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ORIGINAL PAPER

Short-term changes in biomass partitioning of two full-sib clones of *Pinus taeda* L. under differing fertilizer regimes over 4 months

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Abstract Clonal forestry is a reality in the southeastern United States due to improvements in somatic embryogenesis of *Pinus taeda* L. Differences in below-ground carbon (C) allocation between individual genotypes could alter C sequestration and cycling in these clonal plantations. Biomass partitioning may vary between clones and in response to management practices, like fertilization. Our objective was to quantify differences in biomass partitioning due to fertilization in contrasting clones of *P. taeda* produced from the same full-sib cross. A two (clone)-by two (fertilizer)-by-four (sequential harvest) factorial randomized complete block design was replicated eight times in a greenhouse for 4 months. Trees were destructively harvested monthly following fertilizer application, so changes in biomass partitioning could be determined. Both clones responded to fertilizer with a short-term reduction in root:shoot ratio and increase in foliar biomass. These changes were ephemeral, returning to control levels within 4 months. Fertilizer responses in below-ground partitioning were due to allometric differences in one clone, but were only attributable to altered rates of development in the other. Ephemeral changes in biomass partitioning in response to fertilizer application were consistent with a theory of short-term physiological response to increased nutrient availability fueling long-term fertilizer growth responses.

Keywords Allometry • Carbon allocation • Intensive silviculture • Varietal forestry

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Introduction

Clonal plantations are now becoming common in the southeastern USA as improvements in somatic embryogenesis make possible the mass production of Pinus taeda (L.) clones (Stelzer and Goldfarb 1997; Whetten and Kellison 2010). Fertilizer application is also a common practice in plantations, with average growth increases across the southeast of ~25% due to mid-rotation additions of nitrogen (N) and phosphorus (P) (Fox et al. 2007b). In order to maximize growth potential in clonal plantations to justify more expensive genetic material at planting, fertilization and other intensive silvicultural practices will need to be applied (Dougherty 2007). However, differences in morphology and development among clones may require different silvicultural prescriptions for different clones (Roth et al. 2007; Tyree et al. 2009b).

A two-phase model of single application fertilizer-induced growth response was posited by Gough and Seiler (2004) and Gough et al. (2004). The first phase describes the physiological mechanisms by which plants respond in the short-term to fertilizer application. Immediately following fertilizer application, root respiration increases to accommodate increased nutrient uptake. Nutrients, particularly N, are allocated to leaves, increasing photosynthetic rates and increasing the rate of photosynthate accumulation. This additional photosynthate is then allocated to the production of new leaf area. Nutrients are

then retranslocated to the new leaf area, and photosynthetic rates return to pre-fertilization levels. At this point, the fertilizer growth response transitions to the second phase. In the second phase, there are few if any physiological differences between fertilized and unfertilized trees. However, fertilized trees have already developed greater leaf area due to phase 1 short-term physiological responses. It is this morphological difference that then continues to drive the fertilizer growth response in phase 2, as has been described repeatedly in the literature (Albaugh et al. 1998, 2004; Colbert et al. 1990; Fox et al. 2007a; Jokela et al. 2004).

While the above-described model appears to work well when the average of a large number of genotypes is considered, it fails to consistently explain fertilizer growth response in clonal populations. In a field trial examining eight clones, some genotypes demonstrated the two-phase response described above, while others did not (King et al. 2008). Similar differences in physiological response to fertilizer application have been observed in studies of paired clones both in greenhouse and in field trials (Tyree et al. 2009a, b). While these studies all assessed changes over time in photosynthetic response to fertilizer application, none assessed dynamic changes in biomass partitioning. Examination of changes in biomass partitioning over time may explain why photosynthetic rates do not consistently account for short-term clonal physiological responses to single-dose fertilizer applications that eventually lead to increased leaf area and long-term growth responses.

Biomass partitioning changes naturally as trees grow larger. Thus, fertilizer application may alter partitioning through its effect on growth rates, but it may also cause allometric shifts as a direct response to a resource-availability gradient. Some studies of open-pollinated *P. taeda* have shown a resource-availability effect of small magnitude directly on allometry (Jokela and Martin 2000; King et al. 1999), while others have not (Coyle and Coleman 2005; Coyle et al. 2008; Ledig et al. 1970; Samuelson et al. 2004). Results are mixed when the effect of genetics on biomass partitioning is considered. In one study, different families or provenances did not appear to differ in partitioning patterns under similar environments (Retzlaff et al. 2001). However, in another, families with the greatest stem mass and height displayed the greatest allocation to root biomass under low N environments, but the least under high N (Li et al. 1991). This would seem to indicate that high performing families had greater plasticity in allometry, which allowed them to better acclimate to the environment. Shifts in root–shoot ratios appeared to be one causal mechanism of genotypic differences in the observed fertilizer growth response in the study by Li et al. (1991).

However, all these results are based upon averages of many individual genotypes, and thus may not be expected to show similar outcomes to studies of a small number of individual genotypes replicated over larger areas, as would be found in a clonal plantation. Field observations among different clones reveal significantly different growth rates (Baltunis et al. 2007; Bitoki 2008; Paul et al. 1997). Additionally, pairs of clones have displayed different biomass partitioning patterns across a range of resource availabilities, indicating that partitioning may be at least partially responsible for observed variability in growth responses to treatments (Tyree et al. 2009a, b). Much more information on biomass partitioning in a large number of clones is needed to determine whether allometric variation between individual genotypes is of sufficient magnitude to require individual consideration of each genotype, or of genotypes with similar allometry.

