Fertilization reduced stomatal conductance but not photosynthesis of *Pinus taeda* which compensated for lower water availability in regards to growth

Adam O. Maggard ^{a,*}, Rodney E. Will ^a, Duncan S. Wilson ^b, Cassandra R. Meek ^a, Jason G. Vogel ^{c,1}

^aDepartment of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078, USA; * Corresponding author. E-address: adam.maggard@okstate.edu

^bSouth Central Climate Science Center, University of Oklahoma, Norman, OK 73019, USA

^cDepartment of Ecosystem Science and Management, Texas A&M University, College Station, TX 77843, USA

ABSTRACT

Loblolly pine (*Pinus taeda* L.) is the most commercially important tree species in the southeastern USA. Projected increases in regional temperature and drought severity likely will cause reductions in productivity, in particular for the western edge of the loblolly pine 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 southeastern Oklahoma. 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 %) but caused less negative (3 %) midday leaf water potential. Throughfall reduction reduced stomatal conductance (12 %) and net photosynthesis (9 %) and these responses were related to more negative midday leaf water potentials (11 %). These results indicate that fertilization increased water use efficiency in that fertilized treatments maintained similar net photosynthetic rates as nonfertilized treatments even though stomatal conductance was lower. The net

effect was that fertilization increased growth of throughfall reduction treatments such that productivity was similar to ambient throughfall treatments not receiving fertilization.

Keywords

leaf gas exchange, throughfall reduction, fertilization, *Pinus taeda*, loblolly pine

1. Introduction

Over fifty percent of pine plantations in the southeastern USA are loblolly pine (Oswalt et al. 2014), 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 the 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 USA (Oswalt et al. 2014). However, in years to come, changes in 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 4.0 °C by the latter half of this century (Collins et al. 2013). Little change in total precipitation, but 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. Combined with higher vapor pressure deficits (VPD) resulting from higher temperature, predicted trends in precipitation could increase the severity of drought (Breshears et al. 2013, Will et al. 2013).

The western edge of the range of loblolly pine in southeastern Oklahoma, USA experiences higher growing season temperatures and VPD than the rest of the southeastern USA (Seager et al. 2015, Walsh et al. 2014). As a result, loblolly pine planted in this area are more apt to experience water stress. Plantations in this area are especially at risk for future losses in production, in part, because the water stress decreases leaf gas exchange and can lead to reduced tree growth.

Longer-term responses to water limitation frequently involve a trade-off with tree productivity because tree stature and canopy leaf area growth slows to decrease transpirational water loss and tree water stress (e.g., Kramer and Boyer 1995). In addition to these morphological changes, shorter-term physiological changes occur in response to water stress. For instance, stomatal conductance and carbon

assimilation typically decline (e.g., Ni and Pallardy 1992). Stomatal closure to limit water loss is among the earliest responses to water deficit, otherwise severe water stress leads to cell and tissue dehydration, xylem cavitation, and potential death (Chaves 1991). As stomatal conductance is reduced, net photosynthesis also declines from a reduction in the rate of CO₂ diffusion into the leaf (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 of adenosine triphosphate (ATP) synthesis and ribulose-1,5-bisphosphate (RuBP) regeneration (Tezara et al. 2002, Flexas and Medrano 2002, Hu et al. 2010). Reduced rates of photosynthesis due to stomatal and non-stomatal responses have the potential to affect 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, especially once the trees begin to reach crown closure, which is typically after 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 in pine plantations. Applying fertilizer, particularly combinations of N and P, increase the 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 whole-crown carbon fixation (Teskey et al. 1994, Vose and Allen 1988, Albaugh et al. 1998) but at the same time, increases the potential for transpiration and tree water use.

Fertilization treatments that increase leaf area index (LAI) also increase growth, and this response is enhanced 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

impact leaf-level gas exchange of 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 fertilization and irrigation are well documented for loblolly pine plantations (e.g., Ewers et al. 1999, Allen et al. 2005, Samuelson and Stokes 2006, Samuelson et al. 2008b), with fertilization generally having larger effects on productivity than irrigation. However, few studies have examined the effects of fertilization and water stress on the physiology and growth of loblolly pine stands (Tang et al. 2004, Samuelson et al. 2014). A better understanding of the interactive effects of nutrient availability and water stress on the physiological processes and growth of loblolly pine stands is needed to better predict tree and stand responses to a potentially warmer and drier climate and ultimately guide future management practices to sustain loblolly pine productivity. Knowledge of how the benefits of fertilizer are affected by drought will aid in determining its role in future forest management.

The objective of this study was to determine the effects of nutrient amendment and soil water availability on leaf gas exchange, LAI, and tree 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 would increase LAI and exacerbate water stress effects on leaf gas exchange and stand growth. Therefore, positive effects of fertilizer on growth would be minimized under throughfall reduction and fertilization.

