Exploring patterns of thermal acclimation of leaf respiration in a marsh-mangrove ecotone

Statement of work
Project description
Coastal wetland ecosystems, which are dominated by marsh grasses and mangroves, are important habitat for wildlife and make a large contribution to global net primary productivity and carbon (C) cycling despite covering a small proportion of the earth's surface. However, rising temperatures have the potential to modify rates of C uptake, storage, and use in these important ecosystems, which may determine whether they keep pace with rising seas and persist under more frequent and intense storms. Yet, our understanding of C cycling processes over space and time and in response to temperature remain limited for these ecosystems at the terrestrial-aquatic interface. **The overall aim of this project** is to utilize a climate warming experiment replicated at Northern and Southern sites in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTNMERR) on the northern Atlantic coast of Florida to improve our understanding of temperature controls of respiratory CO_2 fluxes in marsh and mangrove species. **The specific objectives of my project are to:**

- Examine changes in the short-term temperature response of leaf respiration (CO_2 efflux) over space (two sites) and time (seasons) in a dominant C_4 marsh grass species (*Spartina alterniflora*) and C_3 mangrove species (*Avicennia germinans*).
- Determine whether leaf respiration 'acclimates' to seasonal temperature changes across sites that differ in seasonality, and whether respiratory responses to temperature across seasons and sites differs between *S. alterniflora* and *A. germinans*.
- Use passive warming chambers at each site to determine whether experimental warming alters respiratory responses to temporal and spatial changes in temperature in both species.
- Provide new data for parameterizing and advancing the representation of temperature controls of C cycling processes in coastal wetlands, which will advance predictions of coastal wetland responses to climate change

Background
Marsh and mangrove ecosystems (i.e. coastal wetlands) are among the most valuable ecosystems on the planet, providing habitat and refuge to a diversity of terrestrial, aquatic, and avian species, while also preventing erosion, regulating nutrient cycling, and providing recreation for humans (Costanza et al. 1997). Despite being limited in extent, these ecosystems also play a major role in the global C cycle (Lugo & Snedeker 1974, Bouillon et al. 2008). 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 (Duarte et al. 2013). On average, saltmarsh ecosystems produce an estimated 1100 g C m^{-2} yr^{-1} at the local scale and contribute 0.02 to 0.44 Pg (1×10^{12} kg) C yr^{-1} to global net primary productivity. Large variation in estimates of saltmarsh primary productivity are partly attributed to uncertainty regarding their rates of C uptake, storage and use (Duarte et al. 2013). At the local scale mangrove ecosystems have similar rates of average primary production but contribute 0.14 to 0.15 Pg C yr^{-1} to global net primary productivity. The high C storage capacity of these ecosystems can be attributed to the fact that much of the C fixed by photosynthesis remains sequestered for millennia due to slow decomposition rates, anoxic conditions, and a relatively high fraction of total biomass allocated to roots and rhizomes. Syntheses of available data suggest that these ecosystems play an important role in the global C cycle, yet there is considerable uncertainty about CO_2 fluxes and their sensitivity to changing temperatures (Duarte 2013, Adame et al. 2018).

This uncertainty stems from several sources:
1. Data on seasonal changes in leaf CO_2 exchange rates remain limited for marsh and mangrove species in tropical and subtropical climates.
2. Climate warming impacts on leaf CO_2 exchange rates in tropical and subtropical marsh-mangrove ecosystems are virtually unknown.
3. Marsh and mangrove species capacity for thermal acclimation of leaf physiology remains understudied and difficult to predict, despite the importance of thermal acclimation in modifying the temperature response of wetland C cycling processes.

The research outlined in this proposal will address these uncertainties and improve our understanding of temperature controls of CO_2 fluxes in coastal wetland ecosystems.

Respiratory responses to temperature and climate warming
At the global scale, the annual respiratory CO_2 fluxes from plants is roughly half the annual CO_2 flux from photosynthesis (IPCC 2013), and roughly 50% of plant respiration comes from leaves (Atkin et al. 2014). Leaf respiration (R, typically measured in darkness) is co-limited by the capacity of respiratory enzymes, adenylate supply, and the supply of carbohydrates produced by photosynthesis that are also required for growth, maintenance, and defense. Temperature strongly influences rates of R given that the activity of respiratory enzymes are temperature-dependent. In the short-term, R increases with increasing measurement temperature before reaching an optimum, which typically ranges from 50 to 60 °C (Aspinwall et al. 2017). There is considerable evidence that R may 'acclimate' to changes in growth temperature; a process termed 'thermal acclimation' (Atkin & Tjoelker 2003, Aspinwall et al. 2016). Thermal acclimation is a reversible process that is thought to optimize the balance between C uptake and use. Measurements of the short-term temperature responses of R are used to reveal the nature of thermal acclimation. Thermal acclimation of R typically occurs via a change in R measured at a set temperature (e.g. 25 °C) or a change in the temperature sensitivity of R (i.e. Q_{10}, Atkin & Tjoelker 2003). In a synthesis of studies, Slot & Kitajima (2015) found a significant downregulation of R (at a set temperature) in response to increasing growth temperature in 73% of the cases examined. A reduction in the Q_{10} of R in response to warmer growth temperatures is less common among studies (Slot & Kitajima 2015).

