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Photosynthetic light responses of planted pin oak (*Quercus palustris*) and swamp white oak (*Quercus bicolor)* seedlings growing in two light environments and across a hydrological gradient

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Abstract
Stress from low light availability and flooding can reduce photosynthesis and growth rates of trees, contributing to possible regeneration challenges in bottomland hardwood forests. This study investigated the photosynthetic light responses of pin oak (*Quercus palustris* Muenchh.) and swamp white oak (*Quercus bicolor* Willd.) seedlings growing in two light environments and across a hydrological gradient. Our findings revealed that neither light environment nor soil moisture availability had a significant impact on leaf mass per area, light compensation point, dark respiration rate, or apparent quantum yield. However, light availability strongly affected photosynthetic rates of the oak seedlings, with greatest photosynthetic rates observed in high light environments. The more light-demanding pin oak exhibited lower rate of photosynthesis in response to decreased light availability compared to swamp white oak. The different light requirements of these oak species could directly influence the length of time seedlings can remain in the understory before they can be released. Additionally, the effect of soil moisture stress on foliar nitrogen and photosynthesis was more pronounced in low light environments. We observed a direct relationship between foliar nitrogen and light availability, as well as a positive correlation between photosynthetic rate and foliar nitrogen. Thus, any decrease in foliar nitrogen resulting from low light environments and soil inundation could adversely affect photosynthetic capacity and subsequent development of oak seedlings in bottomland hardwood forests.

Keywords Photosynthetic light response · Physiology · Morphology · Hydrological gradient · Light environments · Bottomland forests

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Introduction

Tree regeneration in bottomland forests is significantly challenged by limited sunlight due to dense overstory canopy and anoxic soil conditions during flooding. These stressors impede plant growth and productivity (Grime 1977; Gull et al. 2019) and can limit the establishment of desirable species such as oaks (*Quercus* spp.) in bottomland forests of the eastern United States. Insufficient light reduces photosynthesis, stunts growth, and alters plant morphology, affecting competitive dynamics among understory species (Gardiner and Krauss 2001; Gardiner 2002). Soil flooding exacerbates these challenges by impairing root respiration and nutrient uptake, further complicating the survival of sensitive species (Rupngam and Messiga 2024). The interplay of light and moisture stress shape the composition and structure of understory woody species in bottomland ecosystems, with oak species exhibiting varying tolerance to flooding that influence their establishment and growth (Gardiner 2001). Adopting silvicultural practices to enhance light availability can alleviate the adverse effects of flooding and promote a more diverse and resilient understory (Gardiner and Krauss 2001). Understanding the species-specific physiological and morphological responses to these stressors is essential for informing effective management strategies aimed at enhancing regeneration success.

Understory plants adapt to low light environments through anatomical, morphological, and physiological changes to efficiently capture sunlight. For example, shade leaves are typically larger and thinner, with higher chlorophyll concentrations than sun leaves (Boardman 1977). Additionally, shade leaves exhibit a lower light compensation point, lower dark respiration rates, higher apparent quantum yields, and lower maximum photosynthetic rates compared to those acclimated to high light conditions (Hamerlynck and Knapp 1994; Kubiske and Pregitzer 1996). Despite these adaptations, trees in low light environments tend to have lower nitrogen concentrations and photosynthetic rates than those in high light conditions, a trend observed in both hardwoods and conifers (Bond et al. 1999; Gardiner and Krauss 2001; Gardiner et al. 2009).

Bottomland forests are characterized by high water tables, soil inundation, and flooding at various times of the year, which subject trees to anoxic soil conditions. In response, these trees develop anatomical and morphological acclimations that enable them to maintain physiological functions such as photosynthesis (Kozlowski 1997). Research indicates that these stressed trees often increase leaf mass per unit area while suffering declines in parameters such as apparent quantum yield, dark respiration, and maximum photosynthetic rate (Beckman et al. 1992; Gardiner and Krauss 2001; Mielke and Schaffer 2010). Oak species stressed by anoxic roots experience reduced stomatal conductance of CO_2, which limits carbon assimilation required to maintain their growth (Grime 1977). Stomatal control is crucial for regulating gas exchange under saturated soils, with low oxygen intolerant species exhibiting prolonged reductions in stomatal conductance than more tolerant ones (Kozlowski and Pallardy 1979; Pezeshki and Chambers 1986; Gravatt and Kirby 1998). Flood tolerant species adopt several adaptations to cope with oxygen deficiency, including aerenchyma for efficient oxygen transport, hypertrophied lenticels on stems and roots for oxygen absorption, and adventitious roots that compensate for decaying primary root systems (Kozlowski and Pallardy 2002).

Oak regeneration is a common challenge in the bottomland hardwood forests of the Midwest and southeastern United States, primarily due to a complex interaction of environmen-

tal factors including light availability and flooding regimes (Collins and Battaglia 2008; Hayford et al. 2023). These forests often have dense overstory canopies that limit light availability for understory oak seedlings, essential for successful regeneration. In addition, the frequent flooding characteristic of these ecosystems creates anaerobic soil conditions that further hinder the establishment and growth of oak species (Collins and Battaglia 2008).

