

Fertilization but not irrigation influences hydraulic traits in plantation-grown loblolly pine

Lisa J. Samuelson^{a,*}, Marianne G. Farris^a, Tom A. Stokes^a, Mark D. Coleman^b

^a School of Forestry and Wildlife Sciences, 3301 SFWS Building, Auburn University, Auburn, AL 36849, United States

^b USDA Forest Service-Savannah River, P.O. Box 700, Southern Research Station, New Ellenton, SC 29809, United States

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Abstract

The goal of the study was to explore hydraulic traits in a 4-year-old loblolly pine (*Pinus taeda* L.) plantation to better understand plasticity of this species to resource availability. The influence of a factorial combination of irrigation (130 mm year⁻¹ versus 494 mm year⁻¹) and fertilization (0 kg N ha⁻¹ year⁻¹ versus 80 kg N ha⁻¹ year⁻¹) on stand-level transpiration, canopy stomatal conductance (G_S), sapwood area to leaf area ratio ($A_S:A_L$), root area to leaf area ratio ($A_R:A_L$), and leaf specific hydraulic conductance (G_L) was examined. The study site was located on the Savannah River Site near Aiken, SC. Transpiration was measured continuously over 11 months using thermal dissipation probes. Transpiration per unit ground area summed over the 11 months increased from 223 to 420 mm with fertilization, and transpiration per unit leaf area also increased with fertilization, suggesting increased growth efficiency. Fertilization increased $A_S:A_L$, G_L , G_S and leaf water potential but had no effect on $A_R:A_L$. Less aboveground woody biomass allocation to branches or greater growth efficiency may account for higher $A_S:A_L$ in fertilized trees. Irrigation had no influence on hydraulic traits and the influence of fertilization on hydraulic traits was independent of irrigation. Fertilization increased growth whereas irrigation had little effect on growth and fertilization effects. These results indicate that hydraulic traits of loblolly pine are sensitive to nitrogen availability.

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1. Introduction

Plant hydraulic architecture is important to understanding regulation of plant water loss, long-term acclimation to climate and resource availability, and subsequent carbon assimilation and growth in trees (Whitehead et al., 1984a, 1984b; Whitehead, 1998; Köstner et al., 2002; Tyree, 2003; McCulloh and Sperry, 2005). Hydraulic traits such as the sapwood area to leaf area ratio ($A_S:A_L$) and whole tree conductance per unit sapwood area (G) are coordinated with canopy stomatal conductance (G_S) to minimize plant water loss and avoid hydraulic failure. This coordination has been described by the simple hydraulic model:

$$A_S : A_L = \frac{G_S D}{G \Delta \Psi} \quad (1)$$

where $\Delta \Psi$ is the gradient in water potential from soil to leaf and D is the vapor pressure deficit between the leaf and air (White-

head et al., 1984b; Sperry, 2000; Ewers et al., 2005; Simonin et al., 2006). Hydraulic architecture has been shown to be plastic to changes in climate and resource availability, and with tree and stand development (Whitehead and Beadle, 2004), but changes in hydraulic traits in response to resource availability are often inconsistent and reflect variability among species and growing conditions. For example, higher G_S and whole tree conductance per unit leaf area (G_L) in *Populus deltoides* Bart. ex Marsh in response to fertilization were associated with an increase in $A_S:A_L$ (Samuelson et al., 2007). Similarly, fertilization increased $A_S:A_L$ in *Pinus sylvestris* L. (Mörling and Valinger, 1999) and Vanninen et al. (1996) reported higher $A_S:A_L$ in *P. sylvestris* on more fertile sites. In contrast, periodic and annual fertilization had no influence on $A_S:A_L$ in *Pinus contorta* Dougl. var. *latifolia* Engelm. (Amponsah et al., 2004). Irrigation decreased $A_S:A_L$ but increased sapwood permeability in *Eucalyptus globulus* Labill. and *Eucalyptus nitens* (Deane and Maiden) Maiden (White et al., 1998). Increased $A_S:A_L$ has been reported for drier habitats (Mencuccini and Grace, 1995). However, in drought adapted *Pinus palustris* Mill., $A_S:A_L$ was lower on xeric than mesic sites but G_S and G_L were similar

* Corresponding author. Tel.: +1 334 844 1040; fax: +1 334 844 1084.

E-mail address: samuelj@auburn.edu (L.J. Samuelson).

between sites presumably because the root area to leaf area ratio ($A_R:A_L$) was higher on xeric sites (Addington et al., 2006). Sterck et al. (2008) observed lower $A_S:A_L$ and reduced G_L in *Quercus pubescens* Willd. and *P. sylvestris* on a dry compared to wet site. Variability in the relationship between resource availability and hydraulic traits may be related to resource driven changes in tree and stand ontogeny. The ratio $A_S:A_L$ generally increases with tree size to compensate for hydraulic constraints on water transport but $A_S:A_L$ has been shown to both increase and decrease with height depending on species, stand age, and canopy position (Phillips et al., 2003; Ewers et al., 2005; Simonin et al., 2006).

