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Matthew Sturchio
University of North Florida, n01411578@unf.edu

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PLANT PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL CHANGE IN A MARSH-MANGROVE ECOTONE

by
Matthew Sturchio

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Abstract

Globally, photosynthesis (*A*) and autotrophic respiration (*R*) are the two largest physiological processes responsible for CO_2 flux. Coastal wetland ecosystems are responsible for some of the highest rates of C sequestration. Marsh grass and mangrove habitats responsible for this service are important in supporting biodiversity and preventing shoreline erosion, yet little is known about how this vegetation will respond physiologically to effects of climate and global change. In the first chapter a warming experiment was used to determine whether a C_4 marsh grass (*Spartina alterniflora*) and a C_3 mangrove (*Avicennia germinans*) acclimate leaf *R* to seasonal changes in temperature and experimental warming, and whether variation in leaf N (proxy for enzyme concentrations) explains temperature acclimation patterns in both species. In the second chapter a long-term fertilization experiment was conducted to determine whether *Avicennia* alters parameters of *A* and *R* over time in response to nutrient enrichment of nitrogen (N) or phosphorus (P), and whether the response to pulse enrichment of nutrients is temporary or leaves a legacy effect. In chapter 1, *Avicennia* generally increased respiratory capacity as growth temperatures increased, but *Spartina* acclimated to warmer temperatures by reducing respiratory capacity. Although temperature acclimation of *R* differed between *Spartina* and *Avicennia*, changes in N_{mass} explained temperature acclimation patterns in both species. In chapter 2, N addition increased *R* capacity (R_{mass}^{25}), CO_2 assimilation (A_{sat}), and the maximum rate of Rubisco carboxylation (V_{cmax}). The effects of N addition on photosynthesis were short-lived, but the effect of N addition on leaf *R* was consistent over time, indicating a possible legacy effect of *R* but not *A*. This thesis provides new insight into temperature controls of leaf *R* in marsh and mangrove species, which may aid predictions of CO_2 fluxes from coastal wetlands. This research also improves our understanding of the short- and potentially long-term impacts of N and P enrichment on mangrove physiology.

Introduction

Vegetated coastal ecosystems are plant communities that exist at the intersection of ocean and land. These communities are composed of plant species that tolerate both tidal inundation and fluctuating salinity. At higher latitudes of North America where climate is temperate, vegetated coastal communities are comprised of mostly marsh grasses including *Spartina alterniflora* (smooth cordgrass) and *Spartina patens* (saltmeadow cordgrass) and a small number of forb and shrub species. In the subtropics and tropics, coastal vegetative communities are made up of a variety of halophytic mangrove species including *Avicennia germinans* (black mangrove) *Rhizophora mangle* (red mangrove), and *Laguncularia racemosa* (white mangrove). The ecotone represents a highly productive coastal wetland transitional zone where the southern limit of temperate saltmarsh habitat converges with the northern limit of tropical mangrove habitat. Temperature, especially minimum winter temperatures, are a key determinant of the location of the marsh-mangrove ecotone (Osland et al. 2013; Cavanaugh et al. 2014, 2015; Saintilan et al. 2019).

Coastal vegetation serves as the primary habitat for a number of aquatic and terrestrial organisms. Coastal birds utilize these habitats as a food source, building material, and rookeries. Many economically important fish species of recreational or commercial importance utilize tidal marshes through different stages of development (Mumby et al. 2004; Johnson & Swenarton 2016). Finger lakes of intercoastal waterways have calm waters and are an ideal location for juvenile fish to feed and grow before spending their adult life at sea. In summer months, fish, crabs, and snails use the shade from marsh grasses and mangroves to stay cool. This shading also reduces the amount of evaporation that occurs underneath plant canopies which decreases salinity and enables further vegetative recruitment (Peterson & Bell 2018).

Coastal vegetation also serves as an important defense against the impacts of sea level rise (SLR). Coastal vegetation plays an important role in stabilizing sediments so that suspended solids become fixed in soils rather than entering the water column (Wieski et al 2010; Doughty et al. 2017). This fixation is referred to as soil accretion and is an important driver of wetland elevation gain (Coldren et al. 2016; Coldren et al. 2019). Specific accretion rates of marsh and mangrove communities vary in different regions of the world but are correlated with sediment availability. Vegetative growth and root production also increases C inputs belowground which can increase C storage and wetland elevation. If the rate of accretion and elevation gain is rapid enough to keep pace with the rate of SLR, coastal vegetation may limit the loss of coastline from SLR (Morris et al. 2002; Cahoon et al. 2020; Chapman et al. 2021). These habitats also alleviate the impacts of storms and excessive boat traffic by dissipating wave energy and reducing erosion of wetland soils (Guannel et al. 2016). All coastal vegetation plays a role in reducing erosion caused by wave action, although there is evidence that mangrove habitats are more effective at attenuating waves in shorter distances than marsh habitat (Doughty et al. 2017).

These systems also act as natural filters that purify water entering estuaries (Mitsch and Gosselink 2008). As water enters the coastal wetland through river tributaries, groundwater movement, and overland flow is slowed by the physical resistance imparted by wetland vegetation (Morgan et al. 2009). Suspended sediments are then deposited in layers on the soil surface, simultaneously increasing elevation and adding nutrients to an extremely nutrient-limited ecosystem. The ability of these systems to filter nutrients also improves water quality and protects coastal economies from harmful algal blooms (HAB's) (Barbier et al. 2011). Reduction of HAB's promotes healthy growth of submerged vegetative communities (e.g. seagrasses) that would otherwise be shaded out by surface algal blooms. When all the goods and services provided by vegetated coastal ecosystems are accounted for, models estimate that these vegetated coastal wetlands have an *annual* economic value of $10,000-$20,000 per hectare (Kirwan and Megonigal, 2013).

Coastal vegetative ecosystems also play an important role in the global C cycle. At the global scale these ecosystems deposit a similar amount of C belowground as terrestrial forests, despite covering just 3% of the area of terrestrial forests (Lugo & Snedeker 1974, Bouillon et al. 2008, Duarte et al. 2013). On average, marsh and mangrove ecosystems store more carbon per unit area (593 Mg C ha^{-}1 and 956 Mg C ha^{-1}, respectively) than many tropical rainforests (241 Mg C ha^{-1}) (Donato et al. 2011; Alongi et al. 2014). Coastal vegetation is an effective carbon sink for several reasons. First, like terrestrial plants, coastal vegetation sequesters C within living biomass (e.g., leaves, roots, woody material) and non-living biomass (e.g., leaf litter and dead wood) (Duarte et al. 2005, 2013; Mcleod et al. 2011). However, unlike terrestrial soils, mangrove and salt marsh sediments accrete vertically in response to sea level rise which effectively eliminates C saturation (McLeod et al. 2011). This vertical accretion in combination with anaerobic wetland soils creates an environment of burial and slow turnover. For example, it has been estimated that coastal vegetation sequesters C much longer (centuries vs. millennia) than the world's most productive tropical rainforests (Chambers et al. 2001; McLeod et al. 2011; Alongi et al. 2014; Alongi and Mukhopadhyay 2015).

In many locations throughout the planet, habitat destruction, environmental degradation, and climate change are threatening or altering the health and function of these valuable coastal ecosystems (Friess et al. 2019). For example, 25 to 50% of global mangrove extent is estimated to have been lost over the last 50 years due to habitat conversion and degradation (Valiela et al. 2001, Alongi 2002). In a number of locations (United States, Australia) climate warming is contributing to a reduction in freeze events at mangrove range limits, facilitating poleward movement of mangroves into saltmarsh habitat (Cavanaugh et al. 2014, Kelleway et al., 2017; Osland et al., 2013; 2016a; Perry & Mendelssohn, 2009; Saintilan et al., 2014; Feller et al. 2017). The encroachment of mangroves is fundamentally altering the structure and function of these ecosystems (Doughty et al. 2016, others). There is also uncertainty about whether, or for how long, marsh and mangrove ecosystems can gain elevation to keep pace with SLR (Friess et al. 2019, Lovelock et al. 2015, Schuerch et al. 2018). Marsh equilibrium models (MEMs) originally created to assess vulnerability of coastal marshes to SLR have recently been adjusted to include predictions for mangrove forests susceptibility to rising seas (Cahoon et al. 2020). These models predict that by 2100 marsh ecosystems will be lost under a business-as-usual SLR forecast (Morris et al. 2002, 2016). A key conservation solution for SLR is ‘marsh migration’ which would allow for salt marshes and mangrove forests to build vertically along a natural elevation gradient, thus sustaining their ecosystem service functions under SLR scenarios (Schuerch et al. 2018).

In addition to SLR, coastal ecosystems are threatened by other aspects of global change. Global mean temperatures are expected to rise 1–4°C by 2050, with the amount of warming varying among regions (IPCC 2013, Ciais et al. 2013). Warmer temperatures could alter rates of C uptake, use and storage in coastal wetlands, with implications for climate-carbon cycle feedbacks (Atwood et al. 2017). However, there is considerable uncertainty about temperature controls of C cycling processes over space and time in coastal wetlands (Duarte 2017, Adame et al. 2018). This uncertainty stems from several sources including but not limited to: 1) limited data on leaf CO_2 fluxes in relation to seasonal or latitudinal variation in temperature for marsh and mangrove species in tropical and subtropical climates, 2) Climate warming impacts on leaf photosynthetic or respiratory processes in tropical and subtropical marsh-mangrove ecosystems are virtually unknown, and 3) Marsh and mangrove species capacity for 'thermal acclimation' of leaf physiology remain understudied and difficult to predict (Aspinwall et al. 2021), despite the importance of thermal acclimation in modifying the temperature response of wetland C cycling processes. New work is needed to address these uncertainties and improve our quantitative and predictive understanding of coastal C cycling responses to temperature, potential feedbacks between temperature and C cycling, and the representation of these processes in earth system models (ESMs) that predict future climate.

Human activities have also resulted in large and persistent nutrient inputs into coastal habitats resulting in eutrophication and harmful cyanobacteria blooms. These events are increasingly frequent, creating hypoxic conditions which negatively impact the natural biodiversity of coastal ecosystems. In general, nutrient enrichment and N deposition are expected to stimulate growth and productivity of marsh grasses and mangroves (Feller et al. 2003, Darby and Turner 2008, Davis et al. 2017). Similar to most nutrient limited ecosystems, early studies showed that mangrove responses to N and P additions are dependent upon the limiting nutrient in the system (Feller et al. 2007). Some also have suggested that *A. germinans* is a superior competitor to marsh grass species when nutrients are limited (Simpson et al. 2013). In a new study, Dangremond et al. (2020) found that N-enriched mangroves grew taller, increased their canopies, increased leaf chlorophyll content, and had higher leaf N concentrations relative to P-enriched and control trees. However, important questions remain. For instance, few studies have examined how long-term nutrient (N and P) enrichment impacts photosynthetic biochemistry or respiratory capacity of mangrove foliage. We also have a limited understanding of the short-term responses of photosynthesis and respiration to nutrient pulses in coastal wetlands. These knowledge gaps hinder our ability to predict photosynthetic and respiratory processes along spatial or temporal gradients in nutrient enrichment or incorporate the impacts of nutrient enrichment on vegetation function in models that simulate or project C and N cycling over space and time in coastal wetlands.

The overarching aim of this thesis is to investigate patterns of thermal acclimation of leaf respiration over space and time in coastal plant species, and the long and short-term physiological response of mangroves to N and P additions. In the first chapter we tested whether a C_4 marsh grass species (*Spartina alterniflora*) and C_3 mangrove species (*Avicennia germinans*) growing in a marsh-mangrove ecotone show evidence of seasonal temperature acclimation of leaf *R*, and whether seasonal temperature acclimation of leaf *R* is consistent in plants growing under ambient and experimentally warmed conditions, as well as across sites at the northern and southern edge of the ecotone. We accomplished this by repeatedly measuring the short-term temperature responses of leaf *R* over a 10-month period in marsh and mangrove plants grown *in situ* under ambient temperatures and experimental warming at two sites within the ecotone. We also determined leaf N on a subset of leaves and tested whether leaf N scales positively with *Spartina* and *Avicennia* leaf *R*, over time and across sites and treatments. We hypothesized that seasonal acclimation of *R* would be consistent across ambient and warmed treatments, and across northern and southern sites, demonstrating convergent temperature acclimation of leaf *R*. In the second chapter we determined the long-term and short-term impacts of N and P additions on photosynthetic and respiratory processes in black mangrove (*Avicennia germinans*). Ultimately, this work will improve our understanding of coastal wetland vegetation responses to environmental change.

Chapter 1

Patterns of thermal acclimation of leaf respiration in a marsh-mangrove ecotone

Abstract

Leaf respiration (*R*) is an important parameter in terrestrial biosphere models and associated earth system models. Yet, spatiotemporal patterns of leaf *R*, its temperature sensitivity, and temperature acclimation of leaf *R* have not been widely studied in coastal marsh and mangrove species. Here we examined seasonal temperature acclimation of leaf *R* in a C_4 marsh species (*Spartina alterniflora*) and C_3 mangrove species (*Avicennia germinans*) growing under ambient temperatures and experimental warming at northern and southern sites within a marsh-mangrove ecotone in North Florida. We also tested whether variation in leaf N (proxy for enzyme concentrations) explain temperature acclimation patterns in both species. In *Spartina*, respiratory capacity (*R* per unit mass at 25 °C, R_{mass}^{25}) and the temperature sensitivity of *R* (Q_{10}) declined as seasonal temperatures increased, indicating seasonal temperature acclimation, and did so consistently across treatments and sites. Across sites and treatments, leaf N per unit mass (N_{mass}) showed a strong positive relationship with R_{mass}^{25}. In *Avicennia*, R_{mass}^{25} showed no relationship with prevailing seasonal temperatures at the northern site but increased as seasonal temperatures increased at the southern site. Warming caused a small increase in R_{mass}^{25} at both sites. Nonetheless, the Q_{10} of *R* decreased as seasonal temperatures increased and did so consistently across sites and treatments. Importantly, N_{mass} also showed a strong positive relationship with R_{mass}^{25} in *Avicennia*. Therefore, although temperature acclimation of *R* differed between *Spartina* and *Avicennia*, changes in N_{mass} explained temporal and spatial variation in R_{mass}^{25} in both species. These results improve our quantitative and predictive understanding of

temperature controls of leaf respiration in coastal plant species and provide new data for improving representation of these processes in larger-scale models.

Introduction

Coastal marsh and mangrove ecosystems make a large contribution to global primary production and carbon (C) cycling despite covering a small proportion of the earth's surface. (Lugo & Snedeker 1974, Bouillon et al. 2008, Donato et al. 2011, McCleod et al. 2011, Lu et al. 2017). For instance, each year these coastal wetlands deposit a similar amount of organic C belowground as terrestrial forests, despite covering less than 3% of the area of forests (Lugo & Snedeker 1974, Bouillon et al. 2008, Duarte et al. 2013). The high C storage capacity of coastal wetlands can be attributed to their effectiveness in trapping and burying sediment from terrestrial and marine sources, often referred to as blue carbon (Nellemann et al. 2009). Much of the C fixed by photosynthesis remains sequestered for millennia due to slow decomposition rates, anaerobic conditions, and a relatively high fraction of total biomass allocated to roots and rhizomes (Duarte et al. 2005; Mcleod et al. 2011, Howard et al. 2017). However, global mean temperatures are expected to rise 1–4°C by 2050 (IPCC 2013, Ciais et al. 2013) resulting in higher sea levels, more frequent and intense storms, and altered precipitation patterns, all of which could affect the composition, structure, C storage and persistence of coastal wetlands (Lovelock et al. 2016, Friess et al. 2019). Rising temperatures, in particular, will have direct effects on photosynthesis and respiration, with important consequences for coastal C cycling and vegetation – climate feedbacks. New work is required to improve our understanding of temperature controls of C cycling processes over space and time for marsh and mangrove ecosystems.

At the global scale, autotrophic respiration represents the second largest flux of CO_2 second only to photosynthesis (IPCC 2013, Ciais et al. 2013). On an annual basis, roughly half of global terrestrial gross primary production (GPP) by plants is returned to the atmosphere via autotrophic respiration (Piao et al. 2013). Plant respiration is estimated to account for a similar proportion (~50%) of GPP in marsh and mangrove ecosystems (Alongi et al. 2020). At the global scale, roughly 50% of plant respiration comes from leaves (Atkin et al. 2007, Atkin et al. 2015). As a result, leaf mitochondrial respiration (*R*, typically measured in darkness as CO_2 efflux) is an important parameter for simulating C cycling in terrestrial biosphere models (TBMs) and associated earth system models (Fisher et al. 2014, Atkin et al. 2015). Leaf *R* and photosynthetic capacity are closely coupled (O'Leary et al. 2019, Wright et al. 2004, Wang et al. 2020). Respiration plays an important role in nitrate reduction, phloem loading, and turnover of phospholipid membranes and proteins. Rubisco accounts for a large fraction of leaf protein (Evans 1989, Hikosaka and Shigeno 2009, Aspinwall et al. 2019) and thus leaf *R* plays an important role in maintaining photosynthetic capacity under natural conditions. Across a wide range of species and systems, leaf *R* at 25 °C scales positively with maximum rates of Rubisco carboxylation (V_{cmax}) at 25 °C (Atkin et al. 2015). Total leaf N represents total photosynthetic and respiratory enzyme content, and leaf N also positively scales positively with both V_{cmax} and *R* at 25 °C across a wide range of species and growth environments (Reich et al. 2008, Atkin et al. 2015). For these reasons, many land surface models predict functional-type specific rates of *R* using either estimates of V_{cmax} at 25 °C or measurements of leaf N on an area or mass-basis (Fisher et al. 2014, Lawrence et al. 2019).

