Effects of warming and elevated CO_2 on stomatal conductance and chlorophyll fluorescence of C_3 and C_4 coastal wetland species

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Understanding Plant Physiological Responses of Coastal Wetland Species to Climate Change

Coastal wetland communities provide valuable ecosystem services such as erosion prevention, soil accretion, carbon sequestration, and essential habitat for coastal wildlife, but are some of the most vulnerable to the threats of climate change.

Experiments investigating the impact of elevated CO_2 (eCO_2) have shown enhanced photosynthetic rates, reductions in stomatal conductance, and increased water use efficiency in a variety of species, which generally leads to an increase in plant productivity. In cold climates limited by growing season temperatures, experimental warming of air and soil can positively affect gas-exchange rates and plant productivity due to enhanced metabolic rates early in spring and an overall increase in the length of the growing season. However, during droughts or portions of the growing season when low-salinity soil water is limiting due to low precipitation and/or high rates of evapotranspiration, any positive effects of warming can be dampened or even eliminated.

While the individual effects of warming and eCO_2 are relatively well-understood, few manipulative studies have directly assessed their interactive effects on plant communities, despite model analyses suggesting that these factors will interact and affect species in ways that are not necessarily predictable given the results of single-factor experiments.

Warming and eCO_2 Experiment: SMARTX

Objective: To quantify the effects of climate warming and eCO_2 on physiological traits of dominant C_3 and C_4 species in a tidal, brackish coastal wetland.

The Salt Marsh Accretion Response to Temperature eXperiment (SMARTX) was established within the Global Change Research Wetland at the Smithsonian Environmental Research Center in 2016 (Fig 1).

Figure 1. Aerial view of the study site location. Plots were built into existing plant communities: the first is a C_3 sedge community that consists primarily of *Schoenoplectus americanus*, while the second is primarily comprised of two C_4 grasses, *Spartina patens* and *Distichlis spicata*. The higher elevation areas of the marsh that flood during 10-20% of high tides are dominated by the C_4 grasses, the lower elevation areas that flood during 30-60% of high tides are dominated by the C_3 sedge.

The experiment consists of six replicate transects, three in the C_3 sedge community and three in the C_4 grass community. Each transect contains four 2 x 2 m plots: an unheated ambient plot, and plots that are heated to 1.7, 3.4, and 5.1 °C above ambient (Fig 2).

- Warming is carried out using vertical resistance cables belowground (which warm to a soil depth of 1.5 m) and infrared heaters aboveground

In the C_3 community, there are six additional plots, each consisting of an open-top, eCO_2 chamber, three at ambient temperatures and three warmed to +5.1 °C (Fig 2).

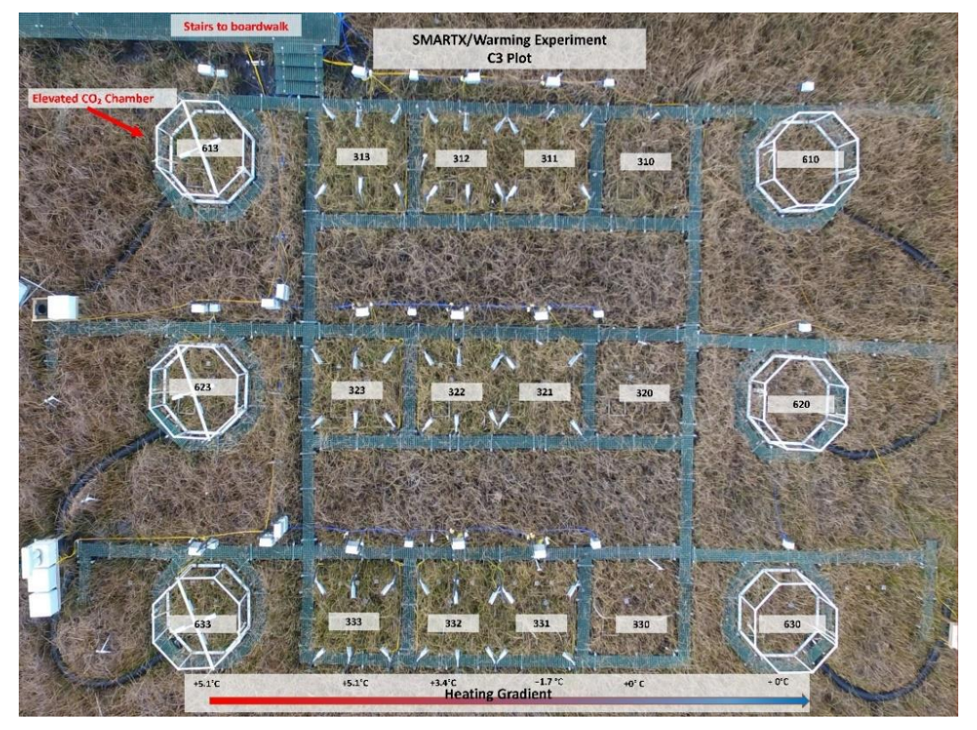
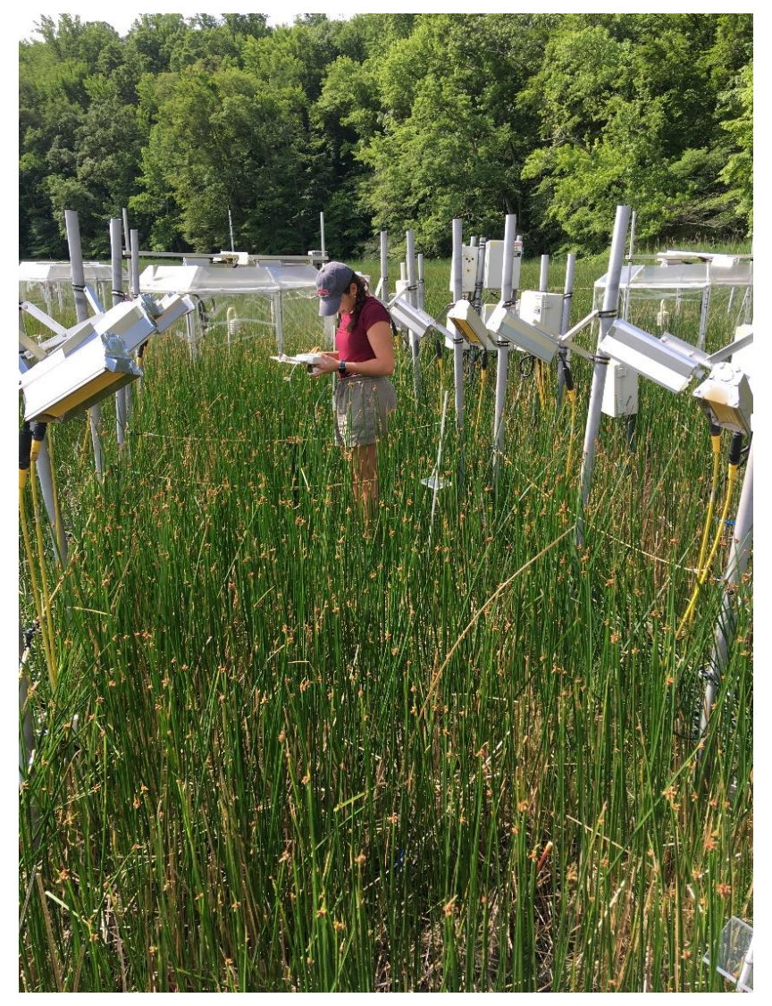