This project was part of a broader research initiative aimed at enhancing oak regeneration management in bottomland hardwood forests (Rives et al. 2020; Hayford et al. 2024). In the late 1990s, a study was initiated at Deer Ridge Conservation Area in Lewis County, MO, to investigate the impacts of various overstory harvest treatments on stand development of bottomland forests, with particular interest in natural oak regeneration. Initial findings revealed that these overstory treatments led to rapid growth of soft-mast species without increasing oak populations. These results suggested the need for additional treatments to increase oak abundance in the developing stands. In 2017, bare-root pin oak (*Quercus palustris* Muenchh.) and swamp white oak (*Quercus bicolor* Willd.) seedlings were planted to increase the oak population through enrichment planting in the stands that had previously been harvested and underplanting in the stands that had received no cut. For all planted seedlings, three levels of midstory release treatments were applied after planting in 2017, including control (no release), light release, and heavy release. A recent report by Hayford et al. (2024) details the effects of these silvicultural release treatments on oak regeneration within this forest type. The goal of this study was to investigate effects of light environments and soil moisture levels on the photosynthetic light response of the planted pin oak and swamp white oak seedlings. The specific objectives were to determine the (1) effects of ambient light environments (low versus high) and a range of soil moisture conditions on leaf mass per area (LMA), photosynthetic response variables (net photosynthesis at light saturation-A_{max}, light compensation point-LCP, dark respiration rate-R_d, and apparent quantum yield-Φ) and chemical variables (nitrogen concentration per unit dry mass-N_{mass}, nitrogen concentration per unit area-N_{area}) of pin oak and swamp white oak; and (2) relationships between chemical variables (N_{mass}, and N_{area}) and net photosynthesis at light saturation (A_{max}) of pin oak and swamp white oak. By evaluating these physiological traits, we can ascertain how light availability and soil moisture conditions influence photosynthetic efficiency and growth rates, ultimately guiding effective silvicultural interventions to promote oak regeneration in bottomland forests.

Methods

Study area

The research was conducted in a 220-ha mixed bottomland hardwood forest located at Deer Ridge Conservation Area (DRCA) (40°10’30’’N, 91°48’0’’W) in Lewis County, northeastern Missouri. DRCA encompasses one of the largest remaining tracts of bottomland forest in northeastern Missouri and is situated adjacent to a channelized section of the North Fabius River (Gwaze and Elliott 2011). This area featured contiguous blocks (>20 ha) of bottomland forest, and its state ownership facilitated long-term access and control of management activities. The predominant soil types in the study area included Blackoar silt loam (fine-silty, mixed, superactive, mesic Fluvaquentic Hapludolls), Fatima silt loam (fine-

silty, mixed, superactive, mesic Fluvaquentic Hapludolls), and Kickapoo fine sandy loam (coarse-loamy, mixed, superactive, nonacid, mesic Typic Udifluvents). All these soils were developed from alluvial deposits but differ in drainage class, and typically found on gentle slopes ranging from 0 to 3.5%. The site was prone to annual flooding, leaving some low-lying areas permanently inundated with water. Over a 30-year span (1991–2020), the mean annual temperature and precipitation levels for the area were 11.7 °C and 1,007 mm, respectively (PRISM Climate Group, prism.oregonstate.edu). The forest is primarily composed of tree species such as silver maple (*Acer saccharinum* L.), American elm (*Ulmus americana* L.), American sycamore (*Platanus occidentalis* L.), green ash (*Fraxinus pennsylvanica* Marsh.), boxelder (*Acer negundo* L.), eastern cottonwood (*Populus deltoides* W. Bartram ex Marshall), pin oak, bur oak (*Quercus macrocarpa* Michx.), and swamp white oak (Rives et al. 2020; Hayford et al. 2024).

Past treatments and planting

The design for this study was based on a previous study that has been thoroughly described by Hayford et al. (2024). In the late 1990s, a study was installed at DRCA to evaluate effects of overstory harvests on stand development of bottomland forests, with particular interest in natural oak regeneration. The overstory treatments included clearcut with reserves (canopy retention of up to 2.3 m^2 ha^{-1}), basal area retention (canopy retention of 4.6 to 6.9 m^2 ha^{-1}), and uncut control (no harvest; canopy 32.0 m^2 ha^{-1}). Canopy removal resulted in rapid growth of undesired soft-mast species without increasing the abundance of oak reproduction. These early results suggested that these methods alone would not maintain or increase the abundance of oaks in the developing stands. To increase the oak component, three levels of midstory release treatments (untreated control, light release, and heavy release) were added to the experimental design in 2017 to release planted pin oak and swamp white oak seedlings, in a completely randomized split-plot design. The overstory treatments (main-plots) were each replicated in five stands (15 main plot units total), and each was subdivided into the three split-plots (45 split-plot experimental units total). A total of 25–40 oak seedling pairs were planted in each of 45 experimental units in 2017. Each seedling pair consisted of one swamp white oak and one pin oak planted adjacent to one another at 1 m spacing.