2. Materials and Methods

2.1 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 plantation productivity and carbon sequestration. Throughfall reduction 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 in a loblolly pine stand near Broken Bow, OK beginning in the 5th growing season and continuing through the 7th growing season. The study site has an elevation of 150 m, latitude 34°01'52.0"N, and longitude 94°49'18.2"W. The soil is a 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 is typical for forests on the uplands of the western and southern upper Coastal Plain and consists 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 is 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 °C, 23.8 °C, and 9.7 °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, 680 g ha⁻¹ of Chopper[®] (27.6 % imazapyr) (BASF Corporation, Florham Park, NJ, USA) plus 2.8 l ha⁻¹ of glyphosate (53.8 % active ingredient) was broadcast in August 2007 followed by prescribed burning in October 2007. The study site was subsoiled along its 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 obtained from Albert Engstrom Forest Regeneration Center operated by Oklahoma Forestry Services. Broadcast application of 420 g ha⁻¹ of Arsenal® (27.6 % imazapyr) (BASF Corporation, Florham Park, NJ, USA) and 175 g ha⁻¹ of Oust Extra® (56.25 % Sulfometuron methyl, 15.0 % Metsulfuron methyl) (E.I. Du Pont De Nemours and Company, Wilmington, DE, USA) was used for woody plant and herbaceous weed control in March 2008. Spacing was approximately 2 m x 3 m for a target density of 1650 trees ha⁻¹.

2.2 Experimental Design

The study comprised four complete blocks that were designated based on proximity. Each block consisted of four treatment plots in a 2 x 2 factorial combination of fertilization and throughfall reduction (16 plots total). Each treatment plot was greater than 0.10 ha containing an interior measurement plot between 0.034 and 0.041 ha. Fertilization treatments included no fertilizer and a fertilizer treatment 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 were applied as a mix of urea (432 kg ha⁻¹) and diammonium phosphate (140 kg ha⁻¹), and K was applied as potassium chloride (56 kg ha⁻¹). Micronutrients were applied as 22.4 kg ha⁻¹ of a granular mix (Southeast Mix, Cameron Chemicals, Inc., Virginia Beach, VA, USA), 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 in all plots 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 opaque, 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 heights of approximately 1.2 m to 0.6 m. Excluders were installed two rows beyond the measurement plot in the larger treatments 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.

2.3 Growth

Tree size was measured in January 2012 prior to treatment application (following the 4th growing season) and in December 2012 (following the 5th growing season), December 2013 (following the 6th growing season), and December 2014 (following the 7th growing season). Measurements included diameter at breast height (DBH) and total 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 4, 5, and 6 using a height pole. Height was measured following the 7th growing season using a hypsometer (Laser Technology, Inc., Centennial, CO, USA). Annual increments of 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 on the measurement plots died.

2.4 LAI

Stand-level, projected LAI was measured with a LAI2000 plant canopy analyzer (LiCor, Inc., Lincoln, NE, USA) approximately every four weeks during the 7th growing season from April 2014 through March 2015. Lower crown branches below throughfall excluders influenced the LAI2000 measurements prior to

the 7th growing season. The LAI2000 data were corrected using empirical litter trap data (twelve 0.5 m² traps per plot) by the product of LAI2000 data and a multiplier of 0.745. 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 diagonally across treatment plots from a randomly selected starting point at the edge of each plot. Readings were taken along transects 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.

2.5 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 a LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, USA). Measured variables included net photosynthesis (P_{net}), stomatal conductance (g_s), and intercellular CO₂ 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. Fascicles 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 sequentially 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 CO₂ concentration were held constant inside the leaf chamber at 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 400 $\mu\text{mol mol}^{-1}$, 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 °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. Additionally, one fascicle was sampled from measurement trees for midday leaf water potential (ψ_L) measurements and were collected in unison as those for leaf gas exchange measurements. Midday ψ_L was measured using a pressure chamber (PMS, Instrument Corp., Corvallis, OR, USA).

The stable carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$) and nitrogen concentration of foliage was estimated for the foliage that had been measured for leaf gas exchange. Foliage was dried at 60 °C, bulked by measurement 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. The $^{13}\text{C}/^{12}\text{C}$ ratios of the foliage were normalized relative to the known $^{13}\text{C}/^{12}\text{C}$ ratio of the reference material, Pee Dee Belemnite, and were expressed in delta (‰) units. The IRMS run was calibrated using glutamic acid 40 and 41 and the internal precision determined with two plant reference materials. From these, the estimated standard deviation was 0.025 ‰ for $\delta^{13}\text{C}$.

240

241 *2.6 Soil moisture*

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

246

247 *2.7 Statistical analysis*

248 Plot means for all data were calculated ($n = 4$). Total (5th through 7th growing season) height, DBH, and
249 basal area growth were analyzed as a 2 x 2 factorial combination of the main effects fertilization and
250 throughfall reduction. For all other measurements, treatment effects were analyzed as a repeated
251 measures analysis (Proc Mixed, SAS Inc., Cary, NC, USA) with block as a random factor and treatments as
252 fixed factors in a 2 x 2 factorial combination. Separation of treatment means was determined by
253 differences of least square means comparisons using the Slice option in SAS. Treatment effects were
254 considered significant at $P < 0.05$.

