Global Change Biology

Global Change Biology (2010) **16**, 1338–1350, doi: 10.1111/j.1365-2486.2009.02042.x

Grass invasion of a hardwood forest is associated with declines in belowground carbon pools

MICHAEL S. STRICKLAND*, JAYNA L. DEVORE†, JOHN C. MAERZ† and MARK A. BRADFORD*‡

*Odum School of Ecology, University of Georgia, Athens, GA 30602, USA, †Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602, USA, ‡School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA.

Abstract

Invasive plant species affect a range of ecosystem processes but their impact on belowground carbon (C) pools is relatively unexplored. This is particularly true for grass invasions of forested ecosystems. Such invasions may alter both the quantity and quality of forest floor inputs. Dependent on both, two theories, 'priming' and 'preferential substrate utilization', suggest these changes may decrease, increase, or leave unchanged native plant-derived soil C. Decreases are expected under 'priming' theory due to increased soil microbial activity. Under 'preferential substrate utilization', either an increase or no change is expected because the invasive plant's inputs are used by the microbial community instead of soil C. Here, we examine how *Microstegium vimineum* affects belowground C-cycling in a southeastern US forest. Following predictions of priming theory, *M. vimineum*'s presence is associated with decreases in native-derived, C pools. For example, in September 2006 *M. vimineum* is associated with 24%, 34%, 36%, and 72% declines in total organic, particulate organic matter, mineralizable (a measure of microbially-available C), and microbial biomass C, respectively. Soil C derived from *M. vimineum* does not compensate for these decreases, meaning that the sum of native- plus invasive-derived C pools is smaller than native-derived pools in uninvaded plots. Supporting our inferences that C-cycling accelerates under invasion, the microbial community is more active per unit biomass: added ^{13}C-glucose is respired more rapidly in invaded plots. Our work suggests that this invader may accelerate C-cycling in forest soils and deplete C stocks. The paucity of studies investigating impacts of grass invasion on C-cycling in forests highlights the need to study further *M. vimineum* and other invasive grasses to assess their impacts on C sink strength and forest fertility.

Keywords: annual grass, carbon sequestration, carbon sink, exotic species, Japanese stiltgrass, *Microstegium vimineum*, Nepalese browntop, preferential substrate utilization, priming effects, stable isotopes

Received 23 April 2009; revised version received 20 July 2009 and accepted 22 July 2009

Introduction

Invasive plants alter ecosystem processes and properties (Mack *et al*., 2000; Liao *et al*., 2008). These impacts can be the result of specific traits, novel to the recipient community. For example, nitrogen (N)-fixing invasive species alter decomposition rates and soil fertility in ecosystems lacking native N-fixers (Vitousek *et al*., 1987; Vitousek & Walker, 1989; Allison & Vitousek, 2004). Similarly, the introduction of invasive grass species can increase fire disturbance and intensity, altering carbon (C)-cycling rates (Mack *et al*., 2001; Mack & D'Antonio, 2003; Bradley *et al*., 2006). We know far less about the effects of those invasive species that do not exhibit particular traits such as N-fixation. Consequently, it is difficult to predict whether invasive plants will, in general, significantly alter ecosystem processes and/or the communities they invade.

Blumenthal (2006) noted that many invasive plants are 'high-resource' species. That is, they show lower investment in defensive compounds and tend to be of higher chemical quality (e.g. lower C : N) than native plants. Others have made similar observations (Pattison *et al*., 1998; Baruch & Goldstein, 1999; D'Antonio & Corbin, 2003). Evidence also suggests that invasive

Correspondence: Michael S. Strickland, tel. +1 706 542 7829, fax +1 706 542 3344, e-mail: strickm@uga.edu

plant presence is frequently associated with increased detrital inputs (Ehrenfeld, 2003; Liao *et al*., 2008). While such changes may not be universal, together they highlight the possibility that many invaders might alter ecosystem processes simply via changes in the quantity and/or quality of detrital inputs (D'Antonio & Corbin, 2003). Indeed, changes in the composition of native detrital resources have been shown to affect decomposition (Ball *et al*., 2008; Harguindeguy *et al*., 2008). The impacts of altered detrital quality and quantity on belowground processes and properties, and specifically soil C, is an area of debate in soil theory (Bradford *et al*., 2008c) and are receiving little attention in the context of plant invasions (Hook *et al*., 2004; Valery *et al*., 2004; Batten *et al*., 2005; Litton *et al*., 2006, 2008).

On one hand evidence suggests that increased degradability of C inputs, as perceived by the microbial community (Strickland *et al*., 2009), will have a negligible and in some cases a positive impact on belowground C pools (Dalenberg & Jager, 1981; Wu *et al*., 1993; Fontaine & Barot, 2005). This is referred to as 'preferential substrate utilization' theory and is based on the premise that high-quality C sources, such as leaf litter and rhizodeposits, will be utilized before lower-quality soil C sources (Fontaine *et al*., 2004a; Fontaine & Barot, 2005). Although the mechanism leading to preferential substrate utilization is debatable, it is assumed that if novel inputs are of both a high enough quality and quantity in comparison to background inputs then the microbial community will shift toward organisms specializing on less recalcitrant compounds (Blagodatskaya & Kuzyakov, 2008; Bradford *et al*., 2008c). Under this expectation the component of the microbial community responsible for the degradation of recalcitrant soil C sources may go unchanged, leading to little change in soil C pools (Fontaine *et al*., 2003). Under more extreme cases, those organisms specializing on recalcitrant resources (e.g. soil C) will be out-competed by those specializing on less recalcitrant compounds, leading to a decline in the former's populations and an increase in the size of soil C pools (Fontaine *et al*., 2003). In contrast, 'priming effects' theory posits that high-quality inputs give rise to disproportionate impacts on soil C (Fontaine *et al*., 2003, 2004b; Dijkstra & Cheng, 2007). Priming is theorized to occur because high-quality inputs stimulate microbial activity, in particular groups specializing in the decomposition of recalcitrant soil C. The result is enhanced degradation of belowground C pools, leading to declines in their size (Fontaine *et al*., 2004b). It seems reasonable to assume that the impact of invasive plants on belowground C pools in native ecosystems will be dependent on the quality and quantity of invasive inputs relative to native inputs, where either preferential substrate utilization or priming effects are realized. Given that both theories suggest a change in soil C-cycling, altered C inputs with plant invasions will likely alter a site's fertility, drought tolerance, C storage potential, and possibly the aboveground-belowground interactions between organisms (Lal, 2004; Lal *et al*., 2007).

