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Warming impacts on photosynthetic processes in dominant plant species in a subtropical forest

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Abstract

Climate warming could shift some subtropical regions to a tropical climate in the next 30 years. Yet, climate warming impacts on subtropical species and ecosystems remain unclear. We conducted a passive warming experiment in a subtropical forest in Florida, USA, to determine warming impacts on four species differing in their climatic distribution, growth form, and functional type: *Serenoa repens* (palm), *Andropogon glomeratus* (C₄ grass), *Pinus palustris* (needled evergreen tree), and *Quercus laevis* (broadleaved deciduous tree). We hypothesized that warming would have neutral-positive effects on photosynthetic processes in monocot species with warmer climatic distributions or adaptations to warmer temperatures, but negative effects on photosynthesis in tree species. We also hypothesized that periods of low soil moisture would alter photosynthetic responses to warming. In both monocot species, warming had no significant effect on net photosynthesis (A) or stomatal conductance (g_s) measured at prevailing temperatures, or photosynthetic capacity measured at a common temperature. In *P. palustris*, warming reduced A (-15%) and g_s (-28%), and caused small reductions in Rubisco carboxylation and RuBP regeneration. Warming had little effect on photosynthetic processes in *Q. laevis*. Interestingly, *A. glomeratus* showed little sensitivity to reduced soil moisture, and all C₃ species reduced A and g_s as soil moisture declined and did so consistently across temperature treatments. In subtropical forests of the southeastern U.S., we conclude that climate warming may have neutral or slightly positive effects on the performance of grasses and broadleaved species but negative effects on *P. palustris* seedlings, foreshadowing possible changes in community and ecosystem properties.

Keywords: climate warming, C₄ photosynthesis, ecotone, longleaf pine, thermal acclimation, xeric

Introduction

Global mean surface temperatures increased 0.85 °C between 1880 and 2012 (IPCC, 2013), contributing to changes in species distributions, shifts in plant community composition, and alterations of ecosystem structure and function (Hughes 2000, Bertrand et al. 2011, Lenoir et al. 2008, Kelly & Goulden 2008, Elmendorf et al. 2015). Further warming of 2 to 4 °C is expected by the middle of the century (IPCC 2013). Warming impacts on many species and communities remain difficult to predict, and may depend upon variation in life history traits, local adaptation, and physiological tolerances of component species (Shi et al. 2015, Li et al. 2016). Furthermore, the magnitude of warming, ecosystem structure, and a host of biotic and abiotic factors can modify community and ecosystem responses to warming (Van Bogaert et al. 2011, Coomes et al. 2014, Tingstad et al. 2015). New experiments are needed to provide insight into

warming impacts on plant species, communities, and ecosystems in understudied regions and potential modifying factors.

Ecotones are boundary areas where vegetation types change or species with different climatic distributions overlap. Experiments and observational studies in ecotones help reveal differential responses to warming among component species and may help predict changes in community and ecosystem attributes with further warming (Harsch et al. 2009, Løkken et al. 2020, Cavanaugh et al. 2014). Most studies have focused on elevational transects or high latitude (boreal, tundra) ecotones; areas where vegetation transitions are especially clear, rates of warming are higher, and ecosystem responses to warming are more pronounced (Reich et al. 2015, Carroll et al. 2017, Pretzsch et al. 2020). There have been relatively few warming experiments in ecosystems located in transitional areas between temperate and tropical climates. This is notable given that some of the most productive and species-rich ecosystems on the planet occur in these regions (Running et al. 2004, Kreft & Jetz 2007). Although rates of warming are generally slower in these regions compared to higher latitude regions, small changes in temperature could still have significant impacts. Modest warming could reduce the performance of species growing close to their warmest geographic or physiological limit (Wertin et al. 2011, Drake et al. 2015), improve the performance of species growing below their temperature optimum (Kauppi et al. 2014), and promote the influx of species from tropical regions resulting in community ‘thermophilization’ (Feely et al. 2020).

Changes in water availability (i.e. soil moisture) could enhance, diminish, or possibly reverse positive effects of climate warming on plant function and productivity. Warmer temperatures could also increase evapotranspiration and reduce soil water availability (Xu et al. 2013, but see Zavaleta et al. 2003), resulting in diminished or negative responses to climate warming. However, direct tests of climate warming effects along temporal or spatial soil moisture gradients are rare. According to the ‘law of the minimum’ and multiple limitation theory (Bloom et al. 1985), warming may alleviate temperature limitation of enzyme activity when temperatures are below the species optimum, resulting in higher photosynthetic rates and greater carbon available for growth, respiration, and defence. Warming should alleviate low temperature limitations until water becomes limiting, triggering stomatal closure and limitation of CO₂ for photosynthesis. Indeed, there is evidence that low soil moisture diminishes the positive effects of climate warming on photosynthesis and productivity in high latitude tree species growing in cold, seasonal climates (D’Orangeville et al. 2018, Reich et al. 2018, Zhang et al. 2020). Whether similar patterns exist in warmer and less seasonal lower latitude ecosystems is unclear (Carter et al. 2020). In these ecosystems, species may experience low temperature limitations less frequently, and warming might reduce carbon uptake and productivity of some species when temperatures are high, even if water is not limiting.

Many plants acclimate to warmer growth temperatures through a series of adjustments, including an increase in the short-term temperature optimum of photosynthesis and reduced rates of Rubisco carboxylation (V_{cmax}) or RuBP regeneration (J_{max}) at a common measurement temperature (Gunderson et al. 2010, Sendall et al. 2015, Kumarathunge et al. 2019). But these adjustments vary among species and environments and have variable impacts on realized photosynthesis at warmer growth temperatures (see Way & Yamori 2014). In some species and environments, a ‘detractive adjustment’ occurs when thermal acclimation results in lower net photosynthesis (A) under warmer temperatures. In other cases, a ‘constructive adjustment’ occurs when thermal acclimation results in higher A under warmer temperatures. Additional studies that investigate thermal acclimation responses and impacts on realized A would improve our understanding of the consequences of photosynthetic temperature acclimation (Carter et al. 2021). More broadly, there is a continued need to examine physiological responses to warming in different plant functional types and species in underrepresented regions. For example, there is uncertainty about whether C_3 and C_4 species differ in their capacity to acclimate to temperature changes (Yamori et al. 2014, Smith & Dukes 2017), and the extent to which species representing different functional types and growth forms growing together in a community differ in their response to warmer temperatures remains understudied (de Valpine & Harte 2001, Hoepfner & Dukes 2012, Volder et al. 2013, Wertin et al. 2015). Addressing these uncertainties is important given that community and ecosystem responses to warming will depend on the aggregate response of different functional types and individual species.

The southeastern U.S. lies at the boundary of temperate and tropical climates and is classified as ‘warm temperate’ or ‘humid subtropical’ (Beck et al. 2018). The region contains the highest diversity of plant species in North America (Keil & Chase 2019, Weakley 2020), and warm-wet growing seasons contribute to higher rates of primary production than many regions in the U.S. (Turner et al. 1995). Mean annual air temperatures in the region have increased $\sim 1^\circ\text{C}$ since 1970 (Karl et al. 2009). This rate of warming is low compared to higher latitude regions of North America (IPCC 2013). However, average temperatures in the region are expected to increase 1 to 3 $^\circ\text{C}$ over the next 30 to 50 years with higher minimum temperatures and more extremely hot days (Carter et al. 2018). Although there are signs that warming is driving ‘tropicalization’ in coastal wetland communities in the southeastern USA. (Cavanaugh et al. 2019), warming impacts on terrestrial ecosystems in the region remains uncertain.

The objective of this study was to determine the effects of experimental warming on leaf physiology and biomass production in subtropical forest species and the potential modifying effects of soil moisture. To accomplish this, we grew seedlings of four species with contrasting climatic distributions, growth forms, and functional strategies (*Andropogon glomeratus* (C_4 grass), *Serenoa repens* (woody shrub palm), *Pinus palustris* (needled evergreen tree), *Quercus laevis* (broadleaved deciduous tree)) in a 1.5-year-long passive warming experiment (mean daily air temperature $+1.0^\circ\text{C}$, mean daytime

air temperature +2.5 °C) in a subtropical forest in northeast Florida. We repeatedly measured leaf gas-exchange at prevailing soil moisture and leaf temperatures on seedlings of each species grown under ambient and warm temperatures, and determined warming effects on photosynthetic biochemistry, total dry mass production, and biomass allocation after the experiment. Aggregated over time, we hypothesized that warming would have negligible or positive effects on photosynthesis and productivity in monocot species with warmer climatic distributions or adaptations to warmer temperatures (*A. glomeratus*, *S. repens*). Yet, warming would generally reduce photosynthesis and productivity in tree species with more temperate distributions (*P. palustris*, *Q. laevis*). We also hypothesized that periods of low soil moisture would negate or reverse the positive effects of warming on photosynthesis in warm-adapted species and exacerbate the negative effects of warming on photosynthesis in tree species.

