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HIGHLIGHTED STUDENT RESEARCH

Plant–microbial competition for nitrogen increases microbial activities and carbon loss in invaded soils

Matthew E. Craig[1,2] · **Jennifer M. Fraterrigo**[1]

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Abstract Many invasive plant species show high rates of nutrient acquisition relative to their competitors. Yet the mechanisms underlying this phenomenon, and its implications for ecosystem functioning, are poorly understood, particularly in nutrient-limited systems. Here, we test the hypothesis that an invasive plant species (*Microstegium vimineum*) enhances its rate of nitrogen (N) acquisition by outcompeting soil organic matter-degrading microbes for N, which in turn accelerates soil N and carbon (C) cycling. We estimated plant cover as an indicator of plant N acquisition rate and quantified plant tissue N, soil C and N content and transformations, and extracellular enzyme activities in invaded and uninvaded plots. Under low ambient N availability, invaded plots had 77% higher plant cover and lower tissue C:N ratios, suggesting that invasion increased rates of plant N acquisition. Concurrent with this pattern, we observed significantly higher mass-specific enzyme activities in invaded plots as well as 71% higher long-term N availability, 21% lower short-term N availability, and 16% lower particulate organic matter N. A structural equation model showed that these changes were interrelated and associated with 27% lower particulate organic matter C in invaded areas. Our findings suggest that acquisition of N by this plant species enhances microbial N demand, leading to an increased flux of N from organic to inorganic forms and a loss of soil C. We conclude that high N acquisition rates by invasive plants can drive changes in soil N cycling that are linked to effects on soil C.

Keywords Invasive plants · Nutrient uptake · Soil carbon · Enzyme activities · Nitrogen limitation

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We find evidence that intensified competition for a limiting resource enhances the microbial breakdown of organic matter in soils occupied by invasive plants, ultimately leading to a shift in soil nutrients from organic to inorganic forms and a decrease in soil carbon storage. This work proposes a mechanism by which invasive plants might enhance nutrient acquisition in nutrient-limited soils and is one of the first investigations to demonstrate linkages between carbon and nutrient cycling in invaded systems.

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✉ Matthew E. Craig
maecraig@indiana.edu

[1] Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1102 S Goodwin, Urbana, IL 61801, USA

[2] Present Address: Department of Biology, Indiana University, 1001 E Third St, Bloomington, IN 47405, USA

Introduction

The strong competitive ability of non-native plant species is considered a key factor promoting successful invasion potential (Roy 1990; Vila and Weiner 2004). Supporting this premise, many invasive plant species show higher rates of aboveground biomass accumulation than their native competitors (Liao et al. 2008; van Kleunen et al. 2010; Vilà et al. 2011; Bottollier-Curtet et al. 2013). This phenomenon occurs even in nutrient-poor soils (Funk and Vitousek 2007), raising the question of how invaders achieve high productivity and thus competitive dominance in resource-limited environments. A leading explanation is that invasive species have higher resource-use efficiency (RUE) relative to natives. Indeed, many invaders enhance carbon (C) gain per unit nitrogen (N) by preferentially allocating nutrients

to photosynthetic tissues (James 2008; Fridley 2012; Heberling and Fridley 2013) or by more efficiently using resources on short timescales (Funk and Vitousek 2007). To be productive under resource-limiting conditions, an alternative strategy is to optimize resource acquisition (Funk et al. 2016), a strategy that should trade off with RUE (Reich 2014). For example, Laungani and Knops (2009) found that the invasive tree species *Pinus strobus* acquired substantially more C than co-occurring native grasses, and this effect was a result of greater long-term N acquisition rather than higher RUE. This strategy appears to be common among invasive plant species, which frequently increase the flux of nutrients from soils to aboveground biomass (Castro-Díez et al. 2014), enhance plant nutrient pools (Liao et al. 2008), and deplete soil nutrient concentrations (Jo et al. 2015). Yet it remains unclear how invasive plants achieve high rates of resource uptake in resource-limited environments and what the consequences are for ecosystem functioning.

There is increasing evidence that specialized root traits enhance the capacity of non N-fixing invasive plants to acquire N. Across 14 non-native and ten native temperate forest species, Jo et al. (2015) found that invasive species had greater specific root length and fine root production, traits associated with high N acquisition (Craine 2011; McCormack et al. 2015). Associations with symbiotic fungi can also play an important role. Some invasive species enhance nutrient acquisition by associating with arbuscular mycorrhizal fungi (AMF; Pringle et al. 2009; Lee et al. 2014), which are highly effective nutrient scavengers. However, although these factors can enhance acquisition of inorganic N, the bulk of soil N is stored in organic forms which must be mineralized before uptake by plants and AMF in temperate systems. This suggests that invasive species might increase their rate of N acquisition by accelerating the decomposition of litter inputs and/or soil organic matter (SOM), a process mediated by the soil microbial community. Whereas many studies have examined how invasion alters litter decomposition (Allison and Vitousek 2004; Ashton et al. 2005; Arthur et al. 2012), relatively few studies have investigated invasion effects on SOM. To elucidate how invasive plant species achieve high rates of resource uptake in resource-limited environments, improved understanding of how plant invasion affects the activities of SOM-degrading microbes is needed.

Regardless of invasion status, microbial degradation of SOM is catalyzed by extracellular enzymes, the production of which is strongly regulated by microbial N demand (e.g., Deforest et al. 2004). When inorganic N supply is insufficient to sustain microbial growth, SOM-degrading microbes can increase their production of N-acquiring extracellular enzymes (Hamman et al. 1997; Geisseler et al. 2010), leading to increases in the amount of inorganic N available for plant and microbial uptake. Downregulation of extracellular enzyme production occurs when microbes immobilize this N, and results in the reduced supply of inorganic N (Geisseler et al. 2010; Piscitelli et al. 2011).

Although such microbial regulation of N cycling is common in many temperate systems (Knops et al. 2002), growing evidence suggests that some plants can subvert microbial regulation of N cycling (Chapman et al. 2006). Notably, plants showing high rates of N acquisition, like many invasive species, can outcompete microbes for inorganic N (Kuzyakov and Xu 2013; Moreau et al. 2015), thereby intensifying N limitation of SOM-degrading microbes and suppressing the downregulation of enzyme synthesis (Cheng et al. 2012). Under these conditions, N is expected to shift from the SOM to plant biomass over the growing season. There is evidence that plant invasion results in elevated enzyme activities (e.g., Kourtev et al. 2002, 2003), as well as faster N cycling and SOM decomposition rates (Liao et al. 2008; Castro-Díez et al. 2014), yet the mechanistic link between these patterns is only partly understood (Hawkes et al. 2005). Moreover, we know little about how changes in N cycling affect soil C cycling in invaded systems.

Because C is lost as CO_2 when SOM is degraded, increased plant–microbe competition for N may drive C loss in N-limited soils (Craine et al. 2007). This could explain why in some cases soil C declines even though aboveground biomass increases following plant invasion (Tamura and Tharayil 2014; Craig et al. 2015). Previous work has attributed this loss of C to priming effects (i.e., the stimulation of microbial activity by high-quality C inputs) in invaded soils (Strickland et al. 2010). Priming is typically associated with increased microbial biomass (Fontaine et al. 2004; Blagodatskaya et al. 2007), however, and many studies report that microbial biomass decreases with invasion (e.g., Scott et al. 2001; Strickland et al. 2010). If plant invasion intensifies the microbial communities' demand for N, then we would expect both reduced microbial biomass because of microbial N limitation (Gallardo and Schlesinger 1992; Hart and Stark 1997) and soil C loss because of higher rates of SOM decomposition (Craine et al. 2007). The input of high-quality C by invasive plants may strengthen this effect because such compounds provide energy and substrate for enzyme synthesis (Geisseler et al. 2010). Thus, we hypothesize that invasive plants enhance their acquisition of N in N-limited ecosystems in part by outcompeting SOM-degrading microbes for inorganic N, leading to increased soil inorganic N availability, and decreased soil organic N (SON) and C (SOC).

We tested this hypothesis by carrying out an integrated investigation of the effects of a well-studied invasive grass, *Microstegium vimineum* (Trin.) A. Camus, on microbial activities and soil C and N cycling in a temperate forest.

