Impacts of Drought and 40% Throughfall Reduction on Water Relations of a Longleaf Pine Stand

by

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Abstract

Projections of increased frequency and severity of climatic- induced drought have raised concerns about the health, productivity and composition of forests in the southeastern United States. Longleaf pine (*Pinus palustris* Mill.) is a native species that thrives on sites that are frequently disturbed by fire and is thought to be more tolerant of drought stress than other southern pines, making it a suitable species to withstand future climate conditions. However, the limits and mechanisms of that presumed drought tolerance are not known. *In-situ* manipulation of water availability was used in this study to examine the effects of drought on water relations in a longleaf pine plantation. Specifically, the effects of a 40% reduction in throughfall precipitation on leaf water potential, sap flux density, whole-tree hydraulic conductance, and productivity were investigated. Following the installation of the throughfall reduction treatment in May 2016, a prolonged natural drought occurred wherein little to no precipitation fell on the site from September 2016 until the end of November 2016. Throughfall reduction did not influence leaf water potential or productivity during the 2016 natural drought but did reduce sap flux density and whole-tree hydraulic conductance. As natural drought conditions alleviated in 2017, the 40% throughfall reduction treatment did reduce sap flux density, but did not significantly affect leaf water potential, whole-tree hydraulic conductance, or productivity. Longleaf pine survived severe water deficit and throughfall reduction by reducing transpiration but not growth, and thus may be a suitable species for drought prone sites.

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List of Abbreviations

^{12}C	Carbon-12 isotope
^{13}C	Carbon-13 isotope
A_{SURF}	Needle surface area
BA	Basal area
C	Soil capacitance
C_p	Specific heat of air
D	Vapor pressure deficit
DBH	Diameter at breast height
D_L	Needle diameter
δT	Temperature difference
δT_{max}	Maximum temperature difference
F	Geometrical factor
ha	Hectare
J	Energy, joules
J_v	Sap flux density
J_Σ	Sap flux density, daily total
K	Whole-tree hydraulic conductance
K_G	Dimensionless flux index (Grainier 1985)

L	Needle length
LAI	Leaf Area Index
min	Minute
n	Number of needles in fascicle bundle
NSC	Nonstructural Carbon
p	Density of air
PDSI	Palmer Drought Severity Index
R	Resistance
R'	Relative water content
SLA	Specific Leaf Area
SLW	Specific Leaf Weight
TDP	Thermal Dissipation Probe
TR_0	Ambient throughfall reduction treatment
TR_{40}	40% throughfall reduction treatment
V	Voltage at time t
V_f	Applied voltage
V_i	Starting voltage
WUE	Water Use Efficiency
α	Alpha level
$\delta^{13}C$	Molar abundance ratio of carbon-13 isotope
$\Delta\Psi$	Water potential gradient
ε	Dielectric permittivity
ε_0	Permittivity of free space
θ	Soil volumetric water content

θ_0 Ambient treatment soil volumetric water content

θ_{40} Throughfall reduction treatment soil volumetric water content

θ_5 Soil volumetric water content at 5 cm

θ_{15} Soil volumetric water content at 15 cm

θ_{50} Soil volumetric water content at 50 cm

θ_{100} Soil volumetric water content at 100 cm

Ψ_L Leaf water potential

Ψ_{MD} Midday leaf water potential

Ψ_{PD} Predawn leaf water potential

Ψ_{SOIL} Soil water potential

1 Introduction

1.1 Background

1.1.1 Longleaf Pine Ecosystem

Longleaf pine (*Pinus palustris* Mill.) once covered approximately 37 million ha of the Southeast [the majority of which was predominantly longleaf pine (Frost, 2006)], today longleaf pine forests have been reduced to 1.2 million ha (Van Lear *et al.*, 2005). Industry, agriculture, predation, and changes to ecosystem drivers in longleaf pine's natural range contributed to the reduction (Frost, 2006). Commonly used for turpentine and timber production, the majority of virgin forests had been impacted by commercial operations by the 1920's (Frost, 2006). As human populations dispersed and increased across the region, wildfire was reduced to protect property. Fire is a critical component to the longleaf pine ecosystem, and its removal from the landscape significantly decreased longleaf pine populations (Jose *et al.*, 2006). Currently, longleaf pine stands make up 2% of the timberland in the southeastern United States (Oswalt *et al.*, 2012).

An open canopy and a fire-regulated understory characterize this species. Typical longleaf pine stand structure requires frequent fire to maintain low, savannah-like densities (Gilliam & Platt, 1999). Longleaf pine is intolerant of competition, so regular burning is important to reduce encroaching hardwoods and other pine species (Boyer, 1990). Fire aids longleaf pine regeneration, as longleaf pine seeds require bare soil to germinate (Boyer, 1990).

Burning decreases ground cover, increasing the odds dispersed longleaf pine seeds will fall to favorable sites. As a disturbance mediated ecosystem, the continued presence and dominance of adapted species depends on the recurrence of conditions that make the species' normal range inhospitable to competitors. Without regular fire, the longleaf pine ecosystem is successional to a mixed-hardwood species composition (Van Lear *et al.*, 2005).

Interest in longleaf pine restoration has been increased over the last two decades (Van Lear *et al.*, 2005; McIntyre *et al.*, 2018). As much as 352,000 ha of longleaf pine have been planted in the Coastal Plain subregion, which the species formerly dominated (Jose *et al.*, 2006), and is where most of current longleaf pine forested area (89%) occurs (Oswalt *et al.*, 2012). In 2007, a coalition of private and public groups developed America's Longleaf Restoration Initiative (ALRI), which set a goal to increase longleaf pine coverage by 1.5 million ha by 2025 (McIntyre *et al.*, 2018). The longleaf pine forest type increased by 85,500 ha between 2010 and 2015, although those gains have been offset by decreases of longleaf/oak forest type (McIntyre *et al.*, 2018). Further, the longleaf pine ecosystem is valued for its plant and animal biodiversity (Outcalt, 2000). From a management perspective, the longleaf pine savannah is most importantly facultative habitat for the endangered red-cockaded woodpecker (*Leuconotopicus borealis* Vieillot). Thus, habitat alteration restrictions included in the Endangered Species Act have aided longleaf pine conservation efforts.

While much of the restoration interest is ecological, longleaf pine stands can yield economic value. Longleaf pine grows with good form, as it is self-pruning and grows straight, yielding long, clear boles that are highly valuable as pole timber (Boyer, 1990; Outcalt, 2000). Longleaf pine needles are often used in landscaping applications, providing mid-rotation income to landowners (Outcalt, 2000). Game species are provided with good habitat due to

planting density and the open understory of a well-managed longleaf pine stand (Outcalt, 2000). Therefore, longleaf pine restoration is beneficial to many different interest groups.

1.1.2 Climate Change and Drought

Increases in anthropogenic CO_2 since the Industrial Revolution have created alterations to climate patterns (IPCC, 2014). Through photosynthesis, southeastern forests remove CO_2 from the atmosphere and were estimated in 2010 to be a store of 12.4 billion tons of carbon (Wear & Gries, 2013). In the United States, carbon sequestration by forests offset an estimated 20% of carbon emitted between 2001 and 2005 (Lu *et al.*, 2015). The southeastern US is expected to lose between 4.5 million and 9.3 million ha (7% - 13%) of forested area to development over the next 40 years, with some sub-regions losing up to 21% (Wear & Gries, 2013). Planted pine is the only forest cover type that is expected to increase in area over the same time period. The forest industry contributed $230.6 billion to the economic output of the Southeast in 2013 and supports about 1 million jobs (Boby *et al.*, 2014). Nationally, the SE is a leading source of softwood timber. In 2007, 60% of all timber produced in the US came from the SE (Wear & Gries, 2013). As an important component of the economy and carbon emission mitigation, therefore it is vital to understand the potential effects that projections of future climate may have on these forest ecosystems.

Mild droughts can be common in the Southeast during late summer (Hanson & Weltzin, 2000). Tree ring analysis shows that long term (~20 years) droughts have occurred in the past 1000 years (Seager *et al.*, 2009). Although drought is a natural part of forest ecosystems, climate change will alter the typical disturbance regimes (Dale *et al.*, 2001). Peak summer temperatures in the Southeast are expected to increase by 0.5-1.5° C over the next two decades (IPCC, 2013). Additionally, summer precipitation in the region has become increasingly variable as rain

events have become less frequent and more intense (Wang *et al.*, 2010). During the last thirty years, the percentage of summer days without rain has increased from approximately 35% to 45%, while the percentage of days of high rain events (where more than 10 mm of precipitation fell in a day) increased from ~12% to 18% (Wang *et al.*, 2010). Changes in summer precipitation patterns may have considerable impacts on natural systems as it alters water availability during growing season (Wehner, 2004; Li *et al.*, 2007). Alterations to precipitation patterns and increases in temperature may lead to increased drought frequency.

1.1.3 Forest Response to Drought

Drought has been linked to tree mortality, and Allen *et al.* (2010) suggested that some forests may already be experiencing climate change related mortality events. In Texas in 2007, a 75% reduction of standard precipitation resulted in a severe year-long drought (Moore *et al.*, 2016). The widespread tree mortality that followed released substantial amounts of stored carbon, partially negating the mitigation value of the affected forests (Moore *et al.*, 2016). Trees that survive a drought may not recover to pre-disturbance levels of productivity, and therefore are increasingly vulnerable to mortality during subsequent droughts. For example, Berdanier & Clark (2015) reported that the majority of trees not able to return to pre-drought growth rates following a multiyear drought in North Carolina died during a subsequent drought a full ten years later.

The predicted rise in temperature will increase D, creating a greater evaporative demand on trees, which will further decrease available soil moisture (Will *et al.*, 2013). Vapor pressure deficit is a factor in stomatal closure and water usage, and thus important in understanding the response of trees to higher temperatures (McDowell *et al.*, 2013). Smaller trees may not survive low soil moisture, but larger trees with more established root systems may be less prone to

mortality (Dale *et al.*, 2001). Conversely, some literature suggests the crowns of larger trees (and the associated high water demand) make larger trees more susceptible to whole mortality than traditionally thought (Moore *et al.*, 2016).

The response of forests to climate change is the cumulative response of individuals to changes in their environment. If the individual can endure the changes it is considered "resistant" (Hodgson *et al.*, 2015). In plants, this is done by minimizing the damage the stress causes (Lambers *et al.*, 2008) or by limiting reductions in productivity (Mitchel 2016). If the plant species is not resistant but can avoid mortality, it has the potential to recover to pre-disturbance levels of productivity. The relative amount of time required to make that return is the "resilience" of that species (Mitchell *et al.*, 2016).

In light of the projected increase in temperatures over the coming decades, there has been interest in planting more drought-resistant species (Clark *et al.*, 2016). Tree ring chronologies show that longleaf pine growth more correlated with Palmer Drought Severity Index (PDSI) than temperature or precipitation alone, indicating that longleaf pine productivity is specifically impacted by drought and not just high temperatures or low precipitation (Henderson & Grissino-Mayer, 2009). However, like any tree species, longleaf pine is not completely immune to long-term water deficit effects. Drought can impact stomatal activity for as much as a year after the drought ends, altering carbon sequestration (Starr *et al.*, 2016).

1.1.4 Water Relations During Water Deficit

Leaf water potential is used as a measure of plant water status. As evapotranspiration occurs, water is moved from the soil through the hydraulic pathway of the tree and into the atmosphere through the stomata of the leaf (Lambers *et al.*, 2008). Water potential is by

convention a negative value, as it is hydrostatic pressure resulting from a suction tension rather than a positive pressure such as turgor (Lambers *et al.*, 2008). Therefore, in conventional terms, Ψ_L under well-watered conditions is "high" (i.e. negative, but relatively close to zero). When θ is low, it is more difficult for water to move from the soil matrix into the root of the plant (Lambers *et al.*, 2008). Under the same amount of evaporative demand, the resultant Ψ_L would be less (more negative) than the Ψ_L under well-watered conditions. Water potential is a valuable measure not only used to assess water status, but to describe a plant species' drought response and vulnerability to drought-induced mortality (McDowell *et al.*, 2008; Choat *et al.*, 2012; Klein, 2014; Steppe, 2018). Longleaf pine is commonly found on sandy or upland sites where water can be scarce, and it would therefore be expected that longleaf pine experiences low Ψ_L. However, in a study of mature (57-years-old on average) longleaf pine trees during drought in southwest Georgia, Ψ_L was as low as -0.8 MPa for predawn measurements and -1.7 MPa for midday measurements (Addington *et al.*, 2004).

By measuring water potential at its highest point (Ψ_{PD}) and at its lowest point (Ψ_{MD}), the gradient of xylem tension experienced by the tree during the day can be determined ($\Delta\Psi = \Psi_{PD} - \Psi_{MD}$). The $\Delta\Psi$ is useful for describing plant responses when soil water becomes less available (Meinzer *et al.*, 2014; Garcia-Forner *et al.*, 2015; Roman *et al.*, 2015). Across a season, Gonzalez-Benecke *et al.* (2011) reported a mean $\Delta\Psi$ in longleaf pine of 0.94 MPa. We observed similar values of $\Delta\Psi$, except for the period at end of the drought in 2016 when $\Delta\Psi$ declined sharply and subsequently recovered. The $\Delta\Psi$ becomes important as soil moisture decreases and/or D increases. If $\Delta\Psi$ becomes too large, the water within the plant's xylem can embolize, creating air bubbles that block the xylem and limit water transport further up the tree (Brodribb & Hill, 2000). Embolisms and their effects can accumulate, appreciably decreasing

the tree's ability to transport water with repeated drought exposure (Gaylord *et al.*, 2015). Stomatal closure in response to low Ψ_L is a strategy to avoid hydraulic failure but closure decreases photosynthesis and carbon accumulation (Brodribb, 2003). Lower carbon intake may lead to "carbon starvation" or contribute to a tree's mortality by predation or disease, even in drought-adapted pine species (McDowell *et al.*, 2008; Berdanier & Clark, 2015; Gaylord *et al.*, 2015; Anderegg *et al.*, 2016).

