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RESEARCH ARTICLE

Long-Term Soil Warming Drives Different Belowground Responses in Arbuscular Mycorrhizal and Ectomycorrhizal Trees

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ABSTRACT

The ability of trees to acquire soil nutrients under future climate conditions will influence forest composition and function in a warmer world. Rarely are multiple belowground carbon allocation pathways measured simultaneously in large global change experiments, restricting our understanding of how trees may shift their allocation of resources to different nutrient acquisition mechanisms under future climates. Leveraging a 20-year soil warming experiment, we show that ectomycorrhizal (EM) trees reduce mycorrhizal colonization and root exudation while increasing fine root biomass, while arbuscular mycorrhizal (AM) trees largely maintained their belowground carbon allocation patterns in warmer soils. We suggest that AM trees may be better adapted to thrive under global warming due to higher rates of nitrogen mineralization in warmer soils and the ability of their mycorrhizal symbiont to acquire mineralized inorganic nutrients, whereas EM trees may need to alter their belowground carbon allocation patterns to remain competitive as global temperatures rise.

1 | Introduction

The terrestrial carbon (C) sink is vulnerable to global warming. Climate warming can increase C fixation by increasing plant productivity (Rustad et al. 2001), but the way in which fixed plant C is translocated to soil dictates how long it will be sequestered in terrestrial ecosystems (Waring et al. 2020). Enhanced soil respiration in response to warming can also offset C gains from enhanced productivity (Melillo et al. 2011; Rustad et al. 2001). The ability of terrestrial ecosystems to sequester and store C in a warming world will largely depend on how warming affects the balance of fluxes in and out of the soil C sink.

Plant belowground carbon allocation mediates C fluxes both in and out of the soil. The root nutrient acquisition strategies that trees use to forage for soil resources can be broken down into at least three broad categories: exploration (allocating C to fine root system expansion), symbiosis (allocating C to mycorrhizal fungi), and exudation (allocating C to low-molecular weight root exudates). First, plants can acquire nutrients via direct root uptake – C allocation to *exploration* via fine root growth

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would increase the likelihood of direct nutrient uptake (Gross, Peters, and Pregitzer 1993). Plants can also rely on mycorrhizal associations with their roots to acquire nutrients – C allocation to *symbiosis* would increase mycorrhizal acquisition of soil nitrogen (N) and phosphorus (P) (Read 1991). Additionally, plants can release root exudates (low-molecular weight organic C compounds) to induce microbial N and P mineralization – C allocation to *exudation* would induce this microbial priming effect resulting in mineralization of soil organic matter (SOM) and associated nutrients (Brzostek et al. 2013; Kuzyakov, Friedel, and Stahr 2000; Meier, Finzi, and Phillips 2017). The fungal collaboration gradient (Bergmann et al. 2020) proposes that plants exhibit a range of belowground C allocation patterns which vary in reliance on microbial symbiosis, with C allocation to exploration and C allocation to symbiosis lying on opposite ends of this range. Exudation is rarely measured in situ but may occupy an intermediate space between exploration and symbiosis, where plants rely on soil microbial communities for nutrient acquisition without being in direct symbiosis with them (i.e., the soil microbes do not directly colonize the plant root tissue). If plant C allocation belowground is constrained, these various root strategies may be expected to trade off depending on how advantageous each is to the plant under a given set of environmental conditions.

Belowground C allocation patterns can regulate ecosystem C cycling. For example, C allocation to root exploration would likely increase soil respiration by increasing overall root biomass and root respiration (Yan et al. 2022), but could also increase SOM formation via root litter inputs (Rasse, Rumpel, and Dignac 2005). Carbon allocation to symbiosis could increase mycorrhizal biomass and necromass turnover but could also drive higher soil heterotrophic respiration rates via mycorrhizal respiration (Tomè et al. 2016). Finally, C allocation to exudation would increase C flux into the soil on a per root basis, but could also drive greater heterotrophic respiration via the priming effect (Chari and Taylor 2022). Thus, trade-offs in C allocation to various belowground pathways in response to global change are likely to affect ecosystem C fluxes. Importantly, these C allocation pathways could partially overlap. For example, an increase in total root biomass combined with a decrease in the root exudation rate may result in no net change in root exudation per unit soil area.

Plants use a combination of root strategies to acquire soil nutrients, but the prevailing C allocation pathway of different species could be dictated by the composition of elemental nutrients in the ecosystem, otherwise known as the ecosystem nutrient economy (Phillips, Brzostek, and Midgley 2013). Temperate forest tree species primarily uptake inorganic N in the form of ammonium (NH_4^+) or nitrate (NO_3^-) (Nadelhoffer, Aber, and Melillo 1984), so the concentration of N in inorganic versus organic pools could dictate the dominant C allocation pathway. We hypothesize that, in an inorganic nutrient economy, plants may be incentivized towards exploration where their roots directly uptake mineralized N, whereas in an organic nutrient economy, symbiosis or exudation, which enhance microbial N mineralization, may be favored. Different mycorrhizal symbioses may also be advantaged or disadvantaged in different nutrient economies (Phillips, Brzostek, and Midgley 2013; Read 1991), which can feed back on soil C dynamics (Keller et al. 2021). Arbuscular mycorrhizal (AM) fungi are better suited for "scavenging" inorganic nutrients, whereas some ectomycorrhizal (EM) fungi have the ability to obtain N from organic forms (Read 1991). Thus, we expect AM-associated trees may increase C allocation to symbiosis in an inorganic nutrient economy, while EM-associated trees may increase C allocation to symbiosis in an organic nutrient economy.