We studied an advancing invasion of *Microstegium vimineum* (Trin.) A. Camus (commonly named Japanese stiltgrass or Nepalese browntop) to measure associated changes in soil C cycling and pools. *M. vimineum* is an annual C_4 grass, which produces relatively little root biomass (Ehrenfeld *et al*., 2001; Claridge & Franklin, 2002), and invades the understory of a variety of forest types across a large portion of the southern and eastern United States . The US Department of Agriculture reports *M. vimineum* invasions in 25 states including all states east of the Mississippi River except Vermont, New Hampshire, and Maine (http:/plants.usda.gov/). Research in northeastern US forests demonstrates that *M. vimineum* alters soil properties, including the activities of microbial exoenzymes that may affect soil C-cycling (Kourtev *et al*., 1998, 2002; Ehrenfeld *et al*., 2001). As yet no work has examined how *M. vimineum* invasion impacts belowground C pools. In fact, little is known about the impact of grass invasions on soil C in forest ecosystems (Kourtev *et al*., 2003; Mack & D'Antonio, 2003; Litton *et al*., 2008). A recent meta-analysis of invasive plant effects on C and N cycling shows only 23% of 94 studies measured belowground C, and of those only two looked at the effect of grass invasion in forests (Liao *et al*., 2008). The first examined how perennial grass replacement of forest, due to increased fire occurrence, impacted soil C (Mack & D'Antonio, 2003). The second, a laboratory study, compared changes in soil C between invasive understory species (including *M. vimineum*) and a single native understory species (Kourtev *et al*., 2003). Mack & D'Antonio (2003) found no significant difference in mineralizable soil C between forest sites where an exotic grass had and had not been removed but did note a greater percentage of mineralizable soil C in the invaded sites during the dry season. Kourtev *et al*. (2003) found that soils planted with *M. vimineum*, when compared with soils planted with a native understory species, had a greater percentage of soil organic matter. A third study (Litton *et al*., 2008) assessed the impacts of invasive grass removal on soil C and found decreased rates of soil C flux where the grass was removed but noted no changes in soil C pools between removal and invaded plots. Despite *M. vimineum*'s presence in many eastern US forest understories and a recent call for research investigating such understory invaders (Martin *et al*., 2009), we are aware of no assessment that compares

the impact on soil C pools of uninvaded and grass-invaded areas within intact forest ecosystems.

Our objectives were to (1) compare the size of multiple soil C and N pools across an active *M. vimineum* invasion front, (2) measure the contribution of native plant and *M. vimineum* C to soil C pools, and (3) evaluate differences in soil C pools across the invasion front with changes in soil microbial activity. In our study system, *M. vimineum*'s C inputs can be distinguished from native inputs using stable C isotope ratios because *M. vimineum* is the only species to use the C_4 photosynthetic pathway. Our overarching hypothesis was that *M. vimineum* invasion would alter belowground C cycling and we expected this would be explained by one of two hypotheses. Specifically, we expected that in invaded plots either the predictions of (H_1) preferential substrate utilization theory would be observed through no change or an increase in native plant-derived soil C pools, or (H_2) priming effect theory would be observed by decreases in soil C pools. A significant strength of our study is the investigation of an active invasion front in an intact forest ecosystem. At the same time this 'observational' approach necessitates consideration of alternative mechanisms that may contribute to any observed responses; we explore these through additional measurements and in the 'Discussion'.

Materials and methods

Site description

Twelve 2 m × 2 m study plots divided into six pairs (one uninvaded and one invaded) were established across the edges of a rapidly progressing *M. vimineum* invasion in a riparian forest within the Whitehall Experimental Forest, Athens, GA, USA (33°53.27′N, 83°21.93′W). The site is a former wood lot, managed as mixed hardwood for the past 70 years (R. Jackson, personal communication). There is little evidence that this particular site was farmed but nearly all of the surrounding land was (R. Jackson, personal communication). Soils are a sandy loam in the Madison series (Soil Survey Staff, 2009). Bulk density was consistent across the site at 1.08 g cm^{-3}. Anecdotal reports indicate *M. vimineum* established within the Whitehall Experimental Forest ~ 15 years ago and this likely holds for our site. Further, *M. vimineum* probably went unnoticed until it formed dense cover and so pinpointing the exact invasion date is difficult (Martin *et al*., 2009). Notably, the invaded plot in each pair appeared to have developed from a discrete, invaded patch and remained discrete while our work was conducted in 2006 and 2007. In the 2008 growing season, patches were expanding to form a contiguous cover of *M. vimineum*. No confounding issues related to invasive earthworms were noted between invaded and uninvaded sites (see 'Results'). The forest overstory was composed of *Acer rubrum*, *Quercus nigra*, *Platanus occidentalis*, and *Liquidambar styraciflua*. The uninvaded areas of the study site were generally depauperate in understory plants, representing <5% total cover of various species (see Bradford *et al*., 2009). In the invaded areas, *M. vimineum* covered >90% of the understory with an average dry green biomass of ~63.04 g m^{-2} (unpublished data).

M. vimineum began invading the uninvaded plots towards the end of our study in 2007 and by 2008 had invaded all the control plots. This suggests that our uninvaded plots were hospitable to *M. vimineum* and it had simply not invaded these areas yet. This is an important strength of our study. We deliberately worked across an advancing invasion to minimize the likelihood that invaded and uninvaded areas might exhibit differences in soil C cycling because of historical contingencies other than the *M. vimineum* invasion. We recognize, however, that our study design is not a controlled experiment and our inferences should be interpreted with this in mind. Further, although our sample plots are spatially independent and our results pronounced, they are limited to the description of a single forest. Whether they are common to other sites or to other grass invasions of forest understories is unknown.

Standard sampling regime

Standard sampling consisted of taking three individual A horizon soil cores (8 cm diametres, 0–10 cm depth) from each plot using a stratified random approach. Soils were sieved (4 mm), homogenized, and stored at + 5 °C until analyzed. Standard samples were taken six times (12 September 2006, 13 November 2006, 19 January 2007, 14 February 2007, 8 May 2007, and 24 July 2007). *M. vimineum* was actively growing during the months of September, May, and July but had senesced by November and seeds did not germinate before the January and February samplings.

