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Increased root-derived carbon buffers soil carbon loss under simultaneous warming and nitrogen addition

Thomas J. Muratore[1] | **Nikhil R. Chari**[2,3] | **Richard P. Phillips**[4,5] | **Benton N. Taylor**[2,3] | **Melissa A. Knorr**[1] | **Serita D. Frey**[1]

[1]Center for Soil Biogeochemistry and Microbial Ecology, Department of Natural Resources and the Environment, University of New Hampshire, Durham, New Hampshire, USA

[2]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA

[3]Arnold Arboretum of Harvard University, Boston, Massachusetts, USA

[4]Department of Biology, Indiana University, Bloomington, Indiana, USA

[5]Indiana University, Bloomington, Indiana, USA

Correspondence
Thomas J. Muratore
Email: thomas.muratore@unh.edu

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Abstract

Plant roots are primary drivers of soil organic matter dynamics, mediating belowground carbon (C) inputs, stabilization, and losses. Yet, how global changes such as rising temperatures and altered nitrogen (N) availability interact to affect these dynamics has rarely been tested empirically in the field. Here, we quantify how inputs to soil organic matter from fine-root production, root exudates, and root-associated fungi respond to long-term (16 years) soil warming (+5°C), nitrogen (N) enrichment (+5 g N m^{-2} $year^{-1}$), and their combination in a temperate hardwood forest. Warming alone reduced root-derived C inputs by 21% and increased microbial respiration by 46%, resulting in a net soil C loss of 135 g C m^{-2} $year^{-1}$. In contrast, N enrichment increased root-derived soil organic carbon (SOC) accumulation by 47% and reduced root respiration by 40%, contributing to a near-neutral soil C balance. When combined, warming × N addition increased root-derived SOC fourfold (from 70 to 281 g C m^{-2} $year^{-1}$), fully offsetting warming-induced C losses and maintaining soil C stocks at control levels. Root-derived SOC accumulation was positively related to fine-root production ($r^2 = 0.42$) and to maple:oak exudate ratios ($r^2 = 0.31$), highlighting species-specific control over C stabilization. These findings demonstrate that interacting global change factors can have balancing effects on root C allocation and microbial losses, highlighting soil N availability as a critical control determining whether warming accelerates SOC depletion or stabilizes new root-derived C.

KEYWORDS
ecosystem ecology, exudates, fine-root production, global change, nitrogen, soil organic carbon, soil warming, stable isotope

INTRODUCTION

Carbon (C) fluxes from roots to soil play an important role in regulating Earth's climate system by sequestering atmospheric CO_2-C as soil organic matter (SOM) or accelerating losses of native SOM (Poirier et al., 2018). Global change may disrupt the balance between root C inputs and soil CO_2 efflux, obscuring whether altered SOM accumulation and decomposition will amplify or dampen soil organic carbon (SOC) feedbacks to the climate system (Knorr et al., 2024). For instance, soil warming enhances losses of SOC by stimulating microbial-mediated SOM decomposition, which increases soil CO_2 efflux (Knorr et al., 2024; Melillo et al., 2017), whereas nitrogen (N) enrichment can suppress microbial activity and CO_2 efflux, leading to gains in SOC (Frey et al., 2014; Zak et al., 2008). Whether simultaneous global change factors affect root inputs (Bradford et al., 2016) and soil CO_2 losses in ways that can be predicted from single-factor responses is largely unknown (Song et al., 2019). Both warming and N enrichment may enhance root C inputs (Li et al., 2015; Wang et al., 2021); however, there is limited evidence on whether such increases lead to net gains in SOC or are offset by enhanced decomposition. Given that warming enhances soil N availability and that N strongly influences soil C storage due to the narrow C:N ratio of SOM, a clearer understanding of how warming and soil N fertility interact to affect SOC accumulation is needed (Rillig et al., 2019).

Plants allocate 1%–20% of their net primary productivity (NPP) belowground to facilitate nutrient acquisition (Jackson et al., 1997; Ouimette et al., 2020), but the effects of root-derived C inputs on soil C balance are highly variable. In addition to allocating C to absorptive roots, plants release C to soil as rhizodeposition (e.g., exudates) and provision C to mycorrhizal fungi, which provide a source of new SOC via root/mycorrhizal turnover and exudates (Keller et al., 2021). However, root-derived C can also contribute to SOC loss (Cheng & Kuzyakov, 2005). Root exudates are typically low molecular weight, high energy yielding organic compounds that can accelerate SOM decomposition via rhizosphere priming (Cheng & Kuzyakov, 2005; Yan et al., 2023), resulting in a net loss of SOC as microbes mineralize native SOM to meet their N demands (Dijkstra et al., 2013). Thus, global changes that alter C allocation belowground have the potential to increase or decrease SOC depending on the fate of root-derived inputs.

The magnitude of root C inputs and their distribution among different allocation pathways are strongly influenced by soil N availability, the most growth-limiting nutrient in temperate forests (Bae et al., 2015). The extent to which plants and microbes rely on SOM-N in response to soil warming or N addition may therefore determine whether roots drive net SOC accumulation or loss. Accordingly, how N addition and soil warming shift root C impacts on SOC stocks will rely on an interaction between the proliferation of root biomass, specific root exudation rates, the response of rhizosphere microbes, and soil N availability. This is an understudied area of climate–carbon feedback research because belowground interactions are difficult to investigate in situ, leaving key uncertainty in the magnitude and consequences of root C inputs under soil warming and N addition (Song et al., 2019).

Here, we investigate root systems of mature trees that have been exposed to long-term (16 years) soil warming (+5°C), N addition (+5 g N m^{-2} $year^{-1}$), or their combination at the Harvard Forest Long-Term Ecological Research (LTER) site. We quantified root C inputs (originating from fine-root production, exudates, and root-associated hyphae) and investigated their relationship to CO_2 efflux (from root and microbial respiration), the balance of which drives soil C storage. Previous work at our site showed that the single-factor treatments behaved as expected: long-term soil warming increased soil respiratory losses and reduced soil C stocks, whereas N enrichment reduced respiration and tended to increase soil C (Knorr et al., 2024). However, when applied together, warming and N addition produced an interactive response in which respiratory C losses exceeded either single-factor effect, despite no detectable decline in soil C storage (Knorr et al., 2024). This suggests that increased root C inputs and root-derived SOC under one or both treatments may offset SOC losses, even as warming × N addition stimulates microbial activity. We test this hypothesis here.

METHODS

Site description

Our study was conducted at the Soil Warming × Nitrogen Addition (SWaN) experiment (Contosta et al., 2011) located on the Prospect Hill Tract of Harvard Forest in Petersham, Massachusetts (42°50′ N, 72°18′ W). The experiment consists of twenty-four 3 × 3 m plots arranged in a full-factorial design with control, N addition, warming, and combined heated × N treatments. Each treatment is replicated six times, except for the warming treatment, which has five replicates due to a heating system failure in one plot in 2009. Soil warming has been continuously maintained at +5°C above ambient since 2006 using buried resistance heating cables installed at 10 cm depth and spaced 20 cm apart. The warming system maintains approximately +5°C heating throughout the upper 20 cm of the mineral soil. Nitrogen addition began concurrently in 2006, with plots receiving 50 kg N ha^{-1} $year^{-1}$ as aqueous NH_4NO_3, applied monthly during the growing season (May–October). The combination treatment (Heated × N) has been simultaneously warmed (+5°C) and fertilized (50 kg N ha^{-1} year^{-1}) since experiment inception. Soils are classified as the Gloucester series (fine-loamy, mixed, mesic Typic Dystrochrepts). The forest canopy is dominated by ectomycorrhizal (EM) red oak (*Quercus rubra*, 52% basal area) and arbuscular mycorrhizal (AM) red maple (*Acer rubrum*, 22%). Mean annual temperature is 7°C, and the site receives 1100 mm of annual precipitation, with long-term trends showing increases of +0.02°C and + 6.9 mm year^{-1} from 1964to 2015 (Boose & Gould, 2024; Finzi et al., 2020).