While nutrient availability is the primary driver of productivity in loblolly pine (Jokela et al., 2004), growth and hydraulic characteristics may depend to some extent on water availability. For example, fertilization increased growth but decreased $A_S:A_L$, G_L and G_S relative to the control treatment in 15-year-old loblolly pine, whereas fertilization plus irrigation resulted in the greatest basal area growth, increased $A_S:A_L$ and no reductions in G_L and G_S (Ewers et al., 2000). Samuelson and Stokes (2006) demonstrated that exceptionally high leaf area index (LAI) and high productivity in 5-year-old loblolly pine in response to fertilization plus irrigation was possible because $A_S:A_L$ was maintained with accelerated stand development, although the study was not a factorial design. In this study we examined hydraulic traits in response to a factorial combination of fertilization and irrigation in 4-year-old loblolly pine. Our goal was to better understand the influence of water versus nutrient availability on relationships between hydraulic traits, physiological function and growth in rapidly growing plantations. Specific objectives were to determine: (1) if increased resource availability would result in modification of hydraulic traits, and (2) whether the influence of fertilization on transpiration and hydraulic traits was dependent on water availability. The results of this study were evaluated in the context of previous research on the plant water relations of loblolly pine. Special attention was given to the plasticity of this species to resource availability and the applicability of Eq. (1) to capture the dynamic behavior of loblolly pine to alterations in water and nitrogen availability.

2. Methods

2.1. Study design

The 24.4-ha experimental plantation was established in 2000 on the U.S. Department of Energy Savannah River Site, a National Environmental Research Park, located near Aiken, SC in the Carolina Sand Hill physiographic region (33°23'N, 81°40'E). Mean annual temperature and precipitation are 20 °C and 1190 mm, respectively, with extended droughts common throughout the growing season. During 2000–2003, average annual rainfall at the site was 982 mm, and over this 11-month study conducted in 2003 precipitation totaled 1212 mm (Fig. 1). The soil is a loamy, siliceous, semiactive, thermic Grossarenic Paleudult (Blanton series). The site was farmed until the 1950s and then converted to *P. palustris* and loblolly

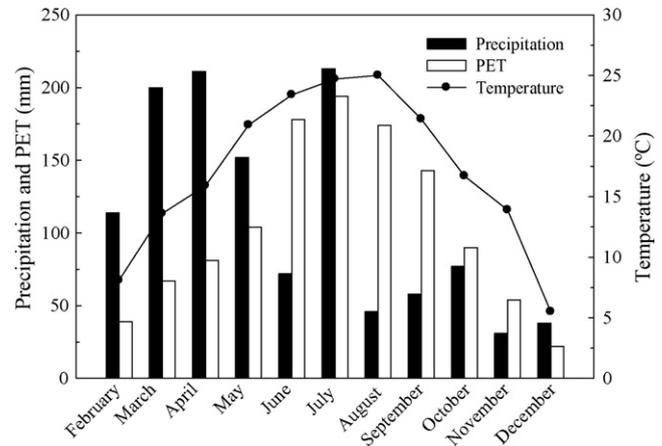


Fig. 1. Monthly precipitation, potential evapotranspiration (PET) and 24 h average temperature over the study duration.

pine stands harvested in 1999. Soil, stumps and small debris from the harvest were homogenized to a depth of 30 cm and dolomite lime was applied at a rate of 3.4 Mg ha⁻¹ to increase soil pH to 6.5.

The site was planted with bare root loblolly pine seedlings (International Paper family 7–56) in February 2000 on 2.5 m × 3 m spacing (1333 trees ha⁻¹). The experimental design was a 2 × 2 factorial combination of irrigation and fertilization replicated in three blocks. The 0.22-ha treatment plots included a central 0.04-ha measurement plot with 54 trees. Irrigated treatment plots received up to 5 mm of water daily through drip irrigation lines to meet evaporative demand and average regional daily evaporation for the period between April and October. An average 494 mm year⁻¹ of water was supplied to irrigated plots during the 2000–2003 growing seasons. All treatments were applied from April 1 to September 30. Fertilized treatments received 40 kg N ha⁻¹ year⁻¹ in 2000 and 2001 and 80 kg N ha⁻¹ year⁻¹ in 2002 and 2003 to meet increasing growth demands. Other nutrients were applied in balance with N at the following rates (kg ha⁻¹ year⁻¹) in years 1 and 2 and doubled in years 3 and 4: P, 22; K, 42; B, 1.57; Mn, 0.35; Zn, 0.21; Cu, 0.07; Mo, 0.00071 (Coleman et al., 2004); the main exception being that P was not included in years 2 and 4. To maintain experimental control, 5 mm of water a week was applied to non-irrigated plots, since the same amount was required to deliver fertilizer and flush drip tubes in fertilized plots. Consequently, an additional 130 mm of water was delivered to non-irrigated treatment plots annually. Lime application during site preparation met Ca and Mg requirements. Weeds were controlled on all plots using a pre-emergent (oxyfluorfen) and glyphosate as needed. Permethrin (Ambush[®], Zeneca Ag Products, Wilmington, DE, USA) was applied for control of Nantucket pine tip moth. An on-site standard weather station (Dynamet, Dynamax, Houston, TX) was used to measure and record environmental data and included software to calculate potential evapotranspiration following Van Bavel (1996). More details on treatments and the experimental design are given in Coleman et al. (2004).

