Aquatic Weeds: One Man's Trash is Another Man's Treasure

Yuting Fu (yutingfu@ufl.edu), Jehangir H. Bhadha and Ramdas G. Kanissery


Water lettuce
Pistia stratiodes


Water hyacinth
Eichhornia crassipes


Filamentous algae
Lyngbya wollei


Duckweed
Lemna minor L.


Muckgrass
Chara Species

ALLELOPATHY

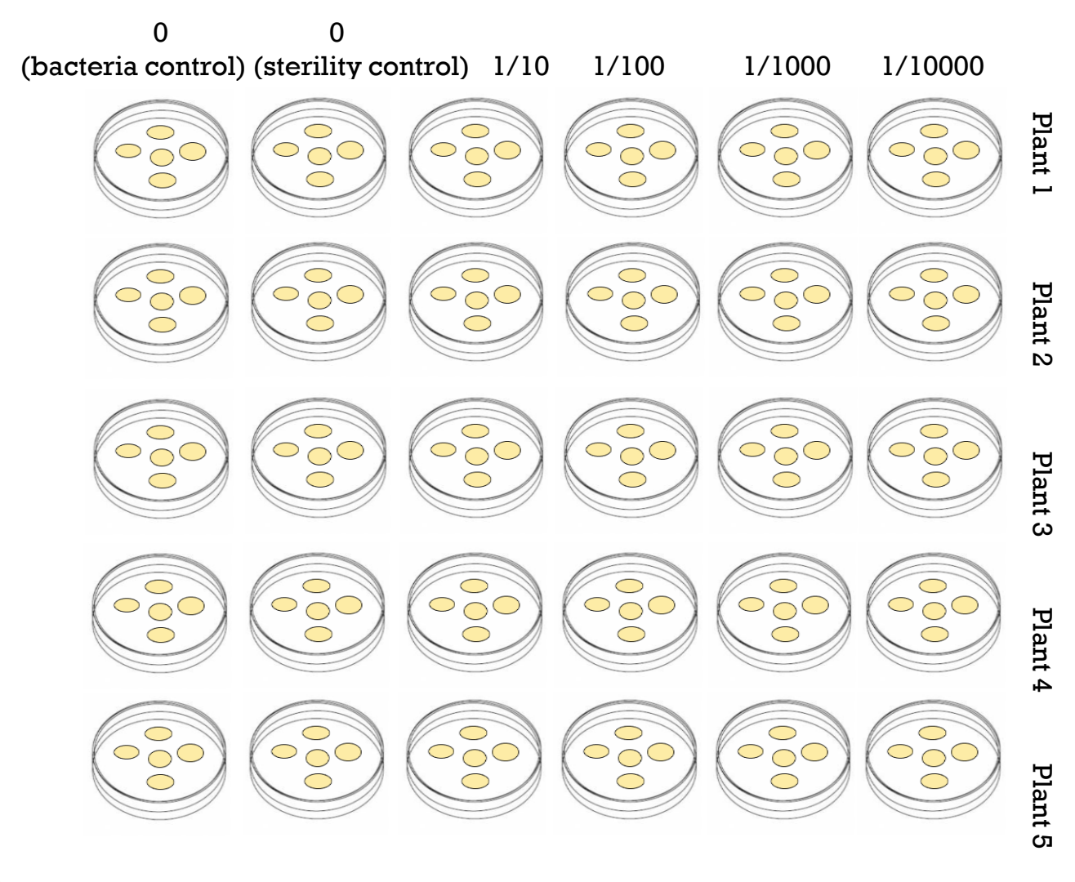
Refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelochemicals, from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems. (Fraenkel et al. 1959 *Science)*

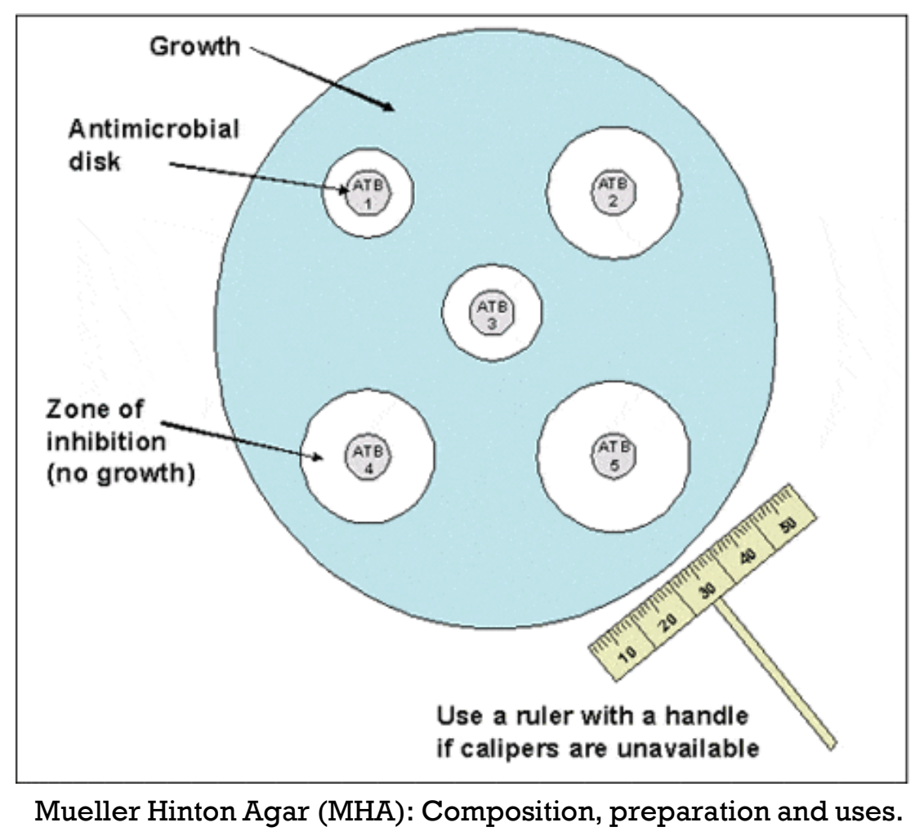
Antimicrobics

- Antibiotics are molecules that are produced by one microorganism that kill (bactericidal) or inhibit (bacteriostatic) other microorganisms.
- *Eichhornia crassipes* is an invasive weed known to out-compete native plants and found to have good antimicrobial, anticancerous and antioxidant activities. (Aboul-Enein et al. 2014 *BMC Compl and Alt Medicine*.)
- High biomass production of water hyacinth corresponded with large amounts of phenolic allelochemicals in the water, which may also help in the process of invasion.
- A series of 100 Rwandese medicinal plants, used by traditional healers to treat infections, were screened for antibacterial, antifungal properties. (Vlietinck et al. 1995 *Ethnopharmacology*)
- Since plants have been reported to show antimicrobial properties, **OBJECTIVE 1** of this study is to research antimicrobial potential of aquatic plants.

Experimental design

1. 5.0 g powdered aquatic plants were extracted with 50 ml hot DI water, shake for 5 minutes;
2. Filter the solution, dilute for different extract concentration (0 control, 1/10, 1/100, 1/1000, and 1/10000);
3. Diagnostic sensitivity test agar was used for bacteria media, the agar was sterilized by autoclaving and 25 ml portions were dispensed in presterilized Petri dishes;
4. The hole-plate diffusion method was used. Two sets of controls were used. One was the organism control and another was to check for sterility;
5. The agar plates were homogeneously inoculated with an inoculum consisting of about 106 microorganisms/ml phosphate buffered saline (PBS) and 12-mm cores of agar were removed from five positions on the seeded agar dish;
6. Four wells were aseptically filled up with 0.2 ml of plant extract, whereas the fifth hole filled with 0.2 ml of neomycine (500 t~g/ml) or nystatine (500 units/ml) in physiological Tris-buffer (pH 7.0);
7. Holding at 4°C for 1 h and incubation for 1 day at 37°C;
8. Then measure the diameters (mm) of the inhibition zones.





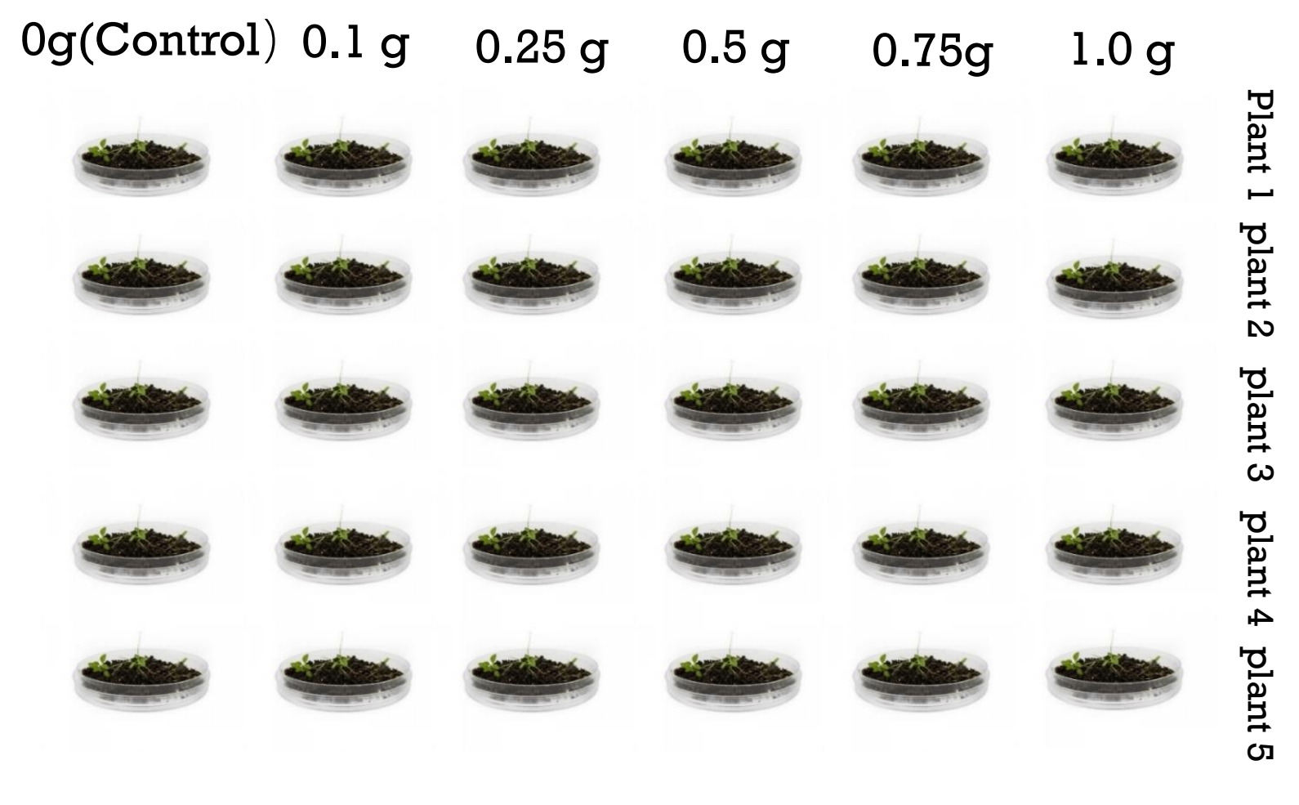
Mueller Hinton Agar (MHA): Composition, preparation and uses. Retrieved from: https://goo.gl/images/7pGpdm