Leaf *R* is determined by a series of enzymatic reactions in the cytosol and mitochondria and is therefore temperature dependent. In the short-term (minutes, hours) leaf *R* increases quasiexponentially with increasing measurement temperature before reaching an optimum, typically between 50 and 60 °C (O'Sullivan et al. 2017, Aspinwall et al. 2017). This temperature sensitivity has led to the prediction that climate warming will accelerate the positive feedback between CO_2 and temperature. However, there is considerable evidence that plants may 'acclimate' to changes in growth temperature by modifying the short-term temperature response of *R*. In most cases, thermal acclimation of *R* results in partial or complete homeostasis of leaf *R* across changing growth temperatures. At the plant scale, thermal acclimation helps balance the supply of ATP and carbon skeletons for growth while reducing the C cost of maintenance *R* (Atkin and Tjoelker 2003, Atkin et al. 2005). Acclimation can occur within a few days of a change in growth temperature (Bolstad et al. 2003, Lee et al. 2005, Aspinwall et al. 2016). Many plants acclimate to increasing growth temperatures by reducing *R* at a set measurement temperature (i.e. 25 °C, Slot and Kitajima 2015). This type of acclimation is referred to as Type II acclimation and is considered a change in respiratory capacity (Atkin and Tjoelker 2003). Less commonly, thermal acclimation can result in a reduction in the short-term temperature sensitivity of *R* (Q_{10}, activation energy, Slot and Kitajima 2015). This type of acclimation is referred to as Type I acclimation (Atkin and Tjoelker 2003). Type II acclimation may be the result of changes in respiratory enzyme concentrations, while Type I acclimation is probably the result of regulatory changes in respiratory enzymes (Atkin et al. 2005). Across sites and species, patterns of thermal acclimation of *R* are generally associated with changes in leaf N and adjustments in photosynthetic capacity (e.g. V_{cmax}, Atkin et al. 2015, Wang et al. 2020). Similar coupling of *R*, V_{cmax}, and leaf N across different growth temperatures is sometimes observed at the local scale with individual species (Tjoelker et al. 1999, Lee et al. 2005, Crous et al. 2017).

Thermal acclimation of *R* has important implications for C cycle responses to temperature at local and global scales, as well as climate C cycle feedbacks. Assuming acclimation results in partial or complete homeostasis of *R* with changing growth temperature, the long-term temperature sensitivity of *R* would be reduced over time, dampening the positive feedback between CO_2 and temperature (King et al. 2006, Smith and Dukes 2013). Without acclimation, *R* would increase sharply as temperature increases, weakening ecosystem C storage, and accelerating the positive CO_2 - temperature feedback (Atkin and Tjoelker 2003, Atkin et al. 2014, Heskel et al. 2014). Modelling experiments that have incorporated thermal acclimation of photosynthesis or *R* have demonstrated that acclimation significantly alters estimates of C pools and fluxes. Lombardozzi et al. (2015) found that simulating thermal acclimation of photosynthesis and *R* in models resulted in a 20 Pg increase in terrestrial C pools by the end of the 21st century. Smith et al. (2016) also found that accounting for thermal acclimation improved model performance and suggested that acclimation results in higher net C storage and uptake than currently predicted.

Although leaf *R* and patterns and mechanisms of thermal acclimation of leaf *R* are better understood and better represented in TBMs, important knowledge gaps and data uncertainties remain. Direct measures of leaf *R* or its temperature sensitivity over space and time are relatively rare for coastal wetland species, and only a handful of studies have quantified thermal acclimation of leaf *R* in mangrove species (see Akaji et al. 2019, Aspinwall et al. 2021) and none have examined thermal acclimation in marsh grasses. As a result, respiratory responses of coastal plants to spatiotemporal variation in temperature and projections of coastal wetland C cycling over space remain uncertain. It is also unclear whether seasonal acclimation and acclimation to climate warming are equivalent, suggesting a common mechanism. Evidence for thermal acclimation of *R* largely comes from two different types of studies: field studies of plants responding to seasonal variation in temperature (Atkin et al. 2000; Lee et al. 2005; Ow et al. 2008, 2010; Tjoelker et al. 2008, 2009); and warming studies conducted under controlled conditions on small plants, usually for short time periods (Bolstad et al., 2003; Bruhn et al., 2007; Cheesman & Winter, 2013; Drake et al., 2015). Two separate experiments with tree species from warm-temperate and boreal climates have indicated that seasonal temperature acclimation and acclimation to climate warming may be equivalent (Aspinwall et al. 2016, Reich et al., 2016), and physiological responses to climate warming might be predicted from studies of physiological responses to seasonal temperature changes. There is also evidence that acclimation-over-time and acclimation-across-space may result in similar changes in leaf *R* in response to changes in temperature (Vanderwel et al. 2015). New studies are required to determine whether convergent temperature acclimation of *R* is common and generalizable across disparate ecosystems and communities, including coastal wetlands. Finally, it remains unclear whether thermal acclimation capacity differs between C_3 and C_4 plants; both are common in coastal wetlands. It has been hypothesized that C_4 plants may show limited capacity for thermal acclimation due to a more complex physiology (Yamori et al. 2014). Support for this hypothesis is mixed, however, with C_4 plants sometimes showing similar or comparable acclimation responses to C_3 species (Smith and Dukes 2017).

The marsh-mangrove ecotone on the northern Atlantic coast of Florida (USA) provides an opportunity to address these uncertainties. The ecotone represents a highly productive coastal wetland transitional zone where the southern limit of temperate saltmarsh habitat converges with the northern limit of tropical mangrove habitat. The purpose of this study was to test whether C_4 marsh grass species (*Spartina alterniflora*) and C_3 mangrove species (*Avicennia germinans*) growing in a coastal wetland show evidence of seasonal temperature acclimation of leaf *R*, and whether seasonal temperature acclimation of leaf *R* is consistent in plants growing under ambient and experimentally warmed conditions, as well as across sites at the northern and southern edge of the ecotone. We accomplished this by repeatedly measuring the short-term temperature responses of leaf *R* over a 10-month period in marsh and mangrove plants grown *in situ* under ambient temperatures and experimental warming at two sites within the ecotone. For each species, we then developed empirical models describing the relationship between prevailing (seasonal) air temperature and *R* at 25 °C and the Q_{10} of *R*. We tested whether site or experimental warming altered the relationship between prevailing air temperature and *R* at 25 °C or the Q_{10} of *R*. We also determined leaf N on a subset of leaves and tested whether leaf N scales positively with marsh grass and mangrove leaf *R*, over time and across sites and treatments. We hypothesized that both species should show declining leaf *R* at 25 °C (and/or Q_{10}) with increasing growth temperature, demonstrating seasonal acclimation, but the decline would be stronger for the C_3 mangrove species than the C_4 marsh grass. We also hypothesized that seasonal acclimation of *R* would be consistent across ambient and warmed treatments, and across northern and southern sites, demonstrating convergent temperature acclimation of leaf *R*.

Materials and methods

Study sites and experimental design

This study took place at two sites in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTMNERR) on the Atlantic coast of northeast Florida, near St. Augustine. The estuarine vegetation in GTMNERR represents a marsh-mangrove ecotone. The southern limit of saltmarsh habitat converges and overlaps with the northern limit of mangrove habitat in Florida, although mangroves are increasingly common north of GTMNERR. The northern site (NS) was located roughly 14 km north of St. Augustine along the Tolomato River near the northern edge of GTMNERR (30°00'41.9"N 81°20'39.2"W). The southern site (SS) was located roughly 20 km south of St. Augustine, 34 km to the south of the northern site, and just north of the Matanzas Inlet (29°43'38.3"N 81°14'25.0"W). The NS and SS have similar elevation, and average annual precipitation at the GTMNERR reserve is 1317 mm (Chapman et al. 2021). Mean annual temperature (2001–2018) at St. Augustine is 20.8 °C. The highest monthly mean daily max temperature (July) is 31.97 °C, and the lowest monthly mean daily low temperature (January) is 11.13 °C (NOAA). *Spartina alterniflora* (smooth cordgrass) and *Avicennia germinans* (black mangrove) are common at both sites although *A. germinans* trees are generally larger and more abundant at SS. *Spartina alterniflora* is a C_4 grass that dominates temperate estuaries along the east coast of North America and subtropical estuaries in the Gulf of Mexico and northern Florida. *Avicennia germinans* is a broadly distributed mangrove species native to warm-temperate, subtropical, and tropical regions of the Americas and Africa. On the Pacific coast, its distribution stretches from Mexico to Peru. On the Atlantic coast, its distribution stretches from North Florida to southern Brazil. All mangroves selected for this study ranged from 4-11 years old in 2018 with an average age of 7.4 years and 7.2 years at SS and NS respectively (Chapman et al. 2021).

The study included six replicates of four treatment plots at each site. The four treatments were: *A. germinans* ambient, *A. germinans* warmed, *S. alterniflora* ambient, and *S. alterniflora* warmed. Treatment plots were randomly positioned within a one-hectare area at each site. The warmed plots were enclosed in 1.5m × 1.5m × 1.5m chambers, framed with PVC and wrapped in 6 mil polyethylene greenhouse film (Greenhouse Megastore, Danville, IL, USA) that transmits ~90% of photosynthetically active radiation. The chamber top was left open to allow for air circulation and natural rainfall. The chambers 'trap' radiation causing the chamber air to passively warm. This passive warming design was chosen based on ease of construction, portability, and durability. In the event of a tropical storm, we removed the polyethylene film from the PVC frames so that chambers would not become airborne. In comparison to warming experiments with infrared heaters, our design did not necessarily change the canopy surface temperature, but instead increased the air temperature. Infrared (active) heating experiments may increase canopy surface temperature without increasing air temperature (Smith et al. 2020). This differentiation is important given that air temperatures are expected to increase while canopy temperatures may or may not depending on transpirational cooling (e.g. Drake et al. 2018). A limitation of our passive warming design was that warming could not be applied at night or during cloudy conditions. Air temperature (T_{air}) and relative humidity (RH) were measured every 15 minutes in the center of two ambient and warmed treatment plots using an air temperature/RH sensor covered in a solar radiation shield (HOBO MX2302 External Temperature/RH Sensor, Onset Computer Corp., Bourne, MA). Gaps in Figure 1 weather data were a result of HOBO logger failure in warmed and ambient plots. A dual failure scenario left us with no way to precisely gap fill temperature data. However, when HOBO failure was limited to ambient temperature measurements, data were gap filled using nearby GTMPCMET (Guana Tolomato Matanzas Pellicer Creek Meteorological) weather station data. Data loggers were reset prior to sampling dates to ensure temperatures were accurately recorded.

Over the course of the experiment, mean daily (24-hr) T_{air} was on average 0.66 °C and 0.63 °C higher in the warmed treatment than mean daily T_{air} in the ambient treatment at the SS (Figure S1c) and NS (Figure S1a), respectively (SS ambient mean daily $T_{\mathrm{air}} = 22.9 \pm 5.1$

(standard deviation) °C, warmed mean daily T_{air} = 23.6 ± 5.4 °C; NS ambient mean daily T_{air} = 22.8 ± 5.1 °C, warmed mean daily T_{air} = 23.5 ± 5.3). Daily maximum T_{air} was on average 3.2 °C and 3.1 °C higher in the warmed treatment than the ambient treatment at the SS (Figure S1d) and NS (Figure S1b), respectively (SS ambient daily max T_{air} = 27.5 ± 5.2 °C, warmed daily max T_{air} = 30.7 ± 5.8 °C; NS ambient daily max T_{air} = 28.1 ± 5.2 ± °C, warmed mean daily T_{air} = 31.2 ± 5.8 °C). Warming of this magnitude (1-3 °C) is expected by 2050 throughout the southeast U.S. (Ashfaq et al. 2016, Diem et al. 2017). The daily mean relative humidity (RH) at both sites was similar. SS daily mean RH was 85.3 ± 8.4% (Figure 1b) and NS daily mean RH was 83.1 ± 7.1% (Figure 1e). Overall, average RH was similar in the ambient and warmed plots at both sites. As a result of high RH, the daily mean VPD was <1 at both sites (Figure 1 c, f), with only small differences in VPD between warmed and ambient treatment plots (Figure 1f).

Temperature response of leaf respiration

Measurements of the short-term temperature response of leaf dark respiration (*R*) were conducted at 6 timepoints (October, December, February, April, June, July) over a 10-month period. Each monthly campaign of leaf collection was split over a maximum of two days at each site (four days total). An equal number of leaves from each species and treatment were selected during each campaign to avoid sampling-date bias. On each sampling day we collected two recent and fully developed upper canopy leaves from *S. alterniflora* and three recent and fully developed upper canopy leaves from *A. germinans*. Leaves were collected pre-dawn (04:30 – 06:00 local time) to avoid activation of photosynthesis. The excised leaves were placed in Ziplock bags with moist paper and transferred to the lab in complete darkness. Previous studies have found no effect of leaf removal on leaf *R* measurements (O'Sullivan et al. 2013; Aspinwall et al. 2017; 2019). Moreover, all measurements were completed the same day as leaf collection and we found no evidence that leaf *R* changed with time since leaf removal. Leaf area (cm^2) of the measured leaves was determined with a leaf area meter (LI-3000C, LI-COR BioSciences, Lincoln, NE, USA) just prior to measurements of leaf *R*. Leaf area data were used to calculate *R* per unit area (R_{area} μmol m^{-2} s^{-1}).

Short-term temperature response curves of leaf *R* were completed by sealing excised leaves inside a large gas-exchange chamber (3010-GWK1, Heinz Walz GmbH, Effeltrich, Germany) connected to a portable infrared gas-analyzer (LI-6400XT, LiCor Inc, Lincoln, NE, USA). The large gas-exchange chamber was set at 20 °C, while the flow rate and reference CO_2 (both controlled by the infrared gas-analyzer) were set at 500 mol s^{-1} and 410 μmol mol^{-1}, respectively. T_{leaf} on the abaxial surface of the leaf was continuously measured with a small-gauge copper constantan thermocouple wire attached to a LI-6400XT external thermocouple adaptor (LI6400–13, LI-COR BioSciences). To facilitate measurements of *R*, the airflow from the chamber was connected to the 'sample' gas line of the LI-6400XT fitted with an empty and closed 2×3 cm cuvette. The incoming air was dried before entering the 3010-GWK1 chamber by routing the incoming air through the LI-6400XT desiccant column. Once rates of *R* reached steady-state at 20 °C (~5 min) the temperature of the leaf was increased from 20°C - 40°C at a rate of 1°C per minute using the chamber software (GFS-Win, Heinz Walz GmbH) while continuously (30 second interval) measuring R_{area} (Figure S2). After temperature response measurements were completed, leaves were dried at 70 °C for ~72 hours after which leaf dry mass measurements were 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 (Figure S3). Leaf tissue was then homogenized and dried at 105°C for ~16 hours, stored under desiccation, and leaf N per unit mass (N_{mass}, % or mg N g^{-1}) was determined using a combustion elemental analyzer (Rapid max N, Elementar Americas Inc., Ronkonkoma, NY, USA). Leaf N per unit area (N_{area}, g N m^{-2}) was calculated as the product of %N and LMA.

Modelling 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). To determine the appropriate model for our data, we compared the suitability of three different algorithms following the approach of Aspinwall et al. (2017): (1) a log polynomial function, which describes the non-linear relationship between natural log transformed *R* and T_{leaf} (O'Sullivan et al. 2013; Heskel et al. 2016), (2) an exponential function with a single Q_{10} value which assumes that the temperature sensitivity of *R* is constant across the entire measurement temperature range (e.g. Tjoelker et al. 2001), and (3) a modified Arrhenius function, which accounts for a non-exponential increase in *R* with increasing T_{leaf} (Lloyd and Taylor 1994). The polynomial function is written as:

(1) $ln\,R = a + bT + cT^2$ or

(2) $R = e^{a+bT+cT^2}$

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 Eqn 2 can be used to estimate the Q_{10} of *R* at any T_{leaf}: $Q_{10} = e^{10\times(b+2cT)}$. Among these algorithms, the polynomial (Equation 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}. Thus, 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 (Table S1). Repeated estimates of R_{area}^{25}, R_{mass}^{25}, and Q_{10} over time provide a direct way of determining the direction and magnitude of seasonal thermal acclimation (Aspinwall et al. 2019).

Data analysis

All analyses were performed using R version 3.43 (R Core Team 2017). As a preliminary step in our analysis, we carried out variance partitioning to determine the proportion of the overall variance in each trait (e.g. R_{area}^{25}) explained by different experimental factors (species, measurement dates, site, treatment). The variance partitioning results indicated that 'species' accounted for the majority of the variance in most traits (16-68%), while other factors generally explained a much smaller proportion of the variance (0-7%) (Figure S4). Therefore, the remainder of the analysis was conducted separately for each species.

For each species, mixed-effect models were used to test the fixed effects of time (measurement date), temperature treatment (ambient, warmed), site (SS, NS) and their respective interactions on R_{area}^{25}, R_{mass}^{25}, Q_{10}, LMA, N_{mass}, and N_{area}. For consistency, we analyzed estimates of R_{area}^{25}, R_{mass}^{25}, and Q_{10} derived from our temperature response models rather than observations at 25 °C, although estimates and observed rates were nearly identical given the high resolution of our temperature response data. Homogeneity of variance for model results were tested using Levene's and Shapiro-Wilk tests. Data were log or square-root transformed as necessary.

For each species, analysis of covariance (ANCOVA) was used to test relationships between R_{area}^{25}, R_{mass}^{25}, Q_{10}, LMA, N_{mass}, and N_{area} values and prevailing mean daily air temperature of the preceding 7 days (mean daily T_{air}) and determine whether relationships differed between sites or treatments. If seasonal temperature acclimation of leaf *R* is occurring, we expect negative linear relationships between mean daily T_{air} and R_{area}^{25}, R_{mass}^{25}, or the Q_{10} of *R* (e.g. Aspinwall et al. 2016). The strength of acclimation is determined by the slope of this relationship; stronger acclimation is indicated by a more negative slope estimate. In this model, site and treatment were treated as factors and mean daily T_{air} a covariate. A significant ($P<0.05$) interaction between mean daily T_{air} and site or treatment indicated that site or treatment affected the relationship between mean daily T_{air} and the response variable (e.g. R_{area}^{25}), and different slope parameters were required for each site or treatment. If site, treatment, and mean daily T_{air} were significant ($P<0.10$), with no interactions, equations with different intercepts for each site or treatment, but a common slope, were fit to the data. If only mean daily T_{air} was significant, one equation describing the relationship between mean daily T_{air} and the response variable was fit to data from both sites and treatments. We also used ANCOVA to test whether leaf N (N_{mass}, N_{area}) scales positively with respiratory capacity (R_{area}^{25}, R_{mass}^{25}), and whether site or treatment changes the relationship between leaf N and respiratory capacity.

