Half-sibling loblolly pine clones exhibited intraspecific variation, a $G \times E$ interaction, and differences in stable isotope composition in response to soil moisture availability

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ABSTRACT

We examined the response of three loblolly pine half-sibling clones (hereafter referred to as Clone 1, 2, and 3) to soil moisture availability in a greenhouse study. Three soil moisture treatments were imposed. Two were constant soil moisture treatments at either high (−0.3 MPa) or low (−1.5 MPa) soil water potential. The third was an alternating wet-dry treatment that alternated between one week at high (−0.3 MPa) and three weeks at low (−1.5 MPa) soil water potential, which simulated several natural wetting-drying cycles. At intervals throughout the experiment we measured biomass accumulation (via sequential harvesting), change in height, pre-dawn needle water potential, afternoon needle water potential, dark respiration, and morning and afternoon gas exchange (photosynthesis, stomatal conductance, intercellular CO₂, and transpiration). Carbon allocation to root and shoot biomass, stomatal density, and carbon isotope composition were measured at the end of the experiment.

All clones accumulated more biomass in the high soil moisture treatment than in the low soil moisture treatment, but there was a genetic by environment ($G \times E$) interaction: Clone 1 accumulated more biomass than the other clones in the low soil moisture treatment and less than the others in the high soil moisture treatment. Stable carbon isotope composition, which we interpreted as a time-integrated index of water use efficiency, was significantly higher (indicating greater water use efficiency) in Clone 2, compared to Clones 1 and 3 across all soil moisture treatments except in the wet-dry treatment, where Clone 1 and 3 were not significantly different. A strong negative correlation was found between stable carbon isotope composition and biomass accumulation. Gas exchange parameters and needle water potential were higher in the high soil moisture treatment compared to the other treatments across all clones. Morning measurements of gas exchange were higher in Clones 2 and 3 than in Clone 1 across all treatments. Stomatal density varied significantly among clones but not among treatments. These results demonstrated that closely related clones differed in their growth and physiological responses to moisture availability, suggesting that clonal variation could be used to breed for increased drought resistance or to identify drought resistant genotypes for selective deployment.

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1. Introduction

A recent analysis of 17 climate models (Cook et al., 2015) predicted future drier conditions in much of North America, including the southeastern USA. A drier climate in the southeastern USA will likely have a deleterious effect on the productivity of loblolly pine (*Pinus*

taeda L.), a species of major economic importance in the region (Schultz, 1997). Selection for drought resistant genotypes, and water relations traits that may affect drought resistance, could help maintain or improve loblolly pine production in the future. Loblolly pine exhibits significant genetic (Eckert et al., 2010) and physiological (Teskey et al., 1987) diversity associated with temperature and moisture regimes across its native range. Although genetically improved loblolly pine half-sibling families, full-sibling families and clonal varieties have been widely planted across the

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southeastern USA (McKeand et al., 2003), there has been little genetic selection for drought resistance.

Intraspecific variation in drought resistance and water relations has been observed in loblolly pine (Aspinwall et al., 2011a; Bongarten and Teskey, 1986, 1987; Bulfe and Fernández, 2016; Kisse et al., 1985; Teskey et al., 1987; Van Buijtenen, 1966; Wells and Wakeley, 1966). For example, the “Lost Pines” of East Texas is a genetically unique population (Al-Rabab'ah and Williams, 2004) that has persisted in a climate that is moisture-limited compared to typical climates in other parts of the loblolly pine native range (Bilan et al., 1977). The “Lost Pines” population has experienced selective pressures resulting in adaptations that confer drought resistance. Seedlings from the “Lost Pines” population exhibited less drought-induced mortality than their counterparts from wetter regions when grown in a common garden (Goddard and Brown, 1959; Zobel and Goddard, 1955). When compared to wetter provenances in common garden, loblolly pine seedlings from drier provenances allocated more carbon to below-ground biomass and showed differences in root architecture (Bilan et al., 1977; Bongarten and Teskey, 1987; Cannell et al., 1978; Seiler and Johnson, 1988; van Buijtenen et al., 1976), and had lower stomatal density and a greater reduction in transpiration in response to moisture deficit (Bilan et al., 1977; Seiler and Johnson, 1988; van Buijtenen et al., 1976).

Loblolly pine displays many drought resistance traits. Compared with well-watered conditions, under water deficit the species has exhibited differences in carbon allocation to root and leaf biomass and altered leaf morphology, a reduction in leaf surface area (Wells and Wakeley, 1966), lower stomatal conductance and transpiration (Newton et al., 1991), increased leaf cuticular wax and lower cuticular transpiration (Oppenheimer and Shomer-Ilan, 1963), and osmotic adjustment in needles (Seiler and Johnson, 1985), shoots (Bongarten and Teskey, 1986), and roots (Meier et al., 1992). Loblolly pine can also acclimate to moisture stress. When exposed to drought conditions, loblolly pine seedlings that were pre-conditioned to moisture stress were capable of photosynthesizing at lower needle water potential and exhibited higher water use efficiency than their counterparts that were not pre-conditioned to moisture stress (Seiler and Johnson, 1988).

Genetically different loblolly pine populations could exhibit physiological differences in their response to drought, or genetic by environment ($G \times E$) interaction, that could be used to select clones, genotypes, and traits better suited to dry conditions. When $G \times E$ interactions were studied in loblolly pine genotypes across different climatic conditions, soil types, and silvicultural inputs, it was concluded that while $G \times E$ interactions occurred, they were not of substantial interest, as productive families tended to maintain high productivity across all sites and treatments (McKeand et al., 2006). Likewise, a field study comparing growth of 1200 clones from 61 half-sibling families found few $G \times E$ interactions among clonal populations (Baltunis et al., 2006). However, others have demonstrated significant $G \times E$ interactions among loblolly pine genotypes subjected to varying silvicultural treatments (Roth et al., 2007). Furthermore, $G \times E$ interactions for loblolly pine growth traits were found to be more common among clones than among full-sibling families (Isik et al., 2003). Increased use of clonal varieties may necessitate the evaluation of $G \times E$ interactions for future tree improvement.

