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RESEARCH ARTICLE

Effects of methodological difference on fine root production, mortality and decomposition estimates differ between functional types in a planted loblolly pine forest

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Abstract

Background and aims Fine roots can be functionally classified into an absorptive fine root pool (AFR) and a transport fine root pool (TFR). Different methods give significantly different fine root production, mortality and decomposition estimates. However, how methodological difference affects fine root estimates has not been assessed by functional type, impeding accurate construction of fine root C budgets.

Methods We used dynamic-flow model, a model based on measurements of litterbags and soil cores, and balanced-hybrid model, a model based on measurements of minirhizotrons and soil cores, to quantify AFT and TFR estimates in a planted loblolly pine forest.

Results Annual production, mortality, and decomposition were comparable between AFRs and TFRs when measured using the dynamic-flow model ($P > 0.1$) but significantly higher for AFRs than for TFRs when measured using the balanced-hybrid model ($P < 0.05$). Annual production, mortality and decomposition estimates using the balanced-hybrid model were 75%, 71% and 69% higher than those using the dynamic-flow model, respectively, for AFRs, but 12%, 6% and 5% higher than those using the dynamic-flow model, respectively, for TFRs. The balanced-hybrid model yielded more reliable AFR and TFR estimates than the dynamic-flow model by directly measuring fine root production and mortality dynamics.

Conclusion The balanced-hybrid model has greater estimation accuracy than the dynamics-flow model. The methodological difference has greater effects on AFR than TFR estimates. The choice of method is critical for quantifying AFR and TFR contributions to fine root C budget.

Keywords Fine root · Production · Mortality · Decomposition · Method · Loblolly pine

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Introduction

Fine roots are the most physiologically active component of the below-ground plant system (McCormack et al. 2015). At the ecosystem scale, fine root

production accounted for up to 63% of forest net primary production (Vogt 1991; Litton et al. 2007). Fine root mortality contributed to nearly half of organic carbon (C) input into the soil in some boreal forests (Ding et al. 2019), while fine root decomposition (i.e., amount of dead fine roots decomposed) represented around 10% of soil heterotrophic C emissions in a planted loblolly pine forest (Li et al. 2020a). Accurate measurements of fine root production, mortality and decomposition in forests are critical for quantifying forest C allocation and cycling and parameterizing climate change models (Woodward and Osborne 2000; Ghimire et al. 2016).

In most root production and mortality studies, fine roots are simply defined as distal roots with diameters < 2 mm (Hendricks et al. 2006; Osawa and Aizawa 2012; Li et al. 2013). Recent studies have shown that the hierarchical root system is morphologically, chemically and functionally heterogeneous and can be further partitioned into two pools: absorptive fine roots (AFRs) and transport fine roots (TFRs) (McCormack et al. 2015; Kou et al. 2018). AFRs represent the most distal roots and are involved primarily in the absorption of soil resources, whereas TFRs occur higher in the branching hierarchy and function mainly in resource transportation and storage. Compared with TFRs, AFRs have relatively higher nitrogen (N) concentrations and shorter lifespans (McCormack et al. 2015). Studying fine roots as two functional pools instead of a single diameter-based pool can provide a more accurate characterization of fine root processes and higher estimation accuracy of C allocation to the root system (Sun et al. 2012; Kou et al. 2018; Li et al. 2020b).

Ingrowth core and soil core methods, which are both low cost and ready-to-use, have been extensively applied to assess fine root production and mortality (Vogt et al. 1998; Brunner et al. 2013; Addo-Danso et al. 2016). In the ingrowth core method, the estimates are based on the amount of fine roots growing into pre-established root-free soil cores, while in the soil core method, the estimates are based on temporal changes in standing fine root biomass (live fine root mass) and necromass (dead fine root mass). However, both methods cannot capture fine root mortality and decomposition dynamics during sampling intervals, resulting in great uncertainties in the estimates (Osawa and Aizawa 2012). To better quantify fine root production, mortality and decomposition, several improved soil core models have been developed in which fine root biomass and necromass dynamics and mass loss rate (i.e., percentage of fine root mass loss per unit of time period) have been integrated into mass balance equations (Santantonio and Grace 1987; Osawa and Aizawa 2012). Dynamic-flow model is a new improved soil core model (Li and Lange 2015) that has the same model structure as those in Santantonio and Grace (1987) and Osawa and Aizawa (2012). Compared to the models in Santantonio and Grace (1987) and Osawa and Aizawa (2012), the dynamic-flow model should be theoretically more reliable by assuming a decreasing instead of constant fine root mass loss rate over time. The reason is that fine root mass loss rate has been found to decrease with time (Fan and Guo 2010; Lin et al. 2011). The deceleration of fine root mass loss rate is mainly due to a decrease in labile component concentrations and an increase in recalcitrant component concentrations in decomposing fine roots over time (Fan and Guo 2010; Lin et al. 2011).

