The Effects of Fertilization and Four Years of Throughfall Reduction on Leaf Physiology of Loblolly Pine (*Pinus taeda* L.)

by

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Abstract

Loblolly pine (*Pinus taeda* L.) is the most widely planted pine species in the southeastern United States. Increased frequency of drought associated with projected climate change may negatively impact loblolly pine productivity. The objective of this study was to evaluate the main and interactive effects of reduced water availability, achieved by throughfall reduction, and one-time fertilization on factors that affect loblolly pine productivity. Leaf-level physiology, and growth were monitored over the third and fourth year of treatment. After four years of throughfall reduction treatment, no interactive treatment effects on light-saturated net photosynthesis (P_{net}), stomatal conductance (g_s), or growth were observed. Neither fertilization nor throughfall reduction treatment effected growth efficiency, midday leaf water potential (Ψ_L), $\Delta\Psi_L$, or leaf-specific hydraulic conductance. Fertilization, regardless of throughfall reduction treatment, increased all growth variables except for height, increased radiation use efficiency of stemwood production (ε_{stem}), increased intercepted photosynthetically active radiation, and increased leaf area index. However, fertilization decreased P_{net} and g_s likely due to increased leaf evaporative demand associated with higher LAI. Throughfall reduction decreased LAI and, in 2014, throughfall reduction reduced the leaf area to sapwood area ratio (A_L:A_S) thereby increasing water supply per unit foliage resulting in no effect of throughfall reduction on leaf-level gas exchange. Conversely, both treatments decreased $\Delta^{13}C$ discrimination indicating higher water use efficiency (WUE) and reduced g_s in response to throughfall reduction treatment not detected by instantaneous leaf-level gas exchange measurements. The results of this study indicate that while fertilization can increase growth regardless of throughfall reduction, higher LAI with fertilization may increase water use and increase short-term leaf-level drought susceptibility. In a more drought prone future climate, management strategies that utilize fertilization to increase growth may result in diminished fertilization responses due to the impact of fertilization on leaf-level drought susceptibility. In order to maintain future productivity, management strategies such as density management may be required to reduce fertilization's impact on water use.

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List of Abbreviations

$\Delta^{13}C$	Carbon isotope ^{13}C discrimination
$\Delta\Psi_L$	Difference between predawn and midday water potential
$\delta^{13}C$	Relative abundance of ^{13}C to ^{12}C
δ_a	Relative abundance of ^{13}C in the atmosphere
δ_L	Relative abundance of ^{13}C in the leaf tissue
ε_{stem}	Radiation use efficiency of stemwood production
Ψ_L	Leaf water potential
A_L:A_s	Leaf area to sapwood area ratio
APAR	Absorbed photosynthetically active radiation
AR (1)	Autoregressive covariance structure
A_s	Sapwood area
ANOVA	Analysis of variance
B	Boron
C	Carbon
Cu	Copper
DAP	Diammonium phosphate
DBH	Diameter at breast height
DIB	Diameter of the inner bark
DOB	Diameter of the outer bark

E_g	Transpiration on a ground area basis
E_L	Transpiration on a leaf area basis
GE	Growth efficiency
G_s	Canopy stomatal conductance
g_s	Leaf-level stomatal conductance
IPAR	Intercepted photosynthetically active radiation
IPCC	Intergovernmental panel on climate change
K	Potassium
K_{leaf}	Leaf-specific hydraulic conductance
LAI	Leaf area index
Mn	Manganese
N	Nitrogen
P	Phosphorous
PAR	Photosynthetically active radiation
PDSI	Palmer drought severity index
PINEMAP	Pine integrated network: education, mitigation, and adaptation project
P_{net}	Leaf-level net photosynthesis
S	Sulfur
SAS	Statistical analysis system
SLA	Specific leaf area
U.S.	United States

VWC	Volumetric water content
WUE	Water use efficiency
Zn	Zinc

1.0 Project Background

Climate change, as defined by the Intergovernmental Panel on Climate Change (IPCC), is a change in the state of the climate that can be identified by changes in its mean or in the variability of its properties, and that persists for an extended period of time (Pachauri and Reisinger 2007). Climate projections indicate that the southeastern United States (U.S.) is entering a period where annual temperatures are likely to be higher, precipitation events will become more intense and infrequent, and summer time droughts will become more commonplace (Christensen et al. 2007; Kunkle et al. 2012). Average annual temperatures over the past 30 years have increased 1-2 °C (Kunkle et al. 2012) and this trend is projected to continue over the next century with average annual increases of 2-6 °C (Christensen et al. 2007; Kunkle et al. 2012). Increased annual temperatures will likely lead to a longer growing season and increased potential evapotranspiration likely resulting in higher water use by plants and a greater risk of soil water shortage (Keenan 2015). Total annual precipitation is projected to decrease by approximately 10%, however some climate change scenarios indicate that this estimate could be as high as 30% (Christensen et al. 2007). Conversely, the frequency of heavy precipitation events, defined as a rain event where 25.4 mm or more of rain falls in a single day, is likely to increase due to the ability of a warmer atmosphere to retain more moisture (Kunkel et al. 2012). Precipitation that falls at a rapid rate is mostly lost as run off and will not penetrate the soil, leading to a net decrease in soil moisture (Kunkel et al. 2012). Increased evapotranspiration resulting from a warmer climate coupled with decreased soil moisture will lead to an increase in drought severity. Increased severity and commonality of drought in the Southeast may negatively impact the productivity and carbon sequestration potential of southeastern forests (Noormets et al. 2010; Wear and Greis 2012).

Rising atmospheric temperatures have been closely linked to the increasing concentrations of CO_2 and greenhouse gasses in the atmosphere over the past few centuries (Stocker et al. 2013). The impacts that climate change may have on forest productivity have highlighted the need to develop mitigation strategies to reduce CO_2 in the atmosphere. In the Southeast, high density planting of economically valuable fast growing species has been proposed as a strategy which could be used to aid in climate change mitigation by sequestering carbon while simultaneously providing an economic incentive (Schwenke et al. 2012; Susaeta et al. 2014). Southeastern forests provide 60% of total U.S. wood products (Conner et al. 2002) and the U.S. provides 17% of the total world production of roundwood timber (Prestemon et al. 2015). The wood products industry employs 10% of the total manufacturing labor in the Southeast and accounts for 8% of the total manufacturing income (Weir and Greis 2012). In the southeastern U.S. forest land makes up 60% of total land area with 48% of total forest land occupied by planted or naturally regenerated pine trees (*Pinus* spp.) (Conner et al. 2002; Wear and Greis 2002). The forests of the contiguous U.S. store approximately 12 Pg of carbon (Turner et al. 1995). Southeastern forests sequester approximately 130 Tg of carbon per year in standing live biomass and forest soils, compensating for 23% of the total annual U.S. CO_2 emissions (Han et al. 2007). In the southeastern U.S., loblolly pine (*Pinus taeda* L.) represents the most widely planted plantation species. Nutrient management, increasing initial stocking density, and planting improved genetic stock has increased the productivity of pine plantations over the past 60 years (Fox et al. 2007). Emerging research suggests that management strategies with the objective of increasing productivity might not be compatible with the objective of increasing carbon sequestration (Schweke et al. 2012; O’Hara et al. 2013; Susaeta et al. 2014). High density stands sequester higher total amounts of carbon, but at a slower rate than less dense stands (Susaeta et al. 2014). Some studies have argued that maximizing merchantable productivity through intensive management may reduce the soil carbon pool, and that highly managed forests can contain 50% less carbon stock than natural forests (Noormets et al. 2015). Understanding how the interaction between management and climate affects forest productivity is critical for understanding the productivity and mitigation potential of pine plantations and southeastern forests in a future climate.

Due to the importance of loblolly pine as an economic species and in order to investigate the effects of climate variability on loblolly pine productivity, the United States Department of Agriculture's National Institute of Food and Agriculture (NIFA) funded the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP). PINEMAP was funded through an Agricultural and Food Research Initiative (AFRI) with the purpose of investigating the effects of fertilization and reduced precipitation on loblolly pine in the Southeast. PINEMAP is comprised of 11 southeastern land grant universities, including Auburn University, as well as 8 regional university-corporate-governmental research cooperatives. PINEMAP's overall goal is to create, synthesize, and disseminate the knowledge necessary to enable southern pine landowners to manage forests to increase carbon sequestration, increase the efficiency of fertilizer inputs, and to adapt forest management practices and plant improved tree varieties to increase forest resilience in a future more variable climate. The overall goal of the research described in this thesis was to explore the interactive effects of reduced precipitation, achieved by artificial throughfall reduction, and fertilization, on physiological characteristics that affect the productivity of loblolly pine.

2.0 The Effects of Fertilization and Four Years of Throughfall Reduction on Leaf Physiology of Loblolly Pine (*Pinus Taeda* L.)

2.1 Introduction

Southeastern forests provide approximately 17% of the world's supply of roundwood timber, more than any other single country (Prestemon et al. 2015), and support an industry that provides 2% of the Southeast's gross regional product (Brandeis and Hadges 2015). The southeastern forest industry is supported by approximately 16 million ha of pine plantations (Wear and Gries 2012; Abt and Abt 2013). Loblolly pine is the most widely planted pine plantation species, representing over 50% of standing pine volume (Fox et al. 2007; Wear and Gries 2012). Maximizing the productivity of southeastern pine plantations has been the focus of over 60 years of research that has documented that the productivity of southeastern pine plantations can be improved by the planting of improved genetic families, and the management of limited resources such as nutrient availability (Albaugh et al. 2004; Fox et al. 2007; Wear and Gries 2012). The future productivity of southeastern pine plantations and the continued efficiency of nutrient management practices are uncertain due to projected future variability in the Southeast's climate (Noormets et al. 2010; Wear and Gries 2012). Climate projections indicate that the southeastern U.S. will likely experience higher annual temperatures and more frequent and intense summer time droughts (Christensen et al. 2007; Kunkle et al. 2012) which could negatively impact forest productivity over the next century (Noormets et al. 2010; Wear and Gries 2012). To ensure the future productivity of southeastern forests, land managers will need to consider how current management strategies for increasing productivity will interact with a more variable future climate (Kennan 2015).

Site water availability is influenced by evapotranspiration, the soil's ability to retain water, and the amount of precipitation (Landsberg and Gower 1997). Dendrochronology studies have shown that the timing and frequency of precipitation is more closely related to tree growth than the total amount annual of precipitation (Johnsen et al. 2014). A lack of water can lead to reductions in aboveground growth by shifting biomass production from above to belowground growth (Waring 1983; Albaugh et al. 1998), decreasing foliage retention or its size (Poorter and Garnier 1999), or reducing leaf-level gas exchange (Seiler and Johnson 1985; Teskey et al. 1987). Shifting production away from aboveground growth and towards increased root development can allow trees to gain access to previously unavailable water (Bongarten and Teskey 1987), but this shift in allocation patterns can lower growth efficiency (Albaugh et al. 1998). Growth efficiency (GE), defined as incremental stemwood biomass production per unit of leaf area, represents an integrated measure of resource allocation, photosynthetic efficiency of the canopy, and respiration cost of respiring tissue. A similar metric, radiation use efficiency of stemwood production (ε_{stem}), is defined as incremental stemwood biomass production per unit of absorbed photosynthetically active radiation (APAR). Both GE and ε_{stem} represent the efficiency of biomass production, and both can be altered by nutrient and water availability (Waring 1983; Albaugh et al. 1998; Allen et al. 2005; Campoe et al. 2013). Because stomata regulate the flux of both CO_2 and water vapor, reductions in soil moisture can reduce stomatal conductance (g_s) and net photosynthesis (P_{net}), which can result in reduced productivity during extreme or prolonged drought (Teskey et al. 1987; Salisbury and Ross 1992). Severe drought can continue to negatively affect growth several years after the drought event ends because drought can reduce foliage retention, production, and canopy-level photosynthesis, which causes recovery to be delayed. For example, Galiano et al. (2011) determined that reduced radial growth in *Pinus sylvestris* (Mill) four years after an extreme drought was due to foliar abscission, reduced levels of canopy-level photosynthesis, and depletion of carbon reserves which together constituted a negative feedback effect and hindered drought recovery. To lessen the demand on carbon reserves, trees may increase their water use efficiency in order to sustain positive growth (Zhang et al. 1996). Water use efficiency (WUE) can be defined as the ratio of carbon assimilated to the rate of water lost through transpiration. When exposed to water stress, stomata will close to reduce water loss which also reduces the internal concentration of CO_2 within the leaf (C_i). Rubisco, the enzyme that catalyzes the carboxylation of RuBP with CO_2, discriminates against the heavier ^{13}C isotope in favor of the lighter more abundant ^{12}C isotope. Discrimination against ^{13}C ($\Delta^{13}C$) decreases when C_i is low as when stomata are closed. Higher ratios of photosynthesis to transpiration (WUE) are correlated with lower $\Delta^{13}C$. Because $\Delta^{13}C$ is related to stomatal conductance through C_i, $\Delta^{13}C$ can provide a measurement of how WUE is affected by water stress over time. Zhang et al. (1996) found that water stress decreased $\Delta^{13}C$ in *Pseudotsuga menziesii* (Mirb) and *Larix occidentalis* (Nutt) and that higher WUE was positively correlated with growth during water stressed conditions.

Nutrient limitation in loblolly pine plantations across the southeastern U.S. has been well documented as a constraint on productivity (Albaugh 1998; 2004; Jokela 2004). In 2004, approximately 485,000 ha of southeastern pine plantations were fertilized (Fox et al. 2007) and from 2000 to 2004 approximately 2.45×10^8 kg year^{-1} of fertilizer was applied across southeastern forests (Albaugh et al. 2007). Fertilization increases aboveground growth by either increasing leaf area production, increasing leaf-level P_{net}, or shifting biomass production from root development to aboveground growth (Vose and Allen 1988; McGrady and Jokela 1998; Allen et al. 2005). Higher GE and ε_{stem} with fertilization typically occurs through increased P_{net}, or by decreased biomass partitioning to roots if water is otherwise not limiting (Fox et al. 2007; Jokela et al. 2004). Higher leaf area from fertilization may also cause self-shading, which can lead to decreased production efficiency due to decreased efficiency of light capture and photosynthesis at canopy closure (Waring 1983). It has been hypothesized that enhancement of P_{net}, from fertilization is due to higher carboxylation efficiency (i.e. the efficiency at which Rubisco can bind CO_2 and RuBP) and higher total amounts of rubisco as a result of increased foliar nitrogen concentrations (Gough et al. 2004b). This has been seen in *Pinus sylvestris* (Mill) where four years of fertilization caused higher concentrations of nitrogen per unit leaf area, higher amounts of Rubisco and chlorophyll, and increased carboxylation efficiency (Warren et al. 2003). However, the physiological processes resulting in enhanced photosynthesis following fertilization are inconsistent because the response of photosynthesis to fertilization is not always positive. For example, inconsistent responses of P_{net} to fertilization were found in seedlings (King et al. 2008) as well as in 14-year-old loblolly pine (Gough et al. 2004a), where positive, insignificant, and even negatives responses of P_{net} to fertilization were reported. Negative responses of P_{net} to fertilization have been attributed to reduced g_s as a result of enhanced leaf area production resulting in increased leaf evaporative water loss (Munger et al. 2003; Gough et al. 2004a). Because fertilization can affect P_{net} and g_s, fertilization can impact WUE. However, because increased WUE can be caused by either increased carboxylation efficiency or decreased g_s, increased WUE with fertilization must be carefully interpreted. Ripullone et al. (2004) determined that increased WUE in *Pseudotsuga menziesii* (Mirb) and poplar (*Populus* spp.)

seedlings with fertilization was due to increased photosynthetic rates whereas Choi et al. (2005) suggested that improved WUE in 8-year-old loblolly pine was the result of decreased g_s.

Multiple studies have investigated the impacts of increased nutrient and decreased water availability on tree physiological function and growth and have found conflicting results. Some studies have indicated that the interaction between increased nutrient and decreased water availability may increase the severity of drought effects on tree physiology and growth, while others have found that this interaction may be negligible. In a more drought prone climate fertilization may increase a tree's vulnerability to drought and the positive gains that would otherwise be realized with fertilization may be reduced or negated. One possible mechanism resulting in higher drought sensitivity with fertilization is the alteration of hydraulic architecture through increased leaf area. For example, Ewers et al. (2000) suggested that a higher leaf area to sapwood area ratio (A_L:A_S) and decreased leaf-specific hydraulic conductance (K_{leaf}) in response to fertilization could decrease g_s by reducing the water supply to foliage. In addition, increased growth due to higher leaf area may result in wider xylem elements with higher specific conductivity and while wider xylem elements are capable of transporting higher quantities of water, they can be more vulnerable to cavitation and more sensitive to decreasing water availability (Sperry 2011; Hacke et al. 2010). Some studies have found no interactive effect of fertilization and reduced water availability on xylem characteristics and growth, or leaf-level gas exchange. For example, there was no interactive effect of fertilization and throughfall reduction treatment on annual height, ring width, or tracheid number or development in the stem in *Picea abies* (L. Karst) (Jyske et al. 2009). No interactive effects of fertilization and throughfall reduction on leaf-level gas exchange, leaf area, or growth were observed with a 30% reduction in throughfall in loblolly pine (Samuelson et al. 2014). In a similar study with 100% throughfall exclusion, Tang et al. (2004) found no interactive effects of fertilization and throughfall exclusion on leaf-level gas exchange, however at the canopy level, fertilization increased whole-canopy photosynthesis, LAI, and annual foliage mass in the ambient throughfall treatment while fertilization had no influence on canopy-level physiology under 100% throughfall exclusion.