Bioherbicides

- Weeds have been considered the most troublesome abiotic factor causing yield reduction since the beginning of agriculture.
- The use of allelopathy for weed control had considerable importance in last few decades, due to issues regarding the sustainability of chemical weed control methods.
- It is found that Phytotoxic chemicals released by different aquatic weeds might have a significant inhibitory influence on germination, growth and yield of field crops. (Abbas et al. 2016 *Planta Daninha*)
- Residues of aquatic weeds have allelopathic effects which can affect the growth of next season crop plants especially wheat in rice-wheat cropping system through their water soluble allelochemicals. (Abbas. et al. 2016 *Planta Daninha*)
- The experiment of allelopathic effects on root growth of rice, lambsquarters, and other plants shows that *Pistia stratiotes L.* and *Lyngbya wollei* as amendments, had an overall negative effect on germination of all the species. (Bhadha et al. 2014 *Sustain Ag Research*)
- However, aquatic weeds might also be used as a potential organic alternative to chemical weed-control, due to the higher susceptibility of terrestrial weeds to the phototoxic chemicals released by aquatic weeds.
- Based on the fact those aquatic plants commonly have effects on algae and other plant species, **OBJECTIVE 2** of this study is to assess allelopathic effects of aquatic plants on common weeds.

Experimental design

1. 10 seeds of amaranth and nutsedge were planted in 5 groups of petri dishes;
2. Six rates of 0.0 g, 0.1 g, 0.25 g, 0.5g, 0.75g, 1.0 g grounded powder of aquatic plants will be added to the seeds;.
3. Petri dishes were sealed with Parafilm paper and placed in growth chamber with day/night temperature of 25 °C, and 78% relative humidity for 30 days;
4. After 30 days, we will access the germination (root length) and biomass.



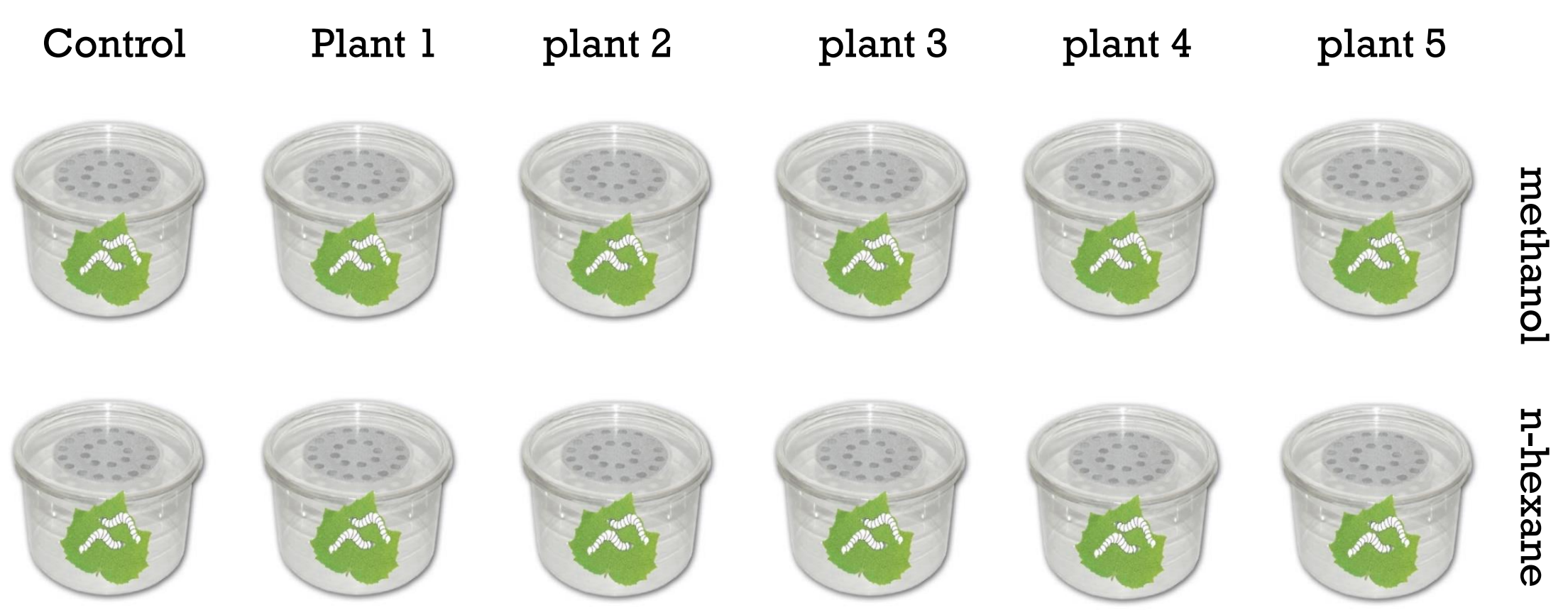
Bhadha et al. 2014
Sustainable Ag. Research

Insecticides

- Florida's warm and humid climate is ideal for the development of pest populations.
- In the past, people used synthetic insecticides. The problems were that they have negative effects on soil properties, the microbial ecological environment, and nutrients cycling, and human beings.
- The search of alternative means of pest control is warranted to minimize the use of synthetic chemicals. Plant-derived chemicals offer great potential for pesticides because they are biodegradable and comparatively safer for the environment. (Petroski and Stanley, 2009 *Agric Food Chem*)
- Plants products have been successfully exploited as insecticides, insect repellents, and insect antifeedants. The most successful example is pyrethrin extracted from Chrysanthemum species as an insecticide. (Duke et al.1990 *Advances in New Crops*)
- It is proved that methanol and n-hexane extracts of aerial parts of E. crassipes significantly reduce the feeding rate of polyphagous insect pest, S. litura. (Lenora et al. 2017 *Asian J of Plant Science and Research*)
- There is still paucity of information on insecticidal potential of aquatic plants. Hence, **OBJECTIVE 3** of this study is to explore insecticidal potential of aquatic plants.

Experimental design

1. 100 g of aquatic powdered plant were subjected to hot extraction using soxhlet apparatus with methanol and n-hexane as solvents;
2. After distillation, extracts were recovered from the solvent by subjecting to rotary evaporator. The same procedure was replicated thrice to get optimum extract;
3. 200 mg of the methanol and n-hexane extracts was made up to 10 ml (20,000 ppm) and these samples were used for the study;
4. Larvae of rice-cotton cutworms were used in this study; the culture was maintained on Ricinus communis L. in container at room temperature (25±2℃) ;
5. 3-4 h starved worms were introduced into the container where pre-treated leaves with extracts and control leaves with no treatment were placed and the worms were allowed to feed;
6. After treatment the leaves were spread in a platform and images were taken into the software called QWin using a CCD camera;
7. Calculate the leaf consumed areas.



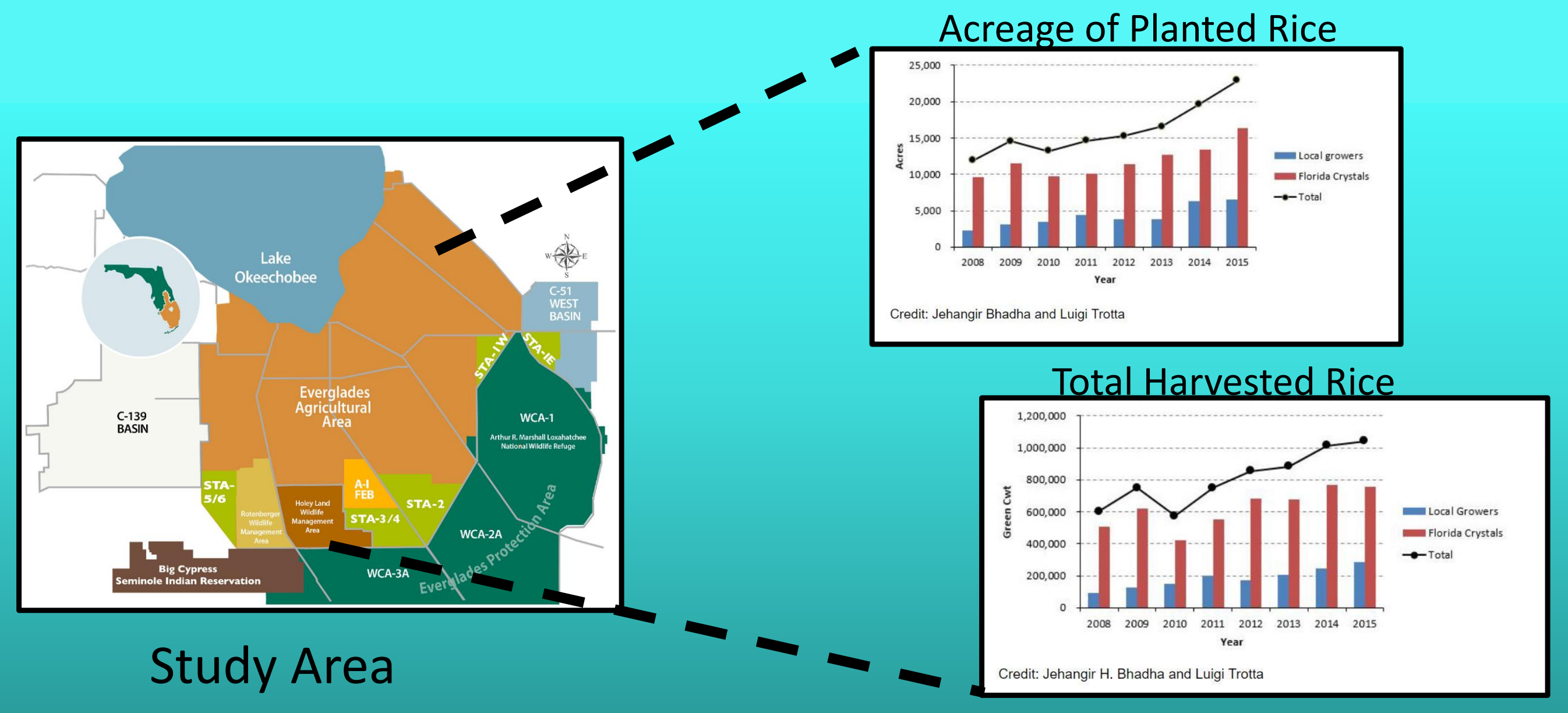
Digging a Little Deeper: An analysis on Environmental Education in Soil and Water Science for Rice Production

