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Contrasting Effects of Nitrogen Addition on Leaf Photosynthesis and Respiration in Black Mangrove in North Florida

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Abstract

Nutrient enrichment is a major driver of environmental change in mangrove ecosystems. Yet, nutrient enrichment impacts on physiological processes that regulate CO_2 and water fluxes between mangrove vegetation and the atmosphere remain unclear. We measured peak growing season photosynthesis (A) and respiration (R) in black mangrove (*Avicennia germinans*) leaves that had been subjected to long-term (8-year) nutrient enrichment (added N, added P, control) in north Florida. Previous results from this site indicated that *Avicennia* productivity was N-limited, but not P-limited. Thus, we expected that N addition would increase light saturated net photosynthesis at ambient CO_2 (A_{net}), intrinsic water-use efficiency (iWUE), maximum rate of Rubisco carboxylation (V_{cmax}), and leaf dark respiration (R), while P addition would have little effect on any aspect of photosynthesis or respiration. We expected that increased photosynthesis and respiration would be most apparent immediately after N addition and in newly formed leaves. Indeed, A_{net} and V_{cmax} increased just after N addition in the N addition treatment; these increases were limited to leaves formed just after N addition. Nonetheless, over time, photosynthetic parameters and iWUE were similar across treatments. Interestingly, R measured at 25 °C increased with N addition; this effect was consistent across time points. P addition had little effect on R. Across treatments and time points, $V_{cmax,25}$ (V_{cmax} standardized to 25 °C) showed no relationship with R at 25 °C, but the maximum rate of electron transport for RuBP regeneration standardized to 25 °C ($J_{max,25}$) increased with R at 25 °C. We conclude that N addition may have small, short-lived effects on photosynthetic processes, but sustained effects on leaf R in N-limited mangrove ecosystems.

Keywords Coastal wetlands · *Avicennia germinans* · Nutrient enrichment · Carbon cycle

Introduction

Mangrove ecosystems are generally nutrient-poor and sensitive to nutrient enrichment from agricultural runoff, atmospheric deposition, and other sources (Kathiresan and Bingham 2001; Holtgrieve et al. 2011; Reef et al. 2010; Stevens 2019, Castaneda-Moya et al. 2020). Nutrient enrichment experiments have demonstrated that mangrove productivity can be strongly stimulated by nitrogen (N) addition (Feller et al. 2003a; Lovelock and Feller 2003; Lovelock et al. 2007; Naidoo 2009; Simpson et al. 2013; Dangremond et al. 2020), phosphorus (P) addition (Lin and Sternberg 1992; Koch and Snedaker 1997, Medina et al. 2010), or combined N and P addition (Feller 1995; Feller et al. 2003b; Lovelock et al. 2004; Weaver and Armitage 2020). What remains unclear, however, is how nutrient enrichment impacts physiological processes that regulate CO_2 fluxes between mangrove vegetation and the atmosphere, whether effects are transitory or sustained,

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and whether leaves of different ages respond differently to nutrient enrichment. These gaps are notable considering the disproportionally large role of mangrove ecosystems in global carbon (C) and nutrient cycling (Lugo and Snedaker 1974, Bouillon et al. 2008; Donato et al. 2011; McLeod et al. 2011). Further work is required to improve our understanding of nutrient enrichment impacts on mangrove physiology and potential consequences for mangrove ecosystem C cycling.

Photosynthesis (*A*) is an important regulator of plant growth and function and represents the largest flux of CO_2 between vegetation and the atmosphere at the global scale (Canadell et al. 2007; IPCC 2021; Keenan and Williams 2018). Under current atmospheric conditions, the maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, V_{cmax}) and the maximum rate of electron transport required to regenerate ribulose-1,5-bisphosphate (J_{max}) are the dominant biochemical determinants of *A* (Farquhar et al. 1980). Many terrestrial biosphere models (TBMs) rely on estimates of V_{cmax} and J_{max} to simulate C fluxes (Zaehle et al. 2005; Bonan et al. 2011; Clark et al. 2011; Lawrence et al. 2019).

Nutrient enrichment is expected to increase photosynthesis given that nitrogen (N) and phosphorus (P) are major components of photosynthetic proteins, energy intermediates, and cell and plastid membranes. A recent meta-analysis across hundreds of terrestrial species showed that N addition generally increases *A* (12.6%), leaf N per unit mass and area (18.4% and 14.3%, respectively), and stomatal conductance to water vapor (g_s, 7.5%), with little change in intrinsic water-use efficiency (A/g_s; Liang et al. 2020). However, among individual studies, photosynthetic responses to N addition range from negative (Mao et al. 2018) to strongly positive (Manter et al. 2005; Zhang et al. 2021). Nutrient enrichment impacts on *A* can also be transient (e.g., Gough et al. 2004). The effects on N addition on V_{cmax} are inconsistent across species and studies (Liang et al. 2020), even though leaf N generally increases with N addition and often scales positively with V_{cmax} (Manter et al. 2005; Kattge et al. 2009; Walker et al. 2014). Studies have also found that P addition increases *A* in some species and systems (Warren and Adams 2002; Li et al. 2016), and low P can limit the response of photosynthesis to increasing leaf N (Reich et al. 2009).

A handful of studies have examined the impacts of nutrient enrichment on mangrove photosynthetic processes. On the east coast of Florida, Lovelock and Feller (2003) found that N addition increased *A* in *Avicennia germinans* but not in *Laguncularia racemosa*. In Panama, Lovelock et al. (2004) found no effect of N or P additions on *A* in *Rhizophora mangle*. Lovelock et al. (2006a) found that P fertilization increased *A* in fringe but not dwarf *R. mangle*. Lovelock et al. (2006b) found that P fertilization increased *A* in *Avicennia germinans* in Belize, but not in *A. germinans* in Florida. Under moderate (30 ppt) to high (60 ppt) salinity (Martin et al. 2010; Chapman et al. 2021) conditions typical of coastal wetlands, N addition can increase iWUE by increasing leaf N and net C fixation when g_s is low (Reef et al. 2010; Martin et al. 2010). Overall, the effects of nutrient enrichment on mangrove *A* vary among studies, and photosynthetic responses to N or P additions are often smaller than or not clearly linked with biomass responses (e.g., Lovelock et al. 2004). Moreover, no mangrove studies have determined nutrient enrichment impacts on V_{cmax}, and whether the impacts are transitory or sustained or vary between leaves of different ages. There is evidence that N addition increases leaf N concentrations in mangroves (Lovelock and Feller 2003; Lovelock et al. 2004; Dangremond et al. 2020) which could ultimately result in increased light saturated photosynthesis at ambient CO_2 (A_{net}), V_{cmax}, and possibly J_{max}. If these increases occur uniformly across leaves of various cohorts, it could improve our quantitative and predictive understanding of N mobility in ecotonal mangrove systems. In general, very few studies have estimated V_{cmax} or J_{max} on any mangrove species, regardless of the growth conditions (Aspinwall et al. 2021).

