ECOLOGICAL
APPLICATIONS
ECOLOGICAL SOCIETY OF AMERICA

Independent effects of tree diversity on aboveground and soil carbon pools after six years of experimental afforestation

Reb L. Bryant[1,2] | Shan Kothari[3,4] | Jeannine Cavender-Bares[4,5] |
Stephanie J. Curran[6] | Jake J. Grossman[7] | Sarah E. Hobbie[5] |
Charlotte Nash[8] | Grace C. Neumiller[9] | Craig R. See[5,10]

[1]Department of Ecology & Evolutionary Biology, University of Kansas, Lawrence, Kansas, USA

[2]Kansas Biological Survey and Center for Ecological Research, University of Kansas, Lawrence, Kansas, USA

[3]Centre d'étude de la forêt, Université du Québec à Montréal, Montréal, Quebec, Canada

[4]Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, USA

[5]Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota, USA

[6]Department of Biology, St. Olaf College, Northfield, Minnesota, USA

[7]Department of Biology and Department of Environmental Studies, St. Olaf College, Northfield, Minnesota, USA

[8]Biology Department, Bowdoin College, Brunswick, Maine, USA

[9]Minneapolis-St. Paul Long Term Ecological Research Site, University of Minnesota, St. Paul, Minnesota, USA

[10]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota, USA

Correspondence
Reb L. Bryant
Email: reb.bryant@ku.edu

Abstract

Planting diverse forests has been proposed as a means to increase long-term carbon (C) sequestration while providing many co-benefits. Positive tree diversity–productivity relationships are well established, suggesting more diverse forests will lead to greater aboveground C sequestration. However, the effects of tree diversity on belowground C storage have the potential to either complement or offset aboveground gains, especially during early stages of afforestation when potential exists for large losses in soil C due to soil decomposition. Thus, experimental tests of the effects of planted tree biodiversity on changes in whole-ecosystem C balance are needed. Here, we present changes in above- and belowground C pools 6 years after the initiation of the Forests and Biodiversity experiment (FAB1), consisting of high-density plots of one, two, five, or 12 tree species planted in a common garden. The trees included a diverse range of native species, including both needle-leaf conifer and broadleaf angiosperm species, and both ectomycorrhizal and arbuscular mycorrhizal species. We quantified the effects of species richness, phylogenetic diversity, and functional diversity on aboveground woody C, as well as on mineral soil C accumulation, fine root C, and soil aggregation. Surprisingly, changes in aboveground woody C pools were uncorrelated to changes in mineral soil C pools, suggesting that variation in soil C accumulation was not driven by the quantity of plant litter inputs. Aboveground woody C accumulation was strongly driven by species and functional identity; however, plots with higher species richness and functional diversity accumulated more C in aboveground wood than expected based on monocultures. We also found weak but significant effects of tree species richness, identity, and mycorrhizal type on soil C accumulation. To assess the role of the microbial community in mediating these effects, we further compared changes in soil C pools to phospholipid fatty acid (PLFA) profiles. Soil C pools and accumulation were more strongly correlated with specific microbial clades than with total microbial biomass or

Reb L. Bryant, Shan Kothari, and Craig R. See are equal contributors; all other authors are alphabetically ordered.

plant diversity. Our results highlight rapidly emerging and microbially mediated effects of tree biodiversity on soil C storage in the early years of afforestation that are independent of gains in aboveground woody biomass.

KEYWORDS

carbon sequestration, functional diversity, leaf type, mycorrhizal type, nutrient cycling, soil aggregation, species richness

INTRODUCTION

Current warming due to rising atmospheric carbon dioxide has prompted a call for "nature-based climate solutions" that increase ecosystem carbon (C) sequestration, which includes managing forests for C storage (Bastin et al., 2019; Fargione et al., 2018). Although tree species vary greatly in their initial productivity (Bukoski et al., 2022), there is strong evidence that forest biodiversity increases long-term aboveground productivity, resilience, and other important ecosystem functions based on both observational (Liang et al., 2016) and experimental (Feng et al., 2022; Grossman et al., 2017; Huang et al., 2018) studies. As a result, there is growing consensus that future afforestation efforts should prioritize tree biodiversity (Liu et al., 2018; Messier et al., 2022). Critically, the impacts of planted tree diversity on belowground C storage are less clear (X. Chen et al., 2020; Ma et al., 2020), despite the fact that the bulk of C is stored below ground in most forests (Pan et al., 2011). Thus, a better understanding of planted diversity effects on whole-system C pools (i.e., above- and belowground) is needed to inform current afforestation efforts (Bossio et al., 2020; Mayer et al., 2020), especially during the early years of afforestation when large losses in soil C are commonly observed (Deng et al., 2014; Potvin et al., 2011).

Tree diversity can influence soil C accumulation through multiple causal pathways, and these two variables are often (Augusto & Boča, 2022; Chen et al., 2023; S. Chen, Wang, et al., 2018; X. Chen et al., 2020; Fanin et al., 2022) but not always positively related (Beugnon et al., 2023; Martin-Guay et al., 2022; Strukelj et al., 2021). Soil C is ultimately derived from new inputs of plant litter, root exudates, and mycorrhizal hyphae (Augusto & Boča, 2022; Mayer et al., 2020; See et al., 2022), and the quantity of these C fluxes to soils depend on primary productivity. Although the positive effect of tree diversity on productivity should increase the total flux of C into soils (i.e., total above- and belowground plant litter C flux; Ma & Chen, 2016; Zheng et al., 2019), evidence that diversity influences belowground productivity is equivocal (Archambault et al., 2019; Martin-Guay et al., 2020; Schuster, 2023). Beyond affecting the total flux of C

into soils, plant diversity exerts strong effects on soil microbial communities (C. Chen et al., 2019; Cline et al. 2018; Grossman, Butterfield, et al., 2019; Khlifa et al., 2017; Strukelj et al., 2021; but see Rivest et al., 2019), which may, in turn, affect the residence time of soil C. Tree composition influences microbial community function directly via litter substrate chemistry (Angst et al., 2019; Bray et al., 2012) and indirectly via interactions between mycorrhizal fungi and soil saprotrophs (Fernandez & Kennedy, 2016; Frey, 2019). Microbial decomposition represents a dominant pathway for soil C loss, and the deposition of microbial residues (i.e., necromass) represents a large proportion of soil C (Sokol et al., 2022). Thus, tree species effects on microbial processes may be just as important for net soil C storage as tree species effects on the quantity of the C flux into soils.

Beyond species richness, tree functional identity (e.g., mycotype, leaf type) can also be helpful in understanding C sequestration. There have been recent calls for common garden experiments that can tease apart tree species and functional group effects from diversity effects on soil C (Mayer et al., 2020). Mounting evidence suggests that arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) trees may promote different C cycling dynamics through different microbial associations, litter quality, and soil properties (Heděnec et al., 2023; Phillips et al., 2013; Read & Perez-Moreno, 2003). In particular, AM fungi have garnered attention for their role in the formation and stability of soil aggregates (Rillig, 2004; Rillig et al., 2015; Wright & Upadhyaya, 1998), although EcM fungi and other saprotrophs have recently also been implicated in soil aggregation (Dick et al., 2022; Filialuna & Cripps, 2021; Zheng et al., 2014). Furthermore, AM trees may lead to faster accumulation of mineral-associated organic matter with a long residence time than EcM trees in the same soil (Craig et al., 2018, 2022). Finally, mycorrhizal type has been increasingly linked to differential diversity effects on aboveground productivity via plant–soil feedbacks (Bennett et al., 2017; Crawford et al., 2019; Deng et al., 2023; Dietrich et al., 2023), which may also affect belowground productivity and soil C accumulation.

In addition to mycotype, leaf type (broadleaf vs. needle-leaf) also affects belowground C accumulation.

Broadleaf forests accumulate soil organic C faster in mineral soils, while needle-leaf forests sequester C faster in organic horizons, and these vertical spatial patterns may lead to complementary effects where both groups are present (Laganière et al., 2010). Likewise, broadleaf and needle-leaf trees also differ in litter chemistry (Kothari et al., 2023), and the presence of multiple leaf types in litter has been linked with changes in microbial community composition (Chapman et al., 2013; Grossman, Butterfield, et al., 2019) and decomposition rates (Liu et al., 2020, but see Porre et al., 2020) in comparison with litter of a single leaf type. However, many of these studies are correlational, and it can be difficult to tease apart interactions between functional groups that have considerable within-group variation in functional traits (Chen, Ding, et al., 2018; Mueller et al., 2015; Rożek et al., 2023). Thus, co-located experimental plantings (i.e., common garden experiments) are needed to understand the impacts of tree functional identity and diversity on soil C sequestration, especially in relation to aboveground woody C gains.

One way that tree diversity may influence soil C accumulation is through aggregate formation. Water-stable soil aggregates can protect organic matter from microbial decomposition (Six et al., 2004). While this process is partially controlled by abiotic factors like soil texture and chemistry, biotic factors are also important (Regelink et al., 2015; Six et al., 2004). Plants influence aggregation through their root structures and distribution, root exudates, and through their effects on the soil microbial community (Amézketa, 1999; Angers & Caron, 1998; Reid & Goss, 1981; Rillig et al., 2002; Scott, 1998). Therefore, tree diversity could impact aggregation if it changes root biomass or structure. Additionally, tree diversity may influence aggregate formation due to its effects on soil microbial communities, which play an important role in aggregate formation and stability (Baumert et al., 2018; Lehmann et al., 2017; Tisdall, 1994). However, few studies have investigated the impacts of tree diversity on aggregation, and knowledge about drivers of soil aggregation comes largely from grasslands and agricultural fields rather than forests.

In this study, we analyzed changes in above- and belowground C pools 6 years after planting in a tree biodiversity experiment planted in sandy soils in central Minnesota, USA. Trees were chosen to represent a functionally and phylogenetically diverse range of native species, including both needle-leaf conifers and broadleaf angiosperms, and both EcM and AM species. Our primary objective was to assess the relationship between changes in above- and belowground C storage during the early stages of afforestation. Our secondary objective was to quantify the effects of planted species richness, functional diversity, and phylogenetic diversity on above- and belowground C pools. Finally, we used microbial biomarkers to assess the role of microbial composition, as influenced by the planted tree diversity, in mediating soil C accumulation.

METHODS

Site description

Our study site was the Forests and Biodiversity experiment (FAB1) at Cedar Creek Ecosystem Science Reserve, East Bethel, Minnesota, USA (Grossman et al., 2017). Cedar Creek has a mesic continental climate with warm summers, cold winters, a mean annual temperature of ~7°C, and a mean annual precipitation of 780 mm (Pellegrini et al., 2021). The soils are characteristic of the Anoka Sand Plain, where upland regions (including FAB1) have well-drained soils comprising fine- and medium-textured sands (Johnston et al., 1996).

FAB1 is part of the IDENT network of tree diversity experiments (Tobner et al., 2014). Here, we briefly summarize the experimental design laid out in Grossman et al. (2017). Trees were planted in a common garden in an abandoned agricultural old field in spring 2013 to disentangle the effects of tree taxonomic, functional, and phylogenetic diversity on ecosystem function. The preexisting herbaceous biomass at the site was burned off before planting, although grasses and forbs (primarily AM-associated) were allowed to reestablish after planting; plots were lightly weeded on occasion until 2016. In each 4 × 4 m plot, 64 saplings were planted in a 0.5 m grid. The species pool included eight deciduous, broadleaf angiosperms (*Acer negundo*, *Acer rubrum*, *Betula papyrifera*, *Quercus alba*, *Quercus ellipsoidalis*, *Quercus macrocarpa*, *Quercus rubra*, and *Tilia americana*) and four evergreen, needleleaf conifers (*Juniperus virginiana*, *Pinus banksiana*, *Pinus resinosa*, *Pinus strobus*). Three species (*A. negundo*, *A. rubrum*, and *J. virginiana*) associate with AM fungi, while the rest associate with EcM fungi. Plot compositions included monocultures and various combinations of two, five, or all 12 species. In multispecies plots, species were planted in equal or nearly equal abundance. Each monoculture and two-species plot was replicated across each of three blocks separated by 4.5 m buffers, and the 12-species combination was replicated ten times across the experiment. A layer of non-native mulch was spread across the ground to reduce understory growth, and commercial mycorrhizal inoculum including both AM and EcM species was added to each seedling (Grossman et al., 2017). Dead seedlings were replanted in spring 2014 and 2015; mortality rates since the final replanting have ranged from 0% to 80% (median 13%) across plots.

When we sampled in 2019, the degree of canopy closure varied widely. In late summer 2018 we measured the fraction of light reaching 5 cm above the ground with an AccuPAR LP-80 ceptometer (METER Group, Pullman, WA, USA) in a sample of 110 plots on overcast days. The median light penetration was 1.4% in needle-leaf conifer plots (10th–90th percentile: 0.4%–2.8%), 2.7% in mixed plots (10th–90th percentile: 0.5%–4.9%), 9.8% in broadleaf angiosperm plots with *B. papyrifera* (10th–90th percentile: 4.3%–25.4%), and 30.8% in broadleaf angiosperm plots without *B. papyrifera* (10th–90th percentile: 13.7%–53.3%). More open plots tended to have more AM-associated herbaceous vegetation in the understory.

Soil sampling and processing

In July 2019, we collected samples from the top 20 cm of mineral soil from 66 plots: 36 monocultures, 10 bicultures, 10 five-species, and 10 12-species plantings. We chose the bicultures to capture planted variation in species, leaf type (broadleaf angiosperm vs. needle-leaf conifer) and mycotype (AM vs. EcM). In each plot we systematically sampled the plot center, and at 1.7 m diagonally from each corner, then composited all 5 cores in the field. Samples were immediately weighed and sieved to 2 mm, taking care to remove all roots. A subsample of the soil was taken to determine the gravimetric moisture content by weighing the subsample, drying the soil at 105°C, and reweighing. A separate subsample was used to measure pH in a 2:1 water and soil solution. Another subsample was oven-dried and ground, after which the C and N concentrations were determined through dry combustion on an elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA).