Figure 2. Experimental design of the C_3 sedge community including: a) three replicate warming transects ranging from ambient to +5.1 °C above ambient, and b) six additional eCO_2 plots with target atmospheric conditions of 750-800 ppm. Plot design is identical in the C_4 community, but does not contain any eCO_2 plots.

Methods

A well-established limitation of working with the dominant plant species in the GCReW site (the C_3 sedge, in particular) is that they do not lend themselves to leaf-level gas-exchange measurements with commonly-used physiological equipment. Due to these limitations, we chose to focus our efforts on making relatively simple, *in situ* measurements

- In 2017-22, stomatal conductance (g_s) was measured between the hours of 08:00 and 14:00 on warm, sunny days across the growing seasons.
- In 2018, maximum quantum efficiency of PSII photochemistry (F_v/F_m) was measured between the predawn hours of 01:00 and 05:00, typically following warm, sunny days. This metric can be used to estimate the stress level of a plant.
- In 2019-22, we measured light-response curves using a light curve program of the FluoroPen FP 110 between the hours of 08:00 and 14:00 on warm, sunny days.
  - Leaves were dark-adapted for 30 minutes, then exposed to actinic light intensities of 0, 100, 200, 300, 500, and 1000 mmol m^{-2} s^{-1} in successive 30 s steps.
  - Curves were used to calculate F_v/F_m, the maximum rate of photosynthetic electron transport of PSII (ETR_{max}), and the light the level at which ETR saturates ($PPFD_{sat}$).



Warming and eCO_2 Reduce Rates of Stomatal Conductance (g_s)

Warming had a significant negative effect on g_s of both C_3 and C_4 species (Fig 3; $p < 0.001$), although the C_3 sedge showed a more consistent negative response to warming when Tukey-Kramer HSD tests were run within year. This was expected, given that warmer growing conditions tend to favor C_4 species over C_3 species due to the elimination of photorespiration that occurs in C_4 species by concentrating CO_2 around Rubisco and eliminating O_2 competition for its active site .

The eCO_2 treatment caused a significant reduction in g_s of 15% on average for the C_3 sedge, whether they were growing under ambient or +5.1 °C temperatures ($p < 0.05$). However, because of the increase in CO_2 supply in eCO_2 treatments, plants tend to have enhanced photosynthetic and growth rates in spite of the limitation of lower g_s.