This study examined biomass accumulation and physiological responses to three soil moisture treatments of three half-sibling clones of loblolly pine. Differences in responses can be more easily identified among clones because there is less biological variation among individuals in the same clone than among individuals in populations or families. Experiments with clones have facilitated more precise determination of genetic and phenotypic variability in *Populus deltoides* (Funk et al., 2007). In addition, the use of clones has resulted in greater stand uniformity, productivity, and genetic

gain in loblolly pine (Isik et al., 2003). Traits we examined include gas exchange, instantaneous water use efficiency, leaf carbon isotope composition, and carbon allocation. The objective of this study was to examine variation in productivity, and some of the physiological factors that contribute to productivity, under well-watered, water-deficit, and cycling wet-dry conditions among a select group of highly productive and closely related clones. We had two hypotheses: 1) a genetic by environment ($G \times E$) interaction will occur in which some clones, relative to others, will have higher productivity in one moisture regime and lower productivity in another; and 2) clones will differ in water use efficiency, gas exchange, and carbon allocation, reflecting different strategies to maximize drought resistance and productivity.

2. Methods

2.1. Plant material

The experiment was conducted on two-year-old ramets of three half-sibling clones of loblolly pine (ArborGen Inc., Summerville, SC, USA) bred from populations in the Atlantic Coastal Plain. Ramets, obtained as containerized stock, were transplanted into 4.3 L tree pots (CP612R, Steuwe and Son, Inc., Tangent, OR, USA) in an artificial soil consisting of a mix of crushed fritted clay (Turface Field and Fairway, Profile Products LLC, Buffalo, IL, USA) and fine sand (3:1, v:v). Drainage in the pots was increased by drilling five holes (diameter 0.9 cm) in the base of each pot in addition to the two existing holes (diameter 1.5 cm). Ramets were grown in a greenhouse at the Whitehall Experimental Forest in Athens, GA, USA, at approximately ambient humidity and air temperature. Air temperature in the greenhouse was measured every five minutes with a shielded thermocouple and recorded with a data logger (CR23X, Campbell Scientific, Logan, UT, USA). Average greenhouse air temperature over the course of the experiment was 25.6 °C. Ramets were planted and fertilized in late March 2013 and grown under well-watered conditions for approximately 11 weeks. During this time, the ramets were watered daily by hand to field capacity and fertilized once with 40 g of 15-9-12 NPK extended release fertilizer (Osmocote Plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA) and 0.2 g of chelated iron (Sprint 138, Becker Underwood, Ames, IA, USA).

2.2. Experimental design

The experiment was set up as a split-plot design with clone as the whole-plot factor and moisture regime as the sub-plot factor with three replications. Each block (replicate) was on a separate greenhouse bench. Fifty-seven ramets were randomly chosen from each clone for assignment to each of the three blocks. Within each block $\times$ clone combination, each of three soil moisture treatments (high, low, and alternating soil moisture) was randomly assigned to a subplot of 19 ramets, resulting in a total of 27 clone $\times$ treatment $\times$ replicate combinations (hereafter called “plots”). To minimize any potential greenhouse location effects, the blocks were rotated among the benches at four-week intervals. One of the replicates of the low soil moisture treatment in Clone 2 was removed from the analysis due to a malfunction in the irrigation system.

2.3. Experimental treatments

Three irrigation treatment levels based on soil water potential were established to control plant-available water (hereafter referred to as soil moisture), one at constant high water potential (−0.3 MPa; high soil moisture treatment), one at constant low water potential (−1.5 MPa; low soil moisture treatment), and a treatment that simulated natural wetting-drying cycles (alternating soil moisture

treatment), in which soil moisture was maintained at high water potential (-0.3 MPa) for a 1-week period, followed by a 3-week period in which the soil was allowed to dry down to, and then maintained at, -1.5 MPa soil water potential. Ramets underwent these treatments for a 12-week period from June 15, 2013 to September 7, 2013. Three entire wet-dry cycles of the alternating soil moisture treatment were completed during the experiment.

Soil moisture treatments were maintained by controlling soil volumetric water content using the technique of [Nemali and van Iersel \(2006\)](#). Soil water content values coinciding with water potential of -1.5 MPa and -0.3 MPa were predetermined by developing moisture release curves with a dew point hydrometer (WP4C, Decagon Devices, Inc, Pullman, WA, USA). Soil volumetric water content was measured by averaging the output of two soil moisture sensors (EC-5, Decagon Devices, Pullman, WA, USA) in each clone $\times$ treatment $\times$ replicate combination. The sensors were randomly assigned to two pots per plot and placed in a horizontal orientation, 8.5 cm above the base of the pot. Data were recorded with a data logger (CR23X, Campbell Scientific, Logan, UT, USA) at five-minute intervals. Soil moisture was controlled separately in each of the 27 clone $\times$ treatment $\times$ replicate plots. Drip irrigation for each plot was provided by a dedicated solenoid (Model 57100, Orbit, North Salt Lake, UT, USA). Pressure-compensating precision drip emitters (Model 01WPCJL2-B, Netafim USA, Fresno, CA) supplied each pot with a metered pulse of water (dispensed at a rate of 1.893 L h^{-1}) and a drip ring was used to distribute the water evenly to the soil surface. The irrigation system was controlled by the data logger program as follows: When measured soil moisture in a plot decreased below a specific water content set point coinciding with the moisture treatment for that plot (-1.5 MPa or -0.3 MPa), the data logger signaled the plot solenoid to give a 15 s irrigation pulse, which increased soil moisture by a small amount. The measurement and signaling sequence was repeated at five minute intervals, resulting in a series of small moisture pulses that continuously maintained volumetric water content near the set point.

2.4. Measurements

Biomass accumulation measurements were made by sequential harvests. Harvests were conducted at the start of the experiment and then at four-week intervals. At each harvest, four individuals from each clone $\times$ treatment $\times$ replicate combination were harvested and their roots and shoots were separated. All tissues were dried at 65°C to a constant mass. Allometric analyses were conducted on dry mass to determine carbon allocation to roots and shoots. Height and diameter were measured on each ramet in the entire experiment immediately prior to each harvest. Stem diameter was measured at the base of each ramet just above the soil surface.

