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Accounting for Carbon Flux to Mycorrhizal Fungi May Resolve Discrepancies in Forest Carbon Budgets

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ABSTRACT

Carbon (C) fluxes among different components of plant growth are important to forest ecosystem C cycling and are strongly influenced by species composition and resource availability. Although mycorrhizal fungi are crucial for nutrient acquisition and can receive a large fraction of annual net primary production, most studies do not explicitly include carbon flux to mycorrhizal fungi in ecosystem C budgets. We measured annual production of plant components (foliage, wood, fine roots) and mycorrhizal fungi across temperate forest stands varying in species composition. Production of mycorrhizal fungi was estimated using both mass balance and isotopic techniques. Total plant production varied from about 600 g C m^{-2} y^{-1} in nearly pure deciduous broadleaf stands down to about 300 g C m^{-2} y^{-1} in conifer-dominated stands. In contrast, the production of mycorrhizal fungi was highest in conifer-dominated stands, varying from less than 25 g C m^{-2} y^{-1} in deciduous broadleaf stands to more than 175 g C m^{-2} y^{-1} in nearly pure conifer stands. Isotopic data indicated that both tree species composition and ecosystem nitrogen (N) availability influenced rates of fungal production. The large investment in mycorrhizal fungi in low-N, conifer-dominated stands demonstrated that a full accounting of ecosystem carbon fluxes to plant and fungal components may help resolve current discrepancies observed in broadscale forest carbon budgets, especially across forest types.

Key words: carbon allocation; forest carbon; mycorrhizal fungi; total belowground carbon flux (TBCF); Bartlett Experimental Forest.

HIGHLIGHTS

- The production of plant components decreased while mycorrhizal fungi increased in forests with

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Author Contributions APO, SVO, EAH, LCL and MAV designed the study; RBS and RJR provided truffle abundance data; RBS, RJR, APO, SVO and EAH provided soil and root δ^{15}N data from the truffle plots; LCL and SJT led field efforts for the collection of aboveground production and belowground fine root production, respectively. APO wrote the manuscript, with all authors contributing significantly to revisions.

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increasing conifer tree dominance.
- Both tree species and resource availability influenced the production of mycorrhizal fungi.
- Mycorrhizal fungi are important components of ecosystem carbon budgets and may account for prior discrepancies in forest carbon fluxes in broadscale datasets.

INTRODUCTION

The flux of carbon to different components of plant growth plays an important role in forest ecosystem carbon (C) cycling. The proportion of gross primary production allocated to ephemeral versus long-lived tissues and aboveground versus belowground components influences the residence time of C in forests and the magnitude of ecosystem services afforded (for example, timber production). At broad scales, C fluxes to different components of plant production vary with climate, the availability of growth limiting resources and species composition. Typically, the ratio of the annual flux of carbon to fine roots versus to aboveground components is greater in cold, nutrient-poor sites than in warm, high-fertility sites (Litton and others 2007; Litton and Giardina 2008; Vicca and others 2012). This shift in tree-level C allocation is thought to primarily reflect a trade-off between nitrogen (N) limitation and light limitation (Dybzinski and others 2011). Additionally, because species vary in their resource acquisition strategies and allometric constraints, species composition has an important influence on the C flux to different components of forest production. Often, the ratio of C allocated to foliar and stem tissues compared to root tissues is lower in stands dominated by gymnosperm compared to angiosperm species (Gower and others 2001).

Despite efforts to quantify broadscale patterns of C fluxes in forests, our understanding of ecosystem C fluxes is far from complete and several important discrepancies remain. Some of these discrepancies in broadscale C budgets result from an incomplete accounting of the C flux to different components of forest growth, with mycorrhizal fungi being a particularly important omission (Chapin III and others 2009). Mycorrhizal fungi are ubiquitous, and nearly all temperate and boreal forest trees associate with either ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) fungi. Both broad groups of mycorrhizal fungi provide trees with soil N and other nutrients necessary for growth and receive photosynthetically fixed C in return. It is typically thought that ECM fungi have higher carbon demands, more extensive hyphae and stronger capabilities to break down soil organic matter than AM fungi (Read and Perez-Moreno 2003); however low-biomass ECM exploration types and AM fungi may be functionally similar.

The C demand of fungi may be an important component of forest C budgets. For example, in a fertilization study in *Pinus radiata* stands, Ryan and others (1996) accounted for 100% of total belowground carbon flux (TBCF) in fertilized plots, but found that 43% of TBCF was ''missing'' in control stands. The ''missing'' TBCF was later attributed to production of mycorrhizal fungi, and exudates, which were presumably related to greater plant nutrient demands (Waring and Running 2010). Similarly, across 49 sites, biomass production was 58% of gross primary production (GPP) in forests with high nutrient availability and 42% in forests with low nutrient availability (Vicca and others 2012). They suggested that the lower biomass production was not merely an increase in plant respiration at low-nutrient sites, but due to the omission of mycorrhizal fungi in ecosystem C budgets. Furthermore, Litton and others (2007) found that a higher proportion of GPP was allocated belowground in colder, slower-growing forests than in warmer, more productive forests. However, root production often comprised a much smaller fraction of the total belowground carbon flux (TBCF) at colder, more nutrient-poor sites (Litton and Giardina 2008), presumably due to the omission of C allocation to root symbionts.

Studies in forests have estimated the annual production of mycorrhizal fungi to be up to several hundred g C m^{-2} y^{-1} and 20% of GPP (for example, Vogt and others 1982; Fogel and Hunt 1983; Godbold and others 2006; Hendricks and others 2006; Hobbie 2006; Allen and others 2010; Allen and Kitajima 2014). Despite this, surprisingly few studies have attempted to quantify fungal production in forest C budgets. Most recent estimates of fungal production are from monodominant *Picea abies* or *Pinus sylvestris* stands in Scandinavia (Ekblad and others 2013) and have measured fungal ingrowth into field-incubated bags of acid-washed quartz sand—an important approach that may underestimate mycorrhizal production when compared with other approaches (Hendricks and others 2006; Neumann and Matzner 2013; Wallander and others 2013).

The importance of obtaining estimates of the production of mycorrhizal fungi goes beyond improving our ability to close forest ecosystem C budgets. Mycorrhizal fungi also play key roles in the C cycle of terrestrial ecosystems by: (1) influencing plant C uptake via mediation of N uptake from soils (Heijden and others 2008) and (2) influencing soil organic carbon (SOC) accumulation and turnover though the quantity and quality (recalcitrance) of fungal necromass (Clemmensen and others 2015) and through competition with free-living, saprotrophic decomposers (Fernandez and Kennedy 2016). Explicitly including mycorrhizal fungi into an earth system model greatly improved predictions of forest N uptake dynamics in temperate forests including several Free Air CO_2 Enrichment (FACE) experiments (Brzostek and others 2017). However, because estimates of fungal biomass production are limited, fungal biomass production is often not included in modeling efforts, precluding fungal feedbacks to soil carbon dynamics and ecosystem carbon storage.

