Short-term impacts of soil amendments on belowground C cycling and soil nutrition in two contrasting Pinus taeda L. genotypes

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ARTICLE INFO

Article history:
Received 8 May 2011
Received in revised form 22 June 2011
Accepted 26 June 2011
Available online 20 July 2011

Keywords:
Carbon sequestration
Fertilization
Logging residue incorporation
Microbial biomass
Soil respiration

ABSTRACT

We monitored two Pinus taeda L. genotypes, planted in 170-L lysimeters, subjected to different combinations of fertilization and logging residue (LR) incorporation for 1 year. The objectives were to elucidate how soil amendments modified soil biological properties and belowground C cycling, and secondly, to determine if planting of contrasting genotypes have a detectable impact on total soil CO₂ flux (Fₜ). LR incorporation resulted in decreased bulk density, increased total soil porosity, and increased total soil C and N contained within the fine-soil fraction. For most of the experiment we found no consistent differences between genotypes; however, on the final two sampling dates a pattern emerged of one clone showing greater Fₜ. If this pattern continues or becomes stronger with increased occupation of soil by roots it may have an influence on total net C exchange. Increased C loss by way of Fₜ and soil leaching made up approximately 7% of total C incorporated as LR. Conservative estimates using a constant rate of decomposition showed that it would take a minimum of 15 years to fully decompose the incorporated LR. Our data suggest that moderate rates of LR incorporation following harvesting over multiple rotations could increase SOM without negatively impacting plant growth, which could increase soil C sequestration.

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1. Introduction

The combination of improved site resource management and planting of superior genotypes (Schultz, 1997; Fox et al., 2004) has enabled intensively managed southern pine plantations to become some of the most productive forests in the world (Allen et al., 2005). Currently, managed southern pine forests occupy more than 13 million hectares and are forecast to increase to 22 million hectares by the year 2040 (Wear and Greis, 2002). This may provide great opportunity to manage these forests to sequester large amounts of C in both soil and plant biomass (Johnson et al., 2001). Management of logging debris following forest harvest is one possible avenue for increasing C sequestration and improving long-term site productivity.

Incorporation of nutrient rich logging residue (Ouro et al., 2001) at the time of site preparation has been shown to improve soil physical (e.g., bulk density, soil porosity, and strength; Powers et al., 2005), chemical (e.g., soil C and nutrients; Ouro et al., 2001; Smokander et al., 2010), and biological properties (e.g., microbial biomass and activity; Houghton et al., 1983; Aggangan et al., 1999; Li et al., 2004). This idea (see review by Buford et al., 1988; Johnson and Curtis, 2001) may both improve site quality by increasing soil organic matter (SOM) and increase soil C sequestration in the long-term. There are possible negative effects to LR incorporation. For instance, high C:N ratio organic material into the mineral soil may result in short-term immobilization of nutrients (i.e., N) resulting in a decreased tree growth. A short-term greenhouse experiment focusing on plant gas exchange and biomass partitioning found that LR incorporation, at low rates (equivalent to 25 Mg.o.d. ha⁻¹), had little effect on overall tree growth (Tyree et al., 2009b). Another study found that contrasting Pinus taeda clones grown in the field responded differently to LR incorporation with one clone being negatively affected while the other clone showed no effect (Tyree et al., 2009a). In addition to possible effects on plant productivity, the incorporation of LR into the mineral soil would likely accelerate decomposition rates possibly negating any possible increases to soil C (LundmarkThelin and Johansson, 1997; Ouro et al., 2001; Perez-Hortalon et al., 2001).

In this experiment we monitored two P. taeda genotypes, planted in 170 L lysimeters and subjected to different combinations of fertilization and logging residue (LR) incorporation for 1 year. Specifically, our objectives were to:

Ol: elucidate how soil amendments modified soil physical properties and macro nutrient concentrations.
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H1: LR will improve soil physical properties and macro nutrients by decreasing soil strength and bulk density and increasing soil C and nutrients.

H2: LR will increase microbial populations, heterotrophic activity, and decomposition rates leading to increased C loss as total soil respiration from the soil surface (Fs) and soil leaching. Consistent with other studies of young P. taeda, fertilization will have the opposite effect on these soil biological properties (Lee and Jose, 2003; Gough and Selle, 2004; Blazier et al., 2005; Tyree et al., 2008).

H3: Differences in growth and allocation patterns between clones will translate into detectable differences in belowground C cycling.

