Root-derived inputs are major contributors to soil carbon in temperate forests

Adrienne Keller[1], Edward Brzostek[2], Matthew Craig[3], Joshua Fisher[4], and Richard Phillips[5]

[1]Indiana University Bloomington
[2]West Virginia University
[3]Oak Ridge National Laboratory
[4]California Institute of Technology
[5]Indiana University

May 18, 2020

Abstract

Roots promote the formation of slow-cycling soil carbon (C), yet we have limited understanding of the magnitude and controls on this flux. We hypothesized that root-derived inputs from ectomycorrhizal (ECM)-associated trees would be greater than those from arbuscular mycorrhizal (AM)-associated trees, and that soils receiving the greatest inputs would promote greater root-derived C accumulation in mineral-associated pools. We installed δ13C-enriched ingrowth cores across mycorrhizal gradients in six Eastern U.S. forests (n = 54 plots). Counter to our hypothesis, root-derived C was 54% greater in AM versus ECM-dominated plots, resulting in 175% more root-derived C in mineral-associated, slow-cycling pools in AM compared to ECM plots. Notably, root-derived soil C was comparable in magnitude to leaf litter inputs and aboveground net primary production. Our results suggest that variation in root-derived C inputs due to tree mycorrhizal dominance may be a key control of soil C dynamics in forests.

TITLE: Root-derived inputs are major contributors to soil carbon in temperate forests

AUTHORS:

Adrienne B. Keller[1,2], Edward R. Brzostek[3], Matthew E. Craig[4], Joshua B. Fisher[5], Richard P. Phillips[1]

[1]Department of Biology, Indiana University Bloomington; [2]Department of Ecology, Evolution and Behavior, University of Minnesota Twin Cities;[3]Department of Biology, West Virginia University;[4]Environmental Sciences Division and Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA; [5]Jet Propulsion Laboratory, California Institute of Technology Joint Institute for Regional Earth System Science and Engineering, University of California at Los Angeles

Adrienne B. Keller: kellerab@umn.edu; Edward R. Brozstek: erbrzostek@mix.wvu.edu; Matthew E. Craig: craigme@ornl.gov; Joshua B. Fisher: joshbfisher@gmail.com; Richard P. Phillips: rpp6@indiana.edu

AUTHOR CONTRIBUTIONS:

All authors contributed to planning the study. ABK, EB and MEC conducted the field work. ABK carried out the laboratory analyses and wrote the first draft of the manuscript. All authors provided feedback on the final manuscript draft.

DATA ACCESSIBILITY:

Upon acceptance, data from this manuscript will be archived in Figshare.

SHORT RUNNING TITLE:

Root inputs to forest soil carbon pools

KEYWORDS: belowground carbon allocation, mycorrhizal association, rhizodeposition, root exudation

TYPE OF ARTICLE: Letter

WORD COUNT:

Number of words in abstract: 146

Number of words in the main text: 4871

Number of references: 49

Number of figures: 4

Number of tables: 2

Number of text boxes: 0

CORRESPONDING AUTHOR:

Adrienne B. Keller

kellerab@umn.edu

4762 Manzanita St

Eugene, OR 97405

651-485-5822

ABSTRACT

Roots promote the formation of slow-cycling soil carbon (C), yet we have limited understanding of the magnitude and controls on this flux. We hypothesized that root-derived inputs from ectomycorrhizal (ECM)-associated trees would be greater than those from arbuscular mycorrhizal (AM)-associated trees, and that soils receiving the greatest inputs would promote greater root-derived C accumulation in mineral-associated pools. We installed δ^{13}C-enriched ingrowth cores across mycorrhizal gradients in six Eastern U.S. forests (n = 54 plots). Counter to our hypothesis, root-derived C was 54% greater in AM versus ECM-dominated plots, resulting in 175% more root-derived C in mineral-associated, slow-cycling pools in AM compared to ECM plots. Notably, root-derived soil C was comparable in magnitude to leaf litter inputs and aboveground net primary production. Our results suggest that variation in root-derived C inputs due to tree mycorrhizal dominance may be a key control of soil C dynamics in forests.

MAIN BODY

Introduction

Plants allocate a substantial amount of carbon (C) belowground (Gill & Finzi 2016), with important consequences for soil C storage. Root-derived inputs have been hypothesized to control soil organic matter dynamics by promoting soil C formation (Rasse *et al.* 2005; Clemmensen *et al.* 2013), stabilization (Jackson

*et al.*2017), and turnover (Cheng & Kuzyakov 2005). Thus, roots have the potential to both increase and decrease soil C stocks. Despite this, we know remarkably little about the magnitude of these fluxes, their controls, and the consequences of belowground inputs for soil C stabilization owing to difficulties of tracking belowground inputs. Given the importance of soil C storage in regulating global C cycling and mitigating the effects of rising atmospheric CO_2, it is critical to constrain estimates of belowground C supply and understand the fate of root-derived C fluxes in heterogeneous forests (Schmidt *et al.* 2011; Iversen *et al.* 2017).

While estimates of plant-derived C inputs to soil remain sparse, the total amount of C allocated belowground by plants is more commonly studied and can vary across climates, edaphic conditions and species. Previous work has revealed broad latitudinal patterns in total belowground C allocation, with 65% of GPP allocated belowground in boreal forests compared to only 30% in tropical forests (Gill & Finzi 2016). These patterns mirrored soil fertility gradients, such that partitioning of C belowground was inversely related to soil N:P. In contrast, Vicca et al. (2012) found that partitioning to belowground C was greater in nutrient-rich forests, likely due to underestimation of belowground C fluxes in nutrient-poor forests (e.g. allocation to mycorrhizal partners and root exudates which can be extremely difficult to quantify). Within a single site, there can be considerable interspecific variation in root traits (Valverde-Barrantes *et al.*2013), which may drive species-specific variation in belowground C allocation and nutrient uptake (Keller & Phillips 2019b). Moreover, there is little evidence of a direct, linear relationship between plant productivity and soil C accumulation, reflecting our poor understanding of the fate of belowground C allocation (Jackson *et al.* 2017). The degree to which belowground C allocation predicts C accumulation in soil has not been tested but is critical to understanding plant community effects on ecosystem C cycling and feedbacks to climate.

