

Nitrogen uptake and preference in a forest understory following invasion by an exotic grass

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Received: 8 November 2010 / Accepted: 12 May 2011
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Abstract Plant–soil interactions have been proposed as a causative mechanism explaining how invasive plant species impact ecosystem processes. We evaluate whether an invasive plant influences plant and soil-microbe acquisition of nitrogen to elucidate the mechanistic pathways by which invaders might alter N availability. Using a ^{15}N tracer, we quantify differences in nitrogen uptake and allocation in communities with and without *Microstegium vimineum*, a shade-tolerant, C_4 grass that is rapidly invading the understories of eastern US deciduous forests. We further investigate if plants or the microbial biomass exhibit preferences for certain nitrogen forms (glycine, nitrate, and ammonium) to gain insight into nitrogen partitioning in invaded communities. Understory native plants and *M. vimineum* took up similar amounts of added nitrogen but allocated it differently, with native plants allocating primarily to roots and *M. vimineum* allocating most nitrogen to shoots. Plant nitrogen uptake was higher in invaded communities due primarily to the increase in understory biomass when *M. vimineum* was present, but for the microbial biomass, nitrogen uptake did not vary with invasion status. This translated to a significant reduction ($P < 0.001$) in the ratio of microbial biomass to plant biomass nitrogen uptake, which suggests that, although the demand for nitrogen has

intensified, microbes continue to be effective nitrogen competitors. The microbial biomass exhibited a strong preference for ammonium over glycine and nitrate, regardless of invasion status. By comparison, native plants showed no nitrogen preferences and *M. vimineum* preferred inorganic nitrogen species. We interpret our findings as evidence that invasion by *M. vimineum* leads to changes in the partitioning of nitrogen above and belowground in forest understories, and to decreases in the microbial biomass, but it does not affect the outcome of plant–microbe–nitrogen interactions, possibly due to functional shifts in the microbial community as a result of invasion.

Keywords Plant–microbe competition · Plant–soil feedbacks · Resource partitioning · Stable isotopes

Introduction

The effects of exotic plant invasions on soil nutrient availability are well documented, but the mechanistic pathways by which invaders promote changes in nutrient availability are only partly understood (Ehrenfeld 2003; Levine et al. 2003; Liao et al. 2008). Much emphasis has been placed on the importance of litter chemistry differences as the proximate cause of changes in soil nitrogen (N) availability via effects on decomposition (Allison and Vitousek 2004; Ashton et al. 2005; Liao et al. 2008). Additionally, several studies suggest that increased soil N availability due to rapid litter turnover enhances the fitness of invaders relative to native species, reinforcing invader dominance (Ehrenfeld et al. 2001; Scott et al. 2001; Rodgers et al. 2008). However, microbial communities are a key link between plant nutrient dynamics and nutrient supply rates and mediate many nutrient transformations, particularly those

Communicated by Tim Seastedt.

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involving N (Knops et al. 2002). While there is growing appreciation for the role of positive plant–soil feedbacks in promoting invader dominance (Callaway et al. 2004; Bever et al. 2010; Inderjit and van der Putten 2010), further integration of above and belowground nutrient dynamics is needed to determine the processes underlying invader impacts on nutrient availability (Ehrenfeld et al. 2005).

Recent studies demonstrating effects of invaders on the diversity and activity of microbial communities suggest that invaders may engender positive plant–soil feedbacks that increase resource availability by altering plant–microbe competition for nutrients (Kourtev et al. 2002; Hawkes et al. 2005). For example, Hawkes et al. (2005) found that exotic grasses increased nitrification rates by stimulating the soil nitrifying community and speculated that ammonia-oxidizing bacteria and heterotrophic microbes were able to obtain a greater fraction of mineral N when associated with exotic compared to native grasses. Kourtev et al. (2002) showed that soils under exotic plants associated with increased nitrification also had higher activities of N-related enzymes and proposed that the soil biota were N limited. These hypotheses are consistent with current views on plant–microbe N interactions, which suggest that, while microbes are more effective than plants at taking up N at the microscale, plants ‘win’ at the macroscale (Kaye and Hart 1997; Hodge et al. 2000; Schimel and Bennett 2004). Moreover, because plants retain N much longer than microbes, plants will outcompete microbes for N even if they access only a small fraction of the bioavailable N turning over through the soil (Kaye and Hart 1997; Schimel and Bennett 2004).

Plant invaders associated with altered soil nutrient availability often have physiological and morphological traits that enhance N acquisition and N-use efficiency (Vitousek 1990; Ehrenfeld 2003); consequently, invasion may intensify plant–microbe N competition. Alternatively, microbial N demand may increase because invaders prime microbial activity via root exudates of high quality carbon (Strickland et al. 2010, 2011). In either case, higher N demand could lead to increased N partitioning if plants and microbes utilize different chemical forms of N or access them with different efficiencies. Examining how invaders differ from natives in N uptake and use in addition to belowground nutrient dynamics may thus be important for understanding shifts in soil N availability.

Forest understories provide a useful setting in which to investigate these hypotheses. In the past few decades, the integrity of many forest understory communities has been compromised by the invasion of exotic species. While closed-canopy forests were once thought to be resistant to invasion (Crawley 1987; Von Holle et al. 2003), a growing number of studies show that they are readily invaded by shade-tolerant species (Webb and Kaunzinger 1993;

Woods 1993; Martin 1999; Webb et al. 2000; Howard et al. 2004; Martin et al. 2004; Gilbert and Lechowicz 2005). A recent review indicated that at least 139 exotic plant species are known to have invaded deeply shaded forest understories that have not undergone substantial disturbance (Martin et al. 2009).