At the global scale, roughly half of the C fixed by plants returns to the atmosphere each year via respiration (Piao et al. 2013), and approximately half of plant respiration comes from leaves (Atkin et al. 2007). Leaf dark respiration (*R*) is co-limited by the supply of carbohydrates from photosynthesis, respiratory protein concentrations, and adenylate demand (Ryan et al. 1996; Atkin and Tjoelker 2003; O'Leary et al. 2017). Leaf *R* is linked with photosynthesis given that *R* supports processes that maintain photosynthesis (Penning de Vries 1975; Amthor 1984; Wang et al. 2020). TBMs estimate leaf *R* as a proportion of V_{cmax} or predict *R* based on empirical relationships between leaf N and *R* at a set measurement temperature (Atkin et al. 2015), and although patterns of *R* vary across vegetation types, there is evidence for strong relationships between N and *R* in coastal wetland vegetation (Sturchio et al. 2022). Most studies have found that N addition increases leaf *R*, likely due to higher maintenance costs associated with protein turnover (Brix 1971; Penning de Vries 1975; Van de Weg et al. 2013). P is also an important component of respiratory enzymes and is needed for phosphorylation of ADP during respiration, which could explain why leaf *R* scales positively with leaf P across species and environments (Meir et al. 2001; Weerasinghe et al. 2014; Atkin et al. 2015). However, in some fertilization experiments, leaf *R* does not increase with P addition, unless N is also added (Heskel et al. 2014). In experiments with mangroves, P addition may or may not result in increased leaf P concentrations (Lovelock et al. 2004; Dangremond et al. 2020), and it unclear whether leaf *R* changes with P addition.

We determined the impacts of nutrient enrichment (N or P addition) on photosynthetic and respiratory processes in black mangroves (*Avicennia germinans*) growing in a long-term fertilization study in a coastal wetland in north Florida. The experiment included three treatments: control, added N, added P. N and P were added annually. Previous results indicated that N addition increased percent leaf N (+ 10%) and aboveground productivity (+ 350%), but percent leaf P and productivity did not change with P addition (Dangremond et al. 2020).

In summer 2020, on three dates prior to the annual N or P addition, we measured A_{net}, g_s, iWUE, and the CO_2 response of A to estimate V_{cmax} and J_{max}. We also measured the instantaneous temperature response of leaf R on each date to determine respiratory capacity and the temperature sensitivity of R. These data were used to assess the "long-term" impacts of nutrient enrichment on leaf physiology. Roughly 20 days after the annual N or P addition, we again measured A_{net}, g_s, and iWUE, and determined V_{cmax}, J_{max}, leaf R, and the temperature sensitivity of leaf R. Measurements collected immediately after N or P addition were made on two sets of leaves: those formed prior to annual N or P addition (older leaves) and those formed after annual N or P addition (newer leaves). These data were used to determine the "short-term" impacts of N or P addition on A and R, and to test whether responses differed between leaves formed before and after N or P addition. Given previous results from this experiment (Dangremond et al. 2020), we expected an overall increase in A_{net}, iWUE, V_{cmax}, and R with N addition, and no effect of P addition on any aspect of A or R. We expected that increases in A_{net}, iWUE, V_{cmax}, and R would be largest immediately following annual N addition, and in leaves formed after N addition, assuming that N would be preferentially allocated to new leaves. Given the role of R in supporting photosynthesis, we also tested whether photosynthetic capacity increased with respiratory capacity, and whether the relationship between photosynthetic capacity and respiratory capacity differed among treatments.

Materials and Methods

Site Description

This study took place in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR) on the Atlantic coast of northeast Florida, near St. Augustine. The vegetation in GTMNERR represents a marsh-mangrove ecotone. The southern range of saltmarsh habitat converges and overlaps with the northern limit of mangrove habitat in Florida, although mangroves are increasingly common north of GTMNERR (Cavanaugh et al. 2019). Our study site (29°43′38.3″N 81°14′25.0″W) was located ~ 20 km south of St. Augustine and just north of the Matanzas Inlet. Average annual precipitation is 1317 mm (Chapman et al. 2021). Mean annual temperature (2001–2018) is 20.8 °C. Mean daily maximum temperature (July) is 32.0 °C, mean daily low temperature (January) is 11.1 °C, and the absolute low was 5.3 °C (NOAA-NCDC). The vegetation is dominated by low-stature, shrubby *A. germinans* trees, less than 2 m tall, with an understory of halophytic succulents, *Batis maritima* and *Sarcocornia perennis* (see Fig. S1). *Spartina alterniflora*, a C_4 marsh grass, is also present but patchy at the site (Fig. S1).

Experimental Design

As described by Dangremond et al. (2020), 24 *A. germinans* trees were selected for a fertilization experiment at the site. Trees were 62 to 87 cm (mean = 70.7 ± 1.53 cm) in height at the start of the experiment (2012). Trees were assigned to one of three treatments (8 replicates per treatment): control (no fertilization), added N, or added P. Treatments were randomly assigned to trees, ensuring that trees were located at least 5 m away from each other. Trees were fertilized annually with 300 g of fertilizer [N as NH_4 (45:0:0; N:P:K) or P as P_2O_5 (0:45:0; N:P:K)]. Surface broadcasting was not used when applying N or P, as there was no way to assure that fertilizer treatments could be contained to target trees. Instead, fertilizer was administered through two (3 cm wide × 30 cm deep) soil cores (150 g per core) inserted on opposite sides of the target tree. One hundred fifty grams of N or P fertilizer was placed in the core hole before sealing. For control trees, holes were cored and sealed, but no fertilizer was added. These methods have been used in similar fertilization experiments in Florida (Feller et al. 2003a, b) and Belize (Feller et al. 2007). Prior to the start of our experiment, the most recent fertilization event was applied in October 2019. The 2020 fertilization event occurred during the middle of our experiment (17 August 2020) and was used to test short-term effects of pulse nutrient enrichment. Leaf gas-exchange measurements taken before 17 August 2020 were used to infer long-term effects of N or P addition. Measurements taken immediately after this date were used to infer short-term responses to N or P addition.

Environmental Data

Air temperature (T_{air}) and relative humidity (RH) were measured every 15 min at the site using an air temperature/RH sensor covered in a solar radiation shield (HOBO MX2302 External Temperature/RH Sensor, Onset Computer Corp., Bourne, MA). Mean daily T_{air} of the 7 days preceding physiology measurements ranged from 26.4 to 28.8 °C, and maximum daily T_{air} ranged from 31.3 to 33.7 °C over the experimental period (June–September 2020) (Fig. 1). At the

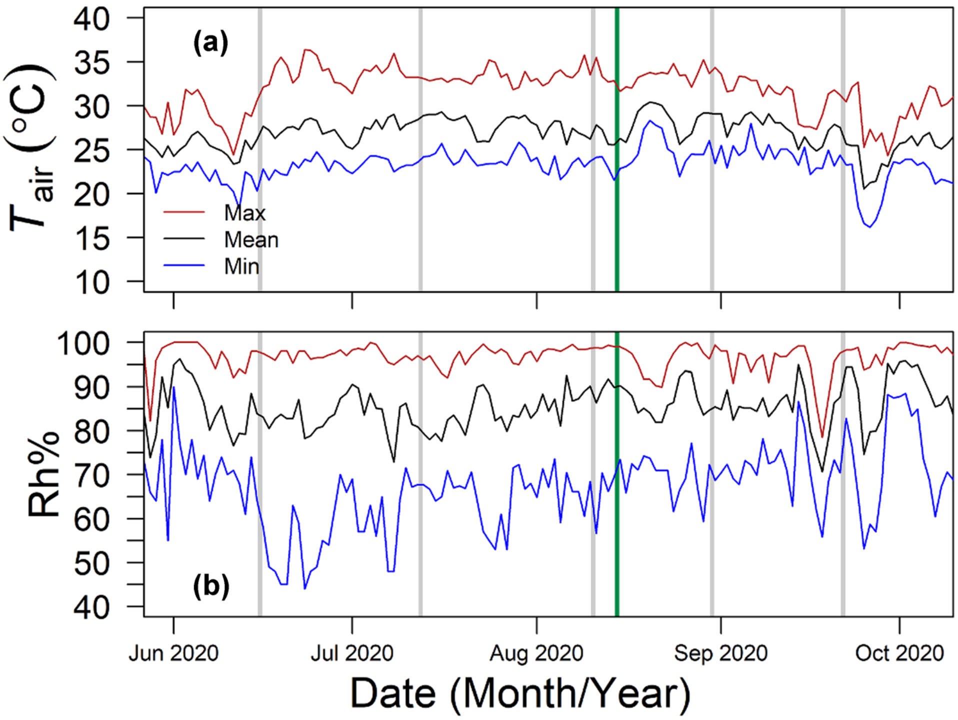

Fig. 1 Maximum, mean, and minimum air temperature (T_{air}) (**a**) and relative humidity (Rh%) (**b**) over the course of the study. Gray vertical lines indicate dates when physiology measurements occurred. The green line indicates the date when annual N or P addition occurred

same site in 2019, Dangremond et al. (2020) reported that pore-water salinity was generally highest during summer (60 ppt) and lower during winter (48 ppt).

