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Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon

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Summary

- Soil organic carbon (SOC) is primarily formed from plant inputs, but the relative carbon (C) contributions from living root inputs (i.e. rhizodeposits) vs litter inputs (i.e. root + shoot litter) are poorly understood. Recent theory suggests that living root inputs exert a disproportionate influence on SOC formation, but few field studies have explicitly tested this by separately tracking living root vs litter inputs as they move through the soil food web and into distinct SOC pools.
- We used a manipulative field experiment with an annual C_4 grass in a forest understory to differentially track its living root vs litter inputs into the soil and to assess net SOC formation over multiple years.
- We show that living root inputs are 2–13 times more efficient than litter inputs in forming both slow-cycling, mineral-associated SOC as well as fast-cycling, particulate organic C. Furthermore, we demonstrate that living root inputs are more efficiently anabolized by the soil microbial community *en route* to the mineral-associated SOC pool (dubbed 'the *in vivo* microbial turnover pathway').
- Overall, our findings provide support for the primacy of living root inputs in forming SOC. However, we also highlight the possibility of nonadditive effects of living root and litter inputs, which may deplete SOC pools despite greater SOC formation rates.

Introduction

Soil organic carbon (SOC), the Earth's largest terrestrial carbon (C) reservoir, is predominantly supplied by plant inputs to the soil. Yet the link between plant C and SOC is the most poorly understood portion of the terrestrial C cycle, as substantial uncertainty surrounds the relative contributions of root vs shoot inputs to soil food webs and SOC pools (Pollierer *et al.*, 2007; Jackson *et al.*, 2017). This is a critical knowledge gap, as the type and proportion of above- and below-ground plant C inputs entering the soil can be dramatically altered by a suite of global change factors, such as CO_2 enrichment (e.g. Phillips *et al.*, 2012), plant invasions (e.g. Peltzer *et al.*, 2010) and nitrogen (N) deposition (e.g. Dijkstra *et al.*, 2004). Predicting the future behavior of the SOC pool must therefore be premised on a basic, empirical understanding of which sources of plant material are efficiently incorporated into protected, mineral-associated SOC pools, vs those which are more rapidly mineralized to CO_2 by the soil microbial community (Harden *et al.*, 2017).

A growing body of work emphasizes the importance of root-related inputs as sources of C to the SOC pool (Schmidt *et al.*, 2011; Clemmensen *et al.*, 2013; Jackson *et al.*, 2017). This perspective directly contrasts with the historical view of SOC formation that focused primarily on the contributions from above-ground structural plant litter, deposited as layers of organic material to the soil surface (Rasse *et al.*, 2005). In the root-centric view of SOC formation, much attention has specifically been focused on living root inputs – most notably, the organic compounds which enter continuously during the growing season into the surrounding rhizosphere soil (i.e. rhizodeposition) (van Hees *et al.*, 2002; Högberg & Read, 2006; Bradford *et al.*, 2008). Even though living root inputs generally constitute only a relatively small proportion of a plant's total photoassimilate relative to root and shoot structural litter (Pausch & Kuzyakov, 2017), they are posited to exert a disproportionate influence on the formation of slower-cycling, mineral-associated SOC. Structural litter inputs, by contrast, are thought to preferentially supply the faster-cycling, particulate organic C pool (Cotrufo *et al.*, 2015).

The combination of the biochemical quality of living root inputs, and the direct and intimate associations they experience with rhizosphere microbes and mineral surfaces, are theorized to promote more efficient formation of SOC per unit of C than from either shoot or root structural litter inputs (Rasse *et al.*, 2005; Jackson *et al.*, 2017). For example, labile dissolved organic C (DOC) compounds (e.g. simple sugars) in rhizodeposition are rapidly anabolized by the dense rhizosphere microbial community – a key precursor to the formation of microbially derived, mineral-associated SOC (Bradford *et al.*, 2013; Cotrufo *et al.*,

2013; Kallenbach *et al.*, 2016). Thus, through the '*in vivo* microbial turnover pathway' of SOC formation (*sensu* Liang *et al.*, 2017), these living root compounds should efficiently form mineral-associated SOC via organo-mineral associations with the surrounding mineral soil (Rasse *et al.*, 2005; Bradford *et al.*, 2013; Pett-Ridge & Firestone, 2017). At the same time, however, several types of living root input (e.g. oxalic acid in root exudates) are also known to potentially accelerate the decomposition of SOC via a rhizosphere priming effect (Finzi *et al.*, 2015; Keiluweit *et al.*, 2015). To accurately quantify the net C contribution of living root inputs vs litter inputs into the mineral soil, it is therefore necessary to differentially track these two sources of input into the soil over time scales that are relevant to the processes of SOC formation and loss.

Yet, there is a notable lack of field studies that track the full C flux of living root inputs vs shoot and root litter through the soil food web and into distinct SOC pools (Paterson *et al.*, 2009). In large part, this is because it is notoriously difficult to measure the full C contributions from living roots over a plant's life cycle (Pausch & Kuzyakov, 2017). One of the main approaches used to quantify living root inputs is ^{13}C pulse-labeling, which captures the fate of rhizodeposition during a defined time frame. While pulse-labeling provides high-resolution insight into the transfer of living root C through different soil pathways (Phillips & Fahey, 2005), it is tied to the period of labeling, and thus does not provide data on a whole plant's C flux (Kuzyakov & Schneckenberger, 2006; Paterson *et al.*, 2009). An alternative approach has been the use of a uniform label either through continuous, steady state labeling (Dijkstra & Cheng, 2007), or using the ^{13}C natural abundance differences found between C_3 and C_4 plants (Katterer *et al.*, 2011; Ghafoor *et al.*, 2017). However, few studies have capitalized on this latter approach to explicitly separate living root vs root and shoot litter inputs into soil C pools over multiple years – especially outside of an agricultural context (Jackson *et al.*, 2017).

Here, we conducted a field experiment with a C_4 annual grass in a temperate forest ecosystem dominated by C_3 plants. We manipulated the living roots and litter inputs of the C_4 grass over several of its life cycles, to track its C inputs through time. Specifically, we used the invasive forest understory grass *Microstegium vimineum* (Trin.) A. Camus, which is an ideal study species for several reasons. First, as an annual plant with a small and shallow root biomass (*c.* 2–8% of total biomass) and minimal fine roots (Ehrenfeld *et al.*, 2001), its entire litter inputs (i.e. shoot + root litter) can be easily manipulated. Second, within the eastern US forests which it invades, its C has been shown to be readily used by the soil microbial community and the soil food web (Bradford *et al.*, 2012; Tang *et al.*, 2012), and is thus an effective model plant through which to investigate forest soil C cycling processes (Paterson *et al.*, 2009). Third, because *M. vimineum* is a C_4 plant that often invades forest stands which consist entirely of C_3 vegetation, stable isotope ratios can be used to precisely track its carbon inputs through the soil microbial biomass, soil fauna, and into SOC pools (Strickland *et al.*, 2010; Kramer *et al.*, 2012; Tang *et al.*, 2012).

For this study, we isolated the living root inputs vs litter inputs (shoot + root) of *M. vimineum* by establishing three different manipulative treatments: (1) *M. vimineum* living root and litter inputs (i.e. unaltered plants), (2) *M. vimineum* living root inputs only (i.e. living plants, removed post-senescence) and (3) *M. vimineum* litter inputs only (i.e. senesced root + shoot litter) (see Fig. 1). Through this design, we sought to untangle the relative importance of living root vs litter inputs in supplying soil food webs and SOC pools. We predicted that, consistent with emerging theory (Cotrufo *et al.*, 2015; Jackson *et al.*, 2017), we would observe two primary pathways of plant C transfer to the soil: living root inputs would preferentially supply the more protected, mineral-associated SOC pool via the soil microbial biomass, whereas root and shoot litter inputs would predominantly supply the particular organic carbon (POC) pool and the decomposer food web.

Materials and Methods

Site and experimental design

We conducted the study in a mixed hardwood, temperate forest in North Branford, Connecticut, USA (41°21′N, 72°47′W; 11.2°C mean annual temperature, 119.7 cm mean annual precipitation). Dominant tree species include *Acer rubrum* L., *A. saccharum* Marsh., *Carya ovata* (Mill.) K. Koch, *Liriodendron tulipifera* L., *Quercus alba* L. and *Q. rubra* L. Soils at this site are Inceptisols predominantly in the Holyoke series, and classified as loamy, mesic Lithic Dystrudepts. The forest stand was selected because it did not contain the non-native C_4 grass *Microstegium vimineum* at the start of the experiment, but it was in the path of an advancing *M. vimineum* invasion. As such, we could experimentally seed *M. vimineum* into the forest stand and consequently manipulate its presence or absence, without ethical concerns of spreading an invasive plant.

Within this stand, we established six experimental blocks along a 500 m transect, spaced *c.* 100 m apart. Each block consisted of three experimental treatments (see Fig. 1), which included: (1) 'Living Roots + Litter' plots, that is undisturbed *M. vimineum* plants, which seeded and re-grew each year; (2) 'Living Roots Only' plots, that is living *M. vimineum* plants, whose shoot + root litter inputs were removed via hand-weeding immediately after seed drop and senescence; and (3) 'Litter Only' plots, that is the senesced shoot + root *M. vimineum* litter from Treatment 2, which we translocated to the soil surface of the 'Litter Only' plots (i.e. Treatment 3) every fall for the duration of the experiment (Fig. 1).

