RESPONSE OF MID-ROTATION LOBLOLLY PINE (*PINUS TAEDA* L.) TO FERTIIZATION AND REDUCED WATER AVAILABILITY IN SOUTHEASTERN OKLAHOMA

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Abstract: Loblolly pine (*Pinus taeda* L.) is the most commercially important tree species in the southeastern United States and driver of timber productivity for the region. The positive of loblolly pine to forest management applications such as fertilization and competition control have led to such increases in productivity. However, a better understanding of impacts from a more variable climate on loblolly pine growth and productivity is needed. Across the southeastern United States, a shift towards a warmer and drier climate is predicted to occur. Increasing temperatures and more variable precipitation is expected to impact southern pine plantation growth and productivity. This impact is projected to stem from larger precipitation events with longer dry down periods which will ultimately lead to an increased intensity and duration of drought. Therefore, the interactive effects of fertilization and reduced water availability, as well as long-term moderate drought on a mid-rotation loblolly pine plantation in southeastern Oklahoma were studied. The first study examined the effects of nutrient availability and decreased soil water availability on leaf gas exchange, LAI, and stand growth. Results showed that fertilization increased productivity of throughfall reduction stands such that it was similar to ambient throughfall stands not receiving fertilization. Fertilization caused a reduction in stomatal conductance while net photosynthesis rates maintained, indicating increased water use efficiency. The second study examined the effects of nutrient availability and decreased soil water availability on stand-level water use efficiency of stem volume production by 18 %. The final study examined the effects of long-term moderate drought on leaf gas exchange, whole-tree water use, and individual tree growth. Results showed that throughfall exclusion did not significantly reduce leaf gas exchange, water use, or tree growth. However, throughfall exclusion significantly reduced leaf biomass. Results from all three studies indicate that fertilization can be beneficial in loblolly pine plantations experiencing reduced water availability on the western limits of its commercial range by increasing water use efficiency and that long-term moderate drought may cause reduced leaf area to conserve water rather than reducing leaf-level water use.

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CHAPTER I

INTRODUCTION

The southeastern United States produces approximately 60 percent of all timber in the country (Smith et al. 2009). Of the approximately 83 million hectares of forest land in the Southeast, 16 million hectares (19 percent) is planted pine (Wear and Greis 2012). Of the planted pine, loblolly pine (*Pinus taeda* L.) is the most extensively planted because of its ability to grow on a variety soil types and its favorable response to silvicultural treatments (Burns and Honkala. 1990). Intensive forest management practices such as fertilization, competition control, and improved genetics over the years has increased loblolly pine productivity. One of the most important factors that limit loblolly pine productivity is soil nutrient availability (Jokela et al. 2004). Therefore, the use of fertilizer as a management tool is common on most loblolly pine plantations. The increased productivity through intensive management practices has enabled loblolly pine to become the most important commercial tree species in the region and vital to the forests products economy in the southeastern United States.

In years to come, it's likely that decreased precipitation will affect loblolly pine plantations across the southeastern United States. The U.S. Global Change Research program has projected that temperature in the region will increase between 2.5 to 4 ^{O}C through the end of the century (Karl et al. 2009). A temperature increase of this magnitude is likely to affect loblolly pine productivity. Schmidtling (1994) speculated that increases of this magnitude would cause a decline in productivity of loblolly pine by a minimum of 10%. Growing seasons for loblolly pine are expected to be affected the most by rising temperatures, with an increase in the number of extremely hot days showing faster temperature increases than the during the summer months (Collins et al. 2013). Precipitation predictions are more variable. Based on information from the Southern Forest Futures Project, precipitation projections are variable based on four separate models, but the overall trend is drier with more sporadic rainfall events likely (Wear and Greis 2012). Similarly, the Intergovernmental Panel on Climate Change (IPCC) predicts decreases in precipitation across the Southeast as a result of an increase in dry days and more intense, but sporadic rainfall events (Collins et al. 2013). Tree response to water limitation frequently involves a trade-off with plant productivity, as tree stature and canopy leaf area are minimized to reduce transpirational loss and tree water stress (Kramer and Boyer 1995). Such response to prevailing precipitation regimes establish the potential for intensified precipitation regimes to alter patterns of resource availability and gain, influence competitive interactions, and lead to changes in productivity and survival. Therefore, a better understanding of how climate affects loblolly pine plantation growth and productivity is needed.

In association with the United States Department of Agriculture – National Institute of Food and Agriculture funded Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP) I studied the effects of fertilization and reduced water availability on a mid-rotation loblolly pine plantation in southeastern Oklahoma. The overall goal of PINEMAP is to create, synthesize, and disseminate the information necessary to adapt forest management practices in order to increase forest resiliency under a changing climate. This information will provide the knowledge necessary for southern pine landowners and forest managers to increase carbon sequestration, increase fertilizer efficiency, and maintain productivity

I studied the response of mid-rotation loblolly pine to fertilization and reduced water availability, as well as long-term moderate drought. The first study focused on determining the effects of nutrient availability and reduced precipitation throughfall on leaf gas exchange, leaf area index (LAI), and stand growth. The second study focused on determining the effects of nutrient availability and reduced precipitation throughfall on tree and plot-level water use, water use efficiency, and growth efficiency. The third study focused on determining the effects of long-term moderate drought on leaf gas exchange, whole-tree water use, and individual tree growth. The first two studies incorporated fertilizer application and a moderate reduction in precipitation throughfall and the third study focused on complete exclusion of precipitation throughfall without the application of fertilization.

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CHAPTER II

FERTILIZATION CAN COMPENSATE FOR REDUCED WATER AVAILABILITY ON LOBLOLLY PINE (*PINUS TAEDA* L.) NET PHOTOSYNTHESIS AND PRODUCTIVITY IN SOUTHEASTERN OKLAHOMA, USA

Abstract

Loblolly pine (*Pinus taeda* L.) is the most commercially important tree species in the southeastern USA and has potential to increase region-wide carbon sequestration. However, projected increases in regional temperature and drought severity will likely cause more extreme impacts for the western edge of the loblolly pine commercial range. To determine the effects of nutrient availability and reduced water availability on leaf gas exchange, leaf area index (LAI), and tree growth, we examined the interactive effects of fertilization (one time application of 224 kg N ha^{-1}, 28 kg P ha^{-1}, 56 kg K ha^{-1} and micronutrients) and reduced throughfall (approximate 30 % reduction) over growing seasons 5-7 for a loblolly pine plantation in southeast, Oklahoma. Across all plots, throughfall reduction reduced volumetric soil water content from 11.3 % to 8.8 % and fertilization increased foliar nitrogen concentration from 1.13 to 1.27 mg g^{-1}. Fertilization increased LAI (10 %) and subsequently tree growth while reducing stomatal conductance (7 %) and leading to

less negative (3 %) mid-day leaf water potential. Throughfall reduction reduced stomatal conductance (12 %) and net photosynthesis (9 %) which was related to more negative mid-day leaf water potentials (11 %). These results indicate that fertilization increased water use efficiency such that leaf-level photosynthesis was not decreased by throughfall reduction even though fertilization increased LAI and potential transpiration. The net effect was that fertilization increased productivity of throughfall reduction stands such that it was similar to ambient throughfall stands not receiving fertilization.

Introduction

Over fifty percent of pine plantations in the southeastern USA are loblolly pine (Oswalt et al. 2014), thus making it the most commercially important tree species in this region. Given the extent of planting, approximately 16 million ha (Wear and Greis 2012), pine plantations provide potential to increase region-wide carbon sequestration (Albaugh et al. 2012, Noormets et al. 2015, Will et al. 2015). Over the last half century, increases in pine plantation management and productivity in the southeastern USA have enabled these forests to produce more timber volume than any other region in the United States (Oswalt et al. 2014). However, in years to come, decreased precipitation along with increases in temperature may affect pine plantations throughout the southeastern USA. Average temperature throughout this region is expected to increase by 2.5 to 4 ^{o}C by the latter half of this century (Collins et al. 2013). Although precipitation projections are variable, predictions at the regional level are fairly consistent. Larger and more intense precipitation events along with increased length of dry periods are projected for the southeastern USA (Easterling et al. 2000, Collins et al. 2013, Walsh et al. 2014). This will increase runoff and possibly reduce soil water availability. This combined with higher vapor pressure deficits (VPD) resulting from higher temperature will increase the severity of drought (Breshears et al. 2013, Will et al. 2013).

The effect of water stress on tree physiology can lead to changes in productivity. This may be especially important for the western edge of the commercial range of loblolly pine in southeastern Oklahoma. This area experiences higher growing season temperatures and VPD than the rest of the southeastern USA (Seager et al. 2015) such that loblolly pine planted in this area (beyond their natural range) may be more apt to experience water stress. This edge of the loblolly pine commercial range is projected to experience more extreme climate patterns consisting of higher temperatures and altered precipitation events compared to other parts of the loblolly pine commercial range (Walsh et al. 2014).

Acclimation to water limitation frequently involves a trade-off with tree productivity because tree stature and canopy leaf area growth slows to decrease transpirational loss and tree water stress (e.g., Kramer and Boyer 1995). In addition to these morphological changes, stomatal conductance and carbon assimilation typically decline (e.g., Ni and Pallardy 1992). Stomatal closure, to limit water loss, is among the earliest response to drought, otherwise severe water stress can lead to cell and tissue dehydration, xylem cavitation, and potentially death (Chaves 1991). As stomatal conductance is reduced, net photosynthesis also declines from a reduction in the rate of CO_2 diffusion into the leaf (e.g., Seiler 1984, Teskey et al. 1986, Chaves 2003). Non-stomatal limitations to photosynthesis due to water stress also occur (Boyer 1976, Lawlor 1995) and result from metabolic inhibition (Tezara et al. 2002, Flexas and Medrano 2002, Hu et al. 2010). Stomatal and non-stomatal responses to drought have the potential to alter patterns of resource availability and gain, influence competitive interactions, and lead to changes in productivity and survival.

Soil nutrient availability in loblolly pine stands is considered one of the most important factors affecting productivity (Jokela et al. 2004). Most loblolly pine plantations in the southeastern USA are on soils that cannot meet the demand for nitrogen once the trees begin to reach crown closure, which is typically 5-8 years (Allen et al. 1987). Once trees become nutrient deficient, leaf development and subsequently whole-tree production is reduced compared to trees with an abundance of nutrient resources (Albaugh et al. 2008, Colbert et al. 1990, Vose and Allen 1988). The use of fertilizer as a management tool is common on southern forest plantations. Applying fertilizer, particularly combinations of N and P, increases development of leaf area in southern pine plantations (Albaugh et al. 1998, Will et al. 2002, Gough et al. 2004, Martin and Jokela 2004, Will et al. 2006). Greater leaf area increases overall productivity by increasing carbon fixation (Teskey et al. 1994, Vose and Allen 1988, Albaugh et al. 1998) but at the same time, increases potential transpiration and water use.

Fertilization treatments that increase leaf area index (LAI) increase growth, more so on sites with adequate water availability combined with competition control (Ewers et al. 2000, Albaugh et al. 2004, Jokela et al. 2004). However, fertilization generally does not significantly impact leaf gas exchange in southern pines (e.g., Munger et al. 2003, Tang et al. 2004, Samuelson et al. 2014). More specifically, a poor correlation between leaf nitrogen concentration and net photosynthesis (Will et al. 2001, Munger et al. 2003, Gough et al. 2004) and stomatal conductance (Green and Mitchell 1992) is common in loblolly pine.

Effects of nutrient availability and irrigation have been well documented in loblolly pine stands (e.g., Ewers et al. 1999, Allen et al. 2005, Samuelson and Stokes 2006, Samuelson et al. 2008b). However, few studies have examined effects of nutrient availability and water stress in loblolly pine stands, including photosynthetic and stomatal responses (Tang et al. 2004, Samuelson et al. 2014). A better understanding of interactive effects of nutrient availability and water stress on physiological mechanisms and growth of loblolly pine stands is needed to better understand tree and stand response to a potentially warmer and drier climate as well as ultimately help guide effective management practices for productivity and carbon sequestration in the future. Knowledge of how the benefits of fertilizer are affected by drought will aid in determining its effectiveness in future forest management.

The objective of this study was to determine effects of nutrient availability and decreased soil water availability on leaf gas exchange, LAI, and stand growth. To accomplish this objective, we examined the interactive effects of fertilization and reduced throughfall. Our central hypothesis was that fertilizer added to a loblolly pine plantation in southeastern Oklahoma experiencing throughfall reduction will increase LAI and exacerbate water stress. Further, we hypothesized that increased water stress will result in reduced stomatal conductance and photosynthesis. Therefore, the positive effects of fertilizer on growth may be reduced due to potentially more severe water stress in stands receiving throughfall reduction and fertilization.

Materials and methods

Study site

This experiment was associated with the Pine Integrated Network Education, Mitigation, and Adaptation Project (PINEMAP) focused on the effects of a changing climate on loblolly pine productivity. As part of PINEMAP, throughfall exclusion and fertilization experiments were installed in Oklahoma, Florida, Georgia, and Virginia to cover the full spectrum of rainfall and temperature across the loblolly pine natural range (Will et al. 2015). This specific study describes results from the Oklahoma installation.

The study was conducted on a loblolly pine stand near Broken Bow, OK beginning in the 5th growing season and continuing through the 7th growing season. The study site had an elevation of 150 m, latitude 34^{o}01’52.0’’N, and longitude 94^{o}49’18.2’’W. Soils were very deep, well-drained, fine sandy loam in the Ruston series (Fine-loamy, siliceous, semiactive, thermic Typic Paleudult). Depth to the water table was greater than 2 m. This soil was typical for forests on the uplands of the western and southern upper Coastal Plain and consisted of 3 to 8 percent slopes (http://soilseries.sc.egov.usda.gov, accessed February 2016). The 20-year average annual precipitation for Broken Bow, OK was 1281 mm, with most precipitation occurring in May (average of 160 mm) and least occurring in August (average of 70 mm). Twenty-year average annual, maximum, and minimum temperatures for Broken Bow, OK, were 16.4 ^{o}C, 23.8 ^{o}C, and 9.7 ^{o}C (http://mesonet.org/index.php/weather/daily_data_retrieval, accessed February 2016). Monthly Palmer Drought Severity Index (PDSI) data were downloaded for Climate Division 9 (southeast) in Oklahoma for 2012, 2013, and 2014. (http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp#, accessed February 2016).

Before planting, broadcast herbicide of 680 g ha^{-1} of Chopper ® (27.6 % imazapyr, 72.4 % inert ingredients) (BASF Corporation, Florham Park, NJ, USA) plus 2.8 l ha^{-1} of glyphosate was applied in August 2007 followed by prescribed burn in October 2007. The study site was subsoiled along the contour to depths of 51 to 61 cm using a D8 Caterpillar dozer and attached subsoiling shanks (Caterpillar Corporate, Peoria, IL, USA) in November 2007. The site was planted in January 2008 with improved Western Gulf Tree Improvement Cooperative 1-0 bare-root seedlings originating from a mix of half-sib families. Broadcast herbicide of 420 g ha^{-1} of Arsenal® (27.6 % imazapyr, 72.4 % inert ingredients) (BASF Corporation, Florham Park, NJ, USA) and 175 g ha^{-1} of Oust Extra® (56.25 % Sulfometuron methyl, 15.0 % Metsulfuron methyl, 28.75 % other) (E.I. Du Pont De Nemours and Company, Wilmington, DE, USA) was applied for woody plant and herbaceous weed control in March 2008. Spacing for the site was approximately 2 m x 3 m for an approximate density of 1650 trees ha $^{-1}$.

Experimental design

The study comprised four blocks (16 plots total). Each block consisted of four treatment plots in a 2 x 2 factorial combination of fertilization and throughfall reduction. Each treatment plot was greater than 0.10 ha containing a measurement plot between 0.034 and 0.041 ha. Fertilization treatments included no fertilizer and an optimum nutrition that consisted of 224 kg N ha^{-1}, 28 kg P ha^{-1}, 56 kg K ha^{-1}, as well as micronutrients. N and P consisted of a mix of urea (432 kg ha^{-1}) and diammonium phosphate (140 kg ha^{-1}). K consisted of potassium chloride. Micronutrients consisted of a granular mix (Southeast Mix, Cameron Chemicals, Inc., Virginia Beach, VA, USA) applied at a rate of 22.4 kg ha^{-1}, containing 6% sulfur, 5% boron, 2% copper, 6% manganese, and 5% zinc. Fertilizer was broadcast by hand in April 2012. Competing vegetation was chemically eliminated using directed sprays of glyphosate (2% a.i., Roundup®, Monsanto Company, St. Louis, MO, USA) from spring 2012 through the remainder of the study.

Throughfall treatments included ambient throughfall and a throughfall reduction of approximately 30%. A reduction of 30% throughfall was implemented based on the driest climate predictions for the region (Christensen et al. 2007). Throughfall reduction treatments consisted of throughfall exclusion troughs (excluders) to cover 30% of ground area. Excluders were installed in late spring/early summer 2012 and were built of lumber and covered with clear, U.V. stabilized plastic sheeting consisting of two layers co-extruded polyethylene and one layer of high strength polyester string (Tuff-Scrimm™ Poly 12, Americover Inc., Escondido, CA, USA). Excluders were installed between each row of trees and consisted of two 50 cm wide troughs separated by a 50 cm opening, sloping from approximately 1.2 m to 0.6 m in height. Excluders were installed two rows beyond the internal measurement plot in the larger gross plot. Water was transported by the excluders out of measurement plots. To eliminate adding water to adjacent plots, excluders were fitted with plastic tubing at the ends to carry water out of the research area as needed.

Growth

Tree size was measured in January 2012 prior to treatment application (following the 4th growing season) and in December 2012 (following 5th growing season), December 2013 (following 6th growing season), and December 2014 (following 7th growing season). Measurements included DBH and height. DBH was measured after the 4th, 5th, and 6th growing seasons by averaging two caliper measurements taken at right angles on the stem. Due to increases in tree size, DBH was measured at stand age seven using a diameter tape. Height growth was measured at ages four, five, and six using a height pole. Height was measured following 7th growing season using a hypsometer (Laser Technology, Inc., Centennial, CO, USA). DBH, basal area, and height growth were determined from the difference in successive dormant season measurements. During the experiment, only one tree out of 1007 died.

LAI

Stand-level projected leaf area index was measured with an LAI2000 plant canopy analyzer (LiCor, Inc., Lincoln, NE, USA) approximately every four weeks from April 2014 through November 2015. LAI2000 data were corrected using empirical litter trap data collected for the 2013 foliage cohort (twelve 0.5 m^2 traps per plot). LAI2000 data were multiplied by 0.625 to correct for bias. Due to the size of trees and lower branches initially below throughfall excluders, accurate measurements could not take place until the 7th growing season. Measurements were conducted in diffuse light conditions, within an hour after dawn or an hour before dusk with clear skies, or within 1.5 hours of dawn and dusk with uniformly overcast skies. Measurements were taken across treatment plots from a randomly selected starting point. Readings were taken along diagonal transects between the rows at intervals of 0.5-1.0 m. The sensor was held above the height of the excluder troughs in all plots regardless of treatment. Readings were conducted using a 90° filter opening placed opposite the user with the uppermost 4 rings used for calculation. Approximately 25 subsamples per plot per sample date were measured.

Physiology

From October 2012 to October 2014, measurements of leaf gas exchange were conducted every four to six weeks during the growing season and every six to eight weeks in the winter months using an LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). Measured variables included net photosynthesis (P_{net}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). Leaf gas exchange was measured on five trees per plot (80 total). Measurement trees were randomly selected within ranges of DBH, consisting of two trees in the upper third, two trees in the middle third, and one tree from the lowest third DBH classes. For each tree on each measurement date, two fascicles were sampled that developed in full sun in the upper third of the tree on the south side. Needles were collected by hand in 2012 and 2013 and by a pole pruner in 2014. Fascicles from the first flush of 2012 were measured from October 2012 to July 2013 while the first flush of 2013 was measured from August 2013 to July 2014 and the first flush of 2014 was measured from August 2014 to October 2014. Within sampling dates, measurements were conducted by block to balance diurnal environmental variation across treatments. Measurements were taken between 0900 and 1500 h.

During gas exchange measurements, photosynthetically active radiation and reference CO_2 concentration were held constant inside the leaf chamber at 1800 μmol m^{-2} s^{-1} and 400 μmol mol^{-1}, respectively. For the majority of measurements, relative humidity (Rh) within the chamber (2 x 3 cm) was not controlled and fluctuated with ambient conditions. However, on several measurement days when Rh was high, it was reduced to between 60 and 70% in the chamber to prevent condensation. Temperature in the leaf chamber was kept uniform within blocks on a given sample date. Initial temperature for each block was set approximately 1 ^{o}C above ambient temperature in an attempt to bracket ambient temperature during the measurements within a given block. For each block, one tree of the five from each treatment plot was randomly selected and measured. This was repeated successively until all trees within a block were measured (20 trees). All sided leaf area in the cuvette was calculated based on measuring the radius of one needle per fascicle using a scale loupe. Samples for mid-day leaf water potential (Ψ_L) measurements were collected in unison as those for leaf gas exchange measurements. Mid-day Ψ_L was measured using a pressure chamber (PMS, Instrument Corp., Corvallis, OR, USA).

Stable carbon isotope ratio ($\delta^{13}C$) and nitrogen concentration of foliage that had been measured in the cuvette was dried at 60^{o} C, bulked by tree and foliage cohort, and stored until measurement. Foliage was measured using an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled with Thermo Conflo IV and Thermo Delta V Adavantage stable isotope mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at Texas A&M University Stable Isotopes for Biosphere Science Laboratory.

Soil moisture

Volumetric soil water content (VWC) at 0 to 12 cm depth was measured with a HydroSense Soil Water Measurement System (Campbell Scientific, Inc., Logan, UT, USA). Eight measurements per plot (128 total) were sampled every four to six weeks during the growing season and every six to eight weeks during winter months. Before first measurement, locations were determined by completely random sampling design.

Statistical analysis

Total (5th through 7th growing season) height, DBH, and basal area growth were analyzed as a 2 x 2 factorial combination of main effects fertilization and throughfall reduction ($n = 4$). For all other measurements, treatment effects were analyzed using repeated measures analysis (Proc Mixed, SAS Inc., Cary, NC, USA) with block as a random factor and treatments as fixed factors and the autoregressive 1 (AR1) covariance structure. Plot means for all data were used ($n = 4$) and treatment effects were considered significant at $P < 0.05$.

Results

Climate

Annual precipitation was 1026 mm in 2012, 1312 mm in 2013, and 1289 mm in 2014. In 2012, greatest precipitation occurred in March (184 mm) and least occurred in November (11 mm). In 2013, greatest precipitation occurred in July (239 mm) and least occurred in August (17 mm). In 2014, greatest precipitation occurred in July (263 mm) and least occurred in August (26 mm) (Fig. 1). Average daily air temperature for 2012 was 17.3 °C with average daily maximum and minimum air temperatures of 25.0 °C and 10.1 °C, respectively. Average daily air temperature for 2013 was 15.5 °C with average daily maximum and minimum air temperatures of 22.6 °C and 9.0 °C, respectively. Average daily air temperature for 2014 was 15.0 °C with average daily maximum and minimum air temperatures of 21.9 °C and 8.7 °C, respectively (Fig. 1). PDSI indicated severe to extreme drought (PDSI < -4) from October 2012 through January 2013. PDSI became slightly positive beginning in February 2013 and remained so for the rest of the calendar year. For 2014, PDSI was near normal with January to May slightly negative and the rest of the year slightly positive (Fig. 2).

Soil moisture

Throughfall reduction reduced VWC during wetter periods (Table 1) (Fig. 3). Ambient precipitation plus fertilizer (F) maintained greater VWC than control (C) treatments while the VWC of the fertilized treatment receiving throughfall reduction (TR+F) was similar to the non-fertilized, throughfall reduction (TR) treatment (Table 1) (Fig. 3). Across the entire study period, VWC 0-12 cm averaged 8.5 ± SE 2.5 % for the TR+F treatment, 9.3 ± SE 2.7 % for the TR treatment, 12.7 ± SE 3.2 % for the F treatment, and 9.7 ± SE 2.9 % for the C treatment. Across all plots, VWC at 0-12 cm was reduced from 11.3 % to 8.8 % by throughfall reduction excluders.

Growth

By age 7, average tree height ranged from 6.8 m for F treatment to 6.4 m for TR+F treatment (Fig. 4A). Average tree DBH ranged from 13.1 cm for F treatments to 11.9 cm for TR treatments (Fig. 4B). There were not interactions between throughfall reduction and fertilization for growth (Table 2). Throughfall reduction (TR and TR+F) reduced total height growth over growing seasons 5-7 (Table 2) by 8.2 % compared with ambient precipitation treatments (F and C) (Fig. 5A). Throughfall reduction (TR and TR+F) reduced total DBH growth over growing seasons 5-7 (Table 2) by 6.1 % and fertilization (F and TR+F) increased total DBH growth (Table 2) by 7.3 %, compared with ambient precipitation (F and C) and non-fertilized treatments (TR and C), respectively (Fig. 5B). As basal area is largely a function of DBH, results of BA and DBH were similar. Throughfall reduction reduced total basal area growth over growing seasons 5-7 (Table 2) by 8.8 % and fertilization increased total basal area growth (Table 2) by 11.6 %, compared with ambient precipitation and non-fertilized treatment trees, respectively (Fig. 5C). Fertilization compensated for drier conditions in DBH and BA growth, such that TR+F trees had similar growth as C treatment trees (Fig. 5B).

LAI

Throughfall reduction reduced LAI and fertilization increased LAI (Table 1), compared with ambient throughfall and non-fertilized treatment trees, respectively (Fig. 6). Similar to tree DBH and BA growth response, fertilizer compensated for drier conditions as TR+F stands had higher LAI than both non-fertilized treatment stands (Fig. 6). The date by treatment interaction was not significant. Seasonally, LAI ranged from 1.9 $m^2 m^{-2}$ for TR treatment in winter (December 2014) to 4.4 $m^2 m^{-2}$ for F treatment in summer (August 2015) (Table 1). Overall, average LAI of treatments was 3.2 ± SE 0.4 $m^2 m^{-2}$ for TR+F, 2.8 ± SE 0.4 $m^2 m^{-2}$ for TR, 3.3 ± SE 0.4 $m^2 m^{-2}$ for F, and 3.0 ± SE 0.3 $m^2 m^{-2}$ for C.

Physiology

P_{net}, g_s, and C_i, varied by sampling date (Table 1) and followed similar trends. Throughfall reduction reduced P_{net} (Table 1) (Fig. 7A) and the interaction between date and throughfall reduction was not significant (Table 1). On average, the main effect of throughfall reduction decreased P_{net} by 9 % (Fig. 8A). Fertilization did not significantly affect P_{net} (Fig. 8A). Throughfall reduction and fertilization reduced g_s (Table 1). In general, g_s was reduced more so due to throughfall reduction during wetter periods (Table 1) except for August 2013 when throughfall reduction decreased g_s (Fig. 7B), but soil moisture was low. The August 2013 measurement was the first measurement on the 2013 flush. On dates where throughfall reduction was significant, throughfall reduction decreased g_s 20 % on average. Across all dates, throughfall reduction decreased g_s by 12 %. Fertilization decreased g_s by 7 % on average (Table 1) and date x fertilization was not significant (Fig. 8B). Throughfall reduction and fertilization (Table 1) reduced C_i by 3 and 4 %, respectively (Fig. 7C, 8C).