Belowground C fluxes from the plant to the soil are comprised of root and root-associated fungal turnover as well as rhizodeposition (e.g. sloughed root cells and passive and active exudation), making accurate quantitative estimates of this flux challenging (Pausch *et al.*2013). Traditionally, root turnover has been estimated either using sequential coring or minirhizotron approaches, with the remaining rhizosphere C flux approximated using a mass balance approach (Fahey*et al.* 1999, 2005; Hendricks *et al.* 2006). Root exudation can be directly measured from roots in the field using the cuvette method, whereby living roots are excavated from the soil and exudates are captured *in situ* (Phillips *et al.* 2008). However, these methods are both time and resource intensive, necessitating the need for simpler, time-integrated methods for estimating root-derived soil C inputs. The isotopic ingrowth core method takes advantage of the difference in $\delta^{13}C$ signatures between C_3 and C_4 plants (and consequently, the soils they are growing in), providing a quantitative estimate of root-derived soil C accumulation over the course of the study methods (Hoosbeek *et al.* 2004; Cotrufo *et al.* 2011; Martinez*et al.* 2016). As such, the isotopic ingrowth core method may provide a better estimate of root-derived C inputs compared to traditional methods.

Tree mycorrhizal association has been shown to be an integrative plant functional trait that links plant and soil properties (Phillips *et al.* 2013). In temperate forests, trees associate almost exclusively with one of two groups of mycorrhizal fungi: arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi. Theory predicts that divergent plant and fungal traits between AM and ECM species should lead to distinct patterns of belowground C inputs between mycorrhizal types. Previous work in temperate forests shows AM trees tend to preferentially allocate C to roots whereas ECM trees rely more heavily on hyphal proliferation to acquire scarce nutrients (Chen *et al.* 2016; Cheng *et al.* 2016). Such mycorrhizal type differences in the ratio of root production to total belowground C allocation may have consequences for soil C storage. For example, AM but not ECM plants promoted soil C destabilization in a pot experiment, with larger soil C losses when both roots and hyphae (rather than hyphae alone) were present (Wurzburger and Brookshire 2017). However, field and modeling studies suggest greater rhizodeposition in ECM compared to AM stands, which may explain greater soil C losses in many ECM-dominated forests (Yin et al. 2014, Brzostek et al. 2015, Sulman et al. 2017, but see Averill and Hawkes 2016). Thus, it remains unresolved how plant mycorrhizal type may influence patterns of root-derived soil C as well as the extent to which total belowground soil C inputs mirror root production patterns.

There is increasing evidence that plant mycorrhizal type is also predictive of soil organic matter formation patterns. Labile tissues with fast decay promote microbial activity, including microbial production and turnover. In turn, these products generated during microbial decay are thought to contribute disproportionately to slow-cycling soil organic C pools by forming associations with reactive silts and clays (Grandy & Neff 2008; Schmidt *et al.* 2011; Kallenbach *et al.* 2016). Accordingly, greater mineral-associated organic matter (MAOM) pools have been measured in AM systems (characterized by fast decay) compared to ECM systems (Craig *et al.* 2018; Cotrufo *et al.* 2019). This pattern suggests plant mycorrhizal type may be critical in determining stabilization of soil C. However, most of the empirical and theoretical work documenting these patterns (Sulman *et al.* 2017; Zhu *et al.* 2018; Jo *et al.* 2019) are premised on the idea of leaf litter quality differences between AM and ECM trees (Keller & Phillips 2019a). This is in contrast to studies showing roots may be a primary source of slow-cycling soil C (Sokol & Bradford 2019). Thus, there is a critical need to investigate whether tree mycorrhizal dominance affects belowground C inputs, and whether such differences contribute to MAOM formation patterns.

To this end, we measured total root-derived soil C accumulation and root production across nine-plot gradients of increasing ECM tree dominance within six temperate forests. We asked 1) what is the magnitude of root-derived soil C accumulation? 2) how does root-derived soil C accumulation vary across gradients of ECM tree dominance and 3) to what extent do belowground C inputs influence the formation of mineral-associated soil C? We hypothesized that root-derived inputs from ECM-dominated plots would be greater than those from AM-dominated plots, and that soils receiving the greatest inputs would promote greater C accumulation in MAOM pools. Using an isotopic ingrowth core technique, we found annual root-derived C accumulation was comparable in magnitude to annual aboveground net primary productivity, with greater root-derived C accumulation in AM-dominated compared to ECM-dominated plots. Root-derived C accumulation in MAOM-C pools was also greater in AM compared to ECM plots, providing evidence that mycorrhizal type differences in belowground C inputs can affect soil C stabilization patterns.

Methods

Site descriptions and experimental design

To examine plant community and soil type controls on belowground C fluxes in temperate forests, we worked in six eastern U.S. temperate forests within the Smithsonian Forest Global Earth Observatory (ForestGEO) network (Anderson-Teixeira *et al.* 2015): Harvard forest (HF; 42° 32' N, 72° 11' W) in North-Central Massachusetts, USA; Lilly-Dickey Woods (LDW; 39° 14' N, 86° 13' W) in South-Central Indiana, USA; the Smithsonian Conservation Biology Institute (SCBI; 38° 54' N, 78° 9' W) in Northern Virginia, USA; the Smithsonian Environmental Research Center (SERC; 38° 53' N, 76° 34' W) on the Chesapeake Bay in Maryland, USA; Tyson Research Center (TRC; 38° 31' N, 90° 33' W) in Eastern Missouri, USA; and Wabikon Lake Forest (WLF; 45° 33' N, 88° 48' W) in Northern Wisconsin, USA. At each of the six sites, we established nine 20m × 20m plots spanning a gradient of ECM tree dominance (by basal area) for a total of 54 plots. At each site, the three plots with the lowest ECM tree dominance and the three plots with the highest ECM tree dominance were designated as AM and ECM 'end-member' plots. The remaining three plots per site were designated as AM/ECM 'mixed' plots.