H4: Despite the increased C loss as soil efflux and soil leachate with LR treatments, a percentage of incorporated organic material has the potential to remain for decades.

2. Materials and methods

2.1. Study design

In April 2006, one-year-old P. taeda clones were planted in 170 L plastic containers (93 L x 53 W x 50 cm H) and grown in a greenhouse through July 2007. Greenhouse settings were adjusted to provide plants with summer and winter conditions representative of the southeastern United States (Fig. 1). The study was a randomized complete block design replicated six times. Treatments were arranged in a full factorial (2 x 2 x 2) with two levels of logging residue (LR and No LR), two levels of fertilization (F and NF), and two elite P. taeda clones (C193 and C185). A total of 48 plastic containers (experimental units) were fitted with a single brass spigot for collecting water, and each was passed through a 2 mm sieve and stored at 4°C for no more than 48 h from collection. Each core was weighed to the nearest 1 g (Freeman et al., 1966). Soil pH and exchangeable cation concentration were determined using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA). Total soil C and N concentration contained within the fine-fraction were determined using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA). The remaining soil sample was sent to the Virginia Tech Soil Testing Laboratory (Blacksburg, VA) for soil pH and exchangeable cation concentration (Mehlich 1) according to procedures described by Mullins and Heckendorn (2006). At the conclusion of the experiment, one soil core was taken using a bulk density hammer to a depth of 5 cm at the base of each tree. Non-capillary porosity was determined by subtracting the weight of the soil core after 24 h under 33.3 kPa tension (field capacity) from the weight of the saturated soil core. Soil total porosity was estimated by drying the cores at 105°C and subtracting from saturated weight. Capillary porosity was calculated by subtraction of non-capillary and total porosity. Total bulk density (including coarse fragments) was determined by weighing (±0.1 g) oven dried (105°C) samples of known volume. Average soil strength (kPa) from 0 to 10 cm depth was measured using Field Scout SC 900 soil compaction meter (Spectrum Technologies, Inc., East-Plainfield, IL) and normalized to 10% soil moisture.

2.2. Treatments

Following 6 months of composting, logging residue (LR) was collected from the logging deck of a harvested P. taeda stand in South Carolina (C:N = 128 ± 14; n = 4). The material consisted of bark, needles, and small branches that remained following an onsite processing of merchantable timber. Residue that could pass through a 5 × 10 cm screen was incorporated evenly throughout the entire soil matrix during pot filling at a rate of 4.92 kg LR container ³ (equivalent to 25 Mg dry wt ha ⁻¹). Fertilizer was applied two times over the course of the experiment. Application rates were selected to maintain consistency with a complimentary field experiment (Tyree et al., 2009a). The first application was on July 28, 2006 in the form of diammonium phosphate (DAP) and ammonium nitrate (AN) at an equivalent rate of 200 kg N and 50 kg P ha ⁻¹. The second fertilization took place on March 16, 2007 and was applied in the form of AN, at a rate of 200 kg N ha ⁻¹. Clonal seedlings (C193 and C185) were donated by Mead Westvaco and have been shown, in an accompanying experiment, to differ in their stem to foliage ratio and response to changes in fertility (Tyree et al., 2009a).

2.3. Objective 1: Soil macro nutrients and physical properties

Determination of soil chemical properties was performed on November 2006, February 2007, and June 2007 on the fine-soil fraction (passed through 2 mm sieve). Six to eight random soil cores per experimental unit were taken to a depth of 20 cm using a 2.5 cm diameter push tube. Approximately 15 g air-dried soil was ground to a powder using a Micro-Mill ® grinder (Bel-Art Products, Pequannock, NJ) and 30 ± 5 mg of powdered soil was weighed into 5 × 9 mm pressed tin capsules (Costech Analytical Technologies, Inc., Valencia, CA). Total soil C and N concentration contained within the fine-fraction were determined using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA). The remaining soil sample was sent to the Virginia Tech Soil Testing Laboratory (Blacksburg, VA) for soil pH and exchangeable cation determination (Mehlich 1) according to procedures described by Mullins and Heckendorn (2006). At the conclusion of the experiment, one soil core was taken using a bulk density hammer to a depth of 10 cm at the base of each tree. Non-capillary porosity was determined by subtracting the weight of the soil core after 24 h under 33.3 kPa tension (field capacity) from the weight of the saturated soil core. Soil total porosity was estimated by drying the cores at 105°C and subtracting from saturated weight. Capillary porosity was calculated by subtraction of non-capillary and total porosity. Total bulk density (including coarse fragments) was determined by weighing (±0.1 g) oven dried (105°C) samples of known volume. Average soil strength (kPa) from 0 to 10 cm depth was measured using Field Scout SC 900 soil compaction meter (Spectrum Technologies, Inc., East-Plainfield, IL) and normalized to 10% soil moisture.