Throughfall reduction (Table 1) caused more negative mid-day Ψ_L with the effects increasing over the course of the experiment (Table 1) (Fig. 7D). On dates where throughfall reduction was significant for Ψ_L, the differences were 16 % on average. Across all dates, throughfall reduction decreased Ψ_L by 11 %. Fertilization caused less negative mid-day Ψ_L (Table 1) (Fig. 9) of 3% on average (Fig. 8D).

Throughfall reduction caused less negative $\delta^{13}C$ for the 2013 foliage cohort ($p = 0.01$), but not for the 2012 cohort (developed before treatment) or 2014 cohort (Table 3) (Fig. 9A). Fertilization increased foliar N concentration (Table 3). Fertilization had a stronger effect on foliar N in the 2012 cohort with an increase of 16 % ($p < 0.0001$) compared to 12 % ($p < 0.0001$) and 4 % ($p = 0.04$) for the 2013 and 2014 cohorts, respectively (Table 3) (Fig. 9B). Throughfall reduction did not significantly affect foliar nitrogen concentration, but there was a date x throughfall x fertilization interaction (Table 3) that occurred due to a change among ranking of treatments and years.

Discussion

Our results indicate that throughfall reduction decreased leaf gas exchange as a result of decreased soil moisture availability (supported by lower VWC and more negative leaf water potentials) which probably in turn slowed growth. In contrast, fertilization increased growth and did not affect photosynthetic rate. While stomatal conductance was reduced by fertilization, it was not due to water stress as the trees in the fertilized treatments had less negative water potentials and VWC was not lower on average compared to nonfertilized treatments. The net effect was that fertilization probably increased the efficiency of photosynthesis per water use which may have helped compensate for lower soil moisture such that growth of the TR+F and Control treatments were similar.

As stand-level leaf area increases with resource availability, potential stand water use also increases which would be reflected in lower VWC. For instance, a similar study (Samuelson et al. 2014) found that fertilization reduced average soil moisture in a 7-year-old loblolly pine plantation. The lack of reduced VWC in the F treatment in our study, even though they had greater LAI than the nonfertilized control treatment, could be from random plot-level differences in soil texture causing variability in VWC. In contrast to

Samuelson et al. (2014) who found thoughfall reduction decreased VWC during drier periods, VWC was reduced by throughfall reduction during wetter periods in our study while VWC was similarly low across all treatments during dry periods.

Fertilization increases diameter and height growth of loblolly pine plantations (e.g., Bolstad et al. 1987, Will et al. 2002, Albaugh et al. 2004, Will et al. 2006). Compared to fertilization, increased water availability (irrigation) has a small positive or no effect (Albaugh et al. 2004, Coyle et al. 2008, Samuelson et al. 2008a). In our study, fertilization compensated for reduced throughfall availability on total DBH and total basal area growth such that trees experiencing TR+F had similar growth as C treatment trees. These results were similar to Samuelson et al. (2014), in that fertilization increased mean DBH and basal area growth over the second growing season of treatment. However, in that study, throughfall reduction had no effect on mean DBH, basal area, or mean height growth. Unlike diameter and BA growth, fertilization in our study did not benefit height growth. The only effect on height development was the negative effect of throughfall reduction. Fertilization generally has a greater effect on diameter growth than height growth in loblolly pine (Allen et al. 2005) and the response could depend on the drainage of the site, where fertilization on poorly drained sites increases height growth more so than on moderately to well drained sites (Amateis et al. 2000) characteristic to our site.

Increasing the availability of nutrients through fertilizer application increases LAI in loblolly pine stands (e.g., Albaugh et al. 1998, Will et al. 2002, Will et al. 2006). We found positive effects of fertilization regardless of the negative effects of throughfall reduction treatment (no interaction). Samuelson et al. (2014) found that LAI was higher in fertilized compared to non-fertilized treatments, but throughfall reduction of ~30 % did not affect LAI in a loblolly pine plantation in Georgia. The negative effects of throughfall reduction we found to both LAI and growth could have been due to higher summer temperatures and greater VPD at our site compared sites further east (Seager et al. 2015). Will et al. (2015) reported average August daily temperatures during the study period for our site was, 2.6 ^{o}C warmer than the Georgia site. Aside from our study and Samuelson et al. (2014), there are no published results on throughfall reduction effects on LAI of southern pine. However, previous studies report increases (Ewers et al. 2000, Albaugh 2004, Samuelson et al. 2008b) and no effect of irrigation on LAI (Albaugh 1998, Samuelson et al. 2001, Allen et al. 2005).

In our study, throughfall reduction reduced P_{net}, and this reduction was consistent with water stress, i.e., lower C_i, more negative mid-day Ψ_L and reduced g_s during wetter periods, and less negative $\delta^{13}C$ for the 2013 cohort (the 2013 cohort represented 45 % of gas exchange measurement dates). For dates where throughfall reduction significantly decreased g_s, average monthly precipitation was 151 mm compared to 88 mm average monthly precipitation bracketing dates where g_s was not affected. Reduction in g_s occurred during wetter periods probably because all trees were stressed during the drier periods such that the effects of a 30% reduction in throughfall were largely eliminated. The only exception was August 2013 when soil moisture was low but g_s was significantly different due to throughfall reduction. For this measurement date, the difference in g_s may have resulted from changing sampling from the first flush of 2012 to the first flush of 2013. Leaf gas exchange of current year foliage in loblolly pine is higher than previous year foliage (Will et al. 2001, Radoglou and Teskey 1997) which could have accentuated the differences. Similar studies found contrasting results where P_{net} and g_s were only reduced by throughfall reduction during drought periods (Tang et al. 2004, Samuelson et al. 2014). Similar to LAI and growth, the different response of our study may be a result of higher temperatures and greater VPD during drought periods at our site at the western margin of the loblolly pine commercial range. During drought periods on our site, leaf gas exchange was minimal across all treatments.

The more negative mid-day Ψ_L due to throughfall reduction occurred overtime as a result of throughfall reduction treatment effects compared to sampling dates earlier that were closer to when throughfall reduction treatments were implemented (Fig 7D). The decrease in C_i that we measured in response to reduced throughfall was due to reduced g_s as P_{net} also declined. This response of C_i is due to a continuation of the removal of CO_2 from the intercellular air space and a reduction in resupply of CO_2 from reduced g_s (Green and Mitchell 1992). When comparing the relationship between C_i and P_{net} of loblolly pine under water stress, P_{net} was nearly linearly (Teskey et al. 1986) or curvilinearly (Barber 1986, Green and Mitchel 1992) related to changes in C_i with stomatal limitation accounting for 20-30 % of total limitation of CO_2 diffusion into the leaf under a wide range of environmental conditions (Teskey et al. 1986). When experiencing decreased water availability it has been suggested that mesophyll conductance is more responsive than g_s, limiting CO_2 diffusion for photosynthesis (Teskey et al. 1986, Green and Mitchell 1992, Grassi and Magnani 2005).

Foliar $\delta^{13}C$ for the 2013 cohort for trees experiencing throughfall reduction was more negative than ambient precipitation treatment trees, which indicates less discrimination against $\delta^{13}C$ and increased leaf water use efficiency in 2013 of the throughfall reduction trees. The year 2013 was the wettest of our study (1312 mm) which was preceded by an extremely dry year in 2012. These results relate to our g_s findings where throughfall reduction had the greatest effect during wetter periods. A similar study found contrasting results with less discrimination against $\delta^{13}C$ occurring during a drier year compared to a wetter year (Samuelson et al. 2014) and could again be related to greater temperature and evaporative demand during drier periods at our site.

Fertilization typically does not affect leaf gas exchange in loblolly pine (Zhang et al. 1997, Samuelson et al. 2001, Will et al. 2001, Munger et al. 2003). The same was found in studies similar to ours that included throughfall reduction and fertilization (Tang et al. 2003, Samuelson et al. 2014). While P_{net} was not significantly affected in our study, fertilization reduced g_s, C_i, and increased (less negative) mid-day Ψ_L. This indicates that photosynthesis was similar across a range of nutrient availability but that leaf-level water loss could have been reduced by fertilization. This response could be related to increased foliar N concentration found in our study. Fertilization can lead to increased water use efficiency in loblolly pine seedlings associated with increases foliar N, P_{net}, and decreased g_s (Green and Mitchell 1992, Samuelson et al. 2000, Tyree et al. 2009). The response of mesophyll conductance to fertilization has been positive, where mesophyll limitation is decreased, thus increasing photosynthesis (Green and Mitchell 1992). While we didn't measure mesophyll conductance, it seems likely that a positive response of mesophyll conductance to fertilization allowed trees within fertilized stands to maintain P_{net} rates at reduced g_s. This increases water use efficiency such that fertilization might help sustain carbon gain under drier conditions. When water use efficiency is expressed as P_{net}/g_s, the average increased 6%, from 74.5 to 80.0 μmol mol^{-1}, with fertilization in our study.

Conclusions and management implications

Our results indicate that thoughfall reduction decrease soil moisture and reduces leaf gas exchange and slows growth. However, we found that fertilization can compensate for throughfall reduction. Additive effects of throughfall reduction and fertilization indicate that positive effects of fertilization are not eliminated by throughfall reduction. Because the responses of fertilization and throughfall reduction are additive, differences between the two responses will determine whether growth increases or decreases when both are combined. Contrary to our initial hypothesis, fertilization did not exacerbate the effects of througfall reduction even though leaf area did increase. Rather, fertilization increased water use efficiency. These results indicate that fertilization can be beneficial in loblolly pine plantations experiencing reduced water availability on the western limits of its commercial range and indicates the impact of decreased water availability in loblolly pine growing areas with high growing season temperatures and atmospheric demand for water.

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Tables.

Table 1. *P* values for the effects of date, throughfall reduction (Water) and fertilization (Fert) treatments on volumetric soil water content (VWC), leaf area index (LAI), net photosynthesis (P_{net}), stomatal conductance (g_s), intercellular CO_2 (C_i), and midday leaf water potential (Ψ_L) for the entire measurement period (2012-2014). Bold values indicate significance at $\alpha < 0.05$.

	VWC	LAI	P_{net}	g_s	C_i	Ψ_L
Date	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Water	<0.0001	**0.005**	**<0.0001**	<0.0001	**0.009**	<0.0001
Fert	0.006	**<0.0001**	0.23	**0.01**	**0.001**	**0.02**
Water x Fert	**<0.0001**	0.19	0.64	0.93	0.83	0.86
Date x Water	**0.01**	0.76	0.10	**0.008**	0.52	**0.04**
Date x Fert	0.99	0.90	0.08	0.36	0.98	0.95
Date x Water x Fert	0.81	0.97	0.90	0.79	0.53	0.41

Table 2. *P* values for the effects of block, throughfall reduction (Water) and fertilization (Fert) treatments on total height growth (Height), total DBH growth, and total basal area growth (BA) for the entire measurement period (2012-2014). Bold values indicate significance at α < 0.05.

	Height	DBH	BA
Block	0.34	0.45	0.24
Water	**<0.0001**	**0.0009**	**0.004**
Fert	0.97	**0.0003**	**0.0006**
Water x Fert	0.91	0.87	0.11

Table 3. *P* values for the effects of year, throughfall reduction (Water) and fertilization (Fert) treatments on stable carbon isotope ratio ($\delta^{13}C$) and nitrogen concentration of the foliage, total DBH growth, and total basal area growth (BA) for the 2012-2014 foliage cohorts. Bold values indicate significance at $\alpha < 0.05$.

	$\delta^{13}C$	N
Year	**<0.0001**	**<0.0001**
Water	**0.01**	0.17
Fert	0.07	<0.0001
Water x Fert	0.95	0.83
Year x Water	**0.0002**	0.61
Year x Fert	0.07	<0.0001
Year x Water x Fert	0.45	**0.008**

Figures.

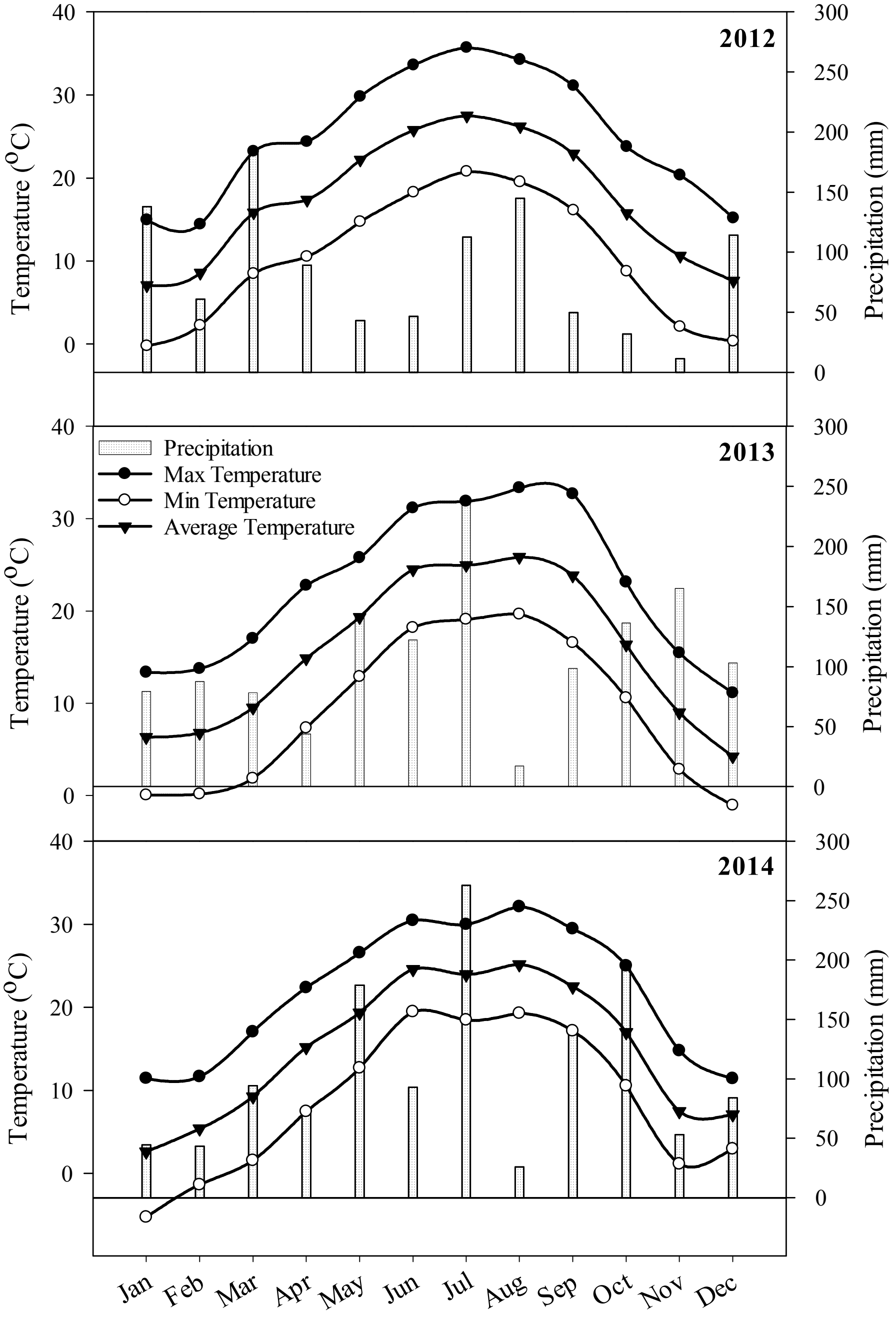

Fig. 1. Monthly precipitation and average maximum, minimum, and mean air temperature for 2012, 2013, and 2014 for Broken Bow Oklahoma, USA.

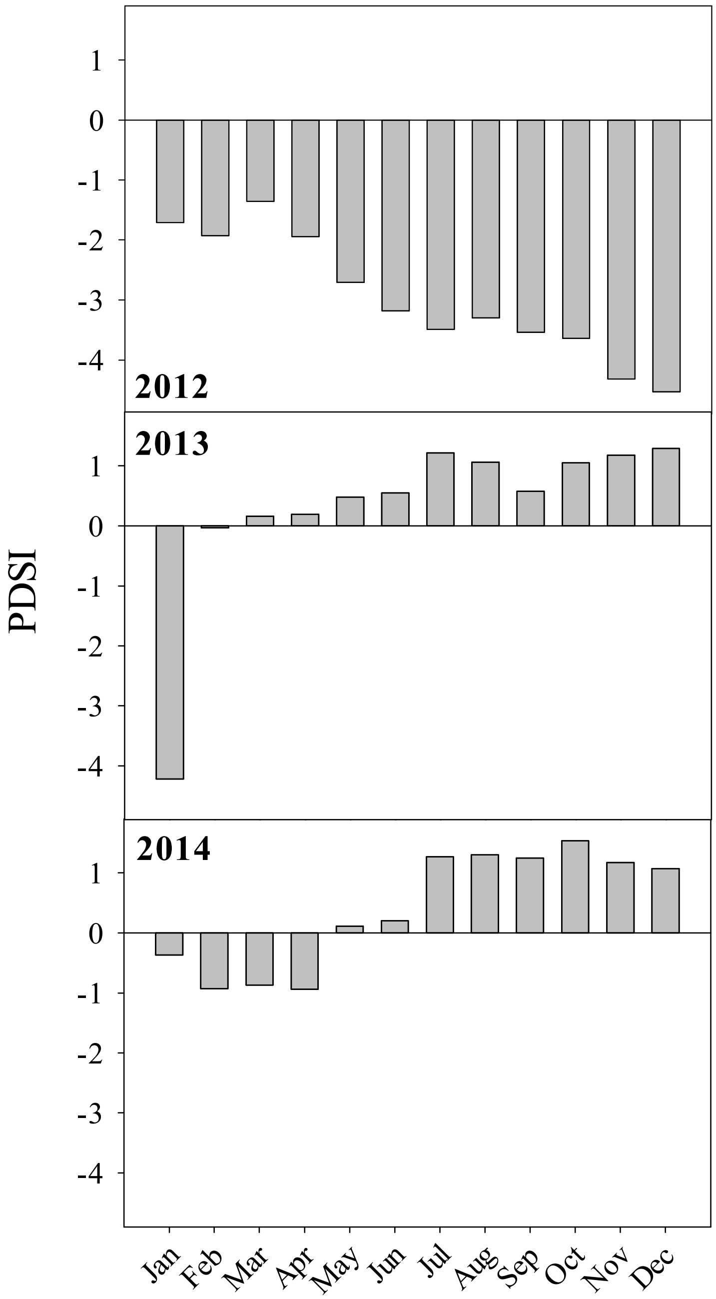

Fig. 2. Monthly Palmer Drought Severity index (PDSI) for Climate Division 9 (southeast) in Oklahoma for 2012, 2013, and 2014.

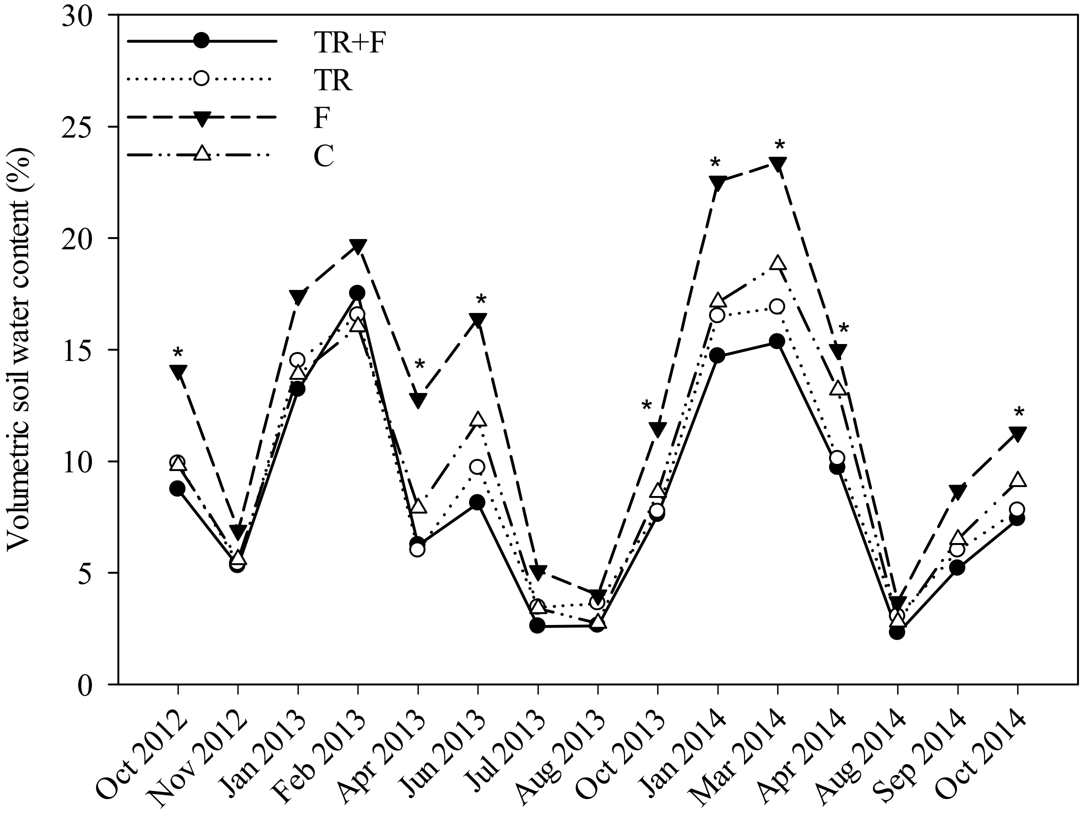

Fig. 3. Mean volumetric soil water content (%) between 0 and 12 cm in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. In cases where there is a significant throughfall reduction x date interaction, an asterisk (*) above the data represents dates that throughfall reduction effect is significant (*n* = 4).

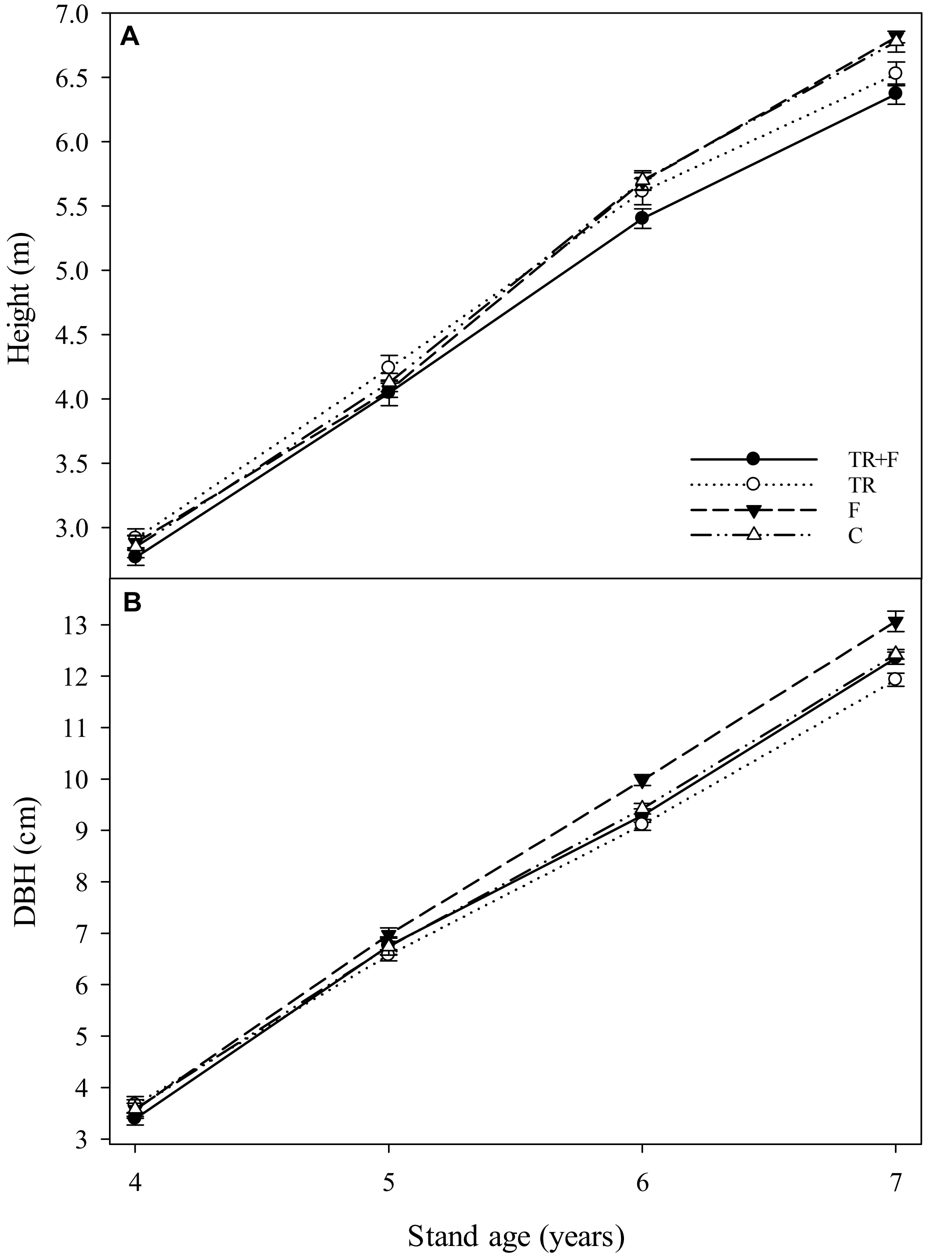

Fig. 4. Mean tree height and DBH by stand age in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments Error bars represent standard error of treatment means (*n* = 4).

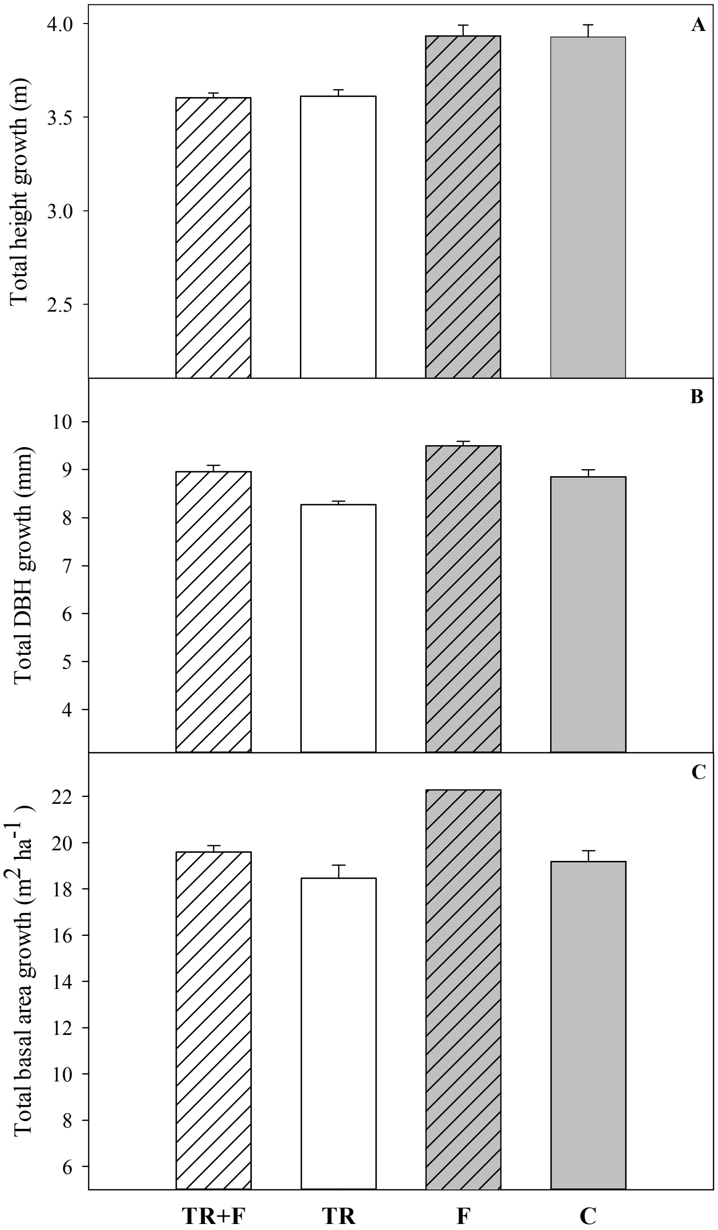

Fig. 5. (A) total height growth, (B) total DBH growth, and (C) total basal area growth over the 5th, 6th, and 7th growing seasons in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments.(*n* = 4). Error bars represent standard error of treatment means (*n* = 4).