The sites vary in climate, soil type, and plant species composition but each host a diversity of AM and ECM associated canopy tree species. For the most dominant tree species at each site, see Table 1. Soils are predominantly Oxyaquic Dystrudepts at HF, Typic Dystrudepts and Typic Hapludults at LDW, Typic Hapludalfs at SCBI, Typic or Aquic Hapludults at SERC, Typic Hapludalfs and Typic Paleudalfs at TRC, and Typic and Alfic Haplorthods at WLF. For site-specific soil properties, see Table 2.

Ingrowth cores

In each plot, we installed five rigid plastic mesh root ingrowth cores (inner diameter 2.27"; Industrial Netting product #RN4465), hereafter referred to as "ingrowth cores", as well as two PVC cores that were impermeable to root and fungal ingrowth, i.e. "control cores". Cores were randomly places throughout the plot. The top and bottom of each ingrowth core was covered with window-screen mesh, while the control cores

were covered with 1-micron mesh to prevent root and fungal ingrowth. Cores were left in the field for two full growing seasons (Spring 2017 — Fall 2018).

To quantify plant-derived belowground C inputs to the soil, we used a dual ingrowth-core isotopic technique similar to Panzacchi et al. (2016). Each ingrowth core was filled with a C_4soil/sand mixture (50:50 by volume) to reduce soil compaction, increase detectability of root-derived C inputs, and enhance root recovery from ingrowth core soils following harvest. The soil was obtained from an agricultural field at the University of Illinois Energy Farm (40º 03' 046" N, 88º 11' 046" W) where soils are silt-loam Arguidolls. The field had been under a corn-soy rotation for >100 years, with corn planted most years including the year prior to soil collection. Given that corn is a C_4 plant, the soil carried a δ13C signature of -16.0 ± 0.15 (mean ± SE, n = 6), which was significantly enriched in ^{13}C compared to the C_3 root material recovered from the ingrowth cores (-28.7 ± 0.15 across all sites, n = 54). Surface soils from the farm field were collected and transported to the laboratory at Indiana University for ingrowth core preparation. There, soils were sieved to 4mm and organic debris was removed. Soils were then mixed with carbonate-free sand in a 50:50 ratio by volume and refrigerated until deployment in the field.

At the beginning of the growing season at each site, ingrowth and control cores were installed in each of the 54 plots. At each core location, a soil core of equal diameter to the ingrowth core was taken, soil was removed and replaced by an ingrowth (or control) core filled with the C_4-soil/sand mixture. Care was taken to minimize disturbance of the surrounding soil to prevent significant air gaps between the installed core and forest soil. After two full growing seasons, cores were carefully extracted and transported back to the laboratory for processing.

Roots were removed from each soil core, washed thoroughly, dried at 60 °C for 48 hours and then weighed to 0.0001g. The C_4 soil from each core was air-dried. Subsequently, all C_4 soil core samples and a subset of root tissue samples were analyzed for total C, N, and $\delta^{13}C$. Specifically, one AM and one ECM end-member plot was selected at each site and root samples from each core within these plots were analyzed, assuming root $\delta^{13}C$ is conserved across plots of similar mycorrhizal dominance within a given site. Root tissue and C_4 soil subsamples were ground to a powder using a 2010 GenoGrinder (SPEX® SamplePrep) and analyzed for total C and $\delta^{13}C$ using an elemental analyzer coupled to a gas-isotope ratio mass spectrometer. Root and C_4 soil samples were analyzed at two facilities (the Purdue Stable Isotope Facility with a PDZ Europa Elemental Analyzer coupled to a Sercon 20-22 IRMS, Cheshire, UK, and the BayCEER Laboratory of Isotope Biogeochemistry with a Carlo Erba 1108 Elemental Analyzer coupled to a delta S Finnigan MAT, Bremen, Germany). A subset of samples was analyzed at both facilities, confirming the two facilities reported comparable results ($R^2 = 0.96$). Isotope ratio values were expressed with the delta notation (δ):

$$\delta^{13}C\ [(R_{sample}/R_{standard} - 1) \times 1000]$$

where R_{sample} and $R_{standard}$ are the^{13}C : ^{12}C sample and standard ratios, respectively, and $R_{standard}$ is referenced to the Vienna Pee Dee Belemnite (VPDB).

Quantifying belowground C inputs

We quantified root production as the total root mass recovered within a given core after two years. Fine roots of temperate trees typically turn-over in one year (McCormack *et al.* 2015), and thus roots recovered in the ingrowth cores likely resulted from one and not two years of production. Consequently, we did not divide our estimates of fine root production by two. However, we acknowledge our root production values reflect the balance between root production and root turnover over a two-year period. We quantified root-derived C accumulation into each core using a two-pool mixing model following Panzacchi et al. (2016). Broadly, as C_3 rhizodeposits are incorporated into the C_4 soil core, the $\delta^{13}C$ signature of the C_4 soil becomes more deplete over time, i.e. the $\delta^{13}C$ signature becomes more similar to that of the C_3 soil. This change in $\delta^{13}C$ of the ingrowth core soil can be used to calculate total root-derived C inputs into the core.

