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REGULAR ARTICLE

Respiratory C fluxes and root exudation differ in two full-sib clones of *Pinus taeda* (L.) under contrasting fertilizer regimes in a greenhouse

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Abstract
Aims We investigated whether changes in respiratory C fluxes, soil CO_2 efflux, or root exudate quantity or quality explained differences in growth rates between closely related clones of *Pinus taeda* (L.).
Methods A factorial design with two clones, fertilized and control treatments, and four sequential harvests was installed in a greenhouse for 121 days.
Results The two clones did show significant differences in respiratory C fluxes, soil CO_2 efflux, and root exudation quantity and quality. While the clones also differed in growth rates, the C fluxes assessed in this paper did not explain how seedlings were able to allocate more C to stem growth in the months following fertilizer application. Changes in root exudation were not consistent with reduced heterotrophic soil CO_2 efflux, which does not appear to be a plant-mediated process.
Conclusions These results indicate that if single genotypes are deployed over large land areas in plantations, dramatic differences between clonal plant-soil interactions may require consideration in ecosystem C budgets. Further, the range of belowground fluxes observed implies that genotype-specific C allocation may make some clones better able to exploit a given resource environment than others.

Keywords Soil CO_2 efflux • Carbon allocation • Intensive silviculture • Varietal forestry

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Introduction

Pinus taeda (L.) plantations span some 13 million hectares across the southeastern United States (Conner and Hartsell 2002), and are responsible for production of a disproportionate amount of timber on a national basis (Adams et al. 2006). These plantations are often fertilized with N and P, with over 6 million hectares already fertilized over the last several decades (Albaugh et al. 2007). Increasingly clonal material is being planted in these plantations in order to increase productivity (Bettinger et al. 2009). An understanding of the ecophysiology of different clones under fertilizer regimes similar to those found in operational plantations is necessary both to understand varying observed clonal growth responses to fertilizer application (King et al. 2008), and to better understand the carbon cycling of this rapidly expanding intensively managed forest ecosystem. The purpose of this paper is to examine how respiratory carbon fluxes and root exudation change in the short-term between closely related clones in response to an operational fertilizer application, and to determine if these changes in ecophysiology are consistent with a theory of short-term growth response to fertilizer application.

Ecosystem respiration, or C emitted by aboveground biomass respiration and soil CO_2 efflux, is one of the primary determinants in whether forest ecosystems are net sources or sinks of C relative to the atmosphere (Valentini et al. 2000). Numerous studies have shown that nutrient

availability affects various respiratory fluxes in forest ecosystems, including foliar, woody, and root biomass respiration, soil microbial respiration, and total soil CO_2 efflux (Phillips and Fahey 2007; Ryan et al. 1996; Vose and Ryan 2002). Fertilizer and irrigation treatments have been shown to affect whether midrotation *P. taeda* plantations on a poor site may be net sources or sinks for C, in part due to treatment effects on ecosystem respiration (Maier et al. 2004). Intra-specific variability in various respiration rates has also been observed in the literature. Lower leaf respiration rates were found in faster growing families of *P. taeda* and *Pinus elliottii* (Englm.) (Samuelson 2000). In another study no difference was observed in foliar respiration among clones of *P. taeda*, although only a relatively small foliar sample (5 fascicles) was measured (King et al. 2008). Clonal variability in respiratory C fluxes could play a significant role in determining differences that have been observed in clonal growth rates (e.g. Paul et al. 1997).

Soil CO_2 efflux (F_S) is the largest respiratory CO_2 flux in forested ecosystems, and is in fact greater in magnitude on average than net primary productivity (Raich and Schlesinger 1992). A number of studies have shown that in *P. taeda* plantations, fertilizer application typically results in reduced F_S rates (Butnor et al. 2003; Giardina et al. 2004; Maier and Kress 2000; Samuelson et al. 2004). However, other studies have shown that F_S increased in either the short-term (< 1 year) or long-term (> 20 year) following fertilizer application (Gough and Seiler 2004; Tyree et al. 2006). Increases in F_S such as these are often attributed to greater root biomass, and thus greater root respiration in fertilized treatments versus controls. However, the heterotrophic component of soil CO_2 efflux (F_H) consistently shows a decline with fertilizer application that has not yet been linked to a specific causal mechanism (Gough and Seiler 2004; Olsson et al. 2005; Tyree et al. 2008). Research in the field on young (e.g. one-year-old) clones has shown no variability in F_S, or its autotrophic or heterotrophic components due to genotype (Tyree et al. 2008). This last study hypothesized that clonal effects may not have manifested yet in one-year-old seedlings, and that any treatment effects would have been difficult to detect in single-tree plots. However, pot-based studies have shown that F_S response to fertilizer application may occur within days (Gough and Seiler 2004). Thus, a pot-based study involving clones would be ideal to detect any short-term changes in belowground respiratory C fluxes following fertilizer application.

The first objective of this paper was to determine whether differences in total ecosystem respiration, aboveground biomass respiration, and F_S would be consistent with observed differences of clonal growth rates. Clones with lower respiratory C fluxes may allocate more C to net primary production, allowing for more rapid short-term growth. Further, we hypothesized that different clonal growth responses to fertilizer application would be consistent with changes in respiratory C fluxes across a nutrient availability gradient. Reduced respiratory C fluxes in response to fertilizer application may be a short-term mechanism by which trees can allocate more C to canopy development, the widely recognized long-term mechanism of fertilizer growth response (Albaugh et al. 1998; Fox et al. 2007; Vose and Allen 1988).

Root exudates are composed of numerous organic and inorganic compounds that are either passively or actively transferred from living roots to the rhizosphere (Nguyen 2003). Among a range of other functions, root exudates are known to increase nutrient availability in the rhizosphere, either through ligand exchange reactions with organic acids (e.g. P) or by increasing rates of microbial mineralization by providing a labile C source (e.g. N) (Dakora and Phillips 2002; Hinsinger 2001; Landi et al. 2006). Estimates of the percentage of GPP allocated to root exudation range from less than 1% up to 4 % (Grayston et al. 1997). While the carbon flux that can be attributed to exudation is of a much lower magnitude than respiratory C fluxes, root exudates remain disproportionately important in governing plant-soil interactions through their role in mediating the biological and chemical nature of the rhizosphere. Differences in root exudation may thus play a critical ecophysiological role in linking plant growth response to fertilizer additions and genotypic variability in fertilizer growth response.

Fertilizer application has been shown to alter both quantity and quality of root exudates. Reduced exudation quantity and reduced total extractable rhizosphere C have been observed with the addition of N fertilizers (Henry et al. 2005; Lagomarsino et al. 2006). Other studies have shown both quantitative and qualitative changes in root exudation in response to a P gradient (Egle et al. 2003; Ratnayake et al. 1978). Generally, exudation rates are greatest under more nutrient deficient conditions, and thus are reduced by fertilizer application. Additionally, studies have also found quantitative and qualitative differences in root exudation between different species and between different cultivars of the same species (Egle et al. 2003). The chemical composition of exudates varies among tree species, even those in the same family or genera (Grayston et al. 1997). However, data pertaining to intra-specific variability of exudate quantity and quality in tree species are lacking in the literature. The second objective of this study was to determine if root exudation quantity and quality varies between clones in response to fertilizer application. Further, we hypothesized that any differences would be consistent with differences in clonal growth responses to fertilizer application, either through the role of exudation as a C sink or through altering nutrient uptake efficiencies between clones.