Prior to planting in 2013, soil samples were collected at depths of 0–15 cm and 15–30 cm in all 66 plots, with bulk density measured at a subset of five plots per block (15 plots total). We used these samples to estimate the initial soil C concentrations to 20 cm based on a weighted average (i.e., $0.75 \times [0\text{–}15 \text{ cm}] + 0.25 \times [15\text{–}30 \text{ cm}]$). We similarly estimated bulk density to 20 cm as a depth-weighted average for each of the 15 measured plots, then calculated block-level means (range 1.33–1.45 among blocks). We calculated the 6 year change in soil C pools as the plot-level change in soil C concentration from 2013 to 2019 multiplied by the block-averaged 2013 bulk density. Mineral soils at this site are extremely sandy (about 94%) and have low organic matter, with bulk density ranging from 1.3 to 1.5 in the top 38 cm of the profile (Grigal et al., 1974). A recent 50-year study of old field succession here found that bulk density was unaffected by time since abandonment, nutrient addition, or changes in soil C (Seabloom et al., 2021). Thus, we

assumed that changes in bulk density were minimal over the 6 years of our study, and far smaller than the changes observed in soil C concentrations.

Fine roots separated during the initial soil sieving process were later carefully washed to remove soil particles and oven-dried at 65°C to a constant mass. Because we were unable to sort roots by species, this pool also included roots of herbaceous species found in the understories of some plots. However, we believe that indirect effects of planting treatments like those on herbaceous vegetation to be of interest in understanding C pools in afforestation. We estimated root C content as 50% of biomass (Thomas & Martin, 2012).

We measured the relative size distribution of water-stable aggregates in each soil sample following a wet-sieving procedure modified from Six (1998). Briefly, 80 g soil samples were submerged in water and sieved through a 250 μm screen for 2 min while gently agitating to determine macroaggregate (250–2000 μm diameter) contents. Soil which passed through this screen was then sieved in a similar fashion through a 53 μm screen. Size fractions were oven-dried (105°C) and weighed to determine the proportional mass of each aggregate fraction.

Aboveground woody C estimation

Beginning in 2013, we surveyed every living tree annually after the growing season concluded. We measured the height of each tree to the nearest 0.5 cm. For trees shorter than 137 cm, we measured the basal diameter (5 cm from the ground) to the nearest 0.05 cm. The first year that a tree crossed the 137 cm threshold, we measured both the basal diameter and the diameter at breast height (dbh; at 137 cm) to the nearest 0.05 cm. In subsequent years, we measured only dbh and not basal diameter, with some exceptions.

Because species in the experiment varied several-fold in their average aboveground relative growth rates (Grossman et al., 2017; Kothari et al., 2021), we adopted different approaches for estimating their aboveground woody C pools in 2019. We divided species into two groups: one in which <65% had a measurable dbh in 2019 (*Acer negundo*, *Acer rubrum*, *Quercus alba*, *Quercus ellipsoidalis*, *Quercus macrocarpa*, *Quercus rubra*, and *Tilia americana*); and another in which >90% of individuals did (*Betula papyrifera*, *Juniperus virginiana*, *Pinus banksiana*, *Pinus resinosa*, and *Pinus strobus*) (no species had between 65% and 90% of individuals with measurable dbh).

For the first group of species, we calculated conical volume based on basal diameter and height. For those trees whose basal diameter was not measured in 2019, we estimated it using species-specific ordinary least-squares

(OLS) regressions built using data combined across years—predicting basal diameter from height and dbh in any year when all three were measured (RMSE/range = 8.4%–16.5% across the 12 species). We multiplied the conical volume by species-specific wood density estimates from Jenkins et al. (2003) to yield biomass.

For the second group of species, we compared each tree's dimensions in 2019 to the species-specific minimum values of height and dbh that Lambert et al. (2005) sampled to build allometric equations for biomass estimation. For trees that exceeded these height and dbh thresholds, we used Lambert et al. (2005)'s species-specific dbh-based equations (*J. virginiana*, *P. resinosa*, and *P. strobus*) or dbh- and height-based equations (*P. banksiana* and *B. papyrifera*) to estimate total woody stem, bark, and branch biomass. For trees that fell short of either threshold, we calculated the conical volume as for the first group of species.

Finally, we multiplied the biomass of all trees by species-specific wood C concentrations (Lamlom & Savidge, 2003) to estimate aboveground woody C. Because C concentration is nearly equal across tissues of any given species (Thomas & Martin, 2012), we assumed that these values applied equally across components of the wood and bark. Although our estimates excluded coarse woody debris from dead branches or trees, we seldom observed any noticeable buildup at this stage of the experiment. Given that the experimental plots were bare of trees just before planting, we assumed the initial aboveground woody C pool to be zero. As a result, the increase in aboveground woody C is simply the amount of aboveground woody C present at the end of 2019.

Net biodiversity effects

The difference between mixture performance and monoculture-based expectations is often called overyielding or the net biodiversity effect (NBE). We calculated NBE of aboveground woody, fine root, and soil C accumulation in mixture plots as the total observed C pool minus the C pool expected based on an average of the constituent species' monoculture plots in the same block, weighted by their planting fraction in the mixture. Because our estimates of aboveground woody C were species-specific, we also additively partitioned aboveground woody NBE into complementarity and selection effects following Loreau and Hector (2001).

Microbial community analysis

In 2016 we collected neutral lipid fatty acid (NLFA) and PLFA data for a subset of the plots sampled in 2019 (described in Grossman, Butterfield, et al., 2019). A total of 33 plots were selected: 15 monocultures (three each of *A. negundo*, *A. rubrum*, *B. papyrifera*, *J. virginiana*, *P. resinosa*), all 10 of the 12-species plots, and 8 AM-EcM bicultures—half of which included angiosperms only, and half of which included conifers only (two each of *A. negundo–Q. alba*, *A. negundo–Q. ellipsoidalis*, *J. virginiana–P. resinosa*, *J. virginiana–P. banksiana*). Briefly, for each plot, we extracted total NLFA and PLFA present from 10 g of soil. Then, we extracted dissolved fatty acids from 2 g of soil, converted them to methyl esters, and ran them on a GC–MS spectrometer. Using 13:0 tridecanoic methyl ester as a standard, we then converted the peak areas associated with 24 microbial lipids to nmol g soil^{-1}. We also calculated the relative abundance of each lipid compared with the total lipid mass in the sample. Grossman, Butterfield, et al. (2019) details the PLFA/NLFAs measured and their associated soil microbial groups.

Statistical analyses

We performed all statistical analyses in R v. 4.2.1 (R Core Team, 2022). We assessed the effects of species richness, functional diversity, and phylogenetic diversity on changes in aboveground wood and soil C pools, as well as fine root C in 2019. As richness-independent measures of functional and phylogenetic diversity, we considered Laliberté and Legendre's (2010) functional dispersion (FDis) and Helmus et al.' (2007) phylogenetic species variability (PSV), as reported by Grossman et al. (2017). In this previous study, FDis was calculated using species traits: wood density, leaf mass per area, leaf N concentration, dominant mycotype, leaf habit, leaf calcium concentration, shade tolerance, drought tolerance, and waterlogging tolerance (Grossman et al., 2017). We set PSV equal to 0 for monocultures. To evaluate which facets of diversity (richness, FDis, and PSV) helped explain aboveground woody, soil, and root total C and NBE, we used a corrected Akaike information criterion (AICc)-based model selection procedure. We compared OLS regression models incorporating all three diversity facets with all models nested within. When the $\Delta AIC_c > 2$ between the dbh top models for a given outcome variable, we selected the better-supported model; otherwise, we report on the most parsimonious model with a $\Delta AIC_c < 2$ from the best-supported model, or the one with lower AIC_c if multiple were equally parsimonious. Due to high skewness in selection and complementarity effects, we used nonparametric two-tailed Wilcoxon signed-rank tests to test whether they differed on average from zero in mixtures.

We performed linear OLS regressions to assess the relationships between different soil properties, microbial

PLFA relative abundance, and C pools. We also performed ANOVAs comparing the soil properties of plots with different phylogenetic and functional groups, including: leaf type (broadleaf angiosperms vs. needle-leaf conifers), mycorrhizal type (AM vs. EcM), and the two most common genera in the experiment (*Quercus*, $n = 4$ species; *Pinus*, $n = 3$ species) binned as categorical predictors. When comparing tree species effects on the relationship between above- and belowground C pools in the monocultures, we performed an ANOVA with species identity, aboveground woody biomass, and the interaction between them with the change in soil C as the response variable. In most regression and ANOVA models, we left out random effects for block because they either resulted in a singular fit or in negligible changes to model parameter estimates. However, we chose to retain a block random intercept in analyses of macroaggregates, soil moisture, and PLFAs because it explained a much larger share of variation and had a stronger effect on model parameters. We carried out mixed-effects modeling in lme4 v. 1.1.32 (Bates et al., 2015), calculating *p*-values with Satterthwaite's degrees of freedom method in lmerTest v. 3.1.3 (Kuznetsova et al., 2017) and marginal and conditional R^2 (R_m^2 and R_c^2) using the method of Nakagawa et al. (2017). We carried out pairwise comparisons between functional groups in emmeans v. 1.8.5 (Lenth, 2022). We used diagnostic plots to evaluate whether model assumptions were met; when they were violated, we carried out robustness checks to examine the models' sensitivity to alternate specifications, as described in Appendix S1.

We combined our hypotheses about the plot-level drivers of aboveground woody C and soil C accumulation using a structural equation model (SEM), implemented using local estimation in piecewiseSEM v. 2.3.0 (Lefcheck, 2016). We sought mainly to test whether the influence of species richness on soil C is mediated in part by aboveground woody C in a holistic causal framework. We took three experimentally manipulated variables to be exogenous—tree species richness (SR), the planted percent of AM-associated trees (%AM), and the planted percent of needle-leaf conifers (%Conifer). From these three variables, we predicted three endogenous variables—aboveground woody C, percent soil macroaggregates, and soil C accumulation. Here, aboveground woody C is taken as a measure of tree size and a proxy for tree inputs to the soil via litter and exudates. We developed an initial causal model based on a combination of a priori reasoning and data analyses described in prior sections, and made minor changes to improve the model's fit as described in Appendix S2: Section S1.

RESULTS

Changes in C pools and their relationship to edaphic factors

We found considerable variation in both above- and belowground C pools across plots. Mineral soil C pools in the top 20 cm varied more than twofold (range 14–33 Mg C ha^{-1}), while aboveground woody C varied by several orders of magnitude (range 0.007–37.5 Mg C ha^{-1}; median 9.10 Mg C ha^{-1}; Table 1). The change in soil C after 6 years of afforestation tended to be positive, with a mean increase of 4.8 Mg ha^{-1}, but ranged widely from −6.0 to 13 Mg ha^{-1}. While the starting soil C pool in 2013 predicted the soil C pool in 2019 ($F_{1,64} = 18.6$, $p < 0.001$, $R^2 = 0.21$; Appendix S2: Figure S1a), it did not significantly predict the change in soil C ($p > 0.1$; Appendix S2: Figure S1b). Surprisingly, the 6-year change in aboveground woody C pools was unrelated to the 6-year change in soil C pools across all plots ($p > 0.7$; Figure 1a). However, when we subset the data to monoculture plots to evaluate tree species identity effects on the change in soil C, we found a significant interaction between species identity and aboveground woody biomass ($F_{11,12} = 3.4$, $p = 0.02$; Appendix S2: Figure S2), suggesting that the relationship between woody biomass and soil C was species-dependent. Fine root C pools varied widely among plots, ranging from 0.232 to 9.59 Mg ha^{-1} (median 1.92 Mg ha^{-1}; Table 1), and there was weak evidence for a positive correlation between fine root pools and the change in soil C pools ($F_{1,60} = 3.0$, $p = 0.09$, $R^2 = 0.03$; Appendix S2: Figure S3a). Lastly, there was evidence for a negative relationship between aboveground woody C and fine root C ($F_{1,61} = 5.3$, $p = 0.02$, $R^2 = 0.06$, Appendix S2: Figure S3b).

We examined three edaphic factors (macroaggregates, soil moisture, and pH) measured in 2019 and their potential relationships with soil C pools in 2013 and 2019. The percent mass of macroaggregates was correlated strongly with soil moisture ($F_{1,41.6} = 71.6$, $p < 0.001$, $R_m^2 = 0.63$, $R_c^2 = 0.71$; Appendix S2: Figure S4b) and more weakly with pH ($F_{1,63} = 4.6$, $p = 0.04$, $R^2 = 0.05$; Appendix S2: Figure S5c). Among these three variables, macroaggregates and soil moisture were strongly structured by experimental block, having the highest values in Block 1 and the lowest in Block 3 (Appendix S2: Figure S6). None of these three variables showed a clear correlation with tree species richness ($p > 0.6$ for all). Macroaggregates were not correlated with the soil C pool in 2013 or 2019 or the change in soil C (all $p > 0.1$). They also were unrelated to the fine root C pool ($p > 0.5$). However, using simple linear regression on the plot level (i.e., not accounting for block), we find a significant positive relationship between

TABLE 1 Average (standard error) of above- and belowground C accumulation and soil aggregate fractions across species monocultures, and binned by species diversity, mycorrhizal type, and leaf type.