The aim of our work was to assess C flux to different components of plant growth in temperate forest stands that span a range of species composition and nutrient availability while explicitly including the production of mycorrhizal fungi in our estimates. We quantified the production of foliage, wood, fine roots and mycorrhizal fungi across stands differing in the abundance of deciduous broadleaf to coniferous tree species. We took several approaches to quantify the production of mycorrhizal fungi, including a mass balance approach and isotopic techniques, along with supporting data from direct estimates of mycorrhizal fungal biomass. Isotopic data provided additional insight into the role of species composition and nutrient availability in regulating the production of mycorrhizal fungi.

Materials and Methods

Net primary production (NPP) of foliage, woody tissues, fine roots, and mycorrhizal fungi, as well as rates of soil respiration and total belowground carbon flux (TBCF), were measured at the Bartlett Experimental Forest (BEF) across stands differing in tree species composition. No consensus exists on the most reliable method to quantify production of mycorrhizal fungi, and estimating C flux to mycorrhizas necessarily requires creative approaches and associated assumptions. To estimate the production of mycorrhizal fungi, we used two independent methods: (1) a mass balance approach and (2) isotopic techniques (Hobbie and Hobbie 2008). Additionally, direct observations of fungal sporocarp biomass were collected to serve as independent support for our measurements of fungal production. We did not include a sensitivity analysis for each approach because uncertainty in some of the assumptions is unknown. Instead, we relied on a comparison of the patterns and magnitudes of multiple independent approaches to constrain estimates of fungal biomass production. Lastly, isotopic data on foliage, fine roots, and soils were used to assess the influence of species composition and nutrient availability on the production of mycorrhizal fungi.

Site Description

Bartlett Experimental Forest (BEF) (44°06′N, 71°3′W) is located within the White Mountain National Forest in north-central New Hampshire, USA. The climate is humid continental with cool summers (mean July temperature, 19 °C) and cold winters (mean January temperature, − 9 °C). Mean annual temperature is 6 °C and mean annual precipitation is 1270 mm (Adams and others 2010). Soils are predominantly well-drained Spodosols and Inceptisols developed on rocky granitic till and glacial outwash.

Our study draws from data collected from three sets of plots at BEF, hereafter referred to as *inventory plots*, *tower plots*, and *truffle plots* (Figure 1 and Table S1). All plots were in mature stands between 100 and 170 years old and varied in species composition. Dominant deciduous broadleaf tree species included *Acer rubrum*, *Fagus grandifolia*, *Acer saccharum*, and *Betula alleghaniensis*, whereas *Tsuga canadensis* and *Picea rubens* were the dominant conifer species.

In 1931, a gridded network of 441 long-term inventory plots (0.1 ha) was established by the U.S.

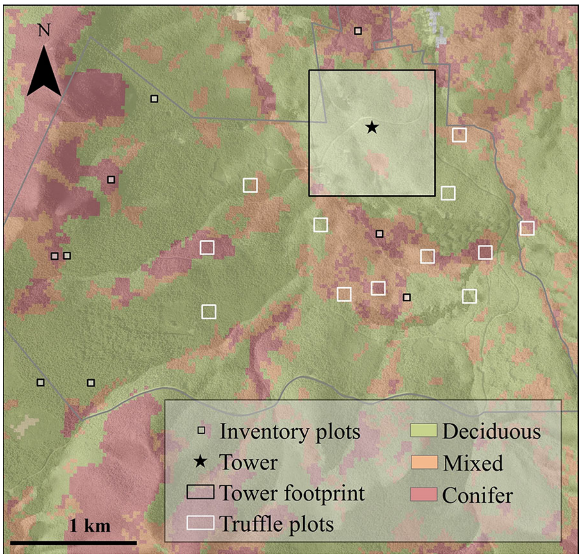

Figure 1. Map of study sites and dominant tree species types at Bartlett Experimental Forest.

Forest Service across BEF, and trees were measured by 2.54 cm (1 in.) diameter classes in 1931–1932, 1939–1940, 1991–1992, and 2001–2003 (Adams and others 2010). Plot elevations ranged from approximately 200–800 m. Previous work at 39 of the long-term plots quantified foliar and woody net primary production and plot-level foliar nitrogen concentration (%N) (Smith and others 2002; Ollinger and Smith 2005). At a subset of these plots (n = 9; *inventory plots*; Figure 1), we supplemented these data with new measurements of soil respiration, branchfall, root production, and stable isotope measurements of foliage, fine roots, and soil.

In 2003, BEF was adopted as a NASA North American Carbon Program (NACP) Tier-2 field research and validation site (Hollinger 2008). A 26.5-m-high tower was installed in a low-elevation northern hardwood stand in November 2003 (http://www.public.ornl.gov/ameriflux/) to make eddy covariance measurements of the forest–atmosphere exchange of carbon dioxide, water, and energy. In 2004, 12 FIA-style (Forest Inventory and Analysis) plots (Bradford and others 2009) were established across a 1 km by 1 km area centered on the flux tower (*tower plots;* Figure 1). Using both eddy covariance and plot-level biometric data, Ouimette and others (2018) assembled a comprehensive C budget for stands within the tower footprint for 2004–2016. Because plot design was meant to estimate C fluxes within the footprint of the flux tower, herein we treated the *tower plots* as a single stand (single data point; Figure 1).

In 2013, Stephens and others (2017) established twelve 1.1-ha sampling grids spanning a range of deciduous broadleaf to conifer-dominated forest stands to measure dynamics of small mammal communities and hypogeous fungal sporocarp (truffle) abundance (*truffle plots*; Figure 1). Soil profiles at the *truffle plots* were also sampled intensively at narrow depth increments (for example, 1–2 cm) to collect roots and soil for isotopic analysis.

Foliar, Wood, and Fine Root Production

Previous studies at BEF quantified net primary production of foliage ($\mathrm{NPP}_{\mathrm{foliage}}$) and woody tissues ($\mathrm{NPP}_{\mathrm{wood}}$), as well as foliar nitrogen (%N) at the *inventory plots* (Smith and others 2002; Ollinger and Smith 2005). We updated aboveground estimates of NPP by including previously unmeasured estimates of branchfall and the production of coarse woody roots following (Ouimette and others 2018). Measurements of foliar, wood, and fine root NPP at the tower plots are reported in Ouimette and others (2018).

