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Positive feedbacks to growth of an invasive grass through alteration of nitrogen cycling

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Abstract Understanding the mechanisms by which invasive plants maintain dominance is essential to achieving long-term restoration goals. While many reports have suggested invasive plants alter resource availability, experimental tests of feedbacks between invasive plants and soil resources are lacking. We used field observations and experimental manipulations to test if the invasive grass *Microstegium vimineum* both causes and benefits from altered soil nitrogen (N) cycling. To quantify *M. vimineum* effects on N dynamics, we compared inorganic N pools and nitrification rates in 20 naturally invaded and uninvaded plots across a range of mixed hardwood forests, and in experimentally invaded and uninvaded common garden plots. Potential nitrification rates were 142 and 63 % greater in invaded than uninvaded plots in forest and common garden soils, respectively. As a result, soil nitrate was the dominant form of inorganic N during peak *M. vimineum* productivity in both studies. To determine the response of *M. vimineum* to altered nitrogen availability, we manipulated the dominant N form (nitrate or ammonium) in greenhouse pots containing *M. vimineum* alone, *M. vimineum* with native species, and native species alone. *M. vimineum* productivity was highest in monocultures receiving nitrate; in contrast, uninvaded native communities showed no response to N form. Notably, the positive response of *M. vimineum* to nitrate was not apparent when grown in competition with natives, suggesting an invader density threshold is required before positive feedbacks occur. Collectively, our results demonstrate that persistence of invasive plants can be promoted by positive feedbacks with soil resources but that the magnitude of feedbacks may depend on interspecific interactions.

Keywords Common garden · Deciduous forest · *Microstegium vimineum* · Nitrification · Plant–soil feedbacks

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Introduction

Plant invasions have long been known to alter community structure and reduce native biodiversity (Mooney and Hobbs 2000). Once established, invasive plants can form monocultures that are slow to be re-colonized by native species. However, the mechanisms contributing to invader persistence are poorly understood (Hawkes 2007). One possible explanation for this phenomenon is that invasive plants alter ecosystem processes in ways that directly or indirectly contribute to their dominance (Bever et al. 2010; Ehrenfeld et al. 2005; Reynolds et al. 2003). To date, most evidence that plant–soil feedbacks facilitate exotic plant invasions has focused on how belowground mutualists and

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pathogens influence the abundance of invasive plants in native communities (Klironomos 2002; Reinhart and Callaway 2006). Variation in plant effects on and responses to nutrient cycling can also feed back to shape plant community composition and contribute to invader persistence, but resource-based feedbacks have received less attention likely because they are difficult to test experimentally (Bever et al. 2010). Because legacy effects of invasive plants may persist long after their removal by restoration practitioners (Suding et al. 2004), understanding the mechanisms driving feedbacks between plant community members and ecosystem processes is critical to the long-term goal of restoring native species.

Most temperate ecosystems are limited by nitrogen (N), and thus alteration of inputs, outputs, or internal transformations of N forms may benefit plant species that can quickly respond to and exploit such changes (Laungani and Knops 2009). A growing body of literature reports differences in N pools and fluxes between exotic-invaded and nearby native-dominated sites (Allison and Vitousek 2004; Evans et al. 2001; Rossiter-Rachor et al. 2009), and some studies provide experimental evidence that invasive plants can cause shifts in N cycling (Ehrenfeld et al. 2001; Farrer and Goldberg 2009; Hawkes et al. 2005). However, there has not yet been a clear demonstration in a temperate ecosystem that a plant species can facilitate its invasive success by altering N cycling. In the tropics, the N-fixing Hawaiian invader, *Myrica faya*, can enrich soil N pools by two orders of magnitude (Corbin and D'Antonio 2004; Vitousek and Walker 1989), thereby altering the competitive balance in favor of fast-growing non-native grasses. Other plant species that do not fix N can also influence N cycling by having traits that can alter relative concentrations of soil N forms through decomposition and nutrient uptake effects on soil N pools (Aanderud and Bledsoe 2009). Despite the growing collection of studies that show invasive plants can elevate nitrification rates (Liao et al. 2008), little is known about the importance of N form changes in driving plant community shifts (e.g., favoring nitrate-preferring species) and engaging in plant–soil feedbacks.

