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REGULAR ARTICLE

Root carbon flow from an invasive plant to belowground foodwebs

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Abstract
Aims Soil foodwebs are based on plant production. This production enters belowground foodwebs via numerous pathways, with root pathways likely dominating supply. Indeed, root exudation may fuel 30–50 % of belowground activity with photosynthate fixed only hours earlier. Yet we have limited knowledge of root fluxes of recent-photosynthate from invasive plants to belowground foodwebs.
Methods Using stable isotopes, we quantify the proportion of recent-photosynthate transferred belowground from the invasive grass *Microstegium vimineum* A. Camus, a widespread invader of forest understory. Given its minimal root biomass (~8 % of individual mass), we expected exudation to contribute little to belowground foodwebs.
Results Within 2 days of ^{13}C-labeling, we recover ~15 % of photosynthate carbon in microbial biomass. Recovery in root and dissolved organic carbon pools is consistently low (<2 %), suggesting these pools operate as 'pipelines' for carbon transport to soil microbes. The recovery of the label in wolf spiders – forest floor predators that feed on soil animals – highlights that root inputs of recent photosynthate can propagate rapidly through belowground foodwebs.
Conclusions Our results suggest that root carbon-exudation, an unexplored process of invasive grass inputs to forest foodwebs, may be an important pathway through which invasive species affect the structure and function of recipient ecosystems.

Keywords Detrital foodweb · Soil foodweb · Soil organisms · Exotic species · *Microstegium vimineum* · Rhizodeposition

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Introduction

Plant carbon enters foodwebs through multiple pathways, including herbivory, decomposition of leaf litter, and microbial assimilation of dissolved compounds exuded by roots (van der Putten et al. 2009). For native plant species, evidence is emerging that root pathways supply a substantial proportion of the carbon consumed by belowground foodwebs (van Hees et al. 2005; Pollierer et al. 2007; Högberg et al. 2010). Indeed, root exudation of recent photosynthetically-fixed carbon fuels a substantial fraction of belowground heterotrophic activity, accounting for as much as 30–50 % of soil respiration (Grayston et al. 1996; Dakora and Phillips 2002; van

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Hees et al. 2005; Högberg and Read 2006; Högberg et al. 2010). The rapid transfer (hours to a few days) of these root exudates to belowground foodwebs has been demonstrated through $^{13}CO_2$ pulse-chase experiments (e.g. Ostle et al. 2000; Leake et al. 2006; Carbone and Trumbore 2007; Högberg et al. 2008; Bahn et al. 2009). Similar evaluations for invasive species are lacking, despite the potential for these inputs to explain observed impacts of invasive species on belowground foodwebs and the ecosystem processes they regulate (e.g. Strickland et al. 2010).

Invasion by the grass *Microstegium vimineum* (Trin.) A. Camus is associated with changes in belowground communities and associated soil carbon and nitrogen cycling (Kourtev et al. 1998; Ehrenfield et al. 2001; Kourtev et al. 2002; Strickland et al. 2010). This shade-tolerant, C_4 annual, is native to southeastern Asia and is spreading throughout the eastern U.S., invading roadsides, trails, waterways, and forest understories (Warren et al. 2011b). In the southeastern U.S., its invasion of forest understories is associated with loss and accelerated cycling of soil carbon (Strickland et al. 2010, 2011). Notably, in this part of *M. vimineum*'s range, it commonly invades understory with minimal plant cover (Warren et al. 2011a). Hence, its invasion of these systems changes the understory structure from one largely devoid of vegetation to one carpeted by plant biomass. The additional detrital input that results from its invasion is primarily composed of aboveground biomass (Ehrenfield et al. 2001; Claridge and Franklin 2002). This is because *M. vimineum* has minimal root biomass (~2–10 % of total plant mass), a growth strategy not unusual for annuals (Monk 1966; Bradford et al. 2007a) (but see Garnier 1992). Its low root biomass would seem to preclude enhanced root inputs, following *M. vimineum* invasion of forests, as a mechanism explaining its impacts on soil carbon dynamics. Yet as an annual *M. vimineum* likely exhibits root traits that optimize resource uptake (Roumet et al. 2006), such as high rates of exudation (van Hees et al. 2005; Bais et al. 2006). If these rates are high they might compensate for its low root biomass, meaning its invasion does augment the supply of recent-photosynthate from roots to belowground foodwebs. However, it is hard to make inferences about this possibility because the majority of studies tracking root inputs to belowground foodwebs focus on native, perennial plant species (e.g. Ostle et al. 2000; Högberg et al. 2008; Wu et al. 2009).

Here, we pulse-label patches of forest understory invaded by *M. vimineum* with CO_2 enriched with the heavier stable isotope of carbon (C^{13}). Across the following week, we track the fate of the ^{13}C label from the leaves and stems, to the roots, the dissolved organic carbon and microbial biomass in soil. We also employ a bioassay using ground-dwelling spiders, to resolve whether the ^{13}C label propagates through the belowground foodweb. The motivation for our study was to rule out *M. vimineum* root exudation as a mechanism explaining losses of soil organic carbon following its invasion (Strickland et al. 2010, 2011). Our working hypothesis is that these losses are instead caused by augmentation of aboveground detrital inputs that stimulate soil carbon cycling. For root exudation not to play a role in this stimulation, we reasoned that the low root biomass of *M. vimineum* at our site (~8 % of total plant mass) would equate to a similar proportion of recent-photosynthate (as ^{13}C-label) being recovered belowground.