The production of fine roots ($<$ 2 mm diameter) was estimated using ingrowth cores. At each of the 9 *inventory plots*, 15 cores (year-long; late October 2013—late October 2014) were installed to a depth of 30 cm (for a total of 135 cores). Within each plot, the 15 ingrowth cores were randomly distributed over an approximately 900-m^2 area. During this same time period, 90 ingrowth cores (year-long) were installed within the *tower plots*. All cores were filled with plot- and soil horizon-specific, root-free soils sieved to 2 mm. This soil was used to fill a volume that had been excavated by a 5.08-cm diameter corer to 30 cm depth. To allow the surrounding soil and roots to recover from disturbance, ingrowth core holes were excavated one month prior to the initiation of ingrowth and held open with a PVC pipe. After this recovery period, PVC pipes were removed and replaced with root-free soil. Prior to filling with soil, three aluminum rods were placed in the core hole to allow for accurate resampling. The central 3.81 cm diameter of the core was sampled to 30 cm after the year-long incubation. Total fine root mass recovered in these samples was assumed to represent annual fine root production, $\mathrm{NPP}_{\mathrm{root}}$ (g C m^{-2} year^{-1}), using a C content of 49% (see Ouimette and others 2018).

Soil Respiration

Soil respiration (Rs) was measured using an infrared gas analyzer in conjunction with static chambers as described in (Ouimette and others 2018). The static chambers consisted of a 25.4-cm PVC collar permanently inserted about 5 cm into the soil. Measurements were made approximately every 3 weeks during the snow-free portion of each year during 2004–2008 at *inventory plots* (six collars per plot), as well as at *tower plots* on 144 chambers across the 1 km^2 flux tower footprint. Continuous soil moisture and temperature measurements were made at 5 cm soil depth at the base of the flux tower.

To derive annual soil CO_2 flux estimates, measured CO_2 flux rates from chambers were fit using a Gauss–Newton optimization method in JMP 13.0 statistical software (SAS 2016) to Q_{10} soil temperature response model (Richardson and others 2006). Model best fits were then applied to continuous (every 30 min) temperature and moisture measurements made at the base of the eddy covariance flux tower (5 cm depth) to calculate annual soil CO_2 flux rates for each plot. Although only snow-free measurements were used to derive best model fits, there was little bias in this approach when compared to snow-covered respiration measurements at the *tower plots* (Ouimette and others 2018).

Production of Mycorrhizal Fungi

Mass Balance Approach to Estimate C Allocation to Mycorrhizas

We estimated the production of mycorrhizal fungi using a mass balance approach that relied on estimating total belowground carbon allocation (TBCF) and several components of plot-level TBCF. First, TBCF was estimated following Raich and Nadelhoffer (1989) and Davidson and others (2002), using measurements of soil respiration (Rs), aboveground litterfall, changes in coarse root biomass (ΔC_{root}), and by assuming that changes in soil carbon stocks (ΔC_{soil}) and leaching losses were minimal compared to other fluxes (Eq. 1) (Giardina and Ryan 2002). Measurements of fine litterfall included foliage as well as woody material with a diameter less than 5 cm (Ouimette and others 2018).

TBCF can also be considered as the sum of its component fluxes (Eq. 2), including C for the production of fine roots (NPP_{froot}), coarse roots (NPP_{croot}), and mycorrhizal fungi (NPP_{fungi}), as well as root plus mycorrhizal respiration (Rs_a), and exudates (Ex) (Chapin III and others 2009). We used plot-level estimates of TBCF from Eq. 1, as well as estimates of component fluxes (specifically, NPP_{froot}, NPP_{croot} and Rs_a), to estimate NPP_{fungi} (Eq. 3). We recognize that these simplifying assumptions ignore both C flux to root or mycorrhizal exudation and also ignore the fraction of measured fine root production that is fungal tissue. Errors from these simplifications are likely offsetting and are addressed in Discussion.

$$\mathrm{TBCF} = \mathrm{Rs} - \mathrm{litterfall} + \Delta C_{root} + (\Delta C_{soil}) + (\mathrm{leaching}) \tag{1}$$

$$\mathrm{TBCF} = \mathrm{NPP}_{froot} + \mathrm{NPP}_{croot} + \mathrm{NPP}_{fungi} + \mathrm{Rs}_a + (\mathrm{Ex}) \tag{2}$$

$$\mathrm{NPP}_{fungi} \approx \mathrm{TBCF} - (\mathrm{NPP}_{froot} + \mathrm{NPP}_{croot} + \mathrm{Rs}_a) \tag{3}$$

To estimate the autotrophic portion of soil respiration (including both root and mycorrhizal sources), we used the Global Database of Soil Respiration Version 3 (Bond-Lamberty and Thomson 2014) to derive a relationship between annual total and annual autotrophic soil respiration. Specifically, we derived a relationship between R_{Sa} and Rs using data from non-experimentally manipulated, temperate forest ecosystems with quality check flags of Q0, Q01, Q02, and Q03 and a logical filter to exclude site years with $Rs_a/Rs > 0.90$ and $Rs_a{:}Rs < 0.10$. Using these criteria, we derived the following relationship between annual R_{Sa} and R_S/ $R_{Sa} = -50.713\ (\pm 23.662) + R_S{*}0.519\ (\pm 0.031)$, ($n = 197$). No significant difference in the relationship between R_{Sa} and R_S was observed between plots dominated by conifer versus broadleaf species ($p = 0.15$), so only a single relationship was used across all plots.

Production of Mycorrhizal Fungi Using Stable Isotope Techniques

Nitrogen stable isotopes, specifically the ratio of $^{15}N/^{14}N$ (referred to as $\delta^{15}N$), have been used to estimate rates of C allocation to ectomycorrhizal fungi (Hobbie and Hobbie 2008). Currently, clear evidence exists for ectomycorrhizas, but not for arbuscular mycorrhizas, that isotopic fractionation during fungal transfer of N to plant hosts decreases plant $\delta^{15}N$ (Hobbie and Högberg 2012). Differences in the $\delta^{15}N$ of plant biomass and soil N available for uptake by plants can be used to estimate C and N exchange between mycorrhizal fungi and their plant hosts (Hobbie and Colpaert 2003; Hobbie and Hobbie 2008; Ouimette and others 2013). We used differences between soil $\delta^{15}N$ and root $\delta^{15}N$ to estimate the transfer ratio, Tr, defined as the fraction of N assimilated by mycorrhizal fungi that is transferred to plant hosts (Eq. 4).