The vast majority of information available on biomass partitioning in both open-pollinated and clonal *P. taeda* pertains to longer-term responses to varying resource availability. Results are typically derived from either (1) a single harvest (e.g., Colbert et al. 1990; Retzlaff et al. 2001) or (2) multiple annual harvests (e.g., Coyle and Coleman 2005; Samuelson et al. 2004). While this information is important to assess effects of treatments on stand development and ecosystem level processes that occur over years, these studies are not designed to address how trees respond physiologically to a single-dose fertilizer application in the short-term. While several studies have incorporated multiple harvests over a single growing season across a N gradient, they have done so to compare only total tree growth response (Griffin et al. 1995) or to ensure a range of seedling sizes appropriate for allometric analyses (Gebauer et al. 1996). Neither of these studies examined the effect of N availability in altering a time series of biomass partitioning. Green et al. (1994) did find significant changes in root–shoot ratio over a 10-day period in response to a combination of drought and fertilizer treatments. While this time series was not long enough for any observable stem growth response to treatments, it does reveal that biomass partitioning can vary in seedlings on the scale of days.

Despite the lack of information on short-term variability in biomass partitioning across a resource-availability gradient, short-term shifts in partitioning in the days, weeks, or months immediately following fertilizer application may explain long-term growth responses. Ephemeral reductions in root allocation could allow greater C allocation to foliar biomass, leading to a long-term growth response to fertilizer application even if partitioning to roots later increased. We hypothesized that (1) biomass partitioning would show a short-term increase in foliar allocation at the expense of root allocation with fertilizer application as a mechanism of short-term fertilizer growth response and that (2) this ephemeral response would vary in magnitude, even between full-sib clones. To address these questions multiple harvests over 4 months following fertilizer application were performed in two full-sibling clones produced from the same parents.

Materials and methods

Study description

This experiment was installed in a greenhouse at Virginia Tech in Blacksburg, Virginia, USA (37.24°N 80.43°W). Trees were potted on April 30, 2009 in homogenized A-horizon soil collected from the USDA Forest Service's Southeast Tree Research and Education Site (SETRES). The soil was a Wakulla series (siliceous, thermic Psammentic Hapludult) that was chosen because its low inherent fertility enabled us to manipulate nutrient availability. Total soil N was 149 mg kg^{-1} as determined on an Elementar CNS Analyzer (Elementar Americas, Inc., Mt. Laurel, New Jersey, USA). Mehlich 1 extractable soil P analyzed by the Virginia Tech Soil Testing and Plant Analysis laboratory was < 2 mg kg^{-1} (below the detection limit). Coarse roots and organic matter were removed from the soil by first passing it through a 1-cm sieve. Large pots (15 × 15 × 38 cm, 8,550 cm^3) were used to attempt to prevent excessive root binding for the duration of the 4-month experiment. All trees were watered daily each morning to prevent soil desiccation throughout the experiment. Soil moisture measured periodically with a TDR probe inserted in the top half of the soil profile averaged 7.8% across all pots during the duration of the experiment, which indicated a well-watered condition in this extremely sandy soil. Temperature was set to a nighttime minimum of 18°C and averaged 25.3°C throughout the experiment, with an average daily minimum of 19°C and a maximum of 36°C. Relative humidity was allowed to vary with atmospheric ambient conditions, and ranged between an average daily minimum of 29% and a maximum of 81%. Day length averaged 12.9 h over the duration of the experiment and was extended during the last 30 days (September 15 to October 15) with artificial sodium lighting turned on daily for ~3 h pre-dawn.

Experimental design

The experiment was a randomized complete block design with a two-by-two-by-four factorial structure replicated eight times (128 trees total). Treatments were clones (GE034 and GE769) provided by ArborGen LLC (Summerville, South Carolina, USA), fertilizer application (fertilized vs. control), and tree-for-time substitution (harvests day 30, 61, 91, and 121). Clones were produced from the same full-sib cross (i.e., they have the same parents) by somatic embryogenesis. These two clones were the first selected by ArborGen in 2005, and are both fast-growing elite selections (Bitoki 2008). Clone 34 is characterized by greater growth rates and a narrower crown with a greater number of smaller branches in comparison to clone 769 (Bitoki 2008). The clonal seedlings were initially grown in containers containing a mixture of peat and vermiculite. They were planted with this root-bound soil media left intact in order to prevent substantial root death and potential tree mortality. At planting, seedlings were 1 year old and averaged 28.1 ± 0.5 (standard error) cm in total height and 6.0 ± 0.1 mm in root collar diameter. Survival rate until planned harvest date was 100%. Fertilizer (N and P) was applied at an operational rate (diammonium phosphate and ammonium nitrate at 225 kg N per hectare, 56 kg elemental P per hectare) on June 16, 2009. The four tree-for-time substitution destructive harvests were conducted monthly on July 16, August 16, September 15, and October 15, 2009. At the beginning of the experiment, when fertilizer was applied, and prior to each destructive harvest heights and basal diameters of all trees were measured to ensure that there was no significant preexisting growth difference between harvest groups and that the tree-for-time substitution assumption was valid. The 8 blocks consisted of 2 greenhouse benches with 4 blocks per bench and 16 trees per block.

Data collection

On each harvest date, pots were carefully overturned to remove the entire tree intact. Roots were washed with tap water, and any remaining plug material was carefully removed by hand. The entire tree was hand dissected into components (foliage, branches, stem, taproot, coarse roots >2 mm diameter, and fine roots <2 mm diameter). Fine root and coarse root data were later combined into a lateral root category due to a low incidence of coarse roots found in the first two harvests (most trees had none). These components were oven dried in paper bags at 65°C for >10 days and weighed to determine biomass partitioning. Results are expressed on both an absolute (g per component) and relative (% of total tree mass) basis in order to examine actual fertilizer and clonal effects versus changes in partitioning normalized to tree size.

Statistics

All analyses were run with SAS software version 9.2 (SAS Institute, Cary, North Carolina, USA). Normality was checked and variables were natural log transformed as necessary, although all reported means and standard errors were untransformed. Q–Q plots were used to examine all outliers using the QQPLOT statement in PROC UNIVARIATE. Absolute biomass components, relative biomass components, and total tree mass were analyzed using ANOVA implemented in PROC MIXED with block as a random effect. The Kenward–Roger method for calculating denominator degrees of freedom was used (Littell et al. 2006). All two-way and three-way effect-by-time interactions were included in the model. If data violated assumptions of homogenous variance, variance was modeled separately for each treatment combination using

the ''group'' option in the ''repeated'' statement of PROC MIXED.

