

Sword Fern Research Project

Progress Report

October 16, 2020



Haven

Ecology & Research

Accomplishments

Research

We completed the initial preparation of all samples. For the metabarcoding samples, preparation involved dissecting a 5 cm section of each rhizome. After removing the crozier-buds and the outer layer of each rhizome, the samples were surface sterilized in a flow hood and minced using a razor blade. From each sample, approximately 4 g of rhizome tissue was prepared for MPG Ranch, and 1 g was preserved for local storage. Four leaf samples (10 pinnae each) were also surface sterilized. Rhizome samples #1 - #36 and leaf samples #25, #28, #33 and #34 were shipped overnight to MPG Ranch on dry ice. All remaining tissue samples (roots, rhizome, stem, leaf) are being stored at -20° C at SoundBio Lab.

MPG Ranch has recently begun the DNA extraction process (Figure 1). At my recommendation, they are using the oom18s and ITS7-ae primers for sequencing oomycetes.

Andres Barrera has nearly completed his analysis of soil physical properties, including bulk density, moisture content and texture. In our preliminary analysis, we analyzed the soil moisture content at each site and the foliar moisture content (FMC) of the ferns (Figure 2). FMC was significantly lower in the symptomatic ferns at Seward Park ($0.98 \text{ g} \cdot \text{g}^{-1} \pm 0.11 \text{ SE}$) compared with the healthy ferns at both Seward Park ($1.67 \text{ g} \cdot \text{g}^{-1} \pm 0.07 \text{ SE}$, $p < 0.001$) and Schmitz Preserve Park ($2.11 \text{ g} \cdot \text{g}^{-1} \pm 0.14 \text{ SE}$, $p < 0.001$).

Superficially, these results may appear to be consistent with the effects of drought. However, soil moisture content was similar between the three treatment groups, with means ranging from $0.10 \text{ g} \cdot \text{g}^{-1}$ to $0.12 \text{ g} \cdot \text{g}^{-1}$ (Figure 3). Additionally, the foliar moisture content of the ferns was not correlated with the soil moisture content of the sites ($r = 0.06$, $p = 0.72$). These results suggest that site conditions that mediate drought stress have little to no influence on the die-off phenomenon.

Project Management

Project management has consisted of a limited amount of coordination and logistics.

Next Steps

Haven Ecology and Research is nearing completion of the data entry. For long-term storage of the remaining tissue samples, I recommend moving them to a -80° C freezer.

MPG Ranch expects the initial sequencing results to be received in January, 2021. They indicated that their lab may be able to analyze the foliar nutrient content of the remaining leaf samples at cost.

Appendix

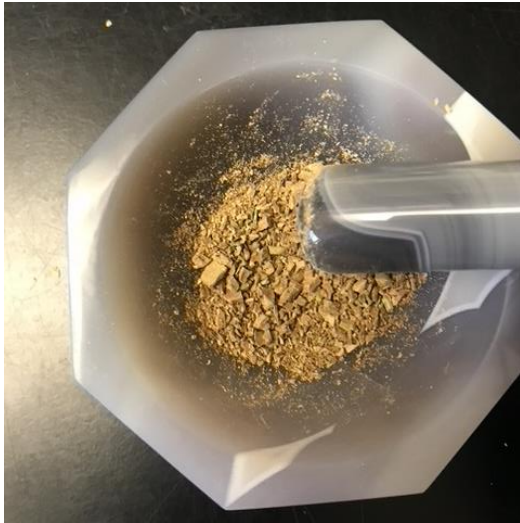


Figure 1: Partially ground freeze-dried rhizome sample at MPG Ranch

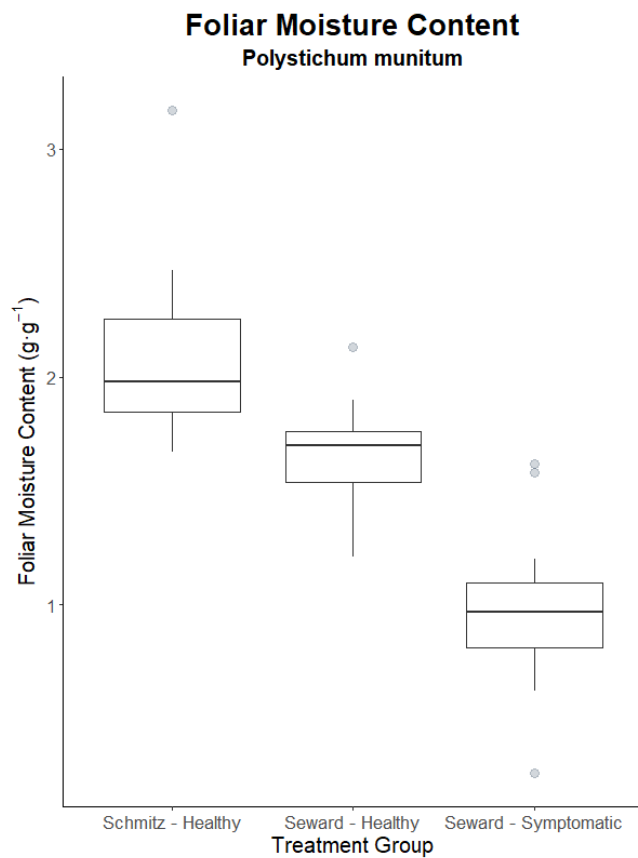


Figure 2: Foliar moisture content of *Polystichum munitum*. Results are displayed as Tukey-style boxplots; bar represents the median value; box represents the interquartile range (IQR); lines represent minimum and maximum values up to 1.5 x IQR; points represent outliers.

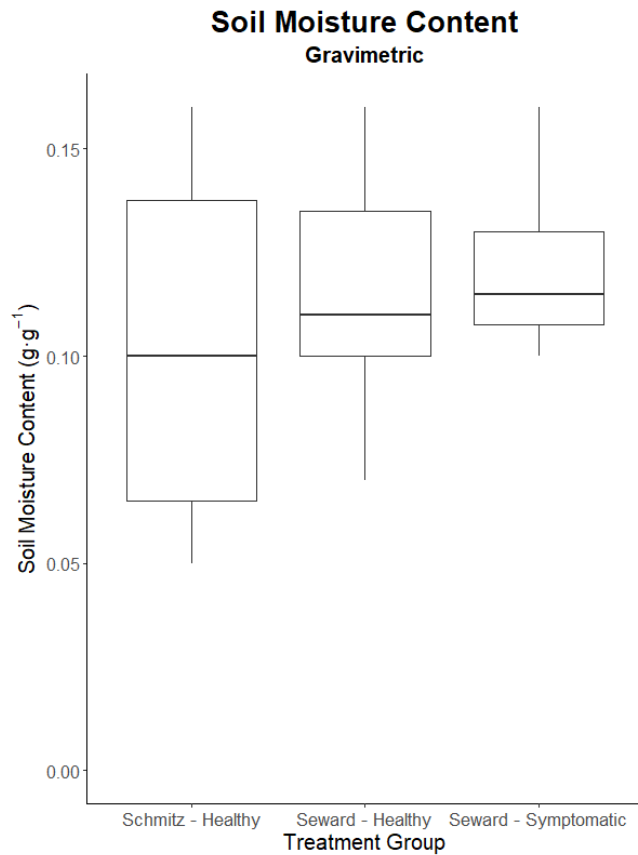


Figure 3: Soil moisture content. Results are displayed as Tukey-style boxplots; bar represents the median value; box represents the interquartile range (IQR); lines represent minimum and maximum values up to $1.5 \times \text{IQR}$; points represent outliers.