Tree Physiology 00, 1–8
doi:10.1093/treephys/tpaa074

Methods paper

An improved method for quantifying total fine root decomposition in plantation forests combining measurements of soil coring and minirhizotrons with a mass balance model

Xuefeng Li[1,3,4], Kevan J. Minick[1], Tonghua Li[1], James C. Williamson[1], Michael Gavazzi[2], Steven McNulty[2] and John S. King[1]

[1]Department of Forestry and Environmental Resources, North Carolina State University, 2820 Faucette Dr., Raleigh, NC 27695, USA; [2]USDA Forest Service, Eastern Forest Environmental Threat Assessment Center, 3041 E. Cornwallis Rd. RTP, NC 27709, USA; [3]Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang City, 110016, China; [4]Corresponding author (lxf.victor@gmail.com)

Received July 26, 2019; accepted May 26, 2020; handling Editor Sean Thomas

Accurate measurement of total fine root decomposition (the amount of dead fine roots decomposed per unit soil volume) is essential for constructing a soil carbon budget. However, the ingrowth/soil core-based models are dependent on the assumptions that fine roots in litterbags/intact cores have the same relative decomposition rate as those in intact soils and that fine root growth and death rates remain constant over time, while minirhizotrons cannot quantify the total fine root decomposition. To improve the accuracy of estimates for total fine root decomposition, we propose a new method (balanced hybrid) with two models that integrate measurements of soil coring and minirhizotrons into a mass balance model. Model input parameters were fine root biomass, necromass and turnover rate for Model 1, and fine root biomass, necromass and death rate for Model 2. We tested the balanced hybrid method in a loblolly pine plantation forest in coastal North Carolina, USA. The total decomposition rate of absorptive fine roots (ARs) (a combination of first- and second-order fine roots) using Models 1 and 2 was 107 ± 13 g m^{-2} $year^{-1}$ and 129 ± 12 g m^{-2} $year^{-1}$, respectively. Monthly total AR decomposition was highest from August to November, which corresponded with the highest monthly total ARs mortality. The ARs imaged by minirhizotrons well represent those growing in intact soils, evident by a significant and positive relationship between the standing biomass and the standing length. The total decomposition estimate in both models was sensitive to changes in fine root biomass, turnover rate and death rate but not to change in necromass. Compared with Model 2, Model 1 can avoid the technical difficulty of deciding dead time of individual fine roots but requires greater time and effort to accurately measure fine root biomass dynamics. The balanced hybrid method is an improved technique for measuring total fine root decomposition in plantation forests in which the estimates are based on empirical data from soil coring and minirhizotrons, moving beyond assumptions of traditional approaches.

Keywords: decomposition, fine root, minirhizotrons, mortality, plantation forests, production, soil coring.

Introduction

Fine roots, the most distal roots and traditionally defined as <2 mm in diameters, receive up to 60% of net primary production of forests (Vogt 1991, Litton et al. 2007). The decomposition of fine roots plays a key role in the soil carbon (C) cycle in forests (Hendricks et al. 2006, McCormack et al. 2015). However, most studies focus on assessing relative fine root decomposition rate (i.e., mass loss rate) and nutrient release rates (Sun et al. 2018, See et al. 2019), while total fine root decomposition (i.e., the amount of dead fine roots decomposed per unit soil volume) is essential for constructing soil C budgets and gaining a deeper mechanistic understanding of C cycling processes has been poorly quantified.

Sequential soil coring is the most reliable approach for measuring fine root standing biomass and necromass, but does not account for the simultaneous processes of root growth, death and decomposition (Vogt et al. 1998, Majdi et al. 2005, Li and Lange 2015). Minirhizotrons allow continuous observation of the growth and death of individual fine roots while minimizing soil disturbance and spatio-temporal variation (Vogt et al. 1998, Majdi et al. 2005, Hendricks et al. 2006). However, the measurements reflect fine root dynamics per minirhizotron image but not per unit soil volume. Moreover, the relative fine root decomposition rate cannot be reliably assessed by analyzing root images captured by minirhizotrons, due to fine roots becoming fragmented (Goebel et al. 2011, personal observations), changes in fine root tissue density over time once they die (Comas et al. 2000), disturbances at the soil/tube interface and difficulty of observing dead roots as a result of ingrowth of new roots and mycorrhizal hyphae (Kume et al. 2018).

