

Rhizoid and root developmental phenotypes in *Ceratopteris richardii* is affected by bacteria

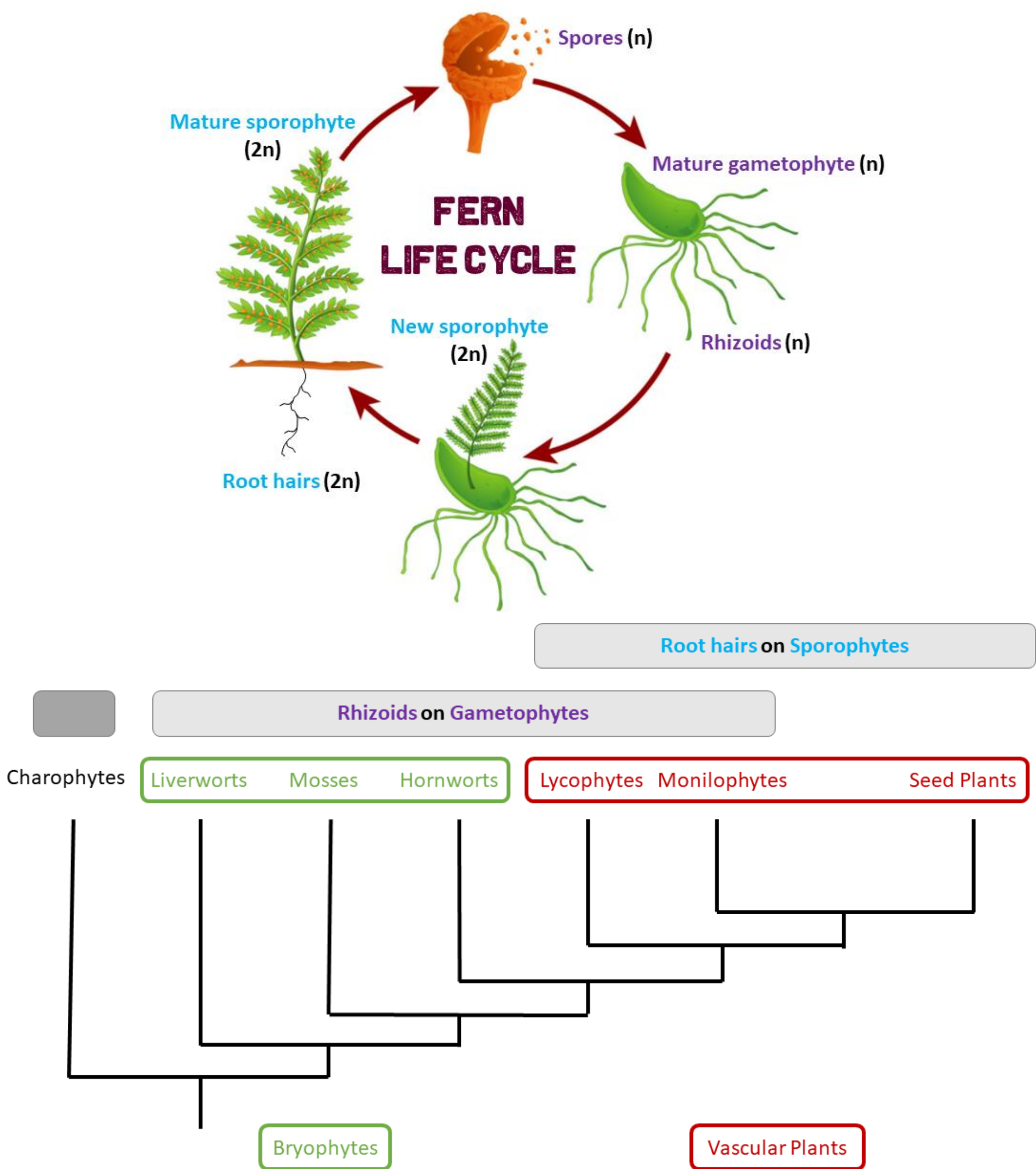


Landen Mayher¹, Jenna Sins¹, Alexis Palmiotto², Nick Devine², Nick Conklin³,
Matt Gacura¹, Mike Ganger¹, and Gary Vanderlaan¹
¹Biology Department, ²School of Engineering and Computing, ³Physics Department
Gannon University, Erie, PA, USA

