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Contrasting genotypes, soil amendments, and their interactive effects on short-term total soil CO_2 efflux in a 3-year-old *Pinus taeda* L. plantation

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ABSTRACT

Intensively managed pine forests in the southeastern United States are considered an important C sink and may play a critical role in offsetting increased global CO_2 emissions. The combination of improved silvicultural methods and the use of superior genotypes are estimated to result in future volume gains of up to 60 percent. However to date, no work has looked at whether selection of elite genotypes could influence soil C dynamics, which could decrease the time necessary for the stand to function as a C sink. We evaluated the effects of contrasting loblolly pine genotypes on total soil surface CO_2 efflux (F_S) and heterotrophic respiration (R_H) under two soil amendment treatments: 1.) fertilization and 2.) logging residue (LR) incorporation. We found an immediate and sustained difference in F_S ($p = 0.05$) and R_H ($p < 0.01$) among our two genotypes throughout the first two years of stand development. Our soil amendment treatments did not significantly change F_S, but did influence R_H. LR increased ($p = 0.05$) R_H while N and P fertilization induced a slight ($p = 0.06$) decrease throughout the study. Our genotypes differed ($p = 0.05$) in their temperature response of F_S, which resulted in an 11% difference in total cumulative C loss from the soil over the duration of the study. We hypothesize that observed treatment effects in F_S and R_H are largely due to differences in belowground C allocation among genotypes, which is supported by others that have looked at fine-root standing crop and turnover on these same genotypes. This work underscores the importance of accounting for differences among genotypes when developing stand-level C estimates.

1. Introduction

Forests are considered an important global C sink (Raich and Schlesinger, 1992). Recent estimates by Pan et al. (2011) show that from 1990 to 2007 the world's forests captured the equivalent of 60% of cumulative fossil fuel emissions during that same period (73 Pg C and 126 Pg C, respectively). It has been suggested that forests, and more specifically managed southern pine forests, may play an important role in offsetting increases in global CO_2 emissions (Johnsen et al., 2001). Currently, southern pine plantations occupy more than 13 million ha and are forecast to increase to 22 million hectares by the year 2040 (Fox et al., 2007; Wear and Greis, 2002). The combination of improved silvicultural methods and the use of superior planting stock has more than tripled volume production of intensively managed southern pine plantations over the last 50 years (Fox et al., 2004; Schultz, 1997), and is estimated to result in future volume gains of up to 60% (Allen et al., 2005; Martin et al., 2005; McKeand et al., 2006). While increased primary productivity would increase the stands ability to function as a C sink, to fully evaluate the total ecosystem C exchange, a measure of C loss through ecosystem respiration is necessary.

Total soil surface CO_2 efflux (F_S) is the sum of autotrophic respiration resulting from maintenance and growth of plant roots, and heterotrophic respiration resulting from the decomposition of soil organic matter by soil microbes (Bond-Lamberty et al., 2004). F_S is the dominant loss of C from terrestrial systems contributing 60–90% of total ecosystem respiration (Bolstad et al., 2004; Law et al., 1999) and the second largest flux of C globally (Raich and Schlesinger, 1992). Consequently, even minor changes in F_S brought about by forest management decisions could have a profound impact on stand level C balance (Maier and Kress, 2000). This has led to an extensive body of research focused on the effects of

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forest management on F_S in southern pine forests. These experiments have advanced our understanding of how management treatments such as: fertilization (Butnor et al., 2003; Gough and Seiler, 2004), forest thinning (Selig et al., 2008; Tang et al., 2005), and site preparation (Tyree et al., 2006) impact F_S rates, however, no studies that we are aware of have compared the effects of planting elite genotypes on F_S in managed southern pine stands.

This research is part of a collaborative effort designed to evaluate the genetic by environmental interaction between two contrasting loblolly pine (*Pinus taeda* L.) genotypes and soil amendments (Maier et al., 2012). These amendments were high C:N (about 700:1) logging residue (LR) incorporated into the mineral soil and fertilization (N and P). Previous work found that the incorporation of LR into the mineral soil led to N immobilization (Tisdale, 2008), which negatively affected growth in one of the *P. taeda* genotypes, but not the other (Tyree et al., 2009). Additionally, differences in fine-root standing crop and turnover were observed between the two genotypes (Pritchard et al., 2010). Our primary objective with this work was to evaluate the effects of contrasting *P. taeda* genotypes on total soil surface CO_2 efflux (F_S) across varying degrees of nutrient availability, as manipulated by soil amendments. Specifically, we hypothesized that:

(H1) F_S will be different among our planted genotypes due to differences in belowground fine-root mortality and overall fine-root standing crop, observed using mini-rhizotrons (Pritchard et al., 2010), which will also have an influence on heterotrophic respiration (i.e., microbial activity);
(H2) the addition of LR would increase F_S rates as a result of increased microbial activity and the addition of N and P fertilizer would result in decreased microbial activity.

