Relationships between Leaf Anatomy and Physiological Functioning of Southern US Oak Species Differing in Flood Tolerance

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Abstract: Research Highlights: Bottomland oaks receive less attention than upland species, however their adaptations to flooding and summer water stress will extend our understanding of the oak genus and links between physiology and leaf anatomy. Background and objectives: Determining links between leaf anatomy and physiology can aid in parameterizing dynamic global vegetation models for oak systems, therefore we sought to (1) compare leaf anatomic, nutrient, and physiological parameters for bottomland oaks differing in flood tolerance, (2) determine correlations across parameters and determine which anatomic and nutrient parameters best predict photosynthetic capacity metrics, and (3) compare these data with reported literature values for oaks across the globe. Materials and Methods: We measured CO₂ response curves (A/C) on leaves from Nuttall, Shumard, swamp chestnut, water and white oak seedlings planted in the Southeastern United States (US) and estimated stomatal size and density, epidermal cell size, vein density, leaf mass per area (LMA) and nitrogen (N) concentrations. Principal component analysis among these leaf anatomic and nutrient parameters was used to determine the best predictors of photosynthetic parameters including Rubisco-limited carboxylation rate (V_{Cmax}) and electron transport limited carboxylation rate (J_{max}). Results: We found that although physiological parameters were similar, flood-tolerant oaks had lower leaf N concentrations and larger, more infrequent stomata than less flood-tolerant species. Leaf epidermal properties were correlated with N concentrations and a principal component capturing this correlation as well as principal components correlated with mesophyll conductance and leaf carbon concentrations were found to best explain variation in V_{Cmax} and J_{max}. These Southeastern US oaks exhibited similar leaf physiological parameters and LMA as oaks reported in the literature but differed in leaf epidermal and stomatal properties as well as leaf N concentrations increasing the reported range of these parameters within the oak genus. Conclusions: Therefore, leaf anatomy and nutrient parameters as opposed to physiology differed across flood tolerance and between bottomland oaks and broader literature values.

Keywords: A/C curves; Quercus; photosynthetic capacity; principal component analysis; structure-function relationships; water use efficiency

1. Introduction

The oak genus is widespread throughout much of North America providing benefits such as timber production and wildlife resources. Ecologically, oaks have been described as having a stress tolerant physiological strategy and compete well under conditions of drought stress [1], increased fire frequency [2] and low nutrient availability [3]. Oaks tolerate drought stress conditions through deep rooting [4] and higher carbon allocation belowground [5,6] and/or withstanding more negative leaf water potentials [7,8] compared with more mesic species. However, the majority of research on
oak physiology has been performed on species that are located on upland sites with species prevalent on bottomland sites receiving much less attention. Due to geomorphological patterns, bottomland floodplain forests tend to be found in the South Central and Southeastern United States (US) on the Gulf and Atlantic Coastal Plains [9]. These sites are characterized by the extent and duration of seasonal flooding with differing species assemblages occurring along a gradient in elevation and flooding. Even among oak species, differences in flooding tolerance exist with overcup oak (Quercus lyrata Walter) and Nuttall oak (Q. texana Buckley) occurring at the swamp edge, water oak (Q. nigra L.) and willow oak (Q. phellos L.) occurring at slightly higher elevations and cherrybark oak (Q. pagoda Raf.), Shumard oak (Q. shumardii Buckland), and swamp chestnut oak (Q. michauxii Nutt.) occurring on better drained ridges [9]. Flood-tolerant species exhibit morphological adaptations including hypertrophied lenticels, adventitious root growth and aerenchyma formation [10]. Nevertheless, soil saturation introduces additional stresses for trees including anaerobic conditions as well as reduced nutrient availability due to nitrate leaching and denitrification [11]. In addition, although seasonal flooding occurs generally during the dormant season, soil texture and topography combined with high temperatures and evaporative demand in the Southern US can also create low water availability conditions during the growing season [12]. Indeed, flooding conditions can lead to root mortality that may exacerbate water stress during the growing season in less flood tolerant species. Therefore, study of oak species typical to bottomland forest conditions and varying flood tolerance can elucidate the physiological strategies that may be necessary to tolerate the unique stress conditions of these sites and increase our understanding of the oak genus as a whole.

Because leaf anatomy and epidermal cell structure determines conductance for carbon dioxide and water vapor to enter and exit the leaf, species adapted to differing environmental growing conditions should alter leaf properties to provide optimal functioning. Ashton and Berlyn [13] found that the more drought tolerant oak species, Q. velutina Lam., exhibited greater plasticity of leaf anatomic properties under differing light conditions and had the lowest stomatal area per unit leaf area while drought intolerant Q. rubra L. exhibited the opposite characteristics. However, Abrams et al. [14] found that wet adapted species had the smallest guard cell lengths and lowest leaf thickness and leaf mass per area compared with xeric and mesic species. Total stomatal area per unit leaf area is a function of both stomatal size and frequency and Franks et al. [15] found in Eucalyptus globulus Labill. that leaves produced smaller, more frequent stomata to increase maximum stomatal conductance and larger, less frequent stomata to decrease maximum stomatal conductance. Smaller stomata have also been shown to respond more rapidly to changes in environmental conditions and are associated with species from drier habitats with greater water use efficiency [16,17]. The size and expansion of leaf epidermal cells will also be affected by environmental conditions such as water stress, temperature, and light levels and will, in turn, affect the final density of stomata [18] as well as leaf venation [19] suggesting that epidermal cell expansion may coordinate water supply (via leaf veins) with water loss (via stomata) [20,21]. Kürschner et al. [22] found that Q. petraea (Matt.) Liebl. leaves grown under high temperature and humidity had lower stomatal densities but larger, less frequent epidermal cells and Beerling and Chaloner [23] found that Q. robur leaves formed under warmer, summer temperatures had lower stomatal densities than leaves formed under cooler temperatures. Kwak et al. [24] found that Q. suhier L. grown under water stress conditions produced leaves with increased stomatal frequency and smaller epidermal cells. Therefore, a combination of leaf epidermal properties may be informative to species’ adaptation to their growing environment.

Given the links between environmental conditions and leaf epidermal properties, leaf anatomy should correlate with physiological functioning to optimize CO2 uptake for a given water loss. These links between leaf structure and function could help inform dynamic global vegetation models in order to predict landscape scale carbon uptake because input parameters including maximum Rubisco-limited carboxylation rate (V_{\text{Cmax}}) and maximum electron transport-limited carboxylation rate (J_{\text{Lmax}}) estimated from A/Ci models [25] can be difficult to estimate as well as standardize across various studies [26,27]. Another parameter derived from A/Ci curves that is used in land surface models (e.g., Integrated Biosphere Simulator [28]; and Ecosystem Demography model [29]) to describe the relationship between stomatal conductance and photosynthesis is the “Ball–Berry"
parameter [30,31]. This parameter is defined as the slope of the linear relationship between stomatal conductance and photosynthetic assimilation scaled with relative humidity and CO$_2$ concentration estimated at the leaf surface. If leaf anatomic properties exhibit significant correlation with physiological parameters of $V_{\text{Cmax}}$ and $J_{\text{max}}$, then leaf anatomy may do a better job of aggregating environmental and physiological information about leaves into parameters that are more easily measured and therefore more readily compared across differing studies than physiological parameters alone.