Understory plant communities also have a major influence on forest nutrient dynamics. Shaped by intense resource competition, the unique life-history characteristics of herbaceous species promote the efficient acquisition, use and cycling of nutrients, which contributes to nutrient conservation at the ecosystem level (Muller 2003; Gilliam 2007). The high foliar nutrient concentrations of herbaceous plants, for instance, facilitate rapid decomposition and incorporation into the organic matter pool (Gagnon et al. 1958; Siccama et al. 1970; Muller 2003). Absorption of nutrients from throughfall by the understory can reduce nutrient leaching during the growing season (Carlisle et al. 1967; Yarie 1980; Andersson 1993). Consequently, native understory communities are thought to enhance overall nutrient availability in forest ecosystems.

Microstegium vimineum is a shade-tolerant, C₄ grass that has invaded forests across a large portion of the southern and eastern United States (USDA and NRCS 2005). Experimental introductions and observational studies of previously invaded sites indicate that invasion by *M. vimineum* significantly lowers native species abundance, richness and diversity (Oswalt et al. 2007; Flory and Clay 2010). While the causative mechanisms driving such changes are unclear (Warren et al. 2011a), previous research indirectly implicates resource competition and belowground dynamics. *Microstegium vimineum* has been shown to increase soil pH and nitrification rates (Ehrenfeld et al. 2001), and alter soil microbial community composition (Kourtev et al. 2002). At the same time, the stems of *M. vimineum* have a relatively high C:N ratio, which enhances N immobilization in its decaying litter and may slow overall N cycling and availability (Ehrenfeld et al. 2001; DeMeester and Richter 2010). The potential for *M. vimineum* to impact native communities by modifying plant–microbe interactions for N has not yet been explored. Given the current and expanding distribution of *M. vimineum*, understanding its influence on forest ecosystems is critically important (Morrison et al. 2007).

In this study, we compared N dynamics between non-invaded areas and areas invaded by *M. vimineum* to understand whether invasion alters N uptake and allocation patterns by understory plants and soil microbes. Using ¹⁵N tracers, we quantified the sizes of multiple N pools (above- and belowground plant, microbial biomass, and soil solution) across local invasion fronts at timescales (50 h and 8 days) relevant to plant–microbe interactions (Hodge et al. 2000; Harrison et al. 2007). We also investigated whether plant and microbial communities exhibited preferences for

dominant forms of N (i.e., organic N, NH_4^+ and NO_3^-) to determine if (1) *M. vimineum* favors nitrate (NO_3^-) over other N species, as has been suggested previously (Ehrenfeld et al. 2001), and/or (2) if other plant species or the microbial community compensate for *M. vimineum* preferences by specializing on different N forms.

Materials and methods

Study site

Our study was conducted within the Whitehall Experimental Forest (WEF), an 800-acre (c. 324-ha) research and teaching site at the University of Georgia, in Clarke County, Georgia, USA (33°57'N, 83°22'W, 150–240 m elevation, 122 cm MAP, 17°C MAT). Soils are sandy clay loams primarily of the Madison series and are classified as Typic Hapludults. The forest overstory at our site (a bottomland hardwood) is comprised of *Acer rubrum*, *Quercus nigra*, *Platanus occidentalis* and *Liquidambar styraciflua*. The understory structure strongly depends on invasion status. In uninvaded plots, the understory is typically depauperate (<5% plant cover). In invaded plots, *M. vimineum* forms dense, continuous cover, with values >90%. The bulk density of the soil is 1.08 g cm⁻³ and is not affected by *M. vimineum* invasion (see Strickland et al. 2010 for further site details). Note that, although a C₄ species, *M. vimineum* has many characteristics of a wetland plant and does not appear to perform well under dry conditions (Warren et al. 2011a, b). Indeed, it also germinates at a similar time (i.e., March) to when the native species flush out in the piedmont of the southeast and—as a product of the elongated growing season—also senesces at an equivalent time (October). If anything, it is differentiated from the native forest understory by employing an annual—as opposed to perennial—life-history strategy.

Within the site, we selected three areas characterized by expanding invasion fronts of *M. vimineum*. Anecdotal reports indicate that *M. vimineum* established within the WEF ~15 years ago and this likely holds for our site. However, the invader probably went unnoticed until it formed dense cover, and so pinpointing the exact invasion date is difficult (Martin et al. 2009). What we do know is that the invasion is active, and in the year (2009) following our study, our non-invaded areas became colonized by *M. vimineum*, suggesting they were not invaded simply because of stochastic processes and so were suitable ‘controls’.

Experimental design and field sampling

We established a block within each of our three areas ahead of and within an *M. vimineum* invasion, placing six PVC

collars (15.4 cm diameter, inserted 5 cm into the soil) in invaded and non-invaded habitat within each block (i.e., 12 collars per block, 36 total). Blocks within the same area had similar resident understory plant community composition, and included *Carex* sp., *Euonymus atropurpureus*, *Euphorbia*, *Gaultheria procumbens*, *Ligustrum* sp., *Parthenocissus quinquefolia*, *Smilax glauca*, and *Viola* sp. (block 1); *Ambrosia* sp., *Ligustrum* sp., *P. quinquefolia*, *Rubus* sp., *Solidago* sp., and *Toxidendron radicans* (block 2); and *E. atropurpureus*, *Ligustrum* sp., *P. quinquefolia*, *S. glauca*, *Solidago* sp., and *Viola* sp. (block 3). Resident species occurred at low densities (<15% of collar) irrespective of invasion status.

We labeled the collars with ¹⁵N-tracers in August 2008. Given the elongated growing season in the southeastern US, August captures peak biomass of many species and occurs prior to seed setting. For ¹⁵N-labeling, we randomly allocated four collars in each block (two invaded and two non-invaded) to one of three N species. We removed any leaf litter within the collar and then added to the surface 500 mL solutions of one of three compounds: ¹⁵N-NH₄Cl, ¹⁵N-NaNO₃, or ¹⁵N-glycine. ¹⁵N content in each substrate was ≥99.1 atom%. All solutions were added at equivalent N concentration (1.118 mg N L⁻¹). The intention behind using such low concentrations was to avoid an ‘N fertilization’ effect and so provide the N species at realistic concentrations (Miller et al. 2007).

