



# Density reduction in loblolly pine (*Pinus taeda* L.) stands to increase tree C assimilation: an approach with the dual $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope signatures in needles

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## Abstract

• **Key message** In the context of increasing droughts related to climate change, our results showed that heavy thinning and/or very low initial planting density can increase  $\text{CO}_2$  assimilation rate in needles, and may be used as a short-term management strategy for loblolly pine plantation across sites prone to drought.

• **Context** The dry summer of 2013 provided us an opportunity to understand the  $\text{CO}_2$  assimilation rate and stomatal conductance after density manipulation treatments using the dual isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) signatures in needles of planted loblolly pine (*Pinus taeda* L.) trees in the southeastern USA.

• **Aims** To our knowledge, this is the first study using the dual isotope approach to examine the physiological response of loblolly pine trees, one of the most widely planted tree species in the world, to stand density manipulation treatments (i.e., thinning intensity and planting density).

• **Methods** In 2001–2003, trees were planted with five different planting densities, 494, 1111, 1729, 2346, and 2964 trees  $\text{ha}^{-1}$  at three sites. In 2009–2011, two thinning treatments (none and moderate thinning) were applied in the 1111 trees  $\text{ha}^{-1}$  plots, whereas three treatments (none, light and heavy thinning) were applied in the 1729 trees  $\text{ha}^{-1}$  plots. Response variables (specific leaf area (SLA), foliar N,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) were measured in February 2014.

• **Results** SLA was lower, while  $\delta^{18}\text{O}$  was higher in the 494 trees  $\text{ha}^{-1}$  plots than the 2964 trees  $\text{ha}^{-1}$  plots without thinning. In plots planted to 1729 trees  $\text{ha}^{-1}$  SLA was lower, while  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were higher following heavy thinning than in the unthinned control. These responses plus increased crown length, DBH, and height following heavy thinning may reflect an increased tree-level  $\text{CO}_2$  assimilation rate.

• **Conclusion** Our results showed that heavy thinning and/or very low initial planting density can be used as a short-term management strategy for loblolly pine plantation across sites prone to drought.

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## 1 Introduction

Drought has been widely reported as a major causal factor of reduced forest productivity (Allen et al. 2010). Forest management options such as manipulating planting density and density reductions with thinning are often proposed to increase drought tolerance (e.g., Sohn et al. 2016). Loblolly pine (*Pinus taeda* L.) is the most widely planted tree species across the southeastern United States (Brender et al. 1981; Baker and Balmer 1983), with nearly 15 million ha of even-aged plantations and an expected increase in plantation area in the future

(Huggett et al. 2013). The natural range of the species stretches across the southeastern United States, which experiences periodic extreme drought conditions (Maxwell and Soulé 2009). These droughts are projected to continue or may even increase in frequency in the future (Seager et al. 2009), and it is still unclear if commercial thinning or other density manipulation strategies are useful silvicultural options to minimize the impacts of increasing drought events expected under climate change.

Tree-level physiological responses to thinning are not consistently discussed in the literature (e.g., Ginn et al. 1991; Sheriff 1996; Tang et al. 1999; McDowell et al. 2003; Sword Sayer et al. 2004; Moreno-Gutiérrez et al. 2011). Thinning has been found effective in decreasing specific leaf area (Giuggiola et al. 2013), decreasing inter-tree competition for above and below ground resources (light, water and nutrients) (Bréda et al. 1995; Selig et al. 2008), increasing foliar N (Di Matteo et al. 2010; Moreno-Gutiérrez et al. 2011), increasing photosynthesis rates ( $A$ ,  $\text{CO}_2$  assimilation) (Tang et al. 1999), and decreasing tree mortality (Elkin et al. 2015; Sohn et al. 2016). The increase in  $A$  with thinning has been associated with a reduction in tree water stress since less trees are using the available water (Bréda et al. 1995). However, canopy opening associated with thinning also increases soil temperature (Matsushima and Chang 2007), and therefore, may increase soil evaporation (Lévesque et al. 2014).

Other studies show an opposite response with thinning increasing water stress and tree dieback (Lagergren et al. 2008; Brooks and Mitchell 2011; Bose et al. 2014). This is possibly due to abrupt changes in stand microclimate conditions such as increases in within-stand radiation, air flow in the canopy, evaporative demand and wind speed (Proe et al. 2001; McDowell et al. 2006). Theoretically, an increasing evaporative demand may cause partial or complete cavitation of the xylem (Adams et al. 2017), eventually reducing water supply to the leaves, therefore reducing the stomatal aperture ( $g_s$ ) and could limit the  $A$  (Bladon et al. 2007). The inconsistent responses to thinning make it difficult to evaluate the effectiveness of a thinning treatment for improving the performance of trees in drought prone forests (Moreno-Gutiérrez et al. 2011).

The stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) is a powerful tool and has successfully been used to evaluate the physiological mechanisms associated with tree growth after commercial thinning (Moreno-Gutiérrez et al. 2011; Giuggiola et al. 2015), which represents the ratio,  $\text{CO}_2$  concentration in the leaf intercellular space ( $c_i$ )/ $\text{CO}_2$  concentration in the atmosphere ( $c_a$ ) (Farquhar et al. 1989). Therefore, the  $\delta^{13}\text{C}$  can be used to interpret changes in intrinsic water use efficiency, defined as the ratio of net photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ) (Farquhar et al. 1982; Farquhar et al. 1989). An increasing  $\delta^{13}\text{C}$  could be due to either (i) reduced  $g_s$  at a constant rate of  $A$  or (ii) increased rate of  $A$  at a constant  $g_s$  (Farquhar et al. 1982; Farquhar et al. 1989).