Previous work by Samuelson et al. (2014) investigated the interactive effects of reduced precipitation achieved by throughfall reduction and increased nutrient availability through one-time fertilization on the productivity of loblolly pine. Over the first two years of treatment, Samuelson et al. (2014) found no interactive treatment effects on leaf-level physiology or growth, but throughfall reduction reduced leaf-level gas exchange, predawn Ψ_L, needle elongation, basal area increment, and stemwood production in a year with less than normal precipitation (2012) and predawn Ψ_L in a year with higher than normal precipitation (2013). However, a companion study by Bartkowiak et al. (2015) found that at the canopy-level, transpiration and whole tree hydraulic conductance were reduced by throughfall reduction only in the fertilized treatment which was attributed to higher A_L:A_S with fertilization that likely reduced the water supply to foliage. The work presented in this thesis builds upon the first two years of the study by exploring the cumulative impact of four years of throughfall reduction and its interaction with higher leaf area associated with fertilization on leaf-level physiology and growth and further investigating the underlying mechanisms important to understanding the interaction between climate and growth. This study contributes to the region wide interdisciplinary project PINEMAP (Pine Integrated Network: Education, Mitigation, and Adaptation Project). The goal of PINEMAP is to enable southern pine landowners to better manage forests to increase carbon sequestration, fertilizer use efficiency, and forest resilience and sustainability under a variable climate. The specific objective of this study was to investigate the long-term main and interactive effects of reduced throughfall (approximate 30% reduction and ambient) and fertilization (one-time application and none) on factors that are related to forest productivity such as: LAI, intercepted radiation, GE and ε_{stem}, as well as leaf-level physiology including gas exchange, water potential (Ψ_L) and leaf-specific hydraulic conductance (K_{leaf}). After four years of throughfall reduction treatment, it was expected that throughfall reduction would reduce g_s, P_{net}, Ψ_L, and K_{leaf}, and decrease $\Delta^{13}C$ and that these effects of reduced water availability on leaf-level physiology would be exacerbated with fertilization treatment due to higher leaf area with fertilization increasing evaporative demand and increasing the A_L:A_S ratio. As a result of decreased P_{net} it was expected that throughfall reduction would reduce GE and ε_{stem} and that reductions in GE and ε_{stem} would be greater with fertilization.

2.2 Methods

2.2.1 Study site and experimental design

This study site was one of four long-term throughfall reduction and fertilization factorial experiments positioned across the native range of loblolly pine (Will et al. 2015). The research presented in this thesis was conducted on the experiment located in Taliaferro County, Georgia (33°37′35″N 82°47′54″W) approximately 13 km southwest of Washington, Georgia. The experiment was implemented on a 44.5 ha loblolly pine plantation owned by Plum Creek Timber Company Inc. The 30-year average annual precipitation for Washington, Georgia is 1109 mm, and the 30-year average daily maximum and minimum temperatures are 22.7 ºC and 10.1 ºC, respectively, with a 30-year annual average temperature of 13.6 ºC. (NOAA National Centers for Environmental Information, Asheville, North Carolina Annual Summaries, Station ID COOP:099157 http://www.ncdc.noaa.gov accessed February 2015). Meteorological data during the study period were collected using an onsite cellular networked weather station (CWB100 Wireless Base Station, Campbell Scientific, Logan, Utah), previously described by Bartkowiak et al. (2015), located in an open clearing in the middle of the stand. The Palmer Drought Severity Index (PDSI) was collected for Georgia Climate Division 3 (http://www1.ncdc.noaa.gov/pub/data/cirs/climdiv/, accessed September 2015). The study site is located near the border between climate division 5 and 3. Although Taliaferro County is located in climate division 5, PDSI values for climate division 3 were chosen because these values better represented on site precipitation patterns. Palmer Drought Severity Index values between 0.49 and -0.49 are defined as “near normal,” negative values below this threshold indicate increasing drought conditions, and positive values above this threshold indicate wetter than normal conditions (Palmer 1965). Palmer Drought Severity Index values from -0.50 to -0.99 are defined as incipient drought, values from -1.00 to -1.99 are defined as mild drought, values from -2.00 to -2.99 are defined as moderate drought, values from -3.00 to -3.99 are defined as severe drought, and values below -4.00 are defined as extreme drought (Palmer 1965).

The predominant soil series at the site was of the Lloyd series, a fine, kaolinitic, thermic Rhodic Kanhapludult. A second soil series, which was isolated to the eastern edge of the site and occupied approximately 5.0% of total site area, was of the Cecil series, a fine, kaolinitic, thermic Typic Kanhapludult (https://websoilsurvey.sc.egov.usda.gov/). Both soils are characterized as well-drained, of moderate permeability, and with medium to rapid runoff (https://soilseries.sc.egov.usda.gov/).

Prior to plantation establishment, the site was a loblolly pine stand with intermixed naturally occurring hardwood species. This stand was clear cut in July 2004 then plowed and bedded with a combination plow in August 2005. In February 2006, the site was hand-planted with one-year-old bare root loblolly pine seedlings on a 3 m by 2 m spacing resulting in an average density of 1544 trees per ha^{-1} at planting. All seedlings were open pollinated, genetically improved second generation seedlings from an orchard in Jessup, Georgia. In 2014, the stand was 9 years of age. Sight index (SI) was determined to be 23.1 m (base age 25 years) (Will et al. 2015).

The experimental design was a randomized complete block 2x2 factorial combination of throughfall reduction and fertilization treatments replicated in four blocks. Each 0.10 ha (34.1 m x 28.0 m) treatment plot contained a 0.03 ha (21.3 m x 14.0 m) measurement plot with approximately 40 trees. A 6.1 m untreated buffer separated adjacent treatment plots.

Fertilization treatments consisted of a no fertilization control and a one-time fertilizer application (224 kg N ha^{-1}, 28 kg P ha^{-1}, 56 kg K ha^{-1}). Fertilizer was broadcast by hand as a granular mix at the base of each tree in March 2012. Nitrogen and P were applied as a mix of 432 kg ha^{-1} urea and 140 kg ha^{-1} diammonium phosphate (DAP), while K was applied as potassium chloride at a rate of 112 kg ha^{-1}. A micronutrient fertilizer was applied as a granular oxysulfate blend (Cameron Chemicals, Southeast Mix, Portsmouth, Virginia) at a rate of 22.4 kg ha^{-1} equating to 1.34 kg S ha^{-1}, 1.12 kg B ha^{-1}, 0.45 kg Cu ha^{-1}, 1.34 kg Mn ha^{-1}, and 1.12 kg Zn ha^{-1}.

Throughfall reduction treatments consisted of an ambient throughfall control and an approximate 30% throughfall reduction, which represents the driest projections for the southeastern U.S. (Christensen et al. 2007). The approximate 30% reduction in throughfall was achieved by constructing throughfall reduction trays which covered approximately 30% of the total plot area and diverted incoming throughfall into the buffer zone. The supporting structures for the trays averaged 1.3 m in height, depending on the topography of each plot, and were sloped to facilitate drainage off the plot. Each structure was 1.5 m wide and held two throughfall reduction trays separated by a 30.5 cm gap. Trays were constructed out of a 12 mil (0.3 mm) laminate with two layers of U.V. stabilized coextruded polyethylene and a high strength cord grid (Poly Scrim 12, Americover Inc., Escondido, California). The amount of throughfall intercepted by the reduction trays was not measured, so the true amount of throughfall reduction is not known. In previous years (2012 and 2013), throughfall reduction had not significantly affected soil moisture in top 0-12 cm at this site, but reduction in predawn water potential under throughfall reduction treatment indicated that soil water availability was affected (Samuelson et al. 2014). In addition, soil moisture in the 0-12 cm of soil was monitored in the three companion long-term throughfall reduction studies and throughfall reduction treatment significantly decreased soil moisture (Will et al. 2015).

2.2.2 Growth

Year-end plot level inventories included measurements of diameter at breast height (DBH) (1.4 m), and height of each tree in the measurement plots. Total outside-bark stem volume per tree was calculated as described by Van Deusen et al. (1981). Sapwood area (A_s) for each tree was calculated using inside bark diameter (DIB). Diameter of inside bark was calculated from:

$$\mathrm{DIB} = 0.842 * \mathrm{DOB} + 0.057 \tag{1}$$

where DIB and DOB are the diameters of the inner and outer bark respectively (Bartkowiak et al. 2015). The leaf area to sapwood area ratio (A_L:A_S) was calculated as the ratio of peak leaf area index (LAI) to total plot sapwood area calculated from the year-end inventory. Stemwood biomass was calculated using the allometric equation by Zhao et al. (2015). Data published previously by Samuelson et al. (2014) were used to calculate incremental growth from 2013 to 2014. Growth efficiency was calculated by dividing annual stemwood biomass production by LAI averaged over February to December 2014 (Allen et al. 2005; Samuelson et al. 2008).

2.2.3 Leaf area index

In 2014, LAI was measured approximately monthly (Table 1) using a pair of LAI2000 Plant Canopy analyzers (Li-COR Inc., Lincoln, Nebraska). In 2015, newer instruments were used (LAI2200C Plant Canopy Analyzers Li-COR Inc., Lincoln, Nebraska). In October and November 2014 (Table 1), the two systems were compared by holding each sensor adjacent to one another and measuring LAI simultaneously. The results from the two systems are shown in

Figure 1. Both systems determined similar LAI values but a correction equation is given for the reader to adjust the 2015 LAI data to the 2014 system (Figure 1).

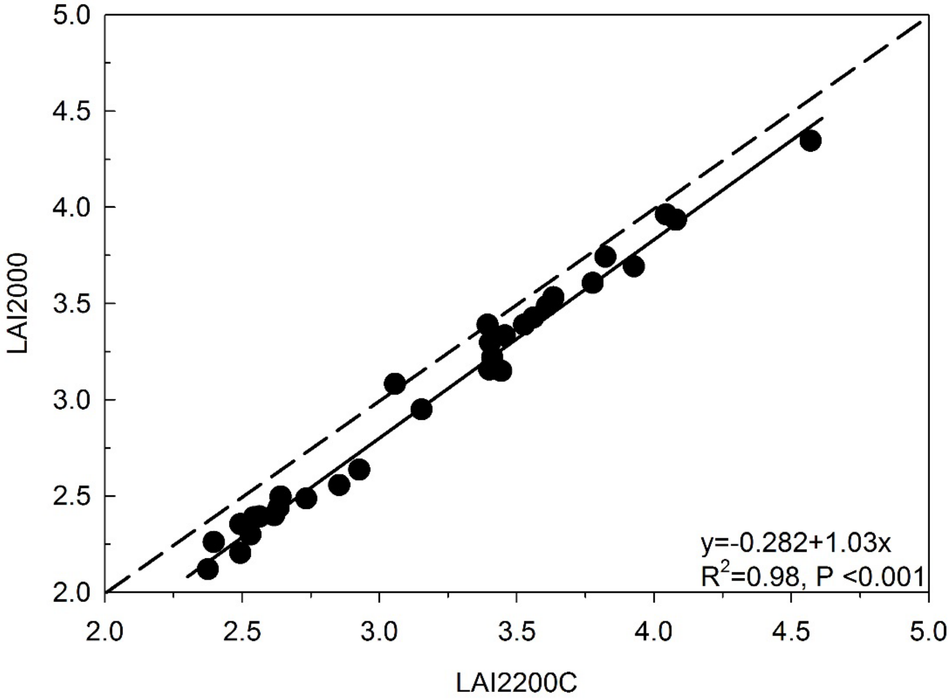

Figure 1. Relationship between measurements of leaf area index (LAI) using the LAI2000 Plant Canopy Analyzer (Li-COR Inc., Lincoln, Nebraska) and the LAI2200C Plant Canopy Analyzer (Li-COR Inc., Lincoln, Nebraska) measured over two months in a 9-year-old loblolly pine plantation. Each point represents a plot average of three transects. The dashed line represents the 1:1 ratio.

To measure LAI, each plant canopy analyzer wand was equipped with a 90º view cap and one sensor was positioned in a clearing outside the stand in order to measure ambient light conditions, while the other sensor was positioned below the canopy. All measurements were made in diffuse light conditions either at dawn starting at apparent sunrise and completing measurements within an hour, or at dusk starting an hour before apparent sunset and completing measurements before actual sunset. All measurements were performed by block and two blocks were typically completed per measurement session. Three trees, one from each inner row, were randomly selected as the starting point of a 4 m diagonal transect across the row. For each measurement, the wand was directed parallel with the row. One measurement was made every 0.5 m along each of these three transects at a height above the throughfall reduction trays. Data from the eight measurements along each transect were then averaged together by plot. Post measurement operations were performed using the FV2200 software (Li-COR Inc., Lincoln, Nebraska) to remove the lowest 5th (68º) light detecting ring to insure that the wand sensor only measured light attenuated by the canopy within the treatment plots and did not include the canopy of trees located outside the treatment plots.

In pine species, optical measurements of LAI may under or overestimate LAI depending on the clumping of foliage and the ratio of branch area to needle area (Deblonde et al. 1994; Sampson and Allen 1995; Pokorny and Marek 2000). In order to provide an additional estimate of LAI, LAI was calculated from needle fall collection. Twelve round 0.5 m^2 litter traps were placed randomly within each plot between trees and between rows, including between and above throughfall reduction trays. Traps were emptied approximately monthly and combined on a plot basis. Non-foliage debris was removed at the time of collection. Litter was dried at 70 °C for a minimum of 72 hours and weighed. Litter collected from March 2013 to February 2014 was assumed to represent the 2012 needle cohort and litter collected from March 2014 to February 2015 was assumed to represent the 2013 needle cohort. Peak all-sided leaf area for 2013 was calculated by multiplying litter weight for each cohort by its respective average specific leaf area (see 2.2.5.2 leaf-level gas exchange) and adding the two together. All-sided leaf area was converted to projected leaf area using a conversion of 3.14 (Grace 1987). Projected leaf area was then corrected for mass lost during decomposition and senescence using a correction factor of 1.13 (Dalla-Tea 1990). Projected leaf area was then expressed on a per area basis for each plot.

2.2.4 Intercepted photosynthetically active radiation

Intercepted photosynthetically active radiation (IPAR) was measured only during the growing season (May to August 2014, and May to August 2015; Table 1) at times when the zenith angle of the sun was between 15º and 30º on cloud-free or mostly-sunny days using a pair of line quantum sensors (LI191, Li-COR Inc., Lincoln, Nebraska). The zenith angle range was limited to 15° to 30° because the path length of solar radiation through the canopy increases as the zenith angle of the sun increases (Will et al. 2005). By performing measurements within a defined range, light attenuation through the canopy was consistent over measurement dates and the confounding influence of sun angle was minimized (Will et al. 2005).

To measure IPAR, one sensor was placed in a clearing adjacent to the site in full ambient sunlight while the second sensor was positioned below the canopy. Three permanent transects were positioned along the three inner rows of each measurement plot with each transect containing ten randomly selected permanent sample points. At each sample point, three non-overlapping measurements were made across the inter-row space. Throughout the study, due to natural deterioration of point markers some sample points were lost. These markers were replaced when possible and did not lead to more than two points being lost per transect or to any apparent increase in the variability in the data. The three measurements per sample point were averaged together for a total of approximately 30 measurements per plot.

Percent IPAR was calculated as the ratio between below canopy transmitted radiation and above canopy ambient radiation. In order to satisfy the linear modeling assumption of constant variance, and to reduce heteroscedasticity in the residuals, all statistical analyses of IPAR were performed on logit transformed data ($Y=\log(IPAR/(1-IPAR))$) (Warton and Hui 2011).

Total absorbed photosynthetically active radiation (APAR) was calculated as the total amount of photosynthetically active radiation (PAR) intercepted from January 1st to September 30th each year. On-site PAR was measured continuously by a quantum sensor (PQS 1 PAR Quantum Sensor, Kipp & Zonen USA Inc., Bohemia, New York), and values were averaged and recorded every 30 minutes. It was assumed IPAR remained constant from January 1st to the first IPAR measurement in May each year (Table 1). A linear spline function (Proc Expand, SAS Inc., Cary, North Carolina) was used to interpolate IPAR for each day between measurements. It was assumed that IPAR did not change significantly from the last measurement in late August (Table 1) to September 30th each year. To calculate total APAR, average 30 minute on-site PAR was summed for each day and multiplied by the respective daily interpolated IPAR and then summed by plot from January 1st through September 30th each year. Radiation use efficiency of stemwood production (ε_{stem}) in 2014 was calculated by dividing annual stemwood biomass production by APAR (Allen et al. 2005).

Table 1. Date of measurements of leaf area index (LAI), intercepted photosynthetically active radiation (IPAR), light-saturated leaf-level photosynthesis (P_{net}), stomatal conductance (g_s), leaf-level water potential (Ψ_L), and soil moisture in 2014 and 2015 in a loblolly pine plantation.