In the first year of the experiment, we established plots using *M. vimineum* seed collected from the advancing invasion in an adjacent forest stand. With these seeds, we planted the 'Living Roots Only' and 'Living Roots + Litter' plots at an approximate density of 450 seeds m^{-2} (the 'Litter Only' plot was not seeded with *M. vimineum* as it contained only detrital root/shoot *M. vimineum* material, as described above). Every experimental block also contained a control plot, kept free of *M. vimineum* ($n = 6$). Soil organic C pools, soil microbial biomass and soil fauna collected from these control plots were used as a control value for isotope mixing equations (described below). Overall, there were 24 total plots (including the control plots), each of which were 2 × 2 m.

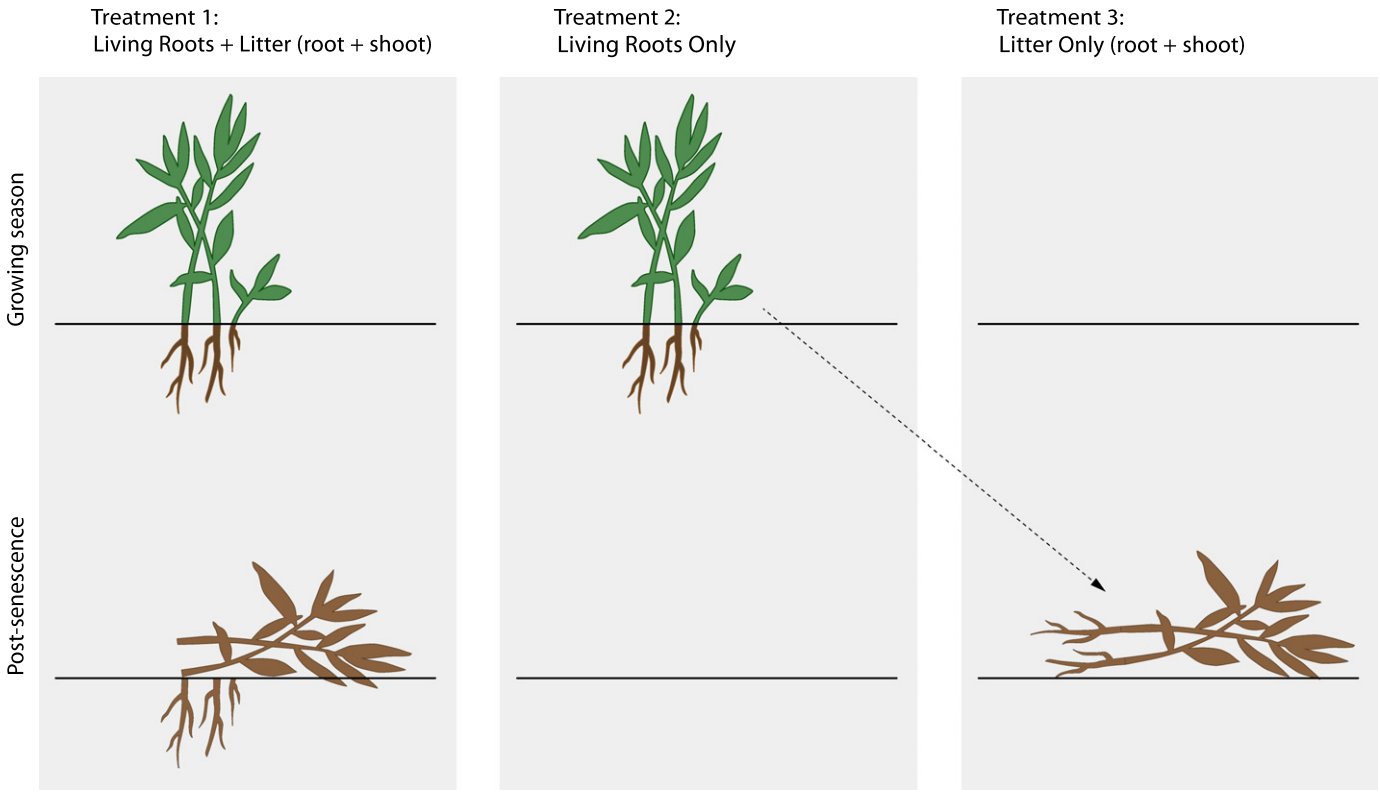

Fig. 1 Experimental design to test how plant carbon (C) from living roots vs litter inputs of *Microstegium vimineum* is incorporated into soil organic carbon (SOC) pools. The diagram shows one of six experimental blocks. During the growing season (upper panel), living root inputs from *M. vimineum* entered the soil in Treatments 1 and 2. Every autumn, after *M. vimineum* senescence and seed-drop (lower panel), shoot and root litter remained in place in Treatment 1, but in Treatment 2 (i.e. 'Living Roots Only') the root and shoot litter was removed and placed on the soil surface of the adjacent plot for Treatment 3 (i.e. 'Litter Only').

In the following years, the density and understory coverage of *M. vimineum* (up to 100% coverage) was roughly equal to the amount observed at the other stands at the site (Sokol *et al.*, 2017). In October 2012, after the first full growing season – and following *M. vimineum* senescence and seed drop – we carefully removed all shoot and root *M. vimineum* plant tissue from the 'Living Roots Only' plots and distributed it evenly across the surface of the 'Litter Only' plots (this transfer was done within a block, not among blocks). We repeated the same process in October 2013 and October 2014. The *M. vimineum* plants in the 'Living Roots + Litter' plots were left unaltered. To confirm the effectiveness of our experimental approach, we measured the quantity of *M. vimineum* roots that remained in the soil after root + shoot removal after the first year of the experiment in the 'Living Roots Only' treatment, and found that only a very small proportion of roots remained (1.0 ± 0.2 g *M. vimineum* roots 6250 cm^{-3}). Importantly, we note that hand weeding of *M. vimineum* from 'Living Roots Only' plots caused some soil disturbance (although it was minimal, due to the plant's very small and shallow root structure), and this caveat is considered in the Discussion section. We kept control plots free of *M. vimineum* through regular hand-weeding. The experiment concluded in October 2015.

Because 2013 was the first full growing season in which 'Litter Only' plots contained litter material, we refer to it throughout as 'year 1' and the subsequent years as 'year 2' (2014) and 'year 3' (2015). Thus, a feature of this experimental design was that the 'Living Roots Only' and 'Living Roots + Litter' treatment were receiving *M. vimineum* C input for an additional year, in the form of living root C inputs, compared to the 'Litter Only' treatment (i.e. they received living root C during the 2012 growing season). We take this difference into account when calculating the efficiency of incorporation of living root vs litter inputs over the course of the whole experiment, as described in detail below (see the Results section).

Plot sampling

We conducted all plot sampling in mid-September, during the peak of *M. vimineum* above-ground biomass growth at the site (Sokol *et al.*, 2017). In years 1, 2 and 3, *M. vimineum* biomass (shoot + root tissue) was sampled in the 'Living Roots Only' and 'Living Roots + Litter' plots. Specifically, within each plot, we harvested the *M. vimineum* root and shoot biomass contained within two 0.25 m^2 quadrats, dried it at 65°C, and weighed it. Also in each plot, we measured soil temperature (10 cm depth) and volumetric soil moisture (12 cm Time Domain Reflectometry probes) at several points throughout the growing season.

We sampled soils in years 1, 2 and 3. In each plot, 20 2-cm-diameter cores were extracted up to 10 cm depth (because in some places we hit rocks) in the A (mineral) horizon. Soil was passed through a 4 mm sieve on site, homogenized, and then all

samples were stored at 4°C in the lab for further analysis. We collected a second set of unsieved soil cores at each plot to calculate bulk density. As the main aim of this study was to track the C inputs from living roots vs litter after several years, we intensively sampled in year 3, when, in addition to SOC pools, we also measured *Microstegium*-derived C in soil microbial biomass (via chloroform-fumigation extraction) and in the soil arthropod decomposer food web. Thus, in year 3, in addition to the soil cores, we harvested two intact blocks (0.25 m^2, 10 cm deep) per plot to collect soil arthropods and estimate *Microstegium*-derived C in the decomposer food web.

Laboratory analysis

All assays described in this section occurred in years 1, 2 and 3, unless explicitly noted.

Soil properties We measured soil pH using a 1 : 1 volumetric ratio of soil to water, on a benchtop pH meter. We determined gravimetric soil moisture by measuring the mass difference between fresh soils and soils that were dried to constant mass at 105°C. For water-holding capacity (WHC), we wet soils to 100-% field capacity, and then calculated the mass difference between wet and dried soils. In year 1, we used air-dried soils to calculate soil texture and bulk density. For texture, we estimated the portion of soil particles that were sand, silt and clay within 50 g of sieved soil, using a simplified version of the hydrometer method (Gee & Or, 2002). We measured bulk density on nonsieved soil cores to correct SOC values for C content per unit volume.