Experimental design of the current study

We randomly selected 6 target oak seedling pairs (pin oak and swamp white oak) from each of 45 experimental units from the previous study in 2021 to monitor their light (photosynthetically active radiation, PAR) and soil moisture environment. AccuPAR LP-80 ceptometers (METER Group, Inc., Pullman, WA, USA) were used to measure PAR reaching the seedling level. Two PAR readings were taken at 90° angles midway between each oak seedling pair. Another ceptometer was placed in an open area adjacent to the study site to measure open-sky PAR in 1-minute intervals. These measurements were taken on uniformly cloudless days between 10:00 and 16:00 at two-week intervals from June to August of 2021. The intent was to sample during a time of day when the sun angle was most constant. Absolute PAR values were converted to relative values as a measure of the light availability to the sampled seedlings using the percent of full sun (%FS):

$$\%FS = \frac{Q_i}{Q_0} \times 100 \quad (1)$$

where Q_i is the averaged PAR reading from the ceptometer and Q_o is the time-matched PAR reading from the datalogger in the open.

Seedlings were assessed in two light environments that included low light (PAR ≤ 5% of full sunlight) and high light (PAR ≥ 20% of full sunlight) (Table 1).

To establish a gradient of hydrologic conditions, we used previously collected data on volumetric water content measured at each target seedling pair across the study to determine the range in soil moisture conditions. Volumetric water content was measured by inserting ML3 ThetaProbe soil moisture sensor (Delta-T Devices Ltd., Cambridge, UK) 6 cm below the soil surface. Two measurements 5 cm apart were taken in the middle of each oak seedling pair for 6 target oak seedling pairs within each split-plot bi-weekly from July 8 through August 9 of 2021. Soil moisture values associated with seedlings identified to be in the two light environments were selected to represent a range of soil moisture conditions. Soil moisture values of selected seedlings ranged from 29.5 to 51.10%. Care was taken to ensure that seedlings selected to represent the two light environments also had varying soil moisture conditions (Table 1). We selected a subset of 40 oak seedlings from the two light environments for subsequent measurements.

Data collection

Leaf-level photosynthetic light response was measured on 40 oak seedlings, comprising 20 pin oaks (10 each in low and high light environments), and 20 swamp white oak (10 each in low and high light environments) in summer 2022 using a portable photosynthesis system (model LI-6400; LI-COR Biosciences Inc., Lincoln, NE). Measurements were made in situ on one sample leaf from each selected oak seedling. Sample leaves were selected from the terminal flush of seedlings in a quiescent stage of stem and leaf growth to reduce sample variation.

Light response curves were developed by measuring net photosynthesis (A; μmol m^{-2} s^{-1}) at ten irradiance levels (Coe and Lin 2018). The leaf chamber environment during measurements was controlled to have a CO_2 concentration of 400 ppm, and leaf temperature of 25 °C. The relative humidity ranged from 50 to 80% in the leaf chamber during measurements. Leaves were acclimated to a photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1} for about 15–20 min for the first measurement, and subsequent measurements were made by decreasing PPFD to 1000, 500, 250, 120, 60, 40, 20, 10, and 0 μmol$m^{-2}s^{-1}$. It

Table 1 Mean ± SE and range values of light environments and soil moisture gradient in which the pin oak and swamp white oak were growing

Variable	Pin oak		Swamp white oak	
	Mean ± SE	Range	Mean ± SE	Range
Light environment (%)				
High	43.50 ± 14.6	24.46–70.59	43.50 ± 14.6	24.46–70.59
Low	2.70 ± 0.74	1.98–4.24	2.70 ± 0.74	1.98–4.24
Soil moisture (%)	40.60 ± 4.82	29.50–51.10	40.60 ± 4.82	29.50–51.10

Mean ± SE values are based on 10 seedlings per species per light environment ($n = 10$)

took approximately 3–5 min to attain stable net photosynthesis at each light level. Measurements were made in descending order because of the lengthy acclimation period required to achieve a constant CO_2 exchange rate by the sample leaves. All light response curve measurements were made over a four-day period between 09:00 and 17:00.

Leaves used for net photosynthesis measurements were harvested, bagged, labeled, and stored in a cooler on ice for return to the laboratory. Leaf area (cm^2) was determined by passing each leaf through a LI-3100 C leaf area meter (LI-COR Biosciences, Inc., Lincoln, NE). The leaf dry mass (g) was also measured after oven drying at 60 °C for 72 h. Leaf mass per area (LMA, g m^{-2}) was calculated as the ratio of dry leaf mass (g) to fresh leaf area (m^2). Nitrogen concentration per unit dry mass (N_{mass}, mg g^{-1}) and nitrogen concentration per unit area (N_{area}, g m^{-2}) were also estimated from dried sample leaves. Total nitrogen concentration of each dried sample leaf was determined by combustion at University of Missouri Plant and Soil Testing Laboratory.

Data analysis

We used nonlinear regression techniques to model photosynthetic light response (A/PPFD) of individual sample leaves. Photosynthesis data from each sample leaf were fit to a Michaelis-Menten model (Gardiner and Krauss 2001):

$$A = \frac{A_{max-g} \times PPFD}{K + PPFD} - R_d \tag{2}$$

where A=net photosynthetic rate or CO_2 assimilation rate, A_{max-g} = gross photosynthesis rate at light saturation, PPFD=photosynthetic photon flux density, K=PPFD required to achieve one half of A_{max-g}, and R_d = dark respiration rate.