Materials and methods

Site description

Research plots were identified within a rapidly progressing *M. vimineum* invasion in a 60–80 year old mixed hardwood forest within the Whitehall Experimental Forest (WEF), Athens, Georgia, USA (N33° 53.27′ W 83°21.93′; 122 cm MAP; 17 °C MAT). The forest overstory at this site is closed and composed of *Acer rubrum*, *Quercus nigra*, *Platanus occidentalis* and *Liquidambar styraciflua.* Soils are sandy clay loams primarily of the Madison series and are classified as Typic Hapludults. The plots were located in habitat typical of that invaded by *M. vimineum* in the southeastern U.S. – specifically, local (<17.5 m) to stream edges and forest road (see Warren et al. 2011a). Diffuse light in the understory was 1.31 % and ranged from 8–28 μmol s^{-1} m^{-2}. As with many southeastern U.S. forests, the non-invaded understory was depauperate (<5 % plant cover). This low native ground cover is likely a product of poor dispersal by many native understory herbs and low soil fertility, which together hinder re-colonization of these forests following historical disturbances such as cotton agriculture. In invaded areas, *M. vimineum* forms a dense, continuous lawn with cover values >90 %.

Anecdotal reports indicate that *M. vimineum* established within the WEF ~15 years ago and this likely holds for our site. However, the invader probably went unnoticed until it formed dense cover and so pinpointing the exact invasion date is difficult (Martin et al. 2009). What we do know is that the invasion is active, and by 2008 it had extended >30 m beyond the invasion front delineated 2 years previously. Further site details are given in Strickland et al. (2010).

^{13}C pulse-labeling

For $^{13}CO_2$ pulse-labeling, three patches of *M. vimineum* were identified within the invasion. The grass forms a dense, monospecific stand, and areal estimates of percentage surface cover were 100 % in each of our experimental patches. Note that given the low understory biomass in non-invaded areas, there are not patches of native understory vegetation dense enough for us to compare to belowground allocation of recent-photosynthate in invaded patches. However, this did not detract from the objectives of our study, which were to determine whether the low root biomass of *M. viminuem* translated to an equally low allocation of recent photosynthate belowground. The belowground pools we resolved were roots, dissolved organic carbon (DOC) and soil microbial biomass. For the foodweb, we focused on resolving ^{13}C-label in the microbial pool because it dominates the heterotrophic biomass belowground (Fierer et al. 2009). Non-invaded plots would have quantified rates of autotrophic $^{13}CO_2$ fixation by soil microbes – a second pathway by which CO_2 might enter belowground foodwebs (e.g. Miltner et al. 2005a). However, rates of autotrophic fixation under optimal, lab conditions are at least 50-times smaller than the rates of belowground allocation we measured (Miltner et al. 2005b), meaning any autotrophic fixation of $^{13}CO_2$ by the microbial biomass will not influence the conclusions of our work (see Discussion).

Labeling was performed at the height of the *M. vimineum* biomass and prior to seed set. Each 1-m^2 patch of *M. vimineum* was bordered with a 10 cm deep wooden frame, cut 3 cm into the soil. Attached to this frame using duct-tape was a chamber constructed of 2 cm dia. PVC piping, covered with PAR-transparent polyester sheeting (Mylar®). The lower edges of the pyramid shaped chambers were 100 cm long and the upper edges, around the opening in the top, were 34 cm long, with the sloping sides giving a total chamber height of 60 cm. The height ensured the full height of the *M. vimineum* canopy was contained within the chamber (Fig. 1) and the opening ensured there was minimal disturbance to environmental conditions (see next).

Each chamber had four PVC inlet lines (2 mm internal dia.) from a compressed gas cylinder containing 99.4 atom% $^{13}CO_2$ at a concentration of 350 μL L^{-1} (Spectra Gases Inc., Branchburg, New Jersey, USA), ensuring CO_2 concentrations in the

Fig. 1 An expanding invasion of *Microstegium vimineum* in a mixed hardwood forest in the southeastern U.S. Shown are two 1-m^2 patches of *M. vimineum* receiving ^{13}C-labeled CO_2 at atmospheric concentrations across 8 h, using an open chamber design. In the foreground the complete coverage of the understory by the invader is evident; in the left mid and background is the depauperate forest floor where the invader is absent

chamber were not elevated over ambient. Between the gas cylinder and the chamber was a manifold of flowmeters, so that flow was regulated through each line at 1 L min^{-1}. Inside the chambers, inlet lines were connected to silicon aquarium airline tubing that was run along the lower edges, and across the middle, of the chamber directly within the *M. vimineum* canopy. The aquarium tubing has many tiny holes, permitting labeled gas to be dispersed finely within the plant canopy. Labeling began at 0800 h and ceased at 1700 h, ~2 h after sunrise and prior to sunset, respectively. Note that by including the 102-cm^2 opening at the top of the chamber, our intention was to minimize disturbance of environmental conditions (e.g. light, CO_2 concentration, temperature, soil moisture) and we monitored these conditions across the 8 h pulsing events. Values were indistinguishable from non-chamber reference points, suggesting that plant allocation and belowground carbon dynamics likely reflect those in undisturbed invaded sites. Including the opening at the top of our chamber did preclude estimating community-level photosynthetic rates for the plots (using the difference in CO_2 concentration between inlet and outlet ports for a closed chamber). However, the focus of our study was the proportional allocation of fixed-carbon to the above- and belowground pools; and we estimated carbon fixed in our 8 h pulsing event by the ^{13}C-contents of the grass immediately after $^{13}CO_2$-pulsing ceased (described below and following Bradford et al. 2007b).