Materials and methods

Study site, experimental design, species

This study took place on the University of North Florida campus in Jacksonville, FL USA (30.2619 °N, 81.5165 °W). The mean annual temperature (1948-2012) is 20.4°C. The highest mean daily maximum temperature (August) is 29.4°C, and the lowest mean daily minimum temperature (January) is 6.7°C (Western Regional Climate Center, 2020, Cooperative Climatological Data Summaries, Retrieved from: <https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?fl4358>). Historically, night-time minimum temperatures fall below 0°C roughly 10-15 days per year (1948-2012), but freezing temperatures are increasingly rare (Cavanaugh et al. 2014). The mean annual precipitation is 1332 mm. Rain predominantly falls during the summer wet season spanning May to September/October. The end of the dry season (October-May) coincides with the onset of high temperatures. The experimental site contained a mixed pine-oak overstory and an understory of broadleaved shrubs and grasses typical of a xeric longleaf pine ecosystem (Peet & Allard 1993). The site was last burned 5-10 years prior to the experiment, although the year and season of burning are unknown, as is the average fire interval. Soils at the site are Leon fine sand (Sandy, siliceous, thermic Aeric Alaquods), characterised by deep sand, low organic matter content and fertility, poor drainage, and slow permeability.

The study was comprised of six blocks, each containing two 1.5 × 1.5 m plots spaced 0.75 m apart. Blocks were randomly positioned in high-light forest gaps within a one-hectare area. Plots within each block were randomly assigned to one of two treatments: ambient or warmed. The warmed plots were enclosed in 1.5 × 1.5 × 1.5 m chambers, framed with 2.5 cm diameter white PVC and wrapped in 6 mil polyethylene greenhouse film (modified after Charles & Dukes 2009). Passive warming chambers were chosen because they require no electricity, are inexpensive, semi-permanent, and easy to deploy at difficult to access field sites. They also provide significant warming by trapping radiation. However, as

discussed elsewhere (e.g., Marion et al. 1997), passive warming chambers have limitations, including modifications of the light environment, lack of night-time warming, and reduced wind speed. We endeavoured to mitigate these limitations. The polyethylene film transmits full spectrum ultraviolet light and ~90% of photosynthetically active radiation. A circular (0.75m diameter) opening was cut into the chamber roof to allow for air circulation (mixing) and natural rainfall. Small slits were cut in the remaining roof area to facilitate additional air movement and rainwater infiltration into the plots. The chambers ‘trap’ radiation causing air temperature to increase. Although minimal warming occurs during cloudy weather or at night, the chambers warm air temperatures rather than canopy surface temperatures (as occurs with infrared heaters), and do not inhibit dew formation (Feng et al. 2021). Control plots were left uncovered. We did not include additional control plots with PVC frames (no polyethylene film) to determine the influence of the PVC frame. We expected that the narrow, white-colored PVC frames would have little influence on the light environment in the control plot, and the addition of these plots would require a 50% increase in the number of sensors and measurements, which was deemed unfeasible. Air temperature (T_{air}) and relative humidity (RH) were measured every 15 min in the center of each treatment plot (0.8 m above the ground) using a shielded air temperature/RH sensor (Model US23 Pro v2, HOBO Instruments Inc.) attached to a wooden post.

Chambers effectively increased T_{air} . Over the course of the experiment, mean daily (24 hr) T_{air} in the warmed treatment was on average 1.0°C higher than mean daily T_{air} in the ambient treatment (ambient mean daily $T_{\text{air}} = 20.8 \pm 5.9$ (standard deviation)°C, warmed mean daily $T_{\text{air}} = 21.8 \pm 6.2$ °C). Mean daytime (08:00 – 18:00 hrs) T_{air} in the warmed treatment was on average 2.5 °C higher than mean daytime T_{air} in the ambient treatment (Figure 1). Warming of this magnitude (1-3°C) is expected by 2050 throughout the southeast U.S. (Carter et al., 2018). Mean daytime RH in the ambient treatment was 5.3% higher than mean daytime RH in the warmed treatment (Figure 1). Mean daytime VPD in the warmed treatment was on average 0.54 kPa higher than mean daytime VPD in the ambient treatment (Figure 1). Volumetric soil water content over 0-15 depth (VWC, m³ m⁻³ or %) was measured in each quadrant of each treatment plot every 2-3 weeks with a handheld time domain reflectometer (TDR) probe (HydroSense II, Campbell Scientific, Logan, UT, USA). VWC was measured on leaf gas-exchange measurement dates (see below for details) as well as several timepoints between leaf gas-exchange measurements. Over time VWC ranged from 10.9 to 0.1%. These VWC values range from below the permanent wilting point (approximately -1.5 MPa) to near field capacity, for sandy soil. No obvious wilting occurred at very low VWC. VWC did not differ between ambient and warmed treatments (Figure 1, $P=0.75$, mean VWC = $5.5 \pm 2.1\%$).

Four species were chosen for this experiment: *Andropogon glomeratus* (bushy bluestem), *Serenoa repens* (saw palmetto), *Pinus palustris* (longleaf pine), and *Quercus laevis* (turkey oak). These

species are common at the site and represent the major growth forms and functional types in forests of the southeastern U.S. *Andropogon glomeratus* is a C₄ NADP-ME type perennial bunchgrass (herbaceous monocot) with a distribution spanning the Atlantic and Gulf Coastal Plains from Texas to North Carolina, and all of Florida (latitude range: 25.2 to 38.0 °N, longitude range: 75.5 to 98.0 °W). *Serenoa repens* is a low-growing evergreen shrub palm (woody monocot) native to all of Florida and warm coastal areas of Louisiana, Mississippi, Georgia, and South Carolina (latitude range: 25.2 to 32.4 °N, longitude range: 80.5 to 89.6 °W). *Pinus palustris* is a needled evergreen tree with a natural distribution that spans the Atlantic and Gulf Coastal Plains of the southeastern U.S, from central Florida to east Texas and north into North Carolina (latitude range: 26.8 to 36.6 °N, longitude range: 77.0 to 95.2 °W). The species occurs on inland montane and piedmont sites, xeric sandhill sites, and poorly drained coastal flatwoods. *Quercus laevis* is a broadleaved deciduous tree species with a distribution from central Florida to southeastern Louisiana and north into southeast Virginia (latitude range: 26.8 to 35.8 °N, longitude range: 77.0 to 89.6 °W). The species is limited to dry pinelands and sandy ridges within its range.

One similarly sized seedling (or rhizome cluster in the case of *A. glomeratus*) of each species was randomly transplanted into one quadrant of each treatment plot on February 21, 2019. Seedlings of *S. repens* and rhizome clusters of *A. glomeratus* were collected from locations within the forest. At transplanting, *S. repens* seedlings contained an average of four leaves with average petiole lengths and leaf widths of 16.7 ± 2.9 cm and 34.0 ± 4.9 cm, respectively. Average petiole length and leaf width did not differ between treatments ($P=0.22$ and $P=0.34$, respectively). *A. glomeratus* rhizome clusters averaged 4.9 ± 1.2 (standard deviation) cm in diameter at planting and did not differ between treatments ($P=0.59$). Natural regeneration of *P. palustris* and *Q. laevis* was very limited so seedlings of these species were sourced from local nurseries. Containerized (164 ml Ray Leach “Cone-tainer”) seedlings of *P. palustris* were sourced from the Florida Forest Service nursery. These seedlings were produced from seed collected throughout north and central Florida. Containerized (1 gallon) seedlings of *Q. laevis* were sourced from a local nursery in northeast Florida (Madison County). These seedlings were produced from seed collected from naturally regenerated mature trees in the same county. At planting, basal diameter (5 cm height) and stem length of *Q. laevis* seedlings averaged 6.3 ± 2.5 mm and 47.1 ± 7.2 cm, respectively, and did not differ between treatments ($P=0.93$, $P=0.91$).