Quantifying belowground carbon inputs

We used a stable isotope ingrowth core method that exploits the δ^{13}C difference between C3 and C4 plants to quantify root- and mycorrhizal-derived carbon inputs. At the SWaN experiment, we installed rigid plastic mesh ingrowth cores (Industrial Netting no. RN4465; 5.77 cm diameter × 10 cm height; 3-mm mesh) into six replicate plots per treatment ($n = 5$ for the heated-only treatment due to cable failure in one plot). Each core was filled with a sand/soil mixture derived from surface soils (0–5 cm) of a long-established C4-dominated field ($\delta^{13}\text{C} = -20.5 \pm 0.17$‰), sieved to <4 mm and incubated field moist for 2 months to incorporate labile C into microbial biomass. To isolate mycorrhizal fungal contributions, we inserted an internal mesh cylinder (2 cm diameter × 7 cm height; 37-μm mesh) to allow hyphal, but not root, ingrowth. Each plot received a control core (no root inputs; $n = 23$), wrapped in 1-μm mesh, which excluded all ingrowth but allowed water movement, serving as a reference for the root ingrowth cores. We also installed solid PVC "no input" cores in an adjacent untreated forest to exclude both root and dissolved organic carbon (DOC) entry. All cores were capped with window screen to prevent aboveground litter contamination. Cores were installed in May 2021 and harvested in October 2022. Air gaps were filled with the soil/sand mixture during installation. Upon harvest, external root fragments were removed, and cores were sealed in plastic bags, stored at 4°C (for ≤3 days), and processed. Hyphae-only inserts were separated from the outer cores, and soils in both compartments were sieved to <2 mm. Fine roots (<2 mm) were removed for biomass estimates, and the soils were then dried at 105°C and finely ground. Dried and ground samples were analyzed for total C and isotopic composition (δ^{13}C) at the Cornell Stable Isotope Laboratory.

Soil δ^{13}C values in control cores (-20.4 ± 0.23‰) did not differ from "no input" cores (-20.4 ± 0.11‰; $p = 0.9887$), and DOC content was low and consistent across treatments (Appendix S1: Figure S1). Because the isotopic composition of DOC inputs could not be directly measured, we used the fraction of DOC recovered in each core type (as a percentage of total C) as a relative indicator of DOC entry. Root tissue recovered from the cores had an average δ^{13}C value of -28.6 ± 0.62‰, significantly lower than the background soil ($p < 0.0001$).

Root contributions to SOC (i.e., root-derived SOC accumulation) were determined using a two-pool mixing model consistent with previous studies (Balesdent et al., 1993; Keller et al., 2021). The change in δ^{13}C between the control and treatment cores (roots + hyphae and hyphae only) was used to calculate total belowground C accumulation. The fraction of soil C (f) that was derived from roots and hyphae was calculated using a two-end member mixing model:

$$f = \frac{\delta^{13}\text{C}_{\text{ingrowth}} - \delta^{13}\text{C}_{\text{control}}}{\delta^{13}\text{C}_{\text{input}} - \delta^{13}\text{C}_{\text{control}}}$$

where $\delta^{13}\text{C}_{\text{ingrowth}}$ is the δ^{13}C of the C_4 soil collected from a treatment core after 2 years in the field; $\delta^{13}\text{C}_{\text{control}}$ is the δ^{13}C value of the soil collected from the root and hyphae-free control core in the same plot as the ingrowth core after 2 years; and $\delta^{13}\text{C}_{\text{input}}$ represents the δ^{13}C weighted average of the maple/oak root tissue inputs recovered in the root + hyphae core or the δ^{13}C of the hyphal inputs in the hyphae-only core. To estimate fungal δ^{13}C, we applied a +2‰ correction to root δ^{13}C values, based on published discrimination offsets (Ekblad et al., 2016). This adjustment accounts for isotopic fractionation that occurs during C transfer from plant roots to mycorrhizal fungi, resulting in a predictable enrichment of fungal tissues relative to their host roots.

Next, the root-derived SOC accumulation rate from roots and hyphal inputs into ingrowth cores (C_{new} g C m^{-2}) was calculated as

$$\text{root} - \text{derived SOC} = \text{Soil C}_{\text{total}} \times \text{f} \times \text{BD} \times \left(\text{core depth} \times 10{,}000\,\text{cm}^2\,\text{m}^{-2}\right),$$

where Soil C_{total} is %C concentration after 2 years of ingrowth of the root-free soil, f is the fraction of belowground derived C, BD is the bulk density of the sand:C_4 soil mixture, and core depth × 10,000 is the conversion factor to transform %C to g C m^{-2}. Annual net fluxes were calculated by dividing root-derived C by the number of growing seasons cores were in the field (i.e., 2 for all cores).

Fine-root production

Fine-root production was estimated using the root + hyphae ingrowth cores installed at each plot location. As described above, the ingrowth cores were retrieved after two growing seasons and processed to quantify newly produced fine roots. Soils were sieved (<2 mm), and live fine roots (<2 mm diameter; 1st–3rd order) were separated using forceps following McCormack et al. (2015). Roots were sorted by genus—*Quercus* (EM-associated) and *Acer* (AM-associated)—based on color, morphology, and mycorrhizal traits, aided by a stereomicroscope. Dead roots were excluded based on discoloration, brittleness, and stele–cortex separation (Persson & Stadenberg, 2009). Cleaned roots were dried at 60°C for 48 h and weighed. Root %C was determined via elemental analysis (Cornell Stable Isotope Facility), and fine-root production was calculated as the mass of live fine roots multiplied by %C and divided by 2 years of core deployment.

Root exudates

Root exudates were collected five times between May and August 2023 using a modified cuvette incubation method (Phillips et al., 2008; full protocol in Chari et al., 2024). At each sampling, intact absorptive roots (~10–15 cm) of *Quercus* and *Acer* spp. were excavated from each treatment plot, rinsed to remove soil, and incubated in glass syringes filled with glass beads and C-free nutrient solution. After 48 h, the solution was replaced and roots were incubated for an additional 24 h before final collection. Three root-free blanks were included during each sampling round to correct for background C. Following incubation, roots were dried at 60°C and weighed to normalize exudation rates. Total organic carbon (TOC) was analyzed as non-purgeable organic carbon (NPOC) using a Shimadzu TOC ASI-V analyzer. Exudation rates (μg C mg^{-1} root day^{-1}) were calculated by multiplying TOC concentration by solution volume, correcting for blank values, and standardizing by root mass and time. Annual ecosystem-scale exudation rates (g C m^{-2} year^{-1}) were estimated by scaling mass-specific exudation to plot-level absorptive root biomass for each species (Appendix S1: Table S1; Muratore et al., 2024).

Total belowground carbon budget

To evaluate the effects of treatment on belowground C fluxes, we constructed a C budget using data from ingrowth cores, root sampling, soil C stocks, and respiration measurements, which enabled consistent comparisons across treatments. While ingrowth cores inherently select for fast-growing, absorptive roots and differ from intact soils, they effectively capture key root-driven processes—such as production and exudation—that reflect the impacts of treatment. This approach provides mechanistic insight into how warming and nitrogen addition influence root C inputs and their transformation into root-derived SOC.

We estimated total root C inputs (g C m^{-2} year^{-1}) as the sum of

$$\text{Root C input} = \text{C}_{\text{roots}} + \text{C}_{\text{hyphae}} + \text{C}_{\text{exudates}},$$

where C_{roots} is fine-root production (g C m^{-2} year^{-1}) as measured in the ingrowth cores, C_{hyphae} is the accumulation of C in the hyphae-only core (g C m^{-2} year^{-1}), and $\text{C}_{\text{exudates}}$ is the estimated annual root exudate input (g C m^{-2} year^{-1}).

Next, we estimated microbial respiration ($R_{\text{microbial}}$; g C m^{-2} year^{-1}) as follows:

$$R_{\text{microbial}} = R_{\text{soil}} - R_{\text{root}}$$

where R_{soil} is annual total soil respiration (g C m^{-2} year^{-1}) and R_{root} is annual ecosystem-level root respiration (g C m^{-2} year^{-1}). Soil respiratory fluxes were obtained from published estimates of total soil respiration (Knorr et al., 2024) and root respiration (Muratore et al., 2024) measured at the SWaN experiment over the timeframe of our study. Briefly, soil CO_2 efflux was measured in situ using permanently installed PVC collars and a portable infrared gas analyzer (LI-8100, LI-COR Biosciences) equipped with a dynamic chamber (Knorr et al., 2024). Root respiration was determined by incubating freshly excavated fine roots that had been severed from the soil and cleaned of adhering material, then measuring CO_2 flux using a closed gas exchange chamber connected to an infrared gas analyzer under controlled temperature conditions (Muratore et al., 2024).

Finally, we balanced plant and microbial inputs with plant and microbial outputs to determine the net C balance (g C m^{-2} year^{-1}) defined as

$$\text{Net Carbon Balance} = (R_{\text{microbial}}) - (\text{Root C inputs} + \text{Root derived SOC}),$$

where $R_{\text{microbial}}$ is the estimated microbial respiration, root C inputs are the belowground NPP productivity, and root-derived SOC is the C that accumulated in the root + hyphae ingrowth cores. We treated *root C inputs* and *root-derived SOC* as distinct terms since the former represents recent belowground NPP (e.g., exudates, root biomass) available for immediate microbial use, while the latter represents more stabilized C from root and hyphal turnover over the two-year core incubation (Phillips et al., 2012). These pools differ in microbial accessibility and turnover time. As plots do not encompass full tree crowns, and individual trees span multiple plots, we assumed litterfall was uniform and excluded it from the model.