Results

Leaf R, leaf N, and LMA over space and time

In *Avicennia*, R_{area}^{25}, R_{mass}^{25}, and N_{area} varied over time, but differences among time points were dependent upon site (significant date × site interactions Table 1, 2). R_{area}^{25} was lower at SS than NS in December 2019 (SS: 0.86 ± 0.04 μmol m^{-2} s^{-1}, NS: 1.09 ± 0.04 μmol m^{-2} s^{-1}), but higher at SS than NS in July 2020 (SS: 1.30 ± 0.04 μmol m^{-2} s^{-1}, NS: 1.05 ± 0.04 μmol m^{-2} s^{-1}, Figure 2a). R_{mass}^{25} was also higher at SS than NS in July 2020 (SS: 7.64 ± 0.36 nmol g^{-1} s^{-1}, NS: 4.36 ± 0.36

nmol g^{-1} s^{-1}, Figure 2c). N_{area} was lower at SS (3.22 ± 0.23 g N m^{-2}) than NS (4.29 ± 0.17 g N m^{-2}) in October 2019 (Figure 2i).

Across both sites, warming increased R_{area}^{25} by 8% (ambient R_{area}^{25} = 1.05 ± 0.02 µmol m^{-2} s^{-1}, warmed R_{area}^{25} = 1.14 ± 0.02 µmol m^{-2} s^{-1}). However, warming effects on R_{mass}^{25} differed between sites (treatment × site interaction, Table 1). Warming increased R_{mass}^{25} by 24% at SS (ambient R_{mass}^{25} = 4.56 ± 0.22 nmol g^{-1} s^{-1}, warmed R_{mass}^{25} = 5.67 ± 0.23 nmol g^{-1} s^{-1}) but did not affect R_{mass}^{25} at NS (mean = 4.43 ± 0.21 nmol g^{-1} s^{-1}).

The Q_{10} of R varied over time (Table 1) and was highest in February (2.25 ± 0.05) and April 2020 (2.26 ± 0.05), and lowest in June 2020 (1.94 ± 0.05, Figure 2e). Q_{10} did not differ between treatments but differed between sites (Table 1). Q_{10} was higher at SS (2.17 ± 0.03) than NS (2.03 ± 0.03). LMA showed a complex pattern; with variation over time depending upon both treatment and site (treatment × date × site, Table 1) (Figure 2g). LMA was highest in warmed plants at NS in July 2020 (272 ± 10.4 g m^{-2}) and October 2019 (277 ± 10.4 g m^{-2}), and lowest in ambient plants at SS in July 2020 (168 ± 10.4 g m^{-2}). N_{mass} did not differ between sites or treatments (Table 2), but was 15% higher in June 2020 (17.1 ± 0.60 g N kg^{-1}) than in February 2020 (14.9 ± 0.60 g N kg^{-1}).

In *Spartina*, R_{area}^{25}, R_{mass}^{25}, and LMA varied over time, but differences among time points were dependent upon site (i.e., date × site interaction, Table 1, Figure 2). In October 2019, R_{area}^{25} was higher at SS (1.71 ± 0.11 µmol m^{-2} s^{-1}) than at NS in February 2020 (1.19 ± 0.10 µmol m^{-2} s^{-1}) (Figure 2b). R_{mass}^{25} was generally higher during winter (December, February) and lower during summer (June, July) (Figure 2). In December 2019, R_{mass}^{25} was significantly lower at SS (9.9 ± 0.5 nmol g^{-1} s^{-1}) than NS (13.6 ± 0.5 nmol g^{-1} s^{-1}). R_{mass}^{25} was similar between sites across all other dates. LMA showed the opposite seasonal pattern of R_{mass}^{25}; lowest in winter and highest during peak growing season. In February 2020, LMA was significantly higher at SS (128 ± 5.9 g m^{-2}) than NS (99 ± 5.9 g m^{-2}). On average, warming reduced R_{mass}^{25} 6.4% across all timepoints (Table 1, ambient R_{mass}^{25}: 10.9 ± 0.2 nmol g^{-1} s^{-1}, warmed R_{mass}^{25}: 10.2 ± 0.2 nmol g^{-1} s^{-1}) but had no effect on R_{area}^{25} or LMA. The Q_{10} of R varied over time and was higher in February 2020 (1.94 ± 0.03) than June 2020 (1.77 ± 0.03) (Figure 2e). The Q_{10} of R was similar across sites and treatments (Table 1). N_{mass} varied over time, but differences among time points were dependent upon temperature treatment (significant date × treatment interaction, Table 2). In February 2020, N_{mass} was significantly lower in warmed plants (15.7 ± 0.6 g N kg^{-1}) than ambient plants (18.3 ± 0.6 g N kg^{-1}) (Figure 2k). N_{area} did not differ between dates, treatments, or sites (and no interactions, Table 2) and averaged (2.04 ± 0.08 g N m^{-2}).

Relationships between T_{air} and leaf R, leaf N, and LMA

For each species, we expected that R_{area}^{25} and R_{mass}^{25} would decline as seasonal temperatures increased, demonstrating seasonal acclimation. We hypothesized that seasonal acclimation would be stronger in *Avicennia* than *Spartina*, but within each species seasonal acclimation would be consistent across sites and treatments. For *Avicennia*, ANCOVA results indicated that the response of R_{area}^{25} and R_{mass}^{25} to prevailing mean daily T_{air} differed between sites (T_{air} × site interaction, Table 3). At SS, we found a *positive* relationship between mean daily T_{air} and R_{area}^{25} and R_{mass}^{25} (Table 4, Figure 3a,c). At NS, R_{area}^{25} and R_{mass}^{25} showed no clear relationship with mean daily T_{air} (Figure 3a,c). In agreement with our ANOVA results above, we found that warming generally increased R_{area}^{25} and R_{mass}^{25} across sites and prevailing mean daily T_{air} (Figure 3a,c). These results provide little evidence for thermal acclimation of respiratory capacity (Type II acclimation, Atkin and Tjoelker 2003) in *Avicennia* growing at their northern range limit.

However, ANCOVA results revealed that the Q_{10} of R decreased as mean daily T_{air} increased (Table 3, Figure 3e), indicating Type I thermal acclimation (Atkin and Tjoelker 2003). This acclimation response was consistent across treatments and sites, although the intercept of the relationship was significantly lower at NS than SS (Table 3, 4; Figure 3e).

In *Avicennia*, LMA decreased as mean daily T_{air} increased, but the slope of the relationship differed between sites (T_{air} × site interaction, Table 3, 4). LMA decreased faster with increasing T_{air} at SS than NS (Table 4, Figure 3g). Similar to our ANOVA results, warming effects on LMA differed between sites (treatment × site interaction, Table 3). Warming generally reduced LMA at SS but did not change LMA at NS. Lastly, N_{mass} increased as mean T_{air} increased and did so consistently across treatments and sites (Table 3, Figure 3i).

Spartina showed clear evidence of Type II and Type I thermal acclimation of R across seasons. Seasonal acclimation patterns were consistent across treatments and sites. Specifically, R_{mass}^{25} decreased with increasing mean daily T_{air}; the intercept of the relationship was lower at SS than NS (Table 4, Figure 3d). This relationship accounts for increasing LMA with T_{air} (Figure 3h) which obscured the relationship between area-based respiratory capacity and T_{air} (Figure 3b). The Q_{10} of R also decreased with increasing mean daily T_{air} (Figure 3f). N_{mass} declined with increasing T_{air}, although the decline in N_{mass} with increasing T_{air} differed between treatments (T_{air} × treatment interaction, Table 3). Across sites, warming reduced the intercept and slope of the relationship between T_{air} and N_{mass} (Figure 3j, Table 4).

Scaling of leaf N and R

In general, we found that leaf N (area- and mass-basis) scaled positively with leaf R (area- and mass-basis) in both species. However, in some cases, relationships between leaf N and R

depended upon treatment or site (Table 5). In *Avicennia*, R_{area}^{25} increased with N_{area} at SS but not NS, and R_{area}^{25} at a given N_{area} was higher under warming than ambient conditions (Table 6, Figure 4a). N_{mass} and R_{mass}^{25} scaled positively although the intercept was higher in plants at SS (Table 5, 6; Figure 4c).

In *Spartina*, the slope of the $N_{\mathrm{area}} - R_{\mathrm{area}}^{25}$ relationship was slightly higher at SS than NS (Table 5,6; Figure 4b). The positive relationship between N_{mass} and R_{mass}^{25} was consistent across treatments and sites (Table 5, 6; Figure 4d). Although temperature acclimation of *R* differed between *Avicennia* and *Spartina*, changes in leaf N explained thermal acclimation patterns in both species.

Discussion

Spatiotemporal patterns of leaf *R*, the temperature sensitivity of *R* (Q_{10}), and temperature acclimation of leaf *R* have been understudied in coastal marsh and mangrove species (Duarte 2017; Adame et al. 2018; Akaji et al. 2019, Aspinwall et al. 2021). To address this knowledge gap, we examined seasonal temperature acclimation of leaf *R* in a C_4 marsh species (*Spartina alterniflora*) and C_3 mangrove species (*Avicennia germinans*) growing under ambient and experimentally warmed temperatures at two sites within a marsh-mangrove ecotone. We also tested whether leaf N scales positively with marsh grass and mangrove leaf *R* over time and across sites and treatments. We hypothesized that both species would reduce leaf *R* at 25 °C (and/or Q_{10}) with as growth temperatures increased over time, demonstrating seasonal temperature acclimation, but the decline would be stronger for the C_3 mangrove species than the C_4 marsh grass. We also hypothesized that seasonal acclimation of *R* would be consistent across treatments and sites. In *Avicennia*, we found that R_{area}^{25} and R_{mass}^{25} showed no relationship with prevailing T_{air} at the north site, increased with T_{air} at the south site, and generally increased with experimental warming across both sites. These results indicated no temperature acclimation of respiratory capacity (no Type II acclimation) in *Avicennia*. Yet, the Q_{10} of R decreased as seasonal temperatures increased (Type I acclimation) and did so consistently across sites and treatments. In *Spartina*, R_{mass}^{25} and the Q_{10} of R both declined as seasonal temperatures increased (demonstrating Type I & II acclimation). These seasonal patterns of temperature acclimation were largely consistent across sites and treatments. These results do not support the expectation for weaker temperature acclimation of R in *Spartina* than *Avicennia*. However, the *Spartina* results supported our expectation of convergent temperature acclimation of R over time, space, and temperature treatments. Although species differed in regulating respiratory capacity over time, sites, and treatments, leaf N scaled positively with leaf R in both species. These results improve our quantitative and predictive understanding of temperature controls of leaf R in coastal wetland species.

In a synthesis of studies, Slot and Kitajima (2015) found a significant downregulation of leaf R in response to increasing growth temperature (Type II acclimation) in 73% of the cases examined. This suggests that temperature acclimation of respiratory capacity is relatively common across species and systems – although coastal wetland plant species were not included in that synthesis. We expected marsh and mangrove species would generally conform to the patterns observed across terrestrial plant species. However, in contrast to our expectations, *Avicennia* generally increased R_{area}^{25} and R_{mass}^{25} with increasing growth temperatures, although no seasonal pattern was observed at the north site. Moreover, we found no evidence for convergent acclimation of respiratory capacity over time, and across sites and treatments in *Avicennia*. This contrasts with results from previous warming experiments with warm-temperate,

temperate, and boreal tree species growing at individual sites (Aspinwall et al. 2016, Reich et al. 2016). This also contrasts with analyses indicating similar changes in leaf *R* with increasing temperature across space and time (Vanderwel et al. 2015). Ultimately, our results indicate that changes in respiratory capacity may not be easily predicted by growth temperature, at least in *Avicennia* growing at its northern range limit.

The increase in respiratory capacity with increasing temperature in *Avicennia* might be explained by temporal variation in salinity, nutrient availability, or the inherent functional strategy of this species. Coastal wetlands are dynamic systems where water levels (i.e. tides) and salinity can vary diurnally and seasonally. Salinity depends on relative proximity to the ocean and freshwater inputs, seasonality of precipitation, and rates of evaporation which change with temperature. In our experiment, the southern site was near an inlet and thus closer to the open ocean while the northern site was further from the ocean and closer to freshwater inputs. This may have resulted in higher salinity at the southern site, particularly during the summer when temperatures and evaporation are high. Indeed, previous work at the southern site indicated that salinity was particularly high during summer (up to 60 ppt) and lower during winter (48 ppt) (Dangremond et al. 2020). Previous studies have also found modest increases in leaf *R* with increasing salinity, presumably due to costs associated with maintaining cellular ion gradients (Lopez-Hofmann et al. 2007, Aspinwall et al. 2021). Thus, the combination of warmer growth temperatures and higher salinity might explain why *R* increased with T_{air} at the southern site, and why thermal acclimation appeared constrained.

However, averaged across sites, respiratory capacity also increased with experimental warming, suggesting that salinity alone is unlikely to explain the increase in leaf *R* with warmer growth temperatures. Instead, coordination between leaf *R* and photosynthetic capacity might explain the positive relationship between growth temperature and respiratory capacity in *Avicennia*. Importantly, we found that variation in respiratory capacity (e.g. $R_{\text{mass}}{}^{25}$) over time and across sites and treatments was largely explained by changes in leaf N. The coupling of leaf N and respiratory capacity could represent enzyme limitation of respiratory capacity (Ryan et al. 1996). However, across other studies leaf N and Rubisco carboxylation (V_{cmax}) are often coupled given that a large fraction of N is allocated to Rubisco. A new theory also indicates that temperature acclimation of leaf *R* is consistent with maintenance of 'optimal' photosynthetic capacity, where respiratory capacity increases to support processes that maintain photosynthesis (e.g. protein turnover, Wang et al. 2020). We hypothesize that respiratory capacity increased with temperature in *Avicennia* due to concomitant changes in photosynthetic capacity, which were reflected in changes in leaf N. If true, *Avicennia* responded to increasing growth temperature by also increasing photosynthetic capacity; a response which is not necessarily consistent with studies of photosynthetic temperature acclimation over space and time (Way and Sage 2008, Way and Yamori 2014, Ali et al. 2015). Further studies are required to determine whether the increase in *R* with growth temperature in *Avicennia* was coupled with increased photosynthetic capacity.

Temporal patterns in leaf *R* and leaf N in *Avicennia* may have also been influenced by nutrient availability, which could vary with air and soil temperature. In general, warmer soil surface temperatures during summer may speed up decomposition and N mineralization (Kirwan and Blum 2011, Gao et al. 2014) which could increase N availability and potentially result in higher leaf N concentrations. Results from a fertilization experiments near our southern site have demonstrated that higher N availability leads to higher leaf N concentrations in *Avicennia* (Simpson et al. 2013, Dangremond et al. 2020). Although seasonal changes in N availability were not quantified in our study, we did find evidence that leaf N concentrations increased with prevailing air temperature across both sites. Therefore, higher N availability during warmer time periods may have resulted in higher leaf N, greater photosynthetic capacity, and coordinated increases in respiratory capacity in *Avicennia* (Wang et al. 2020).

Although respiratory capacity increased with growth temperature in *Avicennia*, indicating no type II temperature acclimation, some temperature acclimation did occur mainly through a reduction in the short-term temperature sensitivity of *R*, also referred to as Type I acclimation (Atkin and Tjoelker 2003, Slot and Kitajima 2015). This acclimation response is less common and results in weaker temperature acclimation than Type II acclimation, but it has been observed in several studies (Atkin et al. 2000, Zaragoza-Castells et al. 2007, Ow et al. 2010). Mechanistic explanations of Type I acclimation remain speculative; however, it has been hypothesized that a reduction in the temperature sensitivity of *R* is the result of regulatory changes in several respiratory enzymes (Atkin et al. 2005, Kruse et al. 2011; 2020). Other studies have identified positive relationships between soluble sugars and the Q_{10} of *R* (Azcón-Bieto et al. 1983, Ow et al. 2010) which could reflect substrate limitation of the maximum catalytic enzyme activity (Atkin and Tjoelker 2003). Although the cause of the reduction in the Q_{10} with increasing temperature is unclear, it is possible that high respiratory demand during summer may have drawn down soluble sugar concentrations in leaves which in turn reduced maximum catalytic activity and the temperature sensitivity of *R*. Whatever the explanation, our results indicate that *Avicennia* may show modest reductions in the temperature sensitivity of *R* as temperatures increase, which could mitigate rising leaf CO_2 efflux as the climate warms.

Compared to C_3 plants, C_4 plants have a more complex photosynthetic apparatus (mesophyll-bundle sheath complex). This complexity has been hypothesized to come at a cost of reduced phenotypic plasticity or lower temperature acclimation capacity in C_4 plants (Sage and McKown 2006, Yamori et al. 2014). Yet, experimental work has found no clear differences in thermal acclimation responses between C_3 and C_4 plant species (Smith and Dukes 2017). We tested the expectation that *Spartina* would show weaker temperature acclimation than *Avicennia* and found no support for this expectation. In fact, we found that *Spartina* reduced R_{mass}^{25} and the Q_{10} of R as growth temperatures increased (Type II acclimation and Type I acclimation) over time and across treatments while *Avicennia* generally showed *increased* respiratory capacity with temperature. Moreover, in *Spartina*, this acclimation response was consistent across sites demonstrating convergent acclimation to changes in growth temperature. This result aligns with patterns of thermal acclimation observed in tree species representing different biomes (Aspinwall et al. 2016, Reich et al. 2016). We conclude that *Spartina*, a dominant C_4 marsh grass, may show rather consistent temperature acclimation of leaf R over space and time, which could reduce the sensitivity of coastal wetland (especially marsh-dominated) C fluxes to changes in temperature, and dampen the positive feedback between warming and rising atmospheric CO_2 (King et al. 2006, Smith & Dukes 2013, Lombardozzi et al. 2015).

Although temperature acclimation of R differed between *Spartina* and *Avicennia*, changes in leaf N (particularly, N_{mass}) explained temporal and spatial variation in respiratory capacity in both species. The relationship between leaf N and leaf R (area- and mass-based) was stronger for *Spartina* than *Avicennia* and was relatively consistent across treatments and sites. Theory suggests that temperature acclimation of R arises through the importance of respiration in maximizing photosynthetic capacity under natural conditions (Wang et al. 2020), where photosynthetic capacity is strongly influenced by concentrations of photosynthetic proteins which represent a large fraction of leaf N. Predictions of this theory have been validated with C_3

plants but not C_4 plants. Even if this prediction holds, our results show clearly contrasting responses of respiratory capacity to seasonal temperature changes in *Avicennia* and *Spartina*. These contrasting responses could be due to factors not directly related to photosynthetic pathway (i.e. C_3 vs C_4). Species differences in leaf N and *R* over time may be in part related to differences in nutrient acquisition or inherent differences in resource investment over seasons. For instance, *Avicennia* is an evergreen species and is known to be very responsive to N enrichment in these systems (Simpson et al. 2013, Dangremond et 2020). At any given time, leaf N may be higher when N availability is higher. In our study, *Avicennia* also generally responded to increasing seasonal temperatures by reducing LMA and increasing leaf N. *Spartina* is also responsive to nutrients (e.g. Mendelssohn 1979) and may also generally increase leaf N with increased N availability (e.g. Gallagher 1975). However, *Spartina* exhibits a strong seasonality to leaf development and leaf N concentrations; LMA generally increases with growth temperature while leaf N generally decreases with growth temperature. These distinct seasonal patterns that may explain the contrasting responses of leaf *R* to prevailing T_{air} between species, but also the close coupling of leaf N and *R* across species.