255

256 **3. Results**

257 *3.1 Climate*

258 Annual precipitation was 1026 mm in 2012, 1312 mm in 2013, and 1289 mm in 2014. In 2012, the
259 greatest precipitation occurred in March (184 mm) and least occurred in November (11 mm). In 2013,
260 the greatest precipitation occurred in July (239 mm) and least occurred in August (17 mm). In 2014, the
261 greatest precipitation occurred in July (263 mm) and least occurred in August (26 mm) (Fig. 1). Average
262 daily air temperature for 2012 was 17.3°C with average daily maximum and minimum air temperatures
263 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 a severe to extreme drought from June 2012 through January 2013, ranging from a PDSI of -3.18 in June to -4.53 in December. PDSI became near normal beginning in February 2013 and remained so for the rest of the calendar year, ranging from a PDSI of -0.03 in February to 1.29 in December. For 2014, PDSI was near normal for the entire calendar year, ranging from a PDSI of -0.37 in January to 1.54 in October (Fig. 2).

3.2 Soil moisture

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

3.3 Growth

By age 7 years, average tree height ranged from 6.4 m for the TR+F treatment to 6.8 m for the F treatment (Fig. 4A) and average tree DBH ranged from 11.9 cm for the TR treatment to 13.1 cm for F treatment (Fig. 4B). 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 treatments reduced total DBH growth over growing seasons 5-7 (Table 2) by 6.1 %

compared to ambient precipitation treatments. Fertilization (F and TR+F) increased total DBH growth (Table 2) by 7.3 %, compared with non-fertilized treatments (TR and C), respectively (Fig. 5B). As basal area is largely a function of DBH in these young plantations, results of BA and DBH were similar. Throughfall reduction reduced total basal area growth over growing seasons 5-7 by 8.8 % compared to ambient precipitation treatments (Table 2) and fertilization increased total basal area growth by 11.6 % (Table 2) compared to non-fertilized treatments, (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).

3.4 LAI

Throughfall reduction reduced LAI compared to ambient throughfall treatment stands and fertilization increased LAI compared to nonfertilized treatment stands (Table 1), (Fig. 6). Similar to tree DBH and BA growth responses, fertilization compensated for drier conditions as TR+F stands had similar LAI compared to C treatment stands (Fig. 6). Seasonally, LAI ranged from $1.9 \text{ m}^2 \text{ m}^{-2}$ for the TR treatment in winter (December 2014) to $4.2 \text{ m}^2 \text{ m}^{-2}$ for the F treatment in summer (July 2014) (Table 1). Over the duration of the study, average values of LAI were was $2.8 \pm 0.3 \text{ m}^2 \text{ m}^{-2}$ SE for C, $3.1 \pm 0.3 \text{ m}^2 \text{ m}^{-2}$ SE for F, $2.6 \pm 0.3 \text{ m}^2 \text{ m}^{-2}$ SE for TR, and $2.9 \pm 0.3 \text{ m}^2 \text{ m}^{-2}$ SE for TR+F plots.

3.5 Physiology

The leaf-level gas exchange variables, P_{net} , g_s , and C_i , varied significantly 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, throughfall reduction decreased P_{net} by 9 % (Fig. 8A). Fertilization did not significantly affect P_{net} on a leaf-level basis (Fig. 8A). Throughfall reduction and fertilization independently reduced g_s (Table 1). In general, g_s was reduced more so by throughfall reduction during wetter periods compared to drier periods which resulted in a

significant throughfall reduction x date interaction (Table 1) (Fig. 7B). An exception was that throughfall reduction decreased g_s during the August 2013 measurement which was a time when soil moisture was low. This date corresponded to the first measurement of the 2013 foliage cohort. On dates where the 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 the date x fertilization interaction was not significant (Fig. 8B). Throughfall reduction and fertilization (Table 1) significantly reduced C_i by 3 and 4%, respectively (Fig. 7C, 8C).

Throughfall reduction (Table 1) caused more negative midday ψ_L with the effects increasing over the course of the experiment (Table 1) (Fig. 7D). On dates when 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 midday 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 the 2014 cohort (Table 3) (Fig. 9A). Fertilization increased foliar N concentration overall (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% ($p = 0.04$) increases in 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 in ranking among treatments and years.

4. Discussion

Our results indicate that throughfall reduction (TR) decreased leaf gas exchange as a result of decreased soil moisture availability which in turn, probably slowed growth. In contrast, fertilization (F) increased growth but did not affect leaf-level 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 the soil VWC was greater on average compared to nonfertilized treatments. The net effect was that fertilization increased the water use efficiency (WUE) defined as the rate of P_{net} divided by g_s . An increase in WUE may have compensated for low soil moisture such that growth of the TR+F and Control treatments were similar.