Soil warming enhances microbial enzymatic rates, resulting in increased concentrations of inorganic N (NH_4^+ and NO_3^-) in the soil (Butler et al. 2012; Rustad et al. 2001). Warming-induced shifts towards an inorganic nutrient economy could favor C allocation to root exploration to take advantage of more available inorganic nutrients (Kwatcho Kengdo et al. 2022; Wang et al. 2021), which could in turn affect ecosystem C cycling. For example, a shift in C allocation from exudation to exploration could decrease heterotrophic soil respiration rates due to reduced exudation-induced priming of soil heterotrophic microbes, while enhancing autotrophic root respiration rates due to increased fine root biomass. But these changes may also be mediated by mycorrhizal type. In warmer soils with more inorganic nutrients, AM trees which are adapted to an inorganic nutrient economy may be less incentivized to shift their C allocation patterns than EM trees which are adapted to an organic nutrient economy. Thus, warming could exert unique effects on the C allocation patterns of AM and EM trees, and tree composition under warming may shift to favor species with more successful nutrient acquisition strategies.

Here, we quantified how AM and EM belowground C allocation patterns respond to long-term soil warming. For root exploration, we measured changes in absorptive and transport fine root biomass. For root exudation, we measured the specific C exudation rate in situ. For symbiosis, we measured the mycorrhizal colonization rates of root tissue. Additionally, we present morphological measurements and paired measurements of root exudation and respiration from adjacent root systems. We hypothesized that plants would shift their C allocation towards inorganic nutrient acquisition strategies (exploration, symbiosis in AM) and away from organic nutrient acquisition strategies (exudation, symbiosis in EM) in response to warming.

2 | Methods

2.1 | Field Site

Samples were collected at the Barre Woods Soil Warming Study located at the Harvard Forest Long-term Ecological Research (LTER) site in Petersham, MA, USA (42°28′ N, 72°10′ W). The site is a 100-year-old closed-canopy temperate mixed hardwood forest with *Quercus rubra* (42% basal area) being the dominant EM tree species and *Acer rubrum* (29% basal area) being the dominant AM tree species (Melillo et al. 2011). There are also minor contributions of *Quercus velutina* (EM) and *Fraxinus americana* (AM). The soils are characterized as Typic Dystrochrepts; mean annual temperature is 7.5°C and mean annual precipitation is 1119 mm y^{-1} (Giasson et al. 2013). The soil warming experiment consists of a 30 × 30 m control plot and a 30 × 30 m warmed plot heated to 5°C above ambient by underground cables, with a 5 m buffer

between the two plots, and each plot is split into 36 5 × 5 m sub-plots (Figure S1). The plots were established in the even-aged forest stand in 2001. A detailed description of the experimental design can be found in Melillo et al. (2011).

Six sub-plots containing both mature *Q. rubra* and *A. rubrum* trees were randomly selected from the 16 innermost sub-plots in both the control and warmed plots (Figure S1). Two locations within each of these sub-plots were randomly selected and flagged for sampling ($n = 12$ sampling locations per treatment).

2.2 | Root Biomass

In July 2021, two soil samples from each sub-plot were taken from the organic horizon using a 10 × 10 cm template and a serrated knife. The depth of each organic horizon was measured to the nearest mm. Plot replicates were not bulked, and all samples were processed and analyzed separately. Thus, the full sampling design was two treatments × six sub-plots per treatment × two replicates per sub-plot. Soils were transported to the laboratory, stored at 4°C, and processed within 1 week. Roots were placed onto a clean 2 mm sieve and removed from the soil in 5-min intervals, recording the mass returned after each interval until a period of stabilized diminishing returns was reached. During the process, live root tissues were separated into either absorptive (1st, 2nd, and 3rd order) or transport root (4th and 5th order) functional types based on branching order (McCormack et al. 2015). Absorptive and transport roots were further divided into AM or EM categories based on color, morphology, and presence of EM or AM associations. Specifically, EM roots were identified based on a darker color, root branching angle, mycorrhizal colonization, and the number of root tips. In contrast, AM roots were identified based on lighter-colored, larger-diameter roots. The presence or absence of ectomycorrhiza was used as secondary confirmation of EM and AM roots, respectively. Dead roots were identified based on their dark discoloration, poor adhesion between the stele and cortex, and brittleness, and removed from further analyses (Persson and Stadenberg 2009). Separated live fine roots were cleaned with water to remove organic debris, dried at 60°C, and weighed to determine root biomass ($g\,m^{-2}$) for each functional class.

2.3 | Root Exudate Collection

Root exudates were collected four different times at 2-week intervals during June and July of 2022 (Table S1). Exudates were collected using a modification of the cuvette method (Phillips et al. 2008). At each sampling location, intact EM and AM root systems (~10–15 cm in length) were gently removed from the O horizon soil while maintaining connection to the larger root system. Tree mycorrhizal type was identified by root morphology in situ and again for confirmation after exudate collection was completed. The individual tree species were not identified. Root systems were rinsed with DI water and cleaned with tweezers to remove visible soil particles. After cleaning, the still-intact root systems were placed in 40 mL glass syringes, which were then filled with glass beads (0.75–1.00 mm diam.) and C-free nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.2 mM $MgSO_4 \cdot 7H_2O$, 0.3 mM $CaHPO_4$) to "water holding capacity" (i.e., until solution came out the base of the syringe). Syringes were sealed around the root in resealable plastic bags and covered in leaf litter for ~48 h (exact time recorded). After 48 h, the nutrient solution was extracted via vacuum, then the syringe was rinsed twice with nutrient solution and replaced with a new nutrient solution for ~24 h (exact time recorded).

After 24 h, the root was cut at the top of the syringe, and the solution was extracted under vacuum into glass TOC vials along with two additional DI water extractions to maximize C removal. Sample vials were stored at −80°C until analysis. Roots were stored at −20°C until analysis for root traits. Three blank syringes that did not include roots were also prepared each sampling round for background C levels.