Soil carbon pools We tracked the accumulation of *Microstegium*-derived C into two distinct C pools: the mineral-associated soil organic carbon (MASOC) pool and the POC pool. The MASOC pool consists of relatively low molecular weight plant- and microbe-derived C compounds, which sorb to mineral surfaces or to existing organo-mineral structures (Lehmann & Kleber, 2015). While not all C in the MASOC pool is well protected from microbial decomposition (e.g. Keiluweit *et al.*, 2015), this C pool is considered overall to be slower-cycling than the POC pool, and contains the subset of C that is most stable over long time scales (Lehmann & Kleber, 2015). The POC pool is faster-cycling, and generally contains larger molecular weight compounds that are in the midst of the decomposition process.

We separated SOC into these two pools using a physical fractionation method, where soil samples (10 g air-dried soil) were shaken with 30 ml of the chemical dispersant sodium hexametaphosphate (NaHMP) for 18 h. Following the shaking period, samples were passed through a 53 µm sieve, which included an extensive rinsing with distilled water to ensure particles below the threshold size had passed through. The fraction that passed through the sieve was classified as MASOC, whereas the fraction retained in the sieve (> 53 µm) was defined as the POC pool (Paul *et al.*, 2001). Both fractions were dried at 65°C; in addition, a subsample of air-dried soil was dried at 105°C for air-dry to oven-dry weight corrections. Dried samples were ball-milled to a fine powder and analyzed for elemental %C and %N (Costech ESC 4010 Elemental Analyzer, Valencia, CA, USA) and $\delta^{13}C$ (continuous-flow isotope-ratio mass spectrometer, precision ±0.2%; Thermo Delta Plus Advantage, San Jose, CA, USA). The laboratory working standards included the glutamic acids 'USGS 40' and 'USGS 41', which were calibrated against the international standard Vienna Pee Dee Belemnite (Yale Analytical and Stable Isotope Facility, New Haven, CT, USA).

We estimated the proportion of *Microstegium*-derived-derived C in POC and MASOC pools by comparing the difference in the $\delta^{13}C$ values of these soil fractions to values in the block-specific control plot (e.g. Kramer *et al.*, 2012). We also measured plot-specific *M. vimineum* $\delta^{13}C$ isotope values from dried and ball-milled samples of plant tissue. Across plots, the average $\delta^{13}C$ of *M. vimineum* was $-13.5 \pm 0.1‰$, while the average $\delta^{13}C$ of C_3 tissue was $-26.8 \pm 0.3‰$. We calculated the amount of *Microstegium*-derived C using the following mixing equation:

$$C_{Microstegium-\text{derived}} = C_{\text{pool}} \times (\delta^{13}C_{\text{treatment}} - \delta^{13}C_{\text{control}}) / (\delta^{13}C_{M.vimineum} - \delta^{13}C_{\text{control}})$$

where C_{pool} is the pool size of the SOC fraction (MASOC or POC), $\delta^{13}C_{\text{treatment}}$ is the $\delta^{13}C$ value of the SOC fraction in the specific treatment ('Living Roots Only', 'Litter Only', 'Living Roots + Litter'), $\delta^{13}C_{\text{control}}$ is the $\delta^{13}C$ of the SOC fraction of the block-specific control plot, and $\delta^{13}C_{M.vimineum}$ is the plot-specific value of the *M. vimineum* plant itself (Ineson *et al.*, 1996). We defined the remainder of C in an SOC fraction that was not *Microstegium*-derived C as C_3-derived Carbon stocks (both *Microstegium*-derived SOC and total SOC) were calculated using the bulk density values, and are presented as g of C per m^2 to 10 cm depth (Kramer *et al.*, 2012).

In addition to these C pools, we also measured the labile, 'microbially available C pool', through a lab-based C mineralization assay over a 30 d period (Paul *et al.*, 2001; Bradford *et al.*, 2008). This assay provides a rough estimate of the size of the bioavailable C pool within the soil. Specifically, soil samples were maintained at 20°C and 65% WHC in 50 ml centrifuge tubes for 30 d, and CO_2 efflux was measured using an infrared gas analyzer (IRGA: Li-Cor Biosciences, Lincoln, NE, USA, Model 8100) on days 1, 3, 5, 10, 20 and 30. We plotted CO_2 efflux values through time, and calculated total microbially available C (g CO_2-C d^{-1}) as the area under the curve.

Soil microbial biomass We used two techniques to measure soil microbial biomass: the first to gain a proxy of active microbial biomass (via a substrate-induced respiration technique), and the second to track the movement of *Microstegium*-derived C through the microbial biomass C (MBC) pool (via chloroform-fumigation extraction). For the substrate-induced respiration (SIR) technique, a soil sample (4 g dry weight equivalent) was mixed with excess, easily degradable C substrate (yeast extract), followed by an incubation period of 4 h at 20°C (Anderson & Domsch, 1978; West & Sparling, 1986; Fierer *et al.*, 2003). We then measured respiration (CO_2 efflux) using an infrared gas

analyzer (see above); higher CO_2 efflux values (g C-CO_2 g^{-1} soil h^{-1}) serve as an index for a larger active microbial biomass.

In year 3, we used a chloroform-fumigation extraction procedure to measure the proportion of *Microstegium*-derived C in both the MBC and DOC pools (Vance *et al.*, 1987). We used a modified version of this procedure, as described by Fierer (2003), whereby two subsamples of soil (5 g dry weight equivalent) from each microcosm were shaken in 0.5 M K_2SO_4 for 4 h. One subsample was exposed to chloroform-fumigation (which measures MBC + DOC) and the other was not, thus serving as a nonfumigated control (DOC only). After shaking, the supernatant from each tube was poured through a #42 Whatman filter. Liquid extracts were analyzed for total C on a total organic carbon (TOC) analyzer (Shimadzu, Columbia, MD, USA), and for $\delta^{13}C$ on a Thermo GasBench coupled to a ThermoDelta Plus XP isotope ratio mass spectrometer (Thermo Fischer Scientific, Waltham, MA, USA). The MBC pool was calculated as the difference in organic C between the fumigated and unfumigated samples, divided by a correction factor (k_{EC}) of 0.45 (Beck *et al.*, 1997). The proportion of *Microstegium*-derived C in the MBC and DOC pools was calculated using the same mixing equation as described above, using the block-specific control plot as the $\delta^{13}C_{control}$ value.

Soil decomposer food web The two intact blocks from each plot, harvested in year 3, were immediately brought back to the lab for processing. We designated one intact block for macrofauna collection (i.e. > 2 mm) and one intact block for mesofauna collection (i.e. 0.1–2 mm). The first intact block, used for soil mesofauna extraction, was immediately placed within a Tullgren funnel and exposed to light for 72 h (McBrayer *et al.*, 1977). Extracted mesofauna samples were stored in 70% ethanol and then sorted under a dissecting microscope into the following three groups: Collembola (springtails), Oribatida (decomposer mites) and predatory mites (Acari: Mesostigmata and predatory Prostigmata), as these were the most abundant and well-represented groups across all plots. We broadly categorize the first two groups as detritivores/microbivores, and the latter group as generalist predators (Coleman *et al.*, 2017).

The second intact block was carefully hand-sorted for soil macrofauna; *c.* 1 h was spent per sample. Fauna were generally separated by taxonomic order, although with some further separation for distinct functional groups, and some at coarser distinctions (i.e. class) when appropriate, as described below. After arthropod sorting, multiple individuals were pooled together by taxonomic/functional grouping. Because we were primarily interested in comparing the extent of *Microstegium*-derived C enrichment across treatments, we only present data from faunal groups which were recovered in at least two of the six plots for each of the treatments (including the control plot, as these were necessary for baseline values). Based on these criteria, we present data on the following groups: Coleoptera (beetle) adults, soil-inhabiting Coleoptera (beetle) larvae, Araneae (spiders) and Isopoda (woodlice). The mean number of individuals (± SE) recovered in a single intact block for the mesofauna and macrofauna groups are shown in Supporting Information Table S1.

Samples were dried at 65°C, hand-milled with a mortar and pestle, and run for element %C and ^{13}C, as described above. Following Pollierer *et al.* (2007), we present the plot-specific $\delta^{13}C$ value for each arthropod group (i.e. the pooled individuals from a single arthropod group from a single plot) relative to the mean $\delta^{13}C$ value of the corresponding arthropod group from the control plots, to determine the relative biomass incorporation of *Microstegium*-derived C. We refer to this term throughout as the 'control-adjusted $\delta^{13}C$ value.'

Statistical analysis

We conduced all analyses using linear mixed models (LMMs) in R v.3.3.2 (R Core Team, 2017). We ran two different sets of LMMs: the first set was for response variables that were measured in years 1, 2 and 3, whereas the second set was for response variables that were only measured in year 3. Both sets of models included 'Treatment' as a fixed effect (i.e. 'Living Roots Only', 'Litter Only' and 'Living Roots + Litter') and 'Block' as a random effect.

