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Partitioning Longleaf Pine Soil Respiration into Its Heterotrophic and Autotrophic Components through Root Exclusion

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Abstract: Rapid and accurate estimations of the heterotrophic and autotrophic components of total soil respiration (R_s) are important for calculating forest carbon budgets and for understanding carbon dynamics associated with natural and management-related disturbances. The objective of this study was to use deep (60 cm) root exclusion tubes and paired control (*i.e.*, no root exclusion) collars to estimate heterotrophic respiration (R_h) and R_s , respectively, in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands in western Georgia. Root biomass was measured in root exclusion tubes and control collars after 102–104 days of incubation and fine root biomass loss from root exclusion was used to quantify root decay. Mean R_s from control collars was 3.3 micromol·CO₂·m⁻²·s⁻¹. Root exclusion tubes decreased R_s , providing an estimate of R_h . Mean R_h was 2.7 micromol·CO₂·m⁻²·s⁻¹ when uncorrected by pretreatment variation, root decay, or soil moisture compared to 2.1 micromol·CO₂·m⁻²·s⁻¹ when R_h was corrected for root decay. The corresponding ratio of R_h to R_s ranged from 66% to 82%, depending on the estimation method. This study provides an estimate of R_h in longleaf pine forests, and demonstrates the potential for deep root exclusion tubes to provide relatively rapid assessments (*i.e.*, ~40 days post-treatment) of R_h in similar forests. The range in R_h to R_s is comparable to other reports for similar temperate coniferous ecosystems.

Keywords: soil CO₂ efflux; roots; *Pinus palustris*; soil moisture

1. Introduction

Anthropogenic emission of greenhouse gasses and concomitant global climate changes have fostered an impetus for the modeling and quantifying of global carbon stocks and balances within terrestrial ecosystems. The overall carbon balance of an ecosystem is dependent upon carbon assimilation (*i.e.*, photosynthesis) being larger in magnitude than the carbon lost from ecosystem respiration, oxidation from fire, and carbon removal (e.g., biomass harvesting, herbivory, run-off, and leaching) [1]. Soil heterotrophic respiration (R_h) is the largest heterotrophic component of ecosystem respiration and is often compared with net primary productivity to estimate whether forests are carbon sinks or sources [1,2]. Carbon sources for R_h from free-living soil microbes include soil organic matter, dead plant matter, and exudates from roots and R_h thus occurs in both root-free bulk soil and the root-affected rhizosphere [3]. In some woody species, including pines, CO₂ is also released by the metabolism of root exudates by ectomycorrhizal symbionts [4,5]. Mycorrhizally sourced CO₂ is also a component of R_h , however because of the interaction between roots and microbes in the

rhizosphere, partitioning total R_s into its autotrophic (*i.e.*, root-derived) and heterotrophic components is challenging.

Methods that have been developed to partition R_s each have inherent strengths and weaknesses [3,6]. One common technique uses trenching to sever existing roots in order to cut off the photosynthetic carbon allocation pathway, which then theoretically leaves residual heterotrophic activity in the soil and allows for direct measurement of R_h [7]. With this technique, plots that are trenched are often fairly large and in place for a year or more before R_h is measured [8]. The downside of using root exclusion techniques such as trenching includes: (1) modification to the soil environment due to changes in evapotranspirational demand from root-severed soil and run-off and leaching patterns; (2) an increase in R_h due to the newly deceased root tissues in the root-severed soil; (3) residual autotrophic respiration (R_a) may continue in severed roots with large carbohydrate reserves; and (4) eventual changes in the microbial community composition as labile substrates diminish and microbes that decompose recalcitrant organic material become more dominant [3,6–9]. In order to reduce some of these artifacts, a technique to measure R_h from root-excluded soils utilizing small-diameter root exclusion tubes has been developed in which root exclusion tubes are pushed through otherwise undisturbed soil, severing the existing roots and excluding root ingrowth [8]. Although some of the methodological artifacts of trenching remain a limitation with root exclusion tubes, such as potential changes to the soil hydrology, small root exclusion tubes are easier to install than the effort and expense necessary for trenching and they can be installed with higher replication and staggered over time to determine how the profile of R_h changes seasonally or in response to disturbances. In addition, the smaller volume of root-excluded soil as compared to trenching should reduce the total available carbohydrate reserves and allow R_h to be more readily estimated after installation, thus reducing the potential for changes in soil environment and microbial community composition [8,10].