More broadly, our results indicate that leaf N may be an effective predictor of foliar C fluxes in dominant coastal wetland species, irrespective of temperature, just as leaf N is used as a predictor of leaf *R* in land surface models that predict terrestrial C fluxes over space and time (Atkin et al. 2015, Fisher et al. 2014, Lawrence et al. 2019). In fact, in our species and across our sites, it appears that leaf N may explain more variation in leaf *R* than prevailing growth temperature. 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. The data presented here offer an opportunity to improve representation and parameterization of CO_2 exchange between coastal vegetation and the atmosphere in land surface models.

Our results provide new information about respiratory C fluxes in marsh and mangrove vegetation. They suggest that C_4 marsh grasses and C_3 mangrove species growing together may show contrasting respiratory responses to temperature, yet both species show a positive relationship between respiratory capacity and leaf N. The data presented here may be useful in modelling ecological processes and physiological feedbacks for coastal marsh-mangrove ecosystems under current and future climate warming scenarios. Future studies that explore plant-soil and plant-air interactions in coastal wetlands, will further advance our understanding of C fluxes in these systems.

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Table 1. Results of a three-way analysis of variance testing the main and interactive effects of temperature treatment (T), measurement date (D), and site (S) on rates of leaf dark respiration at per unit area and per unit mass at 25 °C (R_{area}^{25}, R_{mass}^{25}), the temperature sensitivity of R (Q_{10}), and leaf dry mass per unit area (LMA) in *Spartina alterniflora* and *Avicennia germinans*. Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Species	***Dependent Variables***	*df*	R_{area}^{25}	R_{mass}^{25}	Q_{10}	**LMA**
Avicennia	Treatment (T)	1	**9.05****	**6.79***	0.39	1.95
	Date (D)	5	**10.00*****	**10.09*****	**9.88*****	**13.29*****
	Site (S)	1	0.50	**9.38****	**19.38*****	**32.77*****
	T x D	5	0.35	0.34	0.63	0.83
	T x S	1	1.92	**5.27***	0.57	**6.18***
	D x S	5	**5.96*****	**7.57*****	1.39	**3.87****
	T x D x S	5	0.48	0.86	0.32	**2.41***
Spartina	Treatment (T)	1	1.75	**4.83***	2.76	0.04
	Date (D)	5	**3.17***	**15.67*****	**4.62*****	**22.57*****
	Site (S)	1	0.69	**12.03*****	0.62	2.88
	T x D	5	0.58	0.51	0.73	0.54
	T x S	1	0.42	2.68	2.43	0.42
	D x S	5	**2.33***	**3.53****	0.82	**4.29****
	T x D x S	5	0.49	0.22	0.66	0.37

Table 2. Results of a three-way analysis of variance testing the main and interactive effects of temperature treatment (T), measurement date (D), and site (S) on concentration of leaf nitrogen on per unit area and per unit mass (N_{mass}, N_{area}) in *Avicennia germinans* and *Spartina alterniflora*. Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at *P*<0.05, *P*<0.01, and *P*<0.001, respectively.

Species	***Dependent Variables***	*df*	N_{mass}	N_{area}
Avicennia	Treatment (T)	1	0.83	0.05
	Date (D)	2	**3.34***	0.08
	Site (S)	1	0.44	**9.18****
	T x D	2	0.14	1.34
	T x S	1	0.20	3.46
	D x S	2	0.57	**3.69***
	T x D x S	2	0.42	0.09
Spartina	Treatment (T)	1	0.41	0.02
	Date (D)	2	**15.32*****	2.03
	Site (S)	1	1.82	1.23
	T x D	2	**5.55****	2.20
	T x S	1	0.00	0.42
	D x S	2	0.86	1.68
	T x D x S	2	0.43	0.21

Table 3. The relationship between preceding 7 day daily mean temperature and leaf physiological traits for *S. alterniflora and A. germinans* at both sites, with and without experimental warming. Leaf respiration per unit area at 25°C (R_{area}^{25}); Leaf respiration per unit mass at 25°C (R_{mass}^{25}); The temperature sensitivity (Q_{10}) of (R_{mass}^{25}); Leaf mass per unit area (LMA); leaf N per unit area (N_{area}); leaf N per unit mass (N_{mass}). Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

Species	***Dependent Variables***	*df*	R_{area}^{25}	R_{mass}^{25}	Q_{10}	**LMA**	N_{mass}	N_{area}
Avicennia	Air (A)	1	**6.23***	**26.38*****	**20.81*****	**44.62*****	**5.80***	0.16
	Treatment (T)	1	**6.85****	**4.94***	0.13	0.98	0.76	0.12
	Site (S)	1	0.24	**6.90****	**23.80*****	**23.26*****	0.42	**9.04****
	A x T	1	0.72	0.04	0.14	0.19	0.50	2.84
	A x S	1	**18.19*****	**25.24*****	0.64	**12.08*****	0.34	0.03
	T x S	1	1.44	3.89	0.22	**4.62***	0.23	2.84
	A x T x S	1	0.23	0.76	0.35	2.10	0.16	0.00
Spartina	Air (A)	1	1.07	**86.88*****	**14.52*****	**59.77*****	**34.93*****	0.63
	Treatment (T)	1	1.59	3.02	2.17	0.08	0.06	0.00
	Site (S)	1	0.39	**6.18***	1.51	0.51	2.11	1.05
	A x T	1	1.06	3.23	2.38	0.05	**12.74*****	3.65
	A x S	1	0.36	2.98	1.69	3.17	0.42	3.31
	T x S	1	0.11	1.71	1.75	0.43	0.00	0.59
	A x T x S	1	1.24	0.31	1.06	0.50	0.37	0.95

Table 4. The relationship between preceding 7 day daily mean temperature and leaf physiological traits for *A. germinans* and *S. alterniflora* at both sites, with and without experimental warming. Leaf respiration per unit area at 25°C (R_{area}^{25}); Leaf respiration per unit mass at 25°C (R_{mass}^{25}); The temperature sensitivity (Q_{10}) of (R_{mass}^{25}); Leaf mass per unit area (LMA); leaf N per unit area (N_{area}); leaf N per unit mass (N_{mass}). Slopes and intercepts are only shown for significant ($p<0.05$) relationships.

Species	***Variable***	***Site***	***Treatment***	***Intercept***	***Slope***	***R^2***
Avicennia	R_{area}^{25}	North	Overall	1.169	-0.003	***0.21***
		South	Overall	0.350	0.033	
		Both	Ambient	0.845	0.010	
			Warmed	0.927	0.010	
	R_{mass}^{25}	North	Overall	3.769	0.029	***0.34***
		South	Overall	-2.509	0.335	
		Both	Ambient	1.230	0.146	
			Warmed	1.739	0.146	
	Q_{10}	North	Overall	2.508	-0.023	***0.26***
		South	Overall	2.758	-0.023	
	LMA	North	Overall	299.52	-2.131	***0.40***
		South	Overall	374.68	-6.473	
	N_{mass}	Both	Overall	9.493	0.288	***0.12***
Spartina	R_{mass}^{25}	North	Overall	21.34	-0.444	***0.45***
		South	Overall	20.12	-0.444	
	Q_{10}	Both	Overall	2.525	-0.028	***0.16***
	LMA	Both	Overall	88.18	2.336	***0.33***
	N_{mass}	Both	Ambient	32.36	-0.7500	***0.47***
			Warmed	19.31	-0.1797	

Table 5. Results of a two-way analysis of co-variance testing the main and interactive effects of leaf nitrogen concentration per unit area and per unit mass (N_{mass}, N_{area}) on R_{area}^{25} and R_{mass}^{25} in *Avicennia germinans* and *Spartina alterniflora*. Degrees of freedom (df) and F-values are presented for each factor and reponse variable. F-values with '*', '**' and '***' are significant at P<0.05, P<0.01, and P<0.001, respectively.

	Dependent Variables	***Avicennia germinans***	***Spartina alterniflora***
R_{area}^{25}	N_{area} (N_a)	**5.77***	**154.20*****
	Treatment (T)	**4.18***	2.59
	Site (S)	0.17	0.00
	N_a x T	0.02	0.21
	N_a x S	**7.54****	**5.41***
	T x S	1.37	2.06
	N_a x T x S	0.30	0.20
R_{mass}^{25}	N_{mass} (N_m)	**50.73*****	**51.25*****
	Treatment (T)	3.47	3.10
	Site (S)	**4.51***	0.82
	N_m x T	0.36	0.03
	N_m x S	3.24	0.82
	T x S	3.47	3.46
	N_m x T x S	0.63	0.41

Table 6. Results of a two-way analysis of co-variance testing the main and interactive effects of leaf nitrogen concentration per unit area and per unit mass (N_{mass}, N_{area}) on R_{area}^{25} and R_{mass}^{25} in *Avicennia germinans* and *Spartina alterniflora*. Slopes and intercepts are only shown for significant ($p<0.05$) relationships.

Species	***Variable***	***Site***	***Treatment***	***Intercept***	***Slope***	***R^2***
Avicennia	R_{area}^{25}	North	Overall	1.039	0.017	***0.25***
		South	Overall	0.446	0.179	
		Both	Ambient	0.761	0.077	
			Warmed	0.848	0.077	
	R_{mass}^{25}	North	Overall	0.179	0.267	***0.53***
		South	Overall	0.631	0.267	
Spartina	R_{area}^{25}	North	Overall	0.392	0.499	***0.74***
		South	Overall	-0.177	0.785	
	R_{mass}^{25}	Both	Overall	1.835	0.568	***0.51***

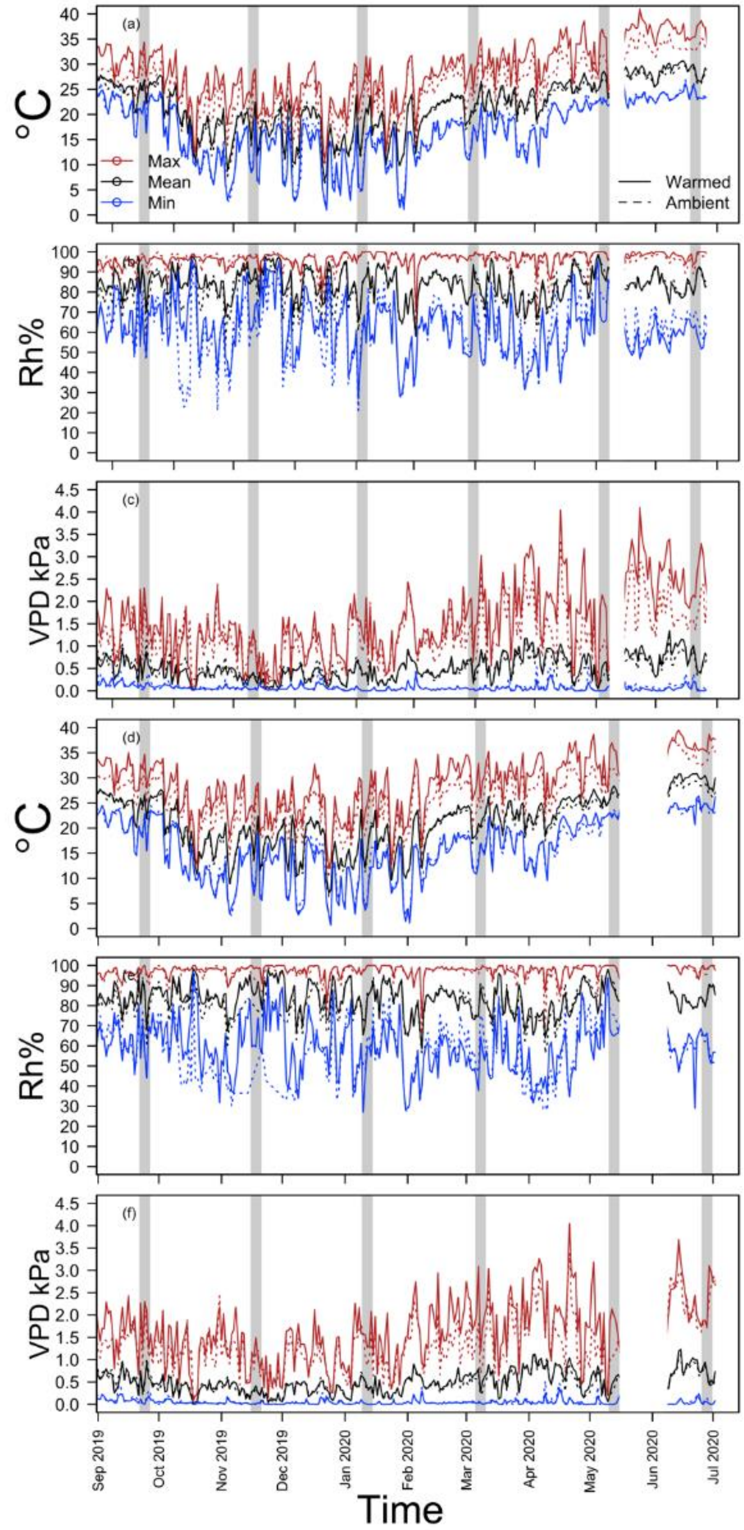

Figure1. South Site temperature, relative humidity, and vapor pressure deficit over the course of the study (Panels a, b, c). North Site temperature, relative humidity, and vapor pressure deficit over the course of the study (Panels d, e, f). Grey rectangles signify timepoints of sampling.

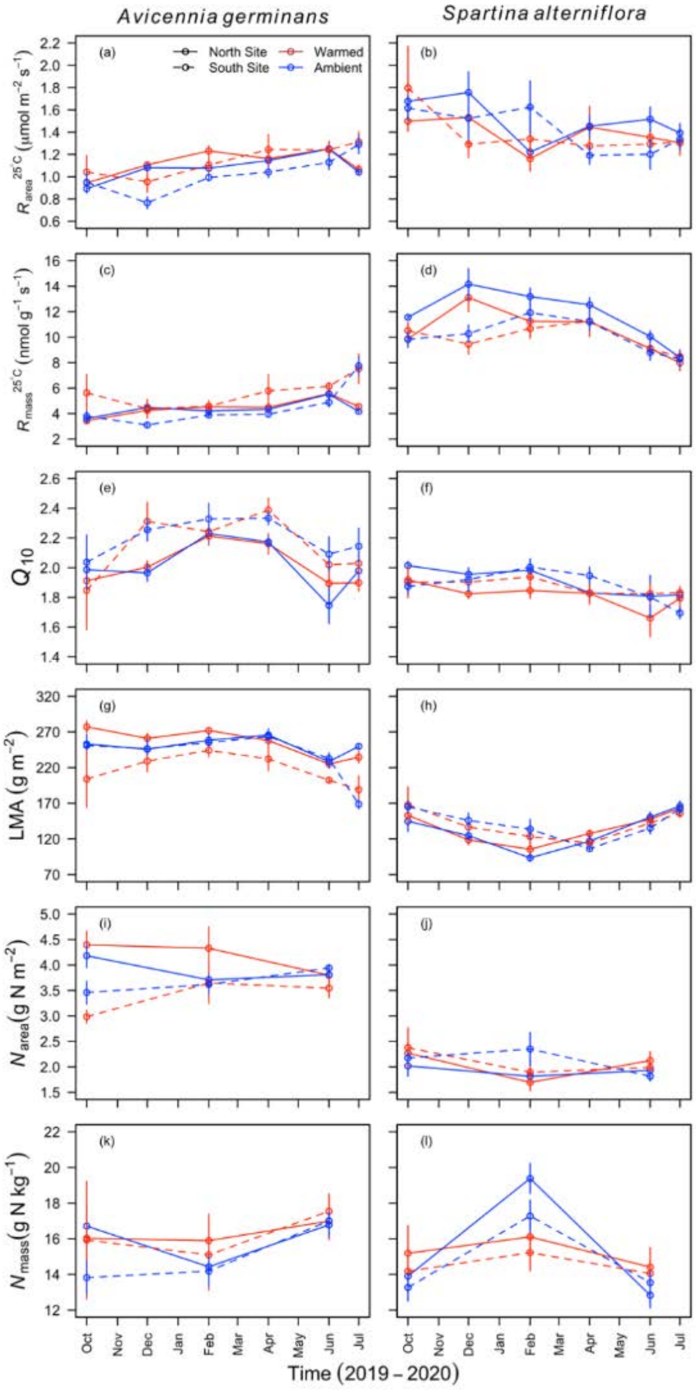

Figure 2. Means of leaf physiological traits across collection dates (2019-2020) for *Avicennia germinans* (a, c, e, g, i, k) and *Spartina alterniflora* (b, d, f, h, j, l) at two sites, with and without experimental warming. Leaf respiration per unit area at 25°C (panels a, b) Leaf respiration per unit mass at 25°C (panels c, d); The temperature sensitivity (Q_{10}) of R_{mass}^{25} (panels e, f); Leaf mass per unit area (LMA) (panels g, h); leaf N per unit area (N_{area}) (panels I, j); leaf N per unit mass (N_{mass}) (panels k, l).