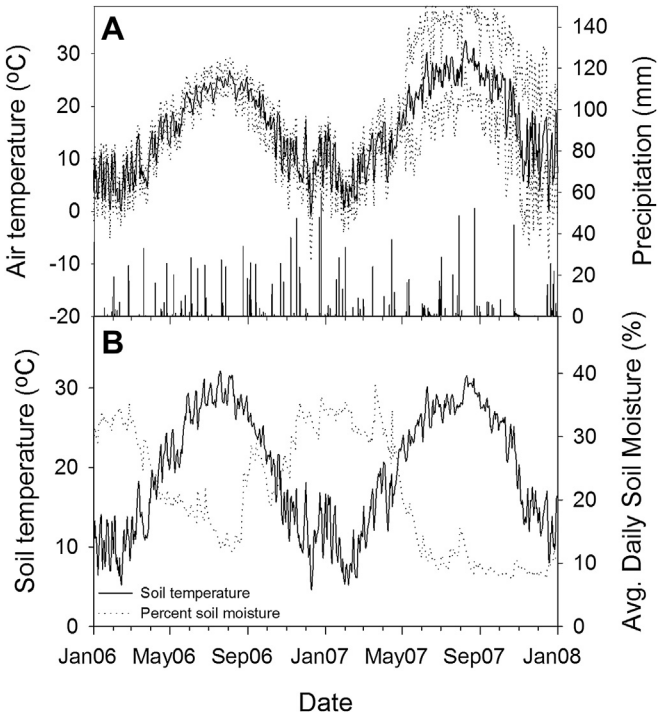

Fig. 1. (A) Average, minimum, and maximum (solid, upper, and lower dotted lines, respectively) daily air temperature (°C), total daily precipitation (bars) measured continuously using an onsite weather station. (B) average daily soil temperature (15 cm depth), and percent soil moisture measured using four dataloggers (CR10x; Campbell Scientific Inc.) located throughout the site between January 2006 and January 2008 for the Cross study site located in Berkeley Co., SC.

2. Materials and methods

2.1. Site location, climate, and stand history

The study site is located in Berkeley County, SC (33° 16′ 50″ N, 80° 10′ 9″ W) at an elevation of 24 m above mean sea level. Average annual temperature was 14.6 °C and 17.4 °C with an average daily maximum of 17.3 °C and 25.2 °C and an average daily minimum of 11.7 °C and 11.2 °C for the 2006 and 2007 year, respectively. Highest daily average temperature was 26.8 °C and 32.5 °C occurring in August 2006 and August 2007, respectively, and a low of −0.9 °C and 0.4 °C occurring in December 2006 and February 2007, respectively (Fig. 1A). Total precipitation was 902 mm in 2006 and 749 mm in 2007 spread evenly throughout the year, which was well below the average of 1200 mm recorded between 1949 and 1973 (Long, 1980). The dominant soil series was a Seagate series (sandy over loamy, siliceous, active, thermic Typic Haplohumods). Harvest of the previous 21-year-old *P. taeda* stand took place in May 2004 and the site was sheared of residual material in July 2004. Logging residue treatments were applied in October 2004, and site preparation (bedding) took place in early November 2004. *P. taeda* genotypes were planted in January 2005 and data for this study were collected between January 2006 and January 2008.

2.2. Study design and treatments

The study was a split-plot, randomized complete block design replicated three times with the whole-plot treatments arranged as a full two by two factorial. Each 0.18 ha plot (48 × 38 m) contained approximately 243 container grown, clonal *P. taeda* seedlings planted in nine rows at a 1.8 m spacing within rows and a 4.3 m spacing between row centers. Two levels of logging residue (LR) and two genotypes ("broad crown" and "narrow crown") served as the whole-plot treatments. The two levels of LR were no LR incorporated (NoLR) and LR incorporated into the mineral soil (LR) at a rate of 25 Mg ha^{-1} (residue weights are presented as oven-dried at 70 °C), which was concentrated onto the beds (equivalent to 75 Mg ha^{-1}) (Maier et al., 2012). Both LR treatments also incorporated the residual forest floor of approximately 25 Mg ha^{-1}. The two *P. taeda* genotypes chosen both exhibit increased aboveground productivity but have different growth efficiencies (i.e., stem growth per unit leaf area). The "narrow crown" genotype (NC) has been shown to allocate more of its resources to stem growth while the "broad crown" genotype (BC) carries more leaf area and thus would require more N (assuming similar foliar N concentrations; Tyree et al., 2009).

Each whole-plot was split into two 13 m^2 split-plots located at opposite ends of the whole-plot, which served as the experimental unit ($EU = 24$). Each split-plot consisted of six seedlings (four measurement trees with one buffer tree on each side), and received one of two fertilizer treatments. No nutrient additions (NF) or N and P fertilization (F) in the form of diammonium phosphate (DAP) and ammonium nitrate (applied in the spring at a rate totaling 209 kg N and 116 kg P ha^{-1} in 2006 and 200 kg N ha^{-1} applied in 2007. Complete competition control was maintained within the measurement plots throughout the study using chemical (2.5% glyphosate) and mechanical control.