Abstract

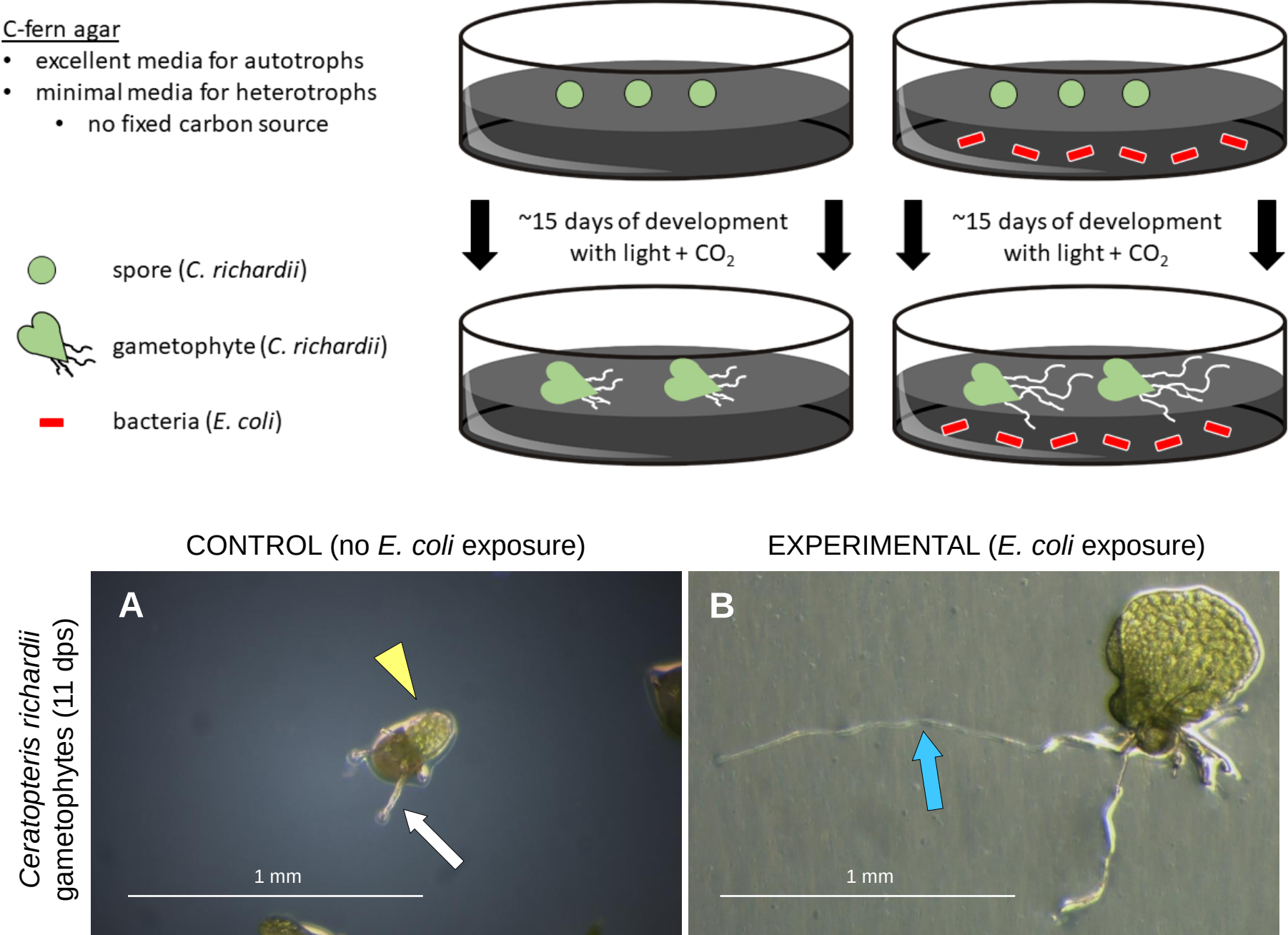
The evolution of terrestrial plants is an approximately 500-million-year-old story. Although all land plants execute an alternation of generations between a haploid gametophyte and a diploid sporophyte stage, the amount of time spent in each of these multicellular stages varies greatly across clades. Additionally, plants coevolved with soil microbes. Of all land plants, ferns uniquely generate rhizoids as well as roots during gametophyte and sporophyte stages, respectively. Here we examine the effects of bacterial exposure in an aquatic fern (*Ceratopteris richardii*) during rhizoid & root developmental windows. We are also seeking novel, commensalistic microbes for these phenotypes via an aerial surveillance approach.

