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Research paper

Spatial variability in tree-ring carbon isotope discrimination in response to local drought across the entire loblolly pine natural range

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Considering the temporal responses of carbon isotope discrimination ($\Delta^{13}\text{C}$) to local water availability in the spatial analysis of $\Delta^{13}\text{C}$ is essential for evaluating the contribution of environmental and genetic facets of plant $\Delta^{13}\text{C}$. Using tree-ring $\Delta^{13}\text{C}$ from years with contrasting water availability at 76 locations across the natural range of loblolly pine, we decomposed site-level $\Delta^{13}\text{C}$ signals to maximum $\Delta^{13}\text{C}$ in well-watered conditions ($\Delta^{13}\text{C}_{\text{max}}$) and isotopic drought sensitivity (m) as a change in $\Delta^{13}\text{C}$ per unit change of Palmer's Drought Severity Index (PDSI). Site water status, especially the tree lifetime average PDSI, was the primary factor affecting $\Delta^{13}\text{C}_{\text{max}}$. The strong spatial correlation exhibited by m was related to both genetic and environmental factors. The long-term average water availability during the period relevant to trees as indicated by lifetime average PDSI correlated with $\Delta^{13}\text{C}_{\text{max}}$, suggesting acclimation in tree gas-exchange traits, independent of incident water availability. The positive correlation between lifetime average PDSI and m indicated that loblolly pines were more sensitive to drought at mesic than xeric sites. The m was found to relate to a plant's stomatal control and may be employed as a genetic indicator of efficient water use strategies. Partitioning $\Delta^{13}\text{C}$ to $\Delta^{13}\text{C}_{\text{max}}$ and m provided a new angle for understanding sources of variation in plant $\Delta^{13}\text{C}$, with several fundamental and applied implications.

Keywords: carbon isotope composition, intrinsic water use efficiency, *Pinus taeda*, water relations, water stress.

Introduction

Physiological drought, defined as ‘sustained periods of anomalously low water availability’ (Phillips et al. 2016), is the primary environmental factor limiting ecosystem productivity in most of Earth’s terrestrial biomes. Precipitation in much of the temperate zone, including the southeastern USA, is projected to become more variable with fewer but heavier rainfall events, resulting in severe drought (IPCC 2019). This change in climate is expected to have a profound impact on forest ecosystems (Vose et al. 2016). Our knowledge of drought effects on gas exchange processes at the leaf level is critical to understand the drought impact on ecosystems and to predict its consequences under future climate change scenarios. Moderate and short-term soil water limitation or high atmospheric demand triggers the closure of stomata (Ward et al. 2015), sometimes reducing mesophyll conductance (g_m , Brodribb and Holbrook 2003) and photosynthetic rate. Long-term drought can alter xylem conductivity (Domec et al. 2015), photosynthetic machinery (Fleta-Soriano and Munné-Bosch 2016, Menezes-Silva et al. 2017), leaf structure and biomass allocation (Ryan 2011). Most of those changes affect gas exchange processes at the leaf level and reduce carbon assimilation (Henckel 1964, Martin-StPaul et al. 2012, Zhou et al. 2015).

Stable isotope carbon discrimination ($\Delta^{13}\text{C}$) of plant tissues is one of the most powerful tools in studying leaf-level gas exchange processes. The discrimination integrates fractionations in the diffusion of $^{13}\text{CO}_2$ from the atmosphere to chloroplast stroma, carboxylation, day respiration and photorespiration (Farquhar et al. 1982). Despite the complex controls in these processes, $\Delta^{13}\text{C}$ is primarily determined by the ratio of intercellular to ambient CO_2 concentration and was commonly treated as a proxy for intrinsic water use efficiency (iWUE; Farquhar et al. 1989). Given its genetic basis (Marguerit et al. 2014, Bartholomé et al. 2015) with a varying degree of heritability (Baltunis et al. 2008), $\Delta^{13}\text{C}$ is used in crop breeding programs to select genotypes with high WUE (Condon et al. 2004). In addition, variations in $\Delta^{13}\text{C}$ reflect the effects of environmental conditions on gas exchange processes (Cernusak et al. 2013). The main influencing variables include water availability (Leavitt 1993), altitude (Körner et al. 1991, Hultine and Marshall 2000), irradiance (Farquhar et al. 1989), atmospheric CO_2 concentration (Schubert and Jahren 2012) and soil nutrient supply (Duursma and Marshall 2006).

Given the strong effect of water availability on gas exchange processes, it is not surprising that it is often found to be the primary modifier of $\Delta^{13}\text{C}$ (Cernusak et al. 2013). A rich body of literature reported an increase in $\Delta^{13}\text{C}$ with increasing water availability (Leavitt 1993). Specially, measured or modeled proxies of soil water balance, such as soil water potential (e.g., McNulty and Swank 1995, van Leeuwen et al. 2010) and drought indices (Tei et al. 2013, 2015, Xu et al. 2017), were

able to explain a large portion of variation in $\Delta^{13}\text{C}$ at site levels. For instance, around 70% of the year-to-year $\delta^{13}\text{C}$ variation in *Fagus sylvatica* was explained by fluctuations in extractable soil water content (Dupouey et al. 1993), which integrates a number of environmental variables (e.g., temperature, precipitation and soil texture) by soil water balance (Walcroft et al. 1997). Given the large temporal variations in local hydroclimate conditions (Domec et al. 2015), we may divide the sources of variation in site-level plant $\Delta^{13}\text{C}$ into two parts: the fast-changing water availability during the time window when source carbon of the sampling tissues was produced, and the less variable variables such as tree genotype, irradiance and soil nutrient supply.

While a linear dependence of plant $\Delta^{13}\text{C}$ on proxies of water availability has often reported, there is considerable variation among relationships obtained from different sites and species (e.g., Warren et al. 2001). Accounting for the effect of water availability in a spatial analysis without considering the inter-site differences may be the reason why some earlier studies have been unable to link wood $\delta^{13}\text{C}$ to drought (e.g., Weitner et al. 2007). One approach to address this issue is to use coefficients obtained from site-level regression analysis in a spatial analysis. To our knowledge, this approach has not been implemented to date. In fact, many regional studies along various environmental gradients have ignored local water availability altogether, implicitly assuming that between-site differences are the primary drivers of $\Delta^{13}\text{C}$ (e.g., Körner et al. 1991, Schulze et al. 1998, Schulze et al. 2014). Consequently, it is plausible that a part of the unexplained variance in global syntheses (Diefendorf et al. 2010, Kohn 2010, Cornwell et al. 2018, Basu et al. 2019) may be attributable to ignoring the effect of incident water availability. We postulate that separating $\Delta^{13}\text{C}$ responses to spatial and temporal dimensions is necessary and in fact a prerequisite for exploring the effect of additional factors (e.g., genetics, which may vary spatially).

The current study was set up to analyze the drought response of $\Delta^{13}\text{C}$ throughout the native range of loblolly pine (*Pinus taeda* L.). Loblolly pine is a drought-sensitive tree species (Stransky and Wilson 1964, Oren et al. 1998, Lin et al. 2019) and the most important commercial tree species in the USA (Will et al. 2015). Representing about 35% of southeastern forests, of which half are plantations (Wear and Greis 2012), loblolly pine contributes 36% of forest carbon sequestration in the conterminous USA (Noormets et al. 2015). As forest health and productivity are critical to the local economy and livelihoods, the climate change impacts on the pine resource are of significant interest.

We quantified drought severity using the Palmer Drought Severity Index (PDSI), a metric widely used in drought monitoring of North America (Svoboda et al. 2002). It evaluates the drought severity of an area during a given period by

comparing actual precipitation with model predictions of water demand (Palmer 1965, Heim 2002). It was developed for 'time and space comparisons of drought severity' (Palmer 1965) and thus is a normalized dimensionless metric (see Table S1 available as Supplementary data at *Tree Physiology* Online for the classifications for wet and dry conditions). Here, using PDSI as a proxy for water availability, our objectives were to (i) quantify the drought responses in $\Delta^{13}\text{C}$ of loblolly pine from 76 plantations across this species' natural range in the southeastern USA and (ii) partition the differences in drought responses to climatic, soil, site-specific and genetic causes.