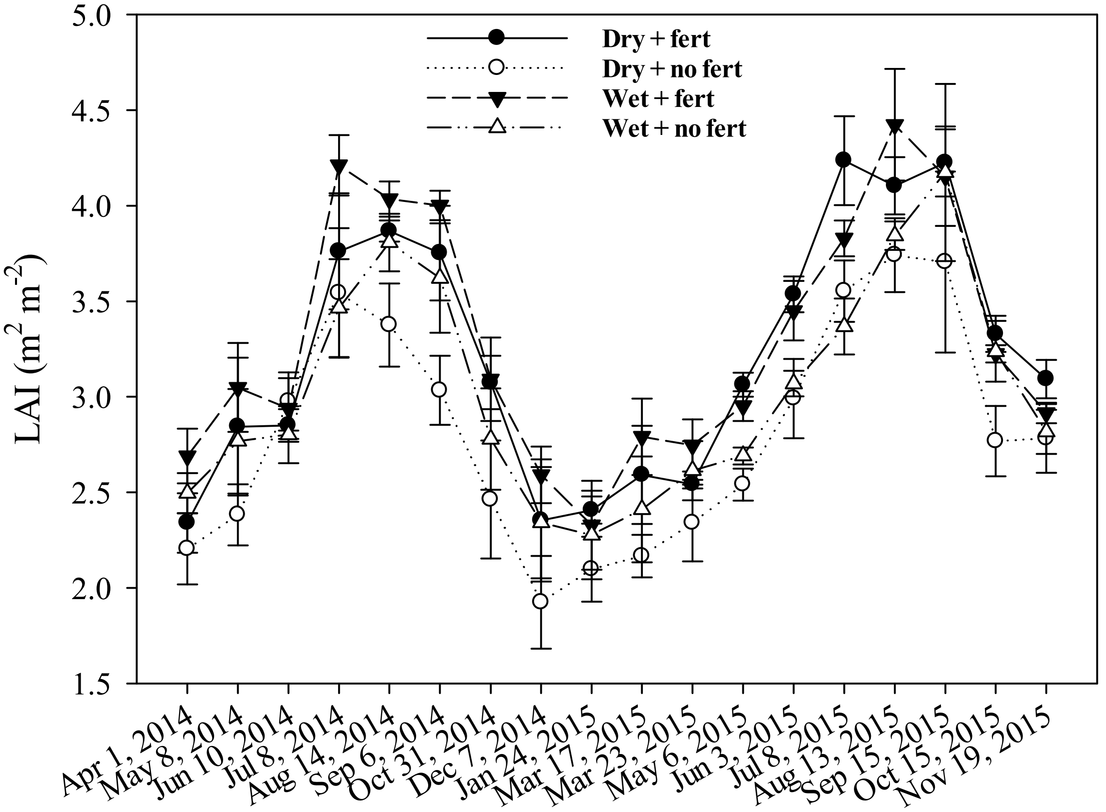

Fig. 6. Mean leaf area index (LAI) in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. Error bars represent standard error of treatment means (*n* = 4)

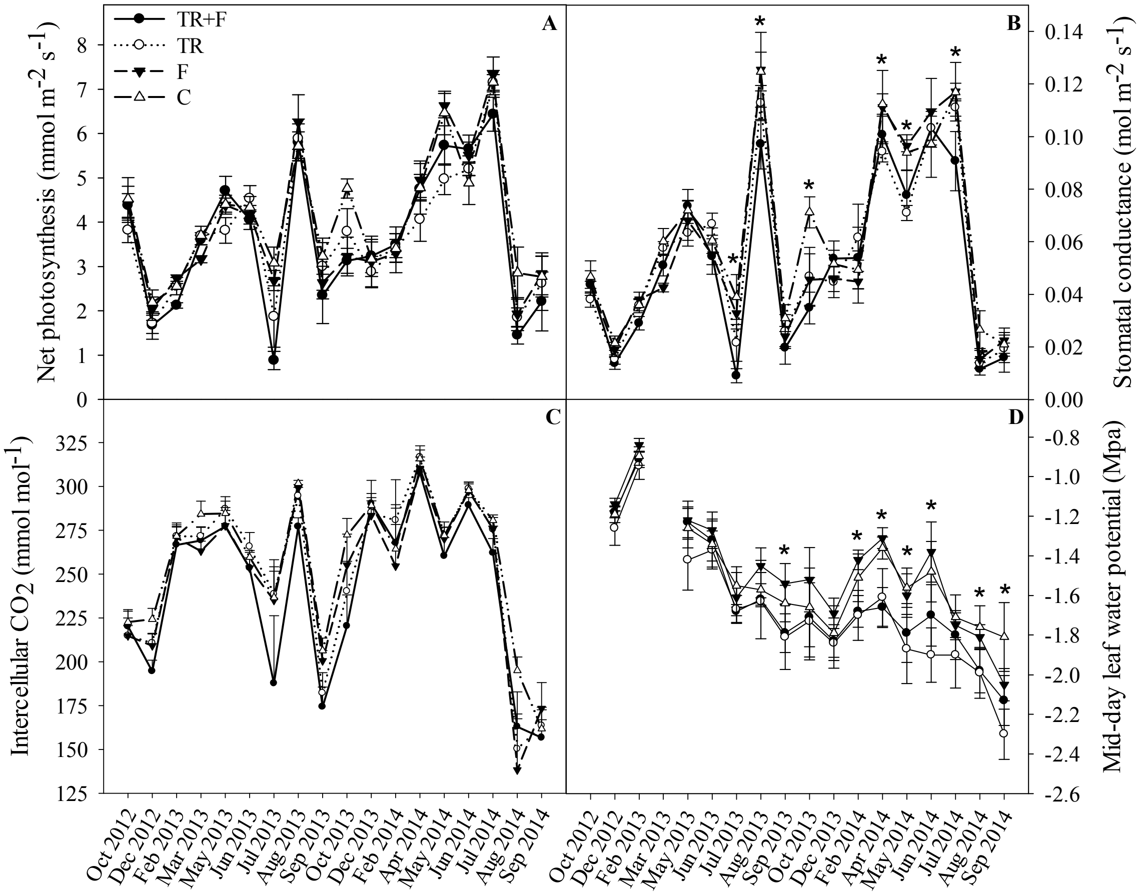

Fig. 7. (A) Mean net photosynthesis (P_{net}), (B) mean stomatal conductance (g_s), (C) intercellular CO_2 (C_i), (D) mean mid-day leaf water potential (Ψ_L) in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control - ambient throughfall plus no fertilization (C) treatments. In cases where there is a significant throughfall reduction x date interaction, an asterisk (*) above the data represents dates that throughfall reduction effect is significant. Error bars represent standard error of treatment means (*n* = 4).

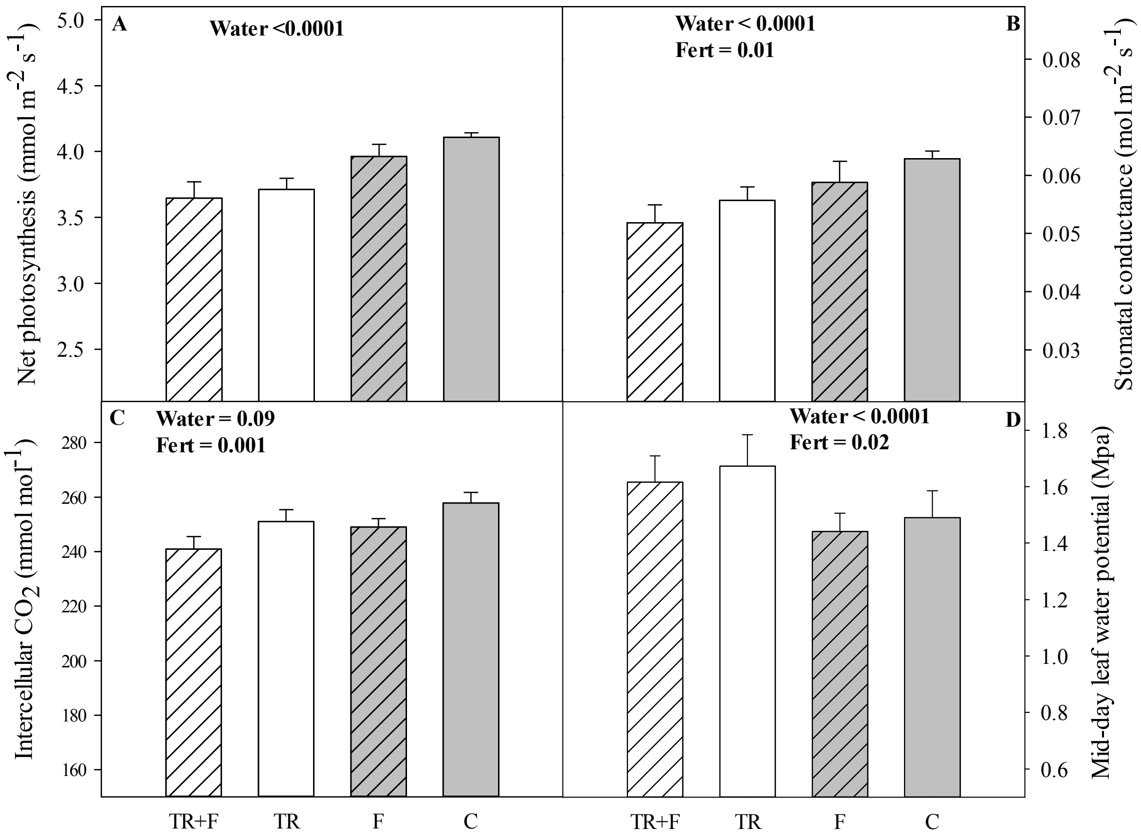

Fig. 8. Total overall (A) mean net photosynthesis (P_{net}), (B) mean stomatal conductance (g_s), (C) intercellular CO_2 (C_i), (D) mean mid-day leaf water potential (Ψ_L) in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control - ambient throughfall plus no fertilization (C) treatments. Error bars represent standard error of treatment means (*n* = 4).

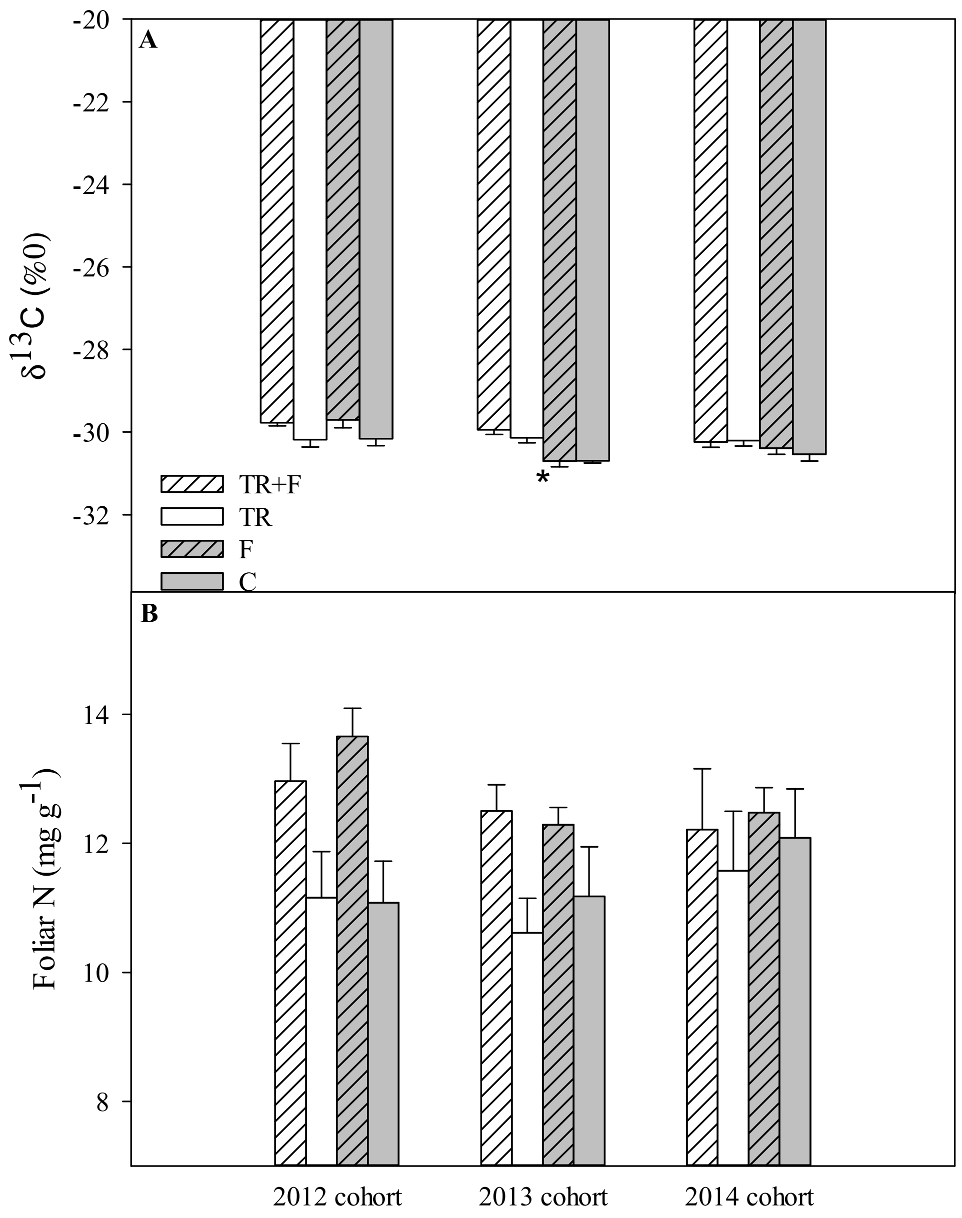

Fig. 9. Mean (A) stable carbon isotope ratio ($\delta^{13}C$) (‰) and (B) nitrogen concentration (mg/g) of foliage by year in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control - ambient throughfall plus no fertilization (C) treatments. . In cases where there is a significant throughfall reduction x date interaction, an asterisk (*) above the data represents dates that throughfall reduction effect is significant. Error bars represent standard error of treatment means (*n* = 4).

CHAPTER III

EFFECTS OF DECREASED WATER AVAILABILITY AND FERTILIZATION ON WATER USE AND GROWTH EFFICIENCY OF LOBLOLLY PINE (*PINUS TAEDA* L.) STANDS AT THE WESTERN EDGE OF THE COMMERCIAL RANGE

Abstract

Over half the standing timber volume in the southeastern US is composed of loblolly pine (*Pinus taeda* L.), making it the most important tree species in the region. Future climate change may impact productivity of these forests through reductions in water availability. To determine the effects of nutrient availability and decreased water availability on stand-level water use efficiency and growth efficiency we examined the interactive effects of fertilization (one time application of 224 kg N ha^{-1}, 28 kg P ha^{-1}, 56 kg K ha^{-1} and micronutrients) and reduced throughfall (approximate 30 % reduction) on whole-tree water use, stand-level canopy transpiration, LAI, and volume growth. This study was conducted over the 6th and 7th growing seasons (2013-2014) of a loblolly pine plantation in southeast, Oklahoma. Across all plots, throughfall reduction reduced VWC from 13.6 % to 10.9 % from 0-12 cm soil depth and from 22.3 % to 19.9 % from 12-45 cm soil

depth and reduced stand volume growth from 20.9 m^3 ha^{-1} to 17.9 m^3 ha^{-1}. Across all treatments, fertilization increased LAI by 12 %, stand volume growth increased from 18.3 m^3 ha^{-1} to 20.5 m^3 ha^{-1}, and increased water use efficiency of stem volume production by 18 %. These results indicate that fertilization can benefit stand growth of loblolly pine plantations in areas with higher growing season temperatures and greater VPD, such as the western edge of the loblolly pine commercial range in part by increasing the efficiency of water use.

Introduction

The Southeast is considered the wood basket of the United States (Schultz 1997), producing more timber volume than any other region in the country (Oswalt et al. 2014). Over half the standing pine volume in this region is contained in loblolly pine (*Pinus taeda* L.) plantations, making loblolly pine the most important tree species in the region (Wear and Greis 2012). However, the ability to maintain productivity of loblolly pine plantations in the future is unclear as these forests are predicted to experience more variable precipitation events and increased temperature. Precipitation events are projected to become more extreme with increased intensity and longer periods of dry days between events (Easterling et al. 2000, Collins et al. 2013, Walsh et al. 2014). By the end of the century, a 2.5 to 4 ^{o}C increase in average temperature is projected across the region (Collins et al. 2013).

If projections come true, the change in climate may impact productivity of these forests through reductions in water availability (Johnson et al. 2014) due to increased runoff of the more extreme events and higher potential evapotranspiration (PET) from increased temperature and vapor pressure deficits (Breshears et al. 2013, Collins et al. 2013, Seager et al. 2015). There is a greater potential for impacts for the western edge of the commercial range of loblolly pine in southeastern Oklahoma which is beyond their natural range. This area already experiences higher growing season temperatures and VPD than the most of the southeastern USA (Seager et al. 2015). This, combined with projections of higher increases in temperature and greater summertime drought compared the rest of the region (Collins et al. 2013) may lead to greater water stress experienced by loblolly pine planted in this area.

Loblolly pine productivity in the southeastern United States is largely driven by intensive silvicultural practices such as site preparation, competition control, and fertilization (Fox et al. 2007). Fertilization, specifically combinations of N and P, increases leaf area, carbon fixation, and productivity of these plantations (Teskey 1986, Vose and Allen 1988, Albaugh et al. 1998), and can increase growth efficiency, i.e., stem growth per unit of leaf (Will et al. 2002, Albaugh et al. 2004, Fox et al. 2007). Greater leaf area index (LAI) can lead to reduced soil water availability from increased interception of precipitation (Stogsdill et al. 1989) as well as increased water use. Evapotranspiration (ET) is the largest water output in forests and is closely related to LAI (Law et al. 2002, Sun et al. 2011). Increasing LAI increases canopy transpiration as a result of increased hydraulic conductance when water is not limiting (Oren et al. 1986, Ewers et al. 1999, Samuelson et al. 2008). Canopy transpiration can account for between 50-70 % of ET in mid-rotation loblolly pine plantations (Cao et al. 2006, Gonzalez-Benecke and Martin 2010, Domec et al. 2012). Fertilization added to non-water stressed loblolly pine plantations could also reduce belowground carbon allocation to fine roots

(Albaugh et al. 1998), which could increase stand vulnerability during periods of reduced water availability (McNulty et al. 2014), possibly as a result of limited water uptake capacity (Hubbard et al. 2004).

Until recently (Tang et al. 2004, Samuelson et al. 2014, Bartkowiak et al. 2015, Ward et al. 2015), research on water availability and water use in loblolly pine plantations focused on the benefits of irrigation (e.g., Pataki et al. 1998, Ewers et al. 2000, Albaugh et al. 2004), rather than throughfall reduction. Irrigation generally either had no effect (Samuelson et al. 2008) or caused moderate increases in canopy transpiration (Albaugh et al. 2004, Samuelson and Stokes 2006). The general lack of substantial increases in canopy transpiration in regards to irrigation could be attributed to greater precipitation and higher relative humidity for areas of these previous studies, which may exceed potential transpiration for these sites (Samuelson et al. 2008, Domec et al. 2012). However, recent research reports a decline in canopy transpiration from reduced water availability in mid-rotation loblolly pine plantations (Bartkowiak et al. 2015, Ward et al. 2015).

Understanding how fertilization and water availability affect growth efficiency (stem volume per unit of leaf) is important to further understand and predict tree and stand growth. Previous studies on southern pines found that nitrogen fertilization increases growth efficiency (Vose and Allen 1988, Albaugh et al. 1998), or have found effects of nitrogen fertilization on growth efficiency is dependent on stand age or tree size, with initial increases in growth efficiency in younger stands and decreased growth efficiency in older stands (Jokela and Martin 2000, Will et al. 2002). The effects of fertilization and reduced water availability on efficiency of southern pine growth, specifically loblolly pine, are minimal and have showed contrasting results. For instance, Samuelson et al. (2014) reported no effects of fertilization or throughfall reduction on growth efficiency (slope of the relationship between annual stemwood production and peak projected LAI) of a 7-year-old loblolly pine plantation in Georgia. In a similar study, Ward et al. (2015) found water use efficiency (stem volume production per sum of annual canopy transpiration) increased with both fertilization and throughfall reduction, and increased the greatest in a treatment of fertilization plus throughfall reduction in a 10-year-old loblolly pine plantation in Virginia. When water use efficiency was calculated as net photosynthesis divided by leaf-level transpiration for an 18-year-old loblolly plantation in Louisiana, throughfall reduction caused increased water use efficiency during wetter periods, whereas fertilization had no effect on water use efficiency (Tang et al. 2004).

A better understanding of how fertilized stands with greater leaf area index and subsequently stand water use and growth will respond to reduced water availability and the negative effects of drought on productivity are needed. This is especially important for commercial range loblolly pine planted along their western limits. This information is essential for understanding potential impacts on volume growth and potentially provide insight on carbon sequestration and ultimately help guide effective management practices necessary for sustaining productivity of loblolly pine plantations under a changing climate.

The objective of this study was to determine the interactive effects of nutrient availability and decreased water availability on stand-level water use efficiency (stem growth per unit of water use) and growth efficiency (stem growth per unit of LAI) by examining whole-tree water use, stand-level canopy transpiration, LAI, and volume growth. To accomplish this objective, we examined the interactive effects of fertilization and reduced throughfall. Our central hypothesis was that fertilizer added to a loblolly pine plantation in southeastern Oklahoma will increase LAI and throughfall reduction will reduce whole-tree and stand-level water use. We further hypothesized that fertilization will increase stand volume growth and compensate for throughfall reduction effects by increasing water use efficiency and growth efficiency, resulting in stand volume growth similar to that of stands not experiencing throughfall reduction or fertilization.

Materials and methods

Study site

The study was located on a loblolly pine stand near Broken Bow, OK (34°01'52.0''N, 94°49'18.2''W) that was part of the Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP) (www.pinemap.org) Tier III range-wide experiment (Will et al. 2015). The research was conducted beginning in the 6th growing season (2013) and continued through the 7th growing season (2014). The study site had an elevation of 150 m and soils were very deep, well-drained, fine sandy loam in the Ruston series (Fine-loamy, siliceous, semiactive, thermic Typic Paleudult). Depth to the water table was greater than 2 m. This soil was typical for forests on the uplands of the western and southern upper Coastal Plain and consisted of 3 to 8 percent slopes (http://soilseries.sc.egov.usda.gov). On average over the last twenty years (1994 – 2014) for Broken Bow, OK, May has been the wettest month (160 mm) and August the driest

(70 mm). Average annual precipitation over this period was 1281 mm. Over the last twenty years, August with average maximum temperature of 34.7 °C has been the warmest month and January has been the coldest month with average minimum temperature of -1.0 °C for Broken Bow, OK (http://mesonet.org/index.php/weather/daily_data_retrieval, accessed March 2016).

Before planting, broadcast herbicide of 680 g ha^{-1} of Chopper ® (27.6 % imazapyr, 72.4 % inert ingredients) (BASF Corporation, Florham Park, NJ, USA) plus 2.8 l ha^{-1} of glyphosate was applied in August 2017 followed by prescribed burn in October 2007. The study site was subsoiled along the contour to depths of 51 to 61 cm using a D8 Caterpillar dozer and attached subsoiling shanks (Caterpillar Corporate, Peoria, IL, USA) in November 2007. The site was planted in January 2008 with improved Western Gulf Tree Improvement Cooperative 1-0 bare-root seedlings originating from a mix of half-sib families. Broadcast herbicide of 420 g ha^{-1} of Arsenal® (27.6 % imazapyr, 72.4 % inert ingredients) (BASF Corporation, Florham Park, NJ, USA) and 175 g ha^{-1} of Oust Extra® (56.25 % Sulfometuron methyl, 15.0 % Metsulfuron methyl, 28.75 % other) (E.I. Du Pont De Nemours and Company, Wilmington, DE, USA) was applied for woody plant and herbaceous weed control in March 2008. Spacing for the site was approximately 2 m x 3 m for an approximate density of 1650 trees ha $^{-1}$.

Experimental design

The study consisted of four treatments replicated four times in a 2 x 2 factorial combination of fertilization and throughfall reduction. The treatment plots were a minimum of 0.10 ha and contained measurement plots between 0.034 and 0.041 ha. Fertilization treatments included no fertilizer and a combination of 224 kg N ha^{-1}, 28 kg P ha^{-1}, 56 kg K ha^{-1}, as well as micronutrients. N and P consisted of a mix of urea (432 kg ha^{-1}) and diammonium phosphate (140 kg ha^{-1}). K consisted of potassium chloride. Micronutrients consisted of a granular mix (Southeast Mix, Cameron Chemicals, Inc., Virginia Beach, VA, USA) applied at a rate of 22.4 kg ha^{-1}, containing 6% sulfur, 5% boron, 2% copper, 6% manganese, and 5% zinc. Fertilizer was broadcast by hand throughout treatments in April 2012. Competing vegetation was chemically eliminated beginning in spring of 2012 using directed sprays of glyphosate (2% a.i., Roundup®, Monsanto Company, St. Louis, MO, USA).

Throughfall treatments consisted of ambient throughfall and throughfall exclusion troughs (excluders) based on the driest climate predictions at the time for the region (Christensen et al. 2007), designed to remove approximately 30 % of precipitation throughfall. Excluders were installed between early and mid-summer 2012 and consisted of two 50 cm wide troughs separated by a 50 cm opening with a height sloping approximately from 1.2 m to 0.6 m, covering ~30 % of ground area. Excluders were built of lumber and covered with clear, U.V. stabilized plastic sheeting consisting of two layers co-extruded polyethylene and one layer of high strength polyester string (Tuff-Scrimm™ Poly 12, Americover Inc., Escondido, CA, USA).