Natural log transformed regressions of biomass components versus total tree mass were utilized to determine if differences in observed partitioning were due to treatment effects directly on allometry or on growth rates. Regression equations were in the form:

$$\ln(y) = a + k \ln(x) \quad (1)$$

where '$\ln(x)$' is the natural logarithm of total tree biomass, '$\ln(y)$' is the natural logarithm of the biomass component being analyzed, and 'a' and 'k' are regression coefficients (Ledig et al. 1970). Sample populations with significantly different values of 'k' have different allocation patterns after accounting for changes in allometry due to growth rate. For instance, a population with a greater value of 'k' for root–shoot ratio will partition more biomass below ground as they grow larger versus a population with a lower value of 'k'. Even if 'k' does not significantly differ among populations, significant differences in 'a' may also indicate distinct patterns of biomass partitioning. For example, a population with a greater value of 'a' despite similar 'k' will always allocate more to the given tissue when compared at any similar overall tree size with a population with a lower value of 'a'.

While most studies employing allometric regressions only test the slope, 'k' (Coyle and Coleman 2005) or test 'a' and 'k' independently using ANCOVA (e.g., Colbert et al. 1990; Jokela and Martin 2000), we employed the technique of conditional error (Swindel 1970) to simultaneously test the full matrix of regression coefficients. Conditional error calculates an F statistic by comparing the sum of squares of errors from a combined model using data from multiple sample populations and the reduced models for each sample population separately. A significant p value indicates that the reduced models have significantly different matrices of regression coefficients, and thus should not be combined into a single model. We utilized this procedure to run pair-wise comparisons of all clone-by-fertilizer treatment combinations with a Bonferroni correction for a family-wise error rate of $\alpha = 0.05$.

Results

Fertilizer response in all biomass components

Over the course of the experiment fertilizer application increased absolute growth of each of the five biomass components and of total tree biomass, as indicated by fertilizer-by-harvest-date interactions ($p < 0.01$; Table 1) and results depicted in the upper panels of Fig. 1. At the day 30 harvest fertilized trees had 10% lower total tree mass, although this difference was not significant ($p > 0.10$) for any component but lateral roots ($p < 0.01$). However, by the day 121 harvest, fertilized trees showed greater average mass versus unfertilized trees of 53% for total tree mass, 52% for foliar mass, 99% for branch mass, 41% for stem mass, 77% for taproot mass, and 44% for lateral root mass ($p < 0.01$; Fig. 1). The only clone-by-fertilizer interaction based on absolute mass data was observed for the branch component ($p < 0.10$). While clone 34 showed a 132% increase in branch mass due to fertilizer application, clone 769 only displayed an 80% increase. The lack of other significant interactions in terms of absolute mass indicates that aside from the branch component, both clones responded similarly to fertilizer application.

To examine normalized changes in partitioning, we also examined partitioning relative to tree size. When biomass partitioning was considered from a relative, rather than an absolute perspective, there were fewer effects attributable to fertilizer application. Lateral root fraction averaged 3.2% less in fertilized trees across all dates ($p < 0.01$), reaching a low of 5.6% by the day 91 harvest ($p < 0.01$; Fig. 1 lower panel). The tap root fraction did not vary among fertilizer treatments across all dates or on any individual date ($p > 0.10$). While stem and branch fractions were not different in the fertilizer treatment across all dates ($p > 0.10$), they were affected on the fourth harvest individually, with 1.7% less allocation to stem and 1.3% more allocation to branches in control versus fertilizer treatments ($p < 0.10$). The foliage fraction showed a clone-by-fertilizer-by-harvest interaction, which is discussed in detail below.

Ephemeral shifts in partitioning vary between clones

When absolute mass was considered, the full-sib clones were different from one another in every biomass component considered with the exception of foliar mass ($p < 0.05$; Fig. 2). Clone 769 had greater mass of most components at almost all dates (Fig. 2, upper panel). For example, for the day 121 harvest, clone 769 had 15% greater total tree mass, 40% greater branch mass, 68% greater taproot mass, and 27% greater lateral root mass than clone 34 ($p < 0.05$). While at the first harvest date, clone 769 had 23.8% greater stem mass ($p < 0.05$), by day 121, the more rapid stem growth of clone 34 brought it within 5% of the stem mass of clone 769, a difference that was no longer significant ($p < 0.10$). No observed clonal differences in absolute foliar mass across dates or on any individual date were observed ($p < 0.10$; Fig. 2).

We did observe several differences between these full-sib clones in relative biomass partitioning response to fertilizer application. While the clone-by-fertilizer-by-harvest interaction for root–shoot ratio was not significant ($p < 0.10$), an interaction was observed at the day 30 harvest ($p < 0.01$; Fig. 3). While clone 769 had less than a 3%mean difference between fertilizer and control trees, clone 34

Table 1 ANOVA results for both absolute and relative components of tree biomass, as well as total tree biomass and root–shoot ratio