$$\mathrm{Tr} = 1 - \frac{\left(\delta^{15}N_{AvailN} - \delta^{15}N_{Root}\right)}{\Delta_f} \tag{4}$$

where $\delta^{15}N_{AvailN}$ is the $\delta^{15}N$ of soil N available for uptake, $\delta^{15}N_{Root}$ is the $\delta^{15}N$ of plant root tissue, and Δ_f is the isotopic fractionation factor during the transfer of N from mycorrhizal fungi to plant hosts (Figure S1). Because soil $\delta^{15}N$ varies strongly with depth (Hobbie and Ouimette 2009), we collected $\delta^{15}N_{Root}$ and $\delta^{15}N_{AvailN}$ from constrained (1–2 cm thick) soil layers and used the average difference between soil and root $\delta^{15}N$ from all layers collected between 0 and 12 cm to calculate Tr. We assumed the $\delta^{15}N_{AvailN}$ was equivalent to bulk soil $\delta^{15}N$ (sieved to 2 mm) based on the similarity of concurrent measurements of $\delta^{15}N$ of NH_4^+ and bulk soil (Figure S2), and the assumption that the $\delta^{15}N$ of dissolved organic nitrogen, the potential intermediary between soil organic matter and NH_4^+, also resembled bulk soil $\delta^{15}N$. Additionally, due to the presence of fungal material on lower (1st–3rd) fine root orders, we used only fourth- and fifth-order fine root material to obtain $\delta^{15}N_{Root}$ data (see Ouimette and others 2013).

The calculated Tr was then used in conjunction with annual plant N uptake (N_{plant}), measurements of the carbon-to-nitrogen ratio of fungal tissue ($C{:}N_{fungi}$), and the basal area weighted fraction of ectomycorrhizal (ECM) tree species (f_{ECM}), to estimate annual C allocation to mycorrhizal fungi (C_{fungi}) (Eq. 5).

$$C_{fungi} = \left(\frac{1}{Tr} - 1\right) \times N_{plant} \times (C{:}N)_{fungi} \times f_{ECM} \quad (5)$$

Annual plant N uptake was calculated using N fluxes in foliage (the product of litterfall mass and litterfall %N), wood (the product of wood NPP and wood %N), and fine roots (the product of root NPP and root %N).

Direct Estimates of Fungal Biomass

We used the average standing biomass of mycorrhizal truffles (hypogeous sporocarps) during the growing season at BEF as an independent, tangible index of the investment in mycorrhizal fungi by plant hosts. We recognize that truffle biomass represents only a fraction of total fungal biomass and that standing biomass can be affected by rapid turnover or loss through fungivory, as well as overwintering of sporocarps (Castellano and Stephens 2017; Stephens and others 2017). However, given the similarity of truffle species across plots, the stability of truffle standing biomass across the growing season, and the up to 40 times difference in fungal biomass across plots, we considered differences in average growing season truffle biomass to be a valuable index for the carbon invested in mycorrhizal fungi.

Details of truffle sampling can be found in Stephens and others (2017). Briefly, truffles were collected at 12 sampling grids in 2014 at 16 plots (*truffle plots*). Each plot was composed of 4 subplots (4 m^2) that were sampled to a depth of 10 cm or until mineral soil was reached. Each of the 4 subplots was sampled in either June, July, August or September/early October for a total of 64 subplots and 256 m^2 in each grid. The average dry weight of truffles from all plots was used to convert truffle counts to truffle biomass.

Foliar, Root, and Soil $\delta^{15}N$

We measured foliar, root, and soil $\delta^{15}N$ to assess the influence of species composition and nutrient availability on the production of mycorrhizal fungi. Foliar, root, and soil $\delta^{15}N$ have been used as indicators of relative measures of ecosystem N limitation and the importance of mycorrhizal fungi to plant N acquisition (Amundson and others 2003; Craine and others 2009). Here we defined three species groups based on fungal and leaf type (ECM conifer, ECM deciduous broadleaf, and arbuscular mycorrhizal (AM) deciduous broadleaf). We compared foliar and root $\delta^{15}N$ across species groups, as well as to soil $\delta^{15}N$, to qualitatively assess the influence of species composition and stand N availability on the production of mycorrhizal fungi across our gradient of plots. We assumed that greater decreases in plant $\delta^{15}N$ of a species group relative to other species groups and soil was indicative of greater reliance on mycorrhizal fungi (for example, Hobbie and Colpaert 2003; Hobbie and Hobbie 2008; Craine and others 2009).

For this analysis, we made measurements of the $\delta^{15}N$ of soil (sieved to 2 mm) and roots from the three species groups from a subset of 6 *inventory plots*. Additionally, we used paired soil and root (not separated by species group) from *truffle plots*. All roots analyzed for $\delta^{15}N$ were fourth- and fifth-order roots free of mycorrhizal fungi and soil. Foliar $\delta^{15}N$ was also collected at the tower and inventory plots. Because foliar $\delta^{15}N$ varies strongly across species, samples of the same four species were collected at all plots. Specifically, leaves of five individuals each of *Picea rubens*, *Tsuga canadensis*, *Fagus grandifolia*, and *Acer rubrum* were collected at all 10 plots. At three plots, *Acer rubrum* was unavailable and was replaced with *Acer saccharum*.

All samples for $\delta^{15}N$ and %N analysis were dried at 60 °C, ground, and analyzed at the University of New Hampshire Stable Isotope Lab (www.isotope.unh.edu) on an Elementar Americas Pyrocube elemental analyzer coupled to a GeoVision isotope ratio mass spectrometer. The measurement uncertainty of the instrument as determined by repeated analyses of in-house QA/QC standards was less than $\pm$ 0.20‰ ($\pm 1\sigma$) for $\delta^{15}N$ (see SI for details).

Species Composition and Ecosystem N Availability

Species composition was quantified using fractional abundance of live growing season foliar biomass. The fractional abundance of foliar biomass of each species was quantified using a point quadrat approach described in Smith and Martin (2001). In addition, growing season canopy %N, annual net mineralization and nitrification rates, and soil C:N were previously measured at a subset of inventory plots (Ollinger and others 2002; Ollinger and Smith 2005). Because we lacked measurements of soil N cycling rates at all of our sampled plots, we used the fractional abundance of foliar biomass as a metric of both species composition and the availability of soil nutrients. Ollinger and others (2002) demonstrated a strong relationship between species composition, foliar %N, soil C/N, and soil N cycling rates at a range of plots at BEF and in the White Mountain National Forest region. In the present study, the conifer fraction of live foliar mass ranged from 0.01 to 0.98 across plots used in this study and was strongly correlated with foliar %N (see. Figures S3a and S3b).