Materials and methods

Study area

The natural range of loblolly pine in the southeastern USA is defined by annual actual evapotranspiration of 1050 mm to the south, 737 mm to the west and 813 mm to the north, along the Atlantic and Gulf of Mexico coasts (Schultz 1997; Figure 1a and b). For the current study, we sampled 76 loblolly pine plantations from 10 states (AL, AR, FL, GA, LA, MS, NC, SC, TX and VA) encompassing this natural range. The study sites were selected based on the availability of biomass and productivity data contributed to the Terra-C database (<http://terracc.ifas.ufl.edu/>) by different university–industry co-operatives. For 67 sites with understory vegetation measurements, the majority (65 sites) had no understory vegetation or understory biomass below 1.0 Mg C ha^{-1} due to competition control.

Experimental design and isotopic analysis

At each of the 76 sites, eight trees from unfertilized areas were cored at breast height from 2012 through 2014. Cores were oven-dried at 60°C , mounted, sanded smooth and dated using the identification of signature years (Speer 2010). Ring width was measured to the nearest 0.001 mm using a tree-ring measuring system (Velmex Inc., Bloomfield, NY) and cross-dated using the COFECHA program (Holmes 1983).

PDSI was used to identify wet and dry years for each site. Monthly PDSI values from each climatological division of the natural range of loblolly pine were obtained from the State Climate Office of North Carolina, USA. Two years with contrasting wetness were chosen based on both yearly and summer (May through August) PDSI. As the ages of the sampling tree were <30 years (Figure 1c), the juvenile effect of $\delta^{13}\text{C}$ (McCarroll and Loader 2004) was probably unavoidable. Given the current study's purpose was to contrast recent wet and dry years at each site, this confounding effect is likely much smaller than for analysis of long-term trends. The time between wet and dry years at all sites was no more than 3 years, with 61 sites (80%) having the contrast years adjacent to each other or separated by a single year. The PDSI of the dry year from 69 sites (91%) was more negative than -2.0 , indicating at

least moderate drought. Most of the sites (72%) experienced severe or extreme drought during the dry year ($\text{PDSI} < -3.0$, Table S1 available as Supplementary data at *Tree Physiology* Online), while the minimal PDSI was -5.39 . The PDSI of wet years ranged from -1.40 to 4.17 , with an average of 0.85 . Due to the climatic conditions of the sampling areas in recent years and the limitation from tree age, the PDSI of wet years at 10 sites fell in the 'incipient drought' category ($\text{PDSI} = -0.99$ to -0.5 ; Table S1 available as Supplementary data at *Tree Physiology* Online), while the wet year at two sites fell into the category of 'mild drought' ($\text{PDSI} = -1.99$ to -1 , Table S1 available as Supplementary data at *Tree Physiology* Online).

Loblolly pine growth rings are delineated by distinct bands of light earlywood (produced in spring and early summer) and dark latewood (produced in late summer and fall). Compared to earlywood, which may be produced using stored carbohydrates from the previous year, latewood better represents conditions of the current year growth (Monserud and Marshall 2001, McCarroll and Loader 2004). As we were interested in drought response of trees in the current year, latewood was separated for α -cellulose extraction. α -Cellulose was extracted according to Wieloch et al. (2011), with an additional acetone pretreatment step (Lin et al. 2017). The ^{13}C stable isotope ratios ($\delta^{13}\text{C}$) of the extracted α -cellulose were determined at the Cornell University Stable Isotope Laboratory (<http://www.cobsil.com>). The within-run isotopic precision using quality control standards was 0.2‰ (K. Sparks, 2015; personal communication).

The $\Delta^{13}\text{C}$ was calculated using $\delta^{13}\text{C}$ values of the extracted α -cellulose ($\delta^{13}\text{C}_p$) and of the atmosphere ($\delta^{13}\text{C}_a$) as follows:

$$\Delta^{13}\text{C} (\text{‰, VPDB}) = \frac{\delta^{13}\text{C}_a - \delta^{13}\text{C}_p}{1 + \delta^{13}\text{C}_p/1000} \quad (1)$$

Yearly average of $\delta^{13}\text{C}_a$ was calculated from the monthly values measured at the Scripps CO_2 Program at La Jolla Pier, California (http://scrippsco2.ucsd.edu/data/atmospheric_co2/ljo), of the sampling year. The $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ data used in this study were available in the Terra-C database (<http://terracc.ifas.ufl.edu/>).

Data sources and conversions

To explore the drivers of the range-wide variability of tree-ring $\Delta^{13}\text{C}$ of loblolly pine, we extracted edaphic and climate variables from publicly available databases. We extracted soil bulk density, clay content, sand content, silt content, saturated hydraulic conductivity (K_s), available water capacity, organic matter content, effective cation exchange capacity (ECEC) and pH from the Gridded Soil Survey Geographic (gSSURGO) database (<http://websoilsurvey.sc.egov.usda.gov/App/WebSoiISurvey.aspx>) using site geographic coordinates. Total nitrogen deposition rates were extracted from National Atmospheric Deposition Program (<http://nadp.slh.wisc.edu/committees/tde>