	Plot type	Aboveground woody biomass C (Mg ha^{-1})	Fine root C (g/m^2)	Change in soil C (kg/ha)	Macro-aggregates (% >250 μm)	Micro-aggregates (% 53–250 μm)	% soil <53 μm
Monocultures	Acer negundo	0.08 (0.06)	1.68 (0.20)	2.0 (0.3)	73.2 (4.3)	25.8 (4.3)	1.0 (0.1)
	Acer rubrum	0.3 (0.1)	3.64 (1.39)	8.8 (2.1)	73.5 (8.3)	25.6 (7.9)	0.9 (0.4)
	Betula papyrifera	10.7 (2.0)	2.6 (0.6)	4.8 (1.1)	74.8 (5.8)	24.5 (5.6)	0.7 (0.2)
	Juniperus virginiana	12.7 (2.1)	2.2 (0.3)	7.9 (1.9)	74.8 (3.6)	24 (3.6)	1.3 (0.7)
	Pinus banksiana	31.5 (3.7)	1.7 (0.6)	5.8 (0.4)	69.7 (4.9)	28.9 (4.8)	1.4 (0.2)
	Pinus resinosa	20.6 (0.7)	2.1 (0.4)	2.6 (0.7)	68.3 (4.9)	29.8 (4.6)	1.9 (0.3)
	Pinus strobus	31.0 (5.6)	1.1 (0.5)	−0.4 (1.6)	73.4 (5.6)	25.8 (5.3)	0.8 (0.4)
	Quercus alba	0.5 (0.1)	1.5 (0.5)	3.7 (0.9)	80.7 (2.6)	18.7 (2.4)	0.6 (0.2)
	Quercus ellipsoidalis	0.6 (0.1)	2.1 (0.5)	1.4 (3.3)	77.9 (3.3)	21.1 (2.6)	1.0 (0.7)
	Quercus rubra	1.2 (0.3)	1.7 (0.2)	6.3 (2.4)	77.2 (6.6)	22.1 (6.4)	0.8 (0.2)
	Quercus macrocarpa	1.0 (0.2)	2.4 (0.2)	2.9 (1.4)	76 (4.5)	23.4 (4.3)	0.6 (0.3)
	Tilia americana	1.3 (0.3)	1.7 (0.4)	3.2 (2.1)	75.8 (5.7)	23.3 (5.4)	1.0 (0.3)
Species richness	One	9.3 (2.0)	2.0 (0.2)	4.1 (0.6)	74.6 (1.4)	24.4 (1.3)	1.0 (0.1)
	Two	11.2 (3.8)	3.1 (0.9)	5.3 (1.0)	68.8 (2.4)	29.4 (2.4)	1.8 (0.2)
	Five	12.9 (2.2)	1.9 (0.2)	4.3 (1.4)	74.5 (2.6)	24.0 (2.6)	1.5 (0.2)
	Twelve	13.0 (1.1)	2.1 (0.5)	7.4 (1.2)	72.2 (2.2)	26.6 (2.1)	1.3 (0.3)
Mycorrhizal type	AM	4.3 (2.2)	2.4 (1.3)	6.3 (1.3)	73.8 (2.9)	25.1 (2.8)	1.1 (0.2)
	EcM	10.9 (2.5)	1.9 (0.2)	3.4 (0.6)	75.0 (1.5)	24.1 (1.4)	1.0 (0.1)
	Mixed	11.2 (3.8)	3.1 (0.9)	5.3 (1.0)	71.5 (1.5)	27.0 (1.4)	1.5 (0.1)
Leaf form (phylogenetic-functional groups)	Broadleaf angiosperms	1.7 (0.6)	2.5 (0.3)	5.6 (1.0)	75.0 (1.6)	24.0 (1.5)	1.0 (0.1)
	Needle-leaf conifers	23.9 (2.0)	1.7 (0.2)	4.5 (0.9)	70.4 (1.7)	28.0 (1.7)	1.5 (0.2)
	Mixed	13.5 (1.1)	2.0 (0.3)	4.5 (0.6)	73.2 (1.7)	25.4 (1.6)	1.4 (0.2)

Note: The No. plots varied according to plot type. Monocultures included three plots per species. For species richness, there were 36 one-species, 10 two-species, 10 five-species, and 10 Twelve-species plots. For mycorrhizal type, there were 9 AMF, 28 EcM, and 29 mixed plots. For leaf form, there were 30 broadleaf angiosperm, 17 needle-leaf conifer, and 19 mixed plots.

Abbreviations: AM, arbuscular mycorrhizal; EcM, ectomycorrhizal.

the percentage of soil C and macroaggregates in both years ($p < 0.05$ for both; Appendix S2: Figure S7). Soil moisture was positively correlated with soil C pools in 2013 ($F_{1,33.8} = 15.2$, $p < 0.001$, $R_m^2 = 0.20$, $R_c^2 = 0.23$) and 2019 ($F_{1,64} = 17.9$, $p < 0.001$, $R^2 = 0.21$; Appendix S2: Figure S4a), but not the change in soil C ($p > 0.1$). Soil pH was not associated with the initial soil C pool in 2013 ($p > 0.5$), but was positively (albeit weakly) associated with soil C pools in 2019 ($F_{1,64} = 5.0$, $p = 0.03$, $R^2 = 0.06$) and with change in soil C ($F_{1,64} = 9.6$, $p < 0.01$, $R^2 = 0.12$, Appendix S2: Figure S5a,b).

Tree biodiversity effects on above- and belowground C accumulation

Among species in monoculture, aboveground woody C ranged by orders of magnitude from 0.08 Mg C ha^{-1} for

A. negundo to 31.5 Mg C ha^{-1} for P. banksiana, and was generally higher among needle-leaves than broadleaves (Table 1). We tested species richness, FDis, and PSV as potential explanatory variables for aboveground woody C. Across all FAB1 plots, the model chosen by our AIC$_c$-based model selection procedure for explaining aboveground woody C ($F_{2,137} = 19.4$; $p < 0.001$; $R^2 = 0.21$) retained both FDis ($t(137) = -3.7$; $p < 0.001$) and PSV ($t(137) = 5.9$; $p < 0.001$). In this model, an increase in FDis from the 10th to the 90th percentile among all plots would be predicted to decrease aboveground woody C by 11.7 Mg ha^{-1} while a similar increase in PSV would be predicted to increase aboveground woody C by 19.1 Mg ha^{-1}. The two facets have a strong positive correlation ($F_{1,138} = 263.7$; $p < 0.001$; $R^2 = 0.65$), so their opposing effects offset each other to some degree, and the positive effect of PSV was larger in magnitude. Thus, tree species identity had the greatest effect on aboveground
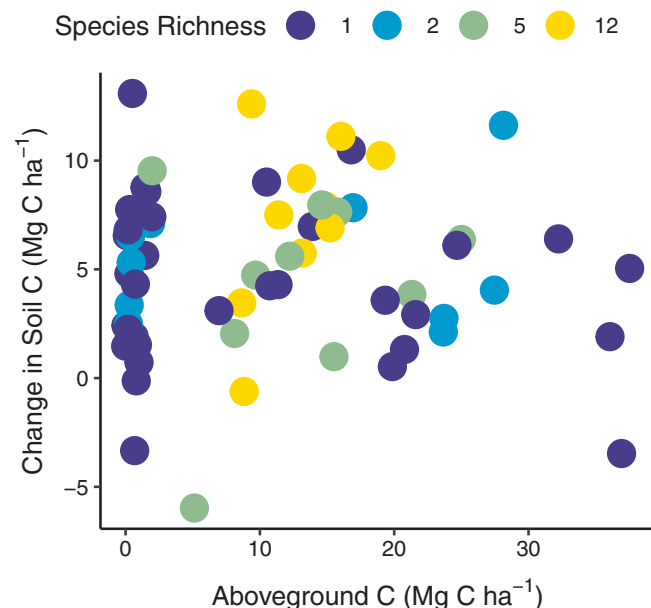
Species Richness ● 1 ● 2 ● 5 ● 12

FIGURE 1 Relationship between changes in aboveground woody C and changes in soil C after 6 years in the FAB1 experiment. There is no detectable relationship between rates of C accumulation in these two pools.

woody C, as evidenced by differences among monocultures, but there was also evidence for a positive effect of tree diversity.

Calculating NBE allowed us to separate the effect of richness from identity. Mixture plots showed a positive average NBE for aboveground woody C ($t(103) = 3.0$; $p = 0.003$; mean of 1.1 Mg ha^{-1}). The model selected for explaining aboveground woody NBE ($F_{2,101} = 10.3$; $p < 0.001$; $R^2 = 0.15$) retained species richness ($t(101) = 2.1$; $p = 0.04$) and FDis ($t(101) = 2.8$; $p = 0.006$). Based on parameter estimates, each additional species would, on average, increase NBE by 0.25 Mg C ha^{-1} (Figure 2b), and an increase in FDis from the 10th to the 90th percentile among multispecies plots would increase NBE by 2.75 Mg C ha^{-1}. On average, there was some evidence for positive complementarity effects ($p = 0.058$) but no evidence for non-zero selection effects ($p > 0.7$).

We found detectable effects of tree species richness on soil C sequestration after 6 years of planting. On average, the change in soil C pools since planting increased by 0.27 Mg C ha^{-1} for each additional species ($F_{1,64} = 5.3$, $p = 0.02$, $R^2 = 0.06$; Figure 2c). The selected model did not include FDis or PSV, but each had a marginally significant effect when considered alone (FDis: $F_{1,64} = 3.0$, $p = 0.09$, $R^2 = 0.03$; PSV: $F_{1,64} = 3.2$, $p = 0.08$, $R^2 = 0.03$; Appendix S2: Figure S8a,b). There was weak evidence that mixtures showed positive NBE on average in the change in soil C ($t(29) = 1.95$; $p = 0.06$;

mean of 1.40 Mg C ha^{-1}). The selected model did not retain any facet of diversity in explaining NBE for change in soil C, but there was weak evidence for an effect of species richness ($F_{1,28} = 3.6$, $p = 0.07$; $R^2 = 0.08$). Based on the model incorporating richness, each additional species would on average increase NBE by 0.31 Mg C ha^{-1} (Figure 2d).

There was no evidence for a relationship between fine root C and any facet of diversity ($p > 0.4$; Figure 2e). In general, mixtures did not show a significantly non-zero NBE in fine root C ($p > 0.1$; Figure 2f). The best-supported model for explaining NBE only retained PSV ($F_{1,26} = 6.2$, $p = 0.02$; $R^2 = 0.16$), which was, in fact, negatively correlated with fine root NBE, such that an increase from the 10th to 90th percentile in PSV among multispecies plots with root samples would cause a decrease of 2.16 Mg ha^{-1} in root C NBE.

Tree composition and soil C accumulation

We found similar effects of leaf type (broadleaf angiosperm, needle-leaf conifer, or both) and genus (*Quercus* vs. *Pinus*) on the soil properties we measured. Neither leaf type nor genus had detectable effects on the change in soil C ($p > 0.5$ for both; Figure 3a,b) or root C ($p > 0.1$ for both; Appendix S2: Figure S9a,b). However, soils in plots containing broadleaf trees had a higher percentage of macroaggregates ($F_{2,60.01} = 5.8$, $p < 0.01$, Figure 3d). This same pattern held true in *Quercus* spp. plots in contrast to *Pinus* sp. plots ($F_{1,17} = 10.9$, $p < 0.01$, Figure 3e). Soil moisture followed the same pattern as macroaggregates, with higher soil moisture in broadleaf plots ($F_{2,63} = 6.4$, $p < 0.01$, Appendix S2: Figure S9a) relative to needle-leaf, and in *Quercus* spp. plots ($F_{1,19} = 18.8$, $p < 0.001$, Appendix S2: Figure S9b) relative to *Pinus* plots. In contrast to patterns observed with leaf type and tree genus, we found that soil C accumulation varied by mycotype ($F_{2,63} = 3.2$, $p < 0.05$), with a trend of greater soil C accumulation in plots containing AM trees (i.e., AM monocultures or bicultures) than EM trees (Figure 3c). However, we found no detectable difference in macroaggregates (Figure 3f) or root C (Appendix S2: Figure S9c) between mycotypes ($p > 0.1$ for both).