The ability to move water from the soil and out through the leaves is very important to the survival of the tree, so J_V, the amount of flow through a given area over a given area of time, is used to assess water movement (Vandegehuchte & Steppe, 2013). As J_V is measured continuously, it can be used to understand changes in plant water use throughout various time scales (day, season, year, etc.). In longleaf pine on sandy soil in Georgia and Florida, J_V averaged 1.9 mol m^{-2} s^{-1} at midday in trees ranging from 35 cm to 42 cm in diameter (Ford *et al.*, 2004; Gonzalez-Benecke *et al.*, 2011). Sap flux density is used with $\Delta\Psi$ to calculate K ,or the amount of water that flows through a given tree at a given time and xylem tension (Brodribb & Cochard, 2009). As embolisms accumulate, less water can flow, and K will be reduced. In temperate conifers, a 50% loss of the tree's intrinsic ability to move water (its hydraulic conductivity) can be lethal (Brodribb & Cochard, 2009; O'Brien *et al.*, 2017). Hydraulic conductance in longleaf pine has been estimated as approximately 0.4 mol m^{-2} s^{-1} MPa^{-1} when θ at 50 cm depth was less than 10% (Addington *et al.*, 2004, 2006; Gonzalez-Benecke *et al.*, 2011).

Low θ is a common result of prolonged drought. As θ declines, soil water becomes more unavailable to trees as Ψ_{SOIL} decreases. Trees will adjust their stomatal behavior on a physiological level to cope with less available water in an attempt to survive the drought. Trees

have two water-use strategies to resist effects of water deficit: either shut down or maintain stomatal conductance. Shutting down conductance (isohydric response) limits water loss, but also limits photosynthesis and carbohydrate production, thereby increasing the risk of carbon starvation (McDowell *et al.*, 2008; Allen *et al.*, 2010). Isohydric plants demonstrate increased stomatal sensitivity to changes in D in order to maintain a tolerable tension in the water column (Sperry & Love, 2015). Stomatal sensitivity to D is common in *Pinus* species (Addington *et al.*, 2004; Domec *et al.*, 2009; Poyatos *et al.*, 2013). Stomatal closure can alter the role of forests as a carbon sink, turning them into a source of carbon through respiration, and diminishing their mitigating benefits to CO_2 emissions (Starr *et al.*, 2016). Lower stomatal conductance means less carbon assimilation, and possibly reductions in productivity. Additionally, isohydric species have been observed allocating more carbon to non-structural carbohydrates (such as soluble sugars and starches) in the roots than to growth (Kannenberg *et al.*, 2017). Carbohydrate stores in stems are also used to compensate for lower C assimilation in isohydric species (Kannenberg *et al.*, 2017).

There have been several different ways of describing isohydric/anisohydric behavior (Tardieu & Simonneau, 1998; Klein, 2014; Maritnez-Vilalta *et al.*, 2014; Meinzer *et al.*, 2016; Hochberg *et al.*, 2017; Lavoie-Lamoureux *et al.*, 2017). A typical isohydric species response is to control stomata to strictly limit low xylem water potential to avoid embolism (Tardieu & Simonneau, 1998; McDowell *et al.*, 2008). Perfectly isohydric trees under drought will reduce stomatal conductance and decrease sensitivity to D as soil water potential declines (Roman *et al.*, 2015). Other definitions of isohydry also use leaf water responses to changes in available moisture in the environment. Definitions of isohydry include: the maintenance of constant Ψ_{MD} with decreasing θ (Tardieu & Simonneau, 1998), relatively small $\Delta\Psi$ (Klein, 2014), or

where the slope of $\Psi_{MD}/\Psi_{PD} < 1$ (Maritnez-Vilalta *et al.*, 2014; Meinzer *et al.*, 2016). It is expected that reductions in stomatal conductance will occur to prevent dangerously low Ψ_L, and $\Delta\Psi$ will decrease as soil water potential declines (Roman *et al.*, 2015). The risk accompanying isohydric strategy lies in carbon accumulation, as trees limit stomatal conductance and therefore photosynthesis and may face carbon starvation if the drought is prolonged (McDowell *et al.*, 2008). The anisohydric response to drought is typified by maintaining stomatal conductance and allowing $\Delta\Psi$ to increase with declining θ (McDowell *et al.*, 2008; Roman *et al.*, 2015). As with isohydry, there are numerous definitions of anisohydry. Anisohydric behavior has been defined as decreasing Ψ_{MD} with declining Ψ_{SOIL} (Tardieu & Simonneau, 1998), relatively large $\Delta\Psi$ (Klein, 2014), and where the slope of $\Psi_{MD}/\Psi_{PD} > 1$ (Maritnez-Vilalta *et al.*, 2014; Meinzer *et al.*, 2016). Stomata of anisohydric species are not particularly sensitive to changes in D (Franks *et al.*, 2007). The perfectly anisohydric tree would, under drought stress, risk hydraulic failure in the xylem for continued carbon accumulation (McDowell *et al.*, 2008).

It is important to state that most tree species do not fall neatly into one category or the other, but fall in a continuum of responses that individual species can express (Klein, 2014; Maritnez-Vilalta *et al.*, 2014; Martínez-Vilalta & Garcia-Forner, 2016). Additionally, the direct mechanisms of drought-related mortality are not known, and the paths to mortality suggested in McDowell et *al.* (2008) are not wholly accepted (Sala *et al.*, 2010). Other research puts the risks and rewards of each strategy into question. In studies of Western ecosystems under drought, stomatal conductance was not found to be different between classically isohydric and anisohydric species, indicating that neither would be more carbon limited during drought than the other (Garcia-Forner *et al.*, 2016a). The isohydric conifer species also showed more

evidence of embolism than the anisohydric species, despite tight control of Ψ_{MD}. Other comparisons have also shown little differences in growth rate between isohydric and anisohydric species during drought (Garcia-Forner *et al.*, 2016b). Others have suggested that stomatal regulation of Ψ_L cannot be used as an indicator of future drought mortality, as stomatal sensitivity is relative to the tensions an individual species is accustom to experiencing (Martínez-Vilalta & Garcia-Forner, 2016). Furthermore, some have suggested abandoning the isohydric/anisohydric concept altogether in favor of examining maximum transpiration, maximum K, and Ψ_L at hydraulic failure (Hochberg *et al.*, 2017).

Tree species that employ a more isohydric strategy can be disadvantaged when exposed to recurring drought as lower carbon accumulation can make less able to recover when drought conditions have abated, and less competitive for belowground resources (Salazar-Tortosa *et al.*, 2018). While pines are commonly considered isohydric (Zweifel *et al.*, 2009), xeric pine species have been observed using less-conservative water use strategies than mesic pine species, and have had higher growth and less mortality during drought (Salazar-Tortosa *et al.*, 2018)

1.2 Objectives

The primary objective of this study was to investigate the effects of increased drought severity (as compared to ambient conditions) on longleaf pine water relations and longleaf pine resilience. Changes in leaf water potential (Ψ_L), sap flux density (J_V), and whole-tree hydraulic conductance (K) will be monitored in response to environmental variables such as soil moisture (θ) and vapor pressure deficit (D). Responses of Ψ_L, J_V, and K to θ and D will be used to better understand the physiological mechanisms that may allow longleaf pine to survive prolonged drought, as well as the potential limits of those mechanisms. This information is valuable to land managers making decisions on existing mature stands, as well as selecting species for establishing new plantations. A 40% throughfall precipitation reduction treatment was applied to an 11-year old longleaf pine plantation located on well-drained soil to simulate drought on a site at the extreme fringes of drought vulnerability.

2 Impact of Drought and 40% Throughfall Reduction on Water Relations in a Longleaf Pine Stand

2.1 Introduction

Climate change is expected to increase the frequency and severity of droughts in the southeastern United States over the next half century (Wang *et al.*, 2010). Additionally, air temperatures are expected to increase by 0.5-1.5° C over the next twenty years (IPCC, 2013). The increase in temperature will likely increase evaporative demand and increase hydraulic stress on trees in the region (Will *et al.*, 2013). Pine trees make up a large part of the forested cover in the SE, and are an economically important source of timber for the region (Wear & Gries, 2013; Boby *et al.*, 2014) Increased climatic stress can lead to large mortality events, so it is important to understand the potential physiological responses trees might exhibit to avoid mortality (Moore *et al.*, 2016). Longleaf pine (*Pinus palustris* Mill.) is a native southern pine species that is commonly found on sandy, well drained sites and is thought to be more tolerant of drought than other southern pine species. While several studies have compared productivity and leaf physiology on mesic and xeric sites (Sheffield *et al.*, 2003; Addington *et al.*, 2006; Wright *et al.*, 2013), a controlled study of drought effects on longleaf pine in the field has not been done. To investigate the effects of prolonged periods of decreased water availability on longleaf pine, a 40% throughfall reduction treatment was installed in an 11-year-old longleaf pine plantation in western Georgia. To evaluate the effect of prolonged drought

on longleaf pine water relations, we monitored leaf water potential (Ψ_L), sap flux density (J_v), and whole-tree hydraulic conductance (K) for their response to vapor pressure deficit (D) and soil moisture (θ).

2.1.1 Objectives and Hypotheses

The overall objective of this research is to examine the effects of a 40% throughfall reduction on the recovery of longleaf pine leaf and whole-tree hydraulic parameters: Ψ_L, J_v, and K. Hypotheses to be tested include: (1) a 40% throughfall reduction will decrease (θ), leading to a decrease in predawn leaf water potential (Ψ_{PD}) and midday leaf water potential (Ψ_{MD}), and the gradient between Ψ_{PD} and Ψ_{MD} ($\Delta\Psi$) will be maintained through stomatal control of Ψ_{MD}, (2) the carbon limitation associated with an isohydric response to 40% throughfall reduction will decrease aboveground growth in longleaf pine, and (3) a 40% throughfall reduction will compound the effects of natural drought, leading to slower recovery of J_v and K following natural drought, thereby decreasing drought resilience. This study is part of a long-term study investigating longleaf pine growth and changes in climate.

2.2 Materials and Methods

2.2.1 Site Description

The study was established in an 11-year-old longleaf pine plantation in Marion County, Georgia (32.553° N, 84.476° W) owned by the Georgia Department of Natural Resources and managed by The Nature Conservancy (Figure 2.2.1). Planting was done in 2005, at an approximate spacing of 2.6 m x 2.6 m. Nature Conservancy employees performed a prescribed burned in January 2016. The site lies in the Sand Hills ecoregion, where soils are typically of the Entisol order (Griffith *et al.*, 2001). The study is located near the boundary between the Piedmont and eastern Coastal Plain subregions (Figure 2.2.1) (Wear & Gries, 2013). Soils are Lakeland Series (2-5% slopes), which are deep, sandy, and excessively drained Thermic, coated Quartzipsamments (Soil Survey Staff, 2016). Site elevation is 211 m.

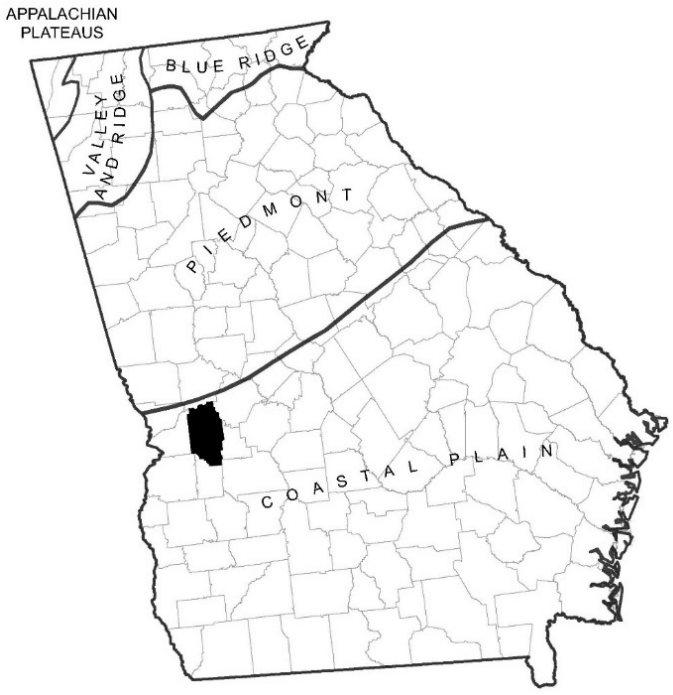

Figure 2.2.1. Research site location in Marion County, Georgia.

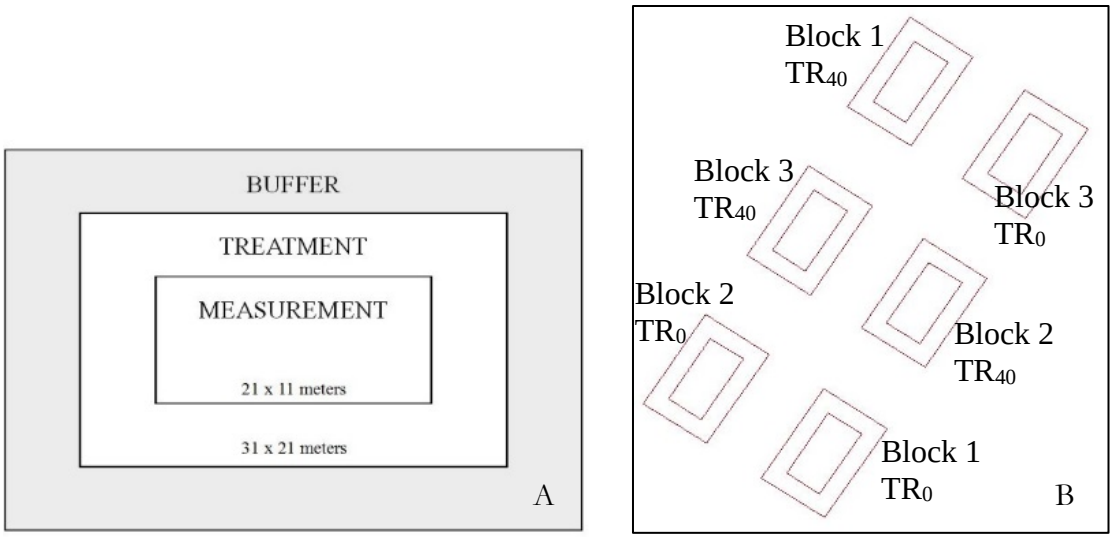

Figure 2.2.2. Nested treatment and measurement plot design (A) and plot layout (B).