The objectives of this study were (1) Compare leaf physiological, anatomic, and nutrient properties across oak seedlings varying in flood tolerance and planted in the Southeastern US Gulf Coastal Plain including Nuttall and water oak which tend to occur on lower elevation sites, tolerate flooding and likely experience less moisture stress, Shumard and swamp chestnut oak which occur on higher elevation sites and are less tolerant of flooding but potentially are more tolerant of moisture stress, and white oak (Q. alba) which exhibits a wide ecological range. We hypothesize that species prevalent in better drained sites (Shumard oak, swamp chestnut oak, white oak) will exhibit parameters related to drought tolerance (smaller, more frequent stomata, higher leaf mass per area (LMA), higher water use efficiency) compared with Nuttall oak and water oak which tolerate more poorly-drained sites. (2) Determine if leaf anatomic and nutrient properties are correlated with one another across oak species. We hypothesize that, due to correlation between using water and nitrogen efficiently in photosynthetic reactions, leaf stomatal properties will correlate with leaf nitrogen properties. (3) Use principal component analysis to generate predictor variables from leaf anatomic and nutrient properties and develop models to predict $V_{\text{Cmax}}$ and $J_{\text{max}}$. We hypothesize that both leaf N content and leaf stomatal parameters will be important in predicting $V_{\text{Cmax}}$ and $J_{\text{max}}$. (4) Compare physiological and anatomic parameters in these five oak species found in the Southeastern US with literature values for oak species measured in locations across the globe. We hypothesize that, given the need for these species to tolerate flooded conditions that may not be found in other systems, leaf properties of Southeastern US oaks in this study will differ from literature values. These data will allow for a comparison of several less-studied bottomland oak species with upland oaks to determine where these southern oak species fit within the oak genus in terms of leaf anatomy and physiological functioning. This may shed light on recent oak decline throughout the eastern US since oak abundance has decreased to a much greater extent in Central upland forests and midwestern forest/prairie transitions of the US compared with southern bottomland forests [32]. Overall, the results of this study will determine how well easily measured anatomic and nutrient parameters are correlated with physiological functioning in oak species across a flood tolerance range.

2. Materials and Methods

This study was conducted at six sites in Southeastern Mississippi (MS), each less than 70 km from the Gulf of Mexico with three located in George County, two in Stone County, and one in Pearl River County, MS. Sites were characterized as fine sandy loams but varied in terms of drainage from well drained to poorly drained soils (Web Soil Survey; https://websoilsurvey.sc.egov.usda.gov/). The region has a subtropical, humid climate with mild winters and hot summers and temperatures average 9.9 °C in winter (DJF) and 27.1 °C in summer (JJA). The region receives about 1565 mm of precipitation annually. Five oak species ranging in distribution and habitat preference were included in this study; two from the white oak group (white oak and swamp chestnut oak) and three from the red oak group (Nuttall oak, Shumard oak, and water oak). White oak has the largest distribution of the measured species with a range size of about 2.4 million km$^2$ [33] and occurring throughout the eastern US from Canada to the Gulf Coast. It prefers dry upland sites or well-drained bottomland sites [34]. Shumard oak has the next most northerly distribution of the species studied extending into Missouri and Indiana but is largely absent from the Appalachian Mountain region. It prefers well drained alluvial soils [34]. Swamp chestnut oak has a more southerly distribution than white oak and Shumard oak extending across the Southeastern US from the Gulf Coast to Maryland and Southern Illinois and Indiana with an overall distribution of almost 900,000 km$^2$ [33]. It prefers moist soils and well-drained bottomland sites [34]. Water oak has a distribution that is similar to swamp chestnut
oak but slightly more southern extending in the Southeastern US from Tennessee to the Gulf Coast and along the Atlantic Coast to Delaware with an overall range area of over 1 million km² [33]. It is found on moist upland and wet bottomland sites [34]. Nuttall oak has the smallest distribution of the studied species at almost 83,000 km² [33] and occurring in the South Central US in Eastern Texas, Louisiana, Arkansas, Mississippi, and Western Alabama. It prefers wet, alluvial soils [34]. Individuals used for this study were bareroot and containerized seedlings that were planted in February and March prior to the growing season in which they were measured. Each site was cleared of previous herbaceous vegetation prior to planting.

In the growing seasons (May–July) of 2016 and 2018, response of photosynthetic assimilation (A) to changing leaf internal CO₂ concentrations (Ci), or A/Ci curves, were measured on 110 oak seedlings (14 Nuttall oaks, 27 Shumard oaks, 12 swamp chestnut oaks, 28 water oaks, 29 white oaks) from the six study sites using a LI-COR 6400 XTP portable photosynthesis system (LI-COR Biosciences Inc., Lincoln, NE, USA) with a red/blue light source. One year old bare root and containerized seedlings obtained from local nurseries were planted at study sites in the dormant season before the measurement period [35,36]. Individuals measured for this study were chosen at random from seedlings that appeared to be in good health and represent both bareroot and containerized seedlings of similar age for each species. Temperatures in the leaf chamber during measurements averaged about 25.2 °C and relative humidity about 41%. A/Ci curves were produced by setting light levels to saturating conditions of 1500 μmol m⁻² s⁻¹ and varying CO₂ concentrations in the leaf chamber beginning at 400 ppm, progressively dropping to 50 ppm and then returning to 400 ppm and increasing incrementally until a level of 800 ppm was reached. From the initial measurements on each curve (at ambient CO₂ concentration and saturating light levels), net photosynthetic assimilation (A; μmol CO₂ m⁻² s⁻¹), transpiration (E; mmol H₂O m⁻² s⁻¹), and stomatal conductance (gs; mol H₂O m⁻² s⁻¹) values were noted and water use efficiency parameters were calculated. Water use efficiency (WUE; μmol CO₂ mmol H₂O⁻¹) was calculated as the ratio of photosynthesis and transpiration and intrinsic water use efficiency (iWUE; μmol CO₂ mol H₂O⁻¹) was calculated as the ratio of photosynthesis and stomatal conductance.

A/Ci curves were analyzed using the Microsoft excel solver program developed by Sharkey et al. [37] based on theory and equations from Farquhar et al. [25]. This program fits nonlinear regression equations to the measured data to estimate parameters that describe the shape of these curves including the maximum Rubisco-limited carboxylation rate (V_Cmax), the maximum electron transport-limited carboxylation rate (J_max), and the triose phosphate utilization limited-carboxylation rate (TPU). This program also estimates daytime respiration rates (R_day) and mesophyll conductance (g_m) from the A/Ci curve data. All parameters are presented based on a common temperature of 25 °C.

Data from A/Ci curves were also used to estimate the Ball–Berry parameter (m) [30,38] based on the following equation:

\[
g_s = m \times \frac{A \times rh_s}{C_s - \Gamma} + g_0 \tag{1}
\]

where \(rh_s\) is relative humidity at the leaf surface, \(C_s\) is CO₂ concentration at the leaf surface (μmol mol⁻¹), and \(\Gamma\) is the CO₂ compensation point (μmol mol⁻¹) of the corresponding A/Ci curve and was calculated as the x-intercept of a linear regression fitted to the initial points on each A/Ci curve.