Total organic carbon (TOC) was measured from exudate samples as non-purgeable organic carbon (NPOC) on a Shimadzu TOC ASI-V analyzer. Total organic carbon (in μg/mL) was multiplied by the sample volume and standardized by root surface area or biomass and incubation time to calculate specific root exudation rates. Average background C levels from blank cuvettes were subtracted from exudate samples before standardization by root traits to determine root biomass-specific and root surface area-specific exudation rates.

2.4 | Root Respiration

Root respiration was measured at the time of exudate collection on a paired root descended from the same transport root. The roots were severed and immediately confirmed for mycorrhizal functional type (AM vs. EM) by root morphology. Roots were carefully cleaned with tweezers to remove organic debris. Cleaned EM and AM roots were individually inserted into a glass chamber (175 mL) connected using tubing (19 mL) to a LI-COR-8100a field infrared gas analyzer (IRGA) that collected CO_2 concentration every 1 s for a 5-min interval. Each interval had a 15 s chamber pre-purge and a 15 s chamber post-purge. Roots were sampled within 15 min of separation from the larger root system.

We calculated root respiration by fitting a linear model to the relationship between time and CO_2 concentration (ppm) within the glass chamber, and we then converted the slope of the linear model to CO_2 flux per unit time. We divided root respiration measurements by the dry root biomass of the sample to represent mass-specific root respiration or root surface area to represent area-specific root respiration (see *Root trait analyses*).

2.5 | Root Trait Analyses

Roots were scanned in grayscale on a PlusTek OpticSlim A3 series scanner, and scanned images were analyzed for root traits using RhizoVision Explorer software. RhizoVision settings were optimized by the user to capture root traits most accurately. We calibrated the software with strips of paper of known length and width. All RhizoVision analyses were completed by the same

user (N.R.C.) to control for user bias. Total root surface area measurements from RhizoVision software were used to calculate area-based exudation and respiration fluxes. After scanning, roots were dried at 60°C and root biomass was measured. Specific root length was calculated by dividing total root length by root biomass.

2.6 | Mycorrhizal Colonization Rates

Four 10 × 10 cm sections of organic horizon soil were collected in April 2023 from each sub-plot where we measured exudation and respiration rates ($n = 24$). We extracted all fine roots manually from the samples by submerging them in water and separated fine roots into AM and EM fractions by morphology as detailed above (see *Root biomass*). Roots were stored in 60% ethanol at 4°C prior to colonization measurements. For EM colonization, 10 root segments from each soil sample were randomly selected, and the presence/absence of mycorrhizal colonization for 20 tips on each root segment (200 tips total) was scored using a dissecting microscope (Zeiss Discovery, Jena, Ger). EM colonization rate is expressed as a percentage of tips colonized. For AM colonization, roots were cut into 2 cm fragments, and 10 fragments per sample were stained with Trypan blue as per Brundrett et al. (1996). The presence/absence of AM arbuscules, vesicles, or coils was scored each millimeter along the root fragment using a visible light microscope (Zeiss Axioimager, Jena, Ger) at 40× magnification (i.e., 20 scores per segment). The AM colonization rate is expressed as the average percent tissue colonization across the 10 fragments per sample. All colonization measurements were completed by the same user (N.R.C.) to control for user bias.

2.7 | Statistical Analyses

All statistical analyses were carried out in R statistical software (R Core Team 2022). We ran linear mixed-effects models using root exudation or root respiration as the response variable and warming treatment and mycorrhizal type as fixed effects. We compared four models: one without random effects, one with plot number as a random effect, one with sampling week as a random effect, and one with both plot and week as random effects (libraries *lme4* and *nlme*; Bates et al. 2015; Pinheiro and Bates 2004). We found that none of the random effects improved the model AIC for area-based and massed-based root exudation and root respiration rates (i.e., did not decrease AIC by more than 2 points). For this reason, we omitted random effects of plot and week from our data analysis and proceeded with two-way analysis of variance (ANOVA) models. We used a contrast matrix to restrict comparisons between the following groups of interest: Control-AM:Control-EM, Warmed-AM:Warmed-EM, Control-AM:Warmed-AM, Control-EM:Warmed-EM (library *multcomp*; Hothorn, Bretz, and Westfall 2008). For root exudation and respiration, we omitted values < 0 on the basis that these measurements were from relatively small roots and were thus likely below the detection limit rather than exhibiting C uptake (Figure S2). We did not omit any values > 0 from our analysis. Here, we present any significant effects of warming and mycorrhizal type as well as any significant group comparisons in the case of interactive effects. We used linear models to determine relationships between root exudation and respiration and specific root length and exudation.

Additionally, we used principal component analyses (PCAs) for AM and EM trees to holistically compare how each tree-mycorrhizal association altered a suite of root strategies in response to warming (library *vegan*; Oksanen et al. 2013). We used root traits representative of exploration (absorptive and transport fine root biomass), symbiosis (mycorrhizal colonization), and exudation (specific root exudation) belowground C allocation. We used a PERMANOVA model to determine whether warming treatments clustered differently in belowground C allocation space for each tree-mycorrhizal type. In all cases, we assessed significance at $\alpha = 0.05$.

3 | Results

3.1 | Structural Root Allocation

In the control plot, there was no significant difference between total fine root biomass of AM and EM trees (despite *Q. rubra* being dominant aboveground), while under warming, total fine root biomass of EM trees was nearly three times higher than AM fine biomass (Figure 1a; $p \leq 0.01$). Notably, basal area was similar between control and warmed plots for both *Q. rubra* and *A. rubrum*, with *Q. rubra* encompassing 21.6 and 21.9 $cm^2\,m^{-2}$ in the control and warmed plots, respectively, and *A. rubrum* encompassing 18.0 and 14.6 $cm^2\,m^{-2}$. Both specific root length (SRL; Figure 1b; $p \leq 0.001$) and average fine root diameter (Figure 1c; $p \leq 0.01$) were higher in AM than EM tree species, indicating differences in species morphology. Specifically, AM trees had a higher average root diameter (Figure 1a) and a lower root tissue density (Figure S3). However, mean AM SRL also increased by 25% under warming (Figure 1b; $p \leq 0.05$), while EM SRL was not significantly affected by warming.