Sampling and isotope analyses

Immediately following cessation of labeling, a 0.25 m×0.25 m quadrat of *M. vimineum* leaves, stems and roots was harvested per plot to estimate the ^{13}C-label fixed and retained by the plants across the 8 h labeling event. Given the shallow rooting depth of the species (from the surface to ~3 cm depth), roots were obtained with minimal soil disturbance by gently unearthing each stem. Samples were hand sorted, roots washed clean of soil, and then placed at 65 °C until constant mass. After weighing, samples were ball-milled to a fine powder and total carbon and stable isotope ratios determined using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy) coupled to a continuous-flow isotope-ratio mass spectrometer (Thermo, San Jose, CA, USA). For the IRMS analytical precision was ±0.1 $\delta^{13}C$‰ and working standards were calibrated to PDB (Pee Dee Belemnite; the standard based on a marine limestone fossil whose ^{13}C:^{12}C ratio gives 0 ‰ on the $\delta^{13}C$ scale) using NIST-SRM 1577b Bovine Liver as a reference.

Given that the study employed an isotopic tracer enriched in ^{13}C above natural abundance values, atom% and not delta values were used to calculate the mass of ^{13}C label in the plant and soil carbon pools (see Fry 2006). Specifically, mass of ^{13}C label assimilated by the plants was determined by subtracting the atom% ^{13}C values of unlabeled materials (leaves, stem or root from a non-labeled patch) from labeled materials, and then multiplying the total carbon mass values for these plant parts by the calculated atom% excess ^{13}C values. Calculation of atom% excess values corrects for the natural abundance content of ^{13}C (~1 %) in organic samples that would otherwise be treated as a component of the isotopic tracer (Fry 2006).

Eighteen hours after cessation of pulse labeling, a second 0.25×0.25 m quadrat of *M. vimineum* leaves, stems and roots was harvested within each plot. In addition to plant sampling, soils were removed in the same quadrat to 5 cm depth, immediately passed through a 2-mm sieve, screened for fine roots that passed the sieve, and then analyzed for DOC and microbial biomass carbon. These assays were performed immediately following soil sieving. Plant, microbial biomass and DOC determinations were repeated 42 and 168 h following cessation of the pulse-labeling. To extract DOC, soils were shaken with 0.5 M K_2SO_4 for 4 h and then filtered using Whatman #42 papers. Microbial biomass carbon was estimated using a modified, chloroform-fumigation extraction method as described in Fierer and Schimel (2002, 2003). The method controls for potential soil moisture differences by using soil slurries, and compares the flush of DOC in fumigated samples against non-fumigated controls. Note that this method involves bubbling the extracted samples with CO_2-free air to drive off chloroform. A consequence of this is that no $^{13}CO_2$ could be dissolved and hence contaminating our DOC or microbial biomass samples.

To determine the total carbon and ^{13}C contents of the DOC and microbial biomass pools, the liquid extracts were introduced to the IRMS via a total organic carbon (TOC) analyzer. Just as with the plant material, DOC and microbial biomass values were derived for unlabeled samples taken adjacent to the plots to provide natural abundance atom%

values for calculation of ^{13}C label amounts. Specifically: $C_{label} = C_{pool} * (Atom\%_{post} - Atom\%_{pre})$, where C_{label} is the mass of ^{13}C label in the pool of interest (e.g. DOC), C_{pool} is the mass of C in the pool (calculated from mass*%total C), $Atom\%_{pre}$ is the atom% ^{13}C value for the pool before the labeling event in an invaded patch adjacent to the experimental plot, and $Atom\%_{post}$ is the atom% ^{13}C value for the pool at a specific time (e.g. 0 or 18 h) after the labeling event. Given that *M. vimineum* has a C_4-photosynthetic value (e.g. leaf material from the site: n=12; mean $\delta^{13}C \pm 1SE = -14.30 \pm 0.311$ ‰), and the native plant species at the site have C_3-photosynthetic values (~−30 ‰), it has a proportional content of ^{13}C greater than the native species. Constraining our calculations with natural abundance values from *M. vimineum* invaded patches was therefore necessary to avoid over-estimating incorporation of the ^{13}C label into the plant, soil and spider pools.