Data analysis and statistics

Analysis of variance (ANOVA) was used to determine the effects of treatment (N addition, heat, heat ×N), tree species (oak or maple), and their interactions on fine-root production, ecosystem-level exudation rate, root + hyphae belowground C accumulation, and hyphae-derived belowground C accumulation. Within-plot replicates were averaged to obtain one plot measure for each response variable sampled. Normality assumptions of ANOVA were evaluated with a Shapiro–Wilk test, and a square root transformation of variables was used to improve model fit when ANOVA model assumptions were not met. A separate mixed-effects model was used to assess the fixed effect of treatment (N addition, heat, heat × N) and random effects of sampling date and plot number on root exudation rates. Additional post hoc comparisons were made between treatments using Tukey's honestly significant difference test using the "emmeans" package in R (R Core Team, 2022). We performed a pairwise *t*-test to test for the effect of the net C balance and the root effect relative to the control for a given treatment. The log response ratio (LRR; Figure 1b) was calculated between root + hyphae and hyphae-only cores as ln[(root-derived SOC + ε)/(hyphae-derived SOC + ε)], where ε is a small constant set to the 5th percentile of positive hyphal flux values added to prevent undefined values for zero fluxes. Finally, we ran a linear model to test for the effect of treatment (heat, N addition, heat × N), Plant C flux (fine-root production or ecosystem root exudation), and tree species (maple or oak) on root-derived SOC. We performed three separate Pearson correlations between the predictors of fine-root production, specific exudation rate (maple and oaks), and the oak:maple ecosystem root exudation ratio on belowground C accumulation measured in the root + hyphae cores. In all cases, we assessed significance at $\alpha = 0.05$. All analyses were performed in R version 4.2.0 (R Core Team, 2022).

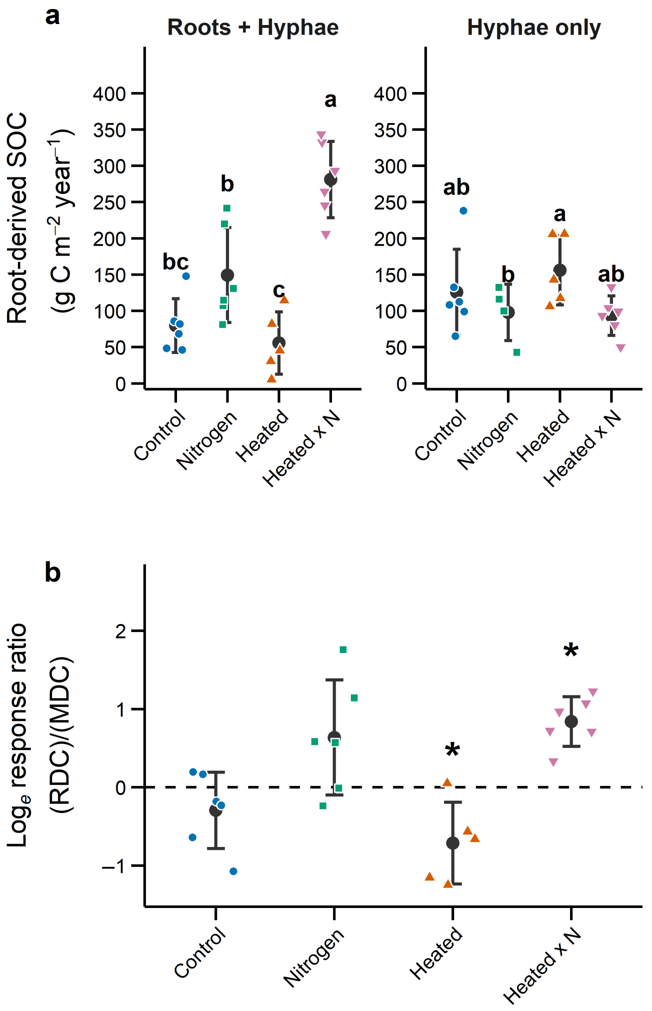

FIGURE 1 Root contributions to belowground carbon accumulation. (a) Soil carbon (C) accumulation in cores containing both roots and hyphae (*root + hyphae*) versus hyphae-only across treatments. Statistical results are reported in Appendix S1: Table S2. Different letters indicate significant pairwise differences among treatments ($\alpha < 0.05$; Appendix S1: Table S3). (b) Root effect expressed as the percent difference in C accumulation between core types; values >0 indicate roots enhanced C accumulation, whereas values <0 suggest root-driven C loss. Asterisks denote significant differences from the control ($\alpha < 0.05$; Appendix S1: Table S4 for pairwise test results). Points represent treatment means and error bars show one standard deviation. Sample sizes: Control ($n = 6$), Nitrogen ($n = 6$), Heated ($n = 5$), Heated × N ($n = 6$).

RESULTS

To assess the contributions of roots on SOC formation (i.e., root-derived SOC), we took advantage of differences in the δ^{13}C signature between root C inputs and that of the soil used to construct the ingrowth cores. Under control conditions, root-derived SOC was 70 g C m^{-2} year^{-1}, compared with 149 g C m^{-2} year^{-1} in the N addition treatment and 55 g C m^{-2} year^{-1} in the heated treatment (Figure 1a). Although neither N addition ($t_{38} = -2.38$, $p = 0.098$) or warming ($t_{38} = 0.78$, $p = 0.86$) differed significantly from the control, they were significantly different from each other, with nearly threefold more root-derived C under N addition than under warming ($t_{38} = -3.05$, $p = 0.021$). When both treatments were applied simultaneously, root-derived C increased to 281 g C m^{-2} year^{-1}—a fourfold increase relative to the control ($t_{38} = -6.82$, $p < 0.001$)—driven by a strong interactive effect of warming and N addition (Figure 1a). Hyphae-derived C declined under N addition ($F_{1,38} = 8.62$, $p = 0.008$) and was significantly lower than under warming ($t_{38} = 2.97$, $p = 0.026$). By comparing C accumulation between root + hyphae and hyphae-only cores, we found that the strong increase in SOC under the combined treatment was driven primarily by root inputs rather than fungal inputs (Figure 1b; response ratio >0). The presence of living roots reduced SOC accumulation in heated soils ($t_4 = -3.26$, $p = 0.031$), but this effect was reversed under the combined heating × N treatment ($t_5 = 3.86$, $p = 0.012$).

Mass-specific exudation rates varied significantly with species (Figure 2a; $F_{1,182} = 16.43$, $p < 0.001$), with oak roots exhibiting markedly lower mass-based exudation rates relative to maple ($t_{182} = 4.05$; $p < 0.001$). When scaled to the ecosystem using absorptive root biomass from the site (Muratore et al., 2024), similar amounts of maple and oak exudates were observed in control plots (Figure 2b; $t_{32} = -0.294$; $p = 0.77$). However, there was a significant three-way interaction among warming, N addition, and tree species ($F_{1,32} = 15.11$, $p < 0.001$), indicating that the combined treatment (heated × N) altered the relative species contributions. Under warming ($t_{32} = -3.59$, $p = 0.0011$) and N addition ($t_{32} = -3.19$, $p = 0.0031$) alone, oak exudation exceeded that of maple, but under the combined treatment oak ecosystem exudation rates returned to control levels ($t_{32} = 1.49$, $p = 0.146$), leading to a more balanced contribution from both species (Figure 2b). Finally, oak fine-root production significantly increased under N addition ($F_{1,38} = 16.52$, $p < 0.001$), but not under warming (Figure 2c; $F_{1,38} = 0.31$, $p = 0.580$).

Across all plots, root-derived SOC accumulation was positively related to fine-root production (Figure 3a; $t_{21} = 3.89$, $p = 0.0008$, $r^2 = 0.42$), independent of tree species. In contrast, relationships between mass-based root exudation and root-derived SOC were species specific. Maple exudation rates were positively correlated with root-derived SOC in the ingrowth cores (Figure 3b; $t_{17} = 1.96$, $p = 0.0667$, $r^2 = 0.18$), whereas oak exudation showed a negative, though marginally insignificant, relationship (Figure 3c; $t_{18} = -2.02$, $p = 0.0583$, $r^2 = -0.19$). When exudate inputs from both species were analyzed together as a ratio, this relationship was strengthened: higher maple-to-oak exudation ratios corresponded with greater root-derived SOC (Figure 3d; $t_{17} = 2.64$, $p = 0.017$, $r^2 = 0.29$).

DISCUSSION

Our results demonstrate that concurrent global change factors can have offsetting effects on root-derived C fluxes, with critical consequences for SOC dynamics. In warmed soils, N availability dictated whether roots acted as net contributors to SOC or accelerants of SOC loss. Warming alone led to net SOC loss, as increased microbial respiration outpaced relatively low root C inputs in N-limited soils. In contrast, N addition alone resulted in no net change in SOC by enhancing root SOC inputs while maintaining or slightly reducing microbial respiration. Under combined warming and N addition (heated × N), root C inputs increased enough to offset the elevated microbial respiration caused by warming, resulting in a neutral SOC balance. These results provide mechanistic evidence for the role of soil N availability as a primary driver of temperate forest soil C cycling in response to warming soils.