First, the fraction of soil C derived from root inputs (F_{rd}; unitless) was calculated using a two-end member mixing model:

$F_{rd} = (\delta^{13}C_{ingrowth} - \delta^{13}C_{control}) / (\delta^{13}C_{root} - \delta^{13}C_{control})$

where $\delta^{13}C_{ingrowth}$ is the $\delta^{13}C$ of C_4 soil collected from an individual ingrowth cores after two years in the field and $\delta^{13}C_{control}$ is the average $\delta^{13}C$ of C_4 soil collected from two PVC control cores from the same plot as the ingrowth core after two years in the field. We estimated root $\delta^{13}C$ for AM/ECM 'mixed' plots as the mean of site-specific AM and ECM $\delta^{13}C$ values. For any given plot, $\delta^{13}C_{root}$was estimated as the mean $\delta^{13}C$ for a given mycorrhizal plot-type at a given site. The net root-derived C inputs ($C_{rd\text{-}net}$; g C m^{-2}) into surface soils (0-15 cm) was calculated as:

$C_{rd\text{-}net} = [?] * [C] * F_{rd} * 75000$

where [?] is the initial C_4 soil bulk density (g cm^{-3}), [C] is the C content (%) of the core after two years in the field, and 75000 is the conversion factor to transform % C to g C m^{-2} to a depth of 15 cm. Bulk density (1.21 g mL^{-1}) was measured on the initial C_4 soil: sand mixture and is thus constant across all plots. To estimate an annual net flux (for comparison to annual aboveground net primary production), we divided root-derived C by the number of years cores were in the field (*i.e.* two years at all sites).

Several assumptions are worth noting with this approach. First, the $\delta^{13}C$ signature is assumed to be uniform throughout the soil core, such that various C fractions (e.g. mineral-associated vs. particulate C) of the C_4 soil which may turnover at different rates are assumed to have identical $\delta^{13}C$ values. While recent crop rotations between C_4 and C_3 plants where the ingrowth C_4 soil was harvested could theoretically create heterogeneity in soil $\delta^{13}C$, any such differences are likely small given the long-term dominance of C_4 corn production at the site. Second, diffusion of dissolved organic C either laterally or vertically through the ingrowth cores could also deplete $\delta^{13}C$ signatures, resulting in artificially high estimates of root-derived soil C. However, vertical diffusion should be similar between ingrowth and control cores, and previous estimates of $\delta^{13}C$ depletion within ingrowth cores due to lateral diffusion suggest the effect is negligible (Phillips, R.P. *unpublished data*).

Soil C fractionations

We separated soil organic matter into MAOM and particulate organic matter (POM) to evaluate mechanisms underlying the persistence of root-derived C in ingrowth cores. Due to costs, we selected three of the six sites and processed all samples from the six end-member plots at each site. For each sample, we used a standard size fractionation procedure (Cambardella & Elliott 1992). Briefly, we dispersed 10 g soil samples in 30 mL of 5% (w/v) sodium hexametaphosphate for 20 h on a reciprocal shaker at 180 rpm and washed slurries through a 53-um sieve using a stream of DI water. Organic matter collected on the sieve was considered POM and the fine fraction that passed through the sieve was considered MAOM. Both fractions were dried (80 °C), weighed and ground to a powder. The MAOM fraction was analyzed for C concentrations and $\delta^{13}C$ at the Purdue Stable Isotope Facility as described above. Root-derived MAOM-C (MAOM-C_{rdc}; mg MAOM-C/g bulk soil) was then calculated as:

$\text{MAOM-}C_{rdc} = \frac{MAOM-C\ (mg)}{bulk\ soil\ (g)} * F_{rd}$

Aboveground net primary productivity and littermass estimates

We estimated aboveground net primary productivity (ANPP) for each plot by scaling tree diameter (diameter at breast height, DBH) to total aboveground biomass using species-specific allometric equations and calculating annual increment growth. ANPP includes all aboveground wood and non-woody production. Specifically, for plots within the ForestGEO forest dynamics plots where long-term tree census data are available, we calculated DBH growth using the two most recent censuses. This included all plots at SCBI (2013, 2018 census), SERC (2014, 2019 census), TRC (2013, 2019 census) and WLF (2013, 2018 census), as well as three HF plots (2013, 2019 census). For plots outside the forest dynamics plots, we measured DBH growth over one year (in 2019 for HF, in 2018 for LDW). In all cases, only trees alive and > 10 cm DBH in both censuses were included. In doing so, we recognize we are ignoring any new recruitment or death between measurements. DBH measurements were converted to total aboveground biomass using species-specific allometric equations following Gonzalez-Akre et al. (2016) or, when not available, from the allodb

R package (https://github.com/forestgeo/allodb, Gonzalez-Akre, pers. comm) Equations are provided in SI Table 1. Annual increment growth of aboveground biomass C (g C m^2yr^{-1}) was then calculated for each tree using a 0.47 conversion factor for biomass to C and summed at the plot-level to estimate annual aboveground net primary productivity (ANPP).

Plot-specific litter mass was measured in 2017 at all plots. Specifically, two to three litter collectors were placed in each plot, covering a total average area of ˜0.5-1.0 m^{-2} per plot. All litter, including leaves, woody debris and reproductive material, was collected two to three times over the course of senescence. Litter was dried at 60 °C for at least 72 hours and then weighed to 0.01g. Plot litter mass C inputs were calculated as the total mass of all litter collected in a given plot in 2017 and converted to units C using a 0.47 conversion factor for biomass to C.

Data analysis

We used multiple linear regression to assess the effects of site, ECM dominance and the interaction between the two on root-derived C or root production separately. To focus on differences between AM and ECM-dominated plots, we also conducted two-way ANOVA tests using just the six end-member plots (AM or ECM-dominated). Site, plot type and the interaction between the two were included as fixed factors and root production or root-derived C were dependent variables. In the case of root-derived C, no significant interaction between site and plot type was observed and was subsequently removed as an independent variable. In the case of root-derived MAOM-C, only plot type was significant and a Student's t-test was used to compared group differences. Student's t-tests were also used to assess global plot type differences. Tukey's post-hoc tests were used to evaluate pair-wise differences in each case. All analyses were carried out using R version 3.6.1 (R Core Team, 2019).

Results

Across six temperate forests, we quantified total root-derived C accumulation and root production in 54 plots spanning a gradient of AM-associated vs. ECM-associated tree dominance. Overall, we found that both total root-derived soil C and root-derived MAOM-C were greater in AM-dominated plots while root production was greatest in ECM-dominated plots. Importantly, annual root-derived soil C accumulation was greater than aboveground litter mass C inputs and rivaled ANPP in magnitude, highlighting the importance of this often poorly quantified component of ecosystem C budgets.

