Canadian Journal of Forest Research
Revue canadienne de recherche forestière

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Journal:	<i>Canadian Journal of Forest Research</i>
Manuscript ID	cjfr-2015-0097.R2
Manuscript Type:	Article
Date Submitted by the Author:	26-Sep-2015
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Keyword:	soil carbon, leaf litter, root, Pinus taeda L., soil size fraction

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Article Title:

Effects of loblolly pine litter, forest floor and root exclusion on mineral soil carbon in a Florida Spodosol

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Article Title:**Effects of loblolly pine litter, forest floor and root exclusion on mineral soil carbon in a Florida Spodosol****Abstract**

Quantifying soil organic carbon (SOC) inputs in the surface soil is a critical component for assessing the potential for C sequestration of managed pine forests. This study used a sequential exclusion of aboveground litter inputs (L=litter exclusion) and above plus belowground inputs (LR= litter and root exclusion) to segregate C sources contributing to the development and maintenance of SOC in the surface soil supporting juvenile loblolly pine (*Pinus taeda* L.) in its rapid growth phase. The study spanned the 7th to 10th year of stand growth. Soil physical size fractions (>2 mm, ≤2mm, 2000-250 µm, 250-150 µm, 150-53 µm, and <53 µm) were used to investigate the change in native SOC over time in the untreated control plots (UC=untreated control) and the effects of exclusion treatments. An accretion rate of 4.6 Mg SOC ha⁻¹ soil yr⁻¹ was observed in the fine earth fraction (≤2 mm), reflecting the rapid phase of stand growth. The accretion was primarily observed in the upper 10 cm of the soil. Treatment effects were most apparent in soil bulk density, SOC of the fine earth, and 150-53 µm size fractions. In general, changes in SOC observed in the L treatment was an intermediate increase between the UC and LR treatments; where only the removal of roots provided no change in SOC and was significantly different from the control ($p = 0.05$). We conclude that a major contributor to the maintenance and increase of SOC in

39 this fast-growing pine ecosystem was due to root turnover (60%), with 40% due to
40 above-ground litter inputs.

41

Draft

42

43 **Abbreviations**

44 BD bulk density

45 C carbon

46 cm centimeters

47 d day

48 ha hectare

49 Kg kilogram

50 L aboveground litter exclusion treatment

51 LOI loss-on-ignition

52 LR above- and belowground litter exclusion treatment

53 m meter

54 Mg megagram

55 μg microgram

56 mm millimeter

57 NaOH sodium hydroxide

58 s.e standard error

59 SOC soil organic carbon

60 UC untreated control

61 **Key Words**62 Carbon accretion, leaf litter, mineralization, *Pinus taeda* L., soil size fraction

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Introduction

Soil organic carbon (SOC) content is a keystone soil quality parameter and plays a significant role in the global carbon cycle. Yet, the role of root turnover and leaf litter in the maintenance and accretion of SOC across a range of forest/soil types is not well documented. The lower Coastal Plain of the southeastern United States supports 12 million ha of natural and planted loblolly pine (*Pinus taeda* L.) stands (Baker and Langdon 1990), making it a dominant cover type in in this region. In addition, there are 5.7 million ha of Spodosols (Adegbidi et al. 2002) covering the lower Coastal Plain, which contain some of the largest soil organic carbon stocks in the southeastern United States (Stone et al. 1993). These soils frequently support highly productive pine plantations through intensive silvicultural practices, i.e. genetic selection, fertilization, and understory suppression (Vogel et al. 2011). In contrast, these cover type/soil type systems' potential for increasing belowground C storage has been described as limited (Shan et al. 2001), due to the excessively sandy, weak-structured soil surface horizons (Richter et al. 1999; Six et al. 2002).

Recent investigations have studied the nature of SOC pools in these forested soils. Sarkhot et al. (2007a, b) revealed the presence of sand-sized aggregates, with increasing degrees of stability and identifiable C pools of various chemical composition. They reported that only some SOC pools were responsive to management in the short term. Therefore, understanding the development and maintenance of SOC under this cover type/soil type may capitalize not only on the economic worth of pine commercial products, but also include the value of SOC storage.

The quantitative contributions of C sources to the formation of SOC pools is being addressed (Rasse et al. 2005) with specific reference to climate change scenarios. Recent advances in the understanding of terrestrial C cycling, particularly below-ground processes, have come from the employment of long-term exclusion studies, the use of stable isotopes, isotopic labeling and molecular-markers (Johnston et al. 2004; Kuzyakov 2011). The simple approach of using prolonged forest floor removal has been useful in estimating the role leaf litter plays in the formation and maintenance of stable SOC pools. Garten (2009) used prolonged O horizon removal (4.5 years) to show that the aboveground litter of a Tennessee temperate hardwood forest was not a significant source of SOC. Likewise, results from another litter and root manipulation study in place for 5 years in a northeastern hardwood forest, indicated that SOC was not affected by either aboveground litter additions or litter removals (Nadelhoffer et al. 2004).

The potential for aboveground sources of C to contribute to SOC is limited when there is not a mechanism for their biomass to be incorporated into the soil (Quideau et al. 2001). In contrast, large amounts of photosynthetically fixed C are drawn into the soil matrix through root growth and exudations (Farrar et al. 2003, Godbold et al. 2006). A study of intensively managed slash pine (*Pinus elliottii* var. *elliottii* Engelm.) plantations showed that 10% of the total annual primary production was devoted to fine root production, while fine roots only constituted about 2% of the total tree biomass (Shan et al. 2001), indicating significant turnover rates and inputs of organic carbon to the soil. The microenvironment of fine roots supports large microbial populations and their persistent cell fragment residues (Kindler et al. 2006 and 2009; Simpson et al. 2007; Miltner et al. 2011). Additionally, excretions from root tips and mycorrhizae have

hydrophobic properties that are important for soil aggregate formation and stability (Oades 1978; Czarnes et al. 2000).