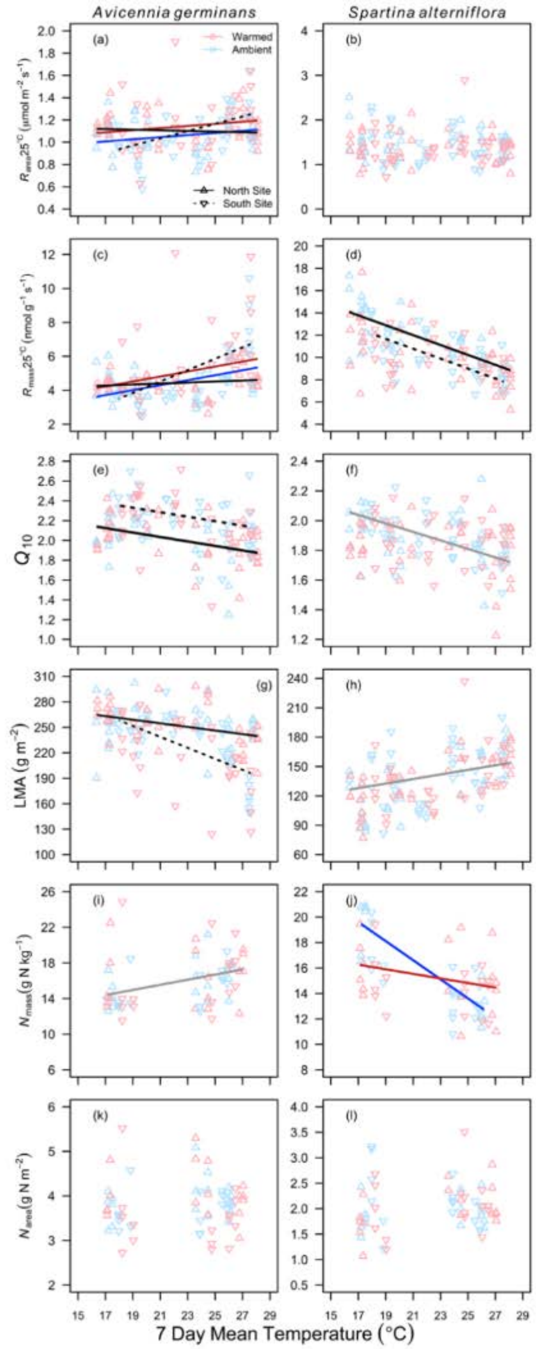

Figure 3. The relationship between preceding 7 day daily mean temperature and leaf physiological traits for *Avicennia germinans* (a, c, e, g, i, k) and *Spartina alterniflora* (b, d, f, h, j, l) at two sites, with and without experimental warming. Leaf respiration per unit area at 25°C (R_{area}^{25}) (panels a, b); Leaf respiration per unit mass at 25°C (R_{mass}^{25}) (panels c, d); The temperature sensitivity (Q_{10}) of R_{mass}^{25} (panels e, f); Leaf mass per unit area (LMA) (panels g, h); Leaf N per unit mass (N_{mass}) (panels i, j); Leaf N per unit area (N_{area}) (panels k, l). Gray solid lines signify a common relationship across sites and to warming. Black solid and dashed lines signify different relationships between sites. Red and blue lines are shown where differences in the effect of warming were consistent across sites.

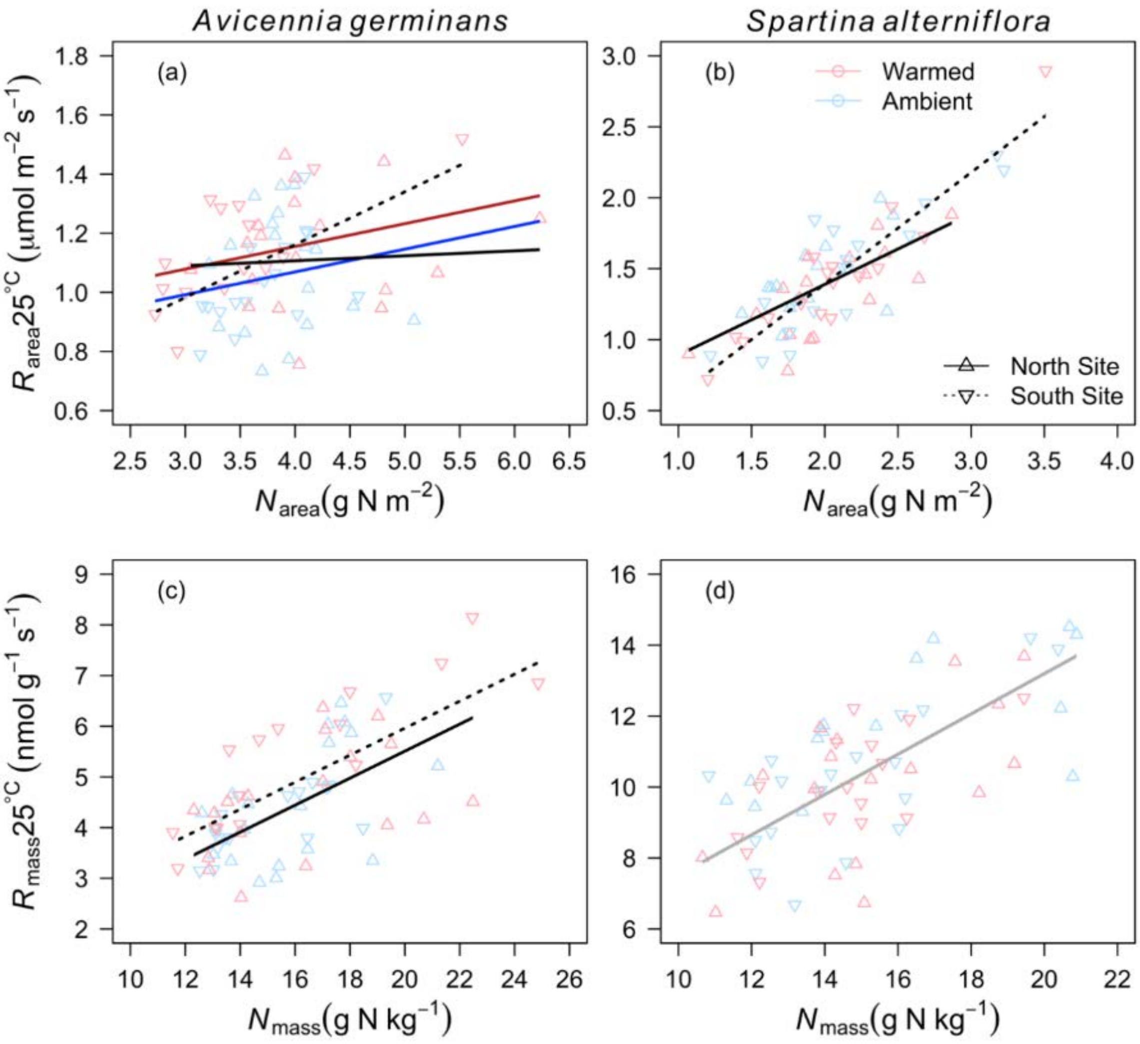

Figure 4. The relationship between leaf N concentration and leaf respiration for *Avicennia germinans* (panels a, c) and *Spartina alterniflora* (panels b, d) at both sites, with and without warming. Leaf respiration per unit area at 25°C (R_{area}^{25}) relationship to leaf N per unit area (N_{area}) (panels a, b). Leaf respiration per unit mass at 25°C (R_{mass}^{25}) relationship to leaf N per unit mass (N_{mass}) (panels c, d). Gray solid lines signify a common relationship across sites and to warming. Black solid and dashed lines signify different relationships between sites. Red and blue lines are shown where differences in the effect of warming were consistent across sites.

Chapter 2

Long-term and short-term impacts of nutrient enrichment on photosynthetic and respiratory processes in black mangrove (*Avicennia germinans*) in North Florida

Abstract

At the global scale, photosynthesis (*A*) and autotrophic respiration (*R*) are the two largest physiological processes responsible for CO_2 flux. The carbon (C) and nutrient cycles are interactively coupled, therefore by measuring the effect of nutrient enrichment on parameters of *A* and *R* we may gain a better understanding of plant allocation and use of C. Here we tested whether nitrogen (N) or phosphorus (P) inputs altered parameters of *A* and *R* in black mangrove (*Avicennia germinans*) over time, whether the application of nutrients (N or P) resulted in short-term differences in *A* and *R*, and whether leaf age (new vs. old) affected rates of *A* and *R* differently between fertilization treatments. We accomplished this by repeatedly measuring short-term temperature responses of leaf *R* and CO_2 response of *A* on N treated, P treated, and un-treated *Avicennia* over a 4-month period *in situ*. We found that N treated trees showed a short-term increase in A_{sat} and V_{cmax} in response to pulse enrichment. N treated trees also showed a consistently high R_{mass}^{25} over time indicating a legacy effect. Across all fertilization treatments, old leaves had 17% lower R_{area}^{25}, 34% lower R_{mass}^{25}, and 20% higher LMA than new leaves, yet leaf age had no consistent effect on parameters of *A*. These results provide mechanistic support for existing hypotheses of mangrove nutrition and simplify future predictions of CO_2 flux and associated physiological feedbacks for mangrove ecosystems experiencing increased nutrient input.

Introduction

Coastal wetland ecosystems play an important role in the global Nitrogen (N) and Carbon (C) cycle. In particular, mangrove forests store up to 4× more C per unit area than tropical rainforests (Donato et al. 2011, Alongi et al. 2014) and play a vital role in supporting the trophic relay that drives N transformation, use, and storage in coastal wetlands (Nagelkerken et al. 2008). However, continued anthropogenic pressures (e.g., agriculture, residential development, and recreation) have increased nutrient, especially N and P, inputs into coastal wetland ecosystems (Meybeck 2003, Holtgrieve et al. 2011, Ward et al. 2020). Atmospheric N deposition is also expected to increase at lower latitudes (Stevens et al. 2019) where mangrove vegetation is common and where mangrove deforestation rates are increasing (Friess et al. 2019). Increased nutrient inputs may impact coastal wetlands by accelerating rates of resource uptake and use or by changing the structure and composition of coastal plant communities (Simpson et al. 2013, Dangremond et al. 2020, Mozdzer et al. 2020). Nutrient additions may also have direct effects on photosynthesis (*A*) and respiration (*R*); key regulators of plant growth and function and the two largest fluxes of CO_2 between vegetation and the atmosphere at the global scale (Canadell et al. 2007, IPCC 2013). Thus, nutrient enrichment is expected to have important consequences for mangrove vegetation and as well as interactions between coastal vegetation and the atmosphere (Janssens & Luyssaert 2009, Fleischer et al. 2013, Fleischer et al. 2019).

Coastal wetlands are generally oligotrophic, and most mangrove soils are extremely nutrient-poor (Kathiresan and Bingham 2001). As a result, several studies have examined how nutrient enrichment affects mangrove productivity (e.g. Feller 1995, Lovelock et al. 2005, Feller et al. 2007, Reef et al. 2010). These experiments have demonstrated that mangrove productivity can be N-limited (Feller et al. 2003a, Lovelock and Feller 2003, Lovelock et al. 2007, Naidoo

2009, Simpson et al. 2013, Dangremond et al. 2020), P-limited (Lin and Sternberg 1992, Koch 1997, Medina et al. 2010), or both N- and P-limited (Feller 1995, Feller et al. 2003b, Lovelock et al. 2004, Weaver and Armitage 2020) depending upon the species and local edaphic conditions. What remains unclear, however, is how nutrient enrichment impacts mangrove *A* and *R* over both short and long timescales. New studies that examine mangrove photosynthetic and respiratory responses to nutrient enrichment could improve our basic understanding of nutrient impacts on mangrove physiology and aid predictions of nutrient enrichment impacts on CO_2 exchange between mangrove vegetation and the atmosphere.

In situ A under natural conditions is determined by both physical (i.e. stomatal) limitations of CO_2 diffusion and biochemical limitations to CO_2 fixation and RuBP regeneration. The maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, V_{cmax}), and the maximum rate of electron transport (J_{max}) required to regenerate ribulose-1,5-bisphosphate, are two key biochemical determinants of *A* (Farquhar et al. 1980). Many terrestrial biosphere models (TBMs) rely on accurate 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). A recent meta-analysis across hundreds of terrestrial species showed that N addition (fertilization) 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 water-use efficiency (Liang et al. 2020). Other broad meta-analyses have also found significant increases in leaf N following N addition (Ostertag and DiManno 2016). However, among individual studies, photosynthetic responses to N enrichment range from negative (Mao et al. 2018) to moderately or strongly positive (Teskey et al. 1994, Manter et al. 2005, Zhang et al. 2020). The effects of nutrient addition on *A* can also be transient (Gough et al. 2004). Moreover, 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} (and net photosynthesis) given that Rubisco represents a substantial fraction of leaf N (Evans 1989, Reich et al. 1999, Wilson et al. 2000, Wright et al. 2004, 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). P availability influences photosynthesis by affecting membrane solubility, NADPH, and ATP production which can in turn effect reduction of phosphoglyceric acid and regeneration of RuBP (Marschner 1995; Taiz and Zeiger 2010). There is some evidence that P scales positively with V_{cmax} (Domingues et al. 2010) and less so with J_{max} (Walker et al. 2014).

Only 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 additions 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 *Rhizophora mangle*. Lovelock et al. (2006b) found that P fertilization increased A in *Avicennia germinans* in Belize, but not in *Avicennia germinans* in Florida. Fertilization can also impact intrinsic water use efficiency (WUE) of mangrove foliage, quantified as the ratio of A to stomatal conductance to water vapor (g_s). Under moderate to high salinity conditions typical of coastal wetlands, N addition can increase WUE by increasing leaf N and 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 in magnitude or not clearly linked with biomass responses (e.g. Lovelock et al. 2004). This fits with general patterns across species where nutrient enrichment increases biomass and leaf area much more than A (Liang et al. 2020). However, no studies have examined how nutrient enrichment impacts V_{cmax} or J_{max} in mangroves, or whether N and P have different effects on photosynthetic parameters. There is evidence that N additions increase leaf N concentrations in mangroves (Lovelock and Feller 2003, Lovelock et al. 2004, Dangremond et al. 2020) which could result in increased V_{cmax} and possibly J_{max}, although the change in J_{max} may depend on leaf thickness or leaf P (Walker et al. 2014). 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). New studies that estimate V_{cmax} and J_{max} over time or under varying conditions could improve C flux estimates in coastal wetlands, and their representation in TBMs.

About half of plant R comes from leaves (Atkin et al. 2007). Leaf R (measured in darkness as CO_2 efflux) is co-limited by the supply of carbohydrates from photosynthesis, respiratory enzyme concentrations, and adenylate demand (Ryan et al. 1996, Atkin and Tjoelker 2003). Leaf R is also linked with photosynthesis given that R is used to support processes that maintain photosynthesis (e.g. protein turnover, Wang et al. 2020). In TBMs, leaf R is estimated as proportion of V_{cmax} or is predicted based on empirical relationships between leaf N and R at a set measurement temperature (Atkin et al. 2015). Although there are exceptions, previous studies have generally found that N addition results in higher leaf N and leaf R (Brix 1971, Manter et al. 2005, 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 applied in combination with N (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 P addition effects leaf *R*. More broadly, respiratory responses to nutrient enrichment or any aspect of environmental change have not been widely studied in mangroves (Burchett et al. 1989, Lopez-Hoffman et al. 2007, Akaji et al. 2019, Aspinwall et al. 2021). Likewise, it is unclear whether nutrient enrichment alters the temperature sensitivity of leaf *R* in any species.

The purpose of this study was to determine the long-term and short-term 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. The study was established in 2012 and included three treatments: control, added N, or added P. Previous results indicated that leaf N and productivity increased with added N, but leaf P and productivity did not change with added P (Dangremond et al. 2020). In summer 2020, on three dates prior to the annual addition of N or P, we measured *in situ* A, g_s, WUE, and the CO_2 response of A to determine V_{cmax} and J_{max}. We also measured the short-term temperature response of leaf *R* on each date to determine respiratory capacity and the temperature sensitivity of *R*. These data were used to determine the long-term impacts of nutrient enrichment on leaf physiology. Then, roughly 20 days after the annual addition (pulse) of N or P, we again measured A, g_s, and WUE, and determined V_{cmax}, J_{max}, leaf *R* and the temperature sensitivity of leaf *R*. Measurements made after the pulse of N or P were made on two sets of leaves: those formed prior to the N or P pulse and those formed after the N or P pulse. These data were used to determine the response of photosynthetic and respiratory processes to the N or P pulse, and test whether responses differed between leaves formed before and after the N or P pulse. Given that *Avicennia* productivity at the site was primarily N-limited and N addition increased leaf N

(Dangremond et al. 2020), we expected modest increases in A, WUE, V_{cmax}, and R with long-term N addition, and larger increases in A, WUE, V_{cmax} and R following the N pulse. We also expected that the effect of the N pulse on leaf physiology would be most apparent in leaves formed after the N pulse. Given that *Avicennia* showed no growth response to P addition at the site, we expected no long-term or short-term response of photosynthetic or respiratory processes to P addition.

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 estuarine vegetation in GTMNERR represents a marsh-mangrove ecotone. The southern limit of saltmarsh habitat converges and overlaps with the northern limit of mangrove habitat in Florida, although mangroves are increasingly common north of GTMNERR (Doughty et al. 2016). Our study site (N29°43′, W81°14′) was located roughly 20 km south of St. Augustine and just north of the Matanzas Inlet. Average annual precipitation at the GTMNERR reserve is 1317 mm (Chapman et al. 2021). Mean annual temperature (2001–2018) at St. Augustine is 20.8 °C. The highest monthly mean daily max temperature (July) is 31.97 °C, and the lowest monthly mean daily low temperature (January) is 11.13 °C (NOAA). 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*. *Avicennia germinans* is a broadly distributed mangrove species native to warm-temperate, subtropical, and tropical regions of the Americas and Africa.

Experimental Design

As described by Dangremond et al. (2020), 24 *A. germinans* trees ranging from 62 to 87 cm (mean = 70.7 ± 1.53 cm) in height were selected to be used for an *in-situ* fertilization experiment at the site described above. Trees were assigned to one of three treatments (8 replicates per treatment): control (no fertilization), added nitrogen (N), or added phosphorus (P). Due to methods limitations and time constraints we randomly selected 6 trees per treatment for our study. Treatments were randomly assigned to trees, ensuring that trees were located at least 5m 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)], with the most recent fertilization event applied during October 2019. A second fertilization event occurred during the middle of this experiment (August 17th 2020). Physiology measurements taken before August 17th 2020 were used to infer long-term effects of N or P addition. Measurements taken after this date were used to infer responses to the pulse of added N or P. 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, nutrient enrichment was administered through two (3 cm wide x 30 cm deep) soil cores (150 g per core) inserted on opposing sides of the target tree. 150 g of N or P fertilizer was placed in the core hole before sealing the hole. 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. 2003) and in Belize (Feller et al. 2007).