Some researchers have suggested using the oxygen isotope ( $\delta^{18}\text{O}$ ) along with  $\delta^{13}\text{C}$  (Scheidtger et al. 2000; Grams et al. 2007), since  $\delta^{18}\text{O}$  is specifically related to isotopic signature of source water, which includes isotopic composition of the soil water and the leaf water enrichment in response to transpiration (Dawson et al. 2002; Roden and Siegwolf 2012). The  $\delta^{18}\text{O}$  of plant tissue is proportional to water vapor pressure in the atmosphere ( $e_a$ ) and water vapor pressure inside the leaf intercellular space ( $e_i$ ) (Farquhar and Lloyd 1993; Barbour 2007). This indicates that  $\delta^{18}\text{O}$  has an inverse relationship with  $g_s$ , when evaporative demand in the environment and source water  $\delta^{18}\text{O}$  remain constant (Farquhar et al. 2007). Therefore, sites with greater evaporative demand may have higher  $\delta^{18}\text{O}$  in plant materials. Since  $\delta^{18}\text{O}$  is unaffected by  $A$ , it can distinguish the independent effect of  $g_s$  and  $A$  on  $\delta^{13}\text{C}$  (Farquhar et al. 1982; Farquhar et al. 2007; Roden and Farquhar 2012). However, recent studies reported operational limitations associated with the dual isotope approach, especially the assumption that the environmental influences on source water  $\delta^{18}\text{O}$ , atmospheric vapor  $\delta^{18}\text{O}$ , ambient humidity, and leaf temperature must be remained constant over the monitoring period and must be constant across treatments examined (Roden and Siegwolf 2012; Roden and Farquhar 2012).

Several studies have used simultaneous measurements of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  to explain tree-level physiological responses to stand density manipulation treatments in drought prone sites in Europe (e.g., Grams et al. 2007; Moreno-Gutiérrez et al. 2011) as well as in North America (e.g., McDowell et al. 2003; Powers et al. 2008), but a study has yet to be conducted in loblolly pine plantations. The knowledge gap is important considering the projection of climate-change associated increased drought frequency and the intensive management of loblolly pine in the southeastern USA.

The objective of this study was to evaluate the effects of planting density and thinning intensity on specific leaf area (SLA), foliar N, foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in loblolly pine trees in the Upper West Gulf Coastal Plain of southeastern Arkansas and northeastern Louisiana, USA in 10–12 year stands, thinned 2–4 years prior to sampling. We hypothesized that (i) trees in higher density treatments usually receive less light, therefore, SLA increase with the increasing planting density, and decrease with the increasing thinning intensity (Hager and Sterba 1985; Giuggiola et al. 2013), (ii) thinning will increase resource (light, water and nutrients) availability to remaining trees, effectively causing an increase in the rate of  $A$  and  $g_s$  and photosynthesis. Therefore, we expected no difference in foliar  $\delta^{13}\text{C}$  due to the equal changes in  $g_s$  and  $A$ , and  $\delta^{18}\text{O}$  will be lower due to the increased  $g_s$  in treatments with low plant density (due to thinning or low initial planting density) than treatments with high plant density (McDowell et al. 2003; Sohn et al. 2014), however,  $g_s$  often has a minor effect on  $\delta^{18}\text{O}$  in needles and the changes in foliar  $\delta^{18}\text{O}$  are primarily

driven by transpiration or the evaporative demand in the atmosphere as well as from mixing of the leaf water with xylem water (Kahmen et al. 2008; Roden and Siegwolf 2012; Bögelein et al. 2017), and (iii) low planting density and thinning will increase foliar N content (López-Serrano et al. 2005), and that enriched foliar N will have a positive association with the increased  $\delta^{13}\text{C}$  in low planting density and/or thinning treatment (i.e., an increased foliar N will increase the photosynthesis rate) (Warren et al. 2001; Moreno-Gutiérrez et al. 2011).

## 2 Methods

### 2.1 Study area

Samples were collected from three sites in the Upper West Gulf Coastal Plain of southeastern Arkansas and northeastern Louisiana, USA: Drew County, Arkansas (33° 32' N, 91° 37' W), Ashley County, Arkansas (33° 15' N, 91° 46' W), and Union Parish, Louisiana (33° 0'19, 92° 24'). Soils at the Drew and Ashley sites are classified as the Henry series (Coarse-silty, mixed, active, thermic typic fragiaqualfs), poorly drained, with > 50 cm depth to a restrictive layer. Soils at the Union site are classified as the Sacul series (fine, mixed, active, thermic aquic hapludults), moderately well drained, and < 50 cm depth to a restrictive layer (Web Soil Survey 2014). Elevations are 47, 60, and 67 m above sea level at the Ashley, Drew, and Union sites, respectively. The sites are located in an area of humid subtropical climate, with a mean annual temperature of 17.5 °C and mean annual precipitation of 1358 mm (NOAA: <http://www.ncdc.noaa.gov>) in Monticello, AR (33° 38' N 91° 45' W). July and August are the hottest and driest months of the year, with average maximum temperature of 33.3 °C and average monthly precipitation of 82 mm. Moderate to severe drought conditions were common across the region in the summer (2013) before foliage was sampled for isotope analysis. For example, the total precipitation from July to August of 2013 was 79 mm while the long-term (1980–2014) total precipitation was 168 mm (<http://droughtmonitor.unl.edu>).

### 2.2 Experimental design

#### 2.2.1 Planting density

The three study sites (Drew, Ashley and Union) were established in 2001, 2002 and 2003, respectively. At each study site, one replicate of five planting density treatments was installed including 494, 1111, 1729, 2346, and 2964 trees  $\text{ha}^{-1}$ . At the Drew and Ashley sites, soils were bedded, followed by planting and a broadcast application of sulfometuron methyl herbicide and directed applications of glyphosate

herbicide, as needed, to maintain weed-free conditions for the first year. At the Union site, imazapyr and glyphosate herbicides were used to prepare the site, followed by planting and follow-up direct applications of glyphosate to maintain weed free conditions for the first year. All study sites were planted with loblolly pine using the best open-pollinated family for that location as determined by the landowner. Each planting spot was double planted to ensure survival, with one seedling cut in the autumn following planting. Fertilization included 44.8 kg  $\text{ha}^{-1}$  N + 56.0 kg  $\text{ha}^{-1}$  P during year 1, and 224.1 kg  $\text{ha}^{-1}$  N + 22.4 kg  $\text{ha}^{-1}$  P during year 10. All three sites were treated for Nantucket pine tip moth (*Rhyacionia frustrana*) with tebufenozide and permethrin during the first 2 years, spraying each tree monthly between March and October.

