

Grass Invasions Across a Regional Gradient are Associated with Declines in Belowground Carbon Pools

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ABSTRACT

The composition of plant communities everywhere now likely comprises alien as well as native species, and those aliens that become invasive have wide-ranging impacts on the structure and function of recipient ecosystems. These impacts include perturbations to soil carbon (C) cycling, but the direction and magnitude of impacts are species and climate dependent, making it difficult to generalize whether a specific invader will promote losses or gains in soil C stocks. Generalizations of a specific invader's impacts are necessary; however, because the range of an invader can encompass thousands of square kilometers, meaning their effects can have broad, regional consequences. To quantify broad-scale and context-dependent impacts of a specific invader, multi-site investigations that capture and measure local and regional environmental heterogeneity are necessary. Using this approach, we show that a widespread grass invader of forest understories is associated with declines in soil C during infilling (spreading within the invaded range). Across the 36 study sites, total soil C stocks

declined ($P = 0.113$) by approximately 12% (estimated mean \pm SD, uninvaded: $2,429 \pm 512.9$ vs. invaded: $2,140 \pm 520.7$ g C m⁻²). The decline in total soil C is driven by a significant ($P = 0.047$) reduction in the native-derived, mineral-associated soil C fraction. This fraction, whose mass and slow turnover makes it an important C store, is approximately 15% lower in invaded (estimated mean \pm SD: $1,560 \pm 400.4$ g C m⁻²) than uninvaded plots ($1,826 \pm 398.1$ g C m⁻²). Notably, declines in this C fraction are only apparent at 21 of the sites, reflecting how environmental heterogeneity in other variables (specifically pH, soil moisture, and clay content) are important to quantify to determine invader impacts across a region. The 26% decline in microbial biomass with invasion ($P = 0.011$; estimated mean \pm SD, uninvaded: 10.05 ± 1.79 vs. invaded: 7.40 ± 1.80 g C m⁻²) is also dependent on site characteristics (pH), and reductions are greater where the invader occurs at higher densities. Reductions in microbial biomass and soil C with invasion suggest that grass invasion will alter soil C cycling and decrease forest-C stores across the study region, although invader effects at a specific-site will be dependent on environmental context.

Key words: soil organic matter; soil carbon storage; carbon cycling; microbial biomass; exotic species; alien species; *Microstegium vimineum*; linear mixed models; environmental heterogeneity; context dependency.

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INTRODUCTION

Exotic plant invasions cause changes in the aboveground structure and function of ecosystems (Vilà and others 2011). Belowground impacts may be equally extensive (Ehrenfeld 2003; Liao and others 2008; Vilà and others 2011), but they are not readily observed because the habitat of roots, decomposers, and other belowground organisms are opaque. A general mechanism of invasive plant impact on belowground organisms and processes is likely mediated through changes in the quality and quantity of detrital and root inputs. Relative to natives, invasive species often have enhanced nutrient acquisition (Blumenthal 2006), root exudation (Bradford and others 2012), and greater biomass (Ehrenfeld 2003; Liao and others 2008), leading to increases in the quality and quantity of organic inputs, which in turn stimulate the activities of belowground microbes and fauna (Kuznyakov 2010). These belowground organisms are the agents of soil organic carbon (SOC) formation and degradation, and changes in the stocks of SOC following invasion might induce pronounced, long-term changes in ecosystem structure and function. Soil carbon (C) is considered a fundamental ecosystem property, not only because it is a massive C store, but because it is also critical to site fertility given the role that SOC plays in structuring soils for aeration and drainage, the retention and provision of nutrients and water, and in providing food and habitat for belowground biota (Schmidt and others 2011). As a result, quantifying the impacts of plant invasion on SOC stocks might identify ecosystems where invasion will decrease C storage, as well as alter productivity and system susceptibility to disturbances such as drought.

Meta-analyses of plant invasion effects show mean increases in SOC pools and soil microbial biomass (Liao and others 2008; Vilà and others 2011). This relationship might suggest that invasive plants generally enhance resource availability to belowground food webs. However, these analyses also reveal pronounced variability across studies, with almost as many invasions showing declines as increases in SOC and microbes (Vilà and others 2011). In addition, the overall positive effects of invasion on SOC and microbes can shift to neutral or negative when the analyses consider individual plant functional groups (for example, woody vs. herbaceous, N-fixing vs. non-fixing) and ecosystem types (for example, forests vs. grasslands) (Liao and others 2008). This variability makes it difficult to generalize how a specific invasive plant in a specific

ecosystem type will affect SOC stocks. Variability is not a trivial matter: the range of invasive species can encompass thousands of square kilometers so that the ecosystem impacts of any one invader might have broad, regional consequences. Yet most studies of invasive plant impacts are conducted at one to a few sites (for example, Strickland and others 2010), enabling detailed investigations of mechanisms of impact but not necessarily extrapolation of the impacts themselves across the range of habitats that the species invades. A complementary approach, then, to meta-analyses and single-site studies, are multi-site investigations. If the multi-site investigations capture local and regional environmental heterogeneity, they produce data that can be used to quantify regional impacts and identify context dependencies that result from environmental variation (for example, Jackson and others 2002). Such studies have elucidated the performance of invasive plant species across a region (for example, MacDougall and others 2006; Warren and others 2011a), but similar approaches toward understanding ecosystem impacts of invasion appear rare.