Environmental Data

Air temperature (T_{air}) and relative humidity (RH) were measured every 15 minutes 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 air temperature (mean daily T_{air}) of the 7 days preceding physiology measurements ranged from 26.4 ºC to 28.8 ºC, and the maximum daily T_{air} ranged from 31.3 to 33.7 ºC over the experimental period (June-September 2020) (Figure S5). At the same study 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

In situ rates of leaf gas-exchange were measured five times between June 2020 and September 2020 to determine the effects of long term and pulse additions of N and P on leaf physiology of *A. germinans*. The number of measurements varied per measurement date depending on weather; afternoon thunderstorm are common in north Florida during summer. On each date, two portable cross-calibrated photosynthsis systems (LI-6800, LiCor., Inc, Lincoln NE, USA) were used to measure CO_2 response of leaf-level net photosynthesis (A-C_i). A roughly equal number of C, N, and P plots were measured by each photosynthesis system on each date.

Both LI-6800s were fitted with a 3 × 3 cm cuvette head and a red-blue LED light source. For all measurements, flow rate was held constant at 500 µmol s^{-1}. To help moderate temperature fluctations in the LI-6800 system, 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 (standard deviation) °C across all measurement dates. Relative humidity conditions in the chamber were controlled near ambient external conditions but also varied depending upon water vapor fluxes from the leaf. There was no shade at our site, so light conditions within the cuvette were set at a photosynthetic photon flux density of 1800 µmol m^{-2} s^{-1}, which approximates full-sun conditions. Each CO_2 response measurement began with steady-state measurements of light-saturated net photosynthesis (A_{sat}, µmol m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}), and intrinsic water use efficiency (WUE, umol mol^{-1}) 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 cuvette.

All A-C_i measurements occurred between 9:00 and 15:00 local time and were made on recently mature, fully expanded, upper canopy leaves of *A. germinans*. The one-sided surface area (cm^2) of leaves within the chamber was determined by measuring the 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_{sat} and intercellular CO_2 (C_i) at a series of reference [CO_2]: 300, 250, 100, 50, 0, 420, 650, 800, 1200, and 1500 µmol mol^{-1}. On the final collection dates (27, 28 September) we recorded A-C_i measurements (using the same methods as listed above) on leaves formed prior to the pulse of N or P, as well as leaves formed after the pulse of N or P.

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 R version 3.2.1 (R-Development-Core-Team, 2009) and the model was fit using the *Plantecophys* package (Duursma 2015) following the methods of Smith and Dukes (2017). The model estimates the maximum rate of Rubisco carboxylation (V_{cmax}; µmol m^{-2} s^{-1}) and the rate of electron transport for RuBP regeneration (J_{max}; µmol m^{-2} s^{-1}). V_{cmax} and J_{max} provide estimates of the biochemical capacity of photosynthesis. Using estimates of V_{cmax} from each A-C_i curve, we also estimated stomatal limitations (L) to photosynthesis. L was calculated following Farquhar and Sharkey (1982) by comparing observed A_{sat} with the predicted rate of A_{sat} if stomatal limitation was zero (i.e., C_i = C_a). The predicted ate of A_{sat} when L = 0 was back-calculated from predicted V_{cmax} using the model of Farquhar et al. (1980).

Temperature response of leaf respiration

Measurements of the short-term temperature response of leaf dark respiration (*R*, measured as CO_2 efflux per unit leaf area) were conducted at five timepoints (June 21st, July 19th, August 16th, September 5th, September 27th). We measured the same leaves used for measurements of the CO_2 response of photosynthesis. Leaves were collected pre-dawn (04:30 – 06:00 local time) to avoid activation of photosynthesis. The excised leaves were placed in Ziplock bags with moist paper and transferred to the lab in complete darkness. Previous studies have found no effect of leaf removal on leaf *R* measurements (O'Sullivan et al. 2013; Aspinwall et al. 2017; 2019). Moreover, all measurements were completed the same day as leaf collection, and we found no evidence that leaf *R* changed with time since leaf removal. Leaf area (cm^2) of the measured leaves was determined with a leaf area meter (LI-3000C, LI-COR BioSciences, Lincoln, NE, USA) just prior to measurements of leaf *R*. Leaf area data were used to calculate *R* per unit area (R_{area} µmol m^{-2} s^{-1}).

Short-term temperature response curves of leaf *R* were completed by sealing excised leaves randomly in one of three infrared gas analyzers (one LI-6400XT, two LI-6800s, LI-COR, Inc., Lincoln, NE, USA) fitted with large chambers (LI-6400-22L or LI-6800-24, LI-COR, Inc.). The large chambers increased CO_2 differentials (sample CO_2−reference CO_2) without leak artifacts without leak artifacts (Drake et al. 2015, Jahnke and Krewitt 2002), which improves measurement accuracy. 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 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. Once rates of *R* reached steady state (~10 min) 5 spot measurements of leaf *R* were logged. Once R_{area} of each leaf was measured at 15 °C, we repeated measurements of *R* in stepwise fashion at a series of higher temperatures; 20, 27, 35 and 40°C. After temperature response measurements were completed, leaves were dried at 70 °C for ~72 hours after which leaf dry mass measurements were 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.

Modelling 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). To determine the appropriate model for our data, we compared the suitability of three different algorithms following the approach of Aspinwall et al. (2017): (1) a log polynomial function, which describes the non-linear relationship between natural log transformed *R* and T_{leaf} (O'Sullivan et al. 2013; Heskel et al. 2016), (2) an exponential function with a single Q_{10} value which assumes that the temperature sensitivity of *R* is constant across the entire measurement temperature range (e.g. Tjoelker et al. 2001), and (3) a modified Arrhenius function, which accounts for a non-exponential increase in *R* with increasing T_{leaf} (Lloyd and Taylor 1994). The polynomial function is written as:

(1) $ln\ R = a + bT + cT^2$ or

(2) $R = e^{a+bT+cT^2}$

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 Eqn 2 can be used to estimate the Q_{10} of *R* at any T_{leaf}: $Q_{10} = e^{10\times(b+2cT)}$.

Among these algorithms, the polynomial (Equation 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 $T_{leaf.}$ Thus, 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. Repeated estimates of R_{area}^{25}, R_{mass}^{25}, and Q_{10} over time provide a direct way of determining the direction and magnitude of respiratory capacity (Aspinwall et al. 2019).

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 (control, added N, added P), and their respective interaction (Date x Treatment) on photosynthetic and respiratory parameters (e.g. A_{sat}, 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 fertilization), fertilization treatment, or their interactions (Application x 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 pulse, formed after N or P pulse), fertilization treatment, or their interaction (Leaf Age × Treatment) had any effect on photosynthetic or respiratory parameters. Homogeneity of variance for model results were tested using Levene's and Shapiro-Wilk tests. Data were log or square root transformed as necessary.

Results

Impacts of N and P addition on photosynthetic parameters

Compared to the timepoint just prior to the fertilizer addition, trees in the added N treatment showed a modest increase in A_{sat} following the pulse of N (+28%), while trees in the added P and control treatments showed less variation over time (Figure 1). Nonetheless, the effects of date, treatment, and their interaction on A_{sat} were not statistically significant (Table 1). Across time points and treatments A_{sat} averaged 7.9 ± 0.65 µmol m^{-2} s^{-1}. g_s did not differ between fertilizer treatments, but varied among measurement dates (Table 1). g_s was highest on September 27th (0.12 ± 0.01 mol m^{-2} s^{-1}), and lowest on July 19th (0.07 ± 0.01 mol m^{-2} s^{-1}) (Figure 1). WUE did not differ among individual measurement dates, or among fertilizer treatments (Table 1) and averaged 0.09 ± 0.01 µmol mol^{-1}.

V_{cmax} and J_{max} differed among measurement dates (Table 1). V_{cmax} was lowest on August 16th (63.5 ± 6.4 µmol m^{-2} s^{-1}) and highest on September 27th (88.6 ± 5.6 µmol m^{-2} s^{-1}) (Figure1a). Similarly, J_{max} was lowest on August 16th (100 ± 9.2 µmol m^{-2} s^{-1}) and highest on September 27th (136 ± 8.0 µmol m^{-2} s^{-1}) (Figure 1c). Compared to the timepoint just prior to the fertilizer addition, trees in the added N treatment showed a marked increase in V_{cmax} and J_{max} following the pulse of N, while trees in the added P and control treatments showed smaller changes (Figure 1a). Nonetheless, no significant date × treatment interactions were observed for V_{cmax} or J_{max} suggesting that increase in photosynthetic capacity after fertilization was not statistically significant. Stomatal limitation of net photosynthesis (L) was similar across measurement dates and fertilizer treatments and showed not date × treatment interaction (Table 1). On average, L was 0.31 ± 0.03 (Figure 1).

Finally, we tested whether the average values for photosynthetic parameters measured after the N or P pulse (i.e. post-fertilization) differed from average values of photosynthetic parameters aggregated across timepoints before the N or P pulses. We also tested whether leaves formed prior to N or P pulses differed in photosynthetic parameters compared to leaves formed after N or P pulses. Compared to measurements taken before the N pulse, trees in the added N treatment showed relatively large increases in A_{sat}, g_s, V_{cmax}, and to some extent J_{max}, following the N pulse (Figure 1). Trees in the added P treatment showed smaller increases in these parameters following the P pulse (relative to measurements taken before the P pulse), while control trees showed little change in photosynthetic parameters before and after the nutrient pulses. The apparent short-term responses of photosynthetic parameters to N (and to some extent P) pulses were only seen in newly formed leaves (Figure 1). Despite these trends, treatment and timing × treatment interactions were not significant for any photosynthetic parameter, suggesting that photosynthetic parameters showed no significant long-term or short-term response to N or P enrichment. Moreover, leaf age (prior formed leaves, leaves formed after nutrient pulses) had no significant effect on photosynthetic traits and did not interact with treatment (Table 3). Significant differences in A_{sat}, g_s, V_{cmax} and J_{max} were only observed between the pre- and post-fertilization time periods (Table 2). Post-fertilization (September 27th) A_{sat} (9.40 ± 0.64 µmol m^{-2} $s^{-}1$) was 26% higher than A_{sat} averaged across pre-fertilization dates (June – August; 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 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}) (Figure 1b).

Impacts of N and P addition on respiratory parameters

Respiratory parameters were generally consistent across treatments but varied over time (Table 4). R_{area}^{25} was 20% higher on September 27th (1.54 ± 0.06 μmol m^{-2} s^{-1}) than September 5th (1.28 ± 0.06 μmol m^{-2} s^{-1}) (Table 4, Figure 2a). R_{mass}^{25} was higher on August 16th (8.73 ± 0.65 nmol g^{-2} s^{-1}) and September 27th (8.65 ± 0.55 nmol g^{-2} s^{-1}) than September 5th (6.61 ± 0.61 nmol g^{-2} s^{-1}). LMA was higher on June 21st (222 ± 8.8 g m^{-2}) than August 16th (178 ± 10.4 g m^{-2}) or September 27th (186 ± 8.8 g m^{-2}) (Table 4, Figure 2e). Although only marginally significant (p=0.06) trees in the N treatment had higher R_{mass}^{25} (8.56 ± 0.47 nmol g^{-2} s^{-1}) than trees in the P treatment (7.47 ± 0.46 nmol g^{-2} s^{-1}) and control trees (7.24 ± 0.44 nmol g^{-2} s^{-1}) averaged over time (Table 4, Figure 2c). There was no effect of treatment or measurement date on the of Q_{10} of R (Table 4, Figure 2g). On average, the Q_{10} of R was 2.39 ± 0.06.

Leaf respiratory parameters were consistently different between leaf age classes (prior formed leaves, newly formed leaves). Compared to newly formed leaves, leaves formed prior to the fertilization event had 17% lower R_{area}^{25} (prior leaves: 1.28 ± 0.05 μmol m^{-2} s^{-1}, new leaves: 1.54 ± 0.05 μmol m^{-2} s^{-1}), 34% lower R_{mass}^{25} (prior leaves: 5.75 ± 0.52 nmol g^{-2} s^{-1}, new leaves: 8.65 ± 0.50 nmol g^{-2} s^{-1}), and 20% higher LMA (prior leaves: 223 ± 6.6 g m^{-2}, new leaves: 186 ± 6.4 nmol g m^{-2}) (Table 5, Figure 2b,d,f). Unlike photosynthetic traits, there were no significant differences between respiratory parameters measured before and after application of fertilization treatments (N or P) (Table 6, Figure 2b,d,f,h).

Although N treated trees showed increased response of A_{sat}, V_{cmax}, and R_d there were no detectable relationships between parameters of A and R.

Discussion

Mangrove ecosystems have the ability to sequester and store large amounts of C, but little is known about how increased nutrient inputs may affect photosynthetic and respiratory processes that regulate patterns of C allocation, storage, and use in coastal wetland plants (Evans 1989, Feller 2007, Donato et al. 2011, Fernandez-Martinez et al. 2014). To address this knowledge gap, we examined whether black mangrove (*Avicennia germinans*) in a north Florida coastal wetland adjust their photosynthetic and respiratory capacities in response to N or P additions, and whether long-term physiological responses to nutrient enrichment differ from short-term physiological responses. We expected that N treated *Avicennia* would increase *in situ* A_{sat}, photosynthetic capacity (V_{cmax}, J_{max}) and respiratory capacity ($R_{\mathrm{area}}{}^{25}$, $R_{\mathrm{mass}}{}^{25}$) in response to the N pulse (i.e. short-term response), with smaller long-term effects of N addition on photosynthetic and respiratory parameters. Trees at our site previously showed no evidence of P-limitation on aboveground productivity (Dangremond et al. 2020). Therefore, we hypothesized that P addition would have little short-term or long-term effect on photosynthetic or respiratory capacity. In addition, we tested whether responses to nutrient enrichment differed between leaves formed before and after the N or P pulse. We found that pulse N addition led to a short-term increase of photosynthetic parameters and consistently increased $R_{\mathrm{mass}}{}^{25}$ over time. These results indicate that photosynthetic responses to N addition may be short-lived, while respiratory responses to N addition may persist for longer time periods. In support of our hypothesis, we also found that P treatment had little short- or long-term effect on photosynthetic or respiratory parameters. Lastly, we found some evidence that increases in A_{sat} and photosynthetic capacity were limited to leaves formed after the N pulse, although leaf age effects on photosynthetic traits were largely non-significant. Even so, newer leaves had significantly lower LMA and higher respiratory capacity.

These results improve our understanding of nutrient enrichment effects on photosynthetic and respiratory physiology in *Avicennia germinans* growing near its northern range limit.

In a synthesis of studies, Liang et al. (2020) found that N addition increased leaf A per unit area, g_s, WUE, and V_{cmax}. In the present study, N treated trees showed a short-term increase of A_{sat} and V_{cmax} in response to pulse enrichment which partially supports our hypothesis that in an N limited system, N enrichment would result in increased photosynthetic capacity. This positive relationship between N enrichment and increased photosynthetic capacity might be explained by the proportion of N allocated to Rubisco and other bioenergetic proteins (Evans and Clarke 2019). Rubisco accounts for the largest fraction of leaf protein, therefore N addition alleviates limitations imposed on Rubisco carboxylation (V_{cmax}) in an N limited system (Evans 1989, Hikosaka and Shigeno 2009, Aspinwall et al. 2019). Previous work by Dangremond et al. (2020) found that leaf %N increased in response to N addition, supporting the hypothesis that leaf %N, photosynthetic capacity, nutrient limitations are tightly coupled in *Avicennia*.

Many plant types have been shown to reduce their response to consistent nutrient enrichment, resulting in weaker relationships between fertilization and physiological response over time (Liang et al. 2020). Considering that in this study trees received nutrient enrichment annually over 9 years, it is plausible that the overall effect of nutrient enrichment declined as the trees became acclimated to this level of enrichment. It is also plausible that the ratio of fertilizer to total plant biomass has been reduced heavily over time, warranting a proportionally smaller physiological response by year 9 (Linder and Rook 1984). However, as described in Liang et al. (2020), the percent change in structural traits (~ 33%) and physiological traits (~ 5.4%) in response to N addition are not equivalent. A simple explanation for this type of response is that the plant is allocating N to something other than leaf level photosynthesis. There is evidence to support a decoupling of leaf *A* and *R* from N driven increases in biomass (Teskey 1994). Some clues for simplifying the imperfect relationships between N addition, photosynthesis and respiration could be attributed to the storage of nonstructural carbohydrates (NSC) (Collalti et al 2020).

Leaf N has been shown to positively scale with capacity of leaf *A* and *R* (Reich et al 2008, Crous et al. 2018). Our results support the hypothesis that *R* capacity (R_{mass}^{25}), CO_2 assimilation (A_{sat}) and carboxylation of Rubisco (V_{cmax}) would positively scale with each other in response to N enrichment. However, it is important to make a distinction between the response patterns of *A* and *R*. Our results showed short-term response of *A* to pulse additions of N but did not show evidence of a sustained legacy effect over time. Mitochondrial enzymes that regulate *R* require much less N than that of those that regulate *A* (Ghimire et al. 2017). Therefore, N addition may result in higher *R* over longer periods of time than *A*. This is an important result for future mangrove physiology studies because it presents a new gap in our understanding of how nutrient enrichment might illicit short-term response of *A*, legacy effects of *R*, and a possible decoupling of *A* and *R* over time. This result may also be useful in earth system modelling in coastal wetland ecosystems as eutrophication, N deposition, and mangrove expansion progress.

Site conditions play an important role in mediating mangrove responses to nutrients (Reef et al. 2010). As a result of proximity (~2 miles) to the Matanzas inlet, our study site had a high rate of exchange and a low residence time for nutrients, a known driver of N limitation in mangrove ecosystems (Smith 1984; Reef et al 2010). There was also evidence that the site would be N limited from the growth response to N addition reported by Dangremond et al. (2020). Many mangrove nutrition experiments have demonstrated large increases in biomass and physiology related responses as a result of P addition (Feller 1995; Feller et al. 2003, Lovelock et al. 2006, Lovelock et al. 2007). However, just as Dangremond et al. (2020) found limited biomass response to P addition, we found no effect of P addition on leaf level physiology. Our results for P treated and control trees were highly variable which may suggest that influxes of organic N could be responsible for variability over time. However, as leaves aged there were consistent patterns of reduced levels of A and R across treatments.