2.3. Total soil surface CO_2 efflux (F_S)

Manual point-in-time sampling of total soil surface CO_2 efflux (F_S) was performed using a Li-Cor 6200 portable infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska) with a dynamic closed soil

chamber. The cuvette chamber was constructed using PVC piping for walls, a plexi-glass top, and a stainless steel edge on the bottom. The chamber had an internal diameter of 25.5 cm with a total system volume of 6300 cm^3 (Selig et al., 2008; Tyree et al., 2008). Soil respiration measurements were taken on 14 sampling dates starting January 2006 and ending December 2007 (May 2006 measurements were discarded due to machine malfunctions). Measurements were taken at approximately the same location on each date and in the same sequential blocking order between 800 and 1600 h. One measurement was taken at the base of the tree (0.3 m) and the other taken between trees (0.9 m) to better account for spatial variation within the planting bed, however, all measurements were confined to planting beds for each experimental unit ($EU = 24$). Soil CO_2 evolution was measured over a 30 s period and efflux rates calculated as μmols CO_2 m^{-2} s^{-1}. Soil temperature and moisture were measured concurrently with F_S measurements. Soil temperature (TC) 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). Percent volumetric soil water content (SM) 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%.

The relationship between F_S and soil abiotic properties (temperature and moisture) was modeled using linear regression. Significant parameter estimates were used to simulate F_S over the two year study using continuous soil temperature and volumetric soil moisture data (Fig. 1B) collected using four data loggers distributed throughout the study site (CR10x; Campbell Scientific Inc.).

2.4. Index of heterotrophic respiration and microbial biomass C

An index of respiration by heterotrophic soil microbes (R_H) 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 cm^3. Measurements were taken simultaneously with F_S measurements using methods described by Gough and Seiler (2004). Two measurements were made per experimental unit. For each measurement, 12 soil cores were taken to a depth of 20 cm using a 2.5 cm diameter push tube. Soil samples were composited, roots carefully separated by hand, and a subsample of soil placed into an aluminum weigh boat (10 × 2 × 2 cm) that was immediately placed into the 0.25 L cuvette chamber. Once the CO_2 concentration began to steadily rise (typically within one minute), R_H was measured over a 30 s period. Soil samples were transported to the lab, oven-dried for 48 h at 105 °C, and weighed gravimetrically to the nearest 0.01 g. R_H rates are expressed in mmol CO_2 kg^{-1} soil s^{-1}. Data measured in the middle of September 2006 were removed due to excessively high R_H rates, which were associated with high soil moisture and 3.14 cm of rainfall the day prior to measurements. Although counterintuitive relative to intact soils, due to the significant disturbance associated with the removal of the soil core, the mixing, and root removal; extremely wet soils give excessively high respiration values that are not an accurate measure of soil heterotrophic activity.

Microbial biomass C (MBC) was estimated in June and December 2006, and July and December 2007 using chloroform fumigation-extraction procedure described by Jenkinson and Powlson (1976) and later modified by Anderson and Domsch (1978). Briefly, twenty-four random soil cores per plot 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 stored at 4 °C prior to analyses (less than 48 h). Two replicate 25 g fresh soil samples were weighed into 50 mL beakers and each placed into two separate vacuum desiccators. Half the samples were fumigated with ethanol-free chloroform ($CHCl_3$) for 24 h while the other samples were left unfumigated to serve as controls. Following fumigation 100 mL of 0.5 M solution of potassium sulfate (K_2SO_4) was added to each sample, shaken on low for 2 h, allowed to rest for 4 h before being passed through a Whatman #2 filter. A 20 mL subsample of filtered leachate was frozen and shipped to the Analytical Services Laboratory at North Carolina State University, Raleigh, NC for total C using a TOC analyzer (TOC-5050 fitted with an autosampler model ASI-5000, Shimadzu Scientific Instruments, Columbia, Maryland). Microbial biomass C was calculated by subtracting fumigated from unfumigated samples divided by an extraction efficiency constant of 0.45 (Joergensen, 1996).

2.5. Soil chemical properties

Determination of soil chemical properties was performed on samples collected on February 2006 (pre-fertilization), June and December 2006, and July and December 2007 to a depth of 30 cm or to water saturated soil with a 2.5 cm push tube. Approximately 15 g air dried soil was ground to a powder using a Micro-Mill® (Bel-Art Products, Pequannock, NJ) and 30 ± 5 mg of powdered soil weighed into 5 × 9 mm pressed tin capsules (Costech Analytical Technologies, Inc., Valencia, CA). Total soil C and N concentration were determined using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA) at the US Forest Service, Southern Research Station lab in Research Triangle Park, NC.