Morning (9:00–11:00) and afternoon (14:00–16:00) gas exchange measurements were made approximately every two weeks in all plots. Our measurement schedule insured that both high and low water conditions in the alternating soil moisture treatment were equally represented (Supplementary Table 1). To control for time of day, gas exchange measurements were blocked by day over a three-consecutive-day period. A single replicate (block) was measured on each day, within which a single ramet in each clone $\times$ treatment combination (9 per block) was randomly selected at the beginning of each day of measurement. The order in which the replicates were measured over a three-day period was chosen at random, and the order in which the clone $\times$ treatment combinations were measured within a day was also chosen at random. Gas exchange measurements, including net assimilation (A_{net}), stomatal conductance (g_s), transpiration (E) and intercellular CO_2 (C_i) were made with a portable photosynthesis system (LI-6400, Li-Cor Biosciences, Lincoln, NE, USA) fitted with a standard leaf chamber. Instantaneous water use efficiency (WUE_i) was

calculated by dividing A_{net} by E . Conditions in the cuvette were set to $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR and 30°C block temperature, while cuvette humidity was adjusted to approximately match greenhouse humidity at the time of measurement. Three needles of a single attached fascicle were spread an equal distance apart and placed parallel to one another in the cuvette. Gas exchange measurements were expressed on the basis of the total needle surface area, accounting for all sides of the needles, enclosed in the cuvette. Needle surface area of the fascicle enclosed in the cuvette was calculated as described in [Ingwers et al. \(2016\)](#) as:

$$\text{Needle surface area in cuvette} = l(2\pi r + 2Nr) \quad (1)$$

where l = length of needle enclosed in cuvette, r = mean needle radius, and N = number of needles per fascicle (three needles for all measurements). Needle radius was measured to 0.1 mm on each needle with a scaled 10 $\times$ hand lens (PEAK 1983, SPI Supplies, West Chester PA, USA).

Dark respiration (R_d) was similarly measured with the portable photosynthesis system. Measurements were conducted on weeks 2, 5, 8 and 10 on needles of a single dark-acclimated individual in each clone $\times$ treatment $\times$ replicate combination. Trees were dark-acclimated by placing them in a dark growth chamber for >6 h under temperature and humidity conditions that approximately matched greenhouse ambient. Cuvette block temperature was set to 30°C as to be consistent with other gas exchange measurements. Dark respiration was measured between 16:00 and 20:00.

Needle xylem pressure potential, which was interpreted as needle water potential, was measured pre-dawn (Ψ_{PD}) and in the afternoon (Ψ) on same ramets selected for gas exchange measurements (1 per plot, 27 total) using a pressure chamber (Model 600, PMS Instruments Co., Corvallis, OR, USA). For each measurement, two single fully-expanded needles from the most recent fully developed flush were selected and an average of the two needle measurements was used for analysis.

Stable carbon isotope composition ($\delta^{13}\text{C}$) of needle tissue, calculated of the stable carbon ratio ($^{13}\text{C}/^{12}\text{C}$) relative to the international standard, limestone Pee Dee belemnite ([Condon et al., 2002](#)), was measured as a time-integrated index of water use efficiency. $\delta^{13}\text{C}$ values were determined on four individuals in each clone $\times$ treatment $\times$ replicate combination during Week 12 of the experiment. To ensure that tissues for analysis developed under treatment, a bulk sample of developing needles was taken from each ramet. Tissue was dried at 65°C to a constant mass, then finely ground and analyzed for $\delta^{13}\text{C}$ by mass spectrometry at the Stable Isotope and Soil Biology Laboratory, Odum School of Ecology, University of Georgia, Athens, GA, USA.

Stomatal density measurements were made on the inside (adaxial) and outside (abaxial) needle surfaces of four individuals in each clone $\times$ treatment $\times$ replicate combination during Week 10 of the experiment. A single needle was chosen from the most mature fully expanded flush of each ramet and a surface cast was made near the longitudinal center of the needle using a cyanoacrylate-based resin (Loctite Super Glue Liquid, Henkel, Westlake OH, USA). Each cast was photographed at $40\times$ magnification under a light microscope (BX 51, Olympus, Tokyo, Japan) equipped with a digital camera (DP70, Olympus, Tokyo, Japan) and stomatal density was determined by counting the number of stomata using an image-processing program (ImageJ v. 1.48u4, Wayne Rasband, National Institutes of Health, USA). The length and width of the stomata were not measured in this process.

2.5. Biomass calculations and statistical analyses

Statistical analyses were conducted using the SAS procedures described below (version 9.3.1; SAS Institute Inc., Cary, NC, USA).

Statistical differences in biomass accumulation among treatments and clones were assessed with a repeated measures analysis of variance (RMANOVA) using PROC MIXED (Littell et al., 1996) with plot as the experimental unit. Prior to statistical analyses, biomass data were first tested for homogeneity and log transformed to satisfy Shapiro-Wilks normality. Treatment and clonal means were evaluated using Fisher's Least Significant Differences (LSD) test. Simple main effects were tested using the SLICE option in the LSMEANS statement of PROC MIXED (Littell et al., 1996; Schabenberger et al., 2000). Clone 2 was larger than Clones 1 and 3 at the start of the experiment. To account for initial size differences, size, height and diameter from the preceding harvest was used as a covariate in the corresponding analysis.

Allometric relationships were utilized to explore how soil moisture treatments affected carbon allocation to the roots (consisting of fine and coarse root) and shoots (consisting of stem, branch, and needle). Allometric relationships were assessed using the model:

$$\ln y = a + k \ln x \quad (2)$$

where x and y represent tissue or organ components, in this case above- and below-ground biomass, a is the y -intercept and k is the allometric coefficient (Coleman et al., 2004; Coyle et al., 2008; Hunt, 1979; Ledig et al., 1970). Equivalent k values among treatments indicated that differences in allocation were due to development and not treatment conditions (Hunt, 1979).

Carbon isotope composition ($\delta^{13}\text{C}$), the relationship between $\delta^{13}\text{C}$ and biomass accumulation, and stomatal density were analyzed using PROC MIXED (Littell et al., 1996) to assess both clonal and treatment effects. The relationship between $\delta^{13}\text{C}$ and the natural log of final biomass was assessed by analysis of covariance (ANCOVA) and corresponding regression parameters were determined using PROC REG.

Needle physiological traits were assessed with RMANOVA using PROC MIXED (Littell et al., 1996). For all needle physiological traits, except R_d , clone $\times$ treatment means were compared over six separate time intervals (i.e. repeated factor), with each interval coinciding with the wet or dry portion of the three wet-dry cycles in the alternating soil moisture treatment. Measurements were taken weekly or biweekly; however, different sets of measurements (i.e. morning and afternoon gas exchange) were not always taken on the same week (Supplementary Table 1). Week of experiment was used as the repeated factor for R_d . We used the Akaike Information Criterion (AIC) (Akaike, 1974) to determine the optimal covariate structure for each model. A compound symmetry (CS) structure was used to assess pre-dawn Ψ and R_d as well as morning A_{net} , g_s , E and C_i . Afternoon A_{net} , g_s , E , C_i and Ψ were evaluated using the power spatial covariate structure (SP). Treatment means were compared using the Fisher's LSD test. The SLICE tool in the LSMEANS statement of PROC MIXED was used to test simple main effects.