Previous studies have shown that the relationship between leaf age and overall physiological capacity is negative across plant types (Suzuki et al. 1987, Whitehead et al. 2011). Black mangrove leaf lifespan is ~16 months (consistent with many broadleaved tropical and subtropical evergreens), supporting our hypothesis that *Avicennia* would show a negative relationship between leaf age and physiological response to nutrient enrichment (Reich et al. 1992, Reich et al. 1998, Suarez and Medina 2005). Our work supports this hypothesis as R_{area}^{25}, and R_{mass}^{25} were greatly reduced in old *Avicennia* leaves. We found that old leaves had much higher LMA and lower parameters of A and R relative to new leaves. This response might be driven by a reduction in leaf N allocated to Rubisco and a simultaneous increase in leaf N is allocated to cell wall material (Kattge et al. 2011, Onoda et al 2017). Although results of statistical analysis were not significant, old leaves showed reduced photosynthesis consistently across treatment types and time with lower V_{cmax} (old leaves: mean = 79.1 ± 5.6 µmol m^{-2} s^{-1}, new leaves: mean = 87.5 ± 5.7 µmol m^{-2} s^{-1}) and J_{max} (old leaves: mean = 119 ± 6.7 µmol m^{-2} s^{-1}, new leaves: mean = 135 ± 6.9 µmol m^{-2} s^{-1}). Leaves formed before the N (or P) pulse showed no difference in response to application of fertilization indicating that an increase in parameters of A is only measurable in leaves formed after the N pulse, and that response is relatively short lived. This finding may be useful in describing similarities between relationships between leaf age, respiration, and photosynthesis for *Avicennia*, mangroves, and other coastal wetland species.

Our results provide new information on the short-term and long-term impacts of nutrient enrichment on mangrove photosynthetic and respiratory parameters. They suggest that alleviation of N limitation drives short term increases in A and that N addition might drive long-term elevation of R. Our results also suggest that old mangrove leaves reduce rates of R as they increase LMA over time; a result found in most C_3 plants. 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. The information presented here may be useful in modelling C cycle and nutrient cycle feedbacks in coastal wetland ecosystems under current and future nutrient input scenarios. Future studies that explore mangrove physiology of 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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Table 1. Results of a two-way analysis of variance testing the main and interactive effect of fertilization treatment and measurement date on *in situ* rate of leaf net photosynthesis (A_{sat}), stomatal conductance to water vapor (g_s), water use efficiency (WUE), the estimated rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport for RuBP regeneration (J_{max}), and stomatal limitation of net photosynthesis (L). Degrees of freedom (df) and F-values are presented for each factor and reponse variable. F-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

	df	A_{sat}	g_s	WUE	V_{cmax}	J_{max}	L
Treatment (T)	2	0.56	0.01	0.40	0.83	0.68	0.14
Date (D)	3	2.57	**3.60***	2.73	**3.53***	**2.89***	2.50
T x D	6	1.10	0.59	0.55	0.97	0.62	0.71

Table 2. Results of a two-way analysis of variance testing the main and interactive effect of fertilization treatment and timing of fertilizer application (before versus after N or P application on the *in situ* rate of leaf net photosynthesis (A_{sat}), stomatal conductance to water vapor (g_s), water use efficiency (WUE), the estimated rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport for RuBP regeneration (J_{max}), and stomatal limitation of net photosynthesis (L). Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

	df	A_{sat}	g_s	**WUE**	V_{cmax}	J_{max}	L
Treatment (T)	2	0.58	0.02	0.44	0.81	0.57	0.16
Application (A)	1	**6.35***	**6.78***	0.22	**5.64***	0.37	0.05
T x A	2	2.05	1.48	0.11	2.37	0.34	0.11

Table 3. Results of a two-way analysis of variance testing the main and interactive effect of fertilization treatment and leaf age (formed prior to fertlizer pulse, formed after fertlizer pulse) on the *in situ* rate of leaf net photosynthesis (A_{sat}), stomatal conductance to water vapor (g_s), water use efficiency (WUE), the estimated rate of Rubisco carboxylation (V_{cmax}), the maximum rate of electron transport for RuBP regeneration (J_{max}), and stomatal limitation of net photosynthesis (L). Degrees of freedom (df) and F-values are presented for each factor and reponse variable. F-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

	df	A_{sat}	g_s	WUE	V_{cmax}	J_{max}	L
Treatment (T)	2	1.74	0.03	1.14	2.70	0.79	0.00
Leaf age (La)	1	1.26	1.66	0.39	1.43	2.87	0.77
T x La	2	1.11	1.20	0.15	0.80	1.31	0.02

Table 4. Results of a two-way analysis of variance testing the main and interactive effect of nutrient treatment and measurement date 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 of *R* (Q_{10}) in *Avicennia germinans*. Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

	df	R_{area}^{25}	R_{mass}^{25}	LMA	Q_{10}
Treatment (T)	2	1.45	2.91	1.57	1.81
Date (D)	4	**3.27***	**2.79***	**3.25***	2.42
T x D	8	0.91	0.87	1.00	0.90

Table 5. Results of a two-way analysis of variance testing the main and interactive effect of nutrient treatment (T) and leaf age (La) 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 of *R* (Q_{10}) in *Avicennia germinans*. Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

	df	R_{area}^{25}	R_{mass}^{25}	LMA	Q_{10}
Treatment (T)	2	0.58	0.61	0.23	0.78
Leaf age (La)	1	**13.49*** **	**16.46*** **	**16.20*** **	1.61
T x La	2	0.73	0.55	0.21	0.08

Table 6. Results of a two-way analysis of variance testing the main and interactive effect of nutrient treatment (T) and application (A) 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 of *R* (Q_{10}) in *Avicennia germinans*. Degrees of freedom (df) and F-values are presented for each factor and reponse variable. *F*-values with '*', '**' and '***' are significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

	df	R_{area}^{25}	R_{mass}^{25}	LMA	Q_{10}
Treatment (T)	2	1.53	2.60	1.39	1.79
Application (A)	1	0.92	0.01	0.85	0.45
T x A	2	0.28	0.12	0.20	0.11

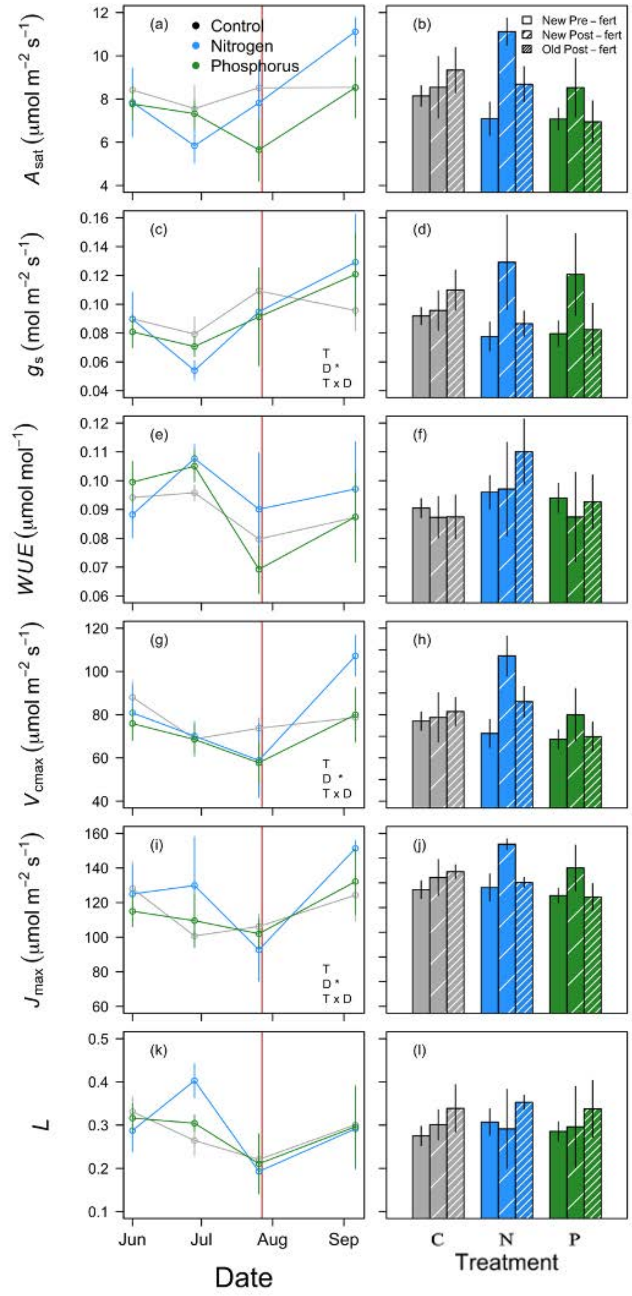


Figure 1. Means (± standard error) values for leaf photosynthetic parameters across collection dates for *Avicennia germinans* (panels a, c, e, g, i, k). Significant main and interactive effects of treatment (T) and date (D) are annotated in the upper right corner of each panel. The red vertical line represents the fertilization event. Signifigant main and interactive effects of fertilization treatment (T) and measurement date (D) on in situ rate of leaf net photosynthesis (A_{sat}) (panels a and b), stomatal conductance to water vapor (g_{s}) (panels c and d), water use efficiency (WUE) (panels e and f) estimated rate of Rubisco carboxylation (V_{cmax}) (panels g and h), electron transport for RuBP regeneration (J_{max}) (panels i and j), and stomatal limitation (L) (panels k and l).

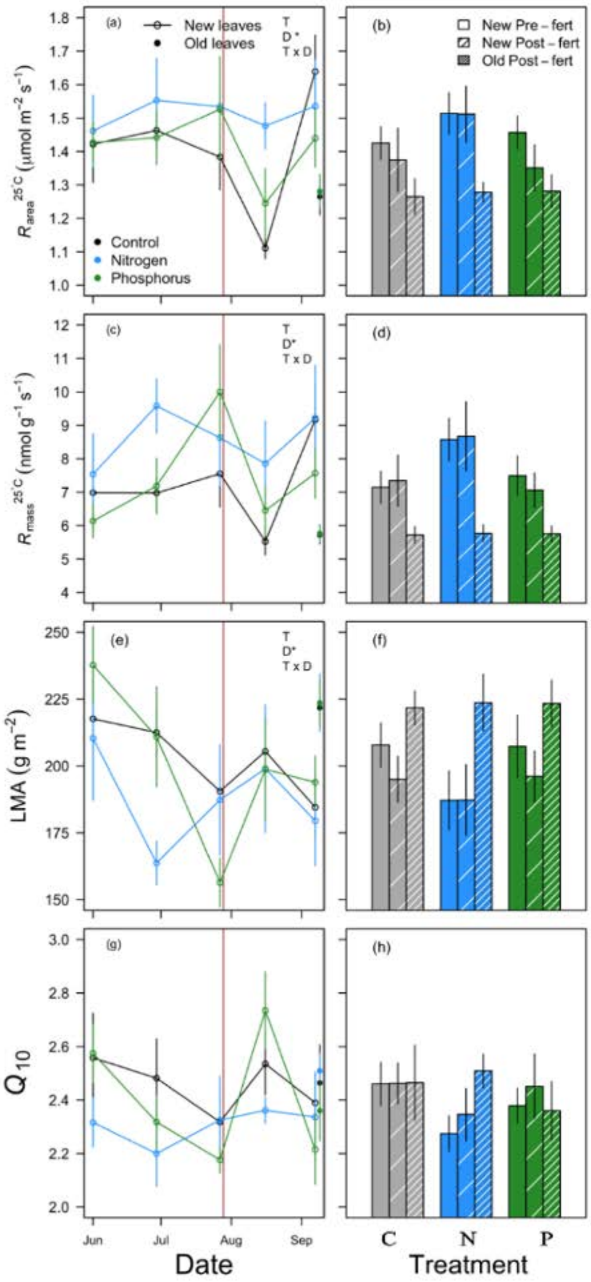


Figure 2. Means of leaf respiratory traits across collection dates for *Avicennia germinans* are shown in panels a, c, e, g. Significant main and interactive effects of treatment (T) and date (D) are annotated in the upper right corner of each panel. The red vertical line indicates the fertilization event. Means of photosynthetic traits Pre and Post fertilization event are shown in panels b, d, f, h. Leaf respiration per unit area at 25°C (panels a and b) Leaf respiration per unit mass at 25°C (panels c and d); Leaf mass per unit area (LMA) (panels e and f); The temperature sensitivity (Q_{10}) of R_{mass}^{25} (panels g and h).

Supplemental Tables and Figures

	October 2019			**December 2019**			**February 2020**			**April 2020**			**June 2020**			**July 2020**		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
S. alterniflora																		
North W	-0.3986 ±0.326	0.0741 ±0.004	-0.0002 ±0.000	-0.2062 ±0.330	0.0753 ±0.004	-0.0003 ±0.000	-0.0729 ±0.379	0.0455 ±0.015	0.0003 ±0.000	-0.3432 ±0.380	0.0761 ±0.012	-0.0003 ±0.000	0.4976 ±0.399	0.0091 ±0.021	0.0008 ±0.000	-0.2591 ±0.303	0.0548 ±0.011	0.0000 ±0.000
North A	-0.6269 ±0.444	0.0983 ±0.003	-0.0006 ±0.000	-0.5400 ±0.332	0.1030 ±0.006	-0.0007 ±0.000	-0.6282 ±0.395	0.0929 ±0.012	-0.0005 ±0.000	-0.2108 ±0.333	0.0720 ±0.004	-0.0002 ±0.000	0.0839 ±0.459	0.0437 ±0.023	0.0003 ±0.000	-0.1486 ±0.005	0.0500 ±0.005	0.0002 ±0.000
South W	-0.5175 ±0.395	0.0927 ±0.009	-0.0006 ±0.000	-0.4064 ±0.327	0.0672 ±0.008	0.0000 ±0.000	-0.5963 ±0.340	0.0855 ±0.012	-0.0004 ±0.000	-0.2298 ±0.374	0.0648 ±0.012	-0.0001 ±0.000	-0.2852 ±0.313	0.0609 ±0.007	0.0000 ±0.000	-0.2750 ±0.294	0.0572 ±0.007	0.0000 ±0.000
South A	-0.4701 ±0.294	0.0847 ±0.008	-0.0004 ±0.000	-0.4363 ±0.332	0.0780 ±0.013	-0.0003 ±0.000	-0.6712 ±0.318	0.1001 ±0.007	-0.0006 ±0.000	-0.6750 ±0.352	0.0905 ±0.007	-0.0005 ±0.000	-0.2474 ±0.313	0.0535 ±0.007	0.0001 ±0.000	0.2513 ±0.288	0.0224 ±0.008	0.0006 ±0.000
A. germinans																		
North W	-0.5076 ±0.306	0.0232 ±0.016	0.0008 ±0.000	-0.4709 ±0.221	0.0302 ±0.006	0.0008 ±0.000	-1.1186 ±0.245	0.0788 ±0.011	0.0000 ±0.000	-0.9562 ±0.250	0.0653 ±0.009	0.0002 ±0.000	-0.1130 ±0.392	0.0232 ±0.017	0.0008 ±0.000	-0.1831 ±0.255	0.0139 ±0.008	0.0010 ±0.000
North A	-0.6151 ±0.270	0.0268 ±0.012	0.0008 ±0.000	-0.3647 ±0.251	0.0243 ±0.008	0.0009 ±0.000	-1.3228 ±0.247	0.0846 ±0.007	0.0000 ±0.000	-1.0407 ±0.248	0.0691 ±0.011	0.0002 ±0.000	0.5286 ±0.380	-0.0199 ±0.020	0.0015 ±0.000	-0.5173 ±0.239	0.0319 ±0.007	0.0007 ±0.000
South W	-0.4381 ±0.525	0.0431 ±0.013	0.0003 ±0.000	-1.3511 ±0.286	0.0789 ±0.011	0.0000 ±0.000	-0.9903 ±0.251	0.0621 ±0.009	0.0004 ±0.000	-1.4821 ±0.262	0.1063 ±0.009	-0.0004 ±0.000	-0.2795 ±0.260	0.0330 ±0.007	0.0007 ±0.000	-0.2570 ±0.308	0.0387 ±0.008	0.0006 ±0.000
South A	-0.7724 ±0.441	0.0419 ±0.020	0.0006 ±0.000	-1.5348 ±0.250	0.0755 ±0.009	0.0001 ±0.000	-1.3524 ±0.278	0.0781 ±0.013	0.0001 ±0.000	-1.3717 ±0.221	0.0813 ±0.007	0.0000 ±0.000	-0.5131 ±0.332	0.0356 ±0.015	0.0008 ±0.000	-0.3717 ±0.349	0.0451 ±0.015	0.0006 ±0.000

Table S1. Log polynomial parameter estimates of *a*, *b*, and *c* for each Species x Treatment (W = warmed, A = ambient) x Site (North or South) x Date combination. Standard error of each estimate is also shown. Grey rectangles signify the dates at which leaf N concentration was measured.

