

**Fertilization Effects on Water Use of 8-year-old  
Loblolly Pine (*Pinus taeda* L.) Vary with Throughfall Treatment**

by

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A thesis submitted to the Graduate Faculty of  
Auburn University  
in partial fulfillment of the  
requirements for the Degree of  
Master of Science

Auburn, Alabama  
May 09, 2015

Keywords: *Pinus taeda* L., sap flow, transpiration, canopy stomatal  
conductance, throughfall exclusion, climate change

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## Abstract

Loblolly pine (*Pinus taeda* L.) plantations in the southern U.S. generate more timber than any other country in the world and therefore reductions in net primary productivity associated with climate variability may have significant economic impacts. As part of PINEMAP ([www.pinemap.org](http://www.pinemap.org)), the objective of this research was to determine whether tree and stand-level water use are influenced by the main and interactive effects of reduced water availability from throughfall reduction and fertilization. We hypothesized that greater leaf area and related soil water depletion in response to fertilization would increase the impact of precipitation reduction on canopy level processes. Sap flow measurements were initiated in January 2013. An interactive effect of fertilization and throughfall treatments on monthly transpiration on a ground area ( $E_G$ ) and leaf area ( $E_L$ ) basis and canopy stomatal conductance ( $G_S$ ) was observed during 2013. Over the one year study period, which was a wetter than normal year, fertilization increased average monthly  $E_G$  in the ambient throughfall treatment from January through July, but fertilization had no effect on  $E_G$  in the throughfall reduction treatment, because of a decrease in  $E_L$  and  $G_S$  in response to fertilization combined with throughfall reduction. These results indicate a more conservative water use strategy, such that greater leaf area associated with fertilization results in a greater sensitivity of canopy-level processes to water availability.

## **Acknowledgements**

The research presented here would have been impossible without my many collaborators, committee members, tree physiology lab mates, and friends and family members. I would first and foremost like to thank my major advisor and committee chair, Dr. Lisa Samuelson, for her guidance, expertise, and encouragement, as well as Dr. Art Chappelka and Dr. Amy Wright for their interest in my development as a research scientist and for serving as members of my graduate committee. Dr. Mary Anne McGuire, Madison Akers, Dr. Bob Teskey, and Dr. Eric Ward provided invaluable assistance and advice both in and out of the field throughout this research project. I am extremely grateful to Tom Stokes and all of my fellow graduate students, including Joe Clark, Charles Pell, John Jackson, Althea ArchMiller, and Lorenzo Ferrari, for their support, advice, and friendship over the past couple years. I would further like to acknowledge Tom Stokes and his father, Al Stokes, as well as Justin Rathel, Jake Blackstock, and my undergraduate research fellow, Stephan Barron, for their help with installing and maintaining an expansive sap flux network. I would like to thank my parents, Lee and Dorothy Spencer, and children, Stanley and Aspen Bartkowiak, for their never-ending consideration and understanding as my time and concentration was fully enveloped in my research. Finally, I would like to thank my wife and cherished friend, Sabrina Bartkowiak, without whose unwavering love and support I would have been lost entirely in this endeavor.

The Pine Integrated Network: Education, Mitigation, and Adaptation project (PINEMAP) is a Coordinated Agricultural Project funded by the USDA National Institute of Food and Agriculture, Award #2011-68002-30185.

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## List of Abbreviations

$-\delta$	Sensitivity of $G_s$ to D
$\lambda$	Latent heat of vaporization
$\gamma$	Psychometric constant
$A_s$	Sapwood area
$A_s:A_L$	Sapwood-to-leaf area ratio
B	Boron
BA	Basal Area
C	Correction factor for radial profile of sap flux
Cu	Copper
$c_p$	Specific heat of air
D	Vapor Pressure Deficit
DAP	Diammonium Phosphate
DBH	Diameter at Breast Height
DIB	Diameter Inside Bark
DOB	Diameter Outside Bark
$E_L$	Canopy transpiration on a leaf area basis
$E_G$	Canopy transpiration on a ground area basis
ET	Evapotranspiration
$ET_0$	Reference Evapotranspiration
$G_s$	Canopy stomatal conductance

$G_{S,ref}$	Reference canopy stomatal conductance at $D = 1.0$ kpa
Ha	Hectare
HT	Height
K	Potassium
LAI	Leaf Area Index
Mn	Manganese
N	Nitrogen
P	Phosphorous
$\rho$	Density of air
PAR	Photosynthetically Active Radiation
PDSI	Palmer Drought Severity Index
PINEMAP	Pine Integrated Network: Education Mitigation and Education Project
$q$	Sap flux density
Q	Sap flow
Ri	Sap flux ratio
S	Sulfur
SAS	Statistical Analysis System
Ta	Air Temperature
VWC	Volumetric Water Content
WUE	Water Use Efficiency
Zn	Zinc

## 1.0 Project Background

The southeastern United States (U.S.) is comprised of more than 25 million hectares of pine plantations (Conner and Hartsell, 2002) from which the U.S. harvests approximately 60% of total domestic wood products, accounting for 15.8% of the global industrial wood supply (Wear and Greis, 2012). Nationally, the forest products industry employs more people than the automotive, chemical or plastics industries, and accounts for 5.5% of the jobs and 7.5% of total industrial output for the region (Wear and Greis, 2012). Loblolly pine (*Pinus taeda* L.) is the dominant plantation pine species in the southeastern U.S. and its productivity has tripled over the last 50 years with enhanced seedling genetics and improved resource management (Fox et al., 2007), specifically that of nutrient availability (Albaugh et al., 2004).

Increasing concentrations of atmospheric carbon dioxide (CO<sub>2</sub>) and other greenhouse gases have been correlated with regional and global rises in temperature and varied precipitation, which has introduced the need to develop mitigation strategies to reduce the impacts of climate variability. Forests throughout the region have the ability to sequester large amounts of carbon through biomass production. Southeastern forests currently store 12 Pg of carbon ( $12 \times 10^{15}$  g C), which amounts to 36% of the sequestered forest carbon in the contiguous US (Turner et al. 1995). Forests in the southeastern U.S. can sequester 76 Tg of carbon ( $76 \times 10^{12}$  g C) annually, equivalent to 13% of regional greenhouse gas emissions (Johnsen et al. 2001, Han et al. 2007), and

have the potential to sequester more through reforestation, afforestation and improved forest management (Albaugh et al., 2012; Aspinwall et al., 2011).

Climate projections indicate the duration and intensity of summertime droughts may increase (IPCC, 2013) with the rate of warming in the southeastern U.S. (Kunkel et al., 2012). Average annual temperature in the southeastern U.S. has increased 1-2°C over the last 30 years (Karl et al., 2009), and the warming trend is projected to continue with an average increase of 2.5-3.5°C over the next century (Kunkel et al., 2012). Total annual precipitation may be reduced by 10% (Kunkel et al., 2012) with the majority of that reduction occurring during the summer (Christensen et al., 2007; IPCC, 2013). Conversely, the frequency of extreme precipitation events (i.e. days with precipitation greater than 25.4 mm) may increase with the water-holding capacity of a warming atmosphere (Kunkel et al., 2012). Precipitation events such as these are likely to result in surface water runoff and therefore less net soil water availability (IPCC, 2013). Shifts in climate patterns and associated changes in temperature and soil water availability may have significant impacts on forest productivity and therefore carbon sequestration in the southeastern U.S. (Noormets et al., 2010; Wear and Greis, 2012).

The National Institute of Food and Agriculture funded the Pine Integrated Network: Education, Mitigation and Adaptation Project (PINEMAP) through an Agriculture and Food Research Initiative (AFRI) grant in order to investigate the influence of reduced precipitation associated with climate change combined with fertilization on loblolly pine plantations throughout the southern United States. The PINEMAP team is comprised of over 120 principle investigators, research technicians,

and graduate students associated with eight major forestry cooperative research programs, nine land grant universities, including Auburn University, the U.S. Forest Service, and climate modeling and adaptation specialists associated with the multi-state Southeastern Climate Consortium and state climate offices. The overarching goal of PINEMAP is to create, synthesize and disseminate the knowledge necessary to enable southern pine landowners to: manage forests to increase carbon sequestration by 15% by 2030 to mitigate atmospheric CO<sub>2</sub>, increase the efficiency of nitrogen and other fertilizer inputs by 10% by 2030, and adapt their forest management approaches to increase resilience in the face of a changing climate.

To achieve this goal, PINEMAP developed six primary aims/objectives: (1) ecophysiology and silviculture, (2) modeling, (3) genetics, (4) economics and management policy, (5) education, and (6) extension. The specific research presented here is a component of the ecophysiology and silviculture aim, which has established a three-tiered monitoring network based on existing cooperative research trials with the goal of developing standardized research methods to quantify carbon, water, and nutrient storage and flux baselines in response to climate and management. The three-tiered monitoring network consists of Tier I “legacy”, Tier II “active”, and Tier III “throughfall exclusion and fertilization” sites [see [www.pinemap.com](http://www.pinemap.com) for a more detailed description of the three-tiered monitoring network]. Four experimental Tier III sites were established at the edges of the loblolly pine distribution that span the full range in precipitation, soil, and potential productivity gradients. In these studies, nutrients and water are manipulated through fertilization and diversion of rain falling

through the forest canopy (throughfall). This approach enables PINEMAP to quantify the response of physiological processes controlling loblolly pine productivity and consequently carbon sequestration to reductions in precipitation associated with climate variability and fertilization.

## **2.0 Fertilization Effects on Water Use of 8-year-old Loblolly Pine (*Pinus taeda* L.) Vary with Throughfall Treatment**

### **2.1 Introduction**

Climate projections for the southeastern United States (U.S.) indicate increased duration and intensity of summertime droughts with the rate of warming (Dai, 2011; Kunkel et al., 2012; IPCC, 2013), although the frequency of extreme precipitation events may increase with the water-holding capacity of a warming atmosphere (Kunkel et al., 2012). Projected changes in global and regional climate may impact the hydrologic cycle of forested ecosystems (Wullschleger and Hanson, 2006), because evapotranspiration (ET), composed of physical evaporation, biological transpiration and interception, is a major component, second only to precipitation, in the water cycle of forested ecosystems (Lu et al., 2004; Cao et al., 2006). In the southeastern U.S., more than half the land area is forested (Wear and Greis, 2012), and 50% to 85% of precipitation is returned to the atmosphere by ET from forested watersheds (Sun et al., 2002). Forest canopy transpiration on a ground area basis ( $E_G$ ) is the primary component of terrestrial water flux, accounting for approximately 47-70% of global forest ET depending on the global ecoregion (Schlesinger and Jasechko, 2014). Canopy transpiration is also linked to carbon sequestration through photosynthesis and gross primary productivity, because stomata regulate the fluxes of carbon dioxide and water vapor (Kim et al., 2014). When

canopy transpiration is expressed on a leaf area basis ( $E_L$ ), it is related to canopy stomatal conductance ( $G_S$ ) and vapor pressure deficit ( $D$ ), as described by:

$$E_L = G_S \left( \frac{D p c_p}{\lambda \gamma} \right) \quad (1)$$

where  $p$  is the density of air ( $1225 \text{ g m}^{-3}$ ),  $c_p$  is the specific heat of air ( $1.01 \text{ J g}^{-1} \text{ K}^{-1}$ ),  $\lambda$  is the latent heat of vaporization of water ( $2465 \text{ J g}^{-1}$ ), and  $\gamma$  is the psychrometric constant ( $65.5 \text{ Pa K}^{-1}$ ).