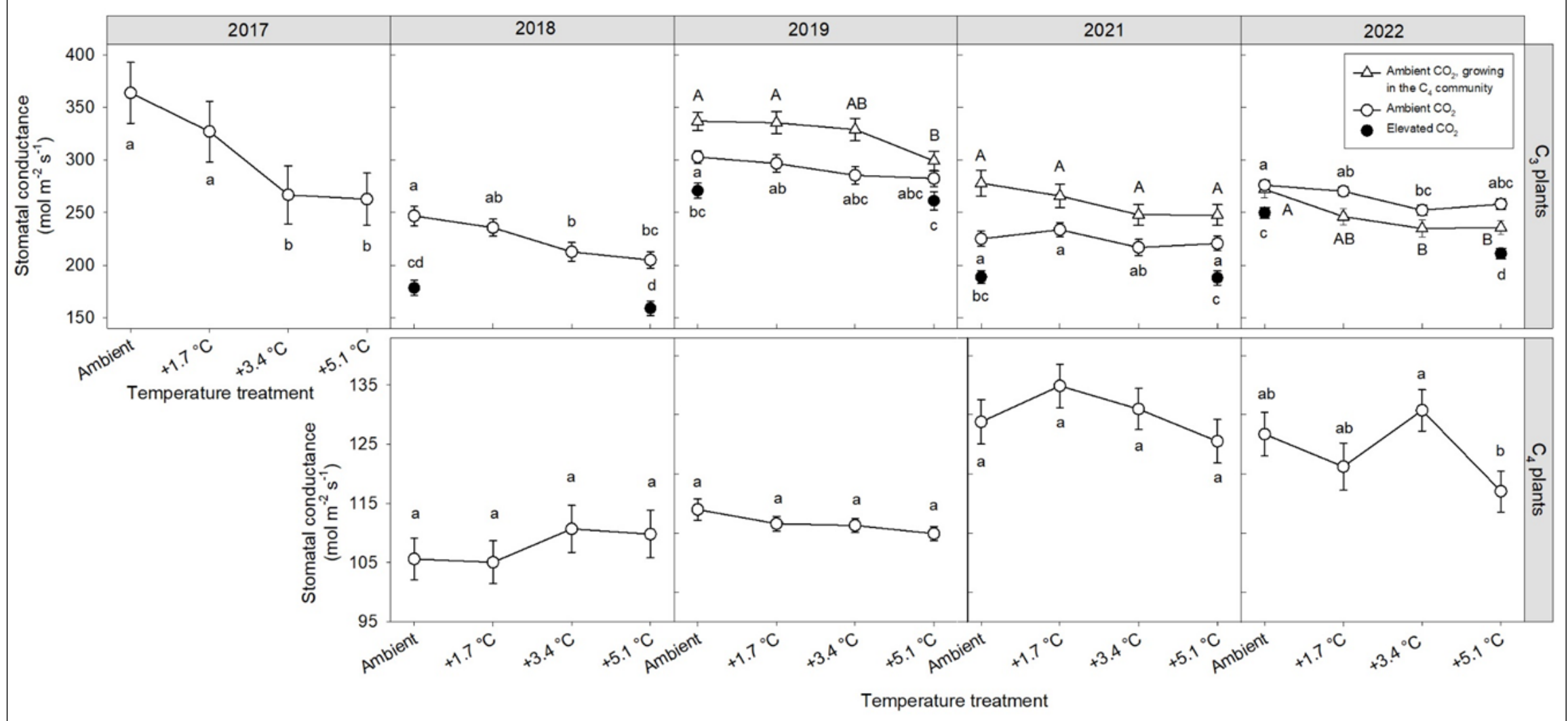
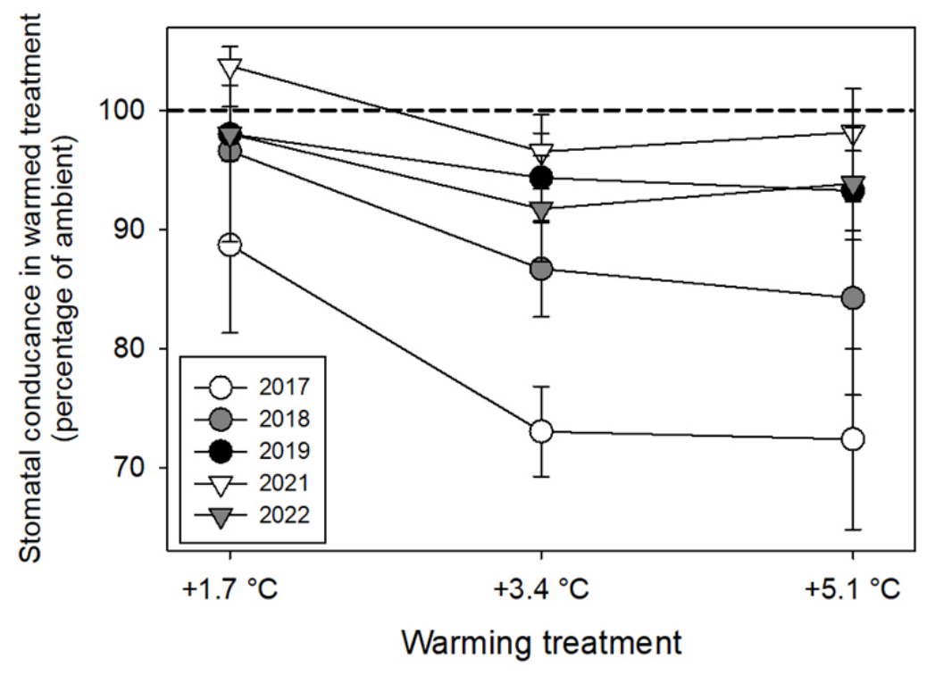


Figure 3. Changes in stomatal conductance in response to warming and CO_2 measured in 2017-2019 and 2021-2022 (n = 273, 1756, 1733, 2443, and 2058, respectively). Open circles represent plants growing under ambient CO_2, closed circles represent plants growing under elevated CO_2, open triangles represent C_3 sedges that began encroaching into C_4 plots beginning in 2019, and error bars represent ± 1SE. Letters show results of Tukey-Kramer HSD tests looking for warming and CO_2 effects within each measurement year; capital letters in the top row show results for C_3 sedges growing in C_4 plots and lowercase letters show results for C_3 sedges in C_3 plots.