2.6. Plant growth

Tree height (H; cm) and basal diameter (D; cm) were measured repeatedly on four trees per experimental and stem volume (cm^3) was calculated by using stem vol = D^2*H. On January 8, 2008 one tree, which most closely fit the mean tree height from each EU, was selected for destructive harvest. The aboveground portion of each seedling was cut 10 cm above ground-line, wrapped in plastic, taken back to the lab, and dissected into needles, branches, and main stem. Belowground plant tissues were sampled by excavating a 1.0 × 1.0 × 0.5 m volume around the main stem. Roots were handpicked from the soil, bagged, and taken with the aboveground tissues to the lab. The root system was separated into tap root and lateral roots and washed. All separated plant parts were oven dried (>two weeks) at a temperature of 65 ± 5 °C then weighed gravimetrically to the nearest gram. The relationship between total biomass and calculated stem volume was determined using simple linear regression. Same-slopes analysis (Analysis of Covariance) showed no difference ($p = 0.77$) among genotypes, which allowed data to be fit with a common allometric equation [Total Biomass = 0.24 (stem volume) + 1657; $r^2 = 0.73$; $n = 24$]. Estimated plant biomass was converted to plant C by using a conversion factor of 0.5 and adjusted to a square meter basis by assuming each 38 m bedded row was one meter wide and contained 27 planted seedlings.

2.7. Data analyses

All subsamples were averaged prior to statistical analyses. Treatment differences for F_S, R_H, soil chemical properties, MBC, plant growth, and modeled F_S were tested by analysis of variance with repeated measures (ANOVARM) using a mixed model (PROC MIXED). Assumptions of normality and equal variance were tested by plotting the residuals and normality curves. Transformation of data was performed as appropriate, however, all data is presented using untransformed values. Covariance structures were chosen using the fit statistics (smaller is better) which are included in the default SAS output. Relationship between F_S and soil abiotic variables was modeled using simple linear regression (PROC REG).

Analysis of covariance (same-slopes analysis) was used to test for differences in slope between genotypes. *P*-values of 0.05 were considered statistically significant; however, *P*-values less than 0.10 were also explored for interesting trends. All data preparation and analyses were performed using the MIXED, GLM, and REG procedures in SAS version 9.3 (SAS, 2012).

3. Results

3.1. Total soil CO_2 efflux

Total soil CO_2 efflux (F_S) measured from the soil surface showed no significant ($p > 0.05$) treatment main effects or genotype by soil amendment interactions. However we did observe a significant genotype by time interaction ($p = 0.05$), which showed differences developing among genotypes during the growing season (late Mar to Oct; Tyree et al., 2009) and diminishing over the dormant season (Fig. 2). Total CO_2 efflux rates were approximately 13% greater in BC relative to NC genotypes (8.99 and 7.97 μmols CO_2 m^{-2} s^{-1}, respectively) when averaged over both growing seasons (2006 & 2007); however, the seasonal F_S pattern was driven primarily by changes in soil temperature and moisture (Figs. 1B and 2).

Regression analysis showed over the two year study that soil temperature alone explained 46 and 31% of the variation ($r^2 = 0.46$ and 0.31) for the BC and NC genotypes, respectively (Fig. 3). The temperature responses (shown by the slope) were statistically different between the two genotypes. The broad crown genotype showed greater sensitivity (steeper slope) to changes in soil temperature relative to the narrow crown genotype (slope 0.40 and 0.28, respectively). Percent volumetric soil moisture ranged from 7 to 35% over the two year study. However, soil moisture was found to be a non-significant regressor, and therefore, was not included in the regression analysis.

3.2. Microbial respiration (R_H) and biomass (MBC)

The incorporation of logging residue (LR) resulted in 26% greater R_H rates relative to NoLR plots (339 ± 13 and 270 ± 13 mmol CO_2 kg^{-1} s^{-1}, respectively; $p = 0.05$, $n = 168$) when averaged over all campaigns. However, a highly significant ($p < 0.01$) LR by time interaction showed that differences were greatest during the growing season (Fig. 4A). We also found a highly significant ($p < 0.01$) genotype by time interaction with the BC genotype maintaining greater R_H rates during the growing season relative to the NC genotype (Fig. 4B). Finally, relative to control plots, the addition of N and P fertilizer slightly decreased R_H by 12% when averaged across all campaigns (324 ± 13 and 286 ± 14 mmol CO_2 kg^{-1} s^{-1}, respectively; $p = 0.06$). Fertilizer did not appear to interact with any other treatments or time (Fig. 4C).

Microbial biomass C results were less clear, however, our data showed a significant ($p = 0.02$) three-way interaction between genotype, fertilizer, and time (Fig. 5). Fertilizer additions decreased MBC, but these trends were weak. In addition, this effect was further compounded by a general difference among genotypes with the BC plots maintaining overall greater MBC relative to NC plots. We found no significant ($p > 0.05$) effect of LR on MBC as either a main effect or as part of an interaction.