Microbial composition and soil C accumulation

Total microbial biomass (measured as total soil PLFA concentration) measured in 2016 was largely unrelated to differences in soil C accumulation and macroaggregate pools across plots in 2019 ($p > 0.2$ for all). However, a few
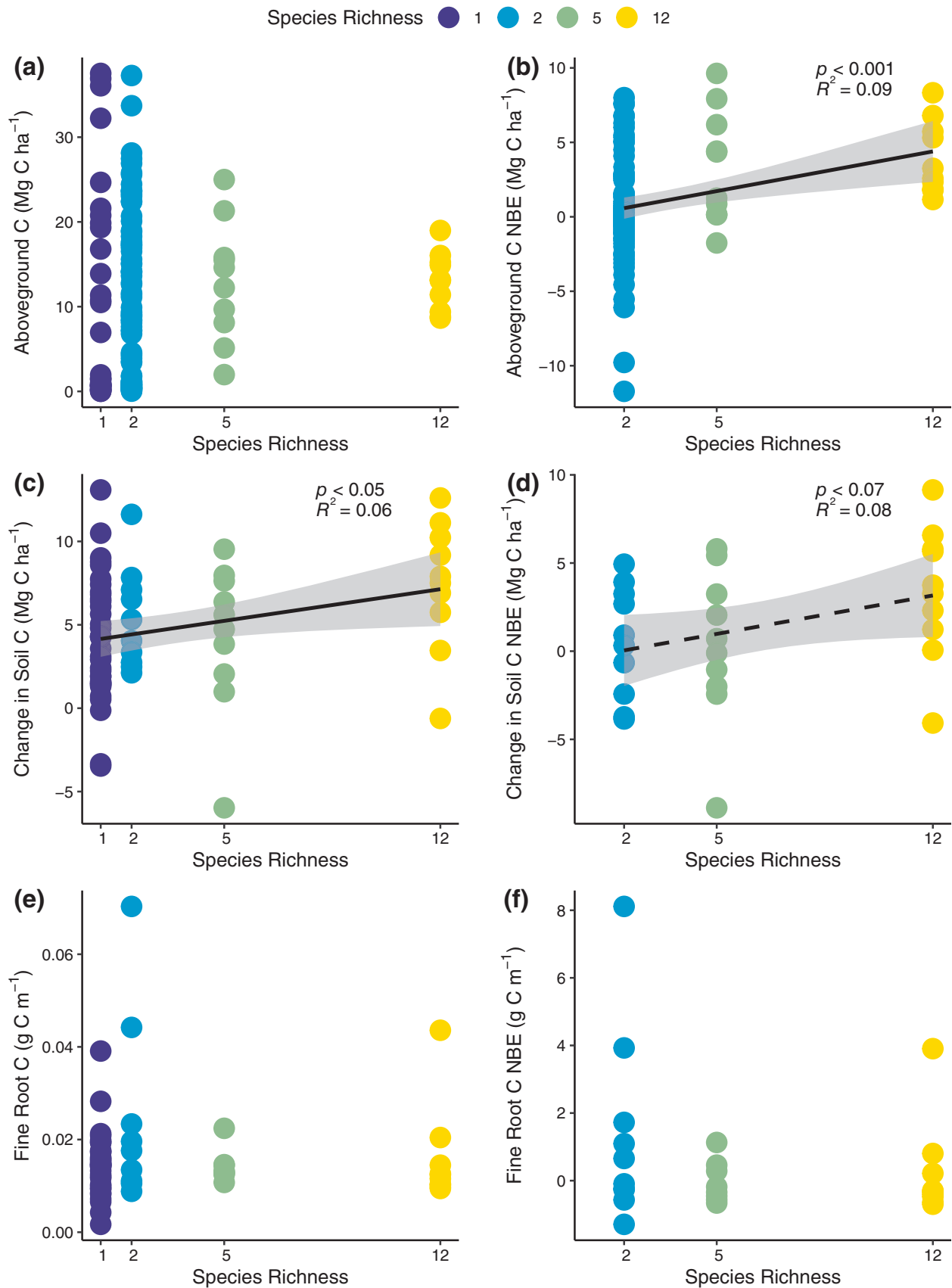
FIGURE 2 Relationships of species richness to different C pools and net biodiversity effects (NBE) across experimental plots. NBE figures (b, d, f) on the right represent plot-level NBE of specific C pools (a, c, e) to the left. Lines and statistics represent simple linear regressions with 95% confidence intervals in the shaded regions.
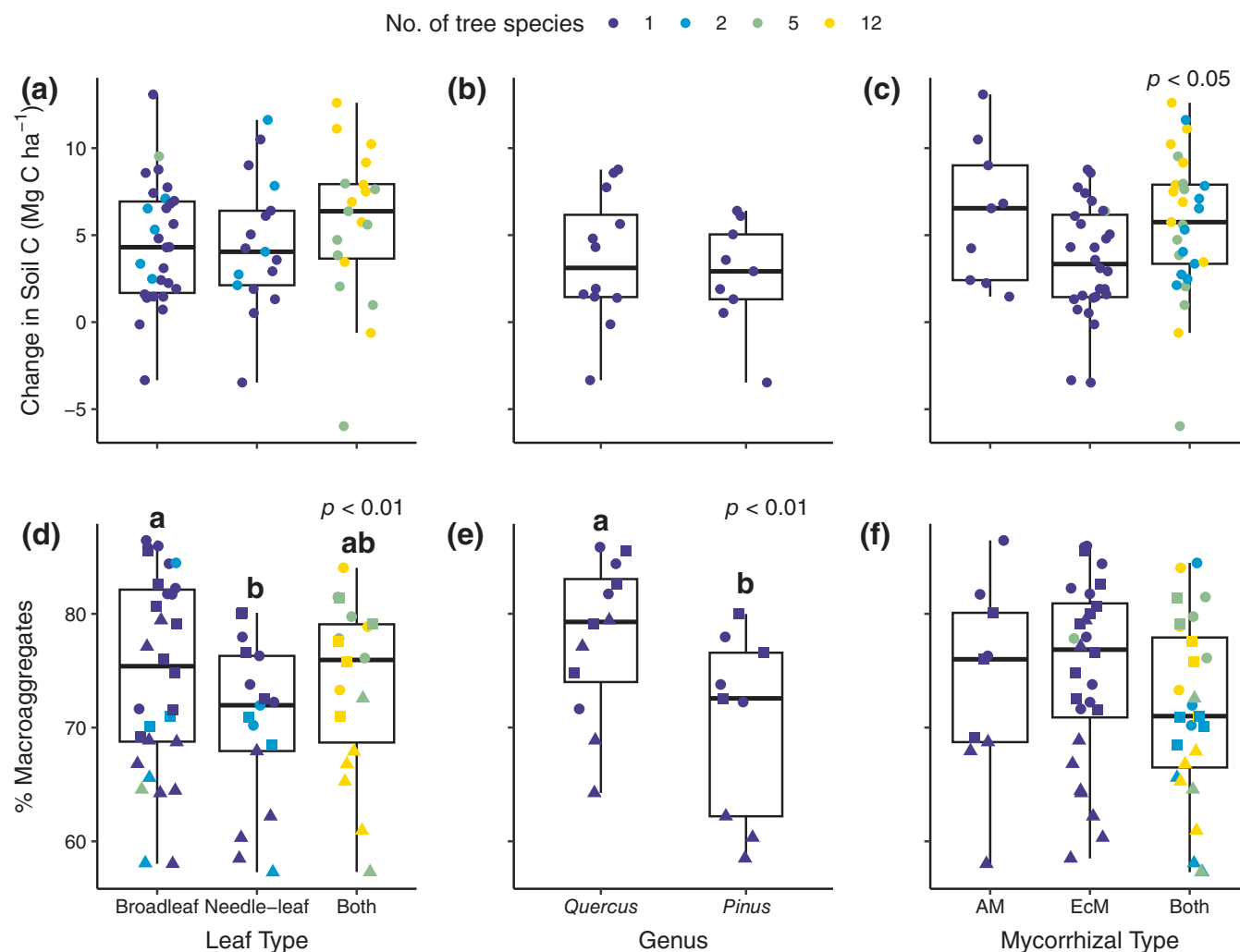
FIGURE 3 Soil C accumulation and % mass of macroaggregates versus plot types. Plots planted with broadleaf angiosperms had significantly higher fractions of macroaggregates than those with solely needle-leaf conifers. Plots with *Quercus* sp. ($n = 4$ species, 12 plots) versus *Pinus* sp. ($n = 3$ species, 9 plots) followed a similar trend. Soil C accumulation over the course of the experiment did not differ significantly by leaf type or genus. Plots with differing mycorrhizal types (arbuscular mycorrhizal [AM] vs. ectomycorrhizal [EcM]) did not significantly vary in percentage of macroaggregates, but did vary in C accumulation. Plots with AM trees or both mycotypes tended to accumulate more soil C than EcM plots though this trend was not significant in pairwise comparisons. Analyses of macroaggregate fractions included block as a random effect, and different shapes here are based on the block a plot is located in.

specific PLFAs positively correlated with soil C. In particular, the concentration of 10Me18:0 (actinomycetes) in the soil showed a strong positive relationship with soil C in 2019 ($F_{1,31} = 12.0$, $p < 0.01$, $R^2 = 0.27$; Figure 4). There was also a marginally significant positive relationship between soil C and the concentration of two Gram-positive bacteria PLFAs: 15:0 iso ($F_{1,29.6} = 3.3$, $p = 0.08$, $R_m^2 = 0.08$, $R_c^2 = 0.26$) and 17:0 anteiso ($F_{1,30.3} = 3.8$, $p = 0.06$, $R_m^2 = 0.11$, $R_c^2 = 0.21$) as shown in Figure 4. Despite correlations with soil C, there was no significant relationship with the change in soil C for any of these groups except for the relative abundance of 18:2ω6,9c, which is a general fungal marker ($F_{1,31} = 5.3$, $p < 0.05$, $R^2 = 0.14$; Figure 4). The proportion of fungal PLFA

showed a marginally positive correlation to an increase in soil C ($F_{1,30.3} = 2.9$, $p = 0.10$, $R_m^2 = 0.08$, $R_c^2 = 0.09$; Appendix S2: Figure S10).

Structural equation modeling of aboveground woody and soil C

Our final SEM (Figure 5) met typical criteria for goodness-of-fit (Fisher's C = 4.182, df = 6, $p > 0.6$; all d-separation tests $p > 0.05$). The model reinforced many of the conclusions we drew from previous analyses. Although there was strong evidence that species richness increased both aboveground woody and soil C
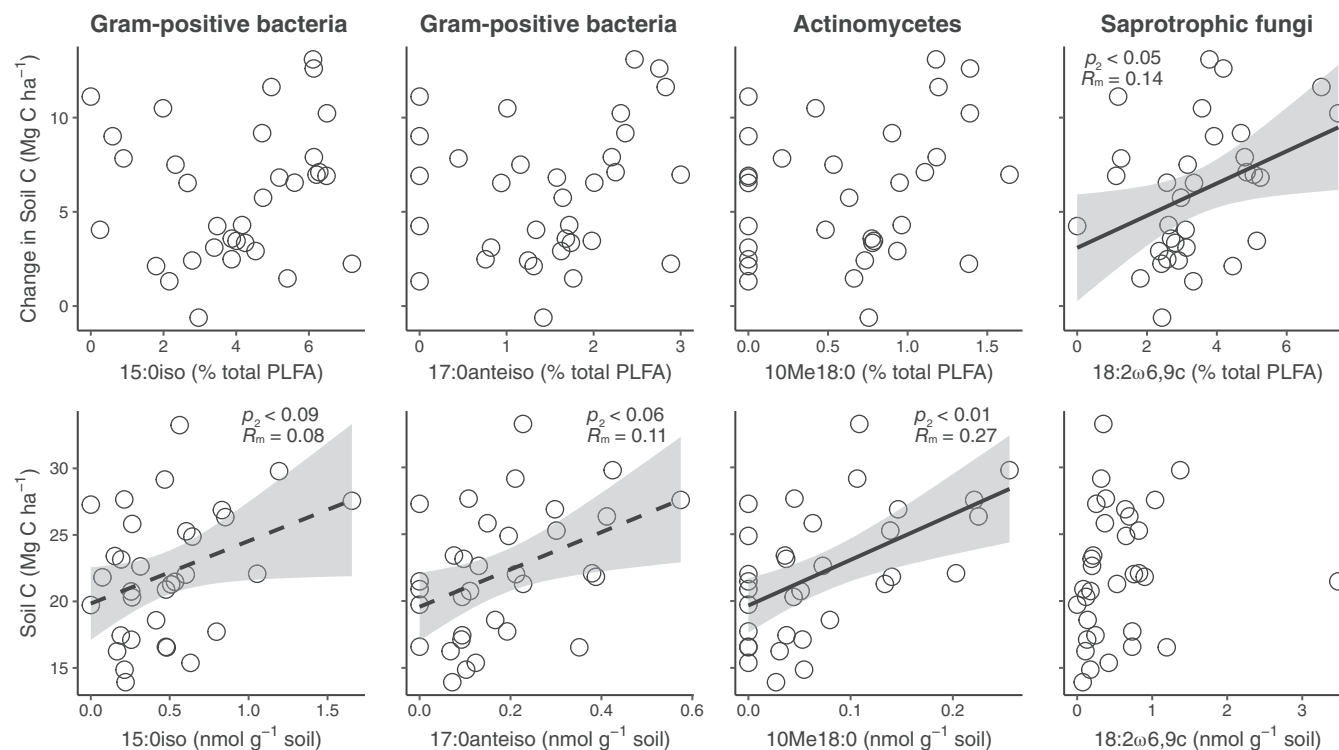
FIGURE 4 Relationships between soil C and four phospholipid fatty acids (PLFAs) extracted from soil. The top panels depict the six-year change in soil C stocks as a function of the relative abundance of each PLFA. Bottom panels depict the relationship between 2019 C stocks and the concentration of the each PLFA in the soil (nmol g^{-1}). Solid regression lines indicate statistical significance at $p < 0.05$, while the dashed lines indicate significance at $p < 0.10$. Shaded areas represent 95% confidence intervals.

accumulation, there was no evidence that aboveground woody C influenced soil C accumulation. As a result, the influence of species richness on soil C was direct relative to other variables in the model. As expected given the high productivity of needle-leaf conifer species, %Conifer had a strong positive influence on aboveground woody C. Both aboveground woody C and soil C accumulation were influenced by %AM, but in opposite directions—aboveground woody C decreased with the presence of more slowly growing AM species, while rates of soil C accumulation increased. Lastly, much of the variation in macroaggregates was explained by the block random effect, but they were also negatively correlated with %Conifer. There was no evidence that macroaggregates contributed to soil C accumulation.

DISCUSSION

There have been recent calls to plant forests to increase ecosystem C sequestration (Bastin et al., 2019), and to diversify these planted forests to ensure their resilience and multifunctionality (Liu et al., 2018; Messier et al., 2022). However, it remains highly uncertain how tree diversity affects whole-ecosystem C balance,

particularly during the early years of afforestation (Mayer et al., 2020). Only a handful of experimental studies have reported changes in soil C in relation to biodiversity during the early stages of afforestation (Table 2), a critical period when large quantities of soil C may be lost from the system (Deng et al., 2014; Potvin et al., 2011; Yanai et al., 2003). We detected positive effects of biodiversity on both above- and belowground C pools after only 6 years of planting in a low C soil. Critically, net gains in aboveground woody C were completely uncorrelated to gains in mineral soil C (Figure 1a). Thus, our findings support the idea that different mechanisms underlie species diversity effects on above versus belowground C in the early stages of afforestation.