Diffusional limitation to photosynthesis (%) was calculated using the following equation:

\[
\text{Diffusional limitation} = 100 \times \left(1 - \frac{A}{A_0}\right) \tag{2}
\]

where \(A_s\) is the photosynthetic assimilation rate calculated if \(C_i = C_s\) of 400 ppm and \(A\) is the measured photosynthetic assimilation rate at \(C_i = 400\) ppm. Therefore, \(A_s\) represents the assimilation rate assuming infinitely large boundary layer, stomatal and mesophyll conductance and was calculated by fitting a nonlinear equation to each A/Ci curve and setting \(x = 400\).
After each A/C curve was measured, leaves were collected, placed in zip-top bags and kept in a cooler until they could be transported back to the lab. Fresh leaves were scanned on a flatbed scanner (CanoScan LiDE 210; Canon; Melville, NY, USA) with a scaling factor so that individual leaf areas (cm²) could be calculated using Image J software version 1.48 (National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/). Then, in discrete locations between the main leaf veins, clear nail polish was applied to the underside of leaves, allowed to dry, removed and placed on a microscope slide with a cover slip. With all nail polish removed, individual leaves were placed into a drying oven at 65 °C for at least three days. Leaf dry mass was then measured and leaf mass per unit area (LMA; g m⁻²) was calculated.

Leaf epidermal peels were viewed using an Olympus Vanox compound light microscope (Waltham, MA, USA) with a digital camera (AmScope FMA050, Irvine, CA, USA) attached. Ten images were obtained under the 20× objective (approximately 330× magnification) for each leaf slide and used to estimate stomatal density (no. mm⁻²) and vein density (%) using Image J software. Vein densities were estimated as the proportion of total area in the microscope field of view occupied by leaf veins. Ten images were also obtained under the 40× objective (approximately 660× magnification) for each leaf and used to estimate stomatal pore length (µm; measured as the distance between where guard cells connect), epidermal cell size (µm²), and stomatal size (µm²) using Image J. Stomatal size was calculated as the cross-sectional area of the stomatal pores within the inner boundary of the guard cells. Approximately ten stomata or epidermal cells were measured from each image. From these measurements, epidermal cell density (no. mm⁻²) was calculated by estimating the area occupied by epidermal cells (total area minus vein area and stomatal area (guard cell apparatus size * density)) and dividing this area by the average size of epidermal cells for each individual leaf. Stomatal pore index was calculated by multiplying stomatal size (µm²) by stomatal density (no. mm⁻²).

Dried leaves from each individual were ground and passed through a 250 µm sieve. The resulting powder was then placed in tin capsules and analyzed for carbon (C) and nitrogen (N) concentration using an elemental combustion analyzer (ECS 4010 CHNO-S Elemental Analyzer, Costech, Valencia, CA, USA). Leaf N concentrations were multiplied by LMA to calculate leaf nitrogen per unit leaf area (Narea; g m⁻²) and net photosynthetic assimilation rates at saturating light and ambient atmospheric CO₂ were divided by Narea to calculate photosynthetic nitrogen use efficiency (PNUE; µmol g⁻¹ s⁻¹).

In order to compare physiological measurements from the Southeastern US bottomland oaks in this study with other species in the oak genus, a literature review was performed using Google Scholar. Data for leaf anatomic and physiological parameters including V\text{Cmax}, J\text{max}, Ball–Berry parameter, diffusional limitation, LMA, stomatal length, stomatal density, epidermal cell density, N\text{area}, and PNUE were extracted from tables, figures and supplementary information from a total of 40 publications (Table 1). If the reviewed literature compared oaks across an age range, only seedling data were used since oaks in the present study were seedlings and if treatments were performed, control data were used. All data were converted to common units. If LMA was not reported in a publication that presented leaf N concentration, LMA from other literature on that species (seedling data, if available) was used to convert N concentration to N\text{area}. Only V\text{Cmax} and J\text{max} data that were reported at a reference temperature of 25 °C were used.

| Citation                  | Parameters          | Species                  |
|---------------------------|---------------------|--------------------------|
| Abrams and Kubiske [39]   | LMA, Stom. dens.    | Q. ellipsoïdalis, Q. macrocarpa, Q. velutina, Q. alba, Q. rubra |
| Ashton and Berlyn [13]    | Stom. dens.         | Q. coccinea, Q. rubra, Q. velutina |
| Bahamonde et al. [40]     | LMA, Stom. dens.    | Q. petraea               |
| Beerling and Chaloner [23]| Stom.dens.          | Q. robur                 |
| Billings et al. [41]      | N\text{area}        | Q. rubra                 |
| Cambi et al. [42]         | V\text{Cmax}, J\text{max}, Diff. lim., LMA, N\text{area} | Q. robur |
| Authors          | Variables | Species                                                                 |
|-----------------|------------|--------------------------------------------------------------------------|
| Coomes et al. [43] | LMA, Stom. dens. | Q. castaneifolia, Q. cerris, Q. georgiana, Q. ilex, Q. nigra, Q. petraea, Q. xanthophylla, Q. rubra, Q. rubra, Q. serrata |
| Contran et al. [44] | VC_{\text{max}}, J_{\text{max}}, Diff. lim. | Q. pubescens                                                              |
| de la Riva et al. [45] | LMA, N_{\text{area}} | Q. coccifera, Q. faginea, Q. ilex                                       |
| Du et al. [46] | N_{\text{area}} | Q. variabilis                                                              |
| Gazal and Kubiske [12] | LMA, Stom. dens. | Q. pagoda, Q. shumardii                                                   |
| Grassi and Magnani [47] | VC_{\text{max}} | Q. rubra                                                                  |
| Gu et al. [27] | VC_{\text{max}}, J_{\text{max}} | Q. alba, Q. velutina                                                       |
| Harley and Baldocchi [48] | Ball-Berry | Q. alba                                                                   |
| Jafarnia et al. [49] | VC_{\text{max}} | Q. brantii                                                                 |
| Juarez-Lopez et al. [50] | VC_{\text{max}}, J_{\text{max}}, Diff. lim., LMA, N_{\text{area}} | Q. ilex, Q. faginea                                                        |
| Kazda et al. [51] | PNUE | Q. robur                                                                   |
| Kurschner et al. [52] | Stom. dens., Epi. cell dens. | Quercus fossil leaves                                                     |
| Kurschner et al. [22] | Stom. dens., Epi. cell dens. | Q. petraea                                                                 |
| Li et al. [53] | VC_{\text{max}}, J_{\text{max}} | Q. myrtifolia, Q. geminata                                                 |
| Manter and Kerrigan [54] | VC_{\text{max}} | Q. garrsana, Q. robra                                                     |
| Mediavilla and Escudero [55] | PNUE | Q. ilex, Q. faginea, Q. pyrenaica                                         |
| Mediavilla et al. [56] | LMA, N_{\text{area}}, PNUE | Q. rotundifolia, Q. faginea                                                |
| Mediavilla et al. [57] | LMA, N_{\text{area}} | Q. pyrenaica, Q. faginea, Q. suber, Q. ilex                               |
| Miller-Rushing et al. [58] | Stom. dens. | Quercus spp.                                                               |
| Peguero-Pina et al. [59] | VC_{\text{max}}, J_{\text{max}}, LMA | Q. crassulae, Q. agrifolia, Q. wilsizeni, Q. coccifera, Q. ilex, Q. suber |
| Pena-Rojas et al. [60] | VC_{\text{max}}, J_{\text{max}}, Diff. lim. | Q. ilex                                                                   |
| Ramirez-Valiente et al. [61] | LMA, N_{\text{area}}, Stom. dens. | Q. oleoides                                                                |
| Renninger et al. [62] | VC_{\text{max}}, J_{\text{max}}, LMA, N_{\text{area}}, PNUE | Q. prinis, Q. velutina                                                     |
| Roussil et al. [63] | VC_{\text{max}}, LMA, N_{\text{area}}, Stom. dens., PNUE | Q. robur                                                                  |
| Rzigui et al. [64] | VC_{\text{max}}, J_{\text{max}}, LMA, Diff. lim. | Q. suber                                                                  |
| Turnbull et al. [65] | VC_{\text{max}}, J_{\text{max}}, LMA, N_{\text{area}}, PNUE | Q. rubra, Q. prinus                                                       |
| Valladares et al. [66] | Stom. dens. | Q. robur                                                                   |
| Watanabe et al. [67] | VC_{\text{max}}, J_{\text{max}}, Diff. lim., LMA, N_{\text{area}} | Q. mongolica                                                              |
| Watanabe et al. [68] | VC_{\text{max}}, J_{\text{max}}, Diff. lim., LMA, N_{\text{area}} | Q. glauca, Q. myrsinaefolia                                               |
| Weber and Gates [69] | Ball-Berry | Q. rubra                                                                   |
| Xing et al. [70] | LMA, N_{\text{area}} | Q. wutaihanica                                                            |
| Xu and Baldocchi [71] | Ball-Berry | Q. douglasii                                                               |
| Zhang et al. [72] | PNUE | Q. guayacifolia, Q. pannosa                                                |
| Zhang et al. [73] | N_{\text{area}} | Q. acutissima, Q. rubra, Q. palustris, Q. texana, Q. falcata, Q. coccinea, Q. virginiana, Q. phellos |