RESULTS

NPP of Plant and Fungal Components

The production of foliage, wood, fine roots, and mycorrhizal fungi differed as a function of species composition at BEF. Annual NPP of foliage, wood (including coarse roots), and fine roots all decreased significantly with increasing conifer abundance (Figure 2A) and decreasing plot-level foliar %N (see Ollinger and Smith 2005 for patterns of wood and foliar NPP vs. foliar %N). The sum of foliar, wood, and fine root NPP, termed $\text{NPP}_{\text{plant}}$, decreased from over 600 g C m^{-2} y^{-1} at deciduous broadleaf-dominated stands to less than 300 g C m^{-2} y^{-1} at conifer-dominated stands (Figure 2B). Although $\text{NPP}_{\text{plant}}$ changed dramatically, the fraction of $\text{NPP}_{\text{plant}}$ allocated to foliage, wood, and fine roots (mean values of 0.19, 0.49, and 0.32, respectively) did not vary significantly across stands when production of mycorrhizal fungi was omitted. Additionally, the fraction of NPP in fine roots and wood were not significantly correlated with one another ($p = 0.43$), whereas the fraction of NPP in fine roots and foliage were negatively correlated with one another ($r^2 = 0.41$, $p < 0.05$).

In contrast to plant components, $\text{NPP}_{\text{fungi}}$ estimated from mass balance and isotopic approaches, as well as direct observations of truffle biomass, all increased with increasing conifer abundance (Figures 3, 4). Using the mass balance and isotopic approaches, estimates of $\text{NPP}_{\text{fungi}}$ increased from less than 25 g C m^{-2} y^{-1} to greater than 175 g C m^{-2} y^{-1} between nearly pure deciduous broadleaf and nearly pure conifer stands (Figure 3). Mean estimates of $\text{NPP}_{\text{fungi}}$ calculated from aver-

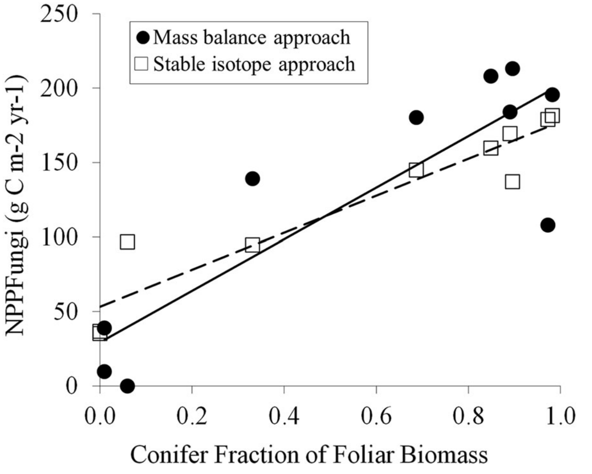

Figure 3. $\text{NPP}_{\text{fungi}}$ estimated from the mass balance (solid regression line; $\text{NPP}_{\text{fungi}} = 149.9x + 58.5$, $r^2 = 0.75$, $p < 0.01$) and isotopic approaches (dashed regression line; $\text{NPP}_{\text{fungi}} = 124.5x + 52.8$, $r^2 = 0.89$, $p < 0.01$) across stands with varying conifer dominance (where x = coniferous fraction of foliar biomass).

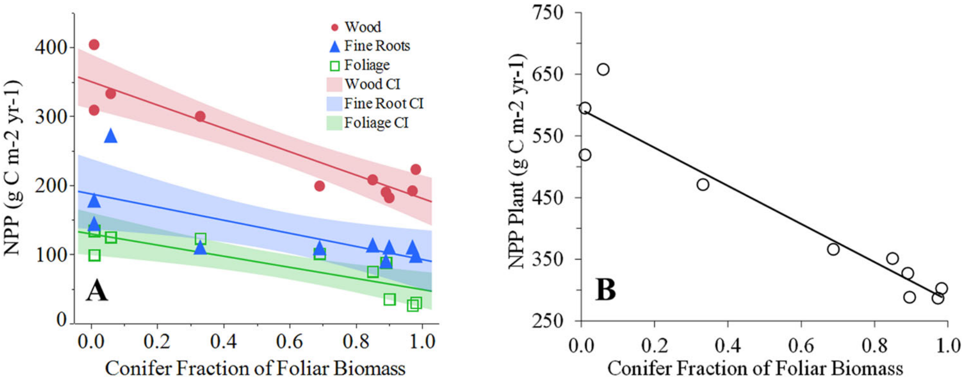

Figure 2. Annual net primary production of foliage, wood, and fine roots (**A**), and total plant production (**B**) across stands varying in the coniferous fraction of foliar biomass. Linear regressions—wood: $r^2 = 0.83$, $p < 0.0001$; fine roots: $r^2 = 0.53$, $p = 0.0174$; foliage: $r^2 = 0.47$, $p = 0.0014$; $\text{NPP}_{\text{plant}}$: $r^2 = 0.91$, $p < 0.0001$. Shaded areas in (**A**) represent 95% confidence intervals of the regressions for wood, fine roots, and foliage. Coarse root NPP is included with wood NPP here.

aging mass balance and isotopic approaches were negatively correlated with $\text{NPP}_{\text{plant}}$ ($r^2 = 0.81$, $p < 0.01$) and positively correlated with the proportion of conifer biomass ($r^2 = 0.91$, $p < 0.01$). Including $\text{NPP}_{\text{fungi}}$ reduced differences in estimates of total NPP across stands. $\text{NPP}_{\text{plant}}$ decreased by 52% across stands with increasing conifer dominance (Figure 2B); however, when including $\text{NPP}_{\text{fungi}}$ total NPP only decreased by 25% across these same plots (Figure 5A). Additionally, when including $\text{NPP}_{\text{fungi}}$ to estimate total NPP, the fraction of NPP allocated to foliage ($r^2 = 0.61$, $p < 0.01$) and wood ($r^2 = 0.65$, $p < 0.01$) decreased with increasing conifer dominance, while the fraction of NPP allocated to mycorrhizal fungi increased significantly ($r^2 = 0.92$, $p < 0.01$; Figure 5B).

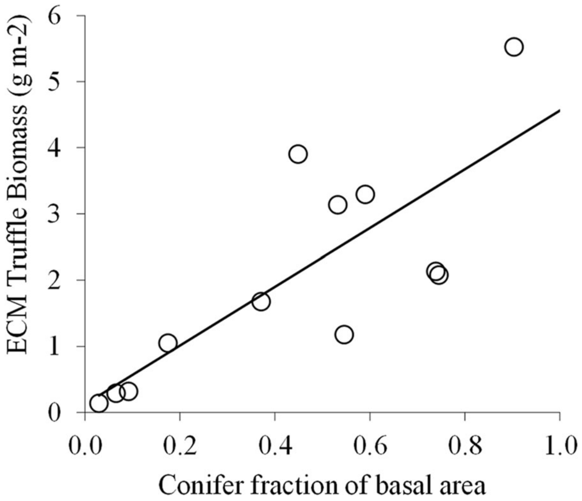

Figure 4. Biomass of ectomycorrhizal truffles across stands varying in conifer dominance (solid regression line; $r^2 = 0.62$, $p = 0.0025$).