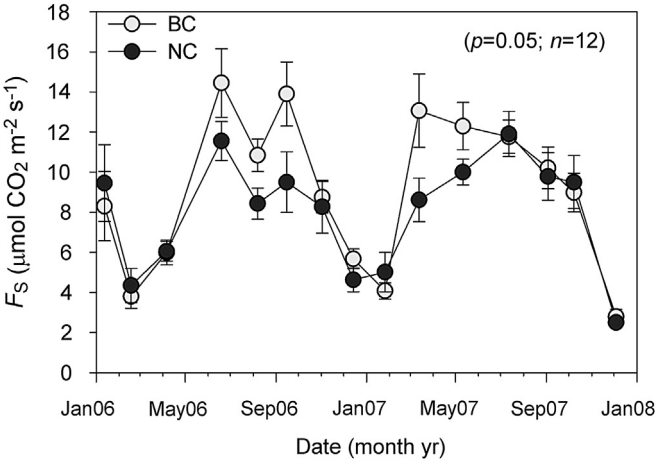

Fig. 2. Total soil surface CO_2 efflux (F_S) among "broad crown" and "narrow crown" loblolly pine genotypes (BC and NC, respectively) measured during the second and third year following planting. Each point is the average of 12 experimental units and the error bars represent ± one standard error of the mean. *P*-value shows the time by genotype two-way interaction tested by ANOVA with repeated measures using PROC MIXED in SAS 9.3.

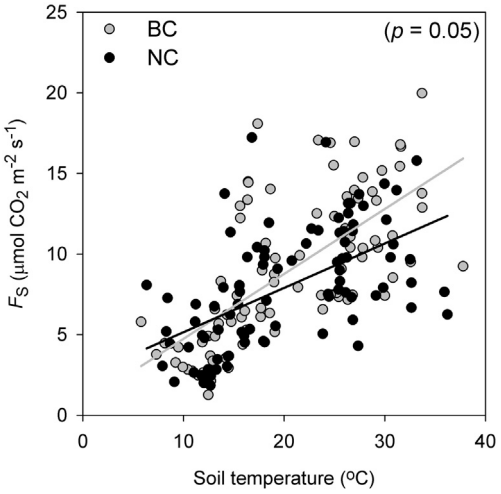

Fig. 3. Relationship between total soil CO_2 efflux (F_S) and soil temperature taken at a depth of 15 cm. Gray and black symbols represent "broad crown" (BC: $y = 0.40x + 0.7$, $r^2 = 0.46$, $p < 0.0001$, $n = 90$) and "narrow crown" (NC: $y = 0.28x + 0.7$, $r^2 = 0.31$, $p < 0.0001$, $n = 90$) genotypes, respectively. Difference in slope among genotypes was tested by Analysis of Covariance (same-slopes analysis) using PROC GLM in SAS 9.3.

3.3. Soil chemical properties

The addition of logging residue (LR) slightly increased total C relative to NoLR (5.24 ± 0.21 and 4.55 ± 0.21%, respectively; $p = 0.06$). The addition of N and P fertilizer resulted in a small increase in total C when LR was present (Table 1). Fertilization with N and P had no significant effect on total soil N contained within the less than 2 mm soil fraction (10 mesh sieve), although, there was a significant ($p = 0.01$) effect of LR on the C:N ratio with the addition of LR resulting in greater C:N relative to NoLR plots (Table 1). Overtime total C and C:N decreased in both soil amendment treatments ($p = 0.04$ and <0.0001, respectively; Table 1).

4. Discussion

4.1. Effects of genotype on F_S

Our hypothesis of a genetic by environment interaction was partially supported by the data. Although we did not find a GxE interaction, we did find a strong genotype by time interaction (Fig. 2). Increased F_S in BC plots was supported by previous work that showed the BC genotype maintained greater fine-root

