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Fern gametophyte response to desiccation is more similar to moss gametophytes than to fern sporophytes

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Introduction

Ferns are important players in various ecosystems. For example, they can act as "ecological filters" by influencing establishment of tree seedlings[1]. Despite their ecological importance, ferns as a group remain understudied. Most previous fern studies have focused on the life stage known as the sporophyte, a vascular plant with stems, leaves and roots. Most sporophytes have anatomical and physiological features that help regulate water loss, like waxy cuticles which cover the leaf surface and slow epidermal conductance to water vapor. However, little is known about ferns' other independent life stage, the gametophyte. In contrast to sporophytes, gametophytes are only a single cell layer thick and smaller (<1cm diameter), lacking cuticles or vascular tissue. Since fern gametophytes lack means to slow water loss, it has been hypothesized that many can tolerate and recover from fluctuation of water availability through desiccation tolerance (DT)[2].

DT is defined as a tissue that can dry to equilibrium with moderately dry air and can revive with rehydration (-100 MPa)[3] . There are two different type of DT: constitutive DT and inducible DT. Gametophytes have been hypothesized to be a constitutive DT, meaning that they are able to tolerate rapid drying and will only have minor damages during rehydration[4]. However, if in fact gametophytes are inducible DT, they would incur significant damage upon rehydration after rapid drying, and instead they would slow drying in order to recover from desiccation[4]. One way to test DT is with dark-adapted chlorophyll fluorescence (F_v/F_m), which can indicate the maximum quantum yield of photosystem II, which is the marker of chloroplast health. Typically, plants will show decreasing F_v/F_m during drying , but DT plants will recover to their original F_v/F_m during rehydration[5]. While some terrestrial fern gametophytes were not DT when desiccated quickly in the lab, we may need field studies to determine whether natural drying speeds result in gametophyte DT.

Soil water potential can indicate the hydration status of the tiny gametophyte, if we assume that the single cell-layered fern gametophyte is in hydraulic equilibrium with the soil. Previous studies have shown a correlation between F_v/F_m and water potential in fern sporophytes during desiccation[5]. However, this correlation did not hold during resurrection of DT fern sporophytes because the F_v/F_m recovery lagged behind rehydration[6].

In this study, we examined F_v/F_m and soil water potential in fern gametophytes versus fern sporophytes and fern gametophytes versus moss gametophytes.

H1: F_v/F_m will differ during seasonal dry down between fern gametophytes and sporophytes, but similar in fern gametophytes and moss gametophytes.
H2: Fern gametophytes F_v/F_m will correlate with the soil water potential during seasonal desiccation, but not during rehydration.

Materials and Methods

Figure 1: We worked at two different sites at Stunt Ranch (Calabasas, CA), one at an exposed site (open canopy) and another at a shady site (closed canopy). There was four species present at this site: *Adiantum jordanii*, *Drypteris arguta*, *pellaea andromedifolia*, *Pentagramma tringularis*

Figure 2: We measured with dark-adapted chlorophyll fluorescence (F_v/F_m) by using a pulse-modulated fluorometer (OS1p,Opti-sciences, Hudson,NH,USA). As its taken in predawn where the plant were taken in their most hydrated state and as dark-adapted, we measured the F_v/F_m once a week until completely desiccated

Figure 3: We used the Scholander-Hammel pressure chameber (model 1001, PMS Instrument Co., Corvallis, Orgeon) to be able to measure the soils water potential that were collected during predawn. Was measured that same morning.