Denominator degrees of freedom (DDFM) were calculated using the Kenward-Roger (Kenward and Roger, 1997) method. A Type-1 error rate of 0.05 was used for all analyses. All figures were created with SigmaPlot graphing software (version 11.0, SysStat Software, San Jose, CA, USA).

3. Results

3.1. Biomass accumulation

Total biomass accumulation differed among clones and the rank order of biomass accumulation among clones differed within soil moisture treatments and harvest dates, resulting in a significant ($p < 0.0001$) clone $\times$ treatment $\times$ time interaction (Fig. 1).

Biomass accumulation was greatest between Week 8 and Week 12 and increased most rapidly in the high soil moisture treatment. Mean biomass accumulation across clones at the end of the experiment was 15.95 (SE 0.96) g in the low soil moisture treatment and 65.15 (SE 2.33) g in the high soil moisture treatment. Mean biomass accumulation in the alternating soil moisture treatment was 26.93 (SE 1.22) g, which was intermediate between the low and high soil moisture treatments. Differences in biomass accumulation among treatments, and clones within treatments, between Week 8 and Week 12 indicated a $G \times E$ interaction (Fig. 2). Within the low soil moisture treatment, Clone 1 grew more than Clones 2 or 3. In contrast, within the high soil moisture treatment, Clone 1 grew less than Clones 2 or 3. Biomass accumulation of Clones 2 and 3 did not differ

within any soil moisture treatment. All three clones had similar biomass accumulation in the alternating soil moisture treatment. Change in height (Fig. 3) showed a similar pattern as biomass accumulation; however, there was a significant clone $\times$ treatment $\times$ initial height interaction. Although stem diameter increased throughout the experiment there were no significant differences among clones or treatments (data not shown).

Fig. 1. Biomass accumulation during the experimental period. Mean biomass accumulation of three loblolly pine clones at three harvest dates. Harvests occurred at 4-week intervals. Solid lines with triangle symbols represent the high soil moisture treatment (-0.3 MPa), dotted lines with circle symbols represent the low soil moisture treatment (-1.5 MPa), and dashed lines with square symbols represent the alternate soil moisture treatment. Unfilled, grey and black symbols represent Clone 1, Clone 2, and Clone 3, respectively. Thin bars represent standard error. Letters denote significant differences in total biomass accumulation throughout the experiment at $\alpha = 0.05$ ($n = 12$).

Fig. 2. Biomass accumulation during last 4-week period of the experiment. Mean biomass accumulation between week 8 and 12. Different letters indicate significant differences across all clones and treatments ($\alpha = 0.05$). Unfilled, grey, and black bars represent the low, alternating, and high soil moisture treatments, respectively. Standard error represented by thin vertical bars ($n = 12$).

3.2. Allometric analysis

The three clones exhibited similar patterns of carbon allocation to root and shoot biomass under the low and alternating soil moisture treatments (Table 1). A difference in allocation among

Fig. 3. Change in height during the experimental period. Height growth of three loblolly pine clones at three harvest dates. Harvests occurred at 4-week intervals. Solid lines with triangle symbols represent the high soil moisture treatment (-0.3 MPa), dotted lines with circle symbols represent the low soil moisture treatment (-1.5 MPa), and dashed lines and square symbols represent the alternating water treatment. Unfilled, grey and black symbols represent Clone 1, Clone 2, and Clone 3, respectively. Thin bars represent standard error. Letters denote significant differences at $\alpha = 0.05$ ($n = 12$).

clones was detected only in the high soil moisture treatment, where Clone 1 allocated relatively less carbon to below-ground biomass than the other two clones. Carbon allocation among treatments varied only within Clone 1, where there was significantly more carbon allocation to below-ground biomass in the alternating soil moisture treatment compared with the low soil moisture treatment.

3.3. Carbon isotope composition

Carbon isotope composition differed among treatments and among clones within treatment (Fig. 4). The $\delta^{13}\text{C}$ value was highest in the low soil moisture treatment, lowest in the high soil moisture treatment, and intermediate in the alternating soil moisture treatment. Clone 2 had higher $\delta^{13}\text{C}$ than Clones 1 and 3 in the high soil moisture treatments, and was higher than Clone 1, but not Clone 3, in the alternating soil moisture treatment. In Clones 1 and 3, $\delta^{13}\text{C}$ did not significantly differ among soil moisture treatments.

Significant negative correlations were found between $\delta^{13}\text{C}$ and biomass accumulation when clones were analyzed across treatments (Fig. 5, Table 2). The slope of the relationship differed between Clones 1 and 2, but not between Clones 1 and 3 or between Clones 2 and 3. The slope of the relationship between $\delta^{13}\text{C}$ and biomass accumulation was similar in Clones 2 and 3, although Clone 2 accumulated more biomass than Clone 3 for any given $\delta^{13}\text{C}$. When $\delta^{13}\text{C}$ was examined within a soil moisture treatment (data from the three clones combined), there was a positive correlation between $\delta^{13}\text{C}$ and biomass accumulation in the high and alternating soil moisture treatments and a negative correlation between $\delta^{13}\text{C}$ and biomass accumulation in the low soil moisture treatment. The relationship in the alternating soil moisture treatment significantly differed from the relationship in the low soil moisture treatment, but not the high soil moisture treatment. Although the regression slopes between treatments were significantly different, R^2 values were low (Table 2).

3.4. Needle physiology (A_{net} , g_s , E , C_i , WUE_i , R_d and Ψ)

Among treatments, mean morning A_{net} , g_s , E , C_i , and predawn Ψ were lowest in the low soil moisture treatment, highest in the high soil moisture treatment, and intermediate in the alternating soil moisture treatment (Table 3). Mean morning WUE_i was highest in the low soil moisture treatment, lowest in the high soil moisture treatment, and intermediate in the alternating soil moisture treatment. All differences among the three treatments were significant except for the intermediate values of WUE_i and C_i in the alternating soil moisture treatment which did not significantly differ from the low or high soil moisture treatments.

Significant differences in A_{net} and E were detected among clones in morning measurements (Table 3). Net assimilation and E were lowest in Clone 1, highest in Clone 3, and intermediate in Clone 2, which did not significantly differ from Clones 1 and 3.

Similar trends were noted in morning g_s and C_i although these differences were nonsignificant. Mean Ψ_{PD} and WUE_i tended to be higher in Clone 1, compared with Clone 2 and 3, but these differences were not significant.