For the spider bioassay, the night prior to the pulse-labeling wolf spiders (Family Lycosidae) were detected in *M. vimeneum* patches using reflectance of their eyes in headlamps and then collected by hand. They were sorted into three size classes based on fresh weight biomass: 1.3–8 mg (small), 9–32 mg (medium), 300–308 mg (large). Five small, three medium and one large spider were introduced into 946 mL enclosures (dia. 7.15 cm) by size class (giving three enclosures per plot). Enclosures were constructed from plastic cylinders inserted 12 cm into the soil of each experimental plot, and sealed at the top using a rubber band and 1-mm screen mesh. Given pronounced intraguild predation, the different spider numbers and size classes per enclosure were employed to avoid cannibalism. Aboveground *M. vimeneum* material was searched for potential foliar herbivores when we initially stocked enclosures (i.e. immediately after the pulse-label) with spiders and at the end of the assay (no herbivores were recovered), ensuring the spiders only source of prey were soil/ litter animals. Wolf spiders actively pursue soil animals in detrital foodwebs (e.g. Collembola), consuming more than the standing biomass of many soil animal groups per annum (Moulder and Reichle 1972; Sanders and Platner 2007). After 168 h post-labeling, spiders were collected, dried, weighed and then milled to fine powder for total carbon and stable isotope ratios determinations. Additional spiders from the size classes were collected at the same time as the bioassay organisms, and immediately prepared for isotope analyses to provide natural abundance values (i.e. unlabeled spiders) from which to calculate ^{13}C-enrichment. Using IRMS, $\delta^{15}N$ values were determined simultaneously with $\delta^{13}C$ to ensure we could distinguish isotopic enrichment due to feeding on ^{13}C-labeled soil animals from starvation (see Oelbermann and Scheu 2002; McCue and Pollock 2008).

Data analysis

We present data in three different formats. First, we show the time-course of the $\delta^{13}C$ values of the plant and soil pools (Fig. 2 Results). Stable isotope pulse-chase data are often presented in this form (e.g. Ostle et al. 2000; Högberg et al. 2008; Wu et al. 2009) because it is easy to discern the increase (and usually decrease) of the ^{13}C label in a pool across time. Although effective at communicating temporal patterns, delta values do not equate to quantitative comparisons between pools of ^{13}C label dynamics (Fry 2006). For example, pools of different sizes may have different $\delta^{13}C$ values but similar amounts of ^{13}C label (and vice-versa) because delta (and atom%) values essentially are proportions. So, we also present data that are mass-balanced for pool size; expressed as absolute amount of ^{13}C label per unit space (Table 1 Results). In mass balancing these data, microbial biomass and DOC pools were expressed as g m^{-2} to 5 cm depth using a soil bulk density for the site of 1.08 g cm^{-3} (Strickland et al. 2010). Lastly, we present ^{13}C label mass data as a proportion of the ^{13}C label recovered from the plants (Fig. 3 Results) at the cessation of the 8 h labeling event: i.e. (mass ^{13}C in pool / mass ^{13}C label in total plant biomass at 0 h)*100, following the approach of Bradford et al. (2007b). This presentation accounts for variation in ^{13}C label fixed given spatial differences between plots in factors such as plant and microbial biomass. Together, the three different expressions of our data permit the dynamics of the label to be fully resolved.

Differences in absolute mass values of the plant and soil pools across time were examined with linear mixed-effects models where carbon pool (e.g. leaf or DOC) and time were treated as fixed effects. Plot was included in the error structure to account for the spatial and temporal nesting of the experimental design that introduces non-independence across data values. That is, by comparing multiple pools within a replicate plot at any one time there is spatial non-independence, and by comparing ^{13}C mass for an individual pool across time within a

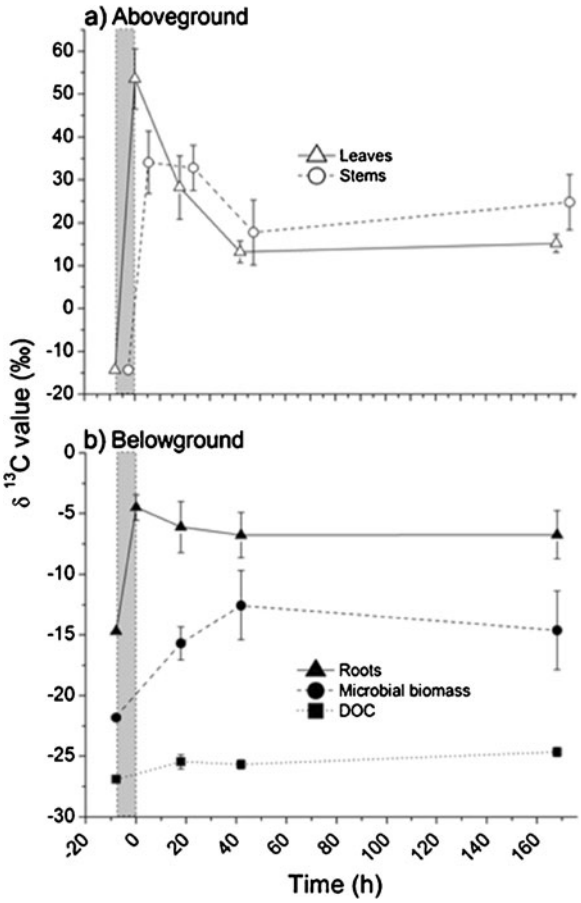

Fig. 2 Enrichment of above- (**a**) and belowground (**b**) pools with ^{13}C across 168 h following labeling with $^{13}CO_2$. The aboveground pools are *Microstegium vimineum* leaves and stems, and the belowground pools are *M. vimineum* roots, microbial biomass, and DOC (dissolved organic carbon). The grey bar indicates the time when the plots were pulse-labeled with $^{13}CO_2$ (an 8 h event). Datapoints preceding 0 h are natural abundance $\delta^{13}C$ values for each pool; points after the pulse event are ^{13}C-enriched. Note that only *M. vimineum* pools were sampled at 0 h. Values are means ± 1 S.E. (n=3). Stems are offset right to ease interpretation