Root-derived C inputs

Root-derived C accumulation decreased with increasing ECM dominance at the plot-level (Fig. 1a; R^2 = 0.09, P = 0.013), and ECM dominance and site together predict root-derived C (R^2 = 0.30, P = 0.004). There was no significant relationship between root-derived C accumulation and root production (Fig. 1b). Moreover, we found no significant relationship between metrics of soil N cycling and root-derived C (data not shown).

Comparing the three most AM-dominated and three most ECM-dominated plots only (i.e. 'end-member plots'), root-derived C was greater in AM plots compared to ECM plots across sites ($F_{1,29}$ = 5.33,P = 0.028) with no statistically significant difference among sites (Fig. 2a). The highest root-derived C inputs were observed in AM plots at HF (418 ± 151 g C m^{-2} yr^{-1}) while the lowest C inputs were observed in ECM plots at TRC (114 ± 28.3 g C m^{-2} yr^{-1}). Annual root-derived C generally exceeded aboveground litter mass C inputs (Fig. 3; SI Fig. 2). The ratio of annual root-derived C: litter mass C was significantly predicted by site ($F_{5,29}$ = 3.60, P =0.012) and plot mycorrhizal type ($F_{1,29}$ = 7.20,P = 0.012), with no significant interaction between site and mycorrhizal type. Root-derived C did not mirror plot ANPP estimates, as indicated by variable root-derived C: ANPP ratios across plots (SI Fig. 1). The amount of root-derived C recovered in MAOM pools was greater in AM compared to ECM plots (P = 0.041; Fig. 4). There was no site or site × plot type effect on root-derived MAOM-C.

Root production

In contrast to the patterns observed with root-derived C inputs, root production was strongly predicted by site and (to a lesser extent) ECM dominance, with a significant site × ECM dominance interaction (R^2 = 0.72, $P < 0.001$). Comparing the end-member plots only, root production varied significantly both by plot mycorrhizal type ($F_{1,24}$= 4.66, $P = 0.041$) and among sites ($F_{5,24} = 15.19$, $P < 0.001$; Fig. 2b). Overall, root production was greater in ECM-dominated plots compared to AM-dominated plots. The highest root production was observed in ECM plots at HF (107 ± 8.94 g C m^{-2} yr^{-1}) while the lowest root production was observed in ECM plots at SCBI (16.4 ± 1.13 g C m^{-2} yr^{-1}). While there were no significant pairwise differences between plot mycorrhizal types within a given site, mean root production values were higher in ECM compared to AM plots at four of the six sites: HF, LDW, SERC, and TRC.

Discussion

Using an isotopic ingrowth core technique, we quantified root-derived soil C accumulation (in the bulk soil and MAOM fraction) and root production across gradients of ECM-associated tree dominance in six temperate forests. We found that root-derived C accumulation does not mirror root production patterns, with greater root-derived C in AM-dominated plots yet greater root production in ECM-dominated plots (Fig. 2). We also recovered more root-derived inputs in the MAOM fraction in AM compared to ECM plots (Fig. 4). Finally, our results highlight the impressive magnitude of root-derived C inputs (199.5 ± 14.7 g C m^{-2} y^{-1}), emphasizing the importance of adequately characterizing this plant-to-soil C flux in order to understand how tree community composition influences ecosystem C cycling (Fig. 3).

Given that root production did not predict root-derived soil C in our study, and that fungal production is typically greater in ECM plots (Clemmensen *et al.* 2013; Cheeke *et al.* 2017), greater root and/or fungal production most likely do not explain the greater root-derived C accumulation in AM plots. Importantly, our measure of root-derived soil C accounts for all root and fungal inputs plus rhizodeposition that persisted in soil after two years. Data quantifying rhizodeposition are less abundant, but previous work has found this flux can be greater in ECM stands (Yin *et al.* 2014) or similar between mycorrhizal types (Keller & Phillips 2019b). Finally, our two-pool mixing model accounts for changes in soil C within each core. This minimizes the effects of mycorrhizal type differences in priming on our estimates of root-derived C. Thus, it is unlikely that the observed variation in root-derived soil C accumulation across the mycorrhizal gradient is principally driven by mycorrhizal type differences in root production or rhizodeposition.

Instead, mycorrhizal type differences in root and fungal turnover between AM and ECM trees may contribute to the greater root-derived C in AM plots. AM plant tissues tend to decay more quickly than those of ECM plants (Keller & Phillips 2019a; See *et al.* 2019), and faster turnover rates of these tissues could result in greater total root-derived C inputs in AM plots when measured over multiple phenological cycles. Mycorrhizal type differences in root turnover may also explain, to some degree, the lack of relationship between root-derived C accumulation and root production (quantified in this study as root biomass recovered from ingrowth cores after two growing seasons). Likewise, turnover of AM fungi can exceed that of ECM fungi by an order of magnitude (Staddon *et al.* 2003; Tedersoo & Bahram 2019). Differences in fungal turnover rates between mycorrhizal types may be particularly important in driving root-derived soil C accumulation as fungal inputs to soil C have been shown to exceed that of both leaf and root litter (Godbold *et al.* 2006).

The greater recovery of root-derived C in AM soils also reflects mycorrhizal-associated differences in soil organic matter formation pathways. Whereas plant inputs to ECM soils tend to accumulate in organic horizons or particulate C pools, there is increasing evidence that AM systems transfer greater amounts of plant-derived C into mineral-associated forms (Cotrufo *et al.* 2019) and our results support this idea (Fig. 4). Faster decomposition of AM inputs leads to more microbial products which are important MAOM precursors (Cotrufo*et al.* 2013). To the extent that MAOM cycles slowly and protects C from microbial decomposers (Grandy and Neff 2008, Bradford et al. 2013, but see Jilling et al. 2018), this could explain the greater root-derived C accumulation in both the bulk soil and MAOM fraction in AM compared to ECM soils. Our ingrowth core method did control for edaphic differences (cores were filled with a uniform soil matrix across all plots and sites) and thus differences in microbial and soil C cycling dynamics driven by edaphic factors were minimized. However, soil C cycling is also driven by distinct plant and microbial traits

which can promote (or reduce) soil C aggregation and stabilization (Cheng & Kuzyakov 2005; Schmidt *et al.* 2011). For example, AM fungi are known to produce an aggregate-promoting glycoprotein (Rillig 2004), while ECM fungi have greater oxidative enzyme capacity to destabilize organic matter (Shah *et al.* 2016). In this way, the observed inverse relationship between root-derived soil C and ECM dominance (Fig. 1a) may reflect differences between mycorrhizal types in their input chemistry, and in their capacity to destabilize soil organic matter to acquire nutrients.