#### 2.2.2 Thinning intensity

Additional treatment plots were established at each site to examine the effects of different thinning intensities. Thinning treatments were applied in plots with two planting densities: 1111 and 1729 trees  $\text{ha}^{-1}$  (Table 1). The timing of thinning was selected to correspond to when the stands reached a stand density index, a composite density metric that incorporates average tree diameter and number of trees per hectare (i.e., Reineke 1933), of approximately 612. This SDI value corresponds to 55% (the threshold where significant mortality starts to occur from self-thinning) of the maximum stand density index of 1111. The 1111 trees  $\text{ha}^{-1}$  plots were thinned to 494 trees  $\text{ha}^{-1}$  (55% density reduction), while the 1729 trees  $\text{ha}^{-1}$  plots were thinned to either 1111 trees  $\text{ha}^{-1}$  (36% density reduction) or 494 trees per  $\text{ha}^{-1}$  (71% density reduction). For consistency, we refer to post-thin density reductions of 36, 55, and 71% as “light”, “moderate”, and “heavy” thinning, respectively. The applied thinning removed smaller, less competitive trees and trees with defects such as stem fusiform rust (*Cronartium fusiforme*) infections, forking, sweep or crook, while also considering tree spacing. At the Drew site, the plots planted to 1729 trees  $\text{ha}^{-1}$  were thinned in spring 2009, while the plots planted to 1111 trees  $\text{ha}^{-1}$  were thinned in spring 2010. At the Ashley site and Union sites, 1729 trees  $\text{ha}^{-1}$  plots were thinned in spring 2010 and the 1111 trees  $\text{ha}^{-1}$  plot were thinned in spring 2011 (Table 1).

The study has a total of eight treatments (five planting densities and three thinning intensities), replicated once at each site. The size of treatment plot varied from 0.09-ha for the plots planted to 2964 trees  $\text{ha}^{-1}$  to 0.22-ha for the plots planted to 494 trees  $\text{ha}^{-1}$ .

### 2.3 Data collection and isotope analysis

Heights and diameters at breast height (dbh) were measured on trees with at least 1.4 m height in early 2013 on the Drew (Age 12) and Union (Age 10) sites and in early 2014 on the Ashley

**Table 1** Description of treatments applied at each of the three study sites

Planting density (stems ha <sup>-1</sup> )	Planted spacing (m × m)	Thinning (% of density removal)	Treatment plot size (ha)	Measurement plot size (ha)	Drew site		Ashley site		Union site	
					Year of planting	Age of thinning	Year of planting	Age of thinning	Year of planting	Age of thinning
<b>Unthinned treatments</b>										
494	4.7 × 4.2	0	0.22	0.08	2001	-	2002	-	2003	-
1111	2.9 × 3.0	0	0.11	0.04	2001	-	2002	-	2003	-
1729	1.9 × 3.0	0	0.10	0.04	2001	-	2002	-	2003	-
2346	1.2 × 2.4	0	0.10	0.04	2001	-	2002	-	2003	-
2964	1.4 × 2.4	0	0.09	0.04	2001	-	2002	-	2003	-
<b>Thinned treatments</b>										
1111	2.9 × 3.0	55	0.11	0.04	2001	9	2002	9	2003	8
1729	1.9 × 3.0	71	0.10	0.04	2001	8	2002	8	2003	7
1729	1.9 × 3.0	36	0.10	0.04	2001	8	2002	8	2003	7

site (age 12). Height was measured to the nearest 0.3 m and diameter to the nearest 0.25 cm. Heights were measured on all trees for the 494 trees ha<sup>-1</sup> planting density, every other tree for the 1111 trees ha<sup>-1</sup> planting density and every third tree for the 1729, 2346, and 2964 trees ha<sup>-1</sup> planting densities. Height to the base of the live crown was measured on all trees that were measured for total height. Tree density per ha and basal area per ha were calculated applying the plot specific per ha expansion factor with surviving trees and plot basal area, respectively.

Foliage samples were collected from three randomly selected trees in each plot in February 2014. We have a total eight plots (each for a treatment) replicated across three sites, which makes a total 72 trees (3 trees\*8 treatments/plots\*3 sites = 72). Collected samples were sun-exposed leaves located at the top-middle part of the crown. Only the last flush of needles formed in 2013 were sampled. Samples were placed in plastic bags and stored on ice in the field. Once back at the lab, foliage samples were placed in a freezer until processed. One hundred needles from each sample were scanned on a flat-bed scanner with a backlight to estimate projected (one-sided) leaf area with the WinSeedle software (Regent Instruments). The scanned needles were then dried in a convection oven at 65 °C for 72 h and weighed to the nearest 1 mg. These data were used to estimate specific leaf area (projected leaf area per unit of dry mass) by tree. After weighing, dried foliage samples were ground in a Wiley mill to pass through a 20-mesh screen and then pulverized into a fine powder using a sterilized mortar and pestle. Samples for δ<sup>13</sup>C analysis were weighed to 0.7 mg, placed in tin capsules, while δ<sup>18</sup>O samples were weighed to 0.3 mg and placed in silver capsules.

Foliage samples were analyzed by the Stable Isotope Facility at the University of Arkansas. δ<sup>13</sup>C composition and N concentration were analyzed with an elemental analyzer by combusting samples in oxygen at 1100 °C, then passed through oxidation and reduction tubes and peaks were separated on a chromatography column. δ<sup>18</sup>O was analyzed with a high temperature conversion elemental analyzer. Samples were combusted at 1300 °C in a reducing environment. Isotope ratios were expressed in delta notation (‰; parts per thousand), where the isotopic composition of the bulk leaf material was expressed relative to internationally accepted reference standards (Dawson et al. 2002). The standard for δ<sup>13</sup>C was PeeDee Belemnite and the standard for δ<sup>18</sup>O was the Vienna-standard mean ocean water. Area-based foliar N content was calculated by multiplying foliar N concentration by the reciprocal of specific leaf area to obtain units of g m<sup>-2</sup>. We used the Carlo Erba NC2500 interfaced to a Delta Plus IRMS (ThermoFisher Bremen Germany) for the δ<sup>13</sup>C and N concentration, and TCEA (Thermo Chemolysis Elemental Analyzer) was interface to a Delta Plus XP for the δ<sup>18</sup>O analysis, with the precisions of 0.15 and 0.30 per mil for δ<sup>13</sup>C and δ<sup>18</sup>O, respectively.