We conducted a regional, multi-site study to understand the impacts of *Microstegium vimineum* (Trin.) A. Camus invasion on SOC stocks and microbial biomass. Ecosystem responses of plant invasion appear to manifest only after major impacts on native species and communities (Vilà and others 2011), and are likely only of regional consequence in latter stages during range infilling when the spatial coverage of an invasive most increases (for example, Muirhead and others 2006). We worked in the southeastern US where *M. vimineum* is actively infilling, at locations within 100 km of one of the earliest US discoveries of *M. vimineum* in the early 1900s (Fairbrothers and Gray 1972). We therefore reasoned that the effects of *M. vimineum* should be pronounced if it does generally accelerate soil C cycling and reduce SOC stocks, as observed at bottomland hardwood sites within our region (Strickland and others 2010, 2011).

Microstegium vimineum is a C₄-grass native to southeastern Asia. It is a shade-tolerant annual, and commonly invades forest understory, where it displaces native vegetation and alters animal assemblages (Cole and Weltzin 2004; Flory and Clay 2009a, b; Oswalt and others 2007). As with plant invasions in general (Liao and others 2008; Vilà and others 2011), it has been shown to have impacts on belowground processes and microbial communities but the few, largely single-site

studies, make generalizations about impacts uncertain (Ehrenfeld and others 2001; Kourtev and others 1998, 2002; Strickland and others 2010, 2011). Generalizations are further hindered because the majority of studies of plant invader impacts on SOC focus on woody species invasion of grasslands; in the meta-analysis by Liao and others (2008), only one field study assessed effects of grass invasion on forest SOC. Although *M. vimineum* is unusual as a shade-tolerant, annual C₄-grass that invades forest understories, its expanding geographic range makes understanding its impacts a priority for effective conservation management.

Although our work was conducted in the vicinity of the historic invasive range of *M. vimineum*, in an area where it is actively infilling, its impacts on SOC stocks may still be hard to detect because there is marked spatial variability in SOC pool sizes at the microhabitat scale and, when considered as a single pool, SOC has a turnover time of decades to centuries (Davidson and Janssens 2006). This means that SOC responses will likely be detectable only where invaders have been established for multiple years. We increased our ability to resolve impacts on soil C cycling by coupling SOC fractionation and stable isotope techniques to quantify faster- and slower-turnover SOC pools and native- versus invader-derived SOC (for example, Bradford and others 2008; Schlesinger and Lichter 2001). In addition, we also quantified effects on the soil microbial biomass, which is the primary biotic agent of SOC degradation and formation (Schmidt and others 2011). Further, across the range of habitat types where *M. vimineum* invades, which include roadsides, riparian zones, and forest understory (Warren and others 2011b), we measured a suite of variables (for example, soil temperature, pH, percent clay) that influence a site's capacity for SOC accumulation to understand context dependencies in impacts. For example, higher temperatures accelerate SOC decomposition, lower pH can slow decomposition, and percent clay determines the carrying capacity for SOC (Baath and others 1980; Conant and others 2011). Our objectives were to determine: (1) whether declines in SOC associated with *M. vimineum* invasion (for example, Strickland and others 2010) are observed at a broader, regional scale and across multiple habitat types; (2) if variation in environmental conditions, such as soil moisture and texture, modify the effects of invasion on SOC; and (3) whether *M. vimineum* invasion and abundance correlate with changes in the soil microbial biomass.

MATERIALS AND METHODS

Study Locations

Our three locations spanned a 100-km climate and ecological gradient from the northern piedmont of Georgia to the southern Appalachian Mountains of North Carolina, USA: (1) Whitehall Experimental Forest (WHF), Athens-Clarke County, GA (33°53'N, 83°21'W; 150–240 m elevation, 122 cm MAP, 17°C MAT); (2) Chattahoochee National Forest (CNF), Habersham County, GA (34°30'N, 83°29'W, 315–450 m elevation, 153 cm MAP, 14°C MAT); and (3) Coweeta Hydrologic Laboratory (CWT), Macon County, NC, USA (35°03'N, 83°25'W; 750–1,025 m elevation, 183 cm MAP, 13°C MAT). Four plots considered part of the CWT location were located nearby in the Little Tennessee Valley (35°04'03"N; 83°23'00"W, 612–622 m elevation) on land managed by the Land Trust for the Little Tennessee. Across these locations, we have observed an advance in local invasion edges (the edges of our *M. vimineum*-invasions advanced ~3 m from summer 2009–2011; unpub. data) and, at the northern location (CWT) of our regional gradient, infilling appears to be the most recent (occurring since 2006). However, given that our study is observational, and that forest understories are traditionally considered resistant to invasion (Martin and others 2009), making monitoring efforts for forest invasives rare, we lack detailed information on time since invasion and subsequent spread of *M. vimineum* across our region.

Site Transects and Field Sampling

The experimental introduction of an invasive species at the regional scale of our research is unfeasible both ethically and logistically (for example, permission from land managers). As such, we substitute space for time to investigate the effects on SOC and microbial biomass of *M. vimineum* invasion. To minimize potential confounding effects of environmental differences across paired invaded and uninvaded plots, we worked locally (1–3 m) either side of discrete invasion edges within what appeared to be continuous habitat (for example, intact canopy). To generate marked variation in the environmental parameters (for example, soil moisture, pH, clay content), we deliberately selected sites in different habitats (for example, wetland edge, bottomland, upland) and, obviously, across our three different locations. Metadata on site and plot characteristics are given in Appendix 1.