3.2 | Root Exudation and Root Respiration

We observed decreased root exudation rates in response to soil warming (Figure 2a,b; $p \leq 0.05$) but no changes in root respiration. Decreased root exudation under warming was driven by EM tree roots ($p \leq 0.05$), with mean area-based and mass-based EM exudation rates reduced by 44% and 58%, respectively, between control and warmed plots. EM tree roots had overall higher surface area-based respiration than AM roots, with no warming effect (Figure 2c; $p \leq 0.05$).

Warming also affected relationships between root exudation and respiration, which varied with mycorrhizal type. Exudation and respiration rates were positively correlated in AM tree species (Figure 3a; $R^2 = 0.31$, $p \leq 0.001$) in the control plot but not in the warmed plot. Relationships between exudation and respiration were not observed in EM tree species (Figure 3b). Additionally, root exudation rates were positively correlated with SRL in EM but not AM tree species (Figure 3c; $R^2 = 0.26$, $p \leq 0.01$). This relationship also was not maintained under warming (Figure 3d). We also note that both of these

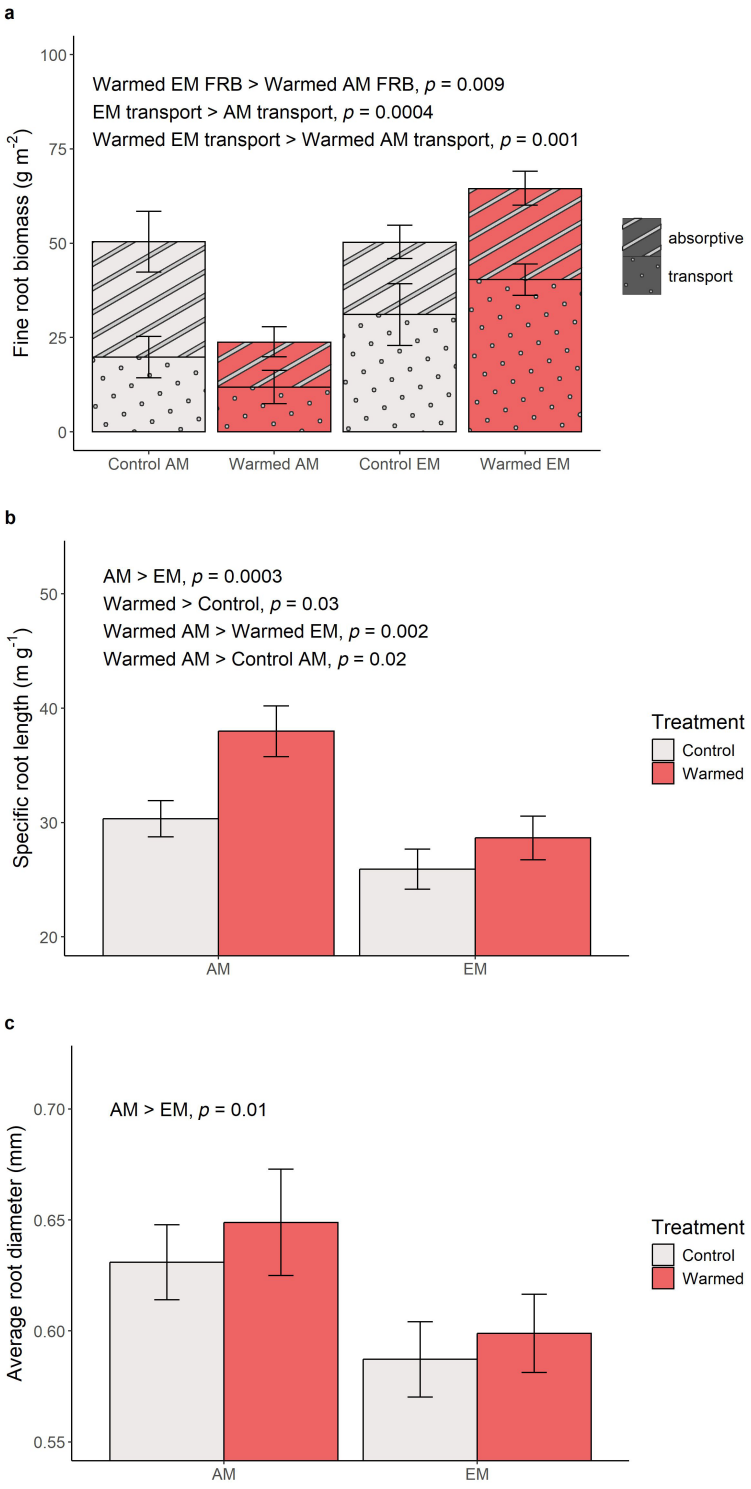

FIGURE 1 | Effects of warming on root biomass (a), specific root length (b), and average root diameter (c) of AM and EM roots in control (grey) and warmed (red) plots. Error bars are standard error.

relationships remain significant when the right-most points are omitted (Figure S4).

3.3 | Mycorrhizal Colonization

There was no response of AM colonization rates to soil warming (Figure 4a), but EM colonization rates were significantly lower in response to warming (Figure 4b; $p \leq 0.001$).