In contrast to the production of mycorrhizal fungi, other metrics of belowground carbon allocation, including TBCF, soil respiration, and estimates of autotrophic respiration, were not correlated to stand-level tree species composition (Figures 6, S5, S6, S7). Although there was little change in TBCF with increasing conifer dominance, there was a large change in the fate of C allocated belowground. The change in the component fluxes of TBCF was primarily driven by a trade-off between fine root NPP and production of mycorrhizal fungi. Similar to whole plant patterns described above, decreased fine root production with increasing conifer dominance was largely offset by increased production of mycorrhizal fungi (Figure 6).

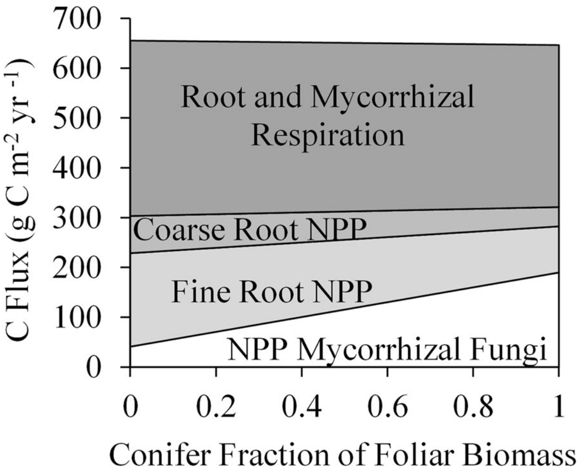

Figure 6. Annual C flux of the components of TBCF across stands with varying in conifer dominance constructed using regression lines from figures S5, S6, and S7. The sum of all components is equivalent to total TBCF.

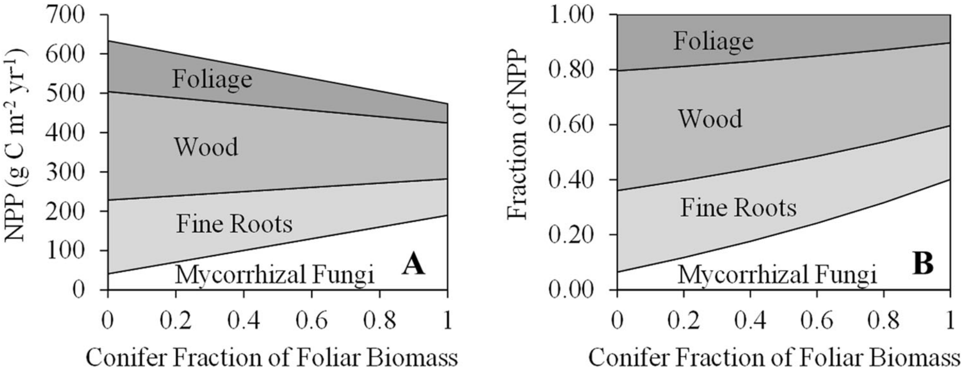

Figure 5. Annual NPP (**A**) and the fraction of total NPP (**B**) of plant and fungal components across stands with varying conifer dominance constructed using regression lines from Figures 2A and 3. Coarse root NPP is included with wood NPP here.

Direct Estimates of Mycorrhizal Biomass

Direct estimates of ECM fungal fruiting body biomass (quantitative ECM truffle surveys) indicated that ECM fungal biomass generally increased with conifer dominance. The number of ECM truffles increased from less than 1500 ha^{-1} at deciduous broadleaf-dominated stands to nearly 60,000 truffles ha^{-1} in conifer-dominated plots, equivalent to 0.14–5.5 g biomass m^{-2} (Figure 4). Fungal fruiting bodies are thought to represent 1–5% of total fungal biomass (Fogel and Hunt 1983), so total fungal production would be considerably greater.

Patterns of δ^{15}N and Associated Estimates of $\mathrm{NPP}_{\mathrm{fungi}}$

Mean stand-level, fine root and foliar δ^{15}N decreased across stands with increasing fractional abundance of coniferous species (Figure 7A). For foliage, changes in stand-level δ^{15}N primarily resulted from decreased δ^{15}N of conifer species across plots with increasing conifer abundance (Figure 7B), coupled with a larger fractional abundance of conifers. For fine roots, across stands that were nearly pure deciduous broadleaf to nearly pure conifer-dominated, fine root δ^{15}N decreased in ECM conifer by − 2.5‰ ($p < 0.01$) and in ECM deciduous broadleaf species by − 1.2‰ ($p < 0.03$), but changed minimally in AM deciduous broadleaf species (+ 0.7‰, $p = 0.11$). Further, fine root δ^{15}N did not vary by species group (for example AM deciduous broadleaf, ECM deciduous broadleaf, ECM conifer species) in deciduous broadleaf-dominated stands ($p > 0.46$ for all species group pairs from matched pairs analyses). In contrast, in conifer-dominated stands, δ^{15}N of ECM deciduous broadleaf and ECM conifer roots were both significantly lower than AM deciduous broadleaf roots, by 1.9‰ ($p < 0.01$) and 2.2‰ ($p < 0.01$), respectively.

DISCUSSION

Comparison with Other C Allocation Studies

At regional to continental scales, GPP and the NPP of plant components generally increase with increasing mean annual temperature (MAT) and mean annual precipitation (MAP) (Litton and others 2007; Luyssaert and others 2007). Similar to these trends in NPP with climate, the production of plant components at BEF (foliage, wood, and fine roots) all significantly increased with increasing N availability and decreasing conifer dominance. However, unlike studies at broader scales (Dybzinski and others 2011), the fraction of NPP allocated to wood versus fine roots were not negatively correlated to one another. Instead, $\mathrm{NPP}_{\mathrm{fungi}}$ and $\mathrm{NPP}_{\mathrm{plant}}$ were negatively and strongly correlated.