Paired invaded and uninvaded plots were established at WHF, CNF, and CWT in May 2009 (12 pairs at each location, $n = 36$ sites and 72 plots total). All sites containing invaded and uninvaded plots were located in temperate hardwood forest edge or understory, with forest ages of at least 60–80 years. Soils were all in the order Ultisol. The plots intersected the edge of *M. vimineum* patches in a straight 6-m line so that three 0.25-m² quadrats fell in adjacent invaded (1, 2, and 3 m from invasion edge) and uninvaded (−1, −2, and −3 m from invasion edge) patches. The plot locations were selected for discrete invasion boundaries—which are what we typically encountered—and we ensured the boundaries occurred within apparently similar habitats (for example, they were not established across roads, habitat boundaries, or land-use shifts). Sampling occurred during the height of the *M. vimineum* growing period (July–August 2009). We systematically sampled plant and soil materials from each site starting with WHF and finishing with CWT. This ordering ensured that *M. vimineum* plants were at a similar stage of growth because it germinates first at WHF and last at CWT as these locations represent the warmer and cooler ends of our gradient.

Microstegium vimineum biomass was harvested from each quadrat by cutting the stems at the soil surface and placing the material in paper bags before drying. The detrital layer (mostly tree leaf litter) was then removed and bagged. We next sampled an 8-cm diameter, 10-cm deep soil core from each quadrat, which encompassed the Oa layer (<1-cm thick across our sites), as well as the surface mineral (A) horizon. Soils were passed through a 4 mm sieve and thoroughly mixed before being placed on ice. A second set of soil cores from the invaded and non-invaded plots were collected but not sieved or placed on ice (for bulk density measures; see “Lab analyses” section). Plant and soil materials from the three quadrats each were composited separately by invaded and uninvaded plots. The samples were returned to the lab at CWT within 12 h of collection. Plant and detrital materials were dried at 65°C to constant mass. Sieved soils were divided and half air-dried, and half stored at +5°C for microbial and other edaphic analyses. The composited but non-sieved soil cores were dried at 105°C.

Volumetric soil moisture (12 cm TDR probes) and temperature (at 8 cm depth) were measured adjacent to each quadrat ($n = 3$) and averaged by plot once in May, July, and September 2009. We averaged these values to provide a mean estimate of soil moisture (%) for the invaded and uninvaded

plots. Given the broad regional and local gradients in temperature and moisture, these analyses were not intended to provide average growing season values but rather to permit a continuous categorization of wetter to drier sites; mirroring the approach used in many population and community studies established across regional and local gradients (for example, Warren and Bradford 2011).

Lab Analyses

Soils stored at +5°C were used to determine gravimetric moisture, pH, and microbial biomass. Gravimetric moisture (g) was determined by drying at 105°C to constant mass and used in C mass balance equations, but not for statistical analysis of SOC responses; instead, we used field volumetric moisture. Soil pH was determined in water (1:1 volumetric ratio of water-to-soil) using a bench-top pH meter. Active microbial biomass was determined using a modified SIR (substrate-induced respiration) technique (Fierer and others 2003). Using this protocol, microbial biomass is measured as $g\ C-CO_2\ g\ soil^{-1}\ h^{-1}$ and was converted to $g\ microbial-C\ g\ soil^{-1}$ following Anderson and Domsch (1978). Non-sieved soil cores were dried at 105°C and used to determine bulk density values for calculating SOC content per unit space (that is, $g\ C\ m^{-2}$ to 10 cm depth); and were corrected for root and stone volume and mass retained on a 2 mm sieve.

The air-dried, sieved soils were used to determine soil texture and SOC values. For texture, silt, sand, and clay contents were estimated using a simplified version of the hydrometer method (Gee and Or 2002). We used the fractionation method described in Bradford and others (2008) to quantify SOC pools. In brief, in duplicate, 10 g of air-dry soil was dispersed with 30 ml of sodium hexametaphosphate via shaking for 18 h. The dispersed sample was rinsed through a 53- μm sieve. Organic matter retained in the sieve with the sand fraction was classified as particulate organic matter (POM) C. Organic matter that passed through the sieve (<53 μm) was classified as silt- and clay-associated organic matter C (mineral-associated C, MIN). The MIN fraction contains primarily microbial-derived C compounds and has a multi-decadal turnover time, and the POM fraction contains primarily plant-derived compounds and has a turnover time of a few years (Grandy and Neff 2008; Schlesinger and Lichter 2001). Both fractions were dried at 105°C to constant mass, weighed and ball-milled to a fine powder in preparation for percent C and $\delta^{13}C$ determinations (Sollins and others 1999). Dried

M. vimineum tissue also was weighed and ball-milled for C isotope determinations. Milled soil and plant samples were analyzed using a Costech ESC 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA) coupled to a Thermo Delta^{Plus} Advantage (San Jose, CA, USA) continuous-flow isotope-ratio mass spectrometer. Analytical precision was $\pm 0.1 \delta^{13}\text{C}\text{‰}$. Working standards were calibrated to V-PDB using PEF and glutamic acids as references.