Results

Desiccation

Exposed canopy — Closed canopy

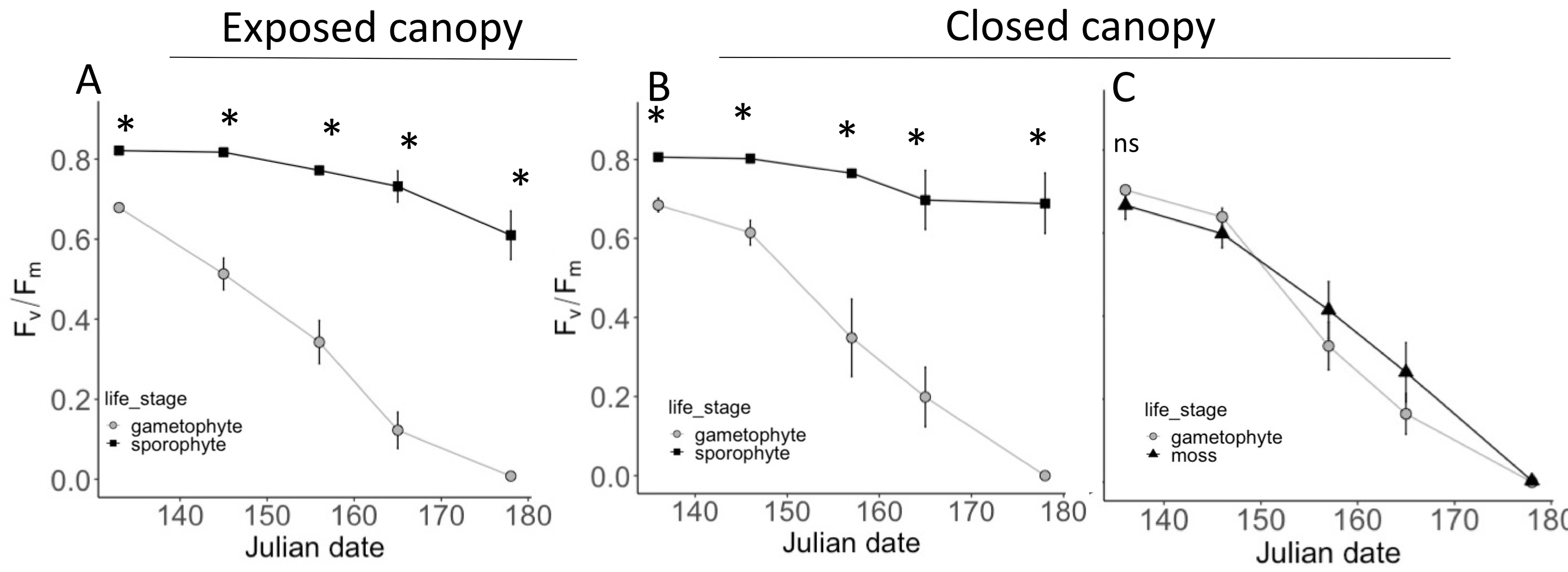

Figure 4: Dark-adapted chlorophyll fluorescence (Fv/Fm) during seasonal desiccation over 32 days in an open-canopy exposed site (A) and a shady closed-canopy site (B, C). We compared Fv/Fm of fern gametophytes to fern sporophytes (A, B) and fern gametophytes to moss gametophytes (C). At each time point, we compared paired fern gametophytes and sporophytes or fern gametophytes and mosses. Asterisks indicate significant difference as shown by a paired student's t test or a paired Wilcox test (p < 0.05). In both the exposed and closed canopy sites, fern gametophytes experienced a faster seasonal decline in F_v/F_m than fern sporophyte leaves (A, B). However, fern gametophytes and moss gametophytes declined F_v/F_m approximately in synchrony during seasonal desiccation (C). Data shown are means +/- SE.

Resurrection

Closed canopy

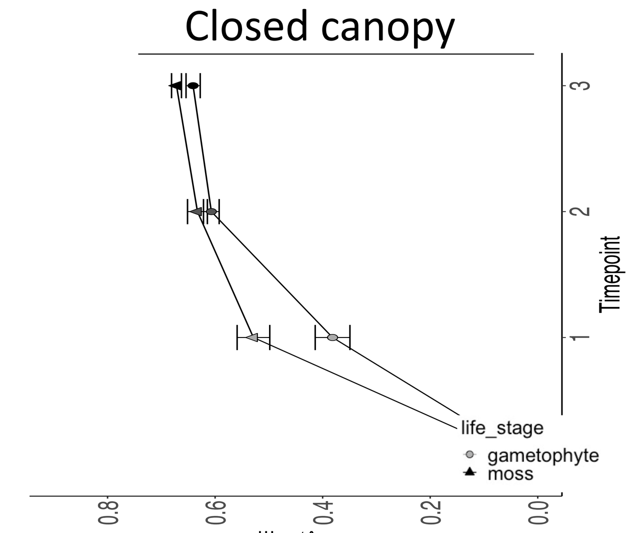

Figure 5: Dark-adapted chlorophyll fluorescence (F_v/F_m) during resurrection over 3 days in a shady closed-canopy site. We compared F_v/F_m of the fern gametophytes to moss gametophytes. Asterisk indicate significant difference as shown by a paired student's t test or a paired wilcox test (p < 0.05). In the closed canopy site both moss and gametophyte have resurrected, and both seem to be synchrony during rehydration