The overall objective of this study was to explore aspects of the short-term development, maintenance, and fate of SOC in the surface mineral soil of a Spodosol supporting an intensively managed loblolly pine plantation. The soil at the surface provides the best perceived opportunity to observe the most significant impact of the O horizon and fine root turnover in SOC dynamics. The specific objectives of this study were to (1) investigate the short-term whole soil and size fraction changes in SOC naturally occurring to characterize the rate of below-ground accretion in surface soil during the rapid growth phase of juvenile loblolly pine; (2) determine the SOC distribution and inherent mineralization rates of different soil size fractions to determine which size fractions are vulnerable to short-term microbial decomposition; and (3) investigate the short-term cumulative effects of above- and belowground C inputs on SOC pools via a sequential exclusion experiment.

Materials and Methods

Experimental Site

The study site was nested within a larger field study owned by Rayonier Inc. and designed by the Forest Biology Research Cooperative at the University of Florida. The site was located northwest of Waldo, FL, USA (29.80° N, 82.21° W). A full description of the study site was provided by Roth et al. (2007). The research was conducted in a densely planted loblolly pine stand. The native ecosystem existing prior to commercial tree production was identified as an upland pine flatwoods ecosystem of the

southeastern U.S. lower Coastal Plain region. It is characterized by flat, wet, low-lying topography with poorly drained, acidic, deep sandy soils. The native ecosystem is maintained by periodic fire events and is described as open, wet pine woodland with a thick understory, and is commonly used for commercial pine plantations. The study site is a typical representation of this specific regional cover/soil type.

The NRCS soil series for the study area was mapped as Pomona fine sand (sandy, siliceous, hyperthermic Ultic Alaquod). In general, the typical soil profile consists of a sandy, A horizon (10YR 3/1) approximately 13 cm thick extending to a lighter colored, leached E horizon (10 YR 6/1 to 7/1) with an approximate thickness of 47 cm. A well-developed Bh (spodic) horizon occurs from 60 to 90 cm below the surface and a Bt (argillic) horizon begins at approximately 130 cm. The soil profile is very strongly acidic, pH ranges from 4.5 to 5.0.

The water table is within 25 to 100 cm from the surface for 6 months or more during most years (Soil Survey Staff, 2011). During the study period, monthly mean temperatures ranged from an average low of 13° C during January and February to an average high of 27° C during June, July, and August (NCDC, 2011). At the initiation of the study in May 2007, the region was experiencing extreme drought conditions. Although these conditions improved over the duration of the study, the study period was classified as having abnormally dry conditions (U.S. Drought Monitor website, 2011). Annual mean precipitation documented at the Gainesville Regional Airport, 13 km from the study site, was 890, 1170, 1008, and 1191 mm for years 2006, 2007, 2008 and 2009, respectively. The long term annual mean is 1242 mm for the years 1960-2010 (NCDC, 2011).

Following the clear cut harvest of the mature pine plantation, the land was bedded and the current loblolly pine stand was planted in January 2000. Of the original study design described by Roth et al. (2007), only the narrow spacing, high culture treatment in each of the three blocks was used in this study. The three study blocks were in close proximity to one another, with the greatest separating distance being approximately 500 m. The planting density was 2,990 stems ha⁻¹, resulting in 128 trees per 0.04 ha treatment plot. The pine stand received understory competition control and 660 kg ha⁻¹ of 10-10-10 N-P-K fertilizer plus micronutrients at the time of planting (Roth et al. 2007). This treatment was managed for optimal growth by using weed control and an intensive fertilizer regime based on annual foliar analyses. This created and maintained an open understory throughout the experimental treatment plot described above. At age 10 yrs volume accumulation averaged approximately 258 m³ ha⁻¹ (Jokela, unpublished data).

In Situ Field Treatments

The data from this study were based on three *in situ* field treatments established within the original replicated narrow spacing, high culture treatment described above. Within each original study treatment, the three *in situ* field treatments were established in the interbed region (the area between bedded planed rows) of the three center rows using a randomized complete block design. Each randomly assigned *in situ* treatment plot was a permanently marked 0.5 m by 1 m area (corners were marked with 1.3 cm diameter PVC pipe). Each of the three interbed regions within a study treatment plot received one randomly placed replication of each of the three *in situ* treatments.

Two of the three *in situ* treatments were designed to prevent C inputs from entering the soil. The first *in situ* treatment eliminated the influence of the aboveground sources of C and is identified as treatment “L”. This was accomplished by removing the entire O horizon at the study initiation and keeping the soil bare during the entirety of the study. An additional 0.5 m of O horizon was removed from the perimeter of the designated treatment plot area to reduce the O horizon’s influence at the edge, giving a 1.5 m by 2 m total area of excluded O horizon. The entire 1.5 m by 2 m bare soil area was covered with shade cloth; aboveground litterfall, plus any sprouting vegetation under the cloth, was removed on a monthly to bimonthly schedule.

The second *in situ* treatment eliminated all inputs from aboveground litter and root growth (treatment “LR”). In addition to O horizon removal and litterfall exclusion, a lined trench surrounded the treatment plot to prohibit new and existing root growth within the plot. This was accomplished using a 40 cm deep trench, 0.5 m from the perimeter of the treatment plot, lining it with aluminum sheet metal, and back filling with soil.

The untreated control (UC) was a marked 0.5 m by 1 m area representing the natural soil state of the loblolly pine plantation. Canopy closure in these stands was reached after approximately five years of growth. At study month 0 the trees were in their 7th year of growth and by the end of the study, 31 months later, they were entering their 10th year.