Variable	Date of Measurement (2014)	Date of Measurement (2015)
LAI	Feb 8th and 9th	Feb 6th and 7th
	Mar 11th and 12th	Mar 26th
	Apr 18th and 20th	May 4th and 5th
	May 28th, 29th, and 30th	Jun 9th and 10th
	Jun 18th and 19th	Jul 13th, 14th, and 15th
	Jul 8th and 9th	Aug 25th and 26th
	Aug 22nd and 23rd	Sep 14th and 15th
	Sep 26th	
	Oct 24th and 25th	
	Dec 9th and 8th	
IPAR	May 6th and 7th	May 7th
	Jun 16th and 19th	Jun 12th
	Jul 9th and 10th	Jul 16th and 17th
	Aug 24th and 25th	Aug 25th and 26th
P_{net}, g_s, Ψ_L, soil moisture	Mar 10th and 11th	Mar 24th and 25th
	Apr 20th and 21st	May 5th and 6th
	May 27th and 28th	Jun 10th and 11th
	Jun 14th and 18th	Jul 14th and 15th
	Jul 7th and 8th	
	Aug 22nd and 23rd	
	Oct 24th and 25th	

2.2.5 Leaf physiology

2.2.5.1 Foliar $\delta^{13}C$ and nutrients

The relative foliar abundance of ^{13}C to ^{12}C ($\delta^{13}C$) was measured on foliage collected in September 2014 on the two current-year flushes. Three trees per plot were randomly selected and shoots from the upper third of the canopy were removed with a pole pruner. Foliage was separated by flush and dried at 70°C for a minimum of 72 hours. Needles were then ground to a fine homogenous powder and samples were analyzed using a continuous flow mass spectrometer at the Duke Environmental Stable Isotope Laboratory (biology.duke.edu/jackson/devil/). Foliar $\delta^{13}C$ was used to calculate foliar ^{13}C isotope discrimination ($\Delta^{13}C$) according to Farquhar et al. (1982):

$$\Delta^{13}\mathrm{C} = \frac{(\delta_a - \delta_L)}{(1 + \delta_L)} \qquad (2)$$

where δ_a is $\delta^{13}C$ of atmospheric CO_2 and δ_L is $\delta^{13}C$ of the leaf tissue. Monthly average atmospheric $\delta^{13}C$ values were collected from the Scripps CO_2 Program (http://scrippsco2.ucsd.edu, accessed June 2015) from the Earth System Research Laboratory Mauna Loa Observatory and average δ_a from the time of bud break to the time of collection was estimated as the average atmospheric $\delta^{13}C$ concentration from March to September 2014.

Foliar nitrogen (N), phosphorous (P), and potassium (K) concentrations were measured on the same foliage collected for foliar $\delta^{13}C$ in September 2014. Samples were prepared in the same manner as $\delta^{13}C$ samples, by drying and grinding the needles to a fine powder. Samples were analyzed at the Auburn University Soil Testing Laboratory (http://www.aces.edu/anr/soillab/) using dry combustion to measure foliar N and inductively coupled argon plasma (ICAP) spectroscopy to measure foliar P and K.

2.2.5.2 Leaf-level gas exchange

In 2014, leaf-level gas exchange measurements were conducted approximately monthly from March to October (Table 1) between 0930 and 1400 hours. In 2015, in order to achieve a more in depth investigation of treatment effects, diurnal measurements of leaf-level gas exchange and leaf water relations were made from March to July. Diurnal measurements were made over three periods in a day: from 0900 to 1100 hours, 1200 to 1400 hours, and 1500 to 1700 hours. All measurements were completed by block over a two day period. The measurement order of blocks was randomly selected. Shoots consisting of sun foliage were detached from the upper third of three randomly selected trees for the 2014 measurements and two trees for the 2015 diurnal measurements in each plot using a pole pruner. Within one minute of shoot detachment (Samuelson et al. 2001) light-saturated (PAR = 1800 µmols m^{-2} s^{-1}) net photosynthesis (P_{net}) and stomatal conductance (g_s) were measured using a LI6400 portable photosynthesis system (Li-COR Inc., Lincoln, Nebraska) on two fascicles of needles removed from the shoot. Measurements were made using a 6 cm^2 (2 cm x 3 cm) leaf chamber equipped with a red/blue LED light source to provide actinic as well as blue light. The ambient CO_2 concentration within the leaf chamber was maintained at 400 ppm and temperature and relative humidity were allowed to fluctuate with ambient conditions. The first foliage flush produced in 2013 was used for gas exchange measurements made March through June 2014 until the first flush of 2014 was fully developed. Once developed, the first flush of 2014 was used for measurements made July 2014 through May 2015. Measurements made June and July 2015 were performed on the first flush of 2015. In order to express gas exchange measurements per unit of photosynthetic surface area present in the leaf chamber at the time of measurement, total all-sided area of both fascicles of needles was calculated following Samuelson et al. (1992).

Following measurements of all-sided leaf area, fascicles were placed in an oven at 70 °C for at least 72 hours. Specific leaf area was calculated as the ratio of green needle area to needle dry weight.

2.2.5.3 Leaf water potential

Predawn leaf water potential (Ψ_L) was measured using a Scholander style pressure chamber (Model 1505, PMS Instrument Company, Albany, Oregon). Measurements of Ψ_L were made on needles removed from shoots cut between 0400 and 0600 hours on the same days (Table 1) and from the same trees selected for gas exchange measurements. In 2014, midday Ψ_L was measured at the same time as each plot's leaf-level gas exchange measurement between 0930 and 1400 hours. In 2015, midday Ψ_L was assumed to be represented by diurnal Ψ_L measurements conducted between 1200 and 1400 hours. For all Ψ_L measurements, detached shoots were placed in a plastic bag with a damp paper towel and stored in a cooler until the measurement could be performed. The difference in predawn and midday water potential ($\Delta\Psi_L$) was calculated for each tree.

2.2.5.4 Leaf-specific hydraulic conductance

Leaf-specific hydraulic conductance (K_{leaf}) was calculated in 2015 using leaf-level transpiration (E_L) measured at midday (1200-1400 hours) and the $\Delta\Psi_L$ for each tree based on Meinzer et al. (1995) where:

$$\mathrm{K_{leaf}} = \mathrm{E_L}/\Delta\Psi_\mathrm{L} \tag{3}$$

Leaf-specific hydraulic conductance is most often derived from sap flow and whole tree transpiration, however, leaf-level gas exchange measurements and canopy level measurements have been shown to be at a near 1:1 ratio in some cases (Ewers et al. 2007; Domec et al. 2009).

2.2.6 Soil moisture

Volumetric soil water content (VWC) was measured concurrently with gas exchange measurements in 2014 (0930 to 1400 hours) and at midday (1200 to 1400 hours) in 2015 (Table 1) using a portable soil moisture meter utilizing the time-domain reflectometry measurement technique (HS2 Hydrosense II, Campbell Scientific, Logan, Utah). Measurements of VWC to a depth of 12 cm were made near each tree selected for gas exchange measurements. Two measurements of VWC were made per tree. One measurement was made equidistant between the measurement tree and a randomly selected adjacent tree, and a second measurement was made on a randomly chosen side of the measurement tree between the rows. These two measurements were averaged. In throughfall reduction plots, the between-row measurements were located beneath the throughfall reduction trays.

2.2.7 Statistical analyses

Data were averaged by year, month, block, and plot with the unit of replication being the treatment plot. A repeated measures ANOVA (Proc Mixed, SAS Inc., Cary, NC) was used to test main and interactive treatment effects for variables that were repeatedly measured. Treatment effects on diurnal measurements were tested by month with time of day as the repeated variable. All repeated measures were performed with block as a random factor and fertilization and throughfall reduction treatments as fixed factors. In order to choose the most appropriate covariance structure for each analysis, the structure with the lowest Akaike information criterion was chosen. The auto regressive structure (AR(1)) was chosen for all repeated measures analyses. Treatment differences for single point in time measurements were tested using an analysis of variance (ANOVA) (Proc GLM, SAS Inc., Cary, NC). All main and interactive treatment effects were considered significant at $\alpha = 0.05$.

2.3 Results

2.3.1 Climate

In 2014 the PDSI ranged from -0.30 in January to -1.75 in September. Incipient drought conditions (index values from -0.50 to -0.99) began in February and continued through May, and mild drought conditions (index values from -1.00 to -1.99) began in June and lasted through December (Figure 2B). Precipitation for the 2014 year totaled 989 mm, which was 11% less than the 30-year average annual precipitation of 1109 mm. Average monthly precipitation was lowest in July at 39 mm and highest in December at 135 mm (Figure 2A). The average monthly minimum and maximum temperatures ranged from -2.0 °C in January to 32.4 °C in July, respectively (Figure 2A).

In 2015, the PDSI ranged from -1.22 in September to -2.10 in July. Mild drought conditions began in January and continued through May, and moderate drought conditions (index values from -2.00 to -2.99) were observed in June and July. Index data was unavailable for October 2015. Total precipitation from January to July was 504 mm, which was 21% lower than the expected 30-year average of 637 mm for the same time period (Figure 2A).

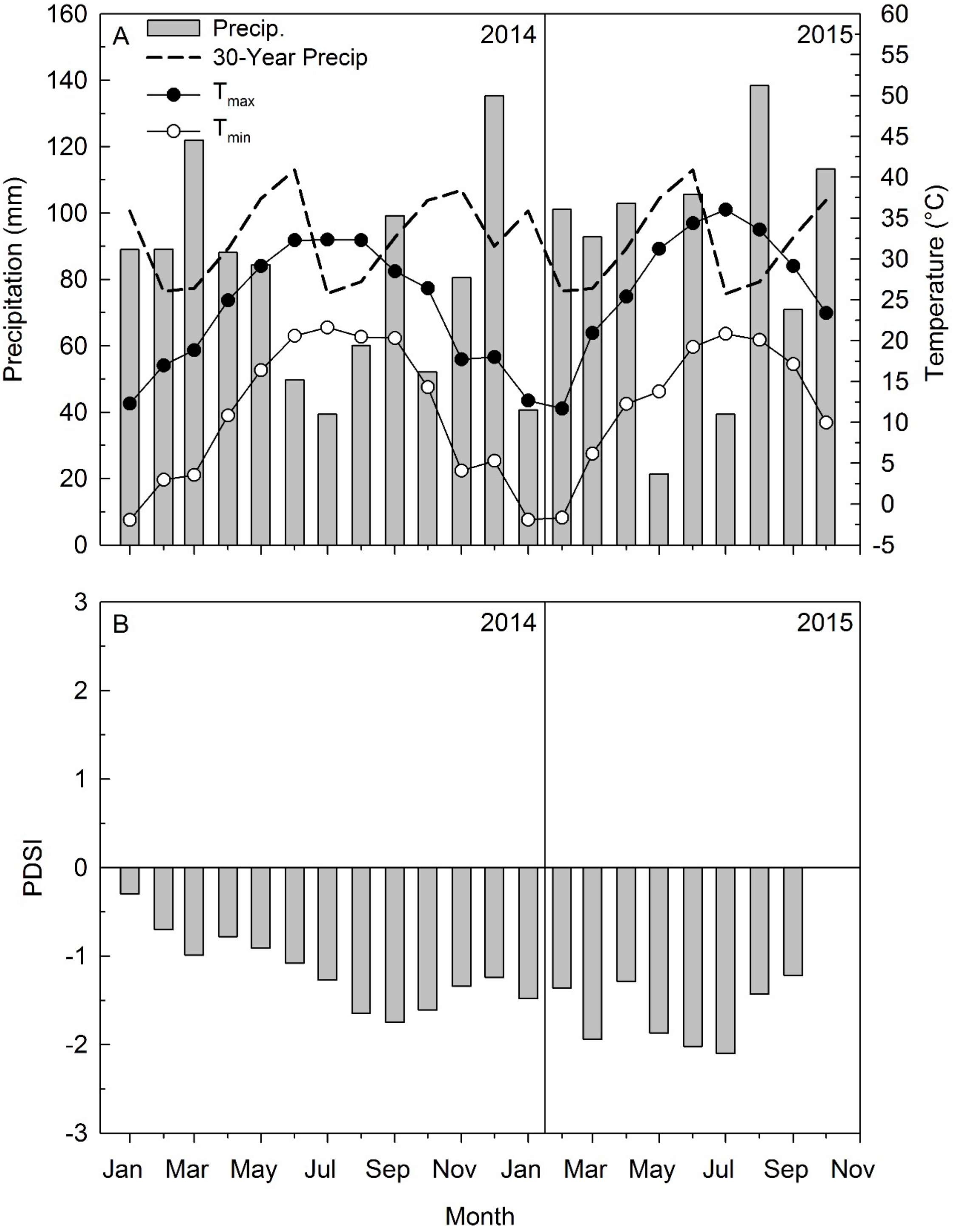

Figure 2. Monthly total precipitation (Precip.), average monthly maximum (T_{max}) and minimum (T_{min}) temperature, and 30-year average monthly precipitation (30-Year Precip.) (A) and the Palmer Drought Severity Index (PDSI) (B) (NOAA NCDC, Georgia Climate Division 3) during 2014 and 2015. (NOAA National Centers for Environmental Information, Asheville, North Carolina).

2.3.2 Soil moisture

There were no two-way or three-way interactive effects of throughfall reduction and fertilization treatment and month on soil moisture in the top 12 cm of soil measured concurrently with leaf-level gas exchange measurements in 2014 (Table 2). Soil moisture averaged across all treatments varied significantly by month (Table 2), ranging from an average of 8.1% in August to an average of 25.8% in March (Figure 3). Over all months, throughfall reduction treatment and fertilization treatment significantly reduced soil moisture (Table 2). Throughfall reduction reduced soil moisture from an average of 15.7% to an average of 13.5% (Figure 3). Fertilization reduced soil moisture from an average of 15.7% to an average of 13.6% (Figure 3).

In 2015, there was a significant three-way interaction between fertilization and throughfall reduction treatments and month of measurement for soil moisture in the top 12 cm of soil (Table 2). Month had a significant effect on soil moisture but month to month variation depended on treatment. With fertilization, soil moisture was similar from May to July regardless of throughfall reduction treatment (Figure 4). In contrast, without fertilization, soil moisture declined from May to June under throughfall reduction but under ambient throughfall reduction soil moisture was similar in May to June and declined in July (Figure 4). Averaged over all months, throughfall reduction treatment reduced soil moisture from an average of 17.1% to an average of 14.4% and fertilization treatment reduced soil moisture from an average of 17.0 % to an average of 14.6% (Figure 3).

Table 2. Observed probability values for the effect of month of measurement, throughfall reduction (TR), and fertilization (Fert) on light-saturated leaf-level photosynthesis (P_{net}), stomatal conductance (g_s), leaf-specific hydraulic conductance (K_{leaf}), predawn, midday, and the difference (Δ) between predawn and midday leaf water potential (Ψ_L), soil moisture, leaf area index (LAI) and intercepted photosynthetically active radiation (IPAR) in a loblolly pine plantation over 2014 and 2015. Measurements performed in 2015 were averaged across the diurnal time periods from 0900 to 1400 hours. Bold values indicate significance at $\alpha = 0.05$. Statistical analyses of IPAR were performed on logit (Y=log(IPAR/(1-IPAR)) transformed data.

	P_{net}	g_s	K_{leaf}	Predawn Ψ_L	Midday Ψ_L	$\Delta\Psi_L$	Soil Moisture	LAI	IPAR
2014									
Month	**<0.001**	**<0.001**	-	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TR	0.623	0.350	-	0.182	0.874	0.558	**0.003**	**0.001**	0.057
Fert	**0.001**	**0.005**	-	0.859	0.103	0.068	**0.004**	**<0.001**	**<0.001**
TR x Fert	0.758	0.449	-	**0.030**	0.567	0.527	0.271	0.295	0.955
Month x TR	0.860	0.609	-	0.325	0.981	0.588	0.564	0.240	0.320
Month x Fert	0.652	0.528	-	0.434	0.951	0.850	0.886	0.448	0.718
Month x TR x Fert	0.315	0.778	-	0.900	0.765	0.808	0.992	0.905	**0.010**
2015									
Month	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TR	0.160	**0.030**	0.427	0.322	0.716	0.963	**0.021**	**<0.001**	**<0.001**
Fert	**<0.001**	**0.011**	0.157	0.549	0.914	0.918	**0.033**	**<0.001**	**<0.001**
TR x Fert	0.528	0.107	0.630	0.900	0.871	0.888	0.628	0.472	0.981
Month x TR	0.954	0.992	0.728	0.072	0.983	0.896	0.091	0.336	0.834
Month x Fert	**0.042**	0.089	0.807	0.246	0.622	0.674	0.163	0.641	0.861
Month x TR x Fert	0.705	0.791	0.660	0.999	0.988	0.983	**0.022**	0.201	0.406

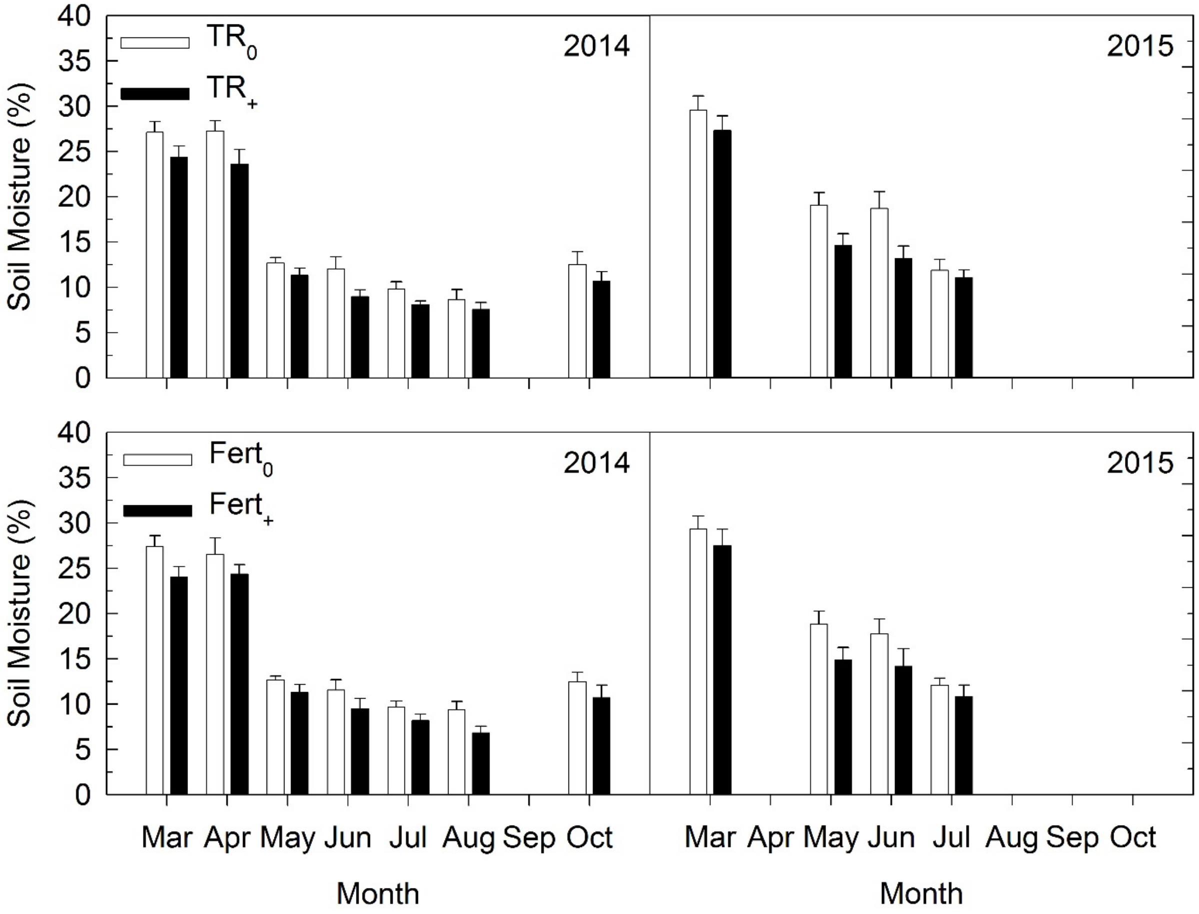

Figure 3. Mean (SE) soil moisture in the top 12 cm of soil in a loblolly pine plantation by month and year in response to throughfall reduction (TR$_0$: no reduction TR$_+$: approximate 30% reduction) and fertilization (Fert$_0$: no fertilization, Fert$_+$: one-time fertilization) treatments.