2.4. Objectives 2 & 3: Total soil CO₂ efflux & microbial activity

Manual point-in-time sampling of total soil CO₂ efflux (Feₐₐ₉) was performed using a Li-Cor 6200 infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) with a dynamic closed soil chamber giving a total system volume of 6300 cm³. Measurements were taken approximately every 1-1.5 months starting June 2006 through June 2007 (11 sampling dates), however, machine leaks required the removal of the June and July 2006 (9 sampling dates). Twenty-four hours prior to Feₐ₉ measurements the soil surface of each pot was lightly scraped to remove any algae, mold, or photosynthesizing vegetation. Measurements were always made in the same sequential blocking order between 1000 and 1600 h. Carbon dioxide evolution was measured over a 30 s period and respiration rates calculated as µmol CO₂ m⁻² s⁻¹. Further details concerning equipment design and methodology can be found in Selig et al. (2008) and Tyree et al. (2008). Soil temperature and moisture were measured concurrently with Feₐ₉ measurements. Soil temperature was measured to the nearest 0.1°C at 15 cm depth using a Digi-sense temperature gauge (model No. 8528-20, Cole-Parmer Instrument Co., Niles, IL) and volumetric soil water content was averaged to a depth of 13 cm using a time domain reflectometer (Hydrosense 620 system, Campbell Scientific Inc., Logan, UT) to the nearest 1%.

Microbial biomass C (MBC) and N (MBN) were estimated in February 2007 (Winter) and June 2007 (Summer) using chloroform fumigation-extraction procedure described by Jenkinson and Powlson (1976) and later modified by Anderson and Domsch (1978). Six to eight random soil cores per experimental unit were taken to a depth of 20 cm using a 2.5 cm diameter push tube. Soil samples were composited and passed through a 2 mm sieve and immediately stored at 4°C for no more than 48 h from collection.
Two replicate 25 g fresh soil samples were weighed into 50 mL beakers and each placed into two vacuum desiccators. Half the samples were fumigated with ethanol-free chloroform (CHCl₃) for 24 h while the other samples were left non-fumigated to serve as controls. Each sample had 100 mL of 0.5 M potassium sulfate (K₂SO₄) solution added and shaken on low for 2 h then allowed to settle for 4 h before being passed through Whatman #2 filter. A 20 mL subsample of filtered leachate was frozen and shipped to Analytical Services Laboratory at North Carolina State University, Raleigh, NC for total C and N using a TOC analyzer (TOC-5050 fitted with an autosampler model ASI-5000, Shimadzu Scientific Instruments, Columbia, Maryland). Microbial biomass C and N was calculated by subtracting complimentary fumigated from non-fumigated samples divided by the extraction coefficient of 0.45 (Joergensen, 1996) and 0.54 (Joergensen and Mueller, 1996) for C and N, respectively.

We used two independent indices to estimate microbial activity. First, an index of heterotrophic respiration (Rₚₙₙ) was measured using a Li-Cor 6250 infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) attached to a 0.25 L cuvette chamber, with a total system volume of 429 cm³. Soil samples were taken to a depth of 20 cm using a 2.5 cm push tube following Fpₚₙₙ measurements using methods described by Gough and Seller (2004) and Tyree et al. (2006, 2008). Second, an index of microbial decomposition measured by the loss of weight of a standard material (Neher et al., 2003; Jurgensen et al., 2006). Two 0.64 cm diameter yellow pine dowels rods were buried in each container, for approximately 1 year starting July 28, 2006, to a depth of 30 cm with the upper 10 cm remaining exposed to the atmosphere. Each dowel rod consisted of four 10 cm sections separated by silicone and held together with 1.5 cm long rubber hosing (0.64 cm inside diameter) to keep each section discrete from the others. Prior to assembly, all dowel rod sections were oven-dried to 65 °C and weighed gravimetrically to the nearest 0.01 g. The ends were covered with silicone to prevent evaporation. At the end of the project (approximately 1 year) the dowels were removed rinsed with weak soap solution (shaken on low for 4 h) followed by rinsing with DI water. Finally, wooden rods were oven-dried for 48 h at a temperature of...
65 °C and weighed. Decomposition was determined by subtracting the final weight from the starting weight for each dowel and averaging the two subsamples for each experimental unit.