Similar to the patterns in morning gas exchange, mean afternoon A_{net} , g_s , E , and Ψ were lowest in the low soil moisture treatment, highest in the high soil moisture treatment, and intermediate in the alternating soil moisture treatment (Table 4). Mean afternoon C_i and WUE_i did not differ among the three treatments. Dark respiration also did not differ among the treatments.

Afternoon Ψ was significantly higher in Clone 1 than in Clones 2 or 3 (Table 4). However, there were no significant differences or trends in mean afternoon A_{net} , g_s , C_i , or E among clones and no

Table 1

Relationship of carbon allocation to root versus shoot biomass. Allometric coefficient, k , which is the slope of the relationship between natural log below-ground biomass and natural log above-ground biomass, compared within clones among treatments and within treatments among clones at the last harvest. Different letters indicate significant differences in clones within a treatment or in treatments within a clone at $\alpha = 0.05$ ($n = 12$).

Analysis	Clone	k -value		
		Low water	Alternating water	High water
Clone effect within treatment	1	0.17	1.47	1.00a
	2	0.74	0.79	1.47b
	3	0.50	0.81	1.68b
		$p = 0.581$	$p = 0.600$	$p = 0.007$
Treatment effect within clone	1	0.17a	1.47b	1.00ab $p = 0.013$
	2	0.74	0.79	1.47 $p = 0.346$
	3	0.50	0.81	1.68 $p = 0.283$

Bold values are significant at $\alpha = 0.05$.

Fig. 4. Carbon isotope composition. Mean $\delta^{13}\text{C}$ among clones and treatments at week 12 of the experiment. Unfilled bars, dotted bars, and slashed bars represent Clone 1, Clone 2 and Clone 3, respectively. Standard error represented by thin vertical bars. Different letters indicate significant differences across all clones and treatments at ($\alpha = 0.05$).

Fig. 5. Relationship between biomass accumulation and $\delta^{13}\text{C}$. Biomass accumulation and $\delta^{13}\text{C}$ across of three clones at three soil moisture treatments ($n = 36$). Unfilled, grey, and black symbols represent Clone 1, Clone 2, and Clone 3, respectively. Circles, squares, and triangles represent the low, alternating and high soil moisture treatments. Solid, dotted and dashed lines indicate Clone 1, Clone 2 and Clone 3. Parameters for the slopes are shown in Table 2.

differences in R_d were observed among clones. No significant clone by treatment interactions were detected in any needle physiological traits.

Stomatal density differed significantly among clones ($p < 0.001$) but not among treatments ($p = 0.362$) (Fig. 6). Stomatal density was highest in Clone 2, lowest in Clone 1, and intermediate in Clone 3. Across clones and treatments, mean stomatal density of the abaxial needle surfaces ($89 \text{ stomata mm}^{-2}$, SE 2) was significantly lower ($p < 0.001$) that of the adaxial surface ($101 \text{ stomata mm}^{-2}$, SE 3). Among treatments, despite no significant differences, there was a general trend in the abaxial needle surface, with the highest stomatal density observed in the low soil moisture treatment and the lowest stomatal density observed in the high soil moisture treatment. Among clones, the stomatal densities of the abaxial and adaxial needle surfaces followed the same trend.

4. Discussion

Our results revealed some differences and some similarities in physiological responses of the three clones to soil moisture treatments. Our first hypothesis was supported by the change in rank order of biomass accumulation (Figs. 1 and 2) and potentially height (Fig. 3) among clones between the low and high soil moisture treatments, i.e., a $G \times E$ interaction, which demonstrated that closely related clones of loblolly pine can respond differently to moisture stress. Although previous work has demonstrated variation in growth among loblolly pine full-sibling (Paul et al., 1997) and half-sibling (Bulfe and Fernández, 2016) families, to the best of our knowledge this is the first $G \times E$ interaction found among loblolly pine clones in response to moisture stress. However, other studies have found $G \times E$ interactions among loblolly pine clones in response to other environmental factors. For example, field studies examining the response of multiple clones of loblolly pine to fertilization have observed $G \times E$ interactions in leaf area (Tyree et al., 2009b), growth (King et al., 2008; Stovall et al., 2013), gas exchange (King et al., 2008) and carbon allocation (Stovall et al., 2013). In addition, a greenhouse study on loblolly pine clones demonstrated that clone $\times$ fertilizer interactions affected growth, allocation, and gas exchange (Tyree et al., 2009a). Silvicultural treatments applied to multiple loblolly pine clones have also resulted in $G \times E$ interactions (Roth et al., 2007). The significant differences in biomass accumulation among treatments in our study, consistent with other studies of the response of loblolly pine to soil moisture treatments (Bauweraerts et al., 2014; Wertin et al., 2012), demonstrated the importance of moisture for loblolly pine biomass accumulation and the potential negative effects of predicted future drought on loblolly pine production. The frequency and severity of drought is expected to

Table 2

Parameter estimates (SE) for the relationship between $\delta^{13}\text{C}$ and the natural log of biomass accumulation. Parameters and SE are the results of a regression analysis. Different letters represent significant differences among slopes determined by mixed model analysis where $\alpha = 0.05$ ($n = 36$). Relationships are of the form $\ln(y) = mx + b$ where y is estimated biomass accumulation, m is the slope, x is $\delta^{13}\text{C}$, and b is the intercept.

Analysis	Subject	Slope	Intercept	R^2	MSE	p -value
Among soil moisture treatments	Low	−1.971 (0.63)a	−22.339 (2.10)	0.231	0.722	0.0035
	Alternating	1.077 (0.51)b	−33.972 (1.86)	0.115	0.344	0.0433
	High	1.067 (0.33)b	−36.601 (1.42)	0.231	0.152	0.0026
Among clones	Clone 1	−2.473 (0.42)a	−21.378 (1.58)	0.502	0.978	<0.0001
	Clone 2	−2.723 (0.24)b	−19.460 (0.94)	0.815	0.473	<0.0001
	Clone 3	−2.730 (0.23)ab	−20.268 (1.42)	0.806	0.412	<0.0001

Table 3

Mean (SE) morning gas exchange (net assimilation, A_{net} ; stomatal conductance, g_s ; intercellular $[\text{CO}_2]$, C_i ; transpiration, E ; instantaneous water use efficiency, WUE_i) and pre-dawn needle water potential (Ψ_{PD}) among clones and soil moisture treatments. Different letters within each group indicate significant differences at $\alpha = 0.05$.