The objective of this study was to estimate the proportion of R_h to R_s in three longleaf pine (*Pinus palustris* Mill. (*P. palustris*)) plantations with small-diameter root exclusion tubes. Net primary productivity and related standing carbon stocks of longleaf pine forests have been previously examined *e.g.*, [11–13]; however, relatively less is known about the magnitude of R_h in longleaf pine forests, thus hindering accurate estimates of the carbon balance of longleaf pine forests. Specifically, the proportion of R_h to total R_s has been estimated in only one known longleaf pine study (85% to 96%) [14]. We hypothesized, based on the previous longleaf pine study [14] and research in another southern conifer, loblolly pine (*Pinus taeda* L. (*P. taeda*)) [10], that R_h would comprise 70% to 90% of R_s . Root exclusion tubes were installed in May 2013 in three 26-year-old longleaf pine stands in western Georgia and compared with paired control R_s measurements to determine the proportion of R_h to total R_s . A recent methodologically similar study with loblolly pine demonstrated that root carbohydrates are metabolized rapidly after severing, and that R_h estimates from root-excluded soil stabilized after 41 days [10]. We attempted to reduce the impacts of residual starch reserves in longleaf pine roots by installing the root exclusion treatment in mid-May during a period of starch depletion [15], and we hypothesized that by late August (~100 days), measurements of R_s from root exclusion tubes would provide a relatively stable estimate of R_h . Because of the excessively drained, sandy soils present in these stands [16], we expected minimal effect of root exclusion tubes on soil moisture. When trenching or small root exclusion tubes are coupled with root biomass measurements, the amount of root decay that has occurred post-treatment can be quantified and used to recalculate more accurate estimates of R_h [7,17]. Thus, we also incorporated an estimation of fine root decay into R_h based upon measurements of root biomass in both the root-excluded and control soil profiles to ameliorate potential impacts of artificially enhanced carbohydrate metabolism after severing.

2. Experimental Section

2.1. Study Site

This study was conducted in three 26-year-old longleaf pine stands located on property in a conservation easement held by The Nature Conservancy within the Chattahoochee Fall Line Wildlife Management area in Talbot County, Georgia, USA (32°34' N & −84°30–36' W; Table 1). These stands were located within the Sand Hills Ecoregion, which is characterized by dry, deep sand and gently rolling hills that are excessively drained and nutrient poor [18]. All three stands were planted in 1988 at a density of 1793 seedlings ha^{−1} and spacing of 1.8 m by 3.0 m, and no known thinning occurred after planting. The 30-year mean annual air temperature for this area is 24.6 °C with mean January and July air temperature of 8.4 °C and 28.1 °C, respectively [19]. The 30-year mean precipitation is 1180 mm, spread evenly throughout the year. Stands 1 and 2 were located in Lakeland sand soil (2% to 5% slopes, excessively drained) and Stand 3 was located in a Troup loamy sand soil (2% to 5% slopes, somewhat excessively drained) [16]. Longleaf pine basal area measured in the experimental plots ranged from 17.3 to 21.4 m²·ha^{−1} and density ranged from 978 to 1567 trees·ha^{−1} (Table 1). Mean diameter at breast height (1.37 m, DBH) of longleaf pine trees ranged from 11.5 to 15.4 cm. Basal area, density, and mean DBH of hardwoods in the stands ranged from 0.0 to 0.2 m²·ha^{−1}, 0 to 89 trees·ha^{−1}, and 4.0 to 4.4 cm, respectively. Understory vegetation was sparse and consisted mainly of bunch grasses, saw palmetto (*Serenoa repens* (W. Bartram) Small), prickly pear cacti (*Opuntia* spp.), and some poison oak (*Toxicodendron pubescens* Mill.). Stands were last burned about one year prior to this study and have been maintained on a frequent (1–3 year) burning regime under The Nature Conservancy's management. No burn records were available previous to the easement held by The Nature Conservancy.

Table 1. Location and forest structural characteristics of the three 26-year-old longleaf pine (LLP; *Pinus palustris* Mill.) stands in Talbot County, Georgia, USA. Note: HW, hardwoods; DBH, diameter at breast height (1.37 m).

	Stand 1	Stand 2	Stand 3
Location & Size			
° N	32°34.469'	32°34.716'	32°34.706'
° W	−84°36.039'	−84°31.371'	−84°30.117'
Size (ha)	75.3	4.0	2.4
Basal Area (m²·ha^{−1})			
Total	18.82	21.48	17.34
LLP	18.60	21.40	17.34
HW	0.22	0.08	0.00
Tree Density (trees·ha^{−1})			
Total	1222	1533	1922
LLP	978	1089	1567
HW	89	33	0
Dead	156	411	356
Mean DBH ± SE (cm)			
LLP	14.89 ± 0.48	15.44 ± 0.35	11.50 ± 0.25
HW	4.39 ± 1.36	4.00 ± 2.70	na

2.2. Sampling Design

In 2013, one 30 m by 30 m experimental plot was established in each of the three stands, and 10 trees were randomly selected from within each stand from a gridded identification system based on row and tree number. At each tree, a temporary R_s collar (PVC, 10 cm diameter, 4.5 cm height) was placed at 1.0 m from that tree at a randomly chosen 45° angle, and a paired temporary R_s collar was

also placed 1.0 m from an adjacent tree at an angle parallel to the first (Figure 1a). Litter was cleared away from the location of collar placement and the collars were gently pounded into the soil to a depth of 2.0 cm below mineral soil. Temporary R_s collar pairs were installed on 10 May and 15 May 2013 and used for initial, pretreatment R_s measurements 24 h after installation.