Microstegium vimineum is an annual, C_4 grass that is native to Asia (Barden 1987). Though it is often found in high-light, high-moisture areas (Warren et al. 2011), it is shade tolerant and found in a variety of habitats (Horton and Neufeld 1998; Huebner 2010; Cheplick and Fox 2011), including N-poor temperate forest understories (Craig et al. 2015). Unlike many invasives, *M. vimineum* has a weak allelopathic potential (Corbett and Morrison 2012). *M. vimineum* can, however, accelerate N cycling rates (Demeester and Richter 2010) and deplete soil C pools (Strickland et al. 2010), though these effects are yet to be measured concurrently. Previously, we observed that the greatest negative effects of *M. vimineum* on soil C occur in areas with relatively high *M. vimineum* biomass and low ambient N availability (Craig et al. 2015), suggesting the need to consider invader abundance and soil N availability to gain a more complete understanding of the mechanisms underlying invasion effects on SOC. Our earlier work also showed that the increase in aboveground biomass that occurs with *M. vimineum* invasion is associated with higher rates of plant N acquisition (Fraterrigo et al. 2011), leading us to use plant cover as an indirect measure of plant N acquisition rate. We expect that *M. vimineum* acquires a large fraction of N from the SOM rather than fresh litter because *M. vimineum* produces slow-decomposing litter (Demeester and Richter 2010) and has little effect on the decomposition of native litter (Craig et al. 2015). In this study, we therefore quantified plant cover (as a proxy for rate of plant N acquisition), and inorganic and organic N dynamics in invaded and uninvaded soils, to determine whether these factors are mechanistically related to previously reported effects of *M. vimineum* on SOC pools and enzyme activities (Craig et al. 2015). We hypothesized that *M. vimineum* intensifies N demand of SOM-degrading microbes by increasing rates of plant N acquisition (i.e., increasing plant cover), thereby accelerating N release from the SOM. We predicted that microbial activity would be greater in invaded soils and associated with lower organic N and C and higher inorganic N. Moreover, we predicted that the magnitude of increase in plant cover would be a key predictor of alterations to microbial activity and coupled effects on soil C and N cycling.

Methods

Study design

We worked in Eastern U.S. temperate forests, where N is broadly considered to limit plant productivity (Vitousek and Howarth 1991), across a region extending from Asheville, NC (35°35′N, 82°33′W) northwest to Pisgah National Forest (35°55′N, 82°47′W). Within this area, we selected twelve forested sites spanning a range of edaphic properties and surrounding land cover, representing the wide range of habitats occupied by *M. vimineum* (e.g., Huebner 2010). Site details are reported in Craig et al. (2015). All sites were located in the French Broad River watershed, and had similar elevations (580–780 m) and hardwood-conifer canopies. Overstory composition at each site was typical of secondary forests in the region and consisted of *Liriodendron tulipifera*, *Pinus strobus*, *Quercus* species, *Juglans nigra*, and *Acer* species. Soils were well-drained loams or sandy loams classified as Typic Hapludults or Typic Dystrudepts. The mean annual temperature and mean annual precipitation (30 year normals) were 13.6 °C and 94 cm, respectively.

In the year prior to sampling, four sets of 1 × 2.5 m^2 paired invaded-uninvaded plots were set up around invasion fronts ($n = 96$ total plots). Plots within a pair were located 1–5 m apart and selected to avoid any obvious environmental gradients. The paired-plot approach assumes that invaded and uninvaded plots differ only in their invasion status. Although we cannot definitively conclude that this assumption held true in our study, we contend that the paired-plot approach was adequate to observe the effects of *M. vimineum* in this study for two reasons. First, in uninvaded areas, we found no evidence of *M. vimineum* thatch, which would have been present had the area previously been invaded. Second, *M. vimineum* is a poor disperser locally, spreading only about one meter per year when unaided by disturbance (Warren et al. 2011). Moreover, evidence from a seed addition experiment suggests that *M. vimineum* can overcome environmental limitation of germination and survival (Warren et al. 2012). Given that many of the populations we studied appeared to expand during the course of our study, our uninvaded plots were likely maintained by seed limitation rather than lack of habitat suitability for *M. vimineum*.

Soil and root sampling

In June and July 2012, we sampled soils with a 2 cm diam. soil probe. In each plot, eight approximately evenly spaced soil cores (30 cm apart, located along a transect bisecting the plot) were collected from the top 10 cm of mineral soils and composited. Samples collected in June were air dried, sieved to 2 mm, and used to determine soil pH (2:1 mL H_2O:g soil) as well as SON and SOC pools (see below). Samples collected in July were rapidly sieved to 4 mm and stored at 5 °C until analysis for inorganic N, microbial biomass, extractable dissolved organic C (DOC), and mineralizable C or −80 °C until analysis for enzyme activities. Since many of these parameters likely vary at short time scales, we chose to measure them on samples collected near peak *M. vimineum* biomass. We chose to sieve to

4 mm rather than 2 mm to facilitate rapid processing of moist soils. In July, additional samples were collected for determination of bulk density and root biomass. Bulk density (0–10 cm depth, 5.08 cm diameter corer) was determined via the core method and averaged from four samples per site. Root biomass was determined by thoroughly picking all roots from samples collected with one 10.16 cm diam. core per plot, washing with deionized water, drying at 55 °C, weighing, and grinding for analysis of C and N content (see below). We also characterized soil moisture and temperature. To acquire an average effect of invasion within each site, we determined soil moisture and temperature in two locations at each plot eight times throughout the year using a 5 cm moisture probe (5TM, Decagon, Pullman, Washington). Gravimetric moisture (105 °C) was also determined to express soil parameters on a dry weight equivalent basis.

Plant cover as a proxy for rates of plant N acquisition

In a previous study, we tracked the fate of isotopically labeled inorganic and organic N species and found higher rates of N acquisition in both the invasive and native understory vegetation of *M. vimineum*-invaded plots (Fraterrigo et al. 2011). Moreover, a substantial fraction of the label was found in the aboveground biomass. For these reasons, we explored using plant cover as a proxy for plant N acquisition rate. We characterized understory plant cover in two 1 m^2 plots within each plot. Two observers working together visually assessed the percentage cover (below 1 m height), rounded to the nearest ten, of seven plant functional groups (non-*M. vimineum* grasses, forbs, woody plants, N-fixers, ferns, mosses, and *M. vimineum*). Cover of all functional types was summed and averaged between the two subplots to calculate total plant cover for each plot. To determine whether plant cover was an adequate proxy for plant N acquisition rate, we first compared *M. vimineum* and total cover to *M. vimineum* aboveground biomass (collected from 625 cm^2 squares adjacent to the study plots; Craig et al. 2015). We then examined root N content and previously reported aboveground biomass N content (Strickland et al. 2010) to infer whether greater *M. vimineum* biomass was associated with enhanced plant N uptake.

Soil inorganic nitrogen

We quantified inorganic N availability using three metrics. We measured the amount of soil inorganic N instantaneously and at a seasonal scale to represent the amount of inorganic N available for uptake by microbes and plants, respectively. Because microbes have short life spans (days) relative to plant roots (months), the instantaneous measure better characterizes the amount of inorganic N available to the microbial community. The seasonal measure better reflects the amount of inorganic N available for plant uptake by accounting for the turnover and release of N from the microbial biomass across the growing season (Kaye and Hart 1997; Kuzyakov and Xu 2013). To measure the amount of instantaneously available inorganic N (hereafter "short-term N availability"), we extracted a ~10 g subsample of soil in the field, immediately after sieving, in pre-weighed cups containing 80 mL of 2 M KCl. To measure the amount of seasonally available inorganic N (hereafter "long-term N availability"), we deployed ion-exchange resin bags for two months (June-Aug) as in Craig et al. (2015). Two resin bags were destroyed during incubation and were not included in data analysis. We also measured net N transformations as an index of microbial N cycling rates and microbial N limitation. We quantified the rates of potential net N mineralization and nitrification in the laboratory by incubating a subsample of soil at 20 °C and 65% water-holding capacity (WHC) for 30 d before extracting in 2 M KCl. All KCl slurries were analyzed for NH_4^+-N and NO_3^--N as in Craig et al. (2015). Potential net N mineralization rates were calculated as the difference in total inorganic N between field- and post-incubation extractions while potential net nitrification rates were calculated as the difference in NO_3^--N.

Soil microbial properties

Microbial biomass C was determined via a simultaneous chloroform fumigation-extraction (CFE) procedure as described in Fierer and Schimel (2003). Dissolved organic C was determined in extracts after shaking soils in 0.5 M K_2SO_4 for 4 h. Active soil microbial biomass was estimated as the pulse of CO_2 following the addition of a high-quality C substrate to soil samples (hereafter substrate-induced respiration; SIR). Substrate-induced respiration was measured by incubating 4 g soil (oven dry equivalent) with 4 mL of autolyzed yeast extract solution (1.2% w/v) at 20 °C for 4 h (Fierer and Schimel 2003). Soil slurries were capped to allow for headspace analysis of CO_2 concentrations at the end of the incubation using infra-red gas analysis (Li-Cor Biosciences, Lincoln, NE). We report uncorrected values for both CFE and SIR.

