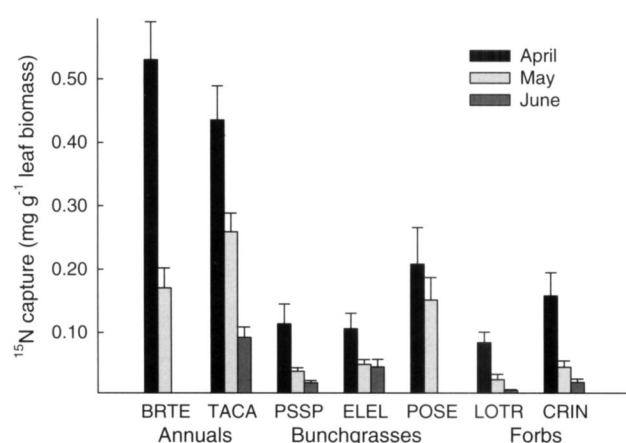

Fig. 3 ^{15}Nitrogen capture per unit biomass by the seven study species following the April, May and June injections. Values are averaged over different depths and chemical form of tracer addition and over the water treatments applied in May and June (mean + SE, $n = 20$ in April and $n = 40$ in May and June)
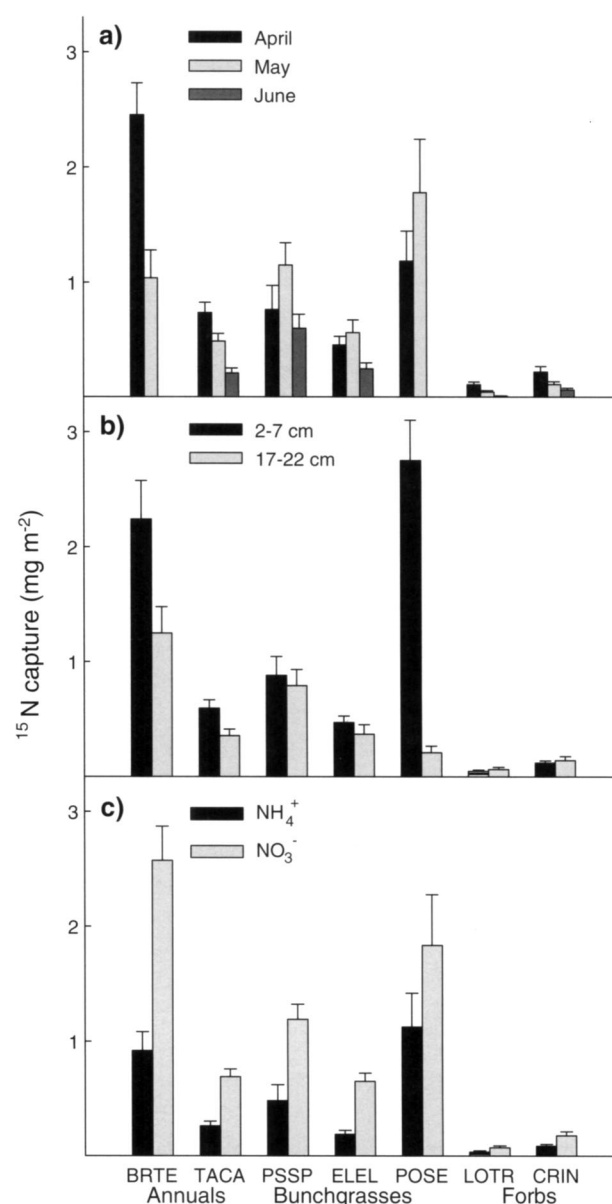

Fig. 4a–c Total ^{15}N capture by the seven study species as affected by the time (**a**), depth (**b**) and form (**c**) of tracer addition. Values in **a** are averaged over the different depths and chemical forms of tracer addition (mean ± SE, $n = 20$). Values in **b** are averaged over the different times and forms of tracer addition (mean ± SE, $n = 40$ for BRTE and POSE and $n = 60$ for the other species). Values in **c** are averaged over the different times and depths of tracer addition (mean ± SE, $n = 40$ for BRTE and POSE and $n = 60$ for the other species)