The apparent quantum yield (Φ) was calculated from the first derivative of Eq. 2:

$$\Phi = A_{max-g} \times \frac{K}{K^2 + 2 \times K \times PPFD + PPFD^2} \tag{3}$$

The light compensation point (LCP; i.e., PPFD where A=0) for each sample leaf was calculated as:

$$LCP = \frac{-K \times R_d}{R_d - A_{max-g}} \tag{4}$$

Net photosynthesis at light saturation (A_{max}) was determined as the difference between A_{max-g} and R_d (Givnish 1988).

To verify the quality of our photosynthetic light response measurements, we went through the following data screening procedures:

i. We plotted the photosynthetic light response measurement observations alongside the model-fitted photosynthetic light response curve for each of the 40 leaves. The purpose of this was to visualize how best the model-fitted photosynthetic light response curve aligned with the photosynthetic light response measurement observations.

ii. We estimated root relative squared error (rrse) to evaluate the model's performance relative to the average of the true values. A lower rrse indicates a better model fit. We estimated rrse for each of the 40 leaves using the equation,

$$rrse = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}} \quad (5)$$

where n = number of observations, y_i = actual value, $\hat{y}_i$ = predicted value, and $\bar{y}$ = average of the actual values.

iii. Standard error and confidence limits were estimated from the photosynthetic light response model fit. We used the standard error and confidence limits to assess the precision of estimated parameters such as $A_{max\text{-}g}$, K, and R_d for each of the 40 leaves.

iv. After reviewing the first three screening procedures above, we noted that the photosynthetic light response measurements of leaves with rrse values ≥ 0.1 showed large standard errors and wide confidence limits for the estimated $A_{max\text{-}g}$, K, and R_d (iii) and a lot of outliers for the plot in (i). We created a histogram of the rrse values to visualize the 0.1 threshold in the context of the data distribution. We also calculated the 0.9, 0.95, and 0.99 quantiles of the rrse value distribution to see how they relate to the 0.1 threshold. We then decided to exclude the 5 leaves (3 pin oaks, 2 swamp white oaks) with rrse values ≥ 0.1 threshold from the subsequent statistical analysis.

We used a generalized linear mixed model with PROC GLIMMIX in SAS 9.3 software (SAS Institute, Inc., Cary, NC) to test the effects of species, light environments, and a range of soil moisture conditions, and their interactions on leaf mass per area (LMA), photosynthetic response variables (A_{max}, $R_{d,}$ LCP, and Φ) and chemical variables (N_{area}, and N_{mass}) (Objective 1). Because the two oak species were paired during planting, with each species sharing a tag number, we used tag number as a random term. The same analysis framework was used in Objective 2 to test the effects of species and chemical variables (N_{mass}, and N_{area}) on net photosynthesis at light saturation (A_{max}). We ran a separate analysis for effect of species and N_{mass}, and species and N_{area} on A_{max}. All pairwise comparisons were evaluated with Tukey's honestly significant difference adjustment. We used an alpha level of 0.05 to assess statistical significance for all analyses. Linear regression analysis in the form $y = a + bx$ was used to further explore relationships among variables of interest; where y is the predicted value, a and b are the estimated regression coefficients, and x is the predictor variable.

Results

Photosynthetic variables and leaf mass per area

Species, light environment, soil moisture, and their interactions did not affect light compensation point (LCP), dark respiration rate (R_d), apparent quantum yield (Φ), and leaf mass per area (LMA) of both pin oak and swamp white oak (Tables 2 and 4). For A_{max}, only the

Table 2 Statistical output of generalized linear mixed model showing the effects of species, light level, soil moisture, and their interactions on net photosynthesis at light saturation (A_{max}), light compensation point (LCP), dark respiration rate (R_d), and apparent quantum yield (Φ)

Variable	Amax (μmol CO_2 m^{-2} s^{-1})		LCP (μmol $m^{-2}s^{-1}$)		Rd (μmol CO_2 m^{-2} s^{-1})		Φ (μmol $m^{-2}s^{-1}$)	
	F-value	p-value	F-value[1]	p-value	F-value[1]	p-value	F-value	p-value
Species	0.41	0.53	0.97	0.34	1.83	0.20	2.50	0.13
Light	0.01	0.91	0.16	0.69	0.00	0.98	2.30	0.14
Species × light	4.45	0.04	0.02	0.88	0.37	0.55	1.25	0.28
Soil moisture	0.00	0.96	1.02	0.32	0.74	0.40	3.87	0.07
Soil moisture × species	0.42	0.53	0.64	0.44	1.55	0.23	3.78	0.07
Soil moisture × light	0.11	0.75	0.35	0.56	0.03	0.86	2.71	0.11
Soil moisture × species × light	4.27	0.05*	0.05	0.83	0.43	0.52	1.21	0.29