Microstegium vimineum (stiltgrass) is a highly invasive, shade-tolerant C4 grass distributed throughout forest and edge habitats in the eastern and Midwestern U.S. (Barden 1987; Cole and Weltzin 2004; USDA and NRCS 2005). Because invaded habitats are generally N-limited, there is considerable interest in whether the persistence of *M. vimineum* can be attributed to its alteration of soil N cycling. Unlike most invasive plants, which have fast decomposing litter (Liao et al. 2008), *M. vimineum* litter decomposes more slowly than native litter (Ehrenfeld et al. 2001; Kourtev et al. 1998). Slow rates of litter decomposition can delay N release and alter competition between plants and microbes for available N. Such changes in N release and plant–microbe competition for N could result in microbial N transformations such that N forms that benefit the invader are more readily available (Hawkes et al. 2005). There have been several reports of elevated levels of nitrate being associated with *M. vimineum* invasions of forest soils (Ehrenfeld et al. 2001; Kourtev et al. 2002, 2003), and a greenhouse study has shown that *M. vimineum* elevates net nitrification potential relative to co-occurring native species (Ehrenfeld et al. 2001). However, it is unknown if *M. vimineum* actually causes increases in soil nitrate pools. Moreover, the degree to which *M. vimineum* productivity is enhanced in the presence of elevated soil nitrate relative to co-occurring native species, and thus contributes to the persistence of *M. vimineum* invasions, is poorly understood (Fraterrigo et al. 2011).

Our overarching objective was to determine if the persistence of *M. vimineum* in mixed hardwood forests was due, at least in part, to positive feedbacks associated with alterations of N cycling. Specifically, we sought to determine if *M. vimineum* was the cause or a consequence of changes in N cycling, and whether such changes in soil N cycling preferentially benefited the invader relative to native plants when grown in monoculture and in competition with one another.

Materials and methods

Field study

To evaluate broad patterns of the effects of *M. vimineum* invasion on soil N, we collected soils from invaded and *M. vimineum*-free plots at Big Oaks National Wildlife Refuge (BONWR), a 20,000 ha former military testing facility in southern Indiana. Most soils at BONWR are fine silty loams (alfisols), developed from loess and with the overlying paleosols developed in till. Three soil cores (5 cm diameter, 0–15 cm depth; AMS, American Falls, ID, USA) were randomly collected from each of 10 naturally *M. vimineum*-invaded and 10 uninvaded plots (10 × 20 m) separated by 50–20 km (most >1 km apart). We intentionally selected plots to encompass the wide variety of habitat types at BONWR including bottomland hardwood forests, successional fields, and oak-dominated mature forests, and we sought to include an equal number of plots from each habitat type. Soils were collected in March, July, and September 2009, divided into 0–5 and 5–15 cm depths, pooled by plot, and sieved through a 2-mm mesh within 48 h of field sampling. The first sampling event in March constituted a smaller sampling effort of 8 plots. Soil samples were collected on the same day for each sampling event to minimize differences in soil moisture. Inorganic N (NH_4^+ and NO_3^-) was extracted immediately from soil

samples with 2 M KCl (10:1) and measured with an auto-analyzer (Lachat; Hach, Loveland, CO, USA). Net nitrification potentials (hereafter "nitrification potentials") were estimated by extracting soil inorganic N from samples after incubating soils (5 g fresh weight) aerobically in the laboratory at 22 °C for 12−14 days. We did not adjust soil moisture prior to the incubations so that we could capture the seasonal differences in soil moisture across the sampling dates. Incubating soils were covered in punctured Parafilm in order to minimize soil moisture loss while allowing for aeration. Soil pH was measured in 8:1 (v/v) extracts of 0.01 mM $CaCl_2$ (VWR sympHony pH meter; VWR International, West Chester, PA, USA). The percentage of organic matter in each sample was determined by loss on ignition using a muffle furnace at 430 °C. Differences in soil NH_4^+, NO_3^-, and total inorganic N concentrations, nitrate:inorganic N ratio, nitrification potential, pH, percent soil moisture, and percent organic matter between invaded and uninvaded plots across the three sampling dates were analyzed using a repeated measures mixed model ANOVA (Proc Mixed; SAS Institute, Cary, NC, USA) with sampling date, invasion, and sampling date × invasion as fixed effects and sampling date repeated. Samples from 0–5 and 5–15 cm depths were averaged for these analyses after determining that they showed similar patterns for all measurements.

Common garden experiment

We conducted a common garden experiment to determine if differences in N cycling observed in the field study were a cause or a consequence of the invasion. The common garden experiment was established at the Indiana University Research and Teaching Preserve's (IURTP) Bayles Road site (39°13′9″N, 86°32′29″W) north of Bloomington, Indiana. The site was previously bottomland hardwood forest, but portions have been maintained as open fields for biological research for the past 60 years. Soils are coarse-silty, mesic, Typic Udifluvents. In a 60 × 60 m forest opening, we established 32 plots (5.25 × 5.25 m each) at 2.5-m spacing and surrounded each plot with 60-cm-tall silt fence (Eco-Systems, Bloomington, IN, USA) buried 10 cm deep to prevent movement of seeds among plots. In 2005, all plots were uniformly planted with a mixture of 12 native grasses, sedges, and forbs (see Flory and Clay 2010a, b for species lists), and many other herbaceous species colonized the plots from the surrounding area over the course of the experiment. In late fall 2005, 8 of 16 plots were sown with locally collected *M. vimineum* at a rate of ∼690 seeds/m^2, corresponding to observed seedling densities in locally invaded sites.