plot introduces temporal non-independence. Mass 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 $P<0.05$. Note that although we follow convention and present $\delta^{13}C$ values (Fig. 2 Results), we do not analyze them for statistical significance. Reasons for this include issues with quantitative comparisons (see previous paragraph) and the fact that – for ^{13}C enrichments greater than natural abundance – the nonlinearity between a $\delta^{13}C$ value and the actual proportion of ^{13}C in a sample (see Fry 2006) makes statistical comparison of $\delta^{13}C$ values erroneous. For the spider bioassay, incorporation of the ^{13}C label was considered significant where the 95 % lower confidence interval did not cross zero (i.e. the enrichment value was significantly above natural abundance spider values).

Results

The $\delta^{13}C$ values of the plant pools show that the 8-h $^{13}CO_2$-pulsing event was successful at enriching the ^{13}C content of the plant parts (Fig. 2). Not surprisingly, the photosynthetic parts of the plant (stem and leaves) showed the highest initial enrichments, with delta values immediately after the pulsing (0 h) at least 45 ‰ higher than the natural abundance values recorded prior to the $^{13}CO_2$ pulse (Fig. 2). Enrichment of the root tissues with ^{13}C was also observed at cessation of the 8-h $^{13}CO_2$-pulsing event, with values increasing from −15 ‰ to −5 ‰ immediately post-labeling (Fig. 2). Delta values then dropped for all plant parts to the 42 h sampling point, but appeared to remain relatively stable between 42 and 168 h, suggesting the fixed carbon had been allocated to biomass or storage components of the plant tissue (Fig. 2).

Given the time required for recent photosynthate to translocate from aboveground plant to roots and then soil, we expected minimal ^{13}C enrichment of the soil microbial biomass and DOC pools at 0 h and so first resolved their $\delta^{13}C$ values 18 h following the $^{13}CO_2$-pulse event. Both pools showed enrichment of their $\delta^{13}C$ values at 18 h, although enrichment of the microbial biomass was ~7 ‰ and for the DOC <2 ‰. In contrast to the plant delta values, mean values for the microbial biomass increased between 18 and 42 h (Fig. 2), suggesting the microbes were net sinks for the carbon label across this time.

For the mass of ^{13}C label per unit space, there were different amounts of ^{13}C label found in the pools ($F_{4,33}=223$, $P<0.001$) and, statistically, the relative difference between pools was consistent across time (Pool × Time interaction: $F_{4,33}=1.51$, $P=0.22$). Specifically, at least four times as much fixed carbon was found aboveground (leaves and stems) than belowground, and the majority (>80 %) of the carbon belowground was found in microbial biomass, as opposed to

Table 1 Amount ^{13}C (mg ^{13}C label m^{-2}) across the pulse-chase study, and total pool sizes (g C m^{-2}), of *Microstegium vimineum* leaves, stems and roots, and soil microbial biomass (MB) and dissolved organic carbon (DOC)

Time (h)	Aboveground ^{13}C pools		Belowground ^{13}C pools			$F_{d.f.}$	P
	Leaves	Stems	Roots	MB	DOC		
0	8.2±0.79	8.5±0.75	0.26±0.01	nd[1]	nd[1]	$636_{2,6}$	<0.001
18	5.1±0.86	8.6±1.59	0.22±0.04	1.9±0.65	0.16±0.06	$43.4_{4,10}$	<0.001
42	3.3±0.29	5.9±1.67	0.20±0.03	2.4±0.20	0.11±0.02	$101_{4,10}$	<0.001
168	3.6±0.23	7.0±1.23	0.20±0.03	1.8±0.41	0.22±0.02	$128_{4,10}$	<0.001
Total[2]	11.01±0.07	16.6±2.16	2.37±0.28	26.9±4.92	9.27±1.14		

[1] not determined; [2] Total carbon (i.e. $^{12}C + ^{13}C$)

F and *P* values report the significance of pool identity on the amount of ^{13}C recovered at each time point. Values are means ± 1 S.E. (n=3)

roots and DOC (Table 1). These patterns held even 168 h post-labeling: for example, of the 2.20 mg ^{13}C label m^{-2} belowground, 1.78 mg (~80 %) was in the microbial biomass (Table 1). For the allocation to the different plant parts, the greater mass of ^{13}C-label aboveground seemed to reflect, in part, the fact that >90 % of the plant biomass was aboveground (Table 1). However, whereas roots made up ~8 % of the plant mass, we only recovered as much as 2 % of the ^{13}C label in them at any one time point, suggesting that roots served more as pipelines for carbon transfer to microbes as opposed to storage bodies.

