**General Field Sampling Methods:**  [Link to google doc of these methods](https://docs.google.com/document/d/17q4FPkG_JLz_Zu54XxfmFsdVUs_l_76AIwmsbfFWdhc/edit?usp=sharing)

Cogongrass trait measurements were taken from the University of Florida Biven’s Arm Research Station (29.6299305, -82.35606) during 2019 May 21st- June 1st (June 1st-4th were left to weighing and organization). There was a heatwave and minor drought during this time. I did not observe heat/ drought stress, but some may have existed.

During this field sampling session, I attempted to constrain stomatal slope via light and humidity curves, specific leaf area, obtain a below ground and aboveground allometry, and take the respiration rate of the rhizomes and fine roots. Traditionally, fine roots represent roots that are smaller than 2 mm in diameter. Rhizomes are a specific type of root. In cogongrass, no live rhizomes encountered were smaller than 2mm and no non-rhizome roots were larger. I refer to fine roots and rhizomes only, despite the fact that in other plants they might not be distinct categories.

Many of my measures are normalized by dry biomass. All drying was done in a drying over set to 60 degrees celsius for at least 48 hours. Larger biomass samples (ie the aboveground samples) were dried for at least a week. Root samples were dried for a minimum of 48 hours.

My two measurement locations in Bivens included a long term garden experiment ([Alba et al, 2017](https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.2729)) and a natural invasion at the northeastern corner of the Bivens property. My aboveground and belowground biomass measurements were done on the 20 invaded treatments of the common garden experiment. Light and humidity (excised and field curves) were done on edge-leaves of the common garden experiment. The specific leaf area and root respiration measurements came from the natural invasion. The natural invasion had not been sprayed or mowed recently.

Light curves and root respiration measurements were done using a LI-COR 6400. Light curves used a standard leaf-chamber portable infrared gas analyzer with a 1098 light source. Root respiration curves were generated using soil respiration chamber with the venting coil blocked by plumber’s putty. The soil respiration chamber plastic cap was modified with a layer of foam (the 9960-112 part from the 6000-09 gasket kit) to be air-tight.

All measurements were taken after calibration. For light curves, calibration included zero-ing the flow rate, CO2 and humidity levels. For soil curves, calibration involved zeroing the CO2 and the humidity.

**Light Curves**

* **General motivation and description**

I wanted to collect light curves for cogongrass in order to constrain stomatal slope. In order to do this, I tried to create light curves where the level of light is slowly ramped down to zero while most other environmental variables are held constant. This is following the technique found in ([Leakey et a,l 2006](https://www.ncbi.nlm.nih.gov/pubmed/16913868); [Wolz et al, 2017](https://www.nature.com/articles/s41559-017-0238-z); [Leakey et al in prep](https://www.evernote.com/shard/s412/sh/b4f2d4d6-1858-4518-9202-5b66df789b5c/89d4a95a57bfe33966d7506e7689d41f)). My methods differ from Leakey’s in three ways. First, there existed no prior work suggesting that excised leaves could reproduce maximum photosynthesis levels ([Leakey et a,l 2006](https://www.ncbi.nlm.nih.gov/pubmed/16913868), Ainsworth et al 2007). Cogongrass is an invasive and highly productive grass, suggesting it might be a candidate for excised leaves reproducing max photosynthesis rates. In my field season I attempted five excised leaf light curves, and was able to produce two curves. I measured field curves separately, to compare to the excised curves. Second, Leakey was able to hold all other environmental variables constant. Because the majority of my curves were take in in vivo, temperature varied as the day progressed and several curves were subject to dramatic humidity swings. To provide additional constraint, two humidity curves were done (on two seperate leaves). One at 1500 photon flux density, and one at 700 photon flux density. Finally, the area for cogongrass leaves was smaller than that of the chamber. The diameter of the leaf was measured, and multiplied by the length of one side of the leaf gasket chamber (). The leaf had a 1.4 cm diameter which was 3.429 . Another leaf had a 1 cm diameter and was run with an area of 2.45. After that, area was set at 3.5 as an approximate area measurement for all leaves. In order to let the stomatal slope stabilize, I set a minimum wait period between two light steps to ten minutes, and a maximum wait period of a half hour. In addition, CO2, humidity and flow rate had to be stable for a full minute before measurements were taken. All light curves but one began at a starting value of 2000 photon flux density before measurement began at 1500 photon flux density. I set the final light curve to start at 2300 based on a conversation with James Estrada that Florida days can easily get up to 2300. The LI-COR was matched before each measurement.