Post addition of the ¹⁵N label, we harvested one collar per N species from each block and habitat at 50 h and 8 days. To harvest a collar, we first removed the *M. vimineum* plants whole by gently unearthing their roots. Given the small biomass and shallow rooting-depth of the species, this caused minimal soil disturbance. Next, we clipped shoots of the native plants at ground level. Following this, in each collar, we removed two, 8-cm-diameter soil cores, each to a depth of 10 cm. Plant materials were returned to the laboratory and dried to constant mass (65°C); at which time *M. vimineum* roots were separated from the above-ground plant biomass. One soil core was stored at 5°C for microbial biomass C and N determinations (below), and from the second core, native understory roots were separated, washed and then treated as other plant materials. Tree roots were separated and discarded. This resulted in a set of aboveground and belowground native understory plant materials from each collar (non-invaded and invaded), which allowed us to make direct comparisons between native understory ¹⁵N uptake under non-invaded and invaded conditions.

Element and isotope analyses

After weighing, dried plant materials were ball-milled to a fine powder and total nitrogen and stable N-isotope ratios

determined using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy) coupled to a continuous-flow isotope-ratio mass spectrometer (IRMS; Thermo, San Jose, CA, USA). For the IRMS, analytical precision was $\pm 0.1 \delta^{15}\text{N}\%$ and working standards were calibrated to atmospheric N_2 .

Soils for dissolved organic C (DOC) and total dissolved N, and microbial biomass C and N determinations were first passed through a 2-mm sieve. DOC and total dissolved N were determined by shaking with 0.5 M K_2SO_4 for 4 h and then filtered (Bradford et al. 2008). DOC concentrations were quantified using a total organic carbon analyzer (Shimadzu, Columbia, MD, USA). Total dissolved N and ^{15}N in this pool was determined using a modified version of the alkaline persulfate oxidation procedure described by Cabrera and Beare (1993) to convert the organic and inorganic N forms in the 0.5 M K_2SO_4 extracts to NO_3^- . Next, the NO_3^- was fixed to acid-washed PTFE discs by shaking at 30°C for 2 days with Devarda's alloy in solutions adjusted to pH 13 with 10 M NaOH. PTFE discs were then rinsed with DI water, dried and their total %N and $\delta^{15}\text{N}$ determined as for the plant materials.

Microbial biomass C and N was estimated using a modified, chloroform–fumigation extraction (CFE) method as described in Fierer and Schimel (2002, 2003). This procedure is essentially the same as described above for the determination of DOC and total dissolved N except for fumigation with ETOH-free chloroform during shaking with K_2SO_4 . The flush of dissolved organic C and N following fumigation allows for the determination of microbial biomass C and N by difference from the non-chloroform samples (soil solution). Raw values for microbial biomass are reported; no correction factors are used.

Given that the study employed isotopic tracers enriched in ^{15}N above natural abundance values, atom% and not delta values were used to calculate the mass of ^{15}N label in the plant and microbial biomass pools (see Fry 2006). Specifically, mass of ^{15}N label assimilated by the biotic pools (e.g., native roots) was determined by subtracting the atom% ^{15}N values of unlabeled pools from labeled pools, and then multiplying the total N mass values for these plant parts by the calculated atom% excess ^{15}N values. We obtained materials for unlabeled pools from additional collars we established in invaded and non-invaded habitat; to the collars, we added DI water without ^{15}N tracer to mimic the conditions the labeled collars experienced. Calculation of atom% excess values corrects for the natural abundance content of ^{15}N in organic samples, that would otherwise be treated as a component of the isotopic tracer (Fry 2006).

Statistical analysis

We used linear mixed effects models to analyze the influence of *M. vimineum* and N species on N uptake. We

measured N uptake in two ways. To determine the absolute difference in N uptake between invaded and non-invaded areas, we computed the percentage of ^{15}N in a pool relative to the amount of ^{15}N added to a collar. Because many exotic species substantially increase the biomass of the plant communities they invade (Liao et al. 2008), we also compared uptake on a per biomass N basis by computing the proportion of ^{15}N in a pool relative to the amount of total N in that pool. Although we derived this variable using a mass balance approach (i.e., through calculation of the mass of ^{15}N label recovered in a pool), it is essentially equivalent to determining atom% excess ^{15}N . We only distinguish the manner in which we derived the variable—by referring to it as proportion of ^{15}N label in a pool relative to total N—to highlight the ecological basis for the derivation (i.e., to evaluate whether biomass differences between natives and *M. vimineum* alone explained the observed patterns). Native plants tend to have slightly lower litter N concentrations compared with *M. vimineum* (Strickland et al. 2010). Scaling in this manner is not equivalent to scaling by biomass per se; however, ^{15}N recovery in *M. vimineum* pools exceeded that in native pools by such a large degree that small differences in tissue chemistry had little effect on comparisons. Nonetheless, we also present biomass data (Table 1).

We treated *M. vimineum* status (present or absent), N species, and time (harvest 1 or harvest 2) as fixed effects, including all interaction terms in our models. Block was treated as a random effect and accounted for fine-scale spatial heterogeneity. All response variables were transformed to conform to assumptions of normality and heteroscedasticity; the latter was confirmed by checking distributions and residuals. Pairwise comparisons of treatment means were performed using Tukey's test. All analyses were conducted using SAS v.9.2. Results were considered statistically significant at $P < 0.05$.

Results

Recovery of ^{15}N

Mean ^{15}N recovery from all resolved pools (i.e., the sum recovered in understory plants, microbes, and the soil solution) was $15 \pm 2\%$ (SE) in invaded areas and $14 \pm 2\%$ in non-invaded areas and did not differ with invasion status ($F_{1,36} = 0.23$, $P = 0.63$). The relatively low recovery values suggest that a substantial amount of ^{15}N may have been taken up by tree roots, which were not resolved in this study, lost from the surface 10 cm at which we worked via leaching, or sorbed to the soil surface in non-extractable forms. Recovery of ^{15}N also decreased with harvest time from approximately 18 to 12% across all treatments ($F_{1,36} = 6.97$, $P = 0.01$).