While root-derived soil C accumulation in forests has been poorly quantified to date, our estimates are similar in magnitude to previous studies using the isotopic ingrowth core technique. Across all plots in our study, root-derived soil C averaged 199 g $m^{-2}y^{-1}$ (ranging from 59 to 500 g $m^{-2}y^{-1}$). In an ECM-dominated 130-year old forest, Martinez et al. (2016) estimated root-derived C inputs to be 303 g m^{-2} y^{-1}, and Panzacchi et al. (2016) reported a similar rate (309 g $m^{-2}y^{-1}$) in a young mixed hardwood plantation. Moreover, mean root-derived C inputs were estimated to be ˜40% and ˜70% of ANPP at these sites, respectively. Across our six temperate forest sites, we found root-derived C to range from 74% (HF) to 157% (SERC) of ANPP. While our ANPP estimates (SI Fig. 1) may be conservative given that we excluded both small, understory trees (i.e. < 10 cm diameter, including individuals that grew into the >10 cm diameter class between measurement periods) and trees that died between measurement periods, annual root-derived C accumulation is still larger on average than leaf litter flux (Fig. 3; SI Fig. 2). This highlights the relative importance of belowground C inputs to soils and suggests that models that presume the primacy of leaf litter fluxes are drivers of soil C dynamics may be underestimating the importance of root dynamics.

Overall, our results suggest the magnitude of root-derived soil C inputs is large and can vary significantly across sites and mycorrhizal types. Importantly, we show direct evidence of distinct plant mycorrhizal type effects on soil C formation. Accurate predictions of ecosystem C cycling in ecosystem and land surface models depend on improved quantification of the belowground C flux from plants to soil C pools, and improved understanding of the factors that control soil C stabilization. Our results suggest that better estimates of root and fungal contributions to stable soil organic matter pools are clearly needed in order to better understand how plant species shifts affect ecosystem C cycling now and in the future.

Acknowledgements

We thank the ForestGeo network and the following individuals and their support teams for access to the sites: Dave Orwig (HF), Bill McShea (SCBI), Sean McMahon (SERC), Jonathan Myers (TRC), and Amy Wolf (WLF). We are grateful to members of the Philips and Brzostek labs for assistance in the field. Funding was provided by the U.S. Department of Energy Office of Biological and Environmental Research, Terrestrial Ecosystem Science Program (Award # DESC0016188), Center for Tropical Forest Science – ForestGEO, and the United States Department of Agriculture, National Institute of Food and Agriculture (no. 2019-67011-29507). JBF contributed to this research from the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. MEC contributed to this research from Oak Ridge National Laboratory which is operated by UT-Battelle, LLC, under contract DE-AC05-00OR22725 with the U.S. Department of Energy.

References

Anderson-Teixeira, K.J., Davies, S.J., Bennett, A.C., Gonzalez-Akre, E.B., Muller-Landau, H.C., Joseph Wright, S., *et al.* (2015). CTFS-ForestGEO: A worldwide network monitoring forests in an era of global change. *Glob. Chang. Biol.* , 21, 528–549.

Averill, C. & Hawkes, C. (2016). Ectomycorrhizal fungi slow soil carbon cycling. *Ecol. Lett.* , 19, 937–947.

Bradford, M.A., Keiser, A.D., Davies, C.A., Mersmann, C.A. & Strickland, M.S. (2013). Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth Author ' s personal copy. *Biogeochemistry* .

Brzostek, E.R., Dragoni, D., Brown, Z.A. & Phillips, R.P. (2015). Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. *New Phytol.* , 206, 1274–1282.

Cambardella, C.A. & Elliott, E.T. (1992). Particulate Soil Organic-Matter Changes across a Grassland Cultivation Sequence.*Soil Sci. Soc. Am. J.* , 56, 777.

Cheeke, T.E., Phillips, R.P., Brzostek, E.R., Rosling, A., Bever, J.D. & Fransson, P. (2017). Dominant mycorrhizal association of trees alters carbon and nutrient cycling by selecting for microbial groups with distinct enzyme function. *New Phytol.* , 214, 1–11.

Chen, W., Koide, R.T., Adams, T.S., DeForest, J.L., Cheng, L. & Eissenstat, D.M. (2016). Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees.*Proc. Natl. Acad. Sci. U. S. A.* , 113, 8741–8746.

Cheng, L., Chen, W., Adams, T.S., Wei, X., Li, L., McCormack, M.L.,*et al.* (2016). Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. *Ecology* , 97, 2815–2823.

Cheng, W. & Kuzyakov, Y. (2005). Root effects on soil organic matter decomposition. In: *Roots and Soil Management: Interactions between Roots and the Soil* . American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., *et al.* (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science (80-.).* , 339, 1615–1618.

Cotrufo, M.F., Alberti, G., Inglima, I. & Marjanovi, H. (2011). Decreased summer drought affects plant productivity and soil carbon dynamics in a Mediterranean woodland. *Biogeosciences* , 2729–2739.

Cotrufo, M.F., Lugato, E., Ranalli, M.G., Haddix, M.L. & Six, J. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter, 12.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K. & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter?*Glob. Chang. Biol.* , 19, 988–95.