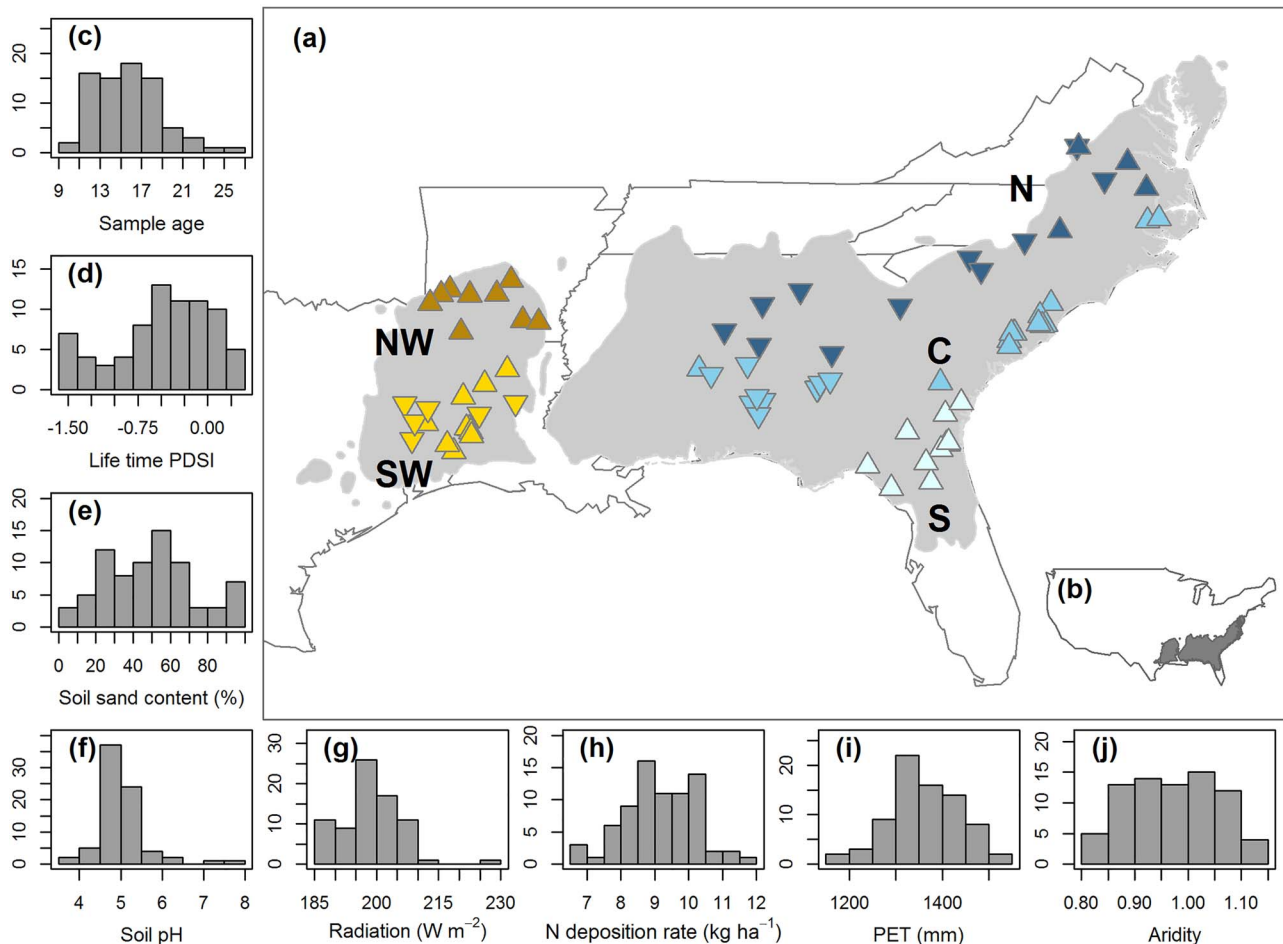


Figure 1. Location and characteristics of loblolly pine sites. (a) The natural distribution of loblolly pine in southeastern USA (shaded area) with state borders (gray lines) and site locations (points). Sites are colored by five genetic regions based on seed source transfer zone: N (northern, dark blue points), C (central, medium blue points), S (southern, light blue points), NW (northwestern, brown points) and SW (southwestern, yellow points). Triangles represent sites with 0–200 cm water table depth, while reversed triangles represent sites with water table depth deeper than 200 cm. (b, inset) map of the continental USA with the natural distribution range of loblolly pine (shaded area). (c) The sampling ages of trees. (d) Average Palmer drought severity index over tree's lifetime (lifetime PDSI). (e) Sand content in soils. (f) Soil pH. (g) Downward surface shortwave radiation. (h) Total nitrogen deposition rate. (i) Mean potential evapotranspiration (PET). (j) Aridity defined as mean annual precipitation divided by PET. The vertical axis represents number of sites for histograms (c) through (j).

[p/tdepmaps/](#)). Given the limited temporal coverage of annual deposition maps, the average nitrogen deposition rates from 2001 to 2010 were used. Thirty-year (1981–2010) normals of precipitation (PPT), mean annual temperature (T) and mean annual dew point temperature (T_d) were obtained from PRISM Climate data (PRISM Climate Group, Oregon State University, <http://prism.oregonstate.edu>). Vapor pressure deficit (VPD) was calculated using T and T_d , according to Murray (1967). Daily downward surface shortwave radiation, wind speed and potential evapotranspiration (PET) from 1981 to 2010 were obtained from gridMET (Abatzoglou 2013; <http://www.climatologylab.org/gridmet.html>), and 30-year normals were calculated for each site. Aridity was calculated as the ratio of PPT and PET. As 95% of our samples spanned from 2007 to 2014 (Table S2 available as Supplementary data at *Tree Physiology Online*) with a change in atmospheric CO_2 concentration of 15 ppm,

the potential change in $\Delta^{13}\text{C}$ due to raising atmospheric CO_2 concentration is small, probably similar to the within-run isotopic precision using quality control standards (0.2‰). As the CO_2 effect also varies by species (Stein et al. 2021) and the global sensitivity by Keeling et al. (2017) may not be appropriate for loblolly pine (Battipaglia et al. 2013), the $\Delta^{13}\text{C}$ values in this study were not corrected for the rising atmospheric CO_2 concentration. Except for total nitrogen deposition rate, which was extracted using ArcGIS 10.5 (ESRI, Redlands, CA), all data processing was completed with the R software (R Core Team 2020). Packages 'maps' (Becker et al. 2016), 'ncdf4' (Pierce 2019), 'raster' (Hijmans 2020) and 'rgdal' (Bivand et al. 2016) were used for data extraction and map visualization.

We explored the regional differences in tree-ring $\Delta^{13}\text{C}$ based on Bailey's ecoregion boundaries (Bailey 1983) and the loblolly pine seed source transfer zone (SSTZ; Schmidtling 2001).

We report the results only for the aggregated SSTZ regions as they better depict regional differences. With the exception of some Atlantic Coastal to interior provenance transfers (Lambeth et al. 2005), commercial loblolly pine plantations are generally planted with locally adapted genotypes. For this reason, the SSTZ regions may serve as a surrogate index of genetic variation across the natural range of the species. Based on sampling locations and site density, we ended up classifying the 76 study sites into five SSTZ regions (Figure 1a): north (N), central (C), south (S), northwest (NW) and southwest (SW). Given the significance of the barrier posed by the Mississippi River (Schmidtling 2001) and the sample size required for regression analysis, we also aggregated the five regions into eastern and western parts of the range.

Data analysis, maximum carbon isotope discrimination and tree lifetime PDSI determination

The relationship between $\Delta^{13}\text{C}$ and water availability at each site can be characterized by a linear function (Dupouey et al. 1993, McNulty and Swank 1995, Xu et al. 2017):

$$\Delta^{13}\text{C} = \text{intercept} + m \cdot \text{water availability index} \quad (2)$$

where the slope m represents $\Delta^{13}\text{C}$ sensitivity to water availability. In this study, a nonlinear response of $\Delta^{13}\text{C}$ to water availability was found. A segmented regression was thus conducted with the 'segmented' package (Muggeo 2008). The segmented regression indicated a two-phase response of $\Delta^{13}\text{C}$ to PDSI: a positive relationship at $\text{PDSI} \leq 1.0$ and no relationship at $\text{PDSI} > 1.0$. Based on this finding, we recoded the wet year PDSI as 1.0 for sites with wet year $\text{PDSI} > 1.0$ (Figure 2) before fitting Equation 2 to all sites (Figure 3). Without adjusting the PDSI values for wet years, m would be underestimated. The regression analysis was conducted using a linear mixed effect model via the 'nlme' package (Pinheiro et al. 2016). PDSI was considered a fixed factor, and individual sites were the random factor. The model was fitted using the restricted maximum likelihood method. We also defined $\Delta^{13}\text{C}$ at $\text{PDSI} = 1.0$ as maximum carbon isotope discrimination ($\Delta^{13}\text{C}_{\text{max}}$; Figure 2), representative of discrimination in well-watered conditions under the physiological range in which the species operates, and calculated $\Delta^{13}\text{C}_{\text{max}}$ for each site using Equation 3 as follows:

$$\Delta^{13}\text{C}_{\text{max}} = \text{intercept} + m \quad (3)$$

The slope of the regression line (m from Equation 2) was termed the isotopic drought sensitivity, the change in tree-ring $\Delta^{13}\text{C}$ per unit change in PDSI (Figure 2).

Given that PDSI is a site-normalized index, $\Delta^{13}\text{C}_{\text{max}}$ and m were comparable across sites. The spatial patterns of $\Delta^{13}\text{C}_{\text{max}}$ and m were tested with Moran's I using the package 'ape'

(Paradis et al. 2004). The effects of genetics (represented by SSTZ) and environment (site, climate and soil) on $\Delta^{13}\text{C}_{\text{max}}$ and m were explored using Pearson's correlation coefficients, multiple regression (MR) and partial least square regression (PLSR) analyses. In particular, we considered a new site parameter, tree lifetime PDSI, which was calculated as the averages of PDSI from planting years (PY) to the averages of the two sampling years (i.e., wet and dry years; Equation 4). The average sampling year (SY) was rounded if it was not an integer.

$$\text{Lifetime PDSI} = \sum_{i=\text{PY}}^{\text{SY}} \text{PDSI}_i / (\text{SY} - \text{PY} + 1) \quad (4)$$

For MR, clusters of variables with multicollinearity were identified using Pearson's correlation coefficients with a cutoff of 0.7, following the suggestion from Dormann et al. (2013). We represented the clusters by selected variables and removed the rest from the datasets for MR analysis. The variable selection for the final regression models was conducted using a stepwise regression based on Bayesian information criterion (BIC), similar to Cornwell et al. (2018). As the fraction of variation explained by MR was low, we conducted PLSR analysis, which is capable of handling multicollinearity, using package 'plsdepot' (Sanchez 2012). We hypothesized that there was no general linear additive relationship between the predictor variables and the response variables over the entire natural range of loblolly pine. We thus examined the effects of stratification by water table depth (shallower or deeper than 200 cm), aridity (higher or lower than 1.0), lifetime PDSI (higher or lower than -0.5) and genetic zones (east and west of the Mississippi River). This was done by fitting the entire and stratified datasets using PLSR. R^2 was used to evaluate if the stratified models outperformed the original ones. As R^2 may also increase due to the reduced sample size from stratification, the stratification was judged as significant if R^2 fell out of the bootstrapped 95% confidence intervals (Guiot 1991). These were obtained by sampling randomly the same number of sites as the stratified models from the entire dataset and fitting PLSR models to the subsamples 1000 times.