The study design was a randomized complete block with two levels of throughfall treatment (TR): ambient throughfall (TR_0) and an approximate 40% reduction of throughfall (TR_{40}) replicated in three blocks. The 40% throughfall reduction represented the 1st quantile of annual precipitation for Americus, Georgia (32.065° N, 84.229° W; 60 km SE from site) over a 100-year period. The 0.07 ha treatment plots had approximately 17 m between plots (Figure 2.2.2). Approximately 70 tree were in each treatment plot. Intensively monitored measurement plots were located in the center 0.02 ha of each plot and encompassed about 25 trees. Blocks were based on similar pre-treatment basal area, which ranged from 17.0 to 20.4 $m^2\ ha^{-1}$ (Table 2.2.1). The size of the 0.02 ha measurement plot was determined by excavating and measuring one root from three trees adjacent to the project site. Roots extended an average of 4.5 m from the base of the tree. By increasing the treatment 5m in all directions

around the measurement plot, trees were not likely able to access water from outside of the treated area.

Rain exclusion trays were installed between tree rows to cover approximately 40% of the total plot area in the TR_{40} treatment. Trays were constructed at an average height of 1 m using 12-mil polyethylene sheeting (Polyscrim 12, Americover Inc., Escondido, California) and pressure treated lumber. Two 0.52 m wide exclusion troughs were built between tree rows, with a 0.48 m gap in-between to reduce soil-moisture banding. Sixteen troughs were installed per plot. Throughfall precipitation was intercepted by trays and collected in large corrugated pipes that carried water a minimum of 20 m off-plot.

Table 2.2.1. Pre-treatment characteristics of measurement plots (n=6) in an 11-year-old longleaf pine plantation. No pretreatment differences were detected.

Block	Treatment	BA ($m^2\,ha^{-1}$)	Density (stems ha^{-1})	Mean DBH (cm)	Mean Height (m)
1	TR_0	20.4	1000	15.9	9.7
	TR_{40}	19.8	1130	14.7	9.3
2	TR_0	15.0	957	13.9	9.6
	TR_{40}	17.0	1000	14.4	9.1
3	TR_0	19.4	1130	14.5	9.6
	TR_{40}	17.5	957	15.0	9.6

2.2.2 Climate

A weather station (6152 Vantage Pro 2 Wireless Weather Station, Davis Instruments, Vernon Hills, Illinois) was used to measure air temperature, wind-speed, and precipitation at thirty-minute intervals for the entirety of the study (Table 2.2.2). The station was set in a 0.65 ha clearing 500 m east of the project site, allowing unobstructed collection of precipitation data. Within the forest canopy, relative humidity and air temperature were measured approximately 2 m high at three locations in-between adjacent plots (HOBO U23 Pro v2

Temperature/Relative Humidity Logger, Onset Computer Corporation, Bourne, Massachusetts).

Table 2.2.2. Measurement frequency, start date, and end date of variables measured during the study. See Table 2.2.3 for specific leaf water potential (Ψ_L) and leaf area index (LAI) measurement dates.

Variable	Frequency	Duration
Canopy Air Temperature	30 mins	April 2016 - September 2017
Canopy Relative Humidity	1 min, averaged and recorded 30 mins	September 2016 - September 2017
Climate	30 mins	April 2016 - September 2017
Growth	Annually	February 2016 - January 2018
LAI	3-4 weeks	February 2017 - September 2017
Precipitation	30 mins	April 2016 - September 2017
Sap Flow	1 min, averaged and recorded 30 mins	June 2016 - September 2017
Sapwood Area	Monthly	June 2016 – September 2017
Soil Moisture	1 min, averaged and recorded 30 mins	June 2016 - September 2017
Specific Leaf Area	Once	November 2016
Whole-Tree Hydraulic Conductance	3-4 weeks	July 2016 - September 2017
Wind Speed	30 mins	April 2016 - September 2017
$\delta^{13}C$	Once	November 2016
Ψ_L	3-4 weeks	June 2016 - September 2017

2.2.3 Soil Moisture

Soil volumetric water content (θ) was used to quantify the amount of water available to the study trees. We installed probes in every plot at three depths: 5 cm, 15 cm, and 50 cm. In every plot, probes were installed in the center of a row, near the center of the plot. Additionally, two plots in one block had a probe inserted at 100 cm. Probes in the treatment plots were placed under the rain exclusion trays to validate that throughfall reduction treatment was effective.

Soil volumetric water content was measured continuously and recorded every 30 minutes in all plots using 10 cm probes (10HS Large Soil Moisture Sensors, S-SMx-M005, Decagon Devices, Inc., Pullman, Washington) inserted at 5 cm, 15 cm, 50 cm, and 100 cm, and data loggers (HOBO Micro Logger Station Data Logger, Onset Computer Corporation, Bourne, Massachusetts). The probes generate an accumulating electromagnetic field in the soil and measure the time required to a reach a particular charge ('10HS Soil Moisture Sensor Manual', 2016). That duration relates to the capacitance of the soil (C) (Equation 1), which in turn relates to the dielectric permittivity (ε) of the soil between the two prongs of the sensor which is analogous to θ (Equation 2) as follows:

$$t = RC\ ln\left[\frac{V - V_f}{V_i - V_f}\right]$$

Equation 1

where R = resistance, V = voltage at time t, V_i = starting voltage and V_f = applied voltage and:

$$C = \varepsilon_0 \varepsilon F$$

Equation 2

where ε_0 is the permittivity of free space and F is a geometrical factor.

The soil moisture sensors that were placed under a throughfall reduction tray measured a drier θ than what the entire plot would have experienced (only 40% of the plot area was covered by trays), and only represent a point measurement of soil moisture. Therefore, the actual θ for the entire treatment plots was better represented by a weighted average of θ. Daily plot averages of θ were calculated in both TR_0 and TR_{40}. Then, by block, a weighted average was made to represent plot-level water potential using ambient treatment volumetric water content (θ_0) and throughfall reduction volumetric water content (θ_{40}):

$$plot - level\ \theta = (0.6 \times \theta_0) + (0.4\ \times \theta_{40})$$

Equation 3

2.2.4 Sap Flux Density

A Dynamax sap flow system with 30 mm thermal dissipation probes (TDP) (TDP-30, Dynamax, Inc., Houston, Texas) was used to determine tree sap flow in four or five trees per measurement plot (30 trees total) (FLGS-TDP Sap Velocity System Model XM1000, Dynamax, Inc., Houston, Texas). Thermal dissipation probes monitor temperature differences between a heated probe and a reference probe (dT). Probes were installed into selected trees at DBH and reflecting wrap was placed around trees to prevent stem heating. Sap flow trees were selected using the quantiles of total method (giving more weight to larger trees), to accurately represent the range of tree basal area in the plot, making the eventual scaling up of the sap flow data more representative of the stand (Martin et al., 1997; Čermák et al., 2004). The quantile of totals method selects trees that represent a portion of a desired biometric parameter; here plot basal area was used.

As water in the xylem flows across the heated probe, heat is carried away from the area around the heated probe, and the difference in temperatures between the heated and reference probe is reduced (Lu *et al.*, 2004). A relatively large difference in temperature between the two probes indicates a low amount of transpiration. When transpiration is low or zero, the difference in temperatures is at its maximum, as there is no movement in the water column to carry heated water away from the heated probe. The heat from the heated probe will radiate outwards and warm the reference probe. At night, once the trees have refilled and water has stopped moving through the tree, dT should theoretically be at its maximum (dT_{max}). However, this was often not the case. To correct instances where dT_{max} was not attained at night, dT_{max} was set by using Baseliner, an open-source software for processing sap flow data (Oishi *et al.*, 2016). To make the correction, a regression line was plotted across a graph of several days' worth of sap flow data. The Baseliner software uses user-defined dT_{max} thresholds to set points for the regression. Granier (1987) showed that dT and dT_{max} were related to a dimensionless parameter K_G (Equation 4) and that K_G could be used to calculate sap flux density (J_v) (Equation 5).

$$K_G = \frac{dT_{max} - dT}{dT}$$

Equation 4

$$J_v = 119 \times 10^{-6} \, K_G^{1.231}$$

Equation 5

2.2.5 Leaf Water Potential and Whole-Tree Hydraulic Conductance

Leaf water potential of sap flow trees was measured every three or four weeks from June 2016 until September 2017 (Table 2.2.3) using a 1505D Pressure Chamber Instrument

(PMS Instruments, Albany, Washington). We collected leaf predawn water potential samples before sunrise and transpiration began, when Ψ_{PD} should be in equilibrium with the water potential of the soil (Ψ_{SOIL}). Midday leaf water potential samples were collected during the middle of the day (1100–1300 EST). Until February 2017, four samples from each one of the sap flow trees (24 trees total) were measured. To aid in sample collection, permanent scaffolding was installed in February 2017. After installation, five samples were collected from at least two sap flow trees per plot, with additional samples taken from a third tree when available (19 trees total). Samples were taken from the most recent mature flush of tufts from as high in the canopy as possible, approximately 8 m high.

Once removed from the tree, samples were placed in a sealable plastic bag containing a moist paper towel and put into a cooler until measured to ensure sample integrity (Boyer, 1995). For each measured tree, the difference between the two water potential measures ($\Delta\Psi = \Psi_{PD} - \Psi_{MD}$) was used in conjunction with J_v (mol m^{-2} s^{-1}) to calculate whole-tree hydraulic conductance, K (mol m^{-2} s^{-1} MPa^{-1}):

$$K = \frac{J_v}{\Psi_{MD} - \Psi_{PD}}$$

Equation 6

Table 2.2.3. Leaf water potential (Ψ_L) and leaf area index (LAI) measurement dates.

Measurement	Year	Dates
Ψ_L	2016	7/20, 8/15, 9/1/, 9/22, 10/13, 11/3, 11/22, 12/7
	2017	2/17, 3/28, 4/18, 5/9, 6/8, 6/28, 7/19, 8/16, 9/8
LAI	2017	2/10, 2/23, 3/27, 4/17, 5/8, 6/7, 6/27, 7/19, 8/16, 9/7

2.2.6 Leaf $\delta^{13}C$ and Specific Leaf Area

Leaf $\delta^{13}C$ was measured on foliage of sap flow trees. Leaf $^{13}C/^{12}C$ fractionation is the preferential selection of the lighter ^{12}C isotope over ^{13}C by Rubisco (Lambers *et al.*, 2008). Rubisco reacts more easily with ^{12}C and will use it more readily than ^{13}C. Rubisco reacts more easily with ^{12}C, and will use it more readily than ^{13}C. However, for isohydric plants, as stomatal closure increases during drought photosynthesis (A) decreases and carbon becomes less available. Consequently, less ^{12}C is available and more ^{13}C will have to be used, despite the lower reactivity with Rubisco and the relative amount of ^{13}C fixed in the leaf increases. A higher proportion of ^{13}C in the plant is therefore an indicator of drought stress. The ratio between ^{13}C and ^{12}C is referred to as the molar abundance ratio (R). The R of a foliage sample was compared the R of a fossil standard to calculate $\delta^{13}C$:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

Equation 7

In October 2016, foliage samples were collected from sunlit foliage in the upper canopy of all sap flow trees and stored on ice until dried at 70° C for 72 hours. Samples were

then finely ground and sent to The University of Arkansas Stable Isotope Laboratory (2016) for $^{13}C/^{12}C$ fractionation analysis.

Length and diameter of five fascicle bundles per sap flow tree were also measured in October 2016. The same tufts that provided samples $^{13}C/^{12}C$ fractionation analysis were used for length and diameters samples. Needle surface area was calculated using:

$$A_{SURF} = \sum_{i=1}^{n} L_i \left(D_L + \frac{\pi D_L}{n} \right)$$

Equation 8

where A_{SURF} = surface area, L = needle length, D_L = needle diameter, and n = number of needles in the fascicle (however, only fascicle bundles with three needles were used). Needles were dried in a 70° C oven for 72 hours, and then weighed. Specific leaf area (SLA) was calculated as the ratio of needle surface area to mass.

2.2.7 LAI and Growth

Treatment effects on leaf area index (LAI) were measured at least once a month (

Table 2.2.2) (LAI-2200C Plant Canopy Analyzer, LI-COR Inc., Lincoln, Nebraska). The LAI-2200C works by comparing the amount of light available to the sensor head under the tree canopy to unobstructed light monitored by a second sensor in an opening (Welles & Cohen, 1996). Five circular optical sensors within each sensor lens detect a conical segment of light (angled 7°, 23°, 38°, 53°, and 68° from the sensor, respectively) that (in the case of the sensor under the canopy) was partially obscured by vegetation.

Measurements in each plot were taken along three transects (approximately 5 m), each starting from the northern side (facing south) and moving diagonally from one row to the adjacent row, ending at nearby tree. Transects varied in length (as the distance from the northern side of the measurement plot to the ending tree in the adjacent row varied). Transect length was divided evenly into five LAI measurement points. In the event a measurement point occurred too close to the scaffolding, that data point was removed from further calculations.

The above-canopy light measurements were automatically collected every 15 seconds from a stationary sensor placed in an opening (the same 0.65 ha clearing 500 m east of the project site where climate data was collected). The sensor was set up far enough away from the tree line to ensure an accurate measurement of above-canopy light conditions. The below-canopy measurements were paired to the open sky measurements using FV2200 v2.0 Software provided by the manufacturer. The software removed data collected by the fifth ring (68°) sensor because it sampled light from parts of the canopy beyond the measurement plot.

We conducted pre-treatment inventory on all plots in the study in 2016. Height (m), crown width (m), and DBH (cm) were measured on every tree within the treatment plot (Table 2.2.1). Annual tree growth was measured to assess productivity and recovery. Height was measured using a laser hypsometer (TruPulse 200, Laser Technology Inc., Centennial, Colorado).

2.2.8 Statistical Analysis

The study was a randomized, complete block design with one treatment (ambient throughfall versus 40% reduction throughfall) replicated in three blocks. Individual leaf water potential measurements (Ψ_{PD} and Ψ_{MD}) were averaged by tree. Because the experimental unit

was the block, variables were averaged by plot across the four trees. Repeated measurements were dependent; therefore, to avoid pseudoreplication, treatment effects were tested using repeated measures ANOVA with block as the random effect (PROC MIXED, SAS Institute Inc., Cary, North Carolina). Covariance structure for each variable was determined by minimizing the Akaike Information Criterion (AIC). For non-repeated measurements, ANOVA was used to test for treatment effects (PROC GLM, SAS Institute Inc., Cary, North Carolina), and were considered significant at $\alpha = 0.05$. Relationships between hydraulic parameters and soil moisture and precipitation were examined using linear and nonlinear regression analysis.