Measurements taken during the seasonal sampling regime consisted of gravimetric soil moisture, pH, soil temperature (10 cm depth), soil CO_2 efflux, substrate induced respiration (SIR; a measure of microbial biomass), and mineralizable C (a measure of microbially available C). Moisture and pH were measured for two analytical repeats per sample. Moisture was determined by drying soil at 105 °C for 24 h and pH using 1 : 1 soil : H_2O by volume. Soil CO_2 efflux, a measure of both heterotrophic and autotrophic respiration, was taken in the field using an infrared gas analyzer (IRGA; Li-Cor

Biosciences, Lincoln, NE, USA, Model LI-8100). Given the small plot sizes, one PVC collar was used per plot (20 cm diametre, inserted 5 cm into the soil), and one measurement per plot was taken during late afternoon. Collars were placed on each sample day and measurements were taken a minimum of 1 h after placement. The SIR method follows Fierer & Schimel (2003) whereby soil slurries are incubated, after a 1 h preincubation with excess substrate, for 4 h at 20 °C.

Mineralizable C was determined using 60-day C-mineralization assays and is often used to estimate the size of the labile soil C pool. It was determined by maintaining soils at 20 °C and 65% water-holding capacity (WHC) for 60 days with periodic determinations of respiration rates using a static incubation technique (Fierer *et al*., 2005a) and infra-red gas analysis of headspace CO_2 concentrations. Mineralizable C was estimated as the area under the curve derived by plotting CO_2 production against time.

Intensive sampling regime

Measurements for the intensive sampling regime were taken before (12 September 2006) and after (13 November 2006) *M. vimineum* senesced. The elongated growing season in the southeastern US meant *M. vimineum* was green in September. We measured the mineral-associated and particulate organic matter (POM) C and N pools, the DOC pool, chloroform fumigation-extracted (CFE) microbial biomass C and N, and extractable inorganic and organic N. The soil fractionation approach, whereby we resolved C pools with different turnover times, provided a powerful approach for detecting changes in soil C that a single blanket measure of total soil C might have obscured (see Bradford *et al*., 2008b, 2008c).

To determine mineral-associated and POM C and N pools, the fractionation method described in Bradford *et al*. (2008c) was used. Briefly, duplicate soil samples (10 g of air-dry soil) from each plot were dispersed with NaHMP (30 mL sample^{-1}) via shaking (18 h) and then passed through a 53 μm sieve. Material <53 μm is considered mineral-associated and material >53 μm is considered POM. Both mineral and POM material were dried (105 °C), ball-milled to a fine powder, and percentage C and N determined using an NA1500 CHN Analyser (Carlo Erba Strumentazione, Milan, Italy). Of these two fractions, mineral-associated C pools are expected to turnover more slowly than POM C pools (Schlesinger & Lichter, 2001). Mineral-associated C pools are presumed to be primarily microbial-derived C whereas POM pools are primarily plant-derived (Grandy & Robertson, 2007). Given the multiannual turnover times of these pools we only measured them during the September sampling; i.e. further changes in pool size would not have been observed across our study.

DOC and organic and inorganic N pools were determined on two analytical repeats per sample. Samples were shaken with 0.5 M K_2SO_4 for 4 h, filtered, and DOC concentrations determined using a Total Organic Carbon Analyzer (Shimadzu, Columbia, MD, USA). Dissolved organic N (DON) and inorganic N pools were quantified on a Lachat autoanalyzer (Milwaukee, WI, USA). The size of the DOC pool is often determined because it is one estimate of labile soil C (Bradford *et al*., 2008c). However, DOC is a mixture of both high- and low-molecular-weight C compounds and so is likely less labile than the C pool resolved using our 60-day mineralization assays.

Microbial biomass C and N was estimated using a modified CFE method as described in Fierer & Schimel (2002) and Fierer & Schimel (2003). They were estimated as the flush of DOC or DON, respectively, following fumigation. Raw values are reported; no correction factors are used. Determining microbial biomass C using the CFE method allowed us to determine the proportion of microbial biomass which was derived from *M. vimineum* (see 'Determination of *M. vimineum* C'). Note that the relationship between SIR and the CFE method for microbial biomass is not necessarily equivalent (Wardle & Ghani, 1995). For example, CFE is expected to estimate total, and SIR active, microbial biomass (Wardle & Ghani, 1995). We used the SIR method for the standard seasonal sampling because it likely provides greater resolution of differences in microbial biomass within a site (Wardle & Ghani, 1995).

Finally, we determined earthworm biomass, litter chemistry, and the fungal-to-bacterial dominance of the microbial community. *M. vimineum* invasion is linked to increased nonnative earthworm biomass in northeastern US forests (Kourtev *et al*., 1998, 1999; Nuzzo *et al*., 2009) so we accounted for this potential confounding issue. Earthworm biomass was determined by hand-extracting earthworms from 20 cm diameter, 20 cm deep soil cores. Hand-extracting is commonly considered the most accurate sampling technique for earthworm biomass (Lee, 1985; Eisenhauer *et al*., 2008), which we report here as wet mass.

Total percentage C, N, cellulose, hemi-cellulose, and lignin were determined for native forest litter, *M. vimineum* leaf material, and *M. vimineum* stem material (Table 1). We determined the ratio of leaf-to-stem material for *M. vimineum*, permitting us to determine whole plant litter chemistry. Total C and N were determined using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy). Fiber concentrations were determined using an Ankom A200 Fiber Analyzer

Table 1 Litter chemistry and percentage of native and *Microstegium vimineum* derived inputs into *M. vimineum* invaded plots

Litter Source	Litter input (%)	C (%)	N (%)	Lignin (%)	Cellulose (%)	Hemi-cellulose (%)
Native	97.25 ± 0.98	48.52 ± 0.72	0.87 ± 0.01	25.58 ± 1.81	18.66 ± 0.23	12.40 ± 0.52
*M. vimineum**	2.75 ± 0.98	42.50	1.10	17.97	25.17	21.03
Leaves		42.33 ± 1.81	1.34 ± 0.06	20.04 ± 2.38	20.54 ± 0.76	20.76 ± 0.54
Stems		42.83 ± 0.09	0.62 ± 0.03	13.99 ± 2.08	34.06 ± 1.09	21.54 ± 0.64

M. vimineum litter was divided into leaf and stem material because of expected differences in chemical recalcitrance. The proportion of *M. vimineum* stem and leaf material was used to calculate whole litter chemistry for *M. vimineum*. Native litter was a mixture of several species found at this site. For litter chemistry the mean ± 1 SE is reported ($n = 3$ analytical repeats). For the percentage of litter inputs the mean ± 1 SE is reported for all six invaded plots.
*Leaves represented 65.74 ± 0.02% of *M. vimineum* litter and stems represented 34.26 ± 0.02% ($n = 3$).