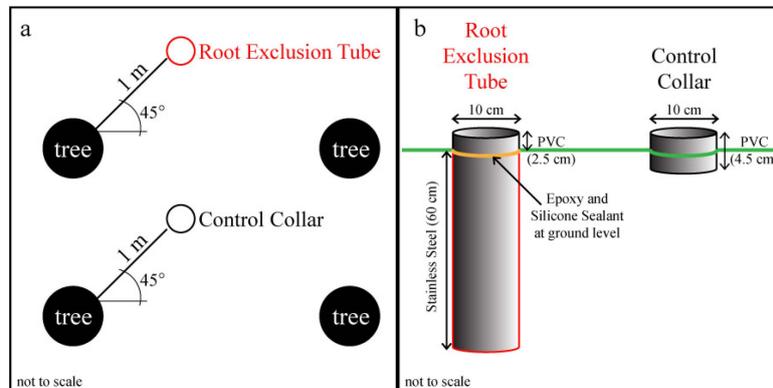


Figure 1. Experimental design schematics showing: (a) the random placement of root exclusion tubes and control collars 1 m from trees at a 45° angle from the tree rows; and (b) the size and composition of the root exclusion tubes and control collars. This study was conducted in three longleaf pine (*Pinus palustris* Mill.) stands.

After initial R_s measurements were made on the temporary R_s collars, root exclusion tubes were installed at the location of one randomly selected temporary R_s collar from each pair. Root exclusion tubes were constructed from a 10 cm diameter stainless steel tube cut to 60 cm length with a sharp, beveled bottom edge (Figure 1b). A small pretreatment test of 1 meter-long root exclusion tubes in an adjacent longleaf pine stand illustrated that 60 cm should be deep enough to exclude all herbaceous and lateral longleaf pine roots and that the beveled bottom edge was able to cut through roots up to about 5 cm in diameter. During installation, tubes were pounded into the ground with a rubber mallet and a board until the tube edge was approximately 2 cm above the soil surface. A 2.5 cm long, 10 cm diameter PVC collar was glued to the exposed top of each root exclusion tube with instant epoxy, and waterproof silicone sealant was applied to create an airtight seal (Figure 1b). After the epoxy and silicone had set, the root exclusion tubes were gently pushed down into the soil until the metal tube edge was flush with the adjacent undisturbed soil, leaving 2.5 cm of PVC collar aboveground. The remaining ten temporary collars per plot became permanent control treatment collars (*i.e.*, no root exclusion). All root exclusion tubes were installed on 14 or 16 May 2013. Litter was removed and live vegetation was clipped from within all collars as needed to maintain bare soil.

2.3. Soil Respiration Measurements

Soil respiration was measured on both root exclusion collars and control collars by placing a LI-COR 6400-09 soil respiration chamber connected to a LI-COR 6400 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE, USA) on the portion of PVC located above the ground. A foam gasket was used on the soil respiration chamber to create an airtight seal with the collars and depth to soil surface within each collar was used to calculate chamber volume for R_s measurements. Ambient CO_2 concentration (CO_{2amb}) was measured at the first block within each plot. Carbon dioxide was scrubbed from the chamber air by passing it through soda lime, and then CO_{2amb} was logged with the LI-COR 6400 console over at least 30 intervals from 5 ppm below ambient CO_{2amb} to 5 ppm above ambient CO_{2amb} . Soil respiration was then derived from linear regression of the logged intervals. Soil respiration was measured at approximately 2-week intervals from 10 May 2013 (first pretreatment measurement) through 26 August 2013; with the exception of mid-August because of heavy rains. All R_s measurements were made from 8:30 a.m. to 1:00 p.m., and the measurement sequence of the

plots and blocks were randomly determined each day. Soil temperature and volumetric soil moisture were measured within 10 cm of the collars at the time of R_s measurements with a 15 cm depth soil temperature probe connected to the LI-COR 6400 and a 20 cm depth time domain reflectometry soil moisture probe (Hydrosense II, Campbell Scientific, Inc., Logan, UT, USA), respectively. After the final R_s measurement was made at each collar on 26 August 2013, soil moisture and soil temperature were measured within each collar to test for treatment effects on the soil environment.

2.4. Soil Sampling

Following the final R_s measurements, soil samples were removed from directly underneath the control collars with a 10 cm diameter soil auger at depths of 0–15 cm, 15–30 cm, 30–45 cm, and 45–60 cm and placed in plastic bags for transport. Root exclusion collars and tubes were pulled out of the ground, sealed with plastic, and transported to the lab for processing. In the laboratory, soil was removed from root exclusion tubes in sections of 15 cm lengths to correspond with soil depths of 0–15 cm, 15–30 cm, 30–45 cm, and 45–60 cm. All soil was air dried and sifted through a 2 mm sieve. Roots were removed, washed, and sorted into fine and coarse size classes (diameter ≤ 2 mm or diameter > 2 mm, respectively) and live *versus* dead based on texture, color and resiliency. Roots were oven dried at 70 °C and weighed.