Microstegium vimineum has a C_4 -photosynthetic pathway and the difference in the C isotope composition from co-occurring native, forest species, which all use the C_3 -pathway in our study region, is sufficient (that is, $> 5\text{‰}$, Staddon 2004) to discriminate whether SOC in the POM and mineral-associated C fractions was derived from native or *M. vimineum* C inputs. Specifically, the amount of C derived from *M. vimineum* was calculated (sensu Ineson and others 1996) as: $C_{M. \text{ vimineum-derived}} = C_{\text{fraction}} \times (\delta^{13}\text{C}_{\text{invaded}} - \delta^{13}\text{C}_{\text{uninvaded}}) / (\delta^{13}\text{C}_{M. \text{ vimineum}} - \delta^{13}\text{C}_{\text{uninvaded}})$, where C_{fraction} is the measured size of the SOC fraction (POM or mineral-associated), $\delta^{13}\text{C}_{\text{invaded}}$ is the $\delta^{13}\text{C}$ value of the fraction where *M. vimineum* is present at a site, $\delta^{13}\text{C}_{\text{uninvaded}}$ is the $\delta^{13}\text{C}$ value of the fraction where *M. vimineum* is absent at a site, and $\delta^{13}\text{C}_{M. \text{ vimineum}}$ is the value for the *M. vimineum* biomass. Where *M. vimineum* was absent, the mean (\pm SE) C isotope value for the POM C was $-27.68 \pm 0.34\text{‰}$ ($n = 36$) and for mineral-associated C it was $-26.75 \pm 0.50\text{‰}$ ($n = 36$). The mean *M. vimineum* value was $-15.32 \pm 1.37\text{‰}$ ($n = 36$). Site-specific isotope values were used when calculating SOC fraction sizes.

Statistical Analysis

Presuming that the clustering of sites at three locations coincides with spatial autocorrelation within the regional gradient, we fitted linear mixed models (LMMs) with location (CWT, CNF, WHF) as a random factor (Bolker and others 2009). We used LMMs assuming a Gaussian error distribution (“identity” link function) to investigate the effects of *M. vimineum* invasion on SOC pools (total, native- and *M. vimineum*-derived POM and MIN) and microbial biomass in the context of multiple fixed effects (for example, percent clay). We entered plot-level *M. vimineum* invasion as a cofactor (that is, invaded vs. uninvaded), and soil moisture, temperature, pH (H^+ concentrations), percent clay and native herbaceous biomass as covariates. We examined soil moisture, temperature and pH because SOC stocks are influenced by

environmental conditions, and percent clay because it determines the carrying capacity for SOC (Conant and others 2011). We also included native herbaceous biomass as an indicator of soil fertility and hence C inputs belowground. *M. vimineum* is influenced by, and may influence, the environmental conditions across plots (Warren and others 2011b), but variation inflation factors below 2.5 indicated that collinearity was low between the model covariables. Interaction terms between the *M. vimineum* invasion factor and the covariates were included in the models, but were removed if not significant (Engqvist 2005). The LMMs were fit using the Laplace approximation in the “lme4” package for the “R” statistical program (R Development Core Team 2009). We used lowest AIC to determine which effects to retain in the models. Because the *F*-statistic is not considered accurate in mixed models, making *P* values anticonservative, we used Markov Chain Monte Carlo (MCMC) sampling (10,000 iterations) in the “languageR” package (Baayen 2007) for the R statistical programming environment (R Development Core Team 2009) to estimate the coefficients and *P* values for retained parameters in the LMMs. From the retained parameters in the LMMs, we used the MCMC intercept and coefficients to construct a multiple-regression equation that we could use to estimate empirically the mass of the various SOC pools across the landscape for the uninvaded and invaded plots. To estimate the error around the SOC mean values, we sampled each coefficient 10,000 times based on the mean and standard deviation for the retained parameter (for example, soil moisture) across our 72 observational plots. This approach accounted for the environmental variation across the landscape and between invaded and uninvaded plots, giving us an empirical estimate of the mean and variance of the different soil C fractions when all other parameters (except invasion) were held equal.

In our best-fit LMM models, we evaluated the slope value of the fixed effects and considered coefficients with $P < 0.05$ significant and coefficients with $P < 0.10$ marginally significant (Hurlbert and Lomabardi 2009). The a priori selection of $\alpha < 0.10$ reflects an effort to better balance model specificity (α) with the reduced detection sensitivity (β) in field studies, particularly the power to detect changes in SOC pools (for example, Carney and others 2007; Strickland and others 2010; Throop and Archer 2008).

There is still considerable uncertainty as to how abundance of invasive plants is linked to impact (Vilà and others 2011) and whether multi-site

studies have the ability to shed light on the relationship. Because we found a significant association between *M. vimineum* invasion and microbial biomass using the LMM approach described for the SOC fractions, we also evaluated the impact of *M. vimineum* biomass within invaded plots on microbial biomass using linear regression.

RESULTS

The best-fit model for the total mass of SOC included all measured variables, but only soil moisture and percent clay explained significant variation across plots (Table 1a). These two variables were positively related to the total mass of SOC (Table 1a) and, although effects of *M. vimineum* invasion (presence or absence) were not significant ($P(\text{MCMC}) = 0.113$), estimated total SOC was 11.9% lower in invaded than uninvaded plots (Table 1a). The lower total SOC with invasion appeared to result from a negative effect of invasion on the slower-cycling, mineral-associated

SOC (that is, MIN), rather than on the faster-cycling POM fraction. Specifically, the best-fit models for both of these fractions retained all measured variables but for the mineral-associated C there was a marginally significant effect of invasion ($P(\text{MCMC}) = 0.087$), whereas the invasion effect on the POM fraction was strongly non-significant ($P(\text{MCMC}) = 0.458$) (Table 1b, c). The estimated POM mass was still 8% lower across invaded plots, but the change in the mineral-associated C fraction with invasion was not only greater (13.2% decline, Table 1b), but this fraction also contains a much greater proportion of the soil C (~75%, Figure 1) and so its response is likely to drive the change in total SOC with invasion.