Recently, models combining fine root biomass and necromass with relative fine root decomposition rates have been developed to quantify total fine root decomposition (Osawa and Aizawa 2012, Li et al. 2013, Li and Lange 2015). Assumptions of these models are that fine root growth and death rates remain constant at each sampling interval and relative fine root decomposition rates in litterbags are the same as those in intact soils. These assumptions could result in large biases in estimates. First, fine root growth and death rates have evident temporal variability rather than remaining constant in forest soils (King et al. 2002, Fukuzawa et al. 2013, McCormack et al. 2014, Kou et al. 2018). Second, litterbags or intact cores may not accurately reflect relative fine root decomposition rates in intact soils because the existence of litterbags greatly alters microbial decomposer community composition (Li et al. 2015) and excludes effects of some soil invertebrate animals (Bokhorst and Wardle 2013). Finally, the intact cores that contain both live and dead roots reflect the relative decomposition rates of newly severed live roots and dead roots, but not fine roots that senesce naturally (Dornbush et al. 2002).

To improve the estimation of total fine root decomposition in forests, we developed a new method (balanced hybrid) with two models. In this approach, fine root turnover rate and death rate in intact soils were estimated using minirhizotrons, while fine root standing biomass and necromass were determined by sequential soil coring. Total fine root decomposition was calculated by integrating fine root turnover rate, death rate, biomass and necromass into a mass balance model. We applied this approach to a loblolly pine (*Pinus taeda* L.) plantation forest located in coastal North Carolina, USA, to test the efficacy of this novel method. Absorptive fine roots (ARs), functionally defined as a combination of first- and second-order roots, are recognized as the most dynamic part of the root system (McCormack et al. 2015, Kou et al. 2018, Li et al. 2019). The relative AR decomposition rate has been determined in many forest ecosystems (Fan and Guo 2010, Xiong et al. 2013), but the total AR decomposition rate has received little attention. Thus, we specifically assessed the dynamics of total AR decomposition rate using the balanced hybrid method to evaluate their contribution to root C budgets of loblolly pine plantation forests.

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) on the lower coastal plain of North Carolina, USA. Mean annual precipitation and temperature for the period 2011–17 was 1320 mm and 12.2 °C, respectively. The area is flat, <5 m above sea level, on Belhaven series histosol (loamy mixed dysic, thermic terric Haplosaprists). The study area was harvested and ditched/drained in the late 19th to the early 20th century and farmed briefly before being converted to commercial loblolly pine timber production. The forest was fertilized with 28–50 kg ha^{-1} of nitrogen (N) and phosphorus (P) at the time of planting and 140–195 kg ha^{-1} N and 28 kg ha^{-1} P at mid-rotation. The soil C and N concentrations at 20-cm depth were 26 and 1.0%, respectively. The mean canopy height, diameter at the breast height and stand age during the study period were about 24 m, 33 cm and 23 years, respectively. For a full site description, refer to Noormets et al. (2010).

Absorptive fine root biomass and necromass measurements

Three plots, 100–800 m apart, were established at random in the forest in 2013. The number of soil cores required was determined according to Bartlett et al. (2001), in which the sample size is dependent on the level of acceptable risk, acceptable margin of error and measured standard deviation. In each plot, eight cylindrical soil cores (3.0 cm diameter, 30 cm depth) were randomly collected in April, July, September and November of 2016 and January and April of 2017 (Figure 1). For the purpose of calculation of root dynamics, two adjacent soil coring dates formed one interval (Figure 1). As a result, there were five soil sampling intervals. Collected soil cores were transported on ice to the lab and then rinsed with clean tap water through a 0.5-mm mesh sieve to isolate roots. The ARs were identified based on their unique morphological features (McCormack et al. 2015, Kou et al. 2018). The first-order roots are the most distal, unbranched roots, and second-order roots begin at the junctions of two first-order roots. The live and dead ARs were separated according to elasticity and cohesion of the stele and periderm. Generally, an AR was regarded to be alive if it was elastic and had an intact stele and periderm. Otherwise it was considered to be dead (Hertel and Leuschner 2002). A microscope was used when the vitality of ARs could not be