In comparison to the soil coring methods, minirhizotrons provide a nondestructive means of studying roots in which clear tubes are inserted into the ground and miniature cameras are used to capture photographic images of roots growing on the tube surface (Hendrick and Pregitzer 1993). This technique allows the continuous monitoring of the growth and death of individual fine roots while overcoming the confounding effect of spatiotemporal variation (McCormack et al. 2014, 2015). Although combining minirhizotrons with soil cores enables the quantification of fine root production and mortality, it still fails to assess fine root decomposition, an important component in soil C fluxes (Hendricks et al. 2006; Addo-Danso et al. 2016). The balanced-hybrid model is an improved minirhizotron-based model which allows for quantifying not only fine root production and mortality but also fine root decomposition by integrating measurements of soil cores and minirhizotrons into mass balance equations (Li et al. 2020a). However, the application of the balanced-hybrid model has been limited to AFRs (Li et al. 2020a), while TFR production, mortality and decomposition, which could account for around 50% of total fine root estimates (Li et al. 2020b), have not been quantified using this method. TFRs have significantly lower N concentrations and are less sensitive to

environmental changes than AFRs (McCormack et al. 2015; Kou et al. 2018). Thus, assessing AFR and TFR estimates separately will help to better characterize fine root C and N cycling processes and their responses to environmental changes, including elevated CO_2, rising temperature and N deposition.

The dynamic-flow model, a method based on measurements of soil cores and litterbags, is inherently different from the balanced-hybrid model, a method based on measurements of soil cores and minirhizotrons, in that fine root mortality rate is derived from separate litterbag and soil core assays in the former, but can be directly measured using minirhizotrons in the latter (Li and Lange 2015; Li et al. 2020a). It has been recommended that multiple methods be used to yield more reliable fine root estimates (Hertel and Leuschner 2002; Hendricks et al. 2006; Addo-Danso et al. 2016). However, AFR and TFR production, mortality and decomposition have not been jointly quantified using both models, thus leading to great uncertainties in fine root C budgets of forest ecosystems.

Loblolly pine (*Pinus taeda* L.) is regarded as the most commercially important tree species for timber in the Southeastern USA (Wear and Greis 2012). It has been estimated that over 1 billion loblolly pine seedlings are planted annually (Wear and Greis 2012). Planted loblolly pine forests cover 11 million hectares in the USA, accounting for 50% of the standing pine volume in the South (Wear and Greis 2012). Since fine roots play a key role in regulating soil C cycling, an improved understanding of AFR and TFR dynamics in planted loblolly pine forests is critical for developing silvicultural and rotation strategies to increase soil C sequestration.

In this study, we used the soil cores, litterbags, and minirhizotrons to assess the biomass and necromass dynamics, mass loss patterns and length production and mortality rates of AFRs and TFRs in a planted loblolly pine forest, and construct two models with those parameters measured. The objectives were to (1) use both the dynamic-flow model and the balanced-hybrid model to quantify AFR and TFR production, mortality, and decomposition in this forest, (2) assess to what extent methodological difference affects AFR and TFR estimates, and (3) determine which method is more reliable.

Materials and methods

Study site

The study was conducted in a commercially managed loblolly pine (*P. taeda* L.) forest (35°48'N 76°40'W) located in the lower coastal plain of Washington County, North Carolina, USA. Mean annual precipitation and temperature for the period 2011–2017 were 1320 mm and 12.2 °C, respectively. The topography of the area is flat (< 5 m above sea level) and on a Belhaven series histosol (loamy mixed dysic thermic terric Haplosaprist). The study area was harvested of trees and ditched/drained in the late 19th to the early 20th century and farmed for about a decade before being converted to a commercial pine plantation forest. The usual rotation period is around 30 years. The forest was fertilized with N and phosphorus at the time of planting and at mid-rotation. The soil C and N concentrations at 20 cm depth were 26% and 1.0%, respectively. Loblolly pine accounts for over 90% of the total biomass, with *Acer rubrum* and *Quercus velutina* representing the remainder. The mean canopy height, diameter at breast height, and stand age during the study period were approximately 24 m, 33 cm, and 23 years, respectively. For a full site description, refer to Noormets et al. (2010). Three plots, 100 to 800 m apart, were established at random in the planted forest in 2013. Each plot measured around 6 m ×9 m, in which only loblolly pine fine roots were studied.