Craig, M.E., Turner, B.L., Liang, C., Clay, K., Johnson, D.J. & Phillips, R.P. (2018). Tree mycorrhizal type predicts within-site variability in the storage and distribution of soil carbon and nitrogen.*Glob. Chang. Biol.* , 24, 3317–3330.

Fahey, T.J., Bledsoe, C.., Day, F.P., Ruess, R. & Smucker, A.J.. (1999). Fine Root Production and Demography. In: *Standard Soil Methods for Long Term Ecological Research* (eds. Robertson, G.P., Coleman, D.C., Bledsoe, C.. & Sollins, P.). pp. 437–455.

Fahey, T.J., Siccama, T.G., Driscoll, C.T. & Likens, G.E. (2005). The biogeochemistry of carbon at Hubbard Brook. *Biogeochemistry* , 75, 109–176.

Gill, A.L. & Finzi, A.C. (2016). Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale.*Ecol. Lett.* , 1419–1428.

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., *et al.* (2006). Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant Soil* , 15–24.

Gonzalez-Akre, E.B., Meakem, V., Eng, C.-Y., Tepley, A.J., Bourg, N.A., McShea, W.J., *et al.* (2016). Patterns of tree mortality in a temperate deciduous forest derived from a large forest dynamics plot.*Ecosphere* , 7.

Grandy, A.S. & Neff, J.C. (2008). Molecular C dynamics downstream : The biochemical decomposition sequence and its impact on soil organic matter structure and function. *Sci. Total Environ.* , 4.

Hendricks, J.J., Hendrick, R.L., Wilson, C.A., Mitchell, R.J., Pecot, S.D. & Guo, D. (2006). Assessing the patterns and controls of fine root dynamics : an empirical test and methodological review. *J. Ecol.* , 94, 40–57.

Hoosbeek, M.R., Lukac, M., Dam, D. Van, Godbold, D.L., Velthorst, E.J., Biondi, F.A., *et al.* (2004). More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): Cause of increased priming effect ? *Global Biogeochem. Cycles* , 18, 1–7.

Iversen, C.M., Mccormack, M.L., Powell, A.S., Blackwood, C.B., Freschet, G.T., Kattge, J., *et al.* (2017). A global Fine-Root Ecology Database to address below-ground challenges in plant ecology. *New Phytol.*

Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G. & Pineiro, G. (2017). The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. *Annu. Rev. Ecol. Evol. Syst.* , 48, annurev-ecolsys-112414-054234.

Jilling, A., Keiluweit, M., Contosta, A.R., Frey, S., Smith, R.G., Tiemann, L., *et al.* (2018). Minerals in the rhizosphere : overlooked mediators of soil nitrogen availability to plants and microbes. *Biogeochemistry* , 103–122.

Jo, I., Fei, S., Oswalt, C.M., Domke, G.M. & Phillips, R.P. (2019). Shifts in dominant tree mycorrhizal associations in response to anthropogenic impacts. *Sci. Adv.* , 5, 1–8.

Kallenbach, C.M., Grandy, A. & Frey, S.D. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nat. Commun.* , 1–10.

Keller, A.B. & Phillips, R.P. (2019a). Leaf litter decay rates differ between mycorrhizal groups in temperate, but not tropical, forests.*New Phytol.* , 222, 556–564.

Keller, A.B. & Phillips, R.P. (2019b). Relationship Between Belowground Carbon Allocation and Nitrogen Uptake in Saplings Varies by Plant Mycorrhizal Type. *Front. For. Glob. Chang.* , 2, 1–10.

Martinez, C., Alberti, G., Cotrufo, M.F., Magnani, F., Zanotelli, D., Camin, F., *et al.* (2016). Belowground carbon allocation patterns as determined by the in-growth soil core 13 C technique across different ecosystem types. *Geoderma* , 263, 140–150.

McCormack, M.L., Crisfield, E., Raczka, B., Schnekenburger, F., Eissenstat, D.M. & Smithwick, E.A.H. (2015). Sensitivity of four ecological models to adjustments in fine root turnover rate. *Ecol. Modell.* , 297, 107–117.

Panzacchi, P., Gioacchini, P., Sauer, T.J. & Tonon, G. (2016). New dual in-growth core isotopic technique to assess the root litter carbon input to the soil. *Geoderma* , 278, 32–39.

Pausch, J., Tian, J. & Riederer, M. (2013). Estimation of rhizodeposition at field scale : upscaling of a 14 C labeling study.*Plant Soil* , 364, 273–285.

Phillips, R.P., Brzostek, E. & Midgley, M.G. (2013). The mycorrhizal-associated nutrient economy : a new framework for predicting carbon – nutrient couplings in temperate forests. *New Phytol.* , 199, 41–51.

Phillips, R.P., Erlitz, Y., Bier, R. & Bernhardt, E.S. (2008). New approach for capturing soluable root exudates in forest soils.*Funct. Ecol.* , 22, 990–999.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. (n.d.). .

Rasse, D.P., Rumpel, C. & Dignac, M.F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* , 269, 341–356.

Rillig, M.C. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Sci.* , 84, 355–363.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. a., *et al.* (2011). Persistence of soil organic matter as an ecosystem property. *Nature* , 478, 49–56.

See, C.R., Mccormack, M.L. & Hobbie, S.E. (2019). Global patterns in fine root decomposition : climate , chemistry , mycorrhizal association and woodiness. *Ecol. Lett.* , 22, 946–953.

Shah, F., Nicolas, C., Bentzer, J., Ellstr, M., Floudas, D., Carleer, R., *et al.* (2016). Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors.*New Phytol.* , 209, 1705–1719.

Sokol, N.W. & Bradford, M.A. (2019). Microbial formation of stable carbon is more efficient from belowground than aboveground input.*Nat. Geosci.* , 12.

Staddon, P.L., Ramsey, C.B., Ostle, N., Ineson, P. & Fitter, A.H. (2003). Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of 14C. *Science* , 300, 1138–1140.