Results

Carbon isotope discrimination and drought response metrics

The $\Delta^{13}\text{C}$ ranged from 15.3 to 22.3‰ across the study area. The average $\Delta^{13}\text{C}$ were 1.2‰ lower during the dry years than during the wet years. As determined by segmented regression (Figure 3a), the threshold PDSI between the well-watered and water-limited conditions was 0.96, with a 95% confidence interval of [0.11, 1.81]. The fitted regression models for the

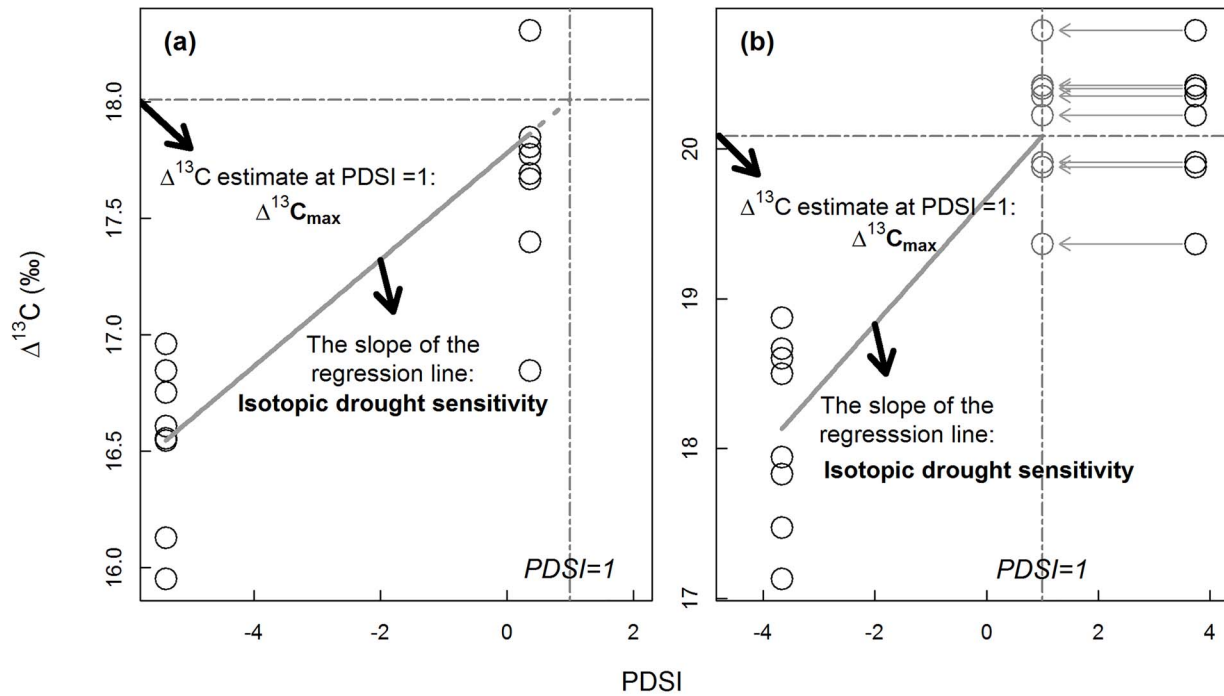


Figure 2. Example datasets to illustrate the maximum carbon isotope discrimination ($\Delta^{13}\text{C}_{\text{max}}$) and isotopic drought sensitivity based on observations (open circles) from two sites (a: Site MRTS_2ndThin_6 from Diboll County, Texas, years 2009 and 2011 and b: Site MRTS-1stThin_15 from Delight County, Arkansas, years 2009 and 2011). The gray arrows and gray circles represent adjusted direction and adjusted data, respectively.

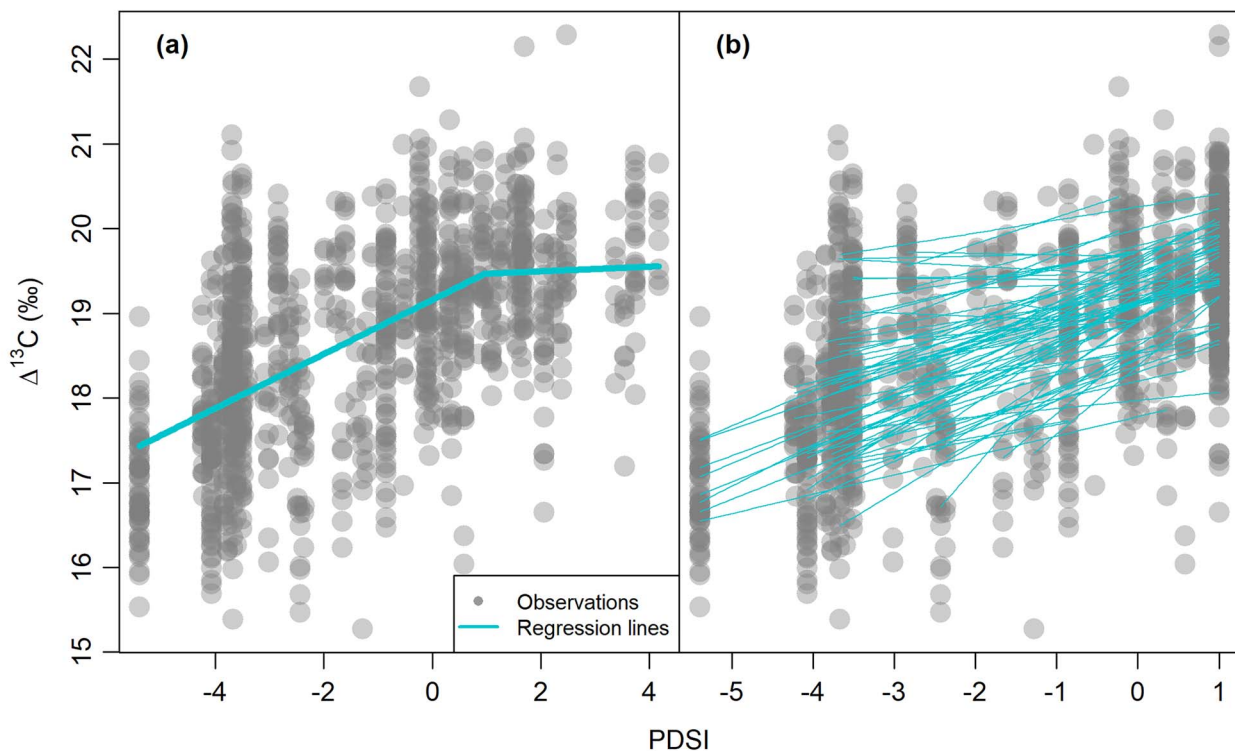


Figure 3. Carbon isotope discrimination ($\Delta^{13}\text{C}$) as a function of Palmer's drought severity index (PDSI): (a) original data with a segmented regression line; (b) adjusted data with regression lines for each site from a linear mixed-effect model.

segments were as follows:

$$\Delta^{13}\text{C} = \begin{cases} 19.16 + 0.32 \cdot \text{PDSI}, & \text{for PDSI} \leq 0.96 \\ 19.45 + 0.03 \cdot \text{PDSI}, & \text{for PDSI} > 0.96 \end{cases} \quad (5)$$

The slope of the first segment was significantly different from zero ($P < 0.0001$) with 95% confidence interval as [0.29, 0.35]. However, the 95% confidence interval of the slope of the second segment ($\text{PDSI} > 0.96$) spanned zero [−0.11, 0.16], meaning that $\Delta^{13}\text{C}$ was effectively unresponsive to PDSI under wet conditions.