Despite variation in the absolute amount of ^{13}C label fixed across the replicate plots (range of 14.566 to 19.785 mg ^{13}C label m^{-2}), the label dynamics were similar to those resolved for the absolute mass values (compare Table 1 and Fig. 3). Not surprisingly, given that they are the sites for photosynthesis, >95 % of the ^{13}C label immediately after the pulse was found in the leaves and stems. By 168 h post-labeling, ~60 % of this fixed carbon was retained aboveground, with ~14 % recovered belowground (Fig. 3). This suggests that across the 168 h following labeling, ~25 % of the fixed carbon was lost to respiration, or leached or translocated below the 5 cm of soil depth we resolved. As much as 17 % of the fixed carbon was found across the belowground pools at any time, with the majority (~15 %) being found in the microbial biomass (Fig. 3). On average, <2 % of the fixed carbon was resolved at any time in the root and DOC pools (Fig. 3).

In contrast to the plant, DOC and microbial biomass pools for which we resolved in situ pool sizes and ^{13}C label amounts, we only incorporated spiders to bioassay whether the ^{13}C label propagated through the belowground foodweb. Although only 0.030± 0.0129 % (mean±95 % CI) of the ^{13}C fixed was recovered in the spiders at 168 h post-labeling, the confidence interval did not overlap zero; demonstrating a statistically significant transfer of the ^{13}C fixed through the belowground foodweb to forest floor predators. However, only large spiders consistently showed ^{13}C-enrichment compared to natural abundance values (i.e. the lower 95 % confidence interval did not cross zero; mean proportion ^{13}C fixed±95 % CI: 0.040±0.0132 %). The enrichment of the large spiders appeared to be the product of assimilation of the ^{13}C-label, as opposed to starvation, because only the $\delta^{13}C$ (natural abundance: −25.34 %, mean enriched sample: −24.56 %, mean shift ± SE: 0.78±0.40 %) and not the $\delta^{15}N$ (natural abundance: 3.48 %, mean enriched sample: 3.30 %, mean shift ± SE: 0.18±0.11 %) value shifted markedly from natural abundance.

Discussion

Using a $^{13}CO_2$ pulse-chase approach we show that for *M. vimineum* as much as 15 % of recent-photosynthate carbon accumulates in the soil microbial biomass (Fig. 3), despite minimal accrual of the ^{13}C label in roots (<2 %; Fig. 3). Use of wolf spiders to bioassay the flow of the ^{13}C label to soil animals, an important prey of the spiders, demonstrates the potential for this carbon to propagate through belowground foodwebs and return to the aboveground. Such flows are expected where root inputs stimulate the activity of the microbial biomass, which in turn should stimulate

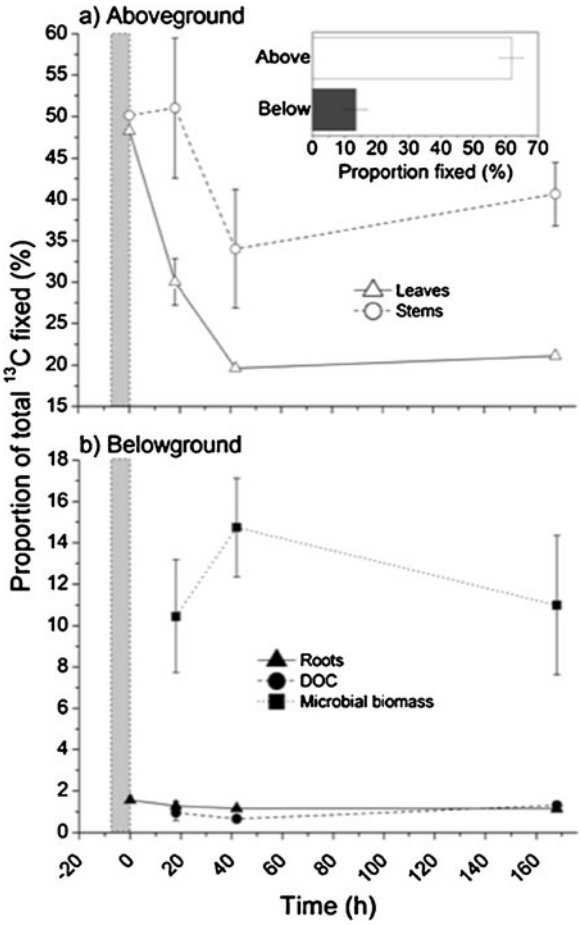

Fig. 3 Proportion of the ^{13}C label fixed by photosynthesis, recovered from the plant material after 8 h of labeling with $^{13}CO_2$, in above- (**a**) and belowground (**b**) pools across 168 h. The aboveground pools are *Microstegium vimineum* leaves and stems, and the belowground pools are *M. vimineum* roots, microbial biomass, and DOC (dissolved organic carbon). The grey bar indicates the time when the plots were pulse-labeled with $^{13}CO_2$ (an 8 h event). Datapoints preceding 0 h are not shown as values for all pools were zero (i.e. no enrichment with the ^{13}C label). Inset in (a) is the proportion of the ^{13}C label fixed that was recovered in above- and belowground pools after 168 h, with the remainder presumably lost via respiration or movement below 5 cm soil depth. Note that only *M. vimineum* pools were sampled at 0 h. Values are means ± 1 S.E. (n=3)

soil animal feeding on microbes (Bardgett and Wardle 2003; Bonkowski 2004). Together, these data show the potential for a widespread invader of forest understories to potentially be an important live-plant (as opposed to detrital) source of carbon to belowground foodwebs, and might explain its observed association with accelerated soil carbon cycling in southeastern U.S. forests (Strickland et al. 2010). Further work is required to establish the quantitative importance of *M. vimineum* as a live-plant source of carbon to belowground foodwebs. Notably Strickland et al. (2010) observed that, at our study site, ~30 % of the microbial biomass carbon was derived from *M. vimineum* during the growing season, and only ~5 % after senescence. Our work and those observations of Strickland et al. (2010) suggest that root inputs of recent photosynthate from *M. vimeneum* could substantially augment carbon supply to belowground foodwebs during the growing season in invaded forests.