As we do not have direct measurement of source water  $\delta^{18}\text{O}$  of the three study sites, micro-climatic factors driving water availability including average daily temperature ( $^{\circ}\text{C}$ ), total annual precipitation (mm) and average daily solar radiation ( $\text{W m}^{-2}$ ) were extracted from  $\sim 1$  km gridded rasters of Daymet Earth Data (<https://daymet.ornl.gov/>), and relative humidity in the air was extracted (%) from PRISM Climate Data (<http://oldprismmap.nacse.org/>) using coordinates of the three study sites. We extracted these data for the period from 1990 to 2013 (the last growth year prior to sample collection) to test the difference across three sites. The results of the one-way ANOVA showed that the three sites were similar in terms of average daily temperature ( $^{\circ}\text{C}$ ), total annual precipitation (mm), average daily solar radiation ( $\text{W m}^{-2}$ ) and average growing season (April–October) relative humidity (%) (Fig. 1).

## 2.4 Statistical analysis

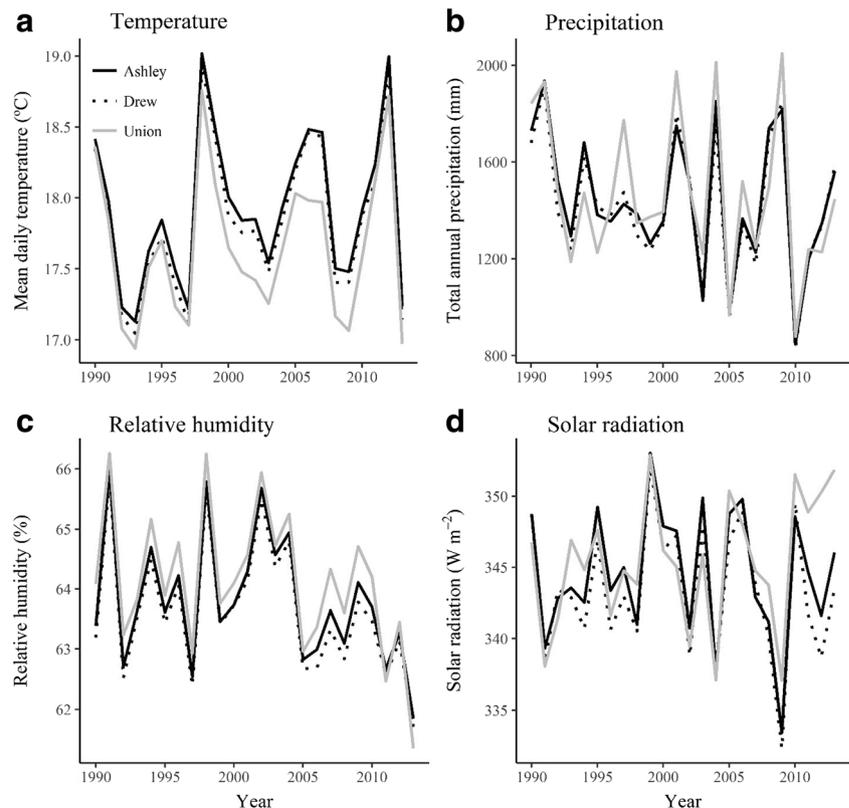
All statistical analysis were performed in R v.3.2.2 (R Development Core Team 2014). Linear mixed effect models (Pinheiro et al. 2014) were used to evaluate the effect of planting density and thinning intensity on the response variables. The response variables were specific leaf area, foliar N content, foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . The effects of planting density and thinning intensity were analyzed separately as thinning treatments were implemented in only two planting density treatments (1111 stems  $\text{ha}^{-1}$  and 1729 stems  $\text{ha}^{-1}$ ). Sites ( $n = 3$ )

were treated as random factors. Both planting density and thinning intensity were considered as discrete variables each with five levels. The planting density of 2964 trees  $\text{ha}^{-1}$  was considered as reference level and compared with four other levels of planting density (494, 1111, 1729 and 2346 trees  $\text{ha}^{-1}$ ). Differences between thinning intensity were tested by means of orthogonal contrasts. Four contrasts were created and executed into the model using the function “contrasts” of R (R Development Core Team 2014). The four contrasts were (1) planting density of 1111 trees  $\text{ha}^{-1}$ : control vs moderate thinning, (2) planting density of 1729 trees  $\text{ha}^{-1}$ : control vs light thinning, (3) planting density of 1729 trees  $\text{ha}^{-1}$ : control vs heavy thinning, and (4) moderate thinning at 1111 trees  $\text{ha}^{-1}$  planting density vs heavy thinning at 1729 trees  $\text{ha}^{-1}$  planting density. We visually verified the assumptions of normality of residuals and homogeneity of the variances. Mean values  $\pm 95\%$  confidence intervals were presented in the figures. We also tested the linear correlation between foliar  $\delta^{13}\text{C}$  and foliar N content using the linear mixed effect models while incorporating random effects associated with sites.

## 3 Results

The average dbh and the crown length were negatively related to stand density (by planting/thinning). Thinning had a substantial effect on tree size (dbh and height), average dbh

**Fig. 1** The trends in micro-climatic conditions (temperature, precipitation, relative humidity and solar radiation) from 1990 to 2013 (year of sample collection) at three study sites



**Table 2** Loblolly pine (*Pinus taeda* L.) stand characteristics (mean  $\pm$  1SD) of eight density manipulation treatments

Planting density (stems ha <sup>-1</sup> )	Thinning (% of density removal)	DBH (cm)	Height (m)	Crown length (m)	Trees per hectare	Basal area (m <sup>2</sup> ha <sup>-1</sup> )	Reineke's SDI (stand density index) at 2013
494	0	25.8 $\pm$ 3.6	13.1 $\pm$ 1.7	9.1 $\pm$ 0.6	450 $\pm$ 30	24.1 $\pm$ 6.1	438 $\pm$ 83
1111	0	20.3 $\pm$ 1.7	13.3 $\pm$ 0.9	7.0 $\pm$ 0.3	994 $\pm$ 46	32.7 $\pm$ 4.7	667 $\pm$ 22
1111	55	22.8 $\pm$ 4.2	13.8 $\pm$ 2.1	8.4 $\pm$ 1.1	462 $\pm$ 33	23.1 $\pm$ 0.1	475 $\pm$ 132
1729	0	17.6 $\pm$ 1.5	13.3 $\pm$ 1.7	6.6 $\pm$ 0.4	1446 $\pm$ 151	35.6 $\pm$ 3.0	770 $\pm$ 27
1729	71	23.3 $\pm$ 2.3	13.7 $\pm$ 1.7	8.8 $\pm$ 0.7	442 $\pm$ 14	19.1 $\pm$ 3.8	352 $\pm$ 66
1729	36	19.3 $\pm$ 2.4	13.3 $\pm$ 2.2	7.2 $\pm$ 0.6	1020 $\pm$ 14	30.6 $\pm$ 6.9	614 $\pm$ 99
2346	0	16.1 $\pm$ 1.9	13.3 $\pm$ 1.9	5.8 $\pm$ 0.3	1909 $\pm$ 124	40.7 $\pm$ 10.1	893 $\pm$ 132
2964	0	14.5 $\pm$ 1.3	13.1 $\pm$ 1.5	5.3 $\pm$ 0.6	2582 $\pm$ 311	43.6 $\pm$ 5.4	1024 $\pm$ 39