Environmental variables

Meteorological variables were measured on site from a weather station located at the top of a tower in an open area centered among plots. Air temperature and relative humidity were measured with a CS215 temperature and relative humidity probe (Campbell Scientific, Logan, UT, USA). Vapor pressure deficit (VPD) was calculated based on Food and Agriculture Organization of the United Nations FAO guidelines for computing crop water requirements (Allen et al. 1998). Average monthly daytime VPD was determined by averaging 15-minute VPD calculations from daily sunrise/sunset times for Broken Bow, OK (Astronomical Applications Dept., U.S. Naval Observatory). Precipitation was measured with a TR-525M remote tipping bucket rainfall sensor (Texas Electronics, Inc., Dallas, TX, USA). Data were recorded continuously every 15 minutes using a CR1000 data logger (Campbell Scientific, Logan, UT, USA). Missing data from malfunctions or power outages were filled using meteorological data from Oklahoma Mesonet environmental monitoring station (Oklahoma Agweather 2016) in Broken Bow, OK (34°02’35.0’’N, 94°37’27.0’’W). There were 37 out of 428 total days gap filled during the two growing seasons. Specifically, 31 days and 6 days for the 5th and 6th growing seasons respectively.

Soil moisture

Volumetric soil water content (VWC) was measured by time domain reflectometry (TDR) using a 1502C metallic cable tester (Tektronix, Inc., Beaverton, OR, USA) taken every four to six weeks during the growing season and every six to eight weeks during winter months. Within each plot, 16 pairs of rods were installed (two pairs at each of eight randomly chosen locations). At each location one pair of rods was placed vertically in the soil 0-12 cm and one pair 0-45 cm. Modeled VWC calculated by calibrating a set of permanent soil moisture probes (model CS655, Campbell Sci., Logan UT) to the TDR periodic measurements across all plots using regression. The permanent probes were placed near the center of each treatment plot in a single replication (rep 3). In these locations, one probe (12 cm rod length) was installed vertically to match the TDR measurements, while others were placed horizontally at 33 and 68 cm. The VWC estimates from these probes were recorded every 15 min to a data logger. To estimate daily VWC per plot the CS655 probes were calibrated to the TDR spot measurements, such that each TDR location had a mapping function that related soil moisture to the soil moisture signal at the continuous locations. Location (eight per plot) by depth (0-12 and 12-45 cm) estimated soil moisture were then estimated using these functions for each day. Daily estimates were averaged to compute a plot level estimate.

Stand volume growth

Tree size was measured in January 2012 prior to treatment application (following the 4th growing season) and in December following each of 5th, 6th, and 7th growing seasons. Measurements included DBH and height. DBH was measured after the 4th, 5th, and 6th growing seasons by averaging two caliper measurements taken at right angles on the stem. Height was measured after the 4th, 5th, and 6th growing seasons using a height pole. DBH and Height was measured following 7th growing season using a diameter tape and hypsometer (Laser Technology, Inc., Centennial, CO, USA), respectively. DBH growth, height growth, and stand volume growth (volume = $0.34864+0.00232*DBH^2*height$; volume in ft^3, DBH in inches, height in ft) (Burkhart 1977) were determined from the difference in successive dormant season measurements. During the experiment, only one tree out of 1007 died.

LAI

Leaf area index was measured using litter traps. Litter was collected approximately every four weeks from September through January each year with less frequent samples collected as needed during other times of year. Loblolly pine retains its needles for approximately a year and a half (Will et al. 2006). Therefore, litter collected from September through January (2012-2013) following the 5th growing season would represent foliage developed during the 4th growing season and on the tree for most of the 5th growing season. Subsequent collections followed similar relationships. Additional collections outside the September to January window made after the preceding March or before the following February were included with that cohort. Litter trap construction consisted of a circular hoop using 2.5 m long section of PEX tubing connected with a two 50.8 mm section of hose (0.5 m^2 area). Vinyl window screen with round edges was stapled to each hoop. Four 508 mm long steel rods were inserted through drilled holes at equal spacing around the tube to function as standing legs. In every plot, each trap was assigned a unique position within the 2 m x 3 m growing space so that all possible positions within the 6 m^2 area were covered. Once assigned positions, traps were randomly assigned to trees for placement. Since the bottom of the live crown was initially below height of excluders, two traps were used when a trap fell under an excluder. One trap was mounted to the excluder and one trap placed directly below near ground level. Combined, these traps captured the equivalent litter for one trap.

Water use

Sap flow density was measured using thermal dissipation probes (TDPs) that consisted of 19 gauge 38.1 mm stainless steel hypodermic needles that were 20 mm in length (Granier 1985) constructed in the tree physiology laboratory at Oklahoma State University. Probes were inserted in to the main trunk below the lowest live branch and ranged from a height of 0.7 m to 0.21 m above ground. Five trees per plot contained one probe (total 80 probes, 16 plots). Measurement trees were randomly selected within ranges of DBH, consisting of two trees in the upper third, two trees in the middle third, and one tree from the lowest third of DBH classes. All probes were initially inserted in May 2012. Probes were monitored through weekly data downloads and screened for malfunctions and errors. Malfunctioning probes were replaced by new probes in the same tree at least 100 mm from the original location. New probes were not expected to provide the same readings as original probes due to variation in sapwood conductivity (Ward et al. 2013, Tateishi et al. 2008), and each replacement was treated as a separate probe. Therefore, the actual number of probes in the study varied in the analysis based on the number of replacements.

The temperature differential between the upper probe (heated at constant 0.2 watts) and lower probe (non-heated) was measured continuously every two minutes beginning from initial probe insertion and recorded by data loggers (model CR1000 or CR10x, Camppbell Sci., Logan UT). Data from April through October of the 6th and 7th growing seasons are presented. Each block contained a data logger that stored data from 20 probes (4 plots per block, 5 trees per plot). Sap flux was calculated according to Granier (1987) by solving the flow index (K) [$K = (\Delta T_{max} - \Delta T)/\Delta T$], where ΔT_{max} is the maximum temperature difference established between the heated and non-heated probes at zero flux and ΔT is the temperature difference between heated and non-heated probes at a given sap flux density and using K to calculate sap flux velocity (V) [$V = 0.000119{*}K^{1.231}$(m s^{-1})]. V was converted to sap flux ($J_s = SA$ x V (m^3 sec^{-1}), where SA is sapwood area in m^2. Finally, these instantaneous measurements were converted to units of sap flux in liters per day, and averaged for each day. Sapwood area for all trees in this study was assumed to be the cross sectional area at probe height minus bark thickness, due to the fact that the trees were 8 years old at the end of the experiment which is under the age when heartwood development begins in loblolly pine (Schultz 1997). This was confirmed by coring five dominant sized trees in the plantation, outside the measurement plots. Due to missing data, average daily (midnight to midnight) tree-level sap flux per basal area increment at breast height data was averaged per plot an imputed using a mixed model in SAS and backfilled for missing dates as total liters per tree per day.

Stand-level canopy transpiration

Transpiration on a ground area basis (E_C, mm d^{-1}) was calculated by dividing tree-level backfilled sap flux data (l m^2 SA at DBH) by basal area increment (BAI) and taking the natural log (ln) of the ratio. Data were then averaged across each tree per plot by date per unit of tree BAI and multiplied by total BAI per plot of using tree-level data as the ratio of sap flux per day per tree.

Efficiency terms

Two indices of growth efficiency were determined from stem volume production (m^3 ha^{-1}), water use efficiency of stem volume production (WUE_{VOL}) and growth efficiency of leaf area index (GE_{LAI}). WUE_{VOL} was calculated as stand-level stem volume growth per plot/total E_C per plot for each year. For 2013, GE_{LAI} was calculated as stand-level stem volume growth per plot/2012+2013 foliage cohort projected LAI per plot. The GE_{LAI} for 2014 was calculated in the same manner. Additionally, water use per unit of LAI (WUE_{LAI}) was calculated for 2013 growing season as 2013 total E_C/2012+2013 cohort projected LAI. The WUE_{LAI} for 2014 was calculated in the same manner.

Statistical analysis

For all measurements, treatment effects were analyzed using repeated measures analysis (Proc Mixed, SAS Inc., Cary, NC, USA) with block as a random factor and treatments as fixed factors and the autoregressive 1 (AR1) covariance structure. For VWC, periodic measurements were modeled for each daily value, but analysis was performed on actual periodic measurements using TDR. When there was a significant interaction involving year, treatment effects within year were tested using SLICE statement in Proc Mixed. Daily tree and plot level water use was summed per week for analysis. There were significant interactions involving year and date(year) so separate analyses were conducted for the 2013 and 2014 growing seasons. Within these year-specific analyses, interactions involving weeks were examined using the SLICE statement Plot means for all data were used ($n = 4$) and treatment effects were considered significant at $P < 0.05$.

Results

Environmental variables

During 2013, average air temperature ranged from 26.1 ^{o}C in August to 4.3 ^{o}C in December with highest average maximum temperature (33.1 ^{o}C) occurring in August and lowest average minimum temperature (-0.9 ^{o}C) occurring in December (Fig. 10). Highest maximum and lowest minimum temperature reached during 2013 were 38.1 ^{o}C (September) and -10.0 ^{o}C (November), respectively (Fig. 10). During 2014, average air temperature ranged from 24.6 ^{o}C (August) to 2.6 ^{o}C (January) with highest average maximum temperature (31.7 ^{o}C) occurring in August and average lowest minimum temperature (-5.3 ^{o}C) occurring in January (Fig. 10). Highest maximum and lowest minimum temperatures reached during 2014 were 36.0 ^{o}C (September) and -13.3 ^{o}C (January), respectively (Fig. 10). During 2013, average monthly daytime VPD ranged from 1.6 kPa in September to 0.25 kPa in December (Fig. 11) and average growing season (April – October) VPD for 2013 was 1.1 kPa. Annual precipitation for 2013 was 1190 mm with the greatest occurring in July (216 mm) and least in August (8 mm) (Fig. 2). Growing season precipitation for 2013 accounted for 64 % of annual precipitation (757 mm). During 2014, average monthly daytime VPD ranged from 0.92 kPa in August to 0.17 kPa in December (Fig. 11) and average growing season VPD was 0.82 kPa. Annual precipitation for 2014 was 1046 mm with the greatest occurring in July (182 mm) and least in August (11 mm) (Fig. 11). Growing season precipitation for 2014 accounted for 70 % of annual precipitation (736 mm).

Soil moisture

From statistical analysis on periodic TDR measurements, the interactive effect between year, date, and throughfall was significant for VWC from 0-12 cm soil depth (Table 4). During 2013, throughfall reduction treatments (TR and TR+F) reduced VWC during wetter periods (Fig. 12A). Ambient precipitation plus fertilizer treatment (F) maintained greater VWC than the fertilized treatment receiving throughfall reduction (TR+F), non-fertilized, throughfall reduction (TR), and control (C) treatments while VWC was similar among the TR+F, TR, and C treatments (Fig. 12A). Across 2013, VWC 0-12 cm averaged 9.0 ± SE 0.9 % for the TR+F treatment, 10.2 ± SE 0.8 % for the TR treatment, 13.5 ± SE 1.0 % for the F treatment, and 10.0 ± SE 1.0 % for the C treatment. For VWC from 0-12 cm during 2014, F treatment maintained greater VWC than the TR+F, TR, and C treatments while VWC was similar among the TR+F, TR, and C treatments (Fig. 12A). Across 2014, VWC 0-12 cm averaged 11.7 ± SE 1.5 % for the TR+F treatment, 12.6 ± SE 1.9 % for the TR treatment, 17.1 ± SE 1.1 % for the F treatment, and 13.3 ± SE 1.8 % for the C treatments.

For VWC from 12-45 cm soil depth, the interactions involving year and date were not significant (Table 4). Across the entire measurement period (2013-2014), a main treatment interaction of throughfall by fertilization was observed. The F treatment maintained greater VWC than C, TR+F, and TR treatments while VWC was similar among the TR+F, TR, and C treatments (Table 4) (Fig. 12B). Across the entire measurement period, VWC 12-45 cm averaged 22.0 ± SE 1.9 % for the TR+F treatment, 22.2 ± SE 2.1 % for the TR treatment, 25.3 ± SE 2.1 % for the F treatment, and 22.4 ± SE 1.8 % for the C treatment.

Stand volume growth

Significant interactive effects for year and throughfall and for year and fertilization occurred for stand volume growth (Table 5). When differences among treatments were considered within years, neither throughfall reduction nor fertilization significantly affected stand volume growth in 2012 (Fig. 13). Throughfall reduction reduced stand volume growth and fertilization increased stand volume growth during both 2013 and 2014 (Fig. 13). During 2013, throughfall reduction reduced stand volume growth by 18.5 % and fertilization increased stand volume growth by 10.2 % (Fig. 13). During 2014, throughfall reduction reduced stand volume growth by 13.4 % and fertilization increased stand volume growth by 12.0 % (Fig. 13).

LAI

Interactive effect of year by fertilization and year x throughfall reduction was significant for LAI (Table 5). Fertilization significantly increases LAI for the 2012 and 2013 cohorts by 33.8 % and 18.3 %, respectively (Fig. 14). Throughfall reduction reduced LAI for the 2013 cohort by 11.5 % (Fig. 14. Across all treatments, LAI increased between the throughout the study foliage cohorts (Table 5). Across all treatments, mean LAI for the 2012, 2013, and 2014 foliage cohorts were 1.2 m^2 m^{-2}, 2.4 m^2 m^{-2}, and 2.7 m^2 m^{-2}, respectively.

Whole-tree water use

The interaction between year, week, and throughfall reduction was significant for WU. Therefore, separate analyses for each year were conducted. During the 2013 growing season, throughfall reduction treatments reduced weekly whole-tree water use

(WU) during a week in May when soil moisture was high, in June and July as soil moisture was high and began to decrease, and again in August as soil moisture decreased from high values during the last week of July. (Table 6) (Fig. 15). Across the 2013 growing season, throughfall reduction reduced WU by 19.6 %. During the 2014 growing season, throughfall reduction reduced WU for two weeks in August as soil moisture declined (Table 6) (Fig. 15).

Water use per tree sapwood area

Significant interactions involving year occurred so analyses were conducted for 2013 and 2014 separately. Significant interactive effects of week x throughfall, week x fertilization, and throughfall x fertilization were observed for weekly WUSA during the 2013 growing season (Table 6). Reductions in WUSA from throughfall reduction were significant because differences tended to be greater when water use rates were high. The throughfall x fertilization interaction was significant across the growing season because the C treatment was consistently higher than the other treatments (Fig. 16). Across the 2013 growing season, WUSA averaged 1159 ± SE 76 cm^3 cm^2 for the C treatment and 818 ± SE 53 cm^3 cm^2, 832 ± SE 75 cm^3 cm^2, and 899 ± SE 68 cm^3 cm^2 for the TR+F, TR, and F treatments, respectively. Interactive effects of week x throughfall and week x fertilization were significant for weekly WUSA during the 2014 growing season (Table 6). Throughfall reduction reduced WUSA for three weeks in August as soil moisture decreased, but also caused an increase WUSA for two weeks in October as a result of high WUSA in the TR treatment (Fig. 16). Fertilization reduced WUSA during the 2014 growing season except during weeks when water use rates were low (Fig. 16). Across the 2014 growing season, fertilization reduced WUSA by 22 %. Across the 2014 growing season, WUSA averaged 736 ± SE 89 cm^3 cm^2 for the TR+F treatment, 1016 ± SE 81 cm^3 cm^2 for the TR treatment, 807 ± SE 74 cm^3 cm^2 for the F treatment, and 954 ± SE 87 cm^3 cm^2 for the C treatment.

Stand-level canopy transpiration

Year by week by treatment interactions were significant for stand-level canopy transpiration per unit ground area (E_C) so that separate analyses were conducted for each year. During the 2013 growing season, interactive effects of week x throughfall, week x fertilization, and week x throughfall x fertilization were significant for mean weekly E_C (Table 6). As E_C decreased following periods of high E_C, the TR+F treatment reduced E_C more so than TR, F, and C treatments and the TR treatment reduced E_C more so than the F and C treatments (Fig. 17). Treatment means for weeks with a significant throughfall by fertilization interaction were 8.0 ± SE 0.95 mm wk^{-1} for the TR+F treatment, 9.6 ± SE 0.66 mm wk^{-1} for the TR treatment, 11.1 ± SE 0.82 mm wk^{-1} for the F treatment, and 11.4 ± SE 0.83 mm wk^{-1} for the C treatment. Overall treatment means for the 2013 growing season were 9.1 ± SE 1.1 mm wk^{-1} for the TR+F treatment, 9.8 ± SE 0.7 mm wk^{-1} for the TR treatment, 9.6 ± SE 0.7 mm wk^{-1} for the F treatment, and 9.7 ± SE 0.7 mm wk^{-1} for the C treatment. During the 2014 growing season, there were also significant interactive effects for week x throughfall and week x throughfall x fertilization observed for E_C. The week by throughfall by fertilization interaction was significant across a four week period at the end of June and beginning of July as E_C was high and starting to decline and during a two week period in August as E_C began a steep decline before treatment means compressed at lower E_C. During the four weeks at the end June and beginning of July, E_C was highest in the TR treatment, second highest in the F treatment and similar for the C and TR+F treatments. During the 2 weeks in August, the E_C of the TR+F treatment was lowest in the TR+F treatment and similar among the others. (Fig. 17). Treatment means across these dates where the interaction between throughfall and fertilization were significant were 13.9 ± SE 1.9 mm wk^{-1} for the TR+F treatment, 17.8 ± SE 1.2 mm wk^{-1} for the TR treatment, 17.0 ± SE 1.5 mm wk^{-1} for the F treatment, and 15.8 ± SE 1.4 mm wk^{-1} for the C treatment. Overall treatment means for the 2014 growing season were 11.3 ± SE 1.6 mm wk^{-1} for the TR+F treatment, 13.4 ± SE 0.9 mm wk^{-1} for the TR treatment, 12.8 ± SE 1.1 mm wk^{-1} for the F treatment, and 12.3 ± SE 1.1 mm wk^{-1} for the C treatment.

Efficiency terms

WUE_{VOL} varied by year (Table 5) as WUE_{VOL} increased in 2014 (0.075 m^3 ha^{-1} mm^{-1}) compared to 2013 (0.066 m^3 ha^{-1} mm^{-1}). Across the entire study period, fertilization increased WUE_{VOL} by 18 % (Table 5) (Fig. 18).The year x fertilization interaction for WUE_{VOL} was not significant (Table 5). A year x throughfall interaction occurred for GE_{LAI}, this could have been driven by the very low GE_{LAI} in the TR+F treatment in 2013 relative to the other treatments (Table 5). Overall, the TR+F treatment had the lowest GE_{LAI} which was significant from both the TR and F treatments (Table 5) (Fig. 19). Across the entire study period, GE_{LAI} averaged 5.3 ± SE 0.6 m^3 ha^{-1} per LAI for the TR+F treatment, 7.3 ± SE 0.9 m^3 ha^{-1} per LAI for the TR treatment, 6.2 ± SE 0.8 m^3 ha^{-1} per LAI for the F treatment, and 6.0 ± SE 0.5 m^3 ha^{-1} per LAI for the C treatment. For $WUE_{LAI,}$ TR treatment was the greatest compared to TR+F, F, and C treatments, which were all similar (Table 5) (Fig. 20). Across the entire study period, WUE_{LAI} averaged 68.9 ± SE 9.0 mm per LAI for the TR+F treatment, 105.9 ± SE 12.5 mm per

LAI for the TR treatment, 74.6 ± SE 4.9 mm per LAI for the F treatment, and 82.8 ± SE 4.9 mm per LAI for the C treatment.

Discussion

In regards to volume growth, our results indicate that fertilization can compensate for throughfall reduction along the western edge of the commercial range. Part of this ability for fertilizer to compensate for throughfall reduction was related to greater leaf area production. However, stem volume growth per unit of LAI was lowest in the TR+F treatment probably because throughfall reduction decreased the efficiency of leaves to produce biomass. Maggard et al. (2016a) found that throughfall reduction decreased leaf-level photosynthetic rates when measured across the same time period as this study. Although water was limited in the TR+F treatment, which may have decreased GE, fertilization increased the amount of stem production per unit of water use. This implies that carbon gain of the fertilized stands was not proportional to LAI, but changes occurred to increase the efficiency of photosynthesis (as it relates to water consumption). This finding supports results from a recent leaf gas exchange study on this same site in which fertilization seemed to compensate for reduced growth from throughfall reduction by increasing water use efficiency of photosynthesis (Maggard et al. 2016a). As water is a finite resource in forest stands that might be less available in the future, this increased volume growth per water use with fertilization may be important to increase productivity in a future, drier climate.

In our study, VWC from 0-12 cm soil depth was reduced by throughfall reduction during wetter periods across 2013, but was reduced across all dates during 2014. The difference in treatment effects between years could be a result of more days with precipitation events during 2014 (60 d) resulting in fewer dry periods where VWC was similarly low across all treatments as during 2013 (47 d) (Fig. 11). In similar throughfall reduction studies, Ward et al. (2015) similar to our results, observed throughfall reduction caused greater separation in VWC from the control treatment following soil recharge events while in contrast, Samuelson et al. (2014) found that throughfall reduction reduced VWC during dry periods. As opposed to VWC from 0-12 cm, VWC from 12-45 cm soil depth was less impacted by dry periods between precipitation events, thus means across all treatments were not reduced to similar values during dry periods such that treatment separation was maintained. As LAI and subsequently growth increased in stands from fertilization, VWC would be expected to be lower from greater water use when stands are fertilized. Increased LAI would also be expected to reduce VWC due to greater interception of precipitation decreasing throughfall (Stogsdill et al. 1989). In that study, it was reported that between 17 and 18 % of precipitation was intercepted by leaf area in an 11-year old loblolly pine plantation in southeast Oklahoma. In our study, VWC was not reduced in the F treatment, even though fertilization increased LAI by 12 %. This variability in VWC could be from random plot-level differences in soil texture.

Fertilization increases volume growth of loblolly pine stands (Albaugh et al. 1998). In our study, fertilization increased volume growth throughout and throughfall reduction reduced volume growth with the exception of the 2012 growing season. The lack of treatment effects on volume growth in 2012 was likely due to timing of treatment application. Fertilization took place in April and excluders were completed in mid-summer, which likely delayed treatment effects. In a similar study, Ward et al. (2015)

found that fertilization increased stem volume increment, but throughfall reduction had no effect over a single growing season which experienced above average precipitation.

For loblolly pine stands, applying fertilizer is widely recognized to increase LAI (e.g., Will et al. 2002, Samuelson and Stokes 2006, Will et al. 2006). In our study, we observed negative effects of throughfall reduction across the entire study period and positive effects of fertilization for the 2012 and 2013 cohorts. For the 2014 foliage cohort, LAI was not significantly increased by fertilization, although LAI was 5 % greater. The negligible difference in LAI due to treatments for the 2014 cohort (three years after application of fertilizer) may have been due to a weakening of the of the effects of a one-time application of fertilizer in addition to the overall higher VWC in 2014 reducing the impact of throughfall reduction on LAI. Effects of fertilization were no longer significant in 2014 (Maggard et al. 2016a) which support a weakening of fertilization effects over time.

In two similar studies, throughfall reduction of ~30 % did not affect LAI over two years for a 7-year old loblolly pine plantation in Georgia or over two years for a 9-year old loblolly pine plantation in Virginia (Samuelson et al. 2014, Ward et al. 2015). Similar to our study, Samuelson et al (2014) found fertilization increased LAI compared to nonfertilized treatments. In contrasts, Ward et al. (2015) found that fertilization did not increase LAI. The reason for the lack of effect was not certain. However, the authors suggested it could be related to the stand not reaching the point where demand for nutrients exceeds current supply across all treatments.

Decreased water availability reduces whole-tree and canopy transpiration in loblolly pine (Ford et al. 2005, Domec et al. 2009, Noormets et al. 2010, Sun et al. 2010) as a result of stomatal closure (Whitehead and Beadle 2004) or reduced leaf area (Hennessey et al. 1992, Pallardy and Rhoades 1997). Across the span of 35 days from the middle of June to the beginning of July in 2013, only 12 mm of precipitation occurred. During this period WU, WUSA, and E_C considerably decreased across all treatments and this decrease was associated with reductions in VWC (Fig.12). However, decreases were greater in the throughfall reduction treatments. Similarly, during the month of August in 2014 only 11 mm of precipitation occurred and WU, WUSA, and EC considerably decreased across all treatments due to reductions in VWC, but decreases were greater with TR+F and appeared to occur at a more rapid pace in the TR+F than other treatments. Treatment effects observed for WU and WUSA across both years were eliminated on dates when WU and WUSA means decreased and were compressed to similar values across treatments. Decreased WU in our study is consistent with reductions in stomatal conductance reported in a recent study by Maggard et al. (2016a) who measured leaf gas exchange on the trees in which we measured water use. A smaller scale (10 total trees) 100 % throughfall exclusion study conducted at an adjacent location did not observe significant reductions in WU or WUSA in exclusion treatment trees (Maggard et al. 2016b).

Fertilization did not affect WU, but did reduce WUSA. The reason for the effect of reduced WUSA resulted from greater sapwood area of the fertilized trees which served to reduce this ratio. The interaction between throughfall reduction and fertilization for WUSA during 2013 was probably due to the greatest WUSA occurring in the C treatment, probably because the C treatment had the least amount of sapwood area and received ambient precipitation.

The interaction between throughfall reduction and fertilization that occurred for E_C was because of higher E_C in the F and TR treatments compared to the TR+F treatment, probably because of greater reductions in stomatal conductance caused by the TR+F treatment in relation to lower soil moisture from throughfall reduction and greater demand for water use from increased LAI from fertilization. Time periods where the throughfall by fertilization interaction was significant were during summer months when VPD is highest. Reduced soil moisture, fertilization, and increased VPD can cause reductions in stomatal conductance (Ewers et al. 2000, Domec et al. 2009, Domec and Johnson 2012, Bartkowiak 2015). In similar throughfall reduction and fertilization studies in Georgia (Bartkowiak et al. 2015) and Virginia (Ward et al. 2015), treatment effects on E_C varied. In Georgia, monthly E_C was reduced by throughfall plus fertilization (Bartkowiak et al. 2015). In Virginia, throughfall reduction and fertilization reduced monthly E_C, with the combined treatment reducing E_C the greatest (Ward et al. 2015). However, LAI did not increase due to fertilization in that study (Ward et al. 2015). The week by throughfall by fertilization interactions for E_C during both years of our study could be due to higher summer temperatures and greater VPD at our site compared sites further east (Seager et al. 2015). Will et al. (2015) reported average August daily maximum temperatures during the study period for our site was, 2.6 ^{o}C warmer than the Georgia site and 3.6 ^{o}C warmer than the Virginia site. Increases in temperature cause VPD and evapotranspiration (ET), thus increasing the potential for tree water stress (Breshears et al. 2013, Will et al. 2013). Therefore, periods when E_C is similar across treatments in our study appears to be both when VWC is higher and the greater atmospheric demand for water at our site is causing similar high E_C across treatments or when VWC is similarly dry across treatments thus causing similarly low E_C across treatments, thus causing a week by treatment interaction.