Loblolly pine is the most extensively planted pine species in the southeastern U.S. (Fox et al., 2007), representing approximately one-half of the standing pine volume in the region (Wear and Greis, 2012). While it is known that variation in environmental conditions such as photosynthetically active radiation (Granier and Breda, 1996), air temperature (Bauweraerts et al., 2013), vapor pressure deficit (Oren et al., 1999), and soil water availability (Domec et al., 2009) as well as forest management (Samuelson and Stokes, 2006) influence loblolly pine  $E_L$  and  $G_S$ , the interactive effect of reduced soil water availability associated with climate variability and fertilization on canopy-level processes is not well understood. The majority of research exploring the impact of water availability on loblolly pine water use has utilized irrigation treatment or natural drought (Pataki et al., 1998; Ewers et al., 2000; Albaugh et al., 2004; Samuelson et al., 2008). Drought has been shown to induce multiple short-term responses in trees, such as stomatal closure and subsequent reduction in  $G_S$  and  $E_L$ , and long-term adjustments in architecture and hydraulic conductance (Ewers et al., 2000). Natural drought has been shown to limit  $G_S$  and reduce carbon assimilation in loblolly pine (Ellsworth, 2000;



Domec et al., 2009). Conversely, Samuelson et al. (2008) determined that increased water availability via irrigation had little impact on  $G_s$  and  $E_L$  of 4-year-old loblolly pine, but in this case water availability was not as limiting as nutrient availability.

The goal of this research was to evaluate the interactive influence of throughfall reduction and fertilization on whole-tree and stand-level water use of 8-year-old loblolly pine to better understand how potential reductions in precipitation associated with climate change combined with fertilization may affect loblolly pine plantations. Increased leaf area in response to fertilization may result in regulation of stomatal conductance to limit water loss via transpiration (Goldstein et al., 2013) or an increase in the sapwood-to-leaf area ratio ( $A_S:A_L$ ) to avoid hydraulic failure (Whitehead, 1998). Concurrent work on this study site reported no interactive influence of throughfall and fertilization treatments on leaf-level physiology (Samuelson et al., 2014). Similarly, Tang et al. (2004) observed no interactive effect of complete throughfall reduction and fertilization on leaf net photosynthetic rate in 18-year-old loblolly pine; however, enhancement of canopy leaf area by fertilization was observed only in the ambient throughfall treatment which suggests that whole tree water use was affected by the interaction between fertilization and soil water availability. Leaf-level measurements are discrete and dependent upon individual leaf physiology, canopy position, and irradiance (Norman, 1980; Baldocchi and Amthor, 2001), whereas continuous sap flux measurements used to describe canopy-level processes may capture treatment effects at broader spatial and temporal scales and enhance understanding of forest productivity response to climate variability.

The specific objectives of this study were to: (1) determine whether whole-tree and stand-level water use are influenced by the main and interactive effects of throughfall reduction and fertilization treatment, and (2) if reduced water availability in response to throughfall manipulation influences the relationship between  $G_s$  and  $D$ . The most important environmental variable to which plant stomata respond is  $D$  (Monteith, 1995). Stomatal closure occurs with increasing  $D$  as a feedback response to leaf and whole-tree water status in order to reduce water use (Lange et al., 1971; Massman and Kaufman 1991; Meinzer et al., 1995; Domec et al., 2012). By limiting  $E_L$ , which would otherwise be increased with  $D$ , stomata closure avoids the decline in plant water potential and prevents excessive dehydration and physiological damage at the whole-tree level (Saliendra et al., 1995). We tested the hypothesis that greater leaf area and subsequent soil water depletion in response to fertilization would increase the impact of throughfall reduction on canopy level processes; more specifically, that trees in the fertilized throughfall treatment would reduce  $G_s$  and  $E_L$  and exhibit greater sensitivity of  $G_s$  to  $D$  in order to limit water loss and avoid drought stress. This study is part of a large interdisciplinary research initiative, the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP ([www.pinemap.org](http://www.pinemap.org))), in which experimental throughfall reduction and fertilization installations have been established at four key locations at the edges of the natural loblolly pine range with the goal of quantifying nutrient, carbon, and water fluxes and baselines in response to climate and management practices (Will et al., 2014).

## 2.2 Materials and Methods

### 2.2.1 Study site and experimental design

The study was installed on land leased from Plum Creek Timber Company, Inc. located in the Georgia Piedmont physiographic region (33°37'N, 82°47'W) in Taliaferro Co., GA. Mean annual high / low temperature and precipitation are 22.7°C / 10.1°C and 1109 mm respectively (1983-2012; NOAA National Weather Service – <http://www.ncdc.noaa.gov/cdo-web/datasets/ANNUAL/locations/ZIP:30673/detail>, accessed February 2014). The Palmer Drought Severity Index (PDSI) was collected for Climate Division 3 in the state of Georgia (<http://www1.ncdc.noaa.gov/pub/data/cirs/climdiv/climdiv-pdsidv-v1.0.0-20140707>, accessed July 2014). The PDSI utilizes monthly precipitation, runoff, soil moisture, and evaporation to determine drought severity, with negative values indicating greater severity in drought (Palmer, 1965; Guttman, 1999). The PDSI values are categorized into ten levels of drought and wet conditions: an exceptional drought defined as a value less than -5.0, extreme drought from -4.0 to -4.9, severe drought from -3.0 to -3.9, moderate drought from -2.0 to -2.9, abnormally dry from -1.0 to -1.9, abnormally wet from 1.0 to 1.9, moderate wet conditions from 2.0 to 2.9, severe wet conditions from 3.0 to 3.9, extreme wet conditions from 4.0 to 4.9, and an exceptional wet period greater than 5.0 (National Drought Mitigation Center, University of Nebraska-Lincoln, U.S. Drought Monitor Classification -

<http://droughtmonitor.unl.edu/AboutUs/ClassificationScheme.aspx>). Reference evapotranspiration ( $ET_0$ ) for the experimental site was acquired from the State Climate Office of North Carolina ([www.nc-climate.ncsu.edu](http://www.nc-climate.ncsu.edu)). The  $ET_0$  method requires radiation, air temperature, air humidity, and wind speed data to estimate evaporation from a reference crop and soil system using the Penman-Monteith Method (Monteith and Unsworth, 1990).

The study site is comprised of three similar soils from the Catula-Cecil (CcB2 and CcD2) and Lloyd (LdB2) series (USDA soil classification - <http://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/home/>, accessed June 2013). The dominant LdB2 complex is a fine, kaolinitic, thermic Rhodic Kandiudult. The remaining CcB2 and CcD2 complexes are fine, kaolinitic, thermic Typic Kandhapludults, which can be more acidic. All soils are well drained with medium to rapid runoff and moderate permeability.

The previous loblolly pine stand was clear cut in 2005 and the land was prepared for planting using a bedding combo plow followed by aerial application of herbicide (Velpar ULW, DuPont Chemical Co.;  $5.97 \text{ kg ha}^{-1}$ ). The site was hand planted with bare root seedlings in 2006 ( $1544 \text{ trees ha}^{-1}$ ) on a 3 m by 2 m spacing. Seedlings were from an open-pollinated, genetically improved, second generation seed source. Following planting, Banded Oust Extra herbicide ( $219 \text{ mL ha}^{-1}$ ) was applied for herbaceous weed control.

The experimental design was a 2 x 2 factorial combination of fertilization and throughfall manipulation treatments replicated in four blocks. The treatment plots were 0.10 ha (34.1 m x 28.0 m) and included a central 0.03 ha (21.3 m x 14.0 m) measurement plot containing approximately 40 trees. Fertilization treatments included no fertilization and a standard, operational fertilization treatment (224 kg N ha<sup>-1</sup>, 28 kg P ha<sup>-1</sup>, 56 kg K ha<sup>-1</sup>) hand broadcast around trees in March, 2012. Nitrogen and P were applied as urea and diammonium phosphate (DAP), and K was applied using potassium chloride. A micronutrient blend that consisted of S-6%, B-5%, Cu-2%, Mn-6%, and Zn-5% by weight (Southeast Mix, Cameron Chemicals, Inc., Portsmouth, VA) was also applied. Throughfall manipulation treatments included an ambient throughfall control and a 30% reduction in throughfall, which corresponds to the driest projections for the region from Christensen et al. (2007). In order to reduce throughfall by approximately 30%, exclusion trays covering 30% of total plot area were installed within each row to divert throughfall from the treatment plots. Supporting structures were built to a height of 1.3 m and a width of 1.5 m. Two throughfall exclusion trays were constructed on top of the supporting structure and separated by a 30.5 cm opening to minimize microclimate effects and soil moisture banding. The trays were covered by a 12 mil (0.3 mm) extrusion laminate with two layers of U.V. stabilized coextruded polyethylene and high strength cord grid (Poly Scrim 12, Global Plastic Sheeting, Vista, Ca).

A cellular networked weather station (CWB100 Wireless Base Station, Campbell Scientific, Inc., Logan, UT, USA), located in an open area in the center of the site, with a data logger (CR1000, Campbell Scientific, Inc., Logan, UT, USA) collected continuous

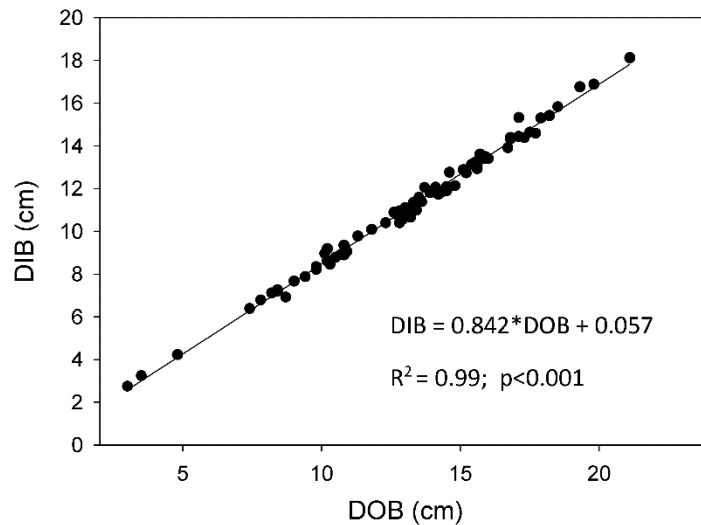
meteorological data over the study period beginning in January 2013, including: precipitation (TR-525I Rain Gauge Tipping Bucket, Texas Electronics Inc., Dallas, TX, USA), photosynthetic active radiation (PQS 1 PAR Quantum Sensor; Kipp & Zonen USA Inc., Bohemia, NY, USA), temperature and relative humidity (CS500-L, Campbell Scientific, Logan, UT, USA), and volumetric water content (VWC; Wireless Soil Water Reflectometer, CWS655, Campbell Scientific, Logan, UT, USA) in the top 12 cm of soil. Ambient throughfall plots contained one randomly located VWC probe while the throughfall exclusion plots had two probes, one randomly located underneath a throughfall exclusion trough and the second between rows. Estimated field capacity is approximately  $0.42 \text{ m}^3 \text{ m}^{-3}$  for clay soils (Saxton and Rawls, 2006). Due to wireless connectivity issues, only 91 days of VWC data were collected over the study period. The largest data gaps in VWC occurred from January 1 - March 1, April 30 - June 19, July 3 - September 25, and November 28 - December 31.