Desiccation

Exposed canopy — Closed canopy

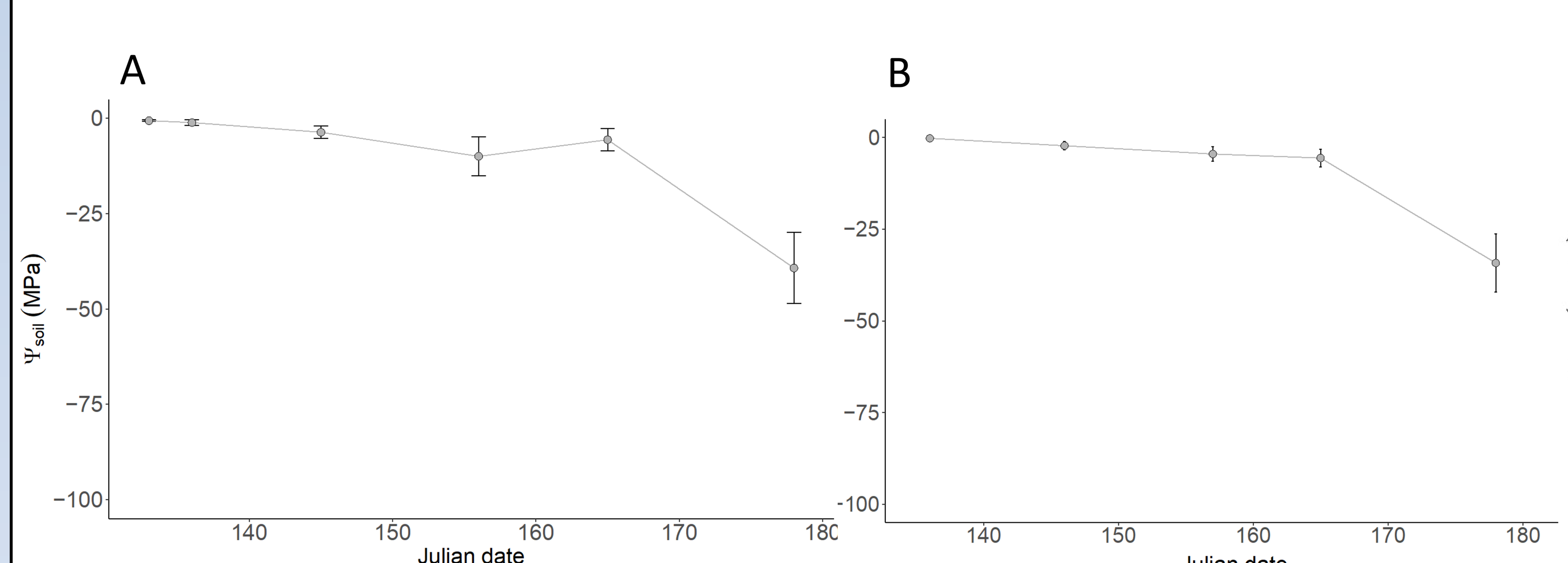

Figure 6: Water potential during seasonal desiccation over 32 days in an open-canopy exposed site (A) and a shady closed-canopy site (B). We measured the water potential of the soil that was near the gametophytes at each different time point (A,B). In both the exposed and closed canopy sites, fern gametophytes didn't experience a fast decline in water potential until the end of seasonal drydown, in both exposed and closed site (A,B). Data shown are means +/- SE.