The first of the models included 'Year' as a fixed effect to account for temporal correlation between plots, as well as a Year × Treatment interaction (i.e. for response variables: SIR 'active' microbial biomass, microbially available C, POC and MASOC). The interaction term was dropped from the model if it was not statistically significant at $P \leq 0.05$. Soil temperature, soil moisture, soil pH and *M. vimineum* biomass were included as covariates, to account for any plot-level or environmental differences which may affect SOC formation or loss. Per cent clay was included as a covariate in the model run for MASOC, as clay content can correlate with MASOC formation (e.g. Schimel *et al.*, 1994). If there was a significant effect of 'Year', we then ran the model separately for each year as a *post-hoc* approach to evaluate within-year treatment effects. For these *post-hoc* models, only *M. vimineum* biomass was included as a covariate because the lower *n* reduced the statistical power and the number of feasible covariates. We conduced multiple comparisons using Tukey's honest significant difference (HSD) test with the *glht* function in the MULTCOMP package (Hothorn *et al.*, 2008).

The second set of models included response variables which were only measured in year 3 (i.e. microbial biomass C, dissolved organic C, and *Microstegium*-derived C within soil mesofauna and soil macrofauna). Again, due to the lower *n*, *M. vimineum* biomass was the only covariate we included in these models. *Post-hoc* tests for multiple comparisons were conducted using Tukey's HSD test.

All residuals were checked for normality and homogeneity of variance. Response variables that did not meet assumptions of normality and homoscedasticity were log-transformed. Statistical significance was determined at $P \leq 0.05$, and marginal significance was defined as $P < 0.1$ (Hurlbert & Lombardi, 2009), chosen *a priori* to balance model specificity with the relatively low sensitivity of field studies to detect changes in SOC pools (e.g. Carney *et al.*, 2007; Throop & Archer, 2008; Kramer *et al.*, 2012).

Results

Microstegium-derived carbon in SOC (years 1–3)

Carbon inputs from 'Living Roots Only' of *M. vimineum* formed significantly more POC compared to C inputs from 'Litter Only' of *M. vimineum* (i.e. root + shoot litter) (coeff. = 5.7, $P < 0.001$). Throughout the experimental period, the greater formation of POC under 'Living Roots Only' relative to 'Litter Only' was generally consistent through time (i.e. there was no significant Year × Treatment interaction; Table S2). Specifically, averaged across 3 yr, the formation of POC from 'Living Roots Only' was greater than from 'Litter Only' by a mean value (± SE) of 153 ± 39%.

By contrast, for the formation of *Microstegium*-derived MASOC, the difference between treatments changed through time (Fig. 2b). This was driven largely by higher MASOC formation from 'Living Roots Only' inputs in year 3 (Year × Treatment interaction, coeff. = 8.8, $P < 0.001$; Table S3). We found no significant differences between treatments in year 1 or year 2. In year 3, the amount of MASOC formed from 'Living Roots Only' inputs was *c.* 106% greater than the amount formed from 'Litter Only' inputs (Tukey's HSD, $P < 0.001$), and also *c.* 47% greater than the amount of MASOC formed under 'Living Roots + Litter' inputs (Tukey's HSD, $P = 0.01$).

Microstegium-derived carbon in microbial biomass (year 3)

In year 3, the 'Living Roots Only' treatment averaged *c.* 660% more *Microstegium*-derived C in the MBC pool relative to the 'Litter Only' treatment (Tukey's HSD, $P < 0.001$) (Fig. 3a). The 'Living Roots + Litter' treatment averaged *c.* 460% more *Microstegium*-derived C in the MBC pool relative to the 'Litter Only' treatment (Tukey's HSD, $P < 0.001$). Notably, there were no statistically significant differences between treatments in the size of the total MBC pool in year 3 (i.e. the sum of *Microstegium*-derived MBC + non-*Microstegium*-derived MBC) (Fig. 3c; Table S4).

For the DOC pool, the 'Living Roots Only' treatment averaged 282% more *Microstegium*-derived C than the 'Litter Only' treatment ($P < 0.001$). The 'Living Roots + Litter' averaged 200-% more *Microstegium*-derived C than the 'Litter Only' treatment, but this difference did not reach statistical significance (Tukey's HSD, $P = 0.1$) (Fig. 3b).

Microstegium-derived carbon in soil fauna (year 3)

Across all treatments, we detected higher $\delta^{13}C$ values for all mesofaunal arthropod taxa (i.e. Collembola, Oribatid mites and predatory mites) relative to the *Microstegium*-free control (i.e. 'control-adjusted $\delta^{13}C$'), indicating consumption of *Microstegium*-derived C among all groups. However, for these mesofaunal groups, we did not detect significant differences between treatments (Table S5).

By contrast, there were significant differences between treatments for several macrofaunal groups (Fig. 4), indicating differences in their biomass incorporation of *Microstegium*-derived C. The control-adjusted $\delta^{13}C$ values were higher for soil-inhabiting Coleoptera larvae in the 'Living Roots + Litter' treatment relative to the 'Litter Only' treatment, by an average of 3.8 ± 1.4‰ (Tukey HSD, $P = 0.01$), and higher in the 'Living Roots Only' treatment relative to the 'Litter Only' treatment, by an average of 2.3 ± 1.1‰ (Tukey's HSD, $P = 0.09$) (Fig. 4a). A similar trend was observed among Coleoptera adults (Fig. 4b). The control-adjusted $\delta^{13}C$ values of Coleoptera adults in the 'Living Roots + Litter' treatment were greater than for those in the 'Litter Only' treatment by a mean value of 1.2 ± 0.6‰ (Tukey's HSD, $P = 0.08$).

The control-adjusted $\delta^{13}C$ values for isopods (woodlice) in the 'Living Roots + Litter' treatment were higher than under 'Litter Only' by an average of 2.6 ± 1.1‰ (Tukey's HSD, $P = 0.06$) (Fig. 4c). Among the Araneae (spiders), the control-adjusted $\delta^{13}C$ values for individuals in the 'Living Roots + Litter' treatment were greater than those in the 'Litter Only' treatment by an average value of 1.2 ± 0.6 ‰ (Tukey's HSD, $P = 0.09$) (Fig. 4d).

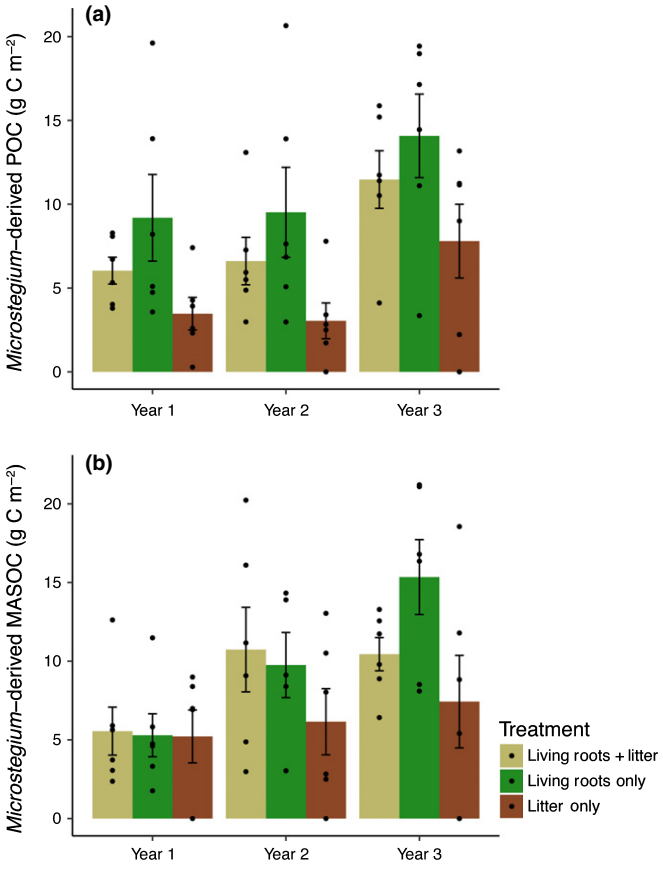

Fig. 2 *Microstegium*-derived soil organic carbon (SOC) formation from living roots compared to litter (i.e. root + shoot litter) in years 1–3. There was significantly greater *Microstegium*-derived C from living roots compared to litter in both the: (a) particulate organic carbon pool (POC) and (b) mineral-associated soil organic carbon pool (MASOC). Bars represent means ± SE ($n = 6$), and individual data points are shown as black opaque circles. For model regression coefficients and Tukey's HSD output for within-year comparisons, see Supporting Information Tables S2 and S3.