In this study, efficiency of stem growth was calculated on both an LAI basis and a water use basis. Fertilization increased WUE_{VOL} regardless of throughfall treatment but not WUE_{LAI} indicating the production of stem volume growth was more efficient due to fertilization on a water use basis, but not a leaf area basis. In fact, WUE_{LAI} was lowest for the TR+F treatment indicating that foliage was less efficient at producing stem volume, probably because of water limitation and stomatal closure. Increased WUE_{VOL} with fertilization could indicate greater leaf level photosynthesis per transpiration. These findings are supported by a study measuring leaf gas exchange on the same site and same trees that we measured. Maggard et al. (2016a) found that throughfall reduction decreased leaf gas exchange and that fertilization decreased stomatal conductance without affecting net photosynthesis. As a result it was suggested that fertilization increased photosynthetic efficiency per leaf-level water use. In a similar throughfall reduction and fertilization study in Virginia, Ward et al. (2015) found that WUE_{VOL} was significantly greater in the TR+F treatment than the C treatment or TR treatment and suggested this response was a result of declined E_C and an increase in stem volume production. Further, effects of throughfall reduction were not significant in that study. In contrast, growth efficiency defined as the slope of the relationship between annual stemwood production and peak projected LAI in a study most similar to ours, found no effects of throughfall reduction or fertilization treatments on growth efficiency

(Samuelson et al. 2014). At that site, throughfall reduction did not decrease photosynthesis while it did in ours which probably contributed to the lower GE_{LAI} of the TR+F in our study. Previous loblolly pine plantation growth efficiency research looking at the effects of fertilization, age, competition control, or a combination have found positive to minimal positive effects of fertilization during the early stages of stand development (Colbert et al. 1990, Albaugh et al. 1998, Jokela and Martin 2000), no effects (Will et al. 2002), or negative fertilization effects on growth efficiency as stand age increased due to larger tree size (Jokela and Martin 2000, Will et al. 2002).

Conclusions and management implications

Consistent with our hypothesis, fertilization increased LAI, growth, water use efficiency in terms of stand-level volume growth while throughfall reduction reduced VWC. Reductions in whole-tree water use and E_C were only observed during periods of reducing VWC. Further, fertilization did not increase growth efficiency per LAI. However, we found that fertilization can compensate for throughfall reduction in regards to stem volume production. Fertilization increased WUE_{VOL} which supports a recent indication of increased leaf-level water use efficiency from fertilization found previously during this experiment. These results indicate that fertilization can benefit stand growth of loblolly pine plantations in areas with higher growing season temperatures and greater VPD, such as the western edge of the loblolly pine commercial range.

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Tables.

Table 4. *P* values for the effects of date, throughfall reduction (Water), and fertilization (Fert) treatments on volumetric soil water content (VWC) at 0-12 cm for 2013 and 2014 and *P* values for the effects of year, Water, and Fert on VWC at 12-45 cm for the entire measurement period (2013-2014). Bold values indicate significance at α < 0.05.

	2013	2014
VWC 0-12 cm		
Date	**<0.0001**	**<0.0001**
Fert	0.02	0.13
Water	0.0002	0.003
Water*Fert	**<0.0001**	**0.02**
Date*Fert	0.22	0.55
Date*Water	**0.0001**	0.16
Date*Water*Fert	0.19	0.54
VWC 12-45 cm	*Entire measurement period*	
Year	**<0.0001**	
Year*Date	**<0.0001**	
Year*Date*Fert	0.91	
Year*Date*Water	0.35	
Year*Date*Water*Fert	0.63	
Fert	0.04	
Water	0.007	
Water*Fert	**0.02**	
Year*Fert	0.73	
Year*Water	0.32	
Year*Water*Fert	0.85	

Table 5. *P* values for the effects of year, throughfall reduction (Water) and fertilization (Fert) treatments on stand volume growth, leaf area index (LAI), water use efficiency of stem volume production (WUE_{VOL}), growth efficiency of leaf area index (GE_{LAI}), and water use efficiency per unit leaf area index (WUE_{LAI}) for the entire measurement period (2012-2014). Bold values indicate significance at α < 0.05.

	Volume growth	LAI	WUE_{VOL}	GE_{LAI}	WUE_{LAI}
Year	**<0.0001**	**<0.0001**	**0.04**	**<0.0001**	0.86
Fert	0.003	0.001	**0.02**	0.31	0.20
Water	0.0001	0.30	0.09	0.20	0.003
Water*Fert	0.05	0.13	0.92	**0.03**	**0.04**
Year*Fert	**0.04**	**0.002**	0.6712	0.21	0.84
Year*Water	**0.01**	**0.0003**	0.60	**0.007**	0.24
Year*Water*Fert	0.40	0.92	0.24	0.19	0.81

Table 6. *P* values for the effects of week, throughfall reduction (Water), and fertilization (Fert) treatments on 2013 whole-tree water use (WU), 2014 WU, 2013 water use per tree sapwood area (WUSA), 2014 WUSA, 2013 stand-level canopy transpiration (E_C), and 2014 E_C. Bold values indicate significance at $\alpha < 0.05$.

	2013 WU	2014 WU	2013 WUSA	2014 WUSA	2013 E_C	2014 E_C
Week	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Water	0.0002	0.61	0.0006	0.95	0.78	0.86
Fert	0.27	0.22	0.02	0.002	0.46	0.34
Water x Fert	0.87	0.19	0.04	0.33	0.58	0.11
Week x Water	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	<0.0001	<0.0001
Week x Fert	0.49	0.94	**0.0007**	**0.0005**	0.0005	0.80
Week x Water x Fert	0.84	0.25	0.12	0.32	**0.005**	**0.0004**

Figures.

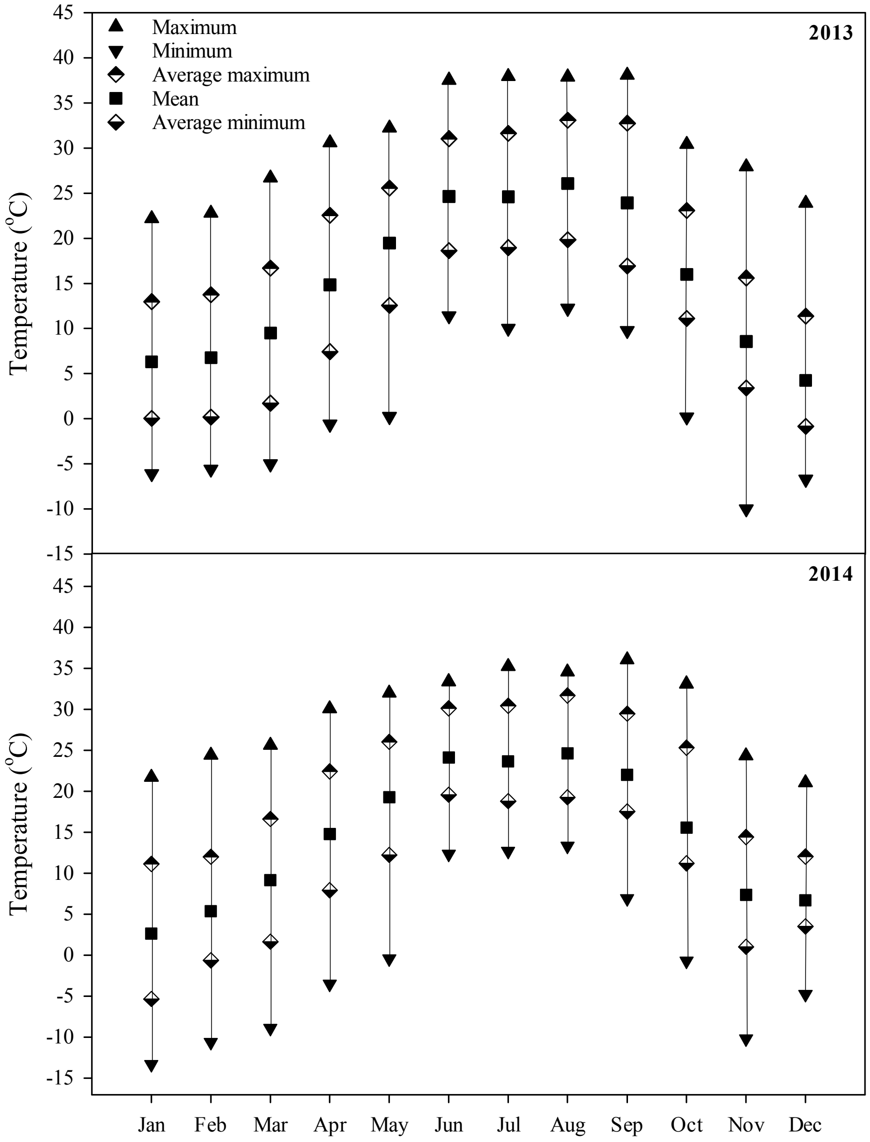

Fig. 10. Study site monthly maximum, average maximum, mean, average minimum, and minimum air temperature during 2013 and 2014 in Broken Bow, Oklahoma.

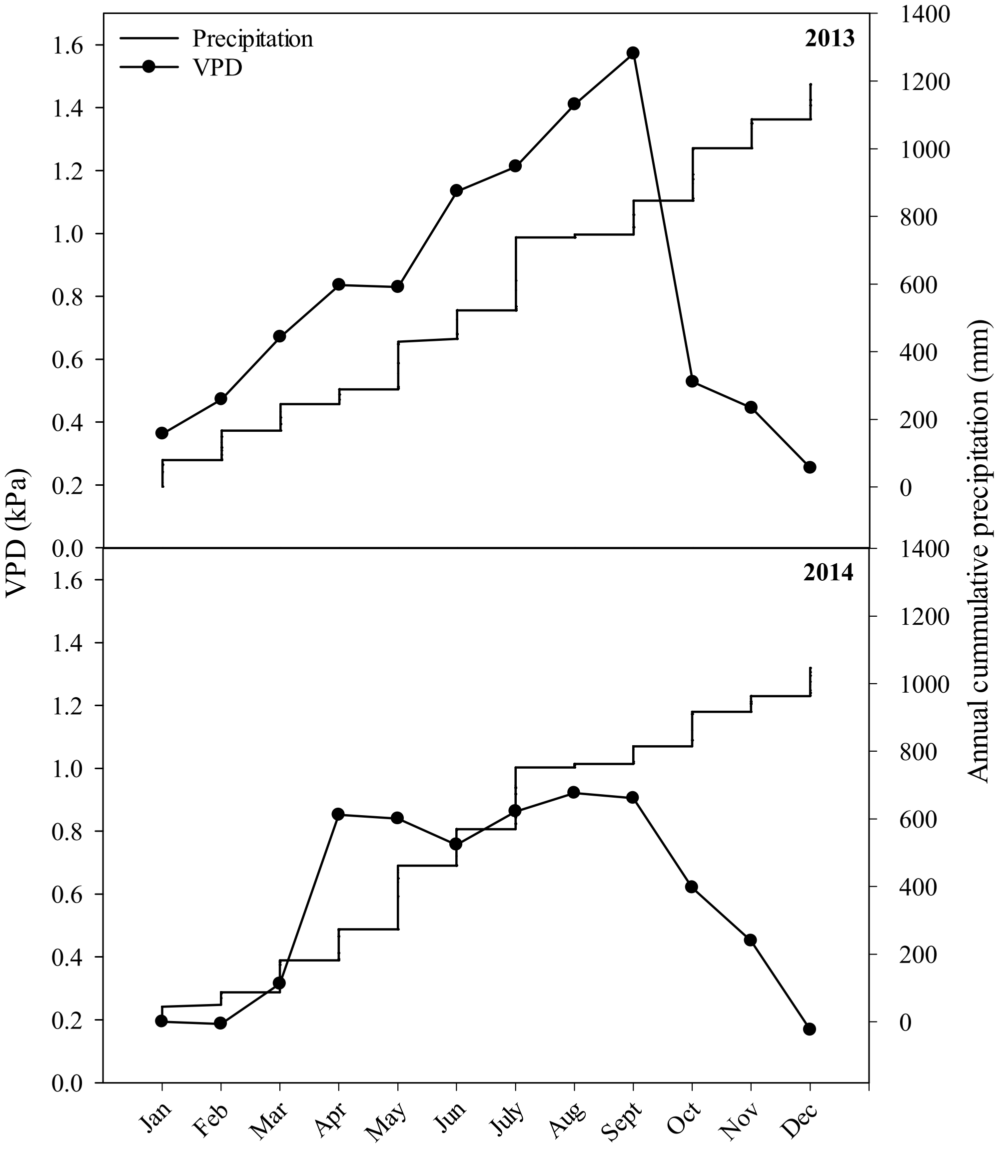

Fig. 11. Average monthly daytime vapor pressure deficit (VPD) and annual cumulative precipitation for 2013 and 2014 for Broken Bow, Oklahoma, USA.

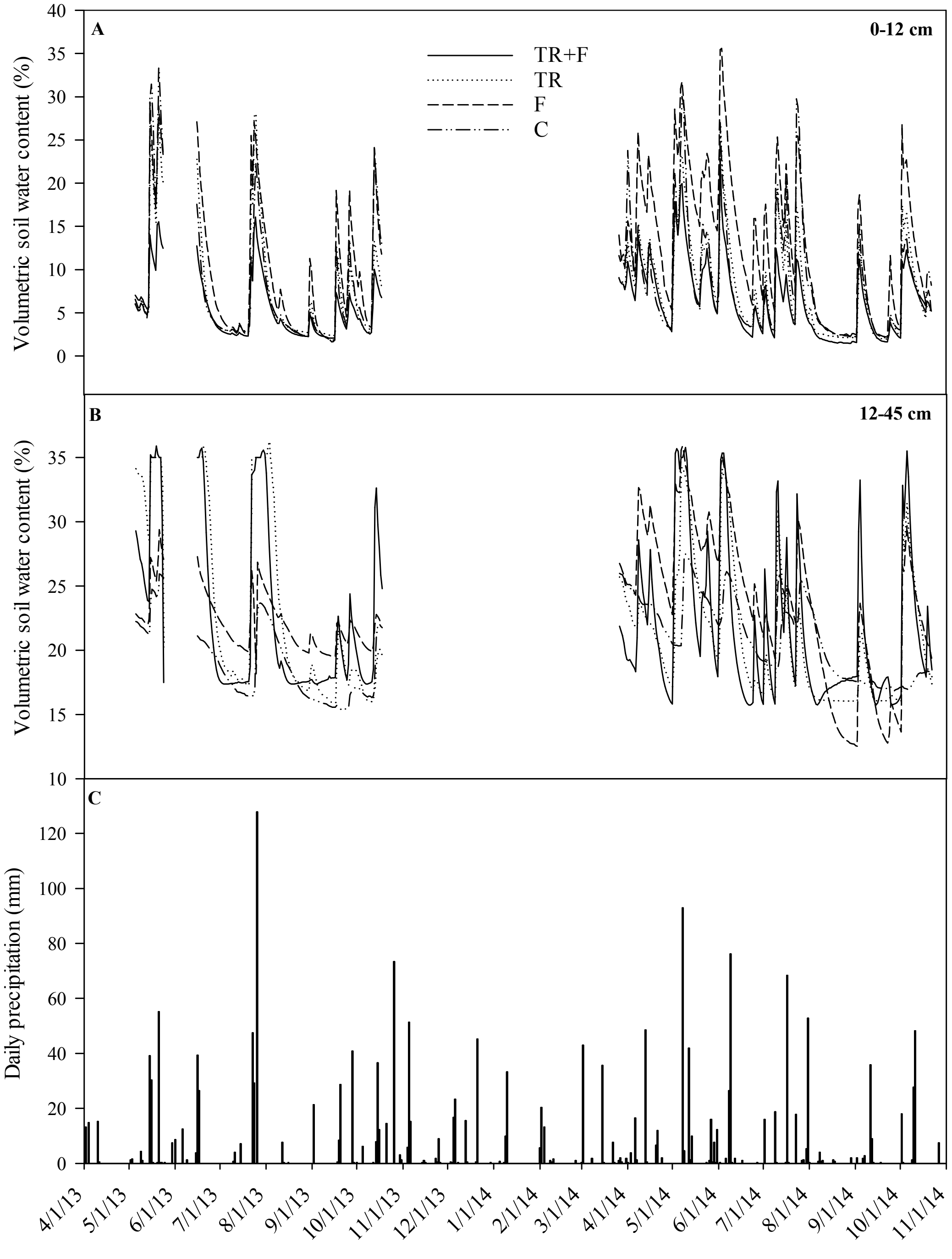

Fig. 12. Daily predicted volumetric soil water content (%) between (A) 0-12 cm soil depth and between (B) 12-45 cm soil depth in response to throughfall reduction plus fertilization (TR+F), throughfall plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments, modeled only for 2013 and 2014 growing season and (C) daily on-site precipitation across the entire study period.

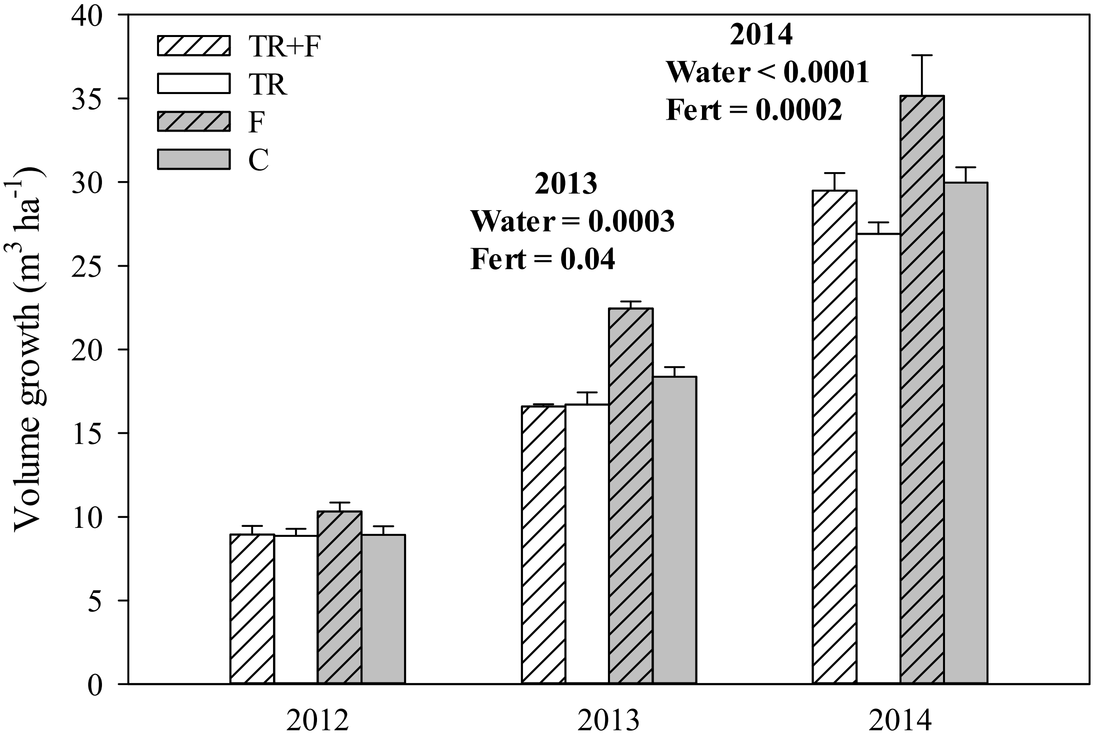

Fig. 13. Stand volume growth over the 2012, 2013, and 2014 growing season in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments ($n = 4$). Error bars represent standard error of treatment means ($n = 4$). P Values represent within year analysis ($p < 0.05$).

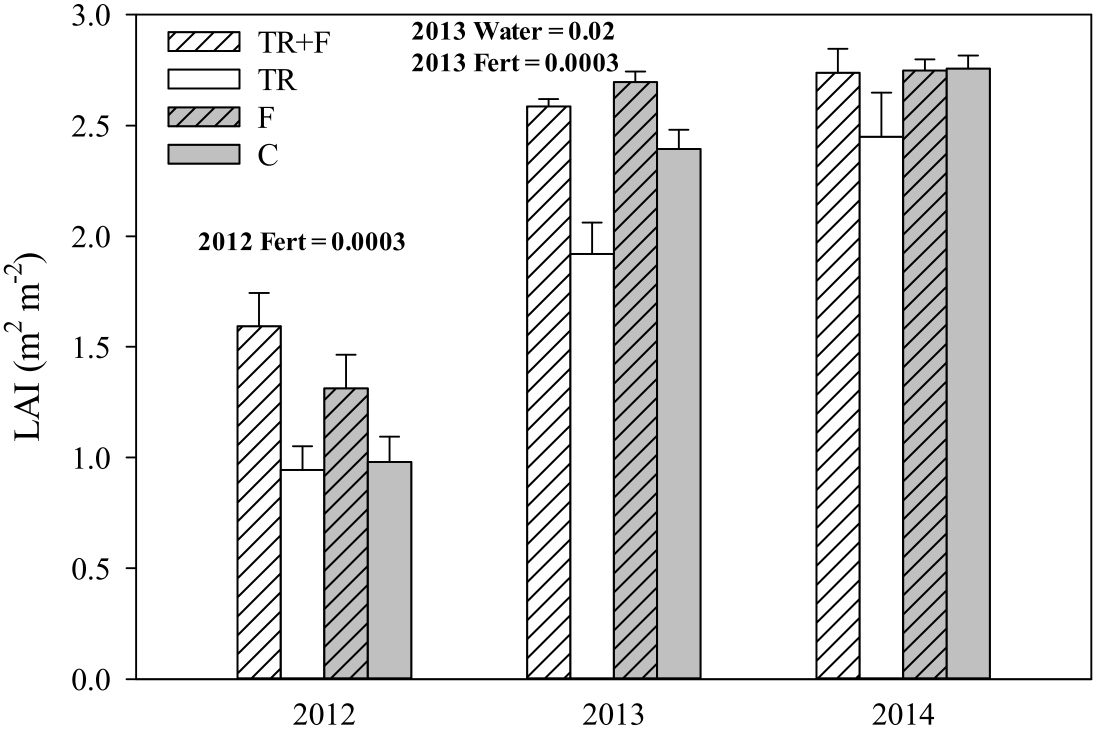

Fig. 14. Mean leaf area index (LAI) for 2012, 2013, and 2014 foliage cohorts in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. Error bars represent standard error of treatment means ($n = 4$). *P* values represent within year analysis ($p < 0.05$).

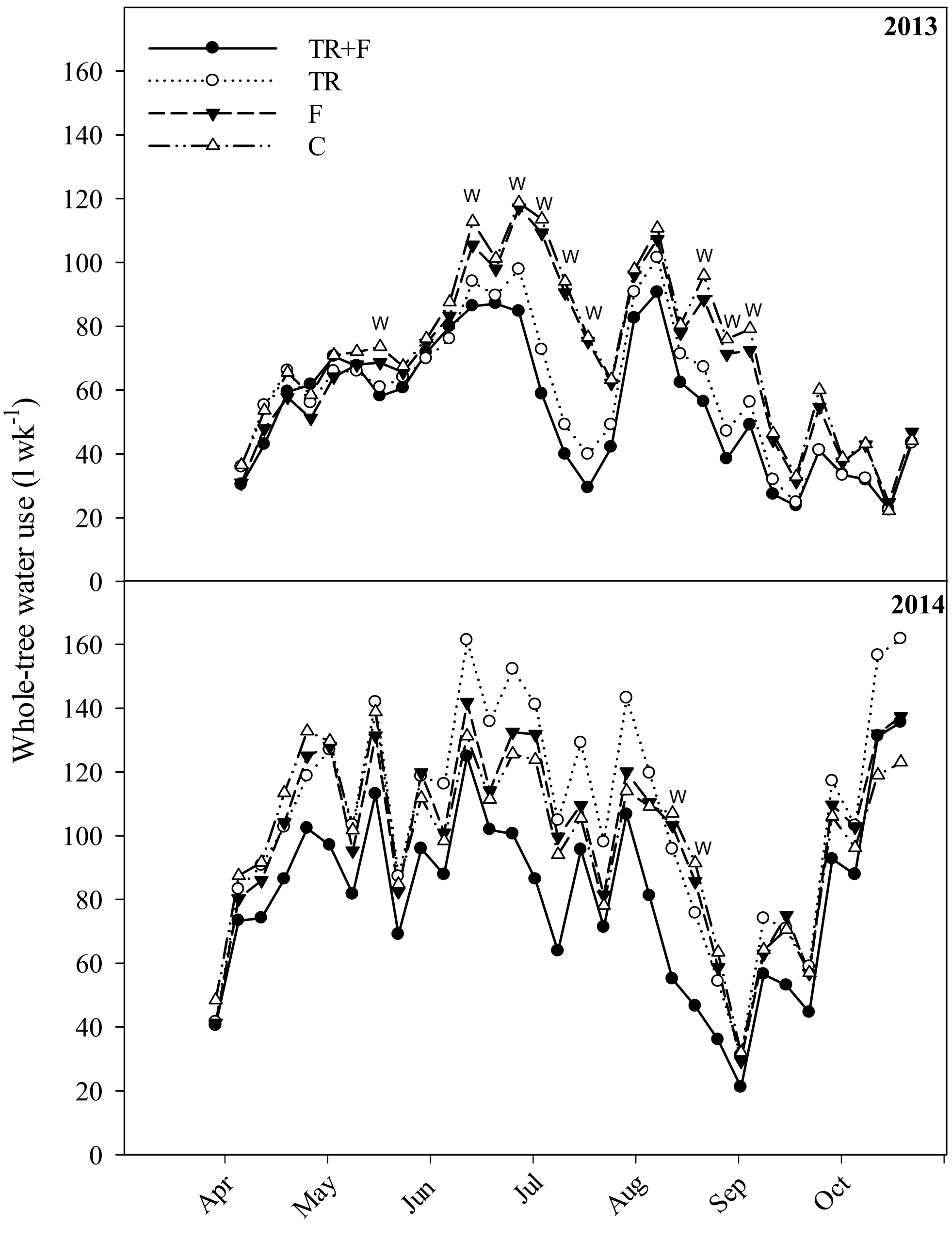

Fig. 15. Weekly whole-tree water use for 2013 and 2014 growing seasons in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. In cases where there is a significant week x throughfall interaction, a (W) above the data represents dates that throughfall reduction effect is significant.