CO_2 Response of Photosynthesis

Six trees in each treatment were randomly chosen for physiology measurements. Daytime leaf gas-exchange was measured on four dates between June 2020 and September 2020. On each date, two portable cross-calibrated photosynthesis systems (LI-6800, LiCor., Inc, Lincoln, NE, USA) were used to measure leaf-level CO_2 response (A-C_i). On some dates (19 July and 16 August), we were unable to measure all six trees due to rapid thunderstorm development. On these dates, we measured at least three trees per treatment. A total of 74 A-C_i curves were completed (56 new leaves, 17 old leaves).

Both LI-6800 s were fitted with a 3 × 3 cm cuvette and accompanying 6800–02 small light source. For all measurements, flow rate was held constant at 500 μmol s^{-1}. The temperature exchanger of each LI-6800 was set to the prevailing midday temperature. Leaf temperature (T_{leaf}) was measured with the built-in leaf temperature thermocouple and averaged 32 ± 1.5 °C (standard deviation) across all measurements. Relative humidity conditions in the chamber were controlled near ambient external conditions but also varied depending upon water vapor fluxes from the leaf (e.g., 84 ± 4.2%). Photosynthetic photon flux density in the chamber was set at 1800 μmol m^{-2} s^{-1}, approximating full-sun conditions. Each A-C_i measurement began with steady-state measurements of light-saturated net photosynthesis (A_{net}), stomatal conductance to water vapor (g_s), and intrinsic water use efficiency (iWUE) quantified as the ratio of A_{net} to g_s at a chamber reference [CO_2] of 420 μmol mol^{-1}. Leaves typically reached steady-state within 5–10 min of being enclosed in the chamber. All measurements occurred between 9:00 and 15:00 local time and were made on recently mature, fully expanded, upper canopy leaves. One-sided surface area (cm^2) of leaves within the chamber was estimated by measuring leaf length and width with a ruler. Leaf gas-exchange data were then back-corrected using the corrected leaf area estimate. A-C_i curves were constructed by measuring A_{net} at 12 reference [CO_2] values between 0 and 1500 μmol mol^{-1} (Fig. S2) in a stepwise fashion (420, 300, 250, 100, 50, 0, 420, 650, 800, 1200, 1500). At each reference value, an auto-matching program limited fluctuations of reference $CO_2 < 0.1$ μmol mol^{-1} before logging to ensure accurate measurements. On the final collection dates (28 September), we returned to leaves previously flagged before annual N or P addition and recorded A-C_i measurements (using the same methods as listed above) for those leaves in addition to leaves formed after annual N or P addition. Each A-C_i curve was parameterized using the Farquhar model of C_3 photosynthesis (Farquhar et al. 1980) using non-linear least squared parameter estimation in R version 3.2.1 (R Core-Team 2013). The model was fit using the *fitaci* function from the *Plantecophys* package (Duursma 2015). The model estimates the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport for RuBP regeneration (J_{max}). The temperature correction function was set to T_{leaf} (leaf temperature) for the first iteration of estimates, and the second iteration of estimates were temperature corrected for 25 °C. Leaf mesophyll conductance to CO_2 was not estimated; thus, V_{cmax} and J_{max} are apparent values that reflect both biochemical limitations and mesophyll conductance (e.g., Salmon et al. 2020). Using estimates of V_{cmax} from each A-C_i curve, we estimated stomatal limitations (L) to photosynthesis. L was calculated following Farquhar and Sharkey (1982) by comparing observed A_{net} with the predicted rate of A_{net} if stomatal limitation was zero (i.e., C_i = atmospheric CO_2). The predicted rate of A_{net} when $L = 0$ was back calculated from predicted V_{cmax} using the model of Farquhar et al. (1980). To account for potential effects of measurement temperature, we also estimated V_{cmax} and J_{max} at 25 °C ($V_{cmax,25}$ and $J_{max,25}$) using a peaked Arrhenius equation (see Medlyn et al. 2002) with assumed activation energies and entropy terms of 58.9 kJ mol^{-1} and 0.629 kJ mol^{-1}, respectively, for V_{cmax} and assumed activation energies and entropy terms of 29.7 kJ mol^{-1} and 0.632 kJ mol^{-1}, respectively, for J_{max}. Deactivation energies for V_{cmax} and J_{max} were held constant at 200 kJ mol^{-1} as in other studies (e.g., Vårhammar et al. 2015).

Temperature Response of Leaf Respiration

Measurements of instantaneous temperature response of leaf dark respiration (R, measured as CO_2 efflux per unit leaf area) were conducted at five dates (21 June, 19 July, 16 August, 5 September, 27 September). Leaves for respiration measurements were collected pre-dawn (04:30–06:00 local time) the morning following the completion of A-C_i measurements. On dates where fewer than 18 A-C_i measurements were completed, leaves from all trees were still collected for temperature response measurements ($n = 99$, 81 new leaves and 18 old leaves). Excised leaves were placed in Ziploc bags with moist paper towels and transferred in darkness to the lab. Measurements were randomized across leaves from each treatment and were completed the same day as leaf collection (within 10 h of excision). Leaf area (cm^2) of the measured leaves was determined with a leaf area meter (LI-3000C, LI-COR BioSciences, Lincoln, NE, USA) to accurately estimate R per unit area (R_{area} μmol m^{-2} s^{-1}).

Temperature response curves of leaf R were completed by sealing leaves in large chambers (LI-6400-22L or LI-6800–24) attached to infrared gas analyzers (one LI-6400XT, two LI-6800 s, LI-COR, Inc., Lincoln, NE, USA). The large chambers increased CO_2 differentials (sample CO_2 – reference CO_2) without leak artifacts. For each gas analyzer, flow rate and reference CO_2 of the air were set at 500 mol s^{-1} and 410 mol μmol^{-1}, respectively. Temperature response curves began by placing gas analyzers inside a temperature-controlled growth cabinet (E41L1, Percival Scientific Inc., Perry, IA, USA) set at 15 °C. The block temperature of the gas analyzers was also set at 15 °C. R_{area} was logged once rates of R reached steady state (~ 10 min). We then repeated measurements in stepwise fashion at a series of higher temperatures (randomizing the order of leaf measurement): 20, 27, 35, and 40 °C. This stepwise approach to measuring the temperature response of leaf R did not allow us to assess potential effects of time since leaf removal of the temperature response of leaf R since time and measurement temperature covary. However, previous studies, including a recent study on the instantaneous temperature response of leaf R in black mangrove (Sturchio et al. 2022), have found no effect of time since leaf removal on leaf R measurements (O'Sullivan et al. 2013; Aspinwall et al. 2017, 2019). Moreover, in our study, nearly 90% of the variation in leaf R was explained by measurement temperature alone ($r^2 = 0.89$). After temperature response measurements were completed, leaves were dried at 70 °C for ~ 72 h. Leaf dry mass was recorded to determine leaf dry mass per unit area (LMA, g m^{-2}). Leaf R per unit mass (R_{mass}, nmol g^{-1} s^{-1}) was calculated by dividing R_{area} (× 1000) by LMA.