* **Excised leaves**

Excised leaves were harvested pre-dawn and cut underwater. Leaves were harvested from common garden experiment edge leaves that had grown out from under the precipitation exclosures. One leaf was done per morning starting around dawn. Leaves suspected of embolism were discarded.

**Table of Excised leaf curves and notes**

|  |  |  |
| --- | --- | --- |
| File name | Notes |  |
| tess-ex-leaf1 | * Humidity as high a 80 * First point of file might be messed up * Was affected by breathing, possibly not a 100% perfect seal. The slope left at 1 min protected from breathing bursts. * Initial CO2 not set at ambient levels that included people being in the space (around 400). When people arrived, the lab co2 levels rose to 450/500 depending. |  |
| tess-ex-leaf5 | * Got high humidity alert * Almost totally saturated desiccant * Humidity was as low as 30% * I think this file contains an excised leaf curve, and a full field curve. |  |

* **Field curves**

Field curves were conducted by running an extension cord out to the edge of the common garden experiment plots. To maintain battery power, the LI-COR was plugged into mains power. Curves were taken during a range of times, from dawn until roughly 2:00 pm. Notes included are the raw notes from my field notebook.

Field curve notes

|  |  |
| --- | --- |
| tess-field-2 | * Set ref CO2 to 450 * Started 10:30 am * Percent humidity as low at 35% * LI-COR died at point 7 of curve * Taken at edge of cogon plot 7 |
| tess-field-curve 5 (Not the same curve as the one attached to tess-ex-leaf5) | * Humidity 45-69 * Maybe baking in heat? |
| tess-field-curve-6 | * Desiccant ran out, was saturated |
| tess -field-curve-7 | * Started 7:30 am * Added ci as a stability metric |

* **Datasets produced**

Ten light curves of indeterminate quality.

**Humidity Curves**

* **General motivation and description**

Humidity curves were taken to try and help constrain stomatal slope. Two curves were taken, both stored in the same file, but corresponding to different leaves. For the first curve, incoming radiation was fixed at 1500 photon flux density. The second was 700 photon flux density. I started at roughly 80% humidity, and kept it steady for about 15 mins. Every 15 minutes, I would use the thumb screw to reduce the humidity by about 20-25%. Humidity reached a minimum of about 10%.

**Biomass allometries**

* **General motivation and description**

I work with a model (The ecosystem demography 2 model aka ED2) that partitions carbon between different pools within a plant. The common garden experiment was planted with a known rhizome density. This biomass dataset provides useful test for how the model partitions cogongrass carbon. In addition, there is a lot of uncertainty in the current representation of cogongrass around the fine root allocation and water conductance. Having information on the below ground to aboveground ration constraints fine root allocation. Because the experiment is a precipitation exclusion experiment, the difference in biomass between the plots could inform how cogongrass is (or isn’t) affected by changes in water availability.

The sampling procedure was as follows. For each plot, a 30 cm^2 quadrat was placed in a subplot location cleared for harvesting (Need description of subplot location). Within the quadrat, three cogongrass leaves were measured for height. All the live tillers were counted, and then harvested with garden shears. The harvested aboveground biomass was put into a drying oven. No completely-dead biomass was included unless it was attached to living biomass. Also only the plants with stems in the 30cm^2 plot itself were included. Many plots had leaves “overhanging” the quadrat. The overhanging leaves were excluded. All Non-cogongrass biomass was harvested and placed in a separate bag. Every unique species of non-cogongrass was counted. Then, a soil core was taken from the center of the plot. Soil cores were taken [using a hammer-style soil corer](https://www.ams-samplers.com/1-1-2-x-6-sst-scs-complete.html). The cores were then stored in a freezer until processing.