### 2.2.2 Aboveground growth

Plot level inventories were conducted annually beginning prior to study initiation (December, 2011). Inventories included diameter measured at breast height (1.3 m, DBH) and height (HT) of all living trees in each measurement plot. Using the growth data for 2013, stand-level water use efficiency (WUE) was calculated over the study period by dividing the amount of carbon produced in the stem wood estimated from annual stem increment ( $\text{g C ha}^{-1}$ ; Samuelson et al., 2014) by the amount of water transpired ( $\text{kg H}_2\text{O ha}^{-1}$ )(Samuelson and Stokes, 2006; Kauwe et al., 2013).

Leaf area index (LAI) was measured approximately every month in diffuse sunlight using two optical sensors (LAI-2000, LI-COR Inc., Lincoln, NE, USA) (Samuelson et al., 2014), one in an opening adjacent to the site and the other below the canopy using a 90° view cap [see Samuelson et al. (2014) for a more detailed measurement description]. Because of month to month variability in mean LAI within a plot, the monthly measurements were smoothed by removing months that deviated from expected phenological patterns of foliage production and senescence (following Sampson et al. (2003)) and interpolating between the adjoining months.

Stem diameter outside bark (Diameter Outside Bark; DOB) near the sap flow probe location (defined in section 2.2.3) was measured monthly and corrected for bark thickness (Diameter Inside Bark; DIB) ( $DIB = 0.842 * DOB + 0.057$ ;  $R^2 = 0.99$ ) (Fig. 1) to calculate individual tree sapwood cross-sectional area ( $A_{S,tree}$ ). The relationship between DOB and DIB was measured July 2014 on five trees per plot using a bark thickness gauge (JIM-GEM bark gauge, Forestry Suppliers, Jackson, MS, USA). Total plot sapwood area ( $A_S$ ) was estimated by applying the average monthly sap flow tree increment in basal area to every tree in the measurement plot and summing across all trees. This approach in calculating  $A_S$  assumes that the range in sap flow tree basal area was representative of the entire plot [see description of sap flow tree selection in section 2.2.3]. The sapwood-to-leaf area ratio ( $A_S:A_L$ ) was calculated as the ratio of monthly  $A_S$  to plot-level LAI.



**Figure 1.** The relationship between outside bark diameter (DOB) and inside bark diameter (DIB) at breast height (1.3 m) in an 8-year-old loblolly pine plantation.

### 2.2.3 Sap flow measurements and transpiration

Five sample trees in each measurement plot (80 trees in total) absent of forks and surrounding gaps were selected for sap flow measurements and represented the range in basal area distribution on each plot. During the study period, three sample trees were replaced with trees of similar size due to disease or probe malfunction. Thermal dissipation probes, modified after Granier (1985), with a 20 mm integrated length were inserted into the sapwood. The outer bark was removed by sanding and two small holes (1.5 and 2.0 mm in diameter) spaced 9 cm apart were drilled into the sapwood approximately 1.3 m above ground level at a northern aspect. To reduce the chances of spreading disease the drill bits were rinsed in bleach (Clorox Co., Oakland, CA). Silicon was applied around the thermocouples in order to seal out moisture and the stem was wrapped with porous Reflectix® insulation (Reflectix Inc., Markleville, IN)



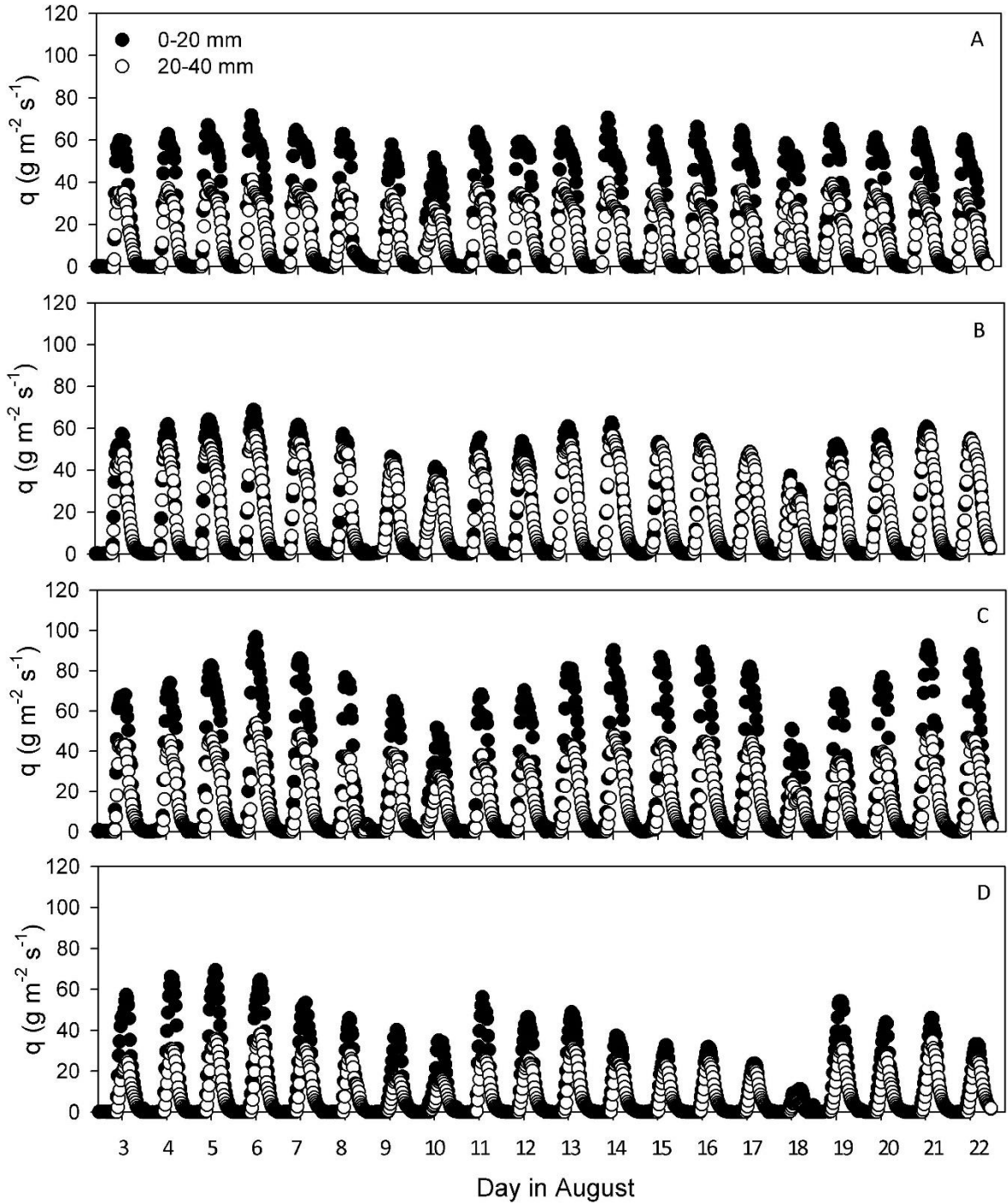
around the probes to reduce thermal gradients. Sap flux density ( $q$ ,  $\text{g m}^{-2} \text{s}^{-1}$ ; as defined by Reid et al., 2005) was recorded every 30 s and 2-min means were stored on a data logger (CR23XPB, Campbell Scientific, Inc., Logan, UT). Sap flux was recorded continuously from January 2013 to December 2013. Two power outages occurred during the study period from January 15-24 and February 24-27. Missing data were gap-filled using monthly regressions developed between  $q$  and  $D$ .

Sap flow ( $Q$ ,  $\text{kg 30-min}^{-1}$ ; as defined by Reid et al., 2005) at the tree level was calculated as the product of  $q$  (summed by 30-min) and  $A_{S,\text{tree}}$  (Granier, 1987). Transpiration on a ground area basis ( $E_G$ ,  $\text{mm}$ ) was estimated by averaging  $Q$  across the five sample trees in a treatment plot, dividing by measurement plot area, and multiplying 30-min average  $Q$  by the ratio of plot basal area to average sap flow tree basal area measured at DBH in each measurement plot (Samuelson and Stokes, 2006; Čermák et al., 2004). Transpiration was calculated on a leaf area basis ( $E_L$ ) by dividing  $E_G$  by projected plot LAI that was converted to all-sided LAI using a conversion factor of 2.36 (Rundel and Yoder, 1998; Domec et al., 2009).

Variation in  $q$  across the radial profile was measured in August during the 2014 growing season (Fig. 2A-D). Sap flow trees were selected to represent the approximate range in quadratic mean DBH estimated from the 2012 inventory data (Table 1). Radial variation was measured at two depths (0-20 mm and 20-40 mm) using probes positioned 5 cm apart horizontally at a north aspect (Phillips et al., 1996). Probe installation in the 20-40 depth interval was accomplished by first drilling a 12 mm

diameter, 20 mm deep hole into the xylem followed by a 1.5 or 2.0 mm diameter (depending on heated or reference probe), 20 mm deep hole into the 20-40 mm depth following Phillips et al. (1996).

A correction coefficient (CF) was calculated to account for variation in  $q$  and subsequent  $Q$  for sapwood depths deeper than 20 mm (adapted from Delzon et al., 2004). Sap flow was calculated for each depth interval (0-20 or 20 mm to pith) from  $q$  from the 0-20 mm or 20-40 mm sensor and the corresponding sapwood area calculated for the 0-20 mm depth and for the 20 mm to the pith. We assumed that all wood was juvenile wood and that the 20-40 mm  $q$  represented  $q$  at deeper depths. The CF was then calculated as the ratio of total 24-hour  $Q$  corrected for the reduction in  $Q$  in the 20-40 mm interval to total 24-hour  $Q$  assuming no radial variation with depth.



**Figure 2.** Diel variation in sap flux density in an 8-year-old loblolly pine plantation ( $q$ ) measured at two sapwood depths (0-20 mm and 20-40 mm) in trees with stem diameter of 9.1 cm in the control (A), 9.7 cm in the throughfall (B), 10.2 cm in the fertilization (C), and 8.1 cm in the combined (D) treatments. Measurements of  $q$  were made from August 3-22 in 2014. Each point represents a 30-min. average of  $q$ .

The daily CF was then averaged over the 19 day measurement period (Table 1). The mean correction coefficient (0.850) was applied to all sap flow tree Q, regardless of sample tree size, prior to scaling up to whole-stand transpiration.

**Table 1.** Mean ( $\pm$ SE) 2012 and 2013 quadratic mean diameter inside bark (DIB), radius inside bark (RIB), the sap flow radial variation correction factor (CF) determined over 19 days (n) in August 2013, and the sample tree DIB and RIB used to calculate CF in the control (C), throughfall (T), fertilization (F), and combined (TF) treatments in a loblolly pine plantation.