Variable	Clone	Fertilizer	C x F	Harvest Date	C x HD	F x HD	C x F x HD
Total tree mass	**0.0001**	**0.0001**	0.6525	**0.0001**	0.5680	**0.0001**	0.5886
Lateral root mass	**0.0176**	0.3049	0.4356	**0.0001**	0.2156	**0.0001**	0.5778
Tap root mass	**0.0001**	**0.0001**	0.6836	**0.0001**	0.2497	**0.0001**	0.3263
Stem mass	**0.0005**	**0.0001**	0.8638	**0.0001**	0.3718	**0.0005**	0.4650
Branch mass	**0.0001**	**0.0008**	**0.0747**	**0.0001**	**0.0308**	**0.0008**	0.4726
Foliar mass	0.8915	**0.0001**	0.4864	**0.0001**	0.8712	**0.0001**	0.7643
Lateral root fraction	0.2232	**0.0001**	0.2106	**0.0099**	**0.0516**	**0.0480**	0.2025
Tap root fraction	**0.0001**	0.1336	0.8587	**0.0026**	0.4671	0.4623	0.5914
Stem fraction	0.7601	0.9993	0.5928	0.2470	**0.0159**	0.1406	0.2297
Branch fraction	**0.0001**	0.1510	**0.0143**	**0.0070**	**0.0280**	0.1774	0.6486
Foliar fraction	**0.0001**	**0.0013**	0.7724	**0.0001**	0.1059	0.1139	**0.0715**
Root-shoot ratio	**0.0001**	**0.0001**	0.1118	**0.0001**	0.1378	**0.0145**	0.1512
Stem-foliage ratio	**0.0001**	0.1380	0.5878	**0.0019**	0.1305	0.8006	0.1865

*p value*s are presented, values <0.10 are shown in bold, $N = 8$

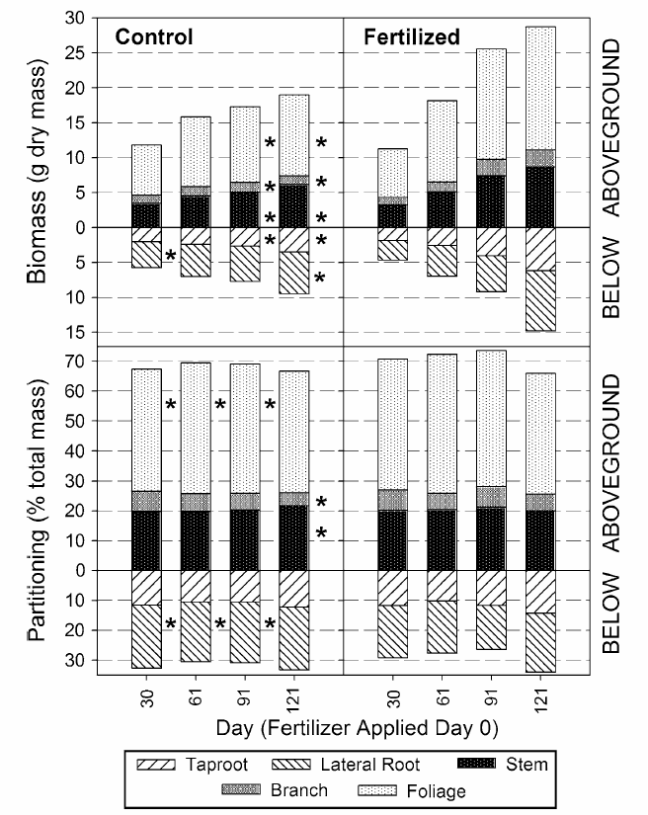

Fig. 1 Fertilizer effects for absolute (*upper panels*) and relative (l*ower panels*) biomass partitioning from four destructive harvests for both clones combined. Above-ground biomass is shown above the axis, below-ground biomass below the axis. Control time series is shown in the *left panels*, fertilized in the *right panels*. An *asterisk* to the right of a biomass component indicates a significant difference between control and fertilizer treatments ($p < 0.10$). $N = 16$, day 0 = June 16, 2009

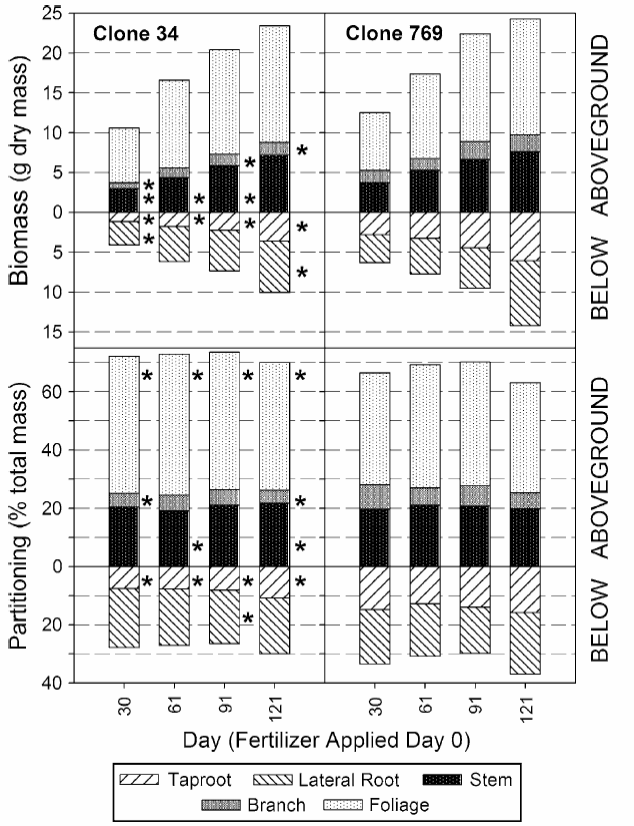

Fig. 2 Clonal effects for absolute (upper panels) and relative (*lower panels*) biomass partitioning from four destructive harvests for both fertilizer treatments combined. Above-ground biomass is shown above the axis, below-ground biomass below the axis. Clone 34 time series is shown in the *left panels*, clone 769 in the *right panels*. An *asterisk* to the right of a biomass component indicates a significant difference between clones ($p < 0.10$). $N = 16$, day 0 = June 16, 2009

showed a 41% mean difference with less allocation to roots in fertilized versus unfertilized trees. There were significantly lower root–shoot ratios in fertilized trees averaged across both clones versus controls for the second and third harvests ($p < 0.10$; Fig. 3). However, reduced partitioning to belowground tissues was only an ephemeral response to fertilizer application, with no significant differences between fertilized and unfertilized root–shoot ratios by the day 121 harvest ($p < 0.10$), although the clones continued to display different root–shoot ratios ($p < 0.01$).