(Ankom, Macedon, NY, USA). Quantitative inputs of *M. vimineum* litter and native forest litter were assessed by taking 0.25 m × 0.25 m quadrats from each plot post-senescence (i.e. November), drying the sample at 65 °C, hand-sorting the material, and determining its mass (Table 1). We recognize that this method may underestimate the invader's inputs if it is cycling more rapidly than native litter inputs. To decrease this potential bias, samples were taken as soon as possible after *M. vimineum* senesced.

Fungal-to-bacterial dominance of the microbial community is often considered an indicator of belowground C processes with fungal-dominated systems associated with greater C stores. We determined these ratios using the qPCR method described by Fierer *et al*. (2005b). Briefly, DNA was isolated from soil (stored at −80 °C) using the MoBIO Power Soil DNA Extraction kit (MoBio Laboratories, Carlsbad, CA, USA) with modifications described in Lauber *et al*. (2008). Standard curves were constructed to estimate bacterial and fungal small-subunit rRNA gene abundances. PCR reactions were performed to generate the ratios of fungal to bacterial gene copy numbers by using a regression equation for each assay relating the cycle threshold (C_t) value to the known number of copies in the standards. All qPCR reactions were run in quadruplicate. Additional details are provided in Lauber *et al*. (2008).

Determination of M. vimineum *derived C*

To establish the amount of *M. vimineum* derived C, we determined the $\delta^{13}C$ value of the following C pools: mineralizable C, CFE microbial biomass, DOC, total soil C, POM C, mineral-associated C, and the litter layer. For mineralizable C, a gas sub-sample taken on the first day of each 60-day incubation was analyzed. The $\delta^{13}C$ value of the CO_2 in the sample was determined using continuous-flow isotope-ratio mass spectrometry (CF-IRMS; Thermo, San Jose, CA, USA). For DOC and CFE biomass a TOC analyzer was coupled to the IRMS and the solid samples were introduced to the IRMS via an elemental analyzer. We estimated the amount of *M. vimineum* derived C in each of the C pools by using the difference in the natural abundance, $\delta^{13}C$ values of either the plant or soil C pools. The pre-invasion $\delta^{13}C$ value for each C pool was determined from plots where *M. vimineum* was absent; all of these pools exhibited a C_3-photosynthetic value ranging from −27.72 ± 0.25‰ (mean ± 1 SE; $n = 6$) for native POM C to −24.29 ± 0.32‰ (mean ± 1 SE; $n = 6$) for the September DOC measurement. *M. vimineum* has a C_4-photosynthetic value ($n = 13$; mean ± 1 SE = −14.31 ± 0.14‰); the difference in the C isotope composition being sufficient to discriminate sources (Staddon, 2004). The amount of C derived from *M. vimineum* was calculated as follows (sensu Ineson *et al*., 1996): $C_{M.\ vimineum\ \text{derived}} = C_{\text{pool}} \times (\delta^{13}C_{\text{invaded}} - \delta^{13}C_{\text{uninvaded}})/(\delta^{13}C_{M.\ vimineum} - \delta^{13}C_{\text{uninvaded}})$, where C_{pool} is the measured size of the pool (total, POM, Mineral, DOC, Microbial C, mineralizable, or litter), $\delta^{13}C_{\text{invaded}}$ is the $\delta^{13}C$ value of the pool in plots where *M. vimineum* is present, $\delta^{13}C_{\text{uninvaded}}$ is the $\delta^{13}C$ value of the pool in plots where *M. vimineum* is absent, and $\delta^{13}C_{M.\ vimineum}$ is the value for *M. vimineum* itself.

^{13}C-glucose pulse-chase

To determine whether or not *M. vimineum*'s presence was associated with more rapidly cycling C pools, we conducted a ^{13}C pulse-chase in November 2006. This involved making additions of ^{13}C-labeled glucose and tracking its mineralization as $^{13}CO_2$. This was accomplished by placing two PVC collars (15.4 cm diametres, inserted 5 cm into the soil) in three plots where *M. vimineum* was present and three where it was absent. Water was added to each core 24 h before sampling to alleviate any differences in water availability. Soil CO_2 efflux rates were determined using a closed-chamber approach (e.g. Bradford *et al*., 2001), where CO_2 concentrations were determined at the start and end of a 45 min capping period. We conducted a pilot study to

determine the appropriate capping time and found that headspace CO_2 concentrations increase linearly from 0 to 45 min; flux rate estimates only began to decrease after 60 min. Although closed-chamber approaches are likely to have caveats associated with them (i.e. underestimated CO_2 efflux rates), they are nonetheless widely used across an array of ecosystem types (Nay *et al*., 1994; Franzluebbers *et al*., 2002). Similar capping times and protocols as described here have been employed by others (Iqbal *et al*., 2008; Mo *et al*., 2008). Headspace samples were taken with 20 mL SGE gas syringes, transported to the laboratory in 12 mL Exetainers, and then CO_2 concentrations were determined using an IRGA (Li-Cor Biosciences, Model LI-7000). A second sample was analyzed using CF-IRMS to determine the stable C isotope composition (see above for details). The initial headspace sampling provided natural abundance values for the isotope mixing equations. After this initial sampling, 1 L of 2.5 mM ^{13}C-labeled glucose solution (99 at%) was added to the collars and permitted to drain. The capping procedure was repeated postaddition at 2, 5, 24, 48, and 72 h, permitting a negative exponential 'decay' of ^{13}C label to be tracked in the soil CO_2 efflux, from which cumulative mineralization was estimated. The contribution of ^{13}C-labeled glucose to soil respiration was estimated using isotope mixing equations similar to those described above.

Statistical analysis

Linear mixed-effects models were used to analyze the effect of *M. vimineum* presence/absence (Pinheiro & Bates, 2000). The presence of *M. vimineum* and month (when applicable) were treated as fixed effects. Pair and plot were treated as random effects with plot nested within pair. The pair identity was included as a blocking variable to account for spatial heterogeneity among paired plots where *M. vimineum* was present and absent. If the removal of this blocking variable resulted in a more parsimonious model, as determined by AIC, then it was dropped from the analysis. Plot identity was included as a random effect to account for repeated sampling across months. When reported, data were $\log_e$-transformed to conform to assumptions of homoscedasticity (verified using model checking). All analyses were conducted using the freeware statistical package R (http://cran.r-project.org/). Results were considered statistically significant at $P<0.05$ and marginally significant at $P<0.10$. Notably, it is acceptable practice to consider changes in soil C pools at $P<0.10$ as statistically significant because of the large spatial variation associated with them (e.g. Carney *et al*., 2007). However, we took a more conservative stance in this work.