Leandra Gonzalez (lgonzalez7@ufl.edu) and Jehangir H. Bhadha

UF IFAS UNIVERSITY of FLORIDA
Soil & Water SCIENCES
Everglades Research & Education Center

Introduction

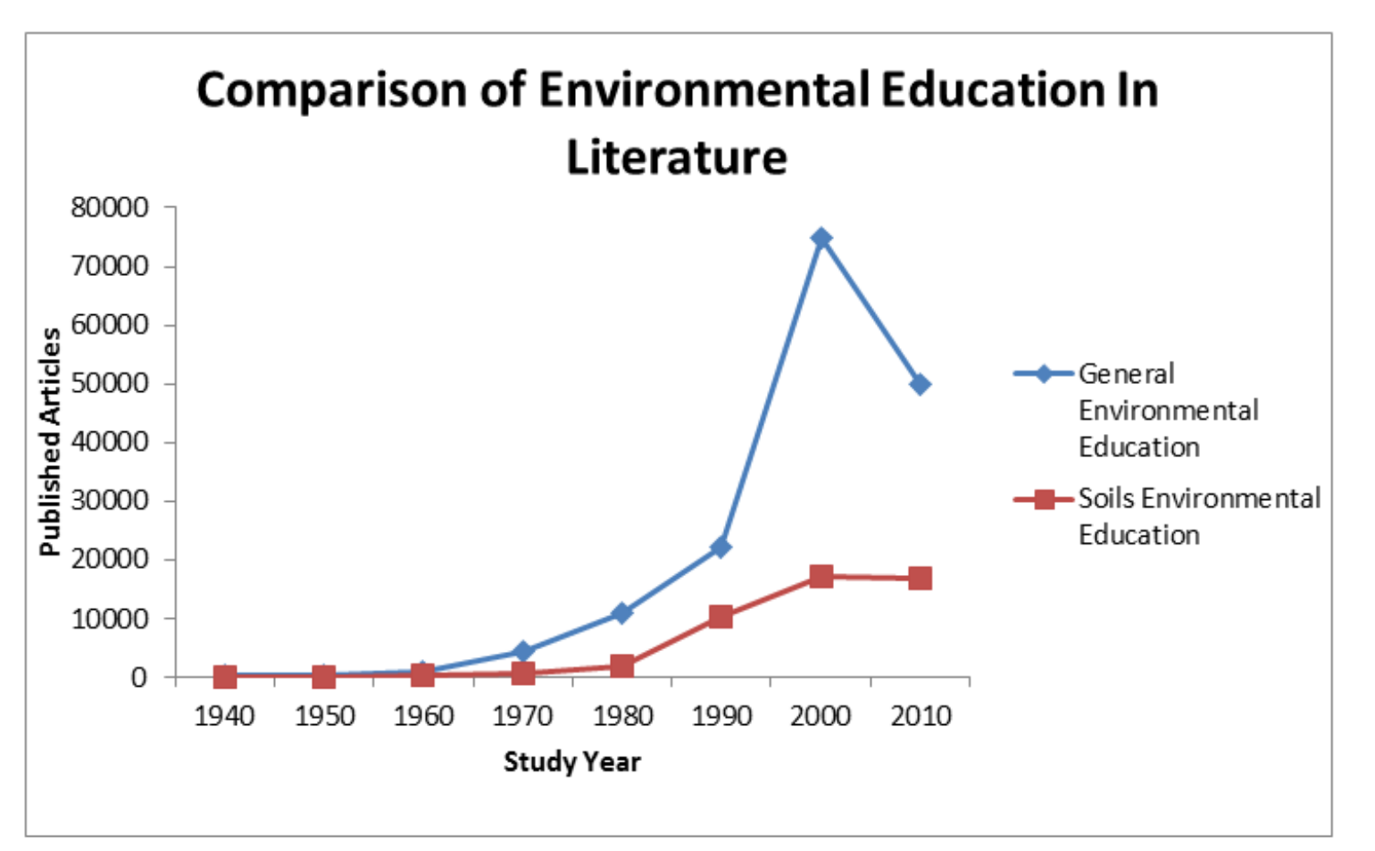
With unprecedented population growth and subsequent intensification of agricultural practices, soils are becoming degraded as time passes. In order to slow this degradation, growers need to have access to information about their soils to ensure best management practices are being used in their operations. This is especially important when addressing soils used to produce rice, being one of the worlds' major food crops, covering approximately 11% of global farmland. To approach this issue, a thorough analysis was conducted to compare environmental education in the growing community.



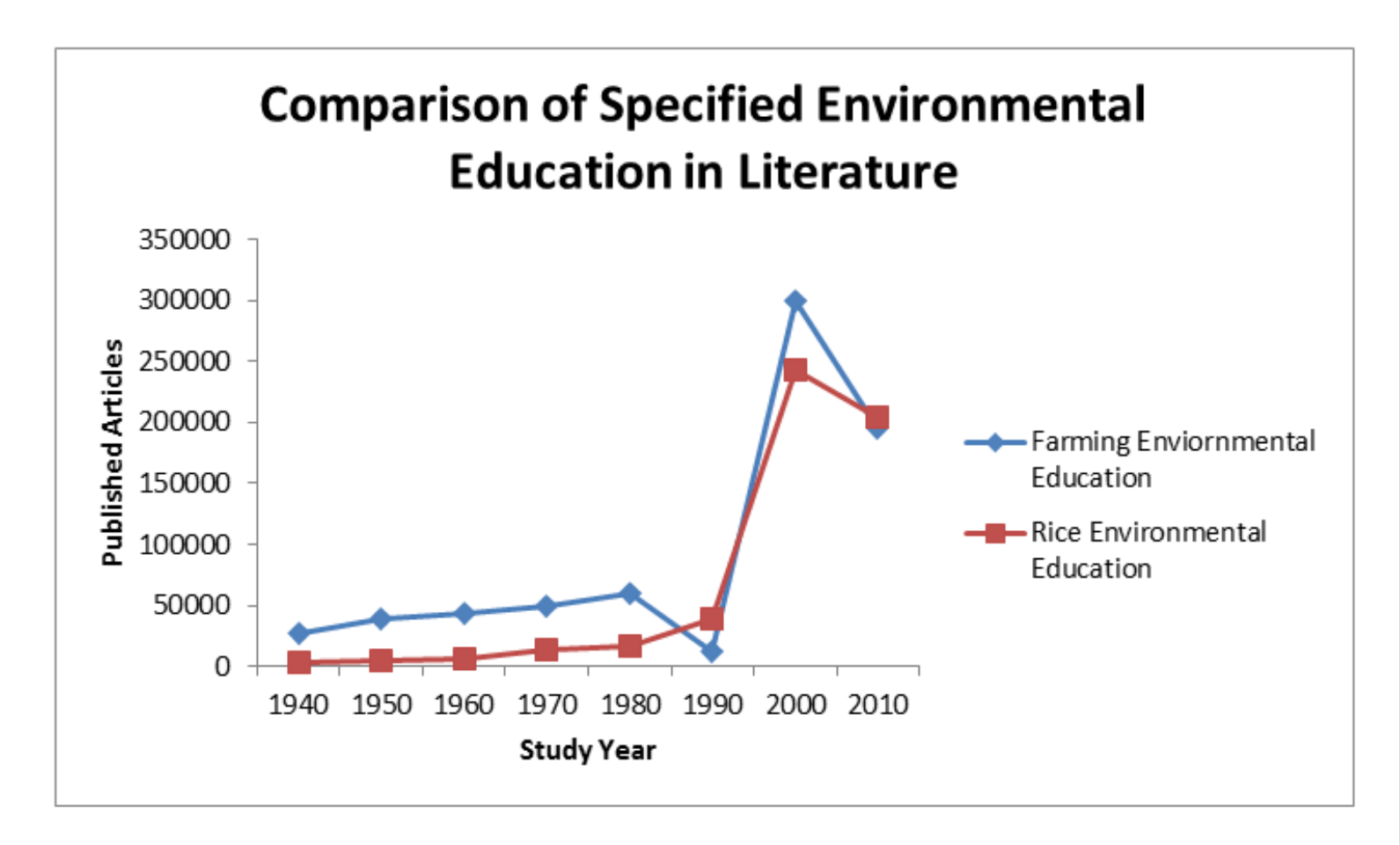
Methods

In order to gain an understanding on the role of environmental education and outreach pertaining to rice production throughout a variety of institutions, two literature assessments were conducted reviewing publications from 1940-2018. Initially, it began with comparing the trends of using environmental education in a general aspect versus using environmental education for soils within published articles. It then focused on comparing environmental education within general farming practices to global rice production in published literature. The occurrence of results were graphed and compared for analysis.

Results



Graph 1: Literature search on " Education outreach soils" VS "Environmental education outreach."



Graph 2: Literature search on "Environmental education rice production" VS "Environmental education farming."