2.3 Results

2.3.1 Climate

During 2016, the site experienced an extreme drought. Beginning September 11, there were 78 continuous days without appreciable rainfall, until rain returned at the end of November (Figure 2.3.1). Mild drought conditions (-1.00 ≥ PDSI ≥ -1.99) were present in the region beginning in March and had increased to severe drought (-3.00 ≥ PDSI ≥ -3.99) by August (Figure 2.3.2). Extreme drought conditions (PDSI ≤ -4.00) were present October through December 2016. Monthly sums of precipitation were low in September, October, and November, with the majority of rainfall occurring in a few large events rather than being spread evenly through multiple events throughout the respective months (Figure 2.3.3). Daily average D ranged from 0.03 KPa in December 2016 to 1.9 KPa in June 2016 (Figure 2.3.3). Total precipitation during the 2016 measurement period was 489.7 mm, and air temperatures ranged from 38.6 °C in June to -4.1 °C in December (Figure 2.3.4).

By January 2017, the extreme drought conditions decreased to moderate drought conditions (Figure 2.3.2). Drought intensified to severe drought in February, when monthly precipitation fell below the 30-year average. Severe drought persisted until May, when above-average precipitation occurred and PDSI increased to an incipient wet spell (0.50 ≤ PDSI ≤ 0.99). By June, PDSI had elevated to slightly wet conditions (1.00 ≤ PDSI ≤ 1.99). The PDSI continued to increase through the end of the measurement period, at which point conditions were moderately wet (2.00 ≤ PDSI ≤ 2.99). Total precipitation during the 2017 measurement period was 1029.2 mm (Figure 2.3.1). Air temperatures during the study in 2017ranged from 35.8 °C in July to -8.7 °C in January and averaged 20.0 °C (Figure 2.3.4). Daily average D during ranged from a low of 0.05 KPa in January to a high of 1.6 KPa in May (Figure 2.3.3).

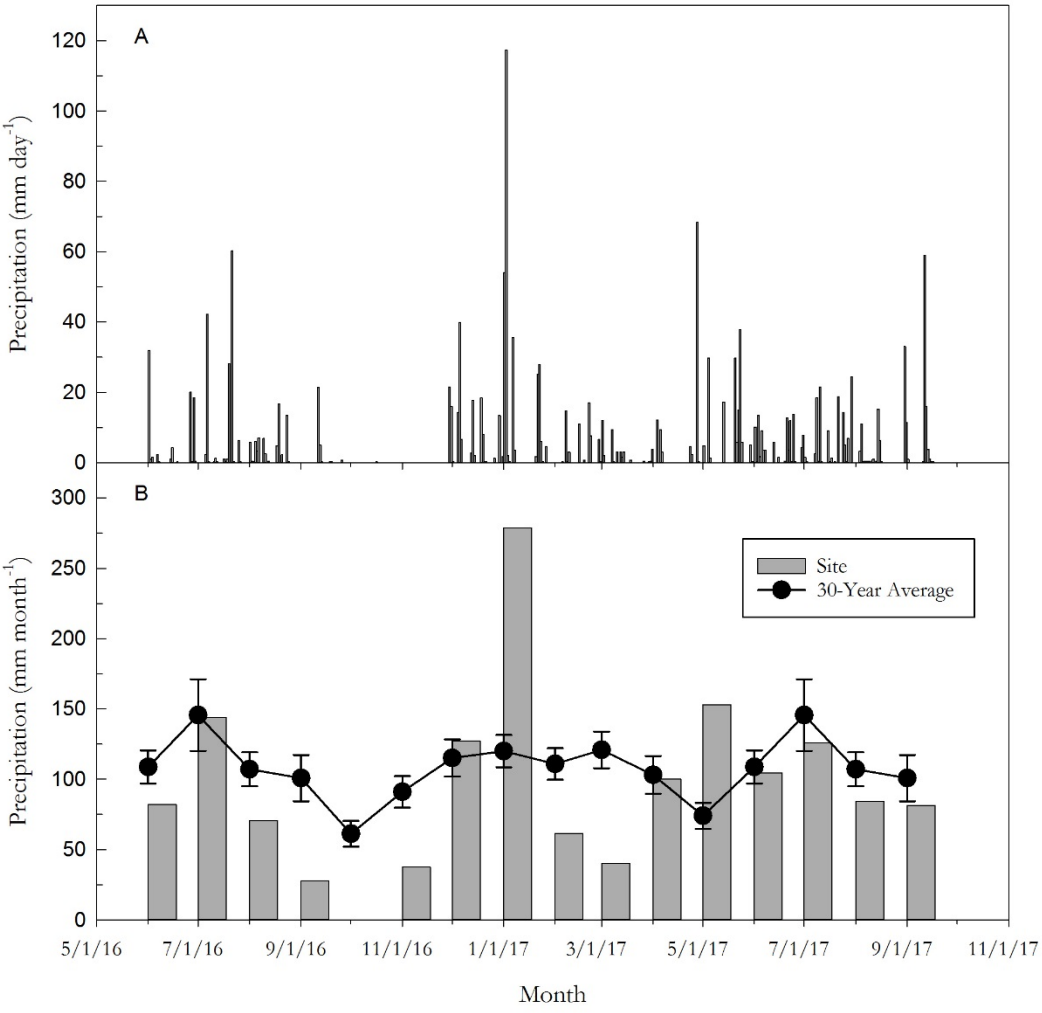

Figure 2.3.1. Daily (A) and monthly (B) total precipitation at the study site in Marion County, Georgia from June 2016 to September 2017. Thirty-year averages (1986-2017) of monthly summed precipitation were calculated using data from nearby Americus, Georgia. Source: NOAA National Centers for Environmental Information. http://www.ncdc.noaa.gov/cag/

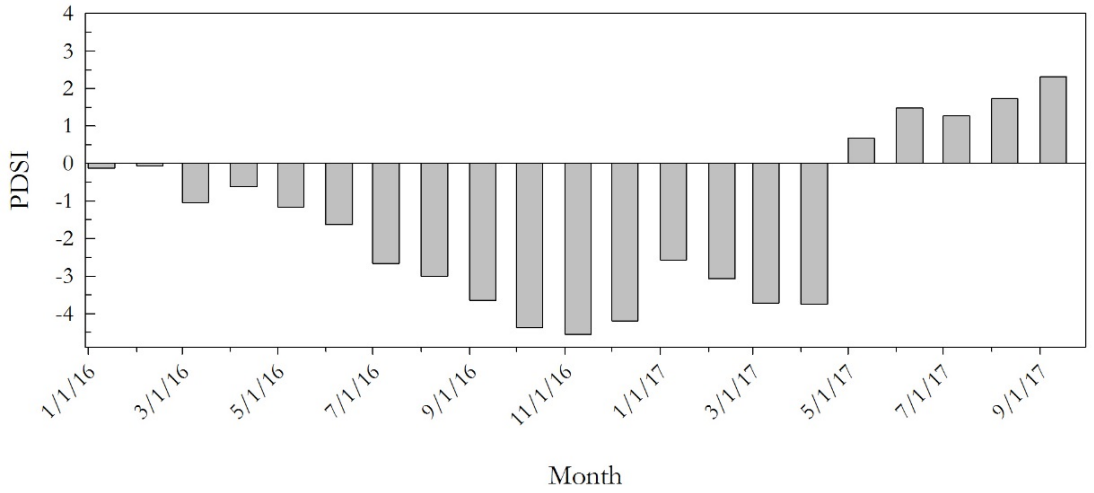

Figure 2.3.2. The Palmer Drought Severity Index (PDSI) for west central Georgia (Climate Division 4) from January 2016 to September 2017. Source: NOAA National Centers for Environmental Information. http://www.ncdc.noaa.gov/cag/

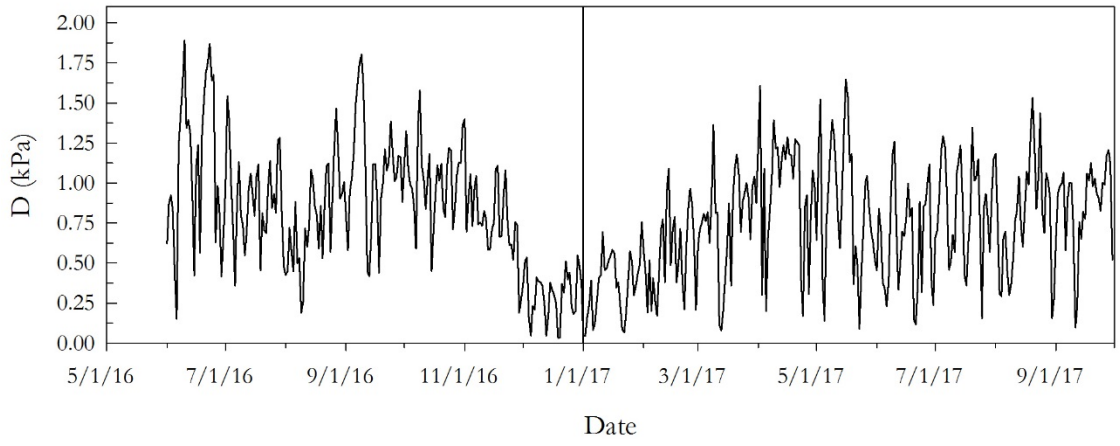

Figure 2.3.3. Daily average vapor pressure deficit (D) at the study site in Marion County, Georgia from June 2016 to September 2017.

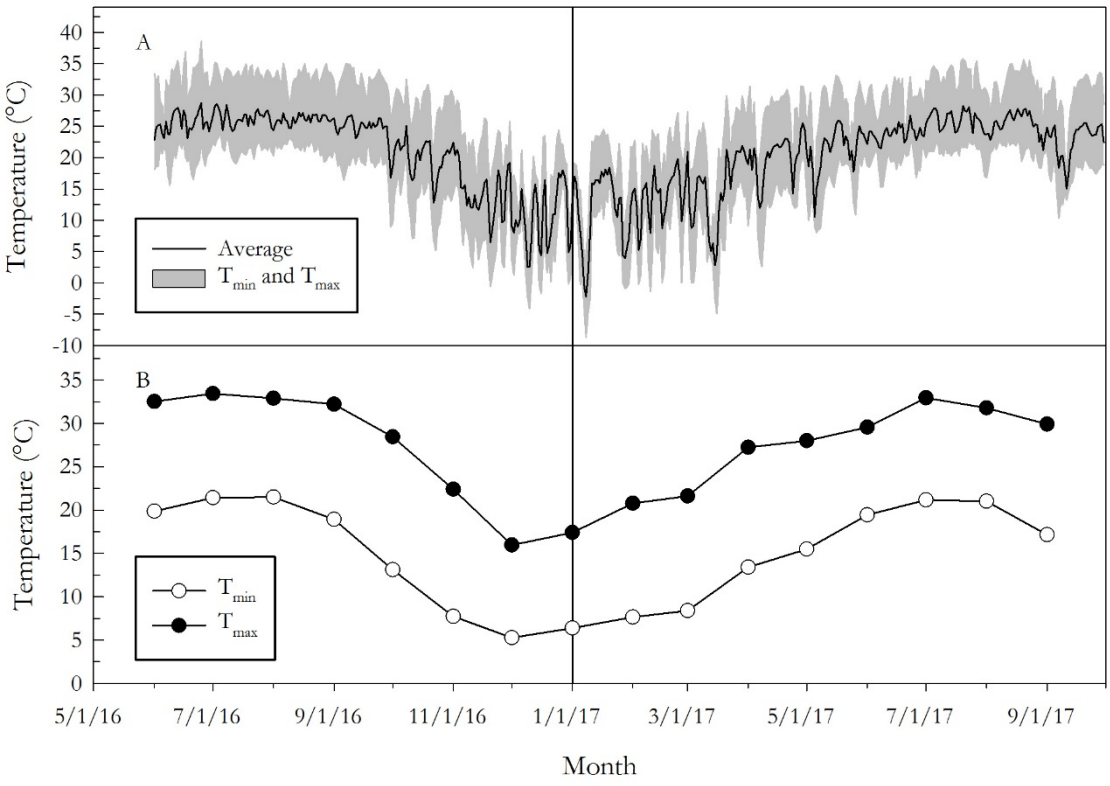

Figure 2.3.4. Daily (A) and monthly (B) minimum (T_{min}), maximum (T_{max}), and mean temperatures at the study site in Marion County, Georgia from June 2016 through September 2017.

2.3.2 Soil Moisture

The lack of precipitation in 2016 affected θ in both treatments. Independent analysis of soil water-retention properties for soil at the site estimated the permanent wilting point at 3.1% (Tom Stokes, personal communication). In both treatments, point and plot-level θ declined to <3% during the drought at all depths (5 cm, 15 cm, 50 cm, and 100 cm), and remained low until precipitation resumed in late November (Figure 2.3.5, 2.3.6). Throughfall reduction trays reduced θ directly under the trays (Figure 2.3.5) and were effective enough to decrease plot-level θ (Figure 2.3.6). Plot-level θ at 5 cm and 15 cm depths in TR_{40} experienced extended periods below the permanent wilting point as early as 20 days before TR_0, and declined to minimum drought-period soil moisture two days before TR_0 (Figure 2.3.6). At the 50 cm depth, plot-level θ in TR_0 declined to the permanent wilting point two days before TR_{40}. It took longer for plot-level θ at 50 cm to recover from the period without precipitation than θ at shallower depths. Volumetric soil moisture at 5 cm and 15 cm depths exceeded the permanent wilting point the same day as the first rain on November 28. At 50 cm, plot-level θ increased past the permanent wilting point on December 4 in TR_0 and December 5 in TR_{40}.

Across all blocks, average plot-level θ in TR_0 during the 2016 measurement period was 4.6%, 4.1%, and 2.8% for 5 cm, 15 cm, and 50 cm depths, respectively (Figure 2.3.6). In TR_{40} in the 2016 measurement, plot-level θ averaged 4.1%, 3.7%, and 2.8% at 5 cm, 15 cm, and 50 cm depths respectively (Figure 2.3.6). During the 2017 measurement period, plot-level θ at 5 cm, 15 cm, and 50 cm averaged 7.2%, 6.8%, and 4.3%, respectively, in TR_0 and 5.9%, 5.7%, and 4.1%, respectively, in TR_{40}.