As LAI increases with nitrogen availability, stand water use also may increase. Whether this translates to lower soil VWC depends on whether there is a compensating change in plant water use, or if the change in LAI alters canopy interception of precipitation or the evaporation from the soil surface. 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 attributed to lower leaf-level stomatal conductance with fertilization, greater evaporation from soil surface in the more open-canopy, nonfertilized stand, or to random plot-level differences in soil texture causing variability in VWC. In contrast to Samuelson et al. (2014) who found throughfall 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 trees in 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 effect or no effect (Albaugh et al. 2004, Coyle et al. 2008, Samuelson et al. 2008a). In our study, fertilization compensated for reduced throughfall effects on total DBH and total basal area growth such that trees in the TR+F treatment had similar growth as trees in the C treatment. 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 (Samuelson et al. 2014), but stand volume growth was reduced (Will et al. 2015). Unlike

diameter and BA growth, fertilization in our study did not benefit height growth but throughfall reduction negatively affected height growth, suggesting that trees at our site were relatively more drought sensitive than the trees in Samuelson et al. (2014) located at a site in Georgia. 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), which is characteristic of 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 on LAI regardless of throughfall reduction. Similarly, Samuelson et al. (2014) found that LAI was higher in fertilized compared to non-fertilized treatments, but their 30 % throughfall reduction did not affect LAI in a loblolly pine plantation in Georgia. Ward et al. (2015) found that neither throughfall reduction nor fertilization affected LAI in a loblolly pine plantation in Virginia. The negative effects of throughfall reduction we found for both LAI and growth could have been due to higher summer temperatures and greater VPD at our site compared to sites further east (Seager et al. 2015). Will et al. (2015) reported average August daily temperatures during the study period for our site were warmer by 2.6°C than the Georgia site and 3.6°C warmer than the Virginia site. Tang et al. (2004) found that throughfall reduction reduced annual foliage mass in a loblolly pine stand in Louisiana. 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 midday 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 the main effect of throughfall reduction significantly decreased g_s , average monthly

precipitation was 151 mm compared to 88 mm for dates where g_s was not affected. Reduction in g_s occurred during wet periods likely because during the dry periods, all trees were similarly stressed such that the differential effects of a 30% reduction in throughfall were largely eliminated. The only exception was in August 2013 when soil moisture was low across all four treatments but g_s was significantly lower due to the main effect of throughfall reduction (TR and TR+F vs. F and C). 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 differences. In addition, there could have been differences in the rate of physiological maturity achieved by new foliage among the four treatments. 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). Different responses to throughfall reduction among studies may be a result of climatic differences such as air temperature and VPD and edaphic differences such as soil water content, soil texture, depth to water table, etc... Additionally, root density and architecture may influence response.

Differences in midday ψ_L between throughfall reduction (more negative) and ambient precipitation (less negative) treatments increased over time as the effects of the throughfall reduction began to manifest. The decrease in C_i that we measured in response to reduced throughfall was disproportionality due to reduced g_s as P_{net} also declined. This decline in C_i was due to a continuation of the removal of CO_2 from the intercellular air space combined with 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). It has been suggested that when trees experience water deficit, mesophyll conductance is more

responsive to water limitation than g_s . As a result, CO_2 diffusion from stomatal cavity to the chloroplast plays a major role in P_{net} where drought frequently occurs (Teskey et al. 1986, Green and Mitchell 1992, Grassi and Magnani 2005).

For the 2013 foliar cohort, $\delta^{13}C$ was greater in the throughfall reduction treatment than the ambient precipitation treatment, which indicates less discrimination against ^{13}C and increased leaf water use efficiency in the throughfall reduction trees. 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-level gas exchange in loblolly pine (Zhang et al. 1997, Samuelson et al. 2001, Will et al. 2001, Munger et al. 2003) and no effect was observed 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 midday ψ_L . This indicates that leaf-level photosynthesis was similar across a range of nutrient availabilities but that leaf-level water loss was reduced by fertilization. This response could be related to the increased foliar N concentration found in our study. Fertilization with N can lead to increased water use efficiency in loblolly pine seedlings due to an associated increase in foliar N and P_{net} and decreases in g_s (Green and Mitchell 1992, Samuelson et al. 2000, Tyree et al. 2009). Mesophyll conductance responds positively to fertilization, thus increasing photosynthesis (Green and Mitchell 1992). While we did not measure mesophyll conductance, it seems likely that a positive response of mesophyll conductance to fertilization allowed trees within the 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 , fertilization increased water use efficiency by 6 %, from 74.5

to 80.0 $\mu\text{mol mol}^{-1}$. We did not measure xylem structure or root quantity, distribution, or architecture, all of which might affect tree water status and response to throughfall reduction or nutrient availability.