2.5. Objective 4: Cumulative C loss

To investigate the effects of soil amendments on cumulative total C loss for the experiment, continuous measures of total soil CO₂ efflux (F_{cont}) were made for 411 days (April 2, 2006 through May 18, 2007) using the Automated Carbon Exchange System (ACES) developed by the USDA Forest Service, Southern Research Station Laboratory at Research Triangle Park, NC (Butnor et al., 2005). The ACES is an open-system infrared gas analyzer unit attached to 15 soil chambers (dimensions are 491 cm² by 10 cm height). Every 150 min the ACES cycles through 15 measurement chambers and one null chamber giving approximately nine cycles per day. The null chamber was used to zero the IRGA every 150 min to account for changes in temperature and pressure.

Every 5 days the ACES chambers were systematically rotated between two identical sets of three replications ensuring that five chambers were allocated to each replication. This approach ensured that each chambers spent equal time at all locations minimizing any chamber bias. This also allowed chambers to be serviced and the soil surface scraped free of any photosynthesizing or respiring vegetation. During this time, raw data was downloaded and checked for errors and machine malfunctions. This involved ensuring chambers: (1) were equilibrating within 10 min, (2) were maintaining a slight (approximately 10%) positive pressure, (3) hosing was free of clogs and leaks, and (4) that thermocouples were functional. Following validation, data was prepared by calculating a daily mean for each chamber. Seven cycles per 24 h period was the minimum number to be considered as representative daily average, otherwise data for that day was deleted. Data collected within 24 h following relocation of chambers were dropped from the data set due to disturbance caused by relocating chambers. Over the 411 day period 79% of the data collected was met the above conditions. All missing dates (approximately 21%) were filled in by averaging the two closest daily values. Treatment effects were tested by averaging daily rates of F_{cont} for the month. Additionally, treatment effects on cumulative monthly C loss were tested by summing daily C loss for each month added to C loss from previous months over the experiment.

Pots were watered to ensure that water never became limiting to plant growth. Ten times over the course of the 411 day experiment, each container was watered beyond pot capacity over the course of approximately 10 days (1-2 gallons per day). This watering scheme allowed time for water to penetrate the soil while preventing pooling on the soil surface. After 10 days water was allowed to drain into leachate catchments for 24 h. Leachate was homogenized by stirring, and a subsample passed through a Whatman #1 filter and poured into 20 mL scintillation vials, which was immediately frozen. Remaining leachate was measured to the nearest centiliter to scale C concentration to total C lost as leachate during that event. Frozen samples were shipped to the Analytical Services Laboratory at North Carolina State University, Raleigh, NC for total C determination using a TOC analyzer (TOC-5050 fitted with an auto-sampler model ASI-5000, Shimadzu Scientific Instruments, Columbia, Maryland).

2.6. Data analyses

Treatment differences for F_{POA}, F_{CON}, and cumulative monthly C loss as F_s and in leachate, R_s, and soil chemical properties were tested using analysis of variance with repeated measures (ANOVARM) using a MIXED model. Covariance structures were selected using AIC fit statistics included in the SAS output. Treatment differences for soil physical properties, MBC, and index of decomposition were analyzed using a general linear model (GLM). For all analyses residuals and the normality curves were plotted to confirm that the data meet assumptions of equal variance and normality for all parameters measured. Values were expressed as untransformed least square means. All data preparation and analyses were performed using SAS statistical software version 9 (SAS, 2006).

3. Results and discussion

3.1. Objective 1: Soil physical properties & macro nutrients

Logging residue decreased soil bulk density by 4% (p < 0.01) and increased percent total soil porosity by 4% (p = 0.01; Table 1). These findings are consistent with what Sanchez et al. (2000, 2003) found in the field following LR incorporation. Our increase in total soil porosity was a function of greater capillary porosity (p = 0.02), and had no apparent effect on non-capillary porosity (p = n.s.). Notably, Sanchez et al. (2000, 2003) observed a decrease in soil strength in the upper soil layers following biomass incorporation, whereas, we observed a 50% increase (p < 0.001) in soil strength with LR. We hypothesized; this increase was likely an artifact of the soil compaction meter coming into direct contact with a fragment of LR and not an accurate indicator of the resistance of mineral soil to root penetration.