Modeling the Temperature Response of Respiration

Nonlinear regression was used to model the temperature response of leaf R. Nonlinear models were fit using R version 3.43 (R Core Team 2017). We compared the suitability of three algorithms: (1) a polynomial function describing the non-linear relationship between *ln*-transformed R and T_{leaf} (Heskel et al. 2016), (2) an exponential function with a single Q_{10} value (Ryan 1991), and (3) a modified Arrhenius function (Lloyd and Taylor 1994). The polynomial function is written as:

$$\ln R = a + bT + cT^2 \tag{1}$$

or

$$R = e^{a+bT+cT^2} \tag{2}$$

where T is T_{leaf} and a is an estimate of $ln\ R$ at 0 °C, b is the slope of temperature response of $ln\ R$ at 0 °C, and c describes any nonlinearity in the temperature response of $ln\ R$ with increasing T_{leaf}. The differential of Eq. 2 can be used to estimate the Q_{10} of R at any T_{leaf}:

$$Q_{10} = e^{10\times(b+2cT)} \tag{3}$$

The polynomial function (Eq. 1) provided the best fit to our data, with a strong linear relationship ($R^2 = 0.998$) between observed and predicted values of $ln\ R$, and residuals values normally distributed around zero with little pattern associated with increasing T_{leaf}. We used the polynomial equation to model the temperature response of R and used coefficients a, b, and c to estimate R_{area} and R_{mass} at 25 °C

(R_{area},25, R_{mass},25), and the Q_{10} of R between 20 and 30 °C for each leaf.

Data Analysis

All analyses were performed using R version 3.43 (R Core Team 2017). A two-way analysis of variance (ANOVA) was used to test the effects of measurement date, fertilization treatment, and their respective interaction on photosynthetic and respiratory parameters (A_{net}, g_s, V_{cmax}, J_{max}, R_{area},25, R_{mass},25, LMA, Q_{10}). A second two-way ANOVA was used to test whether the general timing of fertilizer application (pre vs. post nutrient addition), fertilization treatment, or their interactions (application × treatment) influenced photosynthetic and respiratory parameters. A third two-way ANOVA only included data collected on the final date and was used to test whether leaf age (formed prior to N or P addition, formed after N or P addition), fertilization treatment, or their interaction (leaf age × treatment) had any effect on photosynthetic or respiratory parameters. Homogeneity of variance for model results was tested using Levene's and Shapiro–Wilk tests. Data were log or square root transformed dependent upon heteroscedasticity of residuals.

Analysis of covariance was used to test relationships between photosynthetic capacity ($V_{cmax,25}$ and $J_{max,25}$) and respiratory capacity (R_{area},25 or R_{mass},25) and determine whether relationships differed between treatments. In this model, treatment was a factor and respiratory capacity was a covariate. A significant ($P < 0.05$) interaction between respiratory capacity and fertilizer treatment indicated that different slope parameters were required for each treatment. If treatment and respiratory capacity were significant, with no interaction, equations with different intercepts for treatment, but a common slope, were fit to the data. If only respiratory capacity was significant, one equation was fit to data from all treatments.

Results

Photosynthetic Responses to N and P Addition

Trees in the added N treatment showed higher A_{net} immediately after N addition compared to the date just before N addition, while trees in the added P and control treatments showed relatively similar A_{net} values across all dates (Fig. 2). Nonetheless, the effects of date, treatment, and their interaction on A_{net} were not statistically significant (Table 1). Across dates and treatments, A_{net} averaged 7.9 ± 0.65 μmol m^{-2} s^{-1}. Stomatal conductance did not differ between treatments, but varied among dates (Table 1); g_s was highest on 28 September (0.12 ± 0.01 mol m^{-2} s^{-1}) and lowest on 19 July (0.07 ± 0.01 mol m^{-2} s^{-1}) (Fig. 2). iWUE did not differ among individual dates, or among treatments (Table 1) and averaged 0.09 ± 0.01 μmol mol^{-1}.

Trees in the added N treatment showed higher V_{cmax} and J_{max} immediately after N addition compared to the date before N addition, while trees in the added P and control treatments showed less variation across dates (Fig. 3a, c). However, no significant date × treatment interactions were observed for V_{cmax} or J_{max}. Results were similar when V_{cmax} and J_{max} were standardized to 25 °C (Fig. S3). Averaged across treatments, V_{cmax} was lowest on 16 August (63.5 ± 6.4 μmol m^{-2} s^{-1}) and highest on 28 September (88.6 ± 5.6 μmol m^{-2} s^{-1}) (Fig. 3a). Similarly, J_{max} was lowest on 16 August (100 ± 9.2 μmol m^{-2} s^{-1}) and highest on 28 September (136 ± 8.0 μmol m^{-2} s^{-1}) (Fig. 3c). The ratio of J_{max} to V_{cmax} (J_{max}/V_{cmax}) was similar across dates and treatments (Fig. 3e), as was $J_{max,25}$/$V_{cmax,25}$ (Fig. S4). Averaged across dates and treatments, L was 0.31 ± 0.03 (Table 1, Fig. 3g). V_{cmax} and J_{max} measured at prevailing T_{leaf} showed a strong linear relationship across treatments (Fig. S5). $V_{cmax,25}$ and $J_{max,25}$ were also strongly correlated across treatments on an area and mass basis (Fig. S6).

We observed similar patterns when examining leaf gas-exchange and photosynthetic parameters aggregated across pre-fertilization dates and the post-fertilization measurement date. Trees in the added N treatment increased A_{net}, g_s, V_{cmax}, and to some extent J_{max}, after N addition, relative to values aggregated across the three dates before N or P addition (Figs. 2, and 3). Trees in the added P treatment showed smaller increases in these parameters after P addition (relative to measurements taken before P addition), while control trees showed little change in photosynthetic parameters before and after nutrient additions. The apparent short-term response of photosynthesis to N addition (and to some extent P) was only seen in newly formed leaves (Figs. 2, and 3). Despite these trends, treatment effects and timing × treatment interactions were not significant for any photosynthetic parameter. $V_{cmax,25}$, $J_{max,25}$, and $J_{max,25}$/$V_{cmax,25}$ also showed no significant treatment effect or timing × treatment interaction (Figs. S3, and S4).

Leaf age (leaves formed before nutrient pulses, leaves formed after nutrient pulses) had no significant effect on photosynthetic variables and did not interact with treatment (Table 1). Differences in A_{net}, g_s, V_{cmax}, and J_{max} were only observed between the pre- and post-fertilization dates (Table 1). Post-fertilization (28 September) A_{net} (9.40 ± 0.64 μmol m^{-2} s^{-1}) was 26% higher than A_{net} averaged across pre-fertilization dates (7.43 ± 0.37 μmol m^{-2} s^{-1}). Post-fertilization g_s (0.115 ± 0.010 mol m^{-2} s^{-1}) was also 39% higher than pre-fertilization g_s (0.083 ± 0.006 mol m^{-2} s^{-1}). Post-fertilization