2.5. Data Analysis

The experimental design was a randomized complete block design with collar pairs considered as blocks ($n = 30$) and individual collars considered the experimental unit. To account for random variation between stands and between blocks, treatment effects (*i.e.*, root exclusion tubes *versus* control R_s collar only) were analyzed with a mixed-model approach. Data were analyzed across all measurement replications with date as a random factor to measure the overall treatment effect and at each measurement date to determine the timing at which the root exclusion tubes were significantly different than the control collars. The initial, pretreatment R_s measurements were pooled for analysis. All statistical analysis was done with SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) and with a significance level of $\alpha = 0.05$.

Soil respiration measurements from the control collars were used to estimate total R_s , whereas soil respiration measurements from the root exclusion tubes were used to estimate R_h . In addition to direct measurements of root exclusion tube R_s to estimate R_h , R_s from root exclusion tubes was also corrected for pretreatment R_s variation between collar pairs within blocks and for increased soil moisture and root decay caused by the presence of root exclusion tubes. Root exclusion tube R_h was corrected for pretreatment R_s variation by using the initial within-block difference in R_s as an offset.

The respiration measured from exclusion tubes was corrected for any soil moisture differences between treatments as described by Protingheuer *et al.* [20]. The relationship between R_s from root exclusion tubes and soil moisture on the final day was used to correct R_h based on the volumetric soil moisture measurements made near the root exclusion tubes. Specifically, the difference between soil moisture measured within and adjacent to root exclusion tubes was used to calculate the expected change in R_s due to root exclusion using the fitted relationship between R_s and soil moisture.

Fine root decay was estimated at the block-level using a modified exponential decay function, $M_1 = M_0 \exp(-kt)$, where M_1 is the fine root biomass in the root exclusion tubes, M_0 is the fine root biomass below the control collars, k is the decay rate, and t is the incubation time [7]. The total loss of live fine root biomass was stoichiometrically converted to $\mu\text{mol} \cdot \text{CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ assuming fine roots were 50% carbon by mass [21]. Total live fine root biomass over the entire sampled soil profile (0 to 60 cm depth) was compiled and used for these calculations.

3. Results

3.1. Environmental Conditions

During the duration of the experiment (May 10 through 26 August 2013), daily maximum air temperature ranged from 22.2 to 35.0 °C and daily minimum air temperature ranged from 9.4 to

25.6 °C (Figure 2a) [22]. Daily precipitation ranged from 0.0 to 145.5 mm·day⁻¹ (Figure 2a). Mean soil temperature near collars ranged from 19.9 to 26.2 °C and was not significantly affected by treatment ($F_{1,377} = 2.8$, $p = 0.0953$; Figure 2b). On the final measurement day (26 August 2013), mean soil temperature was 22.8 °C and 22.9 °C within the control collars and root exclusion tubes, respectively (Figure 2b, Table 2). Soil temperature was not significantly different between measurements made within or near the collars for either treatment (Table 2). Mean soil moisture ranged from 2.9% to 7.1% near the control collars and from 3.1% to 6.8% near the root exclusion tubes (Figure 2c), and soil moisture was not significantly affected by treatment across all measurement dates ($F_{1,377} = 0.11$, $p = 0.7391$). On the final measurement day, mean soil moisture was 6.3% and 7.8% within the control collars and root exclusion tubes, respectively (Figure 2c). On the same day, mean soil moisture was significantly higher within the root exclusion tubes compared to near the root exclusion tubes, but soil moisture was not significantly different between locations near *versus* within control collars (Table 2).