Leaf-level physiology

In situ rates of leaf gas-exchange were measured monthly or semi-monthly between May 2019 and July 2020 to determine the effects of experimental warming on leaf physiology of each species. The number of measurement dates varied among species depending on the availability of mature, fully expanded green (non-senescent) leaves/needles (*A. glomeratus* and *P. palustris* = 11 measurement dates, *S. repens* = 12

measurement dates, *Quercus laevis* = 9 measurement dates). On each date, two portable cross-calibrated photosynthesis systems (LI-6800, LiCor., Inc.) were used to record steady-state measurements of light-saturated net photosynthesis (A_{sat} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance to water vapor (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) at a chamber reference $[\text{CO}_2]$ of $420 \mu\text{mol mol}^{-1}$. The ratio of A_{sat}/g_s was calculated as a measure of intrinsic water use efficiency (iWUE, $\mu\text{mol mol}^{-1}$). Leaves typically reached steady-state within 7-10 minutes of being enclosed in the cuvette. Photosynthesis systems were randomly assigned to temperature treatments on each measurement date. Light intensity within the leaf chamber was maintained at $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) using the red/blue LED light source. Flow rate varied between 500 and $600 \mu\text{mol s}^{-1}$. Water vapour inside the leaf chamber was not scrubbed so that RH inside the cuvette approximated ambient conditions. On each measurement date the LI-6800 temperature exchanger was set to the prevailing ambient temperatures inside the ambient and warmed treatment plots. Leaf temperature (T_{leaf}) was measured with the built-in leaf temperature thermocouple. Measurements occurred between 10:00 and 14:00 local time and were made on 1-3 recently mature, fully expanded, upper canopy leaves (*A. glomeratus*, *S. repens*, *Q. laevis*) or needle fascicles (*P. palustris*, three needles per fascicles) per plant. Leaves of *A. glomeratus* and needles of *P. palustris* were placed side by side (non-overlapping) within the cuvette. The one-sided surface area (cm^2) of leaves/needles within the chamber was determined by measuring the length and width of leaves/needles inside the chamber with a ruler. Leaf gas-exchange data were then back-corrected using the corrected leaf area estimate.

On the final date (6 July 2020) we measured the CO_2 response of leaf-level net photosynthesis ($A-C_i$) on similar leaves/needles of all plants. Light conditions within the cuvette were controlled at a PPFD of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ and all measurements were made at a common T_{leaf} of 33 ± 1.0 (standard deviation) $^{\circ}\text{C}$. This was done to separate warming effects on photosynthetic biochemistry from the effect of measurement temperature. Each $A-C_i$ curve began with a steady-state measurement of A_{sat} and g_s at a chamber reference $[\text{CO}_2]$ of $420 \mu\text{mol mol}^{-1}$. For the C_3 plant species (*S. repens*, *P. palustris*, *Q. laevis*), $A-C_i$ curves were constructed by measuring A_{sat} and C_i at a series of reference $[\text{CO}_2]$: 300, 250, 100, 50, 0, 420, 650, 800, 1200, and $1500 \mu\text{mol mol}^{-1}$. Each $A-C_i$ curve was parameterized using the Farquhar model of C_3 photosynthesis (Farquhar et al. 1980). The model estimates the maximum rate of Rubisco carboxylation (V_{cmax} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the rate of electron transport for RuBP regeneration (J_{max} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$). Importantly, we did not measure mesophyll conductance and rely on the simplifying assumption that C_i equals the $[\text{CO}_2]$ in the chloroplasts (as in Farquhar et al. 1980). Therefore, our estimates of V_{cmax} and J_{max} are ‘apparent’ rates that reflect both biochemical limitations of photosynthesis and mesophyll conductance (e.g., Salmon et al. 2020). The model was fit using nonlinear least squared parameter estimation in SAS v9.3 (PROC NLIN, SAS Institute Inc., 2010). For *A. glomeratus* (C_4 species), $A-C_i$ curves were constructed by measuring A_{sat} and C_i at the following series of reference $[\text{CO}_2]$: 300, 150, 50,

100, 200, 300, 340, 380, 500, 800, 1000, and 1200 $\mu\text{mol mol}^{-1}$. $A-C_i$ curves for *A. glomeratus* were parameterized using the equations of Von Caemmerer (2000), with modified code from Smith & Dukes (2017). We used nonlinear least squared parameter estimation in R version 3.2.1 (R-Development-Core-Team, 2013). The maximum rate of phosphoenolpyruvate carboxylase (PEPc) carboxylation (V_{pmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and V_{cmax} were estimated from the CO_2 -limited portion of each curve, and J_{max} was estimated from the light-limited portion of each curve. In *A. glomeratus*, V_{pmax} , V_{cmax} , and J_{max} were also ‘apparent’ rates since mesophyll conductance was not measured and bundle sheath conductance was held constant at $0.003 \text{ mol m}^{-2} \text{s}^{-1} \text{bar}^{-1}$ (Alonso-Cantabrana et al. 2018). If warming resulted in thermal acclimation of leaf photosynthetic capacity, we expected that V_{cmax} , J_{max} , and V_{pmax} (for *A. glomeratus*) measured at a common temperature would be lower in warm-grown plants of each species.

Because $A-C_i$ data were measured at relatively high temperatures and vegetation models often require estimates of photosynthetic parameters at 25°C , we also calculated estimates of V_{pmax} , V_{cmax} , and J_{max} at 25°C . For *A. glomeratus*, we estimated photosynthetic parameters at 25°C using a third-order polynomial function fit to the temperature response data for C_4 plants shown in Smith & Dukes (2017). For the C_3 species, we estimated photosynthetic parameters at 25°C using a peaked Arrhenius equation (see Medlyn et al. 2002) with assumed activation energies and entropy terms of 58.9 kJ mol^{-1} and $0.629 \text{ kJ mol}^{-1}$, respectively, for V_{cmax} and assumed activation energies and entropy terms of 29.7 kJ mol^{-1} and $0.632 \text{ kJ mol}^{-1}$, respectively, for J_{max} . Deactivation energies for V_{cmax} and J_{max} were held constant at 200 kJ mol^{-1} as in other studies (e.g., Vårhammar et al. 2015).

Biomass harvest

All plants of each species were harvested on 8 July 2020, roughly 17 months and two growing seasons after the start of the experiment. For each *A. glomeratus* plant, the entire aboveground portion was cut at 5 cm above the soil surface and transported to the lab in a paper bag. In the field, a 0.064 m^3 ($40 \times 40 \times 40 \text{ cm}$) volume of soil centered on the cluster of cut tillers was excavated and the root system was washed free of soil. A random subsample of ~ 20 leaves were collected from each plant and subsample fresh mass was determined. The area (cm^2) of the subsampled leaves was determined using a portable leaf area meter (LI-3000C, LiCor., Inc.), and the subsampled leaves were dried at 70°C for three days. The resolution of the portable leaf area meter is 1 mm^2 , which may lead to inaccurate estimates of leaf area, especially for narrow needles of *P. palustris*. However, we determined leaf area of plants in both treatments in the same way such that assessment of treatment effects are still valid. Leaf dry mass per unit area (LMA, g m^{-2}) was estimated by dividing subsample leaf dry mass by leaf area. Remaining leaf/tiller and root material were dried at 70°C for seven days. Leaf subsample dry mass was added to the remaining leaf/tiller dry mass to estimate total aboveground dry mass while total belowground dry mass was the sum of root dry

mass. Aboveground biomass was also harvested after the first growing season (25 November 2019) following the same protocol.

Serenoa repens and *P. palustris* required a different protocol since both species produce underground stems. For each plant of these species, the entire plant and soil volume (0.064 m^3) were excavated together and transported to the lab in a plastic bag. In the lab, root and stem biomass were separated, washed free of soil, and dried at 70°C for seven days to determine stem and root dry mass. Palm leaf lamina and pine needles were immediately separated from each stem and weighed to determine total leaf fresh mass. Palm petioles were dried and weighed separately. A small subsample of palm leaf lamina or pine needles (15-30 needles per seedling) were collected from each plant and subsample fresh mass was determined. Lamina/needle subsample area, subsample dry mass, and LMA were determined as described above. Total leaf lamina or needle dry mass was estimated by multiplying the subsample dry matter content (dry mass/fresh mass) by total leaf fresh mass. Total leaf area (m^2) was estimated by multiplying the subsample area to dry mass ratio by the estimate of leaf lamina or needle dry mass. Total leaf mass of *S. repens* was estimated by summing petiole dry mass, leaf lamina dry mass, and leaf lamina subsample dry mass. For consistency, total leaf and stem dry mass were summed to estimate aboveground dry mass while belowground dry mass was the sum of root dry mass.