5. Conclusions and Management Implications

Our results indicate that throughfall reduction decreases soil moisture, 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 the positive effects of fertilization on productivity are not eliminated by throughfall reduction on sites similar to ours. Because the responses of fertilization and throughfall reduction are additive, differences between the two responses will determine whether growth increases or decreases. Contrary to our initial hypothesis, fertilization did not exacerbate the effects of throughfall reduction even though leaf area did increase. Rather, fertilization reduced leaf-level conductance and increased water use efficiency. These results indicate that fertilization may be beneficial in loblolly pine plantations experiencing reduced water availability.

Footnotes

¹ Title page. Author current affiliation address: School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, USA

Acknowledgements

This research was funded by The Pine Integrated Network: Education, Mitigation, and Adaptation Project (PINEMAP), a Coordinated Agriculture Project funded by the USDA National Institute of Food and Agriculture, Award #2011-68002-30185, McIntire-Stennis project OKLO 2929, and the Oklahoma Agricultural Experiment Station. We are deeply grateful to Mr. Ed Hurliman for providing use of his

property to install experiments, Mr. Bob Heinemann and the staff at the Kiamichi Forestry Research Station for help with installations and data collection.

References

Albaugh, T.J., Allen, H.L., Daugherty, P.M., Kress, L.W., and King, J.S. 1998. Leaf area and above-and below-ground growth responses of loblolly pine to nutrient and water additions. *For. Sci.* 44: 317-327.

Albaugh, T.J., Allen, H.L., Dougherty, P.M., and Johnson, K.H. 2004. Long term growth responses on loblolly pine to optimal nutrient and water resource availability. *For. Ecol. Manage.* 192: 3-19.

Albaugh, T.J., Allen, H.L., and Fox, T.R. 2008. Nutrient use and uptake in *Pinus taeda*. *Tree Physiol.* 28: 1083-1098.

Albaugh, T.J., Vance, E.D., Gaudreault, C., Fox, T.R., Allen, H.L., Stape, J.L., and Rubilar, R.A. 2012. Carbon emissions and sequestration from fertilization of pine in the southeastern, United States. *For. Sci.* 58: 419-429.

Allen, H.L. 1987. Forest fertilizers: nutrient amendment, stand productivity, and environmental impact. *J. For.* 85: 37-46.

Allen, C.B., Will, R.E., and Jacobson, M.A. 2005. Production efficiency and radiation use efficiency of four tree species receiving irrigation and fertilization. *For. Sci.* 51: 556-569.

Amateis, R.L., Liu, J., Ducey, M.J., and Allen, H.L. 2000. Modeling response to midrotation nitrogen and phosphorus fertilization in loblolly pine plantations. *South. J. Appl. For.* 24: 207-212.

Barber, B.L. 1986. Nonstomatal effects of water stress on photosynthesis in loblolly pine (*Pinus taeda* L.) seedlings. Ph.D. Dissertation. Auburn University, Auburn, AL.

478 Bolstad, P.V. and Allen, H.L. 1987. Height and diameter growth response in loblolly pine stands following
 479 fertilization. *For. Sci.* 33: 644-653.

480 Boyer, J.S. 1976. Photosynthesis at low water potentials. *Philos. Trans. R. Soc. B* 273: 501-512.

481 Breshears, D.D., Adams, H.D., Eamus, D., McDowell, N.G., Law, D.J., Will, R.E., Williams, A.P., and Zou,
 482 C.B. 2013. The crucial amplifying role of increasing atmospheric moisture demand on tree mortality
 483 associated with regional die off. *Front. Plant Sci.* 4: 266.

484 Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot.* 42: 1-16.

485 Chaves, M.M., Maroco, J.P., and Pereira, J.S. 2003. Understanding plant response to drought-from genes
 486 to the whole plant. *Funct. Plant Biol.* 30: 239-264.

487 Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.T.,
 488 Laprise, R., Magaña Rueda, V., Mearns, L., Menéndez, C.G., Räisänen, J., Rinke, A., Sarr, A., Whetton,
 489 P. 2007. Regional climate projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M.,
 490 Averyt, K.B., Tignor, M., Miller, H.L., (Eds.), *Climate change 2007: The physical basis. Contribution of*
 491 *working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change.*
 492 Cambridge University Press, Cambridge, UK, and New York, pp. 847-940.

493 Colbert, S.R., Jokela, E.J., and Neary, D.G. 1990. Effects of annual fertilization and sustained weed control
 494 on dry matter partitioning, leaf area, and growth efficiency of juvenile loblolly and slash pine. *For.*
 495 *Sci.* 36: 995-1014.

496 Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichet, T., Friedlingstein, P., Gao, X., Gutowski, W.J.,
 497 Johns, T., Krinner, G., *et al.* Long-term climate change: projections, commitments and irreversibility.
 498 In *Climate change 2013: The physical science basis. Contribution of working group I to the fifth*
 499 *assessment report of the Intergovernmental Panel on Climate Change*; Stocker, T.F., Qin, D., Plattner,
 500 G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P.M., Eds.;
 501 Cambridge University Press: Cambridge, UK/New York, NY, USA, 2013; pp. 1039-1106.