The negative effect of warming on C_3 sedges was most significant in the first year of the experiment (2017) and dampened over time as evidenced by a significant Year x Warming interaction term in our statistical model ($p < 0.001$), indicating that *S. americanus* was able to acclimate to the warming treatment after prolonged exposure (Fig 4).

Figure 4. Stomatal conductance of *Schoenoplectus americanus* in +1.7, +3.4, and +5.1 °C above ambient conditions as a percentage of stomatal conductance in the ambient warming treatment. All data are from ambient CO_2 growth conditions. Data are averaged from measurements made from 2017-2022. Symbols represent the mean response to each warming treatment (shown as a percentage of ambient) averaged across transects (n = 3); error bars represent ± 1SE.



Effects of Warming and eCO2 on Chlorophyll Fluorescence Traits

Despite the relatively minimal decline of g_s in response to warming after 2019 (Fig. 4), we observed a negative effect of warming on ETR_{max} in 2022 for C_3 sedges, and both 2021 and 2022 for C_4 grasses ($p < 0.05$, Fig 5). This was surprising, since we expected g_s and ETR_{max} to follow similar patterns because stomatal closure prevents water loss via transpiration, but yields excess light energy which can damage photosynthetic machinery via the generation of reactive oxygen species.

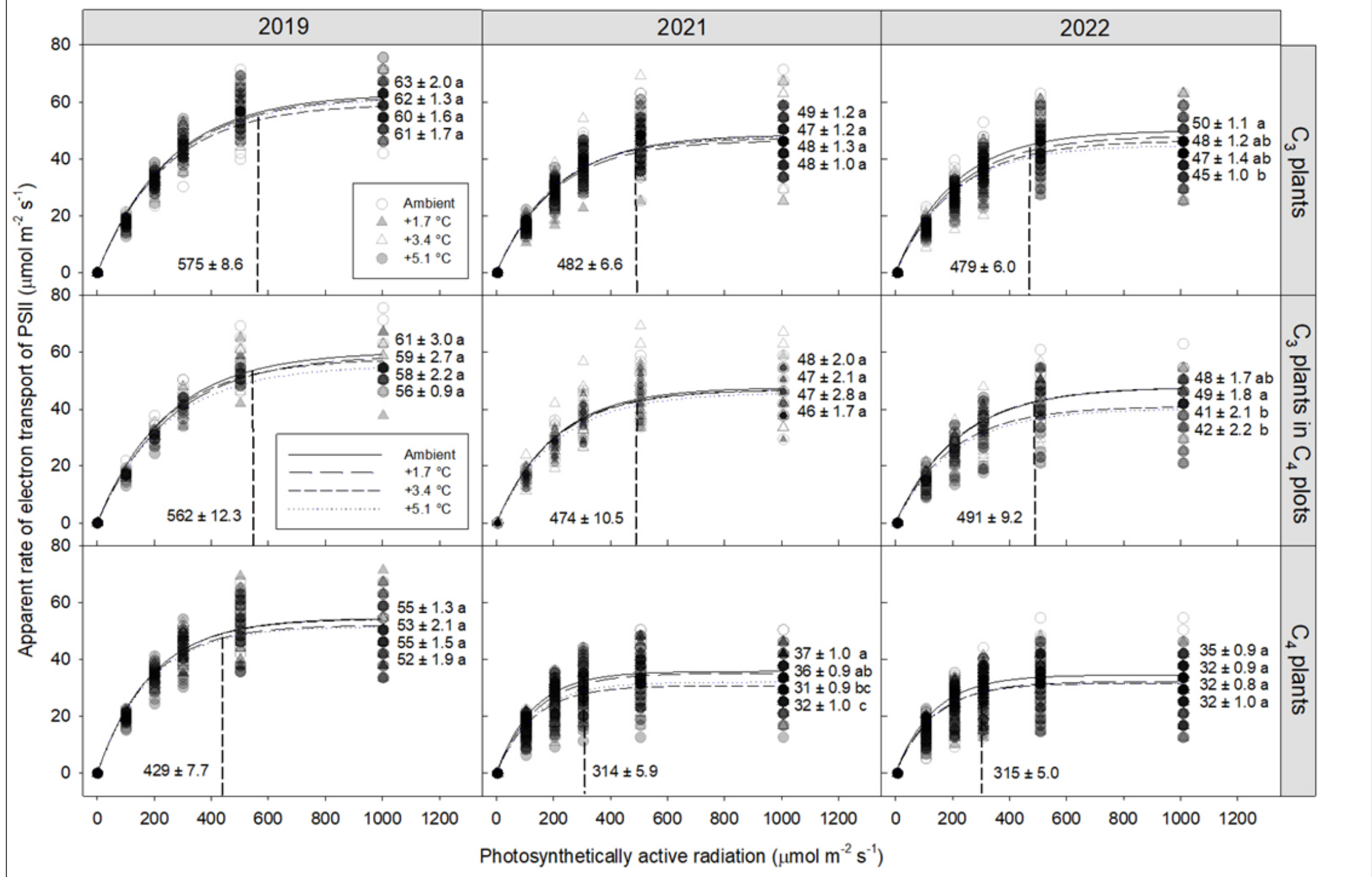


Figure 5. Light response curves of C_3 *S. americanus* and C_4 grasses measured in 2019, 2021, and 2022. Plants were dark-adapted for 30 minutes prior to measurements. The top row of panels represents C_3 plants, the middle row of panels show C_3 plants encroaching into C_4 plots, and the bottom row of panels is C_4 plants. The numbers to the right of the fitted curves give ETR_{max} ± SE for each warming treatment and the dashed vertical lines show $PPFD_{sat}$ ± SE (i.e., PPFD at 90% of ETR_{max}). Only one $PPFD_{sat}$ value is shown for each plant community in a given year because there was no significant effect of warming. Letters show results of Tukey-Kramer HSD tests looking for warming effects for each community within a measurement year.