Sulman, B.N., Brzostek, E.R., Medici, C., Shevliakova, E., Menge, D.N.L. & Phillips, R.P. (2017). Feedbacks between plant N demand and rhizosphere priming depend on type of mycorrhizal association.*Ecol. Lett.* , 20, 1043–1053.

Tedersoo, L. & Bahram, M. (2019). Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. *Biol. Rev.* , 1868, 1857–1880.

Valverde-Barrantes, O.J., Smemo, K. a., Feinstein, L.M., Kershner, M.W. & Blackwood, C.B. (2013). The distribution of below-ground traits is explained by intrinsic species differences and intraspecific plasticity in response to root neighbours. *J. Ecol.* , 101, 933–942.

Wurzburger, N. & Brookshire, E.N.J. (2017). Experimental evidence that mycorrhizal nitrogen strategies affect soil carbon. *Ecology* , 98, 1491–1497.

Yin, H., Wheeler, E. & Phillips, R.P. (2014). Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biol. Biochem.* , 78, 1–9.

Zhu, K., Mccormack, M.L., Lankau, R.A., Egan, J.F. & Wurzburger, N. (2018). Association of ectomycorrhizal trees with high carbon-to-nitrogen ratio soils across temperate forests is driven by smaller nitrogen not larger carbon stocks. *J. Ecol.* , 524–535.

Table 1. Most dominant AM- and ECM-associated tree species at each site.

Site	Dominant AM trees	Dominant ECM trees
HF	*Acer rubrum*, *A. saccharum, Fraxinus americana*	*Pinus strobus, Tsuga canadensis*
LDW	*A. saccaharum*, *Liriodendron tulipifera*	*Quercus montana*, *Q. rubra*
SCBI	*L. tulipifera*	*Q. alba*, *Q. rubra*, *Q. veluntina*
SERC	*Liquidambar styraciflua*, *L. tulipifera*	*Fagus grandifolia*, *Q. alba*
TRC	*F. americana*, *Ulmus rubra*	*Q. alba*, *Q. rubra*, *Q. velutina*
WLF	*A. saccharum*, *F. americana*	*Betula alleghaniensis*, *T. americana*

Table 2. Site edaphic properties (± 1 SD; $n = 9$) at Harvard Forest (HF), Lilly-Dickey Woods (LDW), Smithsonian Conservation Biology Institute (SCBI), Smithsonian Environmental Research Center (SERC), Tyson Research Center (TRC), and Wabikon Lake Forest (WLF).

Site	Sand-Silt-Clay (%)	Soil pH	Soil C	Soil C:N	Consistent O-horizon?
HF	63-29-8	3.7 (0.4)	131 (63)	21 (4)	Y
LDW	15-76-9	4.4 (1.0)	43 (22)	16 (4)	N
SCBI	26-60-14	5.2 (1.0)	34 (7)	14 (3)	N
SERC	50-35-15	4.1 (0.8)	26 (4)	13 (1)	N
TRC	9-82-9	5.6 (0.6)	34 (9)	14 (2)	N
WLF	37-56-7	4.8 (0.5)	81 (32)	14 (2)	Y

Figure 1. Patterns of root-derived C accumulation related to (a) % ECM tree dominance (by basal area) of the plot and (b) root production. Each point represents one plot, with distinct sites depicted with different shaped points. Sites include Harvard Forest (HF), Lilly-Dickey Woods (LDW), Smithsonian Conservation Biology Institute (SCBI), Smithsonian Environmental Research Center (SERC), Tyson Research Center (TRC), and Wabikon Lake Forest (WLF). There is a weak negative relationship between root-derived C accumulation and plot % ECM (R2 = 0.09, P = 0.013) but no relationship between root-derived C accumulation and root production at a given plot. Significant linear regression shown with shaded 95% confidence interval.

Figure 2. (a) Root-derived C accumulation and (b) root production related to plot mycorrhizal type. Bars represent means (with standard error bars shown) for the AM-dominated (red) and ECM-dominated (blue) end-member plots at each site. Sites include Harvard Forest (HF), Lilly-Dickey Woods (LDW), Smithsonian Conservation Biology Institute (SCBI), Smithsonian Environmental Research Center (SERC), Tyson Research Center (TRC), and Wabikon Lake Forest (WLF). Insets show AM vs. ECM cross-site means, with * indicating significant difference between mycorrhizal types for root-derived C accumulation (P = 0.016). Mycorrhizal type, but not site or the interaction between mycorrhizal type and site, significantly predicted root-derived C accumulation (panel a; $P = 0.014$). Mycorrhizal type and site significantly predicted root production (panel b; $P = 0.04$ and $P < 0.001$, respectively), while the interaction between mycorrhizal type and site was marginally significant ($P = 0.09$). There were no significant pairwise differences between mycorrhizal types for any site in either panel a or b.

Figure 3. Ratio of annual root-derived C accumulation to annual aboveground litter mass C at the plot level for the AM (red) and ECM (blue) end-member plots at each site. Sites include Harvard Forest (HF), Lilly-Dickey Woods (LDW), Smithsonian Conservation Biology Institute (SCBI), Smithsonian Environmental Research Center (SERC), Tyson Research Center (TRC), and Wabikon Lake Forest (WLF). The 1:1 line is shown as a dashed grey line for reference. Bars represent plot-level means (with standard error bars shown) for each mycorrhizal type at each site. Site and mycorrhizal type (but not the interaction between the two) significantly predicted annual root-derived C accumulation: litter mass C ($P = 0.003$).

Figure 4. Mean root-derived MAOM-C (mg C/ g bulk soil) for the three AM-dominated (red) and three ECM-dominated (blue) end-member plots at three sites with standard error bars (n = 3 for each group). Mycorrhizal type (P = 0.04) significantly predicted plot-level root-derived MAOM-C, and the inset shows cross-site differences in AM vs. ECM mean root-derived MAOM-C across all sites. * Indicates significant differences between mycorrhizal types ($P < 0.05$). Sites include Harvard Forest (HF), Lilly-Dickey Woods (LD), and Smithsonian Environmental Research Center (SERC).