199

200 *Soil and Litterfall Sampling*

201 Soil samples were taken from the soil surface to 20 cm below the surface, a soil
202 depth that included the mixed A horizon and portions of the underlying E horizon. Soil
203 samples were collected at the initiation of the study in May 2007 from the control plots
204 to establish the initial C content. A 1.9 cm diameter soil push probe was used to collect
205 eight soil samples from 0 to 10 cm and 10 to 20 cm, and composited for each block to
206 provide a covariate for the initial conditions of the treatment plot. Soil samples were
207 subsequently collected after 31 months of *in situ* treatment. To maintain the integrity of
208 the *in situ* plots for future work, three soil subsamples per *in situ* treatment plot were
209 collected with a 6.5 cm diameter aluminum cylinder, separated into 0 to 10 cm and 10 to
210 20 cm soil depths, and composited by soil depth.

211 At the initiation of the study the forest floor was removed from the L and LR plots,
212 oven dried at 70°C and weighed. These *in situ* treatment plots were then covered with
213 shade cloth to prevent litterfall inputs and to buffer any temperature affects due to litter
214 removal. Thermometers placed under the shade cloth and litter layers for the first few
215 months of the study showed no temperature differences by treatment. Litter was
216 collected monthly from the L and LR plots, oven-dried and weighed.

217 An unanticipated commercial pine needle harvest took place in the fall of 2008.
218 The majority of the litter was inadvertently removed from the study areas, including the
219 untreated control plots. However, the commercial process of harvesting pine litter
220 focuses on raking the seasonal needle fall that occurs in the autumn months
221 (September-November) while leaving the more established forest floor intact. In order to

restore the natural condition, newly fallen forest floor material was gathered from a nearby (approximately 300 m) undisturbed loblolly stand of the same age and replaced on the control plots. The initial forest floor mass and measured litterfall were used to reestablish the forest floor on the untreated control plots.

Live Root Observations

To ensure that root exclusions successfully eliminated new root growth, the distribution of fine roots was evaluated at the end of the study in all *in situ* treatment plots. A field method described by Escamilla et al. (1991) based on the relationships among fine root biomass, root length density, and the number of roots crossing a two-dimensional plane was used. A vertical plane of soil was exposed in each treatment plot by trenching. Fine roots protruding from the soil face were counted in a 30 cm horizontal area at 5 cm vertical increments to 20 cm.

Laboratory Methods

Soil fresh mass and moisture content were determined. Bulk density was calculated from the soil volume and mass, and corrected for moisture content. The samples were subsequently air-dried and passed through a 2 mm mesh sieve. One-hundred g of the air-dried fine earth fraction soil was then passed through a column of sieves for 5 minutes, using a horizontal shaker and following the method of Sarkhot et al. (2007a). The sieves fractionated the soil into 2000 to 250 μm , 250 to 150 μm , 150 to 53 μm and <53 μm size fractions. Total organic carbon in the coarse size fraction, the fine earth fraction, and the four mineral soil size fractions was measured by loss-on-

ignition (LOI) and then converted to organic C. The C conversion was based on a pedotransfer function developed from 133 soil samples taken from an adjacent watershed study and described in Azuaje et al. (2012).

Mineralization of SOC in each size fraction ≤ 2 mm was accomplished by using the untreated control soil sampled at 31 months. Twenty grams of air-dried soil from the three larger size fractions and 5 g of the < 53 μm size fraction were moistened to a water holding capacity of -33 kPa, a soil moisture condition relative to field capacity, and placed in a microcosm (container) made of high density polyethylene.

The rate of C mineralization was determined by measuring soil respiration using as alkaline trap method (Anderson, 1982). A liquid based trap of 0.25 M NaOH was securely placed inside the microcosm of the well-mixed, moist soil samples. The microcosms were then placed in a dark, 30°C incubator for a total of 162 d. Moisture content was maintained throughout the study by monitoring the microcosms' weight.

Statistical Methods

Specific measurements of changes in SOC occurring in the UC treatment were analyzed to evaluate the native condition of SOC. The variables evaluated were bulk density, coarse fraction SOC, fine earth fraction SOC, SOC in individual soil size class fractions, and size fraction CO_2 mineralization. The relative percent change over time, defined as the percent change in the variable between the end of the study (T_2) and the beginning of the study (T_1), was used to analyze the effects of the field treatments on bulk density, coarse fraction SOC, fine earth fraction SOC, and SOC in individual soil size class fractions. This approach was required to minimize the uneven variability

determined to be present among treatment plots (Bonate 2000). When necessary the data were log transformed to fit normal distribution patterns prior to statistical analyses.

The PROC MIXED model (SAS; Littell et al., 2006) was used to analyze the data, with the inclusion of the fixed factors of soil depth, fraction, time, treatment and interactions of fixed factors. In addition a random effect of plot was included to model the correlation that exists between the observations made at the two soil depths within each treatment plot. Observations were modelled by an autoregressive error structure. Means were considered significantly different at $\alpha \leq 0.1$. This level of significance was chosen based on soil heterogeneity that is particularly inherent in forest soils, making detection of changes *in situ* SOC more difficult (Haines and Cleveland, 1982; Richter et al. 1999; Conant et al. 2003). Conant et al. (2003) found that resampling micro-plots over short periods of time in forest soil was effective, and that using a lower level of significance was appropriate. When significant main effects and/or interactions were found, multiple mean comparisons and separations were identified using Tukey's HSD test.

Results

By age 7 yr the forest floor had accumulated a total of 12.7 Mg ha⁻¹ of litterfall. The rate of litterfall statistically increased as the stand aged from 7 to 10 yrs ($p = 0.02$). The total litterfall mass collected during Year 8 was 5.7 Mg ha⁻¹, compared to 9.6 Mg ha⁻¹ during Year 10, a 68% increase in annual litterfall. Total litterfall over the entire 31 month study was 22.2 Mg ha⁻¹.