To assess the activity of microbial decomposers, we quantified the activities of four extracellular enzymes: β-glucosidase (BG), β-1,4-*N*–acetylglucosaminidase (NAG), phenol oxidase (PPO), and peroxidase (PER). Potential activities of these hydrolytic and oxidative enzymes are calculated as the amount of substrate cleaved during a brief incubation and assays are thoroughly described in Finzi et al. (2006).

Soil and root organic C and N

We isolated the particulate organic matter (POM) because it is thought to represent a faster-cycling pool of organic matter that is unprotected from decay by soil microbes. POM fractionations were modified from Marriott and Wander (2006). Briefly, 10 g soil was dispersed by shaking with 5% (w/v) sodium hexametaphosphate. The sand fraction was then collected on a 53 µm-mesh fabric, dried at 55 °C and ground with a mortar and pestle for analysis of C and N by combustion (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA, USA). Total organic C and N of each soil and root sample were also determined by combustion. Finally, we quantified mineralizable C as an index of the labile C pool (sensu Strickland et al. 2010). Briefly, soils were maintained for 60 days at 20 °C and 65% WHC. We quantified respiration rates (over a 24 h period) seven times over the course of the incubation and estimated mineralizable C as the area under the curve derived by plotting CO_2 production over time.

Data analysis

While Eastern U.S. temperate forests are generally considered N-limited systems, the forests studied here spanned a wide gradient of soil N availability, which was previously found important for explaining variation in *M. vimineum* impacts on soil C (Craig et al. 2015). Thus, to ensure that our results reflected the mechanisms underlying *M. vimineum* impacts in N-limited systems, we analyzed the effects of *M. vimineum* on N, C, and microbial dynamics in a subset of plots that we determined to have low amounts of available N. To make this determination, we classified paired plots as having "low N" or "high N" availability (sensu Craig et al. 2015) based on whether long-term soil NO_3^- in the uninvaded plots was lower or higher, respectively, than the median value for all uninvaded plots. Statistical analyses were performed on both the "low-N" plots and the total dataset.

To test the hypothesis that microbes are more active on a per unit biomass basis under *M. vimineum*, we expressed measures of microbial activity (enzymes and SIR) relative to CFE microbial biomass (sensu McFarland et al. 2013). Scaled enzyme activities represent relative microbial investment in enzymes, while SIR:CFE is a relative index of the proportion of the microbial biomass that is active rather than dormant. Previous work consistently shows a decline in microbial biomass following *M. vimineum* invasion (Strickland et al. 2010; Kramer et al. 2012), so scaling by microbial biomass also allowed us to isolate the effects of invasion on microbial activity per se.

For both low-N plots and the complete dataset, we tested the effects of invasion on soil properties by fitting linear mixed models with invasion treatment as a fixed factor and site as a random factor. An invasion by site interaction term was also included as a random factor but was dropped as it was never significant. Because this study was primarily concerned with invasion effects, we do not report site effects. For variables that were measured on multiple sampling dates (soil moisture and temperature), we used a repeated measures linear mixed model with invasion, sampling date, and sampling date × invasion as fixed factors, site as a random factor, and sampling date repeated. For all analyses, we tested assumptions of normality (Kolomogrov–Smirnov test) and homogeneity of variance (Levene's test). When necessary, we transformed data (natural log or square root). All analyses were performed using SAS v. 9.4 (Proc Mixed; SAS Institute, Cary, NC, USA).

To integrate and evaluate support for the various potential mechanisms affecting C and N dynamics in invaded areas, we created structural equation models (SEMs) for low-N plots and for all plots, separately. We hypothesized that invasion would be associated with greater total plant cover which, in turn, would alter mass-specific microbial activity. We expected that changes in this variable would affect POM C and the ratio of long-term NO_3^- availability to POM N (LT NO_3^-:POM N), the latter of which represents the change in plant-available, inorganic N relative to organic N. We expected that this ratio would be greater in invaded soils if invasion intensified N demand of SOM-degrading microbes and accelerated N release from the SOM. We focus on NO_3^- rather than total inorganic N because *M. vimineum* prefers this species of N (Kourtev et al. 1999; Lee et al. 2012). Of the five variables in the SEM, mass-specific microbial activity was latent with our five measures of CFE biomass-scaled activities (enzymes and SIR) as indicators while the remaining variables (invasion status, plant cover, POMC, and LT NO_3^-:POM N) were directly observed.

We fit the SEM in two steps as recommended by Grace (2006). First, we fit the measurement model which related the latent variable, mass-specific microbial activity, to its indicators. Upon achieving an adequate fit, we fit full structural models. We fit the data to the hypothesized model using the maximum likelihood estimation method. The resulting parameter estimates were used to generate a model-implied variance–covariance matrix which was compared to the observed variance–covariance matrix. From this comparison, a χ^2 goodness-of-fit statistic was generated and the associated P value was used to assess model fit. A P value >0.05 indicates no significant difference between the model-implied and observed variance–covariance matrix, implying an adequate fit. Thus, no modifications were made to improve the model fit or variance explained as long as this criterion was met. For adequate SEMs, we calculated standardized path coefficients to

compare the relative importance of various pathways. SEM analyses were performed using AMOS 23 (AMOS Development, Spring House, Pennsylvania, USA).

Results

Plant cover and N dynamics

Invasion was associated with 65% higher plant cover across all plots ($F_{1,83} = 175.1$, $P < 0.001$; Fig. S1) and 77% higher cover in low-N plots ($F_{1,36} = 182.4$, $P < 0.001$; Fig. 1). The majority of the differences in plant cover were accounted for by *M. vimineum*, which comprised 52 ± 3% (mean, SE) of total plant cover across all invaded plots and 49 ± 4% of plant cover in low-N invaded plots (Figs. 1, S1). *M. vimineum* cover was positively related to *M. vimineum* biomass samples collected adjacent to plots and reported in Craig et al. (2015; Fig. S2). *M. vimineum* cover was also positively associated with total plant cover (Fig. S3). The cover of *non-M. vimineum* plant functional groups differed between invaded and uninvaded plots, with invaded plots having a greater percentage of grass cover ($F_{1,83} = 5.0$, $P = 0.03$) and uninvaded plots having a greater percentage of woody plant cover ($F_{1,83} = 15.7$, $P < 0.001$; Fig. S4). These patterns were similar in the low-N plots (Fig. S5).

In addition to the differences in total cover, plant tissues collected from invaded plots were more N-rich. Across all plots, root N concentrations were 12.31 ± 0.50 mg N g root^{-1} (mean, SE) in invaded versus 11.70 ± 0.41 mg N g root^{-1} in uninvaded plots ($F_{1,83} = 1.2$, $P = 0.27$) and root C:N values were 35.18 ± 1.52 in invaded versus 38.79 ± 2.03 in uninvaded plots ($F_{1,83} = 2.0$, $P = 0.15$). In low-N plots, root N concentrations were 12.10 ± 0.77 mg N g root^{-1} (mean, SE) in invaded versus 10.99 ± 0.65 mg N g root^{-1} in uninvaded plots ($F_{1,36} = 2.21$, $P = 0.15$) and root C:N values were 33.68 ± 1.75 in invaded versus 41.29 ± 3.72 in uninvaded plots ($F_{1,36} = 4.26$, $P < 0.05$).