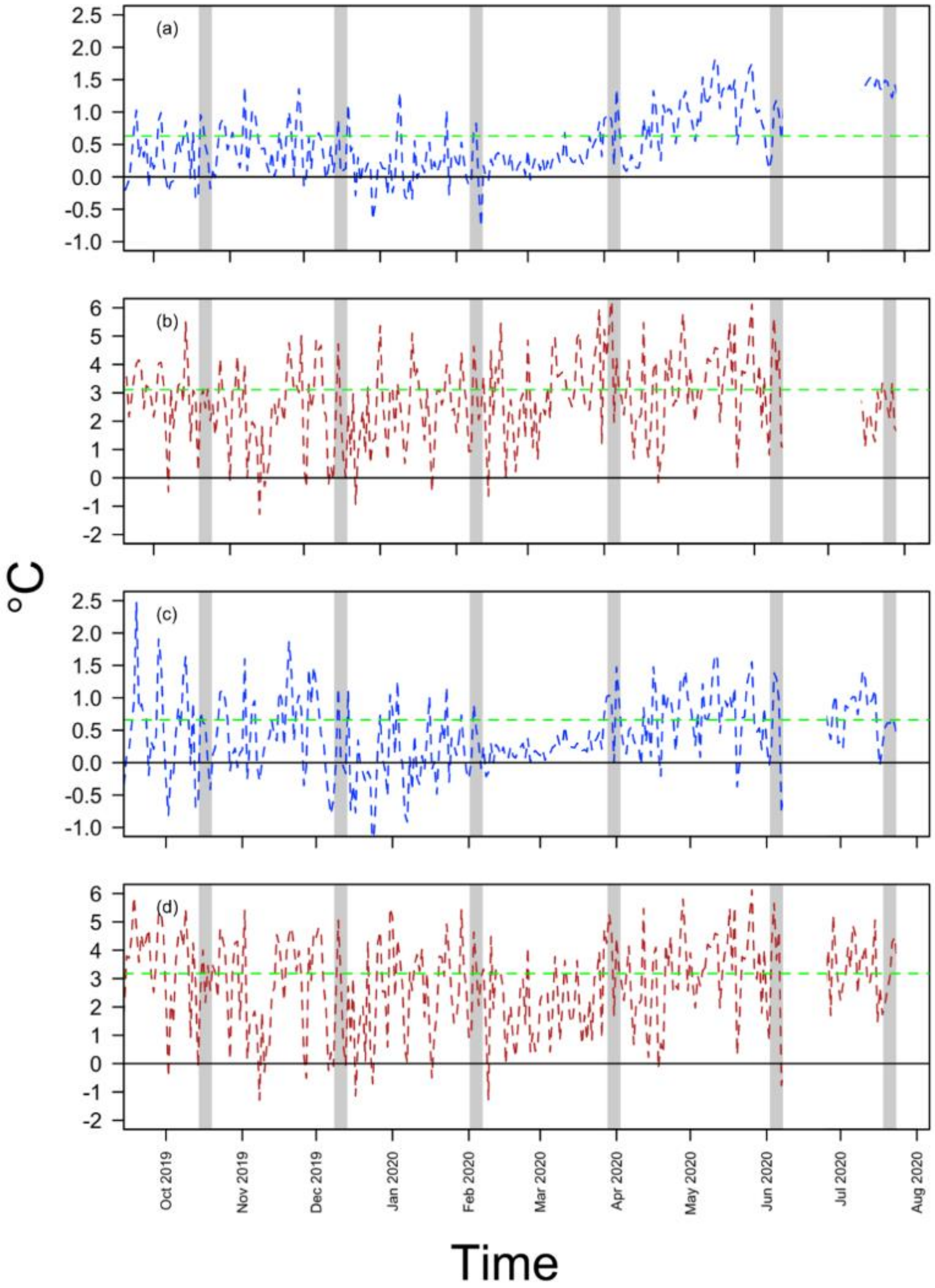


Figure S1. North site difference between Warmed and Ambient plots; daily mean (panel a) and max (panel b). South site difference between Warmed and Ambient plots; daily mean (panel c) and max (panel d).The green dashed lines represent the mean affect of warming over the course of the study.

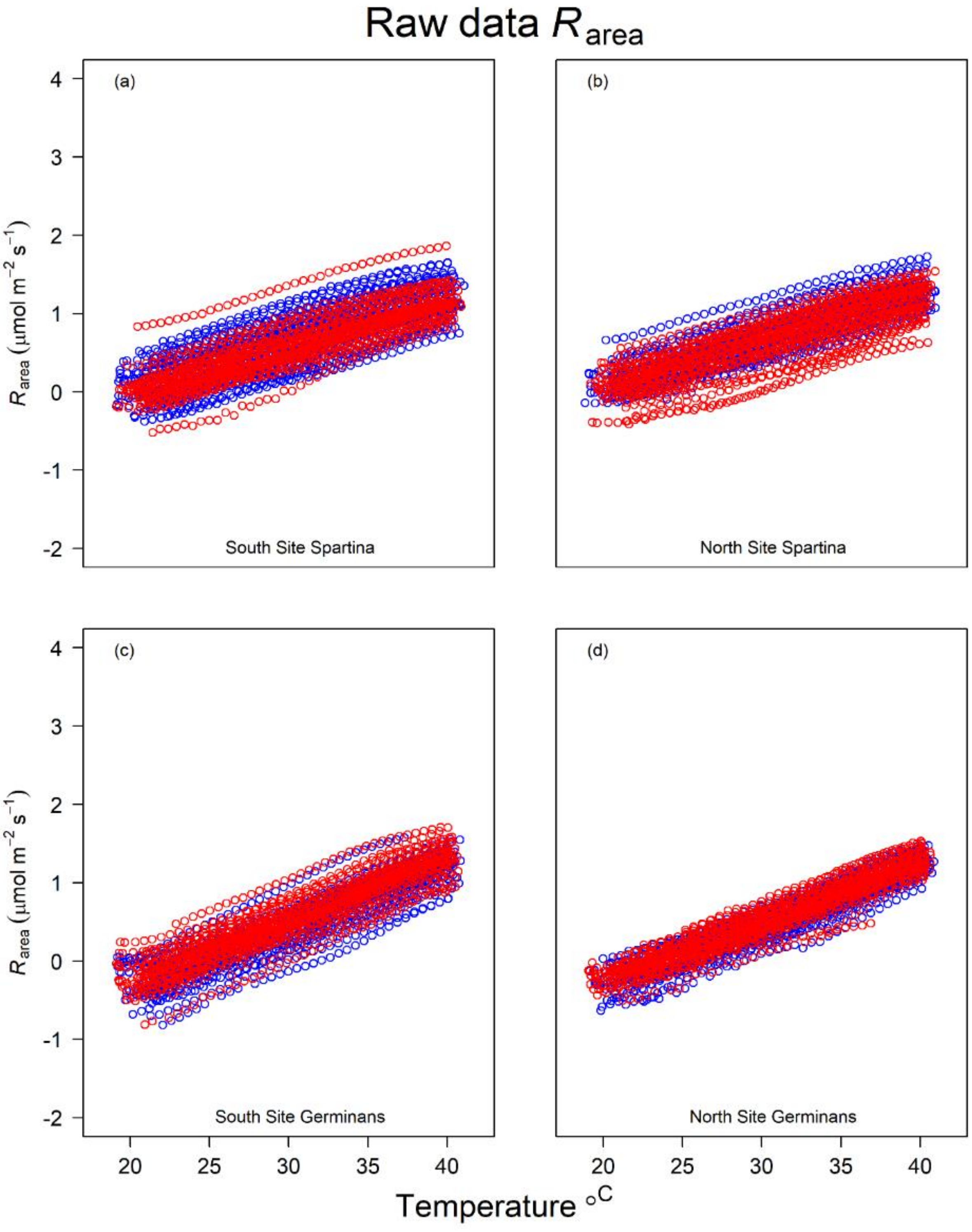
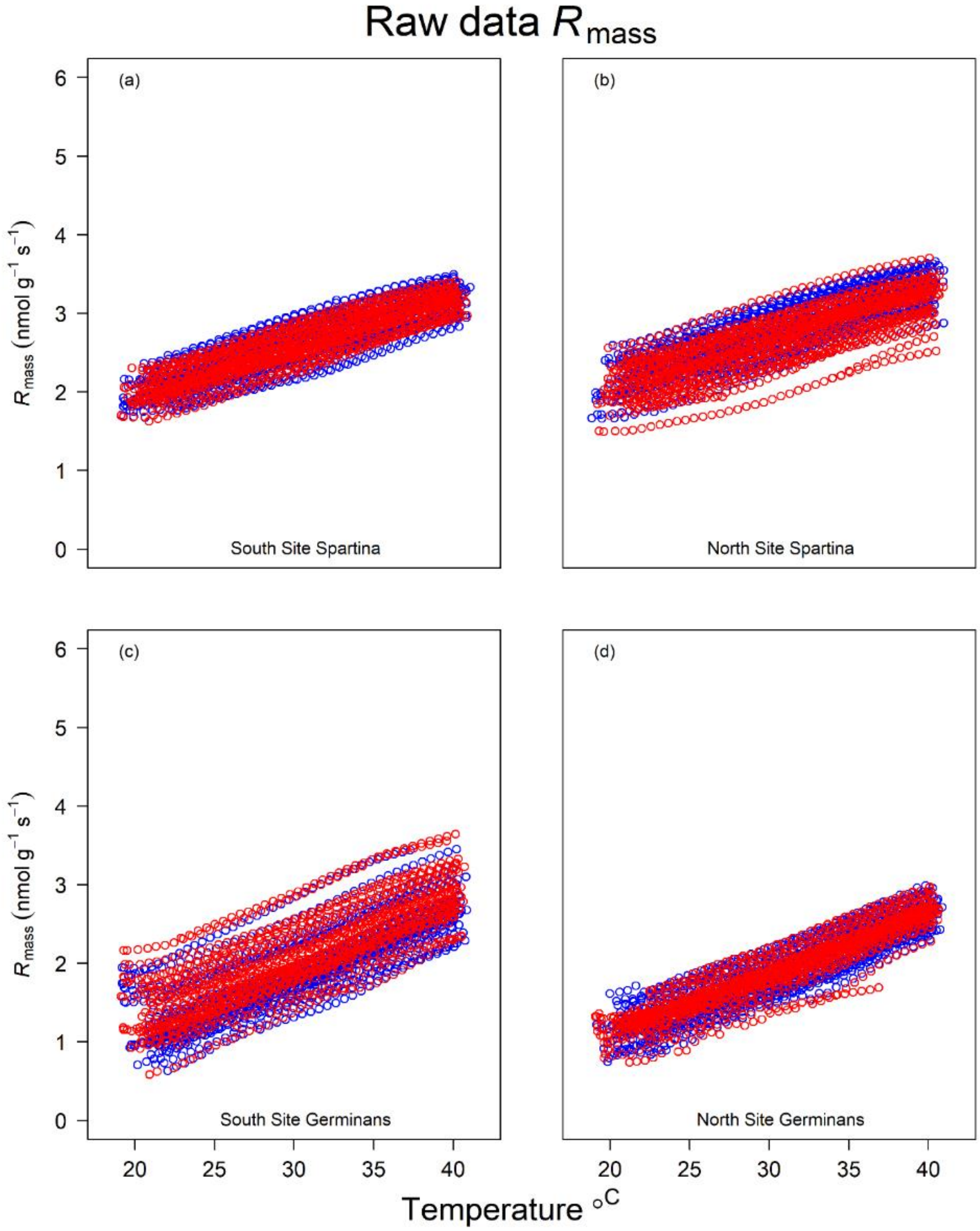

Raw data R_{area}
(a)
(b)
(c)
(d)
South Site Spartina
North Site Spartina
South Site Germinans
North Site Germinans
R_{area} (μmol m^{-2} s^{-1})
4
3
2
1
0
-1
-2
20
25
30
35
40
Temperature °C

Figure S2

Figure S3



Raw data R_{mass}
(a)
(b)
(c)
(d)
R_{mass} (nmol g^{-1} s^{-1})
0
1
2
3
4
5
6
South Site Spartina
North Site Spartina
South Site Germinans
North Site Germinans
20
25
30
35
40
Temperature °C

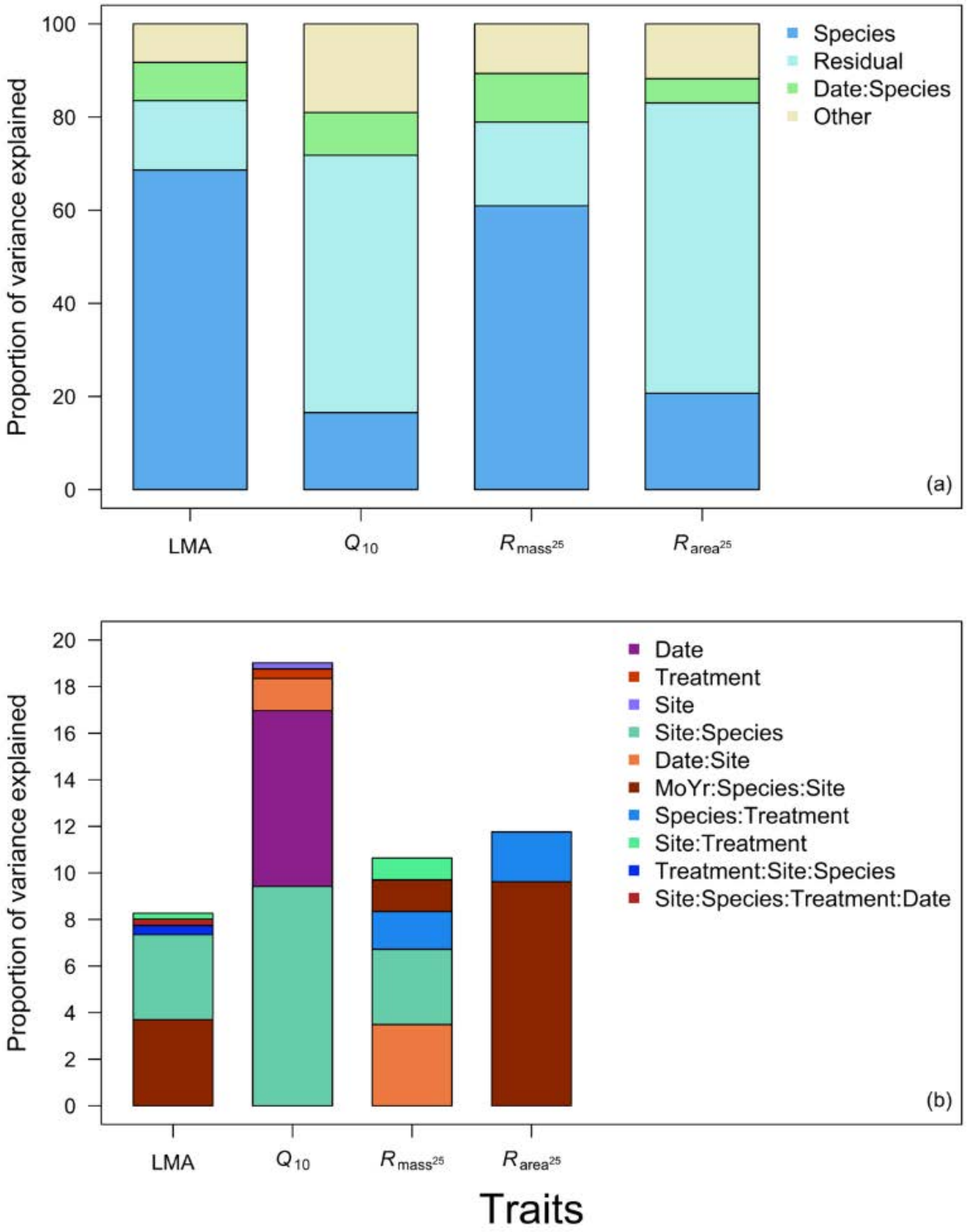


Figure S4. The proportion of variance explained for each of the leaf physiological traits analyzed across all timepoints. Panel a shows main and interactive effects that explain the greatest proportion of variance. Panel b shows what main and interactive effects make up the "Other" section from panel a.

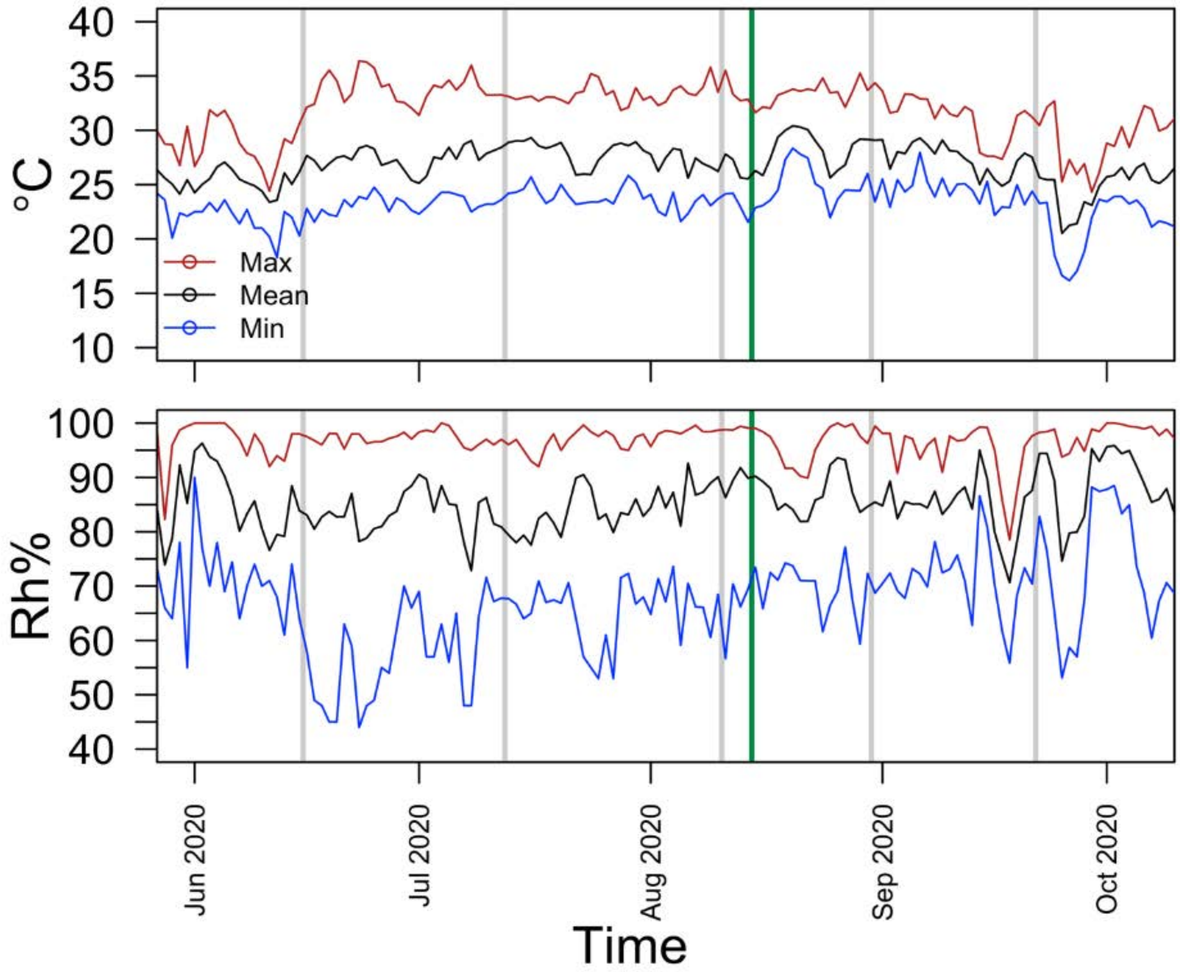


Figure S5. Maximum, mean, and minimum temperature differences over the course of the study. Grey rectangles signify timepoints of sampling. Green rectangle signifies fertilization.

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