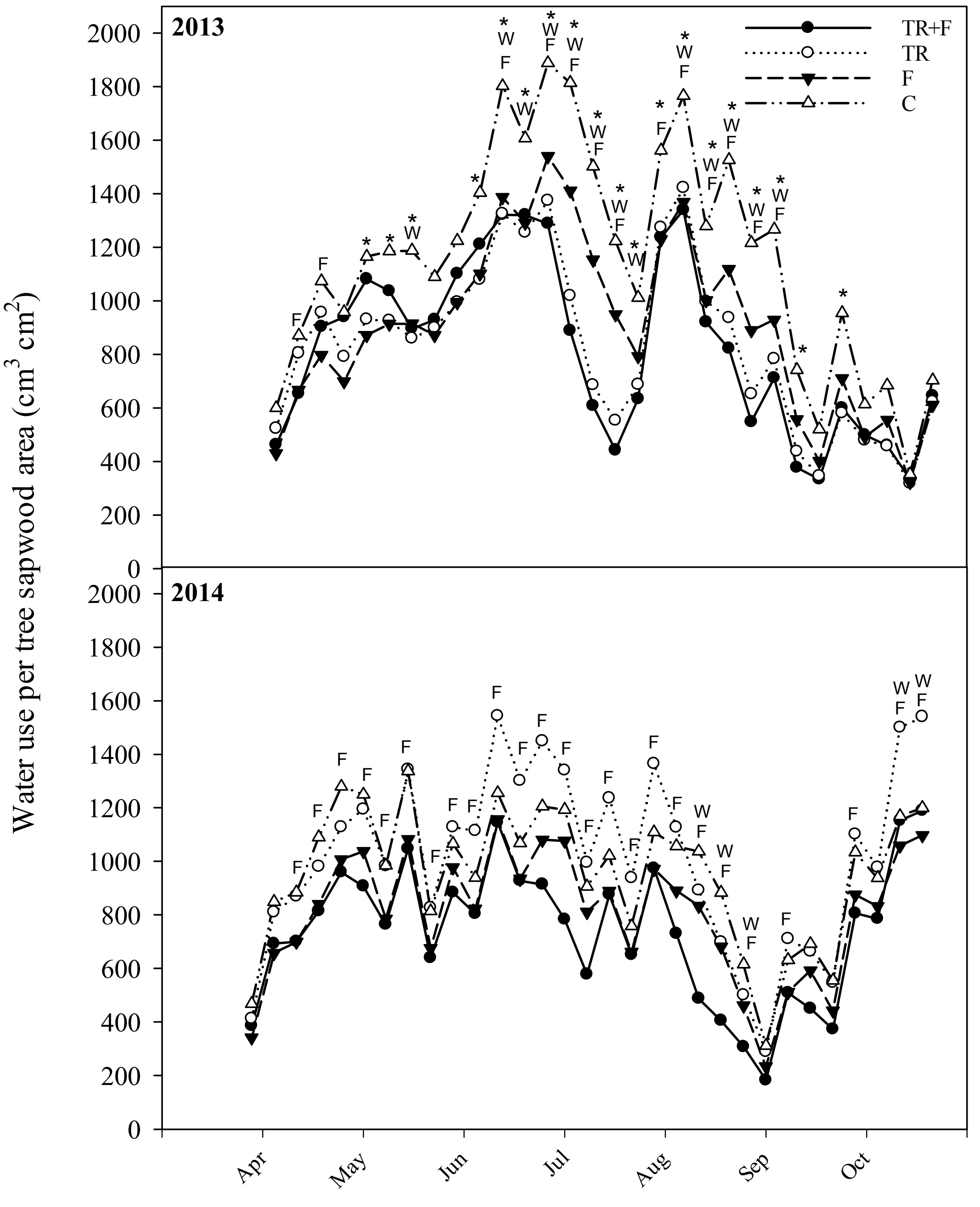

Fig. 16. Weekly water use per tree sapwood area for 2013 and 2014 growing seasons in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. In cases where there is a significant throughfall x fert, week x throughfall, and week x fert interaction a (*), (W), and (F) above the data represents dates that throughfall reduction by fertilization, throughfall reduction, and fertilization interaction is significant, respectively.

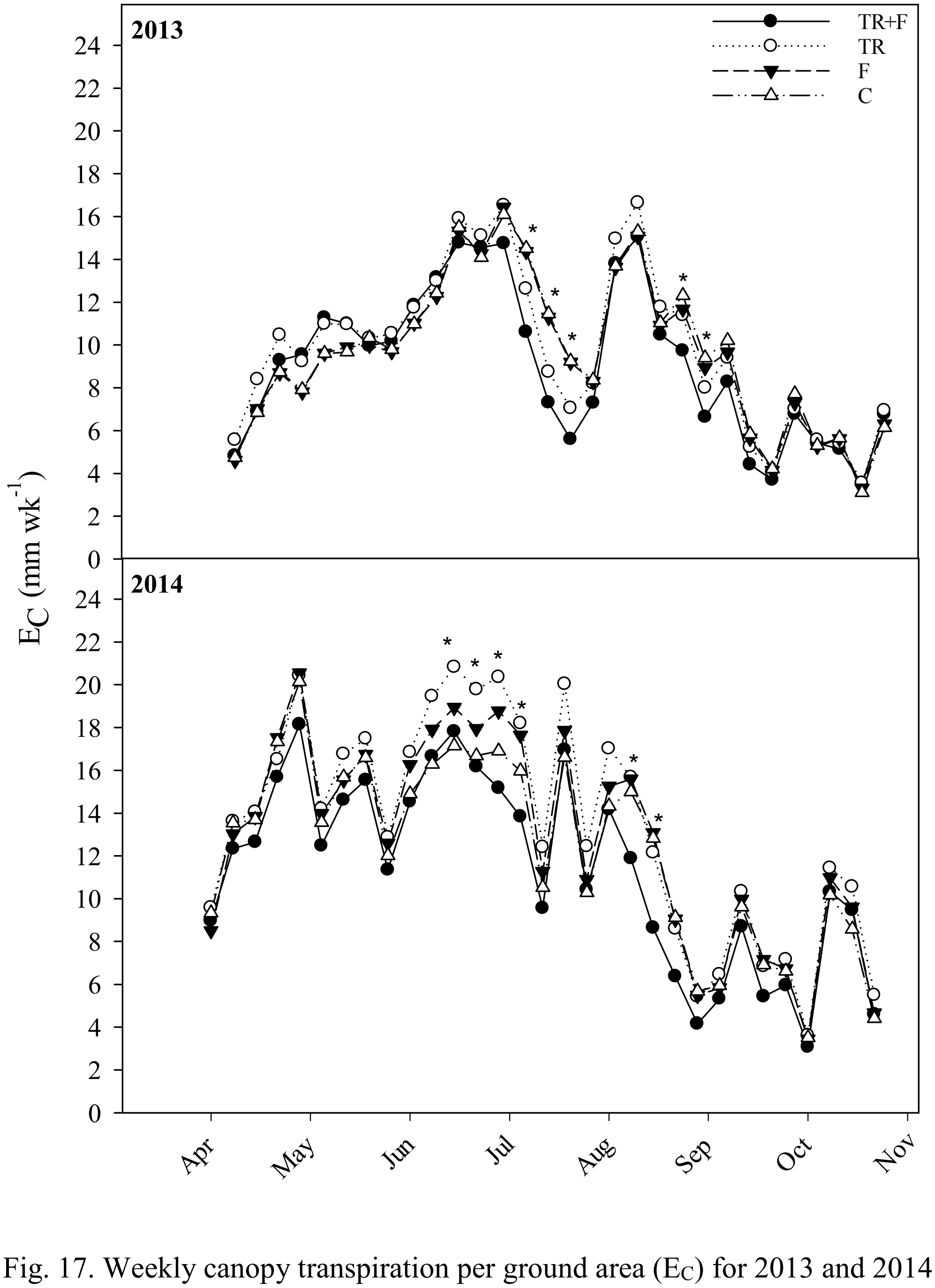

Fig. 17. Weekly canopy transpiration per ground area (E$_C$) for 2013 and 2014 growing seasons in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. In cases where there is a significant week x throughfall x fert interaction, an (*) above the data represents dates that throughfall reduction by fertilization interaction is significant.

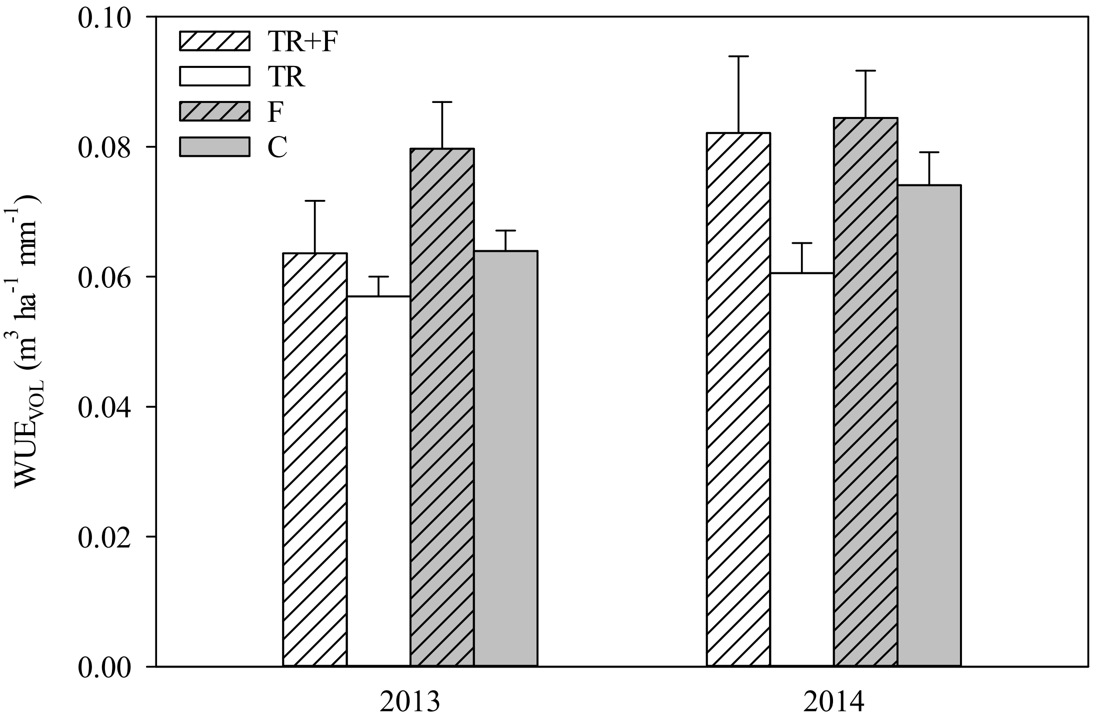

Fig. 18. Water use efficiency of stem volume production (WUE$_{VOL}$) for 2013 and 2014 growing seasons in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. Error bars represent standard error of treatment means (*n* = 4).

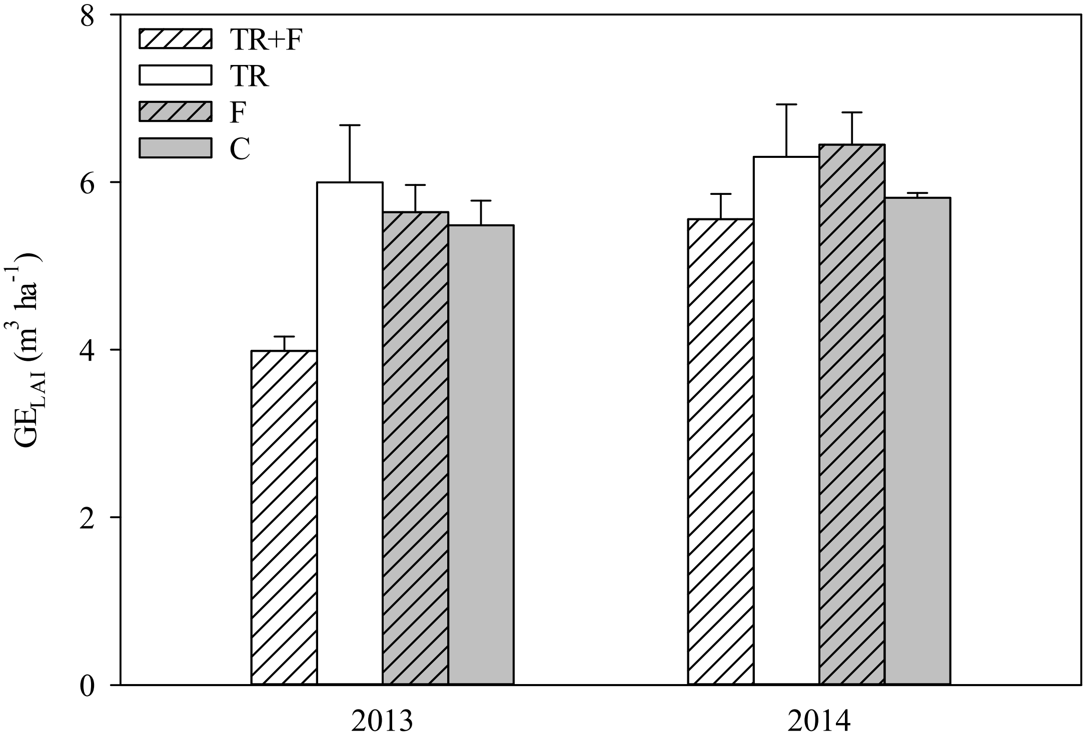

Fig. 19. Growth efficiency of leaf area development (GE_{LAI}) in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. Error bars represent standard error of treatment means (*n* = 4).

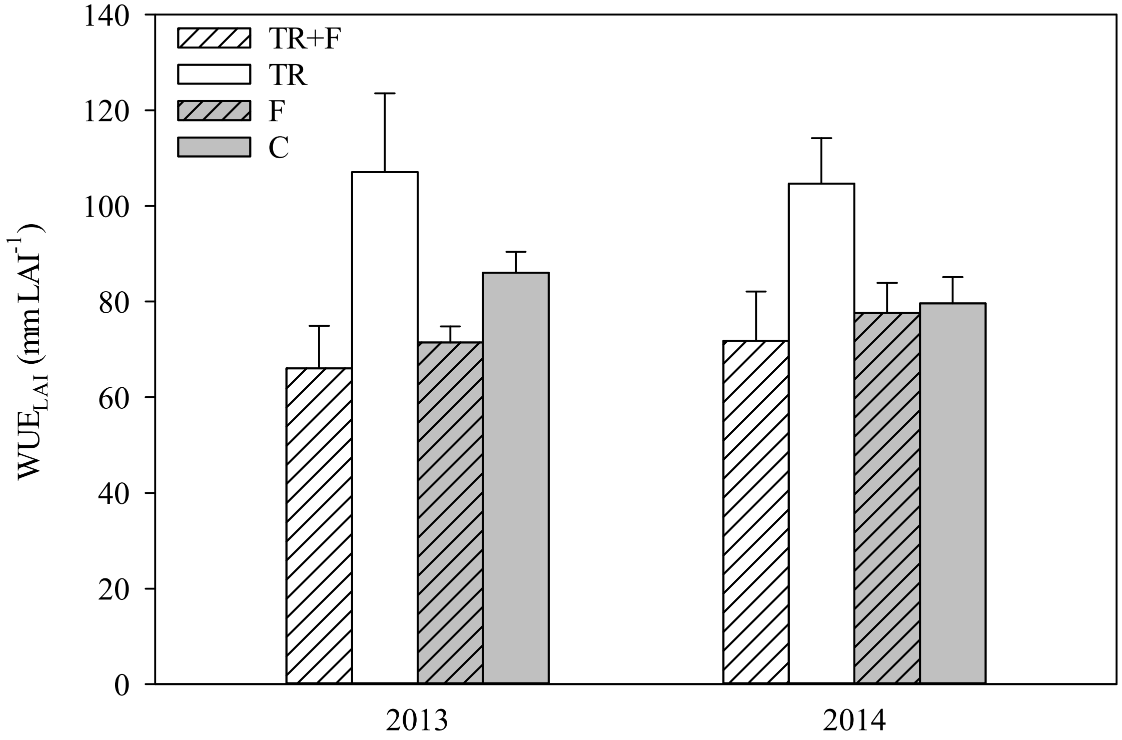

Fig. 20. Water use efficiency of leaf area development (WUE$_{LAI}$) in response to throughfall reduction plus fertilization (TR+F), throughfall reduction plus no fertilization (TR), ambient throughfall plus fertilization (F), and control (C) treatments. Error bars represent standard error of treatment means (*n* = 4).

CHAPTER IV

RESPONSE OF MID-ROTATION LOBLOLLY PINE (*PINUS TAEDA* L.) PHYSIOLOGY AND PRODUCTIVITY TO LONG-TERM INDUCED DROUGHT ON THE WESTERN EDGE OF THE COMMERCIAL RANGE

Abstract

Forests in the southeastern United States contain approximately 30 % of forest carbon in the contiguous USA and loblolly pine plantations are an important part of these forests. However, loblolly pine plantations could be threatened by decreased water availability in the future. To determine effects of long-term drought on leaf gas exchange, whole-tree water use, and individual tree growth, we examined the response of loblolly pine trees to 100 % throughfall exclusion spanning the 6th and 7th growing seasons growing in a loblolly pine plantation in southeastern Oklahoma. Across all trees, throughfall exclusion reduced VWC for soil depth 0-12 cm from 10.8 % to 4.8 % and for 12-45 cm soil depth from 24.2 % to 15.6 %, but did not reduce VWC for soil depth 45-90 cm. Compared to ambient throughfall trees, predawn and midday leaf water potential of the throughfall exlusion trees became more negative, -0.9 MPa vs -1.3 MPa for predawn measurements

and -1.5 MPa vs -1.9 for midday measurements. Throughfall exclusion did not significantly reduce leaf gas exchange, water use, or tree growth. However, throughfall exclusion significantly reduced leaf biomass of trees by 21 %. These results indicate that continuous moderate drought over two years may cause downward shifts in leaf area to conserve water rather than reducing leaf-level water use.

Introduction

Forests in the southeastern United States contain approximately 30 % of forest carbon in the contiguous Unites States (http://fia.fs.fed.us/forestcarbon/, accessed March 2016). Planted pines account for 19 % of all forests in the southeastern US (Wear and Greis 2012) with loblolly pine (*Pinus taeda* L.) plantations accounting for over 50 % of these pine plantations (Oswalt et al. 2014). Therefore, loblolly pine plantations are important carbon pools, but could be threatened by decreased water availability in years to come (Collins et al. 2013, Walsh et al. 2014).

Atmospheric CO_2 is increasing and changes in temperature and precipitation regimes are expected to impact the southeastern USA in coming years (Collins et al. 2013). Increases in intensity and more irregular occurrence of precipitation events across the region are predicted to slightly increase total annual precipitation. However, greater rainfall intensity and increased dry days between events may increase soil runoff, reducing soil water availability (Walsh et al. 2014). Increases in mean annual temperature by the end of this century are predicted to be between 2.5 and 4 ^{o}C for this region (Walsh et al. 2014). Increases in temperature cause greater vapor pressure deficit (VPD) and evapotranspiration (ET), thus increasing the potential for tree water stress (Breshears et al. 2013, Will et al. 2013). Therefore, the combined effects of increased temperature and decreased soil water availability is predicted to increase the frequency and severity of drought (Collins et al. 2013, Walsh et al. 2014). This is especially important for the western edge of the loblolly pine commercial range. High growing season temperatures and VPD, already common to this area (Seager et al. 2015), are expected to increase (Collins et al. 2013), thus increasing the potential of water stress for loblolly pine planted in this area.

Reduced water availability decreases tree growth and can ultimately lead to mortality depending on the longevity and severity of drought events (Kramer and Boyer 1995, Chaves et al. 2003, McDowell et al. 2008). During recent years, drought has been linked to widespread tree mortality events (Fensham et al 2009, Allen et al. 2010, Anderegg et al. 2011). During drought, reduced stomatal conductance or stomatal closure is one of the earliest tree responses (Chaves 1991), which reduces net photosynthesis and subsequently growth (Teskey et al. 1986, Chaves 2003). During longer or more intense droughts, structural modifications can occur, such as reductions in leaf area or leaf biomass (Mencuccini and Grace 1994, Kramer and Boyer 1995, Battaglia et al. 1998). Reduced leaf biomass production due to reduced water availability has also been reported. Hennessey et al. (1992) found reduced leaf biomass production in a loblolly pine stand in southeast Oklahoma in drier years compared to years when water availability was not limited.

Leaf area is commonly studied because it is an important factor in both transpiration and photosynthesis. Trees with greater leaf area typically have greater total transpiration than trees with smaller leaf area. Failure to reduce transpiration rates during drought can harm trees by leading to dehydration and injury, or possibly death (Kramer and Boyer 1995). Changes in stomatal conductance are associated with changes in leaf specific hydraulic conductance (Hubbard et al. 2001), indicating that with increasing leaf area stomatal reductions are essential in order to avoid damaging leaf water potentials (Whitehead and Beadle 2004). During drought, early abscission of leaves can occur which reduces shoot water loss and helps conserve resources (Pallardy and Rhoads 1997).Water stress can lead to early abscission in loblolly pine and shift peak needle fall by up to two months (Hennessey et al. 1992).

Effects of long-term drought (Allen and Breshears 1998, 2007, Dobbertin et al. 2007) and induced drought (Adams et al. 2009, Galvez et al. 2011, Plaut et al. 2013, Pangle et al. 2015) on tree mortality and survival have been documented for numerous species (Adams and Kolb 2005, Breshears et al. 2005, Mueller et al. 2005, Breda et al. 2006, Anderegg et al. 2012, Attia et al. 2015). However, research on the effects of drought on loblolly pine has been focused on seedlings (Teskey et al 1986, Perry et al. 1994, Groninger et al. 1996, Barnes 2002), short-term natural drought (Cregg et al. 1988, Ford et al. 2005, Domec et al. 2009), or induced moderate drought (Tang et al. 2004, Samuelson et al. 2014, Bartkowiak et al. 2015, Ward et al. 2015). During short-term natural drought or induced moderate drought, loblolly pine typically exhibit more negative water potentials and respond by reducing stomatal conductance to avoid water loss (Teskey et al. 1986, Rahman et al. 2003 Samuelson et al. 2014). We currently lack understanding of how long-term drought will impact the physiology and productivity of loblolly pine.

The objective of this study was to determine effects of long-term drought on leaf gas exchange, whole-tree water use, and individual tree growth. To accomplish this objective, we examined the response of individual trees to 100 % throughfall exclusion. Our central hypothesis was that long-term drought would cause more negative leaf water potentials, decrease leaf gas exchange, tree water use, leaf area, and tree growth. Further, we hypothesized that leaf gas exchange would be the most pronounced change such that water use per sapwood area would be much lower with drought. Determining how long-term drought responses differs from short-term drought is important for understanding physiological and structural responses as well as mechanisms that contribute to survival., Further, we hypothesized that long-term drought stress would cause a reduction in annual foliage biomass to avoid harmful leaf water potentials.

Materials and methods

Study site

The study took place from March 2013 through September 2014 which spaned the 6th and 7th growing seasons of a loblolly pine stand near Broken Bow, Oklahoma (N 34°01’ 47”, W 94°49’ 23”). Soils had 3 to 8 percent slopes and consisted of Ruston series (Fine-loamy, siliceous, semiactive, thermic Typic Paleudult). These soil are characterized as very deep, well-drained fine sandy loam typical for forests on the uplands of the western and southern Coastal Plain (http://soilseries.sc.egov.usda.gov, accessed March 2016). Mean annual precipitation for Broken Bow, OK since stand establishment (January 2008) was 1313 mm, with the greatest average monthly precipitation occurring in May (151 mm) and the least occurring in August (79 mm). Annual mean, maximum, and minimum temperatures for Broken Bow, OK since stand establishment were 16.0 ^{o}C, 23.2 ^{o}C, and 9.3 ^{o}C (http://mesonet.org/index.php/weather/daily_data_retrieval, accessed March 2016). Monthly Palmer Drought Severity Index (PDSI) data were downloaded for Climate Division 9 (southeast) in Oklahoma for 2012, 2013, and 2014 (http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp#, accessed March 2016).

Before planting, broadcast herbicide of 680 g ha^{-1} of Chopper ® (27.6 % imazapyr, 72.4 % inert ingredients) (BASF Corporation, Florham Park, NJ, USA) plus 2.8 l ha^{-1} of glyphosate was applied in August 2017 followed by prescribed burn in October 2007. The study site was subsoiled along the contour to depths of 51 to 61 cm using a D8 Caterpillar dozer and attached subsoiling shanks (Caterpillar Corporate, Peoria, IL, USA) in November 2007. The site was planted in January 2008 with improved Western Gulf Tree Improvement Cooperative 1-0 bare-root seedlings originating from a mix of half-sib families. Broadcast herbicide of 420 g ha^{-1} of Arsenal® (27.6 % imazapyr, 72.4 % inert ingredients) (BASF Corporation, Florham Park, NJ, USA) and 175 g ha^{-1} of Oust Extra® (56.25 % Sulfometuron methyl, 15.0 % Metsulfuron methyl, 28.75 % other) (E.I. Du Pont De Nemours and Company, Wilmington, DE, USA) was applied for woody plant and herbaceous weed control in March 2008. Spacing for the site was approximately 2 m x 3 m for an approximate density of 1650 trees ha $^{-1}$.

Experimental design

The study was a randomized complete block design consisting of five blocks. Each block consisted of two trees (10 trees total), one each of 100 % throughfall exclusion (TR_D) and ambient throughfall treatments (TR_W) (Fig. 21). Within the stand, this experiment was located where trees had relatively uniform height and diameter at breast height (DBH) and soils were consistent. The location was on a broad ridge in an effort to minimize belowground water flow and increase chances of above- and belowground water flow away from treatment blocks. To prevent root expansion beyond treatment areas, all 10 trees were trenched. Trenches were excavated to approximately 60 cm. A 2 x 3 m rectangle representing the growing space of each stem was trenched around each treatment tree. Trenches between rows were excavated with ride-on trencher (Ditch Witch RT40, Perry, OK, USA) and trenches between tree spacing were excavated with Zahn walk-behind trencher (Ditch Witch R150, Perry, OK, USA). Trenches were lined with 6 mil heavy duty plastic polyethlene (BLUE HAWK LF, LLC, Mount Mourne, NC, USA) and back-filled with soil. Competing vegetation was chemically eliminated using directed sprays of glyphosate (2% a.i., Roundup®, Monsanto Company, St. Louis, MO, USA) from March 2013 through the remainder of the study.

The TR_D treatment consisted of 3.7 m by 2.7 m excluders built around each tree (Fig. 22). Excluders were installed in February 2013 and were built of lumber and covered with clear 6 mil heavy duty plastic polyethlene (BLUE HAWK LF, LLC, Mount Mourne, NC, USA). Covers were monitored for wear and tear and replaced as needed. Excluders were approximately 1.1 m in height at the tree sloping to approximately 0.8 m to allow precipitation runoff and air flow to circulate underneath. Excluders extended approximately 0.3 m beyond the trenches (Fig. 22). Branches below excluders were removed to prevent shading of the soil surface and from potential damage to excluder covers. Stemflow was diverted onto excluders by securing cone shaped polyethlene around the tree above excluders. Excluders were designed the same way for TR_W trees to minimize possible microclimate differences beneath excluders between treatments. Openings were cut into polyethlene to allow throughfall to reach the soil for TR_W treatments.

Environmental variables

Climate variables were measured on site using meteorological sensors mounted at the top of tower located in an opening within the stand. Sensors included a CS215 air temperature and relative humidity probe (Campbell Scientific, Logan, UT, USA) and a TR-525M remote tipping bucket precipitation sensor (Texas Electronics, Inc., Dallas, TX, USA). Data were recorded continuously every 15 minutes using a CR1000 data logger (Campbell Scientific, Logan, UT, USA). Data gaps from sensor malfunctions or power outages were backfilled using data from the Broken Bow, OK (34°02’35.0’’N, 94°37’27.0’’W) Oklahoma Mesonet environmental monitoring station (http://mesonet.org) via data request from the Oklahoma Climatological Survey. Vapor pressure deficit (VPD, kPa) was calculated from relative humidity and temperature measurements based on Allen et al. (1998). Mean monthly daytime VPD was determined by averaging daily 15-minute VPD calculations between sunrise and sunset times for Broken Bow, OK (Astronomical Applications Dept., U.S. Naval Observatory).

Soil moisture

Volumetric soil water content (VWC) was measured by time domain reflectometry (TDR) using a 1502C metallic TDR cable tester (Tektronix, Inc., Beaverton, OR, USA) taken every seven to ten days during the 6th growing season and every four to six weeks during winter months and throughout the 7th growing season. Beneath each excluder, one pair of two rods of each 0-12 cm, 0-45 cm, and 0-90 cm soil depths were installed on the south side of the tree approximately 0.3 m apart and 0.6 m from the base of the tree. All measurement depths per tree were measured each sampling round.