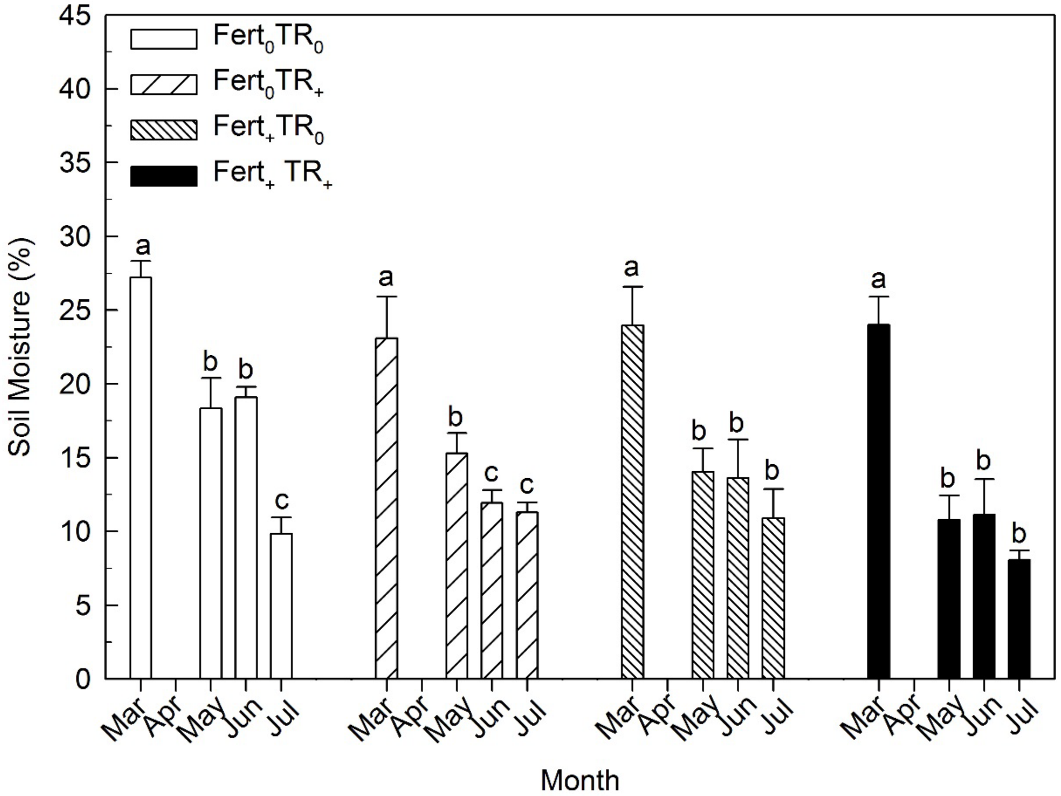

Figure 4. Mean (SE) soil moisture in the top 12 cm of soil by month in 2015 by treatment in a loblolly pine plantation. Lower case letters indicate differences between months within each treatment combination at α=0.05. Treatment abbreviations: Fert$_0$TR$_0$ – no fertilization, ambient throughfall; Fert$_0$TR$_+$ – no fertilization, throughfall reduction; Fert$_+$ TR$_0$ – fertilization, ambient throughfall; Fert$_+$ TR$_+$ – fertilization and throughfall reduction.

2.3.3 Growth

No significant interactive effects of throughfall reduction and fertilization treatments were detected for any growth variable measured in 2014 (Table 3). The only significant effect of throughfall reduction treatment on growth was on A_L:A_S, which was reduced from an average of 0.23 m^2 cm^{-2} to an average of 0.20 m^2 cm^{-2} by throughfall reduction (Table 3). Fertilization increased DBH, basal area, basal area increment, volume, volume increment, stemwood biomass and stemwood production (Table 3). Fertilization increased basal area increment from 3.6 m^2 ha^{-1} yr^{-1} to 4.7 m^2 ha^{-1} yr^{-1}, volume increment from 24.3 m^3 ha^{-1} yr^{-1} to 31.4 m^3 ha^{-1} yr^{-1} and stemwood production from 8.2 Mg ha^{-1} yr^{-1} to 10.7 Mg ha^{-1} yr^{-1} (Table 3). No treatment effects on height were observed.

No main or interactive treatment effects were detected for GE which was calculated as the ratio of annual stemwood production to average LAI (Table 4). Averaged over all treatments, GE was 3.4 Mg ha^{-1} yr^{-1}. No interactive treatment effects on ε_{stem} calculated as the ratio of annual stemwood production to APAR were observed. Fertilization increased ε_{stem} from 0.44 g MJ^{-1} to 0.47 g MJ^{-1} (Table 4). There was no significant effect of throughfall reduction treatment on ε_{stem}.

Table 3. Influence of throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) on mean (SE) stand growth measured in 2014 in a loblolly pine plantation. Bold values indicate significance at $\alpha = 0.05$.

Treatment	Mean DBH (cm)	Mean Height (m)	Basal Area (m^2 ha^{-1})	Basal Area Increment (m^2 ha^{-1} yr^{-1})	Volume (m^3 ha^{-1})	Volume Increment (m^3 ha^{-1} yr^{-1})	Stemwood Biomass (Mg ha^{-1})	Stemwood Production (Mg ha^{-1} yr^{-1})	A_L:A_S (m^2 cm^{-2})
TR_0	14.0 (0.3)	10.3 (0.3)	21.5 (1.0)	4.1 (0.2)	106.4 (6.5)	28.1 (1.8)	31.6 (2.2)	9.6 (0.6)	0.23 (0.01)
TR_+	13.8 (0.3)	10.0 (0.2)	20.9 (0.9)	4.1 (0.2)	101.1 (6.0)	27.6 (1.8)	29.6 (2.0)	9.3 (0.6)	0.20 (0.01)
$Fert_0$	13.3 (0.2)	10.0 (0.3)	19.4 (0.6)	3.6 (0.2)	93.5 (4.7)	24.2 (1.0)	27.4 (1.7)	8.2 (0.4)	0.20 (0.01)
$Fert_+$	14.5 (0.3)	10.3 (0.3)	23.0 (0.7)	4.7 (0.2)	113.9 (5.4)	31.4 (1.3)	33.8 (1.9)	10.7 (0.5)	0.22 (0.01)
P>F									
TR	0.440	0.116	0.482	0.813	0.267	0.787	0.204	0.654	**0.042**
Fert	**0.001**	0.079	**0.001**	**0.002**	**0.001**	**0.003**	**0.002**	**0.004**	0.260
TR x Fert	0.722	0.892	0.847	0.462	0.956	0.742	0.997	0.867	0.783

Table 4. Influence of throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) on mean (SE) growth efficiency (GE) and radiation use efficiency of stem wood production (ε_{stem}) in 2014 in a loblolly pine plantation. Bold values indicate significance at $\alpha = 0.05$.

	GE (Mg ha^{-1} yr^{-1})	ε_{stem} (g MJ^{-1})
TR_0	3.3 (0.1)	0.45 (0.02)
TR_+	3.6 (0.1)	0.47 (0.01)
$Fert_0$	3.4 (0.1)	0.44 (0.01)
$Fert_+$	3.5 (0.1)	0.47 (0.02)
P>F		
TR	0.070	0.159
Fert	0.704	**0.033**
TR x Fert	0.332	0.816

2.3.4 Leaf area index and intercepted photosynthetically active radiation

In 2014, there was a significant three-way interaction between fertilization and throughfall reduction treatments and month of measurement for IPAR (Table 2). Throughfall reduction reduced average IPAR only in fertilized treatments in June and July (Figure 7). No two-way or three-way interactions between throughfall reduction, fertilization, or month were detected for LAI. Leaf area index appeared to increase from September to October, however, LAI did not significantly differ between the two months. The apparent increase was likely due to the nature of using random transects during measurements and does not represent an actual increase in LAI. Throughfall reduction treatment reduced LAI averaged across all months from 2.9 m^2 m^{-2} to 2.6 m^2 m^{-2} (Table 2; Figure 5). Fertilization increased average LAI from 2.4 m^2 m^{-2} to 3.1 m^2 m^{-2} (Table 2; Figure 5). Average IPAR was increased by fertilization from 70.2% to 82.5% (Figure 6). No interactions between throughfall reduction and fertilization were detected for APAR in 2014 (Table 5). Absorbed photosynthetically active radiation was increased from an average of 1881.2 MJ m^{-2} to an average of 2250.8 MJ m^{-2} by fertilization (Table 5). There was no significant effect of throughfall treatment on APAR (Table 5).

In 2015, no interactive effects of treatments or month were observed on LAI or IPAR. Leaf area index and IPAR were decreased by throughfall reduction and increased by fertilization (Table 2). Throughfall reduction treatment reduced LAI from 3.2 m^2 m^{-2} to 2.7 m^2 m^{-2} and IPAR from 83.0% to 76.6% (Table 2; Figure 5). Fertilization increased LAI from 2.7 m^2 m^{-2} to 3.3 m^2 m^{-2} and IPAR from 75.8% to 83.8% (Table 2; Figure 5). No interactions between throughfall reduction and fertilization were detected for APAR in 2015 (Table 5). Absorbed photosynthetically active radiation was increased from an average of 1823.5 MJ m^{-2} to an average of 2069.6 MJ m^{-2} by fertilization (Table 5). There was no significant effect of throughfall treatment on APAR (Table 5).

No interactive treatment effects on litterfall based 2013 LAI were observed (Table 5). Throughfall reduction reduced litterfall based 2013 LAI from 4.6 m^2 m^{-2} to 3.8 m^2 m^{-2}. Litterfall based 2013 LAI was significantly increased by fertilization, from 3.6 m^2 m^{-2} to 4.8 m^2 m^{-2}. Across all treatments, litterfall based 2013 LAI was higher than previously reported values based on optical measurements (Figure 8) (Samuelson et al. 2014).

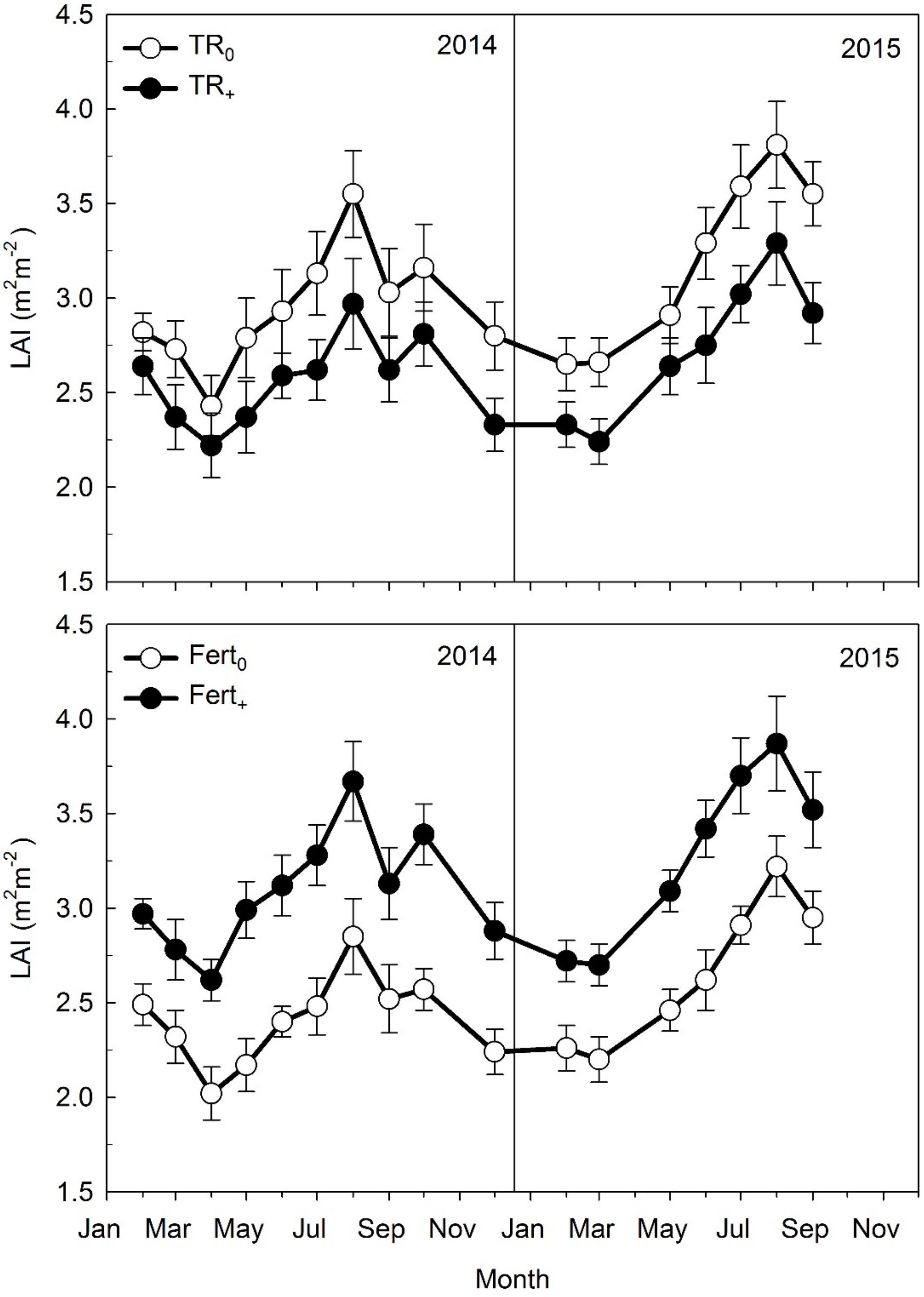

Figure 5. Mean (SE) mean leaf area index (LAI) of a loblolly pine plantation by month and year in response to throughfall reduction (TR$_0$: no reduction TR$_+$: approximate 30% reduction) and fertilization (Fert$_0$: no fertilization, Fert$_+$: one-time fertilization).

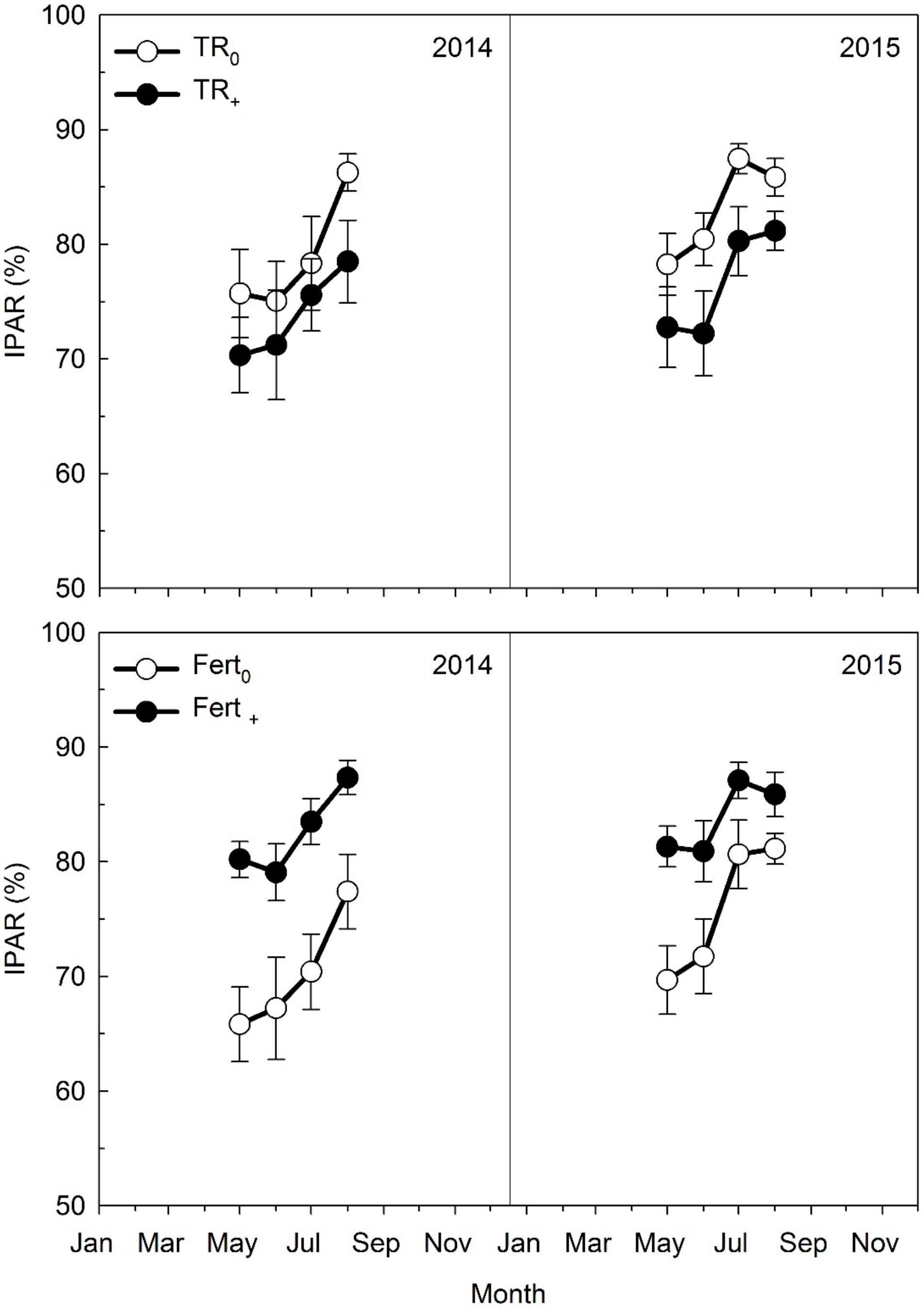

Figure 6. Mean (SE) intercepted photosynthetically active radiation (IPAR) by a loblolly pine plantation by month and year in response to throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) treatments.