In 2009, soil cores (5 cm diameter, 0–15 cm depth; AMS) were taken in March, June, July, and September from three randomly chosen locations within each invaded and uninvaded plot. Samples were bulked by plot and the same soil measurements made on field study samples, described above, were made on these soils. Differences in the eight soil measurements between experimentally invaded and uninvaded plots across four dates during the growing season were analyzed with a repeated measures mixed model ANOVA (Proc Mixed) with sampling date, invasion treatment, and their interaction as fixed effects and sampling date repeated. Samples from 0–5 and 5–15 cm depths were averaged for these analyses after determining that they showed similar patterns for all measurements.

Greenhouse experiment

To experimentally determine if changes in N transformation rates in *M. vimineum*-invaded soils disproportionately promoted *M. vimineum* compared to native species, we established four plant community treatments in 1-gallon (3.785-l) pots (plastic, 16 cm in diameter) in the Indiana University greenhouse in July 2009: (1) unplanted control pots, (2) *M. vimineum* (Mv), (3) *M. vimineum* plus native community (Mv + Nat), and (4) native community (Nat).

Six species that often co-occur with *M. vimineum* were used for our experimental native community with three functional groups represented by two species each: forbs (*Eupatorium perfoliatum* and *Rudbeckia triloba*), grasses (*Elymus virginicus* and *Glyceria striata*), and sedges (*Carex muskingumensis* and *Scirpus atrovirens*). *E. perfoliatum*, *R. triloba*, and *E. virginicus* were grown from seed, and *G. striata*, *S. atrovirens* and *C. muskingumensis* were obtained as seedlings (Heartland Restoration Services, Ft. Wayne, IN, USA). Each experimental native community was composed of two individuals per species (4 individuals represented each functional group), totaling 12 individuals per pot. Individuals were added as young seedlings [4–6 inches (10–15 cm) tall] to experimental pots with their position within the pot fully randomized and mapped for identification at harvest time. Approximately 340 *M. vimineum* seeds by weight were added to each pot receiving the Mv and Mv + Nat plant community treatments to simulate the *M. vimineum* density of nearby natural invasions (Flory et al. 2007).

Soil substrate was collected from a bottomland hardwood forest at the IURTP Bayles Road site where *M. vimineum* is locally absent but present within 200 m. *Juglans nigra*, *Acer negundo*, and *Platanus occidentalis* dominated the forest overstory. After cover vegetation was removed, the top 5 cm of soil was collected and cut with sand (2:1 sand/soil) to facilitate drainage in experimental greenhouse pots.

Pots were provided with 100 ml of one of four aqueous nitrogen treatments three times per week for 12 weeks in a

factorial design: (1) ammonium (AMO), (2) ammonium + N-Serve (AMO + I), (3) nitrate (NIT), and (4) water control (C). Ammonium and nitrate were added as 1 mM $(NH_2)_2SO_4$ and 1 mM $Ca(NO_3)_2$ salt solutions, respectively. N-Serve is a commercial-grade nitrification inhibitor with the active ingredient nitrapyrin (Dow AgroSciences LLC, Indianapolis, IN, USA) which has been shown to be highly effective in reducing net potential nitrification rates in field soils (Shi and Norton 2000). It was used in this experiment to retain N as ammonium in the AMO + I treatment. Five microliters of N-serve dissolved in an aqueous solution with a wetting agent (Coco-Wet; Spray-N-Grow, Rockport, TX, USA) were added weekly to the AMO + I-treated pots. The plant community × nitrogen treatment factorial design included 15 replicates randomized by block. The non-planted treatments had 5 replicates. Block locations were re-randomized in the greenhouse every other week throughout the experiment.

Twelve weeks after the experiment was established, all aboveground plant material was harvested, sorted by species, dried to constant mass, and weighed. Five soil cores (2.5 cm diameter, full container depth; AMS) were taken through the vertical profile of each pot, combined, and sieved through 2-mm mesh. The same soil measurements made on field study samples, described above, were made on these soils. Plant material samples from three randomly chosen blocks were ground separately with a Wiley mill (#60 screen; Thomas Scientific, Swedesboro, NJ, USA) and analyzed for C and N content on an elemental analyzer (ECS 4010; Costech Analytical Technologies, Valencia, CA, USA). Plant N-use efficiency (NUE) was calculated by dividing plant biomass by plant N content (Chapin 1980).