	2012 DIB (cm)	2012 RIB (cm)	2013 DIB (cm)	2013 RIB (cm)	Sample Tree DIB (cm)	Sample Tree RIB (cm)	n	CF
C	8.9 (0.4)	4.5 (0.2)	10.3 (0.3)	5.2 (0.1)	9.9	5.0	19	0.839 (0.002)
T	9.0 (0.4)	4.6 (0.2)	11.2 (0.4)	5.6 (0.2)	9.7	4.8	19	0.966 (0.005)
F	9.3 (0.5)	4.5 (0.2)	10.1 (0.4)	5.1 (0.2)	10.2	5.1	19	0.824 (0.003)
TF	9.1 (0.3)	4.5 (0.1)	10.9 (0.2)	5.5 (0.1)	8.1	4.1	19	0.891 (0.007)

#### 2.2.4 Canopy stomatal conductance

Canopy stomatal conductance ( $G_S$ ) was estimated from  $E_L$  and  $D$  using the simplification of the inversion of the Penman-Monteith model (Monteith and Unsworth, 1990; Samuelson et al. 2006):

$$G_S = \frac{\lambda E_L \gamma}{p c_p D} \quad (3)$$

where  $\lambda$  is the latent heat of vaporization of water (2465 J g<sup>-1</sup>),  $\gamma$  is the psychrometric constant (65.5 Pa K<sup>-1</sup>),  $p$  is the density of air (1225 g m<sup>-3</sup>),  $c_p$  is the specific heat of air (1.01 J g<sup>-1</sup> K<sup>-1</sup>) and  $D$  is the vapor pressure deficit of the air. Values were converted from m s<sup>-1</sup> to mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> following Nobel (2009). To reduce instrumental error to < 10%,  $G_S$  was calculated only when  $D > 0.75$  kPa (Ewers and Oren, 2000). Decreases in  $G_S$  in response to increasing  $D$  has been shown to be proportional to  $G_S$  at low  $D$  under saturating light (Domec et al., 2009). Therefore, the sensitivity of the stomatal response

to  $D$  when photosynthetically active radiation (PAR) was greater than  $1000 \text{ mmol m}^{-2} \text{ s}^{-1}$  (light saturated  $G_s$ ) and  $D > 0.75 \text{ kPa}$  was examined by fitting the data to the functional form:

$$G_s = G_{s,ref} - \delta \log D \quad (4)$$

where  $G_{s,ref}$  is  $G_s$  at  $D = 1 \text{ kPa}$  and  $-\delta$  is the rate of stomatal closure with increasing  $D$  and reflects the sensitivity of  $G_s$  to  $D$  (Oren et al., 1999; Domec et al. 2009). The model was fit to each block and treatment combination by month, for four months, during the period of June through September 2013. These months during the growing season provided range in  $D$  ( $0.75 - 3.75 \text{ kPa}$ ) needed to develop the function (Oren et al., 1999).

#### 2.2.5 Statistical Analysis

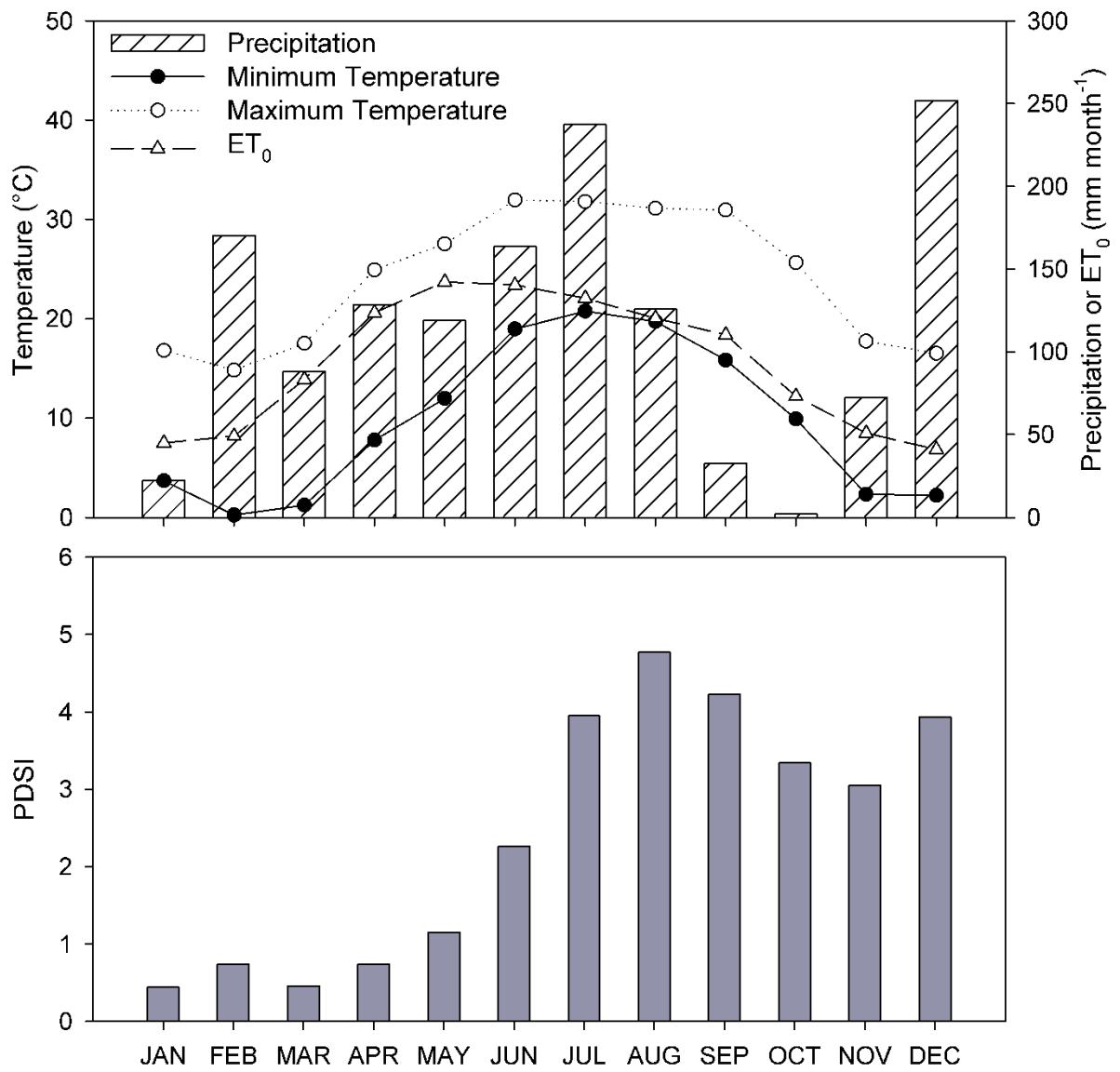
The experimental design was a completely randomized design with four blocks and the experimental unit of replication was the treatment plot. For variables measured repeatedly over time (i.e.  $E_G$ ,  $E_L$ ,  $G_s$ ), the main and interactive effects of fertilization and throughfall exclusion treatments and month were tested using repeated measures ANOVA (Proc Mixed, SAS Institute Inc., Cary, NC). The selected covariate structure for repeated measures analysis was determined graphically (covariance vs. time lag) and by minimizing the Akaike (AIC) and Bayesian (BIC) Information Criterion fit statistics for each variable (Littell et al., 2006). The best fit covariate structure for  $E_G$  and  $E_L$  was unstructured (UN) while the first-order ante dependence (ANTE(1)) covariate structure was the best fit for  $G_s$ . Treatment differences in annual  $E_G$ ,  $E_L$ , and WUE were tested using ANOVA (Proc GLM, SAS Institute Inc., Cary, NC). Treatment differences in

regression coefficients for the model  $G_S = G_{S,ref} - \delta \log D$  were tested using ANOVA with models fit to each block and treatment combination. Main and interactive treatment effects were considered significant at  $\alpha=0.05$ .

## 2.3 Results

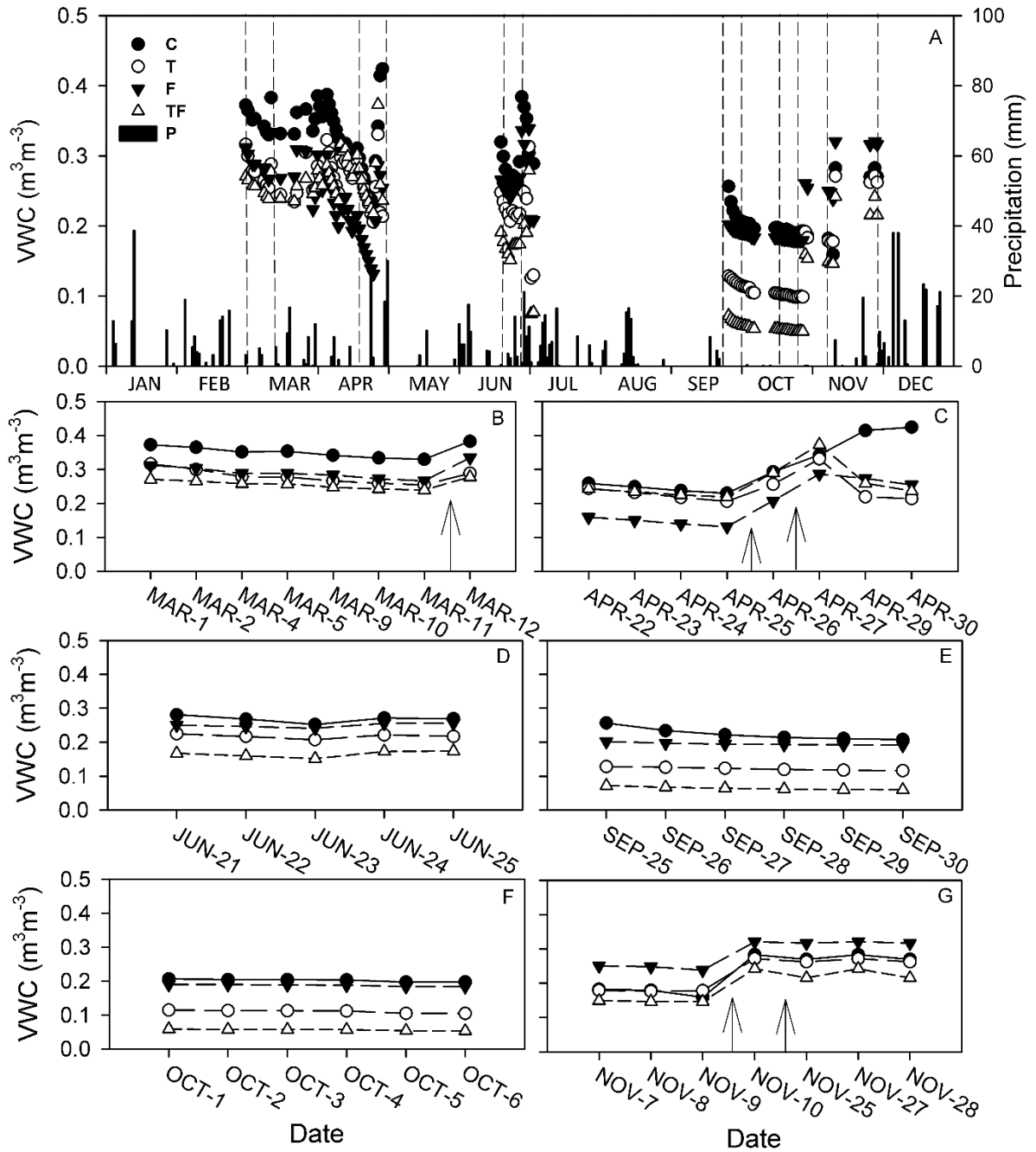
### 2.3.1 Climate

Over 2013, the average 24 hour minimum and maximum temperatures ranged from 0.2°C in February to 32.0°C in July, respectively (Fig. 3). Annual precipitation was 1413 mm and was 304 mm higher than the 30-year normal. Monthly precipitation ranged from 1.8 mm in October to 237.2 mm in July (Fig. 3). Annual  $ET_0$  was 1114 mm and the ratio of annual  $ET_0$  to annual precipitation ( $ET_0:P$ ) was 79%. No drought conditions, defined as a negative PDSI value (see methods section 2.2.1), were indicated but monthly precipitation was lower than the 30-year monthly average for January, March, September, October, and November (Fig. 3). Although not tested statistically, VWC was generally lower in the throughfall exclusion treatment, particularly when combined with fertilization (Fig. 4A-G). Volumetric water content was low in September and October when ambient monthly precipitation was lower than average. In October, average VWC was  $0.06 \text{ m}^3 \text{ m}^{-3}$  in the throughfall reduction treatment compared to  $0.20 \text{ m}^3 \text{ m}^{-3}$  in the control treatment (Fig. 4). A recharge in VWC typically followed precipitation events (Fig. 4A-C and G). The highest observed average VWC was  $0.42 \text{ m}^3 \text{ m}^{-3}$ , which is similar to the average field capacity of  $0.42 \text{ m}^3 \text{ m}^{-3}$  estimated for clay soils (Saxton and Rawls, 2006) (Fig. 4).