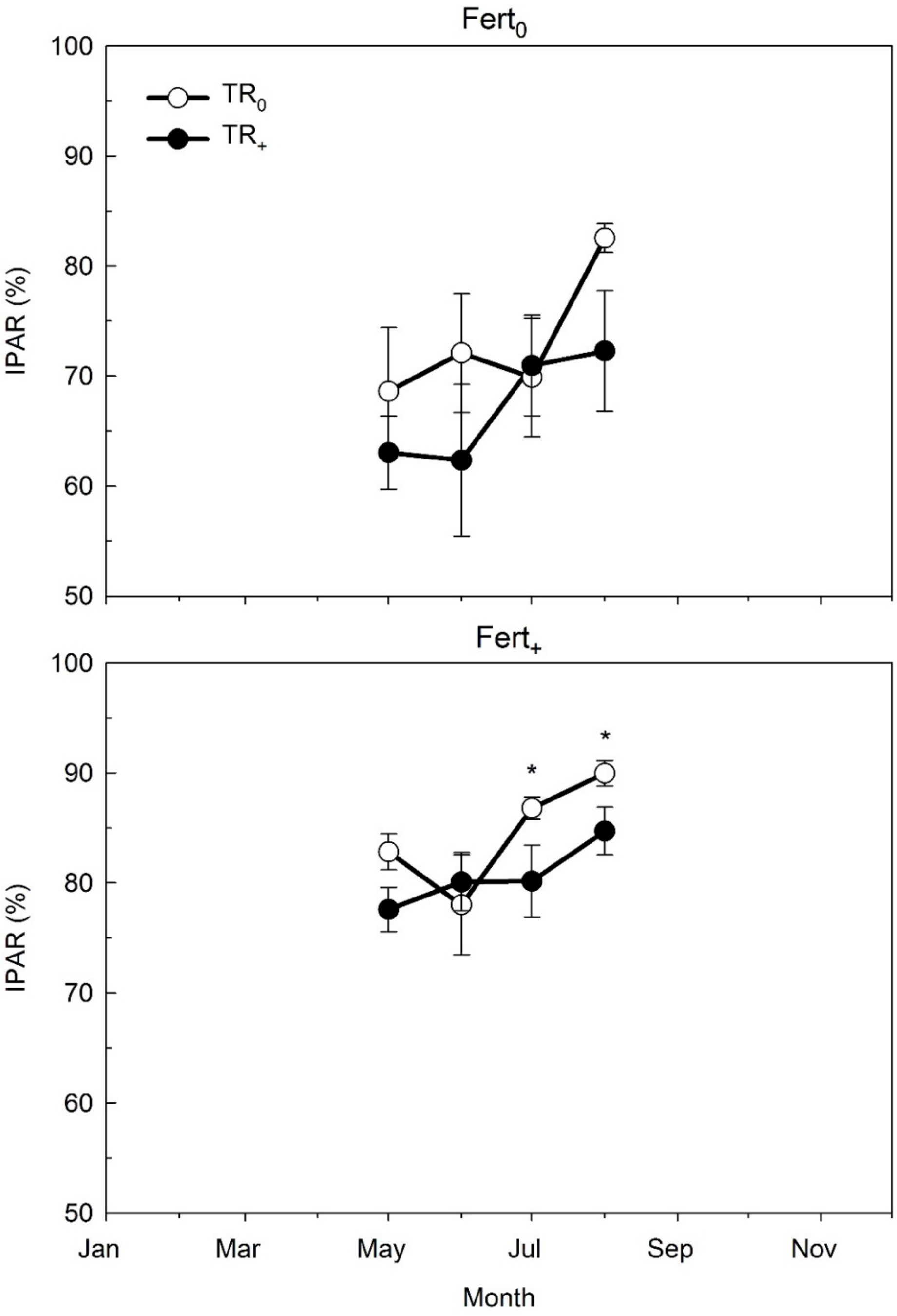

Figure 7. Mean (SE) intercepted photosynthetically active radiation (IPAR) by a loblolly pine plantation by month in 2014 in response to throughfall reduction (TR$_0$: no reduction TR$_+$: approximate 30% reduction) in no fertilization (Fert$_0$) and fertilization (Fert$_+$) treatments. Asterisks indicate a significant effect of throughfall treatment at $\alpha = 0.05$. Statistical analyses were performed on logit (Y=log(IPAR/1-IPAR) transformed data.

Table 5. Influence of throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) on absorbed photosynthetically active radiation (APAR) in 2014, and litterfall based LAI in 2013 in a loblolly pine plantation. Bold values indicate significance at α = 0.05.

Treatment	APAR 2014 (MJ m^{-2})	APAR 2015 (MJ m^{-2})	Litterfall Based LAI 2013 (m^2 m^{-2})
TR_0	2147.8 (82.8)	2035.1 (44.3)	4.6 (0.3)
TR_+	1984.1 (108.1)	1858.0 (84.4)	3.8 (0.3)
$Fert_0$	1881.2 (96.1)	1823.5 (66.6)	3.6 (0.2)
$Fert_+$	2250.8 (38.6)	2069.6 (45.4)	4.8 (0.3)
P>F			
TR	0.175	0.051	**0.004**
Fert	**0.009**	**0.012**	**<0.001**
TR x Fert	0.691	0.584	0.726

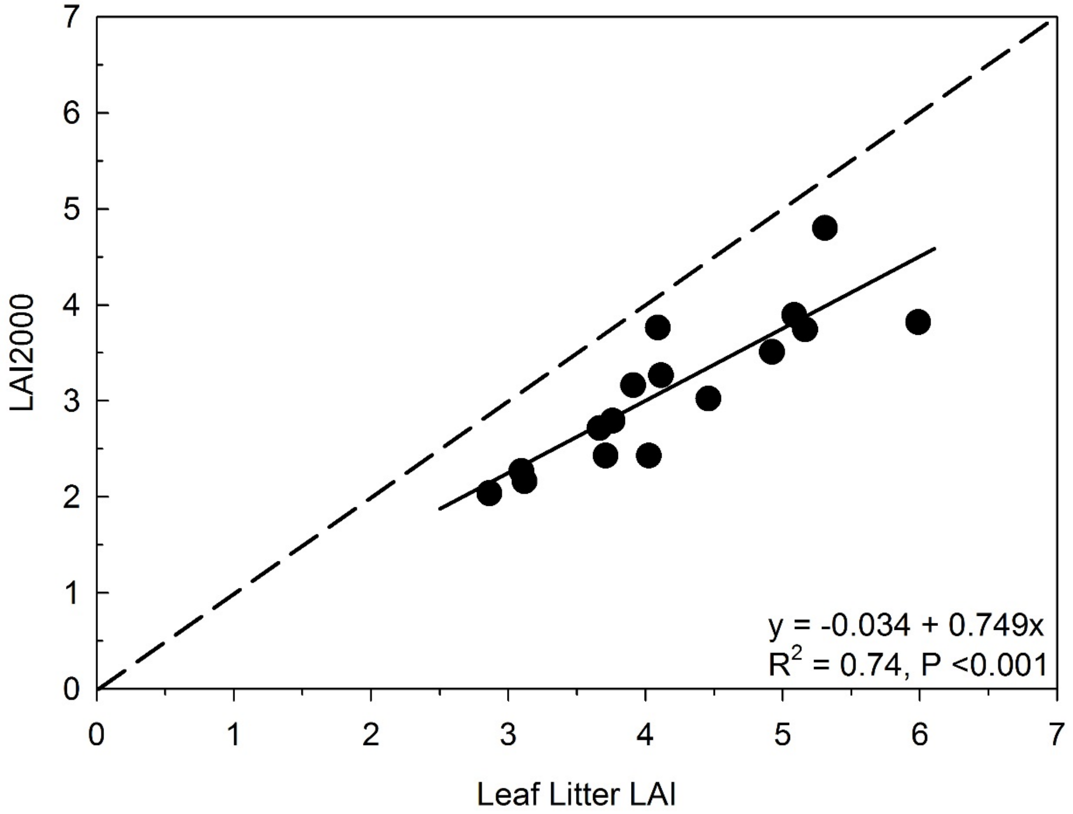

Figure 8. The relationship between peak leaf area index (LAI) measured in 2013 using optical methods (Samuelson et al. 2014) versus LAI calculated from litterfall collection in a loblolly pine planation. The dashed line represents a 1:1 ratio.

2.3.5 Leaf-level gas exchange

No interactive effects between throughfall and fertilization treatment, or month were observed for leaf-level gas exchange measurements in 2014 (Table 2). In 2014, P_{net} and g_s varied significantly by month (Table 2). Averaged across all treatments, P_{net} ranged from 2.9 μmol m^{-2} s^{-1} in August to 5.2 μmol m^{-2} s^{-1} in April, and g_s ranged from 26.3 mmol m^{-2} s^{-1} in August to 82.1 mmol m^{-2} s^{-1} in October. The throughfall reduction treatment had no effect on P_{net} or g_s in 2014 (Table 2). In contrast, in 2014 fertilization treatment reduced P_{net} from 4.4 μmol m^{-2} s^{-1} to 3.9 μmol m^{-2} s^{-1} and g_s from 60.6 mmol m^{-2} s^{-1} to 50.4 mmol m^{-2} s^{-1} (Figure 9).

Diurnal gas exchange measurements in 2015 indicated that there were no interactive effects between fertilization and throughfall reduction treatments and no interactions between time of day and treatment for any month for either P_{net} or g_s (Table 6). Stomatal conductance varied significantly with time of day each month, and P_{net} only varied with time of day in May and June (Table 6; Figure 10). Because no interactive effects among fertilization and throughfall reduction treatments with time of day were observed, diurnal measurements were averaged from 0900 to 1400 hours each month for comparison with the 2014 monthly measurements, which took place between 0930 and 1400 hours. After averaging across the hours, there was an interactive effect of month and fertilization treatment on P_{net} but not g_s (Table 2). Fertilization treatment reduced P_{net} in June and July but not in March or May (Figure 9). Throughfall reduction treatment had a significant effect on g_s; g_s was decreased from an average of 78.8 mmol m^{-2} s^{-1} to 71.0 mmol m^{-2} s^{-1} by throughfall reduction (Figure 9). Throughfall treatment had no effect on P_{net} (Table 2). Fertilization treatment also reduced g_s from an average of 79.5 mmol m^{-2} s^{-1} to 70.3 mmol m^{-2} s^{-1} (Figure 9).

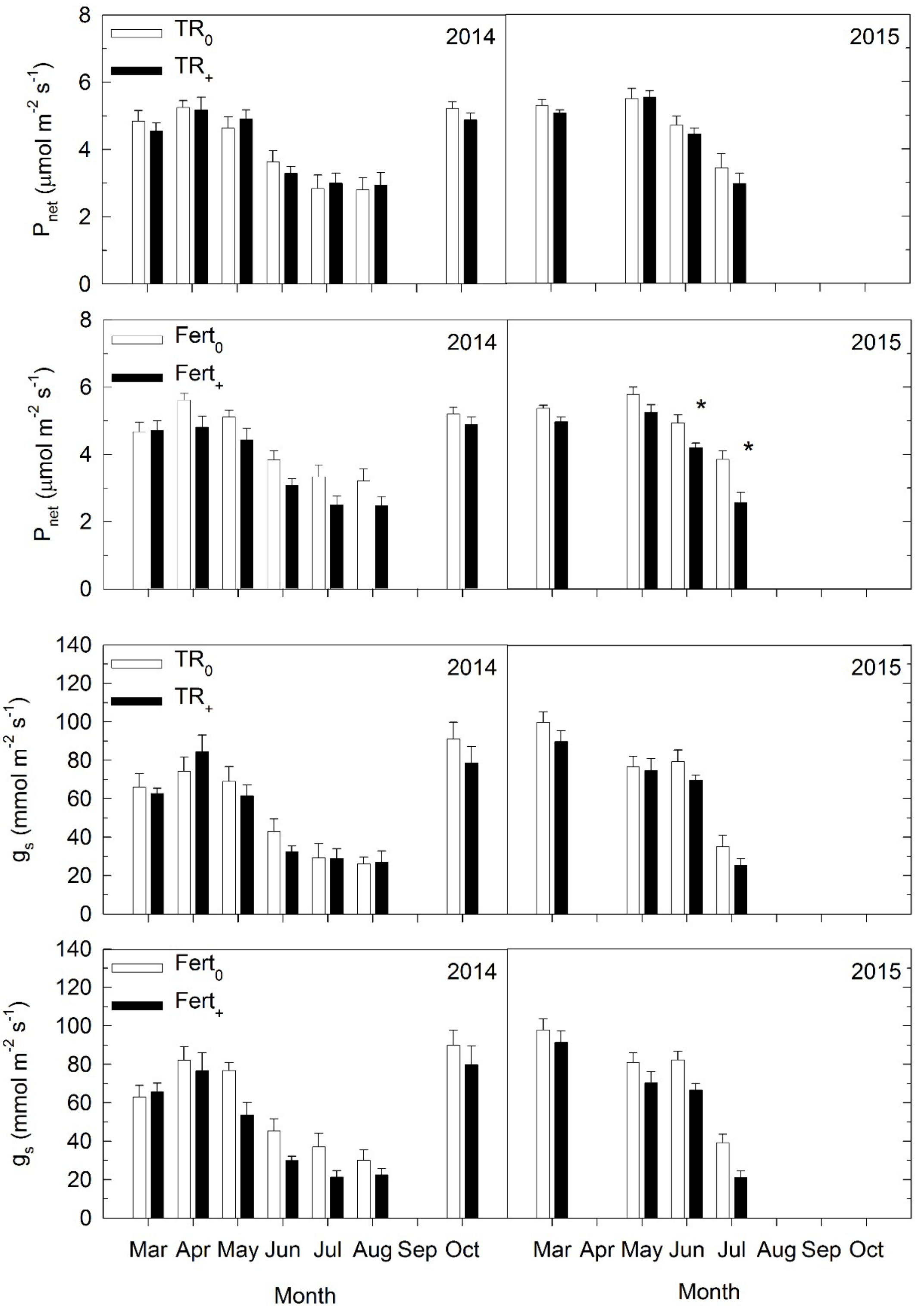

Figure 9. Mean (SE) light-saturated leaf-level photosynthesis (P_{net}) and stomatal conductance (g_s) by month and year in response to throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) treatments in a loblolly pine plantation. Asterisks indicate significant month by treatment interaction at α=0.05.

Table 6. Observed probability values of the effect of time of day, throughfall reduction (TR), and fertilization (Fert) on light-saturated leaf-level photosynthesis (P_{net}), stomatal conductance (g_s), and leaf water potential (Ψ_L) in a loblolly pine plantation in 2015. Bold values indicate significance at $\alpha = 0.05$.

