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# Chaparral fern gametophytes experience microclimates with more moisture and more moderate temperatures than fern sporophytes

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# Chaparral fern gametophytes experience microclimates with more moisture and more moderate temperatures than fern sporophytes


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## Introduction

The chaparral biome found throughout much of the Santa Monica Mountains is characterized by hot, dry summers and cool, mild winters. Chaparrals host a wide range of biodiversity, especially plant life, with plants occupying a wide array of niches to survive in the harsh conditions found in the chaparral, among which are several species of terrestrial ferns that inhabit these mountains[3].

Ferns, as all plants do, experience life cycles based upon alternation of generations, with the sporophyte of a plant producing haploid spores, those spores growing into gametophytes, and those gametophytes producing the gametes that then fuse and grow into more adult sporophytes. In ferns, the sporophyte is typically regarded as the fern itself, while the gametophytes possess relatively simple, small bodies called thalli, lacking a cuticle or complex vascular structures[6]; ferns exhibit an especially unique form of this life cycle, with their gametophytes and sporophytes living independently from each other[2,4].

While the sporophytes demonstrate many unique adaptations that allow their survival in the chaparral[1], the gametophytes lack the body structures that would allow them to possess such adaptations. This has prompted our inquiry as to how these cryptic organisms inhabit the same harsh climatic conditions as their sporophyte counterparts. Desiccation tolerance (DT) is an adaptation that allows plants to reach near-total dryness and resurrect upon later rehydration. Little is known about their ecology or habitat[8]; however, it is thought that desiccation tolerance is the primary lifestyle that these gametophytes exhibit in order to persist through the long, dry summers found in the chaparral[8,9].

Desiccation tolerance is characterized by a plant's vascular tissues being able to dry out completely, reaching water potentials of -100 MPa (most plants have a water potential of -2 MPa), and later being able to resurrect upon rehydration[9]. This lifestyle is thought to be most likely for their survival due to their lack of complex vascular tissues or cuticles to aid in water retention, necessitating survival in a near-absence of water. We observed the fern gametophytes to occupy very specific micro-habitats that provided them with conditions more favorable to their physiology[5,7], with gametophyte habitats possessing a narrower temperature range throughout the day and higher relative humidity on average than that of the sporophytes' habitats. In order to quantify the proposed differences in habitat microclimate, we measured air temperature, leaf temperature, relative humidity, and percent sun, with relative humidity data later being used to calculate the VPD (vapor pressure deficit) of the leaves observed. VPD quantifies the change ambient water undergoes, either via condensation (negative VPD) or evaporation (positive VPD), allowing us to observe the availability of water in the gametophytes' habitat.

**H1:** The microclimate of fern gametophyte habitats will have lower $VPD_{leaf}$, leaf temperature, and light level than that of the sporophyte habitat, which will remain consistent over the course of a full day/night cycle.
**H2:** The microclimate of fern gametophyte habitats will have similar $VPD_{leaf}$, leaf temperature, and light level compared to the microclimate of adjacent mosses.
**H3:** Gametophyte and sporophyte habitats on the island will have different microclimate dynamics relative to each other than those on the mainland.

## Materials and Methods

**Figure 1. (right)** 2 mainland sites at Stunt Ranch in Calabasas, CA were selected, one with an exposed canopy, the other with a closed, shaded canopy. 2 sites on Santa Cruz Island were selected that mimic the conditions of the mainland sites' canopies. Closed canopy sites were divided into gametophyte/sporophyte and gametophyte/moss pairings for observation.