Given the unique $\delta^{13}\text{C}$ value of the *M. vimineum* biomass, we could partition the SOC into native- and invasive-derived C, to reveal the dynamics underlying the changes in total SOC. Across the *M. vimineum*-invaded plots, we found that on average 96% of the SOC (mean \pm SD; $2,240 \pm 856$ g C m⁻²) was derived from native plants and

Table 1. Coefficients Explaining Spatial Variation in Mass of Total SOC and Its Fractions, and the Estimated Size of These Stocks in Uninvaded and *M. vimineum*-Invaded Plots

	Coeff. (MCMC)	P value (MCMC)
(a) Total SOC		
Soil moisture (%)	23.9	<0.001
Soil temperature (°C)	77.1	0.480
Soil pH ([H ⁺])	-1.7E+07	0.130
Clay content (%)	60.0	<0.001
Native biomass (g m ⁻²)	3.75	0.403
Invasion (absent, present)	-282	0.113
Uninvaded mean \pm SD	$2,429 \pm 512.9$ g C m ⁻²	-11.9%
Invaded mean \pm SD	$2,140 \pm 520.7$ g C m ⁻²	
(b) Mineral-associated C		
Soil moisture (%)	10.4	0.047
Soil temperature (°C)	56.0	0.536
Soil pH ([H ⁺])	-2.0E+07	0.015
Clay content (%)	54.9	<0.001
Native biomass (g m ⁻²)	-1.68	0.635
Invasion (absent, present)	-232	0.087
Uninvaded mean \pm SD	$1,836 \pm 410.1$ g C m ⁻²	-13.2%
Invaded mean \pm SD	$1,593 \pm 407.0$ g C m ⁻²	
(c) Particulate organic matter C		
Soil moisture (%)	13.5	<0.001
Soil temperature (°C)	9.46	0.823
Soil pH ([H ⁺])	4.5E+06	0.276
Clay content (%)	4.65	0.416
Native biomass (g m ⁻²)	5.39	0.002
Invasion (absent, present)	-49.3	0.458
Uninvaded mean \pm SD	590 ± 209.1 g C m ⁻²	-8.0%
Invaded mean \pm SD	543 ± 207.9 g C m ⁻²	

Best-fit linear mixed models included all of the measured continuous fixed effects (for example, moisture, percent clay) and *M. vimineum* invasion (a fixed factor), as well as location as a random effect. Empirical coefficients, P values, means and SDs were calculated using 10,000 Monte Carlo (MCMC) iterations. Intercepts for total SOC, mineral, and particulate C were: -413, -155, and -26.5, respectively.

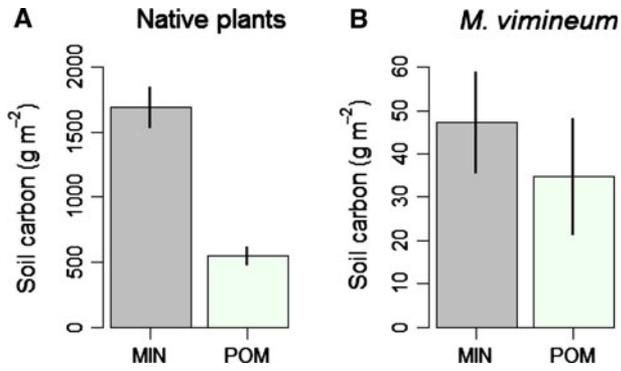


Figure 1. Mass of native- (A) and *M. vimineum*-derived (B) SOC fractions across a regional climate and ecological gradient. Soil C was differentiated into slower- (MIN mineral-associated) and faster-cycling (POM particulate organic matter) fractions. Values are means \pm SD; $n = 36$.

4% from the invasive grass (82 ± 101 g C m⁻²). These relative proportions likely reflect not only the slow turnover of the total SOC pool, but also the fact that understory inputs represent a relatively small fraction of total organic matter inputs to soils under forest or forest edge. Furthermore, the dominance of native-derived C in the SOC suggests that changes in the total SOC are likely the outcome of changes in the native-derived C. When

evaluating this material, we found that the best-fit model for the mass of native-derived SOC in the mineral-associated fraction included all measured variables, but only soil moisture, pH, and percent clay explained significant variation in mineral-associated SOC across plots (Table 2a). These variables were positively related to the mass of the native-derived, mineral-associated fraction, except for pH, where the relationship with increasing acidity was negative (Table 2a). Effects of *M. vimineum* invasion (presence or absence) on the mass of the native-derived, mineral-associated C were significant ($P(\text{MCMC}) = 0.047$) and across uninvaded and invaded plots the estimated mean (\pm SD) mass of this fraction was: $1,826 \pm 398.1$ and $1,560 \pm 400.4$ g C m⁻², respectively (Table 2a). This 14.6% decrease with invasion, however, masked considerable variation across plots in mineral-associated C values, with only 21 of 36 invasions associated with a decrease in native-derived mineral-associated C (Appendix 1).