For *Q. laevis*, the entire aboveground portion of each seedling was cut at the soil surface and transported to the lab where leaf and stem material were separated. In the field, a 0.064 m^3 volume of soil centered on the cut stem was excavated and the entire root system was washed free of soil. All *Q. laevis* seedlings produced a clearly visible lignotuber which was considered part of the belowground root system. Total leaf fresh mass was determined and a subsample of ~10 leaves (~20% of leaf fresh mass) were collected from each seedling. Leaf subsample area, subsample dry mass, LMA, total dry mass, and total leaf area were determined as described above. Stem and root material were dried at 70°C for seven days. Total leaf and stem dry mass were summed to estimate aboveground dry mass while belowground dry mass was the sum of root dry mass. For all species, allocation to different biomass pools, relative to total dry mass, was determined for each plant by calculating leaf mass fraction (LMF, g g^{-1}), stem mass fraction (SMF, main stem + branches, g g^{-1}), aboveground (leaf + tiller/stem) mass fraction (AGMF, g g^{-1}), and root mass fraction (RMF, g g^{-1}). Leaf area ratio (LAR, $\text{cm}^2 \text{ g}^{-1}$) was also calculated for *S. repens*, *P. palustris*, and *Q. laevis* as the ratio of total leaf area to total dry mass.

Statistical analysis

All statistical analyses were performed in SAS v9.3 (SAS Institute Inc., 2010). We separated species in all analyses due to considerable differences in plant size, morphology, growth pattern, and physiology (e.g. tree vs. grass/shrub, C_3 vs C_4). Mixed-effect models (PROC MIXED) were used to test the fixed effects of

time (measurement date), temperature treatment (ambient, warmed) and their interaction on A_{sat} , g_s , and iWUE. Because leaf gas-exchange was measured repeatedly over time on the same plants, we fit a random intercept term for the effect of plant within temperature treatment. When temperature treatment or date \times temperature treatment interactions were statistically significant ($P < 0.05$) or marginally significant ($P < 0.10$) we carried out pairwise comparison of treatment means, overall or at individual measurement dates. Mixed-effect models were also used to test the fixed effects of temperature treatment on photosynthetic parameters (V_{pmax} , V_{cmax} , J_{max} at 33 °C), and seedling biomass production and allocation (e.g. SMF, RMF, LAR), with block considered a random effect.

For each species, analysis of covariance was used to determine whether temperature treatment effects on leaf physiology (A_{sat} , g_s , and iWUE) were dependent upon soil moisture. In this model, temperature treatment was treated as a factor and VWC a covariate (continuous variable). A significant ($P < 0.05$) or marginally significant ($P < 0.10$) interaction between treatment and VWC indicated that temperature treatment affected the relationship between leaf physiology and VWC, resulting in an equation with different slope parameters for each treatment. If treatment and VWC were both significant ($P > 0.10$) equations with different intercepts for each treatment, but a common slope, were fit to the data. If only VWC was significant, one equation describing the relationship between VWC and leaf physiology was fit to data from both treatments. The same approach was used to test whether the long-term temperature response of A_{sat} differed between temperature treatments.

Results

Leaf physiology

Over time and across species and treatments, *in situ* leaf gas-exchange measurements occurred at leaf temperatures (T_{leaf}) between 18 and 37°C (Figure 2). Across all data, soil VWC tended to be low when T_{leaf} was high (Figure S1). On average, T_{leaf} during leaf-gas exchange measurements was 2.7, 2.6, 3.1, and 2.4°C higher in warmed than control treatment plants of *A. glomeratus*, *S. repens*, *P. palustris*, and *Q. laevis*, respectively (Figure 2).

In *A. glomeratus*, warming effects on *in situ* rates of A_{sat} , g_s , and iWUE measured at prevailing T_{leaf} varied over time (date \times treatment interaction, Table 1). Post-hoc comparisons indicated that warming increased A_{sat} in July (+30%) and November (+130%) 2019, but reduced A_{sat} in March 2020 (-47%) (Figure 2e). Warming also increased g_s in October 2019 (+37%) and February 2020 (+51%) but reduced g_s in March 2020 (-19%). Warming reduced iWUE in October 2019 (-39%) and March 2020 (-37%) but increased iWUE in November 2019 (+138%). Aggregated over time, warming resulted in small (non-significant) increases in A_{sat} , g_s , and iWUE in *A. glomeratus* (Table 1, Figure 2).

In *S. repens*, A_{sat} , g_s , and iWUE varied over time and warming resulted in small but non-significant increases in A_{sat} (+15%) and g_s (+25%, Table 1, Figure 2). Warming resulted in a marginally significant reduction in iWUE in *S. repens* (-14%, Table 1, Figure 2n).

Averaged over time, warming caused a significant reduction in A_{sat} and g_s in *P. palustris* (-15% and -28%, respectively, Figure 2). A marginally significant date \times warming interaction occurred for A_{sat} (Table 1), caused by a negative effect of warming during August and September 2019 (-47%), when prevailing temperatures were high, and a positive effect of warming in January 2020 (+39%) when prevailing temperatures were low. iWUE varied over time but was not affected by warming (Table 1, Figure 2o).

In *Q. laevis*, A_{sat} , g_s , and iWUE varied over time and warming caused slight (non-significant) increases in A_{sat} and g_s (Table 1, Figure 2). Warming effects on iWUE varied over time (date \times treatment interaction, Table 1). Post-hoc comparisons showed that warming increased iWUE in November 2019 (+129%) but had little effect otherwise (Figure 2p). Overall, these results provide general support for the expectation that warming would have negligible or positive effects on photosynthesis in monocot species adapted to warmer temperatures. However, we found mixed support for the expectation that warming would reduce photosynthesis in tree species with more temperate distributions given that *P. palustris* showed reduced photosynthesis with warming but *Q. laevis* did not.

In general, warming had little effect on photosynthetic biochemistry (Table 1, Figure 3). In *A. glomeratus*, estimates of V_{pmax} , V_{cmax} , and J_{max} (measured at 33°C) were similar in both temperature treatments (Figure 3) and averaged 332.1 ± 21.5 , 21.2 ± 1.4 , and $136.5 \pm 9.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In *Q. laevis*, estimates of V_{cmax} and J_{max} were also similar between ambient and warmed treatments (Figure 3), and averaged $114.9 \pm 8.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $115.0 \pm 7.6 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In *S. repens* and *P. palustris*, biochemical responses of photosynthesis to warming tended to mirror average responses of A_{sat} to warming. *Serenoa repens* showed small increases in V_{cmax} and J_{max} with warming (+32%, +38%) while *P. palustris* showed small reductions in V_{cmax} and J_{max} with warming (-15%, -19%). Nonetheless, warming effects on photosynthetic parameters were not significant in any species. The average $J_{\text{max}}/V_{\text{cmax}}$ ratio (at 33°C) in *A. glomeratus* was 6.45 ± 0.2 ; substantially higher than $J_{\text{max}}/V_{\text{cmax}}$ in the C_3 species in this study (Figure 3). This is expected given that C_4 species are more commonly light-limited than CO_2 -limited at current atmospheric CO_2 and tend to invest less nitrogen in Rubisco than C_3 species (Sage & Kubien 2007). Warming had no effect on $J_{\text{max}}/V_{\text{cmax}}$ in any species (Table 1, Figure 3). Analysis of photosynthetic parameters corrected to 25 °C yielded similar results (Table S1).

In contrast to our expectation, we found that increasing or decreasing soil moisture did not modify A_{sat} and g_s responses to warming in any species (Table 2, Figure 3). In other words, the slope parameter describing the increase in A_{sat} and g_s with VWC did not differ between temperature treatments

for any species. We note that A_{sat} and g_s showed no relationship with VWC in *A. glomeratus*, reflecting high tolerance of reduced soil moisture (Figure 3). Relationships between A_{sat} and VWC and g_s and VWC also appeared to be similar across all C_3 species (Figure 3). Indeed, additional ANCOVA tests revealed that the slope of the A_{sat} vs VWC and g_s vs VWC relationships did not differ between C_3 species ($P=0.94$, $P=0.92$, Figure 2). Across C_3 species, the relationship between individual measures of A_{sat} and VWC could be described by the linear equation $y = 4.465 + 61.2\text{VWC}$ ($r^2=0.12$, $P<0.0001$). The relationship between individual measures of g_s and VWC could be described by the linear equation $y = 0.0379 + 1.13\text{VWC}$ ($r^2=0.15$, $P<0.0001$). In *A. glomeratus* and *Q. laevis*, iWUE increased as VWC declined, and did so consistently across temperature treatments (Table 2, Figure 3). In *S. repens*, warming did not affect the slope of the iWUE vs VWC relationship but reduced the intercept parameter, indicating lower iWUE at a given VWC (Table 2, Figure 3). iWUE was not associated with VWC in *P. palustris*.