Total SOC, microbially available C and active microbial biomass (year 3)

In year 3, the microbially available C pool under 'Living Roots + Litter' averaged *c.* 41% larger than the 'Litter Only'

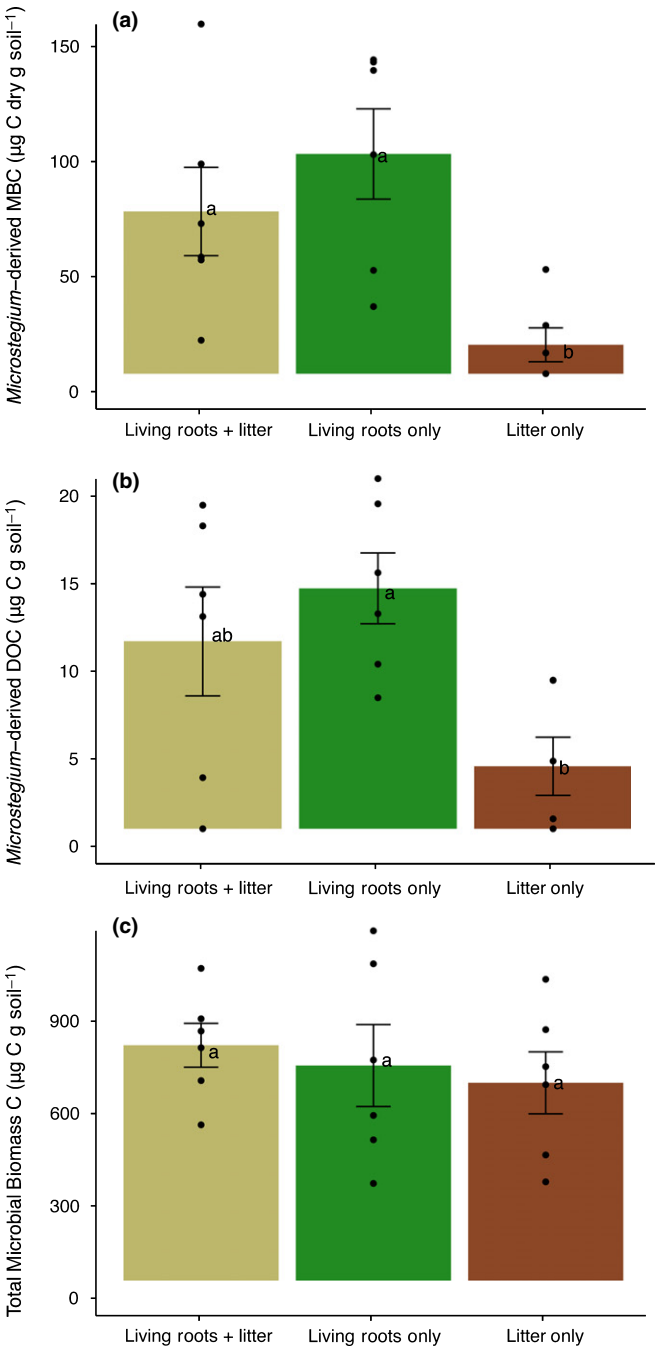

Fig. 3 *Microstegium*-derived carbon in the microbial biomass and dissolved organic carbon (DOC) pools in year 3. Bars represent means ± SE ($n = 6$), and individual data points are shown as black opaque circles. There was a greater quantity of *Microstegium*-derived carbon both in the: (a) microbial biomass carbon pool and (b) the DOC pool under the 'Living Roots' and 'Living Roots + Litter' treatment, relative to 'Litter' alone. (c) By contrast, the total microbial biomass carbon (MBC) pool between the three treatments was not significantly different. Letters indicate differences between means based on Tukey's HSD tests (significance determined at $P \leq 0.05$).

treatment (Tukey's HSD, $P = 0.02$) (Fig. 5a; Table S6). In addition, the active microbial biomass (i.e. SIR microbial biomass) under the 'Living Roots + Litter' treatment was on average *c.* 30% larger than the 'Living Roots Only' treatment (Tukey's HSD, $P = 0.09$) (Fig. 5b; Table S6). Notably, there was also a significant, positive relationship ($r^2 = 0.67$) between the size of the microbially available C pool and the amount of active microbial biomass (Fig. 5c).

There were no significant differences between treatments in the size of the POC pool (i.e. POC from both *Microstegium*-derived and non-*Microstegium*-derived sources), as well as the size of the whole SOC pool (i.e. MASOC + POC from both *Microstegium*-derived and non-*Microstegium*-derived sources) (Fig. 6a,b). However, the total MASOC pool under the 'Living Roots + Litter' treatment declined by *c.* 30% relative to the 'Litter Only' treatment (Tukey's HSD, $P = 0.06$) (Fig. 6c; Table S7).

Efficiency of living root vs litter inputs

To estimate the efficiency by which living root vs litter inputs were retained within the SOC pools of each plot, we calculated the proportion of *Microstegium*-derived C in the SOC pool (i.e. MASOC or POC) at year 3, relative to the amount of *Microstegium*-derived C that entered the soil over the entire experiment (Tables 1, S8). We estimated litter C inputs by summing the total shoot and coarse root biomass of *M. vimineum* in each plot. We estimated living root C inputs by multiplying the biomass value by a conversion factor (see next paragraph for details on the conversion factor). Because we added senesced litter from 'Living Roots Only' plots to the 'Litter Only' plots within an experimental block, we used the same biomass estimates for the 'Living Roots Only' and the 'Litter Only' plots for each experimental block (Table 1). We estimated the total C in plant biomass by multiplying the plot-specific biomass value (g biomass m^{-2}) by the average %C value of *M. vimineum* plant tissue across all plots (45 ± 0.22%).

To calculate living root C input, we derived the conversion factors from estimates in the literature that relate total photoassimilate to living root C contributions to the soil. In a recent meta-analysis, Pausch & Kuzyakov (2017) found that, across 16 studies and 128 datasets, the mean value (± SE) of net rhizodeposition for grassland species was 6.05 ± 0.48% of total photoassimilate (similar values were also found for crop and tree species). The authors also estimated, based on values from Riederer *et al.* (2015), that gross rhizodeposition was *c.* 2.2 times greater than net rhizodeposition. From these values, we therefore estimated that *c.* 13.3% of total *M. vimineum* net photoassimilate (i.e. measured as *M. vimineum* shoot + root biomass) was allocated to gross living root C input. This estimate falls within the range of prior estimates from annual plants (e.g. Marschner, 1986), as annual plants allocate less of their photosynthate below ground than do perennial plants (Grayston *et al.*, 1997; Pausch & Kuzyakov, 2017). This estimate also aligns with Jones *et al.* (2009), who estimated that about 11% of net fixed C is allocated to rhizodeposition. Thus, the conversion factor for 'Living Root Only' plots was 13.3% of the plot-specific biomass C value; the conversion factor for 'Living Root + Litter' plots was 113.3% of the plot-specific biomass C value; and the conversion factor for 'Litter Only' plots was 100% of the plot-specific biomass C value (Tables 1, S8).