Analysis	Subject	A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol mol}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE_i ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	Ψ_{PD} (MPa)
Among soil moisture treatments	Low	3.74(0.40) ^a	0.043(0.007) ^a	208.8(16.4) ^a	1.00(0.11) ^a	3.98(0.35) ^a	−1.10(0.09) ^a
	Alternating	4.94(0.38) ^b	0.063(0.007) ^b	229.2(13.3) ^{ab}	1.46(0.12) ^b	3.46(0.23) ^{ab}	−0.80(0.06) ^b
	High	7.18(0.30) ^c	0.108(0.007) ^c	253.7(8.5) ^b	2.59(0.18) ^c	3.12(0.14) ^b	−0.43(0.03) ^c
		$p < 0.0001$	$p < 0.0001$	$p = 0.0038$	$p < 0.0001$	$p = 0.0103$	$p < 0.0001$
Among clones	Clone 1	4.82(0.34) ^a	0.057(0.006)	215.5(14.4)	1.41(0.13) ^a	3.84(0.26)	−0.67(0.05)
	Clone 2	5.36(0.44) ^{ab}	0.074(0.008)	233.4(15.7)	1.78(0.19) ^{ab}	3.45(0.32)	−0.81(0.09)
	Clone 3	5.85(0.45) ^b	0.086(0.009)	245.6(9.6)	1.94(0.18) ^b	3.21(0.16)	−0.82(0.07)
		$p = 0.0177$	$p = 0.0562$	$p = 0.0586$	$p = 0.0107$	$p = 0.0756$	$p = 0.0517$

Bold values are significant at $\alpha = 0.05$.

Table 4

Mean (SE) afternoon gas exchange (net assimilation, A_{net} ; stomatal conductance, g_s ; intercellular $[\text{CO}_2]$, C_i ; transpiration, E ; instantaneous water use efficiency, WUE_i) as well as needle water potential (Ψ) and dark respiration (R_d) among clones and treatments. Different letters within each group indicate significant differences at $\alpha = 0.05$.

Analysis	Subject	A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol mol}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	WUE_i ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	Ψ (MPa)	R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Among soil moisture treatments	Low	3.00(0.33) ^a	0.037(0.005) ^a	328.5(54.0)	0.86(0.11) ^a	2.06(0.91)	−1.65(0.08) ^a	−0.40(0.07)
	Alternating	4.11(0.39) ^b	0.067(0.007) ^b	280.4(11.59)	1.62(0.19) ^b	2.40(0.24)	−1.26(0.06) ^b	−0.41(0.03)
	High	7.41(0.29) ^c	0.125(0.008) ^c	265.4(6.7)	3.07(0.22) ^c	2.98(0.15)	−0.94(0.05) ^c	−0.55(0.09)
		$p < 0.0001$	$p < 0.001$	$p = 0.4667$	$p < 0.0001$	$p = 0.6072$	$p < 0.0001$	$p = 0.4038$
Among clones	Clone 1	4.99(0.36)	0.073(0.007)	252.0(15.3)	1.79(0.18)	3.19(0.34)	−1.15(0.06) ^a	−0.52(0.05)
	Clone 2	4.93(0.44)	0.080(0.009)	293.3(20.4)	1.96(0.23)	2.30(0.37)	−1.36(0.10) ^b	−0.39(0.06)
	Clone 3	4.81(0.42)	0.081(0.009)	325.2(43.7)	1.92(0.23)	1.98(0.71)	−1.31(0.06) ^b	−0.45(0.08)
		$p = 0.8416$	$p = 0.5118$	$p = 0.1035$	$p = 0.7188$	$p = 0.1066$	$p = 0.0041$	$p = 0.2504$

Bold values are significant at $\alpha = 0.05$.

increase in the future (Seneviratne et al., 2012). Breeders may be able to exploit variation in physiological and morphological responses to drought to develop genotypes and varieties that will be more productive on drier sites and in drier areas.

In contrast to clonal differences in the response of biomass accumulation to high vs. low soil moisture treatments and contrary to our first hypothesis, there were no differences in biomass accumulation among clones in the alternating soil moisture treatment, where all clones showed a similar response (Fig. 1). Relative to the high soil moisture treatment, the reduction in final biomass in the alternating soil moisture treatment was 22% less than the reduction in the low soil moisture treatment. This relative increase in biomass in the alternating soil moisture treatment coincides with the proportion of time (25%) that the ramets were subjected to high water conditions in that treatment, suggesting

that much of biomass accumulation in the alternating soil moisture treatment occurred under the high-water portion of the treatment and that the recovery from moisture stress experienced under the low water portion of that treatment was rapid. Based on the clonal responses to the high and low soil moisture treatments, we suspect that in the alternating soil moisture treatment, Clone 1 may have had more biomass accumulation than Clones 2 and 3 in low soil moisture conditions but less biomass accumulation than Clones 2 and 3 in the high soil moisture conditions, resulting in similar biomass accumulation among all clones in the alternating soil moisture treatment. Trade-offs in drought resistance have also been observed in other tree species. For example, in *Populus × euromaricana* (*Populus deltoides* × *Populus nigra*), genotypes that were found to be relatively more productive were also found to be less drought resistant, while less

Fig. 6. Stomatal Density. Mean stomatal density among clones (A) and treatments (B). black and grey bars coincide with abaxial and adaxial needle surfaces, respectively. Standard error represented by thin vertical bars. Different capital letters indicate significant differences on the abaxial leaf surface, lower case letters indicate significant differences on the adaxial needle surface, * indicates significant differences between abaxial and adaxial needle surfaces at $\alpha = 0.05$ ($n = 36$).

productive genotypes displayed a broad range of drought resistance (Monclus et al., 2006).

Consistent with our second hypothesis, soil moisture affected the ratio of carbon allocation to root vs. shoot biomass among clones and treatments. When comparisons of carbon allocation were made within soil moisture treatments, differences were found among clones, but only in the high soil moisture treatment (Table 1). The variation among clones in carbon allocation in this study is consistent with other studies that have shown variation in either carbon allocation to biomass or root:shoot ratio among loblolly pines from different provenances (Bongarten and Teskey, 1987; Retzlaff et al., 2001), genotypes (Aspinwall et al., 2011b), families (Coyle et al., 2008; Retzlaff et al., 2001) and clones (Aspinwall et al., 2011b; Stovall et al., 2013). Our results are particularly interesting because the clone with more carbon allocation to below-ground biomass, relative to shoot biomass, accumulated more total biomass under high water conditions than the clones with less carbon allocation to below-ground biomass. A possible explanation for this result is that increased carbon allocation to below-ground biomass facilitated better acquisition of nutrients. With regard to carbon allocation in the low and alternating soil moisture treatments, our findings are similar to those of Stovall et al. (2013), which demonstrated significant differences in carbon allocation among loblolly pine clones in a fertilization study, but also found no link between those differences and differences in total biomass accumulation.