Clonal differences in root–shoot ratios were not the result of uniform responses in both tap and lateral root fractions. For instance, while clone 769 had greater lateral root biomass at the first and final harvests ($p < 0.05$), there were no differences in relative partitioning to lateral roots between clones ($p < 0.10$; Fig. 2 lower panel). Conversely, there was no difference in absolute lateral root mass at the third harvest ($p < 0.10$), although clone 34 partitioned 2.2% more of its relative biomass to lateral roots than clone 769 ($p < 0.05$). Despite asynchronous patterns of absolute versus relative partitioning to lateral root biomass, across all four harvest dates clone 769 allocated an average of 5.8% more of its total biomass to taproots ($p < 0.01$), which also resulted in greater tap root mass from an absolute basis on all four dates ($p < 0.01$).

There were also above-ground differences between these full-sib clones in relative biomass partitioning. Branch fraction showed a clone-by-fertilizer interaction across all dates, with clone 34 partitioning 1.2% more of its total biomass to branches in fertilized trees while clone 769 partitioned 0.4% less ($p < 0.05$). Relative stem partitioning varied between the clones over time ($p < 0.05$), with individually significant differences of 2.3% less total mass partitioned to stem by clone 34 at day 61, but 1.6% more at day 121 ($p < 0.10$). A clone-by-fertilizer-by-harvest interaction for foliar fraction was observed, and is depicted in Fig. 4 ($p < 0.10$). While clone 769 maintained a relatively steady difference of ~5% between fertilizer and control treatments, the fertilizer response of clone 34 declined from a 10.6% difference at day 30 to a -6.7% difference at day 121. Thus, while clone 769 did not appear to demonstrate an ephemeral shift in foliar fraction in response to fertilizer application, clone 34 did. When the stem mass to foliar mass ratio was compared, clone 34 had 16.4% less stem mass per foliar mass averaged across all dates ($p < 0.01$; Fig. 5). However, clone 34 increased in total mass by 228% between days 30 and 121, compared to a 205% increase for clone 769, which indicated that while clone 34 may have had lower growth efficiency with respect to stem produced per unit of foliage, it still grew at a more rapid rate. The

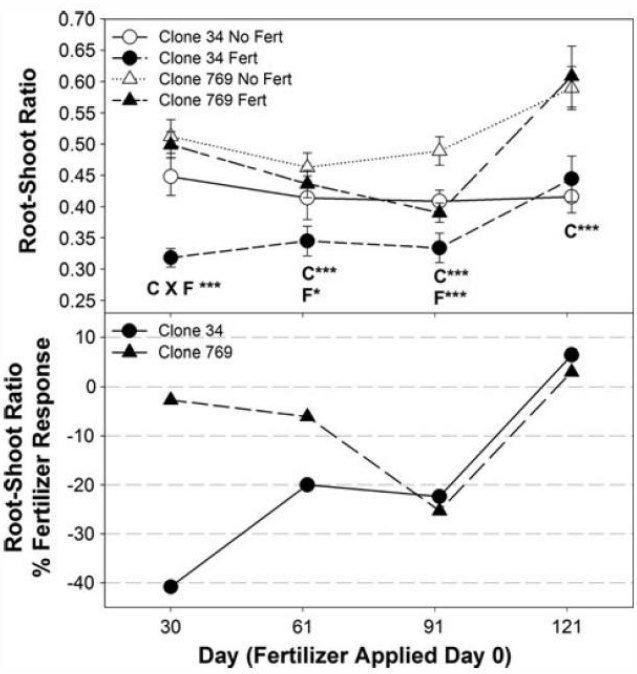

Fig. 3 Root–shoot ratio for two full-sib clones under two fertilizer treatments from each of the four destructive harvests is shown in the *top panel*, while the percent fertilizer response is shown in the *lower panel*. Significant effects are noted, with *C* indicating clone, *F* indicating fertilizer, and *C* × *F* indicating the clone-by-fertilizer interaction (*$p < 0.10$, **$p < 0.05$, ***$p < 0.01$). Standard errors are shown on the *top panel*, *N* = 8. Day 0 = June 16, 2009

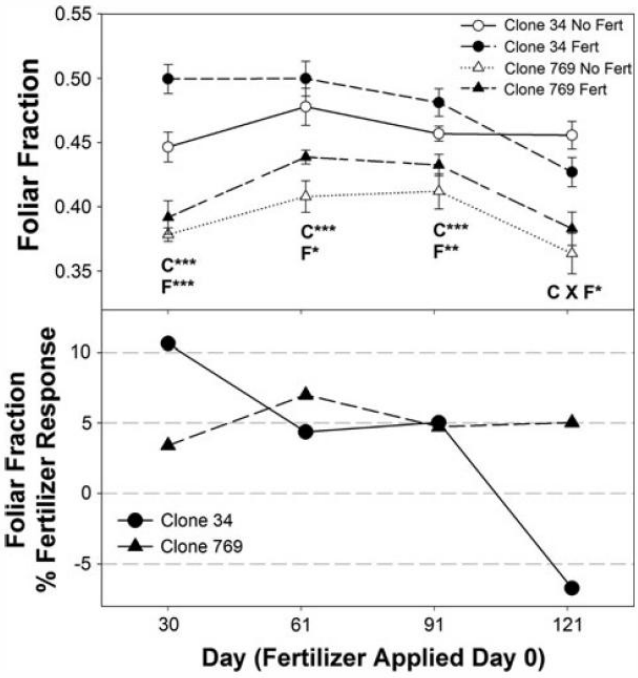

Fig. 4 Foliar mass fraction for two full-sib clones under two fertilizer treatments from each of the four destructive harvests is shown in the *top panel*, while the percent fertilizer response is shown in the *lower panel*. Significant effects are noted, with *C* indicating clone, *F* indicating fertilizer, and *C* × *F* indicating the clone-by-fertilizer interaction (*$p < 0.10$, **$p < 0.05$, ***$p < 0.01$). Standard errors are shown on the *top panel*, *N* = 8. Day 0 = June 16, 2009