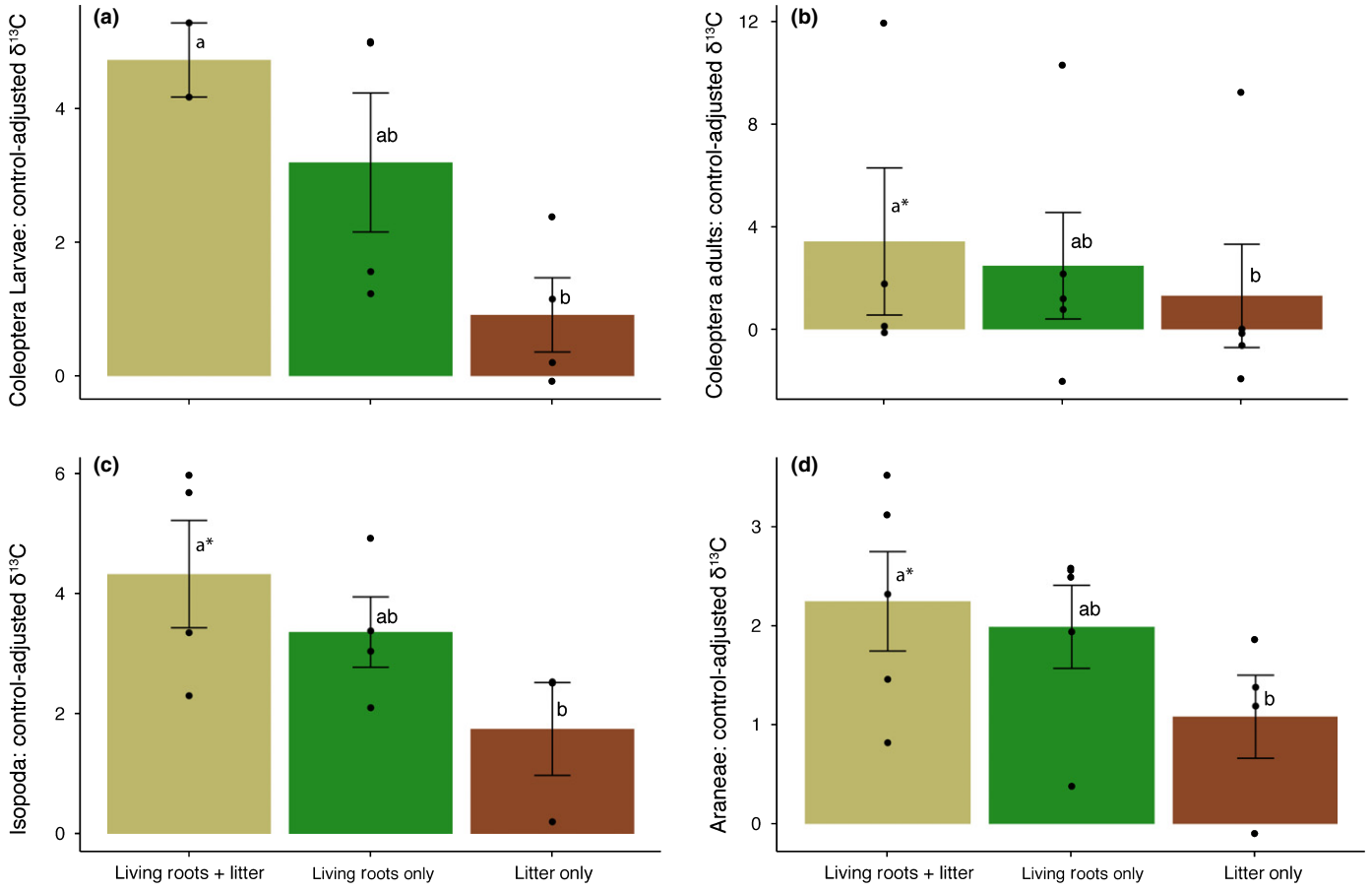

Fig. 4 Differences in the control-adjusted $\delta^{13}C$ values of soil macrofauna groups in year 3. Shown are the plot-specific, 'control-adjusted $\delta^{13}C$ value' for four different soil macrofaunal groups. For each faunal group, we calculated the control-adjusted $\delta^{13}C$ by subtracting the mean $\delta^{13}C$ value of the corresponding faunal group in control plots (i.e. plots with no *Microstegium vimineum*) from the $\delta^{13}C$ value of pooled individuals within each plot. The $\delta^{13}C$ values are all enriched compared to the controls (i.e. positive $\delta^{13}C$ values), indicating consumption of *M. vimineum*. Soil fauna include: (a) soil-inhabiting Coleoptera (beetle) larvae, (b) adult Coleoptera, (c) Isopoda (woodlice) and (d) Araneae (spiders). Individual data points are shown as black opaque circles. Bars represent means ± SE; $n \geq 2$ per treatment (i.e. between two and six plots per treatment contained individuals from the faunal group; see the Materials and Methods section for details). Letters indicate differences between means based on Tukey's HSD tests. $P \leq 0.05$ is significant, $P < 0.1$ is marginally significant and denoted by an additional '*'.

Based on these values, we used two approaches to estimate the percentage of *M. vimineum* living root vs litter inputs that were retained as SOC. For both approaches, we divided the amount of *Microstegium*-derived SOC that formed at the end of the experiment (i.e. either *Microstegium*-derived MASOC or POC) by the estimated amount of *M. vimineum* C input over the entire experimental period (Table 1). We note that one unavoidable feature of our experimental design was that plots with living root inputs received an additional year of C input than did plots with litter inputs, as described in the Materials and Methods section (i.e. 4 yr of living root input compared to 3 yr of litter input). We accounted for this time difference in our efficiency calculations by including an estimate of the sum of all C inputs over the full experimental period (Table 1, column b4).

In the first approach to calculate retention efficiency, we compared the percentage of SOC formed under the 'Living Roots Only' treatment vs the 'Litter Only' treatment. Specifically, an average (± SE) of 35.8 ± 9.4% of living root inputs were retained in the MASOC pool by the end of the experiment, whereas only 2.8 ± 1.3% of litter inputs were retained in the MASOC pool. We observed a similar difference in the POC pool: 32.0 ± 7.2% of living root C was retained as POC, vs 2.5 ± 0.6% of litter inputs (Table 1). To determine the relative efficiency of living root vs litter inputs, we thus divided the percentage retained under 'Living Roots Only' by that retained under 'Litter Only' (i.e. column c in Table 1) (Rasse *et al.*, 2005). Thus, through this first approach, we estimated that, per unit of C, living root inputs were retained both as MASOC and as POC with *c.* 13 times greater efficiency than litter inputs.

Because our experimental design had unavoidable artifacts – such as root litter decaying on the soil surface instead of in the soil – our first approach may be an overestimate of the contributions of living root inputs to SOC pools. Thus, we also used a more conservative approach to estimate the proportion of C retained from living roots. For the second approach, we calculated the amount of C retained under living roots by subtracting the amount of SOC retained under the 'Litter Only' treatment from the 'Living Roots + Litter' treatment (i.e. for the MASOC pool, 10.4–7.4 = 3 g C m^{-2}; see Table 1), and dividing this by the estimated amount of living root C input over the whole

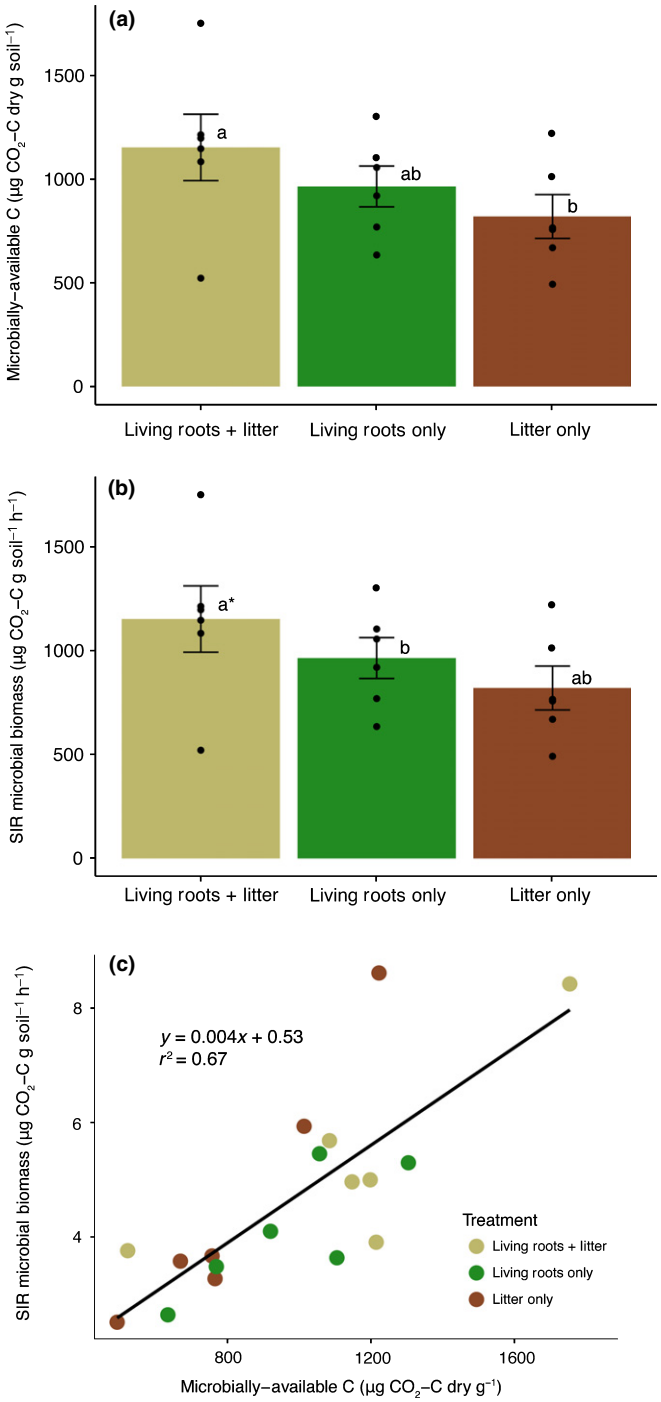

Fig. 5 The microbially available carbon pool and the active microbial biomass in year 3. Bars represent means ± SE ($n = 6$), and individual data points are shown as black opaque circles. The (a) microbially available C pool (measured through a 30 d mineralization assay) and (b) active microbial biomass (measured through substrate-induced respiration, SIR) were both highest in the 'Litter + Living Roots' treatment. (c) There was a strong positive relationship between microbially available C and active microbial biomass ($r^2 = 0.67$, $P < 0.001$). Letters indicate differences between means based on Tukey's HSD tests ($P \leq 0.05$ is significant, $P < 0.1$ is marginally significant and denoted by an additional '*').