**Figure 3.** Monthly summed precipitation and reference evapotranspiration ( $ET_0$ ), average monthly 24-hour minimum and maximum temperature, and the Palmer Drought Severity Index (PDSI) during 2013.





**Figure 4.** Precipitation (P) and volumetric water content (VWC) in the upper 12 cm by control (C), throughfall reduction (T), fertilization (F), and the combined (TF) treatment during 2013 (A) in a 8-year-old loblolly pine plantation. Each data point represents the average daily VWC from periods containing at least one complete block of data. Dashed lines in A indicate time periods selected to illustrate variability in VWC over time (B-G). Arrows indicate a precipitation event in B-G.

### 2.3.2 Aboveground Growth

The growth results were previously reported by Samuelson et al. (2014). In 2012, basal area increment (BAI) was significantly reduced by throughfall reduction, and fertilization increased BAI and peak leaf area index (Samuelson et al., 2014). In 2013, no significant effects of throughfall treatment on growth were observed; however, a trend towards a reduction in height in response to throughfall reduction was observed (Table 2). Diameter at breast height and basal area increment were increased by 8% and 43%, respectively, by fertilization in 2013.

### 2.3.3 Leaf area index and the sapwood to leaf area ratio

A significant effect of fertilization treatment ( $p=0.002$ ) on peak LAI in September 2013 was observed, but the throughfall treatment effect ( $p=0.103$ ) and the interaction between throughfall and fertilization treatments ( $p=0.826$ ) were not significant. Fertilization treatment increased peak LAI from 2.6 to 3.7  $\text{m}^2 \text{m}^{-2}$  (Fig. 5; Table 1). The  $A_S:A_L$  varied significantly by month ( $p<0.001$ ) and with fertilization treatment ( $p=0.043$ ) but no effect of throughfall reduction treatment ( $p=0.376$ ) or interaction effect ( $p=0.949$ ) of throughfall and fertilization treatments was observed. Fertilization decreased average  $A_S:A_L$  from 3.29 to 2.89  $\text{cm}^2 \text{m}^{-2}$ . Seasonal variability in  $A_S:A_L$  followed phenological patterns in LAI; whereby the period of greatest foliar production (May through September) resulted in lower  $A_S:A_L$ .

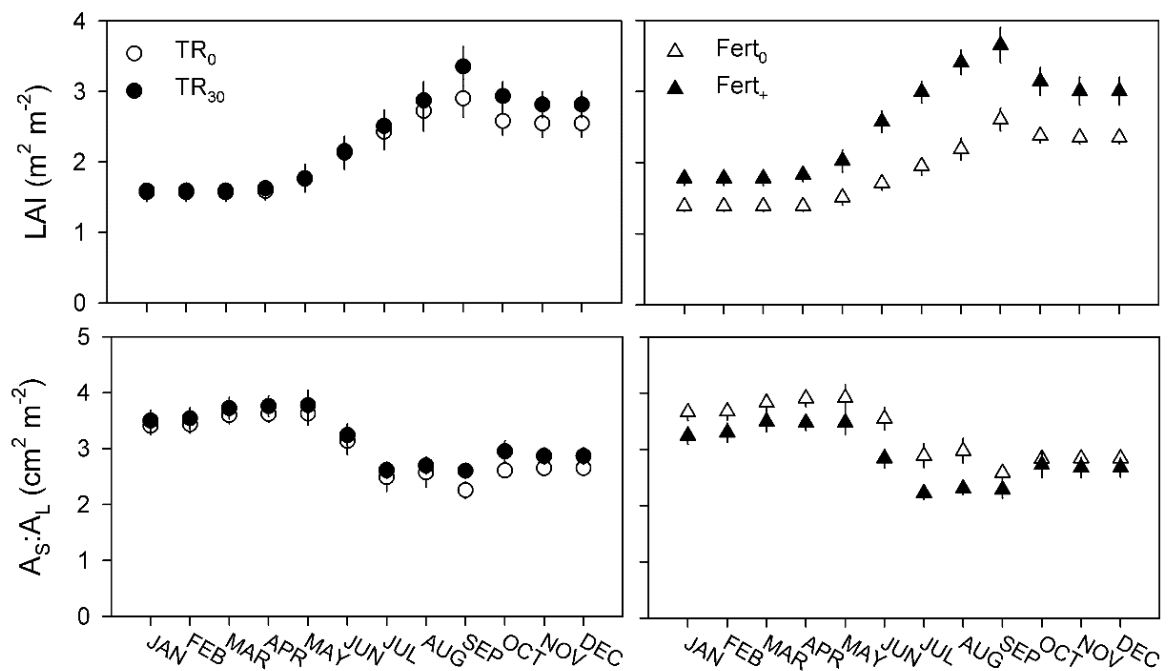
**Table 2.** Mean ( $\pm$ SE) stand characteristics measured in November 2013 in response to throughfall (ambient, TR<sub>0</sub>, and throughfall reduction, TR<sub>30</sub>) and fertilization (no fertilization, Fert<sub>0</sub>, and fertilized, Fert<sub>+</sub>) treatments from Samuelson et al. (2014). The mean and range in diameter in breast height (DBH) of the five sample trees selected for sap flow measurements were measured in November 2013.

Treatment	Mean DBH (cm)	Height (m)	Stand		Stand Basal Area Increment (m <sup>2</sup> ha <sup>-1</sup> yr <sup>-1</sup> )	Leaf Area Index* (m <sup>2</sup> m <sup>-2</sup> )	Sample Tree DBH (cm)	Sample Tree DBH Range (cm)
			Basal Area (m <sup>2</sup> ha <sup>-1</sup> )	Basal Area				
TR <sub>0</sub>	12.6 (0.4)	9.1 (0.3)	17.4 (0.9)	5.0 (0.3)	2.9 (0.3)	13.1 (0.3)	8.2 - 17.5	
TR <sub>30</sub>	12.3 (0.3)	8.8 (0.2)	16.8 (0.7)	4.7 (0.4)	3.4 (0.3)	12.7 (0.4)	7.5 - 18.0	
Fert <sub>0</sub>	12.0 (0.3)	8.8 (0.2)	15.8 (0.6)	4.0 (0.2)	2.6 (0.2)	12.3 (0.3)	8.1 - 18.0	
Fert <sub>+</sub>	12.9 (0.3)	9.1 (0.3)	18.5 (0.7)	5.7 (0.2)	3.7 (0.2)	13.4 (0.4)	7.5 - 17.5	

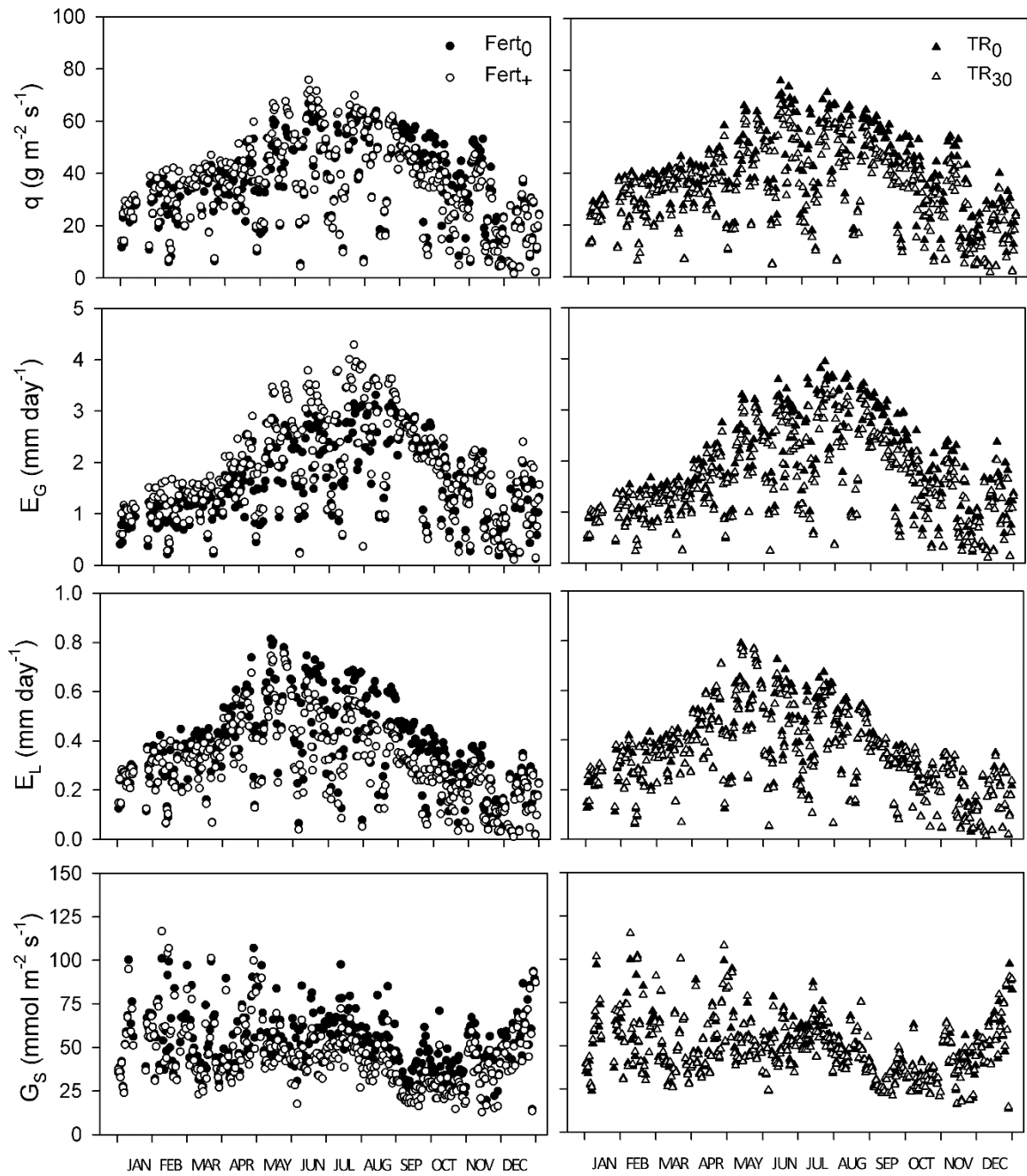
\* Peak Leaf Area Index was in September 2013

### 2.3.4 Sap flux and canopy transpiration

Daily and seasonal patterns in  $q$ ,  $E_G$ , and  $E_L$  are shown in Figure 6. Average daily (defined as the mean of all daylight hours)  $q$  varied seasonally, with higher rates during the growing season (Fig. 6). During the growing season, average daily  $q$  was as high as  $75.8 \text{ g m}^{-2} \text{ s}^{-1}$  (Fig. 6). Summed by day,  $E_G$  and  $E_L$  were on average  $1.8$  and  $0.3 \text{ mm day}^{-1}$ , respectively (Fig. 6). Daily  $E_G$  was as high as  $4.3 \text{ mm day}^{-1}$  in July, and maximum  $E_L$  was  $0.8 \text{ mm day}^{-1}$  in May (Fig. 6).