Point θ (under the trays in TR_{40} plots) also indicate an effect of throughfall reduction on θ once precipitation resumed in November 2016. At 5 cm and 15 cm depths, TR_{40} soils were

slower to show increases in point θ and usually did not reach as high of θ as soils in TR_0 (Figure 2.3.5). When θ in TR_{40} was scaled to the entire plot, differences are less pronounced but were still present (Figure 2.3.6). Often peaks in point θ following precipitation events were higher in TR_0 plots than in TR_{40}. This was especially true at 15 cm in 2016, where point θ had a range of 10.3% in TR_0 plots but only 7.7% in TR_{40} plots.

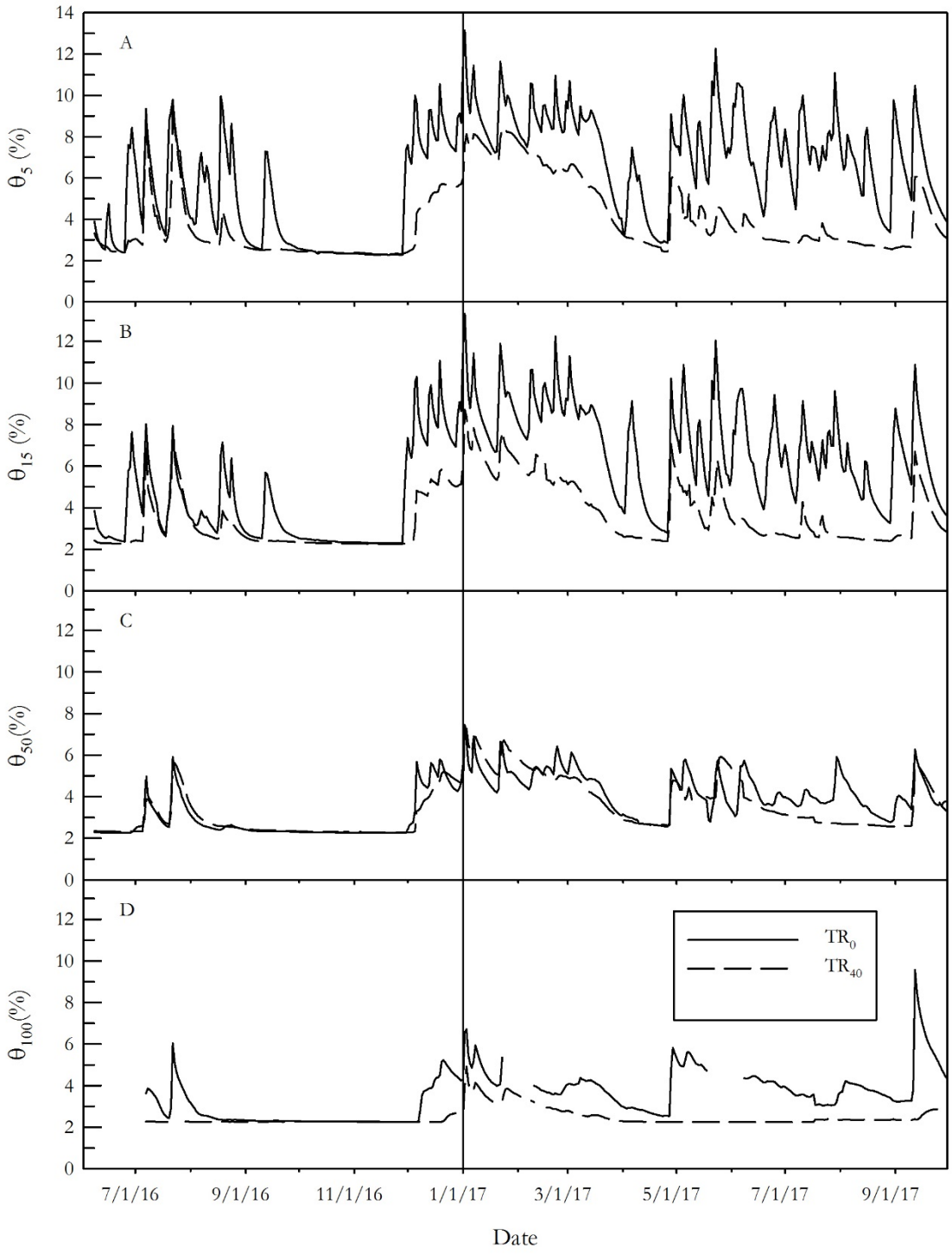

Figure 2.3.5. Mean daily soil moisture (θ) at 5 cm (A), 15 cm (B), 50 cm (C), and 100 cm (D) depths in response to throughfall treatment (TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017.

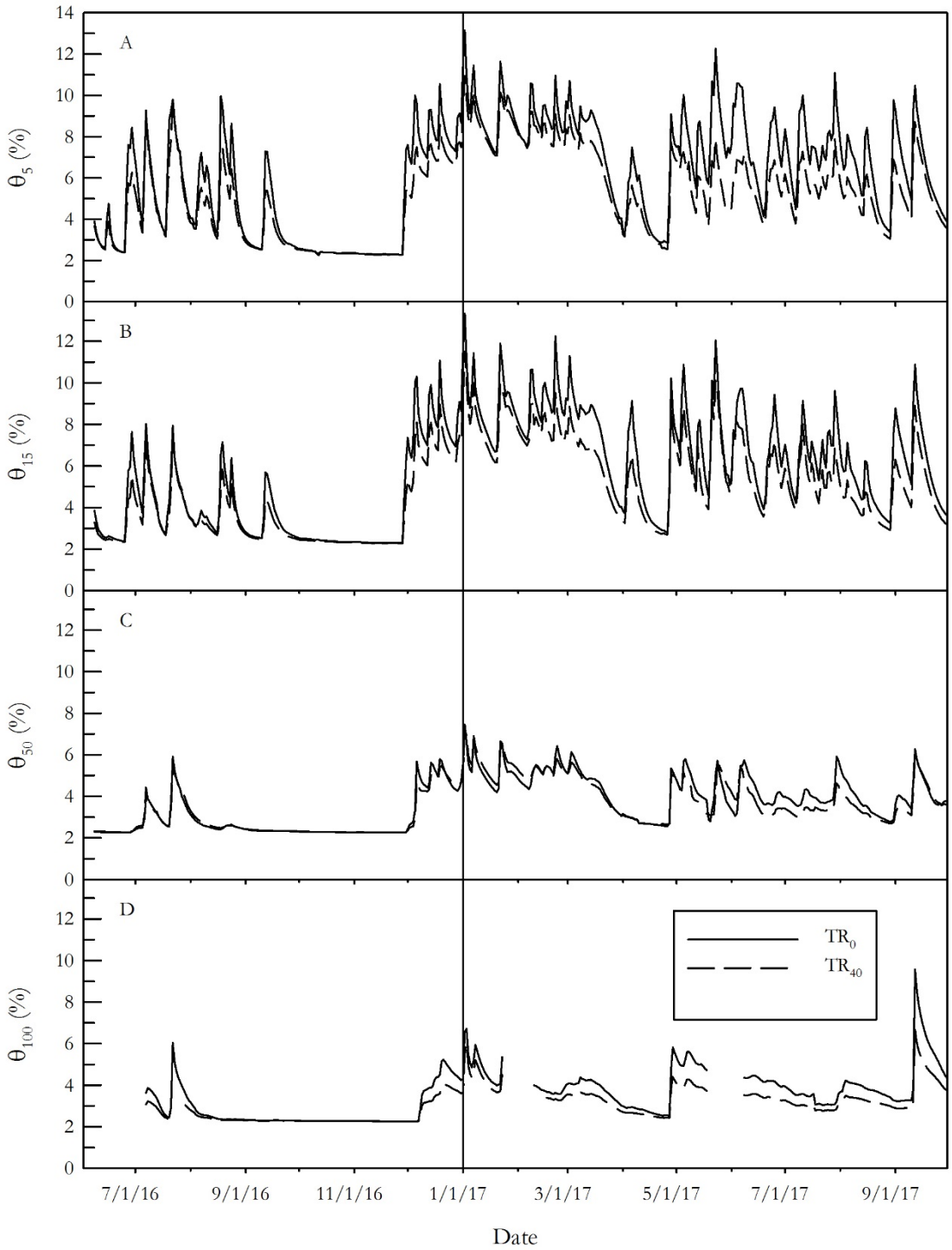

Figure 2.3.6. Mean daily soil moisture (θ) at 5 cm (A), 15 cm (B), 50 cm (C), and 100 cm (D) depths in response to throughfall treatment (TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017. Soil moisture in TR_{40} has been scaled to plot-level.

2.3.3 Water Potential

No significant treatment differences or treatment by date interactions were detected for Ψ_{PD}, Ψ_{MD}, and $\Delta\Psi$ in 2016 and 2017 (Figure 2.3.7). In 2016 during the drought, predawn water potential declined in response to lack of precipitation and reduced soil moisture. The highest average Ψ_{PD} of 2016 was -0.8 MPa, and average Ψ_{PD} eventually declined to as low as -3.2 MPa during the drought. Minimum Ψ_{PD} in 2016 was much lower than the minimum Ψ_{PD} in 2017, which was -1.3 MPa. Midday water potential followed the same pattern as Ψ_{PD}, declining in 2016 from a high of -1.6 MPa to a low of -4.1 MPa as the drought persisted. Minimum Ψ_{MD} was markedly higher in 2017 (-2.1 MPa), however the maximum Ψ_{MD} was comparable (-1.4 MPa). The $\Delta\Psi$ ranged from 0.0 MPa (when J_v was near-zero) to 1.5 MPa in 2016. In 2017, $\Delta\Psi$ was as low as 0.2 MPa (when trees were returning to normal water potential gradients early in the year) and as high as 1.3 MPa.

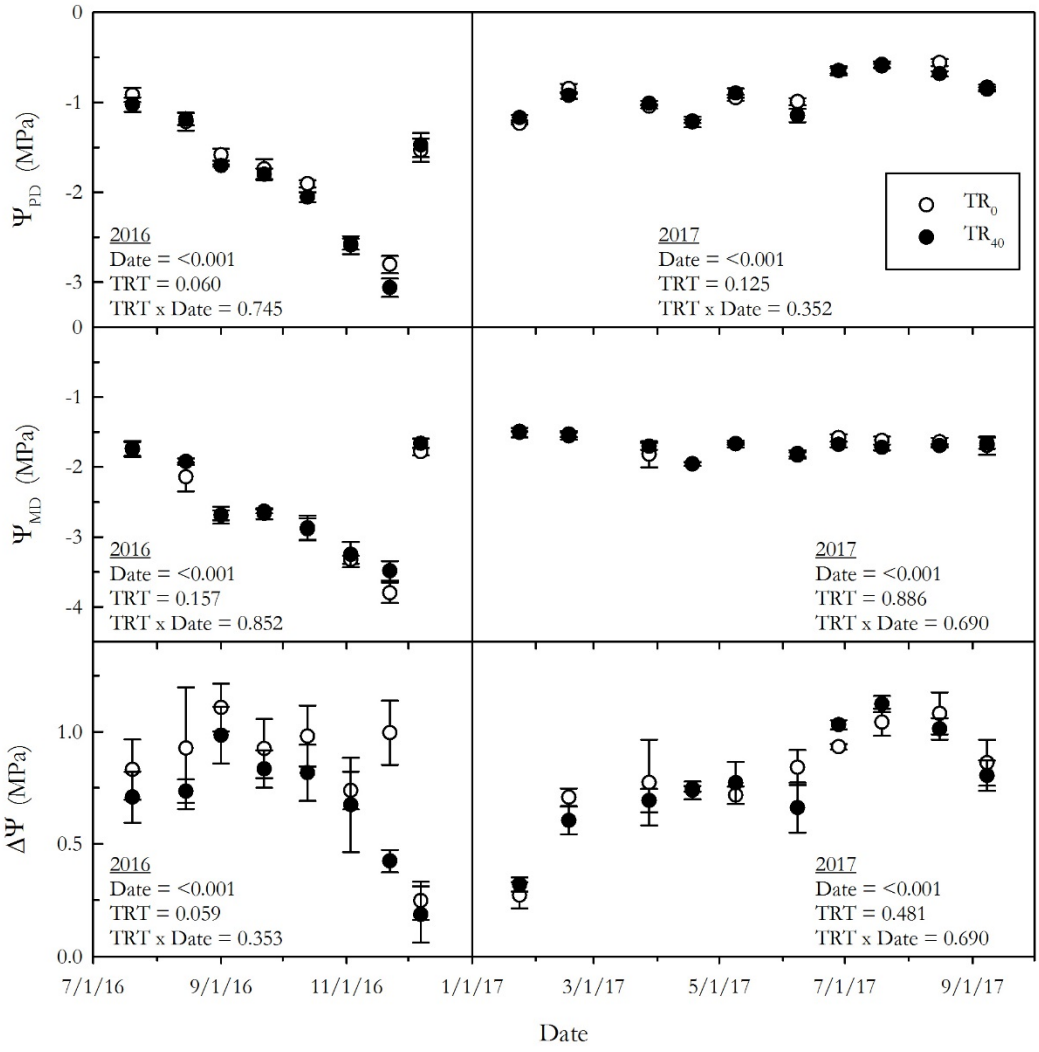

Figure 2.3.7. Mean (± SE) predawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf water potential and the difference between the two ($\Delta\Psi$) in response to throughfall treatment (TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017. Observed probability values for treatment and date effects are shown by year.

Relationships between abiotic variables and Ψ_L were examined, and variation in Ψ_{PD} was best explained by soil moisture at 5 cm depth (θ_5) using a saturation curve ($y = B_1 x/(x + B_2)$), with 71% of the variation in Ψ_{PD} explained by θ_5 (Figure 2.3.8). Tests of the model coefficients fit by plot indicated that throughfall reduction did not affect the response of Ψ_{PD} to θ_5 (B_1, P = 0.423; B_2, P = 0.423). Predawn water potential was unresponsive to declining θ_5 until θ_5 reached 2.7%, at which point Ψ_{PD} and θ_5 became positively related.