2.2. Growth measurements

Stand biometric and biomass data were adapted from Coyle et al. (2008). Growth efficiency was calculated as the 2003 increment in stem mass divided by 2003 foliage mass (current plus 1-year-old foliage). Root sampling was described in detail in Coleman (2007). Briefly, fine roots <5 mm in diameter were collected at five locations in each plot in September 2003. A 5-cm diameter corer was used to remove samples to a soil depth of 45 cm at each of the five locations. Soil in core samples was washed away from roots by elutriation (Gillison's Variety Fabrication, Inc., Benzonia, MI). Live roots were manually separated from dead organic matter and scanned for surface area measurements (WinRHIZO, Regent Instruments Inc., Quebec, Canada). Total root surface area at the stand-level was determined for each plot by summing root values by core and then averaging the five sampling locations.

2.3. Sap flow measurements

Five trees in each treatment plot, selected based on average plot diameter, absence of forks and no missing adjacent trees, were selected for sap flow measurement (Table 1). Thermal dissipation probes based on Granier (1987) of a 20-mm integrating length were inserted into the sapwood approx. 30 cm above groundline at a north aspect of each tree by removing the outer bark and drilling small holes (1.6 mm in diameter) into the sapwood. Any branches below the probes were removed. Insulated constantan wire and a thermally conducting paste were wrapped around the heated probe and aluminum shields were placed around both probes. Silicon was placed around the probes to seal out water and the stem and probes wrapped with porous insulation to reduce thermal gradients. The potential for thermal gradients was tested by turning the probes off several times and no gradients were observed. The voltage difference between thermocouples was converted to a temperature difference between the heated and reference thermocouple and converted to sap flux (q , linear dimension per unit time, as defined by Reid et al. (2005)) based on the empirical calibration by Granier (1985). Sap flux was recorded every 30 s and the 30 min mean stored using a datalogger (DL2e, Delta-T Devices

Ltd., Cambridge, U.K.). Sap flux was recorded continuously from 1 February 2003 to 31 December 2003.

Stem diameter at probe locations was measured monthly to estimate sapwood area. Because of the young age and small size of trees and work by Samuelson and Stokes (2006) trees were considered 100% sapwood. Sap flow (Q , volume flow per unit time, as defined by Reid et al. (2005)) was calculated as the product of q and sapwood area (Granier, 1987). Correction factors for radial variation were not applied because of the small diameter of trees (Delzon et al., 2004). Stand-level transpiration on a ground area basis (E) was estimated by dividing Q by plot area and multiplying by the ratio of total plot basal area based on diameter at breast height (1.3 m, DBH) to average sample tree basal area based on stem diameter at the probes (Čermák et al., 2004). Transpiration per unit leaf area (E_L) was calculated by dividing E by LAI. Leaf area index of each plot was measured monthly with a LAI-2000 plant canopy analyzer (LiCor Inc., Lincoln, NE.).

2.4. Canopy stomatal conductance

Plot-level canopy stomatal conductance was calculated using the following equation:

$$G_S = \frac{\lambda E_L \gamma}{\rho c_p D} \quad (2)$$

where λ is the latent heat of vaporization of water (2465 J g⁻¹), γ is the psychrometric constant (65.5 Pa K⁻¹), ρ is the density of air (1225 g m⁻³), c_p is the specific heat of air (1.01 J g⁻¹ K⁻¹) and E_L is in g m⁻² s⁻¹ (Monteith and Unsworth, 1990). Values were converted from m s⁻¹ to mmol m⁻² s⁻¹ following Nobel (1991). To reduce errors from instrument limitations, G_S was calculated only when $D \geq 1.0$ kPa (Ewers and Oren, 2000). The relationship between hourly G_S at solar irradiances >600 W m⁻² and $D \geq 1$ kPa was described by $G_S = G_{S,\text{ref}} - \delta \ln D$ where $G_{S,\text{ref}}$ is G_S at $D = 1$ and $-\delta$ is the sensitivity of G_S to D (Oren et al., 1999; Oren and Pataki, 2001). The model was fit to each block-treatment combination by month for the months of June–September. Sample sizes for each model ranged from 69 to 101 h and slopes were highly significant ($p < 0.0001$) with R^2 between 0.51 and 0.80.