The mass of the native-derived POM fraction was also influenced by environmental heterogeneity, responding positively to higher soil moisture and native biomass, but did not respond significantly to *M. vimineum* invasion (Table 2b). The mass of the *M. vimineum*-derived SOC was similarly influenced by environmental variation across plots, but in a manner unique from the native-derived SOC. The

Table 2. Coefficients Explaining Spatial Variation in Mass of Native-Derived SOC Fractions, and the Estimated Size of These Stocks in Uninvaded and *M. vimineum*-Invaded Plots

	Coeff.	<i>P</i> value (MCMC)
(a) Mineral-associated C		
Soil moisture (%)	10.1	0.056
Soil temperature (°C)	44.3	0.623
Soil pH ([H ⁺])	-2.0E+07	0.020
Clay content (%)	53.9	<0.001
Native biomass (g m ⁻²)	-1.25	0.723
Invasion (absent, present)	-272	0.047
Uninvaded mean \pm SD	$1,826 \pm 398.1$ g C m ⁻²	-14.6%
Invaded mean \pm SD	$1,560 \pm 400.4$ g C m ⁻²	
(b) Particulate organic matter C		
Soil moisture (%)	12.3	<0.001
Soil temperature (°C)	1.81	0.975
Soil pH ([H ⁺])	4.8E+06	0.200
Clay content (%)	4.49	0.397
Native biomass (g m ⁻²)	4.50	0.004
Invasion (absent, present)	-76.3	0.194
Uninvaded mean \pm SD	587 ± 186.4 g C m ⁻²	-13.0%
Invaded mean \pm SD	511 ± 188.5 g C m ⁻²	

Best-fit linear mixed models included all of the measured continuous fixed effects (for example, moisture, percent clay) and *M. vimineum* invasion (a fixed factor), as well as location as a random effect. Empirical coefficients, *P* values, means, and SDs were calculated using 10,000 Monte Carlo (MCMC) iterations. Intercepts for native mineral and particulate C were: 83.2 and 153, respectively.

Table 3. Coefficients Explaining Spatial Variation in Mass of Invasive-Derived SOC Fractions

	Coeff. (MCMC)	P value (MCMC)
(a) Mineral-associated C		
Soil moisture (%)	1.06	0.102
Soil temperature (°C)	38.9	0.009
Soil pH ([H ⁺])	-6.5E+06	0.089
Clay content (%)	-1.41	0.426
Native biomass (g m ⁻²)	-0.755	0.209
Invasive biomass (g m ⁻²)	-1.05	0.182
(b) Particulate organic matter C		
Soil moisture (%)	1.05	0.022
Soil temperature (°C)	23.0	0.025
Soil pH ([H ⁺])	-2.1E+07	<0.001
Clay content (%)	-1.65	0.238
Native biomass (g m ⁻²)	-0.097	0.825
Invasive biomass (g m ⁻²)	-2.15	0.012
Invasive biomass × pH	1.1E+06	<0.001

Best-fit linear mixed models included all of the measured continuous fixed effects (for example, moisture, percent clay) and *M. vimineum* invasion (a fixed factor), as well as location as a random effect. Empirical coefficients and P values were calculated using 10,000 Monte Carlo (MCMC) iterations.

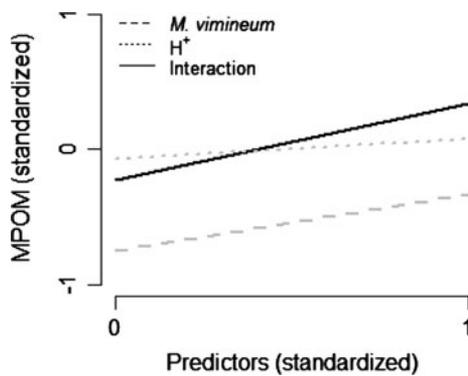


Figure 2. Continuous interaction plot of the effects of soil pH and *M. vimineum* foliar biomass on *M. vimineum*-derived particulate organic matter (MPOM) C. The terms were re-centered for graphing. More acid pH and higher *M. vimineum* biomass result in higher than expected (based on the single factors) formation of *M. vimineum*-derived POM C.

best-fit model for *M. vimineum*-derived, mineral-associated C included a significant, positive relationship with temperature and a negative relationship with acidity, but none of the other variables had a significant influence on this fraction, including the biomass of *M. vimineum* (Table 3a). In contrast, *M. vimineum*-derived POM C was positively related to soil moisture and temperature, and an interaction between *M. vimineum* biomass and pH (Table 3b). The significant interaction indicates that *M. vimineum*-derived POM increases with *M. vimineum* biomass and soil acidity, but increases significantly more where both are highest (Figure 2). That is, the positive relationship

between acidity and *M. vimineum*-derived POM C is accentuated by higher *M. vimineum* biomass (and vice versa).

The soil microbial biomass is both an agent of SOC degradation and formation, and as such we investigated how it responded to *M. vimineum* invasion. Prior to analysis, a single residual (site 33, uninvaded) was removed given that it fell far outside the residual cloud for a plot of microbial biomass C by *M. vimineum* biomass, and influential outliers can undermine statistical analysis (Maindonald and Braun 2003). We confirmed the extreme nature of this outlier using Cook's Distance ($D_i = 1.74$), where a conservative cut-off for these data is $D_i = 0.85$ (Chatterjee and others 2000). The best-fit model to explain microbial biomass only retained H⁺ concentration (coeff. (MCMC) = 2.2E+05, $P(\text{MCMC}) < 0.001$) and presence or absence of the invader (coeff. (MCMC) = -2.65, $P(\text{MCMC}) = 0.011$). Invasion corresponded with a 26.4% decrease in microbial biomass C (MCMC intercept: 11.14; estimated mean \pm SD across plots; uninvaded: 10.05 ± 1.79 g C m⁻², invaded: 7.40 ± 1.80 g C m⁻²). Within invaded plots, this reduction in microbial biomass C under invasion was negatively correlated with *M. vimineum* biomass (Figure 3).