Drivers of aboveground woody C

Aboveground woody C was driven most strongly by species identity in the first 6 years after planting. This finding is consistent with results from earlier stages of this experiment (Grossman et al., 2017; Kothari et al., 2021) and with strong evidence from global analyses that tree species vary considerably in their initial growth rates (Bukoski et al., 2022). Much of the variability could be
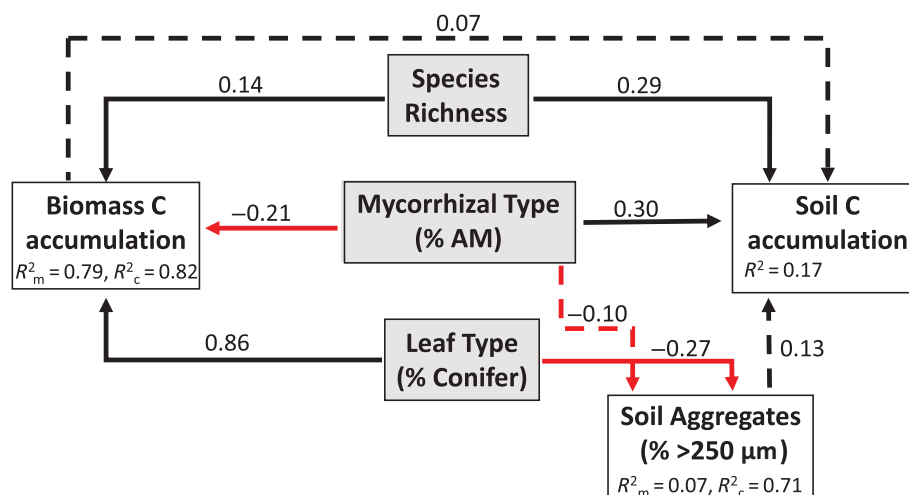
FIGURE 5 Structural equation model depicting the effects of planted tree composition (species richness, mycorrhizal type, and leaf type, in gray boxes) on C accumulation in soil and aboveground woody biomass, and soil aggregate formation. Solid lines denote statistical significance ($p < 0.05$). Red lines indicate a negative effect. All coefficients are standardized. AM, arbuscular mycorrhizal.

explained by leaf type and phylogeny (Table 1); on average, needle-leaf conifer plots accrued an order of magnitude more aboveground woody C than broadleaf angiosperm plots. There was also high variability among broadleaf species, as *B. papyrifera* accumulated more than two orders of magnitude more C per plot than the least productive species, *A. negundo*. While fitting our SEM, we also found that the proportion of AM trees reduced aboveground woody C accrual. This finding makes sense given that AM monocultures had the lowest average biomass within leaf types (i.e., *Acer* spp. among broadleaf angiosperms, *J. virginiana* among needle-leaf conifers), although it is not clear how well it would generalize to more diverse sets of AM species.

We partitioned the effects of diversity from species composition on aboveground woody C by calculating NBE. During the first three growing seasons of FAB1, Grossman et al. (2017) found that, on average, mixtures showed positive NBE, with positive complementarity and nonsignificant selection effects; 4 years later, these results still held. These findings imply that species tended to overperform in mixtures regardless of their monoculture productivity. Complementarity effects can emerge from many mutually inclusive mechanisms (Barry et al., 2019). In this experiment, past research showed that large needle-leaf conifers benefited shade-tolerant broadleaf species by ameliorating abiotic stress associated with high light (Kothari et al., 2021), while evidence for dilution of natural enemies was more variable (Grossman, Cavender-Bares, et al., 2019). Despite the dominant influence of fast-growing conifers on aboveground woody C, there is evidence that interactions in diverse communities produce synergistic effects.

If the strong influence of species identity results from fitness differences due to varying environmental tolerances, it may persist through time. However, if faster growth comes at the cost of higher mortality (Wright et al., 2010) or if canopy closure causes growth to stagnate, the vast gaps between the species may shrink and other factors may become more salient, including richness. Indeed, positive richness effects often strengthen through the initial years of stand development (Feng et al., 2022; Guerrero-Ramírez et al., 2017; Urgoiti et al., 2022). Nevertheless, slow-growing species may continue to be less efficient at using resources, which could explain why transgressive overyielding—in which some mixtures outperform the best-performing monoculture—remains rare in forested ecosystems (Chisholm & Gupta, 2023). If diversity does have benefits, they may have more to do with resilience and multifunctionality than simply maximizing potential aboveground woody C sequestration (Messier et al., 2022). Long-term monitoring will be needed to evaluate how tree identity and diversity effects shift through time.

Drivers of belowground C

While aboveground woody C gains were well explained by tree species growth rates, patterns in belowground C accumulation were less clear due to multiple competing drivers. We found statistically significant effects of species richness and mycotype on mineral soil C accumulation after only 6 years, but these factors only explained a small amount of the total variation in soil C change. This is perhaps not surprising, as soil C accumulation depends on

TABLE 2 Results of experiments examining tree biodiversity and soil C across multiple richness levels in early stages of afforestation.

Site	Study	Impact of richness on soil C	Time since planting	Soil	Climate	No. spp.
La Selva, Costa Rica	Carney & Matson, 2006	Increased C concentrations in three-species plots	9–11 years	Fluventic Dystropept (Cambisol)	Tropical	1, 3, 5
Sardinilla, Panama	Potvin et al., 2011	No clear effect	8 years	Alfisols (Luvisols, Nitisols, or Podzols)	Tropical	1, 3, 6
China	Jiang et al., 2010	No effect	17 years	Ferralsols	Subtropical	1, 2, 3
BEF-China, China	Beugnon et al., 2023; Li et al., 2019	Increased soil C concentration and pools at all 0–20 cm depths after 6 years in a subset of plots; no effect on soil C concentration in 0–10 cm after 9 years	9 years	Cambisols	Subtropical	1, 2, 4, 8, 16
Kaltenborn, Germany	Domisch et al., 2015	Increased C concentrations in top 10 cm of mineral soil, but not other layers	8 years	Arenosols	Temperate	1, 2, 3, 4
BangorDIVERSE, UK	Gunina et al., 2017	No clear effect	10 years	Eutric Fluvic Cambisols	Temperate	1, 2, 3
Zedelgem, Belgium	Hicks et al., 2018	No effect	7 years	Podzols and Gleysols	Temperate	1, 2, 3, 4
IDENT-Montréal, Canada	Martin-Guay et al., 2022	No effect	10 years	Humic Gleysol	Temperate	1, 2, 4
Italy	Palandrani & Alberti, 2020	No effect	19 years	Alluvial mesic Udifluvent (Fluvisol)	Temperate	3, 4, 6, 7, 8, 9
IDENT-SSM, Canada	Strukelj et al., 2021	No effect	4 years	Eutric Arenosols	Temperate	1, 2, 4
ORPHEE, France	Fanin et al., 2022; Strukelj et al., 2021	Increased soil C pools especially at 15-30 cm depth, but depended on water	9 and 10 years	Entic Ortsteinic Podzol	Temperate	1, 2, 3
FAB1, USA	This study	Increased C pools in top 20 cm of mineral soil	6 years	Udipsamments (Gleysols)	Temperate	1, 2, 5, 12
Satakunta, Finland	Domisch et al., 2015	No effect	14 years	Podzols	Boreal	1, 2, 3, 5

Note: Soils are reported as described in the associated study with the equivalent order in the Food and Agriculture Organization of the United Nations (FAO) classification system in parentheses if not originally given.

multiple interacting drivers, including the quantity and quality of plant C inputs, as well as the many biophysical factors governing decomposition rates. What is surprising is that soil C increases were not correlated with increases in aboveground woody biomass, even though both were influenced by species richness.

While we did not directly measure litter inputs, aboveground woody biomass varied by more than 400-fold in 2019, suggesting that these plots experienced a wide range in litterfall C fluxes. The ratio of leaf to aboveground tree biomass varies by growth form and stage (range in needle-leaf saplings = ~0.31–0.08, range in broadleaf saplings = ~0.18–0.03; Jenkins et al., 2003) and our needle-leaf conifer species likely experienced longer leaf longevity (~2–3 years; Reich et al., 1999). However, even a conservative calculation using these parameters suggests that litterfall C flux varied by at least one order of magnitude across these plots. This strongly suggests that differences in the quantity of litter C inputs were not the dominant factor determining mineral soil C

accumulation in this system. Notably, we observed greater soil C accumulation in AM-dominated plots, while we found greater woody C accumulation (and likely C inputs to soil) in EcM-dominated plots.

Greater mineral soil C accumulation in AM plots is consistent with observations in temperate forests in the United States (Craig et al., 2018; Keller et al., 2021; but see Wu et al., 2022), and has multiple possible mechanisms. One possibility is that EcM fungal demand for nutrients led to greater soil C decomposition (Terrer et al., 2021), but we did not observe lower soil C in the high-biomass (EcM-dominated) plots as might be expected if faster growth increased decomposition rates. A second possibility is that AM fungi increased the formation of macroaggregates (Rillig et al., 2002; Rillig et al., 2015; Wright & Upadhyaya, 1998), which increase C storage in sandy soils (Six et al., 2004). However, we did not find higher macroaggregates in soils under AM trees, perhaps due to the presence of AM grasses and forbs in EcM plots with low tree biomass (i.e., *Quercus* species, Table 1; Grossman, Butterfield, et al., 2019). Thus, we did not find strong support that EcM-mediated decomposition effects or AM-mediated soil structure effects contributed to the pattern of greater C accumulation in AM plots.

The greater C accumulation observed in AM soils could have been due to differences in microbial transfer of plant C into soils via microbial residues. AM trees generally have more labile leaf and root litter (Grossman et al., 2020; Keller et al. 2019; See et al., 2019), which may lead to a greater net transfer of C into soil microbial biomass (Cotrufo et al., 2013). As this microbial C pool turns over, a portion of the resulting microbial necromass C may persist as particulate matter for years (Fernandez et al., 2020), or form stable associations with soil minerals (*c.a.* 30% of soil C at this site; DeLancey et al., 2023). Soil fungal community composition has diverged across monocultures in this experiment, as has the abundance of microbial guilds associated with fungal necromass decomposition (Maillard et al., 2023). Divergence in fungal communities, coupled with our finding that a general fungal biomarker was the only microbial PLFA correlated with soil C accumulation during our study, highlights the need for a better understanding of how fungi mediate the flow of C between plants and soils (See et al., 2022; Sokol et al., 2022).

Although we found greater C accumulation under AM trees, we did not find evidence for greater soil aggregation. Instead, we observed greater soil aggregation in broadleaf plots than needle-leaf plots, which in this experiment also corresponds to the phylogenetic division between conifer and angiosperm lineages. The mechanism for leaf-type effects on aggregate formation is likely in part microbial (Merino-Martín et al., 2021; Mueller et al., 2024). In this experiment, Grossman, Butterfield, et al. (2019) previously found that broadleaf-dominated plots contained higher abundances of both bacteria and actinomycetes, which are associated with greater aggregate formation (Forster, 1990; Ren et al., 2022). Similarly, Deng et al. (2018) found that leaf-type drove differences in bacterial communities during reforestation (but see Rożek et al., 2023). Importantly, soil aggregates also varied among blocks, and these pretreatment legacies support the idea that C occluded in aggregates may have a longer residence time than free organic matter, but that aggregates may be slow to form. Still, we were able to detect effects of leaf-type on aggregate fractions after just 6 years. If these differences in aggregate formation persist, they may eventually cause the mineral soils of broadleaf plots to accumulate greater soil C stocks (Deng & Shangguan, 2017; Laganière et al., 2010).

Finally it is highly likely that differences in the physical structure of these plots led to microclimatic effects on decomposition. Plots with higher aboveground woody C had lower soil moisture. Given this variation, the wetter soils in slower-growing broadleaf plots likely experienced higher decomposition rates compared with those with needle-leaf conifer species (Moyano et al., 2013). Somewhat paradoxically, higher soil moisture was also correlated with more macroaggregates and higher soil C concentration due to the high water holding capacity of organic matter, making it difficult to infer causality in the observed relationships between soil moisture and soil C across plots. Regardless, microclimatic variation was strongly mediated by individual species' growth rates during these early years of establishment. As the slowest-growing plots achieve canopy closure, microclimatic variation may begin to dampen (Zhang et al., 2022), and the effects of functional group (e.g., mycotype, leaf type) on soil C may become more salient. Studies on afforestation's impacts on C sequestration could benefit from considering the role of microclimates in mediating the relationship between biodiversity and ecosystem functioning, especially in the face of climate change (de Frenne et al., 2021; Wright et al., 2021).

CONCLUSION

Understanding the effects of tree diversity on whole ecosystem C sequestration is necessary for informing effective reforestation and afforestation strategies. In young stands, after only 6 years of afforestation, we found evidence for positive diversity effects on aboveground woody C and mineral soil C accumulation in a planted tree diversity experiment. Critically, soil C accumulation

was not directly correlated with the quantity of aboveground woody C gains, indicating that diversity effects on soil C were not a function of greater litter inputs alone. Instead, we found greater increases in soil C in plots containing AM fungi-associated tree species. Further, we found that broadleaf species increased soil aggregation relative to needle-leaf conifers, which may lead to longer-term C storage if this effect persists through time. Importantly, our results reflect an experiment in sandy (low C) soils, which allowed for detection of small changes in soil C pools, but our analyses explained only a small quantity of the total variation in soil C accumulation. This highlights the critical need for a better mechanistic understanding of the effects of planted tree diversity on rates of belowground C sequestration, as it has the potential to offset or enhance aboveground C gains.