291 *Objective 1. Short-term changes in SOC content under fast growing pine*

292 Soil bulk density (BD) increased with soil depth from 0.93 g cm⁻³ in the 0-10 cm
293 soil depth to 1.26 g cm⁻³ at 10-20 cm, with no significant change in bulk density over the
294 31 months of study. Bulk densities were used to calculate SOC expressed on a soil
295 volume basis.

296 The coarse fraction (>2 mm) SOC had a significant interaction between sampling
297 time and soil depth ($p = 0.06$). Measurements taken at the initiation of the study were
298 significantly different among soil depths, with the 0-10 cm soil depth containing 2.1 Mg
299 SOC ha⁻¹ and 3.2 Mg SOC ha⁻¹ in the 10-20 cm soil depth. At the end of the study
300 period SOC content was 3.1 and 2.3 Mg SOC ha⁻¹ in the 0 to 10 and 10 to 20 cm soil
301 depths, respectively (Fig 1).

302 The SOC (Mg ha⁻¹) in the fine earth fraction (≤ 2 mm) in the 0-10 cm soil depth
303 increased by 80% from 12.2 to 21.9 Mg SOC ha⁻¹ (s.e. =2.8), while no significant
304 change in SOC was evident at the 10-20 cm soil depth (7.5 to 9.5 Mg SOC ha⁻¹; time x
305 sampling depth interaction, $p = 0.10$; s.e.=2.8; Fig 1). The SOC in soil size fractions,
306 averaged across both sampling times, exhibited some differences among size fractions
307 and soil depth, $p = 0.01$ (Fig 2). SOC was greatest in the 2000-250 μ m fraction of the 0-
308 10 cm layer, while the three smaller size fractions had similar SOC contents. SOC
309 decreased with soil depth for the largest and smallest size fractions. The 2000-250 μ m
310 fraction of the 0-10 cm layer contained 44% of the total soil C in that layer. The most
311 striking difference among size fractions in the 10-20 cm layer was that the 2000-250 μ m
312 fraction was much lower than its 0-10 cm counterpart, and also accounted for less of the
313 total C (30%).

Objective 2. Mineralization potential among soil size fractions

There was an interaction between soil depth and size fraction for specific mineralization rate ($\text{mg C respired g}^{-1} \text{ size fraction C d}^{-1}$) ($p = <0.01$). For the 0-10 cm layer, the specific mineralization rate of the $<53 \mu\text{m}$ fraction was statistically lower (over 25%), with the 150-53 μm fraction intermediate in value (Fig 3). In comparison, mineralization rates at the 10-20 cm layer were similar among all size fractions. Comparing the same size fraction among layers, the significant difference was in the $<53 \mu\text{m}$ fraction, where the 10-20 cm layer was approximately 28% higher than the 0-10 cm layer.

Bulk C mineralization rates were also calculated on a whole soil basis ($\text{mg C respired kg}^{-1} \text{ fine earth fraction d}^{-1}$) to provide the potential relative contribution by each size fraction and soil depth to whole soil ($<2\text{mm}$ fine earth fraction). There was an interaction between soil depth and soil size fraction ($p < 0.01$). The 0-10 cm soil depth had more mineralizable C than the 10-20 cm layer, except for the $<53 \mu\text{m}$ fraction, which was not different between soil depths.

Objective 3. Eliminating fresh C inputs and its effect on SOC development and maintenance

The LR *in situ* treatment significantly reduced the presence of live roots ($p < 0.01$), with a reduction of 75% and 92% at the 0-10 cm and 10-20 cm soil depths, respectively (Fig 4). While root exclusion was not complete, it greatly reduced the presence and hence influence of root turnover on SOC during the study period. Root

numbers in the 10-20 cm depth of the UC treatment were 4 times less than the 0-10 cm depth, illustrating the importance of the near surface soil in fine root development and potential turnover.

At the end of the 31 month study period, the LR treatment had a soil bulk density increase (7%), while the UC treatment remained unchanged. This resulted in significant differences between these two treatments ($p = 0.05$), with the L treatment being intermediate between UC and LR. These bulk densities were used to calculate SOC on a soil volume basis.

The initial distribution of SOC (Mg ha^{-1}) within the three treatments blocks was skewed with higher levels of SOC in the L and LR treatments versus the UC treatment (Table 1). For this reason, treatment effect was based on the relative change in SOC over the 31 month study period. There was no net increase in SOC of the fine earth fraction (≤ 2 mm) in the LR treatment of the mean SOC content in the surface 20 cm. The UC treatment had an increase in the surface 20 cm of approximately 55% in the mean SOC over the 31 month study period (Table1). Statistical analysis of the treatment effects were evaluated on the relative % change of SOC content and differences among treatment were significant ($p = 0.05$) and reported a positive 45% increase in the UC treatment. This change was only evident in the 0-10 cm layer (Table 1). Soil OC in the L treatment was not statistically different than either the UC or LR treatments (Table 1), while the LR treatment demonstrated a reduction in SOC accumulation compared to the UC treatment. In fact, the LR treatment showed no SOC increase over the study period.

The SOC accumulation in the UC treatment was primarily found in the 0-10 cm soil depth, 9.7 Mg ha^{-1} , and secondarily in the 10-20 cm soil depth, 2.0 Mg ha^{-1} . Through evaluation of changes in SOC content over time as effected by treatment and soil depth (Table 1), the influence of litter and roots on the change in SOC observed in the UC plots could be estimated. The above ground litter influence in the 0-10 cm soil depth was estimated from the difference between the change in the UC treatment plots and the L treatment plots, $\Delta\text{UC} - \Delta\text{L} = 3.7 \text{ Mg ha}^{-1}$. This represented 38% of the total change in SOC in the 0-10 cm soil depth over the study period. The influence of roots in the 0-10 cm soil depth was estimated by subtracting change in the L treatment plots from the difference of the UC and LR treatments plots, $(\Delta\text{UC} - \Delta\text{LR}) - \Delta\text{L} = 5.9 \text{ Mg kg SOC ha}^{-1}$; a 61% change of SOC due to the absence of roots.