For each species, we also examined the long-term (realized) temperature response of A_{sat} under ambient and warmed temperatures. A second order polynomial equation provided an adequate fit to the temperature response for all species and treatments. In all species, the intercept, slope, and quadratic term describing the relationship between A_{sat} and T_{leaf} were similar between treatments (all $P>0.10$). Thus, a single equation was used to describe the long-term temperature response of each species (Figure 5). From the fitted equations, the estimated temperature optima (T_{opt}) of A_{sat} was highest in *A. glomeratus* (36.5°C) and lowest in *P. palustris* (23.1°C, Figure 5). The long-term estimated T_{opt} of A_{sat} was 31.6°C in *Q. laevis* (Figure 5). The estimated T_{opt} of A_{sat} in *S. repens* was 26.5°C, but this estimate should be interpreted cautiously given that A_{sat} showed a very broad temperature response with high rates of A_{sat} above the apparent T_{opt} (Figure 5).

Biomass production and allocation

Warming resulted in small increases in total biomass production in *A. glomeratus* (+22%), *S. repens* (+7%), and *Q. laevis* (+11%), but small decreases in total biomass production in *P. palustris* (-14%). This pattern was similar for leaf, stem, and root biomass, as well as total leaf area. In no cases were the effects of warming on biomass production or leaf area significant (Table 3). In general, warming had no significant effect on biomass allocation in any species. However, for *Q. laevis*, warming resulted in marginally significant increases in LAR (+42%) and LMF (+38%), indicating a shift in allocation to leaves relative to stems and roots. For *S. repens*, warming resulted in a marginally significant increase in LMA (+15%).

Discussion

We carried out a warming experiment in a subtropical forest in northeast Florida and assessed the impacts of warming on the physiology and productivity of dominant species with different climatic distributions, growth forms, and functional strategies. We expected that warming would have neutral or positive effects on photosynthesis in species with warmer climatic distributions or physiological adaptations to warmer temperatures (*A. glomeratus*, *S. repens*), but negative effects on photosynthesis in tree species with more temperate distributions (*P. palustris*, *Q. laevis*). Our expectations were partially supported. Aggregated over time, the effects of warming on A_{sat} (and g_s) were not significant but slightly positive in *A. glomeratus* and *S. repens*. Moreover, averaged over time, warming caused a significant reduction in A_{sat} and g_s in *P. palustris*. However, in contrast to our expectation, warming caused a small (non-significant) increase in A_{sat} in *Q. laevis*. We also expected that low soil moisture would negate or reverse the positive effects of warming on photosynthesis in warm-adapted species and exacerbate the negative effects of warming on photosynthesis in tree species. In contrast, we found that *A. glomeratus* showed little sensitivity to reduced VWC, and all three C_3 species reduced A_{sat} and g_s as VWC declined and did so consistently across treatments. We conclude that *P. palustris* seedlings growing in canopy gaps may be vulnerable to climate warming, but warming might have neutral, or slightly positive, impacts on photosynthesis and productivity of dominant monocot species (*A. glomeratus* and *S. repens*) as well as *Q. laevis*.

Species responses to warming

Species' photosynthetic responses to warming should be viewed in relation to (1) the temperature response of photosynthesis, (2) changes in photosynthetic capacity in response to warming, and (3) biomass responses to warming. Combined, these responses provide insight into potential mechanisms and consequences of species photosynthetic responses warming. In *A. glomeratus*, the average T_{leaf} under ambient conditions was below the estimated long-term T_{opt} of A_{sat} (36.5°C), such that warming caused a slight increase in average A_{sat} aggregated over time. The high T_{opt} of A_{sat} in *A. glomeratus* is expected of C_4 plants which are well-adapted to higher temperatures, and often occur in warmer habitats (Sage & Kubien 2007, Yamori et al. 2014). We note that A_{sat} showed a significant date \times treatment interaction in *A. glomeratus* driven by positive effects of warming on A_{sat} in mid-summer and autumn, and negative effects in early spring. Importantly, we observed no warming effects on estimated rates of V_{pmax} , V_{cmax} , or J_{max} . Thus, little adjustment in photosynthetic capacity occurred in *A. glomeratus*, which contributed to little change in A_{sat} with warming. It has been hypothesized that C_4 species are less capable of thermal acclimation than C_3 species (Yamori et al. 2014). However, C_4 plants sometimes show equivalent acclimation responses to warming (Yamori et al. 2014, Sturchio et al. 2021). Our results are similar to those of Dwyer et al. (2007) who found slight positive effects of warming on photosynthetic rates of three

C₄ species. However, the slight increase in A_{sat} with warming we observed in *A. glomeratus* was less than the average increase in A_{sat} with warming averaged across a broader range of C₄ species (Liang et al. 2013). We also found that warming caused a small increase in total biomass production; although biomass production in both treatments was much lower in the second year which could be a legacy of biomass clipping following the first year. Other studies in a related species (*A. gerardii*) also observed slightly positive effects of experimental warming on biomass production (e.g., Sherry et al. 2008).

The physiology of *S. repens* has not been widely studied, despite being common and ecologically important in the southeastern U.S. We expected this woody palm species would respond positively to warming given its occurrence in the warmest habitats in the southeastern U.S., including tropical south Florida. This species showed a very broad photosynthetic temperature response with relatively high rates of A_{sat} above the estimated T_{opt} . Aggregated over time, warming had no significant affect on A_{sat} and g_s although rates increased modestly under warming. We also observed small increases in V_{cmax} and J_{max} (at common measurement temperature) with warming which could represent a ‘constructive adjustment’ of photosynthetic capacity that contributed to small increases in A_{sat} under warming (Way & Yamori 2014). Interestingly, we found that whole-plant LMA measured at the end of the experiment was higher in warmed *S. repens* seedlings than ambient seedlings, indicating increased leaf thickness or density with warming. In contrast, most studies find reductions in LMA with increasing temperature (Poorter et al., 2009). Increased LMA with warming could indicate greater investment in structural components than enzymatic components (Poorter & Villar 1997, Mediavilla et al. 2008). However, there is evidence that leaf N per unit area (N_a) increases with leaf thickness and density (e.g., Niinemets 1999), and V_{cmax} generally increases with increasing N_a (Wilson et al. 2000, Walker et al. 2014). Although we did not measure leaf N concentrations and were unable to examine relationships between LMA and N_a , increased LMA with warming could partly explain increased V_{cmax} . Despite slight increases in photosynthetic capacity and A_{sat} with warming, we found no change in biomass production with warming in *S. repens*. More replicates may be needed in future studies to determine the robustness of species productivity responses to warming. Even so, we conclude that warming might have neutral to slightly positive effects on *S. repens* in north Florida. This conclusion fits with the prediction that *S. repens* habitat will increase slightly with warming (Butler & Larson 2020).

Aggregated over time, we expected that warming would reduce A_{sat} in *P. palustris* and *Q. laevis* given that north Florida is near the southern range limit of both species. Previous studies have also found negative effects of climate warming on photosynthesis and performance of southeastern *Quercus* species at their southern range limit (Wertin et al. 2011). As expected, warming caused a significant reduction in A_{sat} in *P. palustris*. The negative effect of warming on A_{sat} can be partly explained by reduced g_s (increased stomatal limitation), and the observation that the average T_{leaf} under ambient conditions was

above the long-term T_{opt} of A_{sat} (23.1°C), such that warming caused a further reduction in A_{sat} . This T_{opt} estimate is lower than the T_{opt} of photosynthesis in studies with mature *P. palustris* and *Pinus elliotti* (a related sympatric species) at similar sites (Teskey et al. 1994, Powell et al. 2008). There may be differences in the temperature response of A_{sat} between *P. palustris* seedlings and mature trees. Seedling root systems are smaller and more shallow than mature tree root systems, which could limit access to water on xeric sites and increase stomatal limitation of A_{sat} with increasing temperature (Lin et al. 2012), although stomatal limitation of A_{sat} is generally higher in large trees than small trees (Drake et al. 2010). Also, foliar respiration tends to be higher in seedlings than mature trees (Ryan et al. 1994), and may represent an increasing proportion of gross photosynthesis with increasing temperature (Way & Sage 2008), which could result in a lower T_{opt} of A_{sat} in seedlings than mature trees. Importantly, we also observed reduced V_{cmax} and J_{max} with warming. Thus, reduced A_{sat} with warming in *P. palustris* appears to be related to stomatal and biochemical limitations. While the reduction in V_{cmax} and J_{max} is suggestive of thermal acclimation, it may represent a ‘detractive adjustment’ (Way & Yamori 2014). This might also partly explain why *P. palustris* seedlings tended to be smaller under warmer conditions.