AUTHOR CONTRIBUTIONS

Jeannine Cavender-Bares and Sarah E. Hobbie organized the design, setup, and maintenance of FAB1. Craig R. See, Reb L. Bryant, Stephanie J. Curran, Charlotte Nash, and Grace C. Neumiller carried out the design, sampling, processing, and preliminary analysis of the belowground data. Shan Kothari calculated and analyzed the aboveground woody C biomass data. Jake J. Grossman collected and assisted in the analysis of microbial data within this experiment and calculated diversity metrics. Reb L. Bryant, Shan Kothari, and Craig R. See equally contributed to writing the first draft with input from Sarah E. Hobbie. All authors provided substantial input on further drafts of the manuscript.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Bryant et al., 2023) are available in the Environmental Data Initiative (EDI) Data Repository at https://doi.org/10.6073/pasta/dc363389ee514e7384bf348228ee5ced. Microbial data (Grossman, 2019) are available in the EDI Data Repository at https://doi.org/10.6073/pasta/3918fe1d3aab00ab73c4a83372cb82cf. Code (Kothari, 2023) is available in Zenodo at https://doi.org/10.5281/zenodo.10348365.

ORCID

Reb L. Bryant https://orcid.org/0000-0001-7201-8187
Shan Kothari https://orcid.org/0000-0001-9445-5548
Jeannine Cavender-Bares https://orcid.org/0000-0003-3375-9630
Jake J. Grossman https://orcid.org/0000-0001-6468-8551
Sarah E. Hobbie https://orcid.org/0000-0001-5159-031X
Charlotte Nash https://orcid.org/0009-0003-0703-2216
Grace C. Neumiller https://orcid.org/0009-0004-0956-4416
Craig R. See https://orcid.org/0000-0003-4154-8307

REFERENCES

Amézketa, E. 1999. "Soil Aggregate Stability: A Review." *Journal of Sustainable Agriculture* 14(2–3): 83–151. https://doi.org/10.1300/J064v14n02_08.

Angers, D. A., and J. Caron. 1998. "Plant-Induced Changes in Soil Structure: Processes and Feedbacks." *Biogeochemistry* 42(1): 55–72. https://doi.org/10.1023/A:1005944025343.

Angst, Š., L. Harantová, P. Baldrian, G. Angst, T. Cajthaml, P. Straková, J. Blahut, H. Veselá, and J. Frouz. 2019. "Tree Species Identity Alters Decomposition of Understory Litter and Associated Microbial Communities: A Case Study." *Biology and Fertility of Soils* 55(5): 525–538. https://doi.org/10.1007/s00374-019-01360-z.

Archambault, C., A. Paquette, C. Messier, R. Khlifa, A. D. Munson, and I. T. Handa. 2019. "Evergreenness Influences Fine Root Growth More than Tree Diversity in a Common Garden Experiment." *Oecologia* 189: 1027–39. https://doi.org/10.1007/s00442-019-04373-5.

Augusto, L., and A. Bŏca. 2022. "Tree Functional Traits, Forest Biomass, and Tree Species Diversity Interact with Site Properties to Drive Forest Soil Carbon." *Nature Communications* 13(1): 1097. https://doi.org/10.1038/s41467-022-28748-0.

Barry, K. E., L. Mommer, J. van Ruijven, C. Wirth, A. J. Wright, Y. Bai, J. Connolly, et al. 2019. "The Future of Complementarity: Disentangling Causes from Consequences." *Trends in Ecology & Evolution* 34(2): 167–180. https://doi.org/10.1016/j.tree.2018.10.013.

Bennett, J. A., H. Maherali, K. O. Reinhart, Y. Lekberg, M. M. Hart, and J. Klironomos. 2017. "Plant-Soil Feedbacks and Mycorrhizal Type Influence Temperate Forest Population Dynamics." *Science* 355(6321): 181–84. https://doi.org/10.1126/science.aai8212.

Bastin, J.-F., Y. Finegold, C. Garcia, D. Mollicone, M. Rezende, D. Routh, C. M. Zohner, and T. W. Crowther. 2019. "The Global Tree Restoration Potential." *Science* 365(6448): 76–79. https://doi.org/10.1126/science.aax0848.

Baumert, V. L., N. A. Vasilyeva, A. A. Vladimirov, I. C. Meier, I. Kögel-Knabner, and C. W. Mueller. 2018. "Root Exudates Induce Soil Macroaggregation Facilitated by Fungi in Subsoil." *Frontiers in Environmental Science* 6: 140.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67(1): 1–48. https://doi.org/10.18637/jss.v067.i01.

Beugnon, R., W. Bu, H. Bruelheide, A. Davrinche, J. du, S. Haider, M. Kunz, et al. 2023. "Abiotic and Biotic Drivers of Tree Trait Effects on Soil Microbial Biomass and Soil Carbon Concentration." *Ecological Monographs* 93(2): e1563. https://doi.org/10.1002/ecm.1563.

Bossio, D. A., S. C. Cook-Patton, P. W. Ellis, J. Fargione, J. Sanderman, P. Smith, S. Wood, et al. 2020. "The Role of Soil Carbon in Natural Climate Solutions." *Nature Sustainability* 3(5): 391–98. https://doi.org/10.1038/s41893-020-0491-z.

Bray, S. R., K. Kitajima, and M. C. Mack. 2012. "Temporal Dynamics of Microbial Communities on Decomposing Leaf Litter of 10 Plant Species in Relation to Decomposition Rate." *Soil Biology and Biochemistry* 49(June): 30–37. https://doi.org/10.1016/j.soilbio.2012.02.009.

Bryant, R., S. Kothari, J. Cavender-Bares, S. Curran, J. Grossman, S. Hobbie, C. Nash, G. Neumiller, and C. See. 2023. "FAB 1: Forests and Biodiversity Experiment - High Density Diversity Experiment: Carbon Budget Data ver 1." Environmental Data Initiative. https://doi.org/10.6073/pasta/dc363389ee514e7384bf348228ee5ced.

Bukoski, J. J., S. C. Cook-Patton, C. Melikov, H. Ban, J. L. Chen, E. D. Goldman, N. L. Harris, and M. D. Potts. 2022. "Rates and Drivers of Aboveground Carbon Accumulation in Global Monoculture Plantation Forests." *Nature Communications* 13(1): 4206. https://doi.org/10.1038/s41467-022-31380-7.

Carney, K. M., and P. A. Matson. 2006. "The Influence of Tropical Plant Diversity and Composition on Soil Microbial Communities." *Microbial Ecology* 52(2): 226–238. https://doi.org/10.1007/s00248-006-9115-z.

Chapman, S. K., G. S. Newman, S. C. Hart, J. A. Schweitzer, and G. W. Koch. 2013. "Leaf Litter Mixtures Alter Microbial Community Development: Mechanisms for Non-Additive Effects in Litter Decomposition." *PLoS One* 8(4): e62671. https://doi.org/10.1371/journal.pone.0062671.

Chen, C., H. Y. H. Chen, X. Chen, and Z. Huang. 2019. "Meta-Analysis Shows Positive Effects of Plant Diversity on Microbial Biomass and Respiration." *Nature Communications* 10(1): 1332. https://doi.org/10.1038/s41467-019-09258-y.

Chen, S., W. Wang, W. Xu, Y. Wang, H. Wan, D. Chen, Z. Tang, et al. 2018. "Plant Diversity Enhances Productivity and Soil Carbon Storage." *Proceedings of the National Academy of Sciences* 115(16): 4027–32. https://doi.org/10.1073/pnas.1700298114.

Chen, X., Z. Ding, M. Tang, and B. Zhu. 2018. "Greater Variations of Rhizosphere Effects within Mycorrhizal Group than between Mycorrhizal Group in a Temperate Forest." *Soil Biology and Biochemistry* 126(November): 237–246. https://doi.org/10.1016/j.soilbio.2018.08.026.

Chen, X., H. Y. H. Chen, C. Chen, Z. Ma, E. B. Searle, Y. Zaipeng, and Z. Huang. 2020. "Effects of Plant Diversity on Soil Carbon in Diverse Ecosystems: A Global Meta-Analysis." *Biological Reviews* 95(1): 167–183. https://doi.org/10.1111/brv.12554.

Chen, X., A. R. Taylor, P. B. Reich, M. Hisano, H. Y. H. Chen, and S. X. Chang. 2023. "Tree Diversity Increases Decadal Forest Soil Carbon and Nitrogen Accrual." *Nature* 1–8: 94–101. https://doi.org/10.1038/s41586-023-05941-9.

Chisholm, R. A., and T. D. Gupta. 2023. "A Critical Assessment of the Biodiversity–Productivity Relationship in Forests and Implications for Conservation." *Oecologia* 201(4): 887–900. https://doi.org/10.1007/s00442-023-05363-4.

Cline, L. C., S. E. Hobbie, M. D. Madritch, C. R. Buyarski, D. Tilman, and J. M. Cavender-Bares. 2018. "Resource Availability Underlies the Plant-Fungal Diversity Relationship in a Grassland Ecosystem." *Ecology* 99(1): 204–216. https://doi.org/10.1002/ecy.2075.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. "The Microbial Efficiency-Matrix Stabilization (MEMS) Framework Integrates Plant Litter Decomposition with Soil Organic Matter Stabilization: Do Labile Plant Inputs Form Stable Soil Organic Matter?" *Global Change Biology* 19(4): 988–995. https://doi.org/10.1111/gcb.12113.

Craig, M. E., K. M. Geyer, K. V. Beidler, E. R. Brzostek, S. D. Frey, A. Stuart Grandy, C. Liang, and R. P. Phillips. 2022. "Fast-Decaying Plant Litter Enhances Soil Carbon in Temperate Forests but Not through Microbial Physiological Traits." *Nature Communications* 13(1): 1229.

Craig, M. E., B. L. Turner, C. Liang, K. Clay, D. J. Johnson, and R. P. Phillips. 2018. "Tree Mycorrhizal Type Predicts Within-Site Variability in the Storage and Distribution of Soil Organic Matter." *Global Change Biology* 24(8): 3317–30. https://doi.org/10.1111/gcb.14132.

Crawford, K. M., J. T. Bauer, L. S. Comita, M. B. Eppinga, D. J. Johnson, S. A. Mangan, S. A. Queenborough, et al. 2019. "When and Where Plant-Soil Feedback May Promote Plant Coexistence: A Meta-Analysis." *Ecology Letters* 22(8): 1274–84. https://doi.org/10.1111/ele.13278.

DeLancey, L. C., F. Maillard, S. E. Hobbie, and P. G. Kennedy. 2023. "Shifting Fungal Guild Abundances Change Temperate Forest Soil Carbon Stocks." *SSRN.* https://doi.org/10.2139/ssrn.4593790.

Deng, J., Y. Yin, W. Zhu, and Y. Zhou. 2018. "Variations in Soil Bacterial Community Diversity and Structures among Different Revegetation Types in the Baishilazi Nature Reserve." *Frontiers in Microbiology* 9: 284.

Deng, L., G.-b. Liu, and Z.-p. Shangguan. 2014. "Land-Use Conversion and Changing Soil Carbon Stocks in China's 'Grain-for-Green' Program: A Synthesis." *Global Change Biology* 20(11): 3544–56. https://doi.org/10.1111/gcb.12508.

Deng, L., and Z.-p. Shangguan. 2017. "Afforestation Drives Soil Carbon and Nitrogen Changes in China." *Land Degradation & Development* 28(1): 151–165. https://doi.org/10.1002/ldr.2537.

Deng, M., H. Shuijin, L. Guo, L. Jiang, Y. Huang, B. Schmid, C. Liu, et al. 2023. "Tree Mycorrhizal Association Types Control Biodiversity-Productivity Relationship in a Subtropical Forest." *Science Advances* 9(3): eadd4468. https://doi.org/10.1126/sciadv.add4468.

Dick, D. L., T. G. Gardner, J. P. Frene, J. L. Heitman, E. B. Sucre, and Z. H. Leggett. 2022. "Forest Floor Manipulation Effects on the Relationship between Aggregate Stability and Ectomycorrhizal Fungi." *Forest Ecology and Management* 505: 119873. https://doi.org/10.1016/j.foreco.2021.119873.

Dietrich, P., O. Ferlian, Y. Huang, S. Luo, J. Quosh, and N. Eisenhauer. 2023. "Tree Diversity Effects on Productivity Depend on Mycorrhizae and Life Strategies in a Temperate Forest Experiment." *Ecology* 104(2): e3896. https://doi.org/10.1002/ecy.3896.

Domisch, T., L. Finér, S. M. Dawud, L. Vesterdal, and K. Raulund-Rasmussen. 2015. "Does Species Richness Affect Fine Root Biomass and Production in Young Forest Plantations?" *Oecologia* 177: 581–594. https://doi.org/10.1007/s00442-014-3107-3.

Fanin, N., T. L. Maxwell, A. Altinalmazis-Kondylis, L. Bon, C. Meredieu, H. Jactel, M. R. Bakker, and L. Augusto. 2022. "Effects of Mixing Tree Species and Water Availability on Soil Organic Carbon Stocks Are Depth Dependent in a Temperate Podzol." *European Journal of Soil Science* 73(1): e13133. https://doi.org/10.1111/ejss.13133.

Fargione, J. E., S. Bassett, T. Boucher, S. D. Bridgham, R. T. Conant, S. C. Cook-Patton, P. W. Ellis, et al. 2018. "Natural Climate Solutions for the United States." *Science Advances* 4(11): eaat1869. https://doi.org/10.1126/sciadv.aat1869.

Feng, Y., B. Schmid, M. Loreau, D. I. Forrester, S. Fei, J. Zhu, Z. Tang, et al. 2022. "Multispecies Forest Plantations Outyield Monocultures across a Broad Range of Conditions." *Science* 376(6595): 865–68. https://doi.org/10.1126/science.abm6363.