Table 1

Influence of fertilization (F-0: no fertilization, F-1: fertilization) and irrigation (I-0: no irrigation, I-1: irrigation) on stand characteristics of 4-year-old loblolly pine

Treatment	DBH (cm)	Sample DBH (cm)	Basal area (m ² ha ⁻¹)	Height (m)	Aboveground biomass (Mg ha ⁻¹)	Belowground biomass (Mg ha ⁻¹)	Growth efficiency	Root area (m ² m ⁻²)	Branch allocation (%)
F-0	7.6 ± 0.4	7.9 ± 0.2	6.3 ± 0.8	5.3 ± 0.3	18.6 ± 2.4	6.6 ± 0.7	0.69 ± 0.02	1.34 ± 0.23	37.4 ± 1.1
F-1	9.7 ± 0.2	10.0 ± 0.2	10.3 ± 0.3	6.4 ± 0.1	29.5 ± 1.1	11.0 ± 0.4	1.17 ± 0.02	1.63 ± 0.27	31.9 ± 0.7
I-0	8.8 ± 0.6	8.7 ± 0.3	8.5 ± 1.0	5.9 ± 0.3	25.5 ± 3.2	9.3 ± 0.9	0.92 ± 0.12	1.35 ± 0.18	35.6 ± 1.4
I-1	8.6 ± 0.6	9.2 ± 0.3	8.1 ± 1.1	5.7 ± 0.3	22.7 ± 2.7	8.3 ± 1.3	0.93 ± 0.10	1.62 ± 0.31	33.8 ± 1.6
$p > F$									
Fertilization	0.004	<0.001	0.003	0.010	0.007	<0.001	<0.001	0.461	0.004
Irrigation	0.699	0.125	0.601	0.592	0.352	0.216	0.873	0.508	0.178
Fertilization × irrigation	0.492	0.683	0.377	0.486	0.823	0.188	0.192	0.252	0.303

Sample DBH is DBH for sap flow measurement trees. Root area is the surface area of roots <5 mm in diameter. All values are means ± S.E. Observed probability values for treatment effects are indicated. Data were adapted from Coyle et al. (2008) and Coleman (2007).

2.5. Hydraulic traits

The slope of the linear relationship between q per unit sapwood area or leaf area and leaf water potential (Ψ_L) was used to calculate G and G_L (Wullschleger et al., 1998). Leaf water potential was measured on the five sample trees in a plot using a pressure chamber (PMS Instrument Corp., Corvallis, OR) on one fascicle collected from the upper canopy of each sample tree at 0500, 0830, 1200, and 1530 EST hours on 10–12 June, 22–24 July and 12–14 August, 2003. A fascicle was detached from a tree, placed in a plastic bag with a moist paper towel and Ψ_L measured within 2 min of detachment. One block was randomly selected and measured over one day and the order of plot measurement within a block was random. All five trees in a plot were measured before moving to the next plot. To estimate G and G_L for each plot, data were averaged across trees within a plot and pooled by plot across the three measurement months ($n = 15$).

The $A_S:A_L$ ratio was calculated as the ratio of total basal area per plot to plot-level LAI measured monthly. The $A_R:A_L$ ratio was the ratio of root surface area (roots <5 mm in diameter) to LAI in September.

2.6. Statistical analyses

Data were averaged across the five measurement trees in each plot. Analysis of variance (ANOVA) for a factorial design was used to test for irrigation and fertilization main effects and interactions. Block was treated as a random factor and irrigation and fertilization as fixed factors. The relationship between monthly E_L and potential evapotranspiration (PET) was examined using non-linear regression with models fit to each block-treatment combination and parameters tested using ANOVA. For variables measured repeatedly over time, repeated measures analysis of variance was used (Proc Mixed, SAS Inc., Cary, NC). The covariance structure for each variable was determined using AICC (Burnham and Anderson, 1998).

Treatment differences in regression coefficients for the model $G_S = G_{S,ref} - \delta \ln D$ were tested using repeated measures with models fit to each block-treatment combination. To test for treatment differences in the proportionality between $-\delta$ and $G_{S,ref}$, data were pooled across months and the slope ($d - \delta / dG_{S,ref}$) compared between treatments using dummy variable analysis. Effects were considered significant at $\alpha = 0.05$.

3. Results

3.1. Environmental conditions

Total precipitation at the site from February 2003 to December 2003 was 1212 mm and similar to the average annual 1190 mm (Fig. 1). During the study PET totaled 1145 mm (Fig. 1). Mean 24 h temperatures ranged from 6 °C in December to 25 °C in August. Typical maximum D in mid-summer ranged from 3 to 3.6 kPa.

3.2. Growth

Fertilization increased DBH, basal area, height, total aboveground and belowground biomass, and growth efficiency whereas irrigation did not significantly influence any growth variables or interact with fertilizer effects (Table 1). No treatment effects on the area of roots <5 mm in diameter were observed. Fertilization decreased allocation to branch biomass relative to total aboveground woody mass. Leaf area index varied over the months of the study, but no interaction between month of measurement and treatment was detected for LAI (Table 2). Averaged over all months, LAI increased 24% with fertilization, from 1.86 to 2.30 m² m⁻². An interaction between irrigation and fertilization indicated a significant increase in LAI with irrigation only in the fertilized treatment (Table 2). Irrigation increased LAI from 2.14 to 2.46 m² m⁻² in fertilized stands whereas in non-fertilized stands LAI was 1.96 m² m⁻² without irrigation and 1.75 m² m⁻² with irrigation.