Nitrogen availability mediates the balance between root- and hyphal-derived SOC formation under soil warming

Our study tested whether the addition of N to warmed soils promotes greater SOC accumulation from belowground sources (Figure 1a,b). We found that root-derived SOC accumulation increased with N addition relative to cores in the warming treatment and nearly doubled in cores in the combination (heated × N) treatment relative to control and warming alone (Figure 1a). In contrast, hyphae-derived SOC was reduced under N addition compared to warming alone, whereas combining these treatments restored hyphae-derived SOC accumulation to an intermediate level. Thus, under unfertilized conditions (control and warmed only), hyphae-derived SOC constituted the majority of newly accumulated C. When N was added, regardless of heating level (i.e., ambient versus heated), root-derived SOC dominated new SOC accumulation (Figure 1b), highlighting the role of N availability in shifting SOC formation pathways from hyphae- to root-derived sources. Our results from the ingrowth cores provide empirical support for our hypothesis that N addition enhances root-driven SOC formation under warming and does so by altering the balance between root and fungal pathways of SOC accumulation.

Notably, the interactive effect of warming and N addition increased root-derived SOC accumulation beyond what would be expected based on N addition alone. The interactive response suggests that warming and N enrichment jointly modify belowground processes in a nonadditive way, amplifying the root contribution to new SOC formation. The interactive effect observed in the heating × N plots likely arises from two offsetting processes that operate in opposite directions under single-factor treatments. Under warming alone, soils containing both roots and hyphae accumulated less C than those with hyphae alone (response ratio <0; Figure 1b), showing that SOC

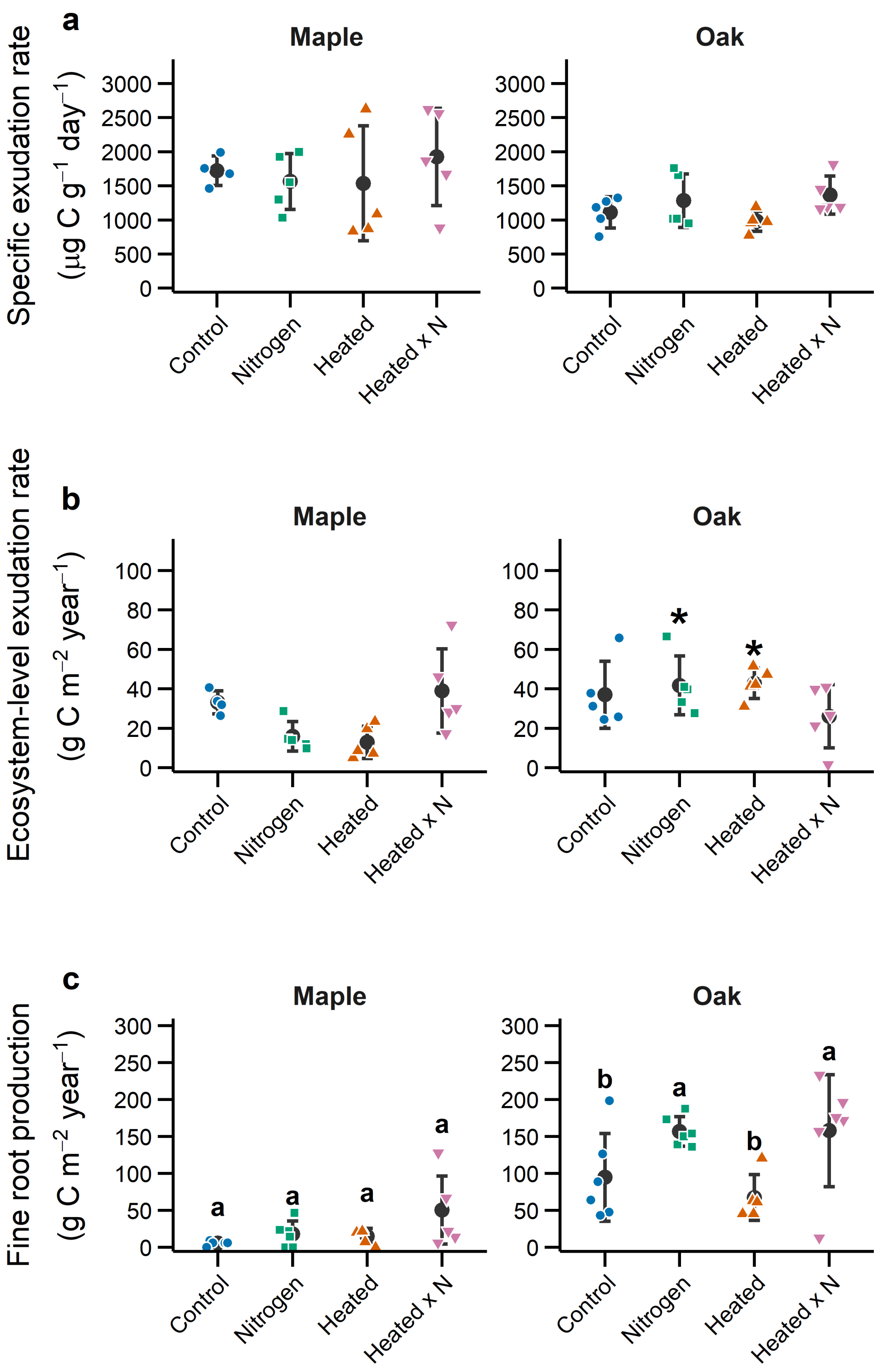

FIGURE 2 Belowground carbon allocation responses to nitrogen addition, soil warming, and their interaction. (a) Mass-based root exudation rates (Appendix S1: Table S5); (b) ecosystem-level exudation (Appendix S1: Table S6), calculated as the product of panel a and absorptive root biomass (Appendix S1: Table S1; Muratore et al., 2024); (c) Fine-root production (Appendix S1: Tables S7 and S8). Different letters indicate significant treatment effects within tree species ($\alpha < 0.05$); asterisks denote significant species differences within treatments. Points represent treatment means and error bars show one standard deviation. Sample sizes: panels a and b ($n = 5$); panels c – Control ($n = 6$), Nitrogen ($n = 6$), Heated ($n = 5$), Heated × N ($n = 6$).

accumulation was greater when only hyphae were present than when roots and hyphae co-occurred. The presence of roots in warmed soils likely stimulated microbial decomposition of newly formed SOM, reducing net SOC accumulation when both inputs are available. This response is consistent with microbial N demand driving