	P_{net}	g_s	Ψ_L
March			
Time	0.360	**<0.001**	**<0.001**
TR	0.134	0.316	0.813
Fert	**0.010**	0.644	0.948
TR x Fert	0.821	0.297	0.979
Time x TR	0.341	0.663	0.988
Time x Fert	0.427	0.076	0.630
Time x TR x Fert	0.826	0.845	0.880
May			
Time	**<0.001**	**<0.001**	**<0.001**
TR	0.967	0.710	0.692
Fert	0.112	0.166	0.137
TR x Fert	0.314	0.147	0.169
Time x TR	0.844	0.747	0.955
Time x Fert	0.765	0.871	0.424
Time x TR x Fert	0.548	0.914	0.670
June			
Time	**0.034**	**0.026**	**<0.001**
TR	0.378	0.269	0.774
Fert	**0.018**	**0.007**	0.349
TR x Fert	0.659	0.574	0.830
Time x TR	0.853	0.871	0.880
Time x Fert	0.816	0.459	0.634
Time x TR x Fert	0.764	0.438	0.925
July			
Time	0.052	**0.040**	**<0.001**
TR	0.309	0.081	0.553
Fert	**0.006**	**0.002**	0.883
TR x Fert	0.369	0.217	0.780
Time x TR	0.140	0.280	0.769
Time x Fert	0.544	0.454	0.857
Time x TR x Fert	0.511	0.797	0.926

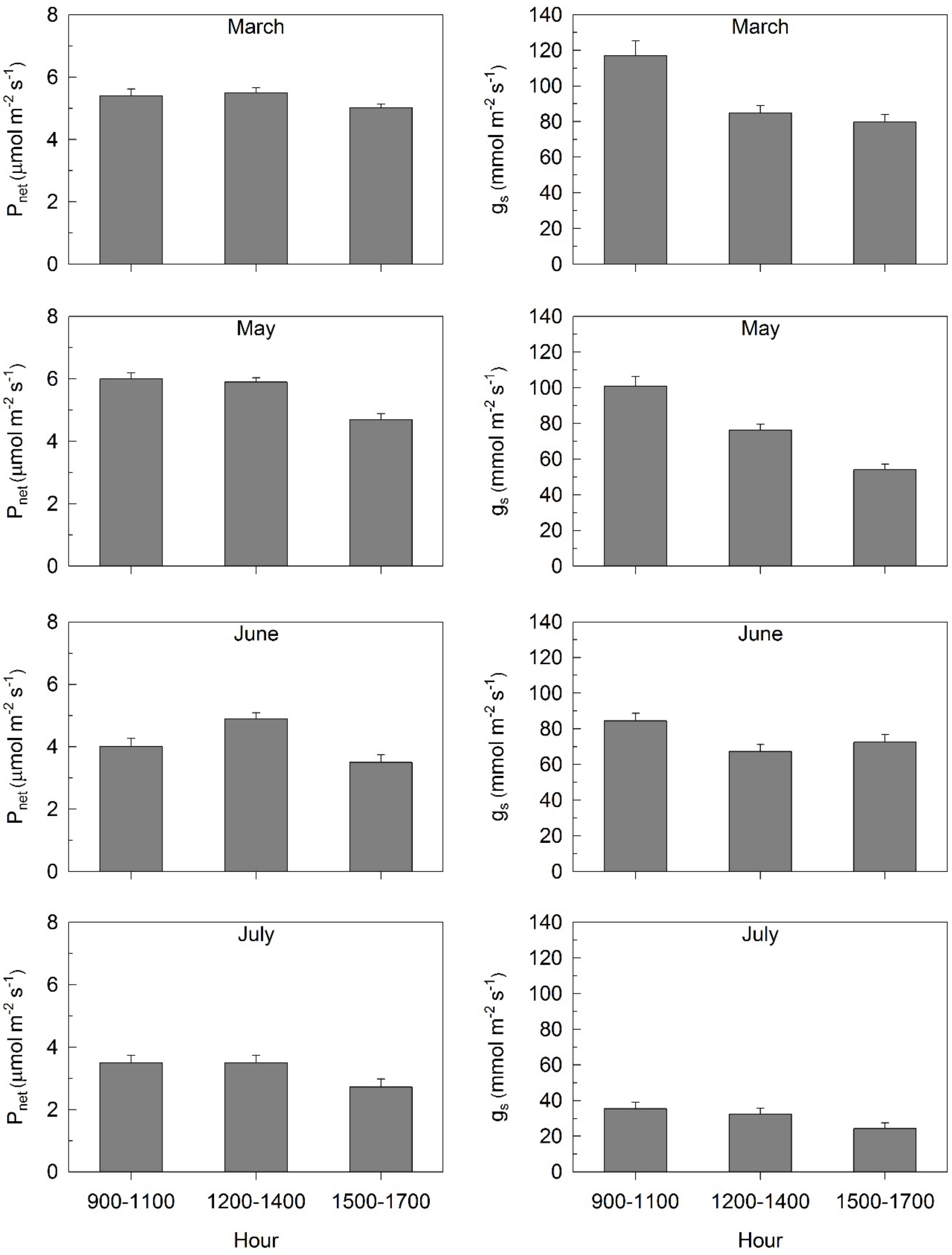

Figure 10. Mean (SE) light-saturated leaf-level photosynthesis (P_{net}) and stomatal conductance (g_s) by hour of measurement and month in 2015 in response to throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) treatments in a loblolly pine plantation. Values are averages across all treatments for a given time period.

2.3.6 Leaf water potential

In 2014 an interactive effect of fertilization and throughfall reduction treatment on predawn Ψ_L was observed (Table 2). Predawn Ψ_L was reduced by throughfall reduction only in the fertilized treatment, from an average of -0.68 MPa to an average of -0.76 MPa (Figure 11). Predawn, midday, and $\Delta\Psi_L$ each varied significantly by month. No fertilization or throughfall reduction treatment effects on midday Ψ_L or $\Delta\Psi_L$ were observed (Table 2; Figure 12; Figure 13). Averaged across all treatments, predawn Ψ_L ranged from -0.48 MPa in March to -1.07 MPa in August. Midday Ψ_L ranged from -1.99 MPa in August to -0.48 MPa in March. The difference between predawn and midday Ψ_L ranged from 1.30 MPa in May to 0.58 MPa in October.

In 2015, no main or interactive treatment effects and no treatment by time of day effects on Ψ_L were observed. Water potential varied with hour of day each month (Table 6; Figure 14). Predawn, midday, and $\Delta\Psi_L$ varied significantly by month but no main or interactive treatment effects were observed for either predawn, midday, or $\Delta\Psi_L$ (Table 2; Figure 12; Figure 13). Averaged across all treatments, predawn Ψ_L ranged from -0.50 MPa in March to -1.13 MPa in July. Midday Ψ_L ranged from -1.42 MPa in June to -2.32 MPa in July. The difference between predawn and midday Ψ_L ranged from 1.33 MPa in June to 0.76 MPa in July.

There were no main or interactive treatment effects on K_{leaf} measured only in 2015 (Table 2). Across all treatments, K_{leaf} varied significantly by month and ranged on average between 0.8 mmol m^{-2} s^{-2} MPa in July and 1.3 mmol m^{-2} s^{-2} MPa in June (Figure 15).

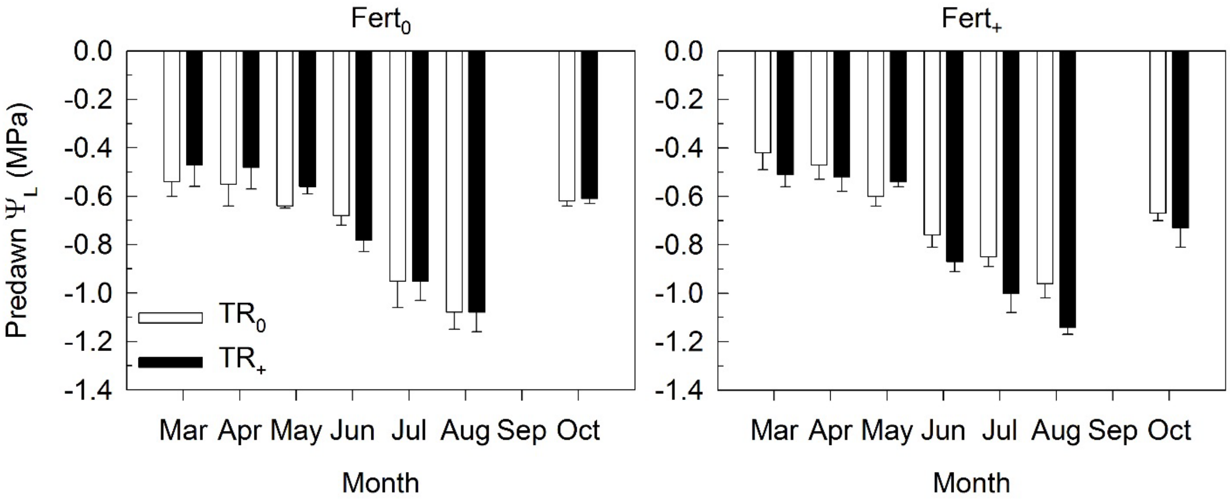

Figure 11. Mean (SE) predawn water potential (Ψ_L) in by month 2014 in response to throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) in no fertilization ($Fert_0$) and fertilization ($Fert_+$) treatments in 2014 in a loblolly pine plantation.

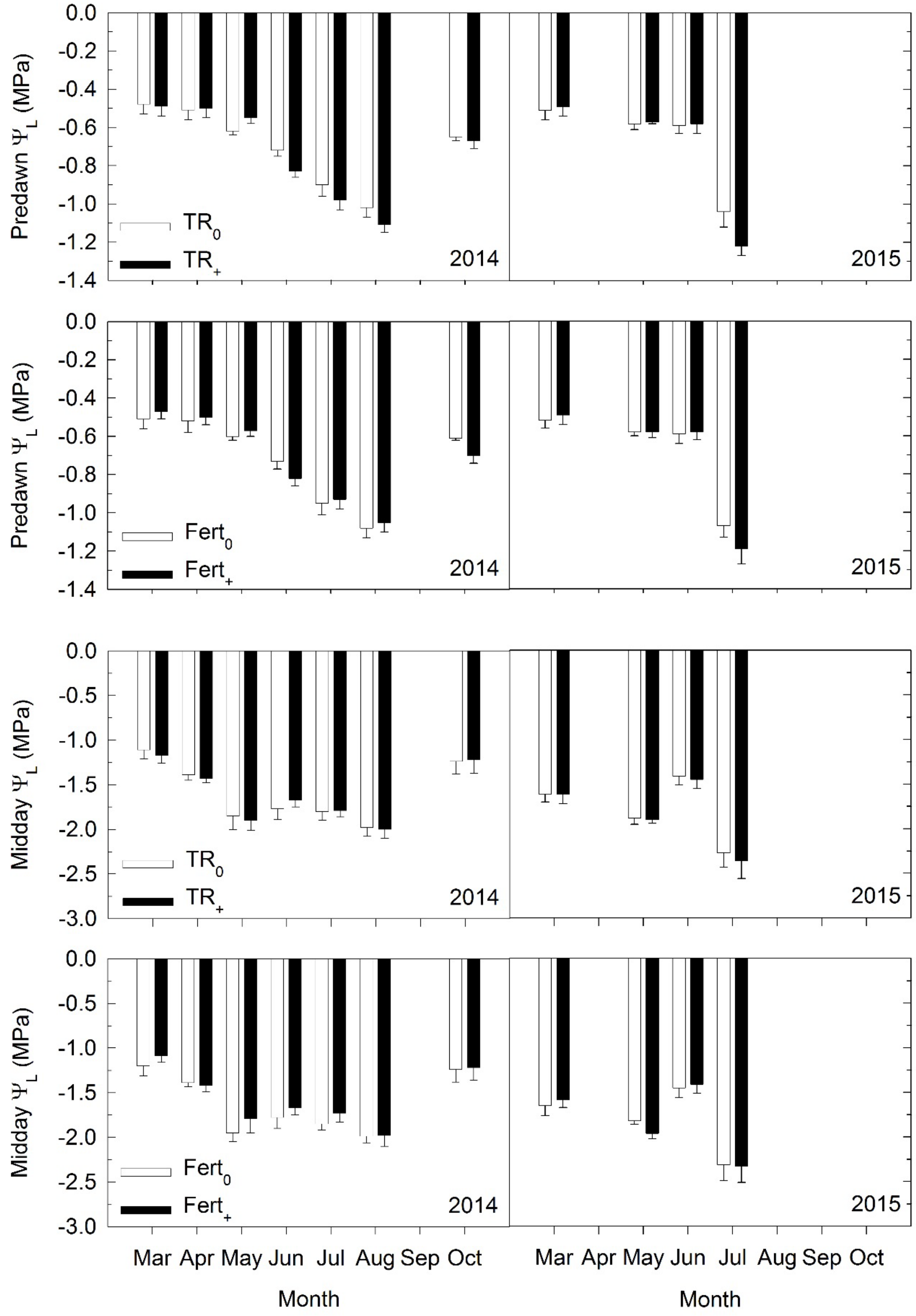

Figure 12. Mean (SE) predawn and midday water potential (Ψ_L) by month and year in response to throughfall reduction (TR$_0$: no reduction TR$_+$: approximate 30% reduction) and fertilization (Fert$_0$: no fertilization, Fert$_+$: one-time fertilization) treatments in a loblolly pine plantation.

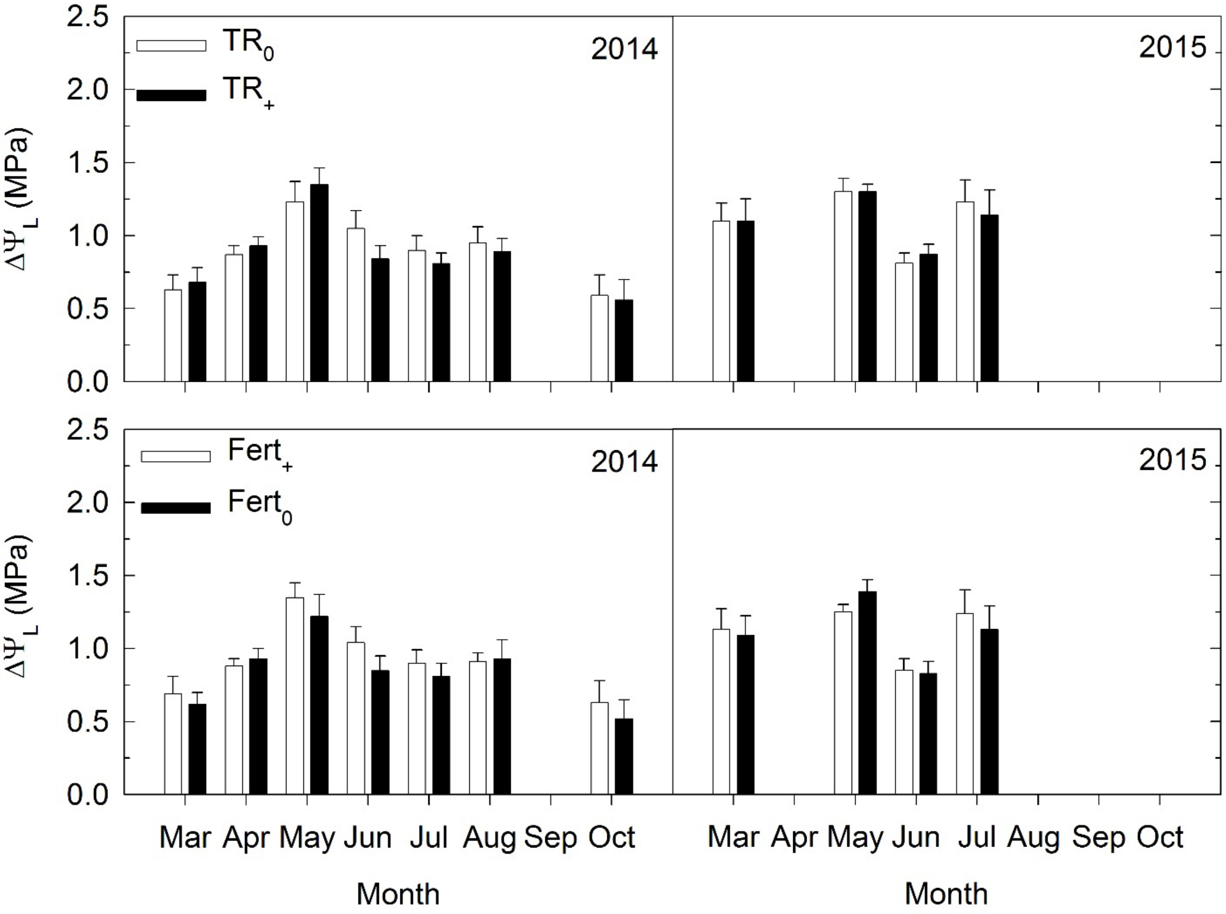

Figure 13. Mean (SE) difference (Δ) between predawn and midday leaf water potential (Ψ_L) by month and year in response to throughfall reduction (TR$_0$: no reduction TR$_+$: approximate 30% reduction) and fertilization (Fert$_0$: no fertilization, Fert$_+$: one-time fertilization) treatments in a loblolly pine plantation.

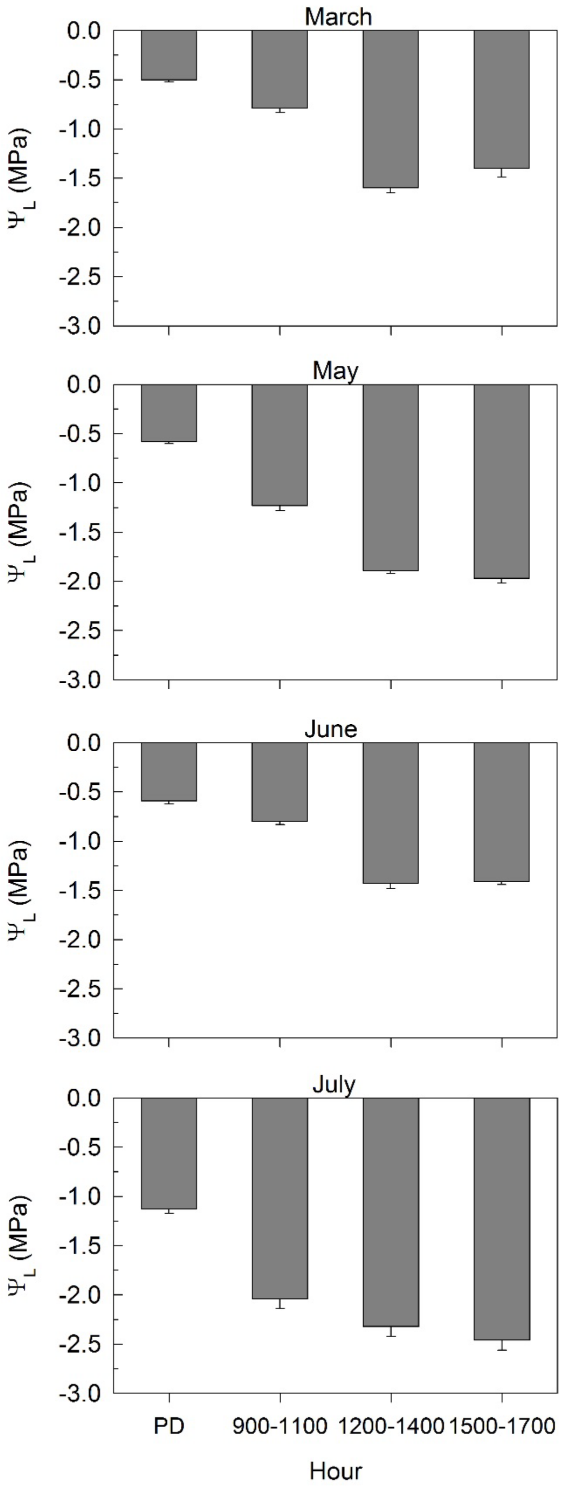

Figure 14. Mean (SE) leaf water potential (Ψ_L) by month and hour of measurement in 2015 in response to throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) treatments in a loblolly pine plantation. Values are averages across all treatments for a given time period.