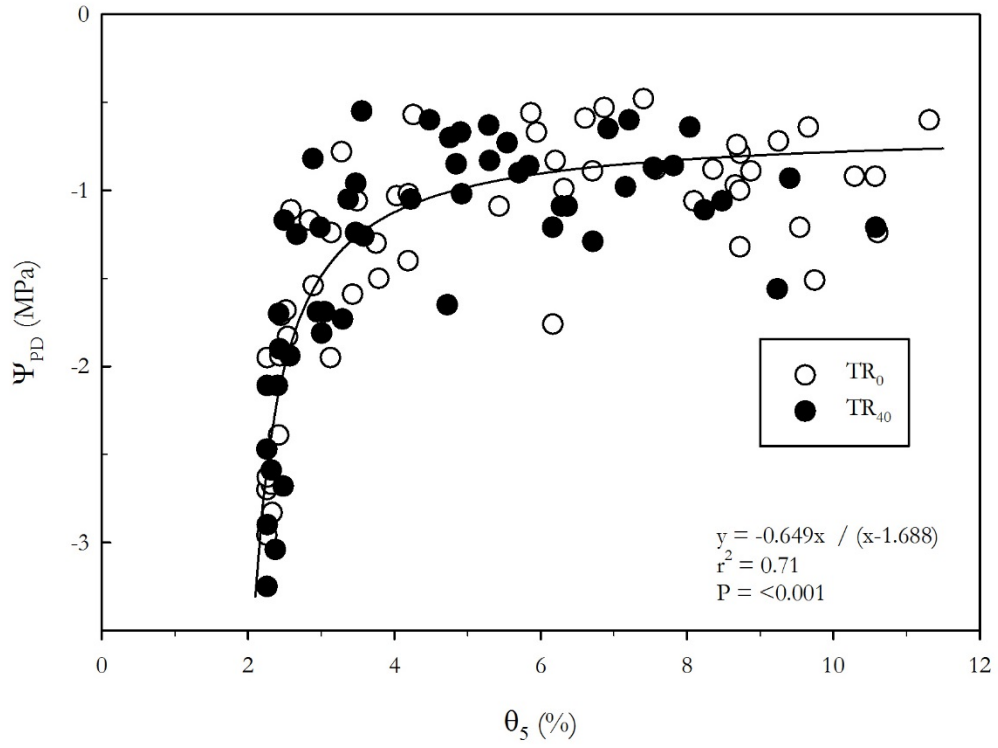

Figure 2.3.8. Predawn leaf water potential (Ψ_{PD}) response to soil moisture (θ) at 5 cm in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017.

Midday leaf water potential was also related (r^2=0.63) to θ_5 (Figure 2.3.9). A power function ($y = B_1(x^{B_2})$), rather than a saturation curve, provided the best fit. No treatment differences in the B_1 (P = 0.089) or B_2 (P = 0.077) coefficients were detected. Midday water potential was unresponsive to θ_5 until θ_5 declined to <4%, then Ψ_{MD} declined rapidly.

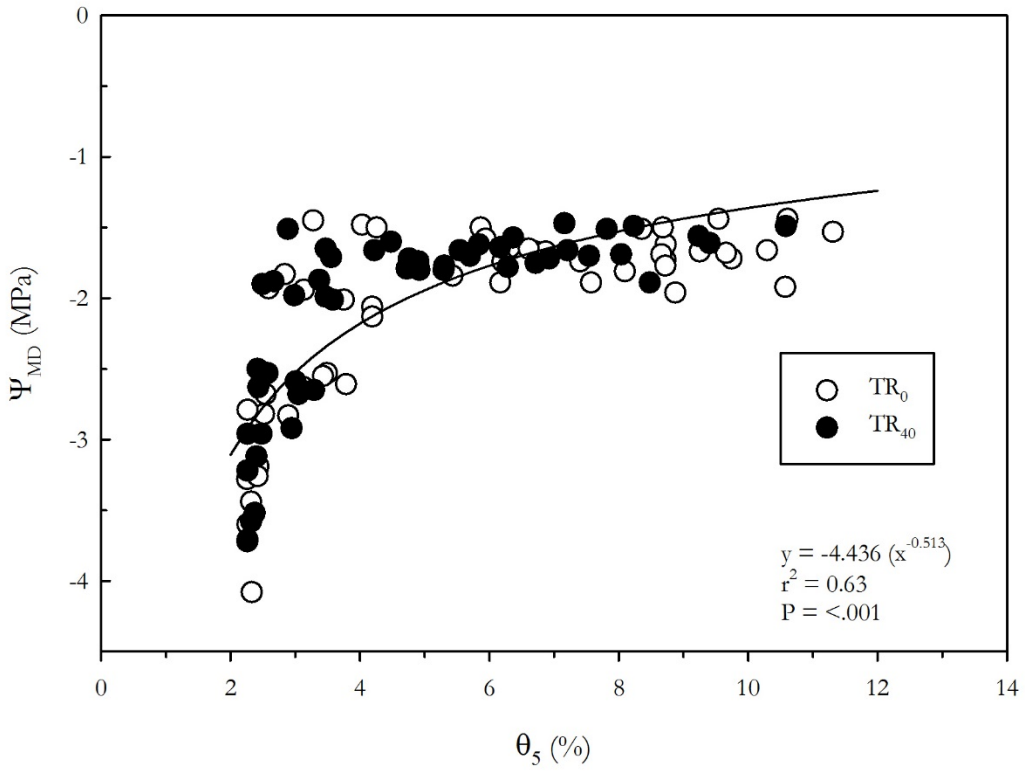

Figure 2.3.9. Midday leaf water potential (Ψ_{MD}) response to plot-level soil moisture (θ) at 5 cm depth in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017.

The relationship between Ψ_{PD} and Ψ_{MD} was also examined (Figure 2.3.10). Data from November 2016 through December 2016 were not included, as Ψ_L on those measurement dates were not regulated by stomatal control (J_V was near zero). A linear regression ($y = B_0 + B_1 x$) was fit to the data. No treatment differences were found for B_0 (P = 0.824) or for B_1 (P = 0.827). The slope of the relationship was 0.85.

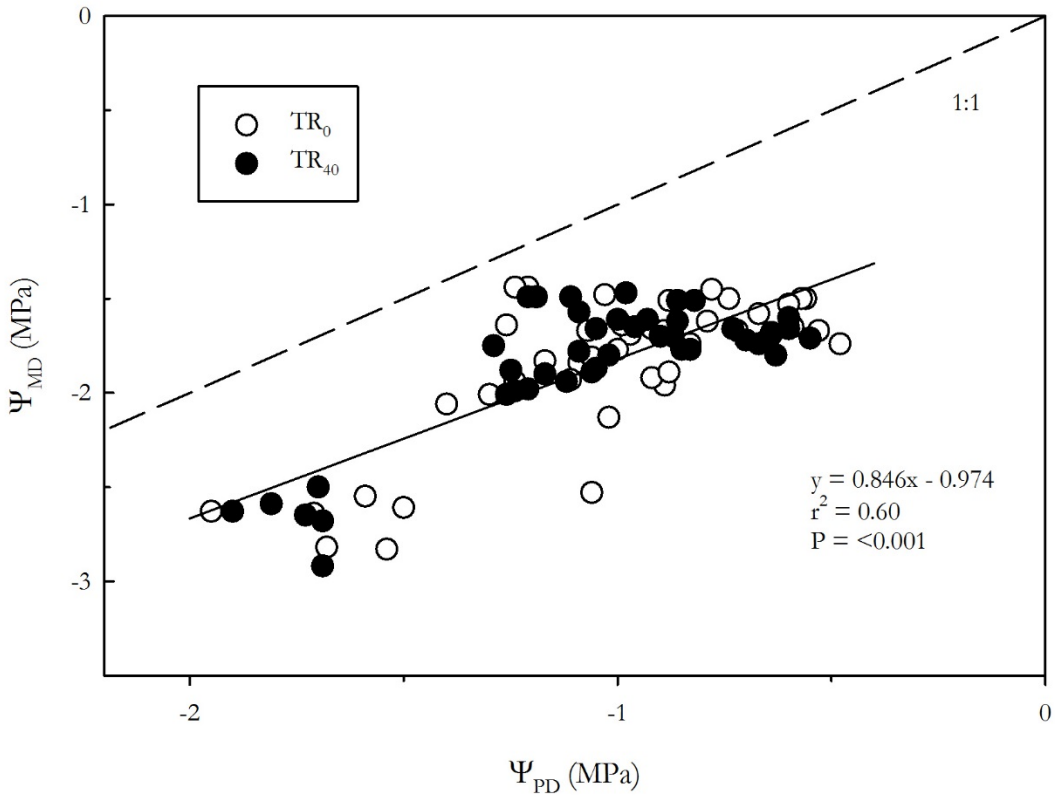

Figure 2.3.10. Midday leaf water potential (Ψ_{MD}) in response to predawn leaf water potential (Ψ_{PD}) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017, but excluding data measured from October 2016 through December 2016. A 1:1 line is included for reference.

2.3.4 Sap Flux Density

In 2016, the prolonged natural drought reduced J_v. Both midday and daily summed J_v declined as the drought progressed, decreasing to near-zero values in the last three measurements of the year (Figure 2.3.11, 2.3.12). Significant treatment differences in midday J_v were detected in 2016 as well as 2017. No significant treatment by date interactions were detected for midday J_v in 2016 or 2017. Throughfall reduction reduced midday J_v by 40% (from 0.9 mol m^{-2} s^{-1} to 0.6 mol m^{-2} s^{-1}) in 2016 and by 14% (from 2.1 mol m^{-2} s^{-1} to 1.8 mol m^{-2} s^{-1}) in 2017.

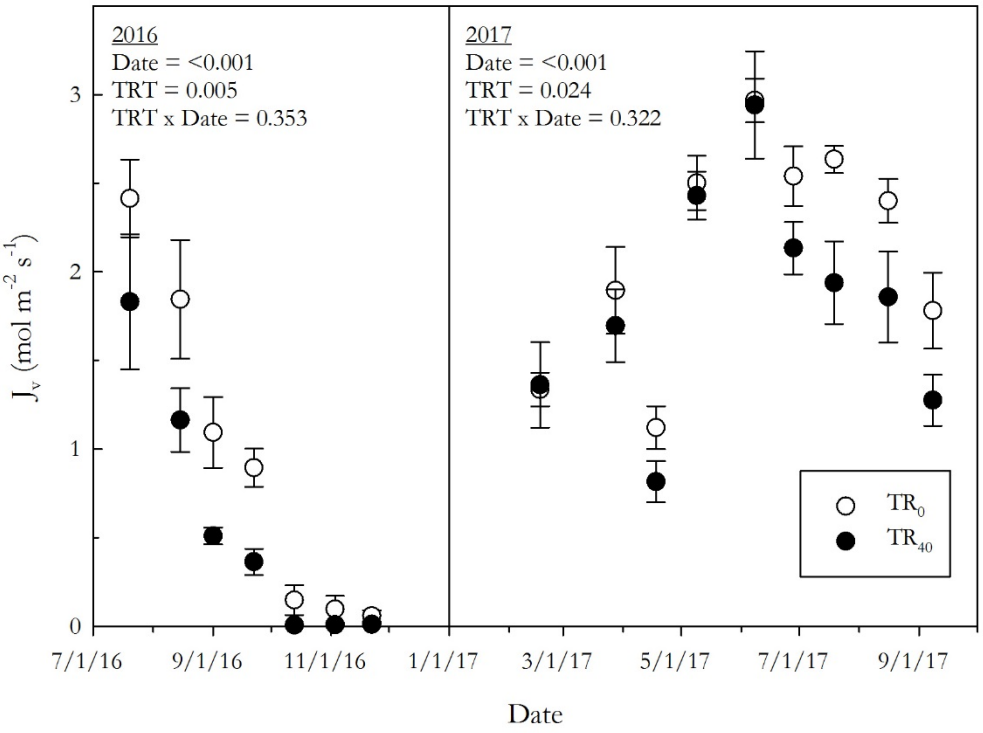

Figure 2.3.11. Mean (± SE) sap flux density (J_v) at midday in response to throughfall treatment (TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017.

In 2016, the daily sum of J_v (J_Σ) ranged from 0.1 kg m^{-2} day^{-1} on October 13 to 1612.2 kg m^{-2} day^{-1} on July 20 (Figure 2.3.12). In 2017, J_Σ ranged from 338.0 kg m^{-2} day^{-1} April 18 to 1919.4 kg m^{-2} day^{-1} on June 8. We detected a significant interaction between treatment and date for J_Σ in both 2016, and 2017. In 2016, TR_{40} reduced J_Σ on July 20, August 15, and September 22 from 23.6% to 70.9%. In 2017, TR_{40} reduced J_Σ on July 19, August 16, and September 8 from 22.7% to 34.4%.

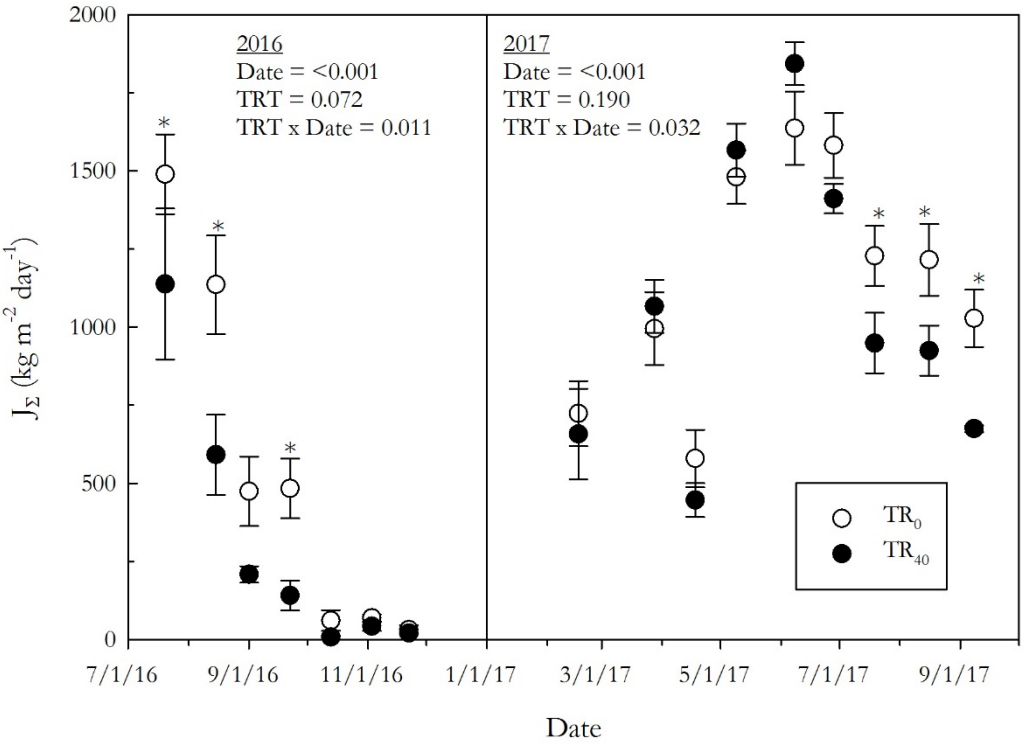

Figure 2.3.12. Mean (± SE) sum of daily sap flux density (J_Σ) in response to throughfall treatment (TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017. * Indicates a significant throughfall treatment effect within a date.