Fine root biomass and necromass measurements

Fine root biomass and necromass were measured using the soil coring method. The number of soil cores required at both plot and stand-level was calculated using the methods in Bartlett et al. (2001) and Dornbush et al. (2002). In each plot, 8 cylindrical soil cores (3.0 cm diameter, 30 cm depth), randomly distributed in space, were collected on each sampling occasion. There were 6 sampling occasions during the period of April 2016 to April 2017, forming 5 soil sampling intervals (Li et al. 2020a) (Table 1). Previous studies have showed that over 90% of fine roots were distributed in the 0–30 cm soil layer in this forest (Noormets et al. 2010; Li et al. 2020a). Collected soil cores were rinsed with clean tap water through a 0.5 mm mesh sieve to extract roots. Loblolly pine fine

Table 1 Minirhizotron-image capturing and soil core sampling dates from 2016 to 2017

Dates	Minirhizotrons	Soil core
25-Apr	†	†
10-May	†	
28-May	†	
30-Jun	†	
12-Jul	†	
28-Jul	†	†
12-Aug	†	
25-Aug	†	
10-Sep	†	
29-Sep	†	†
18-Oct	†	
25-Nov	†	†
5-Dec	†	
25-Jan	†	†
15-Feb	†	
16-Mar	†	
26-Apr	†	†

roots, which accounted for over 95% of total fine root mass in the 0–30 cm soil layer, were sorted out based on morphology. Loblolly pine fine roots with light color and intact stele and periderm were regarded as live roots, while those with dark color and damaged stele and periderm were considered dead. In this study, AFRs represented the first and second-order roots, while TFRs were third-order roots and higher with diameter < 2 mm (Pregitzer et al. 2002; McCormack et al. 2015). The first order roots are the most distal, unbranched roots. The second order roots begin at the junctions of two first order roots, and so on. Live and dead AFRs and TFRs were separated according to the procedures described in Li et al. (2020b). All fine roots were dried at 50 °C to a constant weight and weighed. The measurements of biomass and necromass in the soil cores were scaled to g m^{-2} over a 0–30 cm soil layer.

Litterbag measurements

AFR and TFR mass loss rates were assessed using litterbags. To provide input parameters for the dynamic-flow model, we used four types of fine roots, including live and dead AFRs and TFRs as the decomposing substrates for in situ decomposition experiments. The decomposing substrates were from the fine roots in soil cores collected in July 2016 as they had already been sorted out by functional types and vitality. Each litterbag (20 cm × 3.5 cm, 0.05 mm mesh) was evenly filled with about 0.15 g decomposing substrates and inserted vertically into a 0–20 cm soil layer. This experimental design was intended to have the decomposing substrates distributed evenly in different soil layers. There were 120 litterbags in total, with 30 litterbags per fine root type. The decomposition experiment began on 8 August 2016. The litterbags were collected after 65, 105 and 310 days of incubation. On each sampling occasion, three litterbags of each of the four root types were retrieved from each plot. Roots from the litterbags were rinsed with clean tap water, carefully sorted by type, dried at 50 °C to a constant weight and weighed.

Minirhizotron measurements

A total of 18 acrylic tubes (80 cm long, 6 cm outer diameter) were installed in 2013 at a 45° angle to a vertical soil depth of 50 cm in the three plots (5 to 8 tubes per plot). Root scanning began one year after tube installation to allow the soil around the tubes to stabilize. Only root images taken between late April 2016 to late April 2017 were used as these images co-occurred with soil coring (Li et al. 2020a) (Table 1). There were 17 image-capturing occasions during the study period. Images were collected using a Bartz digital camera with the image capture software BTC I-CAP (Bartz Technology Corp., Carpinteria, CA, USA). Fine root length and diameter were quantified by analyzing the images with WinRHIZO software (Regents Instruments Inc., Quebec, Canada). AFR and TFR length production, mortality and standing length density (mean root length per unit root image area) were calculated based on the image analysis. AFRs and TFRs included both mycorrhizal and non-mycorrhizal fine roots. An AFR or TFR was counted as dead if its diameter shriveled to half the original diameter, it showed signs of deterioration including fragmenting and ectomycorrhizal fungal mantle detachment, or it was consumed by soil animals; otherwise, roots were considered as living (McCormack et al. 2014; Kou et al. 2018).