3.4 | Belowground C Allocation Patterns

We took characteristic measurements of three important belowground C allocation pathways identified as exploration (measured as fine root biomass), symbiosis (measured as mycorrhizal colonization), and exudation (measured as specific root exudation) and used principal component analyses to determine how both AM and EM trees responded to warming treatments in root strategy space. We found that C allocation patterns of AM trees

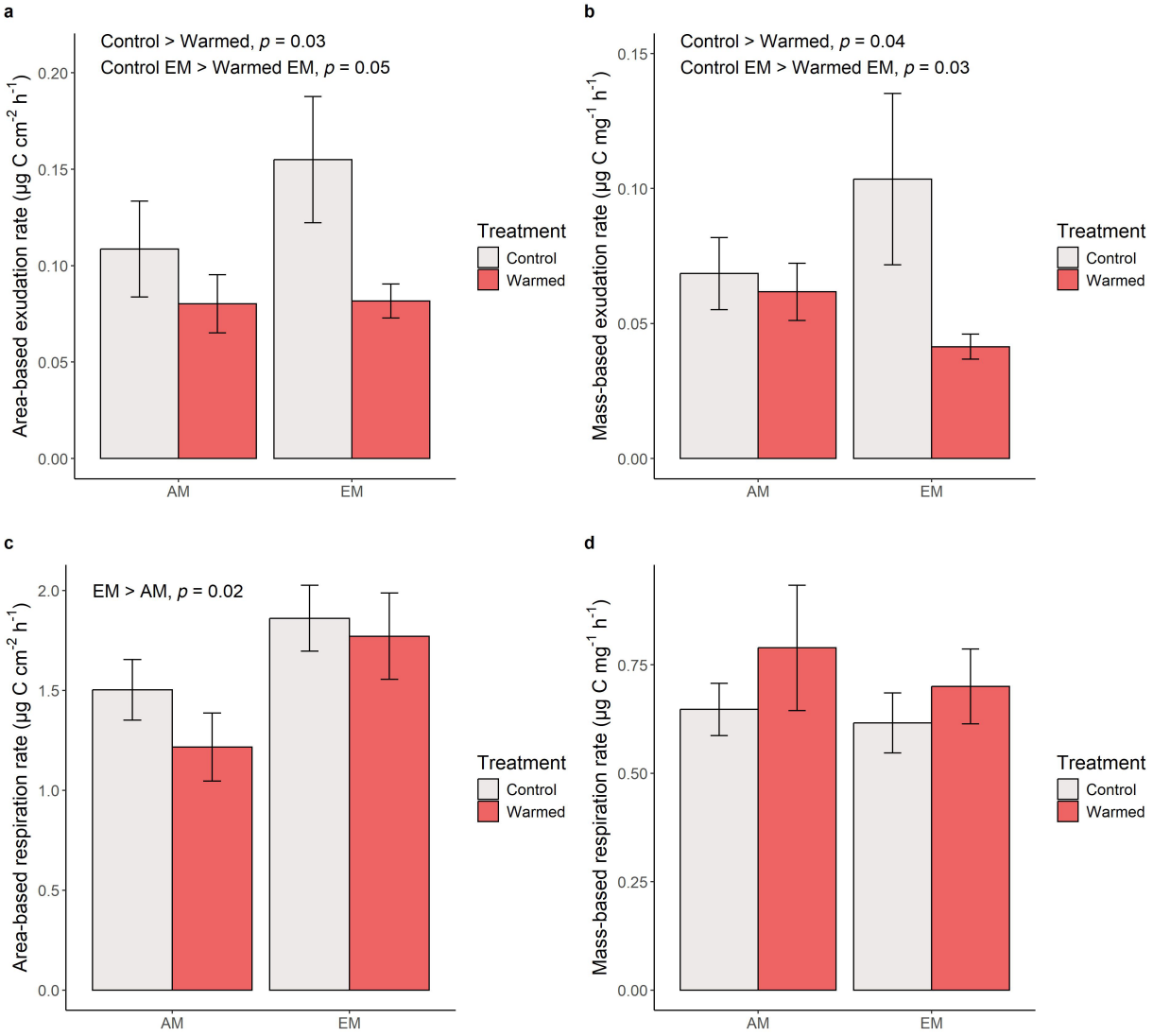

FIGURE 2 | Root exudation (a, b) and root respiration (c, d) from AM and EM tree species in response to soil warming, standardized by root surface area (a, c) and root biomass (b, d). Error bars are standard errors.

did not respond to warming (i.e., AM trees in warmed and control plots clustered similarly, $p = 0.3$; Figure 5a). However, EM trees exhibited unique clustering depending on the warming treatment ($p = 0.03$; Figure 5b), shifting overall foraging strategies away from exudation and symbiosis and towards root biomass exploration in warmed soils.

4 | Discussion

4.1 | Belowground C Allocation Patterns of AM and EM Trees Differ in Response to Warming

Our results provide evidence that trees differentially modify their belowground C allocation patterns under warming depending on their dominant mycorrhizal association. Overall, our data suggest that EM trees may be more likely to reallocate resources away from exudation and symbiosis and towards root exploration under warming. Previous research at our field site found that N mineralization rates increased in the warmed plot (Butler et al. 2012), likely leading to a shift from an organic nutrient economy towards an inorganic nutrient economy where AM associations are likely to provide a greater benefit to the host tree (Phillips, Brzostek, and Midgley 2013). While our data were primarily collected from one AM and one EM tree species, maple and oak are dominant AM and EM tree genera, respectively, in temperate deciduous forests, suggesting that these patterns may be widely conserved.