Results

Of the standard measures taken across September 2006 to July 2007, we observed an average decrease in mineralizable C of 35% in *M. vimineum*'s presence ($F_{1,5}=14.05$; $P<0.05$; Fig. 1a). Average declines in mineralizable C of 36%, 18%, 36%, 39%, 43%, and 37% during the months of September, November, January, February, May, and July, respectively, were noted when the invader was present (Fig. 1a). A significant effect of sampling month was also detected ($F_{5,50}=2.59$; $P<0.05$) but the relative difference between invaded and uninvaded plots was consistent (interaction: $F_{5,50}=1.29$; $P<0.28$). Of the initial mineralizable C (i.e. CO_2 evolved during the first of the 60 incubation days), *M. vimineum* derived inputs accounted for ~10%

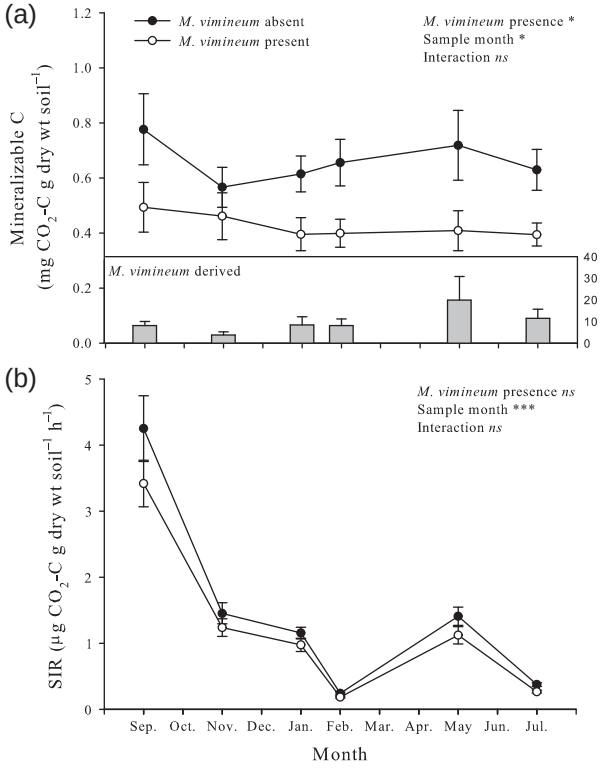

Fig. 1 Mineralizable C (a) and SIR microbial biomass (b) for plots where *M. vimineum* was present and where it was absent ($n=6$). Panel a shows the mean cumulative values ± 1 SE for mineralizable C across each 60-day incubation. Mineralizable C is expected to represent microbially available, labile C. Also shown in (a) is the mean percentage ± 1 SE of CO_2-C derived from *M. vimineum* on the first day of each 60-day incubation for sites where *M. vimineum* was present (subset bar plot). (b) The mean ± 1 SE for SIR microbial biomass which may be an indicator of physiologically active microbial biomass. *Microstegium vimineum* was active during the sampling months of September, May, and July (note it is an annual). $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; *ns*, not significant.

on average across all sampling months (Fig. 1a). A high of ~20% was found for the May sampling and a low of ~4% for the November sampling (Fig. 1a). *M. vimineum*'s presence was not associated with a change in SIR biomass ($F_{1,10} = 3.10$; $P = 0.11$; Fig. 1b).

M. vimineum's presence did not affect soil CO_2 efflux ($F_{1,10} = 1.66$; $P = 0.23$; Fig. 2), but did influence soil moisture ($F_{5,50} = 3.30$; $P < 0.05$), temperature ($F_{5,50} = 2.55$; $P < 0.05$), and pH ($F_{5,50} = 4.17$; $P < 0.01$). Soil CO_2 efflux did vary across sampling month ($F_{5,50} = 63.8$; $P < 0.001$; Fig. 2) with greater efflux during the growing season (i.e. September, May, and July) and lower efflux when plant species were inactive (i.e. November, January, and February). Soil moisture tended to be seasonally more stable in *M. vimineum*'s presence. Soil temperature tended to be lower in *M. vimineum*'s presence in November, January, and February but in September, May, and July sites where it was present had similar or higher temperatures to where it was absent. In general, pH was higher in *M. vimineum*'s presence. This was dependent on sampling month with higher pH values for invaded soils in September, January, and May but similar pH values in the presence/absence of the invader in November, February, and July.

Several soil C and N pools were intensively sampled in September and/or November 2006. For soil C pools, we noted a significant decrease of 64% and 41% (September and November, respectively) in CFE microbial biomass when *M. vimineum* was present ($P < 0.05$; Table 2). Regardless of the invasion status, microbial biomass was marginally greater postsenescence ($F_{1,10} = 3.69$; $P = 0.08$). We also noted marginally significant declines

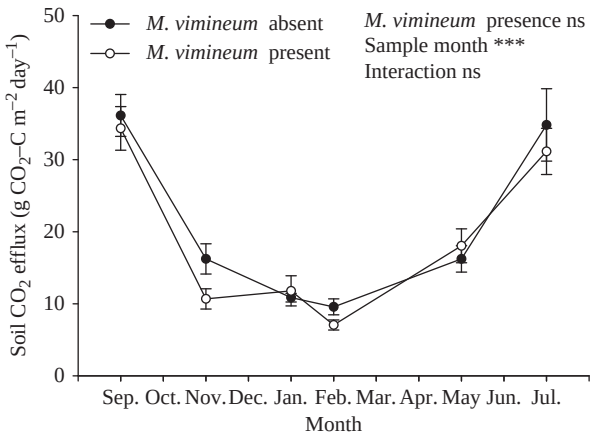

Fig. 2 Soil CO_2 efflux (mean ± 1 SE) measured *in situ* across all sampling dates for plots where *Microstegium vimineum* was present and where it was absent ($n = 6$). This is a measure of both heterotrophic and autotrophic respiration. *M vimineum* was active during the sampling months of September, May, and July (note it is an annual). Symbols are the same as in Fig. 1.

of 22% and 29% in total ($P = 0.09$) and POM-associated organic C ($P = 0.08$; Table 2), respectively, when *M. vimineum* was present. The marginally significant decrease in total soil C was likely due to the decrease in POM C given that total C is the sum of POM and mineral-associated C. No significant change in mineral-associated soil C or DOC was noted ($P > 0.19$ for both; Table 2), There were no obvious differences in any of the measured N pools ($P > 0.12$ in all cases).