Consistent with observations that recent photosynthate is transferred to roots by herbaceous species at timescales <1 day (Ostle et al. 2000; Carbone and Trumbore 2007; Bahn et al. 2009; Wu et al. 2009), ^{13}C enrichment of the root tissues was observed at cessation of the 8-h $^{13}CO_2$-pulsing event (Fig. 2). However, expecting only minimal ^{13}C enrichment of the soil microbial biomass and DOC pools initially, we first resolved their $\delta^{13}C$ values 18 h following the end of the $^{13}CO_2$-pulse event. If we interpolate a linear fit between the pre-pulse and 18-h post-pulse $\delta^{13}C$ values it suggests that marked enrichment might have been observed immediately at the cessation (0 h) of the pulse event in the DOC and microbial biomass (Fig. 2). Further work is required to verify whether recent photosynthate enters belowground foodwebs so rapidly in grass-invaded forests, but the apparent coupling between photosynthesis and soil microbial activity at timescales <1 day highlights the dynamic interplay between above- and belowground carbon cycling (Högberg and Read 2006).

Belowground, ~15 % of the fixed ^{13}C label was found in the microbial biomass, while <2 % on average was resolved in the DOC and roots (Fig. 3). These observations suggest that the DOC and roots serve as conduits of recent-photosynthate carbon to the soil microorganisms. The possibility that chemoautotrophic fixation of $^{13}CO_2$ instead explained the ^{13}C-enrichment of the microbial biomass does not seem plausible because, under ideal lab conditions, microbial CO_2 fixation rates through this pathway are ~55 times lower than needed to explain the enrichment we observed (Miltner et al. 2005a). Further, that the DOC pool accumulated little of the ^{13}C label is consistent with the idea that much of the recent photosynthate entering soils is in the form of low-molecular weight carbon compounds such as sugars, amino acids and carboxylic acids (van Hees et al.

2005). These compounds have high turnover rates (~10 h) in situ likely because microorganisms rapidly assimilate them (van Hees et al. 2005; Boddy et al. 2007; Fischer et al. 2010). Notably, exudation of these compounds is thought to promote uptake of soil nutrients (e.g. Bonkowski 2004), and annual plants exhibit root traits (e.g. low tissue density, high specific root length) consistent with a strategy that optimizes resource uptake (Roumet et al. 2006). These traits, combined with the lower proportional investment in root than shoot biomass by annuals (Monk 1966) (but see Garnier 1992), together seem to provide the most plausible explanation for the accumulation of the ^{13}C label in the microbial and not *M. vimineum* root biomass (Table 1). If *M. vimineum* is exhibiting root traits 'typical' for annuals, then we might expect the pattern of partitioning of recent-photosynthate carbon in soils underlying *M. vimineum* to generalize to other invasive annuals. Further work is required to test this possibility. Indeed, annuals may also have higher mycorrhizal colonization than perennials (Roumet et al. 2006) (but see Vogelsang and Bever 2009), and the rapid turnover, strong sink strength, and regulatory role of mycorrhizal hyphae in transferring carbon to soil foodwebs (Drigo et al. 2010), provides an additional explanation for the substantial allocation of recent photosynthate to the microbial biomass. If this pathway is important, it would likely mean that a fraction of the label recovered in the microbial biomass was directly transferred from root to mycorrhizae, bypassing exudation (e.g. see Epron et al. 2011). We could not find published reports of *M. vimineum*'s mycorrhizal status, but unpublished data suggest they show substantial (>50 %) root length colonization (Luke Flory, pers. comm.). Understanding *M. vimineum*'s mycorrhizal dependency under different environmental contexts will be an important research priority for understanding how it allocates carbon to belowground foodwebs and influences the soil carbon sink.