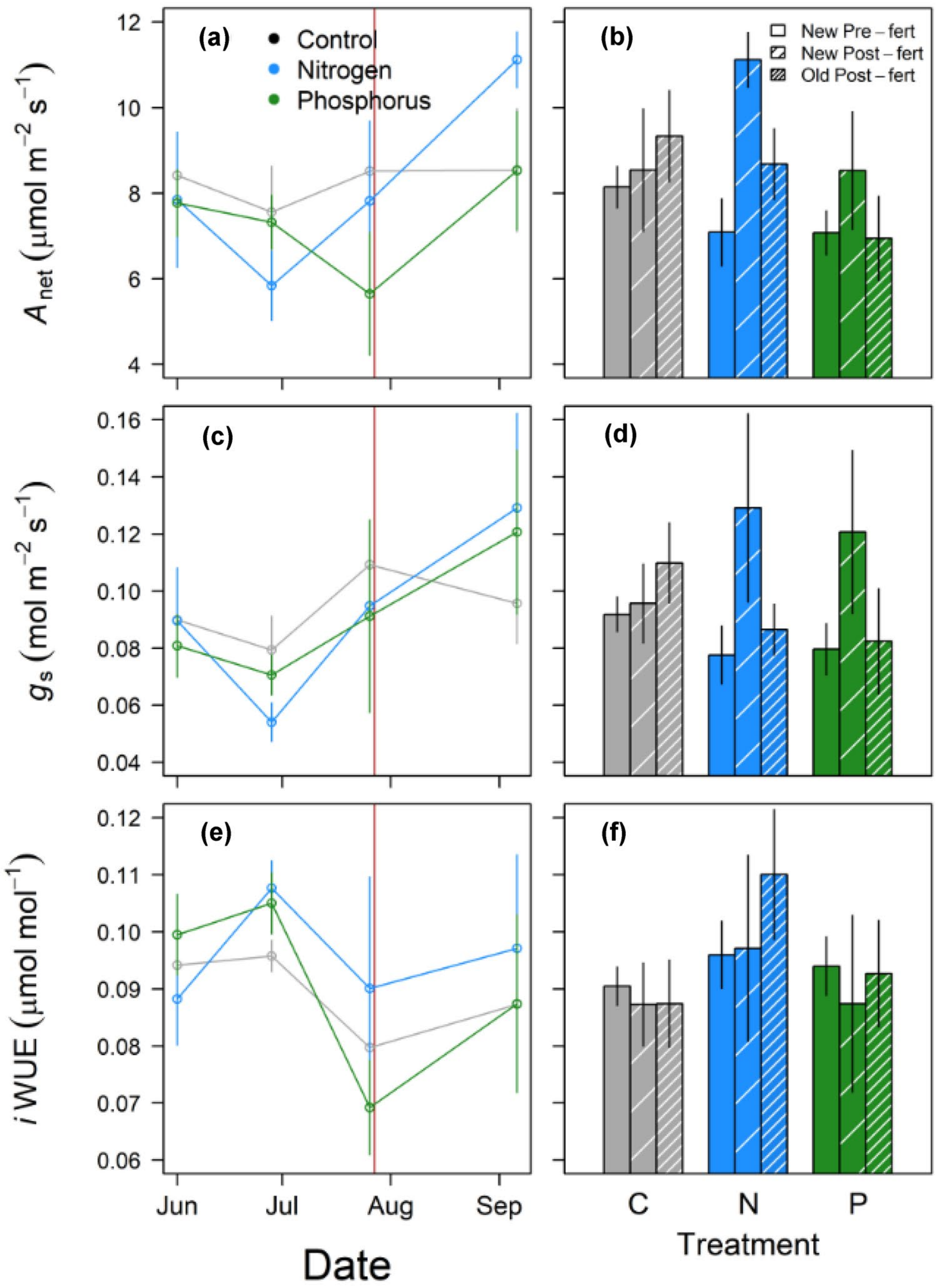

Fig. 2 **a**, **c**, and **e** show mean (± standard error) values for leaf gas-exchange and photosynthetic parameters under control, added N, and added P treatments at four dates. The red vertical line represents the date when annual fertilization was applied. **b**, **d**, and **f** show mean (± standard error) values for leaf gas-exchange and photosynthetic parameters under control, added N, and added P treatments aggregated across time points before the fertilization event (i.e., pre-fert) and after the fertilization event (post-fert) in both recently formed (new) leaves and leaves formed prior to the fertilization event (old leaves). Variables descriptions: A_{net}, light-saturated net photosynthesis; g_s, stomatal conductance to water vapor; iWUE, intrinsic water use efficiency (A_{net}/g_s)

V_{cmax} (88.6 ± 5.6 µmol m^{-2} s^{-1}) was 23% higher than pre-fertilization V_{cmax} (72.3 ± 3.3 µmol m^{-2} s^{-1}) (Fig. 3b). Photosynthetic parameters increased slightly in the added N treatment only, and these small effects were apparently short-lived and only observed in newly formed leaves.

Respiratory Responses to N and P Addition

Respiratory parameters were generally consistent across treatments but varied across dates (Table 2). R_{area},25 was 20% higher on 27 September (1.54 ± 0.06 µmol m^{-2} s^{-1}) than 5 September (1.28 ± 0.06 µmol m^{-2} s^{-1}) (Table 2, Fig. 4a). R_{mass},25 was higher on 16 August (8.73 ± 0.65 nmol g^{-2} s^{-1}) and 27 September (8.65 ± 0.55 nmol g^{-2} s^{-1}) than 5 September (6.61 ± 0.61 nmol g^{-2} s^{-1}). LMA was higher on 21 June (222 ± 8.8 g m^{-2}) than 16 August (178 ± 10.4 g m^{-2}) or 27 September (186 ± 8.8 g m^{-2}) (Table 2, Fig. 4e). Averaged across dates, trees in the added N treatment had higher R_{mass},25 (8.56 ± 0.47 nmol g^{-2} s^{-1}) than trees in the added P treatment (7.47 ± 0.46 nmol g^{-2} s^{-1}) and control trees

Table 1 Results of three separate two-way analyses of variance (ANOVA). The first ANOVA tested the main and interactive effects of fertilization treatment and measurement date (D) on in situ rate of leaf net photosynthesis (A_{net}), stomatal conductance to water vapor (g_s), intrinsic water use efficiency (iWUE), the estimated rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport for RuBP regeneration (J_{max}), their ratio ($J_{\text{max}}/V_{\text{cmax}}$), and stomatal limitation of net photosynthesis (L) in *Avicennia germinans*. The second ANOVA tested the main and interactive effects of fertilization treatment and timing of fertilizer application (A) (before versus after N or P application) on the same traits. The third ANOVA tested the main and interactive effects of fertilization treatment and leaf age (L_a) (formed prior to fertilizer pulse, formed after fertilizer pulse) on the same traits. Degrees of freedom (*df*) and *F*-values are presented for each factor and response variable. *F*-values with "*," "**," and "***" are significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

Trait	Treatment (T)		Date (D)		T×D	
	df	*F*	*df*	*F*	*df*	*F*
A_{net}	2,55	0.56	3	2.57	6	1.10
g_s	2,55	0.01	3	3.60*	6	0.59
iWUE	2,55	0.40	3	2.73	6	0.55
V_{cmax}	2,55	0.83	3	3.53*	6	0.97
J_{max}	2,55	0.68	3	2.89*	6	0.62
$J_{\text{max}}/V_{\text{cmax}}$	2,55	0.36	3	1.34	6	1.05
L	2,55	0.14	3	2.50	6	0.71
Trait	**Treatment (T)**		**Application (A)**		**T×A**	
	df	*F*	*df*	*F*	*df*	*F*
A_{net}	2,55	0.58	1	6.35*	2	2.05
g_s	2,55	0.02	1	6.78*	2	1.48
iWUE	2,55	0.44	1	0.22	2	0.11
V_{cmax}	2,55	0.81	1	5.64*	2	2.37
J_{max}	2,55	0.57	1	0.37	2	0.34
$J_{\text{max}}/V_{\text{cmax}}$	2,55	0.35	1	0.16	2	1.08
L	2,55	0.16	1	0.05	2	0.11
Trait	**Treatment (T)**		**Leaf age (L_a)**		**T×L_a**	
	df	*F*	*df*	*F*	*df*	*F*
A_{net}	2,16	1.74	1	1.26	2	1.11
g_s	2,16	0.03	1	1.66	2	1.20
iWUE	2,16	1.14	1	0.39	2	0.15
V_{cmax}	2,16	2.70	1	1.43	2	0.80
J_{max}	2,16	0.79	1	2.87	2	1.31
$J_{\text{max}}/V_{\text{cmax}}$	2,16	0.36	1	1.36	2	0.49
L	2,16	0.00	1	0.77	2	0.02

(7.24 ± 0.44 nmol g^{-2} s^{-1}) ($P = 0.06$, Table 2, Fig. 4c). There was no effect of treatment or measurement date on Q_{10} (Table 2, Fig. 4g). On average, the Q_{10} was 2.39 ± 0.06.