Discussion

Nitrogen partitioning among native species

We found strong evidence supporting the hypothesis that coexisting species differ in timing, depth and chemical form of N capture, consistent with recent studies in arctic tundra and grasslands (McKane et al. 2002; Kahmen et al.
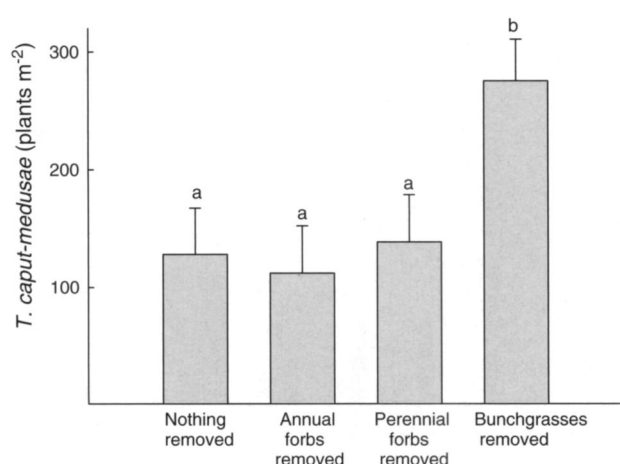
Fig. 5 *Taeniatherum caput-medusae* densities in response to the removal of functional groups (mean + SE, $n = 8$). *Letters indicate differences among treatments* as determined by LS means ($P < 0.05$)

2006). Low soil water availability can decrease plant N capture by inhibiting root activity in dry soil layers and by reducing mineralization of organic matter and mass flow to roots (Nye and Tinker 1977; Fisher et al. 1987). While these factors might be expected to alter N partitioning among species, in our study short-term fluctuations in soil water content influenced the magnitude of N capture but did not differentially affect the pattern of N capture among species. Our results, therefore, extend previous findings by demonstrating that resource partitioning among species can remain consistent under fluctuating environmental conditions that alter resource supply rates.

Dominant and subordinate species were well-differentiated in timing of N capture. Subdominant forbs acquired about 70% of their N in April, while the dominant bunchgrasses acquired about 40% of their N during this period (Fig. 2a,b). Forbs, as expected, had higher rates of leaf production earlier in the growing season but a shorter period of growth than bunchgrasses (Fig. 1). Plant N capture in natural and agricultural systems has been shown to be largely driven by relative growth rates (Siddiqi et al. 1990; Bilbrough and Caldwell 1997; James and Richards 2006). Consistent with previous research in grasslands, it appears that even moderate, differences in rate of leaf production among species can facilitate resource partitioning through the growing season (Fitter 1986; McKane et al. 1990).

The ability of species to acquire different chemical forms of N varied through the season. The most dominant species, *P. spicata*, acquired equivalent proportions of NO_3^- and NH_4^+ in April when gross N-mineralization rates were greatest but acquired almost 80% of N as NO_3^- in May as gross mineralization rates decreased but gross nitrification rates increased (Fig. 2a,b, Table 3). The forbs

in contrast acquired more N as NO_3^- in April but captured equivalent amounts of NO_3^- and NH_4^+ in May when NO_3^- capture by bunchgrasses increased. The potential for species to alter N preference depending on the neighboring environment has been demonstrated (Miller et al. 2007). However, the fact that forbs did not demonstrate greater NH_3^- capture compared to NO_3^- in May suggests that lower NO_3^- capture by forbs in May is a function of greater depletion of NO_3^- by bunchgrasses and not a change in the chemical preference or the availability of inorganic N. At no time did any of the species acquire more NH_4^+ than NO_3^-. Contrary to recent studies in mesic grassland and alpine systems (McKane et al. 2002; Miller and Bowman 2002; Weigelt et al. 2005), these results suggest that fluctuating availability of different chemical N forms is not a strong mechanism facilitating species coexistence in this system.

The apparent preference for NO_3^- demonstrated by the study species could be driven by soil processes. Chemical exchange processes, nitrification and immobilization, can make NH_4^+ less available than NO_3^- (Davidson et al. 1991). These processes limit our ability to differentiate between fundamental and realized N preferences of the study species. Under steady-state nutrition in the greenhouse, however, growth rates of bunchgrasses were higher when supplied with NO_3^- than NH_4^+ (Monaco et al. 2003). This suggests that uptake patterns observed in the field are driven to some extent by species' fundamental preference for NO_3^- over NH_4^+. If competition for NH_4^+ between plants and microbes is more intense in this system than competition for NO_3^- among plants, then there may be little selective pressure on plant species to develop a preference for NH_4^+. This hypothesis is supported by our pool dilution data, showing roughly equivalent rates of nitrification and NO_3^- consumption across the season but a 1.6-fold greater rate of NH_4^+ consumption than mineralization across the season (Table 3).