Leaf anatomical and physiological parameters were compared across oak species using analysis of variance (ANOVA) and Tukey multiple comparison tests in R version 3.1.2 [74]. Linear and non-linear relationships between anatomic and physiological parameters were fitted using SigmaPlot version 13 (Systat Software Inc. San Jose, CA, USA) in order to determine r² and p-values for relationships.

In order to determine which leaf anatomic and nutrient parameters best explained physiological parameters including VC_{\text{max}} and J_{\text{max}}, principal component analysis (PCA) was used since many leaf anatomy and nutrient parameters were highly correlated with one another. The resulting principal components were orthogonal and uncorrelated with each other and were then used as explanatory variables in multiple linear regression models to predict physiological parameters for each oak species. To develop principal components that would be used to predict physiological variables, parameters describing leaf anatomy (leaf area, LMA, vein density, stomatal density, stomatal size, stomatal pore length, epidermal cell size, epidermal cell density, stomatal pore index, and mesophyll conductance) and leaf nutrients (C and N concentration, N_{\text{area}}, and CN ratio) were included in the dataset. The “prcomp” function in R was used to derive principal components with each variable zero centered and scaled by its unit variance. To determine the best fit models to predict VC_{\text{max}} and J_{\text{max}}, all derived principal components were used as explanatory variables as well as their interaction with species in multiple linear regression model selection. Ten candidate best fit models for each physiological parameter were chosen using the regsubsets function in the leaps package [75] in R.
based on adjusted $r^2$ values. This function compares all combinations of terms included in the full model and ranks models based on specified criteria. For each candidate model, cross validation (with 10 folds) using the CVlm function in the DAAG package [76] was used to determine the root mean square error of each model and the model with the lowest RMSE was selected and reported.

3. Results

3.1. Species Comparisons

In general, white oak and water oak tended to exhibit higher photosynthetic capacities than Nuttall, Shumard and swamp chestnut oak with white oak having about 29% higher $V_{\text{Cmax}}$ than Nuttall and Shumard oak and white oak and water oak having 47% and 63% higher $J_{\text{max}}$ and TPU respectively than the other oak species (Table 2).

### Table 2. Mean and standard error (in parentheses) of anatomic and physiological parameters for Nuttall, Shumard, swamp chestnut, water, and white oak planted on the Gulf coastal plain.