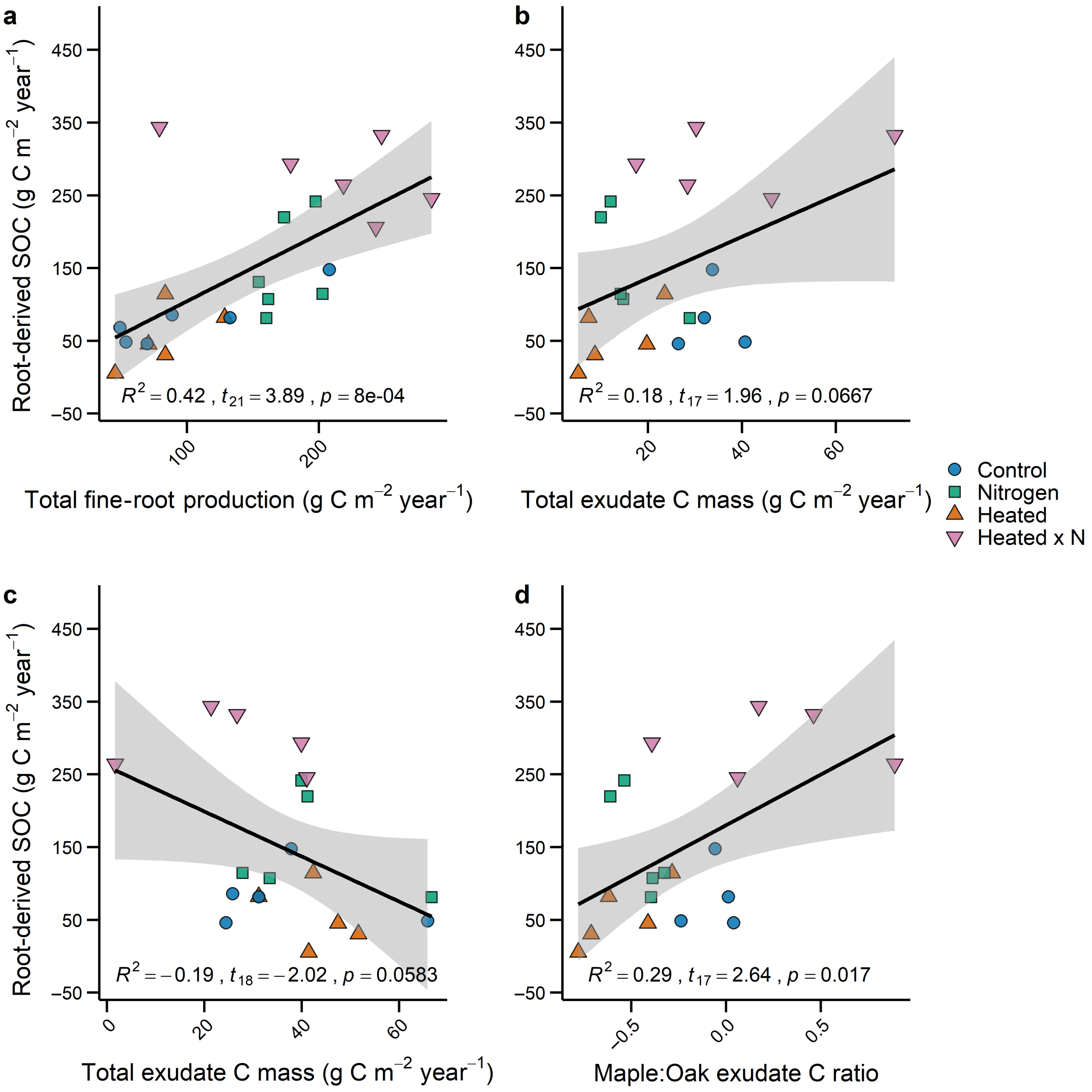

FIGURE 3 Relationships between root-derived soil organic carbon (SOC) accumulation and belowground carbon (C) fluxes. (a) Total fine-root production; (b) total root exudate C mass for red maple; (c) total root exudate C mass for red oak; (d) maple:oak exudate C ratio (ecosystem scale). Lines represent Pearson correlations with 95% confidence intervals (gray shading). Reported R^2, t values, and p values correspond to each correlation. Sample sizes: panel a, $n = 22$; panels b–d, $n = 18$.

positive priming under low-N conditions (Craine et al., 2007). Nitrogen addition promotes greater root-derived SOC but also appears to counteract the priming effect by alleviating microbial SOM-N demand preserving SOC stocks. The resulting interactive effect under heating × N reflects both increased root-derived SOC and reduced microbial decomposition. Any priming that occurred in our experiment would have primarily affected newly formed C3-derived SOC rather than the original C4 material, because all ingrowth core soils were preincubated for several months to mineralize labile C4 substrates before field deployment. Generally, root C inputs outweigh root-induced priming losses, leading to net SOC accumulation through time (Beidler et al., 2023). Most importantly, whether priming occurred does not alter our main result: Root-derived SOC increased most in the combination treatment, and the SOC measured in the cores represents the net effect of 2 years of root ingrowth and decomposition dynamics. Overall, our findings demonstrate that N availability governs the dual role of roots in determining the strength and direction of SOC dynamics, driving whether root activity under warming accelerates SOC loss or promotes its stabilization (Dijkstra et al., 2021).

Root mechanisms underpinning soil carbon accumulation and loss

To explore the processes underlying the observed SOC accumulation in our ingrowth cores, we next examined the belowground C inputs that supply new root C to soil. Specifically, we evaluated how fine-root production and exudation varied among species and treatments, providing mechanistic insight into how N availability and warming interact to shape belowground C allocation. Nitrogen addition consistently enhanced fine-root production (Figure 2c), but this effect was driven by oak species, suggesting that oak fine-root production has a greater sensitivity to N fertilization than maple. This could be attributed to the rooting strategies of oaks, which allocate C to roots dynamically (Cardon et al., 2002; Herrera-Ramírez et al., 2020) and potentially with reduced investment in mycorrhizal fungi (Treseder, 2004). The lack of a comparable response in maple suggests that they may be less affected by N fertilization, at least in terms of root production. Maple trees may support more aboveground biomass under elevated soil N (Averill et al., 2018), though the small size of our plots precluded us from quantifying this response. On a regional scale, Acer spp. have increased in dominance in the eastern United States due to many factors including historical effects of N deposition (Quinn Thomas et al., 2010), and similar effects have been reported from N fertilization experiments of maple saplings (Wheeler et al., 2017) and mature trees (Van Houtven et al., 2019). Despite these species-specific allocation strategies, root-derived SOC uniformly increased with fine-root production across species in our study (Figure 3a), underscoring the importance of root biomass as a direct driver of SOC accumulation. The magnitude of this effect on root-derived SOC accumulation may be associated with root growth and turnover, which enmesh soil particles, leading to aggregate formation (Smucker, 2003), a mechanism that may stabilize SOC even as warming and N alter microbial activity.

Root exudates represent 5% of gross primary productivity at Harvard Forest (Abramoff & Finzi, 2016) but may contribute up to 10–40% of soil respiration by priming microbial metabolism (Kuzyakov, 2002). Chari et al. (2024) found that root exudates decreased under soil warming, which may result from increased inorganic N availability reducing the need for roots to stimulate microbial priming (Liese et al., 2018; Wen, White, et al., 2022). In the present study, we found that mass-specific exudation rates exhibited contrasting responses to N addition and heating that offset each other when applied simultaneously, suggesting that increased inorganic N is not fully responsible for declining exudation rates. However, because the roots used to measure root respiration were excised, our estimates may conservatively reflect treatment effects if the environmental conditions partly drive exudation during measurement. In both studies, declining specific exudation rates were driven by oak tree species (Figure 2a). Despite lower specific root exudation rates for oak, estimates of ecosystem-scale exudation rates for this species (Figure 2b) remain dominant due to a larger standing oak root biomass compared to maple (Muratore et al., 2024).

Root exudate quantity and chemical composition are important for soil C dynamics (Chari & Taylor, 2022). Notably, higher rates of root exudation contribute to greater SOC priming relative to microbial assimilation and turnover by stimulating microbial activity and increasing decomposition of native SOM (Chari & Taylor, 2022). Different exudates, such as simple sugars, amino acids, and organic acids, can uniquely impact microbial assimilation and turnover rates (Bradford et al., 2013; Marschmann et al., 2024). Although maple and oak exhibited similar exudation rates, they had distinct impacts on SOC accumulation, suggesting that the chemical composition of root exudates may play a role in determining whether root C inputs contribute to SOC formation or loss. We observed that higher ecosystem exudation rates for maple were associated with increased root-derived SOC (Figure 3b), while higher oak exudation rates were linked to decreased root-derived SOC (Figure 3c). This relationship became stronger and statistically significant when considering the ratio of maple-to-oak exudation, which integrates both exudation rates and relative root biomass of each species within a plot (Figure 4d). This suggests that root-derived SOC accumulation is not solely driven by exudation rates but by an integrated effect of root biomass and the unique effects of exudates originating from maple versus oaks (Figure 4b). This pattern may be due to (1) maples having a lower exudate flux (driven by lower root biomass), which reduces the priming effect (Chari & Taylor, 2022), and/or (2) the unique chemistry and labile nature of maple root exudates (Smith, 1969) that may inherently promote microbial assimilation and greater soil C accumulation (Wen, Yu, et al., 2022). These tree species-specific effects highlight that root-derived SOC accumulation is not simply a function of C input magnitude but also depends on exudate chemistry. Future studies should investigate exudate composition to improve our understanding of forest C budgets.