Table 2

Transpiration, canopy stomatal conductance, water relations and hydraulic traits of 4-year-old loblolly pine in response to fertilization (F-0: no fertilization, F-1: fertilization), irrigation (I-0: no irrigation, I-1: irrigation) and month

Treatment	E (mm month ⁻¹)	E_L (mm month ⁻¹)	$G_{S,ref}$ (mmol m ⁻² s ⁻¹)	$-\delta$ (mmol m ⁻² s ⁻¹ kPa ⁻¹)	Predawn Ψ_L (MPa)	Mid-day Ψ_L (MPa)	LAI (m ² m ⁻²)	$A_S:A_L$ (cm ² m ⁻²)
F-0	20.2 ± 2.2	11.0 ± 1.3	87.2 ± 8.0	57.3 ± 5.9	-0.51 ± 0.05	-1.30 ± 0.05	1.86 ± 0.12	2.91 ± 0.13
F-1	38.2 ± 3.7	16.2 ± 1.6	124.8 ± 7.0	77.9 ± 4.4	-0.56 ± 0.05	-1.17 ± 0.06	2.30 ± 0.08	3.44 ± 0.13
I-0	28.3 ± 5.3	13.1 ± 2.1	101.0 ± 7.9	65.6 ± 4.8	-0.53 ± 0.05	-1.24 ± 0.05	2.14 ± 0.06*	3.23 ± 0.13
I-1	30.2 ± 4.8	14.0 ± 1.5	111.1 ± 8.9	69.6 ± 6.3	-0.53 ± 0.05	-1.24 ± 0.06	2.46 ± 0.06	3.22 ± 0.13
$p > F$								
Fertilization	<0.001	0.002	0.038	0.040	0.217	0.038	<0.001	0.035
Irrigation	0.643	0.529	0.522	0.657	0.825	0.976	0.642	0.912
Fertilization × irrigation	0.611	0.096	0.286	0.156	0.368	0.835	0.022	0.378
Month	<0.001	<0.001	0.053	0.012	0.001	<0.001	<0.001	<0.001
Month × fertilization	<0.001	0.434	0.136	0.131	0.948	0.650	0.767	0.366
Month × irrigation	0.910	0.888	0.475	0.292	0.698	0.822	0.924	0.916
Month × irrigation × fertilization	0.995	0.688	0.367	0.125	0.998	0.163	0.750	0.896

E and E_L are transpiration on a ground or leaf area basis, $G_{S,ref}$ is the canopy stomatal conductance at a vapor pressure deficit (D) of 1 kPa, $-\delta$ is the sensitivity of G_S to D , Ψ_L is the leaf potential, and LAI is the leaf area index. All values are means ± S.E. Observed probability values for treatment and measurement month effects are indicated. *Denotes irrigation effects in fertilized stands.

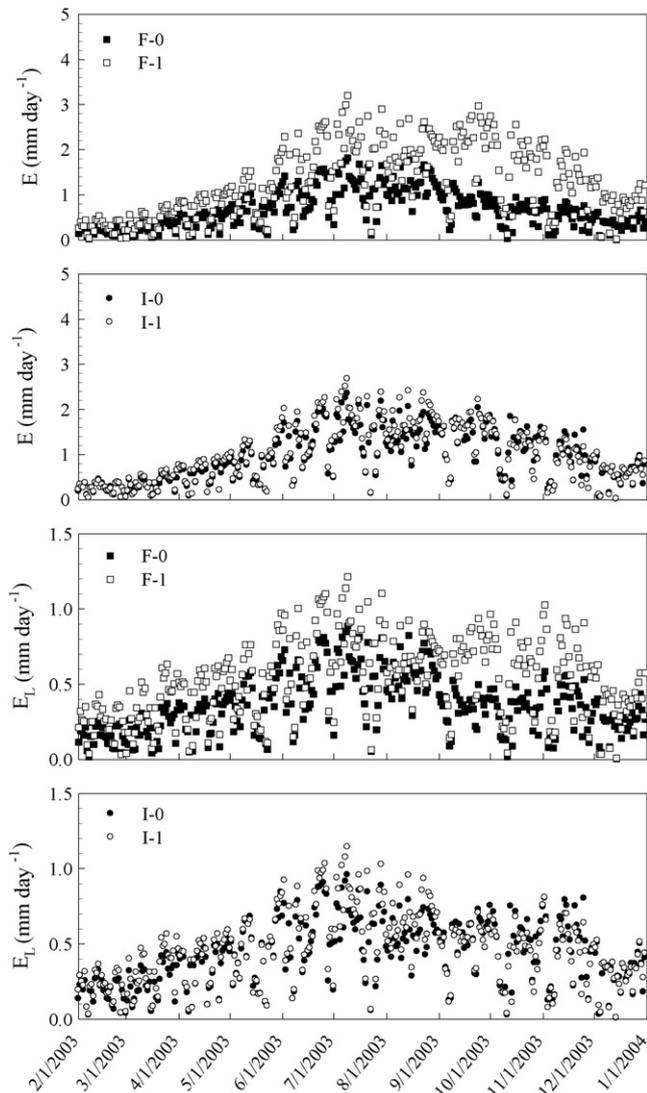


Fig. 2. Mean daily transpiration on a ground area (E) or leaf area basis (E_L) in response to fertilization (F-0: no fertilization, F-1: fertilization) and irrigation (I-0: no irrigation, I-1: irrigation) in 4-year-old loblolly pine from February to December 2003. Each data point represents an average over three blocks by fertilization or irrigation treatment.