Resurrection

Closed canopy

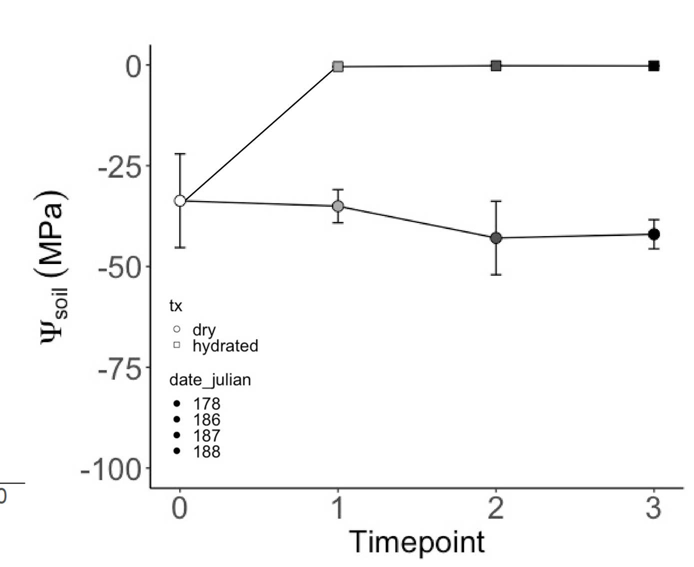

Figure 7: Water potential during resurrection over 4 days in a shady closed- canopy site. We compared both the dry and hydrated fern gametophyte in different time period. In the closed canopy site, we see that the gametophytes appeared to have higher water potential in the soil when it was irrigated compared to the non-irrigated control sites.

Desiccation

Exposed canopy — Closed canopy

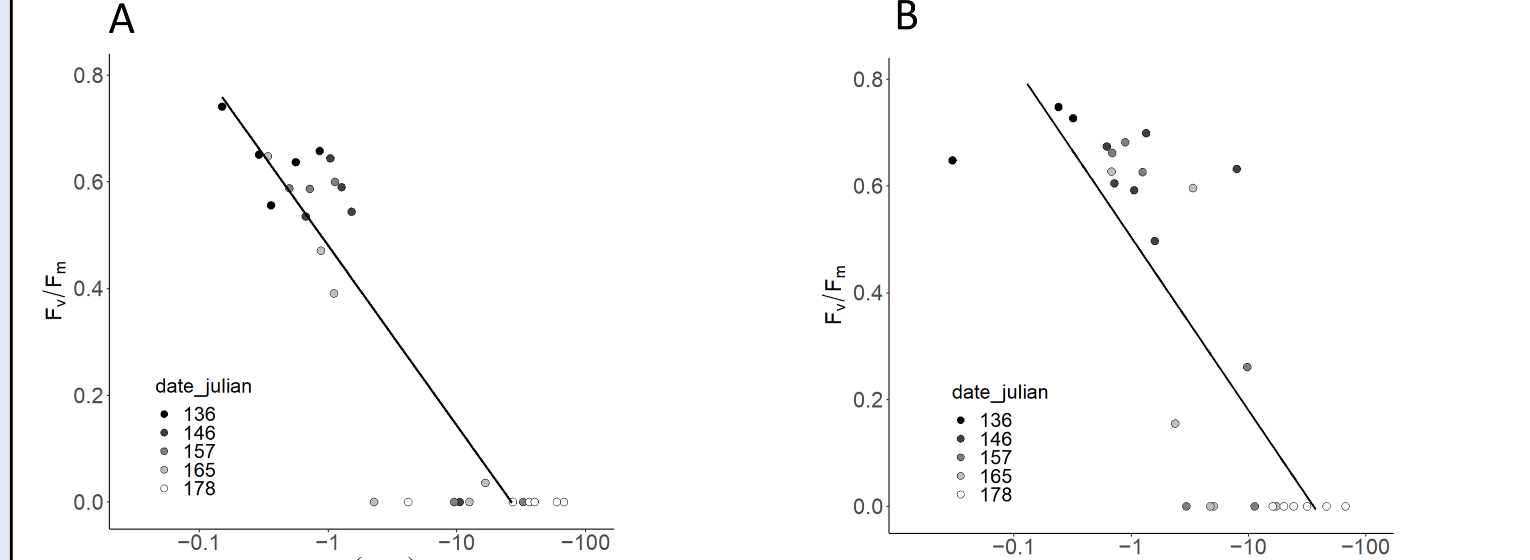

Figure 8: Relationship between dark-adapted chlorophyll fluorescence (F_v/F_m) with the soil water potential over 32 days in an open-canopy exposed site (A) and shady closed-canopy site (B). Soil water potential data was log-transformed to fit model assumptions. In both sites, there was a significant relationship between F_v/F_m and soil water potential as shown by a one-way ANOVA on a linear regression (p < 0.05).