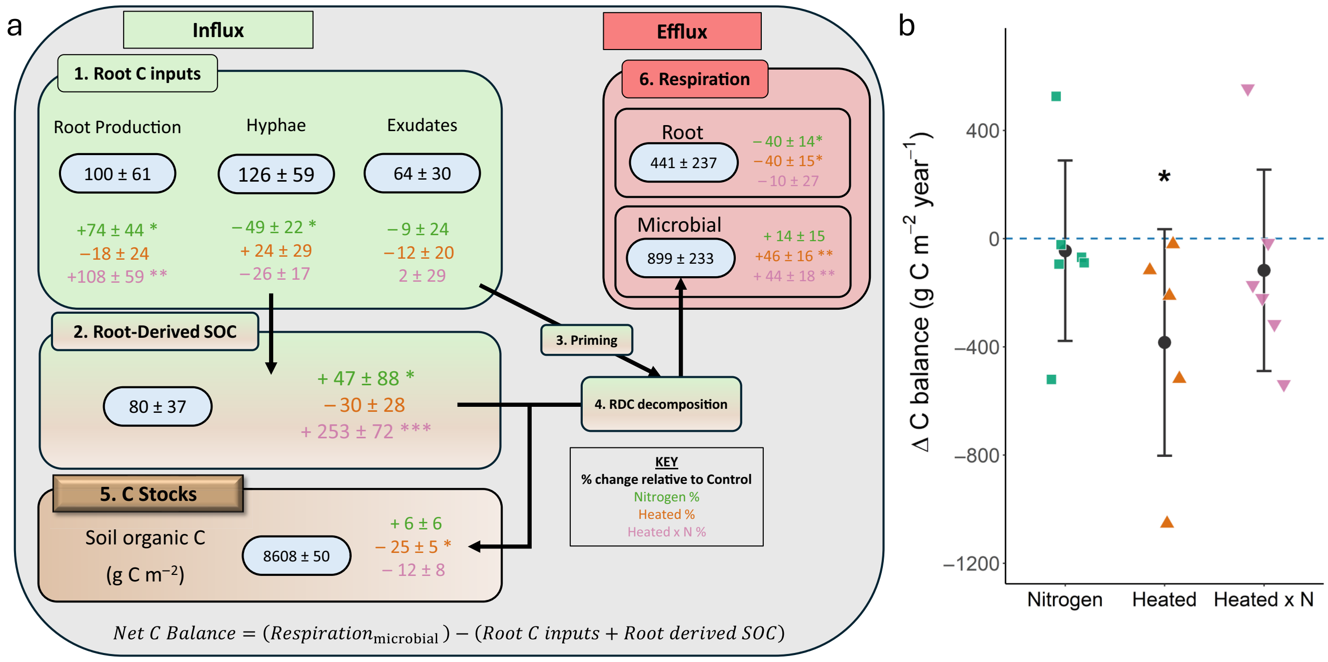

FIGURE 4 Belowground carbon budget at the Harvard Forest Soil Warming × Nitrogen Addition (SWaN) experiment. (a) Conceptual and quantitative framework for belowground carbon (C) fluxes, showing six major components that together describe the soil C balance: (1) Root C inputs represent fresh belowground inputs from plant sources, partitioned into root production, mycorrhizal or hyphal inputs, and root exudates; (2) root-derived soil organic carbon (SOC) represents the amount of root C inputs that persisted over the two growing seasons ingrowth core incubation; (3) priming illustrates how labile root inputs can stimulate decomposition of root-derived SOC via microbial activity; (4) decomposition of root-derived SOC (RDC) contributes to CO_2 flux through microbial respiration; (5) soil C stocks and the net C balance (equation at bottom) represent the long-term balance between influx (root C inputs + root-derived SOC formation) and efflux (root and microbial respiration); and (6) respiration distinguishes between autotrophic (root) and heterotrophic (microbial) CO_2 efflux, where microbial respiration reflects decomposition of root-derived SOC and root C inputs. Values in blue bubbles represent the mean ± standard deviation of each C flux or pool (g C m^{-2} $year^{-1}$) measured in the control plots. Percentages shown beneath each bubble indicate the relative change (mean ± 1 SD) for each treatment—nitrogen addition (green), heated (red), and combined heated × N (purple)—relative to the control. Influxes are derived from field measurements of root production, exudation measured in this study while effluxes (root and microbial respiration) are come from published data sources (Frey, 2026; Knorr et al., 2024; Muratore et al., 2024). Arrows denote potential C flow pathways, not flux magnitude. Asterisks denote significant differences from the control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; Appendix S1: Table S9). (b) Net soil C balance is expressed as $\text{Respiration}_{\text{microbial}}$ − [Root C inputs + Root-derived SOC]) across treatments relative to the control. Zero corresponds to the control mean, with values <0 indicating C loss and values >0 indicating C accumulation relative to the control plots. Asterisks denote significant differences from the control (α < 0.05). Points represent treatment means and error bars show one standard deviation.

Belowground C budget balancing microbial losses and root C inputs under soil warming × N addition

We integrated our isotopic ingrowth core data with long-term measurements of soil respiration and SOC stocks to develop a belowground C budget (Figure 4) to evaluate whether differential root C inputs across our experimental treatments could explain patterns in respiratory and soil C losses previously documented at this site (Knorr et al., 2024). The resulting C balance between microbial respiration, root C inputs, and root-derived SOC (Figure 4a) highlights three distinct trajectories of SOC under global change (Figure 4b). Nitrogen addition produced a nonsignificant ΔC of −51 g C m^{-2} $year^{-1}$ relative to the control. In contrast, soil warming resulted in a significant ΔC deficit (−135 g C m^{-2} $year^{-1}$) associated with a 17% increase in total soil respiration, driven by higher microbial activity (+46%) and reduced root respiration (−40%). These patterns are consistent with the thermal sensitivity of microbial decomposition (Frey et al., 2013; Walker et al., 2018) and reduced microbial carbon-use efficiency under warming (Li et al., 2019). When warming and N were combined (i.e., heated × N treatment), microbial respiration remained elevated (+44%) due to temperature effects, while enhanced root C inputs (+27%) and a fourfold increase in root-derived SOC balanced the increased CO_2 efflux, producing a near-neutral ΔC in soil C (−48 g C m^{-2} $year^{-1}$). By alleviating microbial N limitation, N addition likely reduced SOM mineralization and preserved existing SOM, while simultaneously increasing root production and exudation that replenished soil C pools.

Our budget focuses on the surface horizon where treatment effects on root and microbial activity are strongest because experimental warming and N additions primarily affected the upper mineral soil (~0–20 cm). Most autotrophic respiration in temperate forests originates from fine roots within this depth range (Hanson et al., 2000), and treatment-induced shifts in fine-root biomass at the SWaN site also occur in the organic and upper mineral horizons (Muratore et al., 2024). Accordingly, our "ecosystem-scale" estimates refer to the surface layer that governs the majority of belowground C exchange in this forest. This approach both simplifies depth heterogeneity and sets clear assumptions underlying the budget, allowing us to evaluate whether treatment-driven changes in root C inputs are plausibly large enough to influence net soil C balance.

By directly linking root-derived C inputs, respiration, and SOC storage under different warming and N treatments, our experiment helps explain why soil warming elicits divergent SOC responses across N availability gradients—clarifying that soil N availability governs the balance between microbial C loss and root-driven C accumulation. This is consistent with recent work in a calcareous, C-rich, and nutrient-replete temperate forest, where soil warming triggered a persistent increase in CO_2 efflux without a significant decline in SOC stocks (Schindlbacher et al., 2025). The magnitude of the SOC decline was far smaller than would be expected from the surplus CO_2 released from warmed soils, closely paralleling the combined heated × N response observed at our site (Knorr et al., 2024). In both systems, elevated respiratory fluxes were balanced by enhanced root C inputs and accumulation of root-derived SOC, indicating that plant C supply can compensate for greater microbial losses when nutrient constraints are alleviated. By contrast, in the low-fertility, glaciated soils typical of much of the temperate zone, including our site, warming produced net SOC losses perhaps because limited N availability restricted root C inputs while stimulating microbial mineralization. Together, these findings help reconcile the divergent warming responses reported across sites—from SOC loss in nutrient-poor systems to apparent C neutrality in nutrient-rich ones—and point to soil fertility as a fundamental control on belowground C balance. Integrating soil fertility mechanisms into ecosystem and Earth-system models will be essential for accurately projecting forest C-climate feedback, as fertility governs both the magnitude and direction of root-microbial interactions under warming.

CONCLUSION

Understanding the effects of warming and N addition on soil C cycling is important for accurately predicting SOC feedback to the climate system. Our findings reveal that the addition of N to warmed soils modifies the balance between root C inputs, soil C transformations, and microbial activity, explaining the contrasting SOC responses observed in single-factor warming and N addition experiments (Bai et al., 2023; Frey et al., 2014; Knorr et al., 2024; Melillo et al., 2017). Tree roots play a central role in shaping soil C exchange, with outcomes that depend on N availability: In our study, roots exacerbated SOC loss in heated-only soils but promoted root-derived SOC accumulation under simultaneous warming and N addition. In ecosystems with high N availability, whether naturally or anthropogenically derived through N fertilization or atmospheric N deposition, the expected SOM loss due to warming may be less than anticipated, as enhanced root-derived SOC may offset increased soil CO_2 fluxes to the atmosphere.

Overall, our results support the hypothesis that the combination of warming and N addition enhances root C inputs, mitigating elevated SOC loss to warming alone. We emphasize that increased rates of root-derived SOC accumulation do not necessarily equate to long-term SOC storage. Elevated root-derived SOC was frequently accompanied by increased C loss through root and microbial pathways, as the same root traits that support SOC accumulation were also associated with enhanced microbial activity and turnover. To accurately assess the role of roots in C cycling, it is essential to consider both gains and losses from root-microbial interactions. Root-derived SOC accumulation may act as a transient buffer rather than a stable sink if promptly respired. Future work should investigate the stability of root-derived SOC to determine whether it contributes to stable and persistent SOM formation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Frey, 2026) are available in the Environmental Data Initiative (EDI) repository at https://doi.org/10.6073/pasta/d3ca346465196504f25c2494a6cb6711.