Our hypothesis that differences in root distribution by soil depth between grasses and forbs would allow these groups to partition soil N by depth was partially supported. Forbs and the bunchgrasses *P. spicata* and *E. elymoides* acquired a significant proportion of N from depth, although forbs acquired a greater proportion of N from this pool in April compared to *P. spicata* and *E. elymoides*. On the other hand, the bunchgrass *P. secunda* acquires N mainly from shallow soil layers. Although the ^{15}N labeling showed that *P. secunda* can acquire N at depth, this species is the most shallowly rooted species in this system and is the first perennial species to senesce as upper soil layers dry (Fig. 1). Therefore, while N partitioning by soil depth appears to be a mechanism facilitating coexistence in this system, the degree to which this occurs is limited by a predictable tradeoff between root allocation through the

soil profile and the ability of a species to maintain growth during seasonal drought (Fitter 1986).

Pattern of N capture by invasive annual grasses relative to natives

The annual grasses acquired N mainly as NO_3^- from shallow soil layers in April. Two other important N pools for annual grasses were NO_3^- at depth in April and NO_3^- in shallow soil layers in May. These observations support the large body of work showing high rates of root growth and N capture in spring by annual grasses compared to native perennials (Harris and Wilson 1970; Eissenstat and Caldwell 1988; Bilbrough and Caldwell 1997). However, these results also demonstrate that early in the growing season NO_3^- in deep soil layers is an important N pool for annual grasses. While no native species group preferentially utilized the shallow NO_3^- pool in April, the pool most utilized by annual grasses, the forbs and bunchgrasses demonstrated some degree of overlap in N acquisition patterns with the annual grasses. More importantly, N capture by these groups appeared to overlap annual grasses in different ways. Forbs acquired most of their N in April as NO_3^- from deep soil layers, while grasses acquired most of their N as NO_3^- from shallow soil layers in May. These results suggest that both species groups should play a critical role in reducing N available to invasive annual grasses.

Species total N capture

To determine whether N partitioning is an important mechanism that reduces N availability to invaders, it is necessary to quantify the absolute amount of N sequestered by a species in different soil N pools. Contrary to the relative measures of species' N capture, differences in total N capture among species were not influenced by the time, depth or chemical form in which N was acquired (Fig. 4). Instead, total N capture from different soil N pools was essentially driven by two bunchgrass species, *P. spicata* and *P. secunda*. The forbs, on the other hand, sequestered much less N than these two bunchgrass species. While some of the difference in total N sequestration between bunchgrasses and forbs may be due to greater N storage in roots by forbs, averaged across treatments the bunchgrasses captured ninefold more N than forbs. The magnitude of difference in N capture suggests that these differences in total N capture were not due to differences in N storage alone.

Consistent with recent work in grasslands, these results indicate that at natural levels of species abundance, N sequestration by the plant community is determined by functional group composition and species identity within a functional group (Kahmen et al. 2005, 2006). Large

biomass was the main factor allowing *P. spicata* to sequester high amounts of N, since N capture per unit biomass by this species was largely similar to the other native species (Table 2, Fig. 3). In contrast, *P. secunda* had much lower biomass than *P. spicata* but high N capture per unit biomass, allowing it to be a large sink for N in the community. Nitrogen capture per unit biomass by forbs was comparable to the bunchgrasses *P. spicata* and *E. elymoides*, but forbs had lower biomass, making them a small sink for N in the community. Differences in phenology and root distribution may promote N partitioning and slow rates of competitive exclusion (McKane et al. 1990). However, the degree to which these traits allow coexisting species to exploit different soil N pools are influenced by species differences in biomass and uptake rates per unit biomass.