Root exudation affects CO_2 efflux from the rhizosphere. Inputs of root exudate organic acids into simulated rhizospheres have been linked to changes in microbial N

immobilization and mineralization processes, community structure, and microbial activity (Landi et al. 2006). Up to 36 % of microbial biomass C may be root derived, a major source of which could be exudates (Werth and Kuzyakov 2008). Numerous studies have showed rhizosphere priming effects, or increases in soil organic matter turnover as a result of labile root exudate inputs into soil (Bol et al. 2003; Kuzyakov 2002). Each of these processes mediated by root exudate quantity and quality plays a role in the magnitude of F_H from rhizosphere soils. While there is much tangential evidence to support the hypothesis that F_H may be regulated by root exudate inputs into the rhizosphere, we are not aware of any studies that have yet examined causal links between root exudation and F_H across a resource availability gradient. The final objective of this study was to determine if reduced root exudation as a result of fertilizer application may be the causal mechanism responsible for reductions in F_H routinely observed with fertilizer application (Gough and Seiler 2004; Olsson et al. 2005; Tyree et al. 2008).

Materials and methods

Study description and experimental design

Ramets of two clones were potted on April 30, 2009 in a coarse, nutrient and organic matter deficient soil in a greenhouse at Virginia Tech, Blacksburg, Virginia, USA. The two contrasting clones, GE34 and GE769, were a full-sib pair originally produced by ArborGen in 2005 (Arborgen LLC, Summerville, South Carolina, USA) (Bitoki 2008). Ramets averaging 40 cm in total height and 6.5 mm in root collar diameter were potted in 15-by-15-by-38 cm deep pots (8,550 cm^3) that were sufficiently large to minimize substantial root-binding through the 4 months of this experiment. Seedlings were not root-bound even by the final day 121 harvest. Ramets were planted in their original plugs containing previously fertilized media in order to minimize root mortality and turnover. The soil utilized in this study was the sieved (1-cm mesh) A horizon of a Wakulla series (siliceous, thermic Psammentic Hapludult) obtained from the USDA Forest Service's Southeastern Tree Research and Education Site (SETRES). Seedlings were watered daily to minimize drought stress while also avoiding excessive leaching from the bottom of the pots. Nighttime minimum temperature was set to 18 °C in the greenhouse, and while the vents were set to open during the day at 25 °C daytime temperatures did exceed this frequently.

The ramets were randomly assigned to fertilizer and control treatments, and fertilizer was applied to the selected ramets on June 16, 2009. This date will be referred to as day 0 throughout the remainder of this paper. Fertilizer was applied at an operational rate with diammonium phosphate and ammonium nitrate at 225 kg N per hectare and 56 kg elemental P per hectare. Control seedlings received no fertilizer. Following fertilizer application, ramets from each treatment combination were harvested monthly on July 16, August 16, September 15, and October 15, 2009 (30, 61, 91, and 121 days after fertilization). Thus the experiment was a two-by-two-by-four factorial randomized complete block design replicated eight times (128 seedlings total), with treatments consisting of clone, fertilizer, and sequential harvest, respectively. Some measurements were only made on the final harvest group (day 121 harvest) throughout the experiment. These variables are described below, and may be considered a two-by-two randomized complete block design with repeated measures. Other variables were measured on each seedling at harvest and thus reflect a tree-for-time-substitution assumption.

Biomass and stem growth measurements

At each of the four destructive harvests the entire seedling was partitioned into components. Fine roots were considered those <2 mm diameter, with coarse roots being any root >2 mm diameter. All biomass components were oven-dried at 65 °C for >10 days, and weighed. Detailed analyses of biomass can be found in Stovall et al. (2012). Throughout the experiment ground-line diameter and total height were measured weekly on the final harvest group. A stem volume index was calculated for each seedling with the formula height × (basal diameter)^2. Prior to each destructive harvest heights and basal diameters of all seedlings were measured to ensure that no significant growth differences existed between harvest groups, and that tree-for-time-substitution assumptions were valid. Further data on biomass partitioning and allometry for this study are available in Stovall et al. (2012).

Respiratory C flux and soil CO_2 efflux measurements

Total soil CO_2 efflux (F_S) was assessed in the morning between 10:00 and 12:00 EDT using a small dynamic closed (231 cm^3 volume, 55 cm^2 area) cuvette with no fan. A LI-6200 infrared gas analyzer (IRGA) was used for all respiratory C flux measurements (LiCor Biosciences Inc., Lincoln, Nebraska, USA). The IRGA was zeroed daily immediately prior to the first F_S measurement and a blank reading on a sealed cuvette with no soil was taken to ensure the apparatus was operating correctly. Soil temperature (thermocouple) and volumetric moisture content (TDR) were measured concurrently with efflux for use as covariates in statistical analyses. These measurements were made on 22 separate dates. Procedures were based on those described in Gough and Seiler (2004). For all measurements made with the LI- 6200, rates were calculated to correct for the volume of the respective cuvette.

The heterotrophic component of soil CO_2 efflux (F_H) was measured on all four destructive harvest groups one or two

days prior to harvest. One soil sample (mean 19.9 g, standard error 0.1 g) was taken with a push-tube from the top 17 cm of soil near the edge of each pot to minimize damage to the root system. Roots were removed from the soil by hand, and the soil was then placed in an aluminum boat in a 0.25 L cuvette equipped with a small fan for quantification of F_H. No measurement was taken until the LI-6200 showed a clear and steady increase in CO_2. Once this was observed, change in CO_2 concentration over a 30 s period was used to estimate F_H. Soil was then weighed fresh within the hour, oven dried at 65 °C for >24 h and weighed again dry so that gravimetric moisture content could be calculated and used as a covariate. Rates were expressed on a soil dry mass basis.

The autotrophic component of soil CO_2 efflux (F_A) was measured at days 61, 91, and 121 after fertilizer application on the harvested seedlings. Autotrophic respiration was not measured for the first destructive harvest. Between 13:00 and 16:00 on each harvest date, each pot was carefully overturned to remove the entire seedling intact. Roots were then washed with tap-water, and seedlings were transported to the lab, a process taking no longer than 30 min for each block. A small sample (mean 0.34 g, standard error 0.02 g) of fine roots (< 2 mm) were carefully excised from harvested seedlings immediately after their transport back to the lab, soaked in deionized water, and then F_A was determined in sequential tree order by blocks in a 0.25 L cuvette equipped with a small fan. Roots were then oven dried at 65 °C for >24 h and weighed so that respiration rates could be expressed on a mass basis. Both F_H and F_A are intended for use only as treatment indices, and do not represent rates that would be observed in intact plant-soil systems (Hanson et al. 2000; Tyree et al. 2008). Care was taken to obtain roots with a similar diameter distribution in all treatments for both F_A and exudate measurements.