We observed several responses to warming which suggest that AM trees maintain their belowground C allocation patterns under warming, whereas EM trees alter their C allocation to maximize inorganic nutrient acquisition. First, EM trees downregulated root exudation under warming while AM trees did not (Figure 2a,b). Root exudation has frequently been correlated with activities of enzymes that mine organic N from SOM (Meier, Finzi, and Phillips 2017; Phillips, Finzi, and Bernhardt 2011). Enhanced bulk soil microbial N mineralization in warmer soils may dis-incentivize plants to use exudation as a microbial N mineralization mechanism. This provides evidence that *C allocation to exudation is*

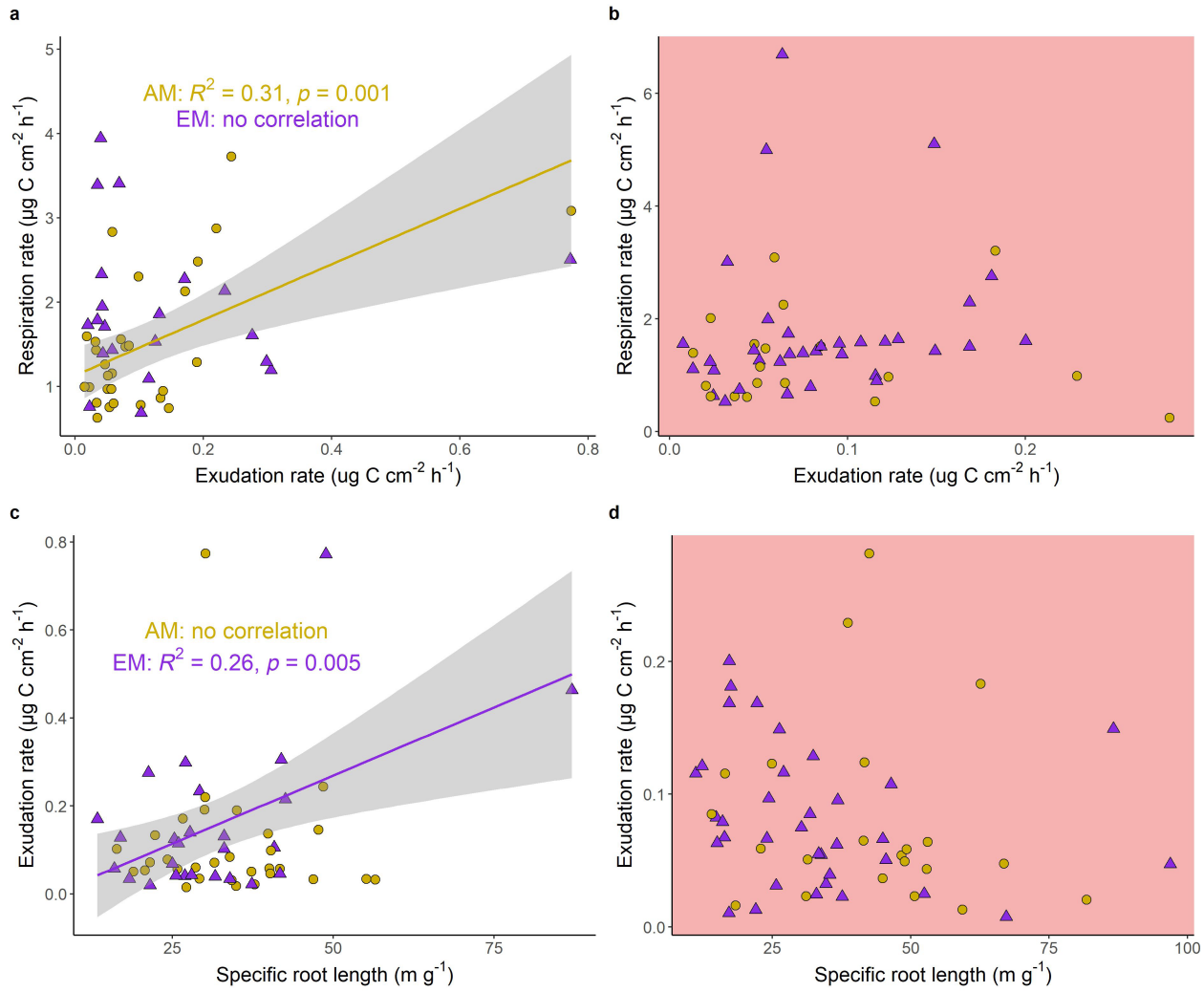

FIGURE 3 | Relationships between exudation and respiration (a, b) and specific root length (SRL) and exudation (c, d) in AM (gold circles) and EM (purple triangles) trees in control (a, c) and warmed (b, d) plots. We note that the relationships observed in (a) and (c) remain significant when the far right points are removed from the data (Figure S4).

downregulated by EM trees under warming. Second, mycorrhizal colonization of EM roots decreased under warming, while mycorrhizal colonization of AM roots did not (Figure 4). EM fungi excel at acquiring organic nutrients, while AM fungi excel at acquiring inorganic nutrients (Read 1991). This response provides evidence that *C allocation to symbiosis is downregulated by EM trees under warming*, as EM fungi are likely to be less efficient in a more inorganic nutrient economy. Third, EM trees exhibited increased fine root biomass relative to AM trees under warming, despite AM trees exhibiting greater aboveground growth (Butler et al. 2012; Figure 1a). This provides evidence that *C allocation to exploration is upregulated by EM trees under warming*, as EM trees favor direct acquisition of inorganic N by roots as opposed to acquisition of organic N by root-associated microbes (Beidler et al. 2021; Keller and Phillips 2019). Specifically, EM trees increased their transport fine root biomass rather than absorptive fine root biomass (Figure 1a), which suggests an investment in root system expansion rather than symbiotic or exudative strategies associated with absorptive roots. Notably, AM trees either maintained or decreased their C allocation to all three belowground C allocation pathways, while increasing aboveground growth (Butler et al. 2012).