Soil N dynamics

Rates of potential net N mineralization were low and we observed negative rates of potential net N mineralization in c. 45% of soils collected from invaded and uninvaded plots (Tables 1 and S1). Across all plots and within low-N plots, nitrogen mineralization assays were dominated by nitrification, and invaded soils tended to have higher potential net nitrification and net N mineralization (Tables 1 and S1). In situ, short-term inorganic N availability, which was predominately (96%) NH_4^+, was 21% lower in invaded than uninvaded soils in the low-N plots ($F_{1,36} = 4.6$, $P = 0.04$; Fig. 2a), and 14% lower across all plots ($F_{1,83} = 4.2$, $P = 0.04$; Fig. S6a). By contrast, long-term inorganic N availability was higher in invaded than uninvaded soils. The largest differences were observed for long-term NO_3^- availability, which was 107% higher in invaded soils across all plots ($F_{1,81} = 8.7$, $P < 0.01$; Fig. S6b), and 17 times higher in invaded soils in the low-N plots ($F_{1,36} = 14.8$, $P < 0.001$; Fig. 2b). There was no difference in long-term NH_4^+ overall

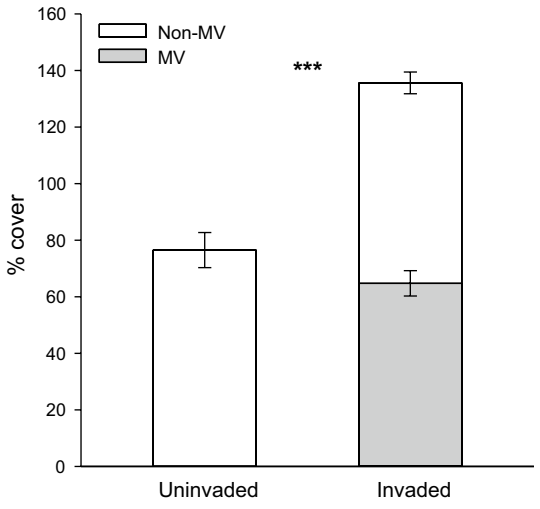

Fig. 1 *Microstegium vimineum* (MV) and other plant cover (non-MV) below 1 m height estimated within a 2 m^2 area in plots where ambient nitrate availability was lower than the median value for the data set. Values are means ± 1 SE ($n = 23$). *Asterisks* indicate significant differences between invaded and uninvaded plots in total plant cover (***$P < 0.001$)

Table 1 Soil properties (mean ± 1 SE) for uninvaded and *Microstegium vimineum*-invaded plots in low-N areas

Variable	Uninvaded	Invaded	F_{df}	P
pH[a]	4.91 ± 0.14	5.24 ± 0.11	$13.4_{1,36}$	**<0.001**
Soil moisture (mL/mL)	0.225 ± 0.006	0.249 ± 0.006	$27.1_{1,302}$	**<0.001**
Soil temperature (°C)	17.47 ± 0.25	17.25 ± 0.22	$0.5_{1,302}$	0.48
Total organic C[a, b]	2461 ± 166	1884 ± 118	$13.3_{1,36}$	**<0.001**
Potential net N mineralization	−2.95 ± 8.50	10.02 ± 7.19	$2.3_{1,36}$	0.14
Potential net nitrification[b]	35.83 ± 8.18	40.20 ± 6.76	$3.2_{1,36}$	0.08
Total organic N	147.2 ± 13.8	122.1 ± 8.7	$7.0_{1,36}$	**0.01**

Significant P values are shown in bold

F values, degrees of freedom, and P values are reported for the main effect of *Microstegium vimineum*. Where significant, P values are shown in bold. Units for N and C pools are g m^{-2} at 10 cm depth. Potential N mineralization and nitrification are shown in μg m^{-2} d^{-1}

[a] Craig et al. (2015)

[b] Statistics reported for natural-logarithm transformed data

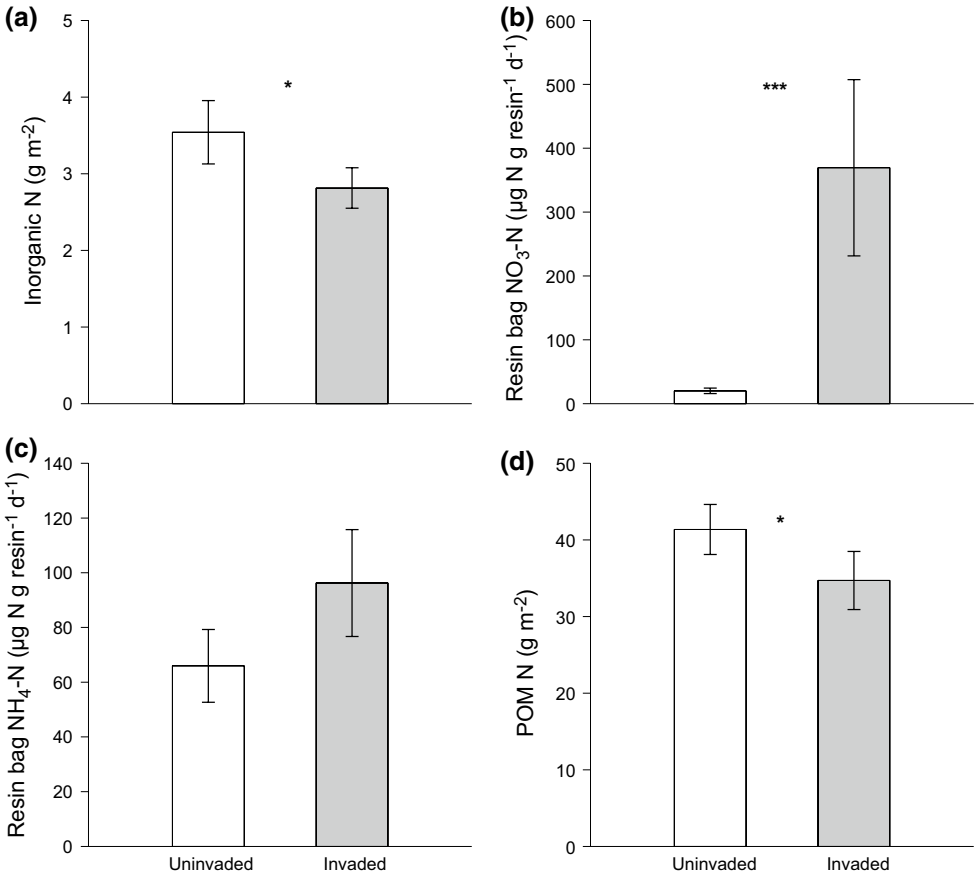

Fig. 2 Short-term nitrogen availability ($NH_4^+ + NO_3^-$) collected from KCl extracts (**a**), long-term NO_3^--N (**b**) and NH_4^+-N (**c**) availability from in situ resin bag incubations, and particulate organic N (**d**) in invaded and uninvaded areas for plots where ambient nitrate availability was lower than the median value for the data set. Resin data for uninvaded plots were previously reported in Craig et al. (2015). Values are means ± 1SE ($n = 23$). *Asterisks* indicate significant differences between invaded and uninvaded plots (*$P < 0.05$, ***$P < 0.001$)

($F_{1,80} = 0.2$, $P = 0.67$; Fig. S6c) or in the low-N plots ($F_{1,35} = 3.1$, $P = 0.09$; Fig. 2c). Organic N content was lower in invaded soils. Compared with uninvaded soils, invaded soils had 24% less POM N ($F_{1,82} = 5.4$, $P = 0.02$; Fig. S6d) and 15% less total organic N (Table S1) across all plots. In low-N plots, POM N was 16% lower ($F_{1,36} = 5.2$, $P = 0.03$; Fig. 2d) and total N was 17% lower (Table 1) in invaded plots.

Microbial activity

Unscaled extracellular enzyme activities and SIR microbial biomass were similar in invaded and uninvaded areas for both the whole dataset and the low-N plots, despite significantly less CFE microbial biomass under *M. vimineum* overall ($F_{1,83} = 14.6$, $P < 0.001$; Fig S7a) and in the low-N plots ($F_{1,36} = 6.4$, $P = 0.02$; Fig. 3a). There was no difference between invaded and uninvaded areas for phenol oxidase ($P > 0.52$), peroxidase ($P > 0.32$), or β-glucosidase ($P > 0.15$) activities or SIR microbial biomass ($P > 0.13$). The exception was NAG, which was non-significantly lower in invaded areas ($P > 0.07$). When enzyme activities and SIR were expressed on a per unit microbial biomass basis, however, striking differences emerged between uninvaded and invaded soils. Specifically, invaded soils showed significantly higher scaled enzyme activities and SIR than uninvaded soils (Fig. S7), particularly in the low-N plots (Fig. 3).

Effects of invasion on soil C and other properties

Invaded soils had significantly less organic C than uninvaded soils. For the low-N plots, we observed 35, 10, 27, and 23% less DOC ($F_{1,36} = 15.4$, $P < 0.001$), mineralizable C ($F_{1,36} = 3.0$, $P = 0.09$), POM C ($F_{1,36} = 11.1$, $P < 0.01$), and total C (Table 1), respectively, in invaded areas (Fig. 4). Similarly, across all plots, we observed 29, 12, 25 and 19% less DOC ($F_{1,83} = 23.6$, $P < 0.001$), mineralizable C ($F_{1,83} = 6.4$, $P = 0.01$), POM C ($F_{1,82} = 11.0$, $P < 0.01$) and total C (Table S1), respectively, when *M. vimineum* was present (Table S1; Fig. S8).