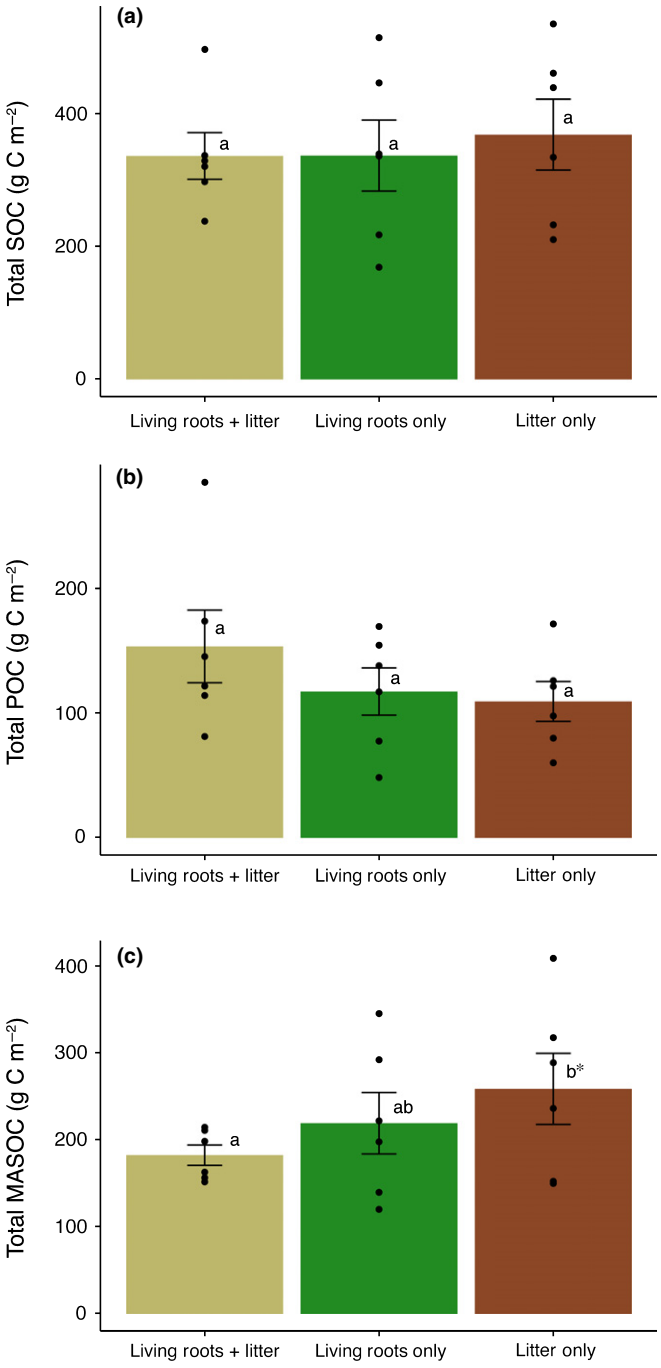

Fig. 6 Total soil organic carbon (SOC) pool sizes in year 3 (i.e. *Microstegium*-derived SOC + native SOC). Bars represent means ± SE ($n = 6$), and individual data points are shown as black opaque circles. There were no significant differences between the three treatments in (a) the total SOC pool (i.e. mineral-associated SOC + particulate organic carbon from both *Microstegium*-derived and C_3-derived C sources), as well as in (b) the particulate organic carbon (POC) pool, specifically. (c) The total mineral-associated SOC (MASOC) pool was reduced under the 'Living Roots + Litter' treatment relative to the 'Litter Only' treatment. Letters indicate differences between means based on Tukey's HSD tests ($P \leq 0.05$ is significant, $P < 0.1$ is marginally significant and denoted by an additional *.

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Table 1 *Microstegium*-derived carbon (C) retained in soil organic carbon pools after year 3

	(a) *Microstegium*-derived C pools		(b) Estimated *Microstegium* C input				(c) % *Microstegium* C retained in SOC pools (mean ± SE)	
Treatment	MASOC (g C m^{-2})	POC (g C m^{-2})	(b1) *Microstegium* biomass (g m^{-2})	(b2) %C in plant tissue	(b3) Conversion factor (%)	(b4) *Microstegium* C input (g C m^{-2})	% retained in MASOC	% retained in POC
Litter Only	7.4 ± 2.9	7.8 ± 2.2	760 ± 64	45	100	342 ± 64	2.8 ± 1.3	2.5 ± 0.6
Living Roots Only	15.3 ± 2.4	14.1 ± 2.5	903 ± 77	45	13.30	54 ± 10.3	35.8 ± 9.4	32.0 ± 7.2
Litter + Living Roots	10.4 ± 1.1	11.5 ± 1.7	699 ± 35	45	113.30	387 ± 44	3.4 ± 0.9	3.8 ± 1.1

The proportion of *Microstegium*-derived living root C vs litter C in the particulate organic carbon (POC) pool and the mineral-associated soil organic carbon pool (MASOC) varied across three experimental treatments (i.e. 'Litter Only', 'Living Roots Only' and 'Living Roots + Litter', $n = 6$) over 3 yr. To calculate the efficiency of incorporation of *Microstegium*-derived C into each SOC pool, we divided the amount of *Microstegium*-derived C from each SOC pool after year 3 (column a) by the estimated *M. vimineum* C input over the full experimental period (column b4), to derive the percentage of *Microstegium*-derived C that was retained in each SOC pool after 3 yr (column c). To calculated the *Microstegium*-derived C input over the experimental period (column b4), we multiplied the cumulative *M. vimineum* biomass (column b1) by the mean %C in *M. vimineum* plant tissue (b2, i.e. 45 ± 0.22%) as well as the conversion factor (column b3, i.e. Pausch & Kuzyakov (2017), Riederer *et al.* (2015)). See article text for more detail on conversion factors. We used plot-specific values for all calculations, and we present the mean values (± SE); all plot-specific values and calculations are shown in Table S8.

experimental period (i.e. 54 g C m^{-2}; see Table 1). This conservative estimate of living root contributions indicated that *c.* 5.6% of living root inputs were retained as MASOC, and 6.9% of living root inputs were retained in the POC pool, relative to 2.8 ± 1.3% and 2.5 ± 0.7% from litter inputs. Similar to the first approach, we then divided the percentage retained from living root inputs by that retained under litter inputs for each SOC pool. Through this second approach, we thus determined that per unit of C, living root inputs were retained as MASOC with 2.0 times greater efficiency, and as POC with 2.8 times greater efficiency, relative to litter inputs.

Discussion

In contrast to our prediction that litter and living root inputs would supply two distinct SOC formation pathways, we found that living roots were instead the overall dominant conduit of C to the mineral soil. Specifically, we predicted that *M. vimineum* living root inputs would predominantly supply the MASOC pool, whereas *M. vimineum* root + shoot litter inputs would primarily supply the faster-cycling, POC pool (Cotrufo *et al.*, 2015). Consistent with our first prediction, we found that after multiple growing seasons of C input from *M. vimineum* living roots and litter, there was *c.* 106% greater formation of *Microstegium*-derived MASOC in the 'Living Roots Only' plots than the 'Litter Only' plots (Fig. 2). Moreover, there was a substantially greater amount of *Microstegium*-derived C in the microbial biomass C pool and the DOC pool from living root inputs relative to litter inputs (Fig. 3), supporting the notion of a 'pipeline' of DOC transfer from living roots (e.g. root exudation), through the microbial biomass, and into the MASOC pool (Bradford *et al.*, 2012; Cotrufo *et al.*, 2015). Notably, and in contrast to our second prediction, there was greater formation of *Microstegium*-derived POC from living root inputs relative to litter inputs. Some prior studies have also shown that root-related inputs (root litter + living root inputs) can drive increases in the POC pool (e.g. Puget & Drinkwater, 2001). Therefore, there is a clear need going forward to distinguish the extent that the POC pool is supplied by labile, living root inputs vs structural inputs from root and shoot litter.

Overall, our work provides field-based empirical support that living root inputs are more efficiently retained than litter inputs (i.e. root + shoot) into MASOC and POC pools. Indeed, we found that, per unit of C, living root inputs were retained as SOC with 2- to 13-fold greater efficiency than litter inputs after 3 yr. This difference is in agreement with recent estimates which have looked at total root inputs (i.e. root litter + living root C) vs shoot inputs (Rasse *et al.*, 2005; Jackson *et al.*, 2017) as C input sources to the SOC. For example, Jackson *et al.* (2017) compiled studies which tracked root vs shoot C primarily using stable isotope tracers (Kong & Six, 2010; Menichetti *et al.*, 2015; Austin *et al.*, 2017; Ghafoor *et al.*, 2017), and all of which had roots grown *in situ*, and found a consistent trend showing the superiority of root-derived inputs as a source of SOC by a mean factor of *c.* 5.6. Our results provide evidence that living root inputs alone (i.e. without root litter inputs) exceed that of total litter inputs by the same wide margins as has been previously documented when comparing total root contributions to shoot inputs (Rasse *et al.*, 2005; Jackson *et al.*, 2017). Thus, according to our data, one possibility is that differences in the efficiencies of SOC formation from root vs shoot inputs are primarily driven by living root inputs.