The effects of exclusion treatments on soil size fractions are shown in Figure 7. Treatment was a significant main effect in the coarse fraction ($>2 \text{ mm}$) ($p = 0.06$), where L showed a significantly greater % increase than LR. The LR SOC decreased during the study but no significant change occurred in the UC or L treatments. The SOC from the 2000-250 μm and 250-150 μm size fractions was not significantly affected by treatment. Over 40% of SOC was lost from the 150-53 μm size fraction due to the LR treatment ($p = 0.02$). There was no treatment effect on SOC in the UC and L plots. The L treatment, due to high variability, did not differ from either the UC or LR treatments.

There was a treatment by soil depth interaction ($p = 0.04$) for the smallest soil size fraction class ($<53 \mu\text{m}$), where the UC treatment more than doubled in SOC content in the 10 to 20 cm soil layer (Fig 5). Both the L and LR treatments had lower increases in

SOC changes, indicating a possible response to litter removal but not to root removal in this soil fraction. There were no treatment effects at the 0-10 cm soil depth.

Discussion

Objective 1. Short-term changes in SOC content under fast growing loblolly pine

Intensive management of genetically improved loblolly pines in the US lower Coastal Plain enhances biomass production throughout the rotation (Martin and Jokela, 2004; Vogel et al. 2011). The rate of net primary production typically peaks during the developmental age range observed in this study. In nearby stands of similar genetic quality that received similar management inputs, aboveground net primary production was reported by Martin and Jokela (2004) to be greatest (approximately $22.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) between ages 8-10 yrs.

Eight to ten years of age is an important phase of stand development for net primary production. The highest rate of increase in annual needlefall occurs during this phase (Gholz et al. 1985; Jokela and Martin 2000) and needlefall rates typically level off at around age 10, when leaf area index culminates. With an assumed correlation between needlefall rate and fine root turnover, one's expectation is that this would also be the phase of greatest SOC accumulation. However, this has never been addressed, particularly in these sandy soils, even though data on relative SOC changes during this phase would be useful to help validate carbon models.

SOC accretion in the fine earth fraction at the 0-20 cm soil depth, $4.6 \text{ Mg SOC ha}^{-1} \text{ soil yr}^{-1}$, was over 50% greater than the estimated SOC accretion averaged over the entire rotation of a loblolly pine stand under similar management, soil type, and

genetic quality ($3.0 \text{ Mg SOC ha}^{-1} \text{ soil yr}^{-1}$ in the 0-20 cm of soil depth; Vogel et al. 2011). Compared to other tree species, these accretion rates appear as high as any and higher than most. Other studies of loblolly pine stands planted following clear cut harvest in the southeastern U.S. lower Coastal Plain region report surface soil C annual accretion rates of 0.5 Mg ha^{-1} in an 11 yr old well drained, low fertility site (Leggett and Kelting 2006) and rates of 6 and $11 \text{ Mg SOC ha}^{-1} \text{ yr}^{-1}$ in the 0-30 cm soil depth of 5 yr old planted loblolly pine stands, without and with understory suppression, respectively, in poorly drained sites with intensive site preparation (disking, bedding, and fertilization (Laiho et al. 2001). The relative high rates reflect the incorporation of the litter layer during stand establishment and the SOC observations were to the 0-30 cm soil depth. Soil moisture, site preparation and stand management appear to be an important factor in determining the fate of SOC development and maintenance.

Compared to forests planted in previous land uses other than clear-cut forest the range of SOC accretion rates were not as high. Young post-agricultural forests in New Jersey accrued at annual rates of 0.1 to $0.3 \text{ Mg SOC ha}^{-1}$ (Lathrop et al. 2011) and $0.07 \text{ Mg SOC ha}^{-1}$ were reported in 34 yr old post-agricultural loblolly pine in the South Carolina Piedmont region (Richter et al. 1995). In the Pacific Northwest of the USA, soils under Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) had reported accretion rates ranging from 0.6 to $4.1 \text{ Mg SOC ha}^{-1}$ (Adams et al. 2005); Scaled estimates of carbon accretion in forest soils of Europe were dependent on how they were modeled but ranged from 0.2 to $0.9 \text{ Mg SOC ha}^{-1}$ (cited in De Vries et al. 2006); while tree species in India on new mine spoils accrued SOC in the first 4-5 years at rates of 0.1 to $1.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ (Singh et al. 2006). A review of long term experiments have shown that

soil carbon can accrete under forests at rates of 0.5 to 2.0 Mg ha⁻¹ y⁻¹ (Dixon et al. 1994). Rates measured in this study put this phase of development for this ecosystem in a range of the higher rates for soil carbon accretion in the reviewed literature and may be a result of intensive site preparation, fertilization, understory control and poorly drained soils.

Objective 2. Carbon distribution and mineralization potential among soil size fractions

The SOC distribution among fractions within a soil depth was similar to other studies on similar soils, being more concentrated at the soil's surface, and greatest in the 2000-250 µm fraction largely as particulate organic matter (Fig 2; Haile et al. 2007b; Sarkhot et al. 2007b). This exemplifies the small amount of reactive clays and oxides in the soil's mineralogy and a concentration of fine roots and biological activity at the soil's surface in these ecosystems (Van Rees and Comerford 1986; Gholz et al. 1986; Sword 1998; Fierer et al. 2003). This further highlights the need to understand these dynamics in the surface mineral soil.

The specific SOC mineralization of each size fraction was evaluated with the intent to better interpret the SOC changes in objective 3 when the carbon sources for maintenance and accretion of SOC were removed (Fig 3). To the extent that specific mineralization rates measured under controlled conditions reflect SOC quality, the results indicated that SOC mineralization rate, hence quality, showed little differences among size fractions within or between soil depths.