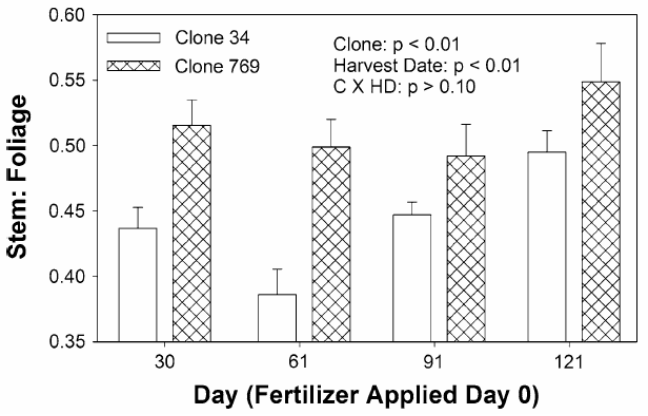

Fig. 5 Clonal effect on the stem-foliage ratio for four destructive harvests. A *lower bar* indicates lower growth efficiency, or unit stem produced per unit foliage. Standard errors are shown, $N = 16$. Day 0 = June 16, 2009

lower root–shoot ratios of clone 34 (e.g., less partitioning below ground) and greater foliar fraction at all dates ($p < 0.01$), appeared to compensate for reduced growth efficiency, resulting in a lack of any difference between clones in absolute stem mass by the final harvest ($p < 0.10$).

Allometry varies between clones

Allometric analyses comparing the natural log transformed data from one biomass component against another indicated that there were no significant differences in regression coefficients among treatment combinations for either the stem or lateral root components ($p < 0.10$; Fig. 6; Table 2). For the tap root component, results indicated that while the within clone allometric response to fertilizer application was only due to treatment effects on growth ($p < 0.10$), these clones produced from the same full-sib cross did have different allometry even after accounting for tree size ($p < 0.05$; Fig. 6; Table 2). When either total root biomass or the root–shoot ratios were considered, a more complex pattern emerged. While changes in partitioning in response to fertilizer application in clone 769 were only attributable to growth ($p < 0.10$), clone 34 shifted its allometry in response to fertilizer application even after accounting for ontogeny ($p < 0.05$; Figs. 6, 7; Table 2). An identical trend was observed for the branch component with one notable exception. When unfertilized, there was no significant trend between natural log transformations of total tree mass and branch mass in clone 769 ($p < 0.10$, $R_2 < 0.05$), indicating very poor ontogenetic control over branch allocation for this clone under conditions of nutrient deficiency. Fertilizer responses in biomass partitioning to foliar mass and stem-foliage ratios were not attributable to changes in allometry within clones ($p < 0.10$; Figs. 6, 7; Table 2).

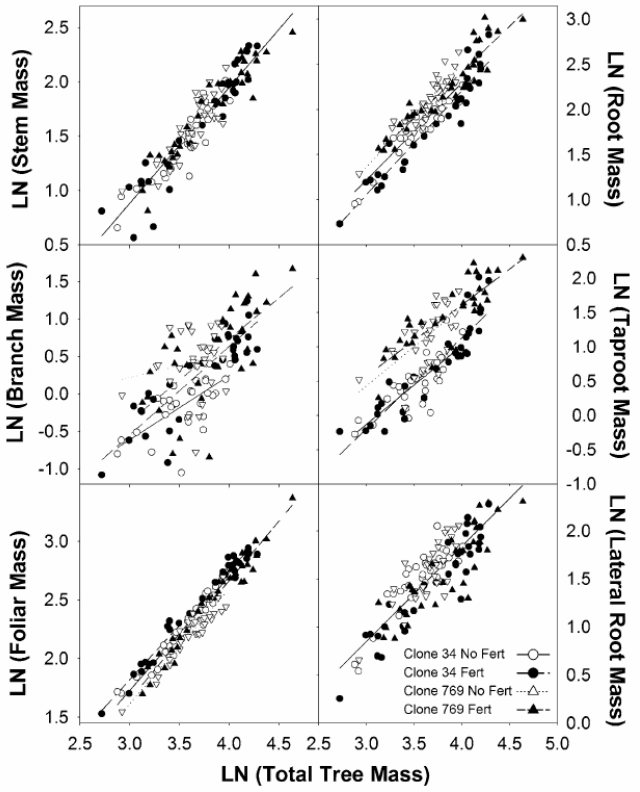

Fig. 6 Natural log transformations of biomass components (y-axis) regressed against total tree mass for trees from all four destructive harvests. Of the six *panels*, two components (stem and lateral root) did not show significant differences between the vectors of regression coefficients, so only one regression for all ($N = 128$) data points is shown. Each of the remaining four *panels* showed significant ($p < 0.05$) differences between regression coefficients, indicating differences in allometry due to treatments when development was considered. For each of these four regressions N = 32. All relevant statistics are presented in Table 2

Discussion

Ephemeral changes in partitioning with fertilizer application

Our first hypothesis, that fertilizer application would result in a short-term change in biomass partitioning, was supported by the data observed in this experiment. Figure 3 clearly shows reduced root–shoot ratios (i.e., less partitioning to roots) at some time in the 4-month post-fertilizer application in both of these clones from the same full-sib cross. This reduced partitioning to roots was consistently of a large magnitude for the first three harvest dates in clone 34, while it was increased in magnitude over the same time period in clone 769. However, this shift in partitioning was ephemeral. By day 121, both clones showed no significant difference between fertilized and unfertilized root–shoot ratios. A single harvest conducted during the dormant season would have concluded that

Table 2 Regression analysis of natural log transformed biomass components versus one another