Additionally, SRL of AM trees increased under warming, while EM SRL did not (Figure 1b). Higher SRL is associated with higher absorptive to transport (A:T) root ratios (Pregitzer et al. 2002). AM fungal colonization is associated with absorptive roots over transport roots (McCormack et al. 2015). While AM colonization rates of absorptive root tissue did not increase significantly under warming, enhanced A:T ratios could facilitate greater mycorrhizal colonization overall. Increased C allocation to mycorrhizae by AM trees under warming would be consistent with the capacity of AM fungi for inorganic nutrient acquisition. Finally, exudation was correlated with SRL in EM tree species in the control plot but not the warmed plot (Figure 3c,d). We expect exudation to be correlated with SRL because exudation is localized at fine root tips (Canarini et al. 2019) and the concentration of first-order root tips increases with SRL (Pregitzer et al. 2002). This relationship has been previously shown to be stronger in EM than AM tree species (Akatsuki and Makita 2020). The absence of this relationship in EM roots under warming suggests

that even root systems with high adsorptive character (high SRL) are shifting their nutrient acquisition mechanisms away from exudation.

When we analyzed the three different C allocation pathways holistically, we observed that EM trees shifted their belowground C allocation patterns under warming while AM C allocation patterns remained the same (Figure 5). These results, coupled with evidence that maple trees in these plots increase their aboveground growth rates more than oaks in response to warming (Butler et al. 2012), suggest that AM trees will be better adapted to acquire soil nutrients in a warmer future. Collectively, our results suggest AM trees may be better equipped to thrive in warmer environments without considerably adjusting their root strategies, whereas EM trees appear to make substantial adjustments to their belowground C allocation patterns in warmer soils.

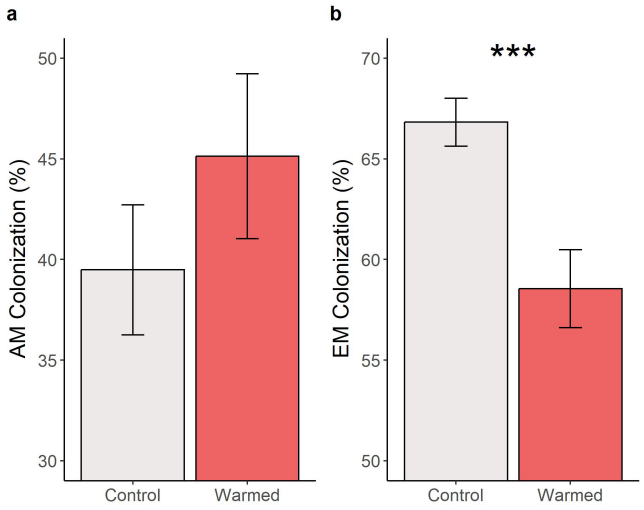

FIGURE 4 | Mycorrhizal colonization rates of (a) AM root systems and (b) EM root systems in response to warming. AM colonization is measured as a percentage of root tissue colonized and EM colonization is measured as percentage of root tips colonized. Error bars are standard error. The asterisks (***) mean that $p < 0.001$.

4.2 | Relationships Between Root Exudation and Respiration Under Warming

We observed decreases in tree root exudation but no change in root respiration in response to long-term soil warming (Figure 2). Our results suggest that tree roots may decrease C export belowground (i.e., exudation) in response to soil warming without changing direct C export to the atmosphere (i.e., respiration), potentially decreasing soil C sequestration capacities. However, decreased root exudation could also preserve soil C by reducing the microbial priming effect by which exudate C inputs stimulate microbial breakdown and respiration of native soil C (Chari and Taylor 2022; Kuzyakov, Friedel, and Stahr 2000). Recent research suggests that root respiration is important in driving soil respiration response to warming (Yan et al. 2022), so root-based fluxes will be particularly relevant for understanding ecosystem-scale C dynamics under global change.

There is a paucity of in situ measurements of root exudation in response to warming. However, the literature that exists suggests an interesting interaction between air and soil warming. In soil warming experiments, root exudation decreases in response to warming (Xiong et al. 2020 and this manuscript). However, infrared air warming experiments (which typically result in smaller changes in soil temperature than soil warming experiments) in tree seedlings have measured increases in root exudation (Yin et al. 2013; Zhang et al. 2016). The contrasting results suggest that enhanced photosynthetic rates due to air warming and enhanced N mineralization due to soil warming have opposing effects on root exudation rates. Enhanced plant growth under air warming may incentivize SOM priming and result in increased exudation, whereas enhanced N mineralization under soil warming may disincentivize priming and decrease exudation. Broadly, we may expect that global change factors which increase N demand (e.g., air warming or elevated CO_2) should increase exudation, whereas global change factors which decrease N demand (e.g., soil warming or N fertilization) should decrease exudation. Measuring root exudation in multifactorial global change experiments to determine which of these effects will dominate in the future is an important next step.