Dark respiration rates were measured at night between 23:00 and 5:00 EDT within two days prior to each destructive harvest. Two distinct measurements were made: 1) total aboveground dark respiration (R_{AG}) and 2) total ecosystem respiration rate (R_{ECO}), which included aboveground biomass, belowground biomass, and the entire potted soil mass. For R_{AG} a large inverted trash-can (volume = 120,000 cm^3) was used as a cuvette. An incision was made along the radius of the lid, so that it could be sealed with weather-stripping around the base of the stem of the seedling being measured without damaging the seedling. All aboveground tissues (needles, branches, stem) were measured together using this technique. A small fan was installed in the cuvette to mix the air volume inside. Due to technical difficulties with the cuvette, aboveground respiration was only measured for harvests 30 and 91 days after fertilizer application. For R_{ECO} measurements, the cuvette used was a large trashcan inverted atop another (total volume = 240,000 cm^3). The entire seedling while still potted was placed in the bottom trashcan, the top trashcan was sealed on top of it, and air was mixed with a small fan. Ambient temperature inside the cuvette was measured with a thermocouple during each measurement so that respiration rates could be standardized to 20 °C assuming a Q_{10} of 2.0 (Ryan 1991). Both rates were expressed on a plant mass basis in order to account for differences in seedling size. Prior to each measurement period, empty, sealed cuvettes yielded zero rates as would be expected.

Root exudation and soil chemical analysis

Root specific exudation rates for a subsample of fine roots (mean 0.78 g, standard error 0.03 g) were determined as per Egle et al. (2003) in the lab beginning no more than 30 min after the harvest of each seedling. Exudation was assessed while the seedling was completely intact, save for a small sample of separate roots that had been excised for determination of F_A. Briefly, this process involved immersing a portion of the washed root system in 75 ml of aerated deionized water for 1 h to allow the roots to equilibrate, then repeating the process with 75 ml of fresh, aerated deionized water for one more hour. After removing roots from the solution, this subsample of fine roots was excised and refrigerated for later morphological analysis. Root metrics (length, surface area) were determined by scanning the root sample taken for exudate measurements and processing images with WinRhizo 5.0A software (Regent Instruments Inc., Quebec, Quebec, Canada).

The second solution was immediately filtered through a number 2 Whatman qualitative filter, and then frozen at -20 °C. Details of the procedure (time of soak, volume of soak solution, adequate concentrations of exudates obtained) were determined in a pilot study conducted prior to the first harvest using 12 ramets of clone 769. Total organic carbon (TOC) was later determined on an Elementar LiquiTOC Analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey, USA). Further analyses of exudate composition were performed by ion chromatography with an AS17C column at a 1 ml/min flow rate through a 25 µl sample loop at 149 mA current. An EG40 Eluent Generator was utilized with an IP25 Isocratic pump, a CD25 Conductivity detector, and a LC25 Chromatography oven (Dionex Corporation, Sunnyvale, California, USA). Anions assessed included acetate, chloride, citrate, formate, lactate, nitrate, oxalate, phosphate, sulfate, and tartrate. All runs were checked for accuracy with standards that were prepared daily.

All soils were sieved through a 2-mm mesh and air dried. Soil C and N were determined on an Elementar CNS Analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey, USA). Other soil nutrients were analyzed by the Virginia Tech Soils Testing Laboratory using a Mehlich I procedure and a Thermo Elemental ICAP 61E (Thermo Scientific, Walthman, Massachusetts, USA) (Mullins and Heckendorn 2009).

Statistical analysis

All analyses were performed in SAS software v. 9.2 (SAS Institute Inc., Cary, North Carolina, USA). All variables were transformed as appropriate to meet assumptions of normality, but all reported means and standard errors are untransformed. Repeated measures analyses utilized covariance structures appropriate for unevenly spaced data (unstructured, compound symmetry, spatial power, spatial Gaussian, and spatial spherical) that were selected by minimizing AIC_C values (Littell et al. 2006). Initial (day -42) seedling size was included as a covariate in all analyses of growth variables. For data from the four harvest dates, all harvest interactions were included in analyses. Block was modeled as a random effect, and PROC MIXED was implemented using the Kenward-Roger method for calculating denominator degrees of freedom (Littell et al. 2006). To meet assumptions of homogenous variance, variance was modeled separately for each treatment combination using the "group" option in the "repeated" statement as necessary.

Root exudate qualitative data for oxalate, citrate, phosphate, and lactate were corrected by changing all non-detect values to the value of half the detection limit for that compound (Smith 1991). As it was not possible to transform these data to normal, the non-parametric Friedman's Chi-Square Test was implemented using PROC FREQ. No analysis was performed for the fertilizer effect on soil P data, since only two of 64 control samples were above the detection limit. Statistical values reported for soil P data pertain only to the fertilized treatment.

Results

Stem growth response to treatments

Both clonal and fertilizer main effects were statistically significant in the repeated measures analysis for stem volume (Table 1). These differences appear to be driven by height growth for the clonal effect and basal diameter growth for the fertilizer effect (Fig. 1). By day 121 fertilized seedlings showed a 53 % increase in volume over controls and a 25 % increase in basal diameter ($p<0.05$). While clone 769 had 50 % greater volume and 10 % greater height at day 0 ($p<0.10$), by day 121 clone 34 had 3 % greater volume and was 5 % taller, although these day 121 differences between clones were no longer statistically significant ($p<0.10$). This indicates that clone 34 is the faster growing of these two clones, since it had less stem volume at day 0 but was not significantly different from clone 769 by day 121. Both clones responded similarly to fertilizer application as is evidenced by lack of significant clone-by-fertilizer interactions for any of the stem growth metrics ($p>0.10$).

Table 1 P-values for stem metrics, respiratory C fluxes, root exudation quantity, and soils data

Variable	Clone	Fertilizer	Clone X fertilizer	Harvest date	Clone X harvest date	Fert. X harvest date	Clone X Fert. X harvest date
tree height	**0.0661**	0.9439	0.7599	–	–	–	–
basal diameter	0.9363	**0.0001**	0.6374	–	–	–	–
stem volume	**0.0207**	**0.0132**	0.7427	–	–	–	–
F_S	0.6381	**0.0001**	**0.0002**	–	–	–	–
F_A per root mass	0.7201	**0.0024**	0.1332	**0.0023**	0.8547	0.4251	0.7429
fine root mass	**0.0152**	0.4468	**0.0973**	**0.0001**	**0.0674**	**0.0001**	0.3088
F_A per tree	0.4636	**0.0038**	**0.0876**	0.3119	0.8411	0.4933	0.6314
F_H	0.5154	**0.0001**	0.1984	**0.0635**	0.6867	0.1269	0.3962
R_{ECO}	**0.0014**	0.4210	0.7487	**0.0001**	0.2450	0.7945	0.2553
R_{AG}	0.1186	**0.0990**	0.6042	**0.0001**	0.2034	0.8625	0.7875
root exudation per mass	**0.0189**	0.2436	0.4513	**0.0001**	0.8019	0.2690	0.1830
root exudation per length	0.1411	0.9904	0.2692	**0.0001**	0.4072	0.3580	**0.0848**
root exudation per area	0.1607	0.5376	0.3202	**0.0001**	0.5123	**0.0401**	**0.0564**
soil pH	**0.0778**	**0.0001**	0.3228	**0.0002**	0.3131	0.1347	0.6248
soil N	0.4411	**0.0001**	0.7230	**0.0001**	0.8696	**0.0125**	0.8391
soil K	0.5439	**0.0009**	0.4738	**0.0135**	0.7579	**0.0154**	0.9764
soil Ca	**0.0419**	0.1984	0.2708	**0.0001**	**0.0944**	**0.0209**	0.6776
soil Mg	0.1001	**0.0398**	0.4273	**0.0001**	0.1115	**0.0007**	0.6331