Influence of functional group removal on *T. caput-medusae* establishment

Bunchgrasses were the only functional group that inhibited *T. caput-medusae* establishment, consistent with the large amount of N sequestered by this group (Fig. 5). Perennial forbs, on the other hand, were minor sinks for N in the community and contributed little to invasion resistance, even though forbs acquired most of their N in April, similar to invasives. These findings support the idea that invasion resistance is largely driven by species or functional groups with the largest biomass or resource acquisition rates (Crawley et al. 1999; Prieur-Richard et al. 2000; Thomsen and D'Antonio 2007). Annual forbs also did not influence invasion resistance despite relatively high aboveground biomass production in early spring (Table 2). Although we did not quantify N sequestration by annual forbs, they have shallower root systems than annual grasses and therefore probably had little ability to interfere with annual grass N capture at depth in April, an important source of N for these invasives. The effects of functional group removal were similar across years, indicating that the contribution of these functional groups to invasion resistance remains consistent despite variations in environmental conditions that may influence ecosystem invasibility and alter species interactions (Davis et al. 2000; Chesson et al. 2004).

While our experimental design did not allow us to evaluate effects of species on invasion resistance, there was some evidence suggesting that resource partitioning among bunchgrasses may be important for N sequestration and invasion resistance. Namely, *P. spicata* and *P. secunda* differed in their ability to exploit N pools in different soil layers, suggesting that growing these species in combination would have the greatest potential to reduce the N available to an invader. The limitations of basing functional groups on taxonomy or coarse differences in

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morphology and physiology have been demonstrated (Wright et al. 2006). In this study we may have been able to more accurately link N sequestration to invasion resistance by basing our groupings on biomass and rooting depth. Regardless of how the species in this system are grouped, however, only two species drove N sequestration by the plant community, supporting the idea that invasion resistance saturates at low species diversity (Wardle 2001).

Conclusion

Linking plant traits to ecosystem properties is a central goal in ecology (Chapin et al. 1997; Lavorel and Garnier 2002). The mass ratio hypothesis proposed by Grime (1998) predicts that the immediate effect of a species trait on ecosystem properties is largely driven by the relative abundance of a species. Consistent with this hypothesis, we observed that bunchgrasses, which were the largest biomass component in this community, not only sequestered the most N from all soil N pools, but was the only group that contributed to invasion resistance. Although perennial forbs differed from bunchgrasses in patterns of N capture, and both perennial and annual forbs differed from bunchgrasses in patterns of biomass production, the relatively low biomass of these groups limited their contribution to invasion resistance. The majority of research investigating invasion resistance has utilized synthesized plant communities. While these studies have been useful in evaluating relative effects of diversity and composition on invader establishment, this approach can underestimate the importance of natural variation in species abundance on invasion resistance (Diaz et al. 2003). Taken together, our results suggest that because of differences in abundance (biomass) and rates of N capture per unit biomass, traits allowing functional groups to partition N do not necessarily allow greater N sequestration by the plant community.

While the amount of N sequestered by a functional group corresponded closely with their ability to resist invasion of the annual grass *T. caput-medusae*, it is important to recognize that multiple factors influence patterns of plant invasion, including climate, propagule pools, disturbance regimes, herbivory and pathogen pressure (Beckstead and Augspurger 2004; Bradford and Lauenroth 2006). Moreover, our experimental approach does not allow us to determine the importance of N partitioning and sequestration for invasion resistance relative to other soil resources such as water or other nutrients (Miller et al. 2006; Newingham and Belnap 2006). Optimal foraging models predict and experimental data demonstrate that plants alter physiology, morphology and

allocation so that multiple resources are limiting (Gleeson and Tilman 1992; Gleeson and Good 2003; James et al. 2005). While the high demand and acquisition costs of N and the extremely low N concentrations in dryland soils suggest an important role of N availability in determining annual grass invasion, our examination of N partitioning and sequestration in this study does not exclude the possibility that partitioning also may occur along another resource axis, and that invasion resistance ultimately depends on the ability of the plant community to sequester multiple resources.

Gaining an understanding of mechanisms of invasion resistance is critical for conservation biology and land management (D'Antonio and Vitousek 1992; Wilcove et al. 1998). While the particular resource or combination of resources facilitating annual grass invasion may change depending on timing and amount of water input and soil chemistry, our results suggest that the main mechanism of invasion resistance likely depends on how species abundance is distributed in the plant community. Namely, in systems where coexisting dominants differ in how they harvest resources, it seems likely that resource partitioning will be a key mechanism contributing to invasion resistance. However, in systems where the bulk of community biomass is determined by one or two species, it seems likely that invasion resistance will mainly be conferred by the resource acquisition traits of the dominant species. Ultimately, experimental manipulations of species and functional group diversity that incorporate realistic variations in species abundance and composition are needed to asses the utility of these predictions.

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