Physiology

Measurements of leaf gas exchange were conducted from March 2013 through September 2014. Measurements were conducted bi-weekly during the 2013 growing season and every four to six weeks during the winter months. For 2014, measurements were conducted approximately every four weeks during the growing season and every six to eight weeks during the winter months. All measurement were conducted using an LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). Measured variables included net photosynthesis (P_{net}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). Leaf gas exchange was measured on all trees (10 total). For each tree on each measurement date, two fascicles were sampled that developed in full sun in the upper third of the tree on the south side. Needles were collected by hand in 2013 and by a pole pruner in 2014. Fascicles from the first flush of 2012 were measured from March to July 2013 while the first flush of 2013 was measured from August 2013 to July 2014 and the first flush of 2014 was measured from August 2014 through September 2014. Within sampling dates, measurements were taken between 1300 and 1500 h.

During gas exchange measurements, photosynthetically active radiation and reference CO_2 concentration were held constant inside the leaf chamber at 1800 μmol m^{-2}

s^{-1} and 400 μmol mol^{-1}, respectively. For the majority of measurements, relative humidity (Rh) within the chamber (2 x 3 cm) was not controlled and fluctuated with ambient conditions. All-sided leaf area in the cuvette was calculated based on measuring the radius of one needle per fascicle using a scale loupe. Samples for midday leaf water potential (Ψ_L) measurements were collected in unison as those for leaf gas exchange measurements. Samples for predawn leaf water potential (Ψ_L) were collected from the same canopy location as those for midday Ψ_L. Measurements of predawn Ψ_L were conducted the mornings before leaf gas exchange measurements between 0400 h and 0600 h, finishing at least 30 minutes before sunrise. Midday Ψ_L and predawn Ψ_L were measured using a pressure chamber (PMS, Instrument Corp., Corvallis, OR, USA).

Stable carbon isotope ratio ($\delta^{13}C$) of foliage that had been measured in the cuvette was dried at 60° C, bulked by tree and foliage cohort, and stored until measurement. Foliage was measured using an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled with Thermo Conflo IV and Thermo Delta V Advantage stable isotope mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at Texas A&M University Stable Isotopes for Biosphere Science Laboratory.

Annual foliage mass

Sampling was conducted on August 20-21, 2015 to capture peak LAI of the 2015 growing season, i.e., both the 2014 and 2015 foliage cohorts. At that point, a small portion of the lower foliage in the 2014 cohort had senesced and that senescence was mostly associated with the death of lower branches from canopy shading.

For each tree, the diameter at insertion (2 cm from the main stem) for all live branches was measured up to a height of 5.5 m. One branch per whorl was randomly selected and harvested with hand clippers and foliage was separated into the 2014 and 2015 cohorts, placed in paper bags, dried at 60 °C, and weighed. For whorls higher than 5.5 m, one branch per whorl was harvested with a pole pruner and its diameter measured. The diameters of the remaining branches were estimated by eye based on the measured diameter of the harvested branch and the relative size difference of the attached branches. Foliage from the main stem was not measured. When a fork occurred (two of ten trees), the smaller of the forks was harvested and all foliage from the fork was collected.

The relationship between branch diameter^2 and foliage biomass was determined for each tree and foliage cohort separately. Based on these plots, the lowest 0 to 3 whorls (depending on tree) were excluded from each regression analysis because the lowest whorls had disproportionately low leaf biomass per branch diameter^2 due to shading effects. These lowest branches had developed to support previous cohorts. After removing the lowest branches from the analysis, the results were linear relationships between foliage biomass and branch diameter^2. The average r^2 was 0.63 and 0.67 for 2014 and 2015 foliage cohorts respectively.

To estimate leaf biomass of each tree and cohort, the leaf biomass of the nonharvested branches was estimated using the regression relationships and the foliage biomass of all branches was summed. For lower branches in whorls that were not included in the regression analysis, biomass of harvested branches was added to the total and biomass of nonharvested branches was estimated relative to the harvested branch for that whorl, i.e., (diameter^2 of branch/diameter^2 of harvested branch)*leaf biomass of harvested branch. For the two trees with forks, forks were not included in the regression analysis and the foliage biomass of the fork was included into the total.

Growth

Tree size was measured in February 2013 prior to treatment application (following the 5th growing season), in January 2014 (following 6th growing season), and in January 2015 (following 7th growing season). Measurements included DBH and height. DBH was measured using a diameter tape. Height growth was measured prior to treatment application and following the 6th growing season using a height pole. Height was measured following 7th growing season using a hypsometer (Laser Technology, Inc., Centennial, CO, USA). DBH, height, and volume growth (volume = $0.34864+0.00232*\mathrm{DBH}^2*\mathrm{height}$; volume in ft^3, DBH in inches, height in ft) (Burkhart 1977) were determined from the difference in successive dormant season measurements.

Water use

Sap flow density was measured using thermal dissipation probes (TDPs) that consisted of 19 gauge 38.1 mm stainless steel hypodermic needles that were cut to 20 mm in length (Granier 1985) constructed in the tree physiology laboratory at Oklahoma State University. Probes were inserted beneath excluders on the north side of each tree. Probe height ranged from 0.73 m to 0.42 m above the ground. All trees contained one probe (total 10 probes). All probes were initially inserted in February 2013. Probes were wrapped with reflective insulation to minimize thermal gradients. Probes were monitored through weekly data downloads and screened for malfunctions and errors. Probes were monitored through weekly data downloads and screened for malfunctions and errors.

Malfunctioning probes were replaced by new probes in the same tree at least 100 mm from the original location. New probes were not expected to provide the same readings as original probes due to variation in sapwood conductivity (Ward et al. 2013, Tateishi et al. 2008), and each replacement was treated as a separate probe. Therefore, the actual number of probes in the study varied in the analysis based on the number of replacements.

The temperature differential between the upper probe (heated at constant 0.2 watts) and lower probe (non-heated) was measured continuously every two minutes beginning from initial probe insertion and recorded by data loggers (model CR1000 or CR10x, Campbell Sci., Logan UT). Data from April through October of the 6th and 7th growing seasons are presented. Each block contained a data logger that stored data from 20 probes (4 plots per block, 5 trees per plot). Sap flux was calculated according to Granier (1987) by solving the flow index (K) [$K = (\Delta T_{max} - \Delta T)/\Delta T$], where ΔT_{max} is the maximum temperature difference established between the heated and non-heated probes at zero flux and ΔT is the temperature difference between heated and non-heated probes at a given sap flux density and using K to calculate sap flux velocity (V) [$V = 0.000119 * K^{1.231}$(m s^{-1})]. V was converted to sap flux ($J_s = SA$ x V (m^3 sec^{-1}), where SA is sapwood area in m^2. Finally, these instantaneous measurements were converted to units of sap flux in liters per day, and averaged for each day. Sapwood area for all trees in this study was assumed to be the cross sectional area at probe height minus bark thickness, due to the fact that the trees were 8 years old at the end of the experiment which is under the age when heartwood development begins in loblolly pine (Schultz 1997). This was confirmed by coring five dominant sized trees in the plantation, outside the measurement plots. Due to missing data, average daily (midnight to midnight) tree-level sap flux per basal area increment at breast height data was averaged per plot an imputed using a mixed model in SAS and backfilled for missing dates as total liters per tree per day.

Statistical analysis

For all measurements, treatment effects were analyzed using repeated measures analysis (Proc Mixed, SAS Inc., Cary, NC, USA) with block as a random factor and treatments as fixed factors and the autoregressive 1 (AR1) covariance structure. When there was a significant interaction involving year, treatment effects within year were tested using SLICE statement in Proc Mixed. Daily tree and plot level water use was summed per week for analysis. As with year, when there was a significant interaction involving week, treatment effects within week were tested using SLICE statement. When there was a main treatment effect interaction, difference of least squares means was used to determine treatment differences. Plot means for all data were used ($n = 5$) and treatment effects were considered significant at $P < 0.05$.

Results

Environmental variables

Mean monthly average temperature was greatest in August in 2013 (26.1 ^{o}C) and 2014 (25.4 ^{o}C) and lowest in December (4.3 ^{o}C) in 2013 and January (4.5 ^{o}C) in 2014. Overall mean monthly temperature for the measurement period (March 2013 – September 2014) was 16.7 ^{o}C (Fig. 21A). Mean monthly daytime VPD was greatest in August in 2013 (1.41 kPa) and 2014 (0.92 kPa) and lowest in December in 2013 (0.25 kPa) and 2014 (0.17 kPa). Overall mean monthly daytime VPD for the measurement period was 0.77 (kPa) (Fig. 21B). Annual precipitation was 1190 mm in 2013 and 1046 mm in 2014. In 2013, greatest precipitation occurred in July (216 mm) and least occurred in August (8.1 mm). In 2014, greatest precipitation occurred in July (182 mm) and least occurred in August (11 mm). Total precipitation over the measurement period was (1982 mm) (Fig. 21C).

Soil moisture

Throughfall exclusion reduced VWC from 0-12 cm soil depth except for dry periods when VWC also decreased in the TR_W treatment, causing treatment differences to compress (Table 7) (Fig. 22). Across the measurement period, throughfall exclusion reduced VWC 0-12 cm soil depth by approximately one half, averaging 4.8 ± SE 1.5 % for the TR_D treatment and 10.8 ± SE 2.9 % for the TR_W treatment. Similar to VWC from 0-12 cm soil depth, throughfall exclusion reduced VWC from 12-45 cm soil depth except for dry periods when VWC also decreased in the TR_W treatment, causing treatment differences to compress (Table 7) (Fig. 22). Across the measurement period, VWC of the TR_D treatment was reduced by approximately one third compared to the TR_W treatment averaging 15.7 ± SE 2.9 % for the TR_D treatment and 24.2 ± SE 3.1 % for the TR_W treatment. Throughfall exclusion had no effect on VWC from 45-90 cm soil depth. A date x treatment interaction for VWC from 45-90 cm was caused by changes in the rank of treatment means (Table 7) (Fig. 22). Average VWC from 45-90 cm across the measurement was 27.4 ± SE 2.8 % and 26.0 ± 2.8 % for the TR_D and TR_W treatments, respectively. Over the last week of October 2013 and first week November 2013, the site received 213 mm of precipitation which is greater than 2.5 times the average monthly precipitation across the study period (73 mm). The intense precipitation over a short period of time caused a recharge in VWC at all depths across both treatments (Fig. 22). However, the TR_D treatment maintained significantly lower VWC than the TR_W treatment.

Annual foliage mass

Averaged across the 2014 and 2015 foliage cohorts, throughfall exclusion decreased foliage mass by 21 % (Table 9) (Fig. 23A). While the foliage biomass was greater for the 2015 than the 2014 foliage cohort, treatment effects were consistent within years (Table 9). Average foliage mass across the measurement period was 3550 ± SE 226 g for the TR_W treatment and 2806 ± SE 232 g for the TR_D treatment. Throughfall exclusion did not significantly affect annual foliage per sapwood area (Table 9) nor was there a significant interaction between throughfall reduction and year (Table 9). (Fig. 23B).

Physiology

P_{net}, g_s, and C_i varied by sampling date (Table 8) and followed similar trends. Throughfall exclusion increased g_s (Table 8) (Fig. 24B) by 12 % on average. Throughfall exclusion did not significantly affect P_{net} (Table 8) (Fig. 24A) or C_i (Table 8) (Fig. 24C). The interaction between sampling date and throughfall reduction was not significant for leaf gas exchange variables (Table 8). Throughfall exclusion (Table 8) caused more negative predawn Ψ_L following the second measurement date once treatments took effect (Table 8) (Fig. 25A). Excluding the first two measurement dates, the differences were 34 % on average. Throughfall exclusion (Table 8) caused more negative midday Ψ_L with the effects increasing over the course of the experiment (Table 8) (Fig. 25B). On dates where throughfall exclusion was significant for midday Ψ_L, the differences were 23 % on average. Across all dates, throughfall exclusion decreased midday Ψ_L by 17 %. $\delta^{13}C$ varied by year (Table 9). Throughfall exclusion did not affect $\delta^{13}C$ for the 2012 (p = 0.89), 2013 (p = 0.29), or 2014 (p = 0.55) foliage cohorts. Average $\delta^{13}C$ was -29.38 ± SE 0.18 ‰ for the 2012 cohort, -29.69 ± SE 0.16 ‰ for the 2013 cohort, and -30.40 ± SE 0.14 ‰ for the 2014 cohort.

Water use

Weekly whole-tree water use (WU) and water use per sapwood area (WUSA) varied by sampling date (Table 8) and followed similar trends. Throughfall exclusion reduced WU by 12 % on average, but this difference was not significant (Table 8) (Fig. 26A). Throughfall exclusion increased WUSA by 6% on average, but this difference was not significant (Table 8) (Fig. 26B).There was no date by treatment interaction for WU (Table 8) or WUSA (Table 8).

Growth

Before treatment application, mean terminal height, DBH, and volume was 4.6 m, 6.8 cm, and 0.012 m^3 for the TR_D treatment and 4.7 m, 6.9 cm, and 0.012 m^3 for the TR_W treatment, respectively.

Throughfall exclusion did not significantly affect total height (Table 9), DBH (Table 9), or volume growth (Table 9). However, all measures of growth were greater for 2014 compared to 2013 (Table 9). Throughfall exclusion showed trends of reduced total height, DBH, and volume growth across the entire study period (Fig. 27A-C). On average, total terminal height growth was 11 % less, total DBH growth was 16 % less, and total volume growth was 25 % less in the TR_D treatment.

Discussion

In support of our hypothesis, long-term throughfall exclusion led to reduced VWC and more negative predawn and midday Ψ_L. In turn, these effects of long-term drought caused a reduction in foliage mass. However, long-term throughfall exclusion did not reduce leaf gas exchange, whole-tree water use, or significantly reduce growth. Given the lack of gas exchange and growth response, the net effect was long-term throughfall exclusion probably caused moderate, sustained water stress. Severe drought stress was probably avoided due to reducing foliage biomass which may have reduced potential water use and by roots accessing water below 45 cm soil depth.

In our study, throughfall exclusion caused a large sustained decrease in predawn and midday Ψ_L. In regards to throughfall reduction experiments on planted pine, both predawn and midday Ψ_L were more negative in our study than reported in previous research (Tang et al. 2004, Samuelson et al. 2014). Mean predawn leaf Ψ_L in our study reached levels more negative than those previously reported for planted loblolly pine during natural drought (Cregg et al. 1988, Domec et al. 2009) but did not quite reach midday levels previously reported (Hacke et al. 2000, Domec et al. 2009). Predawn water potentials represent the equilibrium between soil water and the plant while midday are affected by transpiration rate as controlled by VPD and stomatal conductance. Our midday water potentials could have been less negative than some of those reported because the natural droughts that reported more negative midday water potentials occurred during periods of very high evaporative demand or because the seed source from the Western Gulf region might close their stomate sooner in response to water stress than seed sources from further east in the range.

The excluders were effective at eliminating throughfall. However, deeper soil moisture (45-90 cm depth) did not show a decline. This was probably due to a combination of lower rooting density at that depth and subsurface movement of deeper soil water. In loblolly pine, greater than 95 % of root biomass is in the top 60 cm of the soil of both dry and wet soil (Harris et al. 1977, Brewer and Linnartz 1978), with 70 to 80 % commonly located in the top 20 cm of soil (Box 1968) and the majority of absorptive roots within the top 15 cm of the soil (Schultz 1997). While deeper roots are less common, access to the more available, deeper soil water may have been able to prevent more severe drought stress. Subsurface water moves from areas of higher soil water potential to areas of lower soil water potential. Even though we trenched down to approximately 60 cm, lined the trench with plastic, and placed the site on a ridge, lateral soil water movement may have still occurred, keeping VWC from 45-90 cm soil depth similar throughout the duration of the study. The net effect was that although we eliminated 100% of throughfall, the treatment we imposed was probably represented sustained, moderate drought stress rather than severe drought stress sufficient to cause mortality. This differs from other situations where some fraction of throughfall is removed (Will et al. 2015), in that the surface soil layers never fully recharged with 100% exclusion treatment.

Rather than causing stomatal closure and a reduction in leaf gas exchange, the chronic drought treatment caused a downward shift in leaf area. Morphological changes such as reduced leaf area can occur in some tree species from increased drought severity over longer time scales (Kramer 1983, Battaglia et al. 1998) while, short-term and/or less severe drought cause stomatal closure without affecting foliage mass (Kramer 1983).Trees can employ different strategies during drought, ranging from water-conserving behavior known as isohydric regulation to a riskier, less conservative strategy known as anisohydric regulation (McDowel et al. 2008, Sade et al. 2012). Isohydric species reduce stomatal conductance as soil water content dries and the demand for water in the atmosphere increases during drought conditions, maintaining relatively constant midday leaf water potential. In contrast, anisohydric species allow midday leaf water potential to decline as soil dries during drought, thus maintaining stomatal conductance and CO_2 assimilation (McDowell et al. 2008, Sade et al. 2012, Moshelion et al. 2014). Trees experiencing of long-term, persistent drought in our study did not maintain leaf water potentials or reduce stomatal conductance, which could indicate anisohydric response strategy to long-term drought conditions, but this in turn caused a feedback to reduce leaf area development.

In our study, throughfall exclusion reduced annual foliage mass which included the 2014 and 2015 cohorts, consistent with effects of severe or long-term drought. Current-year loblolly pine needles are influenced by conditions during the previous two years (Hebert and Jack 1998). Annual foliage mass was reduced by 21 %. These results indicate that throughfall reduction reduced annual foliage mass without changing hydraulic conductivity. In comparison, a recent study conducted in the same stand, but different area found that a more moderate reduction in throughfall of approximately 30 % reduced leaf area index by 8.5 % (Maggard et al. 2016b). Similarly, Tang et al. (2004)

reported a 12 % reduction in foliage mass from throughfall reduction in an eighteen year old loblolly pine plantation.

Reductions in VWC often result in reduced g_s to conserve water, subsequently reducing P_{net} and growth in loblolly pine (Teskey et al. 1986, Chaves, et al. 2003). This is consistent with recent loblolly pine throughfall reduction studies (Tang et al. 2004, Samuelson et al. 2014, and Maggard et al. 2016a), as well as drought induced experiments on piῆon pine (*Pinus edulis*) and juniper (*Juniperus monosperma*) (Adams et al. 2009, Pangle et al. 2015). In contrast, throughfall exclusion in this study increased g_s and the lack of differences in P_{net} and C_i in our study could be due to a combination of the significant reduction in annual foliage mass and an anisohydric response to drought conditions.

The partial recharge in VWC observed in late fall 2013 could have prevented throughfall exclusion trees from reaching severe drought stress. However, physiologically this doesn’t appear to be the case. Leaf gas exchange measurements were never significantly different between treatments across the study period. Further, both predawn and midday Ψ_L measurements indicated increased drought stress for the throughfall exclusion trees as the experiment progressed. The increases in g_s, but not P_{net} could be from non-stomatal limitation to water stress such that higher g_s was necessary to maintain P_{net}. Mesophyll conductance has been recognized as more sensitive to water stress than g_s (Green and Mitchell 1992, Grassi and Magnani 2005), which may have triggered such a tradeoff.

Throughfall exclusion did not reduce WU or WUSA in our study which contrasts to recent loblolly pine throughfall reduction studies researching WU (Maggard et al. 2016b) and stand-level transpiration (Bartkowiak et al. 2015, Ward et al. 2015). The lack of effect of throughfall exclusion on WU or WUSA is consistent with our leaf gas exchange results. There were nonsignificant trends of lower WU and higher WUSA in the throughfall exclusion treatment, which is consistent with reduced foliage mass. The increased g_s in the throughfall exclusion treatment could be increasing WU even though foliage mass was reduced, as water use is highly responsive to leaf area and g_s (Sun et al. 2011, Ni and Pallardy 1992).

The fact that WU did not decrease in response to throughfall exclusion is a bit puzzling given the more negative predawn water potentials and much lower VWC in the upper soil surface for the throughfall exclusion trees. Also, lower leaf area and no differences in P_{net} could be expected to cause lower tree growth. While greater g_s could compensate for reduced leaf biomass in regards to water use, there was still a trigger that caused lower leaf biomass development to begin with, which was probably reduced water availability. Hennessey et al. (1992), found that leaf biomass production measured across 5 years was reduced in dry years in which potential evapotranspiration exceeded precipitation during the growing season. However, not significant, there was a trend of reduced growth in the throughfall exclusion treatment across both years of the study period. A possible explanation could be could be relatively small sample size compared to the variation among individual trees.

Conclusions and management implications

Our results indicate that 100 % throughfall exclusion surrounding individual trees decreases soil moisture, increases tree water stress, and reduces foliage biomass. However, contrary to our initial hypothesis, 100 % throughfall exclusion did not reduce leaf gas exchange, water use, or growth. Rather, 100 % throughfall exclusion trees avoided stomatal closure and avoided significantly reduced growth while reducing foliage biomass. In turn, stomatal conductance maintained water use and productivity without reaching catastrophic leaf water potential values. These results indicate that loblolly pine under long-term, moderate drought may change their water conserving strategies and make morphological changes in regards to reduced leaf biomass in order to maintain productivity while avoiding severe and potentially fatal levels of water stress. During long-term, moderate drought morphological water conserving strategies rather than physiological acclimations may be more important for avoiding severe and potentially fatal levels of water stress

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Tables.

Table 7. *P* values for the effects of date, throughfall reduction (Water) and fertilization (Fert) treatments on volumetric soil water content (VWC) from 0-12 cm, VWC from 12-45 cm, and VWC from 45-90 cm for the entire measurement period (2013-2014). Bold values indicate significance at α < 0.05.

	VWC 0-12 cm	VWC 12-45 cm	VWC 45-90 cm
Date	**<0.0001**	**<0.0001**	**<0.0001**
Treatment	<0.0001	<0.0001	0.07
Date x Treatment	**<0.0001**	**<0.0001**	**0.01**

Table 8. *P* values for the effects of date, throughfall reduction (Water) and fertilization (Fert) treatments on net photosynthesis (Pnet), stomatal conductance (gs), intercellular CO2 (Ci), predawn leaf water potential (Ψ_L), midday leaf water potential (Ψ_L), whole-tree water use (WU), and whole-tree water use per sapwood area (WUSA) for the entire measurement period (2013-2014). Bold values indicate significance at α < 0.05.

	P_{net}	g_s	C_i	predawn Ψ_L	midday Ψ_L	WU	WUSA
Date	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Treatment	0.64	0.09	0.12	<0.0001	<0.0001	0.22	0.45
Date x Treat.	0.17	0.24	0.78	**0.0002**	**0.04**	0.99	0.85

Table 9. *P* values for the effects of date, throughfall reduction (Water) and fertilization (Fert) treatments on annual foliage mass, annual foliage mass per sapwood area, stable carbon isotope ratio ($\delta^{13}C$), total height growth, total DBH growth, and total volume growth for the entire measurement period (2013-2014). Bold values indicate significance at $\alpha < 0.05$.

	Foliage mass	Foliage mass per sapwood area	$\delta^{13}C$	Height	DBH	Volume
Year	**0.004**	**0.004**	**<0.0001**	**0.0001**	**0.005**	**<0.0001**
Treatment	**0.047**	0.60	0.34	0.49	0.28	0.13
Year x Treat.	0.50	0.81	0.72	0.91	0.72	0.28

Figures.

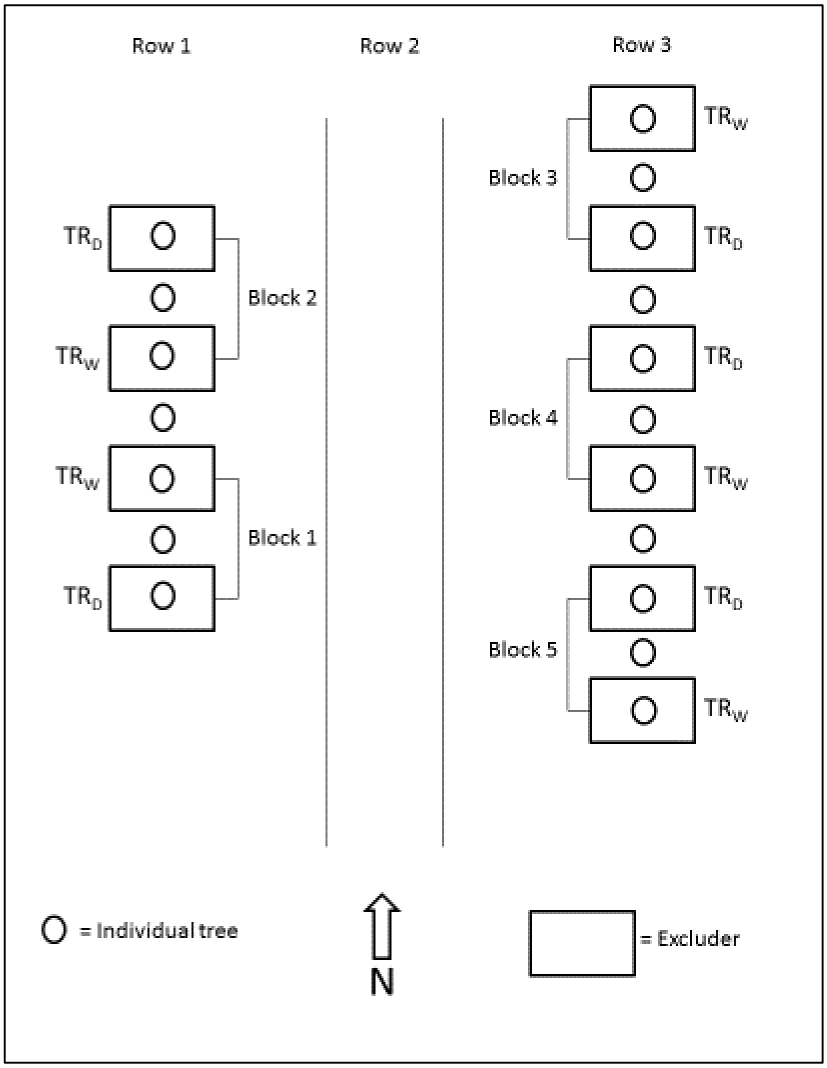

Fig. 21. Diagram of study site showing treatment design and block layout for 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments.

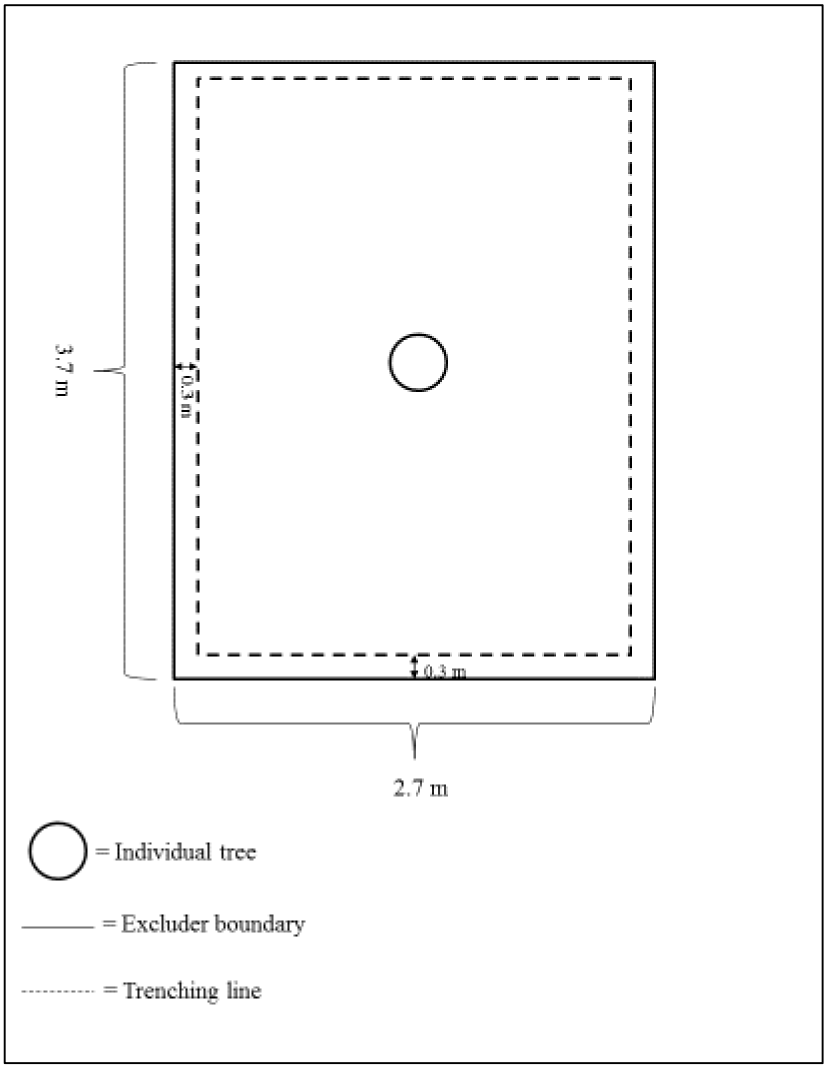

Fig. 22. Diagram of individual tree exclusion showing trenching and excluder design with lengths and widths for trenches and excluders.