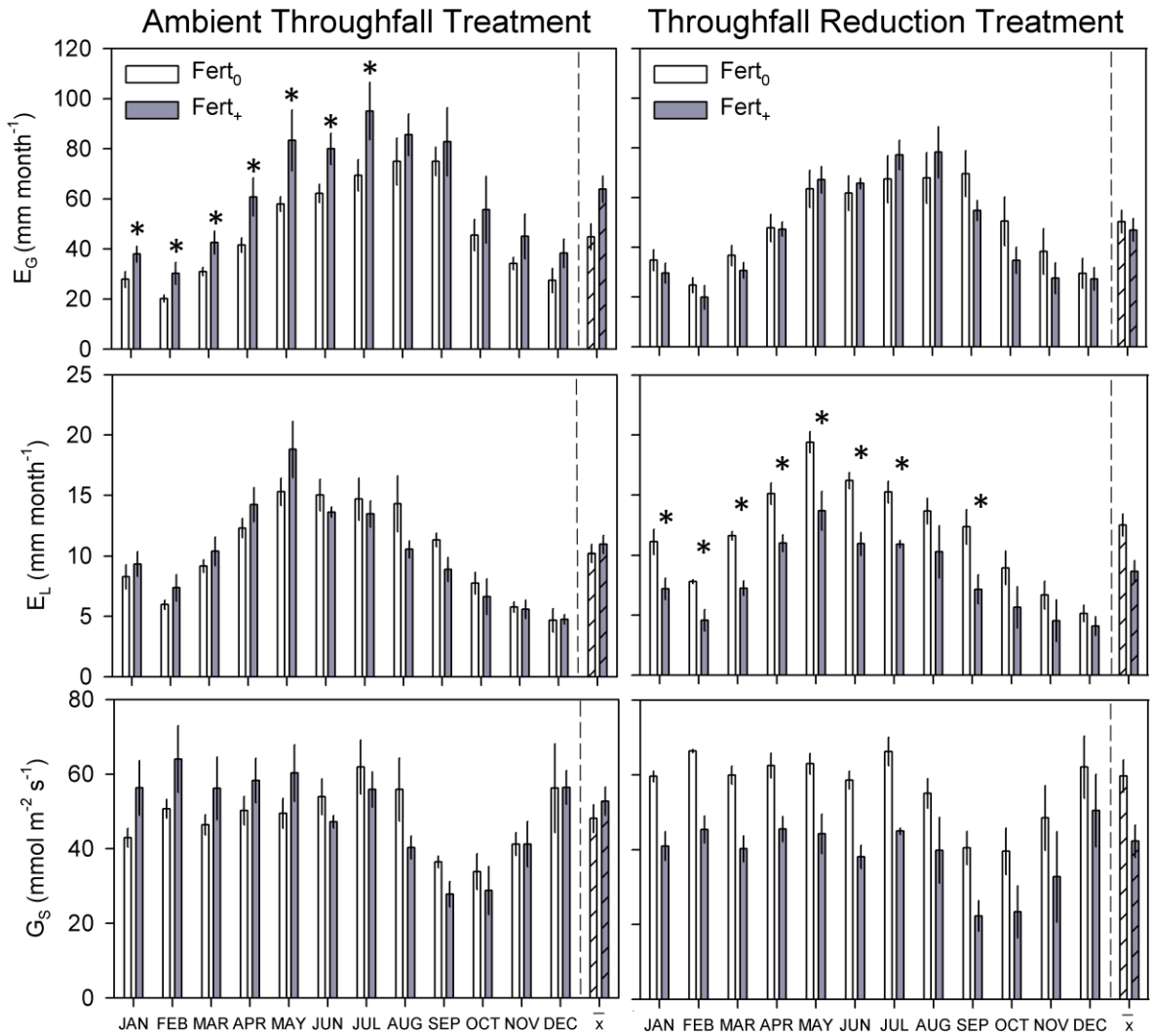
**Figure 5.** The main effects of fertilization (none, Fert<sub>0</sub>, versus fertilization, Fert<sub>+</sub>) and throughfall (ambient, TR<sub>0</sub>, and throughfall reduction, TR<sub>30</sub>) treatments on mean ( $\pm$ SE) monthly leaf area index (LAI) and the sapwood to leaf area ratio ( $A_s:A_L$ ) in 8-year-old loblolly pine. The LAI data were modified from Samuelson et al. (2014).



**Figure 6.** Daily (daylight hours) average sap flux density ( $q$ ), transpiration summed by day on a ground ( $E_G$ ) and leaf area ( $E_L$ ) basis, and daily average canopy stomatal conductance when  $D > 0.1$  kPa ( $G_S$ ) in response to fertilization (none, Fert<sub>0</sub>, and fertilization, Fert<sub>+</sub>) and throughfall reduction (none, TR<sub>0</sub>, versus throughfall reduction, TR<sub>30</sub>) treatments in an 8-year-old loblolly pine plantation from January 2013 through December 2013.

A three-way interactive effect of throughfall and fertilization treatments and month was observed for monthly summed  $E_G$  and  $E_L$  (Table 3). The interaction between throughfall and fertilization treatments and month (Table 3) indicated that fertilization increased monthly  $E_G$  only in the ambient throughfall treatment from January through July (Fig. 7). The interaction between fertilization and throughfall treatments and month (Table 3) indicated that fertilization reduced monthly  $E_L$  only in the throughfall reduction treatment in all months except August, October, November, and December (Fig. 7).

No significant treatment effects on annual  $E_G$  were observed (Table 4). Annual  $E_G$  averaged across all treatments was  $615 \text{ mm yr}^{-1}$ , and the ratio of annual  $E_G$  to  $ET_0$  was 55%. In contrast, a significant interaction between throughfall and fertilization treatments was observed for annual  $E_L$  (Table 4). Annual  $E_L$  was reduced from 143 to  $97 \text{ mm yr}^{-1}$  by fertilization in the throughfall reduction treatment and not affected by fertilization in the ambient throughfall treatment (Table 4). A significant main effect of fertilization was observed for annual WUE (Table 4). Fertilization increased WUE from 1.4 to  $1.8 \text{ g C kg}^{-1} \text{ H}_2\text{O}$  (Table 4).



**Figure 7.** Monthly sums of transpiration on a ground ( $E_G$ ) and leaf ( $E_L$ ) area basis and daily (daylight hours when  $D > 0.1$ ) average canopy stomatal conductance ( $G_S$ ) averaged by month in response fertilization treatment (none, Fert<sub>0</sub>, versus fertilized, Fert<sub>+</sub>) in the ambient throughfall treatment (TR<sub>0</sub>) and the throughfall reduction treatment (TR<sub>30</sub>) in 8-year-old loblolly pine. Mean  $G_S$ ,  $E_G$ , and  $E_L$  in response to fertilization treatment averaged over all months within a throughfall treatment is indicated by  $\bar{x}$ . Asterisks indicate a significant throughfall x fertilization x month interaction in  $E_G$  and  $E_L$  summed by month.

**Table 3.** Observed probability values for the main and interactive effects of month of measurement (M), and throughfall (TR) and fertilization (Fert) treatments on daily (daylight hours) averaged canopy stomatal conductance ( $G_S$ ), reference  $G_S$  at  $D=1$  when  $PAR > 1000$  ( $G_{S,ref}$ ), the sensitivity of  $G_S$  to  $D$  ( $-\delta$ ), and monthly summed transpiration on a ground area ( $E_G$ ) and leaf area ( $E_L$ ) basis in an 8-year-old loblolly pine plantation. Bold values indicate significance at  $\alpha = 0.05$ .

Variable	M	TR	Fert	TR x Fert	M x TR	M x Fert	M x TR x Fert
$G_S$	<b>&lt;0.001</b>	<b>0.007</b>	0.719	<b>0.003</b>	0.998	0.136	0.242
$G_{S,ref}$	<b>&lt;0.001</b>	0.593	<b>0.001</b>	0.194	0.876	0.795	0.205
$-\delta$	<b>&lt;0.001</b>	0.900	<b>&lt;0.001</b>	0.180	0.793	0.735	0.220
$E_G$	<b>&lt;0.001</b>	0.297	0.256	0.135	<b>0.001</b>	<b>0.014</b>	<b>0.001</b>
$E_L$	<b>&lt;0.001</b>	<b>0.021</b>	0.675	<b>0.026</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.009</b>

**Table 4.** Mean ( $\pm$ SE) annual transpiration on a ground area ( $E_G$ ) and leaf area ( $E_L$ ) basis and annual water use efficiency (WUE) in an 8-year-old loblolly pine plantation in response to throughfall (ambient,  $TR_0$ , and throughfall reduction,  $TR_{30}$ ) and fertilization (none,  $Fert_0$ , and fertilized,  $Fert_+$ ) treatments and the associated observed probability values for main effects and interactions. Bold values indicate significance at  $\alpha = 0.05$ .

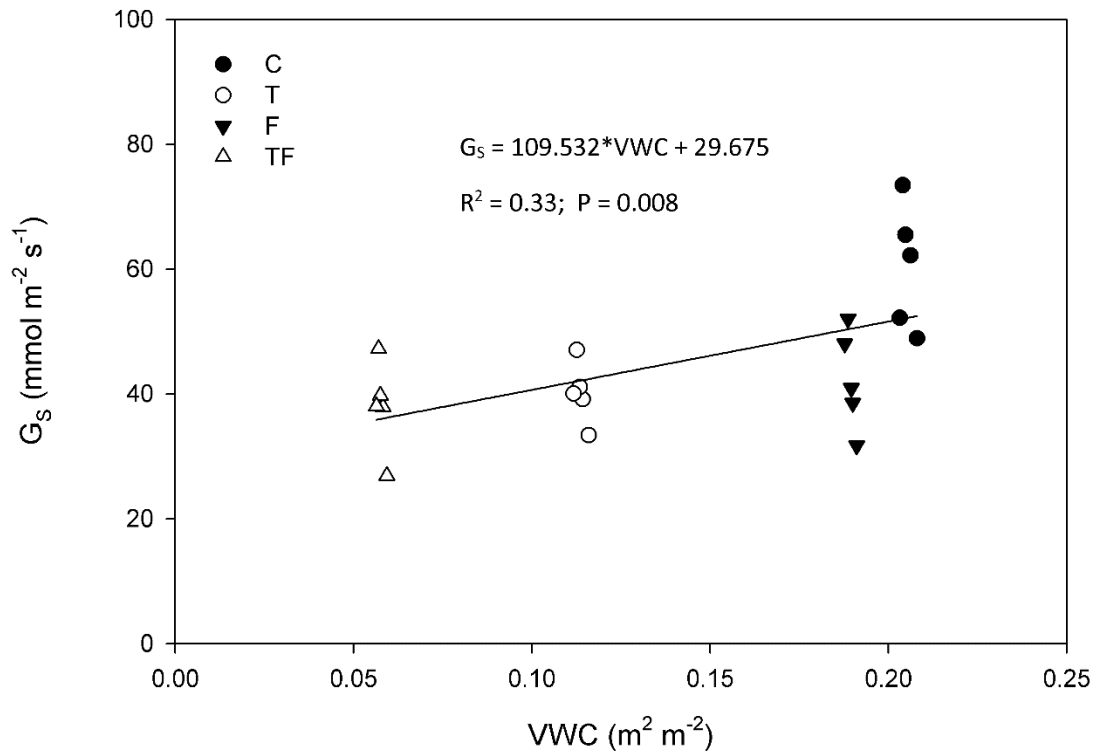
	$E_G$ (mm year <sup>-1</sup> )	$E_L$ (mm year <sup>-1</sup> )	WUE (g C kg <sup>-1</sup> H <sub>2</sub> O )
$TR_0$	652.3 (52.3)	124.1 (5.5)	1.5 (0.1)
$TR_{30}$	577.0 (41.8)	120.3 (10.6)	1.6 (0.2)
$Fert_0$	580.2 (39.7)	134.0 (6.4)	1.4 (0.1)
$Fert_+$	649.1 (54.5)	110.5 (8.0)	1.8 (0.1)
$TR_0 \times Fert_0$	567.2 (26.2)	124.5 (8.5)	1.4 (0.1)
$TR_0 \times Fert_+$	737.4 (85.3)	123.7 (8.1)	1.6 (0.1)
$TR_{30} \times Fert_0$	593.2 (81.1)	143.3 (7.7)	1.3 (0.2)
$TR_{30} \times Fert_+$	560.8 (37.6)	97.4 (10.7)	2.0 (0.2)
<i>P &gt; F</i>			
TR	0.282	0.628	0.455
Fert	0.586	<b>0.013</b>	<b>0.037</b>
TR x Fert	0.158	<b>0.016</b>	0.123