Our results could have important implications for regeneration of *P. palustris*; a foundational tree species in the southeastern U.S. (Van Lear et al. 2005). It is well-established that *P. palustris* seedlings are generally intolerant of shade (Boyer 1979), yet there is also evidence that seedlings growing in open conditions may be more vulnerable to hot, dry conditions. Knapp et al. (2008) showed that *P. palustris* seedling mortality increased in open conditions as soil temperature increased and soil moisture decreased. Moreover, at xeric sites, Loudermilk et al. (2016) found that *P. palustris* seedling survival decreased in canopy gaps, and a moderate density of *Q. laevis* in the mid-story had a facilitative effect on seedling survival and performance. In this way, a healthy and functional cohort of *Q. laevis* may improve *P. palustris* performance and survival (Johnson et al. 2021). Nonetheless, the growth habit of *P. palustris* may partly explain its vulnerability to warmer temperatures at the seedling stage. The species exists in the ‘grass-stage’ for several years, where the stem remains underground, and a thick arrangement of needles protect the apical bud from fire. However, in the grass-stage, *P. palustris* foliage is often in direct or close contact to soil where temperatures can exceed 50°C (Loudermilk et al. 2016). Extreme temperatures could inhibit or permanently damage photosynthetic machinery, increase respiratory costs, and deplete carbon reserves (Aspinwall et al. 2019). Our results suggest a potential narrowing of optimal conditions for *P. palustris* regeneration with climate warming, particularly at xeric sites.

Although warming had no significant effect on A_{sat} in *Q. laevis*, A_{sat} was slightly higher under warming, aggregated over time. The slight increase in A_{sat} could be partly attributed to the observation that the average T_{leaf} under ambient conditions was below the long-term T_{opt} of A_{sat} . The T_{opt} of A_{sat} in *Q. laevis* is within the range of T_{opt} observed in other warm-temperate oak species in the southeastern U.S.

(Gunderson et al. 2010). Although A_{sat} increased slightly with warming, we found no change in V_{cmax} and J_{max} with warming. These results differ slightly from studies across species that have observed reductions in V_{cmax} , J_{max} , or both parameters (measured at a common temperature) with increasing growth temperature (Kattge & Knorr 2007, Aspinwall et al. 2016, Kumarathunge et al. 2019). However, our results are similar to studies that have found no reduction in V_{cmax} and J_{max} with increasing growth temperature, coupled with relative stability of A_{sat} across different growth temperatures (Ow et al. 2010, Slot & Winter 2017, Stefanski et al. 2020). We also found that warming caused a slight increase in total biomass production, mostly driven by increased leaf production. We emphasize that warming of a larger magnitude may induce different responses in each species. Studies that examine species responses to multiple temperature treatments are required to understand potential threshold responses for species performance.

We acknowledge some unavoidable limitations and uncertainties in our study. Passive warming chambers cause small reductions in light intensity and reduce maximum wind speed, and do not cause warming at night. Lower light intensity might limit daily whole-plant C uptake and plant growth. However, *P. palustris* was the only species to show lower growth with warming. We argue that small reductions in light intensity were unlikely to cause reduced growth in *P. palustris* given that previous studies have shown that larger reductions in light are required to reduce *P. palustris* seedling growth (Palik et al. 1997, McGuire et al. 2001, Jose et al. 2003). Lower wind speeds in the chambers could also intensify warming effects by reducing air flow and convective heat transfer. We added openings and slits to the top of the chambers to mitigate this effect. Yet, lower wind speeds are occurring alongside climate warming (McVicar et al. 2012, Zhang et al. 2021), such that passive warming chambers could simulate both aspects of climate change. Previous studies have shown that daytime and nighttime warming treatments can have different impacts on plant function and growth. In *Populus deltoides*, Turnbull et al. (2002) found that daytime warming alone had little effect on leaf respiration or A_{sat} . However, combined night and daytime warming increased leaf respiration and A_{sat} . Increased A_{sat} was caused by greater consumption of carbohydrates by respiration during warmer nights, which stimulated higher demand for carbohydrates and higher photosynthetic capacity. The impacts of nighttime warming on plant growth remain unclear. Theoretically, nighttime warming could increase maintenance costs and reduce growth, yet some studies show increased growth with nighttime warming despite increased respiration (Cheesman & Winter 2013; Krause et al. 2013). Finally, our estimates of warming impacts on apparent rates of photosynthetic capacity could be improved with measures of mesophyll conductance, which could vary among species (e.g., Niinemets et al. 2009).

Climate warming – soil moisture interaction

Soil moisture has been hypothesized to be an important modifier of plant responses to warming. In cool climates, warming effects on A_{sat} might remain positive until water limitations increase stomatal limitation of A_{sat} . Although there are exceptions (Moyes et al. 2013), previous studies have generally found support for this hypothesis (D'Orangeville et al. 2018; Lazarus et al. 2018; Reich et al. 2018). We tested this hypothesis in a subtropical forest where temperature limitation of A_{sat} is probably less-common and found no interaction between soil moisture and temperature treatment. There are a few possible explanations for this result. First, warming effects on leaf gas-exchange were generally small and may not have been strong enough to drive synergistic interactions with soil moisture, which may be the dominant constraint on plant function at our xeric site. Indeed, all three C_3 species reduced A_{sat} and g_s as VWC declined and did so consistently across treatments. Second, unlike previous warming studies that have shown reduced soil moisture under warming (e.g., Reich et al. 2018, Liu et al. 2021), warming did not reduce VWC such that plants in both treatments operated at similar VWC on any given day. This may have contributed to convergent responses to VWC across treatments. Third, high temperature, high VPD, and low VWC conditions tended to co-occur naturally over seasons, which is typical for northern Florida (Figure 1, Figure S1). In much of Florida, maximum daily T_{air} and VPD increase sharply at the end of the dry season (May) when soil moisture is generally low. This means that ambient and warmed plants experienced the driest conditions when prevailing T_{air} and VPD are near their peak. We conclude that soil moisture and warming are unlikely to have synergistic effects in xeric forests in the southeastern U.S. However, additional warming studies across different sites (upland, lowland), microhabitats, or soil moisture treatments would help resolve the potential interactive effects of warming and water availability on plant performance over space and time.

Experimental warming studies have revealed that species adaptation, functional type, and prevailing abiotic and biotic conditions may all be important determinants of warming impacts on plant performance and community and ecosystem properties (Volder et al. 2013, Reich et al. 2018, Dusenge et al., 2020). In a subtropical forest in northeast Florida, we found that warming had neutral, or slightly positive effects on seedling photosynthesis and productivity in warm-adapted monocot species and a sympatric oak species. However, warming negatively affected photosynthesis in *P. palustris* seedlings. Soil moisture did not interact with temperature treatments. Our results highlight the importance of species functional type and biogeography in influencing species physiological responses to warming, especially in the southeastern U.S. Species differences in warming responses observed here could foreshadow changes in community and ecosystem properties. Nonetheless, future experiments should focus on species responses to warming across a broader range of abiotic and biotic conditions, as well as warming impacts on species interactions.

Author contributions

MJA and LD conceived and designed the experiment. MJA, JC, EG, M G-B, and LD collected data. MJA led the data analysis and wrote the manuscript with input from all authors.

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Data availability

Data are freely available here: Aspinwall, Michael (2021): Sawmill Slough Raw data.zip. figshare. Dataset. <https://doi.org/10.6084/m9.figshare.15157707.v1>

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Figure legends

Figure 1. (a) Temporal variation in mean daytime air temperature (T_{air}), (c) mean daytime relative humidity (RH), (e) mean daytime vapor pressure deficit (VPD), and (g) mean volumetric soil water content (VWC). Ambient (blue) and warmed (red) treatment data are shown separately in each panel. Panels (b-h) show the overall distribution (percentage) of T_{air} , RH, VPD, and VWC data in each treatment, as well as the mean and median values.