The dynamic-flow model

AFR and TFR production, mortality and decomposition were determined using the dynamic-flow model based on the measurements of soil cores and litterbags (Li and Lange 2015; Li et al. 2020b). Interval i was any given soil coring interval ($1 \le i$) (year). G_{I-i} and G_{II-i} were the fine roots that died before the start of interval i and in interval i, respectively. The temporal changes in mass remainings of G_{I-i} and G_{II-i} were assessed by the litterbag method with dead and live roots used as decomposing substrates, respectively. The measured data were then fitted to an exponential equation with only two parameters to simulate G_{I-i} and G_{II-i} mass loss patterns:

$$y(t) = y_0 e^{(-\lambda/k)(1-e^{-k\ t})} \tag{1}$$

where $y_{(t)}$ and y_0 are root mass at time t (year) and the start, respectively. The two parameters λ (year^{-1}) and k (year^{-1}) were calculated based on the fine root mass remaining in litterbags collected on all sampling occasions using nonlinear regression. $e^{-k\,t}$ is fine root decomposition rate which is time-dependent. It is the highest at the beginning and decreases over time.

The fine root mortality rate in interval i is assumed to be constant. This is different from the balance-hybrid model in which fine root length mortality rate is directly assessed using minirhizotrons. The total production (g_i), mortality (m_i) and decomposition (d_i) in interval i are calculated by the following equations:

$$g_i = B_i - B_i(0) + m_i \tag{2}$$

$$d_i = m_i - (N_i - N_i(0)) \tag{3}$$

$$N_{I\text{-}i} = N_i\ (0)\ e^{(-\lambda_{I-i}/k_{I-i})(1-e^{-k_{I-i}\ T})} \tag{4}$$

$$N_{II\text{-}i} = N_i - N_{I\text{-}i} \tag{5}$$

$$m_i = \mu_i\, T \tag{6}$$

where $B_i(0)$ and B_i represent the fine root biomass in soil cores sampled at the start and the end of interval i, $N_i(0)$ and N_i represent the fine root necromass at the start and the end of interval i, and N_{II-i} and N_{I-i} are the mass remaining of G_{II-i} and G_{I-i} at end of interval i, T is time length of interval i, and μ_i is fine root decomposition rate in interval i.

Further, μ_i is calculated as.

$$\mu_i = k_{II-i} N_{II-i} \frac{e^{-(\lambda_{II-i}/k_{II-i})e^{-k_{II-i}T}}}{E_1((\lambda_{II-i}/k_{II-i})e^{-k_{II-i}T}) - E_1(\lambda_{II-i}/k_{II-i})} \tag{7}$$

where $E_1(z) = \int_z^\infty \frac{e^{-x}}{x} dx$ is an exponential integral function (Abramowitz and Stegun 1964, ch. 6).

$B_i(0)$, B_i, N_i, N_i (0), N_{II-i}, and N_{I-i} have the unit g·m^{-2} 0–30 cm soil layer^{-1}. g_i, m_i and d_i have the unit g·m^{-2} 0–30 cm soil layer^{-1}. λ_{I-i}, k_{I-i}, λ_{II-i}, and k_{II-i} are decomposition parameters for G_{I-i} and.

G_{II-i}, respectively, which can be calculated using Eq. 1.

The balanced-hybrid model

Fine root production, mortality and decomposition were estimated based on measurements of minirhizotrons and soil cores. Fine root length production (LP_i, m m^{-2} image) and mortality (LM_i, m m^{-2} image) in a given soil coring interval i are estimated from minirhizotron image analysis. LP_i and LM_i are calculated as the length of fine roots that are produced and die in interval i, respectively (Kou et al. 2018).

Fine root length production (TR_i) and mortality rates (DR_i) in the interval are calculated as.

$$TR_i = LP_i/SL_i \tag{8}$$

$$DR_i = LM_i/SL_i \tag{9}$$

where SL_i is the mean standing live fine root length of minirhizotron images captured at the start of interval i (m m^{-2} image).

g_i and m_i are assessed by combining measurements of minirhizotrons and soil cores (Hendricks et al. 2006; Li et al. 2020a):

$$g_i = B_i(0) \times TR_i \tag{10}$$

$$m_i = B_i(0) \times DR_i \tag{11}$$

where $B_i(0)$ is fine root biomass at the start of interval i.

Referencing Eq. 3, d_i can be calculated (Li et al. 2020a).