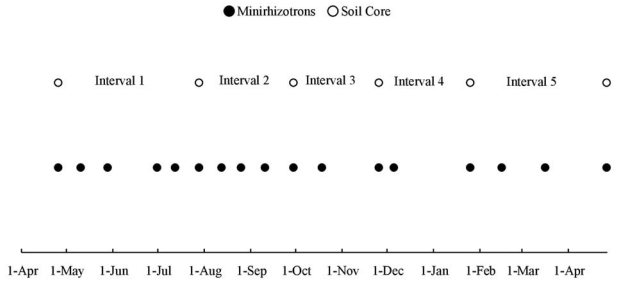

Figure 1. Soil core and root image sampling dates from late April 2016 to late April 2017 in a loblolly pine plantation forest.

visualized with the naked eye. Some ARs were detached from the root branches and were classified based on the described morphological features. For those could not be identified by the morphological features, a cutoff diameter (0.6 mm) developed from intact live and dead fine roots was used to separate ARs from other roots. The detached ARs identified by morphological features and the cutoff diameter represented 9% and 6% of the total AR mass (sum of biomass and necromass), respectively. The metric of AR biomass and necromass in the soil cores was calculated as g m^{-2} for the 0–0.30 m depth. All ARs were dried at 50 °C to a constant weight and weighed.

Absorptive fine root length production and mortality and standing length density (minirhizotrons)

A total of 18 acrylic tubes (80 cm long, 5 cm inner diameter, 6 cm outer diameter) were installed in 2013 at a 45° angle to a vertical soil depth of 50 cm in the three plots (five to eight tubes per plot). We took root images from late April 2016 through late April 2017, which co-occurred with soil coring (Figure 1). The root images were acquired on 17 sampling dates, resulting in three to six times per soil sampling interval during the study period (Figure 1). Images were collected using a Bartz digital camera (Bartz Technology Corp., Carpinteria, CA, USA) with the image capture software BTC I-CAP (Bartz Technology Corporation). The AR length and diameter were quantified by analyzing the images with WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Absorptive fine root length production (m m^{-2} $year^{-1}$), length mortality (m m^{-2} $year^{-1}$) and standing length density (mean AR length per unit root image area) (m m^{-2}) were calculated based on the analysis of the images. An AR was counted as dead when its diameter shriveled to half the original diameter, it became fragmented or its ectomycorrhizal fungal mantle was detached from the root. Otherwise, the AR was considered living (Kou et al. 2018). Absorptive fine roots growing back into the soil, out of the image or covered by mycelia were classified as 'gone' and were not involved in the calculations. Other root parameters, including specific root length (SRL) and root tissue density (RTD), were also assessed as outlined by Majdi and Andersson (2005).

Models

Models 1 and 2 input parameters are listed in Table 1.

Model 1 Absorptive fine root length production (Pr_L, m m^{-2} $year^{-1}$) in a given soil coring interval (year) was estimated using the minirhizotron measurements, according to Kou et al. (2018)

$$\mathbf{Pr}_L = RL_t - RL_0 + ARL_t \quad (1)$$

where RL_0 is the length of live ARs at the start of the interval, RL_t is the length of previously imaged live ARs at the end of the interval and ARL_t is the length of new live ARs in the interval.

AR turnover rate (TR_{live}) in the interval is

$$TR_{live} = \mathbf{Pr}_L / SL_{mean} \quad (2)$$

where SL_{mean} is the mean standing live AR length of minirhizotron images captured at the start of the interval (m m^{-2}).