Our treatments had a significant effect on soil macro nutrients (Table 2). For example, on the final sampling date in June 2007 we found that fertilization resulted in a significant (p < 0.05) decrease in mineral soil K, Ca, and Mg levels. This decrease may have been due to an observed 13% reduction in soil pH in fertilized pots relative to control pots (5.23 and 6.02, respectively; p < 0.0001), increased uptake of K, Ca, and Mg following increased tree growth (Tyree et al., 2009b), or both. The incorporation of LR resulted in a significant (p < 0.05) increase in the C:N ratio on two sampling dates. This is due to an 26% increase in total C contained in the fine-soil fraction (Table 2), which was substantially less than that measured by Sanchez et al. (2000, 2003) in the field. At the conclusion of the study we observed large intact pieces of LR still in the pot, which indicates that after 1 year added LR was relatively resistant to decomposition. The February 2007 sampling date showed that fertilization with N and P did not impact soil C by itself, but when applied in combination with LR, soil C was increased relative to LR being applied alone. This may be a result of decreased microbial activity following fertilization, which has been observed in numerous studies (Nohrstedt et al., 1989; Thindukumaran and Parkinson, 2000; Bizier et al., 2005). Finally, our seedling growth data suggest that although we did observe a decrease in foliar N concentration with LR additions, our rates of LR incorporation did not result in N immobilization to a level that reduced growth (Tyree et al., 2009b).

3.2. Objective 2: Total soil CO₂ efflux & microbial activity

Soil CO₂ efflux data collected using point-in-time sampling (F_{POA}) showed a significant (p < 0.0001) increase with LR incorporation. Our findings are consistent with our hypothesis as well as other studies that have shown increased F_s with LR additions. For example, Aagganan et al. (1999) observed increased F_s with increasing rates of Eucalypt leaf litter incorporation during a 29 week incubation study. Additionally, Perez-Batallon et al. (2001) found increased F_s in plots where LR was incorporated into the top 20 cm of mineral soil relative to treatments where LR was completely removed or left on the soil surface 1 year following harvest in a Pinus radiata stand in NW Spain. A significant LR by
fertilizer by time interaction (p = 0.04) was observed in F_porn (Fig. 2A). When LR was not present the application of fertilizer had little to no effect on F_porn. This may be a result of decreased heterotrophic rates and increased root respiration rates offsetting each other (Tyree et al., 2008). The incorporation of LR increased F_porn, but the effect was diminished when fertilizer was applied (Fig. 2B). We hypothesize that the addition of LR increased microbial activity, which led to increased F_porn, however, the addition of fertilizer decreased microbial populations, activity, or both. During the winter months all treatment effects disappeared, and reappeared following a rise in temperature.

To determine whether changes in F_porn were a function of soil microbial activity we measured heterotrophic respiration. We observed a significant (p = 0.006) LR by fertilizer by time interaction for index of heterotrophic (microbial) respiration. With the exception of one sampling date in October 2006, the application of N and P fertilization decreased R_h with increasing magnitude as the experiment progressed (Fig. 3A and B). Overall, F decreased R_h by 28% relative to NF treatments (0.082 ± 0.005 and 0.11 ± 0.005 μmol CO₂ kg⁻¹ s⁻¹, respectively). In contrast, the incorporation of LR increased R_h relative to control treatments with the difference decreasing over the course of the experiment. However, when fertilizer was applied in the presence of LR it led to an early increase in R_h followed by a decrease in R_h relative to control treatment with time. These findings are consistent with our estimates of F_porn (Fig. 2) as well as findings of others who have measured the effects of fertilization on microbial respiration (Boden et al., 2004; Gough and Seiler, 2004; Blazer et al., 2005; Tyree et al., 2008). Although changes in microbial activity and biomass following nutrient amendments have been widely observed, the mechanisms driving this response are poorly understood. The addition of fertilizer may directly affect microbial populations via pH or osmotic changes (Thirukkumaran and Parkinson, 2000), or indirectly from longer-term changes to aboveground plant growth (Leckie et al., 2004), such as a resoilization of plant C from roots to shoots (Haynes and Gower, 1995; Ryan et al., 1996). Additionally, fertilization has been shown to cause shifts in soil microbial composition (Báth et al., 1978; Wallander and Nylund, 1992; Lilleskov et al., 2002; Nilsson and Wallander, 2003; Bittman et al., 2005).