Model test

The efficacy of the models for estimating the production, mortality, and decomposition was tested by comparing the predicted with the measured AFR and TFR biomass values in July, September, and November 2016 and January 2017. In the dynamic-flow model testing, the two adjacent soil coring intervals were combined into one and fine root production and mortality rates in the new interval were assumed to be constant. Fine root biomass and necromass values at the start and the end of the new interval and fine root mass loss patterns were employed to calculate fine root production and mortality in the new interval. The predicted fine root biomass value in a time point within the new interval ($B_{predicted}$) is:

$$B_{predicted} = B_{start} + t_p \times (g_{new}/t_{new} - m_{new}/t_{new}) \quad (12)$$

where B_{start} is fine root biomass at the start of the new interval, t_p and t_{new} are time length from the start of the new interval to the selected time point and time length of the new interval, respectively, and g_{new} and m_{new} are fine root production and mortality in the new interval, respectively.

The balance-hybrid model testing was the same as that in Li et al. (2020a). The predicted AFR and TFR biomass values in July, September, and November 2016 and January 2017 were calculated according to the procedures described in Hendrick and Pregitzer (1993) and Li e al. (2020a). The estimation accuracy was evaluated using the absolute difference between the predicted and the measured biomass values divided by the measured biomass values.

Statistical analysis

The plots were considered as replicates (n = 3), and data collected within the same plot were averaged before performing statistical analysis. One-way ANOVA was used to assess the difference in means of measured fine root mass loss rates. Post hoc testing of means was conducted using Tukey's HSD. Within each model, paired-t test was performed to evaluate the differences in the production, mortality and decomposition between AFRs and TFRs. The data were log-transformed to normalize variances before analysis when necessary. All data were analyzed using the SPSS statistical software (version 17.0; IBM Corporation, Somers, NY 10,589, USA).

Results

Biomass and necromass

AFR and TFR biomass showed the same temporal patterns, with the highest values in July and the lowest values in January, while AFR and TFR necromass did not show evident peak and trough values during the study period (Fig. 1). AFRs had significantly lower mean biomass than TFRs (67.8 ± 5.3 vs. 88.7 ± 2.9 g m^{-2}; mean ± SE) ($P < 0.05$). The mean necromass of AFRs was lower than that of TFRs (41.2 ± 2.8 vs. 50.4 ± 5.2 g m^{-2}; mean ± SE), but the difference was not significant ($P > 0.05$).

Mass loss rate

Live AFR substrates had significantly higher percent mass remaining than live TFR substrates at the end of the experiment ($P < 0.05$), but dead AFR and TFR substrates had comparable percent mass remaining during the study period ($P > 0.1$; Fig. 2). All live root substrates decomposed significantly faster than dead root substrates ($P < 0.05$; Fig. 2).

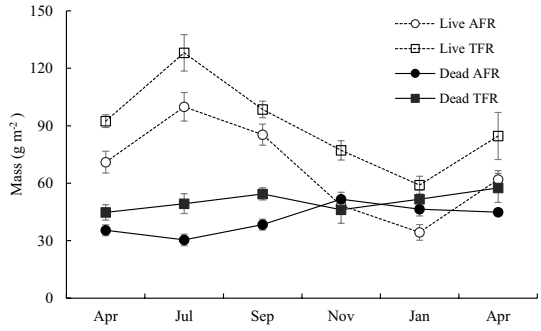

Fig. 1 Absorptive (AFR) and transport (TFR) fine root biomass and necromass dynamics (g m^{-2} for the 0–30 cm soil depth; n = 3; mean ± SE). Note: AFR biomass and necromass have been reported in Li et al. (2020a). We use these values for the purpose of comparison

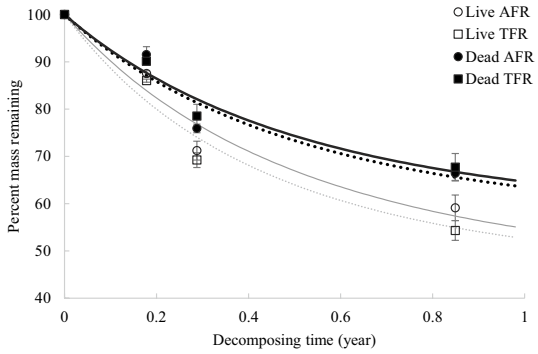

Fig. 2 Mass loss patterns of live and dead absorptive (AFR) and transport (TFR) fine root substrates measured using litterbags in a managed loblolly pine forest (n = 3; mean ± SE; different letters stand for significant difference in means, $P < 0.05$). The lines are the simulated values using Eq. 1. The dark line and dark dotted line represent dead AFRs and dead TFRs, respectively. The gray line and gray dotted line represent live AFRs and live TFRs, respectively

Temporal changes in fine root estimates

Temporal changes in fine root production, mortality and decomposition rates were generally the same between the two models, with greater production in February to July and greater mortality and decomposition occurring in October to November (Fig. 3). Production, mortality, and decomposition estimates using dynamic-flow model were comparable between AFRs and TFRs at all intervals. In contrast, production, mortality, and decomposition estimates using the balanced-hybrid model were significantly higher for AFRs than for TFRs in most intervals.