We found that eCO_2 caused significant reductions in ETR_{max} and $PPFD_{sat}$ of C_3 sedges, whether they were growing under ambient or +5.1 °C temperatures ($P < 0.01$, Fig 6).

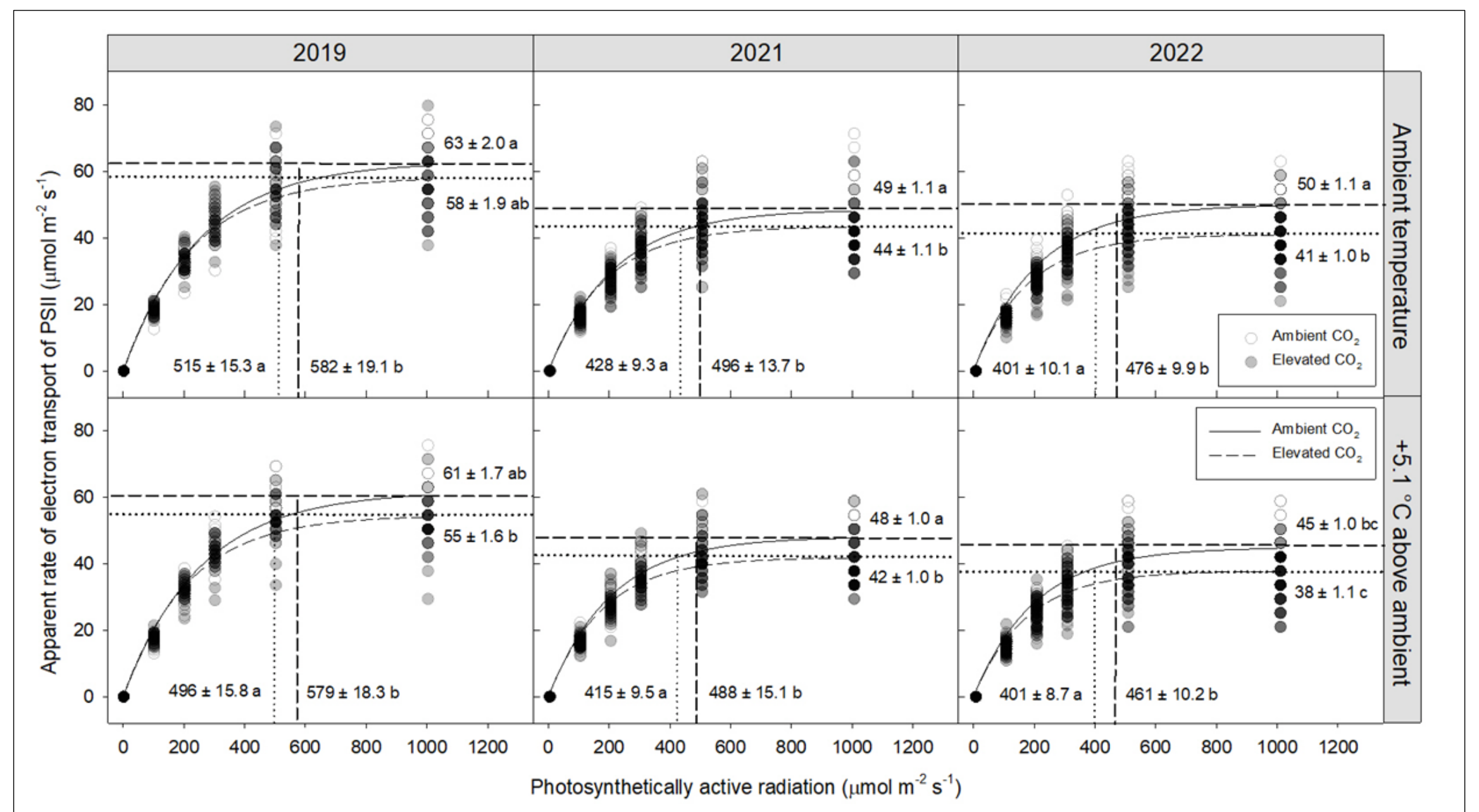
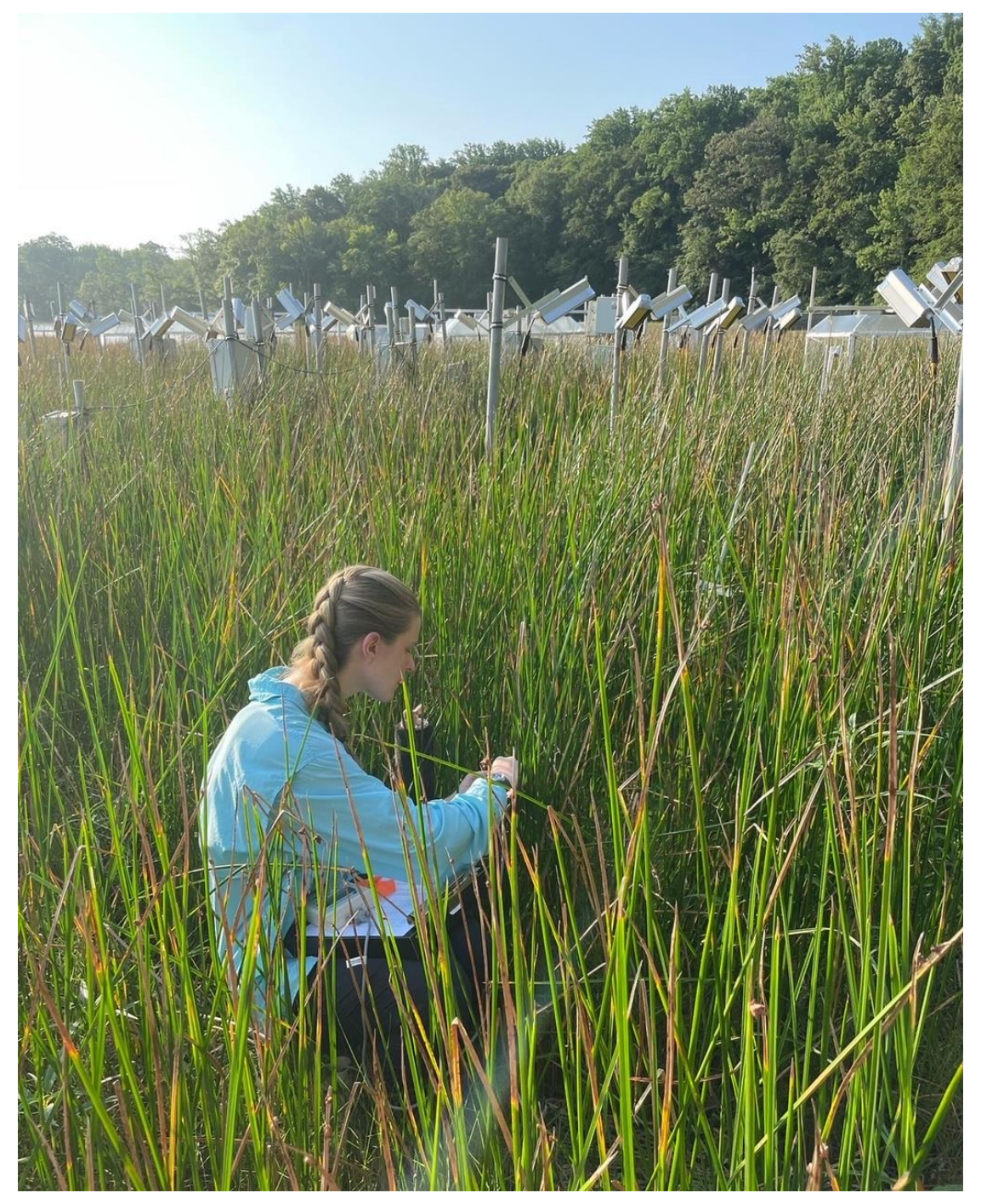
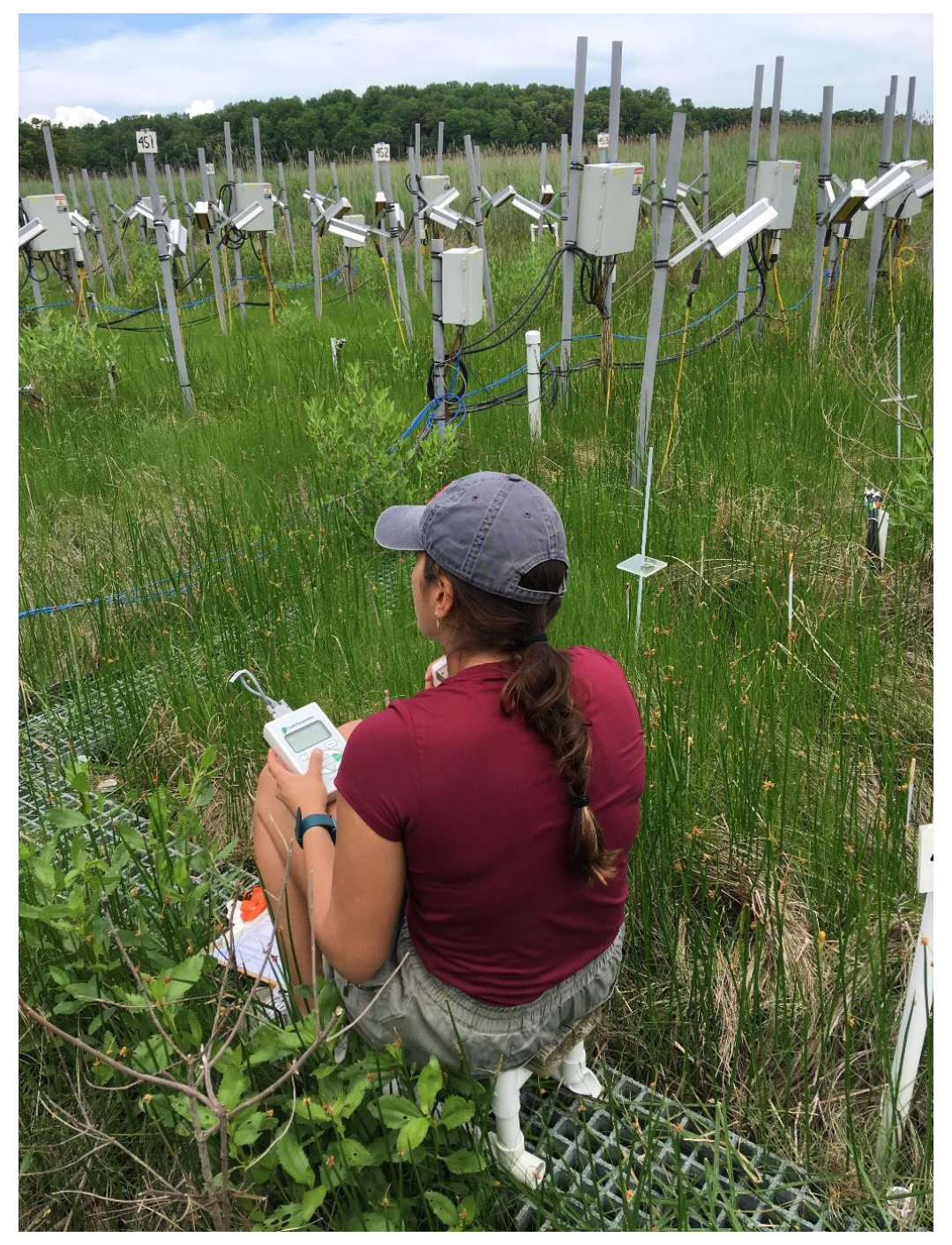