Note. Stand-level measurements of one plot (Union site; 1111 planting density and 55% density removal) were conducted prior to thinning treatment, therefore, this plot was not included in trees per hectare and basal area metrics

increased by 10, 12, and 32% in the light, moderate, and heavy thinning treatments, respectively. Average tree height was similar across treatments (Table 2). For trees from which needle samples were collected, average tree height was similar across treatments and average dbh was greater for treatments with lower density (Table 3). Planting density significantly affected the SLA and foliar  $\delta^{18}\text{O}$ , but not the foliar N concentration, and foliar  $\delta^{13}\text{C}$  (Table 4). SLA was significantly lower, while foliar  $\delta^{18}\text{O}$  was significantly higher in the planting density of 494 trees ha<sup>-1</sup> than in the planting density of 2964 trees ha<sup>-1</sup> (Table 4, and Fig. 2A and B).

Thinning intensity significantly affected the SLA, foliar  $\delta^{13}\text{C}$ , and  $\delta^{18}\text{O}$ , but not the foliar N content (Table 4). In the initial planting density treatment of 1729 trees ha<sup>-1</sup>, SLA was significantly lower, while foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were significantly higher in heavy thinning (71% density removal) than in the unthinned treatment (Table 4, Fig. 3A, B and C). Moderate thinning (55% density removal) at 1111 trees ha<sup>-1</sup> planting density and light thinning (36% removal) at 1729 trees ha<sup>-1</sup> planting density exhibited no difference (Table 4). Foliar N

content had a positive linear association with foliar  $\delta^{13}\text{C}$  ( $R^2 = 0.24$ ,  $p = 0.01$ ) (Fig. 4).

## 4 Discussion

The results of our study indicated that both approaches to reduce stand density, the reduced planting density and increased thinning intensities stimulated growth (i.e., increased crown length, dbh, and height) in residual loblolly pine, increased  $\delta^{18}\text{O}$  in needles, and slightly increased  $\delta^{13}\text{C}$  in needles. The increased tree growth and increased  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in needles may suggest an increased tree-level CO<sub>2</sub> assimilation rate with a reduced or unchanged stomatal conductance (Scheidtger et al. 2000). The increased  $\delta^{18}\text{O}$  in needles is probably due to <sup>18</sup>O enriched source water (Giuggiola et al. 2015), which resulted from an increased evaporative demand through increasing penetration of solar radiation, increasing temperature and wind speed after heavy thinning (Farquhar et al. 2007).

**Table 3** Observed DBH, height, specific leaf area, foliar N concentration, area-based foliar N content,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and WUE<sub>i</sub> (mean  $\pm$  1SD) of sampled trees of eight different density manipulation (planting density and thinning intensity) treatments applied in loblolly pine (*Pinus taeda*) plantation in southeast USA

Planting density (stems ha <sup>-1</sup> )	Thinning (% of removal)	DBH (cm)	Height (m)	Specific leaf area (cm <sup>2</sup> gm <sup>-1</sup> )	Foliar N concentration (mg g <sup>-1</sup> )	Foliar N content (g m <sup>-2</sup> )	Foliar $\delta^{13}\text{C}$ (%)	Foliar $\delta^{18}\text{O}$ (%)
494	0	25.6 $\pm$ 4.4	12.9 $\pm$ 1.2	32.3 $\pm$ 2.6	15.0 $\pm$ 1.6	0.47 $\pm$ 0.08	-30.3 $\pm$ 0.7	27.5 $\pm$ 0.8
1111	0	21.7 $\pm$ 2.6	13.6 $\pm$ 0.8	36.1 $\pm$ 4.4	15.5 $\pm$ 1.9	0.44 $\pm$ 0.09	-30.5 $\pm$ 0.3	27.1 $\pm$ 0.8
1111	55%	23.6 $\pm$ 1.5	14.0 $\pm$ 1.5	33.3 $\pm$ 3.4	15.3 $\pm$ 2.8	0.46 $\pm$ 0.10	-30.2 $\pm$ 0.4	27.5 $\pm$ 0.4
1729	0	18.3 $\pm$ 2.6	13.2 $\pm$ 1.6	36.3 $\pm$ 3.0	17.3 $\pm$ 1.9	0.48 $\pm$ 0.06	-30.5 $\pm$ 0.5	26.8 $\pm$ 0.6
1729	71%	23.9 $\pm$ 2.6	13.8 $\pm$ 1.4	33.2 $\pm$ 3.2	14.9 $\pm$ 1.6	0.45 $\pm$ 0.07	-30.3 $\pm$ 0.5	27.6 $\pm$ 0.4
1729	36%	20.5 $\pm$ 2.3	13.2 $\pm$ 1.9	35.2 $\pm$ 2.6	15.0 $\pm$ 2.3	0.43 $\pm$ 0.04	-30.6 $\pm$ 0.7	26.8 $\pm$ 0.9
2346	0	17.7 $\pm$ 2.5	13.6 $\pm$ 2.0	36.2 $\pm$ 8.7	16.0 $\pm$ 1.9	0.47 $\pm$ 0.14	-30.8 $\pm$ 0.5	26.9 $\pm$ 0.5
2964	0	15.5 $\pm$ 2.8	13.3 $\pm$ 1.3	37.8 $\pm$ 5.3	15.5 $\pm$ 3.5	0.41 $\pm$ 0.10	-30.5 $\pm$ 0.4	26.9 $\pm$ 0.3

Mean  $\pm$  standard deviations are shown ( $n = 9$ ; one tree of planting density treatment 494 tree ha<sup>-1</sup> was excluded due to measurement error)