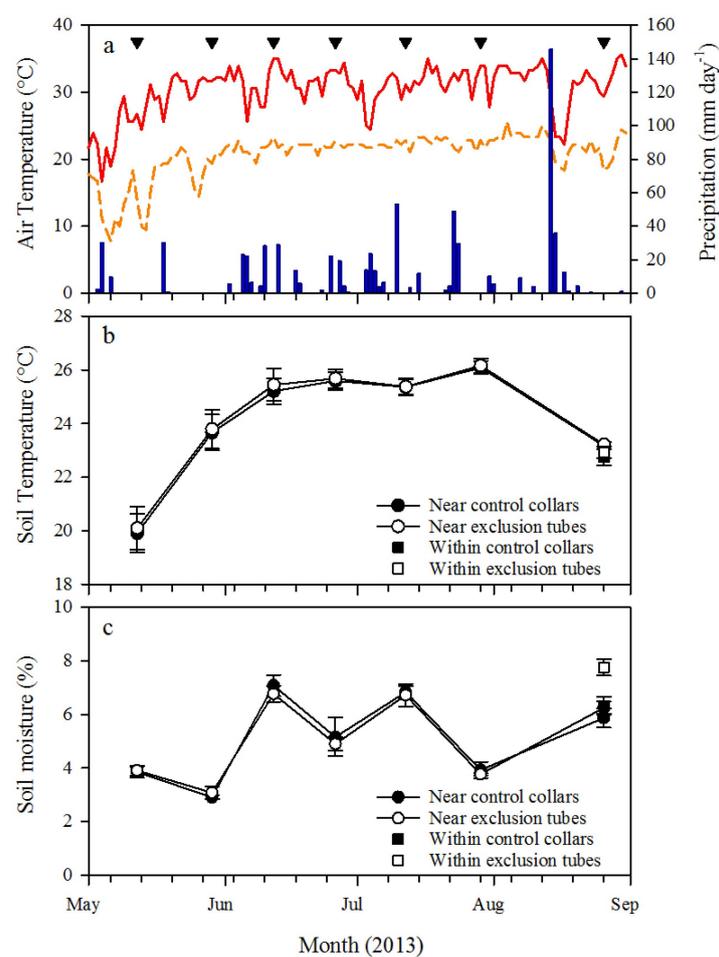


Figure 2. (a) Daily maximum (red solid line) and minimum (orange dashed line) air temperature and precipitation (blue bars) for Columbus Metropolitan Airport Weather Station [22]. Triangles indicate soil respiration sampling dates. (b) Soil temperature and (c) soil moisture by sampling date and treatment measured in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands. Note: Error bars are ± 1 SE (standard error). Initial soil temperature and moisture measurements were pooled from 10 and 15 May 2013.

Table 2. Influence of location on soil temperature and moisture measurements (*i.e.*, within or near soil respiration collars) for control collars and root exclusion tubes measured in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands. Bolded *p*-values are significant at $\alpha = 0.05$.

Location	Soil Temperature \pm SE ($^{\circ}$ C)		Soil Moisture \pm SE (%)	
	Control	Root Exclusion	Control	Root Exclusion
Near	23.2 \pm 0.1	23.2 \pm 0.2	5.9 \pm 0.4	6.3 \pm 0.2
Within	22.8 \pm 0.3	22.9 \pm 0.2	6.3 \pm 0.3	7.8 \pm 0.3
<i>t</i> -value	−1.30	−0.90	1.50	5.05
<i>p</i> -value	0.20	0.38	0.25	<0.01

3.2. Soil and Heterotrophic Respiration

Mean R_h based on uncorrected R_s from root exclusion tubes ranged from 2.7 to 4.1 $\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 3a). Mean control collar R_s ranged from 3.2 to 4.0 $\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figure 3a). Across all the measurement dates, control collar R_s was significantly higher than root exclusion tube R_h corrected by pretreatment R_s variation ($F_{1,377} = 20.17$, $p < 0.0001$) and marginally higher than uncorrected R_h ($F_{1,377} = 3.62$, $p = 0.0577$). Mean R_s was significantly higher than R_h based on uncorrected R_s from root exclusion tubes on the fourth, sixth, and final measurement dates (26 June, 29 July and 26 August 2013; Table 3). Mean R_h corrected by pretreatment R_s variation between collar pairs ranged from 2.5 to 3.9 $\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and was also significantly lower than control collar R_s on the fourth, sixth, and final measurement dates (Figure 3b, Table 3). The estimated ratio of R_h to R_s was 81.9% and 76.9% based on uncorrected R_h and R_h corrected by pretreatment R_s variation, respectively (Table 4).

On the final day, R_s from root exclusion tubes (R_h) was related to volumetric soil moisture measured within the tubes (θ) with the function $R_h = 1.04 + 0.21\theta$ ($F_{1,26} = 5.22$, $p = 0.0308$, $R^2 = 0.17$). Inflated soil moisture within the root exclusion tubes accounted for a mean increase in R_s of $0.01 \pm 0.7 \mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Correction for soil moisture marginally decreased the ratio of R_h to R_s from 82% to 81% and increased the standard error from 8% to 22% (Table 4).