Table 1 Mean biomass, biomass N, and ^{15}N uptake (± 1 SE) of different plant pools at two harvest periods, in non-invaded (*non-inv*) and areas invaded by *M. vimineum* (*inv*)

Pool	Biomass (g)	Biomass N (g/m ²)	% ¹⁵ N recovered		Proportion of ¹⁵ N in pool	
			Harvest 1 (50 h)	Harvest 2 (8 days)	Harvest 1 (50 h)	Harvest 2 (8 days)
Native aboveground, non-inv	0.49 (0.60)	0.41 (0.13)	0.05 (0.03)	0.04 (0.01)	0.004 (0.002)	0.003 (0.001)
Native belowground, non-inv	1.18 (0.61)	2.14 (0.26)	0.54 (0.09)	1.47 (0.31)	0.01 (0.01)	0.03 (0.01)
Native aboveground, inv	0.17 (0.19)	0.15 (0.04)	0.06 (0.03)	0.03 (0.02)	0.01 (0.01)	0.01 (0.01)
Native belowground, inv	0.97 (0.75)	1.64 (0.24)	1.14 (0.36)	1.90 (0.35)	0.04 (0.02)	0.04 (0.01)
<i>M. vimineum</i> aboveground	0.96 (0.67)	1.12 (0.10)	0.62 (0.26)	1.79 (0.50)	0.01 (0.005)	0.05 (0.01)
<i>M. vimineum</i> belowground	0.28 (0.09)	0.09 (0.01)	0.26 (0.11)	0.30 (0.09)	0.09 (0.03)	0.09 (0.02)

Percentage uptake is the amount of ^{15}N in a pool relative to the amount of ^{15}N added to a plot. Proportion of ^{15}N in a pool is relative to the total N in the given pool and accounts for differences in biomass N among pools

Understory plant response

Understory vegetation in areas invaded by *M. vimineum* showed significantly higher uptake than non-invaded areas (Fig. 1). Mean ^{15}N uptake in combined aboveground and belowground plant pools across all treatments, plant species and harvest periods was $1.05 \pm 0.19\%$ in non-invaded areas and $3.05 \pm 0.59\%$ in invaded areas ($F_{1,36} = 16.41$, $P < 0.001$; Fig. 1a). On a per biomass N basis, ^{15}N uptake by the understory vegetation remained 53% higher in invaded areas compared with non-invaded areas ($F_{1,36} = 8.04$, $P = 0.009$; Fig. 1b), indicating that differences in ^{15}N uptake were not solely due to increases in plant biomass accompanying invasion by *M. vimineum*. Time had a significant effect on uptake observed in the total plant pool, such that ^{15}N uptake was 52% higher after 8 days in total ($F_{1,36} = 11.14$, $P = 0.003$) and 43% higher on a per biomass N basis ($F_{1,36} = 13.90$, $P = 0.001$).

Higher uptake in invaded areas was partly due to *M. vimineum*'s presence. Of the ^{15}N recovered in the understory vegetation, 49% was found in the *M. vimineum* pool, with 81% of this ^{15}N in the aboveground compartment. We found similar proportions of ^{15}N (scaled by biomass N) in *M. vimineum* (0.034 ± 0.007) and native species (0.037 ± 0.009) pools in invaded plots, whereas the proportion of ^{15}N in the native species pool averaged 0.015 ± 0.003 in non-invaded areas.

Enhanced ^{15}N uptake by native roots contributed to higher overall uptake in invaded areas. Native belowground biomass took up an average of 34% more ^{15}N in invaded areas compared with non-invaded areas. Accounting for variation in biomass N among treatments, we found that native root uptake was ca. 52% higher in invaded areas. These differences were significant when ^{15}N uptake was scaled by biomass N ($F_{1,36} = 4.35$, $P = 0.04$), and the effect was marginally significant when absolute ^{15}N recovery was considered ($F_{1,36} = 3.37$, $P = 0.076$).

Patterns of ^{15}N allocation varied by species and by time. In native species, belowground pools contained more ^{15}N than aboveground pools at both harvest dates, regardless of invasion status (Table 1). This pattern was reversed in *M. vimineum*, such that aboveground pools contained more ^{15}N than belowground pools (Table 1). Uptake of ^{15}N generally increased with time (Table 1). At harvest 2, there was 65% more ^{15}N in the *M. vimineum* aboveground pool ($F_{1,18} = 10.50$, $P = 0.009$) and 15% more in the belowground pool ($F_{1,18} = 10.50$, $P = 0.009$), as well as 50% more in the native belowground pool ($F_{1,36} = 12.42$, $P = 0.002$). On a per biomass N basis, we found that *M. vimineum* aboveground ($F_{1,18} = 7.53$, $P = 0.02$) and native belowground ($F_{1,36} = 5.06$, $P = 0.03$) pools had higher proportions of ^{15}N at harvest 2 as opposed to harvest 1, whereas the proportion of ^{15}N in the belowground *M. vimineum* pool did not change (Table 1; $F_{1,18} = 0.78$, $P = 0.40$). The amount of ^{15}N contained in the native aboveground pool did not differ with time whether expressed in absolute terms ($F_{1,36} = 0.58$, $P = 0.45$) or relative to biomass N ($F_{1,36} = 0.54$, $P = 0.47$).