To evaluate the ability of nitrogen and plant treatments to alter soil responses, concentrations of NH_4^+, NO_3^-, and total inorganic N, and nitrate:inorganic N ratio were analyzed with a mixed model ANOVA (Proc Mixed) that included nitrogen treatment (C, AMO, AMO + I, NIT) and plant community treatment (unplanted, Mv, Mv + Nat, Nat) as fixed effects and random effects of block and its treatment interactions. In order to more directly address the question of whether *M. vimineum* responds more favorably to nitrate-dominant versus ammonium-dominant N-nutrition relative to a native plant community, plant biomasses (total, *M. vimineum*, native, native forbs, native grasses, native sedges) and the soil responses described above were analyzed without the AMO nitrogen treatment and the unplanted plant community treatment using the same ANOVA model. The AMO treatment was excluded because it did not promote conditions in which ammonium was the dominant form of soil inorganic N, unlike the AMO − I treatment.

We quantified how plant community treatments influenced nitrification potentials under control N conditions and how the relative abundance of *M. vimineum* across a gradient of N availability (i.e., control and ammonium-amended pots) influenced nitrification potentials in order compare these results to the two field experiments. The nitrification potentials of pots that received the control N treatment were analyzed using an ANOVA with plant community treatment as a fixed effect and block as a random effect. Regression was used to examine the relationship between nitrification potentials and *M. vimineum* biomass in mixed community pots (Sigma Plot v.11; Systat Software, San Jose, CA, USA), and for this purpose, nitrification potentials were transformed by adding 0.0015 to make all values positive. Soil pH was analyzed with the ANOVA model used for inorganic N concentrations described above to evaluate the influence of plant community and N treatments.

Plant NUE values were analyzed with a mixed model ANOVA (Proc Mixed) that included nitrogen treatment (C, AMO + I, NIT), plant community (Mv, Mv + Nat, Nat), and plant functional group (M, forbs, grasses, sedges) and their interactions as fixed effects and random effects of block and its treatment interactions.

Results

Field study

Averaged over the growing season, naturally *M. vimineum*-invaded plots had greater soil NO_3^- ($p = 0.0069$), total inorganic N ($p = 0.0020$), nitrate:inorganic N ratios ($p = 0.0152$; Fig. 1a), and nitrification potentials ($p = 0.0470$; Fig. 1b) compared to uninvaded plots (ESM1, 2). Soil NH_4^+ concentration, pH, and percent soil organic matter were not affected by the presence of *M. vimineum* or soil collection date (ESM1, 2). Soil moisture was greater in invaded than in uninvaded plots ($p = 0.0215$), decreased with time ($p < 0.0001$), and showed an invasion × time interaction ($p = 0.0464$; ESM1, 2).

Common garden experiment

Averaged across the growing season, experimentally *M. vimineum*-invaded plots had greater soil total inorganic N concentrations ($p = 0.0039$; ESM1, 2). Soil NO_3^- concentrations exhibited an invasion × time interaction ($p = 0.0022$) with lower and higher concentrations detected in invaded than control plots in May ($p = 0.0005$) and June ($p = 0.0885$), respectively (ESM1, 2). The overall effect of invasion on nitrate: inorganic N ratios was not significant, but values were higher in invaded than uninvaded plots in June ($p = 0.0780$; Fig. 2a; ESM1, 2). All inorganic N pool measurements decreased over the course of the growing season (Fig. 2a, b; ESM1, 2).

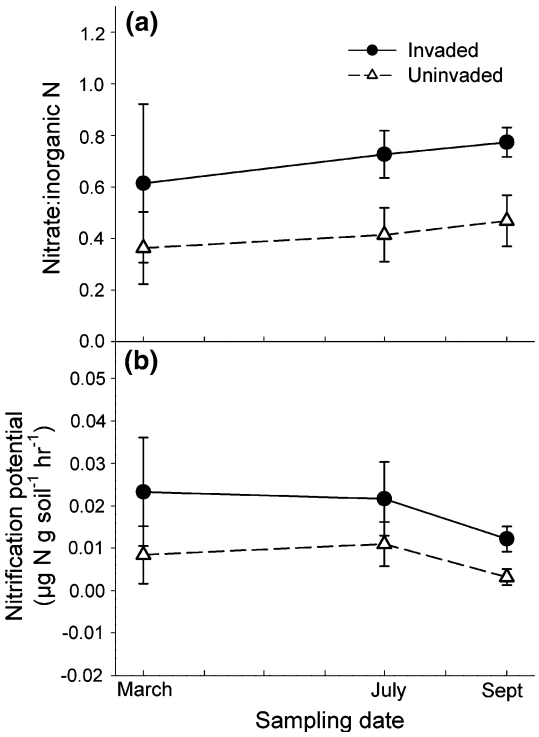

Fig. 1 Seasonal patterns of **a** nitrate:inorganic N ratios and **b** nitrification potentials in *invaded* and *uninvaded* plots at Big Oaks National Wildlife Refuge, IN. Data sampled from 2009 growing season; values are mean ± SE; $n = 10$, except for March $n = 4$