$\Delta^{13}\text{C}_{\text{max}}$, isotopic drought sensitivity and their spatial patterns

As $\text{PDSI} = 1.0$ is the threshold between 'Incipient wet spell' and 'Slightly wet' conditions (Table S1 available as Supplementary data at *Tree Physiology Online*), we chose $\text{PDSI} = 1.0$ instead of 0.96 as the threshold where $\Delta^{13}\text{C}$ stops responding to change in PDSI. After recoding PDSI values for $\text{PDSI} > 1.0$ as $\text{PDSI} = 1.0$ (Figure 2), we calculated the $\Delta^{13}\text{C}_{\text{max}}$ and m as defined in Equations 2 and 3 for each site. The site-specific $\Delta^{13}\text{C}_{\text{max}}$ ranged from 18.0 to 20.8‰, with the mean of 19.4‰. The m varied from −0.14 to 1.11‰ per unit change of PDSI, with the overall mean of 0.31‰, and 56 sites (74%) between 0.10 and 0.50‰ per unit change of PDSI.

Both $\Delta^{13}\text{C}_{\text{max}}$ and m exhibited spatial correlations across sites (Figure 4). The spatial correlation of $\Delta^{13}\text{C}_{\text{max}}$ was marginally significant (Moran's $I = 0.08$, $P = 0.06$, Figure 4a). It was the highest in SC and lowest in the sites along the Gulf Coast. However, the sites with intermediate $\Delta^{13}\text{C}_{\text{max}}$ did not show a distinct spatial arrangement. In contrast, the spatial correlation of m was highly significant (Moran's $I = 0.2$, $P < 0.001$, Figure 4b). The Atlantic coastal plain sites were less drought-sensitive than sites further west, with sites in the SSTZ region S (FL and southeastern GA) being the least sensitive. The most drought-sensitive sites were in the far northeastern part of the range.

Controls of $\Delta^{13}\text{C}_{\text{max}}$

Although the spatial correlation of $\Delta^{13}\text{C}_{\text{max}}$ was only marginally significant, the difference in $\Delta^{13}\text{C}_{\text{max}}$ was significant between SSTZ regions S and C (19.0 vs. 19.6‰, respectively, $P = 0.02$; Figure 5a). Among 19 environmental (site, soil and climate) variables, 7 were found to correlate with $\Delta^{13}\text{C}_{\text{max}}$ at $P < 0.10$ (Table 1). Most of them (lifetime PDSI, sand content, K_s , PET and aridity) were related to water availability at the site. The correlation of $\Delta^{13}\text{C}_{\text{max}}$ with lifetime PDSI was the strongest ($r = 0.29$, $P = 0.01$).

Five clusters of environmental variables with collinearity were identified: (a) soil sand content and silt content; (b) soil sand content and K_s ; (c) downward surface shortwave radiation, T , PET and VPD; (d) PPT and PET and (e) downward surface

shortwave radiation, nitrogen deposition rate and VPD. Soil sand content was selected to represent clusters (a) and (b), PET for clusters (c) and (d) and nitrogen deposition rate for clusters (c) and (e). When considering all genetic and environmental variables together in an MR model, the final model by BIC included only lifetime PDSI, explaining 7% of the variance in $\Delta^{13}\text{C}$ ($P = 0.01$, Table 2).

A PLSR model based on environmental data explained 24% of the variance (Table S4 available as Supplementary data at *Tree Physiology Online*; gray dots in Figure 6a). However, it increased significantly to 53% for a subset of sites with water table depth <200 cm (green triangles in Figure 6a, Table S3 available as Supplementary data at *Tree Physiology Online*) and 49% for sites with water table ≥ 200 cm (yellow reversed triangles in Figure 6a, Table S3 available as Supplementary data at *Tree Physiology Online*), with a combined R^2 for the stratified data as 52%. Stratifications by genetic regions, soil pH, lifetime PDSI or aridity were not significant (Table S3 available as Supplementary data at *Tree Physiology Online*).

Controls of isotopic drought sensitivity

There was a significant spatial correlation in m , strongly correlated with spatial patterns in genetic and environmental variables. The averages among different SSTZ regions differed significantly ($P < 0.001$, Figure 5b). SSTZ regions N, NW and SW were the most sensitive to a unit change in PDSI (>0.36‰ per unit PDSI), while region S was the least sensitive (0.13‰ per unit PDSI). Region C (0.25‰ per unit PDSI) fell in-between.

The parameter m correlated with most (13 out of 19) environmental variables evaluated (Table 1). Variables related to nitrogen deposition rate, irradiance, temperature (T and PET), atmospheric evaporative demand (VPD) and soil pH had the strongest correlation with m ($|r| > 0.34$, $P < 0.01$). Correlation with lifetime PDSI, altitude and aridity were also significant.

The variables that entered the stepwise regression for m were the same as for $\Delta^{13}\text{C}_{\text{max}}$. The final model from the stepwise regression procedure included soil pH and PET (Table 2) and explained 21% of the variability ($P < 0.001$). The fraction of variance of m explained by PLSR across the entire data set was 33% (Table S4 available as Supplementary data at *Tree Physiology Online*; Figure 6b). Stratification by water table depth, aridity, lifetime PDSI or genetic region did not increase model fit significantly (Table S3 available as Supplementary data at *Tree Physiology Online*).

Discussion

Threshold response of $\Delta^{13}\text{C}$ to water availability

Stable isotope carbon discrimination in loblolly pine correlated with PDSI at $\text{PDSI} < 1$, whereas at higher values $\Delta^{13}\text{C}$ was insensitive to PDSI (Figure 3a). This observed threshold response of $\Delta^{13}\text{C}$ to water availability differed from the previously reported