ORCID

Thomas J. Muratore https://orcid.org/0000-0001-9464-1156
Nikhil R. Chari https://orcid.org/0000-0002-2144-9961
Richard P. Phillips https://orcid.org/0000-0002-1345-4138
Benton N. Taylor https://orcid.org/0000-0002-9834-9192
Melissa A. Knorr https://orcid.org/0000-0002-8365-7918
Serita D. Frey https://orcid.org/0000-0002-9221-5919

REFERENCES

Abramoff, R. Z., and A. C. Finzi. 2016. "Seasonality and Partitioning of Root Allocation to Rhizosphere Soils in a Midlatitude Forest." *Ecosphere* 7(11): e01547. https://doi.org/10.1002/ecs2.1547.

Averill, C., M. C. Dietze, and J. M. Bhatnagar. 2018. "Continental-Scale Nitrogen Pollution Is Shifting Forest Mycorrhizal Associations and Soil Carbon Stocks." *Global Change Biology* 24(10): 4544–53. https://doi.org/10.1111/gcb.14368.

Bae, K., T. J. Fahey, R. D. Yanai, and M. Fisk. 2015. "Soil Nitrogen Availability Affects Belowground Carbon Allocation and Soil Respiration in Northern Hardwood Forests of New Hampshire." *Ecosystems* 18(7): 1179–91. https://doi.org/10.1007/s10021-015-9892-7.

Bai, T., P. Wang, Y. Qiu, Y. Zhang, and S. Hu. 2023. "Nitrogen Availability Mediates Soil Carbon Cycling Response to Climate Warming: A Meta-Analysis." *Global Change Biology* 29: 2608–26. https://doi.org/10.1111/gcb.16627.

Balesdent, J., C. Girardin, and A. Mariotti. 1993. "Site-Related ^(13)C of Tree Leaves and Soil Organic Matter in a Temperate Forest." *Ecology* 74(6): 1713–21. https://doi.org/10.2307/1939930.

Beidler, K. V., M. C. Benson, M. E. Craig, Y. Oh, and R. P. Phillips. 2023. "Effects of Root Litter Traits on Soil Organic Matter Dynamics Depend on Decay Stage and Root Branching Order." *Soil Biology and Biochemistry* 180: 109008. https://doi.org/10.1016/j.soilbio.2023.109008.

Boose, E., and E. Gould. 2024. "Harvard Forest Climate Data since 1964 [Dataset]." Environmental Data Initiative. https://doi.org/10.6073/PASTA/305455801DB84257FD4A5FBDB1350BD6

Bradford, M. A., A. D. Keiser, C. A. Davies, C. A. Mersmann, and M. S. Strickland. 2013. "Empirical Evidence that Soil Carbon Formation from Plant Inputs Is Positively Related to Microbial Growth." *Biogeochemistry* 113(1): 271–281. https://doi.org/10.1007/s10533-012-9822-0.

Bradford, M. A., W. R. Wieder, G. B. Bonan, N. Fierer, P. A. Raymond, and T. W. Crowther. 2016. "Managing Uncertainty in Soil Carbon Feedbacks to Climate Change." *Nature Climate Change* 6(8): 751–58. https://doi.org/10.1038/nclimate3071.

Cardon, Z. G., A. D. Czaja, J. L. Funk, and P. L. Vitt. 2002. "Periodic Carbon Flushing to Roots of Quercus Rubra Saplings Affects Soil Respiration and Rhizosphere Microbial Biomass." *Oecologia* 133(2): 215–223. https://doi.org/10.1007/s00442-002-1045-y.

Chari, N. R., and B. N. Taylor. 2022. "Soil Organic Matter Formation and Loss Are Mediated by Root Exudates in a Temperate Forest." *Nature Geoscience* 15: 1011–16. https://doi.org/10.1038/s41561-022-01079-x.

Chari, N. R., T. J. Muratore, S. D. Frey, C. L. Winters, G. Martinez, and B. N. Taylor. 2024. "Long-Term Soil Warming Drives Different Belowground Responses in Arbuscular Mycorrhizal and Ectomycorrhizal Trees." *Global Change Biology* 30: e17550. https://doi.org/10.1111/gcb.17550.

Cheng, W., and Y. Kuzyakov. 2005. "Root Effects on Soil Organic Matter Decomposition." In *Agronomy Monographs*, edited by R. W. Zobel and S. F. Wright, 119–143. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. https://doi.org/10.2134/agronmonogr48.c7.

Contosta, A. R., S. D. Frey, and A. B. Cooper. 2011. "Seasonal Dynamics of Soil Respiration and N Mineralization in Chronically Warmed and Fertilized Soils." *Ecosphere* 2(3): art36. https://doi.org/10.1890/ES10-00133.1.

Craine, J. M., C. Morrow, and N. Fierer. 2007. "Microbial Nitrogen Limitation Increases Decomposition." *Ecology* 88(8): 2105–13. https://doi.org/10.1890/06-1847.1.

Dijkstra, F. A., B. Zhu, and W. Cheng. 2021. "Root Effects on Soil Organic Carbon: A Double-Edged Sword." *New Phytologist* 230(1): 60–65. https://doi.org/10.1111/nph.17082.

Dijkstra, F., Y. Carrillo, E. Pendall, and J. Morgan. 2013. "Rhizosphere Priming: A Nutrient Perspective." *Frontiers in Microbiology* 4: 216. https://doi.org/10.3389/fmicb.2013.00216.

Ekblad, A., A. Mikusinska, G. I. Ågren, L. Menichetti, H. Wallander, R. Vilgalys, A. Bahr, and U. Eriksson. 2016. "Production and Turnover of Ectomycorrhizal Extramatrical Mycelial Biomass and Necromass under Elevated CO_2 and Nitrogen Fertilization." *The New Phytologist* 211(3): 874–885. https://doi.org/10.1111/nph.13961.

Finzi, A. C., M.-A. Giasson, A. A. Barker Plotkin, J. D. Aber, E. R. Boose, E. A. Davidson, M. C. Dietze, et al. 2020. "Carbon Budget of the Harvard Forest Long-Term Ecological Research Site: Pattern, Process, and Response to Global Change." *Ecological Monographs* 90(4): e01423. https://doi.org/10.1002/ecm.1423.

Frey, S. 2026. "Soil Warming Plus Nitrogen Addition Experiment at Harvard Forest since 2006 ver 37." Environmental Data Initiative. https://doi.org/10.6073/pasta/d3ca346465196504f25c2494a6cb6711

Frey, S. D., S. Ollinger, K. Nadelhoffer, R. Bowden, E. Brzostek, A. Burton, B. A. Caldwell, et al. 2014. "Chronic Nitrogen Additions Suppress Decomposition and Sequester Soil Carbon in Temperate Forests." *Biogeochemistry* 121(2): 305–316. https://doi.org/10.1007/s10533-014-0004-0.

Frey, S. D., J. Lee, J. M. Melillo, and J. Six. 2013. "The Temperature Response of Soil Microbial Efficiency and its Feedback to Climate." *Nature Climate Change* 3: 395–98. https://doi.org/10.1038/nclimate1796.

Hanson, P. J., N. T. Edwards, C. T. Garten, and J. A. Andrews. 2000. "Separating Root and Soil Microbial Contributions to Soil Respiration: A Review of Methods and Observations." *Biogeochemistry* 48: 115–146. https://doi.org/10.1023/A:1006244819642.

Herrera-Ramírez, D., J. Muhr, H. Hartmann, C. Römermann, S. Trumbore, and C. A. Sierra. 2020. "Probability Distributions of Nonstructural Carbon Ages and Transit Times Provide Insights into Carbon Allocation Dynamics of Mature Trees." *New Phytologist* 226(5): 1299–1311. https://doi.org/10.1111/nph.16461.

Jackson, R. B., H. A. Mooney, and E.-D. Schulze. 1997. "A Global Budget for Fine Root Biomass, Surface Area, and Nutrient Contents." *Proceedings of the National Academy of Sciences* 94(14): 7362–66. https://doi.org/10.1073/pnas.94.14.7362.

Keller, A. B., E. R. Brzostek, M. E. Craig, J. B. Fisher, and R. P. Phillips. 2021. "Root-Derived Inputs Are Major Contributors to Soil Carbon in Temperate Forests, but Vary by Mycorrhizal Type." *Ecology Letters* 24(4): 626–635. https://doi.org/10.1111/ele.13651.

Knorr, M. A., A. R. Contosta, E. W. Morrison, T. J. Muratore, M. A. Anthony, I. Stoica, K. M. Geyer, M. J. Simpson, and S. D. Frey. 2024. "Unexpected Sustained Soil Carbon Flux in Response to Simultaneous Warming and Nitrogen Enrichment Compared with Single Factors Alone." *Nature Ecology & Evolution* 8: 1–9. https://doi.org/10.1038/s41559-024-02546-x.

Kuzyakov, Y. 2002. "Separating Microbial Respiration of Exudates from Root Respiration in Non-sterile Soils: A Comparison of Four Methods." *Soil Biology and Biochemistry* 34(11): 1621–31. https://doi.org/10.1016/S0038-0717(02)00146-3.