Plant Evolution: Alternation of Generations



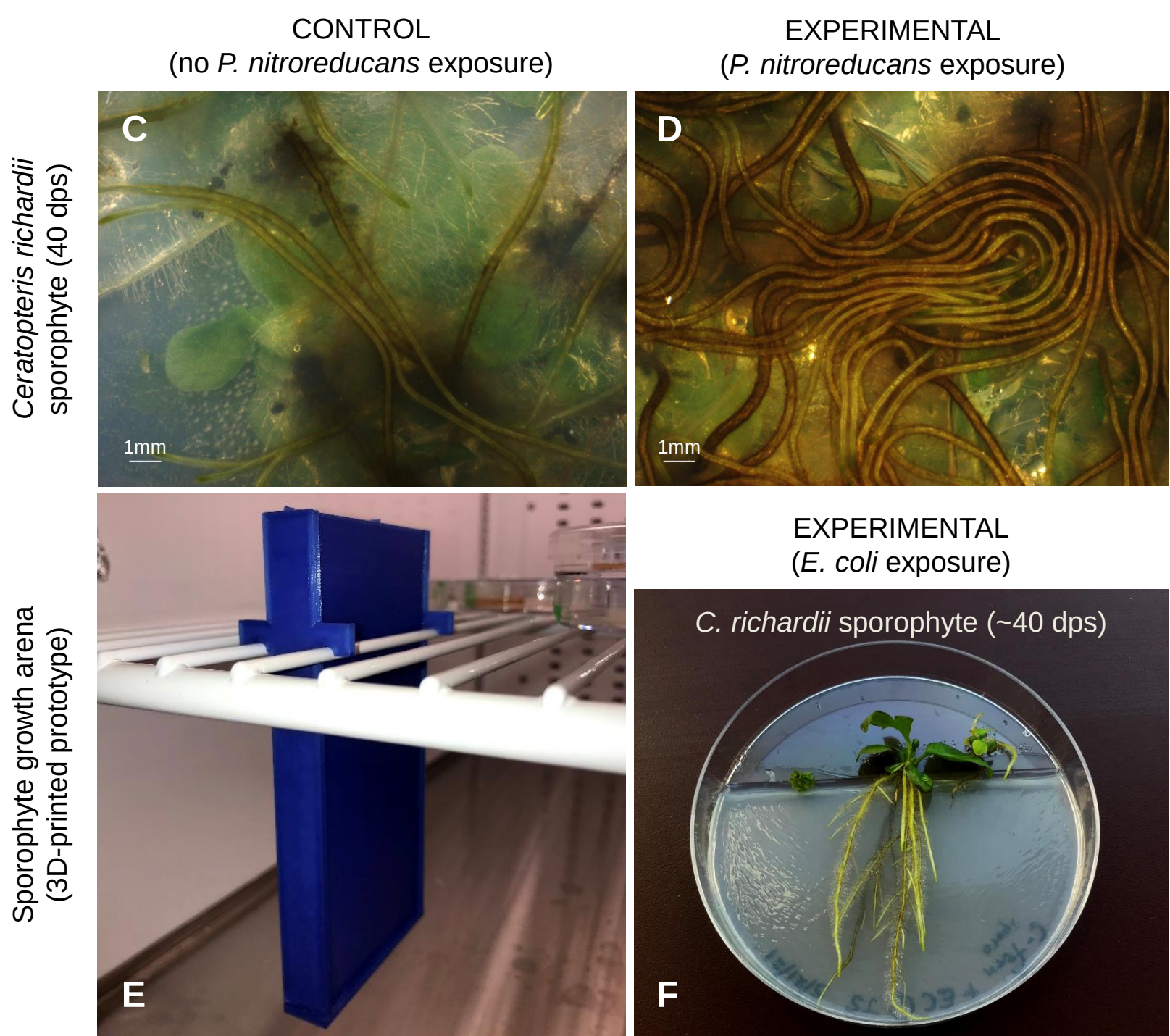
All plant lifecycles execute alternation of generations. For ferns, a meiotically-produced haploid spore germinates into a multicellular gametophyte called a protonema. Flagellated sperm fertilize haploid eggs to form a diploid zygote. Mitoses yield a mature, diploid sporophyte capable of producing haploid spores. All land plants descended with modification from an ancestral charophyte. Bryophytes (mosses) are avascular plants typified by a gametophyte-dominated life-cycle and produce rhizoids but lack roots. All vascular plants in contrast exhibit a sporophyte-dominant life-cycle. Seeded vascular plants (gymnosperms and angiosperms) produce roots but lack rhizoids. Seedless vascular plants (ferns) are a transition organism: gametophyte-stage ferns are bryophyte-like and have rhizoids; sporophyte-stage ferns are angiosperm-like with roots.