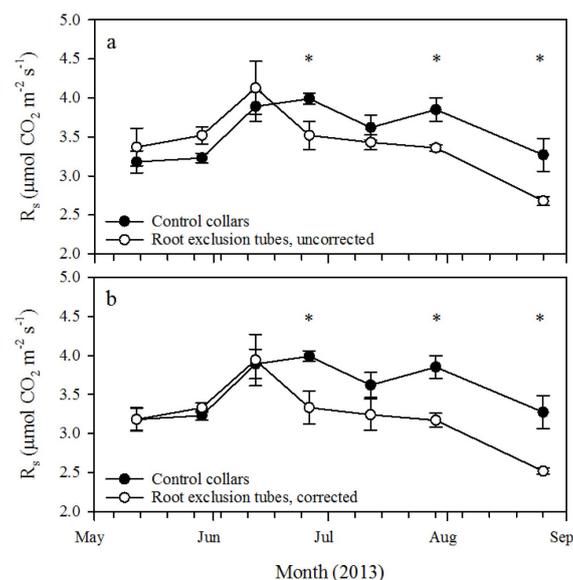


Figure 3. Soil respiration (R_s) from control collar and root exclusion tubes by date measured in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands (a) uncorrected and (b) corrected by pretreatment R_s variation between collar pairs. Note: Asterisks (*) denote significant treatment effects at $\alpha = 0.05$. Error bars are ± 1 SE (standard error).

Table 3. Statistical summary of the influence of treatment on mean soil respiration (R_s) measurements by date in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands. Summaries given for the comparison of R_s from control collars versus R_s from root exclusion tubes uncorrected and corrected for within-block, pretreatment R_s variation. Bolded p -values are significant at $\alpha = 0.05$.

Date	10 May & 15 May	29 May	12 June	26 June	12 July	29 July	26 August
(m/dd/2013)							
Uncorrected							
$F_{1,27}$	1.64	2.10	0.71	5.94	0.76	4.68	7.63
p -value	0.21	0.16	0.41	0.02	0.39	0.04	0.01
Corrected for Within-Block, Pretreatment R_s Variation							
$F_{1,27}$	na	0.30	0.03	13.72	2.39	10.08	11.73
p -value	na	0.59	0.85	<0.01	0.13	<0.01	<0.01

Table 4. Mean heterotrophic respiration (R_h) and the ratio of R_h to total soil respiration (R_s) from control collars by R_h estimation method. Mean R_s from control collars was $3.27 \pm 0.16 \mu\text{mol} \cdot \text{CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Measurements were made in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands.

R_h Estimation Method	$R_h \pm \text{SE}$	$R_h/R_s \pm \text{SE}$
	($\mu\text{mol} \cdot \text{CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	(%)
1. Uncorrected R_s from root exclusion tubes	2.67 ± 0.16	81.9 ± 7.7
2. R_s from root exclusion tubes corrected by pretreatment variation	2.52 ± 0.18	76.9 ± 9.0
3. R_s from root exclusion tubes corrected for soil moisture (θ)	2.66 ± 0.70	81.3 ± 21.8
4. R_s from root exclusion tubes corrected for root decay (k)	2.17 ± 0.37	66.3 ± 17.7

Note: SE is standard error.

3.3. Root Biomass

Root biomass diminished with depth in the soil (Figure 4). The majority of root biomass was within the top 15 cm of soil (55.1%), followed by 15–30 cm, 30–45 cm, and 45–60 cm depths (24.6%, 11.4%, and 8.9%, respectively). In the top 15 cm of soil, live fine and live coarse root categories of biomass were significantly lower in root exclusion tubes than under control collars (Figure 4a,b; Table 5). In both 0–15 cm and 15–30 cm soil depths, dead root biomass was significantly higher in root exclusion tubes than under control collars (Figure 4c; Table 5). Live fine root decay was $k = 0.52 \pm 0.20 \text{ year}^{-1}$ and accounted for an additional flux of CO_2 from root exclusion collars of approximately $0.5 \pm 0.3 \mu\text{mol} \cdot \text{CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Incorporation of root decay decreased the estimated ratio of R_h to R_s to 66.3% (Table 4).

Table 5. Statistical summary of the influence of treatment on mean root biomass by type and depth as measured in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands. Summaries given for the comparison of root biomass from soil beneath control collars versus soil within root exclusion tubes. Bolded p -values are significant at $\alpha = 0.05$.

Depth (cm)	0–15	15–30	30–45	45–60
Live Fine Root Biomass				
$F_{1,27}$	7.17	2.35	0.37	3.16
p -value	0.01	0.14	0.55	0.09
Live Coarse Root Biomass				
$F_{1,27}$	4.82	0.64	0.31	0.01
p -value	0.04	0.43	0.58	0.92
Dead Root Biomass				
$F_{1,27}$	8.42	7.26	0.69	0.11
p -value	<0.01	0.01	0.42	0.74