Table 3

Hydraulic traits for 4-year-old loblolly pine in response to fertilization (F-0: no fertilization, F-1: fertilization) and irrigation (I-0: no irrigation, I-1: irrigation)

Treatment	G (mol $m^{-2} s^{-1} MPa^{-1}$)	G_L (mol $m^{-2} s^{-1} MPa^{-1}$)	$A_R:A_L$ ($m^2 m^{-2}$)	E (mm)	E_L (mm)	a	b
F-0	2.44 ± 0.21	0.61 ± 0.10	0.58 ± 0.13	222.8 ± 23.9	120.9 ± 14.2	19.8 ± 3.0	0.008 ± 0.002
F-1	3.54 ± 0.34	1.14 ± 0.15	0.49 ± 0.08	420.1 ± 41.2	177.7 ± 17.2	24.2 ± 2.3	0.012 ± 0.002
I-0	2.68 ± 0.32	0.76 ± 0.17	0.52 ± 0.08	311.2 ± 58.0	144.4 ± 22.9	21.5 ± 2.6	0.011 ± 0.003
I-1	3.30 ± 0.37	0.99 ± 0.16	0.55 ± 0.13	331.7 ± 52.5	154.2 ± 17.0	23.0 ± 3.2	0.009 ± 0.002
$p > F$							
Fertilization	0.033	0.021	0.585	0.005	0.039	0.192	0.184
Irrigation	0.171	0.234	0.812	0.705	0.666	0.790	0.504
Fertilization \times irrigation	0.541	0.829	0.435	0.678	0.256	0.983	0.276

G and G_L are whole-tree hydraulic conductance per unit sapwood or leaf area, $A_R:A_L$ is the ratio of root surface area (roots <5 mm in diameter) to leaf area, E and E_L are the transpiration per unit ground or leaf area summed over 11 months, and a and b are parameters for the non-linear model ($E_L = a(1 - e^{-b \text{PET}})$) describing the relationship between monthly E_L and monthly potential evaporation transpiration (PET). All values are means \pm S.E. Observed probability values for treatment effects are indicated.

3.3. Transpiration

Daily E and E_L demonstrated typical seasonal patterns with higher rates in summer than in winter months (Fig. 2). In summer, daily E and E_L were as high as 3.2 mm day^{-1} and 1.2 mm day^{-1} , respectively. Accumulated by month, E ranged from 5 mm month^{-1} in winter to 60 mm month^{-1} in summer (Fig. 3). Monthly E and E_L were significantly greater in fertilized than non-fertilized treatments (Table 2). A significant interaction between fertilization treatment and month indicated that enhancement of monthly E by fertilization was greatest in summer and autumn months, as much as 1.4 times higher than in non-fertilized stands (Table 2, Fig. 3). Irrigation had no influence on monthly E or E_L . As reported for other tree species such as *Betula papyrifera* Marsh., *Populus balsamifera* L. and *Picea glauca* (Moench) Voss (Bladon et al., 2006), E_L was non-linearly related to PET in all plots with R^2 ranging from 0.91 to 0.99 (data not shown). Neither fertilization nor irrigation influenced the ultimate value of E_L (parameter a) or the instantaneous slope of E_L for any value of PET (parameter b) (Table 3). Over the duration of the 11-month study, fertilization nearly doubled total E from 222.8 to 420.1 mm and increased E_L by 47% (Table 3). Irrigation had no significant influence on total E and E_L , and no significant interactions between irrigation and fertilization were observed for E and E_L .

3.4. Canopy stomatal conductance

Fertilization significantly increased $G_{S,ref}$ and $-\delta$ (Table 2). No main effects of irrigation or interactions between fertilization, irrigation or month were detected for either variable. Dummy variable analysis indicated that the slope of the relationship between $-\delta$ and $G_{S,ref}$ did not vary significantly with fertilization ($p = 0.144$) or irrigation ($p = 0.639$) and the average slope was 0.602 (Fig. 4).

3.5. Hydraulic traits

Irrigation had no significant influence on $A_S:A_L$, G , G_L , and mid-day or predawn Ψ_L (Tables 2 and 3). In contrast,