Rhizoid Induction (Gametophyte Phenotype)



Pour plates allow us to trap bacteria throughout molten agar, and following solidification, *C. richardii* spores can be sown. Incubation of these sown pour plates within growth chambers allows us to easily test whether the presence of a particular bacterial species can affect fern gametophyte development. **A)** By 11 days-post-sowing (dps), early rhizoids (white arrow) and protonema (yellow arrowhead) are readily visible. **B)** After 11 dps, gametophytes that developed in the presence of *Escherichia coli* exhibit dramatically longer rhizoids (blue arrow).

Root Induction (Sporophyte Phenotype)



For *C. richardii* sporophytes, we tested if *Pseudomonas nitroreducens* can affect root development. Fern spores were initially sown onto pour plates with or without *P. nitroreducens*. After ~15 dps, plates were flooded to allow flagellated sperm to fertilize archegonial chambers. Young sporophytes then developed from such chambers and their roots were imaged about two weeks post-fertilization. **C)** At 40 dps, fern roots that developed in the absence of bacteria are shown. **D)** Exposure to *P. nitroreducens* results in denser, pigmented roots. **E) & F)** To better quantify these root phenotypes, we are currently prototyping several vertical growth arenas.

Fern Commensalist Survey



Media	Growth Target	Selective	Differential
LB	Many bacterial clades	✗	✗
PDA + Strep + Amp	Many fungal clades	✓	✗
FA	Photoautotrophs Cyanobacteria	✓	✗
BAA	Spore-forming rods <i>Clostridium</i> spp. <i>Bacillus</i> spp.	✓	✗
EMB	G- coliforms <i>Enterobacteriaceae</i>	✓	✓
SM110 + Bt-blue	Salt-tolerant cocci <i>Staphylococcus</i> spp.	✓	✓

Previous work at Erie Bluffs examined putative commensalistic microbes (i.e. *P. nitroreducens*) associated with the root structure of a local fern species, *Onoclea sensibilis*. As fern spores and various microbes can be distributed via air, we decided to expand our microbial surveillance from the ground up. We plan to operate a drone at 100 ft altitudinal increments, carrying a payload canister comprising six different bacteriological nutrient media. Following cultivation, microbes will be identified using a combination of DNA sequencing and biochemical assays as well as tested for their ability to alter rhizoid and root development in *C. richardii* gametophytes and sporophytes, respectively.

References

¹Ganger *et al.*, 2019. A soil bacterium alters sex determination and rhizoid development in gametophytes of the fern *Ceratopteris richardii*. **Annals of Botany Company**. 11 (2): plz012.
²Jones and Dolan, 2012. The evolution of root hairs and rhizoids. **Annals of Botany**. 110: 205-212.
³Menand *et al.*, 2007. An ancient mechanism controls the development of cells with a rooting function in land plants. **Science**. 316 (5830): 1477-1480.
⁴Plackett *et al.*, 2018. *LEAFY* maintains apical stem cell activity during shoot development in the fern *Ceratopteris richardii*. **eLIFE**. 7: e39625: 1-34.
⁵Marzec *et al.*, 2015. Root hair development in the grasses: what we already know and what we still need to know. **Plant Physiology**. 168: 407-414.
⁶Rodriguez *et al.*, 2019. Systems biology of plant-microbiome interactions. **Molecular Plant**. 12: 804-821.
⁷Salazar-Henao *et al.*, 2016. The regulation and plasticity of root hair patterning and morphogenesis. **The Company of Biologists | Development**. 143: 1848-1858.
⁸Shibata & Sugimoto, 2019. A gene regulatory network for root hair development. **Journal of Plant Research**. 132: 301-309.
⁹Tam *et al.*, 2015. Conserved regulatory mechanism controls the development of cells with rooting functions in land plants. **PNAS**. 10.1073: e3959-e3968.