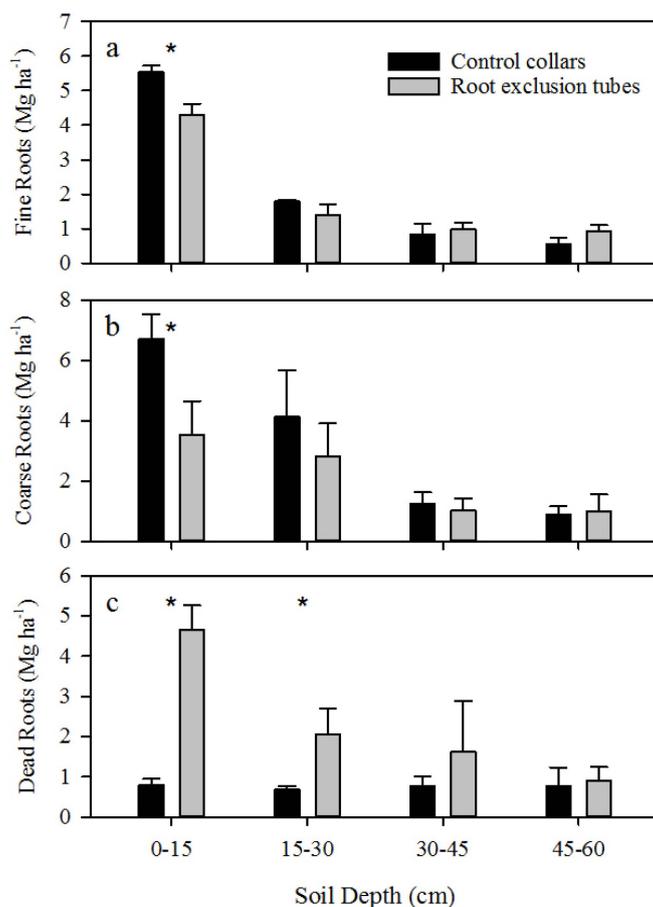


Figure 4. Root biomass by treatment and depth for (a) live fine; (b) live coarse; and (c) dead root categories measured in three 26-year-old longleaf pine (*Pinus palustris* Mill.) stands. Note: Asterisks (*) denote significant treatment effects at $\alpha = 0.05$. Error bars are ± 1 SE (standard error).

4. Discussion

This study utilized small-diameter root exclusion tubes for relatively rapid estimation of R_h in 26-year-old longleaf pine forests in western Georgia. After about 40 days of treatment (*i.e.*, by 26 June 2013), R_s measured from the root exclusion tubes was significantly lower than R_s from the control collars. At that time, the corresponding ratio of R_h to R_s was $83.5\% \pm 5.5\%$ if R_h was corrected for pretreatment R_s variation and $88.2\% \pm 4.8\%$ without correction. This is higher, but not substantially different, than the uncorrected ratio measured at the final measurement date, about 100 days after root exclusion tube installation (*i.e.*, by 26 August 2013). Heim *et al.* [10] similarly found that after 41 days, root exclusion tubes significantly reduced R_s compared to adjacent, undisturbed soil in a nine-year-old loblolly pine plantation with a corresponding uncorrected ratio of R_h to R_s of 79.0%. The results of these two studies indicate that 40 to 50 days of root exclusion may be an adequate time period to isolate R_h from root exclusion treatment tubes.

Rapid measurements of R_s from root severed or trenched soils are recommended by Sayer and Tanner [23] to reduce methodological artifacts such as root decomposition or ingrowth, soil moisture alteration, and microbial community composition changes. Likewise, Vogel and Valentine [8] suggest that measurements of R_s from small root exclusion tubes within three weeks of installation may mitigate effects of root exclusion on soil moisture. However, three weeks of post-treatment incubation was insufficient to detect R_h in longleaf pine, perhaps because of greater carbohydrate stores in longleaf pine roots relative to other species [24,25]. We suggest that the longer incubation did not profoundly change soil moisture dynamics or our overall estimate of R_h to R_s , because soils in this study were

sandy with excessive drainage and the presence of the root exclusion tubes increased soil moisture on the final measurement date by only about 1%. On the other hand, the impact of root severing on the soil microbial community composition and the rate of R_h from ectomycorrhizae associated with longleaf pine roots was untested in this study, so further research is recommended to determine the incubation timeframe that minimizes treatment effects on the soil microbes, including ectomycorrhizae.

Only two known direct estimates of R_h or R_a in longleaf pine forests have been previously published, one with a novel buried root isolation chamber for *in situ* measurement of R_a that cannot be compared to this study as they did not partition R_s into its autotrophic and heterotrophic components [26]. Rather, Cheng *et al.* [26] directly measured R_a on a per root mass basis. The other study provided estimates of the ratio of R_h to R_s based on comparison between root exclusion tubes and control collars after 45 days post-treatment in longleaf pine forests in Georgia [14]. Collins [14] measured R_h with shallow 10 cm deep root exclusion tubes and found that R_h to R_s ranged from 90% to 96% in longleaf pine stands on sandy soils and from 85% to 88% in stands on clayey soils, compared to our 60 cm deep tubes and uncorrected ratio of 82%. In our study, 80% of live roots were in the top 30 cm of the soils and live roots were present down to 60 cm. As such, the relatively shallow root exclusion technique of Collins [14] may have overestimated R_h compared to our estimates.