The most sizeable differences in quality were in the 2000-250 µm fraction of the 0-10cm layer and the <53 µm fraction in the 10-20 cm layer. The explanation for the former difference is that this is the size fraction that receives the greatest amount of

recent SOC from various sources (Sarkhot et al. 2007b) with a high mineralization potential. Yet, this explanation did not hold for the same fraction in the zone just 10 cm below. This study does not provide the reason for this, but it is consistent with the accretion of SOC in this fraction at 10-20 cm (see untreated control treatment in Fig 5) and the lack of accretion, presumably due to enhanced mineralization, in the 0-10 cm layer. It also implies that the SOC input to the 2000-250 μm fraction at the lower layer is of lower quality; a hypothesis that can be tested in future studies.

The <53 μm fraction has been shown to be comprised of more decomposed and recalcitrant material as well as lesser amounts of freshly added organic matter (Sarkhot et al. 2007b), but that does not explain why the two layers would be significantly different for the same size fraction. These data suggest that a better understanding of SOC cycling within and among size fractions, and how SOC moves between size fractions is warranted.

In general the total soil carbon mineralization from each size fraction was a reflection of the total SOC contained in the fractions and relatively homogeneous mineralization quality of the C located in the fractions. The sum of the bulk soil mineralization rates for all the fractions in the 0-20 cm layer, $9.5 \text{ mg kg}^{-1} \text{ d}^{-1}$, was at the upper end of a range (3.4 to $10.6 \text{ mg C kg}^{-1} \text{ d}^{-1}$) reported by Ahn et al. (2009) for SOC located in the top 30 cm of whole soil in similar Coastal Plain Spodosols in north central Florida. Ahn et al. (2009) utilized similar C mineralization assessment methods as this study by incubating soil samples at 35°C at -33 kPa water holding capacity and measuring CO_2 respiration over an 87 d study period.

Objective 3. Eliminating fresh C inputs and its effect on SOC development and maintenance

Contributions of the total C input from various sources into the surface soils supporting loblolly pine plantations are influenced by stand conditions (i.e. genetic quality, stand age, planting density, site fertility, and light and water availability; (Albaugh et al. 1998; King et al. 1999; Burkes et al. 2003)). The contributions can be segregated into C originating from aboveground and belowground sources.

The reported annual litterfall rates for mid-rotation loblolly pine range from 3.0 and 7.0 Mg ha⁻¹, depending on management strategies (Jokela and Martin, 2000). The accumulation of mass in the forest floor is influenced by the long residence time characteristic of pine litter and minimal faunal soil mixing and incorporation of aboveground C residues into the surface mineral soil (Thomas 1968; McBrayer et al. 1977; Piatek and Allen 2001). Most aboveground litter is thought to be utilized by microorganisms that produce enzymes to digest this recalcitrant material (Jorgensen et al. 1980; Berg and McClaugherty 2003; Bardgett et al. 2005). As the forest floor develops with age more advanced stages of decomposition occur and it becomes increasingly populated by fine roots and mycorrhizal fungi (Gholz et al. 1986; Ponge 1991). In the next biotic level observations of meso- and macrofauna in the A horizon of southern U.S. flatwoods pine are primarily mites, ants, and beetle larvae. The frequency of their occurrences has been described as rare when compared to other forests types (Phillips and Fitzpatrick 1999) and characterizes the limited soil mixing events.

The primary C contribution made by the forest floor is presumed to be through DOC leaching. By mid-rotation, the forest floor begins to develop distinct Oi and Oe

horizons. Polglase et al. (1992) reported total labile C concentrations in the Oi and Oe layers as 14% and 6% by weight, respectively.

Utilizing available literature and data from this current study, estimations of annual C inputs and outputs to the surface soil can be made. Forest floor mass at the beginning of this study was 13.0 Mg ha⁻¹. The mean annual aboveground litter additions were 8.6 Mg ha⁻¹ yr⁻¹, which included needles and some coarse woody debris. Loblolly needles have been measured to release approximately 25% of the C within the first year, followed by a long period of slow release (Jorgensen et al. 1980; Gholz et al. 1985). In this study, the maximum annual C release from fresh needlefall would have been approximately 1.1 Mg C ha⁻¹. This assumes that the entire aboveground litter mass was needles, which likely is an overestimation as a small portion of coarse woody debris was collected with the needlefall.

From the available literature of mature loblolly pine stands, the annual contribution of DOC leaching from the bottom of the O horizon was estimated to add 0.3 Mg C ha⁻¹ of DOC (Richter et al. 1995, 1999; Dosskey and Bertsch 1997); a relatively small amount of the total. By mid-rotation, the forest floor of southern pines is only beginning to develop discrete levels of decomposition. If indeed the source of forest floor DOC is from more decomposed material, this may be an over estimation of C input.

Recent studies have questioned the degree of participation of the O horizon in mineral SOC formation. A disconnection between the C of recently added forest litter and the mineral soil has been observed in Tennessee pine-hardwood forests by tracing the decay of C-14 released into the atmosphere from a nearby incinerator (Froberg et al. 2007b). The authors determined that, after four years of various manipulations of C-

14 enriched C additions, only 14% of the total DOC in the top 15 cm originated from the C-14 forest floor litter. Similar results were found with C-13 isotope labeling to trace DOC from fresh C into the soil (Hagedorn et al. 2004). Hagedorn et al. (2004) found that 5-10% of the labeled DOC at the 5-10 cm soil depth had originated from fresh litter and recent rhizodeposition. The authors also determined that recently deposited DOC was preferentially mineralized in a soil incubation study. The majority of DOC that reached the mineral soil surface was thought to originate from the more decomposed Oe horizon, while more recently released DOC was mineralized in the forest floor (Froberg et al. 2007a).