The large investments to mycorrhizal fungi at low N, conifer-dominated stands observed here lends support to the role of mycorrhizas in accounting for C flux discrepancies in broader scale datasets. For example, Chapin III and others (2009) noted that estimates of the production of mycorrhizal fungi were not available in the data compiled by Litton and Giardina (2008), where the fraction of TBCF allocated to root production increased from across stands with MAT ranging from − 5 to 30 °C. This led Chapin III and others (2009) to suggest that increases in the fraction of TBCF allocated to root production at colder compared to

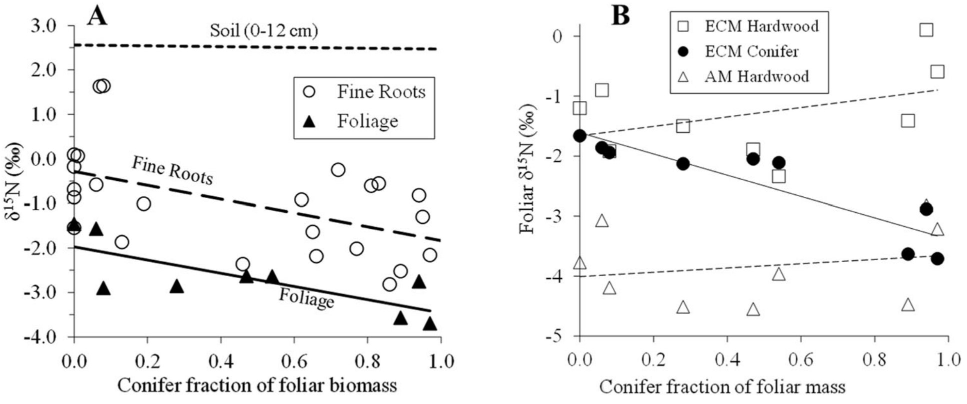

Figure 7. (**A**) Mean plot-level δ^{15}N of soil (dotted regression line; $r^2 = 0.00$, $p = 0.86$), fine roots (dashed regression line; $r^2 = 0.27$, $p = 0.0091$), and foliage (solid regression line; $r^2 = 0.58$, $p = 0.0166$), and (**B**) species-specific foliar δ^{15}N (solid regression line; $r^2 = 0.81$, $p = 0.0010$), across stands varying in conifer dominance.

warmer sites (26–53%) were largely offset by production of mycorrhizas. The increased production of mycorrhizal fungi across deciduous broadleaf to conifer-dominated stands at BEF, equivalent to 12–35% of TBCF, is consistent with increased plant C allocation to mycorrhizal fungi in more nutrient-limited ecosystems (Figures 3, 5, 6).

Additionally, Vicca and others (2012) determined that forests with high-nutrient availability used 16 ± 4% more of their photosynthate for biomass production than forests with low-nutrient availability. They hypothesized that this discrepancy was not due to differences in respiratory fluxes, but rather due to greater carbon allocation to mycorrhizal fungi in forests with low nutrient availability. At stands surrounding the eddy flux tower at BEF, Ouimette and others (2018) indicated that a relatively large estimate of mycorrhizal production (> 100 g C m^{-2} y^{-1}) was needed to close the carbon budget. Similarly, to close the C budget in a deciduous broadleaf-dominated watershed at nearby Hubbard Brook, New Hampshire, USA, Fahey and others (2005) estimated a rhizosphere flux to mycorrhizal fungi and root exudates of 80 g C m^{-2} y^{-1}.

These findings all suggest that the production of mycorrhizal fungi can represent a substantial proportion of forest NPP (up to 30%), especially in low N, conifer-dominated stands (Figures 3, 5), and are consistent with the findings of Gill and Finzi (2016) that the belowground C cost of N acquisition is higher in N-poor boreal systems than in N-rich forests.

Approaches to Estimate NPP$_{\mathrm{fungi}}$

Despite the challenges and uncertainties associated with estimating the production of mycorrhizal fungi, we found consistency in estimates of NPP$_{\mathrm{fungi}}$ between mass balance and isotopic approaches. The significantly larger estimates of NPP$_{\mathrm{fungi}}$ for conifer-dominated stands were also consistent with direct observations of greater mycorrhizal truffle biomass. Although our methods largely avoided sampling artifacts (McDowell and others 2001), the mass balance and isotopic approaches used here required assumptions that could affect our estimates of NPP$_{\mathrm{fungi}}$.

The simplified mass balance approach required several assumptions about root and fungal exudates and components of soil respiration. For instance, the mass balance approach ignored production of exudates from roots and mycorrhizal fungi. Although root and mycorrhizal exudates are important for many soil processes (Finzi and others 2015), annual rates of exudation from field studies are generally less than 25 g C m^{-2} y^{-1} for forest ecosystems (Phillips and others 2011; Yin and others 2014).

Mass balance estimates of NPP$_{\mathrm{fungi}}$ also required estimates of fine root production. It is difficult to assess the biases of using root ingrowth cores at BEF. The use of ingrowth cores can sometimes lead to lower estimates of root production compared to estimates from minirhizotron and sequential coring approaches (Addo-Danso and others 2016). Underestimates of root production would lead to overestimates of NPP$_{\mathrm{fungi}}$. To minimize potential biases associated with ingrowth cores, we allowed a four-week recovery period of the surrounding soil and roots prior to the initiation of ingrowth, as well as an extended (year-long) ingrowth time. Cores were also initiated at the end of the growing season and allowed to overwinter prior to the first growing season of ingrowth.

Several lines of evidence suggest that using ingrowth cores did not lead to large underestimates of fine root production. First, NPP$_{\mathrm{root}}$ estimates reported here using ingrowth cores in deciduous broadleaf-dominated stands (mean of 187 g C m^{-2} y^{-1}) are greater than those for deciduous broadleaf stands at the nearby Hubbard Brook Experimental Forest (90 g C m^{-2} y^{-1}) (Fahey and others 2005) using measurements of root biomass and root turnover from minirhizotrons. Our estimates of root production across all stands (90–272 g C m^{-2} y^{-1}) also resemble fine root production (< 1 mm) estimates reported by Park and others (2008) using minirhizotrons for northeastern U.S. conifer and deciduous broadleaf-dominated stands (42–179 g C m^{-2} y^{-1} assuming fine roots were composed of 49% C). Additionally, patterns in measured fine root production across sites are supported by data from four other years at BEF (with shorter ingrowth times). Root production was greater in the deciduous broadleaf stands than in the conifer stands in all years (Figure S4).

The mass balance approach also ignored the fraction of fungal biomass on fine roots that was tallied as fine root production. In ECM species, as much as 36% of first-order roots can be fungal biomass (Ouimette and others 2013). Since these finer order roots tend have the highest rates of production, up to 25% of measured fine root production can be production of mycorrhizal fungi (Ouimette and others 2013). If 25% of root production were fungal production, our assumptions would underestimate NPP$_{\mathrm{fungi}}$ by up to 25 g C m^{-2} y^{-1} across stands in our study. This uncertainty is opposite in sign, but likely similar in magnitude, to uncertainty associated with the omission of exudates in our mass balance approach.