The belowground biomass was processed by first thawing the soil core, than sieving through a 2mm opening sieve and a 420 micron opening sieve. When possible, the soil was sieved dry, however most soil cores required draining. After that, roots were removed from the soil and put into a drying oven. Rhizomes and fine roots were kept seperate. Roots were rinsed of soil before drying. Roots clearly dead, or non-cogongrass were excluded. Roots with unknown origin were included. Because fine roots can become quite small, the stopping point for root picking was to remove roots until the only roots found were unbranched roots 4mm in length or smaller. After that, roots were removed for an additional ten minutes. Most soil core took approximately two hours of devoted root-removal. The 13th soil core was dropped during the sieving process.

* **Datasets produced**

[Cogongrass Biomass 2019 datasheet](https://docs.google.com/spreadsheets/d/13UInk-VBahbQjMYSJ4J-0MGyOP8MljOAmeUTTN1bjXo/edit?usp=sharing)

**Specific Leaf Area**

* **General motivation and description**

We measured 26 leaves from a natural invasion using a li-cor belt scanner. For most leaves, I took three measures. For two I only got one SLA measure due to time constraints. 11.45 g total biomass.

[Cogongrass SLA Measures](https://docs.google.com/spreadsheets/d/1gyC2fiGEYFduVZcjq4yy-cPVLFloYNoKEnNothb32Z0/edit?usp=sharing)

**Root Respiration**

* **General Motivation and description**

One of the largest uncertainties of the model was root respiration. To constrain root respiration, I based my methodology on Rose Abermoph’s root work ([Abermoph & Finzi, 2016](https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1002/ecs2.1547)). One major difference between my measurements and Rose’s is that Rose was able to demonstrate that severed roots and connected roots had similar levels of respiration. The small size of cogongrass fine roots prohibited making this comparison, but cogongrass is an invasive weed that propogates via rhizomes. Even less than a centimeter lengths of rhizomes are viable for reproduction ([Estrada et al, 2016](https://link.springer.com/article/10.1007%2Fs10530-016-1163-9)), which suggests that the disturbance of removal wouldn’t affect the rhizomes that much.

Stems from the natural cogongrass invasion were dug out with as much soil and root structure as possible. 15-25 stems were dug out and packed into a bin with damp paper-towels and soil. The bin was brought into a lab setting, where at least a gram of wet biomass (Usually 3 grams) of fine roots and rhizomes was weighed out and measure with a soil-respiration chamber. To amplify the signal, fine roots from multiple plants and rhizomes from multiple plants were aggregated in each sample. There are 20 fine root measures and 19 rhizome measures. There are also 10 “combo” measures, where rhizomes were placed with fine roots to amplify the signal even more. Unless there is a “combo” measurement, fine roots and rhizome of the same numbers don’t relate to each other in any way except they were measured near to one anouther in time, and come from the same invasion.

Respiration measures were taken by running the soil respiration program, but on roots placed in a small jar that was then put into a sealed soil chamber (the pressure release venting tube was also sealed). As part of the automatic soil program, a target CO2 is set based on ambient CO2, and then the CO2 level in the chamber are drawn down to below the target and allowed to increase past the target for a number of cycles and based on a delta CO2. I set the target based on measured ambient levels. The number of cycles and the delta CO2 changed depending on the roots I was running, based on when in the experiment I was conducting the measurement. I began with three cycle and a delta CO2 of 3 and. I then added two additional cycle (total of five) and a delta CO2 to 8. After fine-roots-7 I was concerned that I wasn’t logging enough points so I re-ran it with a CO2 delta of three, only to realize that it was a logging options, so I changed it back to 8. The full logg record is still in fine-roots-7, which I re-ran as fine-roots-9. I set the delta CO2 to 20. For both of roots 7 the plumbing hadn’t been changed, so should be discarded. At 11th rhizome switched back to delta 8. I also switched back to 3 cycles. Unclear what happened to #12 fine roots.

File Name Notes

|  |  |
| --- | --- |
| Rhizome-9 | * 5th cycle accidentally opened chamber too soon. |
| rhizome-8 | * At 5th cycle accidentally opened chamber too soon |
| Rhizome 13th | * Accidentally opened the chamber. * Irga not ready alert. At the time, measurement discarded. |
| Fine-roots 20 | * Only two cycles |

[Roots respiration biomass measures](https://docs.google.com/spreadsheets/d/1ST5waqvS9fA_WSwKF0NOD7eBoZRgASVNLfxhPWJXRH0/edit?usp=sharing)