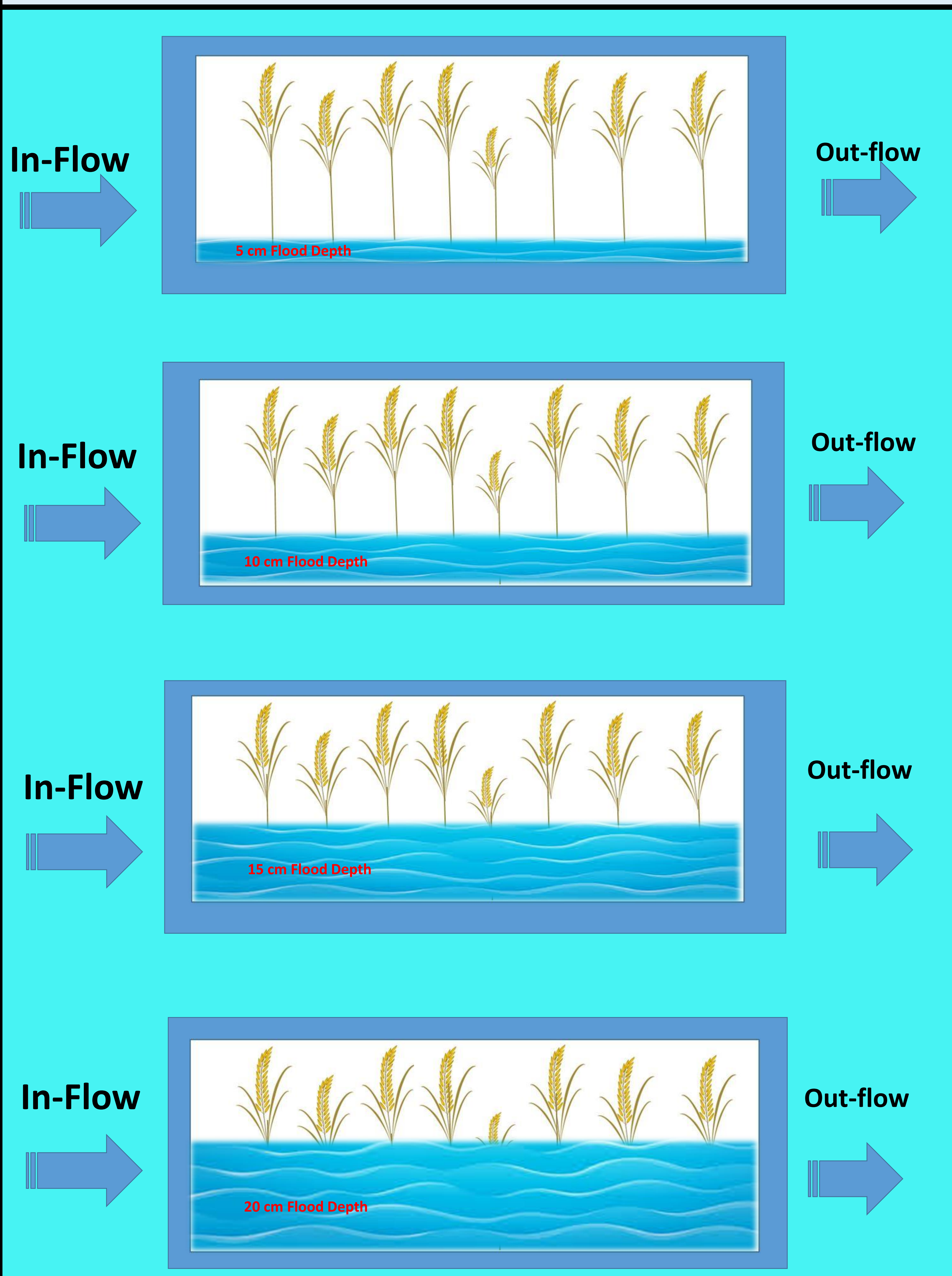
Discussion

Graphs 1 and 2 represent the comparison between publications that have been released from 1940-2018 that has key words including "education outreach soils" (red 1) VS "environmental education outreach" (blue 1) and "environmental education rice production" (red 2) VS "environmental education farming." Though there is a general increasing rate, a gap between the red and blue remains as years progress.

Future Application

To close this gap, scientists can use their knowledge base for practical applications within the field. To build on this, a field study will be conducted to compare rice yields to flood depths, leading individuals to implement more sustainable growing strategies in their organization. From this, a nutrient budget will be developed and outreach services and environmental education will be offered to local growers, community members and other researchers to help bring about the best management practices for rice production in Florida that will contribute to closing the gap between scientific knowledge and environmental education.

Proposed Experimental Design



- Long-grained rice (Taggart) is to be planted with different flood levels through treatments 1-4 at 5cm, 10 cm, 15 cm and 20 cm respectively.
- Drainage water will be analyzed for TP, TN, BOD and TSS concentrations, during the spring-summer growing seasons of 2019 and 2020.
- A phosphorus budget will be developed by calculating the different in Phosphorus concentration between the inflows and outflows of each treatment through water quality sampling, soil sampling and vegetation sampling.
- From developed P budget, education and outreach programs will be created and shared with South Florida farmers to help ensure best management practices for rice production.

Literature:

Bhadha, J.H., Trotta, L., VanWeelden, M. 2016. Trends in Rice Production and Varieties in the Everglades Agricultural Area. University of Florida IFAS EDIS Publication# SL439.
Hartemink Alred, E and McBratney Alex. "A soil science renaissance" *Geoderma, 148* (2): pg. 123-129, 18 September, 2008.
Jury William A and Vaux Henry, "The role of science in solving the world's emerging water problems." *PNAS, 102* (44): pg. 15715-15720, 1 November, 2005.
Wilson, C. "Major world food crops." Global Agroecosystems, August 27, 2018, University of Florida, Gainesville, Florida. Course Lecture.

Investigating Biological Soil Crusts and Nutrient Availability in Citrus Agroecosystems

Clayton J. Nevins, Patrick Inglett, and Sarah Strauss
University of Florida, Soil and Water Sciences Department, Gainesville, Florida

BACKGROUND

- ❖ Biological soil crusts (biocrusts) were recently discovered in Florida citrus agroecosystems (Figure 1).
- ❖ Biocrusts are naturally-occurring phototrophic consortium of microorganisms on the soil surface, common in desert ecosystems, and recently identified in agroecosystems.
- ❖ Biocrusts in desert ecosystems are known to increase inorganic nitrogen (N) availability, enhance soil moisture, and build soil carbon (C) stocks.
- ❖ Understanding the impact of biocrusts on soil physical, chemical, and biological properties has the potential to help optimize citrus management to promote beneficial microorganisms (biocrusts) and increase citrus economic productivity by reducing synthetic fertilizer requirements.

Figure 1: Biocrusts, which resemble a black mat of algae and moss, surround young trees in citrus agroecosystems throughout Florida

OBJECTIVES

- ❖ Develop a comprehensive understanding of how the biocrusts in citrus agroecosystems in Florida impact soil nutrient (N and C) dynamics and soil physiochemical properties (pH and moisture)
- ❖ Determine if biocrusts are a source of N for citrus at N demanding growth stages using stable isotope tracing techniques
- ❖ Investigate soil microbial biomass C and N, and soil microbial community function, diversity, and composition at citrus critical growth stages

SAMPLING SITES

- ❖ Soil cores were sampled from three field sites in Central and Southwest Florida (Figure 2).

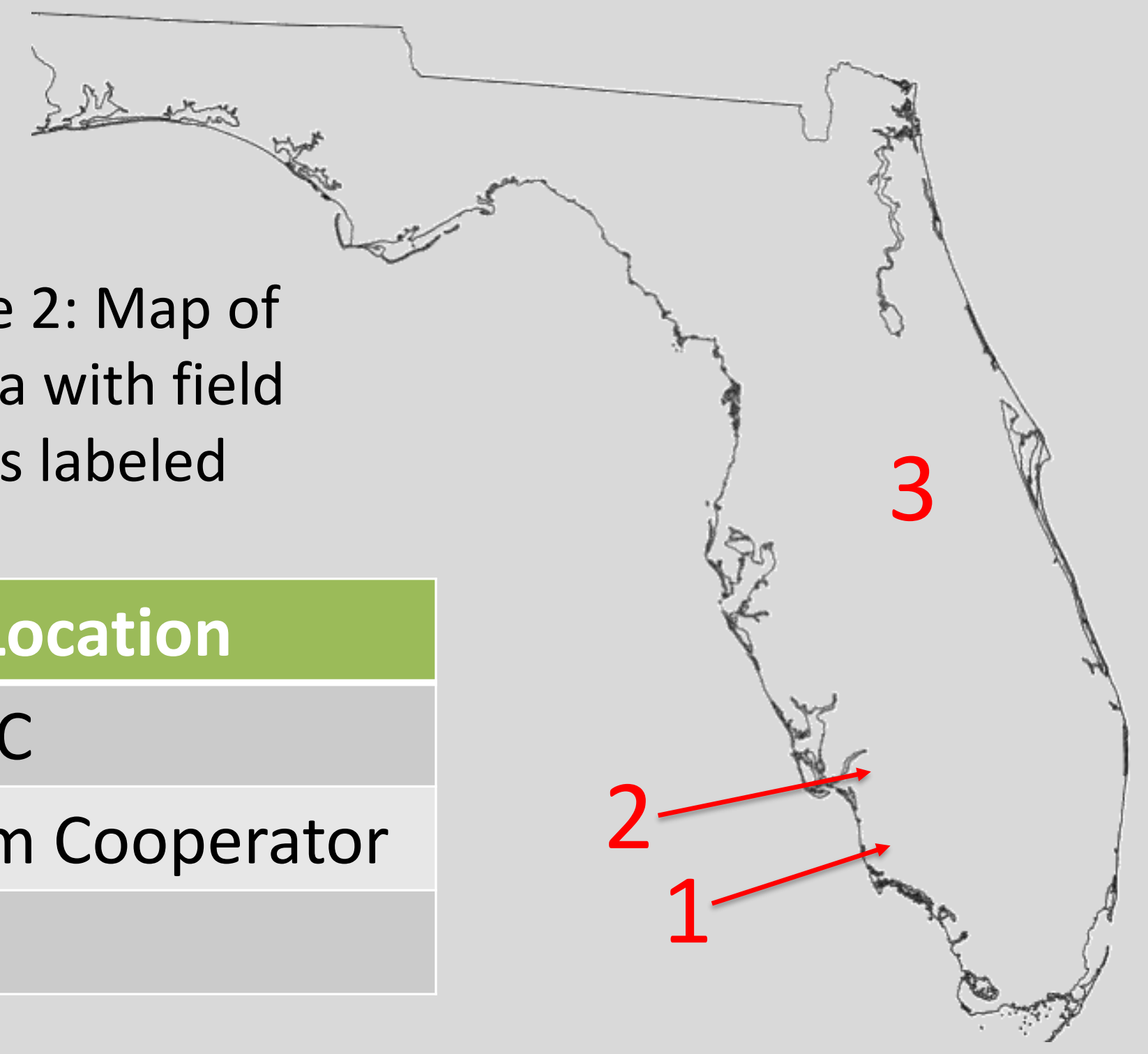


Figure 2: Map of Florida with field sites labeled

	Sampling Date	Location
Site 1	August 30, 2018	SWFREC
Site 2	August 30, 2018	On-farm Cooperator
Site 3	September 13, 2018	CREC