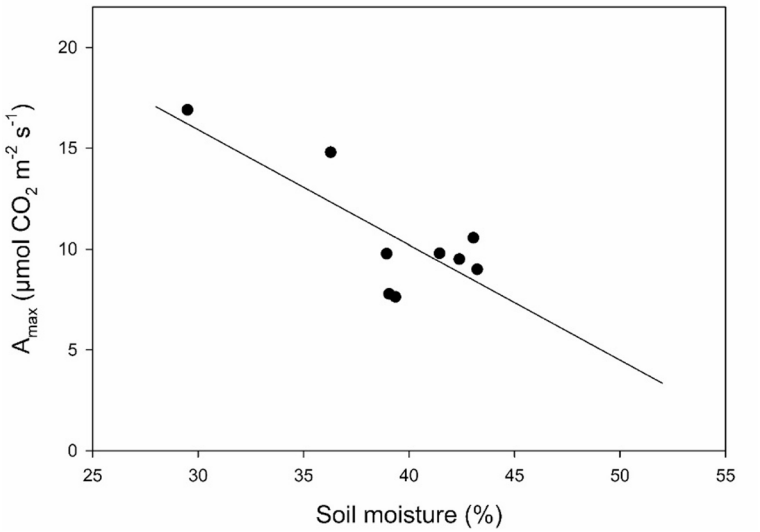

Fig. 1 Relationship between soil moisture and net photosynthesis at light saturation (A_{max}) of swamp white oak at low light conditions ($n=10$). Regression statistics: A_{max} = 33.05–0.57 × soil moisture; $r^2=0.61$; $p=0.01$

soil moisture × species × light interaction was significant. Soil moisture showed no effect on A_{max} of both pin oak and swamp white oak under high light but affected A_{max} of swamp white oak under low light conditions (Fig. 1; Table 2). Soil moisture explained 61% of the variation observed in A_{max} of swamp white oak under low light availability. A_{max} of pin oak was higher under high light than under low light conditions (Table 3; Fig. 2). However, the A_{max} of swamp white oak did not differ between the two light environments. There was no

Table 3 Leaf-level photosynthetic characteristics of pin oak and swamp white oak growing under low and high light environments

Variable	Pin oak		Swamp white oak		*p*-value
	Low light	High light	Low light	High light	
LMA ($g\ m^{-2}$)	34.3 ± 2.6 a	47.8 ± 2.8 a	38.3 ± 2.7 a	49.4 ± 2.6 a	0.12
A_{max} ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	9.3 ± 1.3 b	13.9 ± 1.4 a	10.1 ± 1.4 ab	13.0 ± 1.3 ab	0.04
LCP ($\mu mol\ photons\ m^{-2}\ s^{-1}$)	20.8 ± 8.2 a	36.9 ± 8.6 a	30.0 ± 8.4 a	55.1 ± 8.3 a	0.88
R_d ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	1.3 ± 0.4 a	2.0 ± 0.4 a	1.5 ± 0.4 a	2.5 ± 0.4 a	0.55
Φ (mol-C/mol-photons)	0.06 ± 0.0 a	0.05 ± 0.0 a	0.05 ± 0.0 a	0.04 ± 0.0 a	0.28
N_{mass} ($mg\ g^{-1}$)	33.2 ±1.8 a	33.2 ± 1.9 a	36.5 ± 1.9 a	34.4 ± 1.8 a	0.67
N_{area} ($g\ m^{-2}$)	1.1 ± 0.1 c	1.5 ± 0.1 ab	1.4 ± 0.1 b	1.9 ± 0.1 a	0.04

Values of leaf mass per area (LMA), net photosynthesis at light saturation (A_{max}), light compensation point (LCP), dark respiration rate (R_d), apparent quantum yield (Φ), nitrogen concentration per unit dry mass (N_{mass}), and nitrogen concentration per unit area (N_{area}) are presented as means ± standard error for 10 seedlings per species per light environment ($n = 10$)

Differences within a row are indicated by different letters at p-value ≤ 0.05 according to Tukey's post-hoc test

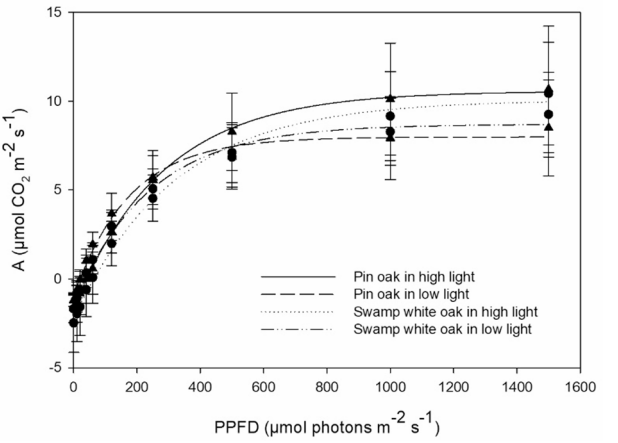

Fig. 2 Net photosynthetic light response of pin oak and swamp white oak seedlings growing under high and low light environments. PPFD is photosynthetic photon flux density and A is net photosynthesis. Each value represents the mean ± standard error for 10 seedlings per species per light environment (n = 10)

difference in A_{max} between pin oak and swamp white oak within any of the light environments (Table 3).