**Table 4** Effect of planting density and thinning intensity (indicated by *p* values) on specific leaf area, foliar N content, foliar  $\delta^{13}\text{C}$ , and  $\delta^{18}\text{O}$  in loblolly pine (*Pinus taeda* L.) trees

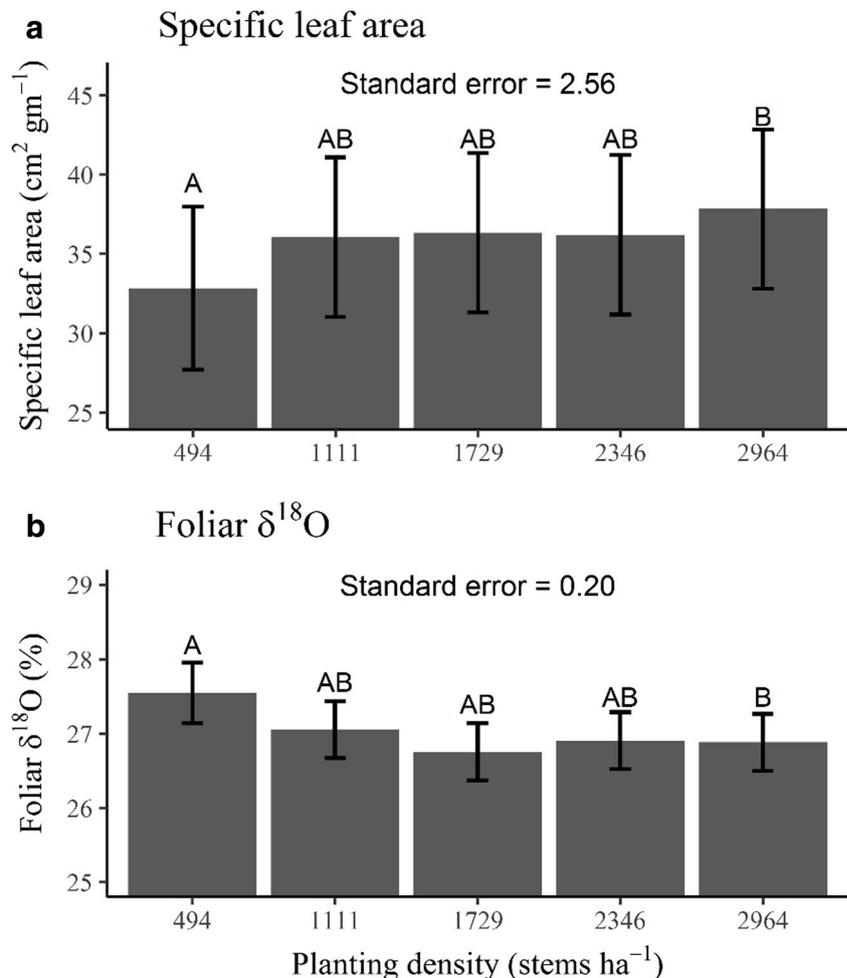
Fixed factors	Planting density				Thinning treatments			
	2964 vs 494	2964 vs 1111	2964 vs 1729	2964 vs 2346	Initial density of 1111 stems $\text{ha}^{-1}$ No-thinning vs moderate thinning (55% density removal)	Initial density of 1729 stems $\text{ha}^{-1}$ No-thinning vs light thinning (36% density removal)	Initial density of 1729 stems $\text{ha}^{-1}$ No-thinning vs heavy thinning (71% density removal)	Two different initial densities Moderate thinning at 1111 trees $\text{ha}^{-1}$ vs heavy thinning at 1729 trees $\text{ha}^{-1}$
Specific leaf area ( $\text{cm}^2 \text{gm}^{-1}$ )	0.021	0.383	0.463	0.423	0.629	0.099	0.043	0.737
Foliar N content ( $\text{g m}^{-2}$ )	0.681	0.985	0.099	0.604	0.373	0.844	0.165	0.597
$\delta^{13}\text{C}$	0.452	0.929	0.903	0.143	0.102	0.578	0.042	0.313
$\delta^{18}\text{O}$	0.025	0.543	0.650	0.940	0.059	0.583	0.002	0.192

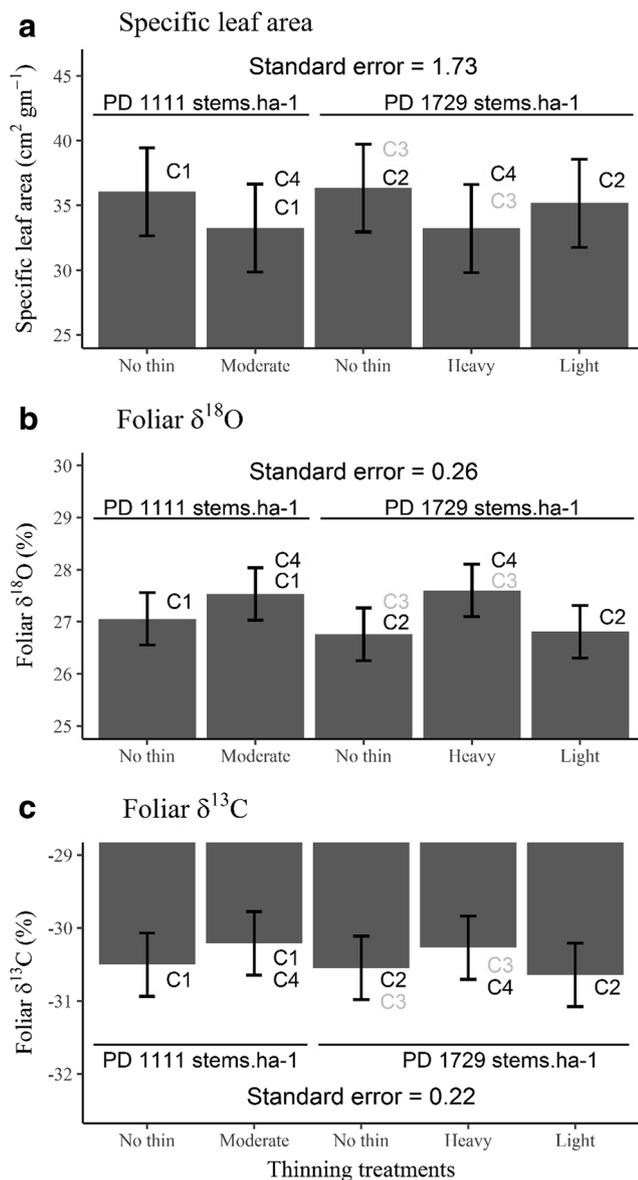
Note. The effects of planting density and thinning intensity were analyzed separately as the thinning treatments were applied in two planting density treatments. The three sites were treated as random effects in the model