Annual fine root estimates

Annual production, mortality, and decomposition were not significantly different between AFRs and TFRs when estimated using the dynamic-flow model, but were significantly higher for AFRs than for TFRs when estimated using the balanced-hybrid model (Fig. 4). Annual AFR production, mortality, and decomposition estimates using the balanced-hybrid model were 75%, 71%, and 69% higher than those using the dynamic-flow model, respectively (Fig. 4). By contrast, annual TFR production, mortality, and decomposition estimates using the balanced-hybrid model were 12%, 6%, and 5% higher

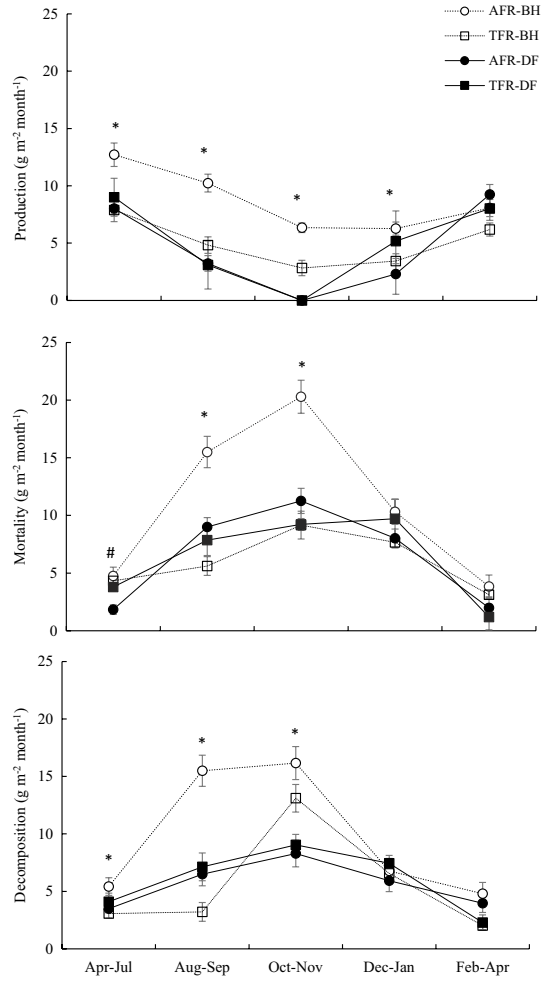

Fig. 3 Temporal changes in production, mortality and decomposition estimates of absorptive (AFR) and transport (TFR) fine roots using balanced-hybrid model (BH) and dynamic-flow model (DF) in a planted loblolly pine forest (n = 3; mean ± SE). * stands for significant difference between AFR and TFR estimates using BH, while # stands for significant difference between AFR and TFR estimates using DF ($P < 0.05$). Note: AFR production, mortality and decomposition estimates using balanced-hybrid model have been reported in Li et al. (2020a). We use these values for the purpose of comparison

than those using the dynamic-flow model, respectively (Fig. 4). Annual fine root (i.e. AFR + TFR) production, mortality, and decomposition were 119 ± 9, 133 ± 7, and 124 ± 11 g m^{-2} (mean ± SE), respectively, when measured using the dynamic-flow model and 172 ± 11, 185 ± 12, and 171 ± 14 g m^{-2} (mean ± SE), respectively, when measured using the balanced-hybrid model.