Fernandez, C. W., and P. G. Kennedy. 2016. "Revisiting the 'Gadgil Effect': Do Interguild Fungal Interactions Control Carbon Cycling in Forest Soils?" *New Phytologist* 209(4): 1382–94. https://doi.org/10.1111/nph.13648.

Fernandez, C. W., C. R. See, and P. G. Kennedy. 2020. "Decelerated Carbon Cycling by Ectomycorrhizal Fungi Is Controlled by Substrate Quality and Community Composition." *New Phytologist* 226(2): 569–582. https://doi.org/10.1111/nph.16269.

Filialuna, O., and C. Cripps. 2021. "Evidence that Pyrophilous Fungi Aggregate Soil after Forest Fire." *Forest Ecology and Management* 498: 119579. https://doi.org/10.1016/j.foreco.2021.119579.

Forster, S. M. 1990. "The Role of Microorganisms in Aggregate Formation and Soil Stabilization: Types of Aggregation." *Arid Land Research and Management* 4(2): 85–98.

Frenne, D., J. L. Pieter, M. Luoto, B. R. Scheffers, F. Zellweger, J. Aalto, M. B. Ashcroft, et al. 2021. "Forest Microclimates and Climate Change: Importance, Drivers and Future Research Agenda." *Global Change Biology* 27(11): 2279–97. https://doi.org/10.1111/gcb.15569.

Frey, S. D. 2019. "Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics." *Annual Review of Ecology, Evolution, and Systematics* 50(1): 237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331.

Grigal, D. F., L. M. Chamberlain, H. R. Finney, V. Wroblewski, and E. R. Gross. 1974. *Soils of the Cedar Creek Natural History Area. Miscellaneous Report 123*. St. Paul: University of Minnesota Agricultural Experimental Station.

Grossman, J. 2019. "FAB 1 Forests and Biodiversity Experiment – High Density Diversity Experiment:Soil Lipid (P/NLFA) and AMF (Spore and Sequence) Data from Selected Plots ver 1." Environmental Data Initiative. https://doi.org/10.6073/pasta/3918fe1d3aab00ab73c4a83372cb82cf.

Grossman, J. J., A. J. Butterfield, J. Cavender-Bares, S. E. Hobbie, P. B. Reich, J. Gutknecht, and P. G. Kennedy. 2019. "Non-Symbiotic Soil Microbes Are more Strongly Influenced by Altered Tree Biodiversity than Arbuscular Mycorrhizal Fungi during Initial Forest Establishment." *FEMS Microbiology Ecology* 95(10): fiz134. https://doi.org/10.1093/femsec/fiz134.

Grossman, J. J., J. Cavender-Bares, and S. E. Hobbie. 2020. "Functional Diversity of Leaf Litter Mixtures Slows Decomposition of Labile but Not Recalcitrant Carbon over Two Years." *Ecological Monographs* 90(3): e01407. https://doi.org/10.1002/ecm.1407.

Grossman, J. J., J. Cavender-Bares, S. E. Hobbie, P. B. Reich, and R. A. Montgomery. 2017. "Species Richness and Traits Predict Overyielding in Stem Growth in an Early-Successional Tree Diversity Experiment." *Ecology* 98(10): 2601–14. https://doi.org/10.1002/ecy.1958.

Grossman, J. J., J. Cavender-Bares, P. B. Reich, R. A. Montgomery, and S. E. Hobbie. 2019. "Neighborhood Diversity Simultaneously Increased and Decreased Susceptibility to Contrasting Herbivores in an Early Stage Forest Diversity Experiment." *Journal of Ecology* 107(3): 1492–1505. https://doi.org/10.1111/1365-2745.13097.

Guerrero-Ramírez, N. R., D. Craven, P. B. Reich, J. J. Ewel, F. Isbell, J. Koricheva, J. A. Parrotta, et al. 2017. "Diversity-Dependent Temporal Divergence of Ecosystem Functioning in Experimental Ecosystems." *Nature Ecology & Evolution* 1(11): 1639–42. https://doi.org/10.1038/s41559-017-0325-1.

Gunina, A., A. R. Smith, D. L. Godbold, D. L. Jones, and Y. Kuzyakov. 2017. "Response of Soil Microbial Community to Afforestation with Pure and Mixed Species." *Plant and Soil* 412(1): 357–368. https://doi.org/10.1007/s11104-016-3073-0.

Heděnec, P., H. Zheng, D. P. Siqueira, Q. Lin, Y. Peng, I. K. Schmidt, T. G. Frøslev, R. Kjøller, J. Rousk, and L. Vesterdal. 2023. "Tree Species Traits and Mycorrhizal Association Shape Soil Microbial Communities via Litter Quality and Species Mediated Soil Properties." *Forest Ecology and Management* 527: 120608. https://doi.org/10.1016/j.foreco.2022.120608.

Helmus, M. R., T. J. Bland, C. K. Williams, and A. R. Ives. 2007. "Phylogenetic Measures of Biodiversity." *The American Naturalist* 169(3): E68–E83. https://doi.org/10.1086/511334.

Hicks, L. C., M. M. Rahman, M. Carnol, K. Verheyen, and J. Rousk. 2018. "The Legacy of Mixed Planting and Precipitation Reduction Treatments on Soil Microbial Activity, Biomass and Community Composition in a Young Tree Plantation." *Soil Biology and Biochemistry* 124: 227–235. https://doi.org/10.1016/j.soilbio.2018.05.027.

Huang, Y., Y. Chen, N. Castro-Izaguirre, M. Baruffol, M. Brezzi, A. Lang, Y. Li, et al. 2018. "Impacts of Species Richness on Productivity in a Large-Scale Subtropical Forest Experiment." *Science* 362(6410): 80–83. https://doi.org/10.1126/science.aat6405.

Jenkins, J. C., D. C. Chojnacky, L. S. Heath, and R. A. Birdsey. 2003. "National-Scale Biomass Estimators for United States

Tree Species." *Forest Science* 49(1): 12–35. https://doi.org/10.1093/forestscience/49.1.12.

Jiang, Y. M., C. R. Chen, Y. Q. Liu, and H. X. Zhihong. 2010. "Soil Soluble Organic Carbon and Nitrogen Pools under Mono- and Mixed Species Forest Ecosystems in Subtropical China." *Journal of Soils and Sediments* 10(6): 1071–81. https://doi.org/10.1007/s11368-010-0191-9.

Johnston, M. H., P. S. Homann, J. K. Engstrom, and D. F. Grigal. 1996. "Changes in Ecosystem Carbon Storage over 40 Years on an Old-Field/Forest Landscape in East-Central Minnesota." *Forest Ecology and Management* 83(1): 17–26. https://doi.org/10.1016/0378-1127(96)03704-8.

Keller, A. B., and R. P. Phillips. 2019. "Leaf Litter Decay Rates Differ between Mycorrhizal Groups in Temperate, but Not Tropical, Forests." *New Phytologist* 222(1): 556–564. https://doi.org/10.1111/nph.15524.

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.

Khlifa, R., A. Paquette, C. Messier, P. B. Reich, and A. D. Munson. 2017. "Do Temperate Tree Species Diversity and Identity Influence Soil Microbial Community Function and Composition?" *Ecology and Evolution* 7(19): 7965–74. https://doi.org/10.1002/ece3.3313.

Kothari, S. 2023. "ShanKothari/FAB-Carbon-Project: DOI Link (v1.0.1)." Zenodo. https://doi.org/10.5281/zenodo.10348365.

Kothari, S., S. E. Hobbie, and J. Cavender-Bares. 2023. "Rapid Estimates of Leaf Litter Chemistry Using Reflectance Spectroscopy." *bioRxiv* https://doi.org/10.1101/2023.11.27.568939.

Kothari, S., R. A. Montgomery, and J. Cavender-Bares. 2021. "Physiological Responses to Light Explain Competition and Facilitation in a Tree Diversity Experiment." *Journal of Ecology* 109(5): 2000–2018. https://doi.org/10.1111/1365-2745.13637.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82(1): 1–26. https://doi.org/10.18637/jss.v082.i13.

Laganière, J., D. A. Angers, and D. Parã. 2010. "Carbon Accumulation in Agricultural Soils after Afforestation: A Meta-Analysis." *Global Change Biology* 16(1): 439–453. https://doi.org/10.1111/j.1365-2486.2009.01930.x.

Laliberté, E., and P. Legendre. 2010. "A Distance-Based Framework for Measuring Functional Diversity from Multiple Traits." *Ecology* 91(1): 299–305. https://doi.org/10.1890/08-2244.1.

Lambert, M.-C., C.-H. Ung, and F. Raulier. 2005. "Canadian National Tree Aboveground Biomass Equations." *Canadian Journal of Forest Research* 35(8): 1996–2018. https://doi.org/10.1139/x05-112.

Lamlom, S. H., and R. A. Savidge. 2003. "A Reassessment of Carbon Content in Wood: Variation within and between 41 North American Species." *Biomass and Bioenergy* 25(4): 381–88. https://doi.org/10.1016/S0961-9534(03)00033-3.

Lefcheck, J. S. 2016. "piecewiseSEM: Piecewise Structural Equation Modelling in r for Ecology, Evolution, and Systematics." *Methods in Ecology and Evolution* 7(5): 573–79. https://doi.org/10.1111/2041-210X.12512.

Lehmann, A., W. Zheng, and M. C. Rillig. 2017. "Soil Biota Contributions to Soil Aggregation." *Nature Ecology & Evolution* 1(12): 1828–35. https://doi.org/10.1038/s41559-017-0344-y.

Lenth, R. V. 2022. "emmeans: Estimated Marginal Means, aka Least-Squares Means." R package version 1.7.2. https://CRAN.R-project.org/package=emmeans.

Li, Y., H. Bruelheide, T. Scholten, B. Schmid, Z. Sun, N. Zhang, B. Wensheng, X. Liu, and K. Ma. 2019. "Early Positive Effects of Tree Species Richness on Soil Organic Carbon Accumulation in a Large-Scale Forest Biodiversity Experiment." *Journal of Plant Ecology* 12(5): 882–893. https://doi.org/10.1093/jpe/rtz026.

Liang, J., T. W. Crowther, N. Picard, S. Wiser, M. Zhou, G. Alberti, E.-D. Schulze, et al. 2016. "Positive Biodiversity-Productivity Relationship Predominant in Global Forests." *Science* 354(6309): aaf8957. https://doi.org/10.1126/science.aaf8957.

Liu, C. L., O. K. Ching, and K. V. Krutovsky. 2018. "Mixed-Species versus Monocultures in Plantation Forestry: Development, Benefits, Ecosystem Services and Perspectives for the Future." *Global Ecology and Conservation* 15: e00419. https://doi.org/10.1016/j.gecco.2018.e00419.

Liu, J., X. Liu, Q. Song, Z. G. Compson, C. J. LeRoy, F. Luan, H. Wang, H. Yalin, and Q. Yang. 2020. "Synergistic Effects: A Common Theme in Mixed-Species Litter Decomposition." *New Phytologist* 227(3): 757–765. https://doi.org/10.1111/nph.16556.

Loreau, M., and A. Hector. 2001. "Partitioning Selection and Complementarity in Biodiversity Experiments." *Nature* 412(6842): 72–76. https://doi.org/10.1038/35083573.

Ma, Z., and H. Y. H. Chen. 2016. "Effects of Species Diversity on Fine Root Productivity in Diverse Ecosystems: A Global Meta-Analysis." *Global Ecology and Biogeography* 25(11): 1387–96. https://doi.org/10.1111/geb.12488.

Ma, Z., H. Y. H. Chen, E. W. Bork, C. N. Carlyle, and S. X. Chang. 2020. "Carbon Accumulation in Agroforestry Systems Is Affected by Tree Species Diversity, Age and Regional Climate: A Global Meta-Analysis." *Global Ecology and Biogeography* 29(10): 1817–28. https://doi.org/10.1111/geb.13145.

Maillard, F., B. Beatty, M. Park, S. Adamczyk, B. Adamczyk, C. R. See, J. Cavender-Bares, S. E. Hobbie, and P. G. Kennedy. 2023. "Microbial Community Attributes Supersede Plant and Soil Parameters in Predicting Fungal Necromass Decomposition Rates in a 12-Tree Species Common Garden Experiment." *Soil Biology and Biochemistry* 184: 109124. https://doi.org/10.1016/j.soilbio.2023.109124.

Martin-Guay, M.-O., M. Belluau, B. Côté, I. T. Handa, M. D. Jewell, R. Khlifa, A. D. Munson, M. Rivest, J. K. Whalen, and D. Rivest. 2022. "Tree Identity and Diversity Directly Affect Soil Moisture and Temperature but Not Soil Carbon Ten Years after Planting." *Ecology and Evolution* 12(1): e8509. https://doi.org/10.1002/ece3.8509.

Martin-Guay, M.-O., A. Paquette, P. B. Reich, and C. Messier. 2020. "Implications of Contrasted Above- and below-Ground Biomass Responses in a Diversity Experiment with Trees." *Journal of Ecology* 108(2): 405–414. https://doi.org/10.1111/1365-2745.13265.

Mayer, M., C. E. Prescott, W. E. A. Abaker, L. Augusto, L. Cécillon, G. W. D. Ferreira, J. James, et al. 2020. "Tamm Review: Influence of Forest Management Activities on Soil Organic Carbon Stocks: A Knowledge Synthesis." *Forest Ecology and Management* 466(June): 118127. https://doi.org/10.1016/j.foreco.2020.118127.

Merino-Martín, L., A. Stokes, H. S. Gweon, L. Moragues-Saitua, S. Staunton, C. Plassard, A. Oliver, Y. Le Bissonnais, and R. I.

Griffiths. 2021. "Interacting Effects of Land Use Type, Microbes and Plant Traits on Soil Aggregate Stability." *Soil Biology and Biochemistry* 154: 108072. https://doi.org/10.1016/j.soilbio.2020.108072.