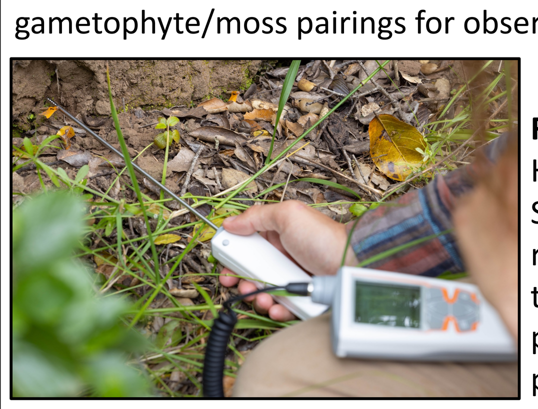
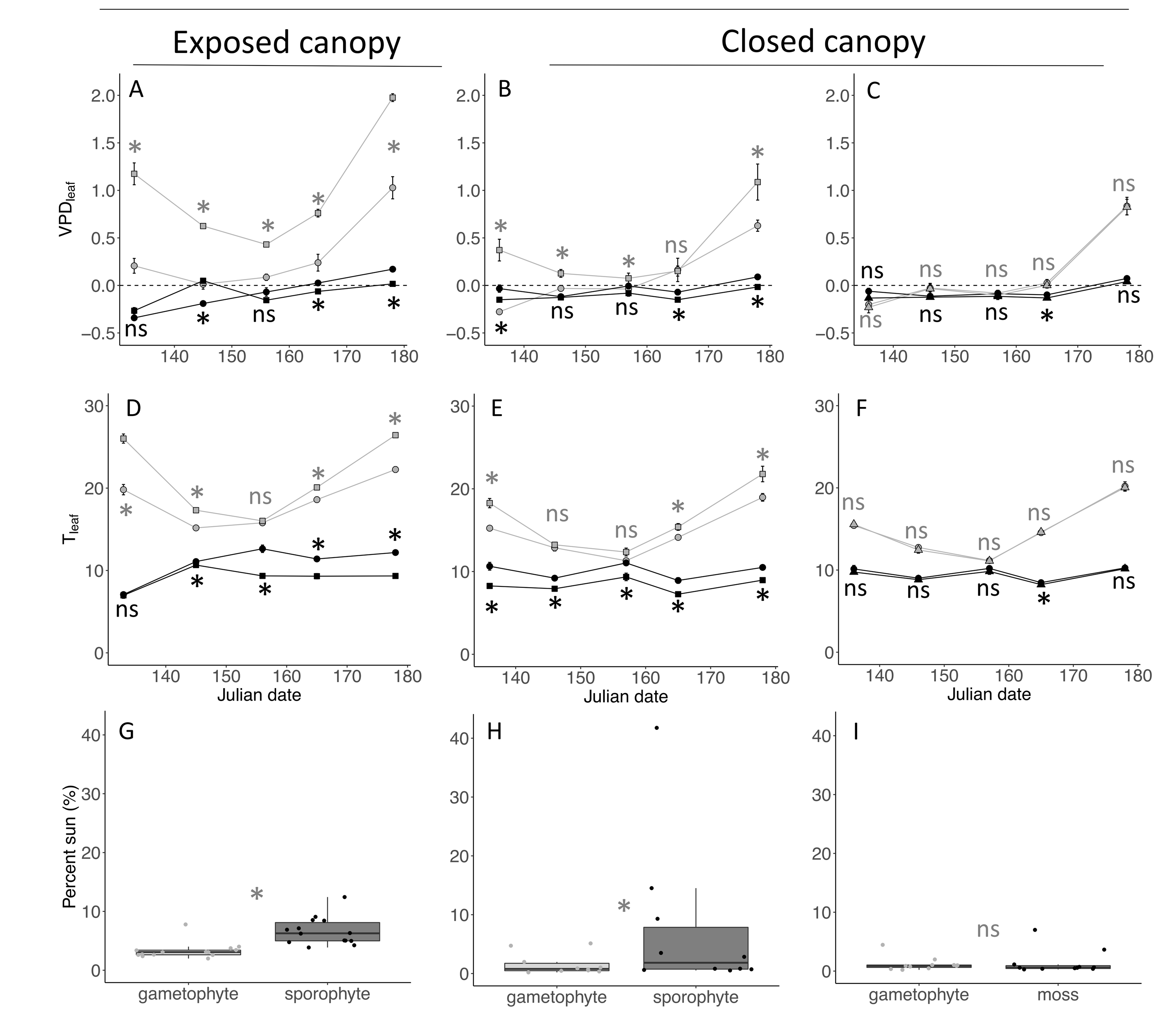


**Figure 2. (left)** Vaisala HUMICAP® Hand-Held Humidity and Temperature Meter HM40 Series used to obtain air temperature and relative humidity. Relative humidity and temperature data were collected at both pre-dawn and midday intervals, while percent sun was gathered only at midday.