SAMPLING STRATEGY

Figure 4 (Below): Partitioning soil into layers

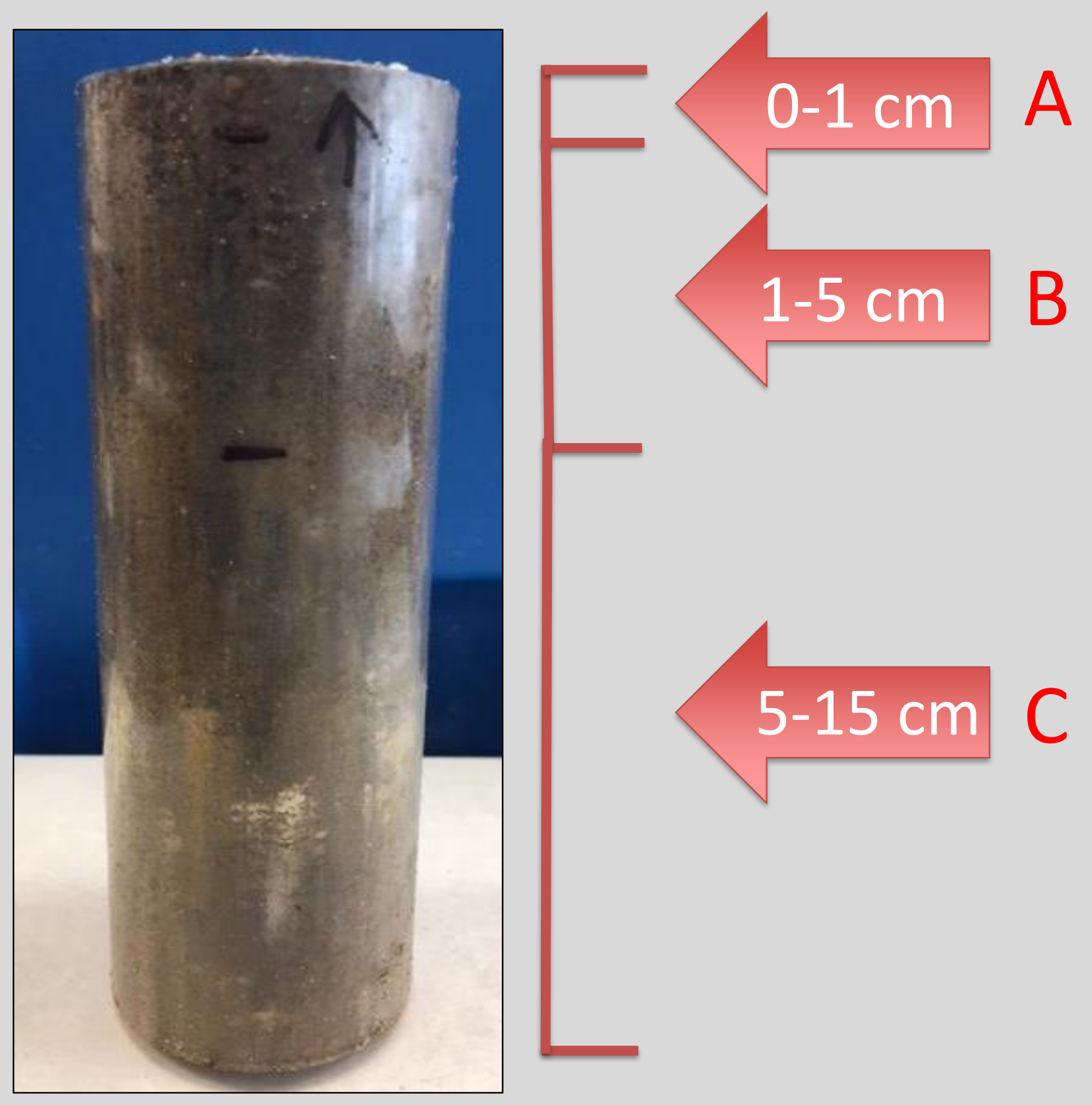
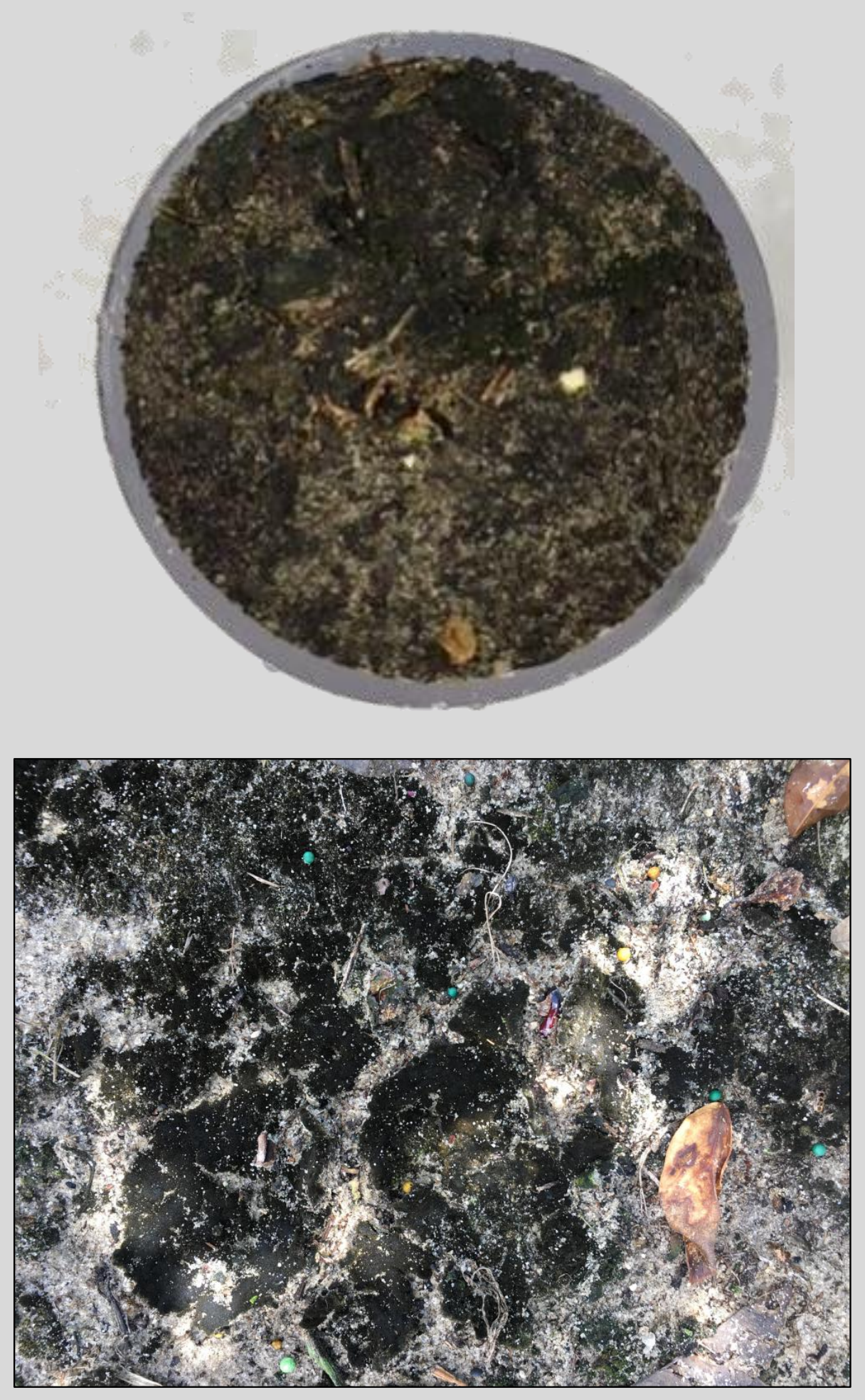


Figure 3 (Left): Soil sampling in citrus groves in Southwest Florida

Figure 5 (Below): Top-down view of soil core



- ❖ Soil cores from areas near citrus trees with biocrust and without biocrust (control) were collected at each sampling site (Figure 4).
- ❖ Soil cores were partitioned into three layers:
  - Layer A- 0-1 cm
  - Layer B- 1-5 cm
  - Layer C- 5-15 cm

PRELIMINARY RESULTS

- ❖ Biocrust soil at Site 3 had significantly higher soil moisture content than control soil within each soil layer (Figure 6).
- ❖ There was significantly more extractable NO_3^--N available in the biocrust soil compared to the control in layers A and B at Site 3 (Figure 7).

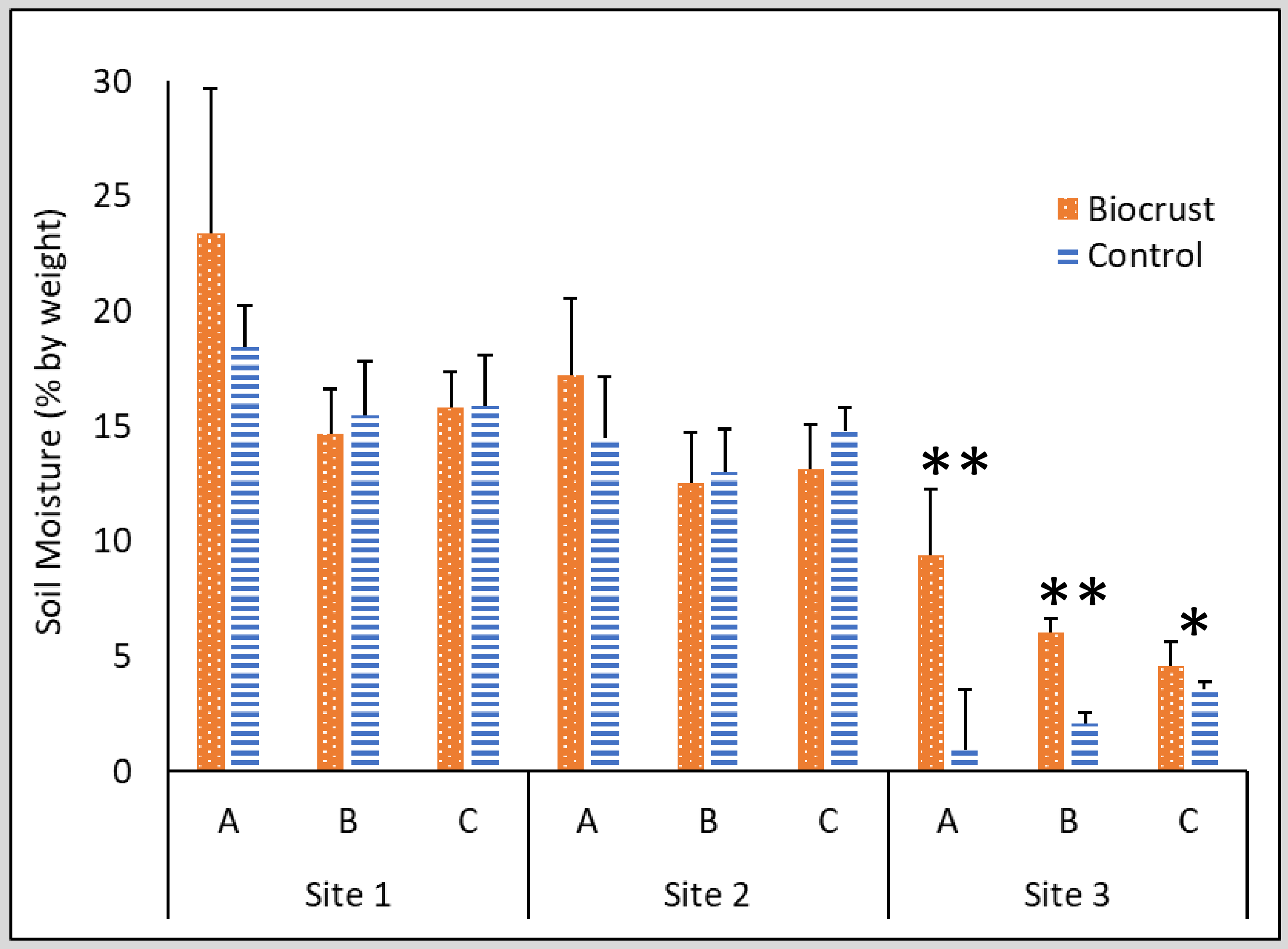


Figure 6: Soil moisture content for each soil core depth at sampling sites (n=6). Significant differences between biocrust and control within site and soil layer identified with **($p < 0.05$), *($p < 0.1$)