We did not measure the ^{13}C concentration of the pulsing chamber CO_2 (it was a mix of atmospheric and tank air) and so cannot estimate the photosynthetic assimilation rate for *M. vimineum*. Future work estimating the rate of recent-photosynthate supply from *M. vimineum* to forest soils will permit evaluation of the magnitude to which *M. vimineum* alters supply rates of this carbon in intact forests. We also did not track the ^{13}C label in soil respiration, and indeed the transfer rates (<1 day) belowground in our study might obscure measurements in the first 24 h due to physical exchange of chamber and soil air ^{12}C- and ^{13}C-CO_2 (see Högberg et al. 2008). Yet our study design does permit relative comparison of the fate of the fixed ^{13}C label with other studies. For example, Wu et al. (2009) estimated that in the 32 days following $^{13}CO_2$ labeling of an alpine meadow that ~30 % of the fixed carbon was lost as respiration from the system. If we use the ^{13}C amounts reported in Table 1 then this is a similar proportion to what we found: of the ^{13}C label recovered in the leaves, stems and roots at 0 h in our study (16.94 mg ^{13}C m^{-2}, Table 1), ~25–30 % was lost by the 42 to 168 h sampling events. Admittedly, our study ran for 7-days post-labeling, whereas the study by Wu et al. (2009) ran for 32 days. Had our study run for an additional 25 days we might have observed larger losses; albeit the amount of ^{13}C label lost from the foliar pools appeared unchanged between 42 and 168 h (Fig. 3) suggesting that, at least for the plant pools, respiratory losses of the carbon label are negligible 2 days post photosynthetic fixation. Assuming this is the case, then it's notable that Wu et al. (2009) found that of the ~70 % carbon remaining after 32 days, ~30 % was aboveground and ~40 % belowground, with the majority (~34 %) of the belowground carbon in roots. In contrast, we found that of the ~70 % remaining in our study that the majority was aboveground (~54 to 62 %) and, of the fraction belowground (~13 to 16 % after 42 h), the majority was in the microbial biomass. The reasons for these differences are most parsimoniously explained by the different plant allocation strategies in the two studies. In the alpine meadow studied by Wu et al. (2009) ~90 % of the plant biomass is roots, while for *M. vimineum* >90 % of the plant biomass is aboveground (Table 1). As opposed to a native vs. invasive comparison, this difference in allocation is probably a result of perennial vs. annual life history strategies; although species identity, age and environmental conditions all influence root: shoot ratios (Wilson 1988; Poorter and Nagel 2000). Studies tracking recent-photosynthate carbon tend to focus on native perennials (e.g. Ostle et al. 2000; Högberg et al. 2008; Wu et al. 2009), highlighting the need for more $^{13}CO_2$ pulse-label studies to resolve belowground dynamics of recent photosynthate across invasives, natives, perennials and annuals. Indeed, there is a growing appreciation that different phenologies and life histories of invaders to natives in recipient ecosystems might be important in

determining community and ecosystem-level impacts (e.g. Wolkovich and Cleland 2010), and it seems plausible that these strategies will influence the relative and absolute amount of recent photosynthate transferred to belowground foodwebs, and the consequent effects on soil carbon cycling.

At the forest site where our pulse-labeling study was performed, previous work associates *M. vimineum* invasion with enhanced soil microbial activity, and consequent loss of soil carbon (Strickland et al. 2010). This association holds across other bottomland, hardwood forests in the southeastern U.S. (Strickland et al. 2011). One motivation for our work was to assess the possibility that root-inputs of recent photosynthate from *M. vimineum* contributed to the enhanced microbial activity under invasions that Strickland et al. (2010) observed. Given the low root biomass of *M. vimenum* we expected to rule out this possibility. Instead, the recovery of ~15 % of the ^{13}C-label in the microbial biomass highlights the potential for root inputs to prime microbial activity, and the belowground foodwebs of which microbes constitute the primary trophic level. Whether *M. vimineum* has similar effects on belowground foodwebs in other parts of its introduced range is unknown. Notably, in many southeastern forests where *M. vimeneum* invades, the understories are largely devoid of understory vegetation (e.g. Warren et al. 2011a). This means that as *M. vimenium* forms the continuous lawns so characteristic of its invasion, it dramatically increases understory biomass. For our study this meant we couldn't pulse-label non-invaded patches of understory because there was little to no vegetation to label. In other parts of its introduced range the understory can be denser (e.g. Flory and Clay 2009), and potentially in these systems *M. vimineum* will not provide a pronounced augmentation of understory carbon. Investigations of soil carbon stocks and the belowground fate of recent photosynthates – across native and invaded understories in other areas of *M. vimineum*'s range – are needed to evaluate whether patterns observed in southeastern U.S. forests can be generalized to other parts of *M. vimineum*'s range.

Despite widespread grass invasions of forest understories (Ehrenfield et al. 2001; Mack and D'Antonio 2003; Litton et al. 2008; Flory and Clay 2009; Strickland et al. 2010), and a recent call for research investigating such understory invaders (Martin et al. 2009), little is known about the impact of grass invasions on carbon cycling in forests (Kourtev et al. 2003; Mack and D'Antonio 2003; Liao et al. 2008; Litton et al. 2008; Strickland et al. 2010). Our observations reveal substantial (~15 % to 5 cm soil depth) allocation of recent photosynthate to belowground foodwebs, suggesting root-carbon supply may be an important pathway through which invasive species are incorporated into native foodwebs. Notably, research to date on root exudation from invasive species has focused on modification of plant competition through allelopathy and changes to the soil environment (Weidenhamer and Callaway 2010). Excepting a study by Hamilton and Frank (2001), which tracked recent photosynthate from a pasture grass of mixed native and introduced origin into soil microbes, exudation as a pathway of carbon flow from exotic plants to native foodwebs has received no attention. Yet transfers of recent, photosynthetically-fixed carbon from the above- to belowground play a pivotal role in structuring the composition and functioning of terrestrial ecosystems (van Hees et al. 2005; Högberg and Read 2006; Paterson et al. 2007). This role and our findings suggest that to understand how invasive species will alter native ecosystems necessitates research on how invasive species modify the rate and magnitude of recent-photosynthate carbon allocation to belowground foodwebs.

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