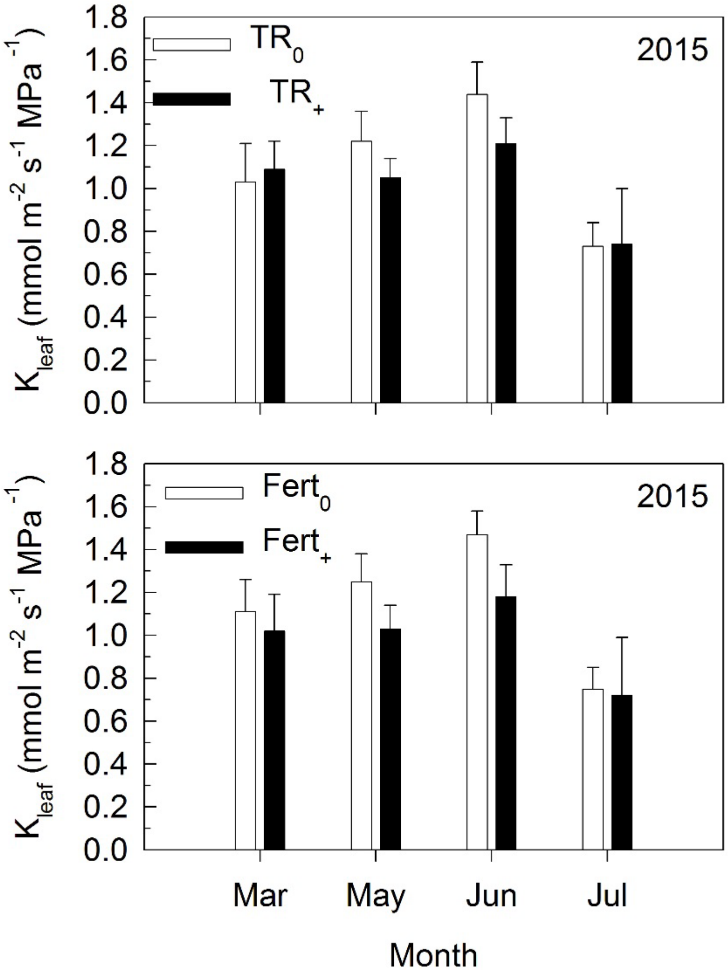

Figure 15. Mean (SE) leaf-specific hydraulic conductance (K_{leaf}) by month in 2015 in response to throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction) and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) treatments in a loblolly pine plantation.

2.3.7 Foliar isotope ^{13}C discrimination, nutrients, and needle morphology

No interactions between throughfall reduction and fertilization treatments or between flush and treatment were observed for $\Delta^{13}C$ measured on foliage collected in September 2014 (Table 7). Carbon 13 isotope discrimination varied significantly by flush. The first flush of 2014 exhibited a higher average $\Delta^{13}C$ (23.5‰) compared to the average $\Delta^{13}C$ of the second flush of 2014 (22.2‰). Throughfall reduction treatment decreased $\Delta^{13}C$, from an average of 23.2‰ to 22.6‰. Fertilization treatment decreased $\Delta^{13}C$ from an average of 23.0‰ to 22.7‰.

No interactions between flush and treatment or between throughfall reduction and fertilization treatments were observed for SLA (Table 7). Specific leaf area and average needle length varied between flushes. Average SLA of the first flush of 2014 was 146.1 cm^2 g^{-1} and average needle length was 16.5 cm. The second flush's average SLA was 176.7 cm^2 g^{-1} and average needle length was 14.3 cm. Throughfall reduction decreased SLA from 165.5 cm^2 g^{-1} to 157.3 cm^2 g^{-1}. No main effect of fertilization on SLA or needle length was observed.

No interactions between flush and treatment or between throughfall reduction and fertilization treatments were observed for any foliar nutrients (Table 7). Foliar nitrogen was higher in the second flush of 2014, averaging 13.5 mg g^{-1}, versus the first flush of 2014, averaging 10.8 mg g^{-1}. Throughfall reduction treatment did not affect foliar N concentration. Fertilization increased foliar N from an average of 11.4 mg g^{-1} to an average of 12.8 mg g^{-1}. There were no main or interactive treatment effects on foliar P or K concentrations. Across all treatments and flushes, P averaged 0.63 mg g^{-1} and K averaged 2.8 mg g^{-1}.

Table 7. Mean (SE) needle length, specific leaf area (SLA), carbon isotope discrimination ($\Delta^{13}C$), and foliar nitrogen (N), phosphorous (P), and potassium (K) content in response to flush, and throughfall reduction (TR_0: no reduction TR_+: approximate 30% reduction), and fertilization ($Fert_0$: no fertilization, $Fert_+$: one-time fertilization) treatments measured in September 2014 in a loblolly pine plantation. Bold values indicate significance at $\alpha = 0.05$.

Treatment	Needle Length (cm)	SLA ($cm^2\ g^{-1}$)	$\Delta^{13}C$ (‰)	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)
Flush1	16.5 (3.2)	146.1 (3.4)	23.5 (0.1)	10.8 (0.3)	0.5 (0.03)	2.9 (0.2)
Flush2	14.3 (3.6)	176.7 (2.0)	22.2 (0.2)	13.5 (0.4)	0.7 (0.03)	2.7 (0.1)
TR_0	15.2 (4.1)	165.5 (3.7)	23.2 (0.2)	12.0 (0.5)	0.6 (0.03)	2.7 (0.2)
TR_+	15.6 (4.7)	157.3 (5.5)	22.6 (0.2)	12.3 (0.5)	0.7 (0.03)	3.0 (0.1)
$Fert_0$	15.3 (3.9)	160.4 (4.5)	23.0 (0.2)	11.4 (0.5)	0.6 (0.03)	2.7 (0.1)
$Fert_+$	15.5 (4.9)	162.4 (5.0)	22.7 (0.2)	12.8 (0.5)	0.7 (0.03)	2.9 (0.1)
P>F						
Flush	**0.003**	**<0.001**	**<0.001**	**<0.001**	0.061	0.331
TR	0.526	**0.034**	**<0.001**	0.447	0.257	0.223
Fert	0.691	0.576	**0.025**	**0.004**	0.405	0.278
TR x Fert	0.951	0.302	0.400	0.302	0.972	0.796
Flush x TR	0.574	0.099	0.100	0.756	0.471	0.819
Flush x Fert	0.122	0.933	0.415	0.688	0.701	0.565
Flush x TR x Fert	0.649	0.087	0.592	0.914	0.920	0.963

2.4 Discussion

No interactive effects of throughfall reduction and fertilization treatment were observed on leaf-level gas exchange; however, fertilization and throughfall reduction treatments independent of one another reduced g_s and P_{net}. In 2012 and 2013, no interactive effects of throughfall reduction and fertilization treatment on leaf-level gas exchange were reported by Samuelson et al. (2014). In addition, no effects of fertilization on leaf-level physiology were reported and throughfall reduction reduced g_s and P_{net} in the dry 2012 year (Samuelson et al. 2014). In contrast, concurrent work on this study found that at the canopy-level in 2013, transpiration per unit ground area (E_G) and per unit leaf area (E_L), and canopy stomatal conductance (Gs) were decreased in response to throughfall reduction in the fertilized treatments but not in the control treatment (Bartkowiak et al. 2015). Similar interactive effects were found by a companion throughfall reduction and fertilization study in Virginia where E_G and G_s were lowest in the combined fertilization and throughfall reduction treatment (Ward et al. 2015). The interactive effects between throughfall reduction and fertilization on canopy-level processes found by Bartkowiak et al. (2015) were attributed to higher A_L:A_S in the fertilized treatment and stomatal control limiting water loss from increased leaf evaporative surface area. No enhancement of LAI with fertilization was observed in the Virginia study and the negative effects of the combined fertilization and throughfall reduction treatment on E_G and G_s were speculated to be the result of lower fine root production and lower root hydraulic conductivity in response to fertilization (Ward et al. 2015). The differences between leaf-level results in this study and results of the canopy-level measurements could be due to leaf-level measurements being discrete single point in time measurements dependent upon individual leaf physiology and canopy location, while canopy-level measurements are continuous and reflect the average treatment response across the canopy. A second companion throughfall reduction and fertilization study in Florida observed no treatment effects on canopy-level gas exchange despite higher LAI with fertilization which was likely due to high annual precipitation and roots having access to a shallow water table (Wightman et al. 2014). In this study, fertilization significantly reduced g_s by 20% in 2014 and 13% in 2015. In addition, fertilization significantly reduced P_{net} by 13% in 2014 and in June and July 2015 by 34%. Reductions in g_s and P_{net} with fertilization were likely due to the 23% and 18% increase in LAI in response to fertilization in 2014 and 2015 respectively. Higher LAI with fertilization likely caused increased leaf-level evaporative demand due to increased evaporative surface area. Reduced gas exchange with higher LAI was observed in 13-year-old loblolly pine where a doubling of LAI with fertilization on a nutrient poor site in North Carolina resulted in reduced E_L and lower G_s unless irrigation was also supplied (Ewers et al. 2001). Regardless of fertilization, throughfall reduction significantly reduced g_s in 2015 by 11% but no significant reduction in g_s with throughfall reduction was observed in 2014. Drier conditions in 2015 compared to 2014 likely resulted in reduced g_s with throughfall reduction. In 2015, total precipitation from January to July was 21% lower than the expected 30-year average where as in 2014 total precipitation for the year was 11% below the 30-year average. In addition, the PDSI indicated that mild drought conditions (PDSI values from -1.00 to -1.99) in 2014 did not start until June whereas in 2015 mild drought conditions began in January and became classified as moderate drought conditions (PDSI values from -2.00 to-2.99) in June. Throughfall reduction may not have impacted leaf-level gas exchange in 2014 because drought conditions were mild, and because 11% lower annual precipitation than the 30-year average was less than what studies reporting reductions in gas exchange in response to drought have observed. For example, in 2012, a 25% reduction in average annual precipitation resulted in a 24% reduction in g_s in response to throughfall reduction independent of fertilization treatment (Samuelson et al. 2014). In a similar throughfall reduction and fertilization study, Tang et al. (2004) observed that 100% throughfall exclusion reduced g_s and P_{net} when precipitation was 66% less that the 30-year average precipitation for August and September. Lower LAI in response to throughfall reduction decreased the A_L:A_S ratio by 15% which likely increased the amount of water available per unit leaf area, possibly explaining the lack of throughfall reduction effect on leaf-level gas exchange in 2014. The A_L:A_S ratio describes the relationship between projected leaf area and the area of sapwood supplying water to the canopy and is directly related to stomatal conductance (Whitehead 1998). Whitehead (1998) proposed that A_L:A_S is directly proportional to the product of sapwood hydraulic conductance and $\Delta\Psi$ and inversely proportional to the product of the vapor pressure deficit of the leaf to the atmosphere and stomatal conductance. Decreased A_L:A_S could result in an increase in g_s in non-water limited conditions. For example, Pataki et al. (2008) reduced leaf area by thinning which decreased the A_L:A_S ratio in 8-year old loblolly pine resulting in increased G_s and g_s. In this study, lower A_L:A_S in response to throughfall reduction treatment likely diminished the effects of throughfall reduction on g_s. Although root growth was not monitored in this study, reductions in g_s in response to throughfall reduction could also have been avoided through increased fine root production. Bongarten and Teskey (1987) found that when loblolly pine seedlings were exposed to repeated drought cycles, dry matter allocation to roots increased significantly. During periods of drought, fine root production may occur deeper in the soil profile where water may be more readily available allowing the canopy greater access of water (Teskey et al. 1986; Sands and Milligan 1990; Gaul et al. 2008).

Although no effect of throughfall reduction on leaf-level gas exchange was observed in 2014, needle morphology was altered in response to throughfall reduction treatment in 2014. Trees respond to limited water supply by altering a range of leaf characteristics such SLA. Specific leaf area was reduced in response to throughfall reduction treatment. Lower SLA in response to reduced water availability could be a "conservation" strategy, which involves increasing leaf dry matter content by retaining minerals or photosynthate, thereby conserving valuable resources (Roden et al. 1900; Choong et al. 1992; Poorter and Garnier 1999). In addition, production of thicker cell walls in some plants has been associated with lower SLA in response to drought and likely represents an adaptation to resist cell collapse and maintain turgidity (Roden et al. 1990; Poorter and Garnier 1999; Niinemets 2001).

Fertilization treatment and throughfall reduction treatment increased WUE, represented by decreased $\Delta^{13}C$, in 2014. Decreased $\Delta^{13}C$ can be caused by decreased diffusion of CO_2 into the stomatal cavity. Reductions in g_s increase the relative abundance of carbon isotope ^{13}C to ^{12}C ($\delta^{13}C$) within the stomatal cavity thereby decreasing ^{13}C discrimination ($\Delta^{13}C$) by Rubisco (Farquhar et al. 1989). The discrimination against ^{13}C by Rubisco is affected by the ratio between the concentration of CO_2 within the stomatal cavity and ambient air (C_i/C_a) across the entire growing season, making $\Delta^{13}C$ a time integrated measurement of g_s. Because g_s was reduced with fertilization, the decrease in $\Delta^{13}C$ with fertilization in 2014 was likely due to reduced diffusion of CO_2 into the leaf. Throughfall reduction treatment decreased $\Delta^{13}C$ as well, which was likely caused by reduced CO_2 diffusion into the stomatal cavity. This reduction in $\Delta^{13}C$ associated with throughfall reduction implies that there were reductions in g_s that were not detected by instantaneous gas exchange measurements. Similar reduction in $\Delta^{13}C$, with fertilization and water stress have been observed in loblolly pine (Choi et al. 2005; Samuelson et al. 2014) as well as other conifers (Korol et al. 1999; Brooks et al. 2009) the mechanisms for which has commonly been due to decreased g_s reducing water loss (Choi et al. 2005). In 2012, throughfall reduction increased the relative abundance of ^{13}C to ^{12}C ($\delta^{13}C$) (lower $\Delta^{13}C$) and higher WUE was accompanied by decreased g_s. In contrast, in 2013, when precipitation was abnormally high, neither throughfall reduction nor fertilization treatments effected $\delta^{13}C$. In water stressed conditions such as in 2012 with low ambient precipitation and 2014 with minor drought conditions and higher stand leaf area compared to 2012, a conservative water use strategy indicated by decreased g_s and decreased $\Delta^{13}C$ was employed. In a review study by Mateinez-vilata et al. (2004) which investigated multiple Pinaceae species, including loblolly pine, $\delta^{13}C$ corresponded to increasing drought stress indicated by a low Ψ_L and low ratio of precipitation to evapotranspiration. In this review, species vulnerable to drought or those that exhibited conservative water use strategies, tended to have higher $\delta^{13}C$ compared to more drought tolerant species.

In 2014, throughfall reduction reduced predawn Ψ_L only in the fertilized plots which could indicate greater drought stress with fertilization. Predawn Ψ_L is often used as a proxy for soil water potential (Ψ_{soil}) because of the assumption that predawn Ψ_L is in equilibrium with the wettest soil that is accessible by the roots (Ritchie and Hinckley 1975). Although no similar interactive effect between fertilization and throughfall reduction on soil moisture was observed in 2014, both throughfall reduction and fertilization reduced soil moisture in the top 12 cm. The regulation of water loss by controlling g_s also regulates Ψ_L (Sperry 2011). Loblolly pine begins reducing g_s early during the onset of drought in order to maintain Ψ_L above levels that would cause hydraulic failure due to severe embolism (Domec et al. 2009). The difference between predawn Ψ_L and midday Ψ_L ($\Delta\Psi_L$) represents the maximum transpiration-induced water potential gradient from roots to shoots and a constant $\Delta\Psi_L$ can be an indicator of strong stomatal control to maintain hydraulic function (Domec et al. 2009; Sperry 2011). The $\Delta\Psi_L$ can be maintained by altering the A_L:A_S ratio, hydraulic conductance, or altering stomatal conductance (Whitehead 1998; Whitehead and Beadle 2004). For example, in 5-year-old loblolly pine higher A_L:A_S in response to fertilization and irrigation treatments maintained a relatively constant $\Delta\Psi_L$ of 0.86 MPa (Samuelson and Stokes 2006). In this study, no main or interactive effects were observed on $\Delta\Psi_L$ and across all treatments $\Delta\Psi_L$ averaged 0.88 MPa in 2014 and 1.11 MPa in 2015. Gonzalez-Beneke et al. (2010) found that an average $\Delta\Psi_L$ of 0.75 MPa was maintained in both irrigated and non-irrigated 5-year-old loblolly pine where canopy stomatal conductance and whole tree hydraulic conductance were both lower without irrigation and a constant $\Delta\Psi_L$ allowed Ψ_L to fluctuate with changing Ψ_{soil} between irrigation treatments. In this study, $\Delta\Psi_L$ was likely unaffected by fertilization or throughfall reduction treatments through a combination of stomatal regulation and changes in the $A_{L:}A_S$ ratio. Although no main or interactive effects of fertilization or throughfall reduction treatment on K_{leaf} were found in this study, a companion study by Bartkowiak et al. (2014) found in 2013 whole-tree hydraulic conductance per unit leaf area was decreased by throughfall reduction in the fertilized treatments from an average of 3.83 mmol m^{-2} s^{-1} MPa^{-1} to 2.76 mmol m^{-2} s^{-1} MPa^{-1}. Compared to Bartkowiak et al. (2014), values in this study were lower, and ranged from an average of 0.8 mmol m^{-2} s^{-1} MPa^{-1} to 1.3 mmol m^{-2} s^{-1} MPa^{-1}. Bartkowiak et al. (2014) determined whole-tree hydraulic conductance per unit leaf area at the canopy level using measurements of sap flux density at midday whereas in this study K_{leaf} was determined from measurements of leaf-level transpiration at midday. Bartkowiak et al. (2014) found fertilization increased A_L:A_S which was coupled with decreased hydraulic conductance and G_s. Differing results of this study could be due in part to the lack of interactive effects on gas exchange and the discrete nature of leaf-level measurements. There was no main or interactive treatment effects observed for midday Ψ_L either year. Midday Ψ_L averaged -1.6 MPa across treatments in 2014 and averaged -1.8 MPa in 2015 which are both above Ψ_{50plc}, the water potential causing 50% loss of conductivity in the stem, proposed for loblolly pine of approximately -3.5 MPa (Martinez-Vilalta et al. 2004). This lack of treatment effect on midday was likely due to decreased g_s and lower A_L:A_S ratio limiting the fluctuation of $\Delta\Psi_L$. These results are consistent with previous studies on hydraulic relations in loblolly pine which have shown that loblolly pine closes its stomata early during the onset of drought (Ewers et al. 2000; Ellsworth 2000; Domec et al. 2009; Goldstein et al. 2013) to reduced water loss, limit xylem embolism, and to keep $\Delta\Psi_L$ relatively constant.