502

503 Coyle, D.R., Coleman, M.D., and Aubrey, D.P. 2008. Above- and below-ground biomass accumulation,
 504 production, and distribution of sweetgum and loblolly pine grown with irrigation and fertilization.
 505 Can. J. For. Res. 38: 1335-1348.

506 Easterling, D.R., Meehl, G.A., Parmesan, C., and Changnon, S. 2000. Climate extremes: observations,
 507 modelling, and impacts. Science 289: 2068-2074.

508 Ewers, B.E., Oren, R., Albaugh, T.J., Dougherty, P.M. 1999. Carry-over effects of water and nutrient
 509 supply on water use of *Pinus taeda*. Ecol. Appl. 9: 513-525.

510 Ewers, B.E., Oren, R., and Sperry, J.S. 2000. Influence of nutrient versus water supply on hydraulic
 511 architecture and water balance in *Pinus taeda*. Plant Cell Environ. 23: 1055-1066.

512 Flexas, J. and Medrano, H. 2002. Drought-inhibition of photosynthesis in C₃ plants: stomatal and non-
 513 stomatal limitations revisited. Ann. Bot. 89: 183-189.

514 Gough, C.M., Seiler, J.R., Johnson, K.H., and Sampson, D.A. 2004. Seasonal photosynthesis in fertilized
 515 and nonfertilized loblolly pine. For. Sci. 50: 1-9.

516 Grassi, G. and Manani, F. 2005. Stomatal, mesophyll conductance and biochemical limitations to
 517 photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell Environ. 28:
 518 834-849.

519 Green, T.H. and Mitchell, R.J. 1992. Effects of nitrogen on the response of loblolly pine to water stress. I.
 520 Photosynthesis and stomatal conductance. New Phytol. 122: 627-633.

521 Hu, L., Wang, Z., and Huang, B. 2010. Diffusion limitations and metabolic factors associated with
 522 inhibition and recovery of photosynthesis from drought stress in a C₃ perennial grass species.
 523 Physiol. Plant. 139: 93-106.

524

525 Jokela, E.J., Dougherty, P.M., and Martin, T.A. 2004. Production dynamics of intensively managed
 526 loblolly pine stands in the southern United States: A synthesis of seven longterm experiments. For.
 527 Ecol. Manage. 192: 117-130.

528 Kramer, P.J. and Boyer, J.S. 1995. Water relations of plants and soils. Academic Press, London, UK. 482 p.

529 Lawlor, D.W. 1995. The effects of water deficit on photosynthesis. In Smirnoff N, Eds. *Environment and*
 530 *plant metabolism. Flexibility and acclimation*. Oxford: BIOS Scientific Publishers.

531 Martin, T.A. and Jokela, E.J. 2004. Stand development and production dynamics of loblolly pine under a
 532 range of cultural treatments in north-central Florida, USA. For. Ecol. Manage. 192: 39-58.

533 Munger, G.T., Will, R.E., and Borders, B.E. 2003. Effects of competition control and annual nitrogen
 534 fertilization on gas exchange of different aged *Pinus taeda*. Can. J. For. Res. 33: 1076-1083.

535 Ni, B.-R. and Pallardy, S.G. 1992. Stomatal and nonstomatal limitations to net photosynthesis in
 536 seedlings of woody angiosperms. Plant Physiol. 99: 1502-1508.

537 Noormets, A., Epron, D., Domec, J.C., McNulty, S.G., Fox, T., Sun, G., King, J.S. 2015. Effects of forest
 538 management on productivity and carbon sequestration: A review and hypothesis. For. Ecol. Manage.
 539 355: 124-140.

540 Oswalt, S.N., Smith, W.B., Miles, P.D., and Pugh, S.A. 2014. Forest resources of the United States, 2012: a
 541 technical document supporting the Forest Service 2015 update of the RPA Assesment. Gen. Tech.
 542 Rep. WO-91. Washington, DC: U.S. Department of Agriculture, Forest Service, Washington Office.
 543 218 p.

544 Radoglou, K. and Teskey, R.O. 1997. Changes in rates of photosynthesis and respiration during needle
 545 development of loblolly pine. Tree Physiol. 17: 485-488.

546 Samuelson, L.J. 2000. Effects of nitrogen on leaf physiology and growth of different families of loblolly
 547 and slash pine. New For. 19: 95-107.

549 Samuelson, L., Stokes, T., Cooksey, T., and McLemore, P. 2001. Production efficiency of loblolly pine and
 550 sweetgum in response to four years of intensive management. *Tree Physiol.* 21: 369-376.

551 Samuelson, L.J. and Stokes, T.A. 2006. Transpiration and canopy stomatal conductance of 5-year-old
 552 loblolly pine in response to intensive management. *For. Sci.* 52: 313-323.

553 Samuelson, L.J., Butnor, J., Maier, C., Stokes, T.A., Johnson, and K., Kane, M. 2008a. Growth and
 554 physiology of loblolly pine in response to long-term resource management: defining growth
 555 potential in the southern United States. *Can. J. For. Res.* 38: 721-732.