Figure 6. Light response curves of C_3 *Schoenoplectus americanus* growing under warming and eCO_2 treatments in 2019, 2021 and 2022. Plants were dark-adapted for 30 minutes prior to the start of measurements. The top row of panels represents plants growing under ambient temperatures and the bottom row of panels represents plants growing in +5.1 °C above ambient. The numbers at the dashed horizontal lines give ETR_{max} (± SD) of aCO_2 plants and the numbers at the dotted horizontal lines give ETR_{max} (± SD) of eCO_2 plants. The dashed vertical lines show $PPFD_{sat}$ (i.e., PPFD at 90% of ETR_{max}) of aCO_2 plants and the numbers at the dotted horizontal lines give $PPFD_{sat}$ of eCO_2 plants. Letters show results of Tukey-Kramer HSD tests looking for warming and CO_2 effects within each measurement year.

We predicted that the combined +5.1 °C eCO_2 treatment would have the most significant effect on plant physiological traits, particularly for the C_3 sedges, but only saw evidence for this in 2022 (Fig 6).





Lower g_s is Related to Declines in Other Leaf Traits

We observed that g_s was positively correlated with chlorophyll fluorescence variables, with higher rates of electron transport (ETR_{max}) and light level at which ETR saturates ($PPFD_{sat}$) attributable to increased CO_2 availability when stomata are open and lower F_v/F_m (*i.e.*, higher levels of plant stress) related to a reduction in evaporative heat loss or an increase in oxidative stress when stomata are closed (Figs 7 and 8).