2.3.5 Whole-Tree Hydraulic Conductance

In 2016, whole-tree hydraulic conductance declined during the drought, and was significantly different between treatments (Figure 2.3.13). In 2016, K in TR_0 was as low as 0.0 mol m^{-2} s^{-1} MPa^{-1} and as high as 2.7 mol m^{-2} s^{-1} MPa^{-1} and was on average 1.0 mol m^{-2} s^{-1} MPa^{-1}. Throughfall reduction lowered K on average by 30.7% in 2016. In TR_{40}, K ranged from 0.0 mol m^{-2} s^{-1} MPa^{-1} to 2.5 mol m^{-2} s^{-1} MPa^{-1} and averaged 0.7 mol m^{-2} s^{-1} MPa^{-1}.

Whole-tree hydraulic conductance was not significantly different in 2017 (P = 0.570). In 2017, K ranged from 1.0 mol m^{-2} s^{-1} MPa^{-1} to 5.6 mol m^{-2} s^{-1} MPa^{-1} and averaged 2.4 mol m^{-2} s^{-1} MPa^{-1}.

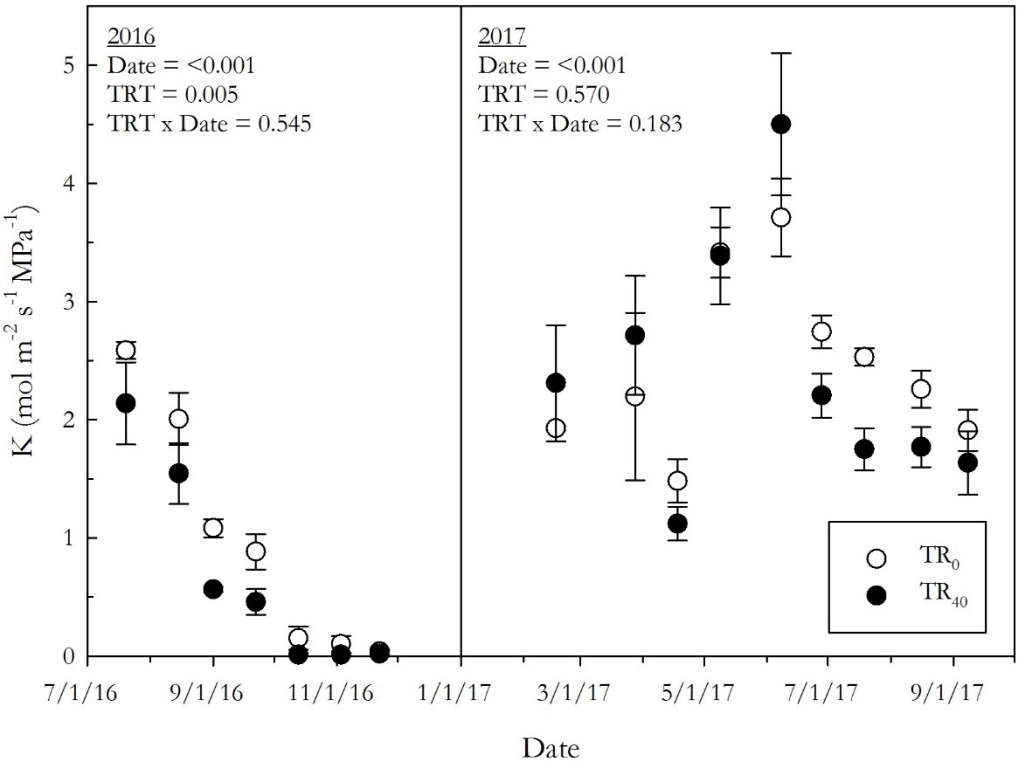

Figure 2.3.13. Mean (± SE) whole-tree hydraulic conductance (K) in response to throughfall treatment (TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 to September 2017.

The response of whole-tree hydraulic to changes in Ψ_{PD} and Ψ_{MD} was examined (Figure 2.3.14, 2.3.15). Relationships were found in the 2016 measurements, but not in the 2017 measurements. In 2016, K was positively related to Ψ_{PD} and Ψ_{MD}. However, water potential continued to decline after K was functionally zero. A regression was fit to K to examine the potential effect of throughfall reduction on the relationship between K and water potential as K declined to zero. There were no treatment differences detected in the slopes (K/Ψ_{PD}, $P = 0.459$; K/Ψ_{MD}, $P = 0.077$) or intercepts (K/Ψ_{PD}, $P = 0.352$; K/Ψ_{MD}, $P = 0.430$) of the relationships. Using data from both treatments, we found that for each 1.0 MPa decrease in Ψ_{PD}, we observed a 2.1 mol m^{-2} s^{-1} MPa^{-1} decrease in K ($P = <0.001$; $r^2 = 0.85$). Midday leaf water potential had less of an effect on K, as we found that for each 1.0 MPa decrease in Ψ_{MD}, we observed a 1.6 mol m^{-2} s^{-1} MPa^{-1} decrease in K ($P = <0.001$; $r^2 = 0.67$).

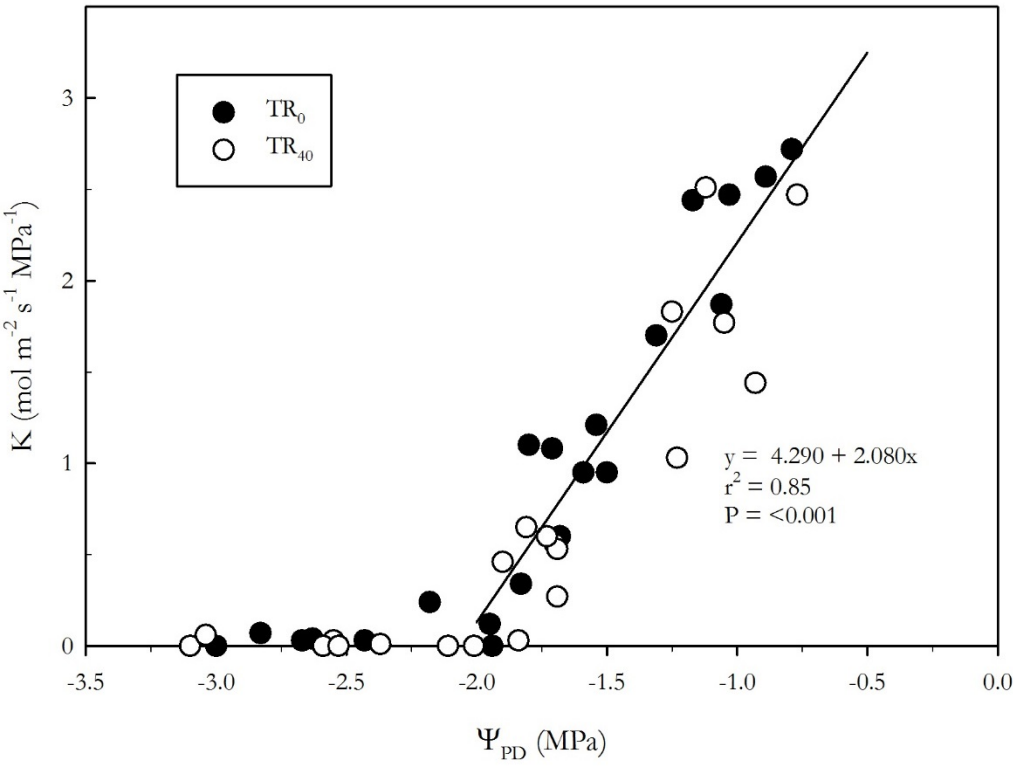

Figure 2.3.14. Whole-tree hydraulic conductance (K) response to predawn water potential (Ψ_{PD}) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 through November 2016.

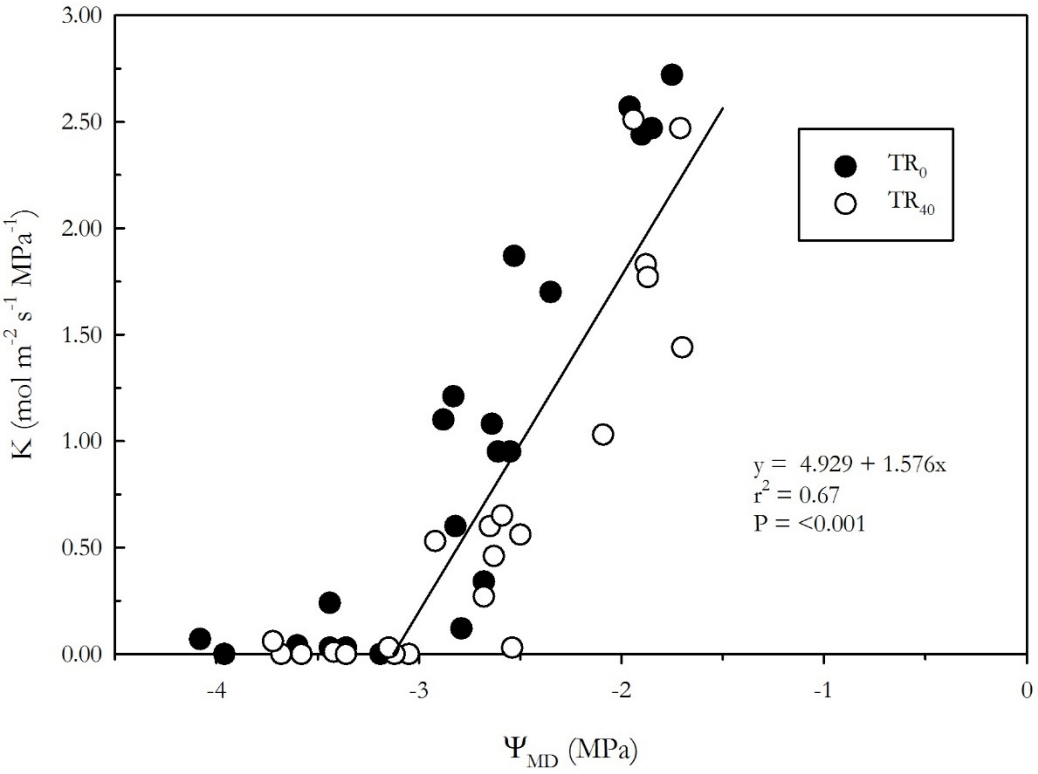

Figure 2.3.15. Whole-tree hydraulic conductance (K) response to midday water potential (Ψ_{MD}) in a longleaf pine plantation in Marion County, Georgia measured from July 2016 through November 2016.

2.3.6 Leaf $\delta^{13}C$ and Specific Leaf Area

Leaf $\delta^{13}C$ did not differ between flushes or treatments, and no interaction between flush and treatment was detected for leaf δ13C (Table 2.3.1). Foliar N concentration was not different between flushes or between treatments, and no interaction transpired between flush and treatment. No differences were detected for needle length between treatments, but was needles were 24.0% longer in the first flush than the second flush. A flush by treatment interaction occurred for specific leaf area. The effect of TR on SLA varied with flush. Treatment did not affect the SLA of the first flush (P = 0.306). In the second flush TR_{40} increased the SLA from 116.6 $cm^2\ g^{-1}$ to 128.9 $cm^2\ g^{-1}$ (P = 0.003), an increase of 10.5%.

Table 2.3.1. Mean (SE) foliar carbon-13 isotope composition ($\delta^{13}C$), foliar nitrogen concentration (N), needle length, and specific leaf area (SLA) and observed probability values for two growing season flushes in response to throughfall treatment (TR; TR_0, ambient throughfall; TR_{40}, throughfall reduction) in a longleaf pine plantation in Marion County, Georgia measured in October 2016.

	$\delta^{13}C$ (‰)	Foliar N (mg g^{-1})	Needle length (mm)	SLA ($cm^2\ g^{-1}$)
Flush				
1	-27.5 (0.2)	1.0 (0.03)	300.0 (5.8)	133.6 (0.1)
2	-27.2 (0.1)	1.0 (0.04)	242.0 (16.7)	122.8 (0.3)
Throughfall treatment				
TR_0	-27.5 (0.1)	1.0 (0.04)	283.3 (17.8)	124.4 (0.4)
TR_{40}	-27.3 (0.2)	1.0 (0.03)	258.6 (16.5)	132.0 (0.2)
P > F				
Flush	0.446	0.800	**0.013**	**0.001**
TR	0.329	0.708	0.976	**0.005**
Flush x TR	0.390	0.451	0.617	**0.039**

2.3.7 LAI and Growth

No significant treatment effects occurred for basal area, density, DBH, or height (Table 2.3.2). Year had a significant effect on basal area, DBH, and height, with 2017 measurements being larger than 2016 measurements. Peak LAI was in August 2017 and was not different between treatments (P = 0.717).

Table 2.3.2. Mean (SE) basal area, density, DBH, height, and maximum leaf area index (LAI) in August in response to throughfall treatment (TR; TR_0, ambient throughfall; TR_{40}, throughfall reduction) and observed probability values in a longleaf pine plantation in Marion County, Georgia.