Variable	*p* value		All data	Clone 34		Clone 769	
				No Fert	Fert	No Fert	Fert
Lateral root versus total mass	>0.10	R^2	0.65	---	---	---	---
		a	-1.48	---	---	---	---
		k	0.94	---	---	---	---
		Group	---	---	---	---	---
Tap root versus total mass	<0.01	R^2	---	0.69	0.86	0.49	0.74
		a	---	-3.00	-3.42	-2.91	-2.10
		k	---	1.15	1.29	1.28	1.05
		Group	---	A	A	B	B
Total root versus total mass	<0.01	R^2	---	0.85	0.93	0.85	0.89
		a	---	-1.40	-1.72	-1.54	-1.16
		k	---	1.06	1.11	1.14	1.00
		Group	---	A	B	A	A
Stem versus total mass	>0.10	R^2	0.90	---	---	---	---
		a	-1.72	---	---	---	---
		k	1.04	---	---	---	---
		Group	---	---	---	---	---
Branches versus total mass	<0.01	R^2	---	0.44	0.77	NS	0.48
		a	---	-2.72	-3.35	NS	-3.14
		k	---	0.85	1.13	NS	1.09
		Group	---	A	B		AB
Foliage versus total mass	<0.01	R^2	---	0.94	0.97	0.82	0.95
		a	---	-0.64	-0.39	-0.74	-0.85
		k	---	0.95	0.89	0.94	0.99
		Group	---	AC	A	B	BC
Root versus shoot	<0.01	R^2	---	0.70	0.85	0.64	0.76
		a	---	-0.76	-1.35	-0.76	-0.52
		k	---	0.96	1.11	1.03	0.92
		Group	---	A	B	C	AC
Stem versus foliage	<0.01	R^2	---	0.80	0.86	0.43	0.88
		a	---	-0.88	-1.24	-0.16	-0.59
		k	---	1.03	1.16	0.78	0.95
		Group	---	AB	A	B	AB

The *p* values presented are derived from a conditional error analysis testing whether the vector of regression coefficients was equal for each clone-by-fertilizer treatment combination. If not significant, only the overall regression R^2 is presented ($N = 128$). If significant all four clone-by-fertilizer group R^2 values are shown ($N = 32$). Allometric coefficient '*k*' and intercept '*a*' are shown for each regression. Letters shown in the 'group' rows indicate significantly different regressions based on pair-wise conditional error tests with a Bonferroni adjustment to the *p* value for a family-wise error rate of 0.05

NS indicates that the regression was not significant (i.e., $p > 0.10$)

fertilizer application did not affect root–shoot ratios in either clone, completely missing short-term reductions to below-ground biomass partitioning.

Figure 4 shows significantly increased allocation to the foliar fraction in both clones during at least one harvest. By the final harvest, foliar fraction in fertilized trees was either not significantly different than controls (clone 769) or showed a reversed trend from the previous three harvests (clone 34). This again indicates an ephemeral shift in partitioning that may explain a portion of the fertilizer growth response, which would be missed based on a dormant season only harvest. Short-term changes in partitioning to both root–shoot ratios and foliar mass are theoretically consistent with the observed 53% total tree fertilizer growth response. A reduction in root partitioning in response to increased nutrient availability allowed an increase in foliar partitioning, and is thus consistent with a model of increasing the photosynthetic capacity of the fertilized trees, allowing for long-term growth response to fertilizer application.

Based on these data, we infer that short-term changes in biomass partitioning, whether due to changes in allometry or growth rate, are real and are a plausible mechanism of short-term growth response to fertilizer application. While several studies have shown that allometry does not change across a resource gradient in *P. taeda*, these studies are all focused on long-term responses to fertilizer treatments among populations consisting of many genotypes (Coyle et al. 2008; Ledig et al. 1970). Reduced below-ground biomass allocation in the weeks and months following fertilizer application allows carbon to be allocated to the more rapid development of a greater foliar biomass in fertilized trees. Once fertilized trees have attained a greater photosynthetic capacity as inferred from greater foliar biomass, root–shoot

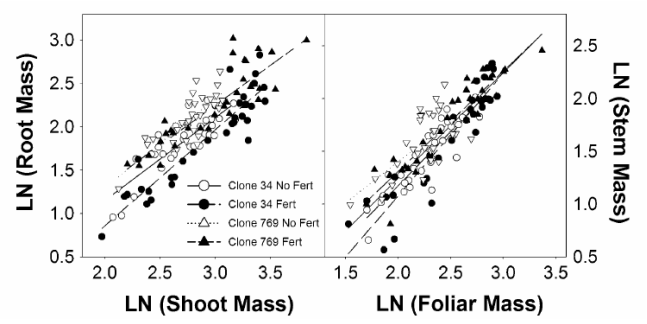

Fig. 7 Natural log transformations of root versus shoot mass (*left panel*) and shoot versus foliar mass (*right panel*) for two full-sib clones under two levels of fertilizer from four destructive harvests. Both panels showed significant ($p < 0.05$) differences between regression coefficients, indicating differences in allometry due to treatments when development was considered. For each of these regressions $N = 32$. All relevant statistics are presented in Table 2

ratios return to levels similar to those of unfertilized controls. However, by this time the canopy-level photosynthetic capacity of fertilized trees has increased, even if no change in photosynthetic rates occurs. This then results in a whole tree growth response to fertilizer application, as we observed, with all plant tissues having a greater mass versus unfertilized controls. Short-term changes in partitioning were consistent with long-term growth responses to fertilizer application, even when considered independently of other potentially contributing physiological responses to fertilizer application observed in other studies (Gough and Seiler 2004; Gough et al. 2004; Tyree et al. 2009a, b). The lack of large longterm changes in partitioning from a relative standpoint is also consistent both in the two-phase model and the literature (Colbert et al. 1990; Coyle and Coleman 2005; King et al. 1999).