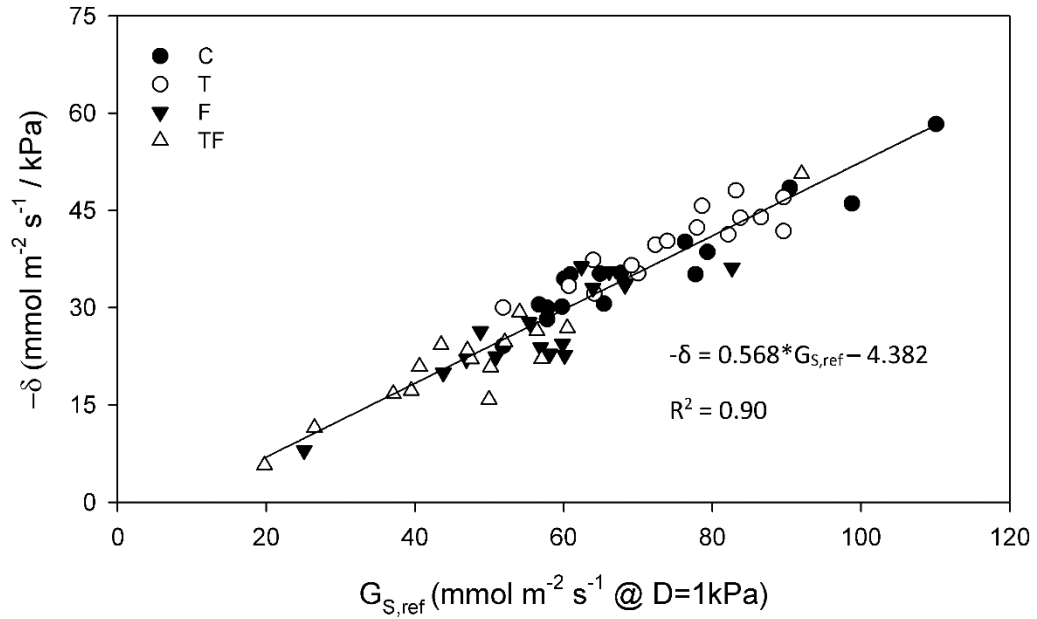
### 2.3.5 Canopy stomatal conductance

Seasonal patterns in daily average (average of daylight hours when  $D > 0.1$  kPa)  $G_S$  are shown in Fig. 6. Daily average  $G_S$  was as high as  $117 \text{ mmol m}^{-2} \text{ s}^{-1}$  during the study period (Fig. 6). A significant interaction effect between throughfall and fertilization treatment was observed for average daily  $G_S$  when averaged by month (Table 3). Fertilization reduced monthly mean  $G_S$  from 60 to  $42 \text{ mmol m}^{-2} \text{ s}^{-1}$  in the throughfall reduction treatment, but fertilization had no effect on  $G_S$  in the ambient throughfall treatment (Fig. 7). Although average monthly  $G_S$  varied by month, the effects of fertilization and throughfall treatment on  $G_S$  did not vary with month (Table 3).

Relationships between  $G_S$  and environmental variables (i.e. VWC, PAR, and D) were explored. No significant relationship between  $G_S$  and PAR were observed in 2013 (data not shown). When pooled across treatments, daily average  $G_S$  (when  $\text{PAR} > 1000 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  and  $D > 0.75$  kPa) demonstrated a positive linear relationship to VWC in October (Fig. 8). Hourly  $G_S$  (limited to when  $\text{PAR} > 1000 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  and  $D > 0.75$  kPa) was significantly and linearly related to the log transformed D all four months in all treatments [ $R^2$  ranged from 0.14 to 0.88 (see appendix Table A1)]. No interactive effect of fertilization and throughfall treatments on  $G_{S,\text{ref}}$  and  $-\delta$  was observed (Table 3). Fertilization decreased  $G_{S,\text{ref}}$  from an average of 73 to  $53 \text{ mmol m}^{-2} \text{ s}^{-1}$  (when  $D = 1$  kPa) and decreased  $-\delta$  from 38 to  $25 \text{ mmol m}^{-1} \text{ s}^{-1} \text{ kPa}^{-1}$ . When pooled across all treatments, the slope of the relationship between  $G_{S,\text{ref}}$  and  $-\delta$  was 0.57 (Fig. 9).



**Figure 8.** The relationship between average daily (daylight hours when  $\text{PAR} > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\text{VPD} > 0.75 \text{ kPa}$ ) canopy stomatal conductance ( $G_s$ ) and average 24-hour volumetric water content (VWC) in October, pooled across control (C), throughfall reduction (T), fertilization (F), and the combined (TF) treatments measured in an 8-year-old loblolly pine plantation. Data points represent one block and treatment combination over a 5 day period.



**Figure 9.** The relationship between the sensitivity ( $-\delta$ ) of daily (daytime hours when PAR > 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and VPD > 0.75 kPa) canopy stomatal conductance ( $G_s$ ) to vapor pressure deficit (D) and  $G_s$  at D = 1 kPa when PAR > 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( $G_{s,ref}$ ) pooled across control (C), throughfall reduction (T), fertilization (F), and combined (TF) treatments and months (June through September) in an 8-year-old loblolly pine plantation. Each data point represents a one month, block and treatment combination.

## 2.4 Discussion

Daily  $E_G$  was as high as  $4.3 \text{ mm day}^{-1}$  and averaged  $2.2 \text{ mm day}^{-1}$  across the growing season, defined as April through October. Average Annual  $E_G$  ranged from 577 to  $652 \text{ mm yr}^{-1}$ . These values are comparable to other reports for loblolly pine plantations. For example, maximum daily  $E_G$  reported for 5 to 12-year-old loblolly pine plantations, with LAI from 1.1 to  $4.6 \text{ m}^2 \text{ m}^{-2}$ , ranged from  $2.4$  to  $4.3 \text{ mm day}^{-1}$  and annual  $E_G$  ranged from 323 to  $930 \text{ mm yr}^{-1}$  (Ewers et al., 1999; Phillips and Oren, 2001; Gonzalez-Benecke and Martin, 2010). Ford et al. (2005) determined that average daily  $E_G$  over the growing season was  $2.2 \text{ mm day}^{-1}$  in 32-year-old loblolly pine during a period of higher than average precipitation during the months of May through September. Ewers et al. (2001) reported a range in  $E_G$  from  $331 \text{ mm yr}^{-1}$  to  $581 \text{ mm yr}^{-1}$  in 12-year-old loblolly pine on well-drained, coarse, sandy soils in North Carolina. Annual  $E_G$  was  $490 \text{ mm yr}^{-1}$  in the control versus  $930 \text{ mm yr}^{-1}$  in response to irrigation treatment in an intensively managed 11-year-old loblolly pine plantation in Georgia (Gonzalez-Benecke and Martin, 2010). Although this study did not employ an irrigation treatment, data were collected during a year with above average rainfall ( $1413 \text{ mm}$  versus the 30-year normal of  $1109 \text{ mm}$ ). Despite higher than average annual precipitation,  $ET_0:P$  was 79%, which is at the higher end of the range reported for the southeastern U.S. (50%-85%), and may indicate relatively high  $ET_0$  (Sun et al., 2002). The ratio of annual  $E_G$  to annual  $ET_0$  ranged from 52% to 59% and averaged 55.2% across all of the treatments, which is

similar to the average ratio of 55% determined for temperate coniferous forests globally (Schlesinger and Jasechko, 2014). The ratio of  $E_G:ET_0$  was within the range of 33% to 65% reported by other studies of similar-aged loblolly pine (Samuelson et al., 2006; Gonzalez et al., 2010).

The hypothesis that the impact of throughfall reduction on canopy level processes would be greater in response to increased leaf area from fertilization was supported by the results of this study. Peak LAI was increased from 2.6 to 3.7  $m^2 m^{-2}$  with fertilization, independent of throughfall treatment, but the increase in LAI resulted in differential impacts of fertilization on stand-level water use depending on throughfall treatment. Fertilization increased average  $E_G$  summed by month from 47.3 to 61.4  $mm month^{-1}$  in the ambient throughfall treatment, and fertilization has been shown to increase  $E_G$  under ambient precipitation conditions (Oren et al., 1986; Meinzer and Grantz, 1991; Köstner et al., 1992; Ewers et al., 2001). For example, Samuelson and Stokes (2006) reported increased LAI in response to fertilization and an increase in annual  $E_G$  from 420 to 528  $mm yr^{-1}$  when soil water availability was not limiting. In 2013, higher  $E_G$  in response to fertilization in the ambient throughfall treatment was most likely a function of increased LAI. In contrast, fertilization had no effect on  $E_G$  in the throughfall reduction treatment even though LAI was increased, most likely because of decreases in  $G_s$  and  $E_L$  with fertilization that occurred with throughfall reduction. Domec et al. (2012) observed no effect of a 40% increase in LAI from fertilization on  $E_G$ , because of a decrease in  $E_L$  ranging from 25% to 40% when water was limiting in a mid-rotation loblolly pine plantation. In 12-year-old loblolly pine, greater LAI in response to

fertilization resulted in reduced  $G_s$  and  $E_L$  that limited water stress, defined as a reduction in leaf water potential (Ewers et al., 2000). A decrease in  $G_s$  and  $E_L$  may be interpreted as a negative feedback response to greater LAI in order to limit water loss by  $E_G$  during water stress, which is consistent with the theory of forest transpiration as a conservative hydrological process (Roberts, 1983). The response of  $G_s$  and  $E_L$  to fertilization in the throughfall reduction treatment may be explained by a combination of: (1) the regulatory nature of an isohydric species, such as loblolly pine (Domec et al., 2009), and (2) carry-over effects caused by previous exposure to drought in 2012 (Samuelson et al., 2014), both of which will be discussed below.