|                     | Nuttall | Shumard | Swamp Chestnut | Water | White |
|---------------------|---------|---------|----------------|-------|-------|
| $V_{\text{Cmax}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 71.0 (6.2)$^a$ | 70.6 (4.2)$^a$ | 88.4 (6.9)$^c$ | 84.2 (3.3)$^a$ | 90.3 (3.9)$^a$ |
| $J_{\text{max}}$ ($\mu$mol m$^{-2}$ s$^{-1}$) | 94.2 (10.1)$^b$ | 101.7 (6.4)$^b$ | 124.0 (11.4)$^c$ | 156.8 (4.1)$^a$ | 157.2 (5.6)$^c$ |
| TPU ($\mu$mol m$^{-2}$ s$^{-1}$) | 6.2 (0.7)$^a$ | 6.7 (0.4)$^a$ | 8.1 (0.8)$^a$ | 11.5 (0.3)$^a$ | 11.3 (0.4)$^a$ |
| Meso. conductance ($g_{\text{m}}$; $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$) | 1.05 (0.16)$^a$ | 1.26 (0.21)$^a$ | 1.47 (0.27)$^a$ | 1.55 (0.29)$^a$ | 1.16 (0.19)$^a$ |
| Diffusional limitation (%) | 25.7 (3.4)$^a$ | 29.2 (1.7)$^a$ | 32.3 (2.8)$^a$ | 41.2 (3.2)$^a$ | 32.2 (3.0)$^a$ |
| Ball–Berry parameter (m; unitless) | 9.6 (0.9)$^{bc}$ | 7.1 (0.7)$^c$ | 6.4 (0.9)$^c$ | 3.9 (0.5)$^b$ | 4.6 (0.8)$^b$ |
| N concentration (%) | 1.61 (0.07)$^b$ | 1.97 (0.12)$^c$ | 2.60 (0.08)$^c$ | 1.67 (0.09)$^a$ | 1.79 (0.13)$^a$ |
| N$_{\text{m}}$ (g m$^{-3}$) | 1.28 (0.06)$^a$ | 1.57 (0.12)$^a$ | 2.35 (0.12)$^a$ | 1.85 (0.10)$^b$ | 1.73 (0.14)$^b$ |
| PNUE ($\mu$mol g$^{-1}$ s$^{-1}$) | 4.76 (0.41)$^a$ | 3.03 (0.35)$^b$ | 2.99 (0.44)$^a$ | 1.82 (0.21)$^b$ | 2.42 (0.31)$^b$ |
| C concentration (%) | 47.4 (0.3)$^a$ | 47.3 (0.3)$^b$ | 47.7 (0.2)$^a$ | 49.0 (0.19)$^a$ | 47.1 (0.15)$^a$ |
| CN ratio | 30.6 (1.7)$^a$ | 26.8 (1.9)$^a$ | 18.5 (0.5)$^a$ | 30.6 (1.7)$^a$ | 29.0 (2.5)$^a$ |
| Leaf area (cm$^2$) | 27.2 (3.1)$^a$ | 29.0 (2.7)$^a$ | 43.9 (4.2)$^a$ | 10.0 (0.9)$^a$ | 30.6 (3.4)$^a$ |
| LMA (g m$^{-2}$) | 79.5 (1.9)$^a$ | 78.6 (2.0)$^a$ | 89.9 (3.4)$^c$ | 110.6 (3.5)$^c$ | 99.6 (3.7)$^a$ |
| Vein density (%) | 11.3 (0.5)$^a$ | 13.8 (0.5)$^b$ | 12.6 (0.6)$^{bc}$ | 14.8 (0.5)$^a$ | 24.3 (0.6)$^a$ |
| Stomatal pore length (μm) | 13.0 (0.2)$^a$ | 10.3 (0.2)$^c$ | 9.3 (0.2)$^a$ | 13.4 (0.3)$^a$ | 11.8 (0.3)$^b$ |
| Stomatal size (μm$^2$) | 85.7 (3.0)$^a$ | 53.2 (1.8)$^b$ | 48.9 (1.8)$^c$ | 83.1 (2.9)$^a$ | 50.8 (2.0)$^a$ |
| Stomatal density (no. mm$^{-2}$) | 625 (34)$^a$ | 772 (28)$^a$ | 1230 (46)$^a$ | 645 (24)$^c$ | 1060 (45)$^c$ |
| Epidermal cell size (μm$^2$) | 202 (9)$^a$ | 163 (5)$^a$ | 105 (4)$^c$ | 214 (6)$^c$ | 127 (6)$^c$ |
| Epidermal cell density (no. mm$^{-2}$) | 3120 (110)$^a$ | 3910 (110)$^c$ | 6590 (260)$^a$ | 2840 (98)$^c$ | 4860 (240)$^c$ |
| Stomatal pore index | 0.053 (0.002)$^{bc}$ | 0.040 (0.002)$^c$ | 0.060 (0.002)$^a$ | 0.053 (0.002)$^{bc}$ | 0.052 (0.001)$^b$ |

$^a$ Different superscript letters signify significant differences at $=0.05$. Abbreviations: Rubisco-limited carboxylation rate ($V_{\text{Cmax}}$), electron transport limited carboxylation rate ($J_{\text{max}}$), triose phosphate limited-carboxylation rate (TPU), photosynthetic nitrogen use efficiency (PNUE), leaf mass per area (LMA).

Mesophyll conductances did not differ significantly across species and averaged about 1.28 $\mu$mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$. However, water oak exhibited diffusional limitations to photosynthesis that were about 50% greater than Nuttall and Shumard oak but were similar to swamp chestnut and white oak (Table 2). Water oak also had the lowest Ball–Berry parameter and Nuttall exhibited the highest. Swamp chestnut oak leaves had the highest N content on a per unit mass (N conc.) and per unit area (N$_{\text{m}}$) basis and the lowest CN ratios with leaf N concentrations being about 45% higher than other measured oak species (Table 2). Leaf nitrogen concentrations were also significantly correlated with
leaf epidermal properties across species displaying a positive correlation with both stomatal density (N conc. = 9.74 \times 10^{-4} \times \text{stom. dens.} + 1.08; Figure 1a) and epidermal cell density (N conc. = 2.12 \times 10^{-4} \times \text{epi. cell dens.} + 1.04; Figure 1b) in particular. Nuttall oaks exhibited the highest photosynthetic nitrogen use efficiencies (PNUE) that were about 85% higher than other oak species (Table 2). Photosynthetic nitrogen use efficiencies were significantly positively correlated with water use efficiency (PNUE = 0.935 \times \text{WUE} + 0.989; Figure 2a), but negatively correlated with calculated diffusional limitations to photosynthesis (PNUE = −0.0620 \times \text{diff. lim.} + 4.94; Figure 2b).

Figure 1. Leaf N concentration (%) vs. (a) stomatal density (no. mm^{-2}; r^2 = 0.21; p < 0.0001) and (b) epidermal cell density (no. mm^{-2}; r^2 = 0.31; p < 0.0001) for Nuttall (dark blue, up triangle), Shumard (green circle), swamp chestnut (yellow square), water (light blue down triangle), and white oak (magenta diamond).

Figure 2. Photosynthetic nitrogen use efficiency (PNUE; \mu\text{mol g}^{-1} \text{s}^{-1}) vs. (a) instantaneous water use efficiency estimated from gas exchange data (water use efficiency (WUE); \mu\text{mol mmol}^{-1}; r^2 = 0.36; p < 0.0001) and (b) diffusional limitation to photosynthesis (%; r^2 = 0.18; p < 0.0001) for Nuttall (dark blue, up triangle), Shumard (green circle), swamp chestnut (yellow square), water (light blue down triangle), and white oak (magenta diamond).

Individual leaf sizes varied fourfold across the measured oaks and LMA differed by about 40% across oak species (Table 2). Across all measured oaks, LMA was linearly and positively correlated
with $J_{\text{max}}$ ($J_{\text{max}} = 1.09 \times \text{LMA} + 28.8$; Figure 3a), TPU ($\text{TPU} = 0.088 \times \text{LMA} + 0.911$; Figure 3b), and vein density ($\text{vein density} = 0.00103 \times \text{LMA} + 0.066$; Figure 3d) and non-linearly, negatively correlated with the Ball–Berry parameter ($\text{Ball–Berry parameter} = 30.0 \times e^{-0.018 \times \text{LMA}}$; Figure 3c). Across species, white oak leaves exhibited the largest vein densities that were significantly larger than all other measured oaks and about twice as large as Nuttall and swamp chestnut oak (Table 2).

![Figure 3](image-url)

**Figure 3.** Relationships between leaf mass per unit area (LMA; g m$^{-2}$) and (a) electron transport limited carboxylation rate ($J_{\text{max}}$; µmol m$^{-2}$ s$^{-1}$; $r^2 = 0.28$; $p < 0.0001$), (b) triose phosphate utilization limited carboxylation rate (TPU; µmol m$^{-2}$ s$^{-1}$; $r^2 = 0.32$; $p < 0.0001$), (c) the Ball–Berry parameter (unitless; $r^2 = 0.21$; $p < 0.0001$) and (d) leaf vein density (cm$^2$ cm$^{-2}$; $r^2 = 0.14$; $p < 0.0001$) for Nuttall (dark blue, up triangle), Shumard (green circle), swamp chestnut (yellow square), water (light blue down triangle), and white oak (magenta diamond).