Within each clone, carbon allocation to root vs shoot biomass did not differ between the low, alternating and high soil moisture treatments except in Clone 1, where there was greater carbon allocation to the roots in the low soil moisture treatment compared to the alternating soil moisture treatment (Table 1). Lack of difference in carbon allocation in response to different soil moisture treatments, as observed in Clones 2 and 3, was also observed in a field study of four-year-old loblolly pine stands (Coyle et al., 2008); but not in 11-year-old loblolly pine stands (Coyle et al., 2016). The greater carbon allocation to below-ground biomass in the low soil moisture treatment in Clone 1 did coincide with greater biomass accumulation in that treatment when compared to the other two clones. These findings suggest that some loblolly pine clones may be able to better cope with moisture stress than others by increasing their below-ground carbon allocation.

The relationships between $\delta^{13}\text{C}$ and biomass accumulation found in this study (Fig. 5, Table 2), suggest that $\delta^{13}\text{C}$ could be used as a predictor for biomass accumulation within a specific genotype. Foliar $\delta^{13}\text{C}$ values have been broadly shown to increase under soil moisture stress in many studies. Increases in $\delta^{13}\text{C}$ have even been observed across broad temperature gradients (heat waves) in *Populus* (Jia et al., 2015). Relationships between $\delta^{13}\text{C}$ and height or biomass have been observed in many coniferous species including *Picea mariana* (Flanagan and Johnsen, 1995; Johnsen et al., 1999), *Picea abies* (Sanders et al., 2016), *P. radiata* (Li et al., 2015), *P. ponderosa* (Cregg et al., 2000), and *P. elliotii* Engelman var. *elliotti* x. *P. caribaea* hybrids (Xu et al., 2000). The relationships between $\delta^{13}\text{C}$ and height or biomass has also been explored in a meta-analysis of 38 forest tree species that found a negative relationship between carbon isotope discrimination (Δ) and height (McDowell et al., 2011), indicating a positive correlation between $\delta^{13}\text{C}$ and height. Another meta-analysis of 34 woody plant species from 16 different genera (Fardusi et al., 2016) found a generally positive correlation between $\delta^{13}\text{C}$ and height and/or biomass in conifers. In *P. taeda*, we found a negative correlation between $\delta^{13}\text{C}$ and biomass which indicates that within species responses may differ from between species responses. Compared to other intraspecific common garden experiments in pine, our results agree with findings in *P. sylvestris* (Cregg and Zhang, 2001) and *P. halepensis* (Voltas et al., 2008), both of which found negative relationships with $\delta^{13}\text{C}$ and growth. The negative relationship reported in this study can be interpreted to indicate that decreases in $\delta^{13}\text{C}$ are concomitantly associated with increased biomass accumulation in loblolly pine in response to available water.

The relationship (slope) between $\delta^{13}\text{C}$ and biomass accumulation differed in Clone 1 relative to Clones 2 and 3 (Fig. 5, Table 2), which demonstrated that there is likely intraspecific variation in the effect of water on biomass accumulation in loblolly pine. The genetic variation in this relationship could theoretically be targeted in future breeding efforts to improve productivity and drought resistance. Notably, Clone 2, with the highest $\delta^{13}\text{C}$, which we interpret as WUE, also had the lowest biomass accumulation in the low soil moisture treatment. A study comparing six different *Populus* genotypes in a common garden also found a similar result; the genotype with the greatest biomass accumulation had the lowest $\delta^{13}\text{C}$ values (Cao et al., 2012). When

two *Populus* genotypes with contrasting levels of drought resistance were compared, it was also found that the faster growing, more drought sensitive genotype had a greater reduction in gas exchange, change in height and higher $\delta^{13}\text{C}$ values under soil moisture stress conditions (Cao et al., 2014). The findings reported here, are consistent with the idea that it is effective use of water, meaning maximal capture of soil moisture for transpiration, and not WUE, that drives productivity under moisture stress conditions (Blum, 2009). Blum (2009) observed that genotypes of row crops that are high yielding under moisture stress conditions also have greater g_s and E under moisture stress conditions, which can result in greater photosynthesis over time but can also reduce WUE.

The rank order of $\delta^{13}\text{C}$ values among the clones was similar in the three soil moisture treatments, (Fig. 4) which highlights the potential screening value of $\delta^{13}\text{C}$ in loblolly pine improvement efforts. Consistency in rank order of carbon isotope discrimination among seed sources was observed among *Pinus ponderosa* in a common garden, also indicating the potential usefulness of screening for $\delta^{13}\text{C}$ in pine (Cregg et al., 2000). As $\delta^{13}\text{C}$ is largely affected by soil moisture, we suspect that trends in $\delta^{13}\text{C}$ among clones may be consistent across nutrient and temperature gradients, suggesting that such screening could be useful in a broad range of conditions. Relationships between $\delta^{13}\text{C}$ and biomass accumulation suggests that $\delta^{13}\text{C}$ screening could be used to identify clones and genotypes that will perform well under specific conditions (specialist) or under a broad range of site conditions (generalist). For example, selecting for genotypes with relatively low $\delta^{13}\text{C}$ values across a broad range of soil moisture conditions could result in the identification of clones and genotypes with relatively high rates of biomass accumulation when grown under high soil moisture conditions. Likewise, clones and genotypes that have relatively high $\delta^{13}\text{C}$ values across a broad range of soil moisture conditions may have higher rates of biomass accumulation on sites with lower soil moisture. Despite differences in $\delta^{13}\text{C}$, no differences in WUE_i were found among the clones. The time-integrative nature of $\delta^{13}\text{C}$ may have allowed for detection of significant differences among clones not evident in instantaneous gas exchange measurements. Future research is needed to verify that the rank order of $\delta^{13}\text{C}$ among genotypes persists across a broad range of environmental conditions as well as through time as the trees mature.

Supporting the second hypothesis, differences were detected in some of the needle physiological traits among the clones (Tables 3 and 4). Clonal variation in gas exchange in loblolly pine has been observed in other studies as well. For example, g_s and E were observed to be lower in some clones, relative to others, in response to fertilization (Tyree et al., 2009a). Differences in light-saturated net photosynthesis were observed among clones in two separate fertilization studies (King et al., 2008; Tyree et al., 2009b). In contrast, differences in gas exchange among three loblolly pine clones in a separate study were found to be largely insignificant (Aspinwall et al., 2011c), suggesting that the clones in this study did not differ in their response to moisture stress.