Microbial and dissolved N response

Microbial biomass carbon (C) was approximately 50% lower in invaded plots than in non-invaded plots. Averaged over time (because the time \times habitat effect was non-significant: $F_{1,12} = 3.25$, $P = 0.12$), microbial biomass C was 43.32 ± 5.95 g C m⁻² in non-invaded and 21.78 ± 6.02 g C m⁻² in invaded plots ($F_{1,12} = 17.58$, $P = 0.006$). Yet uptake of ^{15}N by the microbial biomass did not vary with invasion status, whether compared on an absolute ($F_{1,36} = 1.01$, $P = 0.33$; Fig. 2a) or biomass N basis ($F_{1,36} = 0.01$, $P = 0.91$; Fig. 2b). Notably, the microbial C:N ratio was not significantly affected (habitat: $F_{1,36} = 0.22$, $P = 0.64$) by invasion. Potentially, the marked inter-replicate variation in microbial biomass C obscured any effects on ^{15}N recovery expressed per biomass N. That

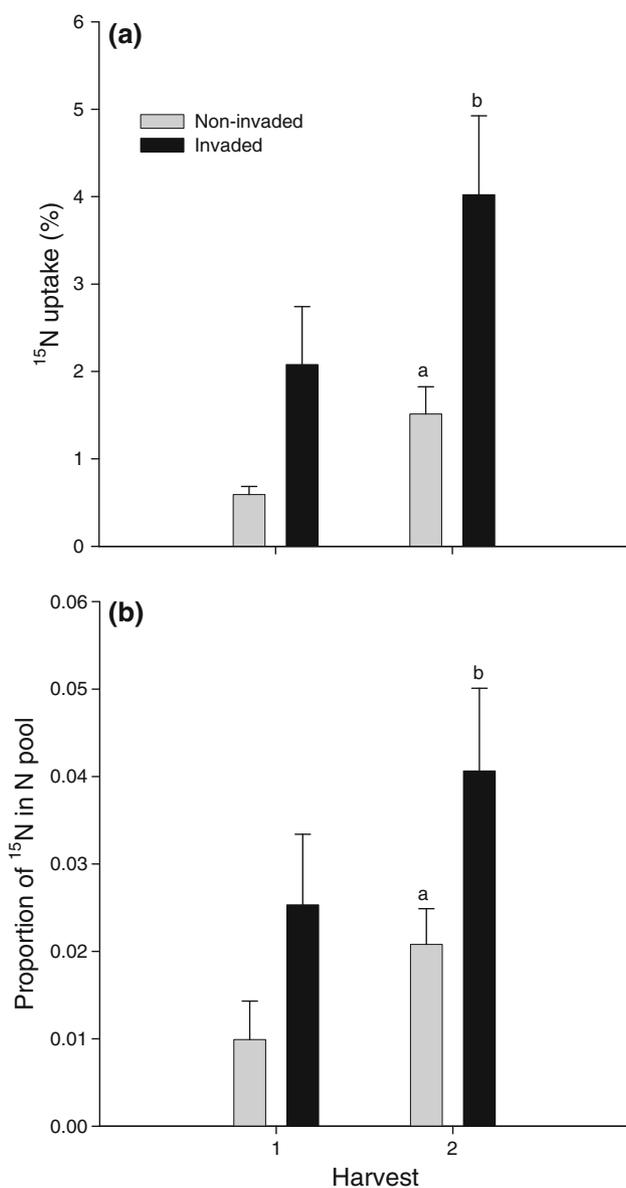


Fig. 1 Mean ^{15}N uptake (+1 SE) by the forest understory vegetation (above + belowground) in areas that were and were not invaded by *M. vimineum* at two times after inoculation (harvest 1: 50 h; harvest 2: 8 days). Data were computed relative to **a** the amount of ^{15}N added to a plot and **b** the amount of total N in the pool. Different lowercase letters above bars indicate significant differences ($P < 0.05$) among pools with respect to ^{15}N uptake

is, we might have expected higher scaled values in invaded habitat because absolute ^{15}N uptake was unaffected by invasion and there was lower microbial biomass in invaded habitat but no effect on microbial C:N ratio. There was a significant effect of time on ^{15}N uptake, such that microbial biomass $^{15}\text{N}\%$ declined between harvest 1 and harvest 2 ($F_{1,36} = 5.11$, $P = 0.03$; Fig. 2).

There was no effect of invasion status on ^{15}N content in the dissolved N pool (i.e., extractable DON, NH_4^+ and NO_3^-), although content decreased significantly with time. Specifi-

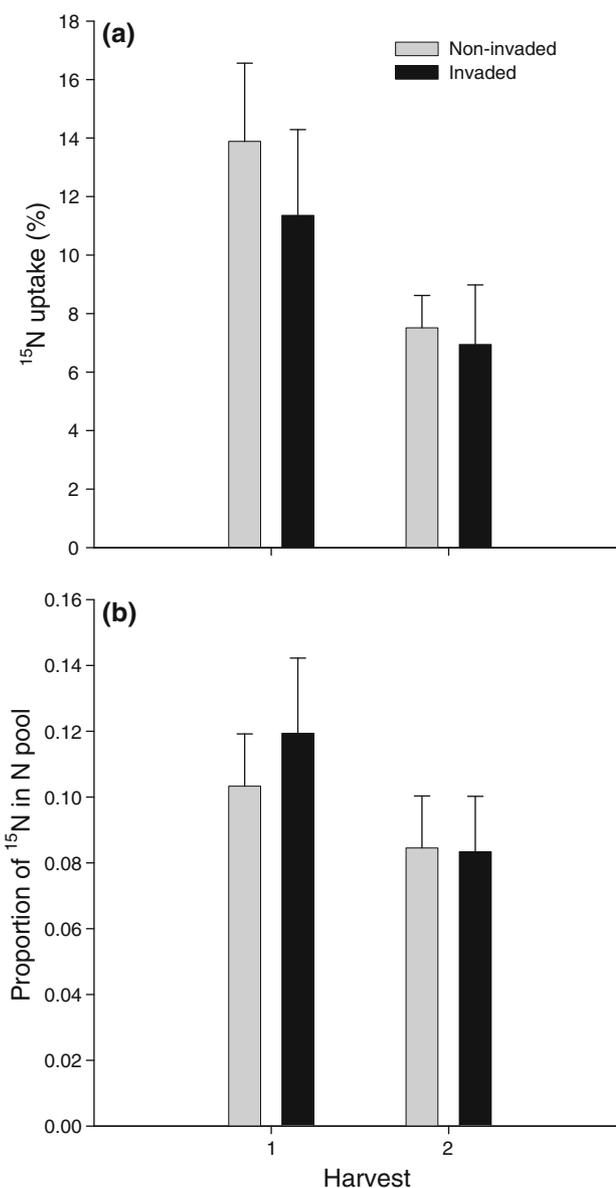


Fig. 2 Mean ^{15}N uptake (+1 SE) by the microbial biomass in areas that were and were not invaded by *M. vimineum*. Data were computed relative to **a** the amount of ^{15}N added to a plot and **b** the amount of total N in the pool