**Figure 3. (left)** Extech 42511 Dual Laser Infrared Thermometer used to obtain leaf temperature.

**Figure 4. (above)** LI-250A Light Meter with an LI-190R Quantum Sensor used to obtain percent sun.

## Results



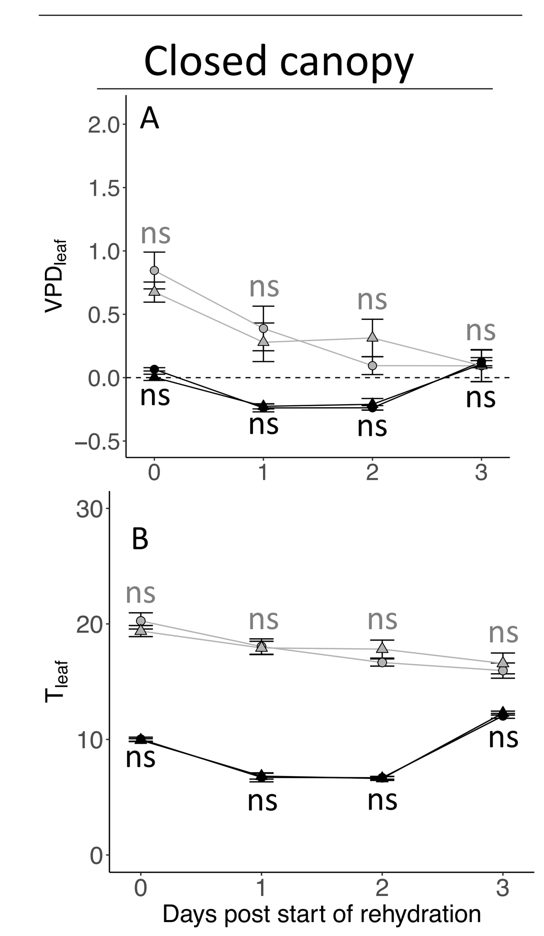




**Figure 2. (above)** Microclimate conditions during irrigation of dry gametophytes and mosses at closed canopy site. Shown are $VPD_{leaf}$ (A) in kPa and $T_{leaf}$ (B) in °C over time in days post-rehydration, with points being means +/- SE. Significance is denoted with an asterisk, $p < 0.05$, obtained via either paired t-test or Wilcox test. Gametophytes are shown with circles and mosses with triangles, with pre-dawn data shown black and midday shown gray.

**Figure 1. (above)** Microclimate conditions during dry-down of exposed canopy site (A, D, and G), closed canopy site pairing gametophytes and sporophytes (B, E, and H), and closed canopy site pairing gametophytes and adjacent mosses (C, F, and I). Conditions shown are $VPD_{leaf}$ (A, B, and C) in kPa, $T_{leaf}$ (D, E, and F) in °C, and percent sun (G, H, and I) in %. Data points are means +/- SE for $VPD_{leaf}$ and $T_{leaf}$. Percent sun is represented with boxplots showing medians and upper/lower quartiles, with dots showing raw data. Significance is denoted with an asterisk, $p < 0.05$, obtained via either paired t-test or Wilcox test. Gametophytes are represented with circles, sporophytes with squares, and mosses with triangles, with pre-dawn data shown black and midday shown gray.