	Basal area (m^2 ha^{-1})	Density (stems ha^{-1})	DBH (cm)	Height (m)	LAI (m^2 m^{-2})
Year					
2016	19.2 (0.9)	1000 (32)	15.4 (0.3)	10.3 (0.1)	-
2017	20.6 (0.8)	978 (33)	16.1 (0.3)	10.9 (0.2)	2.69 (0.09)
Throughfall treatment					
TR_0	20.9 (1.1)	1029 (35)	15.8 (0.4)	10.8 (0.2)	2.73 (0.11)
TR_{40}	19.0 (0.3)	949 (18)	15.7 (0.2)	10.4 (0.2)	2.65 (0.17)
P>F					
Year	**0.002**	0.228	**< 0.001**	**< 0.001**	-
TR	0.288	0.221	0.867	0.157	0.717
Year x TR	0.898	0.670	0.265	0.316	-

2.4 Discussion

We captured longleaf pine's response to prolonged natural drought. In doing so, observed values showed that longleaf pine experiences lower Ψ_L than previously reported. Capturing the lower limits of Ψ_L allowed for a better understanding of where longleaf pine would be on the isohydric/anisohydric gradient. We also observed J_V, J_Σ, and K as they decreased to near zero values in both the ambient and treatment plots as the drought persisted. Despite lowered transpiration during the 2016 drought, no growth differences in TR_0 and TR_{40} were detected.

The trees in our study experienced extremely low water potentials during the 2016 natural drought. Under drought conditions and very low θ, the Ψ_L measurements recorded at out study site were lower than Ψ_L reported in other studies A study of mature (57-years-old on average) longleaf pine trees during drought in southwest Georgia reported Ψ_{PD} as low as -0.8 MPa and Ψ_{MD} as low as -1.7 MPa (Addington *et al.*, 2004). The study site in southwest Georgia was on a site similar to our study conditions, albeit with very different stand structure. Soils at the Addington *et al.* (2004) site were also Typic Quartzipsamment and experienced similarly low θ (0.02 - 0.04 m^3 m^{-3}) during the drought. However, the drought was shorter in duration than the drought that occurred in 2016, and the Addington *et al.* (2004) site experienced some relief in the form of intermediate (albeit low) precipitation events. The trees observed in the Addington *et al.* (2004) study were also older than our study trees, and would have had deeper and more established root systems with which to access water than the trees in our study. In another study of longleaf pine during drought, a 65-year-old mixed longleaf and slash pine (*Pinus elliottii* Engelm.) stand in northeast Florida, Ψ_L precipitation was <50% of historic averages (Gonzalez-Benecke *et al.*, 2011). Soil water potential (to which Ψ_{PD} is

analogous) was estimated at -0.96 MPa, while Ψ_{MD} was -1.83 MPa. Maggard et al. 2016 working with loblolly pine (*Pinus taeda* L.), found a 100% throughfall reduction decreased θ to 4.8% and 15.6% at 0-12 cm and 12-45 cm depths respectively, decreasing Ψ_L to -1.3 MPa during predawn measurements and -1.9 MPa at midday. We observed Ψ_{PD} between -0.8 MPa and -3.2 MPa during the 2016 drought, values much lower than previously reported. The longleaf pine trees in our study also experienced much lower Ψ_{MD} (as low as -4.1 MPa) than were reported in other studies. The Ψ_L values are lower than the critical water potentials of other tree tissues where 50% loss of conductivity occurs. For example, in longleaf pine, 50% loss of conductivity occurs in roots at -1.31 MPa and in branches as -1.81 MPa (Gonzalez-Benecke *et al.*, 2010)

Across a season, Gonzalez-Benecke *et al.* (2011) reported a mean $\Delta\Psi$ in longleaf pine of 0.94 MPa. We observed similar values of $\Delta\Psi$, except for the period at end of the drought in 2016 when $\Delta\Psi$ declined sharply to 0.0 MPa and subsequently recovered.

We observed J_V values similar to other studies of longleaf pine. In longleaf pine on sandy soil in Georgia and Florida, J_V averaged 1.9 mol m^{-2} s^{-1} at midday in trees ranging from 35 cm to 42 cm in diameter (Ford *et al.*, 2004; Gonzalez-Benecke *et al.*, 2011). That value is similar to mean J_V values recorded in this study in 2017 (2.1 mol m^{-2} s^{-1} in TR_0, 1.8 mol m^{-2} s^{-1} in TR_{40}), but a great deal higher than values recorded in 2016 (0.9 mol m^{-2} s^{-1} in TR_0, 0.6 mol m^{-2} s^{-1} in TR_{40}), likely due to drought. We found that midday J_V was reduced relatively more during the 2016 drought year than in 2017. However, in both year TR_{40} reduced average J_V by approximately 0.3 mol m^{-2} s^{-1}. The larger effect size in 2016 is likely because values were so low that any reduction would have a larger overall effect.

Trees in our study had higher rates of K during soil water deficit than other studies. Hydraulic conductance in longleaf pine has been estimated as approximately 0.4 mol m^{-2} s^{-1} MPa^{-1} when θ at 50 cm depth was less than 10% (Addington *et al.*, 2004, 2006; Gonzalez-Benecke *et al.*, 2011). During 2016, at our site soil moisture at 50 cm was never above 6%, and average K values in TR_0 and TR_{40} plots were near double that of other studies (0.7 mol m^{-2} s^{-1} MPa^{-1} in TR_{40} and 1.0 mol m^{-2} s^{-1} MPa^{-1} in TR_0). The difference in K values may be due differences in the age and root profile of study trees, as Addington *et al.*, (2006) found that mature longleaf pine had a greater proportion of roots at depths below 50 cm than at shallower depths. In our study, we found that Ψ_{PD} was best explained by θ_5, and that Ψ_{PD} began to respond to declining θ_5 at -1.7 MPa. Whole-tree hydraulic conductance values became similar to other studies once Ψ_{PD} decreased to around -1.9 MPa. It may be that younger trees are more sensitive to changes in θ at shallow depths (where they have higher root concentrations) than mature trees, which will have established root systems at deeper depths.

We found that longleaf pine's response to prolonged drought was less isohydric than expected. When using classifications of isohydry/anisohydry that relate Ψ_{MD} and Ψ_{PD}, we found that longleaf pine was only partially isohydric ($0 <$ slope of $\Psi_{MD}/\Psi_{PD} < 1$) (Maritnez-Vilalta *et al.*, 2014). In fact, with a slope of 0.85, longleaf pine is closer to a strictly anisohydric response (slope = 1), than a strictly isohydric response (slope = 0). Longleaf pine has been described as having an intermediate (in between isohydric and anisohydric) response to low θ before, but previous observations had not reported as low Ψ_L as we observed (Gonzalez-Benecke *et al.*, 2011). Longleaf pine has a greater proportion of deep (below 0.6 m) roots and higher root to shoot ratio than other southern pine species, which might allow longleaf pine to better compete for water on xeric sites during severe drought (Gonzalez-Benecke *et al.*,

2011; Samuelson *et al.*, 2016). The lack of maintenance of Ψ_{MD} with declining θ suggests that longleaf pine may benefit from continued carbon gain while avoiding fatal cavitation (Gonzalez-Benecke *et al.*, 2011). The carbon gains accumulated by longleaf pine during drought may contribute to improving access to belowground resources, potentially improving future drought resistance.

The water potential responses to declining θ we observed did not follow the expected isohydric pattern. Midday water potential was not held constant as θ decreased. The hypothesis that lower θ due to drought will lower Ψ_{PD} and Ψ_{MD} was supported. During the 2016 measurement period, Ψ_{PD} was initially unresponsive to declining θ_5 but eventually declined when θ_5 reached 2.7%. Similarly, Ψ_{MD} did not respond until θ_5 was less than 4%. We also hypothesized that $\Delta\Psi$ would be maintained as Ψ_{PD} declined, by stomatal control of Ψ_{MD}. That hypothesis was not supported, as we observed a significant effect of date on $\Delta\Psi$ throughout the measurement period, indicating that $\Delta\Psi$ was not consistent as water availability fluctuated. In typical isohydric behavior, $\Delta\Psi$ should decrease as the soil dries and Ψ_{MD} becomes tightly regulated. If declines in K due to embolism are great enough, the $\Delta\Psi$ response to declining soil moisture will become more isohydric (declining with soil moisture), behavior which was observed in this study during the end of the 2016 drought (Sperry, 2000; Roman *et al.*, 2015). Whole-tree hydraulic conductance declined as the 2016 season progressed, but $\Delta\Psi$ was relatively unchanged until K declined to near-zero values in October.

Although drought has been shown to reduce growth in pine trees and increase susceptibility to mortality (Klos et al., 2009), the hypothesis that TR_{40} would reduce aboveground growth was not supported. No treatment differences were detected for any growth variables, however drought effects may take years to impact mortality (Berdanier &

Clark, 2015). Longleaf pine growth is related to PDSI, particularly during the months when the worst of the 2016 drought occurred (Henderson & Grissino-Mayer, 2009). Drought may have limited transpiration and carbon accumulation enough in both treatments that no immediate growth changes were detected. Additionally, growth measurements began in July 2016, after much of the growing season had passed. Drought-related reductions in growth are dependent on the seasonal timing in which a drought occurs (Eilmann *et al.*, 2011). Because the natural drought occurred shortly after site establishment, an immediate baseline for growth rates was not available. We did not observe growth reductions, possibly because the majority of the year's growth may have occurred before the drought became severe. There were also no differences in LAI between treatments, so neither treatment had an advantage in carbon accumulation. Tree cores remain a viable option for future examination of site recovery following the end of the drought.

The hypothesis that TR_{40} will cause slower recovery of J_V and K after natural drought also was not supported. No differences occurred in J_V, J_Σ, or K as values were returning to observed 2016 maximums. However, J_Σ in 2017 suggests the TR_{40} trees may be more sensitive to mild stress following the 2016 drought than TR_0 trees. After precipitation returned at the end of November 2016, there was no immediate difference in J_Σ between treatments. Once the increased stress of the 2017 summer came, TR_{40} reduced average J_Σ. While not conclusive, this may be evidence of cumulative drought effects reducing J_Σ in TR_{40}. Other evidence that TR_{40} may have long-lasting effects on drought-related mortality is found in SLA. We found that TR_{40} increased SLA by 10.5%. Increases in SLA could mean an increase in potential water use, and therefore increase in drought stress (Greenwood *et al.*, 2017). Higher SLA could also mean a reduction in photosynthetic machinery, and as there were no differences in needle

length between treatments, the increase in SLA is likely due to decreases in needle mass. If not recovered, reductions in photosynthetic machinery would lower the amount of accumulated carbon.

In summary, we examined the effect of a 40% throughfall reduction on water relations in a longleaf pine plantation. In 2016, the study area experienced a severe drought. Soil moisture, Ψ_L, J_V, J_Σ, and K decreased in both the ambient and treatment plots as the drought persisted. During the 2016 drought, the 40% throughfall reduction did not affect Ψ_L or J_Σ, but did reduce J_V and K. In 2017, J_V was reduced by TR_{40}, and J_Σ was also reduced on certain measurement days. We found that prolonged natural drought caused longleaf pine to behave less like a perfectly isohydric tree and more like an anisohydric tree. The partially isohydric behavior observed may allow longleaf pine to be more resistant to future drought stress and improve recovery.

2.5 Conclusions

Often during discussions of forest management and drought the focus is on large scale effects over large areas. However, because the conversation surrounding climate change and forests is about collections of individual organisms and not simply features on a landscape, it is important to understand the response of the individual to changes in its environment. Altering tree density to reallocate resources is a common tool of silviculture. Thinning decreases drought mortality risk (Dale *et al.*, 2001). To manage southeastern forests to be more resistant to climate changes, work done by Kerhoulas et al., (2013) suggests a more intense thinning regimen beyond current practices is needed. Lower stand densities not only stimulate the growth of residual trees, but improve drought resilience if the treatment is timed appropriately (Kerhoulas *et al.*, 2013). However, a meta-analysis of drought mitigation through thinning that included sixteen studies on Pinus species (*P. taeda* L., *P. resinosa* Ait., *P. halepensis* Mill., *P. sylvestris* L., *P. nigra* Arnold, P. *ponderosa* Dougl. ex Laws, and P. *canariensis* C.Sm) showed that conifers' resistance to drought was not improved by thinning. Water use efficiency increased, but growth was reduced by 30 - 40%. Thinning increases crown diameter and creates larger rooting systems, increasing evaporative demand, which may offset the stand level benefit of lower tree density (Sohn *et al.*, 2016). Once the drought ended however, the large crowns and root systems of the conifers enabled tree growth to return pre-drought levels within a year, demonstrating the positive effects of thinning on conifer drought resilience. The Berdanier & Clark (2015) example highlights the importance of tree recovery to pre-disturbance levels for future drought survival.

In the Southeast US, managed forests are designed to be as uniform as site conditions will allow, and usually consist of one even-aged species. Pioneer species are used because they

are evolutionarily adapted to natural regeneration and grow easily in a nursery (Pretzsch & Forrester, 2017). While the simplicity of an even aged stand monoculture is what has allowed for many improvements in silviculture techniques over the last century (site index charts, stocking charts, growth and yield models), that simplicity is also a risk. Trees in a monoculture compete for the exact same nutritional and light level resources, so from a stand perspective there is little diversity in how resources are used (Klos *et al.*, 2009). High competition causes monoculture forests to be less resistant to drought stress than more species-rich forests, as different species use resources differently, lowering overall competition (Klos *et al.*, 2009). Increasing species mixture is a potential tool to insulate forests from effects of altered disturbance regimes. By selecting tree species that are more tolerant of drought and other climate-affected disturbances, a forest can be fortified against a wider range of disturbance scenarios (Dale *et al.*, 2001). However, managers are generally not changing their silvicultural practices (Keenan, 2015), and some researchers are suggesting that there isn't cause to alter conifer management in the southeastern United States (Coyle *et al.*, 2015). Temperate forests are at a relatively high risk to species range changes (Choat *et al.*, 2012), which may change management projections and make forestry fiscally unsustainable for some landowners. Diversifying managed forest areas presents opportunities for lower risk, but will require more intense management. Increasing species mix increases the complexity of silvicultural considerations needed to meet goals, and may be a cause for the lack of management changes (Pretzsch & Forrester, 2017). Traditional predictive tools often don't apply to mixed species forests, and interactions between species are often unknown (Pretzsch & Forrester, 2017).

Drought is a serious concern for the forested areas of the southeastern United States. Estimates of forest futures point towards more planted pine stands, which will likely be

loblolly pine, while existing diverse forests decline. Climate change threatens to increase temperatures and alter growing season precipitation patterns, which will test forests resistance and resilience as species respond to decreasing water availability. Longleaf pine may present a management solution, mitigating some effects of climate change through carbon sequestration. It will possibly be more resistant to drought–induced mortality than other southern pine species, and the research presented here suggests resiliency to extended periods of low water availability.

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