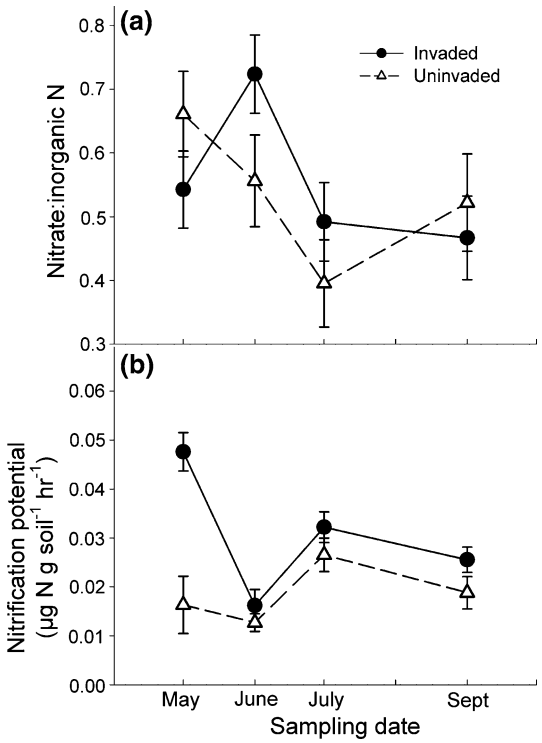

Fig. 2 Seasonal patterns of **a** nitrate:inorganic N ratios and **b** nitrification potentials in *invaded* and *uninvaded* plots at Bayles Road, IN. Data sampled from 2009 growing season; values are mean ± SE; $n = 8$

Higher nitrification potentials were detected in invaded plots averaged over the growing season ($p < 0.0001$; Fig. 2b; ESM1, 2). Nitrification potentials were higher in invaded than in uninvaded plots in May ($p = 0.0005$), but the difference narrowed by June, producing an invasion × time interaction ($p < 0.0001$; Fig. 2b; ESM1, 2).

Soil pH and organic matter increased over time ($p < 0.0005$ and $p = 0.0050$; ESM1, 2) but could not be explained by invasion. Soil moisture varied over time and generated an invasion × time interaction ($p < 0.0001$ and $p < 0.001$; ESM1, 2) with invaded plots tending to be drier at the beginning and wetter toward the end of the growing season than uninvaded plots.

Greenhouse experiment

Across N treatments, unplanted pots had greater soil NO_3^- and total inorganic N concentrations ($p < 0.001$ for NO_3^- and total inorganic N post hoc comparisons against the unplanted treatment; ESM3), and greater nitrate: inorganic N ratios ($p = 0.0492$, $p = 0.0760$, and $p = 0.0699$ for post hoc comparisons against Mv, Mv + Nat, Nat, and the unplanted treatment, respectively; ESM3) than planted pots, whereas soil NH_4^+ concentrations did not differ. Mv pots had greater soil total inorganic N concentrations than Nat pots ($p < 0.0001$; ESM3) while nitrate:inorganic N ratios did not differ.

Across plant community treatments, the total inorganic N concentrations of soils given N treatments (AMO, AMO + I, and NIT) did not differ from one another and were greater than the control ($p < 0.0001$ for post hoc comparisons against the control; ESM3). Soils given the AMO + I treatment had higher NH_4^+ and lower NO_3^- concentrations, and lower nitrate:inorganic N ratios than those given the AMO treatment ($p < 0.0001$, $p = 0.0199$, and $p = 0.0083$; ESM3), showing that the nitrification inhibitor was effective and that our use of the AMO + I treatment to represent ammonium-dominated conditions was appropriate.