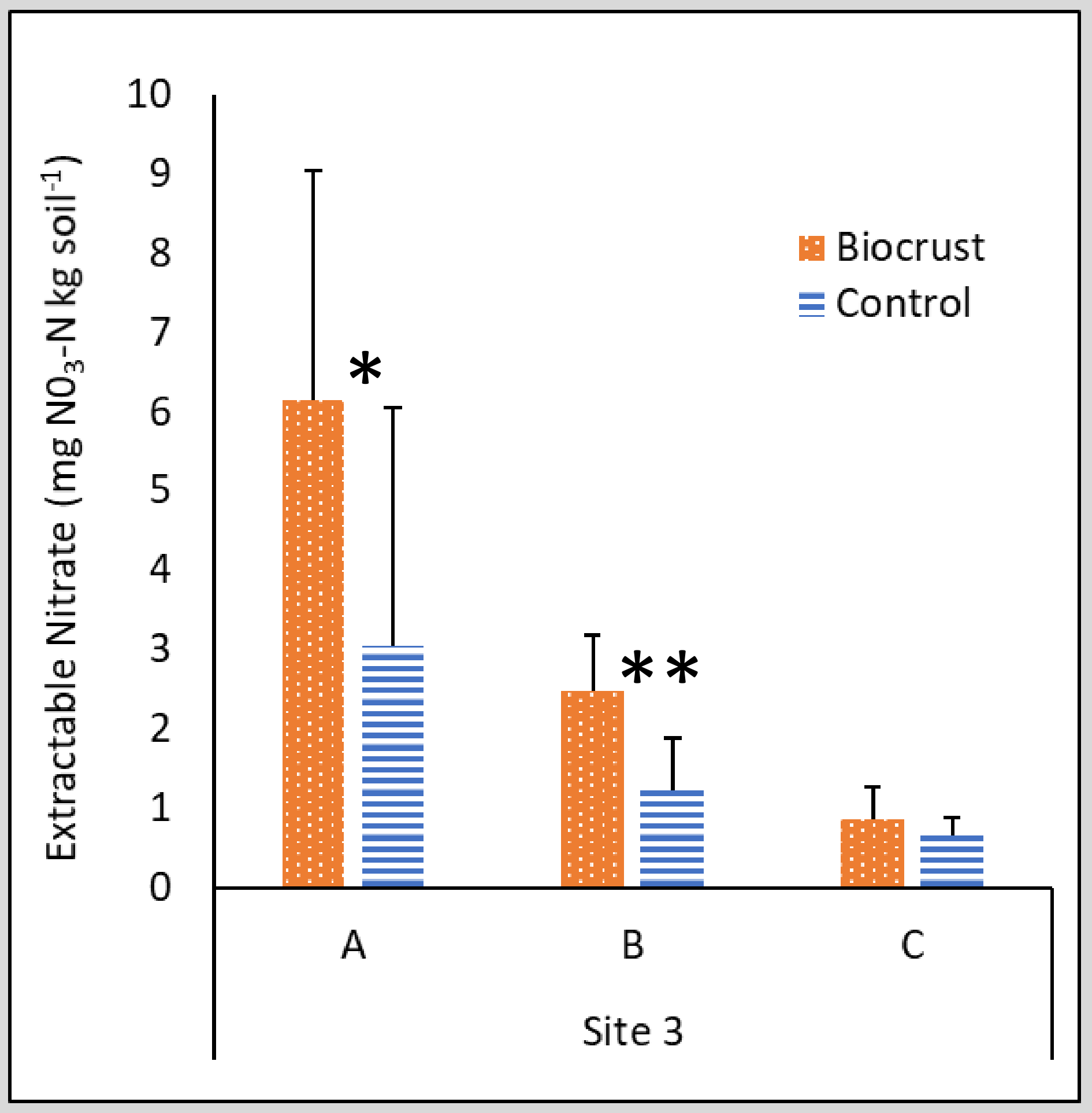
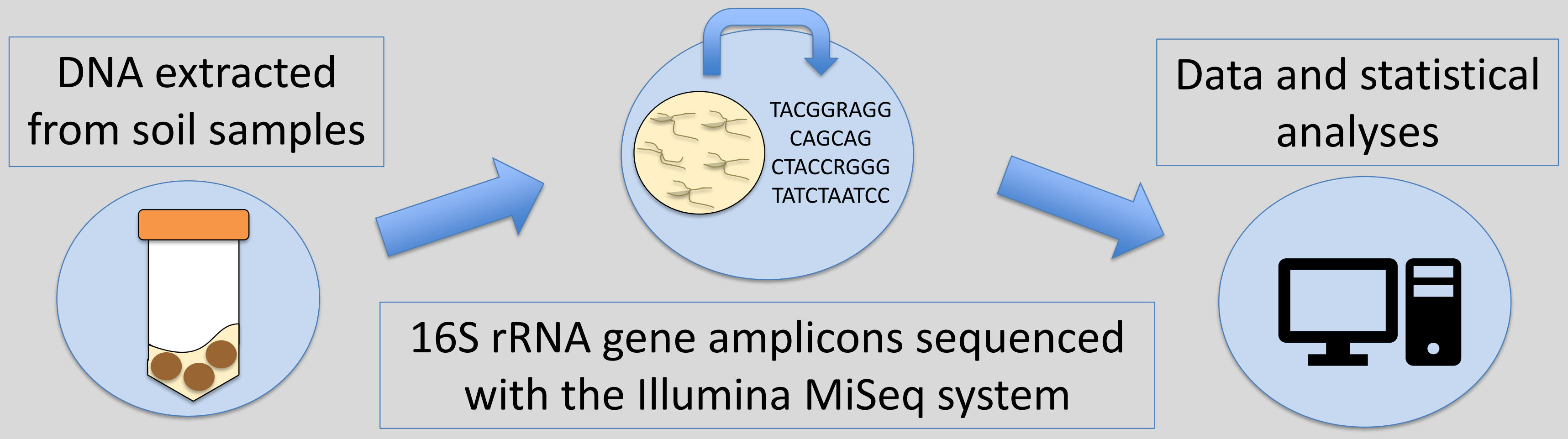


Figure 7: Soil extractable $NO3^-$-N for each soil core depth at Site 3 (n=6). Significant differences between biocrust and control within soil layer identified with **($p < 0.05$), *($p < 0.1$)

- ❖ Overall, results indicate that biocrusts have the ability to elevate soil moisture levels when soils are water limited and increase levels of plant available N in the 5 cm below the crust compared to non-crusted soil.

FUTURE APPROACHES

- ❖ Soil microbial community diversity and composition of biocrust and control soil will be determined using 16S rRNA gene sequencing (Figure 8: Right).
- ❖ Microbial community composition will be correlated with soil nutrient status and bioavailability



DNA extracted from soil samples

16S rRNA gene amplicons sequenced with the Illumina MiSeq system

Data and statistical analyses

ACKNOWLEDGEMENTS

- ❖ Sophia Barbour, Kira Sorochkina, Devin Leonard, and Lindsey Mikell of the Wetlands Biogeochemistry Laboratory at the University of Florida
- ❖ University of Florida Southwest Florida Research and Education Center (SWFREC) Soil Microbiology Laboratory
- ❖ Dr. Lauren Diepenbrock of the Citrus Research and Education Center (CREC)
- ❖ USDA-NIFA Award #: 2018-67019-27797

Nitrogen Fixation Potential and Cyanobacterial Composition of Biocrusts in an Agricultural Ecosystem

Kira Sorochkina[1], Patrick Inglett[*1], Sarah Strauss[*1, 2]

* Faculty advisors; 1 - Soil and Water Sciences Department, Gainesville, Florida; 2 – Southwest Florida Research and Education Center, Immokalee, Florida

Background

- Growers observed biocrusts in citrus groves.
- Biocrust - a microorganism community living in the top centimeter of soil.
  - Extensively studied in arid ecosystems
  - Not studied in a mesic agroecosystem.
  - Have nitrogen fixing cyanobacteria
- Biocrust's potential to provide a natural source of fertilizer for the crops through N fixation could translate into savings by using less artificial fertilizer.