Li, J., G. Wang, M. A. Mayes, S. D. Allison, S. D. Frey, Z. Shi, X.-M. Hu, Y. Luo, and J. M. Melillo. 2019. "Reduced Carbon Use Efficiency and Increased Microbial Turnover with Soil Warming." *Global Change Biology* 25: 900–910. https://doi.org/10.1111/gcb.14517.

Li, W., C. Jin, D.-X. Guan, Q. Wang, A. Wang, F. Yuan, and J. wu. 2015. "*The effects of simulated nitrogen deposition on plant root traits A meta-analysis* [Dataset]."

Liese, R., T. Lübbe, N. W. Albers, and I. C. Meier. 2018. "The Mycorrhizal Type Governs Root Exudation and Nitrogen Uptake of Temperate Tree Species." *Tree Physiology* 38(1): 83–95. https://doi.org/10.1093/treephys/tpx131.

Marschmann, G. L., J. Tang, K. Zhalnina, U. Karaoz, H. Cho, B. Le, J. Pett-Ridge, and E. L. Brodie. 2024. "Predictions of Rhizosphere Microbiome Dynamics with a Genome-Informed and Trait-Based Energy Budget Model." *Nature Microbiology* 9(2): 421–433. https://doi.org/10.1038/s41564-023-01582-w.

McCormack, L., I. A. Dickie, D. M. Eissenstat, T. J. Fahey, C. W. Fernandez, D. Guo, H.-S. Helmisaari, et al. 2015. "Redefining Fine Roots Improves Understanding of below-Ground Contributions to Terrestrial Biosphere Processes." *New Phytologist* 207: 505–518. https://doi.org/10.1111/nph.13363.

Melillo, J. M., S. D. Frey, K. M. DeAngelis, W. J. Werner, M. J. Bernard, F. P. Bowles, G. Pold, M. A. Knorr, and A. S. Grandy. 2017. "Long-Term Pattern and Magnitude of Soil Carbon Feedback to the Climate System in a Warming World." *Science* 358(6359): 101–5. https://doi.org/10.1126/science.aan2874.

Muratore, T. J., M. A. Knorr, M. J. Simpson, R. B. Stephens, R. P. Phillips, and S. D. Frey. 2024. "Response of Root Respiration to Warming and Nitrogen Addition Depends on Tree Species." *Global Change Biology* 30(10): e17530. https://doi.org/10.1111/gcb.17530.

Ouimette, A. P., S. V. Ollinger, L. C. Lepine, R. B. Stephens, R. J. Rowe, M. A. Vadeboncoeur, S. J. Tumber-Davila, and E. A. Hobbie. 2020. "Accounting for Carbon Flux to Mycorrhizal Fungi May Resolve Discrepancies in Forest Carbon Budgets." *Ecosystems* 23(4): 715–729. https://doi.org/10.1007/s10021-019-00440-3.

Persson, H. Å., and I. Stadenberg. 2009. "Spatial Distribution of Fine-Roots in Boreal Forests in Eastern Sweden." *Plant and Soil* 318: 1–14. https://doi.org/10.1007/s11104-008-9811-1.

Phillips, R. P., I. C. Meier, E. S. Bernhardt, A. S. Grandy, K. Wickings, and A. C. Finzi. 2012. "Roots and Fungi Accelerate Carbon and Nitrogen Cycling in Forests Exposed to Elevated CO2." *Ecology Letters* 15(9): 1042–49. https://doi.org/10.1111/j.1461-0248.2012.01827.x.

Phillips, R. P., Y. Erlitz, R. Bier, and E. S. Bernhardt. 2008. "New Approach for Capturing Soluble Root Exudates in Forest Soils." *Functional Ecology* 22(6): 990–99. https://doi.org/10.1111/j.1365-2435.2008.01495.x.

Poirier, V., C. Roumet, and A. D. Munson. 2018. "The Root of the Matter: Linking Root Traits and Soil Organic Matter Stabilization Processes." *Soil Biology and Biochemistry* 120: 246–259. https://doi.org/10.1016/j.soilbio.2018.02.016.

Quinn Thomas, R., C. D. Canham, K. C. Weathers, and C. L. Goodale. 2010. "Increased Tree Carbon Storage in Response to Nitrogen Deposition in the US." *Nature Geoscience* 3(1): 13–17. https://doi.org/10.1038/ngeo721.

R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org.

Rillig, M. C., M. Ryo, A. Lehmann, C. A. Aguilar-Trigueros, S. Buchert, A. Wulf, A. Iwasaki, J. Roy, and G. Yang. 2019. "The Role of Multiple Global Change Factors in Driving Soil Functions and Microbial Biodiversity." *Science* 366(6467): 886–890. https://doi.org/10.1126/science.aay2832.

Schindlbacher, A., S. Kwatcho Kengdo, J. Heinzle, Y. Tian, M. Mayer, J. Gadermaier, C. Shi, et al. 2025. "Increased Belowground Carbon Allocation Reduces Soil Carbon Losses under Long-Term Warming." *Global Change Biology* 31(10): e70561. https://doi.org/10.1111/gcb.70561.

Smith, W. 1969. "Root Exudates of Seedling and Mature Sugar Maple." *Phytopathology* 60: 701–3.

Smucker, A. J. M. 2003. "Root Carbon Contributions to Soil Aggregate Formation and Function." In *Roots: The Dynamic Interface between Plants and the Earth: The 6th Symposium of the International Society of Root Research, 11–15 November 2001, Nagoya, Japan*, edited by J. Abe, 421–26. Dordrecht: Springer. https://doi.org/10.1007/978-94-017-2923-9_41.

Song, J., S. Wan, S. Piao, A. K. Knapp, A. T. Classen, S. Vicca, P. Ciais, et al. 2019. "A Meta-Analysis of 1,119 Manipulative Experiments on Terrestrial Carbon-Cycling Responses to Global Change." *Nature Ecology & Evolution* 3(9): 1309–20. https://doi.org/10.1038/s41559-019-0958-3.

Treseder, K. K. 2004. “A Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric CO2 in Field Studies.” *New Phytologist* 164(2): 347–355. https://doi.org/10.1111/j.1469-8137.2004.01159.x.

Van Houtven, G., J. Phelan, C. Clark, R. D. Sabo, J. Buckley, R. Q. Thomas, K. Horn, and S. D. LeDuc. 2019. “Nitrogen Deposition and Climate Change Effects on Tree Species Composition and Ecosystem Services for a Forest Cohort.” *Ecological Monographs* 89(2): e01345. https://doi.org/10.1002/ecm.1345.

Walker, T. W. N., C. Kaiser, F. Strasser, C. W. Herbold, N. I. W. Leblans, D. Woebken, I. A. Janssens, B. D. Sigurdsson, and A. Richter. 2018. “Microbial Temperature Sensitivity and Biomass Change Explain Soil Carbon Loss with Warming.” *Nature Climate Change* 8: 885–89. https://doi.org/10.1038/s41558-018-0259-x.

Wang, J., C. Defrenne, M. L. McCormack, L. Yang, D. Tian, Y. Luo, E. Hou, et al. 2021. “Fine-Root Functional Trait Responses to Experimental Warming: A Global Meta-Analysis.” *New Phytologist* 230(5): 1856–67. https://doi.org/10.1111/nph.17279.

Wen, T., G.-H. Yu, W.-D. Hong, J. Yuan, G.-Q. Niu, P.-H. Xie, F.-S. Sun, L.-D. Guo, Y. Kuzyakov, and Q.-R. Shen. 2022. “Root Exudate Chemistry Affects Soil Carbon Mobilization Via Microbial Community Reassembly.” *Fundamental Research* 2(5): 697–707. https://doi.org/10.1016/j.fmre.2021.12.016.

Wen, Z., P. J. White, J. Shen, and H. Lambers. 2022. “Linking Root Exudation to Belowground Economic Traits for Resource Acquisition.” *New Phytologist* 233(4): 1620–35. https://doi.org/10.1111/nph.17854.

Wheeler, J. A., S. D. Frey, and K. A. Stinson. 2017. “Tree Seedling Responses to Multiple Environmental Stresses: Interactive Effects of Soil Warming, Nitrogen Fertilization, and Plant Invasion.” *Forest Ecology and Management* 403: 44–51. https://doi.org/10.1016/j.foreco.2017.08.010.

Yan, S., L. Yin, F. A. Dijkstra, P. Wang, and W. Cheng. 2023. “Priming Effect on Soil Carbon Decomposition by Root Exudate Surrogates: A Meta-Analysis.” *Soil Biology and Biochemistry* 178: 108955. https://doi.org/10.1016/j.soilbio.2023.108955.

Zak, D. R., W. E. Holmes, A. J. Burton, K. S. Pregitzer, and A. F. Talhelm. 2008. “Simulated Atmospheric NO3- Deposition Increases Soil Organic Matter by Slowing Decomposition.” *Ecological Applications: A Publication of the Ecological Society of America* 18(8): 2016–27. https://doi.org/10.1890/07-1743.1.

SUPPORTING INFORMATION

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

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