Inputs derived from *M. vimineum* contributed between 1% and 29% of the C found in the belowground pools of invaded plots (Table 2). These inputs did not compensate fully for observed declines in several native plant-derived C pools (Table 2). We noted significant declines in both native-derived CFE microbial biomass C ($P < 0.05$) and POM-associated C ($P < 0.05$), as well as a marginally significant decline in native-derived total soil organic C ($P = 0.07$) in *M. vimineum*'s presence (Table 2). *M. vimineum*'s presence was associated with a 72% decline in native derived CFE microbial biomass C in September and a 43% decline in November (Table 2). Overall, native-derived CFE microbial biomass C tended to increase postsenescence in November ($F_{1,10} = 5.40$; $P < 0.05$). *M. vimineum*'s presence was not associated with a significant decline in native derived DOC ($P = 0.10$; Table 2). Native derived POM-associated soil C was 34% lower and total soil organic C 24% lower in *M. vimineum*'s presence but there was no statistical support for significant changes in mineral-associated soil C (Table 2).

Of the remaining September and November 2006 measures, no differences in earthworm biomass were detected between invaded and uninvaded plots ($F_{1,10} = 0.04$; $P = 0.84$), nor in fungal-to-bacterial ratios ($F_{1,10} = 0.00$; $P = 0.99$). The cumulative amount of ^{13}C-glucose mineralized to $^{13}CO_2$ was, however, lower in sites where *M. vimineum* was absent ($F_{1,2} = 19.6$; $P < 0.05$; Fig. 3). Across time, ^{13}C-glucose mineralized to $^{13}CO_2$ declined exponentially. Significant differences in cumulative values between sites was likely due to higher rates of ^{13}C-glucose mineralized to $^{13}CO_2$ observed in invaded plots during the 2 and 5 h sampling periods (Fig. 3).

Discussion

We evaluated the impact of the widespread invader, *M. vimineum*, on belowground C pools. We expected that if it impacted belowground pools, the mechanism could likely be framed around the theories of preferential substrate utilization or priming. If preferential substrate utilization was the mechanism then our expectation (H_1) was either no change or an increase in belowground C pools, meaning that soil C turnover rates

Table 2 The mean ± 1 SE of the *Total* (native + *Microstegium vimineum*) and *native* forest derived total soil organic C, POM associated C, mineral associated C, DOC, and microbial biomass C for plots where *M. vimineum* is absent and present ($n = 6$)

	Presenescence			Postsenescence				
	M. vimineum absent	*M. vimineum* present	% *M. vimineum* derived C	*M. vimineum* absent	*M. vimineum* present	% *M. vimineum* derived C	F_{df}	*P*-value
Total C								
Total	22.21 ± 3.20	17.24 ± 3.56		NA	NA	NA	$4.56_{1,5}$	*0.086*
Native		16.80 ± 3.50	2.87 ± 0.64	NA	NA	NA	$5.28_{1,5}$	*0.070*
POM C								
Total	8.46 ± 1.24	5.99 ± 1.22		NA	NA	NA	$4.79_{1,5}$	*0.080*
Native		5.60 ± 1.16	6.01 ± 1.34	NA	NA	NA	$6.83_{1,5}$	<0.05
Mineral C								
Total	13.75 ± 2.41	11.25 ± 2.44		NA	NA	NA	$3.37_{1,5}$	0.126
Native		11.13 ± 2.43	1.29 ± 0.51	NA	NA	NA	$3.51_{1,5}$	0.120
DOC								
Total	102.97 ± 12.96	87.10 ± 15.55		113.13 ± 18.32	87.46 ± 21.68		$2.34_{1,5}$	0.186
Native		77.16 ± 14.62	9.80 ± 2.12		85.09 ± 21.47	3.12 ± 1.48	$4.07_{1,5}$	0.100
Microbial biomass C								
Total*	68.56 ± 22.61	25.03 ± 10.48		74.50 ± 16.24	44.20 ± 6.86		$2.34_{1,10}$	<0.05
Native*		19.11 ± 9.57	29.41 ± 7.50		42.55 ± 7.34	5.25 ± 3.63	$10.08_{1,10}$	<0.05

Where applicable, values are shown both before and after *M. vimineum* senesced (i.e. September and November samplings, respectively). Also, shown is the mean percentage ± 1 SE that *M. vimineum* derived C contributed to the total C in each pool. Analyses compared both *Total* (native + *M. vimineum*) pool sizes between plots where *M. vimineum* was present/absent and *Native* (native only) C pool sizes. In uninvaded plots *Total* = *Native* and so a dash is shown in the table for these entries. Total C, POM C, and Mineral C pools were only measured presenescence. F-values, degrees of freedom, and *P*-values are reported for the main effect of *M. vimineum* presence/absence. Where measures were taken both pre- and postsenescence (e.g. DOC) these values are reported for the main effects across the two sampling points. Units for Total, POM, and mineral C pools are $mg\,g^{-1}$ dry wt $soil^{-1}$ and $\mu g\,g^{-1}$ dry wt $soil^{-1}$ for DOC and microbial biomass C. Where significant ($P < 0.05$) *P*-values are reported in bold. Marginally significant ($P < 0.10$) *P*-values are italicized.

*Data were $\log_e$ transformed.