A significant decrease in shallow live roots and increase in shallow dead roots was observed within root exclusion tubes, and not accounting for CO_2 efflux from the death and decay of severed roots may therefore overestimate R_h [7]. Incorporating an estimate of root decay ($k = 0.52 \text{ year}^{-1}$) resulted in an approximate 15% decrease in the proportion of R_h to R_s . Accounting for root decay resulted in a 2% to 24% reduction in R_h to R_s ratios in previous studies reviewed by Subke *et al.* [7], and root decay rates cited in Subke *et al.* [7] ranged from $k = 0.14$ to 0.96 year^{-1} (mean $k = 0.32 \text{ year}^{-1}$). Studies in deciduous ecosystems generally had higher cited root decay rates and larger decreases in the ratio of R_h to R_s after incorporating root decay than coniferous ecosystem studies [7]. Root decay rates cited in other R_s partitioning studies not reviewed by Subke *et al.* [7] were $k = 0.5 \text{ year}^{-1}$ and $k = 0.3 \text{ year}^{-1}$ for a boreal black spruce (*Picea mariana* (Mill.) Britton, Stearns & Poggenb.) forest [17,27] and mountain Norway spruce (*Picea abies* (L.) Karst.) forest [9], respectively. Silver *et al.* [28] found that the assumed decomposition rate, which is the percentage of root C that gets oxidized and respired as CO_2 , can affect the estimated reduction in R_h to R_s ratio after incorporating root decay. We assumed a decomposition fraction of 1.00, like most studies reviewed by Subke *et al.* [7], but this may overestimate the fraction of root C that is respired. For instance, Heim *et al.* [10] found at most a 60% reduction in soluble loblolly pine root nonstructural carbohydrates 90 days after severing.

Subke *et al.* [7] performed a global meta-analysis of the proportion of R_h to R_s based on ecosystem type, biome, stand age, and R_h partitioning technique (e.g., root exclusion including trenching). Subke *et al.* [7] and others [29,30] found that the proportion of R_h to R_s varies with annual R_s levels. To make a comparison to Subke *et al.* [7], we assumed that annual R_s from these longleaf pine stands was similar to those measured in other nearby longleaf pine stands varying in age and structure (12 to $14 \text{ Mg} \cdot \text{C} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$) [31]. Our range in R_h to R_s corrected for root decay is comparable to the mean ratio of R_h to R_s ($60\% \pm 12\%$) from Subke *et al.* [7] across similar temperate coniferous ecosystems with annual R_s from 12 to $14 \text{ Mg} \cdot \text{C} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$. On the other hand, our root decay-corrected ratio is high compared against the mean ratio of R_h to R_s from studies that corrected for root decay in coniferous ecosystems (47%) [7]. However, Subke *et al.* [5] discusses how the majority of root decay-corrected ratios were derived from boreal forests, causing a bias towards lower mean R_h to R_s ratios than would be expected from temperate coniferous ecosystems.

The specific conditions of these longleaf pine ecosystems made this study ideal for a “proof-of-concept” examination of small-diameter root exclusion tubes to partition total belowground R_s into its heterotrophic and autotrophic components. These stands are managed with frequent prescribed burns (every 1–3 years) resulting in negligible litter layers and a reduced understory. Therefore, microbial respiration from the litter layer and herbaceous ingrowth in the root exclusion tubes were minimized, which are both potential sources of error in root exclusion studies [8,23].

As previously discussed, soils in these stands are deep sands, which facilitated the installation of the steel tubes to deeper depths than possible in more clayey soils and reduced the treatment effects on soil compaction and soil moisture. Under these conditions, root exclusion tubes decreased R_s by 18% to 34% and provided an estimate of longleaf pine R_h . However, because estimates of R_h are often specific to the ecosystem and ecological context (e.g., season, soil types, humus form, vegetation composition, management regimes, etc.) [2,7], further research across the native range of longleaf pine, which encompasses a range in soil types, understory composition, and climate, is necessary to gain a more complete understanding of the heterotrophic and autotrophic components of R_s in longleaf pine ecosystems.

5. Conclusions

Small root exclusion tubes were used for relatively rapid assessment of R_h and results highlight the importance of corrections for initial, pretreatment variation in R_s and CO_2 loss from root exclusion tubes resulting from treatment effects on root decay. The ratio of R_h to R_s in longleaf pine varied from approximately 66% to 82%, depending upon the estimation method, with apparent overestimations of R_h occurring when root decay in the root exclusion tubes and pretreatment R_s variation were not considered. Although root exclusion tubes increased soil moisture by about 1%, R_h corrected for soil moisture changed only in uncertainty but not in magnitude. The results of this study demonstrated that small diameter root exclusion tubes might supply reliable estimates of R_h as early as 40 days post-treatment and up to at least 104 days post-treatment. A high replication of small root exclusion tubes staggered over time when coupled with a better understanding of the influence of soil and vegetation type on R_h may provide a means to estimate changes in R_h through time in response to forest management and climate.

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