Total plant biomass and *M. vimineum* biomass varied by plant community treatment ($p < 0.001$ and $p < 0.0001$; Fig. 3a; ESM3), N treatment ($p < 0.0001$ and $p < 0.0001$; Fig. 3a; ESM3), and their interaction ($p = 0.0002$ and $p = 0.001$; Fig. 3a; ESM3). Native community biomass showed plant community and N treatment effects ($p < 0.0001$ and $p < 0.0001$; Fig. 3b), but no treatment interaction effects. Total plant biomass was greatest in Mv, intermediate in Mv + Nat, and least in Nat pots ($p < 0.0001$ for all post hoc comparisons; ESM3). *M. vimineum* accumulated more biomass in the absence of

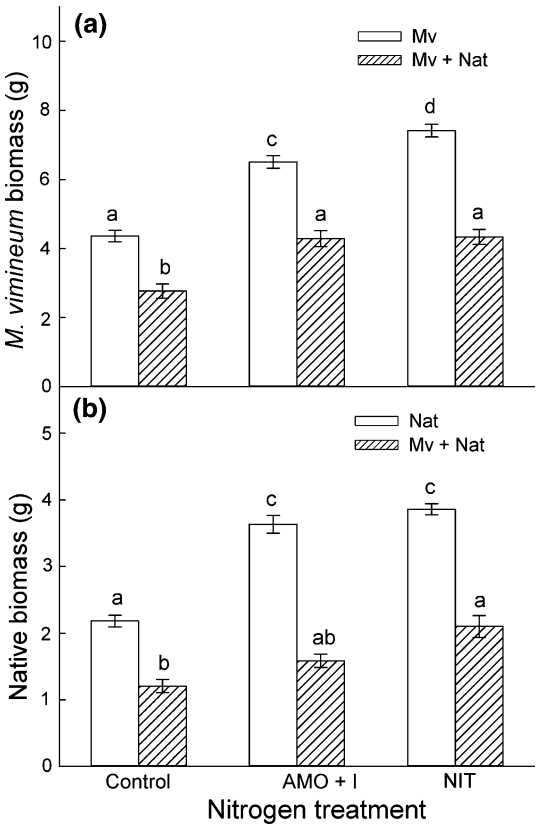

Fig. 3 Effects of nitrogen treatment on **a** *M. vimineum* and **b** native community aboveground biomass grown separately (*Mv* or *Nat*) and in mixed (*Mv* + *Nat*) communities. Treatment codes are *AMO* + *I* ammonium, *NIT* nitrate. *Bars* mean ± SE; $n = 15$; and different *letters* show significant differences

the native community ($p < 0.0001$; Fig. 3a) and the native community accumulated more biomass without *M. vimineum* ($p < 0.0001$; Fig. 3b). Native communities were dominated by forbs at harvest time, making up 59 and 69 % of the native biomass in Nat and Mv + Nat pots, respectively.

Grown in monoculture (Mv) and with the native community (Mv + Nat), *M. vimineum* accumulated more biomass given AMO + I ($p < 0.0001$, +49 %, and $p < 0.0001$, +55 %; Fig. 3a) and NIT ($p < 0.0001$, +70 %, and $p < 0.0001$, +57 %; Fig. 3a) treatments than the control N treatment. *M. vimineum* accumulated 14 % more biomass given NIT than AMO + I ($p = 0.0126$; Fig. 3a) in monoculture (Mv), but its biomass did not differ among NIT and AMO + I treatments in the presence of the native community (Mv + Nat).

Native communities accumulated more biomass given AMO + I than the control N treatment in the absence (Nat, $p = 0.0264$, +67 %; Fig. 3b), but not the presence (Mv + Nat) of *M. vimineum*. Both without and with *M. vimineum*, native communities accumulated more biomass given NIT than the control treatment (Nat $p = 0.0004$, +77 %, and Mv + Nat $p = 0.0192$, +74 %; Fig. 3b) and biomass in NIT and AMO + I treatments did not differ (Fig. 3b). Of the three functional groups represented in the native community, forbs were the only ones that responded to N fertilization in the presence of *M. vimineum* (Mv + Nat), only responding to NIT ($p = 0.0192$).

Among plant community treatments given the control N treatment, nitrification potentials showed no statistically significant differences; however, nitrification potentials tended to be highest in Mv pots, followed in order by Mv + Nat, Nat, and unplanted pots (Fig. 4a; ESM1, 3). The relative abundance of *M. vimineum* in Mv + Nat pots given control and AMO N treatments was positively correlated with nitrification potential ($y = 0.00007e^{0.0533x}$ where y = nitrification potential and x = percent of *M. vimineum* biomass, $p = 0.0039$, $r^2 = 0.27$; Fig. 4b). Unplanted soils had the highest pH values, followed in order by soils given Mv, Mv + Nat, and Nat treatments ($p = 0.0007$; ESM1, 3). Soil pH did not correlate with nitrification potential in any plant treatment.