When harvest effects are not shown, data were collected only on the final harvest group trees over time and were analyzed using repeated measures. When harvest effects are shown, data were collected from each of the four harvest groups at the corresponding monthly destructive harvest following fertilization. P-values <0.10 are indicated in boldface

Respiratory C fluxes and soil CO_2 efflux

R_{ECO} was greater in clone 34 than clone 769 by 32.4 % averaged across all dates ($p<0.01$; Fig. 2). This effect was only significant when tested on individual dates for the 30 and 121 harvests, where clone 34 had 35.5 and 53.1 % greater flux rates, respectively ($p<0.10$). No fertilizer or clone-by-fertilizer effects were observed ($p>0.10$). By contrast, no significant clonal differences were found in R_{AG} ($p>0.10$). R_{AG} only varied among fertilizer treatments when averaged across both measurement dates ($p<0.10$), although this effect was not individually significant on either day ($p>0.10$). R_{AG} was 14.7 % higher across both dates in fertilized ramets. A number of factors could result in this disparity between R_{ECO} and R_{AG} fluxes among treatments. F_S may be sufficiently large to mask any signal from R_{AG} as both are components of R_{ECO}. However, it is not possible to determine what proportion of R_{ECO} was attributable to R_{AG} due to chamber effects. R_{ECO} and R_{AG} measurements should not be directly compared on the same scale, but rather should only be considered as treatment indices (Norman et al. 1997).

F_S response to fertilizer application differed significantly between the two clones over time (Table 1, Fig. 3). In the 10 days immediately following fertilization efflux rates spiked

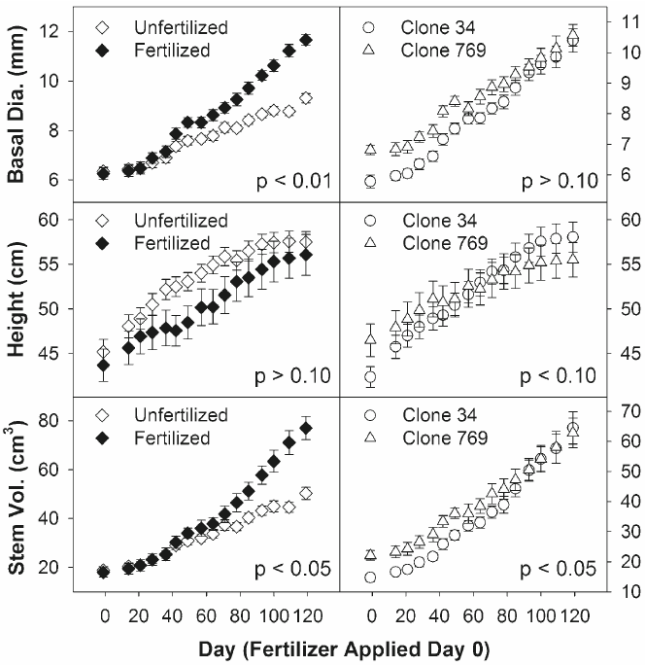

Fig. 1 Fertilizer and clonal effects on stem dimensions of the final harvest group. Standard error bars are shown, *N*=16. Fertilizer was applied day 0=June 16, 2009

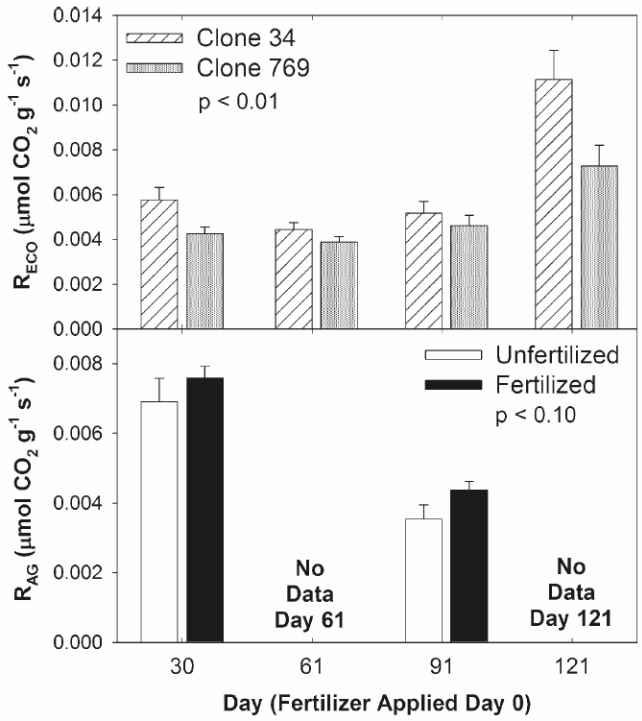

Fig. 2 Clone and fertilizer effects on total ecosystem dark respiration and aboveground plant dark respiration, respectively, measured prior to each of four destructive harvests. Data are not available for R_{AG} for days 60 or 120. Measurements were made with a LICOR 6200 IRGA. Respiration is expressed on a plant biomass basis for both metrics. Standard error bars are shown, *N*=16. Fertilizer was applied day 0 = June 16, 2009

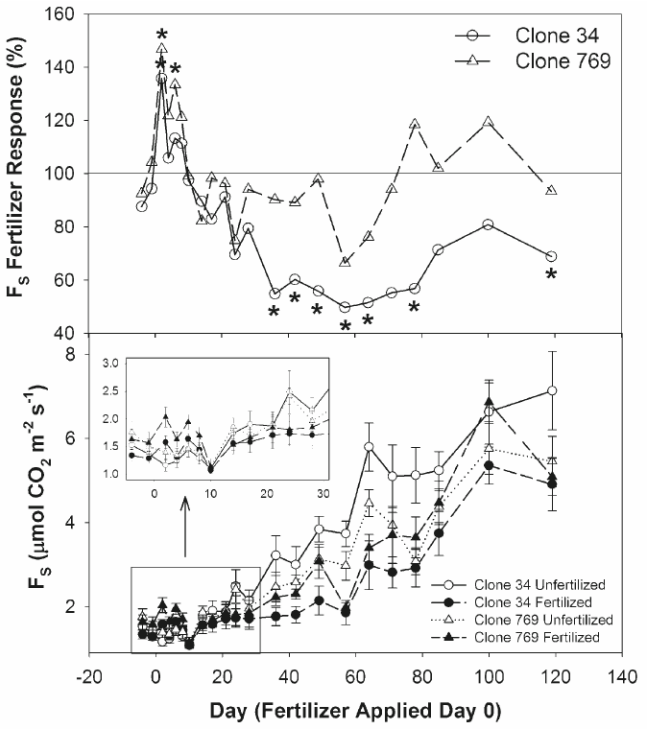

Fig. 3 Clone-by-fertilizer interaction for soil CO_2 efflux from harvest 4 trees (bottom panel). The fertilizer effect for each clone is shown in the top panel, with significant differences for each clone ($p<0.10$) denoted by an asterisks. Measurements were made using a LICOR 6200 with a 231 cm^3 cuvette. Standard errors are shown, *N*=8. Fertilizer was applied day 0=June 16, 2009