Higher afternoon needle water potential was observed in Clone 1 relative to Clones 2 and 3, however, these differences in water status did not affect the rate of gas exchange (Table 4). Equivalent C_i values indicated that stomatal limitation was similar among clones. Dark respiration was not affected by clone or treatment; nor was dark respiration correlated with biomass accumulation, contrary to what was found in a previous study (Aspinwall et al., 2011c). However, differences in R_d in our study may have been too minute to detect with our equipment and measurement procedures, nor did we measure respiratory losses from stems or roots.

Stomatal density provides some intriguing explanations for the differences in rank order of biomass accumulation among the clones in the different soil moisture treatments.

Differences in stomatal density of adaxial and abaxial surfaces of loblolly pine have been previously described (Ingwers et al., 2016); however, intraspecific variation in stomatal density and the effects of stomatal density on water status are not well understood in loblolly pine. Clone 1, which had the most biomass accumulation under low water conditions and the least biomass accumulation under high water conditions, also had the lowest stomatal density (Fig. 6A). Perhaps lower stomatal density in Clone 1 limited water loss via stomatal leakage in low water conditions, while also potentially limiting the maximum rate of gas exchange observed under favorable conditions, such as in the morning, and thus carbon assimilation under high water conditions. In contrast, Clone 2, with the highest stomatal density, may have had more water loss than Clone 1 in low water conditions but also the capacity for higher gas exchange in high water conditions. A_{net} and E were indeed found to be higher in the morning in Clones 2 and 3 compared to Clone 1 (although the difference was not significant in Clone 2 compared to Clone 1). Although not significant, morning g_s tended to be lower in Clone 1 than Clone 2 or 3, further suggesting stomatal density affected the rate of gas exchange. However, no differences were found in gas exchange among clones in the afternoon. Clone 2, with the highest stomatal density, also had the highest $\delta^{13}\text{C}$ values across treatments, perhaps suggesting a mechanistic relationship between the two parameters, for example through mesophyll conductance. The stomatal density in Clone 3 was intermediate between the stomatal density in Clones 1 and 2, as was the amount of biomass accumulation. Similar findings have been reported among *Populus* genotypes. For example, a common garden experiment found that the fastest growing genotype, *Populus cathayana*, had the highest stomatal density and lowest $\delta^{13}\text{C}$ values in the study (Cao et al., 2012). It was also shown that *Populus cathayana*, relative to the more drought resistant *Populus × euromericana*, exhibited lower rates of gas exchange under low soil moisture conditions (Cao et al., 2014). Likewise, in *Populus × euramericana* a negative correlation was found between carbon isotope discrimination (Δ) and stomatal density (Monclus et al., 2006), indicating a positive correlation between $\delta^{13}\text{C}$ and stomatal density. The findings suggest that the higher observed WUE may be due to stomatal density and/or total open area of the stomata and indicate that stomatal density affects gas exchange and by extension WUE. The findings here suggest important correlations between stomatal density, $\delta^{13}\text{C}$ and biomass accumulation that merit further study. Correlations between stomatal density and $\delta^{13}\text{C}$ have been detected in other woody species (Cao et al., 2012; Cregg and Zhang, 2001; Liu et al., 2016; Monclus et al., 2006).

Although not significant, the trend toward high abaxial stomatal density in the low soil moisture treatment and low abaxial stomatal density in the high soil moisture treatment (Fig. 6B) may have been due to differences in needle expansion, with the most expansion in the high soil moisture treatment and the least expansion in the low soil moisture treatment. It is, however, interesting that stomatal densities of the two needle surfaces behaved differently. The data here suggest that in *P. taeda*, the stomatal densities of the abaxial needle surface may be responsive to soil moisture conditions, while the stomatal densities of the adaxial surfaces are not. The data in this study are in agreement with work in the perennial grass, *Leymus chinensis*, which found that moderate moisture stress increased stomatal density and that stomatal density was positively correlated with gas exchange (Xu and Zhou, 2008). It is also possible that while stomatal densities may have changed in response to moisture stress, the total open area of the stomata did not as indicated by a lack of difference in g_s among clones. A general trend has been observed in plants where higher stomatal densities are associated with small stomata and vice versa. For example, a negative correlation has been observed between stomata density and size in *Eucalyptus globulus* (Franks et al.,

2009) and between both adaxial and abaxial stomatal densities and stomata length among *Populus* genotypes (Cao et al., 2012). In our study, it is possible that the plants with lower stomatal densities have larger stomata. A study of five different *Banksia* species has further shown that smaller stomata have a faster response rate (Drake et al., 2013). If the trees with higher stomatal densities in our study, also had smaller, more responsive stomata then these findings would be in agreement with the observed trend among our treatments. To better understand these relationships, future studies should not only study the effects of moisture stress on stomatal density, but also the effects of soil moisture stress on stomatal size (length and opening area). The findings in the present study suggest that stomatal density may play an important role in needle physiology and may be a potential trait to target in future improvement efforts.

5. Conclusion

Biomass accumulation differed by clone, as well as treatment, with a significant interaction between clone and treatment. Clone and treatment also affected carbon allocation to root and shoot biomass and needle physiology, but in contrast to biomass accumulation, no interactions between clone and treatment were detected. Differences in carbon allocation to root vs shoot biomass were observed among clones, but were not related to differences in total biomass accumulation. A strong correlation between $\delta^{13}\text{C}$ and biomass accumulation was found when data were analyzed across treatments, suggesting that $\delta^{13}\text{C}$ might be a useful criterion for selection of genotypes for field testing with the goal of breeding for improved biomass accumulation. Clonal differences in needle gas exchange were detected in the morning, but not in the afternoon, which may account for the differences in biomass accumulation among clones under high water conditions. Finally, differences in stomatal density among the clones may also account for the change in rank order of biomass accumulation of the three clones when compared among soil moisture treatments. Here, the lowest stomatal density may have been advantageous in low water conditions while the highest stomatal density may have been more advantageous in the high soil moisture conditions.

Declaration of interest

Authors declare no conflict of interest.

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Declaration of authors contributions

All authors participated in: project design, data acquisition, analysis, interpretation, and manuscript preparation. MWI: Project conception, drafting of manuscript, data assembly. MAM: Technical expertise, logistical support and critical manuscript revision. DPA: Statistical expertise. ROT: Supervision, funding, administrative support and critical manuscript revision. MWI (milesingwers@gmail.com) takes responsibly for this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.envexpbot.2017.03.010>.

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