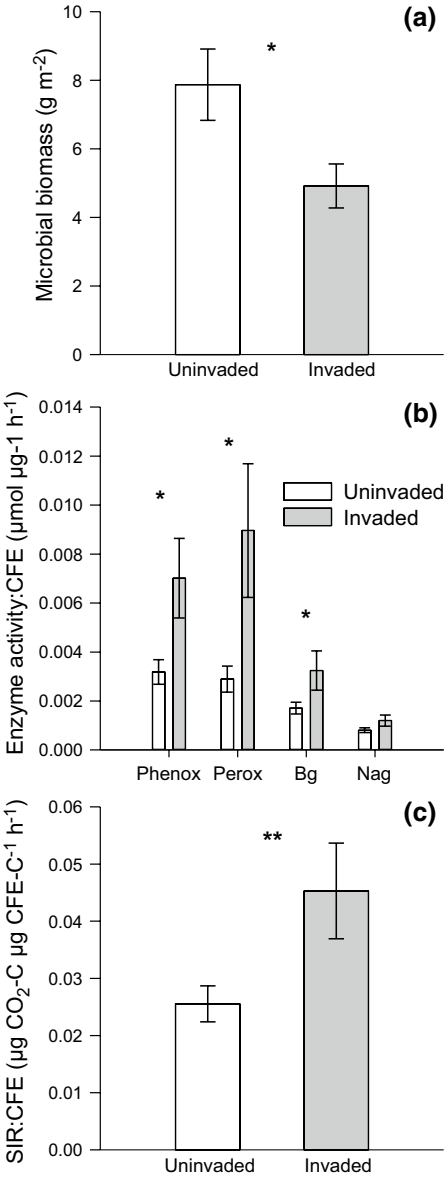

Fig. 3 Microbial biomass C from chloroform fumigation-extraction (**a**), phenol oxidase (phenox), peroxidase (perox), β-glucosidase (bg) and β-1,4-*N*–acetylglucosaminidase (nag) activities expressed on a per unit microbial biomass basis (**b**), and SIR:CFE ratios (**c**) in invaded and uninvaded areas for plots where ambient nitrate availability was lower than the median value for the data set. Microbial biomass data as well as the effect of invasion on mass-specific enzymes previously reported in Craig et al. (2015). Values are means ± 1SE ($n = 23$). *Asterisks* indicate significant differences between invaded and uninvaded plots (*$P < 0.05$, **$P < 0.01$)

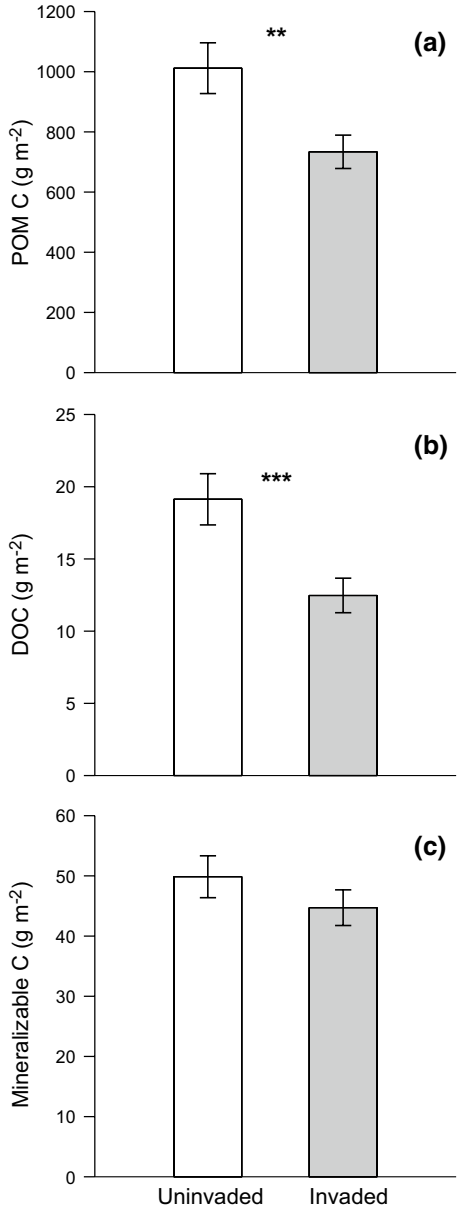

Fig. 4 Particulate organic carbon (**a**) (Craig et al. 2015), dissolved organic carbon (**b**), and mineralizable carbon (**c**) in invaded and uninvaded areas for plots where ambient nitrate availability was lower than the median value for the data set. Values are means ± 1SE ($n = 23$). *Asterisks* indicate significant differences between invaded and uninvaded plots (**$P < 0.01$, ***$P < 0.001$)

Invasion was associated with significant differences in soil pH and soil moisture, but not with soil temperature (Tables 1, S1). On average, soil pH was 0.34 pH units higher in invaded plots ($F_{1,83} = 24.2$, $P < 0.001$). Soil moisture was greater in invaded plots for all sampling dates ($F_{1,643} = 23.8$ $P < 0.001$), though this effect was weaker in the spring.

Structural equation model

Our SEM adequately fits our data for POM C in the low-N plots ($\chi^2 = 24.2$, $P = 0.09$, df = 16; Fig. 5a) and overall ($\chi^2 = 23.2$, $P = 0.11$, df = 16; Fig. S9a) and LT NO_3^-:POM N in the low-N plots ($\chi^2 = 26.2$, $P = 0.05$, df = 16; Fig. 5b) and overall ($\chi^2 = 18.0$, $P = 0.26$, df = 16; Fig. S9b). The SEMs explained 35 and 24% of the variability in each response, respectively, in the low-N plots, and

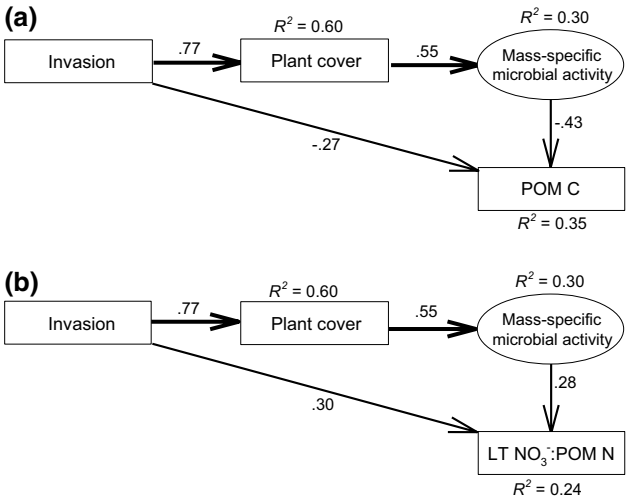

Fig. 5 Results of SEM analysis for particulate organic matter carbon (**a**) and the ratio of long-term NO_3^- to particulate organic matter nitrogen (**b**) in plots where ambient nitrate availability was lower than the median value for the data set ($n = 46$). Thickness of *lines* corresponds to the strength of the relationship. "Mass-specific microbial activity" is a latent variable representing enzyme and substrate-induced respiration on a per unit biomass basis

20% and 13%, respectively, in the overall dataset. Invasion was positively related to total plant cover (low-N: $r = 0.77$, $P < 0.001$; overall: $r = 0.68$, $P < 0.001$) which, in turn, was positively related to mass-specific microbial activities (low-N: $r = 0.55$, $P < 0.001$; overall: $r = 0.46$, $P < 0.001$). Enzyme activities were negatively associated with POM C (low-N: $r = -0.43$, $P = <0.01$; overall: $r = -0.35$, $P < 0.001$) and positively associated with LT NO_3^-: POM N (low-N: $r = 0.28$, $P = 0.05$; overall: $r = 0.24$, $P = 0.07$). In addition, invasion was directly, negatively related to POM C (low-N: $r = -0.27$, $P = 0.04$; overall: $r = -0.19$, $P = 0.04$) and positively related to LT NO_3^-: POM N (low-N: $r = 0.30$, $P = 0.04$; overall: $r = 0.22$, $P = 0.06$).

Discussion

Increasing evidence suggests that some invasive plant species gain competitive advantage in nutrient-limited systems because of their enhanced capacity to acquire soil nutrients (Jo et al. 2015), but the mechanisms by which invasive plants enhance the uptake of limiting nutrients are poorly understood. Here, we found that plots invaded by *M. vimineum* had higher plant cover and lower tissue C:N ratios, and lower amounts of soil inorganic N available in the short term. Mass-specific metabolic activities of the decomposer community were also higher in invaded plots. Our SEM revealed that these patterns were related and partly explained observations of both greater inorganic relative to organic N and less soil C in invaded plots. In addition, the effects of *M. vimineum* on soil N, C, and microbial activities as well as the mechanism reflected in the SEM were stronger in low-N plots supporting that these patterns are related to plant nutrient acquisition in nutrient-limited, and not nutrient-rich, systems. Overall, these findings support the hypothesis that invasive species can enhance N acquisition by outcompeting SOM-degrading microbes for inorganic N, leading to enhanced SOM mineralization. While the confirmation of this mechanism warrants experimental testing, our results nonetheless suggest that invasion effects on both C and N cycling are linked through one mechanism.