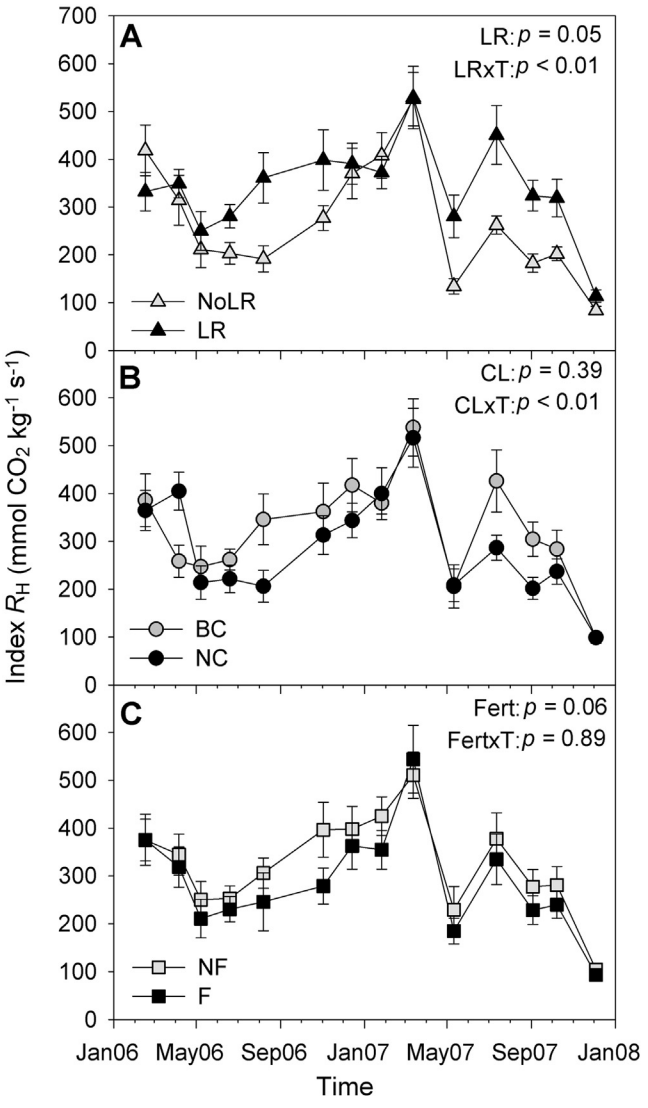

Fig. 4. Index of heterotrophic respiration (R_H) rates among logging residue (LR; Panel A), genotype (Panel B), and fertilizer treatments (Panel C) measured over a two year period. Each point and error bar represents the average of 12 measurements ± one standard error of the mean. *P*-values show treatment main effect and treatment by time (*T*) two-way interaction tested by ANOVA with repeated measures using PROC MIXED in SAS 9.3.

standing crop and fine-root turnover rates relative to NC (Pritchard et al., 2010). Greater fine-root length would lead to increased autotrophic respiration (R_A) assuming the genotypes have similar specific R_A on a unit mass basis. This is supported by Tyree et al. (2008) who found similar specific R_A among six, two-year-old loblolly pine elite genotypes. In addition, we observed greater R_H and MBC in BC plots (Figs. 4B and 5). We hypothesize that differences in R_A and R_H among BC and NC genotypes had an additive effect leading to greater F_S in BC plots. Although we are aware of no field studies that looked at differences in F_S among pine genotypes, our results do agree with findings from a greenhouse study, which utilized two distinct loblolly pine genotypes. That study observed that F_S began to diverge among the two genotypes by the end of the one year study and the authors postulated that differences would increase throughout plant development as genotype differences in biomass allocation became greater (Tyree et al., 2011). Likewise, our genotypic differences in F_S did not emerge until summer of 2006 (Fig. 2) after tree growth began to accelerate (Fig. 6B).

4.2. Cumulative C loss among genotypes

We estimated cumulative C loss over the two-year study period using genotype specific temperature response functions (Fig. 3). This increased temperature response in BC genotypes could be due to greater fine-root standing crop relative to NC plots. Findings by Boone et al. (1998) showed that the temperature sensitivity of autotrophic and rhizosphere respiration to be greater than that of bulk soil. Our soil CO_2 efflux estimates were scaled to the stand level using continuous, micrometeorological data averaged from four locations throughout the study site. Monthly estimates of soil temperature (20.6 and 20.3 °C for BC and NC genotypes, respectively; $p = 0.24$) and moisture (14.4 and 16.7% for BC and NC genotypes respectively; $p = 0.15$) did not differ among our two genotypes so no adjustment was performed to account for differences among our treatments for soil abiotic properties. We found by the end of the two year simulation that BC plots lost approximately 11% more C in the form of F_S than NC plots (6.52 and 5.89 kg C m^{-2}; Fig. 6A). Additionally, we found that NC plots maintained more C onsite in the form of total biomass C (Fig. 6B). Although rather simplified, this exercise demonstrates the need to account for genotypic differences of this magnitude, since it will impact the time necessary for the stand to switch from a C source to a C sink.

Our soil respiration rates are greater than other published values from field studies conducted in southern pine ecosystems (Gough et al., 2005; Maier and Kress, 2000; Pangle and Seiler, 2002; Samuelson et al., 2009). There are a couple of possible explanations for these high rates. First, all measurements were concentrated on the planting beds. This work did not attempt to estimate inter-row efflux rates since treatment differences were unlikely to present themselves this early in the rotation age. Second, our sampling methodology did not use preset collars, which the disturbance associated with cuvette placement could have led to an overestimation of F_S. However, our estimates of F_S are comparable to measurements taken using preset collars on our same plots (Maier, unpublished data) and on studies using a similar soil type (Tyree et al., 2011). Third, measurements were taken on double bedded rows in which logging residue was incorporated into the mineral soil in both LR treatments. This soil mixing has been shown to accelerate rates of organic matter decomposition (Coppens et al., 2007; Holland and Coleman, 1987), therefore, producing higher F_S rates.