556 Samuelson, L.J., Farris, M.G., Stokes, T.A., and Coleman, M.D. 2008b. Fertilization but not irrigation
 557 influences hydraulic traits in plantation-grown loblolly pine. *For. Ecol. Manage.* 255: 3331-3339.

558 Samuelson, L.J., Pell, C.J., Stokes, T.A., Bartkowiak, S.M., Akers, M.K., Kane, M., Markewitz, D., McGuire,
 559 M.A., and Teskey, R.O. 2014. Two-year throughfall and fertilization effects on leaf physiology and
 560 growth of loblolly pine in the Georgia Piedmont. *For. Ecol. Manage.* 330: 29-37.

561 Seager, R., Hooks, A., Williams, A.P., Cook, B., Nakamura, J., *et al.* 2015. Climatology, variability, and
 562 trends in the U.S. vapor pressure deficit, and important fire-related meteorological quantity. *J. Appl.*
 563 *Meteorol. Climatol.* 54: 1121-1141.

564 Seiler, J.R. 1984. Physiological response of loblolly pine seedlings to moisture-stress conditioning and
 565 their subsequent performance during water stress. Ph.D. Dissertation. Virginia Polytechnic Institute
 566 and State University, Blacksburg, VA. 199 p.

567 Tang, Z., Sword Sayer, M.A., Chambers, J.L., and Barnett, J.P. 2004. Interactive effects of fertilization and
 568 throughfall exclusion on the physiological responses and whole-tree carbon uptake of mature
 569 loblolly pine. *Can. J. Bot.* 82: 850-861.

570

571 Teskey, R.O., Fites, J.A., Samuelson, L.J., and Bongarten, B.C. 1986. Stomatal and nonstomatal limitations
 572 to net photosynthesis in *Pinus taeda* L. under different environmental conditions. Tree Physiol. 2:
 573 131-142.

574 Teskey, R.O., Gholz, H.L., and Cropper Jr., W.L. 1994. Influence of climate and fertilization on net
 575 photosynthesis of mature slash pine. Tree Physiol. 14: 1215-1227.

576 Tezara, W., Mitchell, V.J., Driscoll, S.P., and Lawlor, D.W. 2002. Effects of water deficit and its
 577 interactions with CO₂ supply on the biochemistry and physiology of photosynthesis in sunflower. J.
 578 Exp. Bot. 53: 1781-1791.

579 Tyree, M.C., Seiler, J.R., and Maier, C.A. 2009. Short-term impacts of nutrient manipulations on leaf gas
 580 exchange and biomass partitioning in contrasting 2-year-old *Pinus taeda* clones during seedling
 581 establishment. For. Ecol. Manage. 257: 1847-1858.

582 Vose, J.M. and Allen, H.L. 1988. Leaf area, stemwood growth and nutritional relationships in loblolly
 583 pine. For. Sci. 34: 547-563.

584 Walsh, J., Wuebbles, D., Hayhoe, K., Kossin, J., Kunkel, K., Stephens, G., Thorne, P., Vose, R., Wehner, M.,
 585 Willis, J.; *et al.* Ch. 2: Our changing climate. In *Climate Change Impacts in the United States: The*
 586 *Third National Climate Assessment*; Melillo, J.M., Richmond, T.C., Yohe, G.W., Eds.; U.S. Global
 587 Change Research Program: Washington, DC, USA, 2014; pp. 19-67.

588 Ward, E.J., Domec, J.-C., Laviner, M.A., Fox, T.R., Sun, G., McNulty, S., King, J., Noormets, A. 2015.
 589 Fertilization intensifies drought stress: Water use and stomatal conductance of *Pinus taeda* in a
 590 midrotation fertilization and throughfall reduction experiment. For. Ecol. Manage. 355: 72-82.

591 Wear, D.N. and Greis, J.G. 2012. The southern forest futures project: summary report. General Technical
 592 Report SRS-168. USDA Forest Service Southern Research Station, Asheville, NC.