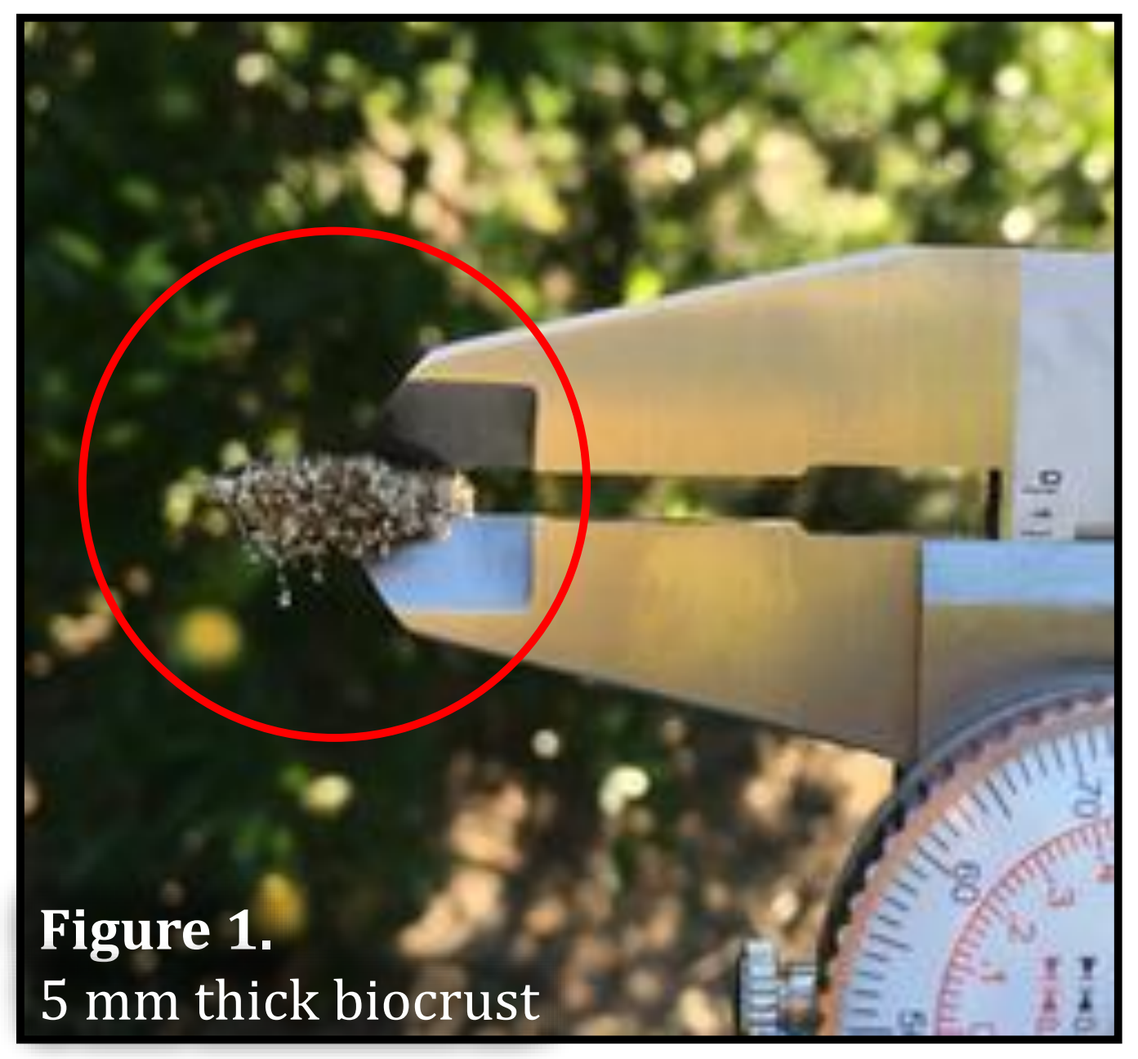


Figure 1.
5 mm thick biocrust

Objectives

- Compare biocrusts from citrus groves to each other and against common characteristics of a typical arid land biocrust through microscopy.
- Design a method to characterize biocrust community composition
- Design a method to characterize agroecosystem biocrust N fixation potential

Preliminary Study

Hypotheses

1. Biocrusts in citrus groves will have cyanobacterial genera analogous to the Southwestern deserts, including N fixing cyanobacteria.
2. The cyanobacterial community composition will vary between different groves.

Overall Collection

- 6 biocrust covered and 6 bare soil samples at three different citrus groves in Florida:
  UF/IFAS SWFREC: 1 year old trees at the Southwest Florida Res. and Ed. Center
  Commercial Grove: 4-5 year old trees at a private large scale grove
  UF/IFAS CREC: 2-3 year old trees at the Citrus Research and Education Center
  - 3 biocrust replicates: taxonomic characterization (microscopy)
  - 2 biocrust replicates: particle analysis (image J software)

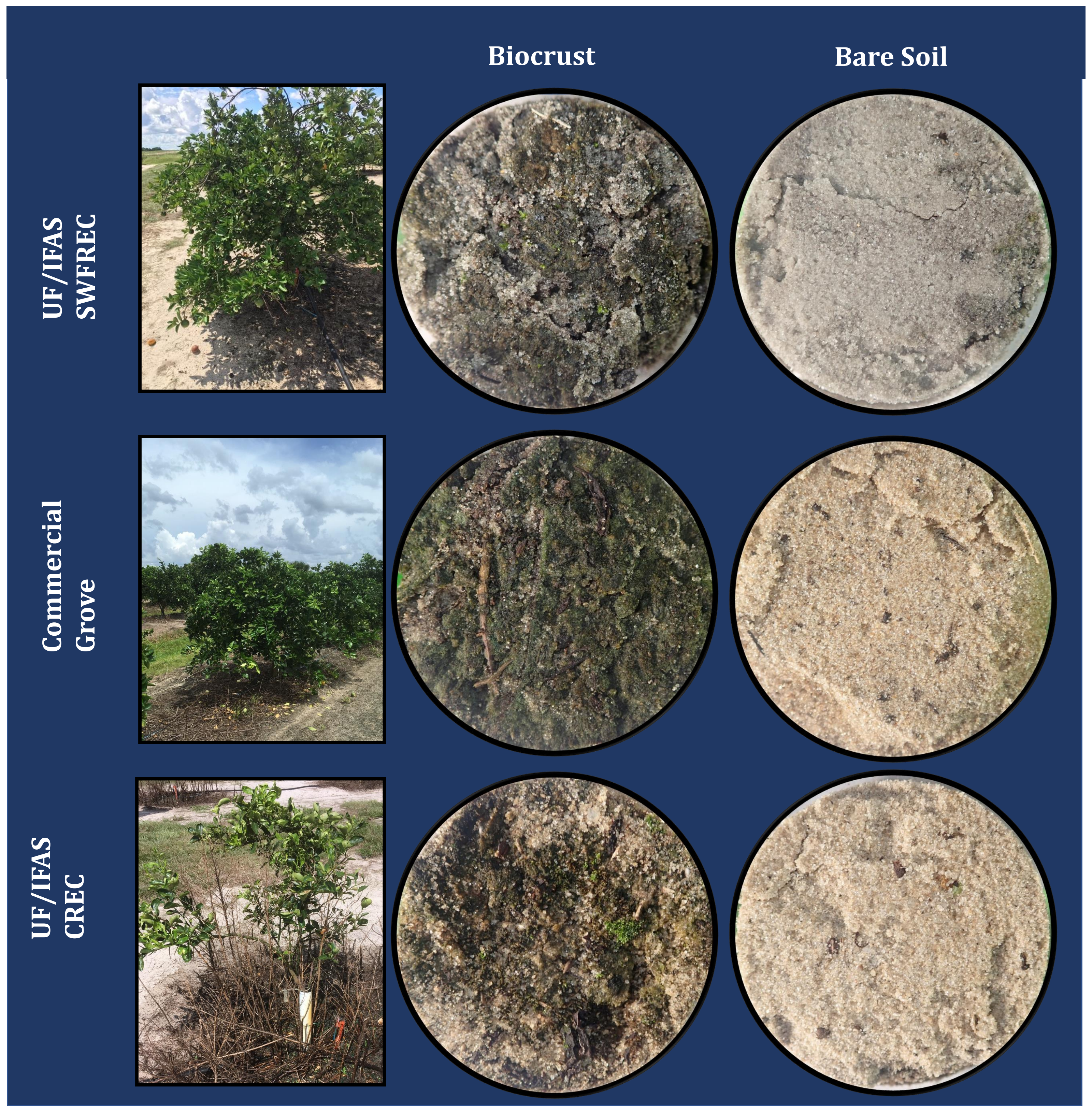


Figure 2.
Citrus trees from 3 different sites and top view of their corresponding samples in petri dishes (5 cm diameter).

Preliminary Results: Microscopy

	UF/IFAS SWFREC	Commercial Grove	UF/IFAS CREC
Filamentous cyanobacteria holding soil particles	✓	✓	✓
Heterocystous N fixing cyanobacteria	✓	✓	Not Observed
N fixing cells visible in heterocystous nitrogen fixing cyanobacteria	Not Observed	✓	Not Observed
Non-heterocystous N fixing cyanobacteria	Not Observed	Not Observed	Not Observed
Total cyanobacterial and algae cell count in a x10 magnification microscopy field	268	1180	293
% Single cell algae in a x10 magnification microscopy field	49 %	47 %	57 %
% Filamentous cyanobacteria in a x10 magnification microscopy field	51 %	53 %	43%
Unique Character	Filamentous *Leptolyngbya* spp.	➢ Abundant detritus ➢ Abundant moss	Unidentified filamentous cyanobacteria

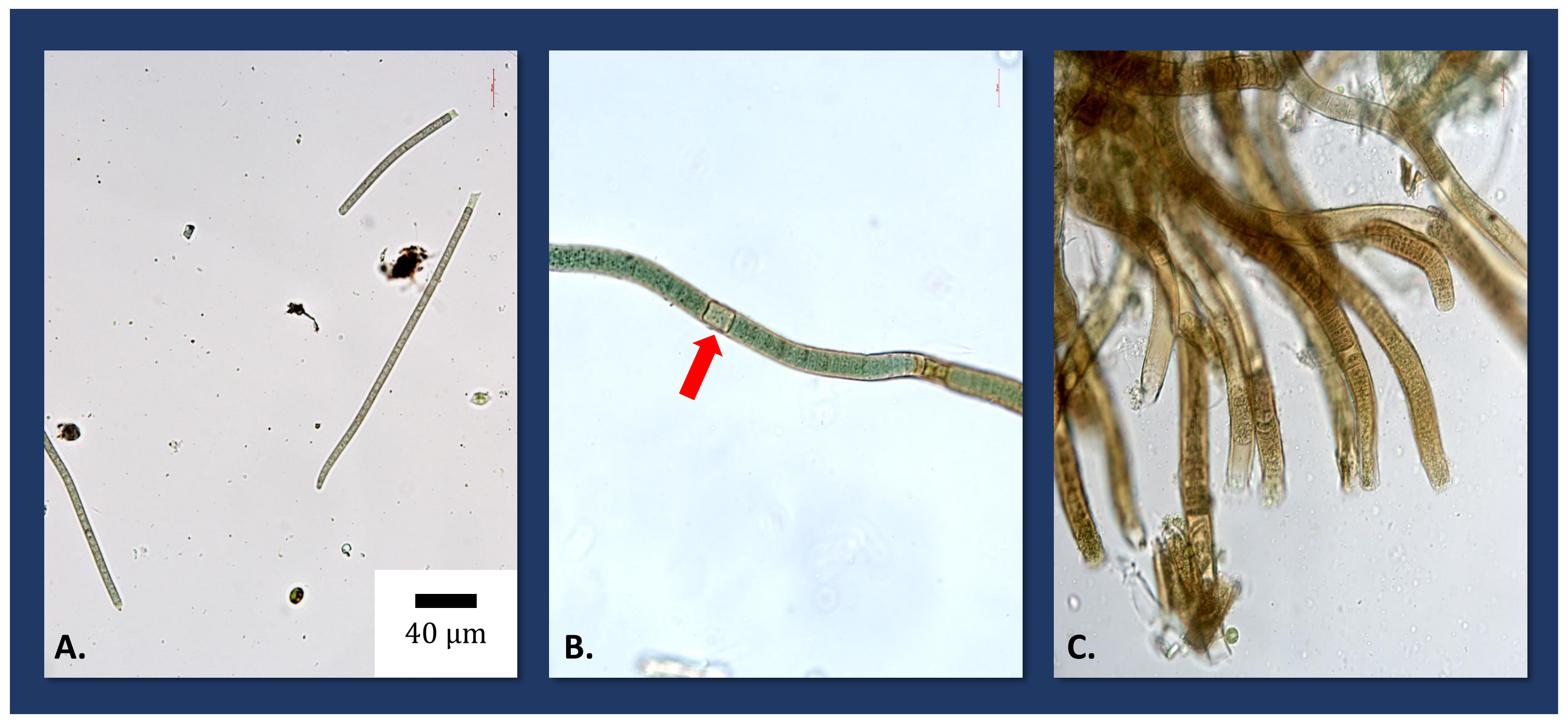


Figure 3.
A. *Leptolyngbya* spp. from UF/IFAS SWFREC; **B.** *Scytonema* spp. with a N fixing cell from Industrial Grove; **C.** Unidentified filamentous cyanobacteria from UF/IFAS CREC.