Total AR production (Pr, g m^{-2} 0.30 m^{-1} soil depth $year^{-1}$) in the interval was assessed by combing minirhizotron image analysis with soil coring measurements (Hendrick and Pregitzer 1993, Hendricks et al. 2006).

$$\mathbf{Pr} = B_0 \times TR_{live} \quad (3)$$

where B_0 (g m^{-2} 0.30 m^{-1} soil depth) is standing AR biomass at the start of the interval.

Based on root mass balance model (Li and Lange 2015), total AR mortality (Mo, g m^{-2} 0.30 m^{-1} soil depth $year^{-1}$) and decomposition (De, g m^{-2} 0.30 m^{-1} soil depth $year^{-1}$) in the interval are

$$Mo = \mathbf{Pr} - (B_t - B_0) \quad (4)$$

$$De = Mo - (N_t - N_0) \quad (5)$$

where B_t (g m^{-2} 0.30 m^{-1} soil depth) is AR biomass at the end of the interval and N_0 (g m^{-2} 0.30 m^{-1} soil depth) and N_t (g m^{-2} 0.30 m^{-1} soil depth) are AR necromass at the start and end of the interval, respectively.

Because B_0, B_t, N_0 and N_t are measurable in soil cores, TR_{live} is estimated by root image analysis, Pr and Mo can be calculated by Eqs (1–4), and thus De can be calculated by Eq. (5).

Model 2 Absorptive fine root length mortality (Mo_L, m m^{-2} $year^{-1}$) in a given soil coring interval (year) can also be estimated from minirhizotron root image analysis.

Mo_L is the length of ARs that died in the interval.

AR death rate (DR_{dead}) in the interval is

$$DR_{dead} = Mo_L / SL_{mean} \quad (6)$$

where SL_{mean} is the mean standing live AR length of minirhizotron images captured at the start of the interval (m m^{-2}).

Table 1. Description of parameters used in Models 1 and 2 for estimating total absorptive root (AR) decomposition in a certain interval (year).

Model	Symbol	Description	Unit
1	B_O	AR biomass at the start of the interval	g m^{-2} m^{-1} soil depth
1	B_t	AR biomass at the end of the interval	g m^{-2} m^{-1} soil depth
1, 2	N_O	AR necromass at the start of the interval	g m^{-2} m^{-1} soil depth
1, 2	N_t	AR necromass at the end of the interval	g m^{-2} m^{-1} soil depth
1, 2	SL_{mean}	Mean standing live AR length	m m^{-2} image
1	Pr_L	AR length production	m m^{-2} image $year^{-1}$
2	Mo_L	AR length mortality	m m^{-2} image $year^{-1}$
1	TR_{live}	AR turnover rate	times $year^{-1}$
2	DR_{dead}	AR death rate	times $year^{-1}$

Mo in the interval was assessed by combing minirhizotron image analysis with soil coring measurements (Hendrick and Pregitzer 1993, Hendricks et al. 2006). Then Mo is

$$Mo = B_O \times DR_{dead} \quad (7)$$

Resorting to Eq. (5), De can be calculated.

Model test

The efficacy of Models 1 and 2 for estimating the total production, mortality and decomposition was tested by comparing the predicted with the measured AR necromass using a subset of data not used for model parameterization. We estimated AR necromass in July 2016 and then compared it with measured AR necromass in July 2016.

First, we combined the intervals from May to July and July to September into one to exclude the measured AR necromass of July 2016 from the estimation.

Second, we calculated TR_{live} and DR_{dead} in the combined interval (i.e., May to September) by analyzing minirhizotron images for the same interval.

Third, we used Models 1 and 2 to estimate mortality from May to September ($Mo_{M\text{-}S}$) based on AR biomass in May and September and TR_{live} and DR_{dead} in the interval from May to September using Eqs (1–7). Thus, the decomposition in the interval from May and September ($De_{M\text{-}S}$) is

$$De_{M-S} = Mo_{M-S} - (N_{Sep} - N_{May}) \quad (8)$$

where N_{Sep} and N_{May} are AR necromass in September and May, respectively.