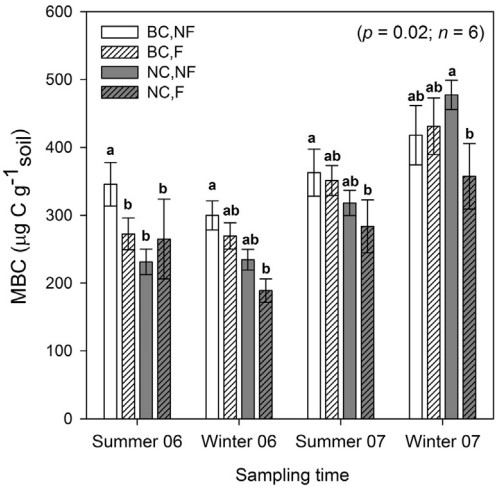

Fig. 5. Microbial biomass C (MBC) for the genotype by fertilizer by time three-way interaction. Small letters indicate significant ($p = 0.10$) differences among genotype by fertilizer treatments for each sampling time using the *pdiff* option within the LS means statement in PROC MIXED, SAS 9.3.

Table 1
Mean (s.e.) for soil chemical properties by soil amendment treatment. Samples were collected to a depth of 20 cm repeatedly over the second and third year following planting of loblolly pine elite genotypes ($n = 6$). Letters indicate statistically significant ($p < 0.10$) differences tested using the *pdiff* option within the LS means statement in PROC MIXED (SAS 9.3).

Soil amendment treatment					
Time	None	LR	F	LR + F	Average
Total soil C (%) in fine-soil fraction (<2 mm)					
Feb 06	4.82 (0.79)	4.39 (0.24)	4.55 (0.43)	5.96 (0.83)	**4.93 (0.32) abc**
Jun 06	5.91 (0.67)	5.52 (0.83)	5.38 (0.63)	5.18 (0.78)	**5.50 (0.35) a**
Dec 06	4.20 (0.57)	5.30 (0.87)	4.66 (0.38)	7.10 (1.28)	**5.32 (0.46) ab**
Jul 07	4.20 (0.51)	5.30 (0.66)	4.22 (0.47)	4.23 (0.25)	**4.49 (0.25) bc**
Dec 07	3.83 (0.24)	5.23 (1.06)	3.67 (0.47)	4.23 (0.36)	**4.24 (0.32) c**
Average	**4.59 (0.28) ab**	**5.15 (0.33) ab**	**4.50 (0.23) a**	**5.34 (0.39) b**	
Total soil N (%) in fine soil fraction (<2 mm)					
Feb 06	0.14 (0.02)	0.11 (0.01)	0.12 (0.01)	0.14 (0.02)	**0.12 (0.01) a**
Jun 06	0.13 (0.02)	0.11 (0.02)	0.14 (0.02)	0.11 (0.02)	**0.12 (0.01) a**
Dec 06	0.10 (0.01)	0.13 (0.02)	0.13 (0.01)	0.16 (0.02)	**0.13 (0.01) a**
Jul 07	0.14 (0.01)	0.16 (0.02)	0.12 (0.01)	0.13 (0.01)	**0.14 (0.01) a**
Dec 07	0.12 (0.01)	0.15 (0.02)	0.12 (0.01)	0.14 (0.01)	**0.13 (0.01) a**
Average	**0.13 (0.01) a**	**0.13 (0.01) a**	**0.12 (0.01) a**	**0.13 (0.01) a**	
Carbon to nitrogen ratio (C:N)					
Feb 06	35.0 (2.79)	41.6 (1.65)	39.7 (2.11)	44.1 (1.29)	**40.1 (1.18) a**
Jun 06	44.8 (2.41)	54.1 (4.62)	41.6 (2.94)	49.2 (2.59)	**47.4 (1.81) b**
Dec 06	40.5 (2.68)	42.5 (3.78)	38.0 (2.44)	45.2 (2.97)	**41.6 (1.51) a**
Jul 07	30.8 (1.98)	34.3 (1.12)	33.9 (1.11)	33.2 (1.19)	**33.1 (0.71) c**
Dec 07	31.2 (1.24)	33.7 (1.40)	30.5 (0.90)	30.8 (0.66)	**31.6 (0.57) c**
Average	**36.5 (1.39) a**	**41.3 (1.82) b**	**36.7 (1.13) a**	**40.5 (1.56) b**	