cally, ^{15}N amount as a proportion of that added was ca. 3% in non-invaded and 4% in invaded areas after 50 h, and 1.5% in both areas after 8 days (time: $F_{1,36} = 21.12$, $P < 0.001$). Similarly, the proportion of ^{15}N in the dissolved N pool relative to total dissolved N was 0.07 and 0.09 after 50 h, and 0.03 and 0.04 after 8 days, in non-invaded and invaded habitats, respectively (time: $F_{1,36} = 18.00$, $P < 0.001$).

Plant–microbe interactions

The ratio of microbial biomass ^{15}N to plant ^{15}N was considerably lower in invaded areas than in non-invaded

areas, suggesting that invasion altered plant–microbe partitioning of N (Fig. 3a). Averaged over time, the ratio of ^{15}N recovered was 17.72 and 9.85 in non-invaded and invaded habitats, respectively ($F_{1,36} = 14.03$, $P < 0.001$), indicating that understory plants captured relatively more N (compared to microbes) in invaded than non-invaded sites. Differences in the ratios based on ^{15}N uptake scaled by biomass N were also significant ($F_{1,36} = 4.80$, $P = 0.04$), but a single value obscured the difference between habitat types when averaged across blocks, such that the average scaled ratio in non-invaded habitats was 11.11, while the average in invaded habitats was 11.09. After removing the outlier (identified using a Dixon test), the ratio in invaded habitats averaged 6.89 and the effect of invasion status was pronounced ($F_{1,36} = 8.95$, $P = 0.007$). Although the invasion \times time interaction was not significant ($P > 0.33$), we did observe that the ratios declined significantly over time (Fig. 3) (absolute values: $F_{1,36} = 16.21$, $P < 0.001$; per biomass N values: $F_{1,36} = 14.68$, $P < 0.001$).

N preferences

We observed marked preferences for certain N forms in the microbial biomass ($F_{2,36} = 7.45$, $P = 0.003$) and *M. vimineum* ($F_{2,36} = 8.38$, $P = 0.007$) after accounting for differences in N pool sizes. As there were no significant time or invasion effects, we present these data pooled across time and invasion status (Fig. 4). A significantly greater proportion of ^{15}N in the microbial biomass pool was derived from NH_4^+ than from glycine ($t_{24,36} = -3.37$, $P = 0.007$) or NO_3^- ($t_{24,36} = 3.32$, $P = 0.008$). For *M. vimineum*, greater proportions of ^{15}N came from both NH_4^+ ($t_{10,24} = -2.81$, $P = 0.04$) and NO_3^- ($t_{10,24} = -2.81$, $P = 0.006$) compared with glycine (Fig. 4). Native plants did not show a general preference for N form ($F_{2,36} = 0.29$, $P = 0.75$) (Fig. 4).

Discussion

We conducted a ^{15}N -tracer experiment to evaluate changes in N allocation patterns for understory plants and microbes following plant invasion to elucidate mechanistic pathways by which invasive species might impact N availability. Additionally, we compared the uptake of different chemical forms of N by the microbial biomass and native and invasive plants to determine if preferences for N forms changed post-invasion. We found that native understory plants took up similar amounts of the added N to *M. vimineum* but allocated it primarily to roots, whereas *M. vimineum* allocated most N to shoots. Although we found lower microbial biomass C, and lower microbial biomass ^{15}N to plant biomass ^{15}N ratios, in invaded sites, absolute uptake of ^{15}N by the microbial biomass was unaffected by invasion