These results must be considered, however, in light of our experimental design, which aimed to explicitly isolate the C inputs from living roots vs root + shoot litter. First, we added root and shoot litter to the soil surface in the 'Litter Only' treatment, which does not accurately simulate how decaying root material enters the mineral soil. When roots decay *in situ,* they are in close contact with soil microbes and mineral surfaces; owing to these intimate associations, decaying roots are prime candidates to form aggregate C or MASOC (Rasse *et al.*, 2005). As with shoot litter, when root litter is deposited above ground, it must first be physically incorporated into the mineral soil by decomposer organisms. Therefore, we may have disadvantaged the efficiency of root litter incorporation in the 'Litter Only' plots, and underestimated the

contribution of root litter relative to living root input. Second, we assumed that decaying root litter inputs (i.e. root turnover) during the growing season were negligible in the 'Living Roots Only' treatment, and thus that living roots were the only source of C within these plots. We made this assumption based on the short life cycle of *M. vimineum* (*c.* 5 months), and its minimal fine root structure (Ehrenfeld *et al.*, 2001). Regardless, possible root turnover during the growing season in the 'Living Roots Only' treatment would have elevated the contributions that we assumed were from living roots alone. Third, by physically removing the root + shoot litter in the 'Living Roots Only' treatment, we disturbed the upper mineral soil. This disturbance should have been relatively minimal, as the root structure of *M. vimineum* is so small and shallow (i.e. 2–8% of total biomass, and extending only a few centimeters into the soil). Yet this soil disturbance may have altered SOC formation in these plots. On the one hand, it may have accelerated the incorporation of living root C into the SOC pool. On the other hand, the disturbance may have stimulated SOC loses, as has been observed with disturbances in forests (Petrenko & Friedland, 2015), through cultivation (Elliott, 1986) or in cropping systems (Guo & Gifford, 2002). Because our experimental design may have led to either an underestimation or overestimation of the role of living root inputs, we used two methods to estimate the efficiency of C incorporation from living root inputs (see above). This range (i.e. 2–13 times more efficient) allows us to capture the spread of how potential artifacts influenced the conclusions that we reached.

Interestingly, we found that the amount of SOC formed from living roots and litter inputs was not summative: the amount of SOC formed under 'Living Roots Only' exceeded that of 'Living Roots + Litter' to the MASOC pool (Fig. 2a). The nonadditivity may be due to some of the experimental artifacts described above, or it might have been due to a microbial priming effect induced by the combined presence of litter and living root inputs (Fontaine *et al.*, 2007; Tamura & Tharayil, 2014). Specifically, the amount of microbially available C (i.e. the labile C pool) was highest in the 'Living Roots + Litter' treatment (Fig. 5a), as was the active microbial biomass (measured via substrate-induced respiration) (Fig. 5b). Due to the strong positive relationship between active microbial biomass and microbially available C (Fig. 5c), it is possible that the enlarged microbially available C pool under 'Living Roots + Litter' elevated the active microbial biomass. This increase in active microbial biomass could have, in turn, metabolized a portion of the newly formed MASOC pool, thus decreasing the net *Microstegium*-derived MASOC pool in the 'Living Roots + Litter' treatment.

Possible priming effects from living roots need to be considered not only for newly formed *Microstegium*-derived SOC, but also for non-*Microstegium*-derived SOC (i.e. C_3-derived SOC), to accurately assess the net effect of living roots on the size of the SOC pool. Indeed, losses in SOC that are stimulated by living roots can exceed the new SOC that their inputs form, leading to reductions in SOC stocks (Dijkstra & Cheng, 2007; Tamura & Tharayil, 2014). Within our plots and the time scale of our work, the size of the total SOC pool (i.e. MASOC + POC from both *Microstegium*-derived and C_3-derived sources) did not significantly differ across treatments (Fig. 6a). Yet when looking at the different SOC fractions, we found that the size of the total MASOC pool under 'Living Roots + Litter' was *c.* 30% lower than the 'Litter Only' treatment (Fig. 6c). This observation suggests that the combined presence of *M. vimineum* living roots and litter not only primed the loss of some of the new MASOC and POC formed by the living roots of *M. vimineum* (Fig. 2), but also decreased the native, C_3-derived MASOC pool (Fig. 6c). In line with this observation, Shahbaz *et al.* (2017) found that with increasing amounts of wheat residue added to the soil – regardless of whether it was shoot or root residue – there was both increased microbial priming of SOC and a reduced proportion of fresh inputs that were retained as SOC. Similar observations have been found in forest ecosystems with the experimental doubling of litter inputs (Sulzman *et al.*, 2005; Pisani *et al.*, 2016). Thus, beyond a certain threshold level of input – especially with the addition of structural litter inputs – it is possible for net SOC formation to be reduced and microbial priming of native SOC to be stimulated.

What is unclear, however, is how these SOC dynamics will play out over the longer term. While the combined presence of *M. vimineum* living roots and litter was associated with a decline in the total MASOC pool relative to the 'Litter Only' treatment, it was also associated with overall more rapid cycling of SOC. Specifically, there was not only greater formation of SOC under 'Living Roots + Litter' relative to the 'Litter Only' treatment (Fig. 2), and greater microbially available C and active microbial biomass (Fig. 5), but also greater usage of *Microstegium*-derived C among several soil fauna groups (Fig. 4). Among detritivore/microbivore and predator macrofauna (i.e. soil-inhabiting beetle larvae, adult beetles, woodlice and spiders), the greater ^{13}C enrichment in the 'Living Roots + Litter' treatment suggests that *Microstegium*-derived C was being used by the soil microbial community and propagating up the food web. Consistent with this finding, Bradford *et al.* (2012) also found that living root C from *M. vimineum* rapidly propagated through the food web and was recovered in surface-dwelling wolf spiders within a week after a ^{13}C pulse-labeling event. This more rapid cycling of C may lead to a continual reduction in MASOC stocks, if priming of old stocks exceeds formation of new MASOC (Dijkstra & Cheng, 2007). However, it is also possible that the priming effects may be transient (Lajtha *et al.*, 2014b) and that MASOC stocks could ultimately increase through time, even while there is more rapid cycling of SOC. Although some manipulative forest litter studies have shed light on longer-term dynamics of root- and shoot-related inputs (e.g. the detrital input and litter removal 'DIRT' experiments (Lajtha *et al.*, 2014a,b)), additional long-term, manipulative studies are clearly needed. The additional studies should aim to understand how living root inputs collectively influence the turnover and stock size of SOC, in addition to C flows through below-ground food webs, both alone and in the presence of litter inputs.

Conclusion

We show that, in a temperate forest ecosystem, the living root inputs of the understory grass *M. vimineum* were retained

between 2 and 13 times more efficiently than litter inputs in both the MASOC pool and the POC pool after several years of input. The dominant effect of living root inputs on net formation of MASOC is commensurate with expectations from the 'DOC-microbial pathway' theory (Cotrufo *et al.*, 2015), whereby labile DOC compounds are efficiently microbially anabolized, turned over and deposited into the MASOC pool. To our knowledge, this is one of the first studies that provides evidence that the efficiency of living root C in forming MASOC is at least partially due to its incorporation via the DOC–microbial pathway.

Our results thus collectively point to the importance of living root inputs in forming SOC. We highlight two key areas for future work, based on our results. First, as *M. vimineum* has such a small and shallow root system, it is important to determine how plants with different above-ground : below-ground biomass ratios, as well as different root traits, will alter the relative importance of living root vs litter inputs. For example, Poirier *et al.* (2018) recently compiled an extensive list of relevant root traits that may affect soil C formation; features such as root branching intensity, root depth distribution and specific root length will certainly influence the importance of root detritus relative to living root inputs. Second, through our design, we could not untangle which specific features of living root inputs were primarily responsible for the greater efficiency of net SOC formation via this pathway as opposed to via litter inputs. These specific features include the chemical composition of living root inputs, as well as their unique pattern-of-entry to the mineral soil relative to litter inputs (Rasse *et al.*, 2005). Deepening our understanding of the specific mechanisms underpinning the living root pathway, and the plant traits with which they are associated, will be critical steps to better represent the root and shoot pathways in soil biogeochemical models, and to predict the effects of altered terrestrial C cycling under global environmental change.

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Author contributions

N.W.S. and M.A.B. conceived the study. N.W.S., M.A.B., S.E.K. and E.K-A. contributed to field and laboratory work. N.W.S. analyzed the data and wrote the initial manuscript, with input from M.A.B. and S.E.K.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Mean number of arthropod individuals extracted per treatment within a single 6250 cm^3 intact soil block, in year 3

Table S2 Results from a linear mixed effect model examining the effects of 'Year' and 'Treatment' on the *Microstegium*-derived particulate organic carbon (POC) pool

Table S3 Results from a linear mixed effect model examining the effects of 'Year' and 'Treatment' on the *Microstegium*-derived mineral-associated soil organic carbon (MASOC) pool

Table S4 Results from a linear mixed effect model examining the effect of 'Treatment' on the total microbial biomass carbon (MBC) pool, the *Microstegium*-derived MBC pool and the *Microstegium*-derived dissolved organic carbon (DOC) pool in year 3

Table S5 Results from a linear mixed effect model examining the effect of 'Treatment' on the control-adjusted $\delta^{13}C$ value of macrofaunal and mesofaunal soil arthropod groups in year 3

Table S6 Results from a linear mixed effect model examining the effect of 'Treatment' on microbially available carbon (measured via a 30 d mineralization assay) and active microbial biomass (measured via substrate-induced respiration) in year 3

Table S7 Results from a linear mixed effect model examining the effect of 'Treatment' on total soil organic carbon pools (i.e. *Microstegium*-derived + non-*Microstegium*-derived C in the MASOC and POC pools)

Table S8 The plot-specific efficiency calculations for the amount of *Microstegium*-derived C retained in the MASOC and POC pools after year 3 (i.e. supporting information for the calculations and values in Table 1)

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