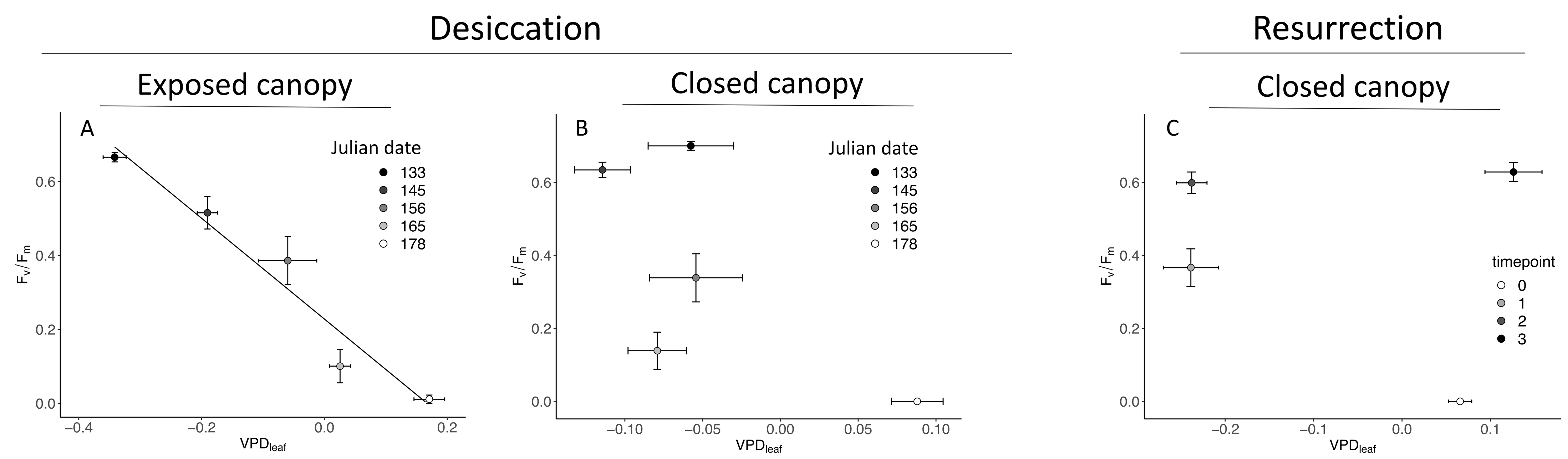



**Figure 3. (above)** Gametophyte-only comparison of $F_v/F_m$ and $VPD_{leaf}$ (in kPa) during dry-down (A and B) and resurrection (C) at exposed (A) and closed (B and C) canopy sites. Points represent means +/- SE of both $F_v/F_m$ and $VPD_{leaf}$ at each time point. Significance is denoted with a fitted line, $p < 0.05$, obtained via linear regression tested with a one-way ANOVA. Gradient from black to white denotes hydrated to dry and vice versa.