Decomposition of dowel rods was also used to estimate microbial activity. Not surprisingly, after 1 year we found a significant (p < 0.0001) difference in percent decomposition between soil depth classes. Overall, dowel rods buried in the 0-10 cm depth class decomposed at the fastest rate and the section of dowel rod left on the surface decomposed the slowest (Fig. 4A). Burying organic material into the soil where temperature and moisture make an ideal environment for soil microbes has been widely shown to increase decomposition rates (Holland and Coleman, 1987; Aggarwal et al., 1998; Ouro et al., 2001; Coppers et al., 2007; Nicolardot et al., 2007). We also observed a significant LR by
fertilizer interaction ($p = 0.003$). There was poor agreement between our decomposition and $R_i$ results. Fertilization increased decomposition and LR decreased decomposition (Fig. 4B), which was opposite to our respiration data. When LR was present, decomposition responded to fertilizer more than if LR was absent. Finally, application of N and P fertilization affected decomposition, but the response was dependent on soil depth ($p < 0.0001$). At the soil surface there was no difference in decomposition rates between F and NF treatments. The largest difference was found in the 0–10 cm depth where F treatments decomposed approximately 9% more than NF treatments; however, the deeper two depth classes both showed about 4.5% more decomposition in F treatments. A possible explanation for this finding include that adding N to a buried C source such as a wooden dowel rod increased decomposition at that point and not in the bulk soil. We found that our MBC data supported our findings in both RII and Fpoint. For example, LR alone had the greatest MBC on both sampling dates, but was slightly reduced when applied in combination with fertilizer (Fig. 5). Across all data LR significantly ($p < 0.0001$) increased MBC approximately 11% relative to treatments that did not receive LR (679 ± 26.4 and 514 ± 24.7 mg C kg$^{-1}$ soil, respectively; $n = 48$). The addition of a C source has been shown to increase microbial populations (Aggangan et al., 1999; Perez-Piqueres et al., 2006). We also found that MBC was slightly greater ($p = 0.05$) when sampled in February 2007 relative to July 2007. We did not observe a consistent effect of fertilizer on MBC ($p = 0.23$), but overall F containers showed a decrease relative to NF treatments (579 ± 23.2 and 617 ± 32.5 mg C kg$^{-1}$ soil, respectively; $n = 48$). Our findings were consistent with others who found decreased MBC with fertilization with N (Lee and Jose, 2003; Blazier et al., 2005). This finding may be due to a 13% decrease in soil pH in fertilized pots. Although not measured as part of this study, fertilization may also lead to a shift in microbial community composition such as a decrease in fungal:bacteria ratio. We concluded that the incorporation of a decomposable substrate into the mineral soil led to an increase in microbial biomass and activity which we hypothesized directly influenced $F_p$.  

3.3 Objective 3: Effect of genotype on total soil CO$_2$ efflux

We did not find an interaction between genotypes and soil amendments as we hypothesized. Overall, there was no differences

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**Fig. 2.** Least squares mean of soil CO$_2$ efflux ($F_{\text{point}}$) for the logging residue (LR) by fertilizer (F) by time three-way interaction (Panel A), percent $F_{\text{point}}$ treatment effect relative to control treatment (Panel B), and clone (CL) by time interaction (Panel C). Data were analyzed using ANOVA with repeated measures in SAS version 9 (error bars represent ±standard error from the mean). Arrows indicate times of fertilization and dashed line represents control treatment.
in $F_{\text{Point}}$ between genotypes, although, there was a significant genotype by time interaction ($p = 0.05$). Both clones showed similar rates of $F_{\text{Point}}$ throughout most of the experiment with differences between clones emerging at the end of the experiment (Fig. 2C). Clone 85 showed increased rates of $F_{\text{Point}}$ relative to CL93 by the end of the experiment, but it is unknown whether these differences would continue to be expressed or disappear as the seedlings matured. We speculated based on biomass partitioning data collected that CL93 would have greater $F_{\text{Point}}$, but this was not the case. Although CL85 had greater overall root mass, CL93
had greater fine-root mass and showed an increase in the fine- to coarse-root ratio at the end of the study (Tyree et al., 2009b). *P. taeda* clones have been shown to differ in their growth and allocation patterns in response to nutrient additions (Li et al., 1991; Retzlaff et al., 2001; King et al., 2008), but no studies that we are aware of have shown clonal differences in $F_s$ in conifer seedlings.