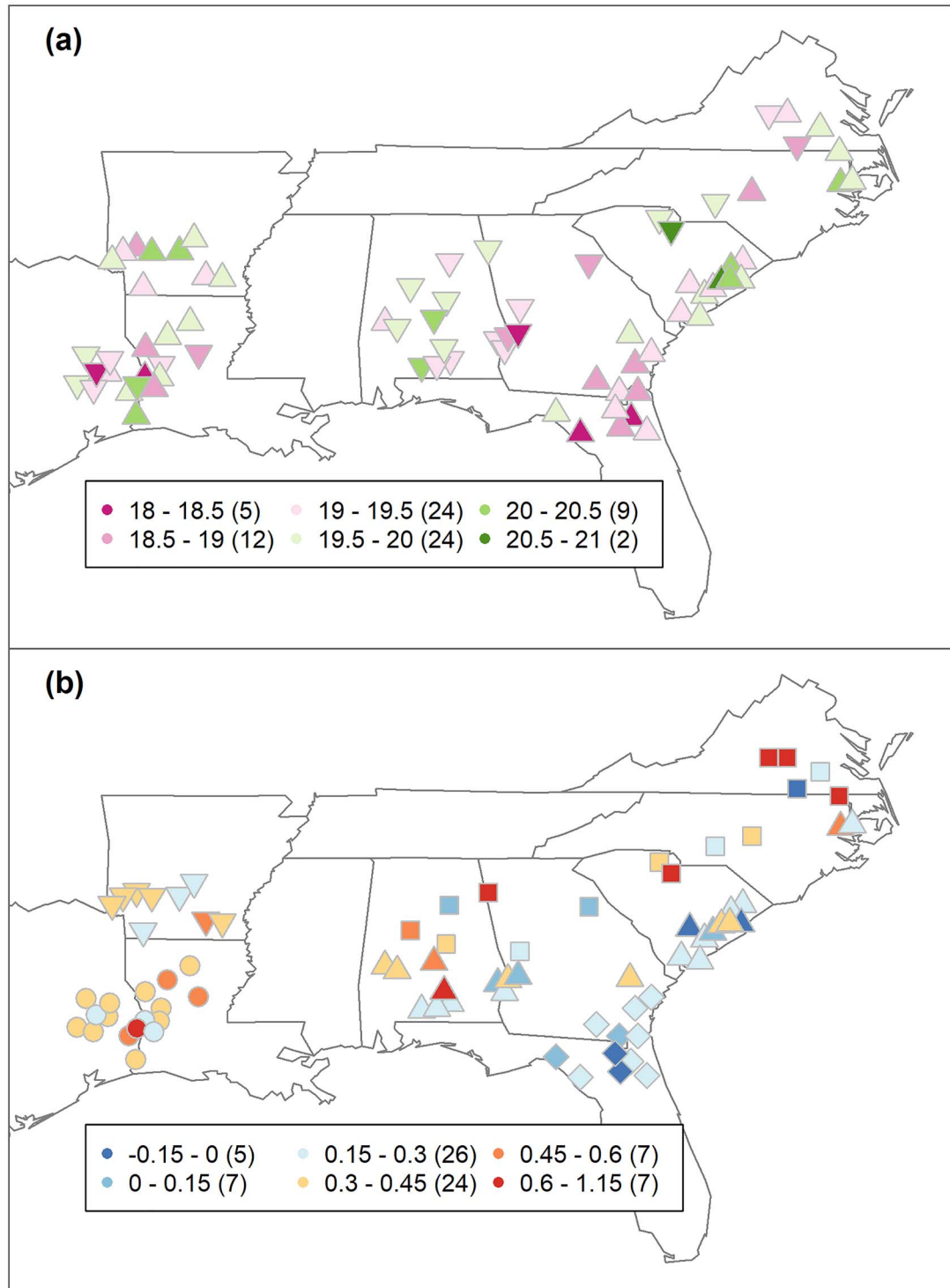


Figure 4. Maximum carbon isotope discrimination (‰, a) and isotopic drought sensitivity (‰ per unit PDSI, b) at 76 loblolly pine plantations in southeastern USA. Triangles represent sites with 0–200 cm water table depth, while reversed triangles represent sites with water table depth greater than 200 cm in panel a. Squares represent sites from northern SSTZ, triangles represent sites from central SSTZ, diamonds represent sites from southern SSTZ, reversed triangles represent northwestern SSTZ and circles represent sites from southwestern SSTZ in panel b. The numbers inside parentheses in the legends indicate the number of sites in each category. Gray lines indicate state borders. The GPS coordinates have been jittered to minimize the overlaps of symbols of nearby sites.

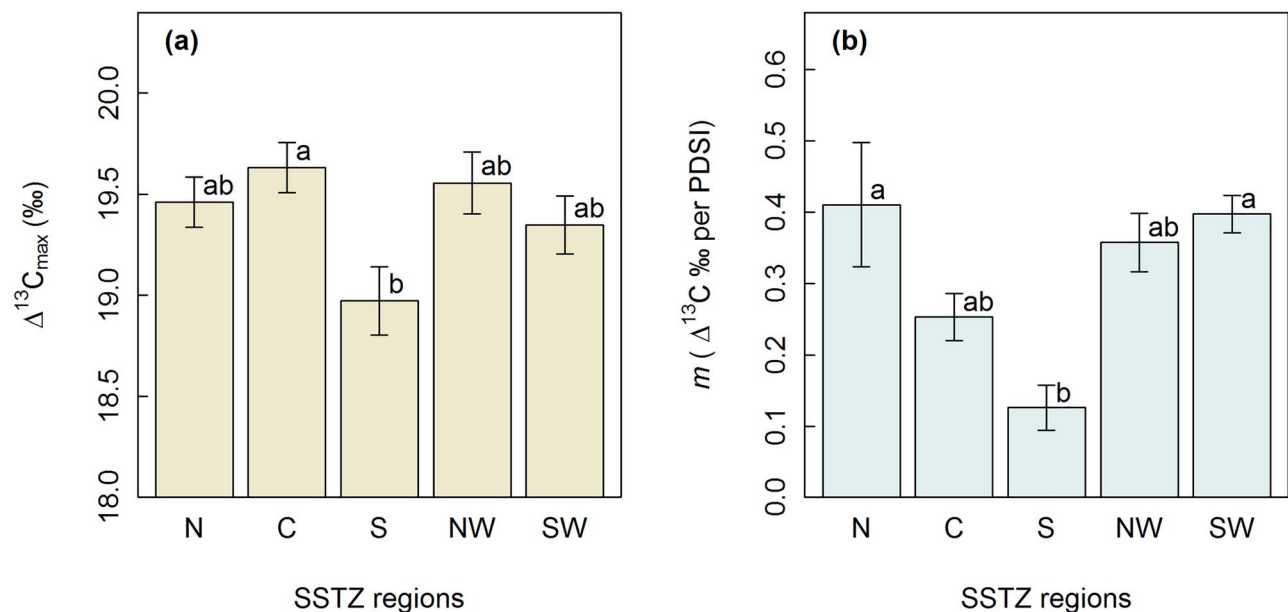


Figure 5. Arithmetic means (bars) and standard errors (whiskers) of maximum carbon isotope discrimination ($\Delta^{13}C_{max}$; a) and isotopic drought sensitivity (m ; b) at different SSTZ regions. N represents northern, C central, S southern, NW northwestern and SW southwestern.

Table 1. Pearson correlation coefficients of maximum carbon isotope discrimination ($\Delta^{13}C_{max}$) and isotopic drought sensitivity (m) with environmental (site, soil and climate) variables.

Category	Variable	$\Delta^{13}C_{max}$	m
Site-related variables	Altitude (m) ¹	−0.09 ^{ns}	0.30 ^{**}
	Sample age ¹	0.21	0.05 ^{ns}
	Lifetime PDSI ¹	0.29 [*]	0.30 ^{**}
Soil variables (from gSSURGO and National Atmospheric Deposition Program)	Sand content (%) ¹	−0.22	−0.24 [*]
	Silt content (%)	0.19 ^{ns}	0.23 [*]
	Bulk density (cm ³ /cm ^{−3}) ¹	0.12 ^{ns}	0.14 ^{ns}
	Organic matter content (%) ¹	0.07 ^{ns}	−0.01 ^{ns}
	Saturated hydraulic conductivity (mm s ^{−1})	−0.24 [*]	−0.26 [*]
	Available water capacity of top 100 cm soils (cm) ¹	0.14 ^{ns}	0.15 ^{ns}
	Water table depth (cm) ²	0.12 ^{ns}	0.34 [*]
	Effective cation exchange capacity (meq 100 g ^{−1}) ¹	0.09 ^{ns}	0.20
	Soil pH ¹	0.05 ^{ns}	0.36 ^{**}
	Nitrogen deposition rate (kg-N ha ^{−1} year ^{−1})	0.12 ^{ns}	0.37 ^{***}
Climate variables (from gridMET and PRISM)	Downward shortwave radiation (W m ^{−2})	−0.22 [*]	−0.36 ^{**}
	Temperature (°C)	−0.19 ^{ns}	−0.34 ^{**}
	Wind speed (m/s) ^{−1}	−0.04 ^{ns}	−0.18 ^{ns}
	Precipitation (mm)	0.03 ^{ns}	0.04 ^{ns}
	VPD (kPa)	−0.14 ^{ns}	−0.40 ^{***}
	Potential evapotranspiration (mm) ¹	−0.25 [*]	−0.39 ^{***}
	Aridity ¹	0.20	0.29 [*]

¹The variables that were used for stepwise regression analysis for $\Delta^{13}C_{max}$ and m . SSTZ regions were also used as an input variable.

²The correlation coefficient was calculated with 48 sites with water table depth shallower than 200 cm, as water table depth deeper than 200 cm was not reported in gSSURGO.

^{ns}Nonsignificant ($P > 0.10$).

^{*}Significant at 5% level.

^{**}Significant at 1% level.

^{***}Significant at 1‰ level.

linear relationship between $\Delta^{13}C$ and proxies of water availability in Equation 2. However, it is consistent with the general understanding of the effects of water availability on plant gas exchange (Walcroft et al. 1997, Tor-ngern et al. 2017). The

Table 2. The final multiple regression models for predicting maximum carbon isotope discrimination ($\Delta^{13}\text{C}_{\text{max}}$) and isotopic drought sensitivity (m) as selected by Bayesian information criterion.