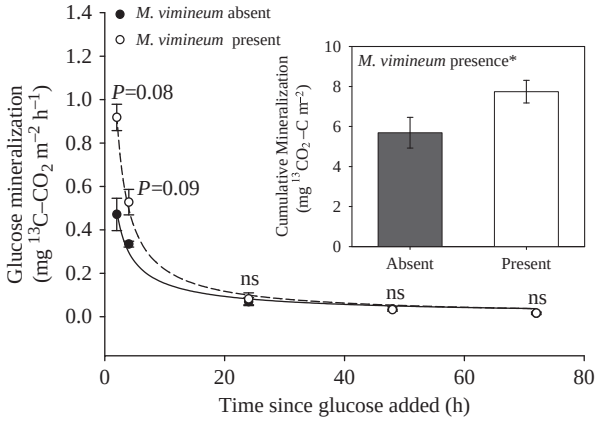

Fig. 3 Results of the ^{13}C-glucose pulse-chase experiment conducted during the November sampling. The panel insert shows the cumulative amount of ^{13}C-glucose mineralized to $^{13}C–CO_2$ (mean ± 1 SE) across 72 h for plots where *M. vimineum* is present and where it is absent ($n = 6$). The main panel shows the time course of glucose mineralization. A marginally significant effect of *M. vimineum* presence was detected at 2 h ($F_{1,2} = 10.5$, $P = 0.084$) and 5 h ($F_{1,2} = 9.98$, $P = 0.087$) but no effect was detected at 24 h ($F_{1,2} = 0.13$, $P = 0.76$), 48 h ($F_{1,2} = 0.01$, $P = 0.94$), or 72 h ($F_{1,2} = 0.11$, $P = 0.77$). Symbols are the same as in Fig. 1.

would likely remain unchanged or decrease (Fontaine *et al*., 2004a; Fontaine & Barot, 2005; Bradford *et al*., 2008c). In contrast, if a priming effect (H_2) was the mechanism then our expectation was that *M. vimineum*'s presence would be associated with a decrease in the size of C pools and an increase in their turnover rates (Fontaine *et al*., 2004b). We observed that *M. vimineum*'s presence was associated with increased turnover rates and declines in several belowground C pools. Our findings suggest that *M. vimineum* invasion is altering soil C cycling via a priming effect. We do recognize that our study was observational and so causation could be attributed to other response variables. Given that neither moisture nor temperature were consistently higher or lower in invaded sites and earthworm biomass was similar across invaded and uninvaded plots we discuss below why priming may be the most plausible explanation. We contrast our results with other studies that appear to have found preferential substrate utilization in grass-invaded forest soils.

We found a marginally significant decrease in both total organic and POM C associated with the invasion (Table 2). The decrease in total soil C was largely due to the decrease in POM C and no statistically significant decrease in mineral-associated C was observed, likely due to its slower turnover rates and hence response times (Schlesinger & Lichter, 2001; Grandy & Robertson, 2007; Bradford *et al*., 2008c). Declines in both POM and total soil C represent an average 29% and 22% decrease, respectively, under *M. vimineum*. If expressed on an areal basis, this equates to a decline of $\sim 375\,g\,m^{-2}$ of total C representing a decrease of $\sim 25\,g\,m^{-2}\,yr^{-1}$, conservatively assuming *M. vimineum* has been present at this site for 15 years. We recognize that we are basing some of our inferences on statistically marginal differences (see Table 1). However, we think this is legitimate given that a power analysis indicated the likelihood of detecting a significant effect associated with a 22% total or 29% POM C decline was extremely low (0.18 and 0.29, respectively) and even lower power with smaller percentage declines (e.g. a 10% decline is associated with a power of 0.08). In both instances achieving the recommended power of 0.80 would have required a sample size of >40 and >20 replicates for total and POM C, respectively (Thomas, 1997).

The lack of power to detect significant changes in soil C pools, given their large spatial variation at fine scales, is a common issue (Throop & Archer, 2008). Recent work by Saby *et al*. (2008) indicates that the ability to detect changes in C pools is largely site specific and annual changes in these pools are difficult to detect. This may have prompted studies conducted at fine spatial and temporal scales to deem changes in soil C pools at $P < 0.10$ as significant (e.g. Carney *et al*., 2007). We followed a more conventional and conservative approach where $P < 0.10$ is considered marginally significant and $P < 0.05$ is considered significant. Given *M. vimineum*'s widespread occurrence and a call for increased emphasis on understory invaders of forest systems (Martin *et al*., 2009), more studies such as ours that assess the impacts of invasion on forest soil C pools are needed. Use of fractionation and stable isotope approaches will, where applicable, increase the ability to resolve changes in soil C stocks and cycling. When conducting future studies, investigators need be aware that the power to detect statistically significant changes in C pools is generally below recommended values when logistically feasible replicate numbers are used. Failure to detect statistically significant effects should not then be interpreted as a null effect of grass invasion on forest C pools. Yet, nor is it evidence for an unmeasured effect. So, whether the nonsignificant but obvious mean decrease in mineral-associated C under invasion that we observed is a 'true effect' of *M. vimineum* invasion is not known. We suggest that more studies and then meta-analysis of their collective results will indicate the significance, direction, and size of the effect on soil C associated with grass invasion of forests. The use of meta-analysis to explore the result of multiple studies using small sample sizes is successfully and widely adopted in other fields (Gurevitch & Hedges, 1999).

In contrast to the mineral-associated C pool, C derived from native sources in total organic and POM C were significantly (at least marginally) lower in the invader's presence. Declines in these pools may indicate that C is cycling faster in plots where the invader is present due to invasive inputs priming its decomposition. For example, native-derived POM C decreased by ~34% and *M. vimineum*-derived C only accounted for ~6% of the pool, highlighting a potential imbalance between POM C decomposition and formation. Further declines will be contingent on whether native-derived POM C has stabilized under the invasion. Future research will need to address such contingencies if we are to understand the long-term impacts of this invader on soil C.

Neither DOC nor DON was significantly impacted by *M. vimineum*'s presence and we found no significant difference in inorganic N pools. This last result contrasts with other studies conducted in the northeastern US which showed that *M. vimineum*'s presence was associated with changes in available NO_3^- and nitrification rates (Kourtev *et al*., 1999, 2003; Ehrenfeld, 2003). We found that *M. vimineum* was not associated with a change in available NO_3^- although soil pH, which typically increases when NO_3^- is the dominant form of plant available N (Nye, 1981; Ehrenfeld, 2003), was greater in the invader's presence. One possible reason for the contrast between our study and studies conducted in northeastern forests may be the association between nonnative earthworm biomass and *M. vimineum*. Nonnative earthworm biomass is often associated with altered N availability and mineralization in soils (Scheu, 1987; Kourtev *et al*., 1999; Eriksen-Hamel & Whalen, 2008) and may have contributed to changes in N pools in invaded northeastern US forests (Bohlen *et al*., 2004; Marhan & Scheu, 2006; Eisenhauer *et al*., 2007). Given earthworm biomass did not differ between our plots, we were able to explore *M. vimineum*'s effect under a uniform earthworm gradient.