Loblolly pine has been described as an isohydric species (Domec et al., 2009), that regulates stomatal conductance to avoid plant water deficit when water availability is limited (Buckley, 2005). In an analysis of 30 relatively mesic tree species, Oren et al. (1999) determined that the sensitivity of  $G_s$ , defined by the rate of stomatal closure in response to increasing D ( $-\delta$ ), is proportional to  $G_s$  at low D ( $G_{s,ref}$ ), with a mean proportionality of 0.60. High  $-\delta$  represents a tradeoff between a rapid reduction in  $G_s$  with increasing D to limit water stress and loss of leaf carbon gain (Ewers et al., 2007). In this study, when pooled across treatments, the slope of  $-\delta/G_{s,ref}$  (0.57) was similar to the 0.60 proportionality reported by Oren et al. (1999) and other studies (Ewers et al., 2007; Samuelson et al., 2008; Ward et al., 2008; Domec et al., 2009). Reduced soil water availability has been shown to decrease  $G_{s,ref}$  and  $-\delta$  in 16-year-old loblolly pine (Domec et al., 2009); however, no effect of throughfall exclusion on  $G_{s,ref}$  or  $-\delta$  was observed in this study, most likely because 2013 was a relatively wet year. A reduction in  $G_{s,ref}$  and  $-\delta$

$\delta$  in response to fertilization was observed, which is consistent with studies of loblolly pine and Norway spruce (*Picea abies*) (Ewers et al., 2000; Ward et al., 2008). For example, Ewers et al. (2000) reported reductions in  $G_{S,ref}$  and  $-\delta$  in fertilized loblolly pine, which was attributed to changes in root hydraulic architecture and whole plant hydraulic conductance rather than a direct response of  $G_S$  to soil or tree water status. Decreased hourly  $G_{S,ref}$  with fertilization across both throughfall treatments suggests a short-term conservative water use strategy in response to fertilization not detected at large temporal scale (i.e. average monthly  $G_S$ ).

Carry-over effects of drought on canopy-level processes have been observed in loblolly pine and other forested ecosystems (Ewers et al., 1999; Ewers et al., 2000; Duursma et al., 2008; Van der Mollen et al., 2011). Rooting density in soil is typically greatest near the soil surface and decreases exponentially with depth (Sands and Mulligan, 1990). During periods of moderate drought, trees may reallocate fine root development to relatively deeper soil profiles where more water may be available (Sands and Mulligan, 1990). Fine roots comprise the largest proportion of the total length of root systems per unit soil volume and contribute the highest rate of water uptake compared to suberized roots (Pregitzer et al., 2002). However, fine roots produced during drought may have smaller tracheid diameters, shorter tracheid lengths, or modified pit membrane structures, possibly due to increased auxin (Torrey, 1976) or gibberellin (van Overbeek, 1966) production, all of which may reduce xylem root vulnerability to cavitation but at the cost of reduced root and whole plant hydraulic conductivity and reduced  $G_S$  (Pregitzer et al., 2000; Ewers et al., 2001). Fine root growth

in loblolly pine occurs during three distinct growth flushes in spring, summer, and late fall (King et al., 2001), because of the mild climate and moderate soil temperatures in the southeastern U.S. (Reed, 1939). In 11-year-old loblolly pine, the average lifespan of fine roots was determined to be 166 days in well-drained, sandy soils in North Carolina (King et al., 2001). The study site experienced a moderate to extreme drought in 2012 and received 25% less precipitation than the 30-year normal (Samuelson et al., 2014). During 2012, fertilized trees may have produced fine roots with altered tracheid structure in the throughfall reduction treatment to reduce the risk of root embolisms, and the related effects on whole-tree hydraulic conductivity and  $G_s$  may have been carried over into the 2013 growing season.

The interaction between throughfall and fertilization treatments observed in canopy water use at age 8 was not expressed in annual stand growth. Nutrient availability, rather than water limitation, has been reported to be the primary driver of plantation productivity in the southeastern U.S. (Albaugh et al., 2004; Jokela et al., 2010). Jokela et al. (2004) identified nutrient availability as the dominant driver of leaf area production and subsequently growth in loblolly pine. Isohydric species may adapt to a decline in water availability by decreasing  $G_s$  (Morán-López et al., 2014) and increasing WUE (Fischer and Turner, 1978), which may maintain growth even when water availability is limited (Zhang et al., 1996). Fertilization with N and P has been shown to improve WUE in *Pinus radiata* (Sands and Mulligan, 1990), *Pseudotsuga mansiesii* (Brix and Mitchell, 1986), and *Eucalyptus grandis* (Clearwater and Meinzer, 2001; Hubbard et al., 2004) by increasing aboveground biomass accumulation. In this



study, fertilization treatment increased WUE from 1.4 to 1.8 g C kg<sup>-1</sup> H<sub>2</sub>O most likely from an increase in annual stem increment from 7.7 to 11.2 Mg ha<sup>-1</sup> yr<sup>-1</sup> in response to fertilization. However, no interaction between fertilization and throughfall treatments was detected for annual WUE, because no treatment interactions were observed for E<sub>G</sub> or annual stem increment. It is likely that the 30% to 31% reduction in average monthly G<sub>S</sub> and E<sub>L</sub>, respectively, limited water use but were not of sufficient magnitude to reduce growth. Stand WUE was higher than previously reported values of 0.6 g C kg<sup>-1</sup> H<sub>2</sub>O for 5-year-old loblolly pine (Samuelson and Stokes, 2006), which may be due to the higher stand density in this study.

In summary, the effect of fertilization on canopy level processes was modified by throughfall treatment. Fertilization increased LAI independently of throughfall treatment during the 2013 study period, but fertilization increased E<sub>G</sub> only in ambient throughfall treatment and not the throughfall reduction treatment. E<sub>G</sub> was not increased in the combined treatment because of a reduction in G<sub>S</sub> and subsequently E<sub>L</sub>, an isohydric strategy to limit water stress in response to increased LAI. Growth was not reduced by throughfall treatment in either fertilization treatment in 2013, which suggests that the isohydric nature of loblolly pine may reduce the negative impacts of drought on growth of fertilized plantations. However, further research over a range in annual precipitation is needed in order to determine long-term physiological and growth responses to climate variability.

## 2.5 Application

The results from this study will be integrated with data and modelling efforts from multiple PINEMAP aims and contribute to the PINEMAP Decision Support System (DSS). The DSS is a web-based platform currently being designed by PINEMAP research and extension specialists that will provide a collection of tools and educational materials to assist professional foresters, extension agents, and private landowners with decisions regarding forest management practices (Aldridge et al., 2014). Management recommendations will focus on the primary goals of PINEMAP and help in the effort to increase carbon sequestration, enhance productivity and fertilizer efficiency, improve forest resilience to climate variability and disturbances (i.e. pests and disease), and contribute to a more robust forest-based economy in the southeastern U.S. For example, the DSS will provide professionals with site specific recommendations on genetic, economic, and climatic factors, such as: (1) the best genetic variety of pine to plant for drought resistance, (2) density management guidelines for optimal growth, and (3) management practices to reduce vulnerability to southern pine beetle outbreaks (Aldridge and Boyles, 2013). Understanding the interactive effects of management (i.e. fertilization) and climate variability (i.e. reduced precipitation) will be critical in guiding the adaptation of forests in the southeastern U.S. for the mitigation of negative climate impacts.

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## Appendix

Table A1. Parameters for the linear model describing the relationship between hourly  $G_s$  (limited to  $PAR > 1000$  and  $D > 0.75$ ) and  $\log D$  ( $G_s = G_{s,ref} - \delta * \log D$ ) for the months of June through September for 8-year-old loblolly pine in response to control (C), throughfall reduction (T), fertilized (F), and combined (TF) treatments.

Month	Block	Trt	$G_{s,ref}$	$-\delta$	n	$P > F$	$R^2$
June	1	C	90.43	48.52	181	< 0.001	0.80
	1	T	86.52	43.98	181	< 0.001	0.77
	1	F	66.10	35.60	181	< 0.001	0.77
	1	TF	54.07	29.33	181	< 0.001	0.83
	2	C	64.89	35.26	181	< 0.001	0.79
	2	T	72.31	39.68	181	< 0.001	0.88
	2	F	59.85	24.44	181	< 0.001	0.38
	2	TF	47.56	22.10	181	< 0.001	0.63
	3	C	76.30	40.18	181	< 0.001	0.78
	3	T	83.15	48.11	181	< 0.001	0.79
	3	F	56.88	23.87	181	< 0.001	0.47
	3	TF	39.50	17.25	181	< 0.001	0.46
	4	C	59.77	30.17	181	< 0.001	0.76
	4	T	77.91	42.39	181	< 0.001	0.80
	4	F	68.24	33.53	181	< 0.001	0.64
	4	TF	60.49	26.91	181	< 0.001	0.56
July	1	C	98.76	46.09	167	< 0.001	0.69
	1	T	89.58	41.82	167	< 0.001	0.77
	1	F	68.26	33.75	167	< 0.001	0.67
	1	TF	56.39	26.46	167	< 0.001	0.73
	2	C	67.67	35.34	167	< 0.001	0.68
	2	T	70.03	35.32	167	< 0.001	0.79
	2	F	58.11	22.89	167	< 0.001	0.50
	2	TF	50.15	20.85	167	< 0.001	0.57
	3	C	77.73	35.19	167	< 0.001	0.68
	3	T	82.13	41.30	167	< 0.001	0.65
	3	F	60.12	22.68	167	< 0.001	0.41
	3	TF	49.96	15.86	167	< 0.001	0.23
	4	C	57.79	28.24	167	< 0.001	0.69
	4	T	73.93	40.31	167	< 0.001	0.79
	4	F	82.64	36.17	167	< 0.001	0.48
	4	TF	57.03	22.12	167	< 0.001	0.47

Table A 1. (Continued)

Month	Block	Trt	$G_{S,ref}$	$-\delta$	n	$P > F$	$R^2$
August	1	C	110.07	58.30	149	< 0.001	0.62
	1	T	89.59	47.07	149	< 0.001	0.78
	1	F	62.39	36.36	149	< 0.001	0.79
	1	TF	92.00	50.63	149	< 0.001	0.84
	2	C	60.90	35.12	149	< 0.001	0.83
	2	T	69.12	36.53	149	< 0.001	0.87
	2	F	46.94	22.05	149	< 0.001	0.68
	2	TF	47.05	23.49	149	< 0.001	0.74
	3	C	79.32	38.62	149	< 0.001	0.80
	3	T	78.61	45.72	149	< 0.001	0.75
	3	F	50.87	22.44	149	< 0.001	0.57
	3	TF	43.51	24.32	149	< 0.001	0.36
	4	C	57.80	30.00	149	< 0.001	0.79
	4	T	63.94	37.35	149	< 0.001	0.82
	4	F	63.93	33.03	149	< 0.001	0.68
	4	TF	40.59	20.91	149	< 0.001	0.59
September	1	C	65.38	30.61	140	< 0.001	0.30
	1	T	83.74	43.88	140	< 0.001	0.73
	1	F	48.81	26.37	140	< 0.001	0.59
	1	TF	52.10	24.71	140	< 0.001	0.48
	2	C	60.09	34.47	140	< 0.001	0.73
	2	T	64.13	32.18	140	< 0.001	0.78
	2	F	25.08	8.00	140	< 0.001	0.14
	2	TF	37.06	16.75	140	< 0.001	0.42
	3	C	51.82	24.11	140	< 0.001	0.67
	3	T	60.68	33.39	140	< 0.001	0.64
	3	F	55.39	27.71	140	< 0.001	0.46
	3	TF	19.75	5.74	140	< 0.001	0.18
	4	C	56.67	30.50	140	< 0.001	0.74
	4	T	51.84	30.04	140	< 0.001	0.76
	4	F	43.79	19.98	140	< 0.001	0.47
	4	TF	26.48	11.52	140	< 0.001	0.38