The increase in plant cover was one of the most qualitatively obvious effects of *M. vimineum* invasion. In a previous study, we found that high aboveground biomass in *M. vimineum*-invaded areas is associated with enhanced plant N acquisition rates (Fraterrigo et al. 2011). Thus, we used plant cover as a proxy for aboveground biomass and, therefore, plant N acquisition rates. We determined that this was an adequate, though imperfect proxy. For example, while we detected a clear positive relationship between *M. vimineum* biomass and *M. vimineum* cover, this relationship explained only 43% of the variation in *M. vimineum* biomass. Nonetheless, we found that the abundance of *M. vimineum* in invaded plots was the primary difference between invaded and uninvaded plots and the increase in total plant cover in invaded plots was due to greater *M. vimineum* cover. Moreover, the structure of the native understory communities was similar in invaded and uninvaded plots, indicating that the observed increase in cover was due to greater plant biomass in invaded plots rather than differences in community structure. Meta-analyses show that invasion results in higher primary productivity at the site level across a wide range of non-native species (Liao et al. 2008). While this has previously been reported for *M. vimineum* in forests with sparse understories (e.g., Bradford et al. 2009; Fraterrigo et al. 2011), we find that this pattern arises even in our study system which has a dense understory and where N availability presumably limits plant productivity, as is generally the case in Eastern U.S. forests. Our N mineralization data suggest that the soils in which we worked were N-poor. For example, we measured net immobilization in about half of our N mineralization assays and even our highest measured potential net N mineralization rate, 0.785 mg kg $soil^{-1}$ d^{-1} (Site 2, uninvaded plot 2 as classified in Craig et al. 2015), would be considered below average relative to other measured rates in the French Broad River watershed (Fraterrigo et al. 2005, 2006). However, N limitation of plant and, especially, microbial growth are difficult to confirm and additional studies on N-related invader impacts in

N-limited systems should verify N status of invaded and uninvaded soils.

In N-limited soils, both an increase in N-use efficiency and an increase in N uptake could lead to enhanced productivity in invaded sites. Invaders of nutrient-limited soils often have high nutrient-use efficiency (Funk and Vitousek 2007). Indeed, previous work has found N-use efficiency to be elevated in *M. vimineum* relative to native plants (Demeester and Richter 2010; Lee et al. 2012). According to whole plant economics, this nutrient-conservative strategy should be associated with poor nutrient acquisition abilities (Reich 2014). However, when examining whether plant economic tradeoffs hold for invasive species, Heberling and Fridley (2013) found that many invaders have strategies that deviate from these predicted tradeoff relationships such that invasive species have traits associated with both enhanced resource conservation and acquisition. *M. vimineum* is likely one of these outliers.

The observed increase in plant cover, together with the lack of decrease in plant tissue N concentrations and decrease in tissue C:N ratios, suggests that invasion by *M. vimineum* enhances rates of plant N acquisition. Whereas nutrient-conservative species would be expected to have lower N concentrations, we did not observe this effect in root tissues collected from invaded plots. Although we did not characterize the chemical composition of aboveground biomass, previous investigations in southeastern temperate forests found that N concentrations were 26% higher in aboveground *M. vimineum* biomass than in the native biomass (Strickland et al. 2010). Moreover, Fraterrigo et al. (2011) conducted a ^{15}N tracer study to evaluate plant–microbe competition for N in plots invaded by *M. vimineum* and in control plots. They found a higher total amount of plant ^{15}N uptake in invaded plots, with *M. viminuem* favoring inorganic forms and allocating it primarily to shoots. The magnitude of this increased N uptake was primarily attributed to the accumulation of N in aboveground biomass in invaded plots. These observations suggest a shift in N partitioning toward greater aboveground storage. Consistent with this pattern, we observed reduced SON and short-term inorganic N availability under *M. vimineum*-dominated vegetation. Moreover, a study at a nearby site found that dissolved organic N and microbial biomass N were 34 and 42% lower, respectively, while microbial C:N was 16% higher under *M. vimineum*-dominated vegetation (Fraterrigo et al. unpubl. data). Taken together, these findings suggest that *M. vimineum* strongly competes for and sequesters N in its biomass.

Microstegium vimineum is known to possess several root traits that may promote high rates of inorganic N uptake from the soil. Previous research suggests that *M. vimineum* enhances the rhizodeposition of C-rich compounds (Strickland et al. 2010): a strategy that plants use to obtain N from either root-associated mutualists or saprotrophic decomposers (e.g., Hamilton and Frank 2001). Bradford et al. (2012) found that about 15% of photosynthate C is recovered in the soil microbial biomass under *M. vimineum* after only two days. AMF may play a key role in this exchange of C for N. AMF are highly effective competitors for inorganic N (Smith and Read 2008), have been shown to deplete soil nutrients (Pringle et al. 2009), and are known to be promoted by *M. vimineum* (Kourtev et al. 2002; Lee et al. 2012). Future work should examine the N relations between AMF, SOM-degrading microbes, and invasive plants.

The effects of *M. vimineum* on soil inorganic N may explain the common finding that the microbial community is more active on a per unit biomass basis under *M. vimineum*-dominated vegetation. Despite significant reductions in CFE microbial biomass in areas invaded by *M. vimineum*, SIR and enzyme activities have been found to either increase or remain unchanged (Kourtev et al. 2002, 2003; Strickland et al. 2010; Fraterrigo et al. 2011). In agreement with these findings, we observed declines in CFE microbial biomass and detected no significant change in SIR or enzyme activities, except for NAG which was marginally lower under *M. vimineum*-dominated vegetation. However, when scaled to microbial biomass, all enzyme activities were higher under *M. vimineum*-dominated vegetation. Furthermore, the SEM shows a positive relationship between plant cover and microbial activity per unit biomass. Other studies have attributed this increase in microbial activity to priming effects. However, priming effects are typically associated with increased microbial biomass (e.g., Blagodatskaya et al. 2007, Drake et al. 2013) whereas we observed substantially lower CFE biomass under *M. vimineum*-dominated vegetation, suggesting that microbial biomass was constrained by other factors. In response to N limitation, microbial communities may decrease their carbon use efficiency and increase their nitrogen use efficiency (Manzoni et al. 2012; Mooshammer et al. 2014) and enhance enzyme production to acquire N from the SOM (Geisseler et al. 2010), which collectively could reduce microbial biomass C.

We propose that the increase in mass-specific microbial activity brought on by enhanced plant–microbe N competition and potentially fueled by root-derived C inputs leads to an increase in N and C mineralization and, ultimately, a loss of SON and SOC as long-term inorganic N availability is enhanced. Supporting this, we found that mass-specific microbial activity was negatively related to SOC and positively related to LT NO_3^-:POM N. Rapid N turnover from leaf litter could also lead to higher soil nutrient availability and primary productivity without depleting SON (Allison and Vitousek 2004; Heneghan et al. 2006; Arthur et al. 2012). However, while it is often assumed that invasive

plants possess rapidly decomposing litter, a recent survey of decomposition rates of 36 invasive and 42 native leaf litters found that this is not a generalizable pattern (Jo et al. 2016). Indeed, *M. vimineum* thatch is known to release N slowly (DeMeester and Richter 2010), so it is unlikely that *M. vimineum* litter would be a substantial N source within the same growing season. Moreover, *M. vimineum* is not known to substantially enhance N release from native litter (Craig et al. 2015). Thus, our observations support that *M. vimineum* acquires N from the SOM rather than the litter layer.

A comparison of the results of the low-N plots to the results of the whole dataset supports that our proposed mechanism is important to invasive plant N acquisition in N-limited systems. Previous work at these sites suggested that the effects of *M. vimineum* on SOC pools and enzyme activities increased with decreasing ambient inorganic N availability (Craig et al. 2015). Here, we found that the magnitude of invasion effects on soil N and the relationships in our SEM tended to be greater for the subset of our plots that had the lowest ambient inorganic N availability. However, the direction of effects was the same for the low-N plots and complete dataset. This suggests that the mechanism outlined in this study tends to increase in importance with N limitation, but also suggests that this mechanism is general to a broad set of conditions in the study region. Moreover, the findings of this study suggest that the context dependence observed in Craig et al. (2015) was, in part, explained by the N dynamics observed in this study.