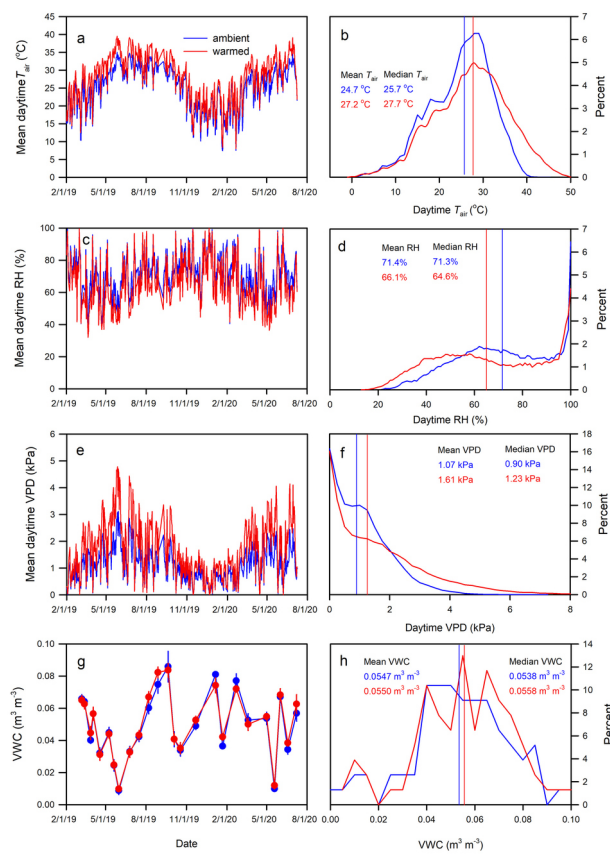
Figure 2. Mean (\pm standard error, $n=6$) values for *in situ* (instantaneous) leaf temperature (T_{leaf}), light-saturated net photosynthesis (A_{sat}), stomatal conductance to water vapor (g_s), and intrinsic water-use efficiency ($iWUE = A_{\text{sat}}/g_s$) over time in four species grown under ambient conditions and experimental warming in a subtropical forest in northeast Florida. *Andropogon glomeratus* is a perennial C_4 grass (monocot). *Serenoa repens* is a small woody palm (monocot) species. *Pinus palustris* is a coniferous tree. *Quercus laevis* is a broadleaved deciduous tree species. When gaps exist in the data, leaves were brown or senesced. Inset Figures show the overall mean (aggregated) values for T_{leaf} , A_{sat} , g_s , and $iWUE$ under ambient and warmed conditions for each species.

Figure 3. Mean (\pm standard error, $n=6$) values for biochemical parameters of photosynthesis in four species grown under ambient conditions (blue bars) and experimental warming (red bars) in a subtropical forest in northeast Florida. V_{pmax} is the maximum rate of phosphoenolpyruvate carboxylase (PEPc) carboxylation in C_4 plants (*Andropogon glomeratus* only). V_{cmax} is the maximum rate of Rubisco carboxylation. J_{max} is the maximum rate of electron transport for RuBP regeneration. All parameters were estimated at a common measurement temperature (leaf temperature = 33 ± 1.0 (standard deviation) $^{\circ}\text{C}$) in the field. For each species, the relative change in each parameter caused by warming is shown above warmed treatment mean.

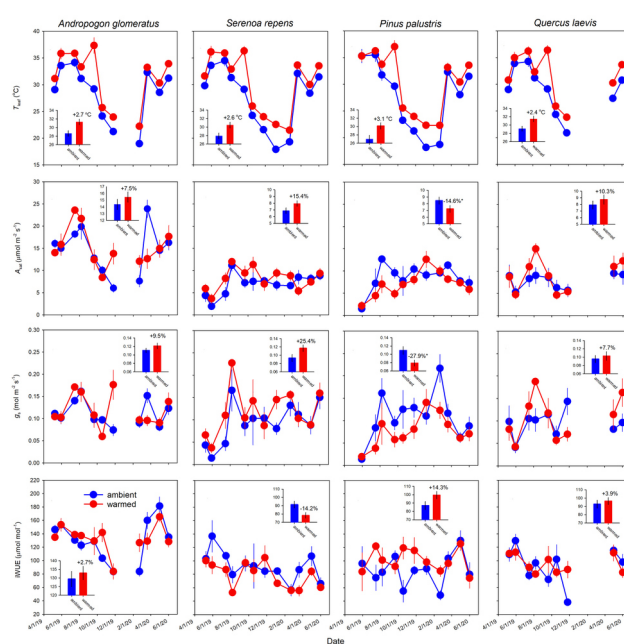
Figure 4. The relationship between volumetric soil water content (VWC) and instantaneous measures of leaf net photosynthesis (A_{sat}), stomatal conductance to water vapor (g_s), and intrinsic water-use efficiency ($iWUE = A_{\text{sat}}/g_s$) in four species grown under ambient conditions (blue symbols) and experimental warming (red symbols) in a subtropical forest in northeast Florida. When experimental warming altered the relationship (intercept, slope) between VWC and gas-exchange variables, separate lines (blue, red) were fit to the data for ambient and warmed treatments. When experimental warming did not influence the relationship between VWC and leaf gas-exchange, we fit a single equation (one line) across data for both treatments. Intercept and slope estimates are provided in each Figure panel when the relationship between VWC and gas-exchange parameters were significant ($P < 0.10$). Where appropriate, model coefficients of determination (r^2) are provided.

Figure 5. The realized long-term relationship between leaf-level light saturated net photosynthesis (A_{sat}) and leaf temperature (T_{leaf}) in four species grown under ambient conditions (blue symbols) and experimental warming (red symbols) in a subtropical forest in northeast Florida. Second order polynomial equations were used to describe the relationship between A_{sat} and T_{leaf} in each species. In all cases, the

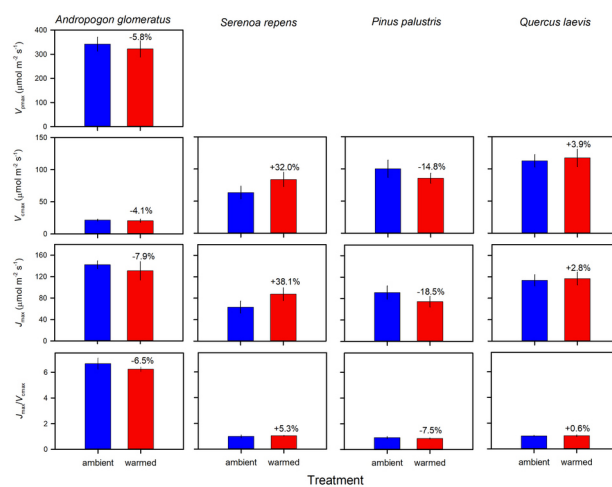
relationship between A_{sat} and T_{leaf} was consistent between treatments. Model coefficients of determination (r^2) and significance (p -values) are provided.



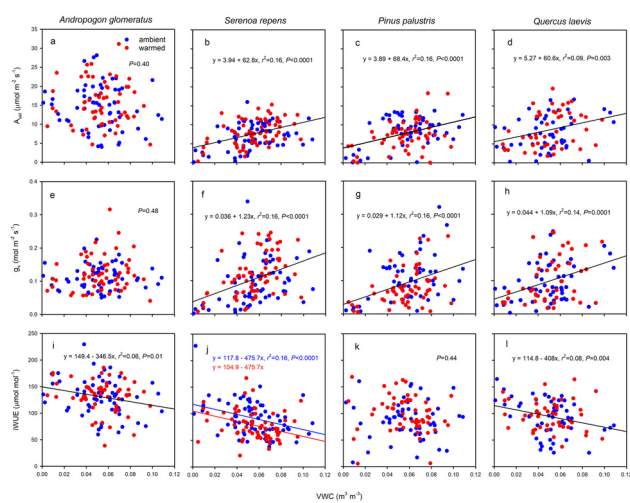
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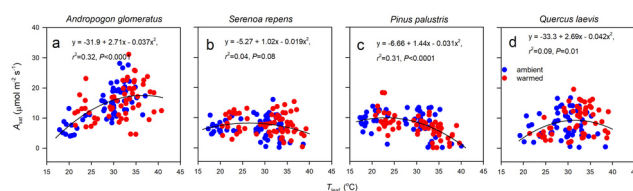
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PPL_13654_Figure 5.JPG

Table 1. Analysis of variance of measurement date, temperature treatment, and date \times temperature treatment effects on leaf-level physiological variables in four species growing in a subtropical forest. Numerator and denominator degree of freedom (df) and F -values are presented for each trait and experimental factor. F -values denoted with “***”, “**”, “*”, and “†” are significant at $P < 0.001$, $P < 0.01$, $P < 0.05$, and $P < 0.1$ respectively.