in fertilized ramets of both clones, reaching a peak on day 2 at approximately 140 % the level of unfertilized ramets. Following day 10, F_S was not significantly affected by fertilizer application in either clone until day 36. From day 36 to day 78 clone 34 consistently and significantly ($p<0.05$) had less than 60 % of the F_S rates in fertilized ramets compared to controls. During this period unfertilized ramets of clone 34 also consistently had the highest efflux rates of any treatment combination, while fertilized ramets consistently had the lowest rates. However, during this same period clone 769 showed no significant F_S response to fertilizer application. While the treatment effect diminished in clone 34 following day 78, fertilized ramets still had less than 80% of the rates observed in controls. Clone 769 continued to show no significant treatment effects between days 78 and 120 ($p<0.10$).

Despite the different clonal responses found in F_S rates, neither F_A nor F_H showed any significant clonal effects across all dates (Table 1). F_H was significantly depressed on days 30, 60, and 90 following fertilizer application ($p<0.05$; Fig. 4). However, on day 120 there was a significant clone-by-fertilizer interaction, with clone 34 showing a 20 % increase while clone 769 showed a 46 % decrease in fertilized ramets ($p< 0.05$). While F_H is only intended as a treatment index, since approximately the same soil mass was contained in each pot these rates can be inferentially scaled to the whole plot level for more direct comparison with F_S data. The lack of consistency between the distinctly different clonal F_S fertilizer responses and F_H would seem to indicate that F_H alone is not driving F_S in this nutrient-deficient coarse sand.

Across all dates root-specific F_A rates were 29 % higher in the fertilizer treatment (Table 1). The only individual date for which this effect was significant was day 60 ($p<0.01$; Fig. 4). As with F_H, there were no significant clonal effects for root-specific F_A. However, there was a significant clone-by-fertilizer effect in fine root biomass across harvest dates ($p<0.10$; Fig. 4). When this was taken into account by

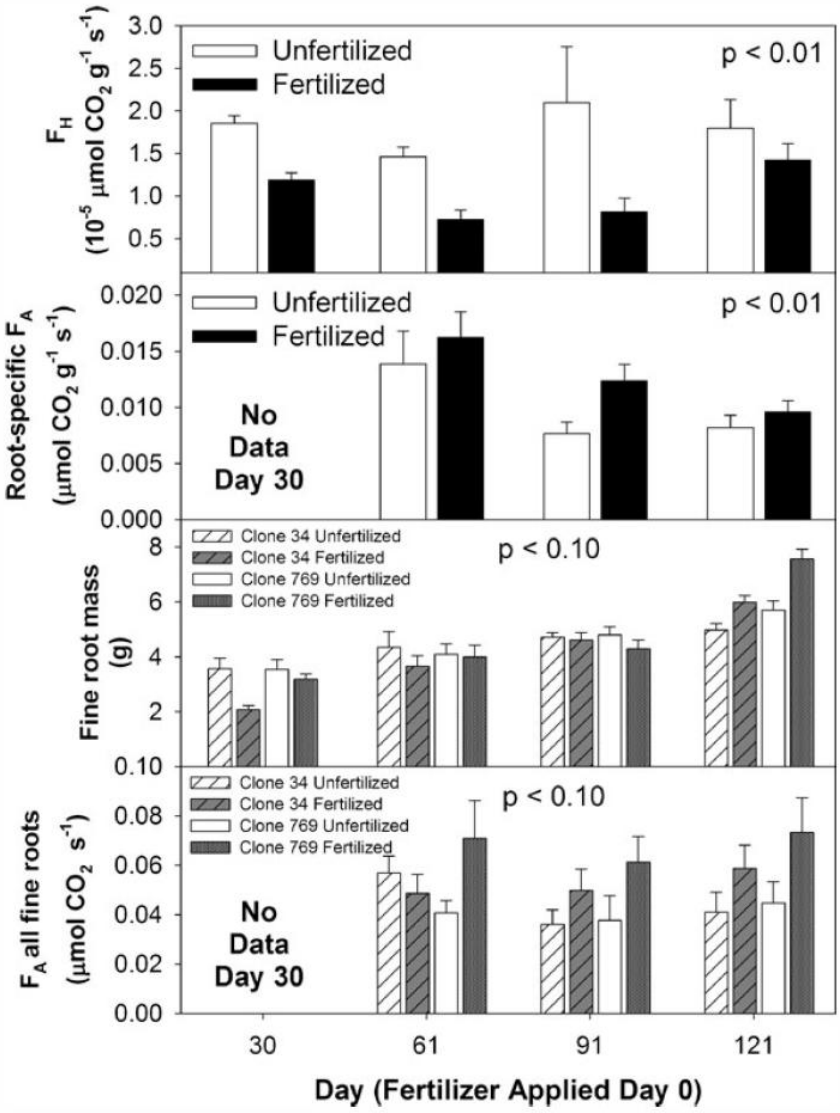

Fig. 4 Fertilizer effects for heterotrophic and autotrophic components of soil CO_2 efflux in the top two panels from four destructive harvest dates. Measurements were made using a LICOR 6200 and are standardized to soil mass and root mass, respectively. The bottom two panels show clone-by-fertilizer interactions for fine root mass and F_A scaled to all fine roots. Standard errors are shown, N=16 for the upper two panels, N=8 for the lower two panels. Fertilizer was applied day 0=June 16, 2009

scaling root-specific F_A rates up to the tree level by multiplying by total fine root biomass, a significant clone-by-fertilizer interaction emerged for F_A ($p<0.10$; Fig. 4). Data shown in Fig. 4 seem to indicate that the clonal differences in F_S attributable to fertilizer are consistent with the combined fertilizer effects on F_H and F_A when scaled to all fine roots. Fertilized ramets of clone 769 had F_A rates scaled to total fine root biomass that were the greatest of any other treatment combination. This substantial increase coupled with reduced F_H rates in fertilized ramets is consistent with the lack of fertilizer effect observed for F_S for clone 769. In clone 34, reduced fine root mass in fertilized ramets at days 30 and 60 lead to reduced F_A when scaled to all fine roots, despite increased root-specific F_A (Fig. 4). While we lack day 30 F_A data, it appears likely that the reduction in F_A found in clone 34, coupled with the reduced F_H observed across both clones combined to cause the substantial and significant reductions observed in F_S for fertilized ramets of clone 34 between days 36 and 78.