In terms of epidermal cell properties, Nuttall oak and water oak had the largest stomata and longest pore lengths which were about 66% larger and 26% longer than the other oaks in this study (Table 2). Swamp chestnut oak had the highest stomatal density which was 16% higher than white oak and almost twice as high as Nuttall, Shumard and water oak (Table 2). Epidermal cells in swamp chestnut oak and white oak were about 44% smaller than Nuttall oak and water oak (Table 2). Epidermal cell densities were highest in swamp chestnut oak which were over twice as large as epidermal cell densities in Nuttall and water oak (Table 2). Across all measured oaks, increasing leaf areas were correlated with greater epidermal cell density (In epidermal cell density = 0.23 $\times$ ln leaf area $+$ 7.56; Figure 4) when both parameters were expressed on a natural log scale. Epidermal cell properties were also significantly correlated with one another with stomatal sizes being non-linearly, negatively correlated with stomatal densities (stom. dens. = 1930 $\times$ $e^{-0.0135 \times \text{stom. size}}$; Figure 5a), linearly and positively correlated with epidermal cell size (epi. cell size = 1.94 $\times$ strom. size + 41.8; Figure 5b) and non-linearly and negatively correlated with epidermal cell density (epi. cell dens. = 9620 $\times$ $e^{-0.0148 \times \text{stom. size}}$; Figure 5d). Stomatal densities were also non-linearly and negatively correlated with epidermal cell sizes (epi. cell size = 349 $\times$ $e^{-0.0009 \times \text{stom. dens.}}$; Figure 5c) and linearly and positively correlated with epidermal cell densities (epi. cell dens. = 3.95 $\times$ strom. dens. + 748; Figure 5e). Epidermal cell sizes and densities were also significantly correlated with one another (data not shown; $y = 12,600 \times e^{-0.0072 \times \text{stom. size}}$; $r^2 = 0.90$; $p < 0.0001$). Because stomatal sizes and densities were negatively correlated across species, stomatal pore indices tended to be similar except for Shumard oak whose stomatal pore index was about 27% smaller than other measured oaks.
Figure 4. Relationship between the natural log of leaf area (ln cm²) and the natural log of epidermal cell density (ln no. mm⁻²; r² = 0.22; p < 0.0001) for Nuttall (dark blue, up triangle), Shumard (green circle), swamp chestnut (yellow square), water (light blue down triangle), and white oak (magenta diamond).

Figure 5. Regressions of leaf epidermal properties including (a) stomatal density (no. mm⁻²) vs. stomatal size (μm²; r² = 0.55; p < 0.0001), (b) epidermal cell size (μm²) vs. stomatal size (r² = 0.58; p < 0.0001), (c) epidermal cell size vs. stomatal density (r² = 0.60; p < 0.0001), (d) epidermal cell density (no. mm⁻²) vs. stomatal size (r² = 0.49; p < 0.0001) and (e) epidermal cell density vs. stomatal density (r² = 0.57; p < 0.0001) for Nuttall (dark blue, up triangle), Shumard (green circle), swamp chestnut (yellow square), water (light blue down triangle), and white oak (magenta diamond).
3.2. Principal Components and Multiple Linear Regression

Because many leaf anatomic, nutrient and physiological parameters were highly correlated with one another, principal components of the data were developed and used as explanatory variables for multiple regression models to predict physiological parameters $V_{C_{\text{max}}}$ and $J_{\text{max}}$. Table 3 presents the principal components (PC) that were significantly correlated with at least one of the physiological parameters and loadings of each leaf anatomic or nutrient variable in each PC.

Table 3. Loadings for each variable used to predict physiological parameters $V_{C_{\text{max}}}$ and $J_{\text{max}}$, for each principal component (P1–13) that was a significant explanatory variable in either of the best fit multiple regression models.

| Variable                  | PC1  | PC5  | PC6  | PC10 |
|---------------------------|------|------|------|------|
| Mesophyll conductance$^2$ | 0.10 | 0.83 | −0.36| −0.03|
| Epidermal cell density    | 0.38 | −0.09| −0.13| 0.00 |
| Epidermal cell size       | −0.38| 0.11 | 0.18 | −0.20|
| Stomatal density          | 0.35 | 0.00 | 0.05 | −0.21|
| Stomatal size             | −0.33| −0.06| −0.09| −0.66|
| Stomatal pore length      | −0.32| 0.04 | −0.31| 0.58 |
| N concentration           | 0.36 | −0.06| 0.11 | 0.01 |
| C concentration           | 0.03 | −0.52| −0.56| −0.01|
| CN ratio                  | −0.33| −0.07| −0.06| 0.13 |

$^1$ Numbers in bold have the largest loadings (usually $> 10.41$) and indicate high covariance between the variable and the principal component. $^2$ Only variables that have high loading in at least one model principal component are shown.

PC1 captured correlation among the leaf epidermal parameters (Figure 5) as well as the correlation between epidermal parameters and leaf N concentration (Figure 1). PC5 loaded positively with mesophyll conductance and negatively with leaf C concentration and therefore describes leaves with large mesophyll conductance and low C content. Additionally, PC6 was negatively correlated with leaf C concentration. PC10 was negatively correlated with stomatal size but positively correlated with stomatal pore length and captures variation in the shape of the stomatal pores.

The best fit models predicting $V_{C_{\text{max}}}$ and $J_{\text{max}}$ from leaf anatomic and nutrient parameters had adjusted $r^2$ values of 0.45 and 0.63 respectively and RMSEs of 20.3 and 31.3 respectively (Table 4). These RMSE values were approximately 25% of mean $V_{C_{\text{max}}}$ and $J_{\text{max}}$ values. Both $V_{C_{\text{max}}}$ and $J_{\text{max}}$ were significantly correlated with PC1 and PC5 which describe the correlation between leaf epidermal anatomy and N concentration and the correlation between mesophyll conductance and leaf C concentration respectively. For $V_{C_{\text{max}}}$ all measured oak species exhibited the same correlation with PC1 whereas species differed with regard to PC5 in which Nuttall and white oak exhibited a strong positive correlation, Shumard and water oak exhibited a small correlation and swamp chestnut oak exhibited a strong negative correlation (Table 4). $V_{C_{\text{max}}}$ was also correlated with PC10 which describes stomatal pore shape and is positively associated with long, narrow stomatal pores. Nuttall, Shumard, water, and white oak exhibited a negative correlation with this PC and swamp chestnut oak exhibited a strong positive correlation. For $J_{\text{max}}$, species responded similarly to PC5, but differed in their correlation to PC1 with Nuttall, Shumard, swamp chestnut and white oak exhibiting a positive correlation and water oak exhibiting a negative correlation.
Table 4. Multiple linear regression model results for $V_{C_{\text{max}}}$ and $J_{\text{max}}$ including coefficients for each species, adjusted $r^2$ values and root mean square error (RMSE) from cross validation.