Last, we estimated mortality in the interval from May to July ($Mo_{M\text{-}J}$) based on AR biomass in May and July and TR_{live} and DR_{dead} in the interval from May to July using Eqs (1–7).

Therefore, AR necromass in July (N_{Jul}) can be calculated as

$$N_{Jul} = N_{May} + Mo_{M-J} - (De_{M-S}/T_{M-S}) \times T_{M-J} \quad (9)$$

where N_{May} is the AR necromass in May and T_{M-S} and T_{M-J} are the time lengths of the interval from May to September and interval May to July, respectively.

Finally, we compared the estimated N_{Jul} with measured N_{Jul} using the soil coring method.

Statistical analysis

The plots were considered as replicates ($n = 3$), and data collected (sub-replicates) within the same plot were averaged before performing statistical analysis. A paired *t*-test was performed to assess the differences in mortality and decomposition estimates between Models 1 and 2. The data were log-transformed to normalize the variance among the estimates of the two models before analysis when necessary. We also analyzed the sensitivity of the decomposition estimates of both models to percent change in the biomass, necromass, AR turnover rate or AR death rate. All data were analyzed using the SPSS statistical software (version 17.0; IBM Corporation, Somers, NY, USA).

Results

Minirhizotron image analysis

The coefficients of variation (CV) for AR length production, length mortality, turnover rate and death rate were 0.57, 0.19, 0.54 and 0.24, respectively, showing that AR growth dynamics had greater variability than did the mortality dynamics (Figure 2A). The mean standing AR length density showed a relatively low temporal variability, with a CV of 0.22 (Figure 2B). The AR turnover rate was the highest in the growing season and the lowest in the non-growing season, while the AR death rate had an opposite temporal pattern (Figure 2C). The SRL and RTD of loblolly pine ARs were 41 ± 4.1 m g^{-1} and 25 ± 1.2 g m^{-3}, respectively.

Biomass and necromass dynamics

Mean AR biomass and necromass in the top 30 cm of soil were 35.4 ± 4.9 and 42.1 ± 3.5 g m^{-2}, respectively. Absorptive fine root biomass had the highest value in July and lowest value in November, whereas Absorptive fine root necromass was the highest in November and lowest in July (Figure 3). AR biomass had a significant positive relationship with live AR standing length ($n = 6$, $r^2 = 0.8$; $P < 0.05$).