Arid Biocrust Traits

- ☐ Microorganismal exudates hold soil particles
- ☐ Dominated by few well known cyanobacteria
- ☐ N fixing cyanobacteria
- ☐ Successional stages:
  1. Cyanobacteria only
  2. Cyanobacteria + Algae
  3. Cyanobacteria + Lichens +Algae
  4. Appearance of moss

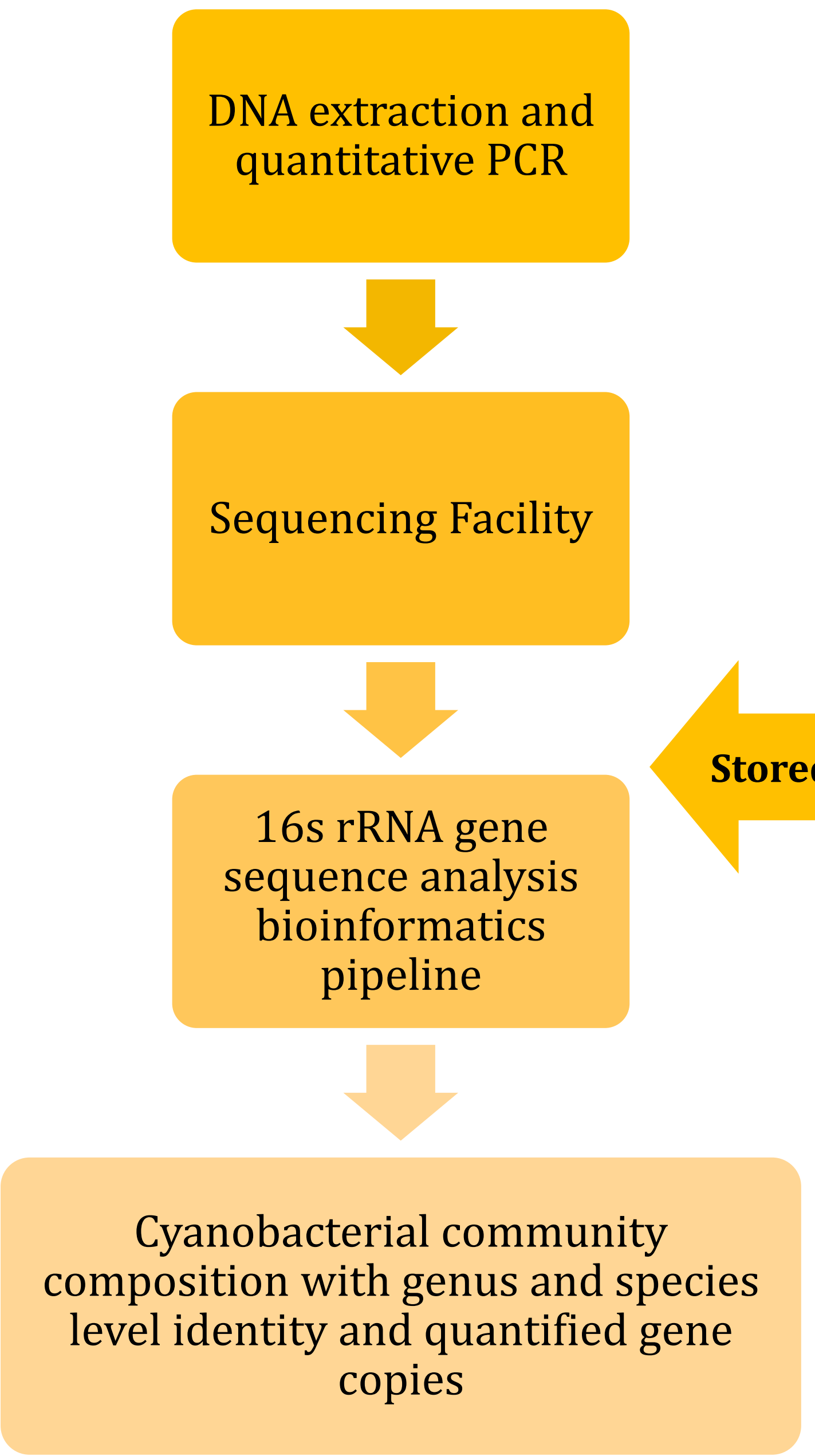
Major Findings

- In comparison to typical arid biocrusts, these biocrusts differ:
  - No *Microcoleus* genera
    - First cyanobacteria to form a biocrust.
  - Higher algae presence
- Appear to differ between different citrus groves
- N fixing heterocystous cyanobacteria are similar to a desert biocrust.

Implications

- Could provide ecosystem services similar to a late successional arid biocrust
- Could amend the soil with a natural fertilizer
- Grove management practices could influence biocrust type.

Future Work



DNA extraction and quantitative PCR

Sequencing Facility

16s rRNA gene sequence analysis bioinformatics pipeline

Cyanobacterial community composition with genus and species level identity and quantified gene copies

Predictions	Preliminary Evidence
The cyanobacterial community composition will significantly vary between different groves.	Microscopically, biocrusts appear to differ between sites.
Biocrusts will have a detectable low range of nitrogen fixation rates due to high available N in artificial fertilizer form.	Low nitrogenase activity has been measured in the biocrusts from other citrus grove sites.

Stored at -80 °C

Figure 4. Field collected sample.

Fresh

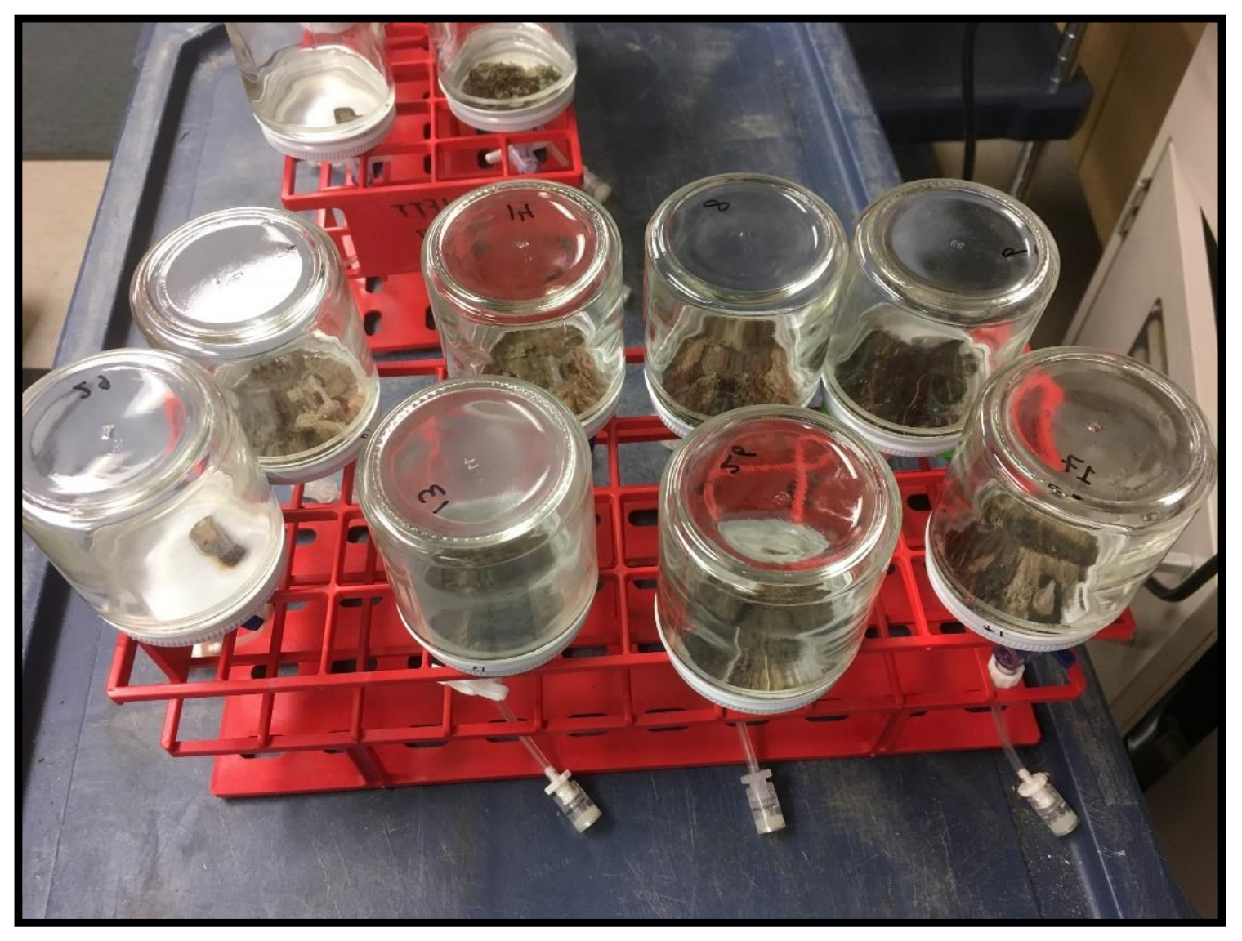


Figure 5. Jars with biocrusts and soil for gas injection/ incubation.

Acetylene and ^{15}N Incubation

- Light and temperature controlled
- Nitrogenase activity
- N_2 fixation rates

Acknowledgements

- Conor MacDonnell and Clayton Nevins of Wetlands Biogeochemistry Laboratory
- Dr. Monika Oli of Microbiology and Cell Sciences Department
- Citrus Research and Education Center
- NIFA Award #: 2018-67019-27797