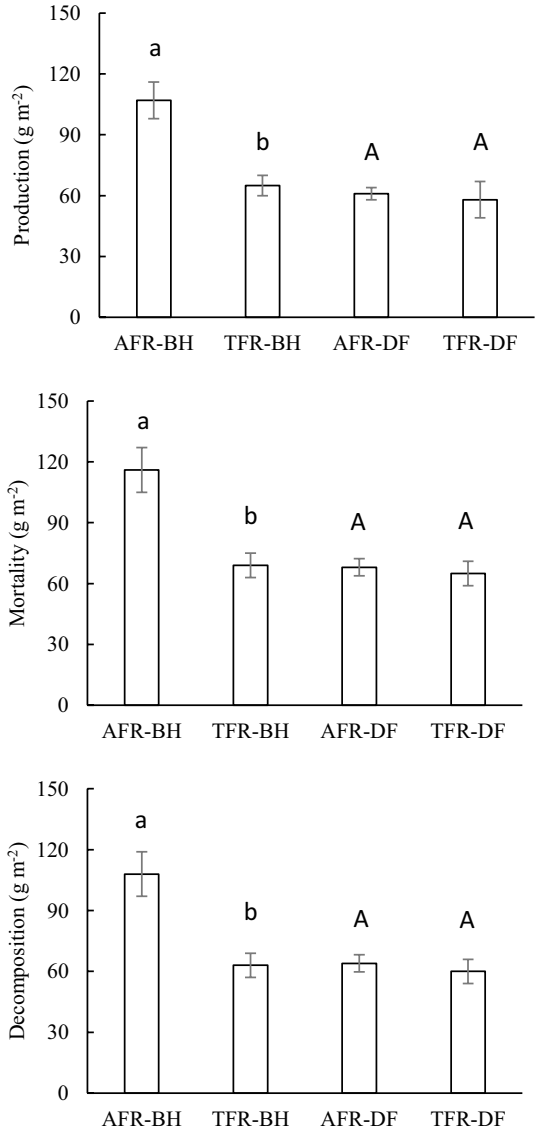

Fig. 4 Annual absorptive (AFR) and transport (TFR) fine root production, mortality and decomposition measured using balanced-hybrid model (BH) and dynamic-flow model (DF) in a planted loblolly pine forest (n = 3; mean ± SE). Different lowercase letters stand for significant difference between AFR and TFR estimates using BH, while different uppercase letter represents significant difference between AFR and TFR estimates using DF ($P < 0.05$)

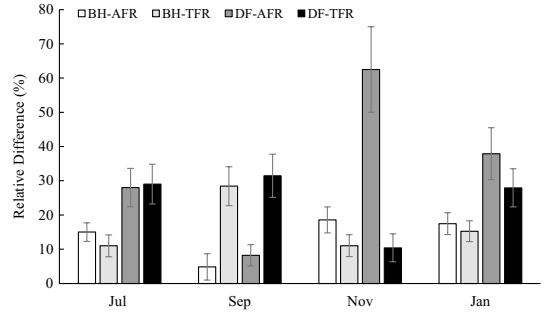

Fig. 5 Percent difference between the measured fine root biomass using soil cores and the predicted fine root biomass using both balanced-hybrid model (BH) and dynamic-flow model (DF) in July, September and November 2016 and January 2017. AFRs and TFRs stands for absorptive and transport fine roots, respectively (n = 3, ± SE).

Model test

The percent difference between measured and predicted fine root biomass ranged from 4 to 62% and differed greatly between models and between functional types (Fig. 5). On average, the measured AFR biomass was 34% and 14% higher than that estimated by the dynamic-flow model and the balanced-hybrid model, respectively, while the measured TFR biomass was 25% and 16% higher than that estimated by the dynamic-flow model and the balanced-hybrid model, respectively, indicating that the balanced-hybrid model is more reliable than the dynamic-flow model.

Discussion

Functional classifications are increasingly being incorporated in the context of fine root dynamics in forests. However, most of the existing studies are based on two-dimensional minirhizotron analysis (McCormack et al. 2015; Kou et al. 2018) and do not include separate measurements of AFR and TFR biomass and necromass dynamics due to great labor and time input (Li et al. 2020b). Failing to assess the biomass and necromass dynamics impedes us from characterizing soil C flux dynamics through AFR and TFR production, mortality and decomposition. The functional-based fine root studies are particularly important to climate change research because AFRs and TFRs are chemically and functionally different and have different responses to environmental changes (Kou et al. 2018). Ignoring these differences between AFRs and TFRs could substantially undermine the predictive capacity of climate change models. In this planted loblolly pine forest, AFRs

had significantly lower biomass than TFRs but made comparable or even significantly greater contributions to total fine root production, mortality and decomposition than TFRs. These results demonstrate that three-dimensional, function-based studies are essential to quantify fine root C budget and understand fine root dynamics, while two-dimensional minirhizotron analysis cannot reflect the differential contributions of AFRs and TFRs to total fine root production, mortality and decomposition.