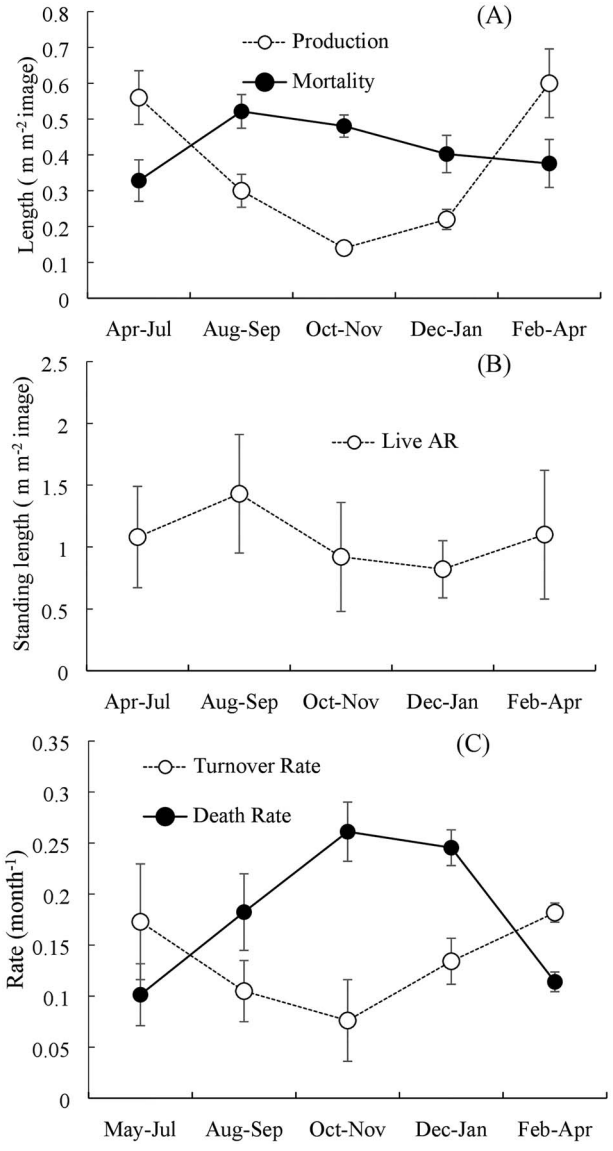

Figure 2. Absorptive fine root (AR) length production and mortality (m m^{-2}), mean standing live AR length (m m^{-2}), AR turnover and death rates ($month^{-1}$) in each soil coring interval from May 2016 to April 2017 ($n = 3$; mean ± SE).

Production, mortality and decomposition

From April 2016 to April 2017, total AR production, mortality and decomposition estimates using Model 1 were 113 ± 9 , 123 ± 12 and 114 ± 13 g m^{-2} $year^{-1}$, respectively, while total AR mortality and decomposition estimates using Model 2 were 138 ± 11 and 129 ± 12 g m^{-2}, respectively. Annual AR mortality and decomposition estimates were not significantly different between the two models. Monthly AR mortality and decomposition showed similar temporal variation, with the highest values appearing in October to November and the lowest values from February to April (Figure 4). By contrast, monthly AR production was highest in April to July and lowest from October to November (Figure 4).

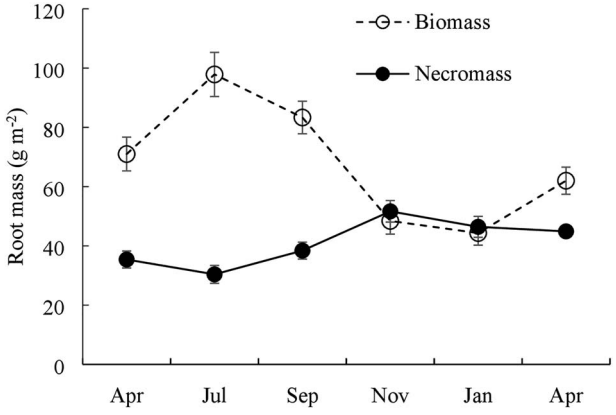

Figure 3. Absorptive fine root (AR) biomass and necromass dynamics (g m^{-2} for the 0–0.30 m soil depth; $n = 3$; mean ± SE).

Model test and sensitivity analysis

The measured necromass in July was 12% higher than that estimated by Model 1 and 8% lower than that estimated by Model 2. Absorptive fine root decomposition estimates were very sensitive to changes in the biomass, AR turnover and death rates, but insensitive to change in necromass in both models (Figure 5).

Discussion

Consistent with previous studies (King et al. 2002, McCormack et al. 2014, Kou et al. 2018), the AR length production and mortality and the AR standing length varied greatly among intervals, resulting in high temporal variability in AR turnover rate and death rates. The standing biomass was significantly and positively related to the standing length, indicating that the ARs growing around the minirhizotron tubes were a good representative of those living in the intact soils. Mean annual AR mortality of the two models represented 34% of mean annual aboveground litterfall (data not shown). Adopting a C concentration of 0.48 g g^{-1} in ARs (King et al. 1997), mean annual decomposition C of the two models was 58.3 g C m^{-2} 0.3 m^{-1} soil horizon, representing about 5% of mean annual soil respiration measured in the same plantation forest (Aguilos et al. 2020). In Model 1, measurements of fine root death rate were not required. This could help overcome the error derived from flawed criteria defining dead fine roots in Model 2 and the previous method combining minirhizotrons and soil coring (Hendrick and Pregitzer 1993, Hendricks et al. 2006), because fine roots may die days before evident signs of deterioration are apparent. However, Model 1 requires a higher measurement accuracy for fine root biomass dynamics. This suggests that more soil cores are needed to be sampled, which results in greater time and effort.