Thermal acclimation of R can modify C cycle responses to temperature at local and global scales, as well as climate - C cycle feedbacks. Without acclimation, rates of R would increase sharply as temperature increases, thereby weakening ecosystem C storage, and accelerating the feedback between C loss and atmospheric warming. However, assuming acclimation follows the patterns highlighted above, the temperature sensitivity of R would be reduced over time, which would effectively dampen the climate - C cycle feedback. The extent to which marsh and mangroves adjust respiratory physiology in response to changing temperatures will go a long way in determining whether these highly productive ecosystems/habitats remain strong C sinks and keep pace with rising seas and more frequent/intense storms.

Approach
The project will utilize the experimental infrastructure of a National Science Foundation (NSF)-funded project led by Dr. Samantha Chapman (Villanova University), which focuses on root production and organic matter decomposition (https://www.wetfeetproject.com/).

The work I will carry out is novel and will not duplicate any ongoing research. The experiment is replicated at a northern and southern site within the GTMNERR (see **Figure 1**). At each site there are six replicates of four treatment plots: mangrove ambient, mangrove warmed, marsh ambient, and marsh warmed. Smooth cordgrass (*Spartina alterniflora*) and black mangrove (*Avicennia germinans*) are the dominant grass and mangrove species in the plots at both sites. Experimental warming (2-3 °C above ambient) is imposed using passive warming chambers. The chambers are 1.5m x 1.5m x
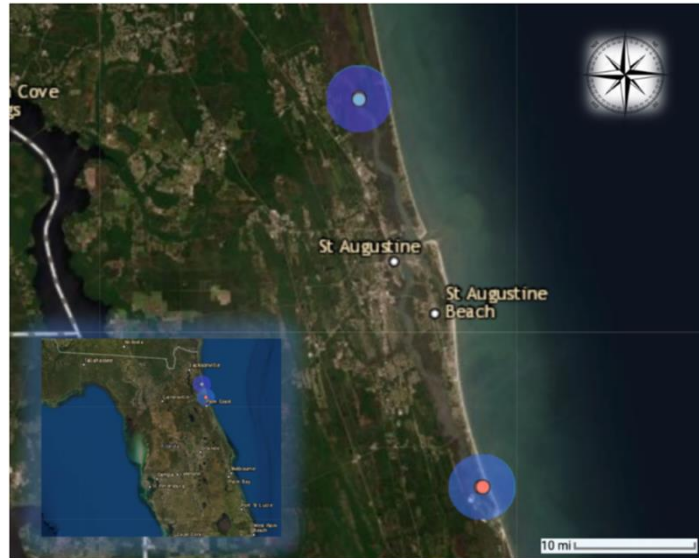
Figure 1. The blue dot represents the north site, the red dot represents the south site. In the lower left corner is a map of Florida displaying where the sites are located relative to the rest of the state.

1.5m in dimension, framed with PVC, wrapped in greenhouse film, and are open on top. The chambers 'trap' radiation causing the chamber air to warm without overheating. Air temperature and relative humidity inside the chambers is continuously measured using HOBO MX2302 External Temperature/RH Sensor Data Loggers (Onset Computer Corp., Bourne, MA). Measurements of salinity and water temperature are recorded at nearby monitoring stations.

Methodology

Leaf samples are collected monthly over the course of a 12-month period from both species at each site and both treatments. Sampling consists of collecting 2-3 leaves per plot during pre-dawn hours to avoid activation of photosynthesis. Leaves are brought back to the Plant Physiological Ecology Lab at University of North Florida (led by Dr. Aspinwall) for analysis. Short-term temperature response curves of leaf respiration are generated by sealing leaves in a Walz GFS-3000 temperature-controlled chamber connected to a LI-6400xt gas analyzer. This system allows us to increase the temperature of the leaf from 20 °- 40 °C at a rate of 1 °C per minute while continuously measuring rates of leaf R as the rate of leaf CO_2 efflux per unit leaf area (see Aspinwall et al. 2017). I will record the surface area and dry mass of the gas exchange leaves to determine leaf dry mass per unit area (LMA, g cm^{-2}). I will determine leaf N concentrations on the measured leaves to estimate leaf N per unit area (N_{area}). A natural log polynomial model will be used to describe the temperature response of leaf R. We will use measurements of leaf R at a set temperature (e.g. 25°C) and the temperature sensitivity of R (i.e. Q_{10}) to test questions about thermal patterns of thermal acclimation. If thermal acclimation occurs, we expect that R at 25 °C will decline as prevailing air temperatures increase (across seasons or under experimental warming).