Root exudate quantity and quality and soil nutrients

Root exudate TOC varied between clones depending on fertilizer status and time since fertilizer application (Table 1). Figure 5 depicts exudate TOC expressed on a root-length basis. Unfertilized ramets of clone 34 had substantially elevated exudation rates (63 % greater) on day 30 ($p<0.05$). Rates were still higher by day 60, but not significantly so versus the other three treatment combinations ($p>0.10$). At days 90 and 120 there were no significant differences among the four treatment combinations. Similar results were observed whether data were expressed on a root-mass or root-surface-area basis (Table 1). This pattern of exudation was inconsistent with the observed reductions in F_H rates across both clones, and thus did not support our hypothesis that reduced root exudation in fertilized ramets would be correlated with reduced F_H.

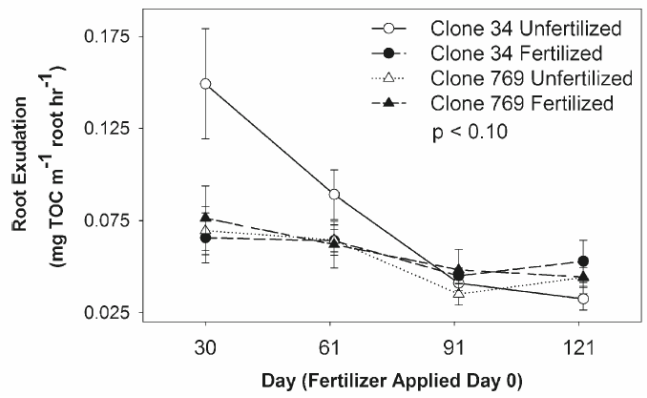

Fig. 5 Total organic carbon in root exudates analyzed with an Elementar LiquiTOC and expressed on a root length basis for each of four destructive harvests. Root exudates were collected per Egle et al. (2003). Standard error bars are shown, *N*=8. Fertilizer was applied day 0=June 16, 2009

Root exudate anion composition varied markedly among treatment groups. Of the anions we assessed through ion chromatography, formate was observed in no samples, acetate in only one, and tartrate in only eight of 128. No data analysis was preformed and no further data are shown for these compounds. Phosphate and citrate were each observed in 22 % of samples, oxalate in 38 %, lactate in 92 %, and sulfate in 100 % (Fig. 6). While we cannot determine whether the phosphate and sulfate we observed originated from root exudates or from the minimal amount of rhizosphere soil we were unable to wash from the roots, both anions have been previously collected in tree root exudates (Nguyen et al. 2003; Qin et al. 2007). Citrate and lactate showed no significant differences among treatment combinations ($p>0.10$), although they did vary in concentration across treatment dates (Table 2, Fig. 6). Sulfate showed a significant clone-by-fertilizer interaction. While sulfate was found at increased concentrations in fertilized ramets of both clones at days 61 and 91, the magnitude of this increase differed between clones. Oxalate showed a significant clone-by-fertilizer interaction across all dates ($p<0.01$) and was present in exudates sampled from a greater number of the control ramets of both clones at greater levels than the fertilized ramets (Fig. 6). Phosphate was observed in 87.5 % of control ramets for both clones at day 30, but only in 50 % or fewer of fertilized ramets (Fig. 6). After day 30, phosphate declined dramatically, and was only observed in five of 32 ramets at day 60, two of 32 at day 90, and none at day 120. At day 30 phosphate concentrations showed a strong clone-by-fertilizer interaction ($p<0.01$), with clone 34 having much greater levels in control ramets than any of the other treatment combinations.

Fertilizer treatments significantly affected soil nutrient concentrations. Soil P was below the detection limit (2.0 ppm) of the Virginia Tech Soil Testing Laboratory Mehlich I procedure for all but two of 64 unfertilized soil samples, and was substantially greater than the detection limit in all 64 fertilized soils (Table 3) (Mullins and Heckendorn 2009). P uptake varied between clones in fertilized ramets, with clone 769 showing significantly higher soil P levels at day 30 while clone 34 had significantly greater soil P at days 91 and 121 ($p<0.05$). Soil N was significantly elevated in fertilized soils at 30, 60, and 90 days post-fertilization ($p<0.01$), but had returned to control levels by day 120 ($p>0.10$). No significant clonal differences were observed for N. Fertilizer application resulted in significantly greater pH across all dates and for each individual date ($p<0.01$; Tables 1 and 3). Additionally there was a small but significant clonal effect at day 30, with clone 769 having slightly greater pH ($p<0.10$). Base cations showed a trend consistent with pH, with lower concentrations observed for K, Mg, and Ca in fertilized ramets at 120 days after fertilization ($p<0.05$). K was also found at significantly lower concentrations in fertilized ramets at day 60. At day