The balanced hybrid method has advantages over the ingrowth/soil core-based models (Osawa and Aizawa 2012,

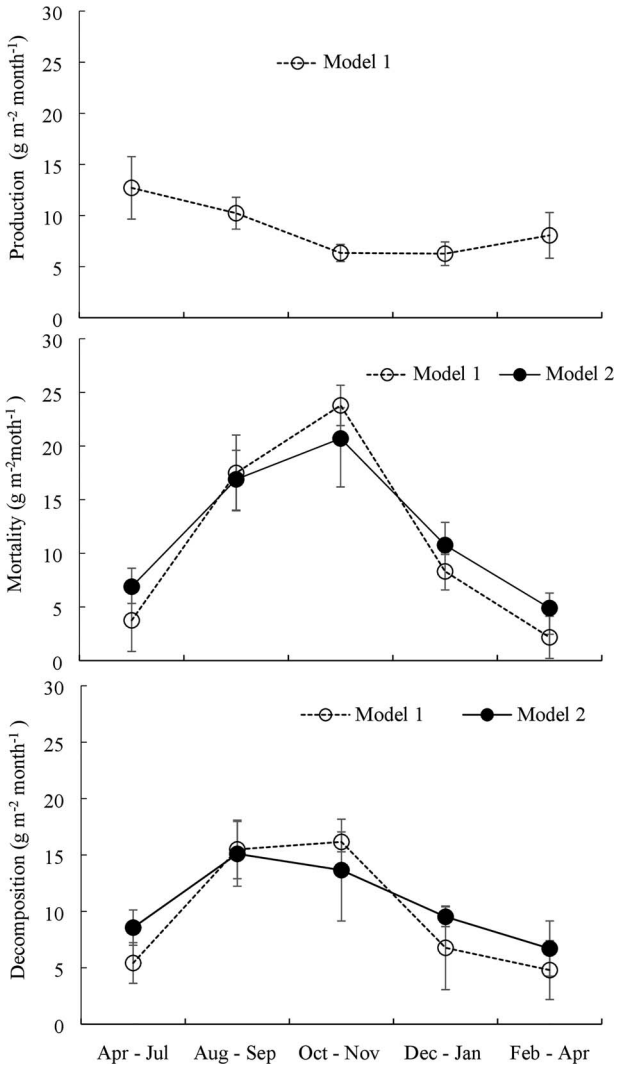

Figure 4. Monthly total absorptive fine root (AR) production, mortality and decomposition (g m^{-2}) for the 0–0.30 m soil depth using Models 1 and 2 in each soil coring interval from May 2016 to April 2017 ($n = 3$; mean ± SE; * stands for significant difference in means $P < 0.05$).