Using these data, I will test whether marsh grasses and mangroves show similar acclimation of leaf respiration to seasonal temperature changes at sites differing in temperature seasonality, and whether acclimation is consistent between plants grown under ambient and warmed conditions. There are several potential outcomes of this research, each of which has important implications.

First, we may find that *S. alterniflora* and *A. germinans* show similar rates of *R* at 25 C and both species show similar patterns of seasonal acclimation of leaf *R* that is equivalent across treatments (**Figure 2a**). This would simplify the results and indicate that respiratory responses to temperature can be easily predicted for both marsh and mangrove species. Another possibility is that *S. alterniflora* shows weaker seasonal acclimation of leaf *R* than *A. germinans*, but patterns of seasonal acclimation are equivalent across treatments in both species (**Figure 2b**). This might mean that separate predictions of respiratory responses to temperature are needed for each species. Yet another possibility is that seasonal acclimation of leaf *R* depends upon both species and treatment (**Figure 2c**). Lastly, I might find that patterns of seasonal acclimation depend upon species, treatment, and site (not shown). Ultimately, by combining the respiration data with leaf nitrogen, salinity, air temperature, and water temperature I can build a predictive model for changes in leaf respiration over space and time.
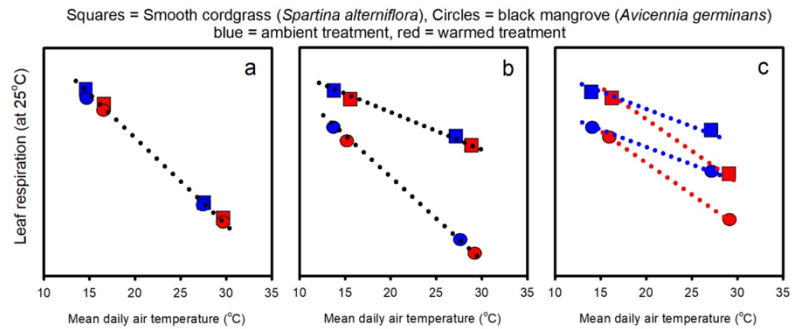

Figure 2. Conceptual figure showing three potential patterns of thermal acclimation of leaf respiration in marsh and mangrove species.

Preliminary Results

Thus far I have measured short-term temperature responses of leaf *R* on all plants at both sites on two dates (October 2019, December 2019). Across both sites, *Spartina* has shown consistent thermal acclimation of leaf *R* in response to both seasonal temperature changes and climate warming. In contrast, at both sites, *Avicennia* showed little evidence of thermal acclimation of leaf respiration in response seasonal temperature changes and warming. These results indicate that thermal acclimation might reduce respiratory C fluxes from marsh grasses in response to warming. However, acclimation was limited in *Avicennia* indicating that respiratory C fluxes from mangroves might increase substantially with climate warming. By continuing to collect this data we may improve predictions of carbon cycling processes in vegetated coastal ecosystems over space and time.

Benefits: global and local impacts

Results from this project will improve our quantitative and predictive understanding of coastal C cycling responses to temperature and potential feedbacks. This is especially important given that global mean temperatures are expected to rise 1-4°C by 2050 (IPCC 2013). Research on temperature effects on C cycling processes in tropical and subtropical coastal wetlands are urgently needed for additional reasons. These ecosystems play a major role in combating sea-level rise and attenuating wave energy from increasingly frequent and intense tropical storms. Understanding the C regulating services of these ecosystems could aid their conservation. The marsh-mangrove ecotone in the southeast United States provides an opportunity to examine the temperature controls of C cycling processes in coastal ecosystems along a tropical-subtropical transition zone and assess potential changes in C cycling processes resulting from climate warming. This project will also provide improved insight for future management of the marsh-mangrove ecotone at GTMNERR. The research reserve is one of the largest and well-managed estuaries on the east coast of Florida. This estuary is a critical nursery for a number of marine species and plays an important role in buffering coastal communities from the effects of rising seas. This work will improve our understanding of C cycling in the reserve and inform

conservation decisions. The preliminary data will be shared at "The State of the Reserve" an event hosted by GTMNERR that is open to the public, where scientists share what projects they are working on at the reserve.

Use of funds and plans for sharing results
Receiving the Coastal Wetlands Scholarship from the Garden Club of America would help me buy supplies (chemical consumables for LI-6400xt gas-exchange system), pay for lab analysis to determine leaf nitrogen concentrations, and pay tuition. The scholarship will fund my travel and presentation of results at two major international conferences: Ecological Society of America (Salt Lake City, UT) and American Geophysical Union (San Francisco, CA) in 2020. The opportunity to attend and present at a nationally recognized conference would allow me to share the results of this important research and highlight the importance of coastal wetland conservation and management.

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