| Explanatory Variable | Nuttall | Shumard | Swamp | Chestnut | Water | White | $r^2$ | RMSE |
|----------------------|---------|---------|--------|----------|-------|-------|-------|-------|
| $V_{C_{\text{max}}}$ | Coefficients | 81.4 | 74 | 72 | 112 | 90 | 0.45 | 20.3 |
| PC1: Cell densities (+) cell sizes (-), N conc. (+) | 5.97 | 5.97 | 5.97 | 5.97 | 5.97 | 5.97 | 5.97 |
| PC5: Mesophyll cond. (+), C conc (-) | 13.8 | 1.52 | -17.1 | 0.25 | 9.17 |
| PC10: Stom. size (-), stom. pore length (+) | -29.3 | -23.5 | 31 | -32.8 | -19.1 |
| $J_{\text{max}}$ | Coefficients | 118 | 104 | 107 | 156 | 143 | 0.63 | 31.3 |
| PC1: Cell densities (+) cell sizes (-), N conc. (+) | 8.03 | 14.2 | 4.55 | -5.05 | 4.99 |
| PC5: Mesophyll cond. (+), C conc (-) | 7.13 | 7.13 | 7.13 | 7.13 | 7.13 |
| PC6: C conc. (-) | 38.7 | -8.37 | 34.5 | -5.85 | -29.1 |

1 Principal components (Table 3) of leaf anatomical and nutrient parameters were used as explanatory variables in models and parameters with the highest loading (and the sign of the loading) in each PC are provided.

$J_{\text{max}}$ was also correlated with PC6 which captures variability in leaf C concentration. However, species differed in their correlation with this parameter with Nuttall and swamp chestnut oak being highly positively correlated and Shumard, water, and white oak exhibiting a negative correlation (Table 4).

3.3. Comparison with Literature Values

The oak seedlings measured in this study displayed similar $V_{C_{\text{max}}}$ compared with literature values with all species falling close to the literature median (Figure 6). $J_{\text{max}}$ of measured oaks was also similar to literature values with three species falling within the 50% data bounds and Nuttall and Shumard oak falling just below this boundary. However measured oaks exhibited large variability in the Ball–Berry parameter with only Nuttall oak located within the 50% data bounds. For Shumard oak, about 90% of the literature values exhibited higher Ball–Berry parameters and other measured oak species were well below the 90% data bounds (Figure 6). For the calculated diffusional limitation to photosynthesis most measured oaks fell within or near the 50% literature data bounds except for water oak which fell above more than 95% of the literature data. For LMA, all measured oaks fell within the 50% literature data bounds. However measured oaks exhibited large variation in leaf epidermal parameters including stomatal density and pore length and epidermal cell density. For stomatal pore length, only Shumard oak fell within the 50% literature data bounds with swamp chestnut oak falling below it and white oak falling above it. Water and Nuttall oak fell above the 95% literature data bounds for stomatal pore size but were within the 50% data bounds for stomatal density (Figure 6). All other measured oaks fell above the 50% literature data bounds for stomatal density with white oak and swamp chestnut oak being above the 90% data bounds. Similar results were seen for epidermal cell density with Nuttall and water oak falling within the 50% literature bounds and other oaks falling well above the 95% data bounds. Nitrogen per unit leaf area ($N_{\text{area}}$) and photosynthetic N use efficiency (PNUE) for measured oaks tended to be on the low end of published literature values although swamp chestnut and Nuttall were near the literature median for $N_{\text{area}}$ and
PNUE respectively. For Narea, water and white oak were below the 50% literature data bounds and Shumard and Nuttall oak were below the 90% data bounds. For PNUE, Shumard and swamp chestnut were below the 50% literature data bounds and water and white oak were below the 90% data bounds (Figure 6).

![Figure 6. Boxplots of physiological and anatomical parameters for Quercus species derived from literature values (Table 1). Means for bottomland species including Nuttall (dark blue up triangle), Shumard (green circle), swamp chestnut (yellow square), water (light blue down triangle), and white oak (magenta diamond) are also presented.](image)

4. Discussion

In terms of species comparisons, we found that physiological parameters (\(V_{\text{cmax}}\), \(J_{\text{max}}\), Ball–Berry parameter, PNUE) and whole leaf anatomical parameters (LMA, vein density) did not vary in relation to flood tolerance of these measured southern bottomland oak species. However, leaf epidermal properties and leaf N concentration did exhibit differences by flood tolerance across species. Leaf N concentrations tended to be the lowest and CN ratios the highest in Nuttall and water oak, the species most tolerant of flood conditions. Since seedlings of these species were planted at sites that also contained the less flood tolerant species, this suggests that this effect had a genetic as opposed to environmental basis. Lower leaf N concentration in flood tolerant species may be related to shallow rooting depths [77] which would be an advantage under soil inundation but could limit N uptake. Additionally, we found that flood tolerant Nuttall and water oak tended to have the largest, least
frequent stomata and the largest, least frequent epidermal cells compared with less flood tolerant species. These differences in stomatal and epidermal cell properties may relate to tradeoffs of either tolerating drought or water logging conditions. The large, infrequent stomata of flood tolerant species may allow for higher transpiration rates during extended inundated conditions as well as provide evaporative cooling during hot summer conditions [1]. Conversely, smaller more frequent stomata may be more beneficial on higher elevation sites that may experience more water stress conditions than lower sites. Smaller, more frequent stomata may respond more quickly to changing environmental conditions [15] that would result in water savings compared to less responsive stomata. Additionally, smaller more frequent epidermal cells may allow for greater trichome densities as another moisture stress adaptation in higher elevation species particularly for swamp chestnut oak which exhibited the highest epidermal cell densities as well as abundant trichomes on the abaxial leaf surface.

As anticipated, leaf epidermal properties exhibited strong correlation with one another across measured oak species with cell sizes and density generally being negatively correlated and stomatal sizes as well as densities being positively correlated with corresponding epidermal cell characteristics. These relationships are expected given the contrasting effects of cell division vs. cell expansion for both epidermal and guard cells [21]. Additionally, we found that across species, larger leaves exhibited higher epidermal cell densities (Figure 4) suggesting that cell division is a stronger driver of leaf size compared with individual cell expansion for these oak species [78]. Interestingly, we also found correlation between leaf epidermal properties and N concentrations across these measured oak species, specifically that N concentrations were positively correlated with stomatal and epidermal cell densities. This may mean that epidermal cell densities mirror internal cell packing and more cells will yield higher N concentrations. Alternatively, N found in cell wall structures may be contributing to overall leaf N and higher cell densities would require more wall material and potentially more N [79]. Contrary to findings of other studies [80,81], PNUE was positively correlated with water use efficiency (WUE) as opposed to a tradeoff in either using water or nitrogen efficiently. This suggests that WUE in these oak seedlings is driven more by increased assimilation as opposed to lower water use and these higher assimilation rates yielded greater PNUE as well. Oaks measured in this study did exhibit a negative correlation between PNUE and the diffusional limitation to photosynthesis suggesting that overall CO₂ movement from the atmosphere to chloroplasts affects efficient use of leaf nitrogen independent of leaf transpiration rates and WUE.