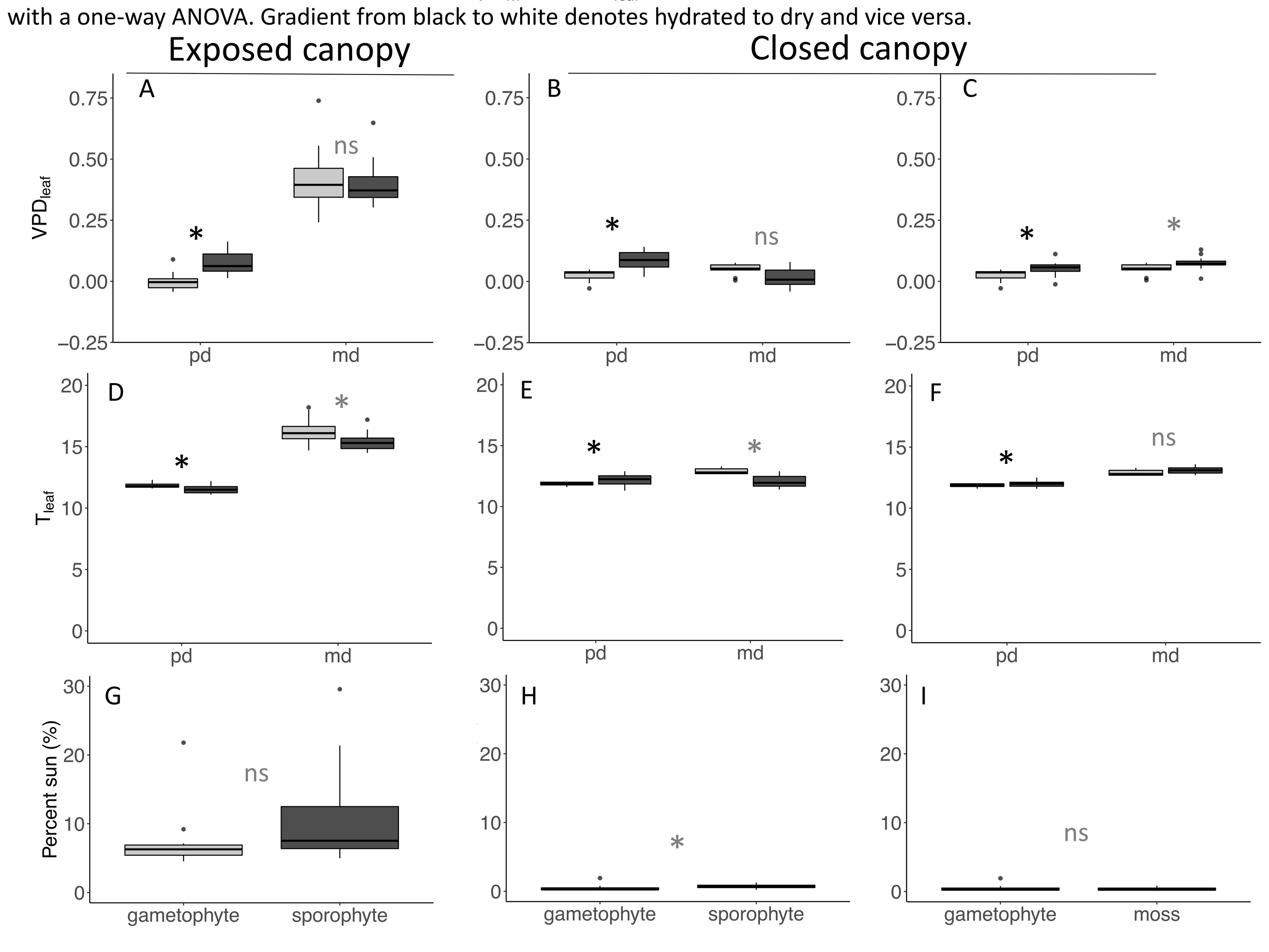



**Figure 4. (left)** Boxplots showing microclimate conditions at exposed (A, D, and G) and closed (B, C, E, F, H, and I) canopy sites on Santa Cruz Island. Gametophytes paired with sporophytes at both sites (A, B, D, E, G, and H) and paired with mosses at closed canopy site (C, F, and I). Conditions shown are $VPD_{leaf}$ (A, B, and C) in kPa, $T_{leaf}$ (D, E, and F) in °C, and percent sun (G, H, and I) in %. Boxplots represent data medians, upper and lower quartiles, the ends of the distributions, and outliers denoted by dots. Significance is denoted with an asterisk, $p < 0.05$, obtained via either paired t-test or Wilcox test. Gametophytes are denoted by gray boxes while sporophytes and mosses respectively are denoted by dark gray boxes. $VPD_{leaf}$ and $T_{leaf}$ shown at both pre-dawn and midday, while percent sun is observed only at midday.

## Discussion

The microclimate of the gametophytes' habitat was, in most instances, significantly different than that of the sporophytes' habitat. Specifically, the gametophytes have a narrower range of leaf temperature that they experience throughout the day than the sporophytes do, along with having a lower average $VPD_{leaf}$ than that of the sporophytes, both at pre-dawn and midday[5]. This ultimately means that the gametophytes experience more moderate temperatures, lower sun exposure, and have wetter surroundings than the sporophytes do over the course of a full day/night cycle. Gametophytes and mosses were observed to have no significant differences in their microclimate conditions, with $VPD_{leaf}$ and leaf temperature, with their microclimates undergoing nearly identical changes over time and having similar levels of sun exposure, which may be attributed to the similar physiology of gametophyte and moss thalli[6]. These observations align well with our first two hypotheses, as we predicted that the gametophyte/sporophyte habitats would be significantly different while the gametophyte/moss would not.

Utilizing our comparison of $F_v/F_m$ (chlorophyll fluorescence) with $VPD_{leaf}$, we observed that in the exposed site, a significant linear relationship was found between increasing $VPD_{leaf}$ and decreasing $F_v/F_m$, which ultimately means that upon the surrounding water reaching the evaporation threshold, plant metabolic function began to decline accordingly. Resurrection trials showed that gametophytes do exhibit DT upon a natural dry-down and rehydration[1,7,9].

In contrast to the mainland observations, different dynamics between gametophytes and sporophytes were observed on the island sites. Gametophytes, while exhibiting significantly different leaf temperatures than the sporophytes, did not consistently have a temperature range they occupied. Similar $VPD_{leaf}$ dynamics were observed between gametophytes and sporophytes on the island compared to the mainland, however, which further enforces the hypothesis that gametophyte physiology necessitates readily available surface moisture.

To our knowledge, this study is the first observed and reported instance of temperate terrestrial fern gametophyte DT *in situ*. It is our hope that this study will pave the way for future studies like it seeking to better understand the cryptic nature of chaparral ferns, their gametophytes, and their wide range of survival strategies.

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Holmlund lab photo taken by Dr. Stephen Davis