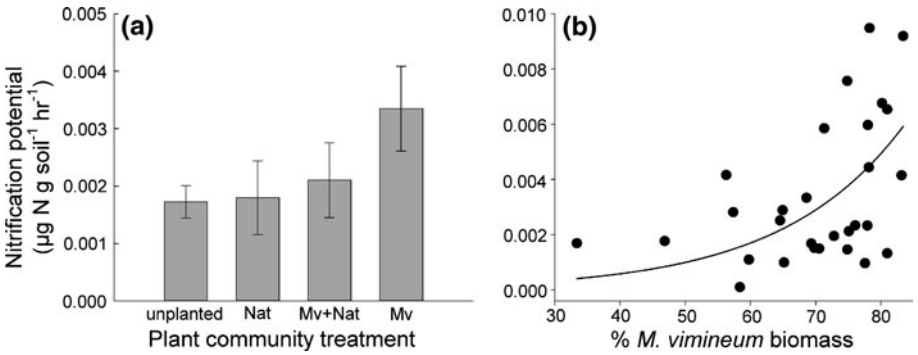

Fig. 4 Effects of **a** plant community treatment and **b** relative abundance of *M. vimineum* in mixed community pots (*Mv* + *Nat*) on nitrification potentials. In (**a**), data are shown for plant community treatments given the control N treatment, and in (**b**) for the mixed plant community treatment given control and AMO N treatments. *Bars* mean ± SE; $n = 15$, except for unplanted $n = 5$; and the *trend line* is ($y = 0.00007e^{0.0533x}$, $p = 0.0039$, $r^2 = 0.27$; $n = 30$)

The NUE of *M. vimineum* and the three native functional groups represented in this study differed significantly ($p = 0.0049$). Across all plant and nutrient treatments, *M. vimineum*'s NUE (115.35 g/g) was more than twice as large as that of the forbs (48.76 g/g), sedges (53.08 g/g), and grasses (48.17 g/g) in native communities.

Discussion

A useful conceptual framework for understanding resource-based feedbacks is one that considers the role of "effects" and "responses" (Eviner and Hawkes 2008; Lavorel and Garnier 2002). Plant effects (e.g., changes in litter and root exudate quantity and quality) influence soil resources through alteration of microbially mediated processes such as decomposition, nitrification, and N_2-fixation. Plant responses (e.g., growth rate, nutrient use efficiency, etc.) are traits that are associated with the response of plants to changing resource quantities and qualities. Accordingly, invasive species may induce positive feedbacks when their effects and responses enhance their ability to maintain dominance in the community (Bever et al. 2010; Ehrenfeld et al. 2005; Reynolds et al. 2003). While this mechanism is often alluded to, it is rarely investigated experimentally. Here, we present evidence that *M. vimineum* alters soil nitrate availability, resulting in greater productivity under high-density invasions. This process represents an under-appreciated mechanism to explain how *M. vimineum* may persist in nutrient-poor habitats.

Enhanced soil nitrate levels have commonly been observed under invasive plants (e.g., Adair and Burke 2010; Hellmann et al. 2011; Rout and Chrzanowski 2009), but experimental tests of whether invaders are the cause or consequence of such conditions are rare. In our experiment, nitrate:inorganic N ratios and nitrification potentials were enhanced in both naturally and experimentally *M. vimineum*-invaded areas during the peak of the growing season (Figs. 1 and 2; ESM1, 2); hence, we conclude that *M. vimineum* was in fact the driver of these changes in N cycling. To our knowledge, this is the first field experiment to demonstrate the direct effect of an invader on soil N cycling. Interestingly, invasion effects measured in experimentally invaded plots were smaller in magnitude and more variable than the naturally invaded field sites (ESM2). This may be due to differences in the time since invasion and successional status between the two sites. Experimental plots had only been established and invaded 4 years prior, while the field study sites included a range of secondary and mature forests with unknown, but likely extended, invasion histories. Moreover, unlike the naturally invaded plots, the uninvaded experimental plots were dominated by grasses due to the lack of canopy closure in these plots. Given that grasses are commonly associated with extravagant N cycling and enhanced nitrification rates (Chapman et al. 2006), the higher baseline nitrification rates in these plots may explain why the *M. vimineum* effects were less pronounced in experimental uninvaded plots.

Elevated nitrification rates in invaded soils have been hypothesized to result from plant-induced changes in soil conditions (e.g., pH) that allow chemoautotrophic nitrifiers to compete more effectively for ammonium (Kourtev et al. 2003), or from the replacement of native species by invasive plants with greater nutrient-use efficiency (Funk and Vitousek 2007). In our study, we observed differences in soil pH in invaded soils only in the greenhouse experiment, and not in the field study or common garden experiment (ESM1, 2, 3). Nitrification potentials tended to be highest in Mv pots, followed in order by Mv + Nat, Nat, and unplanted pots (Fig. 4a; ESM3) and correlated with the relative abundance of *M. vimineum* in mixed community greenhouse pots (Fig. 4b). Given that soil pH did not correlate with nitrification potentials under any plant treatment in the greenhouse experiment, these results do not support the hypothesis that nitrifier activity is sensitive to *M. vimineum*-mediated pH changes.