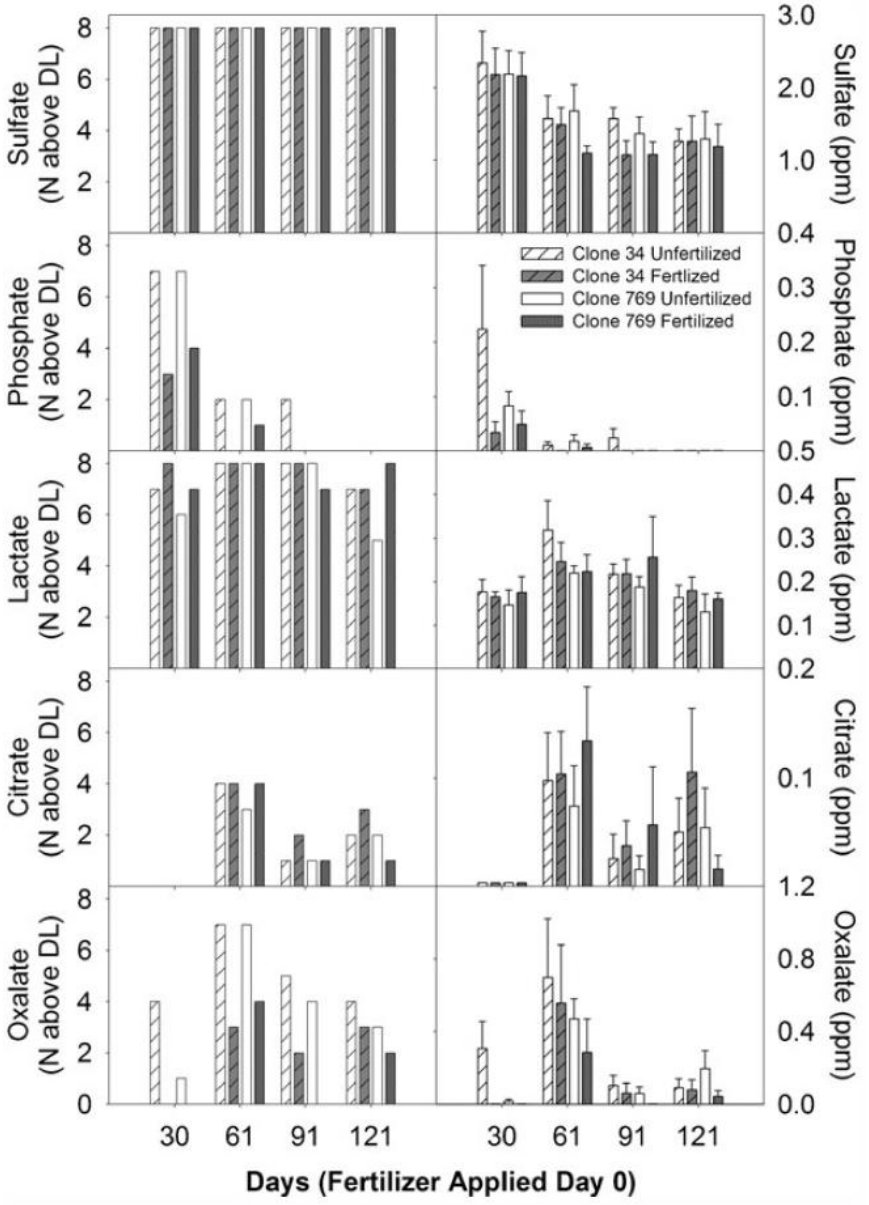

Fig. 6 Root exudate and rhizosphere anion composition analyzed with a Dionex ion chromatograph for each of four destructive harvests. Left panels show number of samples above the detection limit, while the right panels show the mean of each treatment combination. Values below the detection limit were corrected to half the detection limit and are included in data shown in the right panel. Root exudates were collected per Egle et al. (2003). Standard error bars are shown in the right panels, *N*=8. Fertilizer was applied day 0=June 16, 2009

Table 2 P-values for the Friedman's Chi Square non-parametric test for rhizosphere anion composition

Variable	Clone	Fertilizer	Clone X fertilizer	Harvest date	Detection limit (ppm)
lactate	0.1015	0.8997	0.3621	**0.0028**	0.02086
oxalate	**0.0630**	**0.0002**	**0.0008**	**0.0001**	0.00360
citrate	0.6831	0.4142	0.8271	**0.0002**	0.00851
phosphate	0.8273	**0.0030**	**0.0003**	**0.0001**	0.00430
sulfate	0.1336	**0.0455**	**0.0251**	**0.0012**	0.01156

While phosphate and sulfate observed may have originated from root exudation, they may also have been sourced from rhizosphere soil.
P-values<0.10 are indicated in boldface

Table 3 Means and standard errors (in parentheses) for soils data from each of four destructive harvest dates

Variable	Day	34 Unfertilized	769 Unfertilized	34 Fertilized	769 Fertilized
pH	30	4.90 (0.04)	5.03 (0.03)	4.56 (0.07)	4.67 (0.07)
	61	4.89 (0.05)	4.92 (0.04)	4.48 (0.07)	4.56 (0.09)
	91	4.84 (0.05)	4.89 (0.05)	4.61 (0.06)	4.58 (0.08)
	121	5.05 (0.04)	5.19 (0.12)	4.70 (0.05)	4.61 (0.07)
N (%)	30	0.0148 (0.0010)	0.0149 (0.0007)	0.0193 (0.0011)	0.0182 (0.0011)
	61	0.0152 (0.0012)	0.0142 (0.0006)	0.0185 (0.0010)	0.0179 (0.0012)
	91	0.0130 (0.0006)	0.0134 (0.0006)	0.0150 (0.0007)	0.0149 (0.0009)
	121	0.0141 (0.0011)	0.0137 (0.0008)	0.0144 (0.0007)	0.0145 (0.0008)
P (ppm)	30	2.00 (0.00)	2.00 (0.00)	9.63 (1.43)	13.38 (1.45)
	61	2.00 (0.00)	2.00 (0.00)	10.00 (1.38)	10.63 (1.99)
	91	2.00 (0.00)	3.00 (1.00)	8.88 (0.72)	5.75 (0.62)
	121	2.00 (0.00)	2.13 (0.13)	8.50 (0.53)	6.88 (0.85)
K (ppm)	30	5.25 (0.25)	5.25 (0.25)	5.25 (0.31)	6.00 (0.78)
	61	5.25 (0.31)	4.75 (0.25)	4.38 (0.42)	4.25 (0.25)
	91	4.75 (0.31)	5.13 (0.58)	4.63 (0.60)	5.00 (0.73)
	121	5.88 (0.30)	6.38 (0.63)	4.25 (0.25)	4.50 (0.27)
Ca (ppm)	30	41.25 (1.41)	43.63 (0.86)	41.13 (1.44)	48.63 (3.50)
	61	44.00 (1.38)	42.50 (1.49)	43.25 (1.80)	45.00 (1.60)
	91	46.50 (1.69)	49.38 (4.08)	45.13 (1.51)	46.75 (3.23)
	121	53.13 (1.69)	56.13 (2.83)	46.25 (1.21)	47.13 (1.55)
Mg (ppm)	30	11.00 (0.50)	11.75 (0.37)	10.88 (0.48)	12.50 (0.46)
	61	12.13 (0.44)	11.63 (0.50)	12.13 (0.48)	12.50 (0.57)
	91	12.88 (0.55)	13.50 (0.85)	12.50 (0.42)	12.38 (0.42)
	121	15.63 (0.53)	15.75 (0.49)	13.00 (0.46)	13.25 (0.41)

N and P fertilizer was applied at an operational rate on day 0=June 16, 2009 to a Wakulla sand

30 only, clone 769 showed significantly greater concentrations of both Mg and Ca ($p<0.05$).

Discussion

Respiratory C fluxes

Neither R_{ECO} nor R_{AG} responses to fertilizer application in either clone was consistent with our hypothesis of short-term fertilizer growth response via reduced respiratory C fluxes. R_{AG} showed slight but significant increases with fertilizer application, while R_{ECO} showed no effect. Thus, fertilized ramets did not have more C to allocate to greater leaf area as we hypothesized due to reduced respiratory C fluxes. Additionally, while the two clones showed different growth rates, there were no observed clonal differences in R_{AG}. R_{ECO} was greater in clone 34, the faster growing clone, at days 30 and 121, which again is contrary to our hypothesis of short-term growth response. We cannot attribute these clonal effects to a more specific cause, as trends in F_S do not appear to explain the clonal difference in R_{ECO}. While fertilized ramets of clone 34 consistently had the lowest F_S rates of any treatment combination almost all dates, they displayed R_{ECO} rates 19.7 % higher than unfertilized ramets of clone 769 and 21.6 % higher than unfertilized ramets. Unfertilized ramets of clone 34 consistently had both the highest F_S and R_{ECO} rates.

Increased R_{AG} due to fertilizer application has been previously observed in the literature. In one study with *Pinus radiata* (D. Don) clones, it was found that R_{AG} increased in fertilized ramets, yet remained a constant fraction of gross primary production, indicating that the observed fertilizer effect was primarily due to greater overall productivity resulting from fertilizer application (Bown et al. 2009). This is consistent with the findings of Wertin and Teskey (2008), who observed a tight relationship between net photosynthetic rates and foliar dark respiration rates. Maier et al. (2004) observed both increased R_{ECO} and R_{AG} due to fertilizer treatments in a midrotation *P. taeda* plantation, consistent with our results in this study. They also found that R_{AG} comprised a greater fraction of R_{ECO} in fertilized plots, which is consistent with our observed increase in R_{AG} in fertilized ramets coupled with reduced F_S due to fertilizer application, at least in clone 34. Bown et al. did observe a significant clonal effect for R_{AG}, similar to what we observed in R_{ECO} but not R_{AG} (2009). Based on the results of this study and our own it appears that while clonal differences in respiratory C fluxes may not bear a direct causal link to short-term fertilizer growth responses, higher respiratory rates are consistently observed in faster, not slower, growing clones. This is consistent with the 'rising tide lifts all boats' hypothesis of Litton et al. (2007), whereby a greater magnitude of all C fluxes is expected in faster growing individuals.