DISCUSSION

Only a handful of field studies have assessed soil C responses to grass invasion of forests (for example, Bradley and others 2006; Litton and others 2008; Mack and D'Antonio 2003; Strickland and others

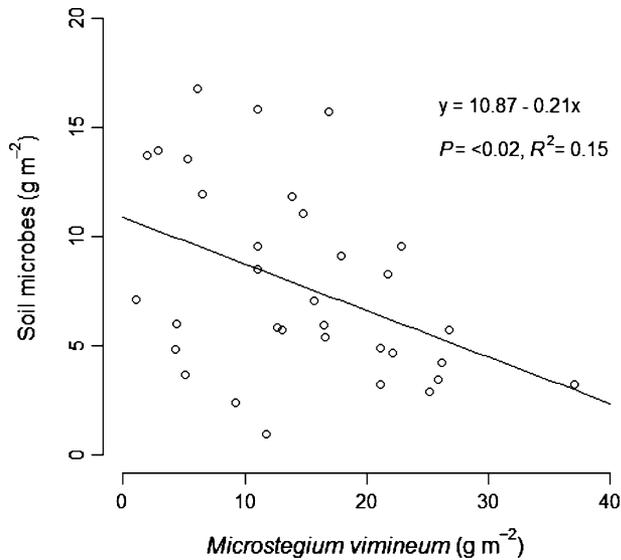


Figure 3. Negative linear relationship between *M. vimineum* and soil microbial biomass. Each point represents one of the 36 *M. vimineum*-invaded plots.

2010; Wolkovich and others 2010) and, as with the majority of invasive plant studies examining impacts on belowground processes, nearly all of the work has been conducted at single sites. We evaluate the association between grass invasion and SOC pools in 36 temperate forest sites spanning a 100-km climate and ecological gradient in the southeastern US, from the Georgia piedmont to mountains in North Carolina. Plant invasions occur within the milieu of environmental heterogeneity inherent in natural systems and we account for these influences by including measurements of abiotic drivers and biotic inputs that may influence, and respond to, *M. vimineum* invasion and its impacts on SOC (Schimel and others 1994; Schmidt and others 2011; Warren and others 2011b). Although *M. vimineum* comprised about 65% of the herbaceous foliar biomass in our invaded plots (Warren and others 2011a), it only accounted on average for about 4% of SOC. However, its presence was associated with declines in SOC, consistent with a single-site study conducted by Strickland and others (2010). Whereas the 12% decrease in total SOC was not significant, there was a significant decrease in the largest SOC fraction (that is, native-derived, mineral-associated C) despite the fact that SOC processes are highly heterogeneous locally and regionally (for example, Liu and others 2011; Saby and others 2008). Certainly, to detect impacts of *M. vimineum* invasion on SOC, it was necessary to account for heterogeneity across sites in other factors that drive SOC processes, and

in our study soil moisture, percent clay, and pH all explained significant variation in the mass of total and native-derived SOC (Tables 1, 2). As in meta-analyses that synthesize the impacts of invasive plant species across multiple habitat types (Liao and others 2008; Vilà and others 2011), the environmental heterogeneity resulted in context dependencies in the impacts of invasion. Specifically, only 21 of the 36 sites showed decreases in native mineral-associated C (Appendix 1), highlighting that to generalize results across a region requires multi-site investigations, such as the one we present here, where variation in controlling factors are measured and included in statistical models.

SOC is a large pool that is heterogeneous even at fine spatial-scales and that turns over slowly. These characteristics substantially reduce the power to detect changes in SOC stocks (Carney and others 2007; Strickland and others 2010; Throop and Archer 2008), and we address these limitations in part through the use of stable isotopes to separate SOC fractions. Unexpectedly, given its decadal-centuries turnover time (Grandy and Neff 2008; Schlesinger and Lichter 2001), we detected a decrease in native-derived mineral-associated C but not the POM C fraction, which turns over at annual-to-decade timescales. Previous studies across bottomland hardwood sites in the southeastern US have shown *M. vimineum* invasion associated with decreases in native POM C, with a mean but not significant decrease in mineral-associated C (Strickland and others 2010, 2011). However, *M. vimineum* invasion has been associated with reductions in soil microbial biomass at a site in our study region (Strickland and others 2010) and we observe a 26% reduction in microbial biomass C with invasion. Given the positive relationship between SOC contents and microbial biomass (Fierer and others 2009), and that microbial biomass both consumes and becomes SOC (Grandy and Neff 2008; Schmidt and others 2011), it is hard to disentangle whether reductions in SOC drive reductions in microbial biomass, whether the opposite is true, or whether both variables are reduced with invasion through an unrelated mechanism. However, mineral-associated C is primarily derived from microbial (as opposed to plant) necromass and secondary compounds (Grandy and Neff 2008), suggesting that reductions in microbial biomass might lead to reductions in the formation rate and hence mass of mineral-associated C. Whatever the mechanism of *M. vimineum* impact on mineral-associated C loss, that it is a stable SOC fraction constituting approximately 75% of the total SOC (across our sites) makes any agent that reduces its

size of concern given the associated reductions in soil fertility and C storage as SOC declines (Lal 2004). Further, microbial biomass forms the basal trophic level in detrital food webs and changes in its mass are likely associated with altered nutrient cycling belowground. Reductions in stable SOC and microbial biomass could therefore lead to reductions in forest-C storage, productivity, and associated ecosystem services, in the southeastern US.