Different methods have been found to yield divergent fine root estimates, but all these methodological comparisons are diameter-based rather than function-based (Hertel and Leuschner 2002; Hendricks et al. 2006; Osawa and Aizawa 2012; Li and Lange 2015). This knowledge gap has prevented accurate identification of the strengths and weaknesses of each method and characterization of the C allocation patterns within the root system. Our study for the first time uses two types of models, a litterbag-based model and a minirhizotron-based model, to assess AFR and TFR production, mortality, and decomposition. AFR estimates were significantly more responsive to methodological difference than TFR estimates, indicating that choice of method matters for assessing AFR and TFR contributions to fine root C fluxes. The smaller AFR and TFR estimates of the dynamic-flow model compared to the balanced-hybrid model can be ascribed to the underestimated AFR and TFR mass loss rates by litterbags. In existing litterbag-based models, including the dynamics-flow model (Osawa and Aizawa 2012; Li and Lange 2015), mortality is positively related to the production and decomposition and fine root mass loss rate is the dominant determinant in mortality estimation. Higher fine root mass loss rate results in greater mortality estimate and therefore greater production and decomposition estimates. Since both models used the same biomass and necromass data, lower mass loss rates were the only cause for the smaller estimates in the dynamic-flow model estimation.

The balanced-hybrid model has greater estimation accuracy than the dynamic-flow model as indicated by the smaller percent differences between measured and predicted biomass values. The two models are inherently different. In the balanced-hybrid model, the relative production and mortality rates at the tube-soil interface are assumed to be representative of those in bulk soil. This assumption has been shown to be very likely in previous studies (Hendrick and Pregitzer 1993; Hendricks et al. 2006; Li et al. 2020a). By contrast, in the dynamic-flow model, the estimation is based on the assumptions that fine root mortality rates remain constant in a certain interval and fine root mass loss patterns in litterbags are the same as those in bulk soil. Both of these assumptions are unrealistic. The mortality rate, particularly AFR mortality rate, has been found to vary greatly among seasons (McCormack et al. 2014; Kou et al. 2018). Further, the decomposer community composition in litterbags is different from those in natural soil (Li et al. 2015; Beidler and Pritchard 2017). Moreover, in the litterbag method, unrepresentative roots are used as the decomposing substrates (Kunkle et al. 2009; Fan and Guo 2010; Sun et al. 2018) and the existence of the litterbags disrupts the interactions between roots, soil fauna and soil microbes (Koide et al. 2011; Li et al. 2015; Beidler and Pritchard 2017; Moore et al. 2020), which substantially reduce the accuracy of the measurements. As a result, there would be greater errors in fine root estimates in the dynamic-flow model than in the balanced-hybrid model. This claim is further supported by the negative production estimates using the dynamic-flow model during October to November 2016 (Fig. 3).

The balanced-hybrid model can continuously track the growth and death of individual AFRs and TFRs while maintaining the rhizosphere associations (McCormack et al. 2015; Beidler and Pritchard 2017), which makes it effective in comparing fine root estimates between functional types. By contrast, the capacity of the dynamic-flow model in distinguishing AFR and TFR estimates has been severely undermined by the disturbances in rhizosphere and a confounding effect of spatiotemporal variation (i.e., the effect of variances in soil environmental conditions on the mass loss rate could cover the inherent difference in decomposability between AFRs and TFRs as AFR and TFR litterbags are placed at different locations in forest soils) (Koide et al. 2011; Sun et al. 2018). Thus, the higher estimates for AFRs than for TFRs in the balanced-hybrid estimation generally reflects the real situation, while the comparable estimates between AFRs and TFRs in the dynamic-flow model estimation could be most likely an error of the model.

Conclusion

AFR and TFR estimates and the estimation accuracy differ greatly between the two methods. The balanced-hybrid model is more reliable than the dynamic-flow model in quantifying AFR and TFR production, mortality, and decomposition by accurately monitoring the individual fine root length production and mortality dynamics while reducing the confounding effect of spatiotemporal variation in the soil environment. The inherent weaknesses of the litterbag method in assessing fine root mass loss rate and the unrealistic assumption on fine root mortality rate greatly undermine the estimation accuracy of the dynamic-flow model. AFR estimates are more sensitive to the model differences than TFR estimates. AFRs and TFRs have different functions and N concentrations in root system. Thus, choosing a reliable method and studying AFRs and TFRs separately are essential for accurately characterizing fine root dynamics and quantifying fine root contributions to forest C and N fluxes.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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