Soil CO_2 efflux and root exudation

Clones 34 and 769 responded differently to fertilizer application with regard to F_S despite being closely related (full-sib) clones. While clone 769 showed almost no

response at all, clone 34 generally had the highest rates when unfertilized, and the lowest when fertilized. The clonal differences we observed in F_S fertilizer response were surprising, given that no previous studies we are aware of have shown an effect of genotype on F_S fertilizer response. Several studies with clonal *Pinus* species have shown no effect of clone or clone-by-fertilizer interactions on F_S (Bown et al. 2009; Tyree et al. 2008). A study of *Betula pendula* (Roth) clones did show clonal differences in F_S response to elevated CO_2 and O_3 that were not entirely consistent with observed root or aboveground biomass responses to treatments.

Clone 34, when unfertilized, showed both the highest F_S rates and the highest root exudate TOC rates. However, these two trends did not appear to be completely synchronous: exudation peaked in the day 30 and 60 harvests, while F_S rates did not become elevated in the unfertilized ramets versus the fertilized ramets until day 36. F_S rates remained elevated through much of the duration of the experiment, while exudation rates dropped to similar levels to the other treatment combinations at days 90 and 120. Previous research in artificial rhizospheres has shown that root exudates can alter rhizosphere microbial communities and diurnal patterns of F_S (Landi et al. 2006). This is one possible operating mechanism that explains our observed temporal asynchrony between exudation and F_S in clone 34. It is also possible that these observations are consistent with a rhizosphere priming effect, whereby addition of a labile C source alters the microbial community, increasing its capacity for later breakdown of soil organic C (Cleveland et al. 2007; Kuzyakov and Bol 2006). Whatever the cause, the F_S and exudate responses of these two clones to fertilizer application remain markedly different. Clone 769, by contrast to clone 34, showed little response in terms of exudate quantity or F_S rates to fertilizer application.

Our hypothesis of reduced root exudation as a possible mechanism of short-term fertilizer growth response does not seem to be supported by the data. While we did observe this trend in clone 34, we did not observe any differences in root exudate TOC in clone 769, which also showed a substantial fertilizer growth response. Other short-term ecophysiological responses of clones that are inconsistent with their growth responses to fertilizer application have previously been observed for net photosynthetic rates in *P. taeda* (King et al. 2008). Conclusions derived from these types of observations can be categorized as either 1) the observed ecophysiological trait is not the primary mechanism of short-term fertilizer growth response or 2) the short-term mechanism of fertilizer growth response is genotype specific, and a generalized theory of response is not feasible for clonal plantation forestry. Without assessing the full C budget of a larger number of contrasting clones over time, it is not possible to distinguish between these two competing hypotheses. This remains an avenue of research worth further pursuit. Our hypothesis that reduced root exudates with fertilizer application could explain observed reductions in F_H were not supported by the data. Despite a clear difference between clones in root exudation, there was no significant clonal effect observed for F_H. Additionally, the observed reduction in F_H with fertilizer application was based on a sample from the edge of the pot, which was primarily bulk, not rhizosphere, soil. Root exudates are often rapidly consumed in the rhizosphere and have a lesser effect on bulk soil (Landi et al. 2006). Our results are consistent with reduced F_H with fertilizer application, which has been observed in numerous studies (Gough and Seiler 2004; Olsson et al. 2005; Tyree et al. 2008), being a direct effect of fertilizer on bulk soil microbial communities without any significant plant mediation involved in this process. Direct effects of fertilizer application on bulk soil microbial communities have been reported previously for laboratory experiments involving fertilizer application in systems lacking growing plants (Thirukkumaran and Parkinson 2000). Root exudation and F_H do not appear to be closely linked in fertilized *P. taeda* plantations.

Root exudate chemistry

In soils containing Al oxides that bind tightly with P, oxalate has been shown to release potentially large quantities of inorganic P through ligand exchange reactions (Fox and Comerford 1992; Fox et al. 1990). The higher concentrations of oxalate observed in the root exudates of unfertilized ramets of primarily clone 34 at days 30 and 61 appear to be consistent with higher levels of phosphate also found in these exudates. Although roots were washed, it was impossible to remove all rhizosphere soil in the short (< 30 min) time-frame available to collect exudates from sampled seedlings. It is likely that greater oxalate concentrations in these exudates had increased labile phosphate in the rhizosphere soil, which we also detected with our sampling protocol. Considering that phosphate was observed above the detection limit in more than twice as many unfertilized ramets as fertilized ramets at both days 30 and 61 and at much higher concentrations despite phosphate having been directly added to the fertilized ramets, it seems highly likely that the observed phosphate was released from the soil as a result of root exudate ligand exchange reactions. However, we should note that a number of previous studies have observed phosphate in root exudates (Nguyen et al. 2003; Pellet et al. 1995; Pellet et al. 1996), although observed releases of phosphate have typically been in low pH soils (< 4.5) under conditions of Al toxicity.

While sulfate is not typically observed in studies of root exudation, one previous study did find that sulfate was released in root exudates of *Populus tremula* (L.) (Qin et al. 2007). This study also speculated that sulfate was released through similar channels as malate and formate as they found a high correlation among the release of these three

anions. While we did observe an increase of sulfate we did not observe formate concentrations above the detection limit in any samples, and did not assess malate. It is possible that the sulfate we observed originated from the rhizosphere soil, and not from root exudation. We did not observe any significant treatment effects for either citrate or lactate, despite the previously observed role of citrate release in P uptake in some species (Grayston et al. 1997).

Conclusions

Respiratory C fluxes, F_S, and root exudation did not change in either of these full-sib clones as predicted by a hypothesis of short-term fertilizer growth response being caused by reduced respiratory C fluxes. We observed greater R_{ECO}, reduced F_S when fertilized, and greater total exudation of higher oxalate and phosphate concentration when unfertilized in clone 34 relative to clone 769. The clones showed different patterns of F_A scaled to all fine roots that explained their different fertilizer F_S responses. Exudation was not consistent with observed reductions in F_H, indicating that F_H response to fertilizer application is probably a direct effect of fertilizer on soil microbial communities and is not the result of plant-mediated processes. Overall, our results show that different clonesmay display unique responses to fertilizer treatments that affect their plant-soil interactions. Respiratory C fluxes may also vary between clones. The range of C cycling processes we observed between these two clones implies that genotype-specific belowground C allocation may allow some clones to better exploit a given resource environment than others. At an ecosystem scale, large clonal plantations comprised of a small number of genotypes may interact with the soil in dramatically dissimilar patterns, potentially resulting in overall changes to stand-scale C budgets. Much more research on this topic will be necessary to scale results to the field.

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