Lastly, the mass balance approach required estimates of autotrophic respiration from roots and mycorrhizas (Rs_a), which was calculated using measured rates of soil respiration (Rs) and a relationship between R_{Sa} and R_S derived from the Global Soil Respiration Database (Bond-Lamberty and Thomson 2014). If the true ratio of Rsa/Rs was smaller than we assumed, then our estimates of Rs_a would underestimate NPP_{fungi} (there would be more ''missing'' TBCF), and vice versa. Although uncertainty in estimates of Rs_a would change the magnitude of NPP_{fungi}, this uncertainty is unlikely to alter the pattern of increasing NPP_{fungi} with increasing conifer dominance. Of the 197 studies in Bond-Lamberty and Thomson (2014) that fit our criteria, the relationship between R_{Sa} and R_S in plots dominated by evergreen versus deciduous species did not differ significantly ($p = 0.15$).

The isotopic approach we used to estimate NPP_{fungi} relied on knowing the $\delta^{15}N$ of plant tissues, the $\delta^{15}N$ of plant-available N in soil, and the fractionation against ^{15}N during transfer of N from mycorrhizal fungi to plant hosts (Δ_f), as well as plant N demand (Hobbie and Hobbie 2008). Of these, plant N demand is fairly well-constrained and has minimal impact on the estimates of NPP_{fungi}. The least well-constrained parameter is Δ_f and was derived from a single culture study using *Pinus sylvetris* seedlings and two strains of ECM fungi (Hobbie and Colpaert 2003). To our knowledge, no other studies exist that allow for calculation of Δ_f. Relatively small changes (for example $\pm 0.5‰$) in Δ_f lead to estimates in NPP_{fungi} that average 38% higher and 21% lower than those using a Δ_f of 5.7‰. Likewise, predictions of NPP_{fungi} are sensitive to relatively small changes (for example $\pm 0.5‰$) in mean plant and soil $\delta^{15}N$. Nevertheless, our estimates of NPP_{fungi} from N stable isotopes compare well with our mass balance approach (Figure 3B). Uncertainty in Δ_f would clearly affect the magnitude of our estimates of NPP_{fungi}, but barring any systematic differences in Δ_f among stands would not alter the pattern we observed in NPP_{fungi}, which was primarily driven by observed isotopic patterns of plant tissues and soils.

Allocation to Mycorrhizal Fungi Within and Across Species

We also used patterns in foliar, fine root, and soil $\delta^{15}N$ to qualitatively assess reliance on ectomycorrhizal fungi by different tree species groups. At broad scales, cold, ECM-dominated sites with low N cycling rates tend to have lower values of foliar $\delta^{15}N$ than warmer, N-rich, AM-dominated sites (Amundson and others 2003; Craine and others 2009). Our results generally agree with these broader scale patterns in $\delta^{15}N$. Plot-level foliar and root $\delta^{15}N$ decreased relative to soil $\delta^{15}N$ across stands at BEF with increasing dominance of ECM conifers (Figure 7). One complication in using plant $\delta^{15}N$ to infer the degree of reliance on ECM fungi is that in temperate forests, contrary to expectations based on mycorrhizal type, foliar $\delta^{15}N$ is sometimes lower in AM (especially *Acer* spp.) than ECM species (Pardo and others 2006). This was the case for foliage but not fine roots in the deciduous broadleaf-dominated stands at BEF. In contrast, we found fine root $\delta^{15}N$ to be significantly lower in ECM than in AM species in conifer-dominated stands.

Interspecific patterns in $\delta^{15}N$ are complicated by differences in the form (and $\delta^{15}N$) of N assimilated (Averill and Finzi 2011), rooting depth and the depth of N acquisition (Hobbie and others 2014), inclusion of fungal tissue during isotopic analysis of roots (Ouimette and others 2013), and intra-plant fractionation (Evans 2001). Comparing plant $\delta^{15}N$ to soil and co-occurring species should normalize many of these confounding factors. In this approach, the significant changes in foliar and fine root $\delta^{15}N$ of the two conifer species sampled at BEF relative to both soil and broadleaf deciduous species within the same stands suggest that the reliance on mycorrhizal fungi (and reciprocal transfer of C to fungi) increased with decreasing N availability primarily in conifer species. The decrease in conifer $\delta^{15}N$ across stands suggests that reliance on ECM fungi is mediated by both the species present (for example, conifer species), as well as edaphic conditions (for example N availability) which varied concurrently with species composition (Figures S3A and S3B).

Recent work has begun to include characteristics of mycorrhizal fungi in trait-based plant frameworks (Chagnon and others 2013; Aguilar-Trigueros and others 2014; Fry and others 2019). In this vein, Powell and others (2009) observed that the extent of root and soil colonization by mycorrhizal fungi may be a conservative trait that developed during evolution of mycorrhizas. At BEF, more C was allocated to mycorrhizal fungi in conifer-dominated stands, suggesting that conifers may allocate more C toward ECM fungal symbionts. Fine roots of coniferous species tend to have lower specific root length than angiosperm species (Reich and others 1998; Comas and Eissenstat

2009; McCormack and others 2012), which suggests a lower potential for soil exploration by conifer roots (Ostonen and others 2007). Instead, the short and thick fine roots of slow growing conifers may have relatively long lifespans (Eissenstat and Yanai 1997; McCormack and others 2012) and may be adapted to serve as centers for mycorrhizal colonization. In a study of 96 woody species from subtropical China, mycorrhizal colonization was strongly and positively correlated to root diameter in both AM and ECM species (Kong and others 2014). Nevertheless, δ^{15}N patterns at BEF suggest that even within species (for example, conifers), C allocation to mycorrhizal fungi increased with decreasing N availability. Intraspecific variation in the degree of C allocation to mycorrhizas would not be consistent with fixed rates of soil and root fungal colonization within host species. Instead, patterns in δ^{15}N at BEF suggest that reliance on ECM fungi is mediated by both the species present (for example, conifer species) and edaphic conditions (for example, N availability).

CONCLUSION

We measured the production of foliage, wood, fine roots, and mycorrhizal fungi across temperate forest stands spanning a range of tree species composition and N availability. As the proportion of conifer species increased across plots, the production of plant components significantly decreased, while the production of mycorrhizal fungi more than doubled. The contrasting patterns in the production of plant and fungal components highlight the importance of including mycorrhizal fungi in ecosystem C budgets and may account for prior discrepancies in forest C flux patterns in broader scale datasets. Although quantifying the production of mycorrhizal fungi is inherently difficult, we found internally consistent estimates of $\mathrm{NPP}_{\mathrm{fungi}}$ in mass balance and isotopic approaches. The significantly larger estimates of $\mathrm{NPP}_{\mathrm{fungi}}$ found at conifer-dominated stands were also consistent with direct observations of greater mycorrhizal truffle biomass at conifer-dominated stands. Isotopic data indicated that both tree species (for example, conifers) and resource availability influenced production of mycorrhizal fungi.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest

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