Response variable and model information	Variable	Coefficient estimate	Standard error	t-value	P-value
$\Delta^{13}\text{C}_{\text{max}}$: $R^2 = 0.07$, $P = 0.01$	Intercept	1.96E+01	0.088	221.39	<0.001
	Lifetime PDSI	3.18E-01	0.124	2.56	0.01
m : $R^2_{\text{adj}} = 0.21$, $P < 0.001$	Intercept	1.04E+00	0.473	2.20	0.03
	Soil pH	1.02E-01	0.037	2.74	<0.01
	PET	-9.15E-04	0.0003	-3.16	<0.01

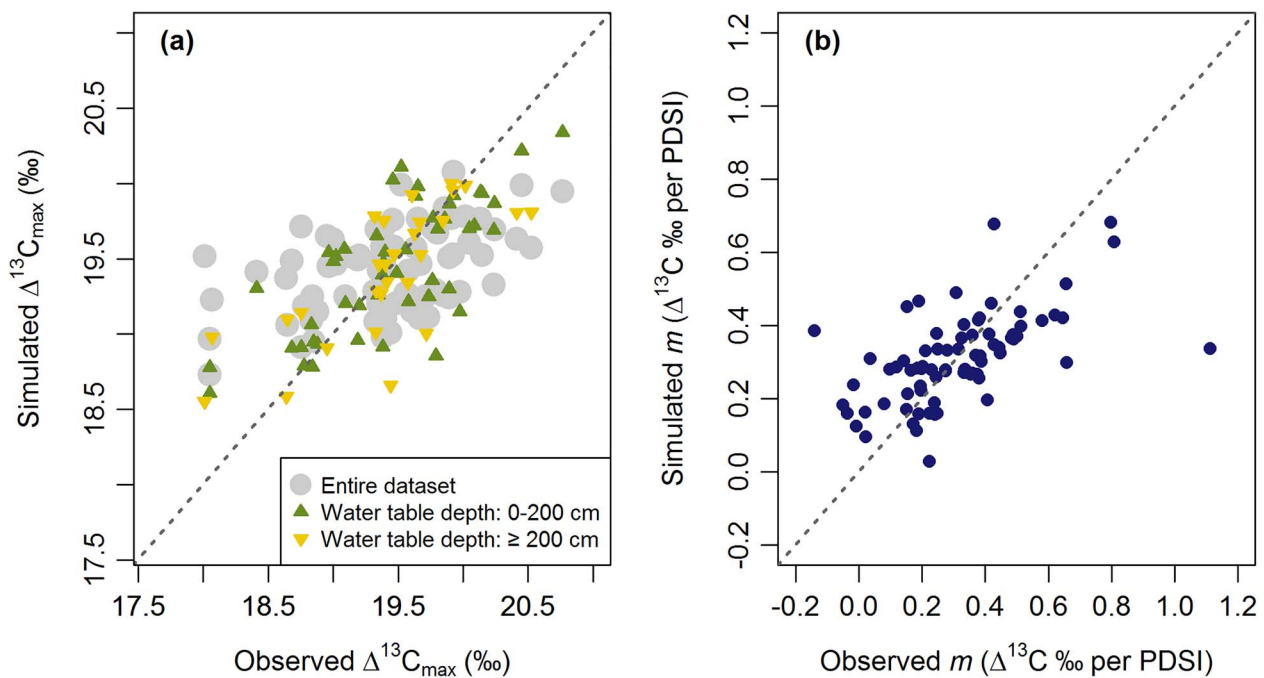


Figure 6. The comparison of observations and simulations of loblolly pine maximum carbon isotope discrimination ($\Delta^{13}\text{C}_{\text{max}}$, a) and isotopic drought sensitivity (m , b) from PLSR models. Dashed line represents 1:1 line. The data from two simulations of $\Delta^{13}\text{C}_{\text{max}}$ were shown in panel a: the first simulation was based on the entire dataset (gray dots) and the second on the water-table-stratified datasets (green triangle and yellow reversed triangles).

detectability of the response threshold in different studies may be a factor of species, the range of environmental conditions and sample size.

The response of $\Delta^{13}\text{C}$ to PDSI was similar to that of canopy conductance to soil moisture (Oren et al. 1998, Gonzalez-Benecke and Martin 2010), suggesting that stomatal constraints predominate variations of $\Delta^{13}\text{C}$ in loblolly pine. While the threshold soil moisture varied from site to site, the corresponding threshold PDSI was around 1.0 across the species' natural range. This convergence indicates a common response of $\Delta^{13}\text{C}$ to different hydroclimate conditions and a generalized framework which can be used for deriving $\Delta^{13}\text{C}_{\text{max}}$ from regression coefficients.

Controls of $\Delta^{13}\text{C}_{\text{max}}$

As $\Delta^{13}\text{C}_{\text{max}}$ is defined as $\Delta^{13}\text{C}$ under well-watered conditions, stomatal conductance is supposed to reach its local maximum,

which is determined by intraspecies genetic variability, hydroclimate conditions and site history (Dow and Bergmann 2014). Other variables that regulate photosynthetic rate and mesophyll conductance are also expected to contribute to the variations in $\Delta^{13}\text{C}_{\text{max}}$. In the current study, downward surface shortwave radiation, sample age (a surrogate for tree height) and different site water status metrics, like lifetime PDSI, soil sand content, K_s , PET and aridity, were found to have significant correlations. The primary role of water availability is also supported by the significant increase in the explained variability in $\Delta^{13}\text{C}_{\text{max}}$ when sites were grouped by water table depth (Figure 6a, Table S3 available as Supplementary data at *Tree Physiology* Online). The effects of water availability and irradiance on $\Delta^{13}\text{C}_{\text{max}}$ are consistent with our understanding of the environmental controls on plant $\Delta^{13}\text{C}$ (Cernusak et al. 2013; but see Note S1 available as Supplementary data at *Tree Physiology* Online for the effect of sample age). The poor correlation between

$\Delta^{13}\text{C}_{\text{max}}$ and nitrogen deposition rate agrees with earlier reports that loblolly pines produced more leaf area but remained similar photosynthetic capacity under fertilization (Samuelson et al. 2001, Gough et al. 2004). Interestingly, $\Delta^{13}\text{C}_{\text{max}}$ was better simulated by PLSR using environmental variables for sites with water table depth shallower than 200 cm (R^2 increased from 0.24 to 0.54, Table S3 available as Supplementary data at *Tree Physiology* Online). As loblolly pine was reported to have roots up to 2–4 m deep (Canadell et al. 1996), it suggests that physiological changes related to rooting depth, such as biomass allocation, may have changed gas exchange strategies of this species.

The $\Delta^{13}\text{C}_{\text{max}}$ correlated most strongly with lifetime PDSI (Table 1), which was also the only variable predicting $\Delta^{13}\text{C}_{\text{max}}$ in the MR model (Table 2). The pivotal impact of lifetime PDSI on $\Delta^{13}\text{C}_{\text{max}}$ was unexpected. We examined site-level PDSI values and found individuals from sites with lifetime PDSI values more negative than -1.0 experienced long-term drought. The correlation between $\Delta^{13}\text{C}_{\text{max}}$ and lifetime PDSI suggests structural and physiological acclimation of trees when exposed to persistent water limitation, which can have long-lasting implications for plant water use and assimilation rate. This finding may complicate the interpretation of tree-ring carbon isotope composition used for paleoclimate reconstruction (Voelker et al. 2014) but may help with ecosystem modeling by providing information on how photosynthetic carbon assimilation responds to environment (Wei et al. 2014, Schönbeck et al. 2021).

Given that adverse environmental conditions during critical or extended periods of development may have lasting effects on organism's development and physiology (Noormets et al. 2008, Correia et al. 2018), we calculated Pearson correlation coefficients between $\Delta^{13}\text{C}_{\text{max}}$ and moving averages of PDSI for different years. The highest correlation was found between $\Delta^{13}\text{C}_{\text{max}}$ and the average PDSI in the first 7 years of establishment ($r = 0.34$, $P = 0.003$, Table S5 available as Supplementary data at *Tree Physiology* Online). This correlation remained significant ($r = 0.31$, $P = 0.02$) even when the 12 youngest sites (9–12 years old) were excluded from the analysis, indicating that water availability during stand establishment and canopy closure has a lasting effect on tree gas exchange traits. Further studies are needed to decipher the mechanism underlying this memory effect.

It has also been shown that plant $\Delta^{13}\text{C}$ can have a strong genetic element (Marguerit et al. 2014, Bartholomé et al. 2015). Although the terms of $\Delta^{13}\text{C}_{\text{max}}$ and m have not been defined previously, by focusing on wet year needles alone, Baltunis et al. (2008) effectively demonstrated a weak but statistically significant heritability for $\Delta^{13}\text{C}$ observed in over 1000 clones in two mesic genetic trial sites from Southern USA, suggesting genotypes of loblolly pine differed in foliar $\Delta^{13}\text{C}_{\text{max}}$. Similarly, in the current study, the spatial correlation of $\Delta^{13}\text{C}_{\text{max}}$ was marginally significant and the difference was significant

only between the extremes (Figure 5a), corroborating the study cited above.