593

- Will, R.E., Barron, G.A., Burkes, E.C., Shiver, B., and Teskey, R.O. 2001. Relationship between intercepted radiation, net photosynthesis, respiration, and rate of stem volume growth of *Pinus taeda* and *Pinus elliottii* stands of different densities. *For. Ecol. Manage.* 154: 155-163.
- Will, R.E., Munger, G.T., Zhang, Y., and Borders, B.E. 2002. Effects of annual fertilization and complete competition control on current annual increment, foliar development, and growth efficiency of different aged *Pinus taeda* stands. *Can. J. For. Res.* 32: 1728-1740.
- Will, R.E., Markewitz, D., Hendrick, R.L., Meason, D.F., Crocker, T.R., and Borders, B.E. 2006. Nitrogen and phosphorus dynamics for a chronosequence of loblolly pine stands receiving fertilization and competition control. *For. Ecol. Manage.* 227: 155-168.
- Will, R.E., Wilson, S.M., Zou, C.B., and Hennessey, T.C. 2013. Increased VPD due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone. *New Phytol.* 200: 366-374.
- Will, R.E., Fox, T., Akers, M., Domec, J.-C., González-Benecke, C., Jokela, E.J., Kane, M., Lavinier, M.A., Lokuta, G., Markewitz, D., McGuire, M.A., Meek, C., Noormets, A., Samuelson, L., Seiler, J., Strahm, B., Teskey, R., Vogel, J., Ward, E., West, J., Wilson, D., and Martin, T.A. 2015. A range-wide experiment to investigate nutrient and soil moisture interactions in loblolly pine plantations. *Forests* 6: 2014-2028.
- Zhang, S., Hennessey, T.C., and Heinemann, R.A. 1997. Acclimation of loblolly pine (*Pinus taeda*) foliage to light intensity as related to leaf nitrogen availability. *Can. J. For. Res.* 27: 1032-1042.

Tables.

Table 1. *P* values for the effects of date, throughfall reduction (TR) 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
TR	<0.0001	0.0006	<0.0001	<0.0001	0.009	<0.0001
Fert	0.006	<0.0001	0.23	0.01	0.001	0.02
TR x Fert	<0.0001	0.58	0.64	0.93	0.83	0.86
Date x TR	0.01	0.94	0.10	0.008	0.52	0.04
Date x Fert	0.99	0.78	0.08	0.36	0.98	0.95
Date x TR x Fert	0.81	0.93	0.90	0.79	0.53	0.41

655 Table 2. *P* values for the effects of block, throughfall reduction (TR) and fertilization (Fert) on height
 656 growth (Height), DBH growth, and basal area growth (BA) measured across entire measurement period
 657 (2012-2014). Bold values indicate significance at $\alpha < 0.05$.

	Height	DBH	BA
TR	<0.0001	0.0009	0.004
Fert	0.97	0.0003	0.0006
TR x Fert	0.91	0.87	0.11

658

659

660

661

662

663

664

665

666

667

668

669

670

671

672

673

674

675

676

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691

692

693

694 Table 3. *P* values for the effects of year, throughfall reduction (TR), and fertilization (Fert) on stable
695 carbon isotope ratio ($\delta^{13}\text{C}$) and nitrogen concentration of the foliage, for the 2012-2014 foliage cohorts.
696 Bold values indicate significance at $\alpha < 0.05$.

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

697

698

699

700

701

702

703

704

705

706

707

708

709

710

711

712

713

714

715

716

717

718

719

720

721

722

723

724

725

726

727

728

730
 731 Fig. 1. Monthly precipitation and average maximum, minimum, and mean air temperature in 2012,
 732 2013, and 2014 for Broken Bow Oklahoma, USA.

Fig. 2. Monthly Palmer Drought Severity index (PDSI) of 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 control, i.e., ambient throughfall and no fertilization (C), ambient throughfall plus fertilization (F), throughfall reduction and no fertilization (TR), and throughfall reduction plus fertilization (TR+F) treatments. An asterisk (*) above the data represents dates that the throughfall reduction effect was significant ($n = 4$).

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

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

Fig. 6. Mean leaf area index (LAI) in response control, i.e., ambient throughfall and no fertilization (C), ambient throughfall plus fertilization (F), throughfall reduction and no fertilization (TR), and throughfall reduction plus fertilization (TR+F) treatments. Error bars represent standard errors 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 midday leaf water potential (Ψ_L) in response to control, i.e., ambient throughfall and no fertilization (C), ambient throughfall plus fertilization (F), throughfall reduction and no fertilization (TR), and throughfall reduction plus fertilization (TR+F) treatments. When there was a significant throughfall reduction x date interaction, an asterisk (*) above the data represents dates that throughfall reduction effect was significant. Error bars represent standard errors of treatment means ($n = 4$).

Fig. 8. Overall (A) mean net photosynthesis (P_{net}), (B) mean stomatal conductance (g_s), (C) intercellular CO_2 (C_i), and (D) mean midday leaf water potential (ψ_L) in response control, i.e., ambient throughfall and no fertilization (C), ambient throughfall plus fertilization (F), throughfall reduction and no fertilization (TR), and throughfall reduction plus fertilization (TR+F) treatments. Error bars represent standard errors of treatment means ($n = 4$).

790

791 Fig. 9. Mean (A) stable carbon isotope ratio ($\delta^{13}C$) and (B) foliar nitrogen concentration by foliage cohort
 792 in response to control, i.e., ambient throughfall and no fertilization (C), ambient throughfall plus
 793 fertilization (F), throughfall reduction and no fertilization (TR), and throughfall reduction plus
 794 fertilization (TR+F) treatments. An asterisk (*) above the data represents dates when the throughfall
 795 reduction effect was significant. Error bars represent standard errors of treatment means ($n = 4$).