Despite the important findings that heavy thinning increased tree-level  $\delta^{13}\text{C}$ , the analysis has some key limitations. There was no direct measurement of xylem or soil

water  $\delta^{18}\text{O}$  and/or gas exchange, which is necessary to assure that pre-evaporated water was not enriched in  $\delta^{18}\text{O}$  (Roden and Siegwolf 2012). The needle temperature

**Fig. 2** Effect of planting density on specific leaf area and  $\delta^{18}\text{O}$  in loblolly pine (*Pinus taeda* L.) trees. Note. Error bar represents mean  $\pm$  95% confidence interval. Non-significant results (Table 3) are not presented. In both figures, 2964 trees  $\text{ha}^{-1}$  stand density treatment was considered as the reference in the analysis, and was significantly different from 494 trees  $\text{ha}^{-1}$ , and differences with other density treatments were non-significant





**Fig. 3** Effect of thinning treatments on specific leaf area, foliar  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in loblolly pine (*Pinus taeda* L.) trees. Note. Error bar represents mean  $\pm$  95% confidence interval, PD = planting density, moderate = 55% density removal, heavy = 71% density removal, and light = 36% density removal. Non-significant results (Table 3) are not presented. C1, C2, C3 and C4 are four contrasts used in the analysis, gray and black represent significant and non-significant differences, respectively

may vary across treatments if the treatments are associated with different solar radiation input and humidity. The differences in needle temperature might affect the outcomes of  $\delta^{18}\text{O}$  ratio. In addition, leaf boundary layer may be changed by the very different planting densities and thinning intensities, and may also affect the isotope ratios (Roden and Siegwolf 2012; Gessler et al. 2014). Light and soil water availability were not directly measured, which are important variables driving photosynthesis and transpiration. A final limitation is that the time since

thinning varies from 2 to 4 years across three study sites, which may contribute to unexplained variance by our model.

The three study sites were similar in terms of major micro-climatic factors (temperature, precipitation, relative humidity and solar radiation) that potentially influence water availability and relative humidity (Fig. 1), and the distances among treatment plots within a site were minimal. Roden and Siegwolf (2012) demonstrated two prerequisites of the dual isotope approach including (i) the mean relative air humidity during the growing season should not be high (< 80%), and (ii) only isohydric tree species with tight stomatal control to changes in vapor pressure deficit should be studied. The average growing season (April–October) relative humidity of our three study sites ranged from 63.8–64.1%, while loblolly pine is a drought avoider species with strong stomatal control (Teskey et al. 1987; Tang et al. 1999; Domec et al. 2009). Therefore, these two assumptions of the dual isotope approach were met.

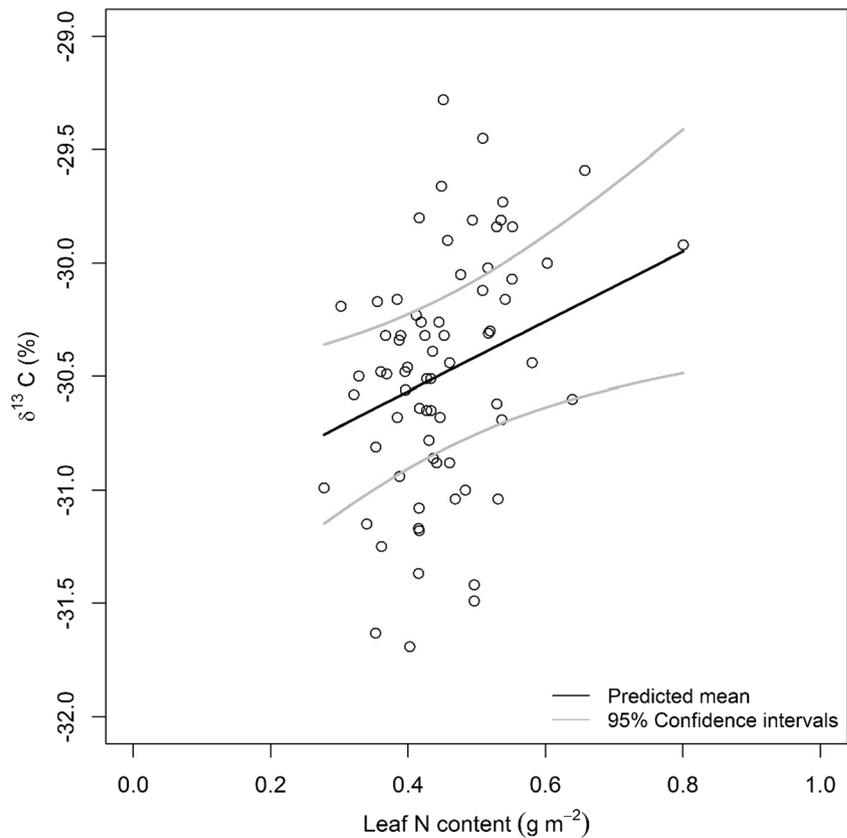
#### 4.1 Specific leaf area

We hypothesized that specific leaf area (SLA) would decrease with increasing thinning intensity and decreasing planting density. However, only heavy thinning (71% density removal) relative to the unthinned—1729 trees ha<sup>-1</sup>, and only the lowest planting density (494 trees ha<sup>-1</sup>) relative to 2964 trees ha<sup>-1</sup> exhibited reduced SLA. Morphologically, leaves with lower SLA have greater mass for a given unit of leaf area (Wilson et al. 1999). Maximum photosynthetic rates decrease as SLA decreases across multiple plant life forms (Reich et al. 1998), but the decrease in photosynthesis at the leaf level may be offset by the increase in total tree leaf area associated with lower stand density (Shelburne et al. 1993), and thus total tree photosynthesis may increase. Although tree leaf area was not measured in this study, the heavy thinning and the low planting density treatment (494 trees ha<sup>-1</sup>) increased crown length (Table 2), suggesting increased light availability to individual tree crowns and greater surface area for light interception.