Biomass partitioning, particularly root–shoot ratios, have been shown to vary throughout the year in a number of species (Cannell and Willett 1976). Typically growth shifts above ground in the late spring to early summer as shoots and new foliage elongate. Root growth is minimal during this period. Later in the growing season, in the late summer to early fall, above-ground growth ceases, allowing C allocation to shift below ground as roots grow throughout the fall and into the winter, depending upon soil temperature and water relations (Barnes 2002; Edwards et al. 1992; Iivonen et al. 2001). In *P. taeda* seedlings, episodic growth phases shift between above-ground and below-ground tissues on a monthly to bi-monthly basis to a lesser extent than larger seasonal growth phases, resulting in varying root–shoot ratios within a single growing season (Drew and Ledig 1980). This variability does not change our conclusions on ephemeral fertilizer partitioning responses, since they are based on simultaneous comparison of fertilized trees with controls throughout the growing season. The difference in root–shoot ratios observed in this study between fertilized and unfertilized trees indicates that resource availability over the short-term, through either its effects on allometry or development rate, may be at least as large a source of variability in root–shoot ratios as seasonal fluctuations in some clones (Figs. 3, 4). It should be noted that the fertilizer responses in this experiment may be of greater magnitude than average, since we used an extremely infertile soil (149 mg kg^{-1} total N, < 2 mg kg^{-1} Mehlich 1 extractable P) in order to increase our ability to detect treatment differences.

Varying clonal partitioning strategies

Our second hypothesis that short-term shifts in partitioning would vary in magnitude between clones was also supported by the data. Clones had similar total growth responses to fertilizer application, with no significant clone-by-fertilizer interactions in either stem mass or total tree mass observed. Clone 769 did have significantly greater whole tree mass in both fertilized and control treatments, and both clones showed several different partitioning responses to fertilizer application. Clone 34 showed a greater magnitude of reduction in the root–shoot ratios over the first two harvest dates versus clone 769 (Fig. 3), despite the fact that they are full-sib to one another. The different partitioning patterns between the two clones appear to be theoretically consistent with the differences in growth rates. Greater partitioning to foliage and less partitioning to roots in clone 34 are consistent with its greater overall growth rate. While it is less efficient from a stem mass per foliar mass production basis, clone 34 may still outperform clone 769 in the long-term due to its lesser below-ground allocation. This is supported by results from the literature comparing the growth of these two clones in a field trial (Bitoki 2008).

Results of this study show that not only did allometry vary substantially between a full-sib pair of clones, but also did the cause of changes in allometry. Allometric analyses of the total root fraction show that while clone 34 responded to fertilizer application by changing its allometry, clone 769 only showed growth rate effects (Table 2). We are unable to determine, based on the time-scale of this experiment, whether the shift in allometry was a short-term or long-term response to fertilizer application. Clones of other species have shown markedly different patterns of biomass partitioning within a single growing season (Scarascia-Mugnozza et al. 1997), and significant differences in allometric coefficients for some biomass components across a resource gradient in the long-term (Coyle and Coleman 2005). While it is possible based on the literature that the allometric shifts we observed may be longer-term differences between these clones, we cannot conclude so without further experiments.

The vast majority of previous studies of allometric shift in response to a resource-availability gradient have been based on genotypic averages (e.g., open-pollinated trees). It is possible that the mixed results found in the literature are

to some extent due to the randomly selected genotypes specific to each study. Studies finding positive results for allometric shifts may have had more genotypes like clone 34, while those finding no allometric shifts may have had more genotypes like clone 769. While we have no direct evidence of this, it is possible given the relatively small number of replications in most studies due to the large investments of time and labor that collecting biomass partitioning data requires. Of the studies on biomass partitioning in *P. taeda* we examined, five had five or fewer replications per treatment per harvest date (Coyle et al. 2008; Green et al. 1994; Li et al. 1991; Retzlaff et al. 2001; Samuelson et al. 2004), five had ten or fewer (Adegbidi et al. 2002; Colbert et al. 1990; Gebauer et al. 1996; Griffin et al. 1995; Jokela and Martin 2000), and only two had more than ten (Albaugh et al. 2006; Gough and Seiler 2004). If genotypes are highly variable in direct allometric shift versus growth rate effects as the source of fertilizer partitioning changes in biomass partitioning, relatively small sample sizes taken from random genotypes could randomly determine the outcome of each individual allometric analysis. As we have demonstrated, even full-sib clones may differ markedly in allometry, indicating that even constraining experiments to full-sib families would still likely result in a range of genotype-specific partitioning strategies.

Regressions across a large number of studies, such as the one shown in Coyle et al. (2008), indicate that changes in the rate of development are the predominant mode of partitioning change found in *P. taeda* across all genotypes. However, the fact that these two full-sib clones behaved so differently when fertilized in terms of allometric shifts versus growth rate effects raises the question of how variable individual genotypes may be. Of the studies, we are aware of examining biomass partitioning in *P. taeda* clones, none have yet attempted to ascribe changes in partitioning to their fundamental causes, changes in allometry or changes associated with growth (Tyree et al. 2009a, b). Further studies with a greater number of clones will be necessary in order to assess how variable *P. taeda* is in its mode of fertilizer response to partitioning.

Conclusion

Short-term changes in biomass partitioning in response to fertilizer application occurred in two full-sib clones of *P. taeda*. Ephemeral reductions in allocation to roots and increases in allocation to foliage in the several months following fertilizer application are consistent with a theory of long-term fertilizer growth response caused by short-term physiological changes. The magnitude of changes in partitioning differed between the two full-sib clones, and was consistent with each clone's observed whole tree growth response to fertilizer. Allometric analysis revealed that in response to fertilizer application one clone only altered growth rates, causing corresponding changes with respect to below-ground biomass partitioning, while the other shifted its allometry. The variable modes of partitioning response to fertilizer application indicate that different clones may have fundamentally different physiological capacities to respond to fertilizer application. These results emphasize the importance of understanding how different clones respond to fertilizer application in order to optimize management and accurately model carbon dynamics in clonal plantations.

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