Our estimates of inorganic N availability provide further evidence that *M. vimineum*, by intensifying microbial N demand, enhances plant opportunities to acquire N. Soil microbes tend to have much shorter lifespans (3–5 days) than roots (1–3 months; Kuzyakov and Xu 2013). Because of this, microbes are often stronger competitors for N over short time scales, but plants win in the long run because roots have multiple opportunities to capture the same atom of N as the microbial biomass turns over and re-releases N (Frank and Groffman 2009; Kuzyakov and Xu 2013). Thus, our short-term measure of soil inorganic N better reflects inorganic N available to microbes while our long-term measure (i.e., resin bags) better reflects inorganic N available to plants across the growing season. Although short-term inorganic N was lower, potentially suppressing the downregulation of extracellular enzyme production, long-term resin bag incubations collected much more inorganic N under *M. vimineum*-dominated vegetation. This suggests that microbial N demand in *M. vimineum*-invaded soils enhances the flux of N from organic to inorganic forms, which plants ultimately have more opportunities to capture. Although we speculate that *M. vimineum* is a strong competitor for inorganic N released during mineralization of SOM, there is evidence that other plant species acquire more N in *M. vimineum*-invaded areas. Fraterrigo et al. (2011) added isotopically labeled organic and inorganic N to invaded and uninvaded plots and found that native plants acquired more N per unit biomass when associated with *M. vimineum*. If *M. vimineum* enhances the flux of N from organic to inorganic forms, as our observations suggest, perhaps native plants were able to access mineralized N more rapidly in invaded plots.

We found that long-term N accumulated primarily as nitrate suggesting that nitrifiers were also competitive for mineralized N. Previous research suggests that many invasive plants, including *M. vimineum*, actively promote both nitrification and nitrate uptake (Ehrenfeld et al. 2001; Hawkes et al. 2005; McLeod et al. 2016). *M. vimineum* enhances nitrifier abundance (Shannon-Firestone et al. 2015), perhaps by ameliorating soil acidity and enabling nitrifiers to compete more effectively for ammonium (Kourtev et al. 2003). In addition, *M. vimineum* has high concentrations of nitrate reductase (Kourtev et al. 1999) and enhances productivity to a greater degree when treated with nitrate rather than ammonium (Lee et al. 2012). Thus, in addition to enhancing the overall supply of inorganic N, invasive plants may pick winners (i.e., nitrifiers) that convert this N to a form they are best suited to assimilate (Hawkes et al. 2005). Regardless of the N form, the observed pattern that long-term inorganic N is enhanced under *M. vimineum*-dominated vegetation conforms to a recent meta-analysis showing that invasion by a wide range of invaders leads to an increase in the flux of N from SOM to the soil, and from the soil to plant tissues (Castro-Díez et al. 2014). Our results suggest that shifts in plant–microbe competition for inorganic N could underpin these patterns.

While our observations support that enhanced plant N demand is partly responsible for altered N and C dynamics in invaded soils, we note that much variation was left unexplained by the SEMs. Experimental manipulations would enhance our understanding of how *M. vimineum* affects biogeochemical cycling in N-limited soils. We take the increase in plant cover without a concomitant decrease in plant tissue N as evidence for enhanced N uptake and accumulation in invaded plots, and the results of our SEM suggest that this leads to altered soil N and C cycling. However, *M. vimineum* might possess other traits that could affect soil N and C cycling. For example, it has been suggested that enhanced uptake of NO_3^+ relative to NH_4^+ by *M. vimineum* could ameliorate soil acidity, leading to more rapid nitrification or decomposition rates (Ehrenfeld et al. 2001; Kramer et al. 2012). This or other unique traits of *M. vimineum* might explain why we observed a significant direct effect (i.e., not mediated through plant cover) of invasion status on soil N and C parameters in our SEM. Experimental manipulation of *M. vimineum* and native plant cover

could help partition the effects of *M. vimineum* per se versus changes in total cover (which might affect the physical properties of a soil regardless of community composition) on soil N and C cycling. Ultimately, multiple effects (e.g., enhanced N uptake, C exudates, lowered soil acidity, and increased plant cover) may work in concert to alter N and C cycling in invaded soils.

To determine the relative importance of our proposed mechanism, two assumptions should be validated. First, we assumed that the increase in plant cover in invaded plots reflected an increase in plant N acquisition rate and, second, we assumed that SOM-degrading microbes were N- and not C-limited. Although these are reasonable assumptions given isotopic evidence that *M. vimineum* enhances plant N uptake and accumulation in aboveground biomass (Fraterrigo et al. 2011) and root exudation of C which is available to soil microbes (Strickland et al. 2010; Bradford et al. 2012), we recommend that future studies couple N manipulations with biogeochemical measurements to address these assumptions. In particular, ^{15}N-labelled inorganic N could be added as a tracer to confirm that *M. vimineum* enhances plot-level N uptake while subplots could be fertilized with N and C to determine whether plant and microbial growth is more limited by N in invaded and uninvaded plots. These manipulations would allow for a more quantitative evaluation of N uptake and N limitation which could then be related to microbial activities and plot-level N and C dynamics.

In conclusion, our findings support the hypothesis that the high rates of N acquisition by invasive plant species enhance competition with SOM-degrading microbes for inorganic N, leading to a suite of biogeochemical changes. Specifically, we found that high plant cover in invaded plots was associated with increased microbial activity and soil inorganic N, and decreased SON and SOC stocks. Moreover, we found that these effects were stronger in low-N soils suggesting that invasive plants can acquire N from the SOM in N-limited systems. Given the broadly observed trend that invasive plant species enhance the competition for and uptake of nutrients (Liao et al. 2008; Jo et al. 2015; Moreau et al. 2015), we propose that the patterns of decreasing organic N and increasing inorganic N observed in this study may be a general effect of invasion and may partly explain alterations of ecosystem C cycling in nutrient-poor soils. Future studies should continue to address the potentially broad relevance of coupled biogeochemical impacts in invaded ecosystems.

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Author contribution statement MEC and JMF conceived and designed the study. MEC carried out the study and analyzed the data. MEC and JMF wrote the manuscript.

References

Allison SD, Vitousek PM (2004) Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. Oecologia 141:612–619

Arthur MA, Bray SR, Kuchle CR, Mcewan RW (2012) The influence of the invasive shrub, *Lonicera maackii*, on leaf decomposition and microbial community dynamics. Plant Ecol 213:1571–1582

Ashton IW, Hyatt LA, Howe KM, Gurevitch J, Manuel T (2005) Invasive species accelerate decomposition and litter nitrogen loss in a mixed deciduous forest. Ecol Appl 15:1263–1272

Barden LS (1987) Invasion of *Microstegium vimineum* (Poaceae), an exotic, annual, shade-tolerant, C4 grass, into a North Carolina floodplain. Am Midl Nat 118:40–45

Blagodatskaya EV, Blagodatsky SA, Anderson T, Kuzyakov Y (2007) Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Appl Soil Ecol 37:95–105

Bottollier-Curtet M, Planty-Tabacchi A-M, Tabacchi E (2013) Competition between young exotic invasive and native dominant plant species: implications for invasions within riparian areas. J Veg Sci 24:1033–1042

Bradford MA, DeVore JL, Maerz JC, McHugh JV, Smith CL, Strickland MS (2009) Native, insect herbivore communities derive a significant proportion of their carbon from a widespread invader of forest understories. Biol Invas 12:721–724

Bradford MA, Strickland MS, DeVore JL, Maerz JC (2012) Root carbon flow from an invasive plant to belowground foodwebs. Plant Soil 359:233–244

Castro-Díez P, Godoy O, Alonso A, Gallardo A, Saldaña A (2014) What explains variation in the impacts of exotic plant invasions on the nitrogen cycle? A meta-analysis. Ecol Lett 17:1–12

Chapman SK, Langley JA, Hart SC, Koch GW (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. New Phytol 169:27–34

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO2. Science 337:1084–1087

Cheplick GP, Fox J (2011) Density-dependent growth and reproduction of *Microstegium vimineum* in contrasting light environments. J Torrey Bot Soc 138:62–72

Corbett BF, Morrison JA (2012) The allelopathic potentials of the non-native invasive plant *Microstegium vimineum* and the native *Ageratina altissima*: two dominant species of the Eastern Forest herb layer. Northeast Nat 19:297–312

Craig ME, Pearson SM, Fraterrigo JM (2015) Grass invasion effects on forest soil carbon depend on landscape-level land use patterns. Ecology 96:2265–2279

Craine JM (2011) Resource strategies of wild plants. Princeton University Press, Princeton

Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88:2105–2113

DeForest JL, Zak DR, Pregitzer KS, Burton AJ (2004) Atmospheric nitrate deposition and the microbial degradation of cellobiose

and vanillin in a northern hardwood forest. Soil Biol Biochem 36:965–971
Demeester JE, Richter DD (2010) Differences in wetland nitrogen cycling between the invasive grass *Microstegium vimineum* and a diverse plant community. Ecol Appl 20:609–619
Drake JE, Darby BA, Giasson M, Kramer MA, Phillips RP, Finzi AC (2013) Stoichiometry constrains microbial response to root exudation- insights from a model and a field experiment in a temperate forest. Biogeosciences 10:821–838
Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of exoctic understory indeciduous forests. Ecol Appl 11:1287–1300
Fierer N, Schimel JP (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sci Soc Am J 67:798–805
Finzi AC, Sinsabaugh RL, Long TM, Osgood MP (2006) Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. Ecosystems 9:215–226
Fontaine S, Bardoux G, Abbadie L, Mariotti A (2004) Carbon input to soil may decrease soil carbon content. Ecol Lett 7:314–320
Frank DA, Groffman PM (2009) Plant rhizospheric N processes: what we don't know and why we should care. Ecology 90:1512–1519
Fraterrigo JM, Turner MG, Pearson SM, Dixon P (2005) Effects of past land use on spatial heterogeneity of soil nutrients in Southern Appalachian forests. Ecol Monogr 75:215–230
Fraterrigo JM, Balser TC, Turner MG (2006) Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology 87:570–579
Fraterrigo JM, Strickland MS, Keiser AD, Bradford MA (2011) Nitrogen uptake and preference in a forest understory following invasion by an exotic grass. Oecologia 167:781–791
Fridley JD (2012) Extended leaf phenology and the autumn niche in deciduous forest invasions. Nature 485:359–362
Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. Nature 446:1079–1081
Funk JL, Standish RJ, Stock WD, Valladares F (2016) Plant functional traits of dominant native and invasive species in Mediterranean-climate ecosystems. Ecology 97:75–83
Gallardo A, Schlesinger WH (1992) Carbon and nitrogen limitations of soil microbial biomass in desert ecosystems. Biogeochemistry 18:1–17
Geisseler D, Horwath WR, Joergensen RG, Ludwig B (2010) Pathways of nitrogen utilization by soil microorganisms: a review. Soil Biol Biochem 42:2058–2067
Grace JB (2006) Structural equation modelling and natural systems. Cambridge University Press, New York
Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402
Hamman OB, Rubia TDL, Martinez J (1997) Effect of carbon and nitrogen limitation on lignin peroxidase and manganese peroxidase production by *Phanerochaete flavido-alba*. J Appl Microbiol 83:751–757
Hart SC, Stark JM (1997) Nitrogen limitation of the microbial biomass in an old-growth forest soil. Ecoscience 4:91–98
Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecol Lett 8:976–985
Heberling JM, Fridley JD (2013) Resource-use strategies of native and invasive plants in Eastern North American forests. New Phytol 200:523–533
Heneghan L, Fatemi F, Umek L, Grady K, Fagen K, Workman M (2006) The invasive shrub European buckthorn (*Rhamnus cathartica*, L.) alters soil properties in Midwestern U.S. woodlands. Appl Soil Ecol 32:142–148
Horton JL, Neufeld HS (1998) Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C4 grass, to variable light environments. Oecologia 114:11–19
Huebner CD (2010) Establishment of an invasive grass in closed-canopy deciduous forests across local and regional environmental gradients. Biol Invas 12:2069–2080
James JJ (2008) Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. J Arid Environ 72:1775–1784
Jo I, DA FridleyJD Frank (2016) More of the same? In situ leaf and root decomposition rates do not vary between 80 native and nonnative deciduous forest species. New Phytol 209:115–122
Jo I, Fridley JD, Frank DA (2015) Linking above- and belowground resource use strategies for native and invasive species of temperate deciduous forests. Biol Invas 17:1545–1554
Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. Trends Ecol Evol 12:139–143
Knops JMH, Bradley KL, Wedin DA (2002) Mechanisms of plant species impacts on ecosystem nitrogen cycling. Ecol Lett 5:454–466
Kourtev PS, Huang WZ, Ehrenfeld JG (1999) Differences in earthworm densities and nitrogen dynamics in soils under exotic and native plant species. Biol Invas 1:237–245
Kourtev PS, Ehrenfeld JG, Häggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166
Kourtev PS, Ehrenfeld JF, Ha M (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biol Biochem 35:895–905
Kramer TD, Warren RJ, Tang Y, Bradford MA (2012) Grass invasions across a regional gradient are associated with declines in belowground carbon pools. Ecosystems 15:1271–1282
Kuzyakov Y, Xu X (2013) Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytol 198:656–669
Laungani R, Knops JMH (2009) Species-driven changes in nitrogen cycling can provide a mechanism for plant invasions. P Natl Acad Sci USA 106:12400–12405
Lee MR, Flory SL, Phillips RP (2012) Positive feedbacks to growth of an invasive grass through alteration of nitrogen cycling. Oecologia 170:457–465
Lee MR, Tu C, Chen X, Hu S (2014) Arbuscular mycorrhizal fungi enhance P uptake and alter plant morphology in the invasive plant *Microstegium vimineum*. Biol Invas 16:1083–1093
Liao C, Peng R, Luo Y, Zhou X, Wu X, Fang C, Chen J, Li B (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytol 177:706–714
Manzoni S, Taylor P, Richter A, Porporato A, Agren GI (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol 196:79–91
Marriott EE, Wander MM (2006) Total and labile soil organic matter in organic and conventional farming systems. Soil Sci Soc Am J 70:950–959
McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari H, Hobbie EA, Iversen CM, Jackson RB, Leppalammi-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M (2015) Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol 207:505–518
McFarland JW, Waldrop MP, Haw M (2013) Extreme CO2 disturbance and the resilience of soil microbial communities. Soil Biol Biochem 65:274–286
McLeod ML, Cleveland CC, Lekberg Y, Maron JL, Philippot L, Bru D, Callaway RM (2016) Exotic invasive plants increase

productivity, abundance of ammonia-oxidizing bacteria and nitrogen availability in intermountain grasslands. J Ecol 104:994–1002

Mooshammer M, Wanek W, Ha I, Fuchslueger L, Hofhansl F, Takriti M, Watzka M, Wild B, Keiblinger KM, Knoltsch A, Zechmeister-boltenstern S, Richter A (2014) Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nat Commun 5:4694

Moreau D, Pivato B, Bru D, Busset H, Deau F, Faivre C, Matejicek A, Stribik F, Philippot L, Mougel C (2015) Plant traits related to nitrogen uptake influence plant-microbe competition. Ecology 96:2300–2310

Piscitelli A, Giardina P, Lettera V, Pezzella C, Sannia G, Faraco V (2011) Induction and transcriptional regulation of laccases in fungi. Curr Genom 12:104–112

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal Symbioses and Plant Invasions. Annu Rev Ecol Evol S 40:699–715

Reich PB (2014) The world-wide "fast–slow" plant economics spectrum: a traits manifesto. J Ecol 102:275–301

Roy J (1990) In search of the characteristics of plant invaders. In: Di Castri F, Hansen AJ, Debussche M (eds) Biological invasions in Europe and the Mediterranean basin. Kluwer Academic Publishers, Dordrecht, pp 333–352

Scott NA, Surinder S, McIntosh PD (2001) Biogeochemical impact of *Hieracium* invasion in New Zealand's grazed tussock grasslands: sustainability implications. Ecol Appl 11:1311–1322

Shannon-Firestone S, Reynolds HL, Phillips RP, Flory SL, Yannarell A (2015) The role of ammonium oxidizing communities in mediating effects of an invasive plant on soil nitrification. Soil Biol Biochem 90:266–274

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier, New York

Strickland MS, Devore JL, Maerz JC, Bradford MA (2010) Grass invasion of a hardwood forest is associated with declines in belowground carbon pools. Glob Change Biol 16:1338–1350

Tamura M, Tharayil N (2014) Plant litter chemistry and microbial priming regulate the accrual, composition and stability of soil carbon in invaded ecosystems. New Phytol 203:110–124

Van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecol Lett 13:235–245

Vila M, Weiner J (2004) Are invasive plant species better competitors than native plant species?: evidence from pair-wise experiments. Oikos 105:229–238

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14:702–708

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115

Warren RJ, Wright JP, Bradford MA (2011) The putative niche requirements and landscape dynamics of *Microstegium vimineum*: an invasive Asian grass. Biol Invasions 13:471–483

Warren RJ, Bahn V, Bradford MA (2012) The interaction between propagule pressure, habitat suitability and density-dependent reproduction in species invasion. Oikos 121:874–881