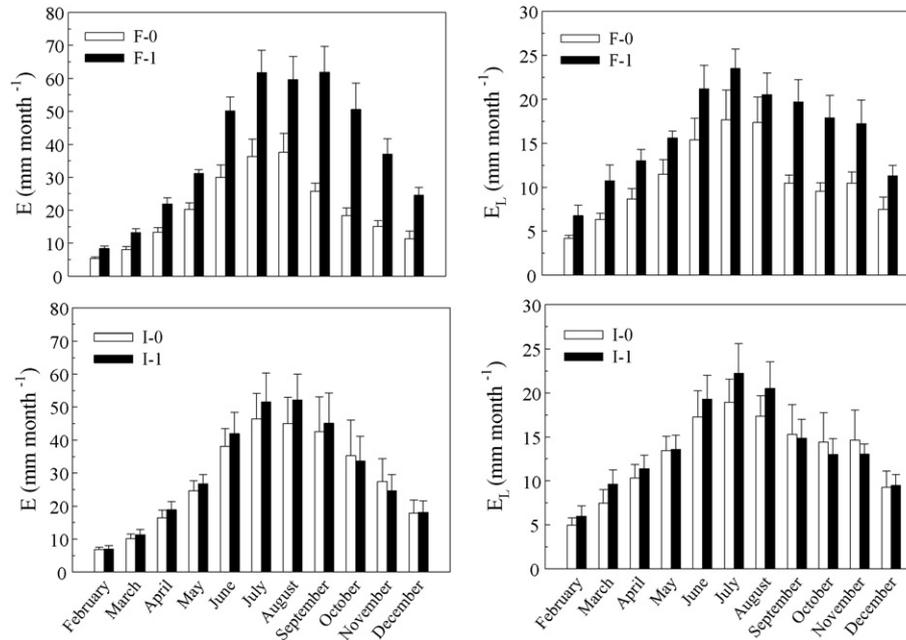


Fig. 3. Mean (\pm S.E.) monthly transpiration on a ground (E) or leaf area basis (E_L) in response to fertilization (F-0: no fertilization, F-1: fertilization) and irrigation (I-0: no irrigation, I-1: irrigation) in 4-year-old loblolly pine from February to December 2003.

fertilization increased $A_S:A_L$, G , and G_L (Table 3). Fertilization had no influence of predawn Ψ_L but mid-day Ψ_L was increased by fertilization (Table 2). No significant treatment effects on $A_R:A_L$ were observed (Table 3).

4. Discussion

Maximum daily E was as high as 3 mm day^{-1} in fertilized stands and within the range reported for 5-year-old loblolly pine in Bainbridge, Georgia under long-term fertilization plus irrigation (Samuelson and Stokes, 2006) and natural stands of loblolly pine ranging in age from 6 to 10 years (Phillips and Oren, 2001). In the above-mentioned Georgia study, family 7–56 was planted at a density of $1070 \text{ trees ha}^{-1}$ and by age 5 had reached a LAI of $3.7 \text{ m}^2 \text{ m}^{-2}$ in fertilized plus irrigated stands compared to $2.5 \text{ m}^2 \text{ m}^{-2}$ in fertilized stands in this study. Over the 11-month study, E was 420 mm in fertilized stands compared to 528 mm in fertilized plus irrigated stands in the Georgia study. When standardized by leaf area, E_L of the same treatments was, notably, 178 mm in both studies. These results highlight the role of LAI in understanding water use in fast growing loblolly pine plantations. In contrast to the Georgia study, LAI did not fully account for the increase in E with fertilization, as indicated by greater monthly and total E_L in fertilized stands. Growth efficiency, or annual increment in stem mass per unit mass of foliage, was higher with fertilization, which indicates that fertilization may have increased foliar photosynthetic efficiency or altered carbon allocation patterns to favor stem growth (Vose and Allen, 1988). Fertilization increased G_L and a positive relationship between G_L and net photosynthetic rate has been proposed (Ryan et al., 2000; Hubbard et al., 2001; Katul et al., 2003). Fertilization did not influence $A_R:A_L$ and up to age 4 there was no effect of

fertilization on the proportion of above versus belowground biomass (Coyle et al., 2008). However, fertilized trees allocated proportionately less aboveground woody biomass to branches than unfertilized trees, which may account for the increase in $A_S:A_L$. Increased stemwood to leaf mass allocation and decreased branch allocation relative to stem were reported for 4-year-old loblolly pine in response to fertilization (Colbert et al., 1990). High allocation to stem, which has low priority for biomass allocation, may reflect trends associated with accelerated stand development (Colbert et al., 1990) or a direct response to optimum nutrient availability (Waring and Schlesinger, 1985).

Higher E , E_L , and $G_{S,\text{ref}}$ in response to fertilization were accompanied by adjustments in hydraulic traits; specifically, increases in $A_S:A_L$, G , and G_L . Positive relationships between G_L and stomatal conductance, and G_S and $A_S:A_L$ have been demonstrated (Wullschlegel et al., 1998; Hubbard et al., 2001; Fischer et al., 2002; Addington et al., 2004; Kim et al., 2008; Ward et al., 2008). Changes in hydraulic traits of pines are most frequently associated with variation in climate and soil water availability (Maherali and DeLucia, 2001) with a limited number of studies on hydraulic changes in response to long-term resource management. Clearwater and Meinzer (2001) reported adjustments in leaf water use efficiency rather than changes in $A_S:A_L$ with fertilization in *Eucalyptus grandis* Hill ex Maiden. Five years of fertilization increased LAI but had little influence on $A_S:A_L$, G_S , and E_L in fast growing *Eucalyptus saligna* Sm. (Hubbard et al., 2004). A comparable increase in $A_S:A_L$ to our study, from 1.61 to $2.11 \text{ cm}^2 \text{ m}^{-2}$, with fertilization in loblolly pine at age 4 but not at age 10 (Jerez et al., 2005) suggests that the relationship between nutrient availability and $A_S:A_L$ depends on stand age and tree structural characteristics, in particular height (Ward et al., 2008).