Leaf respiratory parameters were consistently different between leaf age classes. Compared to newly formed leaves, leaves formed before annual nutrient addition (i.e., older leaves) had 17% lower R_{area},25 (older leaves: 1.28 ± 0.05 μmol m^{-2} s^{-1}, new leaves: 1.54 ± 0.05 μmol m^{-2} s^{-1}) and 34% lower R_{mass},25 (older leaves: 5.75 ± 0.52 nmol g^{-2} s^{-1}, new leaves: 8.65 ± 0.50 nmol g^{-2} s^{-1}). Older leaves had 20% higher LMA than new leaves (older leaves: 223 ± 6.6 g m^{-2}, new leaves: 186 ± 6.4 nmol g m^{-2}) (Table 2, Fig. 4f). In aggregate, respiratory parameters measured before and after N or P addition (i.e., application) were similar (Table 2, and Fig. 4b, d, f, and h).

Relationships Between Photosynthetic and Respiratory Parameters

Across and within treatments, we found no relationship between area-based $V_{\text{cmax,25}}$ and $J_{\text{max,25}}$ and R_{area},25 (Fig. S7a, c). We also found no relationship between mass-based $V_{\text{cmax,25}}$ and R_{mass},25. However, mass-based $J_{\text{max,25}}$ increased with R_{mass},25 and did so consistently across treatments (Fig. S7d).

Discussion

We assessed the impacts of nutrient enrichment (N or P addition) on photosynthetic and respiratory processes in black mangrove (*Avicennia germinans*) growing in a long-term fertilization study in a coastal wetland in north Florida.

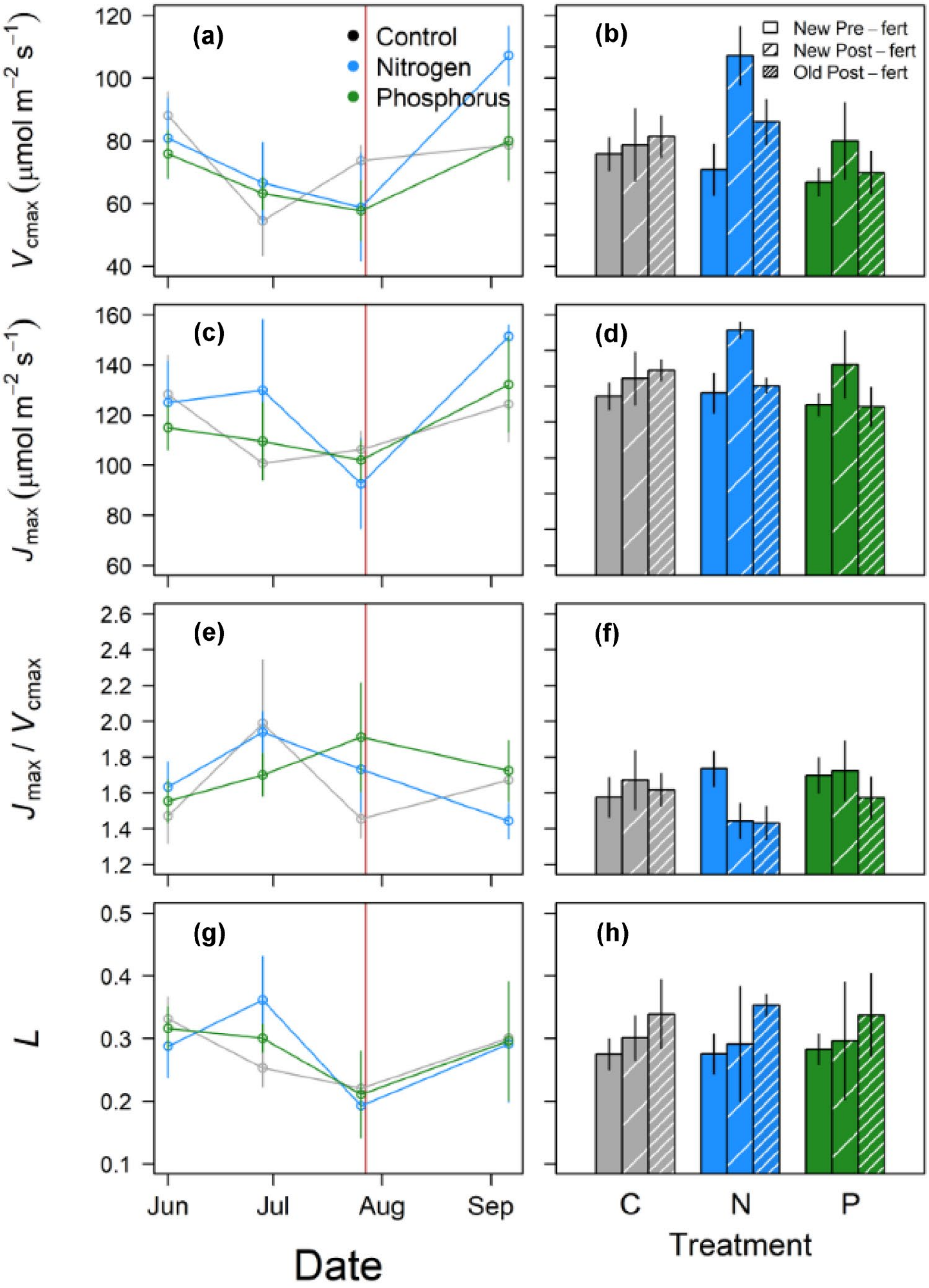

Fig. 3 **a**, **c**, **e**, and **g** show mean (± standard error) values for leaf gas-exchange and photosynthetic parameters under control, added N, and added P treatments at four dates. The red vertical line represents the date when annual fertilization was applied. **b**, **d**, **f**, and **h** show mean (± standard error) values for leaf gas-exchange and photosynthetic parameters under control, added N, and added P treatments aggregated across time points before the fertilization event (i.e., pre-fert) and after the fertilization event (post-fert) in both recently formed (new) leaves and leaves formed prior to the fertilization event (old leaves). Variables descriptions: V_{cmax}, maximum rate of Rubisco carboxylation; J_{max}, maximum rate of electron transport for RuBP regeneration; L, stomatal limitation of net photosynthesis

Averaged over time, we expected an overall increase in A, iWUE, V_{cmax}, J_{max}, and R with N addition, with larger increases in A, iWUE, V_{cmax}, and R immediately following the annual N pulse. Net photosynthesis and photosynthetic capacity were not strongly affected by N addition but increased immediately after the N pulse. We found that increases in A_{net} and photosynthetic capacity were limited to leaves formed after the N pulse, although leaf age effects were largely non-significant. Although N addition had a marginal effect on photosynthetic parameters, we found that N addition increased R_{mass},25. Trees at our site previously showed no evidence of P-limitation on aboveground productivity (Dangremond et al. 2020), and we found support for our hypothesis that P addition would have little effect on photosynthetic or respiratory capacity. We conclude that photosynthetic responses to N addition may be short-lived in N-limited mangrove ecosystems, while respiratory responses to N addition may persist for longer time periods.