The enhanced nitrification rates in *M. vimineum* soils may have increased due to the accelerated turnover of microbial N pools. Elevated nitrification generally arises from increased ammonium supply (e.g., via enhanced gross N mineralization or accelerated microbial turnover) relative to plant demand. Although we did not measure gross N mineralization rates in any of our experiments, the activities of aminopeptidase and phenol oxidase, enzymes involved in microbial N acquisition and soil organic matter degradation, respectively, have been reported to be greater in *M. vimineum* soils (Kourtev et al. 2002). Further, microbial communities in *M. vimineum* soils utilize labile C substrates more quickly than in native soils (Strickland et al. 2010). To the extent that enhanced C assimilation influences the turnover rate of the microbial biomass, soil ammonium pools are also likely to increase (Clarhom 1985). Further, much of this ammonium is likely oxidized to nitrate rather than acquired by plants given the modest N requirements of *M. vimineum* (e.g., the nutrient use efficiency of *M. vimineum* was 2 times greater than that of native plants in this study). This may explain, in part, why experimental additions of ammonium without a nitrification inhibitor increased nitrate pools in *M. vimineum* pots but not in native pots (ESM3).

M. vimineum's ability to increase nitrate:inorganic N ratios and nitrification rates, regardless of the mechanism, may contribute to its dominance in the community because of the way in which *M. vimineum* responds to these

conditions relative to native species. *M. vimineum* was 14 % more productive in nitrate than ammonium-dominated soil conditions (Fig. 3a), whereas native communities showed no response (Fig. 3b). *M. vimineum* has elevated nitrate reductase activity (Ehrenfeld et al. 2001; Kourtev et al. 1998) indicating that the cost of reducing assimilated nitrate may be low for *M. vimineum*. Since our experimental AMO + I and NIT treatments were proxies for soils conditioned by native plant species and *M. vimineum* during peak *M. vimineum* growth, respectively, our results suggest that *M. vimineum* and nitrate-dominated soil conditions may engage in a positive feedback with respect to the native community (Bever et al. 2010, 1997). Interestingly, this effect was reduced when *M. vimineum* and native plants were grown together, indicating that the presence of other competitors for nitrate may mediate the strength of this feedback. This suggests that, while *M. vimineum* may be more effective at using nitrate than ammonium, *M. vimineum* may be unable to benefit from enhanced nitrate availability until some threshold of invader density (between 50 and 100 % plant community composition) has been reached (Fig. 4b).

Resource-based feedbacks are difficult to demonstrate experimentally as processes which affect soil microbial communities and their activities can have strong consequences on the magnitude and direction of feedbacks. In a recent study, Fraterrigo et al. (2011) added ^{15}N tracers to natural invasions of *M. vimineum* and reported that *M. vimineum* had no preference for inorganic N forms. However, because nitrification rates were not experimentally inhibited in this study, some of the added ammonium may have been nitrified prior to uptake. In a greenhouse study using live and sterile soil inoculum from invaded and uninvaded soils, Shannon et al. (2012) reported a negative soil microbial feedback when native plants and *M. vimineum* were grown in competition, but not when grown separately, primarily due to greater native community performance in invaded soil. Given that nitrifiers are generally poor competitors for ammonium in nutrient-poor soils (Hawkes et al. 2005), background levels of soil ammonium can influence the magnitude and direction of plant–soil feedbacks. Hence, future studies that alter the relative abundance of ammonium oxidizer populations (e.g., through experimental N additions and nitrification inhibitors) under field conditions are needed to determine the extent to which alterations of N cycling can drive the persistence of natural invasions.

Conclusions

Although elevated net potential nitrification in invaded soils have been reported previously for *M. vimineum* (Ehrenfeld et al. 2001; Kourtev et al. 2002), no studies to our knowledge have tested whether the invader can drive such changes in field plots. Our study demonstrates that this invader produces a discernable shift in N cycling under natural field conditions. By using both the common garden and greenhouse experiments, we were able to consider both plant effects (i.e., promotion of soil nitrification) and responses (i.e., high nitrate–N assimilation efficiency). Few studies documenting positive plant–soil feedback as a driver of invasions have experimentally tested both of these factors. We show that *M. vimineum* promotes nitrification rates in invaded soil, and suggest that monocultures of invasions are maintained by high soil nitrate concentrations. Such positive plant–soil feedbacks due to microbially mediated nutrient transformations and nutrient availability may be an underappreciated mechanism supporting the persistence of plant invasions.

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