The impacts of plant invasions on SOC dynamics seem idiosyncratic (for example, Liao and others 2008; Litton and others 2008; Strickland and others 2010; Vilà and others 2011; Wolkovich and others 2010), suggesting that habitat-specific impacts of a single invader may not generalize across the range of habitat types in a region. A primary motivation for our study was to test this possibility that single-site impacts are unlikely to generalize across a region. We found that unless heterogeneity across habitats (for example, in percent clay, soil moisture, and pH) is accounted for, single-habitat invasion studies cannot be generalized. However, despite this variation, impacts of *M. vimineum* invasion were additive: that is, there were no statistical interactions with other variables; except for *M. vimineum*-derived POM. This means that regional-scale impacts of plant invasion might be generally predictable, even if locally context dependent. Admittedly, by working in the southeastern US, our study controlled for certain variables such as soil order (all our sites were on ultisols). Similar studies in other parts of *M. vimineum*'s invaded range, that include different soil orders, will be necessary to ascertain whether our results extrapolate to northeastern and midwestern US regions. However, it may still be too early for such investigations to detect an impact on SOC dynamics, given the time ecosystem effects take to develop (Vilà and others 2011) and that their regional-scale consequences likely result from range infilling given the much greater area that is invaded through this process (for example, Muirhead and others 2006). Even though we deliberately worked in *M. vimineum*'s historic invaded range, to capture range-infilling and established invasions, it was evident across our plots that our invasions ranged in age, as did the extent of infilling. In 2011, we found that invasion edges across all three of our locations were advancing (unpub. data), and at the CWT location we first observed *M. vimineum* in 2006. Given that invasion dynamics and impacts likely depend on time since invasion (Václavík and Meentemeyer 2012; Vilà and others 2011), that our observational approach did not account for duration of invasion likely contributed

to variance in our data. Experimental introductions of invasive species would be one solution for defining time since invasion, but regional-scale introductions are often illegal, considered unethical, and can be logistically challenging (for example, obtaining permission from land owners). Instead, we demonstrate that an observational approach, that clusters local sites within regional gradients, can be used to discern regional-scale impacts and environmental-context dependencies.

Despite the fact that we worked within 100 km of one of the earliest US discoveries of *M. vimineum* in the early 1900s (Fairbrothers and Gray 1972), several lines of evidence point toward the belowground impacts of *M. vimineum* being in transition. Three quarters of the native SOC was found in the mineral-associated fraction, and the slow turnover of this fraction probably explained why only 58% of *M. vimineum*-derived SOC was partitioned to it (Figure 1). Next, *M. vimineum*-derived SOC was influenced by a different set of variables than native-derived SOC. For example, percent clay sets an upper-bound to SOC stocks given organo-mineral interactions that stabilize soil C (Schimel and others 1994); so although we expected and observed positive relationships between clay content and native SOC, we did not observe these relationships for *M. vimineum*-derived C (Tables 2, 3). In reviewing plant invasion studies, Vilà and others (2011) concluded that ecosystem effects manifest much later than impacts on communities. Our data support this conclusion and, worryingly, suggest that the impacts of *M. vimineum* invasion on SOC across the southeastern US are still in earlier, transitional stages, despite this region encompassing the historic invaded range. It may be that the ecosystem consequences of invasions will only be realized long after the initial impetus to study them.

There is still considerable uncertainty as to how the abundance of an invader, as opposed to just its presence, modifies its impacts on recipient systems (Vilà and others 2011). Our data suggest that invader abundance can be important in determining impact, with microbial biomass showing greater decreases in invaded plots as *M. vimineum* density increased (Figure 3). Further, *M. vimineum*-derived POM mass was influenced by the invader's density and soil pH (Figure 2). One possible mechanism that could explain the relationship between POM and soil pH is that lower pH can slow decomposition (Baath and others 1980). If lower pH did slow decomposition of organic inputs in our invaded plots, then this might have promoted accumulation of the less-decomposed and less-stable POM C fraction (Figure 2), which in turn reduced

accumulation of the more stable, mineral-associated fraction (Table 3a). Greater *M. vimineum* densities accentuated accumulation of POM C (Figure 2), presumably because this increased *M. vimineum* C inputs to the soil. This creates the potential for an interesting dynamic with time: *M. vimineum* appears to ameliorate soil acidity (Warren and others 2011a, b) and so it may be that the accumulation of *M. vimineum*-derived POM C will be ephemeral, with accelerated losses of this fraction as soil pH becomes less acid with time since invasion. Experimental studies are required to discern such mechanisms but our data do suggest that invader abundance will likely be important in determining the ecosystem consequences of plant invasion.

SOC is a fundamental ecosystem property, representing an important C store whose size is intimately tied to the value of land for provisioning (for example, timber production) and sustaining (for example, nutrient cycling) ecosystem services. Our work demonstrates how clustering local sites across a climate and ecological gradient can help identify general impacts of a widespread and prevalent invasive species on SOC at a regional scale. Coupling our work with similar study designs that evaluate the performance of invasive species across the environmental heterogeneity embedded within a region (for example, Warren and others 2011a) should permit development of spatially explicit models that project invasive density and impact across the regional landscape. Development of these models also demands data on invasive dispersal rates. As we develop these models our data suggest that, at least in the case of *M. vimineum* and the southeastern US, plant invasion is likely decreasing the inherent fertility and C storage of our soils.

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