It is possible, to some degree, that nutrient cycling in the forest floor is decoupled from the mineral soil in southern pine ecosystems. By mid-rotation, the forest floor in loblolly pine has been reported to immobilize nitrogen and phosphorus (Piatek and Allen 2000). This may be an indication of microbial populations in the forest floor utilizing the labile C released from the fresh litter inputs. Results of these findings correspond with radiocarbon data that identified the age of DOC in forested surface soils around 20-30 yrs and its presence in the soil related to microbial processes (Trumbore et al. 1992; Guggenberger et al. 1994; Tegen & Dörr 1996).

When the forest floor was excluded from adding C to the soil in this study, the SOC was suppressed but not significantly different than the untreated control plots. This suppressed effect may be an indication the ecosystem's high precipitation rate and poorly drained soil is elevating the microbial activity in the O horizon (Hass et al. 2010). In more poorly drained landscapes, where the forest floor litter is more consistently moist and under more persistent decomposition, loblolly pine litter may have a greater

543 opportunity as a C source to SOC development. Although data were not presented by
544 block in this study, it was noted the study's most poorly drained block showed the
545 largest negative affect on SOC development in the aboveground exclusion treatment
546 plots.

547 However, the absence of a significant difference between the litter exclusion plots
548 and the untreated control plots supports the perception the O horizon plays a limited
549 role in SOC development at the near surface mineral soil layers. Corresponding forest
550 soil studies that used prolonged O horizon removal in several temperate deciduous
551 forest ecosystems found minimal or absent effects on the surface mineral SOC
552 (Nadelhoffer et al. 2004; Garten 2009; Kramer et al. 2010). In addition, southern pine
553 litter raking studies report surface SOC to be unaffected after five to seven years of
554 consecutive litter removal (Ross et al. 1994; Blazier et al. 2008).

555 In contrast the success of the soil trenching method in inhibiting new fine root
556 growth prevented the SOC accretion that was experienced in the untreated control
557 plots, indicating a primary dependence on subsurface processes. The trenching method
558 may have temporarily elevated the SOC accretion as the existing roots decompose,
559 creating an underestimation of the dependence on subsurface sources of C. Short-term
560 effects of eliminating the above- and belowground inputs of C resulted in higher soil bulk
561 density and a loss of SOC in the coarse ($> 2\text{mm}$) and $150\text{-}53\ \mu\text{m}$ fraction (Table 2, Fig
562 5).

563 This study measured a 55% increase in SOC in the fine earth fraction, primarily at
564 the 0-10 cm soil depth. The measured annual net change of SOC in the 0-20 cm soil
565 depth of $4.6\ \text{Mg C ha}^{-1}$ was more than 3 times higher than the estimated C addition from

fine root turnover (1.4 Mg C ha^{-1}). If this net SOC accretion is as dependent on belowground sources of C, it may suggest fine root production is higher in this ecosystem than other estimates.

The increases of SOC among the 2000-250 μm and 250-150 μm sand size fractions were expected in the control and aboveground litter exclusion plots. These fractions have been characterized as containing large amounts of recently added organic material, significant amounts of particulate matter, and aggregates combined with fine roots and fungal hyphae (Oades 1978; King et al. 2002; Sarkhot et al. 2007a, b). For example, the diameter of loblolly pine ephemeral fine roots range from 2000-400 μm , with turnover rate estimated at 166-300 days depending on root diameter (King et al. 2002). Mycorrhizal associated fine roots also fall within this range, 600-200 μm , but observed turnover rates have been reported to be much slower at 507 days (King et al 2002).

Eliminating new sources of C for an extended period of time revealed a SOC dependence of root inputs to the >2 mm and 150-53 μm fractions. Lateral coarse roots and associated root branches are the primary constituent of the >2 mm size class in these sandy soils. Roots are also suspected as the principle source of C in the 150-53 μm fraction. It is the very fine root class (<1 mm) that are identified as ephemeral and dependent on continual regeneration. The very fine roots are dynamic but not uniform in function and morphology (King et al. 2002); it is the first order roots, the smallest fine roots, that experience the highest rate of turnover (Pregitzer et al. 2002b). First order root activity appeared to be significant in maintaining SOC in the 150-53 μm fraction, possibly as these small roots turnover.

Conclusion

Carbon storage in the sandy soils of the lower Coastal Plain region is particularly sensitive to disturbances and land management. Through a more complete understanding of SOC cycling in managed lower Coastal Plain pine stands, additional strategies can be developed to manage and forecast SOC accumulation and storage through the life of a stand's rotation (18-25 yrs) and through successive rotation plantings. As a dominant ecosystem across the Southeast US, highly productive pine plantations offer opportunities to capitalize on their ability to build SOC in the surface soil despite relatively high C mineralization rates. This research suggests that the juvenile fast-growth stage of tree development is a crucial phase in SOC accretion under intensively managed loblolly pine growing on Spodosols of the lower Coastal Plain. Soil OC accretion during the rapid growth phase represents some of the highest SOC accretion figures found in the literature for forest stands. Root turnover was found to be the most significant source of SOC for maintenance/accretion during this stage, with root turnover representing approximately 60% of the SOC change.

Although forest soil studies are prone to high spatial variability and changes in SOC can be difficult to measure, this study's sampling approach successfully captured changes over time. Future studies will require a focus on minimizing variability to expose more subtle responses and to include further evaluation of above-ground litter's role in SOC development. One approach would be to identify a relevant elementary soil volume for sampling to reduce sampling and maximize information. While this was a

single location study, its favorable comparison to literature and to other carbon studies in similar soil/climatic conditions as cited above speaks to its wider applicability.

Acknowledgements

We would like to thank the University of Florida’s Forest Biology Research Cooperative and Rayonier, Inc. for the opportunity to utilize the research site for this study. We also acknowledge and thank Dr. Salvador Gezan for assistance provided with the statistical analysis.