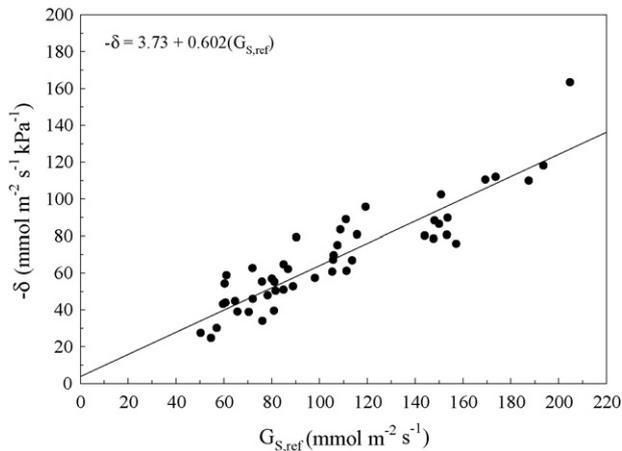


Fig. 4. Relationship between the sensitivity ($-\delta$) of canopy stomatal conductance (G_S) to vapor pressure deficit (D) and G_S at a D of 1 kPa ($G_{S,ref}$) in 4-year-old loblolly pine. Data were pooled across treatments.

Vanninen et al. (1996) proposed that lower $A_S:A_L$ in *P. sylvestris* on less fertile sites was related to lower leaf physiological activity or increased $A_R:A_L$ and thus lower stem transport capacity needs. Higher $G_{S,ref}$ and G_L with fertilization may also be related to changes in sapwood permeability (Brix and Mitchell, 1983) which was not examined. For example, Shelburne and Hedden (1996) observed higher sapwood permeability of loblolly pine in stands on better sites and suggested that high soil resource availability maintained the function of tracheids in the inner sapwood. Both positive (Martínez-Vilalta et al., 2004) and negative (Whitehead et al., 1984a) relationships between $A_S:A_L$ and sapwood hydraulic conductivity have been reported for conifers.

Ewers et al. (2000) reported higher $G_{S,ref}$, G_L , and $A_S:A_L$ in 15-year-old loblolly pine in response to fertilization only when irrigation was supplied but in this study changes in hydraulic traits were independent of irrigation. In Ewers et al. (2000), fertilization both with and without irrigation decreased $A_R:A_L$ and soil water extraction potential, implying greater vulnerability to drought without irrigation (Hacke et al., 2000). Dissimilar results between studies could be related to variation in soil water availability, maintenance of $A_R:A_L$ with fertilization, and differences in stand age and structure.

An analysis by Oren et al. (1999) of 30 tree species including ring- and diffuse-porous deciduous broadleaf species and conifers, revealed a consistent relationship, or slope of approximately 0.60, between G_S at low D ($G_{S,ref}$) and sensitivity of the closure response to increasing D ($-\delta$), despite variation in species growth form and habitat and differences in methodologies between studies. High $G_{S,ref}$ and high $-\delta$ represent a tradeoff between high potential leaf carbon gain at low D and a rapid reduction in G_S with increasing D relative to trees with lower $G_{S,ref}$ (Ewers et al., 2007). The model 0.60 proportionality between $-\delta$ and $G_{S,ref}$ is in theory a result of regulation of minimum Ψ_L to avoid excessive E . As long as stomata regulate Ψ_L near a constant value, the slope of 0.60 is expected, an exception being desert and very drought tolerant trees which may exhibit weaker regulation of Ψ_L (Oren

et al., 1999). Pinaceae family members have been shown to maintain water potentials within narrow limits (Martínez-Vilalta et al., 2004). The 0.60 proportion between $-\delta$ and $G_{S,ref}$ confirmed in this study has been previously reported for mature loblolly pine under varying management intensity and stand conditions (Ewers et al., 2000). It should be noted that many studies including ours suffer from autocorrelation between G_S and D . Higher mid-day Ψ_L and no evidence of increased sensitivity of E_L to PET with fertilization suggest that water loss was regulated at high D to limit transpiration and avoid decreasing Ψ_L . Ewers et al. (2007) observed maintenance of the 0.60 ratio between $-\delta$ and $G_{S,ref}$ and consistent minimum Ψ_L over 2 years in seven tree species despite variation in E and E_L between years. Our results corroborate the model of homeostasis of minimum Ψ_L in trees and the prediction of G_S at any D with measurement of $G_{S,ref}$, as discussed by Ewers et al. (2007).

In summary, the hypothesis that fertilization effects on hydraulic traits would depend on irrigation was not supported, as irrigation had little impact on transpiration and hydraulic attributes. Fertilization increased E , E_L , $A_S:A_L$, G_L , $G_{S,ref}$ and sensitivity of G_S to D , indicating that hydraulic traits of loblolly pine are sensitive to nitrogen availability. Greater basal area and biomass in fertilized stands was associated with changes in hydraulic traits, greater growth efficiency, and decreased branch allocation.

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