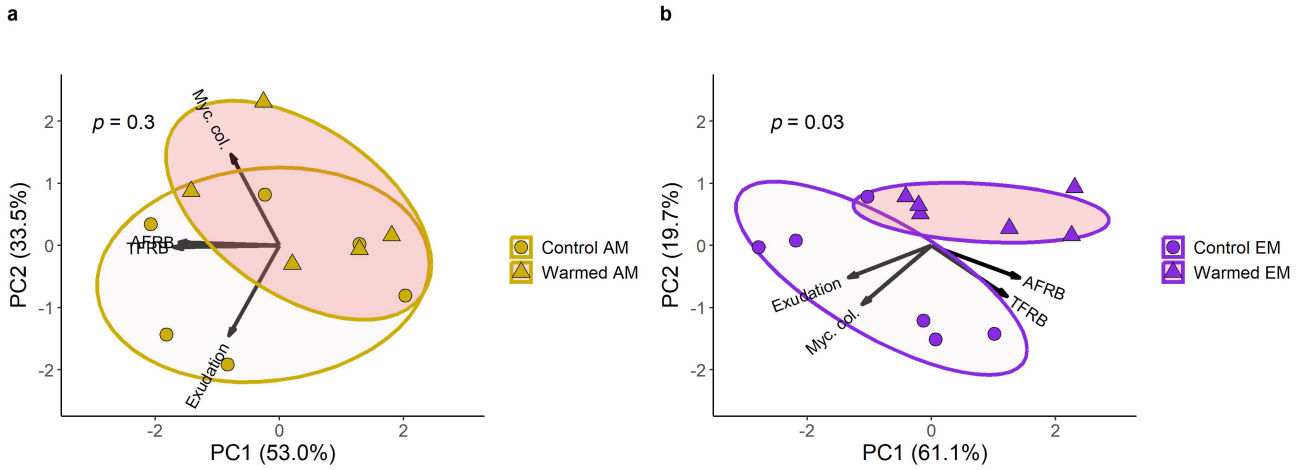

FIGURE 5 | Biplots for principal components analyses of belowground C allocation patterns for (a) AM roots and (b) EM roots. Each dot is from a control sub-plot (shaded white ellipse) and each triangle is from a heated sub-plot (shaded pink ellipse). Arrows show different C allocation pathways, where AFRB and TFRB (absorptive and transport fine root biomass) are "exploratory," myc. col. (mycorrhizal colonization) is "symbiotic," and exudation is "exudative." Ellipses represent 68% confidence intervals.

We also observed relationships between root exudation and respiration rates. Root exudation and respiration were positively correlated in control plots ($R^2 = 0.08$, $p = 0.03$), an effect driven by AM trees ($R^2 = 0.31$, $p = 0.001$) (Figure 3a). Positive relationships between root exudation and respiration have also previously been observed in temperate forests (Sun et al. 2017). However, we observed that this relationship was not maintained under warming (Figure 3b). It is reasonable to assume root exudation and respiration rates should be coupled, as both rates are driven by C export to fine roots and root metabolic activity. The drivers of the decoupling of these metabolisms in response to warming are unknown to us and merit further investigation.

4.3 | Implications for Future Forest Structure

We did not find differences in mass- or area-specific root respiration rates in response to warming, suggesting that specific root respiration acclimated to long-term soil warming at this study site. These results indicate that root contributions to soil respiration under warming in temperate forests may be driven by changes in fine root biomass rather than specific root respiration. While Melillo et al. (2011) found that decreased root biomass under warming reduced root respiration rates per soil area at this study site, here we show that 20 years of soil warming have returned total root biomass back to control levels with important differences in the root biomass allocation patterns of the dominant tree species in the plots. We found fine root biomass (and specifically transport fine root biomass) of EM trees was significantly higher than that of AM trees under warming, perhaps due to the inability of EM trees to effectively compete with AM trees in high N environments and their reinvestment of C into root biomass.

Barre Woods is an EM-dominated forest (*Quercus rubra*), with a significant contribution of the AM tree species, *Acer rubrum*. At the Harvard Forest and elsewhere, there are indications that AM trees may better sustain aboveground growth under warming compared to EM trees (Butler et al. 2012; Lankau, Zhu, and Ordonez 2015), perhaps because AM roots and fungi are better equipped for increasingly inorganic nutrient economies (Querejeta et al. 2021). Our results suggest that EM trees compensate for their decreased mycorrhizal efficiency by increasing C allocation to root exploration. The ability of EM trees to remain competitive aboveground in a warmer future may depend in part on their ability to reallocate C belowground to maximize nutrient acquisition.

In addition to increasing C allocation to root system expansion, we show that EM trees appear to downregulate C allocation to organic root nutrient acquisition strategies such as root exudation and mycorrhizal fungi. In the decreased presence of exudate C inputs, rhizosphere microbial activity may decelerate, reducing soil heterotrophic respiration. This effect would likely interact with well-known positive effects of warming on microbial enzyme kinetics (Lloyd and Taylor 1994). Thus, the net effect of shifting root exudation regimes under warming on soil C dynamics will likely rely on an interaction of the specific exudation rate, the proliferation of root biomass, and the response of rhizosphere microbes to warming.

5 | Conclusion

Soil warming appears to have a profound effect on the belowground dynamics of forest ecosystems that varies by mycorrhizal association. Fine root biomass increased in EM relative to AM trees under warming, indicating investment in root exploration for acquiring inorganic nutrients. On the other hand, root exudation and mycorrhizal colonization rates decreased in EM trees under warming, indicating divestment from exudative and symbiotic strategies for acquiring organic nutrients. Meanwhile, AM trees maintained their belowground C allocation patterns in response to warming, suggesting that AM trees may already be well-adapted to increasingly inorganic nutrient economies under warming. Altered belowground C allocation in a warmer future could have considerable effects on the amount and type of C inputs from plants to soil, and changes in the root strategies of individuals will interact with changes in forest structure to regulate soil C inputs as temperatures rise.

Author Contributions

Nikhil R. Chari: conceptualization, formal analysis, funding acquisition, investigation, methodology, writing – original draft, writing – review and editing. **Thomas J. Muratore:** conceptualization, formal analysis, funding acquisition, investigation, methodology, writing – review and editing. **Serita D. Frey:** funding acquisition, supervision, writing – review and editing. **Cristina L. Winters:** investigation, writing – review and editing. **Gabriela Martinez:** investigation, writing – review and editing. **Benton N. Taylor:** funding acquisition, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study is openly available at doi:10.5061/dryad.tx95x6b6r

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