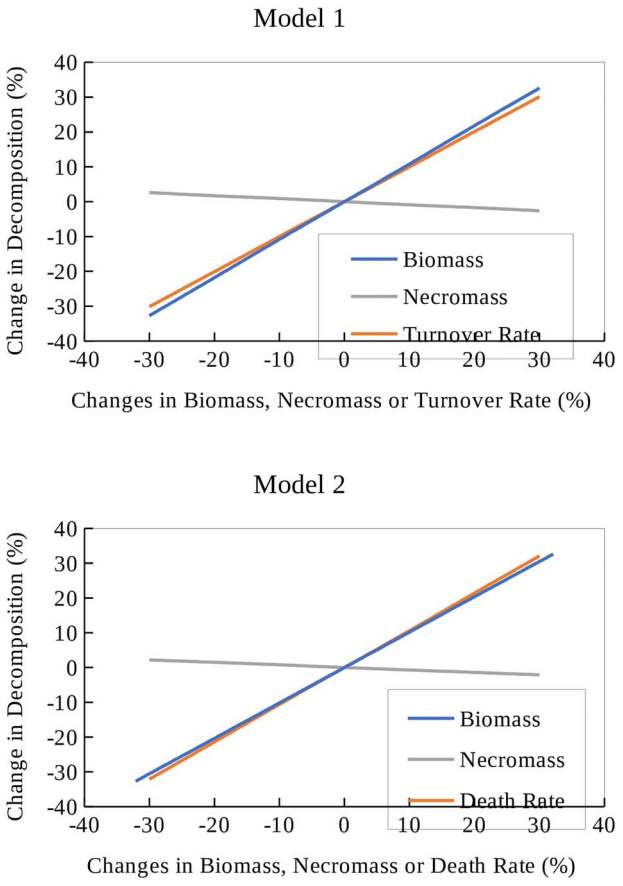

Figure 5. Effects of percent changes in absorptive root biomass, necromass, turnover and death rates on total decomposition estimates.

Li et al. 2013, Li and Lange 2015) in several important ways. First, the estimation of total AR or fine root (diameter <2 mm) decomposition was solely based on AR or fine root biomass, necromass, turnover rate and death rate, avoiding the measurement of relative AR or fine root decomposition rates using the litterbag method and the resulting errors (Li et al. 2015). Second, AR or fine root growth and death dynamics have been observed by minirhizotrons, decreasing the uncertainty induced by ignoring the temporal variations in the previous models. Third, the decomposition estimate was insensitive to change in the necromass. As a result, the negative effect of misidentifying partly decomposed dead roots as organic matter in the estimate of decomposition was greatly reduced. Fourth, soil core sampling frequency can be as low as twice per year, reducing the sampling errors and the labor-intensive root sorting work. Last, the balanced hybrid method enables a root order-based estimation, which is more functionally appropriate than the diameter-based assessments, because the biomass and turnover rates of first- and second-order roots can be reliably measured by both soil coring and minirhizotrons. In theory, the balanced hybrid method is more suitable for the more uniform overstory of managed loblolly pine forests than for mixed forests as the more homogeneous soil physical and chemical properties and species composition in these forests enable more accurate measurements of fine root biomass and necromass and fine root growth and death dynamics. The application of this method to mixed forests is mainly hindered by the large spatial heterogeneity in soil and species composition, which theoretically could be addressed, but in practice would require significantly increased effort compared to the current study.

The key to the successful application of this method is accurate measurements of fine root biomass, turnover rate and death rate. As a consequence, the number of soil cores collected and root image-acquiring frequency must be large. In this loblolly pine forest, the core sample size should be over 20 cores as the CV value did not decline appreciably after 20 cores, while the imaging frequency should be at least twice a month because some ARs were found to die within 3 weeks. Another concern

is how well fine roots observed by minirhizotrons can represent those living in soils (Norby et al. 2004). However, the use of minirhizotrons should be considered as an improvement rather than a compromise as minirhizotrons are the most effective and widely used way of assessing undisturbed root dynamics in soils (Crocker et al. 2003, Norby et al. 2004, Hendricks et al. 2006, Hansson et al. 2013, Kou et al. 2018, Kume et al. 2018) and supported by the good agreement between minirhizotron-observed AR length density and soil core estimates of AR biomass of the current study.

Accurate measurements of total fine root decomposition not only reduce uncertainty in soil C flux estimates but also provide insight into belowground C cycling processes. By integrating fine root biomass, necromass, turnover rate and death rate into mass balance equations, the balanced hybrid method provides an improved mean for the estimation of total fine root decomposition in pine plantation forests, with potential application to other managed or natural forested ecosystems.

Acknowledgments

We thank Jordan Luff, Wen Lin and Yuan Fang for their help with analyzing the minirhizotron images and processing the samples.

Conflict of interest

None declared.

Funding

Primary support was provided by United States Department of Agriculture National Institute of Food and Agriculture (Multi-agency A.5 Carbon Cycle Science Program) award 2014-67003-22068. Additional support was provided by Department of Energy National Institute for Climate Change Research award 08-SC-NICCR-1072, the United States Department of Agriculture Forest Service Eastern Forest Environmental Threat Assessment Center award 13-JV-11330110-081 and Carolinas Integrated Sciences and Assessments award 2013-0190/13–2322.

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