Table 2 Results of three separate two-way analysis of variance (ANOVA). The first ANOVA tested the main and interactive effects of fertilization treatment and measurement date (D) on rates of leaf dark respiration at per unit area and per unit mass at 25 °C (R_{area},25, R_{mass},25), leaf dry mass per unit area (LMA), and the temperature sensitivity (Q_{10}) of R between 20 and 30 °C for each leaf in *Avicennia germinans*. The second ANOVA tested the main and interactive effects of fertilization treatment and timing of fertilizer application (A) (before versus after N or P application) on the same traits. The third ANOVA tested the main and interactive effects of fertilization treatment and leaf age (L_a) (formed prior to fertilizer pulse, formed after fertilizer pulse) on the same traits. Degrees of freedom (*df*) and *F*-values are presented for each factor and response variable. *F*-values with "*," "**," and "***" are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively

Trait	Treatment (T)		Date (D)		T×D	
	df	*F*	*df*	*F*	*df*	*F*
R_{area},25	2, 80	1.45	4	3.27*	8	0.91
R_{mass},25	2, 80	2.91	4	2.79*	8	0.87
LMA	2, 80	1.57	4	3.25*	8	1.00
Q_{10}	2, 80	1.81	4	2.42	8	0.90
Trait	**Treatment (T)**		**Application (A)**		**T×A**	
	df	*F*	*df*	*F*	*df*	*F*
R_{area},25	2, 80	1.53	1	0.92	2	0.28
R_{mass},25	2, 80	2.60	1	0.01	2	0.12
LMA	2, 80	1.39	1	0.85	2	0.20
Q_{10}	2, 80	1.79	1	0.45	2	0.11
Trait	**Treatment (T)**		**Leaf age (L_a)**		**T×L_a**	
	df	*F*	*df*	*F*	*df*	*F*
R_{area},25	2, 17	0.58	1	13.49***	2	0.73
R_{mass},25	2, 17	0.61	1	16.46***	2	0.55
LMA	2, 17	0.23	1	16.20***	2	0.21
Q_{10}	2, 17	0.78	1	1.61	2	0.08

Photosynthetic Responses to Nutrient Addition and Their Relationship with Productivity

The short-term and marginal increase in photosynthesis from N fertilization is somewhat surprising given that N addition caused large increases in mangrove productivity (Dangremond et al. 2020). Why was there no strong effect of N addition on leaf photosynthesis, despite large changes in productivity? There could be several non-mutually exclusive explanations. First, small increases in leaf N and leaf photosynthesis, when scaled across the entire tree crown, could result in greater whole-tree C uptake and partly explain why trees in the added N treatment were substantially larger. Additionally, Dangremond et al. (2020) reported that trees in the added N treatment showed 350% higher shoot biomass than trees in the control treatment after 4 years. However, leaf N concentrations were only ~ 10% higher (on average) in trees in the added N treatment compared to control trees. Meta-analyses of plant responses to N addition have found similar patterns, where growth traits increase more than physiological traits (Liang et al. 2020). In our study, trees in the added N treatment showed a ~ 50% increase in A_{net} and V_{cmax} following the N pulse, but only in newly formed leaves. Given that A_{net} and V_{cmax} were similar among new leaves at dates prior to the N pulse, we hypothesize that increased A_{net} in new leaves was short-lived. Nonetheless, we only measured photosynthesis at one time point following the N pulse and we do not know whether or how photosynthetic parameters responded to N or P pulses over longer time periods (i.e., months).

Another possible explanation for the large differences in productivity among treatments despite small and possibly transitory differences in photosynthesis among treatments could be that the effects of nutrient enrichment on photosynthesis weaken over time as trees grow larger. It is plausible that N addition at the start of the experiment "released" the smaller trees from N limitation by stimulating larger increases in leaf N and photosynthesis across a small number of leaves, resulting in more C available for growth, higher leaf area, more light interception, and accelerated development (e.g., Coleman et al. 2004; Coyle and Coleman 2005). However, the effects of N addition on leaf photosynthesis might diminish as trees become larger. Many plant types have shown reduced responsiveness to constant nutrient enrichment rates, resulting in weaker relationships between fertilization, leaf physiology, and productivity response over time (Liang et al. 2020). Considering these trees received constant amounts of added N and added P annually over

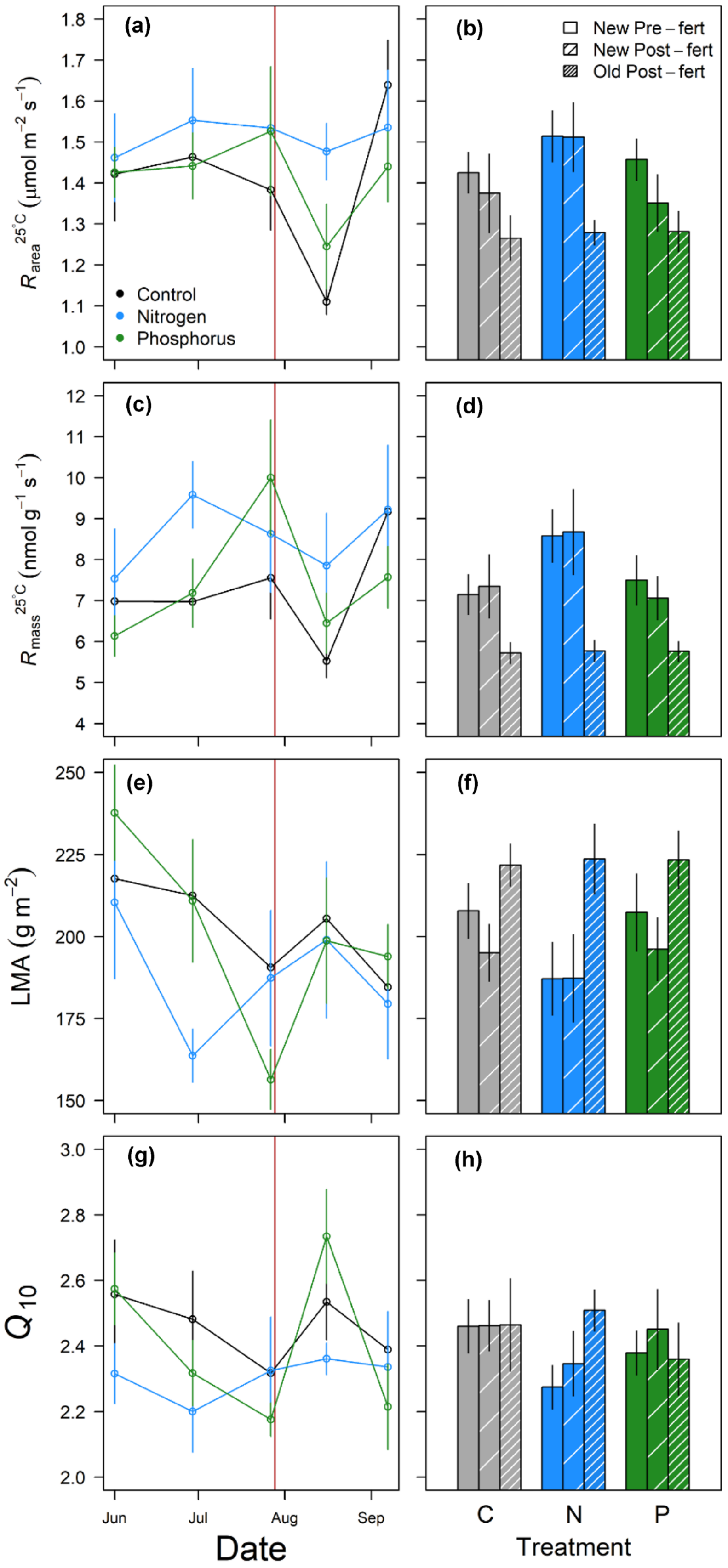

Fig. 4 **a**, **c**, **e**, and **g** show mean (± standard error) values for leaf respiratory parameters under control, added N, and added P treatments at five dates. The red vertical line represents the date when annual fertilization was applied. **b**, **d**, **f**, and **h** show mean (± standard error) values for leaf respiratory parameters under control, added N, and added P treatments aggregated across time points before the fertilization event (i.e., pre-fert) and after the fertilization event (post-fert) in both recently formed (new) leaves and leaves formed prior to the fertilization event (old leaves). Variables descriptions: R_{area},25, leaf respiration per unit area at 25 °C; R_{mass},25, leaf respiration per unit mass at 25 °C; LMA, leaf mass per unit area; Q_{10}, the temperature sensitivity of R between 20 and 30 °C

9 years, it is plausible that nutrient enrichment impacts on photosynthesis declined as trees became larger and resources were spread across more leaf area (Linder and Rook 1984).