Species	Variable	Date		Treatment		Date \times treatment	
		df	F	df	F	df	F
<i>Andropogon glomeratus</i>	A_{sat}	10,85	14.0***	1,10	0.2	10,85	4.6***
	g_s	10,85	6.9***	1,10	0.3	10,85	3.9***
	iWUE	10,85	14.5***	1,10	0.5	10,85	2.4*
	V_{pmax}			1,10	0.2		
	V_{cmax}			1,10	0.1		
	J_{max}			1,10	0.4		
	$J_{\text{max}}/V_{\text{cmax}}$			1,10	0.8		
<i>Serenoa repens</i>	A_{sat}	11,96	8.0***	1,10	2.1	11,96	1.6
	g_s	11,96	12.2***	1,10	3.3	11,96	1.2
	iWUE	11,96	7.1***	1,10	4.5†	11,96	1.5
	V_{cmax}			1,10	1.9		
	J_{max}			1,10	2.1		
	$J_{\text{max}}/V_{\text{cmax}}$			1,10	0.2		
<i>Pinus palustris</i>	A_{sat}	10,76	7.4***	1,10	6.5*	10,76	1.9†
	g_s	10,76	9.2***	1,10	8.4*	10,76	1.2
	iWUE	10,76	2.4*	1,10	2.9	10,76	1.4
	V_{cmax}			1,10	0.9		
	J_{max}			1,10	1.1		
	$J_{\text{max}}/V_{\text{cmax}}$			1,10	0.6		
<i>Quercus laevis</i>	A_{sat}	8,69	4.0**	1,10	1	8,69	0.6
	g_s	8,69	3.3**	1,10	0.3	8,69	1.3
	iWUE	8,69	6.2***	1,10	0.4	8,69	2.7*
	V_{cmax}			1,10	0.1		
	J_{max}			1,10	0.1		
	$J_{\text{max}}/V_{\text{cmax}}$			1,10	0.1		

Variable descriptions: A_{sat} , light-saturated net photosynthetic rate; g_s , stomatal conductance to water vapor; iWUE, intrinsic water use efficiency (A_{sat}/g_s). For all species, g_s and iWUE data were square-root transformed to fulfil assumptions of normality.

Table 2. Results of analysis of covariance (ANCOVA) testing whether the relationship between volumetric soil water content (VWC) and leaf gas-exchange variables is dependent upon temperature treatment (ambient, warmed). For each relationship (species, variable), the number of observations (n), model F -values, and associated probability (p) values are provided.

Species	Variable	n	VWC		Treatment		VWC \times treatment	
			F	$P > F$	F	$P > F$	F	$P > F$
<i>Andropogon glomeratus</i>	A_{sat}	117	0.60	0.44	0.02	0.89	0.32	0.57
	g_s	117	0.63	0.43	0.09	0.76	0.70	0.40
	iWUE	117	6.6	0.01	0.01	0.96	0.04	0.84
<i>Serenoa repens</i>	A_{sat}	130	26.0	<0.0001	0.01	0.94	0.70	0.41
	g_s	130	25.9	<0.0001	0.03	0.86	1.07	0.30
	iWUE	130	15.7	0.0001	4.0	0.05	1.35	0.25
<i>Pinus palustris</i>	A_{sat}	108	20.1	<0.0001	0.83	0.37	0.12	0.73
	g_s	108	20.3	<0.0001	0.81	0.37	0.01	0.97
	iWUE	108	0.56	0.45	1.84	0.18	0.55	0.46
<i>Quercus laevis</i>	A_{sat}	97	9.5	<0.01	0.07	0.79	0.44	0.51
	g_s	97	15.6	0.0002	0.07	0.79	0.27	0.61
	iWUE	97	7.7	0.007	1.00	0.32	1.92	0.17

Variable descriptions: A_{sat} , light-saturated net photosynthetic rate; g_s , stomatal conductance to water vapor; iWUE, intrinsic water use efficiency (A_{sat}/g_s).

Table 3. Mean values (\pm standard error, $n = 6$) for biomass production and allocation in four plant species grown under ambient conditions and experimental warming in a subtropical forest in northeast Florida. [†]Indicates that mean values of biomass traits differ between ambient and warmed treatments at $P < 0.1$.

Variable	<i>Andropogon glomeratus</i>		<i>Serenoa repens</i>		<i>Pinus palustris</i>		<i>Quercus laevis</i>	
	ambient	warmed	ambient	warmed	ambient	warmed	ambient	warmed
Leaf dry mass (g)	---	---	10.2 \pm 1.9	12.0 \pm 2.7	7.3 \pm 2.1	6.8 \pm 2.3	6.8 \pm 1.5	10.5 \pm 2.8
Stem dry mass (g)	---	---	32.1 \pm 6.5	34.8 \pm 8.7	3.0 \pm 0.6	2.2 \pm 0.7	11.2 \pm 1.3	13.3 \pm 3.0
AG dry mass (g)	19.4 \pm 3.5	21.2 \pm 7.6	42.3 \pm 8.0	46.8 \pm 8.9	10.3 \pm 2.6	9.0 \pm 2.8	18.0 \pm 2.4	23.8 \pm 4.7
*AG dry mass (g)	62.6 \pm 7.3	75.7 \pm 15.4	---	---	---	---	---	---
Root dry mass (g)	4.21 \pm 0.35	7.61 \pm 3.12	11.7 \pm 1.2	10.8 \pm 0.5	7.6 \pm 1.9	6.4 \pm 2.4	62.6 \pm 11.2	65.6 \pm 12.6
Total dry mass (g)	23.6 \pm 3.5	28.8 \pm 10.6	54.0 \pm 8.8	57.6 \pm 9.2	17.9 \pm 4.5	15.4 \pm 5.1	80.6 \pm 13.4	89.4 \pm 17.0
Total leaf area (cm ²)	---	---	591 \pm 103	613 \pm 131	481 \pm 126	451 \pm 159	653 \pm 137	1044 \pm 291
LAR (cm ² g ⁻¹)	---	---	11.0 \pm 1.1	10.8 \pm 2.4	26.2 \pm 2.0	28.4 \pm 1.0	7.7 \pm 0.9	10.9 \pm 1.4 [†]
LMA (g m ⁻²)	116.0 \pm 7.7	115.3 \pm 10.0	141.3 \pm 5.2	161.8 \pm 9.3 [†]	147.5 \pm 8.0	154.0 \pm 10.5	103.2 \pm 2.5	103.3 \pm 3.4
LMF (g g ⁻¹)	---	---	0.19 \pm 0.02	0.21 \pm 0.05	0.39 \pm 0.04	0.43 \pm 0.02	0.08 \pm 0.01	0.11 \pm 0.01 [†]
SMF (g g ⁻¹)	---	---	0.59 \pm 0.03	0.58 \pm 0.05	0.18 \pm 0.03	0.17 \pm 0.03	0.16 \pm 0.04	0.15 \pm 0.03
AGMF (g g ⁻¹)	0.79 \pm 0.05	0.76 \pm 0.02	0.77 \pm 0.02	0.79 \pm 0.03	0.57 \pm 0.02	0.60 \pm 0.04	0.24 \pm 0.03	0.26 \pm 0.02
RMF (g g ⁻¹)	0.21 \pm 0.05	0.24 \pm 0.02	0.23 \pm 0.02	0.21 \pm 0.03	0.43 \pm 0.02	0.40 \pm 0.04	0.76 \pm 0.03	0.74 \pm 0.02

Variable descriptions: AG dry mass, aboveground dry mass (leaf + stem/tiller dry mass); LAR, leaf area ratio (LA/Total DM); LMA, leaf dry mass per unit area (leaf dry mass/total leaf area); LMF, leaf mass fraction (Leaf DM/Total DM); SMF, stem mass fraction (Stem DM + Branch DM) / Total DM; RMF, root mass fraction (Root DM/Total DM). *Aboveground biomass measured in November 2019. All other variables were measured at the end of the experiment (July 2020).