Nitrogen concentration response to light and soil moisture by species

Soil moisture affected nitrogen concentration per unit dry mass (N_{mass}), but the effect was not different between the two oak species (Table 4). Soil moisture was inversely related to N_{mass} and explained 21% of its variation (Fig. 3). Neither light nor the interaction of light and soil moisture affected N_{mass} of either oak species (Table 4). Nitrogen concentration per unit area (N_{area}) of swamp white oak decreased with increasing soil moisture under low light availability, and soil moisture accounted for 66% of the variation in N_{area}, but pin oak was not affected (Fig. 4). The N_{area} of both pin oak and swamp white oak showed no response to soil moisture under high light availability. The N_{area} of each of pin oak and swamp white oak

Table 4 Statistical output of generalized linear mixed model showing the effect of species, light level, soil moisture, and their interactions on leaf mass per area (LMA), nitrogen concentration per unit dry mass (N_{mass}), and nitrogen concentration per unit area (N_{area})

Variable	LMA (g m^{-2})		N_{mass} (mg g^{-1})		N_{area} (g m^{-2})	
	F-value	p-value	F-value	p-value	F-value	p-value
Species	2.61	0.12	0.16	0.69	1.51	0.23
Light	0.44	0.52	0.07	0.80	0.13	0.73
Species × light	2.58	0.12	0.19	0.67	4.68	0.04
Soil moisture	3.31	0.08	4.29	0.04*	0.00	0.10
Soil moisture × species	2.34	0.14	0.25	0.62	1.06	0.32
Soil moisture × light	1.05	0.32	0.10	0.76	0.38	0.54
Soil moisture × species × light	2.48	0.13	0.15	0.70	4.56	0.05*

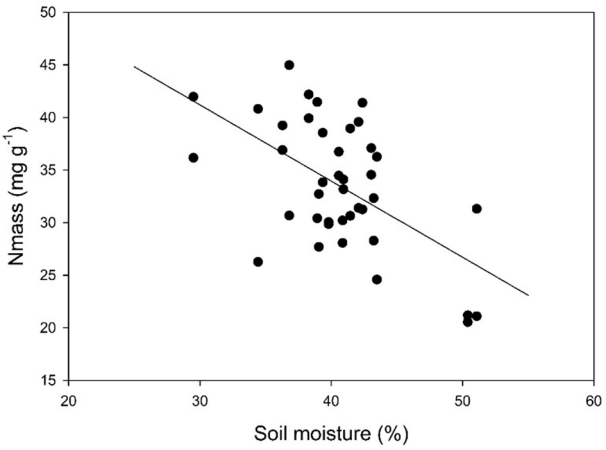

Fig. 3 Relationship between soil moisture and nitrogen concentration per unit dry mass of leaf (N_{mass}) for both pin oak and swamp white oak (combined species $n=40$). Regression statistics are: $N_{mass} = 59.90–0.63 \times$ soil moisture; $R^2=0.21$; $p=0.01$)

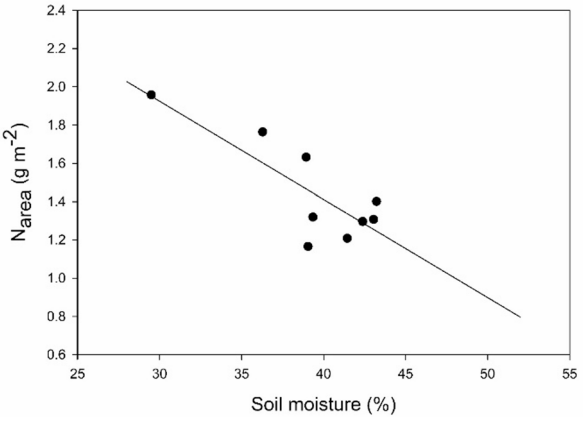

Fig. 4 Relationship between soil moisture and nitrogen concentration per unit area (N_{area}) of swamp white oak at low light conditions ($n=10$). Regression statistics: $N_{area} = 3.46–0.05 \times$soil moisture; $r^2=0.66$; $p=0.01$

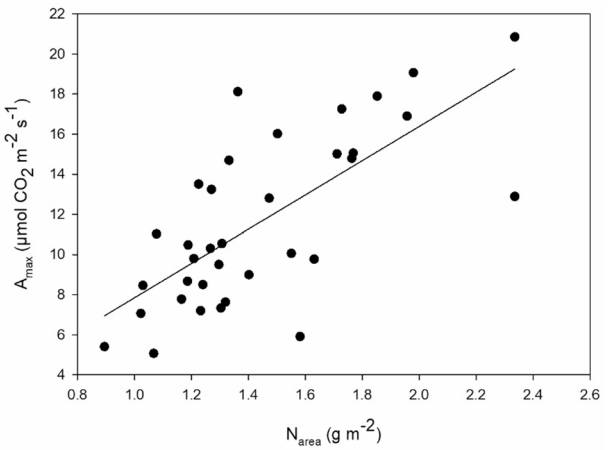

Fig. 5 Relationship between nitrogen concentration per unit area (N_{area}) and net photosynthesis at light saturation (A_{max}) for both pin oak and swamp white oak (combined species $n=40$). Regression statistics: $A_{max} = -0.68+8.53 \times N_{area}$; $R^2=0.50$; $p<0.01$

Table 5 Statistical output of generalized linear mixed model showing the effects of species, nitrogen concentration per unit dry mass (N_{mass}), and nitrogen concentration per unit area (N_{area}) on net photosynthesis at light saturation (A_{max})

Variable	Num DF	Den DF	F-value	*p*-value
N_{mass}				
Species	1	24.12	0.75	0.40
N_{mass}	1	24.63	0.34	0.56
Species × N_{mass}	1	23.5	0.67	0.42
N_{area}				
Species	1	31	1.25	0.27
N_{area}	1	31	44.08	<0.01***
Species × N_{area}	1	31	2.63	0.12

was higher under high light than low light environment (Tables 3 and 4). The N_{area} of swamp white oak was higher than pin oak under low light conditions but there was no difference in N_{area} between the two oak species when under a high light level (Table 3).