Extractable microbial biomass C, both total and native-derived, was lower in plots invaded by *M. vimineum*. Microbial biomass C, determined using a modified CFE method, is expected to measure total active and inactive biomass (Wardle & Ghani, 1995; Bradford *et al*., 2008c). Declines in microbial biomass C have often been associated with declines in soil C pools (Wardle & Ghani, 1995; Bradford *et al*., 2008a, b). Since microbial biomass C is likely to be a responsive belowground C pool then its decrease may be a bellwether of an overall decline in soil C associated with this invasion. We observed a greater proportion of microbial biomass C derived from *M. vimineum* prethan postsenescence (Table 2). Under actively growing *M. vimineum*, the microbial community derived ~30% of its C from the invader (Table 2). This is a remarkable percentage because it is much larger than the aboveground biomass of *M. vimineum* at the study site (relative to native plant biomass), and *M. vimineum* also has a rather superficial root system (Ehrenfeld *et al*., 2001; Claridge & Franklin, 2002) accounting for <10% of total biomass at our sites (unpublished data). That *M. vimineum*-derived C in the microbial biomass was much higher presenescence may suggest that the microbial community is attaining the bulk of this C via root exudates rather than foliar litter. Root exudates are in part composed of an array of low-molecular-weight compounds, such as glucose, which are readily used by microbes (van Hees *et al*., 2005). Such compounds have caused priming effects when applied to soils and may be the direct link between *M. vimineum* invasion and associated declines in soil C (Dalenberg & Jager, 1981; Blagodatskaya *et al*., 2007; Carney *et al*., 2007). Further work is necessary to establish this possibility. Additionally, we noted a decrease in the size of the microbial biomass pool but no concomitant decline in SIR biomass (Table 2; Fig. 1b). SIR biomass differs from CFE biomass because it may indicate physiologically active biomass rather than absolute biomass (Wardle & Ghani, 1995; Bradford *et al*., 2008c). If true then the microbial community under *M. vimineum* was more active on a per unit biomass basis.

The mineralizable C pool, a measure of microbially available C (Bradford *et al*., 2008c), was significantly lower under *M. vimineum* invasion (Fig. 1a). The decrease in this pool may indicate that it is cycling more rapidly. The soil CO_2 efflux data also support the idea that C is cycling more rapidly under *M. vimineum* invasion. Indeed, soil CO_2 efflux did not decrease under *M. vimineum* yet declines in mineralizable C as well as other C pools were observed (Fig. 2; Table 2). This discrepancy may be explained by more rapid turnover of belowground C pools (assuming autotrophic respiration is unchanged). That is, if soil C pools were simply smaller and turnover rates were unchanged then soil CO_2 efflux would be smaller in invaded plots. Instead, CO_2 efflux rates were equivalent in the presence/absence of *M. vimineum*, indicating that labile C pools under *M. vimineum* may be turning over more rapidly (i.e. C atoms have reduced residence times in the soil of invaded plots). To help determine the validity of this proposed mechanism we conducted an *in situ* pulse-chase with ^{13}C-glucose. The more rapid mineralization of this substrate under invasion supports our inference that soil C is turning over faster in invaded plots. Notably the difference in cumulative values was driven by higher rates of glucose mineralization during the first 5 h after glucose addition (Fig. 3).

The results of our study show that *M. vimineum*'s presence was associated with declines in several belowground C pools and these declines were likely caused by priming. *M. vimineum* represents a relatively minor quantitative detritus input (<3% of litter layer biomass immediately after senescence; Table 1) at our site but the higher chemical quality of *M. vimineum* litter (Table 1), in addition to its root exudates, appears to stimulate soil C decomposition. Other studies of invasive plant effects on belowground C pools, similar in context to our own, have observed relatively little change in soil C pools (Stock *et al*., 1995; Mack *et al*., 2001; Mack & D'Antonio, 2003; Hook *et al*., 2004; Valery *et al*., 2004; Bradley *et al*., 2006; Koutika *et al*., 2007; Litton *et al*., 2008). We speculate that what appear to be inconsistencies between our results and those of others may be consistent in the context of preferential substrate utilization vs. priming effects. That is, the impact an invasive plant species has on belowground C pools may depend on the quality and quantity of its C inputs relative to the quality and quantity of native ones. If invasive plant species' inputs are of high chemical quality (i.e. low C : N or low lignin : N) but low quantity relative to native inputs then a priming effect may occur leading to a decrease in belowground C pools. However, if quantity is high then preferential substrate utilization may occur. One key example of this is work conducted by Litton *et al*. (2008). They found that grass invasion in Hawaiian forests increased soil CO_2 efflux, represented a very large increase in litter inputs (i.e. 16–44-fold increase), but did not impact mineral soil C pools. Such results are indicative of preferential substrate utilization. In our study, we found that *M. vimineum*'s presence did not change soil CO_2 efflux, its litter inputs only represented an $\sim$3% increase in total litter inputs, and we noted declines in several soil C pools. These results are indicative of a priming effect, as observed in Carney *et al*. (2007). Furthermore, when considering both Litton *et al*.'s (2008) study and ours there is support for the proposition that the strength of preferential substrate utilization vs. priming may be dependent on the relative increase in inputs (Blagodatskaya & Kuzyakov, 2008; Bradford *et al*., 2008c). Specifically, that priming effects tend to decrease as relative inputs increase. It is also important to recognize that effects may not be consistent across all belowground C pools, with both C input rates and their nutrient content affecting distinct C pools differently (Bradford *et al*., 2008c). By incorporating priming and preferential substrate utilization theories with our current understanding of invasive plants, we may be able to understand and predict how invasive plants affect soil C cycling and long-term soil fertility.

In conclusion, we have demonstrated that at our site *M. vimineum* invasion is associated with declines in the size of several belowground C pools and C gains derived from *M. vimineum* do not offset the loss of native, plant-derived soil C. The loss of C appears to be the result of priming via higher quality *M. vimineum* foliar litter inputs and possibly root exudates, which lead to increased soil C turnover rates where the invader is present. Whether our results can be generalized to *M. vimineum* invasions at other sites is not yet known. Assuming they are then our results indicate that invasions by *M. vimineum* may have long term implications for forest soil fertility, C storage, and potentially the flow of energy through detrital pathways to aboveground components of terrestrial foodwebs.

Acknowledgements

We thank C.A. Davies for his assistance with the ^{13}C-glucose pulse-chase, N. Fierer and C. Lauber for assistance with qPCR, and B.A. Snyder and M. Callaham for assistance with earthworm sampling. We thank three anonymous reviewers whose comments have improved earlier versions of this manuscript. This research was supported by the Andrew W. Mellon Foundation and by National Science Foundation grants to the Coweeta LTER Program.

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