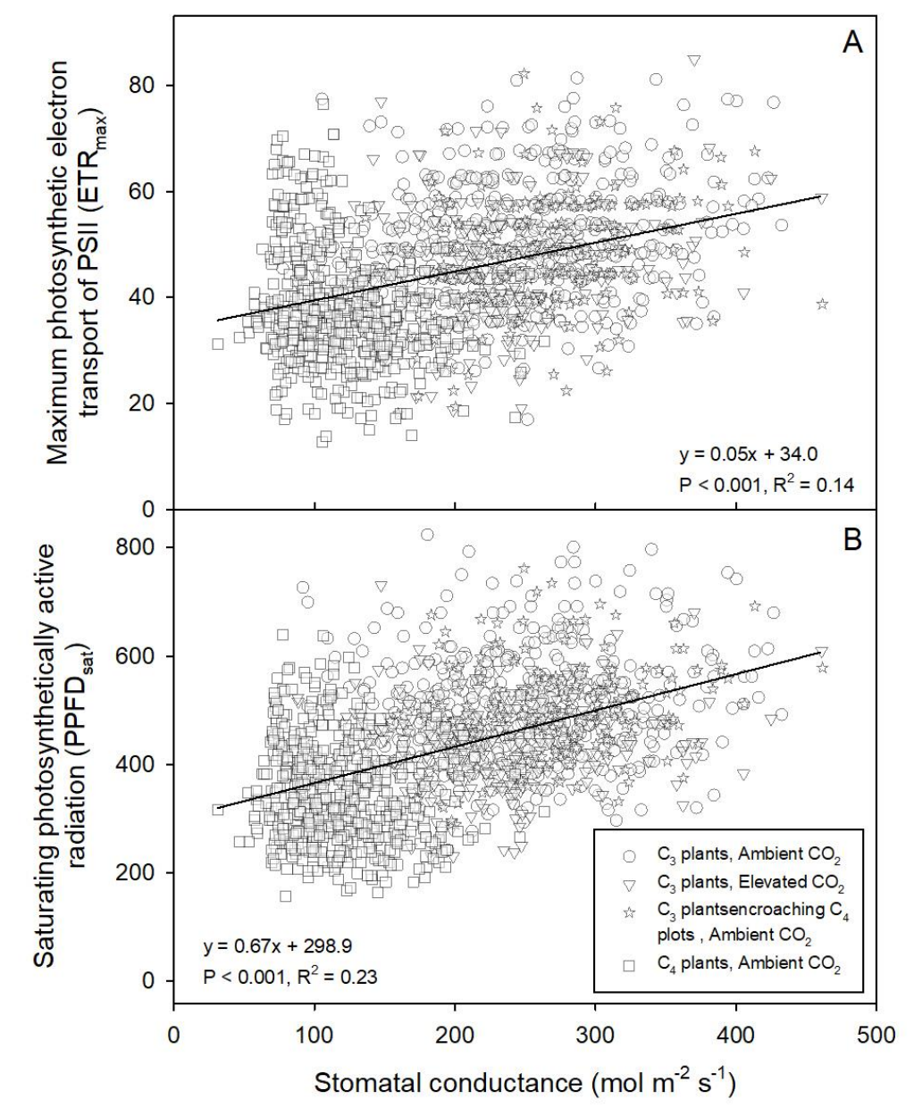


Figure 7. Maximum photosynthetic electron transport of PSII (panel A) and saturating photosynthetically active radiation (panel B) in relation to stomatal conductance. Measurements were made in 2019, 2021, and 2022 (n = 1544).

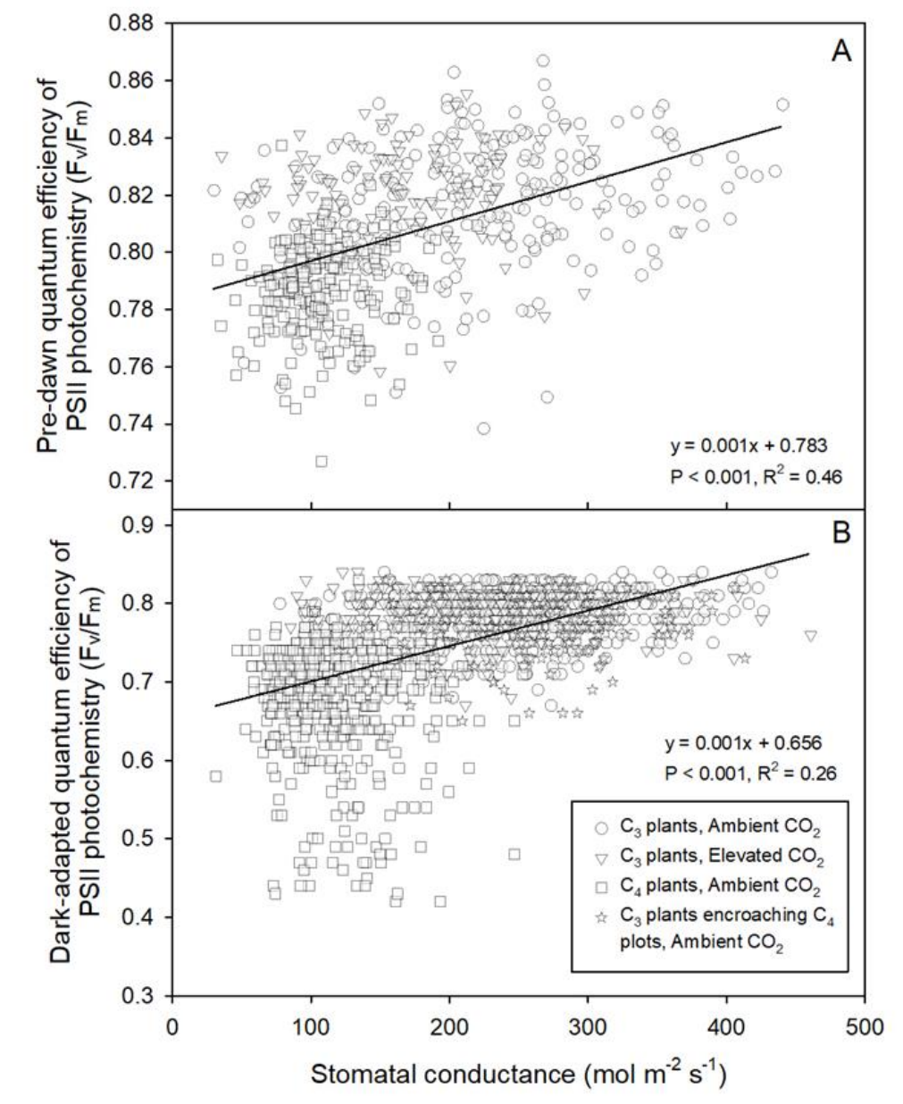


Figure 8. Quantum efficiency of PSII photosynthesis (F_v/Fm) in relation to stomatal conductance. Pre-dawn measurements (panel A) were made in 2018 between the hours of 01:00 and 05:00 (n = 566) and dark-adapted measurements (panel B) were made in 2019-2022 between the hours of 08:00 and 14:00 (n = 1434).

Conclusions

These results are important for predicting future trends in growth of wetland species, which serve as a large carbon sink that may help mitigate the effects of climate change.

More studies evaluating the interaction of climate stressors are needed to better understand mechanisms driving gas-exchange and growth responses of plant communities. For example, this study is helping to fill in some gaps regarding plant responses to warming and eCO_2, but a recent publication investigating the effects of rising temperatures and CO_2 levels found that most ecosystems are becoming deficient in nutrients such as nitrogen, which further complicates making predictions about the health of future ecosystems.

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