4.3. *Effects of soil amendments on F_S and microbial respiration*

In contrast to our hypothesis, we did not observe an increase in F_S with the additional incorporation of logging residue. Our data did show a strong increase in R_H with LR additions (Fig. 4A), but no increase in MBC in LR plots was observed ($p = 0.45$). These findings are consistent with others that have observed increases in decomposition rates following LR additions in conifer forests (LundmarkThelin and Johansson, 1997; Ouro et al., 2001; Perez-Batallon et al., 2001) and agricultural studies (Coppens et al., 2007; Holland and Coleman, 1987; Nicolardot et al., 2007). It is theorized that incorporation of logging residue into the soil provides conditions (temperature and moisture) favoring increased microbial activity as well as putting C substrate in direct contact with soil biota. In contrast to others that found an increase in F_S with LR incorporation (Aggangan et al., 1999; Perez-Batallon et al., 2001; Tyree et al., 2011), we observed no effect. We hypothesize that the short-term decrease in the autotrophic component offset the increase in microbial respiration (R_H) leading to no change in F_S. This is supported by the decrease in both lateral and total belowground biomass with LR additions observed in these plots (Tyree et al., 2009). In addition, the incorporation of the existing forest floor into the beds (approximately 25 Mg ha^{-1}) may have diminished the effects of the additional 75 Mg ha^{-1} of logging residue incorporated in the LR treatments at least in the short-term.

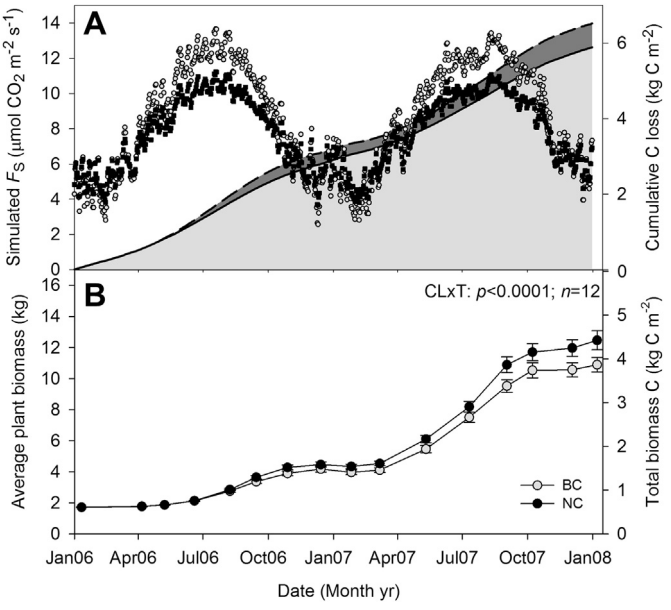

Fig. 6. Panel A: Simulated total soil surface CO_2 efflux (F_S) for "broad crown" (BC; gray circle) and "narrow crown" (NC; black square) genotypes and cumulative respiratory C loss from a loblolly pine plantation during the second and third year following planting. Daily average F_S rates were modeled using temperature response curves (Fig. 3) fit to continuous on-site data loggers located on the bedded rows (Fig. 1B). Cumulative C loss in the form of CO_2 for BC (dark gray shading) and NC (light gray shading) genotypes is the summation of daily average F_S rates estimated from the bedded rows and presented as kilograms of C per square meter. Panel B: Average plant biomass (left *y*-axis) converted to total biomass C per square meter (right *y*-axis) for each genotype. *P*-value shows the time by genotype two-way interaction tested by ANOVA with repeated measures using PROC MIXED in SAS 9.3.

We found no change in F_S with the addition of N and P fertilizer, which is consistent with other studies (Lai et al., 2002; Lee and Jose, 2003; Maier et al., 2004; Pangle and Seiler, 2002; Tyree et al., 2008). Similar to the effects of LR, we hypothesize that the observed decrease in R_H (Fig. 4C) and MBC (Fig. 5) with fertilization was offset by increased plant growth and consequently autotrophic respiration, which has also been concluded by others (Gough and Seiler, 2004; Lee and Jose, 2003). This hypothesis is supported by the increased belowground biomass and total biomass with the addition of N and P observed on these same plots (Tyree et al., 2009). Additionally, others have shown increased root biomass (Albaugh et al., 1998; King et al., 2002; Maier and Kress, 2000; Samuelson, 2000), and a few have shown greater specific root respiration (Gough and Seiler, 2004; Tyree et al., 2008) following nutrient additions in southern pines.

In conclusion, we found an immediate and sustained difference in total soil surface CO_2 efflux (F_S) among our two genotypes during the first two years of stand development. We showed that these two genotypes differed in their response of F_S to changes in soil

temperature, which resulted in an 11% difference in total cumulative C loss from the site in the form of soil surface CO_2 efflux. This underscores the importance of accounting for differences among genotypes when developing stand-level C estimates. We did not observe an effect of logging residue or N and P fertilization on F_S rates over the two years we collected samples, however, our soil amendment treatments did result in significant differences in microbial activity (R_H) and biomass (MBC) among our treatments. We concluded that in both soil amendment treatments that there were offsetting affects between the heterotrophic and autotrophic components that make up F_S. Further work is still needed to better understand how our treatments influence microbial community composition and the soil biological properties in the longer-term.

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