Nitrogen concentration and net photosynthesis at light saturation relationships

The A_{max} of both pin oak and swamp white oak showed a strong linear relationship with N_{area}, with about 50% of the variation in A_{max} explained by N_{area} (Fig. 5; Table 5). However, N_{mass} showed no effect on A_{max} of either oak species (Table 5).

Discussion

Morphological and physiological responses

Oak leaves develop morphological acclimations in response to their environment, primarily reflected in leaf area and leaf mass per area (LMA) (Petersson et al. 2020). Under low light conditions, leaf blade area typically increases and thickness decreases, which results in a lower LMA. Previous studies have reported reduced LMA in low light environments for various bottomland oak species, including Nuttall oak (*Quercus texana* Buckley) (Gar-

diner et al. 2001; Gardiner 2002), and cherrybark oak (*Quercus pagoda* Raf.) (Gardiner and Krauss 2001; Gardiner 2002). In contrast, our study found no significant effect of light environment on LMA (Table 4). However, we did observe a nonsignificant trend of smaller LMA in low light for both pin oak and swamp white oak (Table 3). The lack of significance could stem from differing species-specific responses to light conditions. This could mean that the genetic makeup of the studied oak species dictates a different response to light availability compared to Nuttall oak and cherrybark oak. Additionally, the lack of significance could suggest that our sample size was insufficient or that the light conditions were not extreme enough to elicit a measurable effect. We also found no impact of soil moisture on LMA. This finding differs from that of Gardiner and Krauss (2001), who reported an increase in LMA when cherrybark oak seedlings were subjected to flooding for 30 days. Their study compared inundated and non-inundated soil, while our study examined a range of soil moisture conditions that might not be wide enough to capture significant differences.

At the physiological level, both light availability and soil moisture influenced the instantaneous photosynthetic rates of the oak species studied (Table 2; Fig. 1). Oak leaves exposed to high light conditions exhibited greater photosynthetic rates compared to those in low light environments (Table 3). This is consistent with previous observations in other bottomland oaks, such as cherrybark oak (Gardiner and Krauss 2001) and Nuttall oak (Gardiner et al. 2001; Gardiner 2002). We observed that the more light-demanding oak species exhibited a greater reduction in photosynthesis in response to decreased light availability. Pin oak showed a substantial increase in A_{max} when transitioning from low to high light conditions. Pin oak is classified as shade intolerant, requiring more light for optimal photosynthesis compared to swamp white oak, which is considered intermediate in shade tolerance.

Effects of soil moisture and light availability

The effect of soil moisture on instantaneous photosynthetic rate of the oak seedlings was contingent upon light availability. In high light environments, soil moisture appeared to have no significant impact on photosynthetic rates. However, in low light conditions, we observed a decrease in photosynthetic rates as soil moisture levels increased (Fig. 1). This result can potentially be attributed to combined stress effect of low light and increased soil moisture conditions, which reduced the photosynthetic rate of the oak seedlings.

We found no effect of light environments or soil moisture on other physiological parameters (Table 2). This contrasts with previous studies that have reported reduced Rd, reduced LCP, and increased Φ for oak species growing under low light conditions (Gardiner and Krauss 2001; Gardiner 2002; Gardiner et al. 2009). We speculate that the inherent variability of the individual oak species and their acclimation processes could have masked these expected effects. Our findings are consistent with Gardiner (2002), who reported no effect of light on R_d and LCP for Nuttall oak, as well as on Φ for cherrybark oak. In a greenhouse study conducted in Stoneville, Mississippi, Gardiner and Krauss (2001) also found no differences in either LCP or R_d of cherrybark oak leaves when exposed to full and partial sunlight conditions. In addition, our findings indicated that soil moisture had no impact on LCP, R_d, and Φ of the oak seedlings. Similarly, Gardiner and Krauss (2001) reported no effect of flooding on LCP and R_d for cherrybark oak leaves. However, they observed that soil flooding for 30 days reduced Φ in cherrybark oak seedlings. The flooded seedlings in their study might have experienced similar soil moisture conditions to those in our high-moisture envi-

ronment. However, the non-flooded seedlings in their study may have encountered lower soil moisture conditions than those in our study, potentially explaining the discrepancies in results between the two studies.