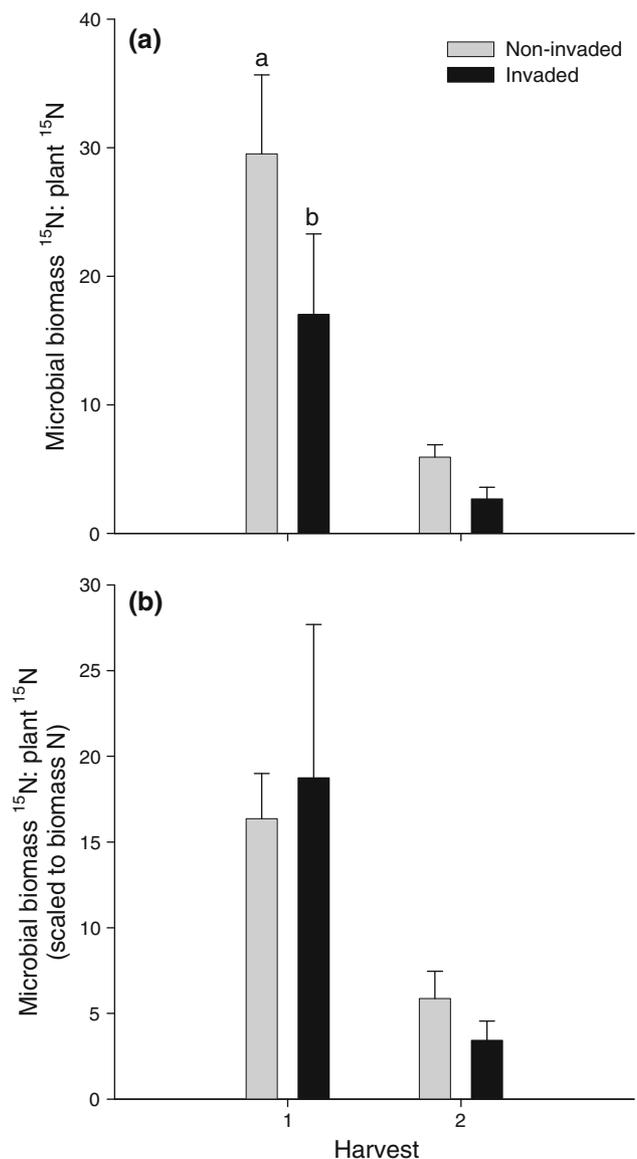


Fig. 3 Mean ratio of microbial biomass ^{15}N to plant ^{15}N (+1 SE) in areas that were and were not invaded by *M. vimineum*. Ratios are based on the relative amount of ^{15}N **a** added to a plot and **b** amount of total N in each pool. Different lowercase letters above bars indicate significant differences ($P < 0.05$) among ratios

status. This suggests that, although the understory plants acquired more of the ^{15}N tracers in invaded plots (primarily because of the increase in understory plant biomass through the addition of *M. vimineum*), the microbes did not ‘lose out’ competitively. Preferences for different N forms were exhibited by the microbial biomass and *M. vimineum*, but not by the native understory plants, and these patterns were unaffected by invasion. Overall, our findings suggest that *M. vimineum* primarily alters N dynamics in forest understories by sequestering additional N in the aboveground biomass of the understory. This finding is not surprising given

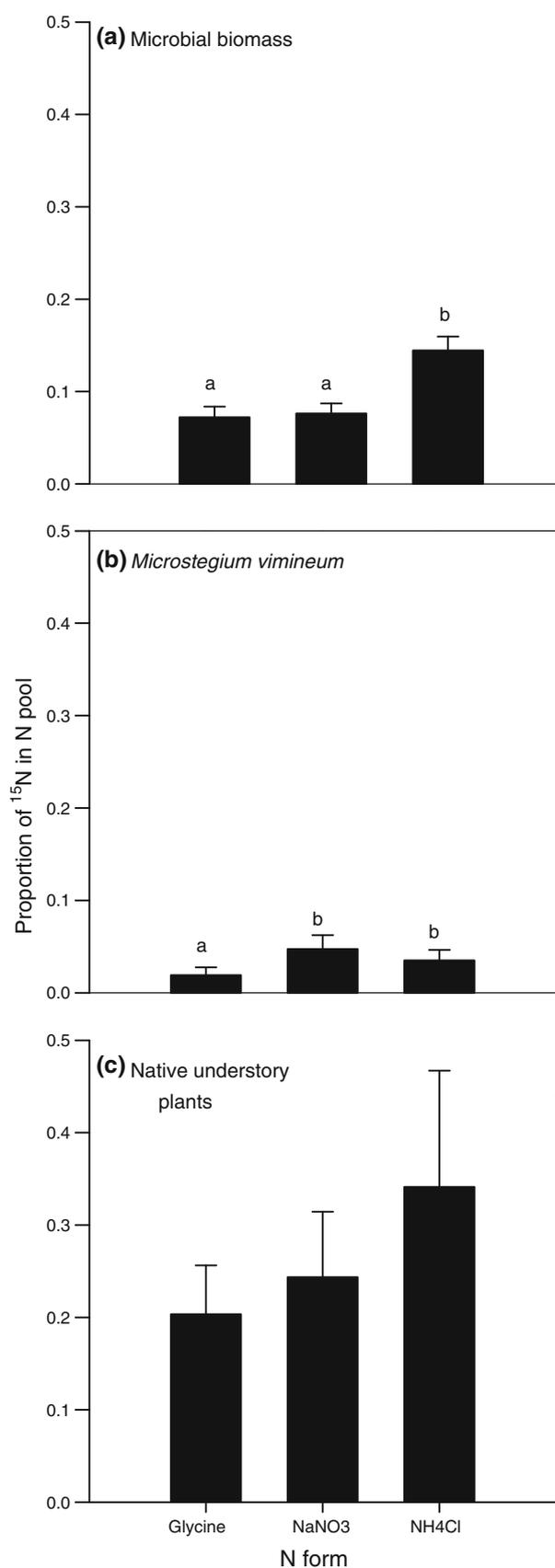


Fig. 4 Preferences for different forms of N by **a** microbial biomass, **b** *M. vimineum*, and **c** native understory plants. Values shown are means + 1 SE and were computed relative to the amount of total N in the pool. Different lowercase letters above bars indicate significant differences ($P < 0.05$) in form of N uptake

the fact that *M. vimineum* is an annual plant invading communities dominated by perennials, which invest a large fraction of biomass to perennating belowground structures. Nonetheless, with observations at our experimental forest that multiple species of invertebrate foliar herbivores in the understory are deriving substantial fractions of their biomass from *M. vimineum* (Bradford et al. 2010), it is possible that this additional allocation of N aboveground will shift N from below- to aboveground foodwebs. Furthermore, above- and belowground litter inputs are thought to be under different controls and to have different fates (Hogberg and Read 2006; Pollierer et al. 2007; Chapin et al. 2009), suggesting that *M. vimineum* invasion could alter forest decomposition dynamics given the simple fact it allocates most of its N aboveground. These possibilities demand further investigation to understand how this invader affects forest N cycling.