3.4. Objective 4: Total C loss through $F_{\text{Cont}}$ and leaching

The incorporation of LR has commonly been shown to increase the rate of decomposition mainly by creating conditions (temperature and moisture) favoring increased microbial activity as well as putting C substrate in direct contact with soil biota. We
hypothesized that incorporation of LR would increase decomposi-
tion as seen by others (LundmarkThelin and Johansson, 1997; Ouro
et al., 2001; Perez-Barralon et al., 2001), but that some fraction
would remain as recalcitrant soil C. To estimate total C loss we con-
tinuously monitored \( F_{\text{cont}} \) using the ACES and C loss through leach-
ing by collecting all leachate and analyzing subsamples for total C.
We found that the incorporation of LR increased C loss through both
avenues, but C loss as leachate made up less than a percent of
total C lost for the year (Fig. 6). We observed a highly significant
\((p < 0.0001)\) increase in cumulative C loss as \( F_{\text{cont}} \) with the addition of
LR (Fig. 6A). Additionally, we observed a slight decrease in \( F_{\text{cont}} \)
with the addition of fertilizer and a difference between genotypes,
but these treatment main effects as well as interactions were not
statistically significant \((p > 0.10)\). Overall, C loss through leaching
made up a minor portion of total C loss from the system, but was
significantly \((p = 0.04)\) increased by the addition of LR (Fig. 6B).
We observed no significant differences between other treatments
main effects or interactions. The incorporation of LR resulted in
an increase of 160 g of C loss through \( F_{\text{cont}} \) and leaching over treat-
ments not receiving LR. Of the 2460 g of C that was incorporated in
the form of LR approximately 93% of the C remained at the end of
the experiment approximately 1 year later. Assuming a constant
rate of decomposition we calculated that it would take more than
15 years for all the incorporated LR to decompose, therefore, reach-
ing a "net zero" point. This simple calculation makes a number of
assumptions that would lead to an underestimation of the years
before reaching the "net zero" point. Firstly, it assumes the increase in
\( F_s \) is due entirely to the decomposition of the added LR. Increases in aboveground productivity attributed to improve-
ments to soil physical and chemical properties would likely
contribute to C inputs and hence increased \( F_s \). Secondly, we also
assumed a constant rate of decomposition which is unlikely. We
believe the rate of decomposition would diminish as we move fur-
nier from the time of disturbance, and as more labile material
would decompose relatively early leaving more stable material
with increased resistance to decay (Paul and Clark, 1996).

4. Conclusion

We conclude that the incorporation of LR into the mineral soil at
rates used in this study would increase soil organic matter, site
quality, and could lead to increased C sequestration on the site.
We observed improved soil physical and chemical properties with
LR incorporation without causing decreases in plant growth.
Increased C loss by way of \( F_{\text{cont}} \) and through leaching made up only
a small percent total C incorporated as LR. The most conservative
estimates showed that it would take 15 years to fully decompose,
although, decreases in decomposition rates with time would sub-
stantially increase that amount of time. When LR was incorpo-
rated, microbial activity and MBC influenced increases in \( F_{\text{pot}} \).
Heterotrophic respiration correspondingly increased with LR and
decreased with fertilizer additions as did MBC. Finally, we did
not find consistent differences between genotypes. For most of
the experiment, \( F_{\text{pot}} \) did not differ between clones, but on the final
two sampling dates a pattern emerged of one clone showing a
higher \( F_{\text{pot}} \). If this pattern continues or becomes stronger with
increased occupation of soil by roots then this could have a signif-
icient influence on net C exchange, but further study on mature
stands is needed.

Acknowledgments

The authors would like to thank US Forest Service, Virginia Tech
Graduate Student Association, and the Virginia Tech, Department
of Forest Resources and Environmental Conservation for partial
funding of this research. Special thanks to John Peterson and
Jeremy Stovall for designing, construction, and filling of Lysimeters.
Thanks to Phil Dougherty and Mead Westvaco for donating clonal
seedlings. Additional thanks to Daniel McInnis for assistance
setting up and running the ACES, and Kurt Johnsen and Karen
Sarsnay for C and N analysis. Thanks to Drs. Mike Aust and Thomas
Fox for their expert advice. Special thanks to Mike Aust and Bobby
Ashburn for their help in obtaining soil for use in this research.
Finally, we would like to thank Jennifer Tyree for her many hours
of help in the greenhouse.

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