Messier, C., J. Bauhus, R. Sousa-Silva, H. Auge, L. Baeten, N. Barsoum, H. Bruelheide, et al. 2022. "For the Sake of Resilience and Multifunctionality, Let's Diversify Planted Forests!" *Conservation Letters* 15(1): e12829. https://doi.org/10.1111/conl.12829.

Moyano, F. E., S. Manzoni, and C. Chenu. 2013. "Responses of Soil Heterotrophic Respiration to Moisture Availability: An Exploration of Processes and Models." *Soil Biology and Biochemistry* 59(April): 72–85. https://doi.org/10.1016/j.soilbio.2013.01.002.

Mueller, C. W., V. Baumert, A. Carminati, A. Germon, M. Holz, I. Kögel-Knabner, S. Peth, et al. 2024. "From Rhizosphere to Detritusphere – Soil Structure Formation Driven by Plant Roots and the Interactions with Soil Biota." *Soil Biology and Biochemistry* 193(June): 109396. https://doi.org/10.1016/j.soilbio.2024.109396.

Mueller, K. E., S. E. Hobbie, J. Chorover, P. B. Reich, N. Eisenhauer, M. J. Castellano, O. A. Chadwick, et al. 2015. "Effects of Litter Traits, Soil Biota, and Soil Chemistry on Soil Carbon Stocks at a Common Garden with 14 Tree Species." *Biogeochemistry* 123(3): 313–327. https://doi.org/10.1007/s10533-015-0083-6.

Nakagawa, S., P. C. D. Johnson, and H. Schielzeth. 2017. "The Coefficient of Determination R2 and Intra-Class Correlation Coefficient from Generalized Linear Mixed-Effects Models Revisited and Expanded." *Journal of the Royal Society Interface* 14(134): 20170213. https://doi.org/10.1098/rsif.2017.0213.

Palandrani, C., and G. Alberti. 2020. "Tree Derived Soil Carbon Is Enhanced by Tree Species Richness and Functional Diversity." *Plant and Soil* 446(1): 457–469. https://doi.org/10.1007/s11104-019-04381-7.

Pan, Y., R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, et al. 2011. "A Large and Persistent Carbon Sink in the World's Forests." *Science* 333(6045): 988–993. https://doi.org/10.1126/science.1201609.

Pellegrini, A. F. A., A. M. Hein, J. Cavender-Bares, R. A. Montgomery, A. Carla Staver, F. Silla, S. E. Hobbie, and P. B. Reich. 2021. "Disease and Fire Interact to Influence Transitions between Savanna–Forest Ecosystems over a Multi-Decadal Experiment." *Ecology Letters* 24(5): 1007–17. https://doi.org/10.1111/ele.13719.

Phillips, R. P., E. Brzostek, and M. G. Midgley. 2013. "The Mycorrhizal-Associated Nutrient Economy: A New Framework for Predicting Carbon-Nutrient Couplings in Temperate Forests." *New Phytologist* 199(1): 41–51. https://doi.org/10.1111/nph.12221.

Porre, R. J., W. van der Werf, G. B. De Deyn, T. J. Stomph, and E. Hoffland. 2020. "Is Litter Decomposition Enhanced in Species Mixtures? A Meta-Analysis." *Soil Biology and Biochemistry* 145(June): 107791. https://doi.org/10.1016/j.soilbio.2020.107791.

Potvin, C., L. Mancilla, N. Buchmann, J. Monteza, T. Moore, M. Murphy, Y. Oelmann, et al. 2011. "An Ecosystem Approach to Biodiversity Effects: Carbon Pools in a Tropical Tree Plantation." *Forest Ecology and Management* 261(10): 1614–24. https://doi.org/10.1016/j.foreco.2010.11.015.

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

Read, D. J., and J. Perez-Moreno. 2003. "Mycorrhizas and Nutrient Cycling in Ecosystems – A Journey towards Relevance?" *New Phytologist* 157(3): 475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x.

Regelink, I. C., C. R. Stoof, S. Rousseva, L. Weng, G. J. Lair, P. Kram, N. P. Nikolaidis, M. Kercheva, S. Banwart, and R. N. J. Comans. 2015. "Linkages between Aggregate Formation, Porosity and Soil Chemical Properties." *Geoderma* 247–248 (June): 24–37. https://doi.org/10.1016/j.geoderma.2015.01.022.

Reich, P. B., D. S. Ellsworth, M. B. Walters, J. M. Vose, C. Gresham, J. C. Volin, and W. D. Bowman. 1999. "Generality of Leaf Trait Relationships: A Test across Six Biomes." *Ecology* 80(6): 1955–69. https://doi.org/10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2.

Reid, J. B., and M. J. Goss. 1981. "Effect of Living Roots of Different Plant Species on the Aggregate Stability of Two Arable Soils." *Journal of Soil Science* 32(4): 521–541. https://doi.org/10.1111/j.1365-2389.1981.tb01727.x.

Ren, C., K. Liu, P. Dou, J. Li, and K. Wang. 2022. "The Changes in Soil Microorganisms and Soil Chemical Properties Affect the Heterogeneity and Stability of Soil Aggregates before and after Grassland Conversion." *Agriculture* 12(2): 307. https://doi.org/10.3390/agriculture12020307.

Rillig, M. C. 2004. "Arbuscular Mycorrhizae, Glomalin, and Soil Aggregation." *Canadian Journal of Soil Science* 84(4): 355–363. https://doi.org/10.4141/S04-003.

Rillig, M. C., C. A. Aguilar-Trigueros, J. Bergmann, E. Verbruggen, S. D. Veresoglou, and A. Lehmann. 2015. "Plant Root and Mycorrhizal Fungal Traits for Understanding Soil Aggregation." *New Phytologist* 205(4): 1385–88. https://doi.org/10.1111/nph.13045.

Rillig, M. C., S. F. Wright, and V. T. Eviner. 2002. "The Role of Arbuscular Mycorrhizal Fungi and Glomalin in Soil Aggregation: Comparing Effects of Five Plant Species." *Plant and Soil* 238(2): 325–333. https://doi.org/10.1023/A:1014483303813.

Rivest, M., J. K. Whalen, and D. Rivest. 2019. "Tree Diversity Is Not Always a Strong Driver of Soil Microbial Diversity: A 7-Yr-Old Diversity Experiment with Trees." *Ecosphere* 10(4): e02685. https://doi.org/10.1002/ecs2.2685.

Rožek, K., D. Chmolowska, I. Odriozola, T. Větrovský, K. Rola, P. Kohout, P. Baldrian, and S. Zubek. 2023. "Soil Fungal and Bacterial Community Structure in Monocultures of Fourteen Tree Species of the Temperate Zone." *Forest Ecology and Management* 530(February): 120751. https://doi.org/10.1016/j.foreco.2022.120751.

Seabloom, E. W., E. T. Borer, S. E. Hobbie, and A. S. MacDougall. 2021. "Soil Nutrients Increase Long-term Soil Carbon Gains Threefold on Retired Farmland." *Global Change Biology* 27 (19): 4909–20. https://doi.org/10.1111/gcb.15778.

Schuster, M. J., L. J. Williams, A. Stefanski, R. Bermudez, M. Belluau, C. Messier, A. Paquette, D. Gravel, and P. B. Reich. 2023. "Patterns of Belowground Overyielding and Fine-Root Biomass in Native and Exotic Angiosperms and Gymnosperms." *Oikos* 2023(1): e08877. https://doi.org/10.1111/oik.08877.

Scott, N. A. 1998. "Soil Aggregation and Organic Matter Mineralization in Forests and Grasslands: Plant Species Effects." *Soil Science Society of America Journal* 62(4): 1081–89. https://doi.org/10.2136/sssaj1998.03615995006200040032x.

See, C. R., A. B. Keller, S. E. Hobbie, P. G. Kennedy, P. K. Weber, and J. Pett-Ridge. 2022. "Hyphae Move Matter and Microbes to Mineral Microsites: Integrating the Hyphosphere into Conceptual Models of Soil Organic Matter Stabilization." *Global Change Biology* 28(8): 2527–40. https://doi.org/10.1111/gcb.16073.

See, C. R., M. L. McCormack, S. E. Hobbie, H. Flores-Moreno, W. L. Silver, and P. G. Kennedy. 2019. "Global Patterns in Fine Root Decomposition: Climate, Chemistry, Mycorrhizal Association and Woodiness." *Ecology Letters* 22(6): 946–953. https://doi.org/10.1111/ele.13248.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. "A History of Research on the Link between (Micro)Aggregates, Soil Biota, and Soil Organic Matter Dynamics." *Soil and Tillage Research* 79(1): 7–31. https://doi.org/10.1016/j.still.2004.03.008.

Six, Johan Walter Urbain Albert. 1998. "Aggregate and Soil Organic Matter Fraction Dynamics in Agroecosystems." Order No. 9922027, Colorado State University https://www2.lib.ku.edu/login?url=https://www.proquest.com/dissertations-theses/aggregate-soil-organic-matter-fraction-dynamics/docview/304434246/se-2.

Sokol, N. W., E. Slessarev, G. L. Marschmann, A. Nicolas, S. J. Blazewicz, E. L. Brodie, M. K. Firestone, et al. 2022. "Life and Death in the Soil Microbiome: How Ecological Processes Influence Biogeochemistry." *Nature Reviews Microbiology* 20(7): 415–430. https://doi.org/10.1038/s41579-022-00695-z.

Strukelj, M., W. Parker, E. Corcket, L. Augusto, R. Khlifa, H. Jactel, and A. D. Munson. 2021. "Tree Species Richness and Water Availability Interact to Affect Soil Microbial Processes." *Soil Biology and Biochemistry* 155(April): 108180. https://doi.org/10.1016/j.soilbio.2021.108180.

Terrer, C., R. P. Phillips, B. A. Hungate, J. Rosende, J. Pett-Ridge, M. E. Craig, K. J. van Groenigen, et al. 2021. "A Trade-off between Plant and Soil Carbon Storage under Elevated CO2." *Nature* 591(7851): 599–603. https://doi.org/10.1038/s41586-021-03306-8.

Thomas, S. C., and A. R. Martin. 2012. "Carbon Content of Tree Tissues: A Synthesis." *Forests* 3(2): 332–352. https://doi.org/10.3390/f3020332.

Tisdall, J. M. 1994. "Possible Role of Soil Microorganisms in Aggregation in Soils." *Plant and Soil* 159(1): 115–121. https://doi.org/10.1007/BF00000100.

Tobner, C. M., A. Paquette, P. B. Reich, D. Gravel, and C. Messier. 2014. "Advancing Biodiversity–Ecosystem Functioning Science Using High-Density Tree-Based Experiments over Functional Diversity Gradients." *Oecologia* 174(3): 609–621. https://doi.org/10.1007/s00442-013-2815-4.

Urgoiti, J., C. Messier, W. S. Keeton, P. B. Reich, D. Gravel, and A. Paquette. 2022. "No Complementarity No Gain—Net Diversity Effects on Tree Productivity Occur Once Complementarity Emerges during Early Stand Development."

Ecology Letters 25(4): 851–862. https://doi.org/10.1111/ele.13959.

Wright, A. J., K. E. Barry, C. J. Lortie, and R. M. Callaway. 2021. "Biodiversity and Ecosystem Functioning: Have our Experiments and Indices Been Underestimating the Role of Facilitation?" *Journal of Ecology* 109(5): 1962–68. https://doi.org/10.1111/1365-2745.13665.

Wright, S. J., K. Kitajima, N. J. B. Kraft, P. B. Reich, I. J. Wright, D. E. Bunker, R. Condit, et al. 2010. "Functional Traits and the Growth–Mortality Trade-off in Tropical Trees." *Ecology* 91(12): 3664–74. https://doi.org/10.1890/09-2335.1.

Wright, S. F., and A. Upadhyaya. 1998. "A Survey of Soils for Aggregate Stability and Glomalin, a Glycoprotein Produced by Hyphae of Arbuscular Mycorrhizal Fungi." *Plant and Soil* 198(1): 97–107. https://doi.org/10.1023/A:1004347701584.

Wu, Y., M. Deng, J. Huang, S. Yang, L. Lulu Guo, J. A. Yang, Z. Peng, W. Liu, and L. Liu. 2022. "Global Patterns in Mycorrhizal Mediation of Soil Carbon Storage, Stability, and Nitrogen Demand: A Meta-Analysis." *Soil Biology and Biochemistry* 166(March): 108578. https://doi.org/10.1016/j.soilbio.2022.108578.

Yanai, R. D., W. S. Currie, and C. L. Goodale. 2003. "Soil Carbon Dynamics after Forest Harvest: An Ecosystem Paradigm Reconsidered." *Ecosystems* 6(3): 197–212.

Zhang, S., D. Landuyt, K. Verheyen, and P. De Frenne. 2022. "Tree Species Mixing Can Amplify Microclimate Offsets in Young Forest Plantations." *Journal of Applied Ecology* 59(6): 1428–39. https://doi.org/10.1111/1365-2664.14158.

Zheng, W., E. Kathryn Morris, and M. C. Rillig. 2014. "Ectomycorrhizal Fungi in Association with *Pinus sylvestris* Seedlings Promote Soil Aggregation and Soil Water Repellency." *Soil Biology and Biochemistry* 78: 326–331. https://doi.org/10.1016/j.soilbio.2014.07.015.

Zheng, Li-Ting, Y. H. Chen Han, and En-Rong Yan. 2019. "Tree Species Diversity Promotes Litterfall Productivity Through Crown Complementarity in Subtropical Forests." *Journal of Ecology* 107(4): 1852–61. https://doi.org/10.1111/1365-2745.13142.

SUPPORTING INFORMATION

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