In invaded plots, average whole-plant ¹⁵N uptake by native plants was ca. 5% higher on an absolute basis than *M. vimineum* uptake. Scaled by biomass N, whole-plant ¹⁵N uptake by native plants was ca. 4.2% higher than *M. vimineum* uptake. These findings are consistent with previous studies showing that native understory plants tend to have a high biomass N content (Muller 2003), particularly compared to *M. vimineum* (DeMeester and Richter 2010). More importantly, we found that N allocation patterns differed substantially between native species and *M. vimineum*. Native plants allocated 23 and 32 times more N belowground than aboveground in non-invaded and invaded plots, respectively, whereas *M. vimineum* allocated 4 times more N aboveground than belowground. Notably, scaling by biomass N did not change the relative proportion of N in above- and belowground compartments of native species, but scaling reversed the pattern for *M. vimineum* due to the low biomass and relatively N-rich status of its roots (Ehrenfeld et al. 2001; DeMeester and Richter 2010). Overall, these patterns support the idea that marked shifts in the relative proportion of N stored above- and belowground in forest understories will accompany *M. vimineum* invasion.

Microstegium vimineum produced a greater amount of biomass than native species, indicating high nitrogen-use efficiency (NUE). DeMeester and Richter (2010) estimated that biomass NUE was ca. 31% higher in *M. vimineum*-dominated plant communities than in diverse wetland plant communities. Supporting this hypothesis, Collins and Wein (1998) found that *M. vimineum* became exceedingly abundant in plots where it was seeded regardless of soil

fertility. High NUE under favorable (i.e., high light) conditions is a general characteristic of C_4 photosynthetic plants, which require three to six times less Rubisco (an N-rich enzyme) to achieve similar or higher photosynthetic rates than C_3 plants (Ehleringer and Monson 1993). Consequently, *M. vimineum* and other invasive species with C_4 photosynthesis may have a significant fitness advantage over C_3 plants in N-limited environments when light is not limiting. Interestingly, for *M. vimineum*, this higher NUE seems to hold under shade (Claridge and Franklin 2002), and this may be because it uses the C_4 strategy to exploit high-intensity sunflecks efficiently (Horton and Neufeld 1998; Cole and Weltzin 2005) to compete under shade (see Warren et al. 2011a).

Although the decline in microbial biomass in invaded plots suggests that *M. vimineum*'s high NUE and low biomass N alter plant–microbe interactions, we found no support for the hypothesis that microbes were outcompeted by plants for N following invasion. Despite lower microbial biomass N to plant biomass N ratios and 50% less microbial biomass in plots where *M. vimineum* was present, microbes took up similar amounts of ^{15}N in non-invaded and invaded plots. Working in our experimental forest, Strickland et al. (2010) found that microbial activity, measured as substrate-induced respiration (SIR), was unchanged following *M. vimineum* invasion despite a decrease in microbial biomass measured with CFE. Kourtev et al. (2002, 2003) found increased SIR as well as increased N-related soil enzyme activity in the presence of *M. vimineum* in both laboratory and field settings. Together with the shift we observed in N allocation to aboveground biomass, these findings suggest that *M. vimineum* invasion intensifies demand for N belowground thus stimulating microbial activity, at least per unit biomass. Litterbag decomposition studies further support this hypothesis, showing that *M. vimineum* litter can immobilize N during decomposition whereas native understory species litter typically releases N, although these changes depend on the nutrient richness of the native litter (Ehrenfeld et al. 2001; DeMeester and Richter 2010). The changes that lead to enhanced N acquisition in the microbial biomass are poorly understood. Indeed, changes in microbial community composition, the relative dominance of different genotypes within populations, and/or microbial physiology could all explain enhanced microbial activity that results in greater N acquisition per unit biomass (Bradford et al. 2008). Interestingly, an experimental study aimed at evaluating *M. vimineum*'s effects on microbial community composition demonstrated significant compositional changes, including a greater abundance of arbuscular mycorrhizal fungi (AMF) and a lower ratio of bacterial to fungal fatty acids in the presence of *M. vimineum* (Kourtev et al. 2003); however, others have not observed these patterns, potentially

because of site or methodological differences (Strickland et al. 2010).

In our study, the microbial biomass preferred NH_4^+ to NO_3^- and glycine. This finding is consistent with other studies showing microbial preference for NH_4^+ , particularly between pH 6 and 7 (Geisseler et al. 2010). *Microstegium vimineum*, on the other hand, appeared to prefer either NH_4^+ or NO_3^- to glycine. We did not inhibit nitrification of the added NH_4^+ , however, so we cannot rule out the possibility that *M. vimineum* took up the NH_4^+ after it was converted to NO_3^- by the microbial biomass. Indeed, others have suggested that *M. vimineum* favors NO_3^- over other N forms (Ehrenfeld et al. 2001). In contrast to *M. vimineum*, we found no evidence that native plants preferred inorganic N to glycine. This may indicate that native species are more flexible than *M. vimineum* in terms of the sources from which they can derive nitrogen.

Microstegium vimineum grows in a diversity of habitats, including forest stands with depauperate understories that we and others have investigated (Strickland et al. 2010; Strickland et al. 2011), stands with dense herbaceous layers (Flory and Clay 2010), and wetlands (DeMeester and Richter 2010). Consequently, studies in other parts of *M. vimineum*'s range are needed to evaluate whether the shift to allocation of N aboveground is a phenomenon common to *M. vimineum*, or whether this shift is context specific.

Overall, our findings demonstrate that *M. vimineum* influences the above- and belowground partitioning of N in forest understories. Although additional N was found aboveground in invaded understories, we found no evidence that this reduced ^{15}N uptake by the microbial biomass. Given the role of understory plants in nutrient retention, what this means for N dynamics of forest foodwebs and soil N stocks demands investigation if we are to predict the long-term impacts of *M. vimineum* on forest fertility and N export.

Acknowledgments This study benefitted from discussions with Robert Warren, Tony Yannarell and Monica Turner. We also thank K. Gross and three anonymous reviewers for their comments. Funding was through the Long-Term Ecological Research (LTER) Program of the National Science Foundation (Grants DEB-0823293 and DEB-9632854) and the Andrew W. Mellon Foundation. The work conducted complied with all current laws and regulations of the US, where the study was conducted.

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