Reduction in loblolly pine growth in response to drought (Klos et al. 2009) is dependent on the severity, length, and seasonality of drought conditions as well as evapotranspiration, the soil's ability to retain water, and stand structure (Landsberg and Gower 1997). Klos et al. (2009) found that across the Southeast the degree to which growth declined in *Pinus* species in response to drought depended on the severity of the drought as well as stand basal area, tree age, species richness and slope. The seasonality of drought, such as drought in the spring and summer, may impact growth more than total annual precipitation (Jordon and Lockaby 1990; Johnsen et al. 2014). For example, a long-term growth study of loblolly pine in Oklahoma, the edge of loblolly pine's natural range, found that over the 16 year study LAI and current annual increment (CAI) were only decreased over three years when total annual precipitation was relatively normal but potential evapotranspiration was greater than precipitation during the growing season (Hennessey et al. 2004). Length and seasonality dependent drought effects on growth were observed by Mendivelso et al. (2014) who determined that in tropical dry forests in Bolivia, tree growth was most sensitive to multiple years of wet season drought or wet to dry season transitional drought. In this study, throughfall reduction reduced LAI and the A_L:A_S ratio, but no other throughfall reduction effects on growth in 2014 were observed. In 2014, the sum of precipitation from March through August was 17% below the 30-year average and the PDSI indicated minor drought conditions (PDSI values from -1.0 to -1.99). Samuelson et al. (2014) found that basal area increment and stemwood production were reduced by throughfall reduction in a previous drought year when PDSI values approached -4.0 and annual precipitation was 25% less than the expected 30-year average. It is likely that drought conditions in 2014 were not severe enough to decrease growth. Reductions in growth associated with drought have been attributed to reduced leaf area through leaf shedding (Gholz et al. 1990; Hennessey et al. 1992), production of smaller needles (Poorter and Garnier 1999; Dougherty et al. 1994), and reductions in P_{net} (Teskey et al. 1987). There were no significant effects of throughfall reduction on P_{net} or needle length, however throughfall reduction likely reduced needle production because LAI was 12% lower in response to throughfall reduction in 2014 and 16% lower in response to throughfall reduction in 2015. In some tree species, leaf production and expansion have been found to be more sensitive to reduced water availability than photosynthesis (Niinemets et al. 1999). Due to clumping of foliage and branching pattern in conifers (McGrady and Jokela 1998, Will et al. 2005), changes in LAI may not lead to equal equivalent changes in IPAR. For example, while LAI was decreased in response to throughfall reduction in 2014, IPAR was reduced by throughfall reduction only in fertilized treatments from June to July. This lack of main effect of throughfall reduction across the entire measurement period could explain why APAR, which was calculated from January to September 2014, was unaffected by throughfall reduction. In 2015, throughfall reduction reduced IPAR by 8%, and while there was no significant effect of throughfall reduction on APAR measured in 2015, there was a trend towards a 12% reduction in APAR with throughfall reduction (Pvalue = 0.051).

Fertilization had the greatest impact on growth, increasing all growth variables except for height. Higher LAI with increased nutrient availability is a well-documented response that typically results in accelerated stand development until canopy closure as a result of higher photosynthetic surface area, greater intercepted radiation, and increased canopy level photosynthesis (Cannell et al. 1987; McGrady and Jokela 1998; Allen et al. 2005). In 2014, fertilized trees continued to have higher levels of N than non-fertilized trees. Foliar N concentration was 12.8 mg g^{-1} in the fertilized treatment which is still above the critical value for loblolly pine of 11.0 mg g^{-1} (Allen 1987). Foliar P and K levels however fell below their critical threshold values of 1.0 mg g^{-1} and 3.5 mg g^{-1}, respectively (Allen 1987). The stand, was age 10 in 2015 and is likely at maximum LAI. Peak LAI in fertilized plots averaged 3.9 m^2 m^{-2} in 2015. Similar peak LAI values were reported in a study by Xu et al. (1998) which found that 12 years after various intensities of site preparation and repeated fertilization, LAI ranged from 1.7 m^2 m^{-2} to 5.2 m^2 m^{-2} and averaged 3.9 m^2 m^{-2} across all treatments on a site located in the Georgia Piedmont. Similar values of peak LAI have been reported in similarly aged loblolly pine studies (Hack et al. 2000; Samuelson et al. 2008). Basal area in fertilized plots in 2014 was 23.0 m^2 ha^{-1}, and in similarly aged loblolly pine stands, a BA range of 20 to 25 m^2 ha^{-1} has corresponded to maximum peak LAI (Albaugh et al. 2004; Martin and Jokela 2004).

Litter fall based measurements of LAI indicated that peak LAI may have been reached at age 8 in 2013. Litter fall based 2013 LAI averaged 4.6 m^2 m^{-2} with fertilization which is similar to litter fall based peak LAI values reported by Martin and Jokela (2004) where LAI in fertilized loblolly pine growing in Florida peaked at approximately 5.0 m^2 m^{-2} between ages 9 to 11. In addition, throughfall reduction reduced litterfall based 2013 LAI where previously there was only a trend towards a reduction with throughfall treatment (Samuelson et al. 2014). Lower predawn and midday Ψ_L in response to throughfall reduction in 2013 could have limited needle production over the course of the growing season resulting in lower LAI. Reduced litter fall based LAI with throughfall reduction treatments indicates that throughfall reduction reduced LAI for three consecutive years across interannual variations in precipitation. Persistently lower LAI in response to throughfall reduction could be a response to limited water availability (Poorter and Garnier 1999) and may have reduced leaf evaporative surface area and leaf evaporative demand. Litter fall based 2013 LAI was higher in all treatments when compared to optical measurements reported by Samuelson et al. (2014). For example, in the fertilized treatment, litterfall based LAI was 26% higher than peak 2013 LAI estimated from optical measurements. Sampson and Allen (1995) found that litterfall and allometric methods of estimating LAI were approximately 33% higher than values measured with optical methods in *Pinus contorta* (Douglas). Results in this study are consistent with the literature that shows that due to clumping of foliage and the ratio of branch area to leaf area, optical methods typically underestimate LAI when compared to litter or destructive based techniques (Breda 2003) and that in conifer species, this underestimation is greater at higher LAI (Deblonde et al. 1994; Sampson and Allen 1995).

No main or interactive effects on GE were observed. Average GE was 3.4 Mg ha^{-1} yr^{-1} and is comparable to results from other studies on loblolly pine. For example, Allen et al. (2005) found that GE ranged from 1.7 Mg ha^{-1} yr^{-1} to 3.7 Mg ha^{-1} yr^{-1} across irrigation and fertilization treatments on a dry upland site in Georgia. At a relatively infertile site in North Carolina, GE in 8-year-old loblolly pine ranged from 1.9 Mg ha^{-1} yr^{-1} to 2.9 Mg ha^{-1} yr^{-1}, and GE increased in response to irrigation and fertilization treatments (Albaugh et al. 2004). The lack of an effect of fertilization or throughfall reduction on GE is similar to the results found by Samuelson et al. (2001; 2004) who found no effect of irrigation or fertilization on GE of three and 6-year-old loblolly pine. Conversely, $\varepsilon_{\mathrm{stem}}$, was increased by fertilization by 6% and across all treatments averaged 0.46 g MJ^{-1}. Similar results were found by Campoe et al. (2013) who found that production efficiency based on annual net primary production (ANPP) ($\varepsilon_{\mathrm{ANPP}}$) ranged from 0.31 g MJ^{-1} to 0.87 g MJ^{-1} in 9-year-old loblolly pine across variably sized trees and that fertilized trees had higher $\varepsilon_{\mathrm{ANPP}}$ than non-fertilized trees. To compare estimates of $\varepsilon_{\mathrm{stem}}$ and $\varepsilon_{\mathrm{ANPP}}$, an approximate multiplication factor of 1.5 is sometimes used (Allen et al. 2005) assuming stem biomass comprises approximately 60% to 70% of total ANPP (King et al. 1999; Samuelson et al. 2004). After this approximate correction, values of $\varepsilon_{\mathrm{stem}}$ still correspond well to the values of $\varepsilon_{\mathrm{ANPP}}$ reported by Campoe et al. (2013). Higher estimates of $\varepsilon_{\mathrm{ANPP},}$ ranging from 1.33 g MJ^{-1} to 1.48 g MJ^{-1}, were reported for 4-year-old loblolly pine (McGrady and Jokela 1998). Allen et al. (2005) calculated $\varepsilon_{\mathrm{stem}}$ by dividing current-year stem biomass growth by APAR, and found that neither irrigation nor fertilization effected $\varepsilon_{\mathrm{stem}}$ and $\varepsilon_{\mathrm{stem}}$ averaged 0.53 g MJ^{-1} across all treatments. Similarly, Dallas-Tea and Jokela (1991) found in 6-year-old loblolly pine in Florida that $\varepsilon_{\mathrm{stem}}$, calculated by dividing aboveground dry matter production and APAR, averaged 0.81 g MJ^{-1}. Increases in GE and $\varepsilon_{\mathrm{stem}}$ have been attributed to enhanced photosynthesis (Waring 1983; Albaugh et al. 2004; Campoe et al. 2013) with fertilization which was not observed in this study. Enhancement of P_{net} with fertilization is typically temporary and occurs when foliar N is severely limiting (Tesky et al. 1987; Gough et al. 2004a) which was not the case in this study. Fertilization's reduction in photosynthesis did not conversely limit GE or $\varepsilon_{\mathrm{stem}}$ indicating that carbon limitation likely did not occur. Radiation use efficiency of stemwood production is dependent on the amount of ambient PAR and total PAR intercepted, the efficient use of intercepted PAR in terms of carbon fixation, and the allocation of this fixed carbon (Waring 1983; Stenberg et al. 1994). It is possible that the higher ε_{stem} in response to fertilization was due to higher efficiency of converting intercepted par into biomass with increased light capture. This relationship of increasing production efficiency at increasing levels of LAI and APAR has been attributed to the observation that the efficiency of light use does not relate linearly with LAI, and has been commonly observed in eucalyptus species (Smethurst et al. 2003; Binkley et al. 2004). Crown structure including branching pattern and leaf clumping may also have been altered by fertilization which may have allowed for more optimum light capture and conversion into stemwood (Stenberg et al. 1994). Higher ε_{stem} in response to fertilization could also be due to allocation of carbon from below to above ground biomass (Albaugh et al. 2004; Maier et al. 2004), however root biomass and belowground carbon allocation was not monitored in this study.

2.5 Conclusions

The results of this study show that throughfall reduction and fertilization treatment independently reduced leaf-level gas exchange and that high LAI associated with fertilization may increase water use and short-term leaf-level drought susceptibility. Although fertilization increased growth, higher LAI decreased P_{net} due to reduced g_s possibly to limit water loss from increased leaf evaporative surface area. The reductions in P_{net} and g_s associated with fertilization were likely not great enough to cause carbon limitation to occur and limit growth efficiency, which allowed fertilization to still increase growth. In addition, decreased $\Delta^{13}C$ with throughfall reduction and fertilization treatments indicated increased WUE which may have helped to maintain growth. Reductions in predawn Ψ_L with throughfall reduction in fertilized treatments were likely in response to higher LAI which increased the susceptibility to water limitation. In 2014, under minor drought conditions, it is possible that lower LAI associated with throughfall reduction may have temporarily reduced drought stress by lowering the leaf area to sapwood area ratio (A_L:A_S) thereby providing greater water per unit leaf area. However, under more severe drought conditions prolonged reduction of g_s coupled with low LAI may result in decreased growth. In the southeastern United States, fertilization is a common silvicultural practice to increase site fertility and to increase the productivity of pine plantations. The results of this study support the extensive literature showing that loblolly pine growth responds positively to increased nutrient availability through increased LAI and intercepted radiation. Although reductions in productivity were not observed in this study, fertilization and throughfall reduction resulted in greater leaf-level drought susceptibility which, in a future more drought prone climate, may diminish the growth enhancement effects of fertilization. Southern pine plantation managers should consider the effects of fertilization on water use and drought susceptibility in order to maintain productivity and to adapt southeastern forests for a more drought prone climate.

2.6 Management applications

Climatic resistance is the ability to avoid negative impacts of the growing environment such as drought, and climatic resilience is the capacity to recover during more favorable conditions (Millar et al. 2007). Results from this study indicated that although fertilization increased growth regardless of reduced water availability, both fertilization and throughfall reduction independently effected physiological indicators of drought stress.

In western and European ecosystems thinning or lower initial stocking density have been shown to reduce the risks associated with drought and enhance climatic resistance and resilience by lowering stand leaf area and water demand (Sala et al. 2005; D’Amato et al. 2011; Magruder et al. 2012; D’Amato et al. 2013; Susaeta et al. 2014; Elkin et al. 2015). Thinning can reduces drought stress by reducing the competition for water but can also enhance growth and lead to higher water use as a stand grows (Nyland 2007; D’Amato et al. 2013). In western Montana, thinned stands of *Pinus ponderosa* (Douglas ex. C. Lawson) displayed higher leaf-level photosynthesis, stomatal conductance and Ψ_L, as well as increased radial growth compared to stands that were not thinned (Sala et al. 2005). In the European Alps, Elkin et al. (2015) determined that thinning reduced drought related mortality of various montane species, and the benefits of thinning lasted approximately 20 years. In southeastern loblolly pine plantations where nutrient availability is typically the greatest factor limiting growth (Jokela et al. 2004; Fox et al. 2007), thinning is typically used to increase light availability rather than reduce water limitation (Nyland 2007). In addition, lower stocking density has been shown to be a possible preemptive management option to reduce future drought impacts. Magruder et al. (2012) reported that stands of *Pinus resinosa* (Sol. ex Aiton) that were planted at a lower stocking density exhibited higher climatic resistance to drought and that growth was less sensitive to variations in precipitation.

The objectives of adapting forests for a future climate, mitigating climate change through enhanced forest carbon sequestration, and maximizing yield may not all be synonymous (D’Amato et al. 2011). Maintaining higher stand density may result in higher carbon stores and therefore enhance mitigation and overall yield, but may lead to higher drought susceptibility (D’Amato et al. 2011). In southeastern loblolly pine plantations, even-aged high density management that maximizes total productivity is commonplace (Fox et al. 2007; Nyland 2007; Wear and Gries 2012). Rapid warming compared to historical trends and changes in precipitation associated with climate change will likely result in local seed sources of loblolly pine being increasingly less adapted for their local planting environment (Schmidtling 1994; Parry et al. 2007). Planting seedlings genetically adapted to future climate conditions has been proposed as a possible adaptation strategy for southeastern loblolly pine plantations to increase climatic resistance through increased drought resistance while maintaining growth and carbon sequestration potential (Johnsen et al. 2014).

In 2007 nearly all loblolly pine plantations in the Southeast were stocked with genetically improved seedling families and from 1968 to 2007 it was estimated that genetically improved loblolly pine plantations sequestered approximately 9865 Tg of carbon equaling to approximately 80% of total carbon sequestered by loblolly pine plantations over that time frame (Aspinwall et al. 2012). Identifying the genes responsible for traits and identifying ideotypes associated with increased growth and drought resistance could be informative for tree improvement programs (Martin et al. 2001; Nelson and Johnsen 2008; Isik 2014). Specific genes and ideotypes associated with drought tolerance (Koralewsk et al. 2013), wood strength (Koralewsk et al. 2013), and xylem formation (Palle et al. 2013) are already being identified. Associating specific genes and families with improved performance and climate resilience requires the investigation of physiological attributes and their interaction with the local environment. For example, Gonzalez-Benecke and Martin (2010) observed that under water limited conditions, loblolly pine families from a South Carolina seed source had lower stomatal conductance, greater stomatal control against water loss, and higher $\delta^{13}C$ and WUE than trees from a Florida seed source. Studies that investigate physiological indicators of drought tolerance at the family level can help assist land managers in making decisions regarding forest management practices and seedling deployment to better manage for increased carbon sequestration, growth, and climatic resilience. As more research about the interactive effects between management options and climate becomes available, programs such as PINEMAP and the PINEMAP Decision Support System (DSS) (Aldridge et al., 2014) that integrate interdisciplinary research with forest management will be critical in adapting forests to climate change.

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