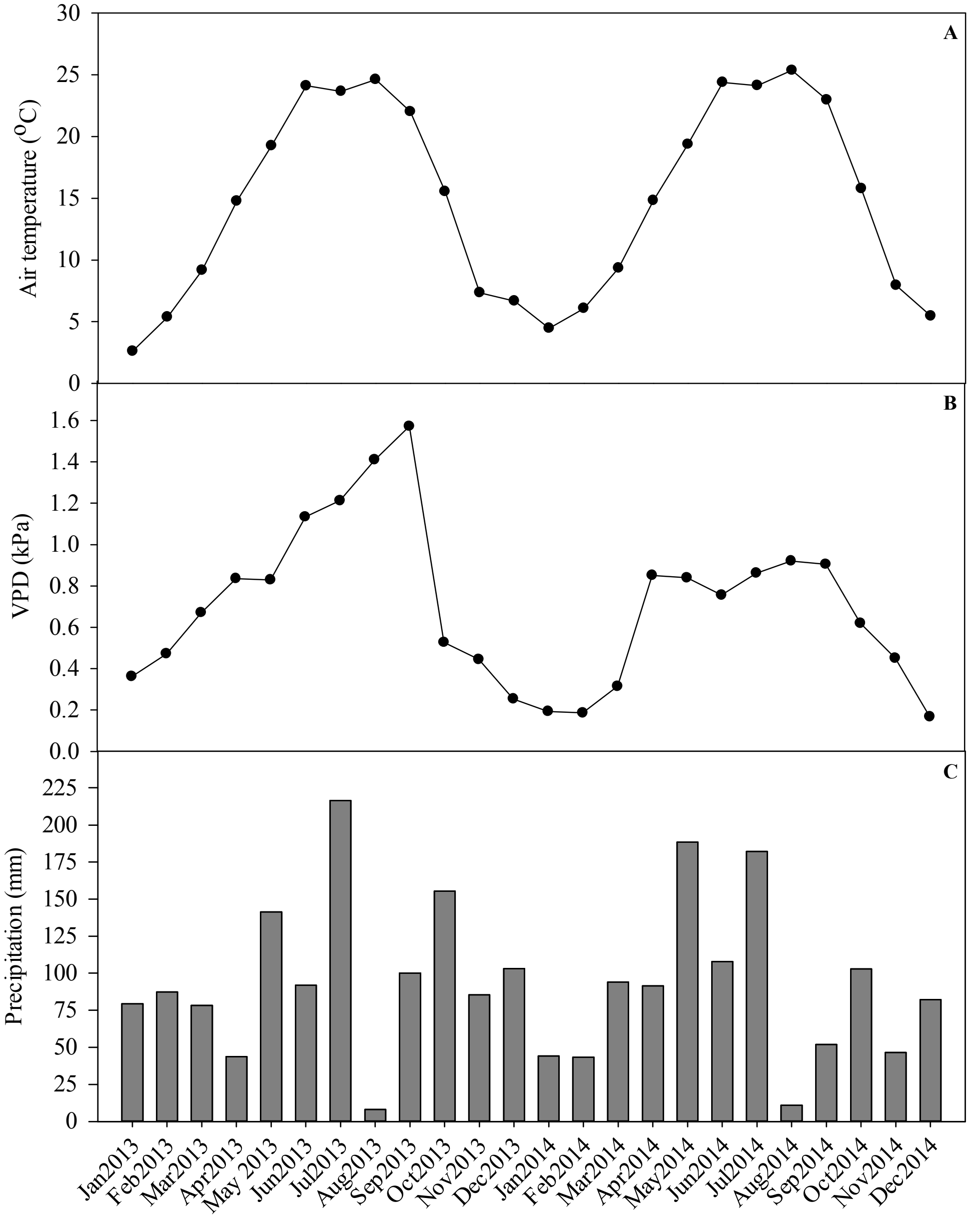

Fig. 23. Mean monthly (A) air temperature, (B) mean monthly daytime vapor pressure deficit (VPD) and (C) total monthly precipitation for 2013 and 2014.

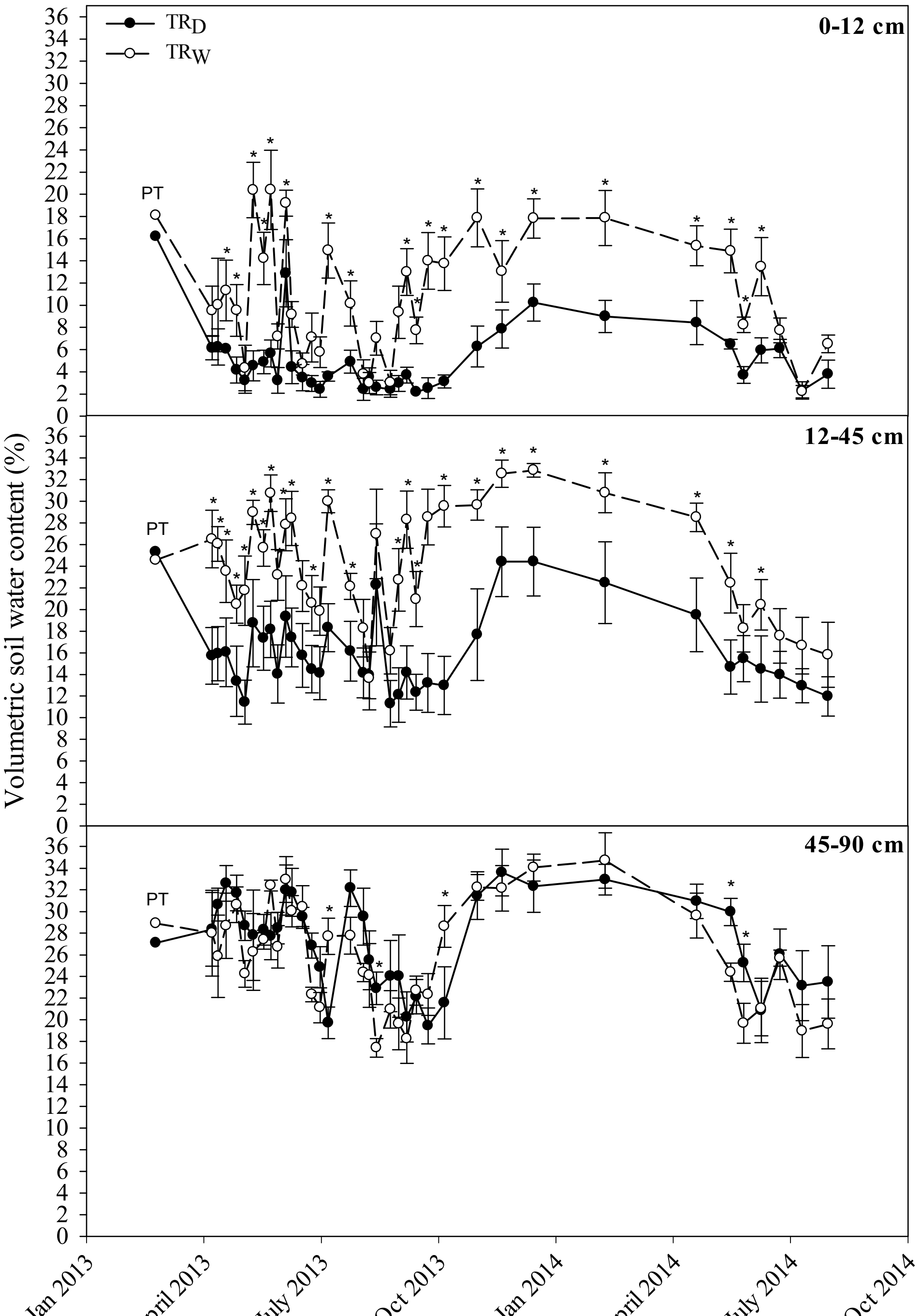

Fig. 24. Mean volumetric soil water content (%) between 0-12 cm, 12-45 cm, and 45-90 cm in response to 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments. An asterisk (*) above the data represents dates that treatments are significantly different ($\alpha < 0.05$ ($n = 5$). Pre-treatment (PT) represents measurement before treatment application.

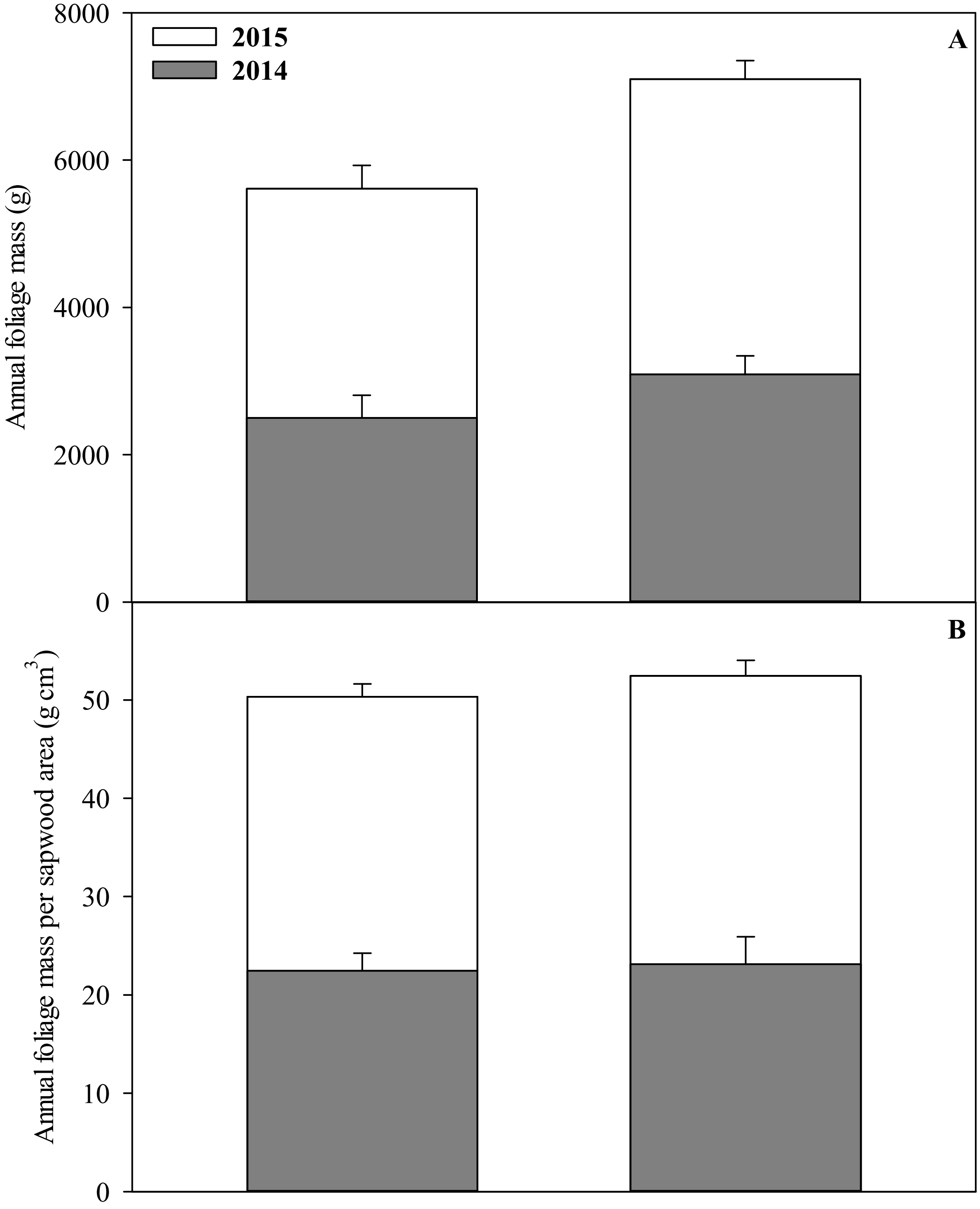

Fig. 25. (A) Annual foliage mass and (B) annual foliage mass per sapwood area for the 2014 and 2015 foliage cohorts in response to 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments. Error bars represent standard error of treatment means (*n* = 5).

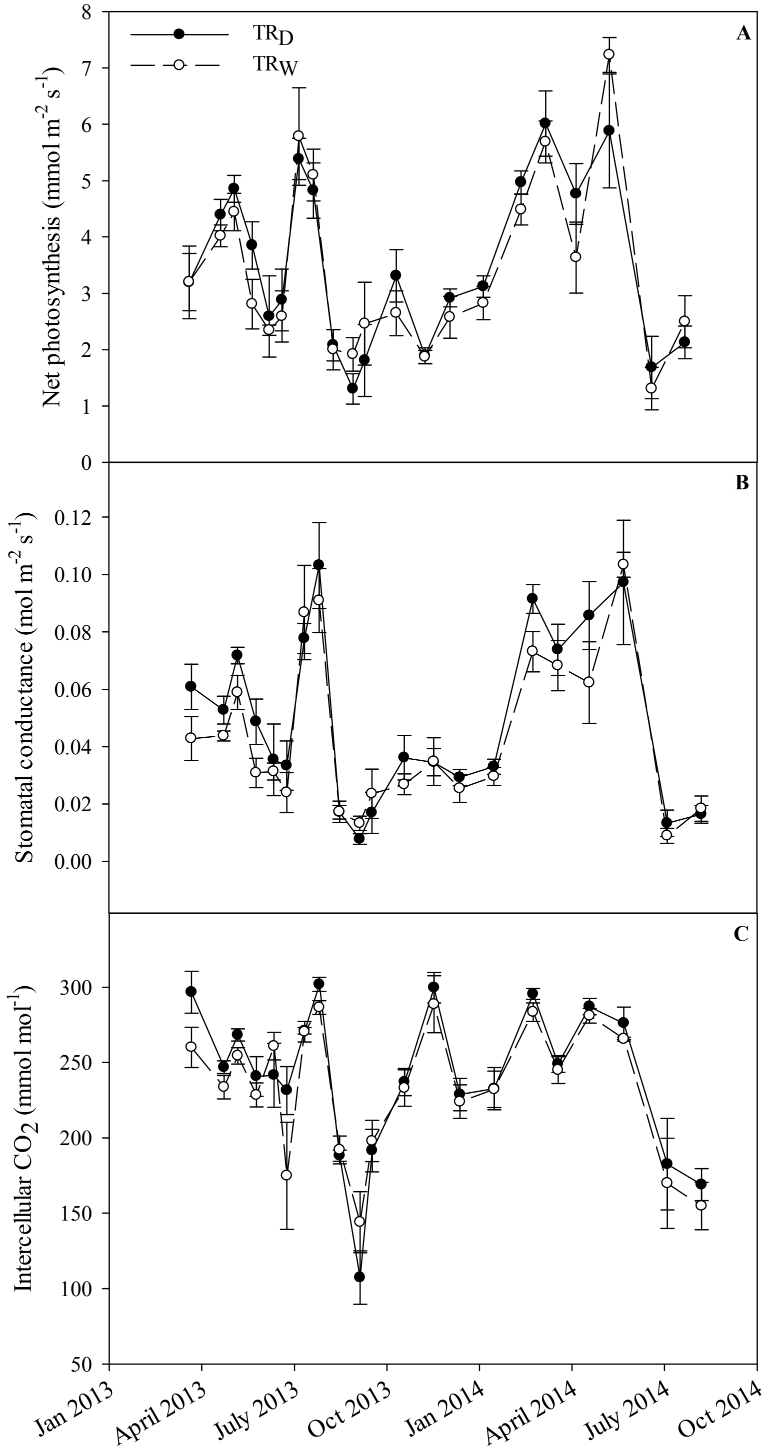

Fig. 26. (A) Mean net photosynthesis (P_{net}), (B) mean stomatal conductance (g_s), (C) intercellular CO_2 (C_i) in response to 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments. Error bars represent standard error of treatment means (*n* = 5).

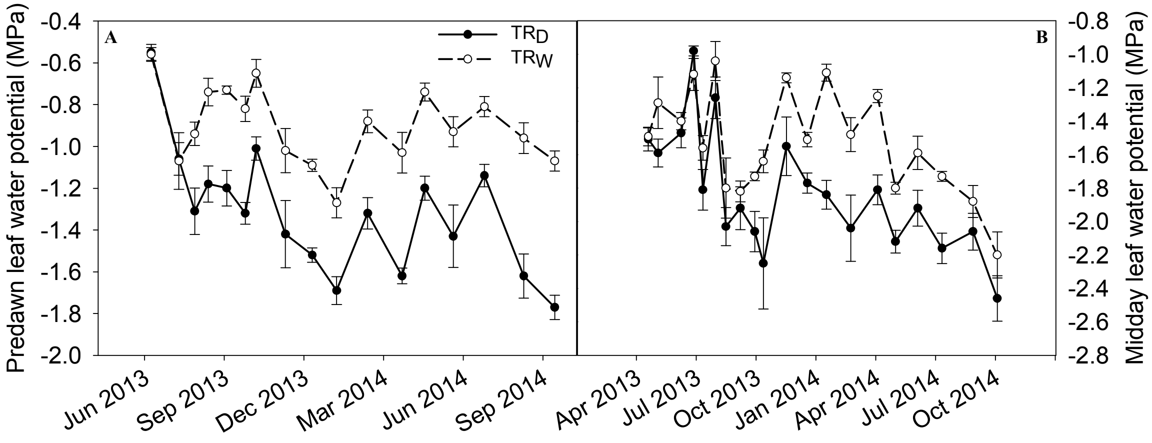

Fig. 27. (A) Mean predawn leaf water potential (Ψ_L), (B) mean midday leaf water potential (Ψ_L) in response to 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments. An asterisk (*) above the data represents dates that TR$_D$ effect is significant. Error bars represent standard error of treatment means (*n* = 5).

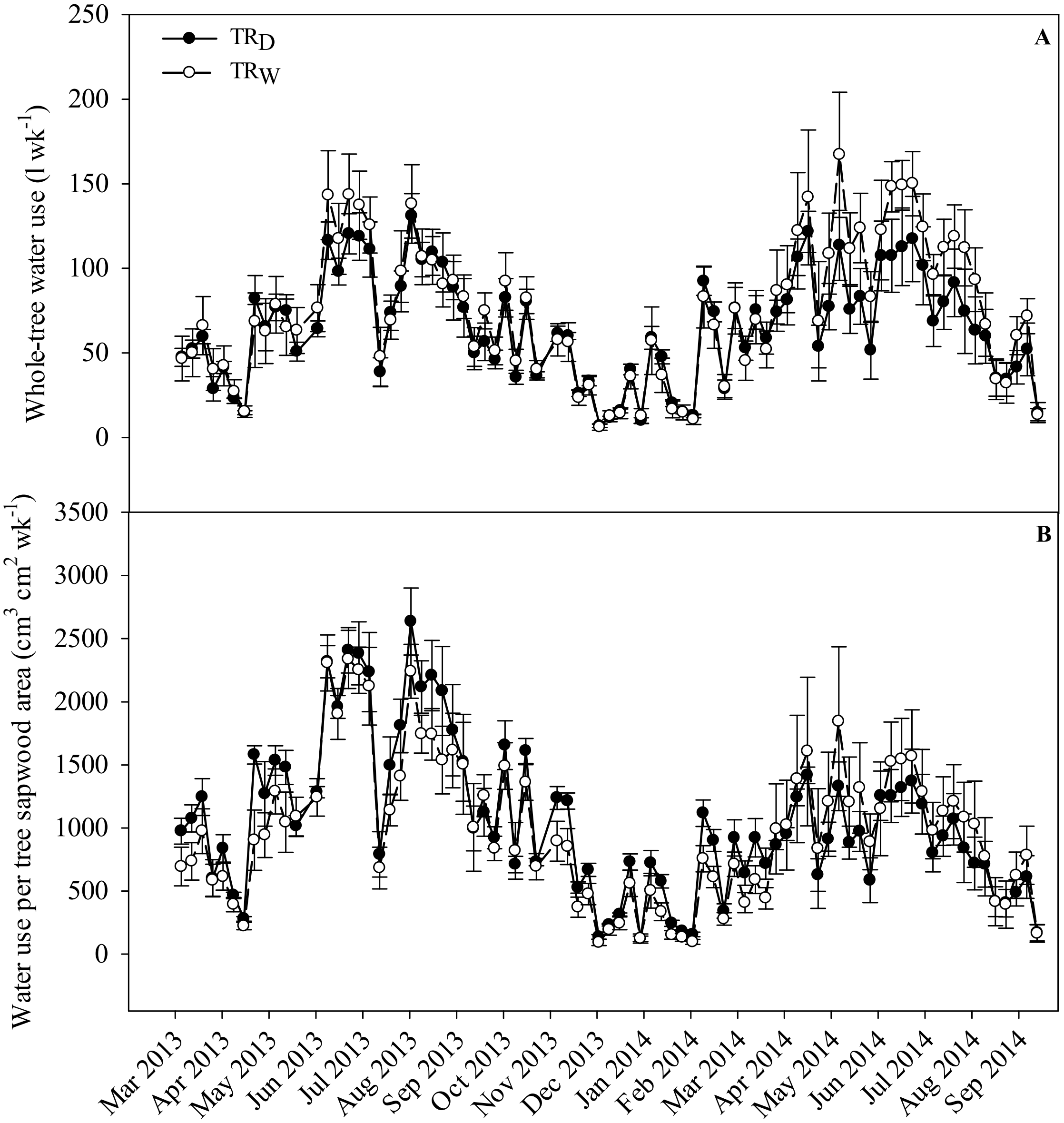

Fig. 28. (A) Mean weekly total whole-tree water use and (B) mean weekly total water use per sapwood area in response to 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments (*n* = 5).

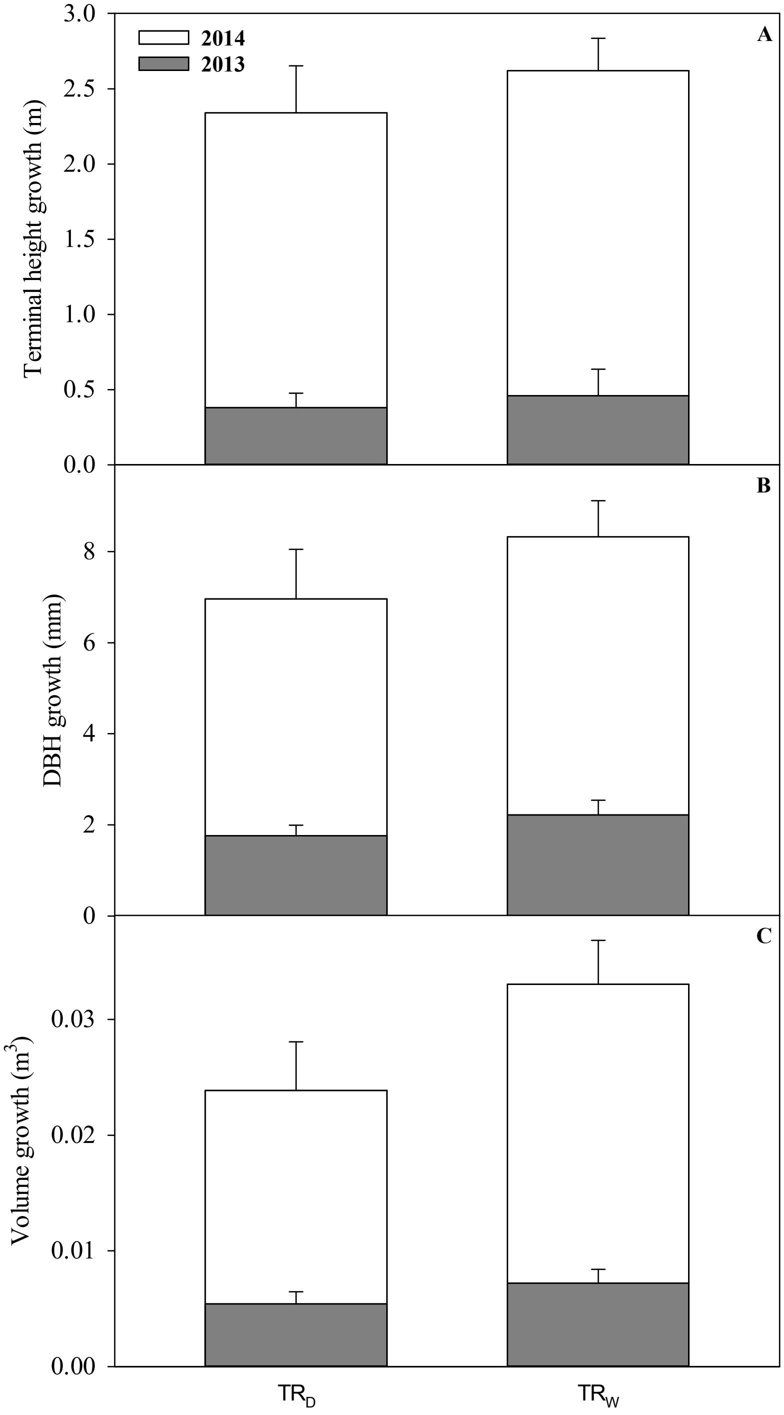

Fig. 29. Total (A) terminal height growth (m), (B) DBH growth, and (C) volume growth (m^3) for 2013 and 2014 in response to 100 % throughfall exclusion (TR$_D$) and ambient throughfall (TR$_W$) treatments. Error bars represent standard error of treatment means (*n* = 5).

CHAPTER V

CONCLUSION

Results of these studies indicate that throughfall reduction decreases soil moisture, reduces leaf gas exchange and slows growth. However, fertilization can compensate for throughfall reduction. Fertilization did not exacerbate the effects of throughfall reduction, but increased water use efficiency. In the first study I found that fertilization decreased stomatal conductance without reducing net photosynthesis indicating a positive nonstomatal response to fertilization. In support of results from the first study, I found that fertilization increased water use efficiency of stem volume production in the second study. These results indicate that fertilization can be beneficial in loblolly pine plantations experiencing reduced water availability in areas with higher growing season temperatures and greater VPD, such as the western limits of its commercial range. In the third study I found loblolly pine under long-term moderate drought may change their water conserving strategies by reducing leaf biomass instead of reducing leaf-level water use.

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