Other explanations for apparent discrepancies between whole-plant growth responses to added N and photosynthetic responses to added N include potential changes in biomass allocation. In terrestrial species grown under relatively uniform conditions, allocation to roots relative to shoots generally decreases as nutrient availability increases and increases as nutrient availability decreases (Shipley and Meziane 2002; Poorter et al. 2012). Increased shoot allocation tends to coincide with increased shoot N concentrations. It is unclear whether similar patterns exist under natural conditions where above and belowground competition varies, and little is known about nutrient enrichment impacts on biomass allocation in mangroves. However, a study by Weaver and Armitage (2020) in the Gulf of Mexico found that combined N and P fertilization reduced the root:shoot ratio of *Avicennia germinans* from 0.80 to 0.42, although this difference was not statistically significant. On the Atlantic coast of Florida, Simpson et al. (2013) found that N addition caused a small (non-significant) reduction in the root:shoot ratio of *Avicennia germinans* seedlings under low competition, but the opposite pattern was observed under high (natural) competition. In potted *Avicennia marina* seedlings, Hayes et al. (2017) found large reductions in the root:shoot ratio with N addition, but field growth trees showed faster rates of root growth under N addition. Leaf N and P were not measured as part of our study, and it is not clear whether N or P addition altered biomass allocation in our study. However, if trees in the N addition treatment allocated more biomass to shoots relative to roots, this might partly explain large increases in aboveground biomass despite small and transitory increases in photosynthesis. Regardless of the mechanism, our results indicate that N addition may have small effects on leaf-scale photosynthesis in mangroves growing in north Florida.

Nutrient Enrichment Effects on Leaf Respiration

Averaged across dates R_{mass},25 was ~ 16% higher in the added N treatment compared to the added P and control treatments. This result supported our hypothesis that leaf R would increase with N addition, but not P addition. Previous work across other species has also found evidence of increased leaf R with N addition (Brix 1971; Manter et al. 2005, Van de Weg et al. 2013). To our knowledge, this is the first direct evidence of increased leaf R with N addition in a mangrove species. This increase in respiratory capacity did not coincide with changes in the temperature sensitivity of leaf R (i.e., Q_{10}). Interestingly, leaf R and photosynthesis were not strongly coupled. Although R_{mass},25 increased with N addition, A_{net} and V_{cmax} only increased in newly formed leaves, just after N addition. We also found no clear relationship between V_{cmax} and leaf R across treatments. This apparent decoupling is somewhat surprising given the role of leaf R in supporting processes involved in photosynthesis. However, leaf R is known to play an important role in other processes including turnover of phospholipid membranes and maintenance of cellular ion gradients (Penning de Vries 1975; Amthor 1984). Across treatments and dates, we found a rather weak relationship between $J_{\mathrm{max,25}}$ and R_{mass},25. Other studies have also found evidence of a linkage between $J_{\mathrm{max,25}}$ and leaf R in tropical trees (Rowland et al. 2015). Although speculative, the $J_{\mathrm{max,25}}$–R_{mass},25 relationship we observed could represent a linkage between thylakoid membrane turnover, electron transport, ATP synthesis, and RuBP regeneration. Alternatively, under stressful conditions (drought, salinity, heat) respiratory enzyme (e.g., alternative oxidase) activity is upregulated to support cell function, resulting in higher sink demand, and increased photosynthetic capacity. Respiratory demand may be particularly high in coastal wetlands like ours where salinity varies and is sometimes much higher than seawater (Dangremond et al. 2020). Under these conditions, leaf R surely plays a role in maintaining cellular ion gradients that maintain cell water status (López -Hoffman et al. 2007). It is possible that N addition allowed for greater maintenance of ion gradients. While the underlying cause of increased leaf R with N addition and the relationships between leaf R and $J_{\mathrm{max,25}}$ remain unclear, these results indicate that leaf R and photosynthesis were not strongly coupled. Such coupling may not be widespread in coastal wetlands, where nutrient and salinity conditions show high spatial and temporal variability.

Leaf Age Effects

Previous studies have generally shown that photosynthesis and respiration decline with leaf age (Suzuki et al. 1987; Whitehead et al. 2011). *Avicennia germinans* has an average leaf lifespan of ~ 16 months (Suarez and Medina 2005; Reef et al. 2010). We hypothesized that photosynthesis and respiratory capacity would decline with leaf age and found support for this hypothesis as R_{area},25 and R_{mass},25 were significantly lower in older *Avicennia* leaves. We also found that LMA was higher in older leaves. This result could reflect a reduction in N allocation to photosynthetic and respiratory proteins and an increase in N allocation to cell wall material as leaves age (Kattge et al. 2009; Onoda et al. 2017). Although results of statistical analysis were not significant, old leaves showed reduced photosynthesis across treatments and over time with lower V_{cmax} and J_{max}. We also found that leaves formed before N (or P) addition showed no response to N or P addition, indicating that increased photosynthesis is probably only apparent in newly formed leaves, and that response is relatively short lived. This finding may be useful in describing general relationships between leaf age, respiration, and photosynthesis for *Avicennia*, mangroves, and other coastal wetland species.

Conclusion

Our results provide new insight into nutrient enrichment impacts on mangrove physiology. They suggest that N addition can cause a small, short-term increase in photosynthetic processes in N-limited mangrove wetlands. However, N addition might trigger sustained increases in leaf *R*. Such decoupling might result in subtle changes in mangrove ecosystem C balance with nutrient enrichment. We also show that older, thicker, and denser mangrove leaves exhibit lower respiratory capacity; a result found in most C_3 plants. It is not clear whether these subtle long-term patterns in physiological response to nutrient enrichment will influence mangrove range expansion. However, as pioneer mangroves continue to push their range limits, the added effect of nutrient enrichment has the potential to increase mangrove resilience to low temperatures due to apparent increases in C assimilation and growth. We note that coastal wetlands are not well-represented in land surface models due to gaps in our understanding of key processes and data limitations. These results may be useful in modeling C cycle and nutrient cycle feedbacks in coastal wetland ecosystems under current and future conditions. Future studies that explore mangrove physiology with full factorial nutrient enrichment experimental designs (N, P, N + P) and legacy effects of nutrient enrichment across multiple sites will further advance our understanding of how nutrient inputs impact mangrove C exchange.

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Author Contribution ICF, MAS, and MJA conceived and designed the experiment. MAS, JC, and MJA collected data. MAS, JC, and MJA led the data analysis with input from all authors. MAS and MJA wrote the manuscript with input from all authors.

Data Availability The datasets used and/or analyzed during the current study are available at https://github.com/Msturchio/WETFERT.

Declarations

Conflict of Interest The authors declare no competing interests.

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