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Table 1. Means of soil organic carbon (SOC) kg ha^{-1} measured in the fine earth fraction (≤ 2 mm) effected by *in situ* field treatment and soil depth (0-10 cm and 10-20 cm) at the study's initiation (T_1) and after 31 months (T_2). Right column shows significant differences in the statistical analysis of the relative % change of soil organic carbon (SOC) at the 0-20 cm soil depth in the fine earth fraction (≤ 2 mm) after 31 months of exclusion treatment (Trt. main effect, $p = 0.05$) Values are LS means of relative % change with standard error bars. Different letters signify mean separations with $\alpha \leq 0.10$.

Table 2. Effects of *in situ* exclusion treatments and soil depth (0-10 cm and 10-20 cm) on soil organic carbon (SOC) kg ha^{-1} at the study's initiation (T_1) and after 31 months (T_2) for each size fractions and the relative % change of SOC of the five physical size soil fractions are compared. Statistical evaluations of each fraction were preformed individually, LS means with standard error bars and probability values are reported in the table. A treatment by soil depth interaction was only found in the $< 53 \mu\text{m}$ fraction was significant and the 0-10 cm and 10-20 cm soil depths. Different letters signify mean separations with $\alpha \leq 0.10$

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Figure 2. Distribution of soil organic carbon (SOC kg ha^{-1}) among soil size fractions averaged across time for two soil depths in an Ultic Alaquod supporting an intensively managed loblolly pine stand in north central Florida. Soil C values reported are LS means with standard error bars, $p = 0.01$. Different letters signify mean separations with $\alpha \leq 0.1$

Figure 3. Specific soil C mineralization (mg g^{-1} size fraction C d^{-1}) of soil size fractions at two depths after 162 d laboratory incubation at 30°C were effected by soil depth and size fraction ($p < 0.01$). Soil C respiration values reported are LS means and standard error bars. Different letters signify mean separations with $\alpha \leq 0.1$

Figure 4. . At the end of the study, a vertical profile inside each of the treatment plots was exposed and live roots counted in area increments of 30 cm across x 10 cm depth. Effects of *in situ* exclusion treatments significantly reduced the

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Figure 5. The relative % change of SOC among five physical size soil fractions after 31 months of exclusion treatment are compared. Statistical evaluations of each fraction were performed individually. Exclusion treatment main effects at the 0-20 cm soil depth are shown for all size fractions except the $>53 \mu\text{m}$ fraction where a treatment by soil depth interaction was found ($p = 0.04$). LS means with standard error bars are reported refer to Table 2 for probability values of treatment main effects. Different letters signify mean separations with $\alpha \leq 0.10$

Effects of Exclusion Treatment on Fine Earth Fraction (≤ 2 mm)					
Treatment	Soil Depth	T ₁ Mean SOC Mg ha ⁻¹	T ₂ Mean SOC Mg ha ⁻¹	T ₂ - T ₁ Mean SOC Mg ha ⁻¹	Relative % Change SOC 0-20 cm Trt $p = 0.05$
UC	0-10 cm	12.2	21.9	9.7	46% ^a
	10-20 cm	7.5	9.5	2.0	
L	0-10 cm	19.5	25.5	6.0	14% ^{ab}
	10-20 cm	15.3	12.3	-3.0	
LR	0-10 cm	19.5	19.6	0.1	-3% ^b
	10-20 cm	11.9	9.5	-2.4	

Table 1. Means of soil organic carbon (SOC) kg ha⁻¹ measured in the fine earth fraction (≤ 2 mm) effected by *in situ* field treatment and soil depth (0-10 cm and 10-20 cm) at the study's initiation (T₁) and after 31 months (T₂). Right column shows significant differences in the statistical analysis of the relative % change of soil organic carbon (SOC) at the 0-20 cm soil depth in the fine earth fraction (≤ 2 mm) after 31 months of exclusion treatment (Trt. main effect, $p = 0.05$) Values are LS means of relative % change with standard error bars. Different letters signify mean separations with $\alpha \leq 0.10$.

Effects of Exclusion Treatment on Five Size Fractions					
Size Fraction	Effect	Soil Depth	Treatment	T ₂ - T ₁ Mg ha ⁻¹	%Change SOC
> 2mm	Trt $p = 0.06$	0-20 cm	UC	-1.5	-5% ^{ab}
			L	1.9	16% ^a
			LR	-2.7	-31% ^b
2000-250 μm	Trt $p = 0.30$	0-20 cm	UC	5.9	46%
			L	3.7	9%
			LR	3.4	16%
250-150 μm	Trt $p = 0.78$	0-20 cm	UC	0.4	22%
			L	1.6	30%
			LR	1.3	21%
150-53 μm	Trt $p = <0.02$	0-20 cm	UC	2.4	38% ^a
			L	-0.2	-5% ^{ab}
			LR	-0.6	-46% ^b
>53 μm	Soil Depth x Trt $p = 0.04$	0-10 cm	UC	-0.1	4% ^b
		10-20 cm		2.5	110% ^a
		0-10 cm	L	1.8	29% ^b
		10-20 cm		1.1	34% ^b
		0-10 cm	LR	0.5	12% ^b
		10-20 cm		1.0	44% ^b

Table 2. Effects of *in situ* exclusion treatments and soil depth (0-10 cm and 10-20 cm) on soil organic carbon (SOC) kg ha⁻¹ at the study's initiation (T₁) and after 31 months (T₂) for each size fractions and the relative % change of SOC of the five physical size soil fractions are compared. Statistical evaluations of each fraction were performed individually, LS means with standard error bars and probability values are reported in the table. A treatment by soil depth interaction was only found in the <53 μm fraction was significant and the 0-10 cm and 10-20 cm soil depths. Different letters signify mean separations with $\alpha \leq 0.10$

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