Resurrection

Closed canopy

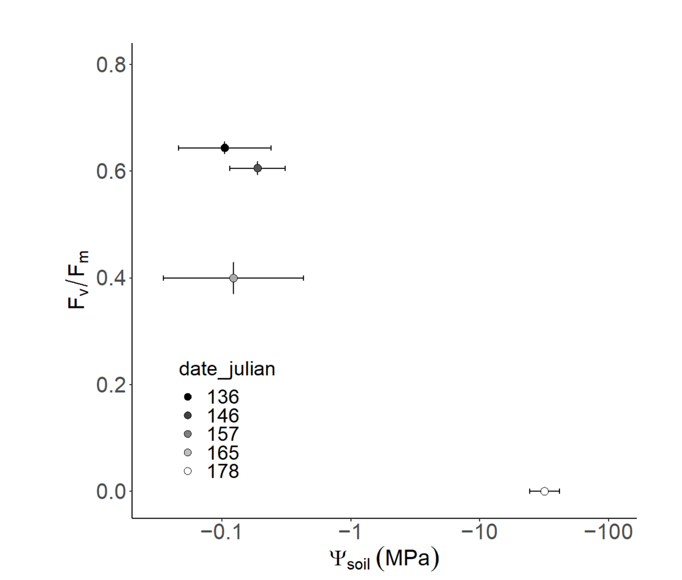

Figure 9: Relationship between dark-adapted chlorophyll fluorescence (F_v/F_m) and the soil water potential over 3 days in a shady closed-canopy site. There was no significant relationship between F_v/F_m and soil water potential as shown by a one-way ANOVA on a linear regression ($F_{1,2}$ = 11.64, p = 0.076).

Discussion

During seasonal desiccation, we found a significant difference in chlorophyll fluorescence (F_v/F_m) between fern gametophytes and sporophytes. These data are consistent with the lack of water conservation abilities of the fern gametophytes, which they have a single cell layer and no cuticle, compared to the fern sporophytes which have a vascular system, cuticles and stomata to regulate water loss. However, the fern gametophytes and moss gametophytes decline F_v/F_m approximately in synchrony, both reaching an F_v/F_m of zero by early July. Since both fern and moss gametophytes share a similar lack of structure to slow water loss, it makes sense that they are both declining at the same rate.

During artificial rehydration in *situ*, the fern gametophytes and mosses were both able to recover over 90% of their initial F_v/F_m, indicating that both fern and moss gametophytes are likely to be desiccation tolerant at these sites. Past studies have shown that mosses are DT, and it appears that in our system, fern gametophyte are also DT just like the mosses, as shown by the fact that both are able to tolerate seasonal desiccation while having little damage during rehydration.

Our relationships between F_v/F_m and soil water potential are consistent with prior studies on fern sporophytes. During desiccation there was a significant relationship between F_v/F_m and soil water potential, but during resurrection this relationship was not significant (but note that the p value was 0.076).

Some of the limitations of our study include the use of artificial irrigation. Since we took these measurements during the summer season, we did not have rain to facilitate natural rehydration. Future studies could examine the natural rehydration process following a seasonal rain event. We were also limited on the amount of soil that we could sample, limiting our ability to pair soil water potential with individual gametophytes. Finally, as chaparral fern gametophyte are small and similar in appearance, we could not ascertain exactly which species that we were measuring. The presence of diverse species could account for unexplained variation in the data.

To our knowledge, this study was the first reported instance of DT in temperate terrestrial gametophytes. This study is also unique as one of the few studies of gametophyte physiology *in situ*. We hope that this research will open the door to further investigate the ecological niche of chaparral fern gametophytes and better understanding their range of habitats. Additionally, as climate changes, we need to understand the capacity of chaparral fern gametophytes to thrive in a warmer or drier climate.

Literature Cited

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