Foliar nitrogen dynamics and photosynthesis

Our results suggested that foliar nitrogen was influenced by soil moisture and light availability (Table 4). The N_{mass} of the oak leaves decreased as soil moisture increased (Fig. 3). Soils become anoxic as soil moisture increases, inhibiting nitrate uptake by the hypoxic roots, and reducing foliar nitrogen (Kozlowski 1997). Lower foliar nitrogen in response to soil inundation can also be attributed to decline in soil nitrate availability through denitrification (Pezeshki 2001). Gardiner and Krauss (2001) reported a similar reduction in N_{mass} for cherrybark oak leaves in flooded soils compared to non-flooded soil under full or partial sunlight conditions in a greenhouse study in Stoneville, Mississippi. Our study also revealed that the impact of soil moisture on N_{area} was contingent on light availability. N_{area} was unaffected by increased soil moisture under high light conditions, but it decreased at low light environments as soil moisture increased (Fig. 4). This contrasts with the findings of Gardiner and Krauss (2001), who observed no impact of 30-day flooding on N_{area} in cherrybark oak growing under full or partial sunlight. However, it is important to note that their definition of partial sunlight (25% of full sunlight) differed significantly from our low light condition, which was less than 5% of full sunlight. Consistent with previous studies (Naidu and DeLucia 1997; Gardiner and Krauss 2001), light availability did not affect N_{mass}, but N_{area} increased under high light conditions. Generally, foliar nitrogen is higher in well-lit environments compared to shaded ones, a trend supported by multiple studies across various North American forest types (Walters and Field 1987; Naidu and DeLucia 1997; Gardiner and Krauss 2001; Gardiner et al. 2001). The relationship between N_{area} and light environment reflects the increase in the carboxylating capacity of sun-adapted leaves, mediated through an increase in the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO)-associated nitrogen pool (Evans 1989; Vincent 2001; Liu and Su 2016).

The A_{max} of the oak leaves showed a positive correlation with N_{area}, with N_{area} accounting for approximately 50% of the variation in A_{max} (Fig. 5). This relationship between photosynthesis and foliar nitrogen has been observed in other oak species, including Nuttall oak (Gardiner et al. 2001), cherrybark oak (Gardiner and Krauss 2001), and pedunculate oak (*Quercus robur* L.) (Gardiner et al. 2009). Nitrogen plays a crucial role in photosynthesis as it is an essential component of the chlorophyll, the photosynthetic enzyme RuBisCO, and the proteins of the Calvin cycle and thylakoids (Givnish 1988; Evans 1989). However, the fact that N_{area} accounted for only 50% of the variance in A_{max} indicates that other factors also influence photosynthetic rates. While RuBisCO is essential for carbon fixation, an increase in nitrogen associated with this enzyme does not always ensure a proportional rise in A_{max}. This is due to the potential allocation of nitrogen to other essential functions within the leaf, including the synthesis of light-harvesting proteins and chlorophyll. Additionally, various environmental stressors, such as low light and soil inundation, can affect the distribution and utilization of nitrogen, thereby limiting its availability for photosynthesis (Kwak et al. 2011; Rupngam and Messiga 2024). A reduction in foliar nitrogen because of these stresses can adversely impact photosynthetic capacity by restricting the leaf's overall function. Consequently, while leaf nitrogen is important, a comprehensive understanding of

photosynthetic dynamics in oaks requires consideration of multiple intrinsic and extrinsic factors that contribute to variability in photosynthetic rates.

Conclusion

The results of this study indicated that the oak seedlings exhibited minimal morphological and physiological acclimations to variations in light and soil moisture availability. Morphological and physiological parameters such as LMA, LCP, R_d, and Φ showed no significant responses to changes in these environmental factors. However, light availability had a strong effect on photosynthetic rates of the oak seedlings, with highest rates observed at 20% or more of full sunlight compared to 5% or less. Notably, the more light-demanding pin oak demonstrated a steeper decline in photosynthetic rates in response to reduced light compared to swamp white oak. This difference in light requirements between the oak species could influence the duration that seedlings can remain in the understory before their release, which is crucial for planning effective silvicultural release operations to maximize survival and growth. Relationships among photosynthetic rate, leaf nitrogen content, and LMA are well documented, but they may not fully distinguish species' traits without context-specific physiological integration. Future research designed to capture continuous diurnal gas exchange and environmental variability could build on the present dataset to provide more comprehensive insights into daily carbon gain and its relationship to leaf traits across species.

Additionally, variations in light availability can mitigate the impacts of soil moisture stress on foliar nitrogen and photosynthesis in both pin oak and swamp white oak seedlings. The effects of soil moisture stress on foliar nitrogen and photosynthesis were more pronounced in low light conditions. These findings reiterate the importance of silvicultural interventions aimed at creating optimum light environment to reduce soil moisture stress and promote the development of oak advance reproduction in bottomland hardwood forests. Moreover, a direct relationship was observed between foliar nitrogen and light availability, and photosynthetic rates positively correlated with foliar nitrogen. Although foliar nitrogen explained half of the variation in photosynthetic rates, this partial relationship underscores the importance of other physiological and environmental factors. Stressors such as limited light availability and soil inundation can disrupt nitrogen allocation within leaves and constrain its ability to support optimal photosynthetic function. As a result, reductions in foliar nitrogen under these conditions may restrict overall photosynthetic performance. To fully understand the dynamics of photosynthesis in oaks, it is essential to consider both intrinsic leaf traits and extrinsic environmental variables that collectively influence photosynthetic variability.

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Author contributions IH - Conceptualization, methodology, data collection, formal analysis, writing, manuscript preparation.BOK - Conceptualization, methodology, funding acquisition, supervision, formal analysis, reviewing, and editing.JDW- Methodology, supervision, formal analysis, reviewing, and editing.JMK - Supervision, reviewing, and editing.BG - Reviewing, and editing.

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Data availability Data will be made available upon request.

Declarations

Competing interests The authors declare no competing interests.

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