In general, most leaf anatomical and nutrient parameters tended to exhibit non-significant correlations with physiological parameters except for LMA which exhibited significant correlation with $J_{\text{max}}$, TPU, and the Ball–Berry parameter. The relationship between LMA and photosynthetic parameters may be species specific or may reflect strategies to specific ecological conditions with other studies finding a positive relationship [65,82] or no significant relationship [83,84] between LMA and photosynthetic rates. However, LMA did not exhibit strong loading in any principal components that were included in the best fit models to estimate $V_{\text{Cmax}}$ and $J_{\text{max}}$. It may be that other parameters including mesophyll conductance and leaf C concentrations capture the same variability in the physiological data as LMA and are potentially a more direct measure of the physiological impacts of LMA. High loading of parameters within the calculated principal components in this study may also shed light on anatomical and leaf nutrient relationships which may not seem directly obvious but were nonetheless shown to be significant factors in modeling physiological parameters. For example, leaf C concentration exhibited high loading in two principal components found to be important in models for both $V_{\text{Cmax}}$ and $J_{\text{max}}$. This suggests that leaf C concentration is capturing variability in leaves that affects physiological functioning and may be correlated with aspects of leaf cell wall structure, composition, and leaf density [42]. These components would affect CO₂ diffusion within the leaf and to the chloroplast which was captured by the strong loading of both mesophyll conductance and leaf C concentration in PC5.

When principal components were used as predictor variables to generate models to predict $V_{\text{Cmax}}$ and $J_{\text{max}}$, best fit models had r² values of 0.45 and 0.63 respectively with cross validated RMSEs that were about 25% of average values. This suggests that some variability in physiology of these oak
seedlings is not being captured by these models. One likely reason for this is the fact that seedlings were measured from multiple sites and different years leading to large variation in soil and environmental conditions in the dataset. Although increasing the variability in the dataset, the timing and spatial scale of the data increase the robustness of the study and the parameters chosen by model selection. For $V_{\text{Cmax}}$, all species exhibited positive correlation with PC1 suggesting a positive correlation between $V_{\text{Cmax}}$ and N concentration. Across species, $V_{\text{Cmax}}$ was correlated with PC5 which represents mesophyll conductance and PC10 which represents stomatal shape, however species differed in the direction of this correlation. For all measured oaks except swamp chestnut oak, $V_{\text{Cmax}}$ was positively correlated with mesophyll conductance and stomatal pores that are more rounded with swamp chestnut oak exhibiting the opposite relationships with $V_{\text{Cmax}}$ being positively correlated with long, narrow stomatal pores and negatively correlated with mesophyll conductance. Swamp chestnut oak likely differs from the other species because it exhibits extensive trichomes on its abaxial surface as well as the largest individual leaf areas which will likely alter the interaction between boundary layer conductance, stomatal conductance, and mesophyll conductance [85]. For $J_{\text{max}}$, all species exhibited a similar positive correlation with PC5 which represents mesophyll conductance but differed in other parameters. All species except water oak were positively associated with PC1 and therefore leaf N concentration but species exhibited opposing correlations between $J_{\text{max}}$ and PC6 which had strong loading in leaf C concentration. For Nuttall and swamp chestnut oak, $J_{\text{max}}$ increased with decreasing leaf C concentration and for the other species, the opposite pattern was seen. This suggests that leaf C concentrations are capturing variability in the leaves from unmeasured parameters that correlate with $J_{\text{max}}$. These unmeasured parameters potentially include leaf cell wall properties that can affect conductance and light attenuation in the leaves. Since these relationships among species do not follow differences in flooding tolerance or other ecological or morphological characteristics, more research is needed to fully explain these relationships.

Oaks grow in a wide variety of environmental conditions; however, a large amount of previous research has focused on their drought tolerance in upland sites. Therefore, comparison of leaf anatomic and physiological parameters of Southern US oak species with varying flood tolerance measured in this study with parameters reported in the literature for oaks from differing ecoregions can shed light on differences that may reflect adaptation to seasonal flooding, and extended summer hot temperatures. Likewise, this comparison can highlight similarities in oak physiology and anatomy across a broader range of species within the genus. For several parameters including $V_{\text{Cmax}}$, $J_{\text{max}}$ and LMA, oaks measured in this study fell within the 50% literature data bounds. However, for other parameters including the Ball–Berry parameter, stomatal and epidermal cell properties, leaf N per unit area and PNU, oaks measured in this study fell outside of the 50% literature data bounds and sometimes outside of the 90% data bounds. This suggests that a wider range of epidermal and leaf N properties can yield similar leaf physiological properties in terms of $V_{\text{Cmax}}$ and $J_{\text{max}}$. In terms of flooding tolerance, flood tolerant Nuttall and water oak had stomatal lengths that were larger than the 90% literature data bounds but fell fairly close to the median literature values for stomatal and epidermal cell densities. In contrast, less flood tolerant species exhibited stomatal and epidermal cell densities that were larger than the 50% and, in some cases, the 90% literature data bounds. In general, oaks measured in this study and growing in the Southern US had lower $N_{\text{area}}$ than literature values with all species except swamp chestnut oak falling below the 50% data bounds. This suggests low nutrient availability for these growing sites potentially due to leaching of nitrate and denitrification during flooded conditions [11]. However, measured individuals in this study also exhibited generally lower PNU than literature values with all species except Nuttall oak falling below the 50% data bounds. This may be due to the fact recently planted seedlings were measured in this study that may exhibit gas exchange limitations due to shallow rooting thereby limiting the overall efficiency of leaf nitrogen use in terms of stomatal limitations to photosynthesis. In total, data from these Southeastern US bottomland species has extended the ranges for published leaf parameters for the oak genus.
5. Conclusions

Overall, this research highlights the importance of leaf structural parameters in discerning significant differences across species as well as the explanatory power of variables including epidermal cell size and density and leaf C concentrations in explaining physiological functioning in terms of $V_{\text{Cmax}}$ and $J_{\text{max}}$. This research also extends the data on physiological, anatomic and leaf nitrogen parameters across the oak genus and provides evidence of some unique attributes of Southeastern US oaks adapted to a range of flooding conditions. Specifically, these oaks exhibited low leaf N concentrations but similar $V_{\text{Cmax}}$ and $J_{\text{max}}$ as oaks reported in the literature suggesting that, in addition to issues of flooding and drought tolerance, low nitrogen availability may be another limiting factor for bottomland systems. In this study, oak species exhibited similar leaf characteristics depending on flood tolerance with flood tolerant Nuttall and water oak having leaves with generally lower leaf N concentrations, larger stomata and epidermal cells and lower cell densities compared with less flood tolerant species. Finally, the use of principal component analysis in this study has shown how leaf anatomy, N concentrations and physiology vary together and helped to elucidate groupings of parameters that may seem disparate, but nonetheless show high explanatory power in estimating physiological functioning. While these models lack predictive power for future studies, they help to define leaf structural parameters that are important, but may be overlooked, including epidermal cell parameters and leaf C concentrations. In total, better understanding of relationships between leaf structure and function will aid in the extrapolation of estimates of physiological functioning to leaves throughout an entire tree crown or across species based on easily measured anatomical parameters, increasing our understanding and modeling of forest functioning.

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