The controls of isotopic drought sensitivity

Among the 76 loblolly pine plantations, we found a strong spatial structure in m (Figure 4b). The pattern aligns with loblolly pine provenances (R. Whetten, 2016; personal communication), which roughly follows the divides based on SSTZ (Schmidtling 2001). This observation is supported by the different m across the five SSTZ regions and the significant differences of m between SSTZ regions S and N/SW (Figure 5b). The effects of genotype and water supply on plant $\Delta^{13}\text{C}$ (or $\delta^{13}\text{C}$) have been studied in manipulated experiments, whereas differences in $\Delta^{13}\text{C}$ (or $\delta^{13}\text{C}$) due to water supply treatments were found to vary among genotypes in some crop species (e.g., White et al. 1990, Ismail and Hall 1992) but not tree species (Zhang and Marshall 1994, Le Roux et al. 1996, Sun et al. 1996, Roupsard et al. 1998, Olivas-García et al. 2000, Aspelmeier and Leuschner 2004, Monclus et al. 2009). In the current study, a clear delineation between genetic and environmental drivers was difficult, as the loblolly pine population structure across its range is associated with underlying environmental variability (Eckert et al. 2010).

Of the environmental variables explored, most showed a statistically significant correlation with m (Table 1). Given the limited range (200 m) of altitude, we suspected its significant correlation with m was due to its covariation with other environmental variables (e.g., with downward surface shortwave radiation, $r = -0.49$, $P < 0.001$; and with K_s , $r = -0.36$, $P = 0.002$). The strong correlation with VPD and soil water availability (lifetime PDSI, soil sand content, K_s and water table depth) indicated the primary role of stomata in affecting m . However, m decreased at sites with high VPD (Table 1), contrary to the physiological relationship between them (Oren et al. 1999), suggesting that such correlation was due to adaptations of local populations. Therefore, it is likely that m was partly affected by tree stomatal sensitivity (Oren et al. 1999), whose variation between genotypes of loblolly pine had been reported by Gonzalez-Benecke and Martin (2010). As stomatal movement is subject to genetic regulation (Bartholomé et al. 2015), m may reflect differences in water use strategies between genotypes. Soil nutrition is usually related to $\Delta^{13}\text{C}$ by its impact on the photosynthetic rate. However, the strong correlation of nitrogen deposition rate, soil pH, soil silt content and ECEC with m indicated that drought-induced changes in $\Delta^{13}\text{C}$ were stronger at better nutrition. This is consistent with the other evidence that nutrient availability may have a direct impact on plant drought tolerance by decreasing the ratio of fine root area to leaf area (Chen et al. 2013, Noormets et al. 2015, Ward et al. 2015). The process facilitates faster growth during ample water availability but exhausts soil moisture reserves faster under drought. Thus, trees with better nutrition

may have a more acute response to the same level of drought stress.

The positive correlation between lifetime PDSI and m indicated that loblolly pines were more sensitive to drought at mesic sites than xeric sites, consistent with similar observations of stomatal conductance responses to drier conditions (Oren et al. 1999, Tor-ngern et al. 2017). When correlations between m and moving averages of PDSI for different years were examined, the highest correlation was found during the recent 5–12 years ($r > 0.30$, $P < 0.01$, Table S5 available as Supplementary data at *Tree Physiology* Online), indicating m was affected by more recent site water status. As our isotopic drought sensitivity measure is novel, the full mechanistic relationship between m and average PDSI in recent years or irradiance is yet to be elucidated.

The implications of $\Delta^{13}\text{C}_{\text{max}}$ and isotopic drought sensitivity

Despite the broad recognition that water availability is often the critical environmental driver of plant $\Delta^{13}\text{C}$ (Cernusak et al. 2013), it is usually not accounted for when analyzing the spatial variability in $\Delta^{13}\text{C}$ (e.g., Cornwell et al. 2018). The two-component approach applied in the current study allowed us to more precisely define the aspects of $\Delta^{13}\text{C}$ evaluated and to compare stands from different ambient conditions. For instance, Cornwell et al. (2018) found that atmospheric pressure, PET and soil pH explained 44% of the variation in plant $\Delta^{13}\text{C}$ on a global scale. In the current study, soil pH correlated strongly with m , but not with $\Delta^{13}\text{C}_{\text{max}}$ (Tables 1 and 2), providing a new angle explaining the effect of soil properties on plant $\Delta^{13}\text{C}$. Although competition was minimized at our study sites due to vegetation control (allowing a better separation of different sources of variation in $\Delta^{13}\text{C}$), this approach could be applied to natural ecosystems as long as the effect of competition is considered in data interpretation. It is also expected that $\Delta^{13}\text{C}_{\text{max}}$ of wood from mature trees might be more responsive to environmental conditions because of a lesser confounding effect of juvenile wood.

The concept of m has unique implications for plant breeding programs. Intrinsic WUE, inferred from plant $\Delta^{13}\text{C}$, is widely used in crop breeding programs to select drought-resistant genotypes (Condon et al. 2004). A determination of iWUE is usually conducted by sampling plant tissues at one time from plants growing in a well-watered environment. The limitation of this approach is that iWUE describes water use efficiency, but it does not describe a drought response or drought resistance. Therefore, if drought resistance is of interest, then m may be more informative about the plant's actual loss of assimilatory potential under water limitations. Therefore, we recommend that both $\Delta^{13}\text{C}_{\text{max}}$ and m be tracked in such genotype selection studies.

Conclusions

Partitioning tree-ring cellulose $\Delta^{13}\text{C}$ data to well-watered baseline, $\Delta^{13}\text{C}_{\text{max}}$, and isotopic drought sensitivity, m , allowed for the comparison of 76 loblolly pine plantations across the species' natural range, varying in genetic makeup, age, climate and edaphic conditions. Both metrics exhibited different degrees of spatial structure, attributable to underlying genetic and environmental variations (see Note S2 available as Supplementary data at *Tree Physiology* Online for methodological and modeling considerations). Findings from this work have important implications for evaluating the potential impacts of global climate change.

The $\Delta^{13}\text{C}_{\text{max}}$ exhibited some spatial structure and was impacted primarily by site water status. As a proxy for long-term water availability, lifetime PDSI was most highly correlated with $\Delta^{13}\text{C}_{\text{max}}$, indicating the profound effects of long-term water status in changing loblolly pines' baseline physiology. In contrast, the parameter m , which describes the slope of $\Delta^{13}\text{C}$ against PDSI, exhibited a highly significant spatial correlation across the range and represents a novel metric in characterizing $\Delta^{13}\text{C}$ dynamics. The Atlantic coastal plain sites in FL and GA were less drought-sensitive than sites further inland. We speculate that this metric measures stomatal sensitivity to water availability, indicating potential use for breeding programs for selecting drought-resistant genotypes.

This two-component approach accounts for temporal changes of $\Delta^{13}\text{C}$ due to local water availability and permits to make cross-site comparisons, allowing us to identify the critical genetic and environmental controls of plant $\Delta^{13}\text{C}$ at different levels. With projections of increasing likelihood and severity of drought in the southeastern USA, loblolly pine is expected to experience more long-term drought stress, the baseline physiology and $\Delta^{13}\text{C}_{\text{max}}$ of this species may continue to shift. In this light, the differences in stomatal sensitivity to water stress, reflected partly by isotopic drought sensitivity, may be used to direct future seed source deployment.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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gSSURGO data. Dr Liangxia Zhang extracted site altitudes and total nitrogen deposition rates.

Conflict of interest

The authors have no conflicts of interest to declare.

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Data and materials availability

The data that support the findings of this study are available in the Terra-C database at <http://terrac.ifas.ufl.edu/>.

Authors' contributions

T.A.M., J.-C.D., T.R.F., J.S.K., S.M., A.N., G.S., L.S., J.V. and R.E.W. designed the study. M.A., J.C., M.A.L., C.M. and W.L. conducted field work. W.L. performed the in-lab measurements and data analysis. W.L. wrote the manuscript with substantial inputs from all authors.

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