#### 4.2 Foliar $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

An elevated  $\delta^{13}\text{C}$  in needles can result from either an increase in  $\text{CO}_2$  assimilation rate at a constant stomatal conductance or from a decrease in stomatal conductance with a constant  $\text{CO}_2$  assimilation rate (Scheidegger et al. 2000). Our results showed that the average crown length and average dbh were higher in trees of low planting density 494 trees ha<sup>-1</sup> relative to trees of higher planting densities, and trees of heavy thinning treatment than trees of the unthinned—1729 trees ha<sup>-1</sup> controls (Table 2). A larger crown length and a larger tree size may indicate an increased  $\text{CO}_2$  assimilation rate through increased photosynthesis by trees of 494 trees ha<sup>-1</sup> planting density than higher

**Fig. 4** Effect of foliar N content on foliar  $\delta^{13}\text{C}$  of loblolly pine trees (*Pinus taeda* L.)



planting densities, and by trees of heavy thinning than trees of unthinned—1729 trees  $\text{ha}^{-1}$  controls. Therefore, the heavy thinning treatment probably increased the light availability for residual trees, and concomitantly increased the rate of photosynthesis (may be indicated by an increased  $\delta^{13}\text{C}$  in needles).

The increased  $\delta^{18}\text{O}$  in needles might be due to an increased soil evaporation (Roden and Siegwolf 2012; Gessler et al. 2014), which was also reported by Giuggiola et al. (2015), while working with a heavy thinning treatment (similar to the heavy thinning treatment of our study) in xeric sites of Switzerland. The higher  $\delta^{18}\text{O}$  in the heavy thinned treatment could be due to an increased atmospheric evaporative demand by allowing greater penetration of solar radiation and wind into the stands. An increased evaporative demand after heavy thinning has also been reported for *Pinus nigra* in central Spain (Martín-Benito et al. 2010), *Pseudotsuga menziesii* in Vancouver Island, Canada (Brooks and Mitchell 2011), and *Populus tremuloides* in boreal Canada (Bladon et al. 2007). In drought prone sites, stand microclimate can be drier and warmer (e.g., Rambo and North 2009; Ma et al. 2010), which may cause a higher air and soil temperature and a lower air humidity. This warming increases water evaporation from soil, and results in  $^{18}\text{O}$  enrichment of soil water as  $^{16}\text{O}$  diffuses more than the  $^{18}\text{O}$  isotope (Farquhar et al. 2007; Sarris et al. 2013). Therefore,  $^{18}\text{O}$  enriched source water may result in  $^{18}\text{O}$

enriched xylem water of trees, leading to a concomitant increase of foliar  $\delta^{18}\text{O}$  ( $^{18}\text{O}/^{16}\text{O}$ ).

On the other hand, if heavy thinning increases soil water availability, trees may have a greater accessibility to shallower water sources that are relatively more  $^{18}\text{O}$  enriched (Ehleringer et al. 1993; Giuggiola et al. 2015). Therefore, similar to the previous explanation,  $^{18}\text{O}$  enriched source water will result in an elevated foliar  $\delta^{18}\text{O}$ . Therefore, the increased foliar  $\delta^{18}\text{O}$  in the heavy thinning treatment could be due to an increased evaporative demand, or to an increased availability of shallow water, or a combination of them (Farquhar et al. 2007; Roden and Siegwolf 2012; Gessler et al. 2014).

The variable physiological responses to density manipulation treatments have been widely reported (see the review of Giuggiola et al. 2015), and a range of tree-, species-, and site-level factors modulate the magnitude and the pattern of responses. For example, McDowell et al. (2003) reported a reduced  $\delta^{13}\text{C}$  due to an elevated stomatal conductance relative to  $\text{CO}_2$  assimilation rate in *Pinus ponderosa* stands after thinning. In contrast, while working on *Pinus radiata* in a thinning experiment, Warren et al. (2001) reported an increased  $\delta^{13}\text{C}$  due to an increased  $\text{CO}_2$  assimilation rate that resulted from an elevated foliar N. Other studies reported no significant effect of thinning on residual tree  $\delta^{13}\text{C}$  (e.g., Sala et al. 2005; McDowell et al. 2006).

### 4.3 Foliar N concentration and $\delta^{13}\text{C}$

A strong relationship between leaf N content and net photosynthetic capacity has been widely reported (e.g., Reich et al. 1995; Wright et al. 2004). We found a positive linear relationship (although weak) between foliar  $\delta^{13}\text{C}$  and foliar N content, suggesting  $\text{CO}_2$  assimilation rate during photosynthesis was affected by foliar N content, and that was independent of density manipulation treatment types. Warren et al. (2001) reported that a greater foliar N content generally results in a greater net  $\text{CO}_2$  assimilation rate during photosynthesis, and a corresponding greater  $\delta^{13}\text{C}$  or intrinsic water use efficiency ( $\text{WUE}_i$ ). We did not detect a statistically significant difference among treatments in terms of foliar N content, therefore, greater  $\delta^{13}\text{C}/\text{WUE}_i$  in heavy thinning relative to unthinned control was probably not due to foliar N content.

## 5 Management implications

Initial planting density and thinning intensity are important silvicultural tools for forest landowners managing loblolly pine across the southeastern US. Preferred planting density and/or final density after thinning is derived from a combination of economic and biologic factors, but little information is available on the physiology and in particular water-use efficiency effects in this key management decision. Our results showed that the heavy thinning (71% of total density) treatment resulted in a higher  $\delta^{13}\text{C}$  relative to the unthinned—1729 trees  $\text{ha}^{-1}$  control. This outcome may indicate an increased photosynthesis, which we hypothesized and is widely reported in the literature (e.g., McDowell et al. 2003; Giuggiola et al. 2015). Therefore, heavy thinning and/or very low initial planting density can be used as a short-term management strategy to increase drought resistance for loblolly pine plantation across sites prone to drought.

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### Compliance with ethical standards

**Data accessibility** A data file named ‘Carbon and oxygen isotope data in needles of loblolly pine’ and an instruction file are available from figshare

Digital Data Repository ([https://figshare.com/articles/Loblolly\\_pine\\_foliar\\_carbon\\_and\\_oxygen\\_data/5663362](https://figshare.com/articles/Loblolly_pine_foliar_carbon_and_oxygen_data/5663362)) (Bose et al. 2017).

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