

HABITAT DISTRIBUTION AND FROND REORIENTATION AS PHOTOPROTECTION  
AND DROUGHT-AVOIDANCE MECHANISMS IN CHRISTMAS FERN  
(*POLYSTICHUM ACROSTICHOIDES*) IN THE SOUTHERN APPALACHIAN  
MOUNTAINS

A Thesis  
by  
DAVID CHRISTIAN NIELSEN

Submitted to the Graduate School  
at Appalachian State University  
in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE

August 2017  
Department of Biology

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## **Abstract**

### **HABITAT DISTRIBUTION AND FROND REORIENTATION AS PHOTOPROTECTION AND DROUGHT-AVOIDANCE MECHANISMS IN CHRISTMAS FERN (*POLYSTICHUM ACROSTICHOIDES*) IN THE SOUTHERN APPALACHIAN MOUNTAINS**

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Christmas fern (*Polystichum acrostichoides* (Michx.) Schott) is a ubiquitous wintergreen herb found in the forests of the Appalachian Mountains, yet it is distributed asymmetrically on the landscape, favoring north-facing slopes and shady stream banks. In late autumn, the fronds of Christmas fern undergo an irreversible reorientation, bending at the base of the stipe and lying flat on the forest floor. These fronds maintain high chlorophyll concentrations throughout winter and are photosynthetically active on warm winter days and in early spring before canopy emergence. In three populations in the Appalachian State University Biological Preserve, I prevented fronds from reorienting using wooden dowels and floral wire, artificially holding them up over winter. The fronds that were prevented from reorienting to a prostrate position suffered severe photoinhibition characterized by extensive leaf necrosis coupled with significant declines in light-saturated gas exchange, chlorophyll fluorescence ( $F_v/F_m$ ), and total chlorophyll, while a control group showed little or no declines as winter progressed. "Surrogate ferns" were constructed and mounted with light

sensors in order to characterize the light environment at the leaf level on north- and south-facing slopes. Inclined fronds experienced much higher light levels than prostrate fronds (oriented horizontally), on both slopes, and the sensor on the south-facing slope experienced 22 days in which light exceeded  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the air temperature at the leaf level was below freezing. Conversely, on the north-facing slope, these conditions only occurred on one day. This result suggests that frond reorientation is sufficient to prevent photoinhibition in overwintering fronds of Christmas fern by reducing winter light, and may be a significant factor limiting its distribution on south-facing slopes where it is bright and cold during winter. Christmas ferns demonstrated remarkable resilience in a controlled dry-down, with little physiological decline as midday water potentials exceeded  $-1.0 \text{ MPa}$  and soil water content approached 0%. Presently, it is unclear how fern water relations contribute to frond reorientation or distribution on the landscape, but the microclimate data suggest that south-facing slopes are not dry enough to induce significant physiological stress on Christmas fern, and that winter light may have more influence. Forecasted changes in local climate may alter the range and distribution of Christmas fern. Our results provide insight into how this species might be affected, with significant ecological implications for understory herbs in our region.

## **Acknowledgments**

This manuscript has benefitted greatly from the vetting and proofreading of my primary academic advisor and committee chair, Dr. Howard S. Neufeld. I am extremely grateful for the opportunity to work with Dr. Neufeld on this project, and I have become a better scientist and student with his guidance and support. I especially need to thank Dr. Neufeld for his patience during the year I took off from the program to build my house.

I am particularly indebted to my wife and colleague, Catherine Alexander. Her expertise in computer programming and instrumentation saved me many days of frustration. Her input, both in the conceptualization and execution of these experiments, was critical in many cases. Again, I need to thank Catherine for her unwavering patience, support, and love during this journey.

I would also like to thank my committee members Drs. Ray S. Williams and Michael D. Madritch for their input and support, as well as their proofreading and vetting of this manuscript. I am indebted to University Greenhouse manager Jerry Meyer and the student volunteers who watered my plants while I was away. I am thankful for the Cratis D. Williams School of Graduate Studies, particularly Sharon Grimes and Charity Chicoine, who helped me with readmission to the program and registration. I am also very grateful for the research and travel grants I received from the Office of Student Research.

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## **Foreword**

This thesis will be submitted to *New Phytologist*, an international peer-reviewed journal; it has been formatted according to the style guide for that journal.

## Introduction

Ferns evolved 360 million years ago and at least 160 million years before angiosperms. Fossil evidence indicates that some species are virtually identical to their ancestors from 180 million years ago or more (Taylor *et al.*, 2009; Li *et al.*, 2014; Rothfels *et al.*, 2015). The ancient and highly conserved physiology of ferns has allowed them to persist in sympatry with seed plants in myriad habitats worldwide, rather than succumb to competitive exclusion (Watkins *et al.* 2007). The persistence of ferns, despite fierce competition, merits ecophysiological investigation. Studies of ferns may yield new information regarding the evolution of early vascular plant physiology and lend insight into how they might respond to anticipated anthropogenic climatic changes (Agrawal *et al.*, 2004; Goldblum & Kwit, 2012; Wiens, 2016).

As basal vascular plants, ferns lack true vessels and secondary xylem typical in angiosperms, and support far less photosynthetically active foliage on a per-xylem-area basis (Pittermann *et al.*, 2011). Most extant ferns have a tracheid-based primary xylem with heavily pitted lateral walls and highly porous pit membranes that are relatively vulnerable to drought-induced air embolisms (cavitation) (Brodersen *et al.*, 2015). This may limit fern distribution to relatively moist, shady habitats, where evaporative demand and the risk of drought is low although there are exceptions and ferns have evolved a wide range drought adaptation strategies (Kessler & Siorak, 2007; Anthelme *et al.*, 2011; McAdam & Brodribb, 2013). For evergreen vs deciduous ferns, the trade-offs may be between having higher

hydraulic conductivities and stomatal conductances, leading to high productivity in high-light environments, but with an increased risk of cavitation due to drought, while shade-adapted evergreen species, with slower growth and lower stomatal conductances, may trade high productivity for greater drought tolerance, a necessity for leaves that must resist winter-desiccation (Brodersen *et al.*, 2012).

Some evidence suggests stomatal responses to perturbations in vapor pressure deficit (VPD), elevated ambient carbon dioxide (CO<sub>2</sub>), and desiccation are more nuanced in angiosperms than in ferns, which have conservative stomata and tend to reduce carbon assimilation (*A*) under mild water stress (Brodrribb *et al.*, 2009; Brodersen *et al.*, 2015). Additionally, ferns regularly reach photosynthetic saturation at light levels less than half that of angiosperms (Franks & Britton-Harper, 2016). These adaptations often confer water-use efficiencies in angiosperms which are orders of magnitude greater than those of ferns (Davies, *et al.*, 2002; Tyree & Zimmermann, 2002; Sperry, 2003). These studies, coupled with observations of fern preference for moist (mesic) and dark habitats, suggest that this clade is very sensitive to drought, and physiological processes are highly dependent on the water status of the plant (Damour *et al.*, 2010; Watkins *et al.*, 2010; McAdam & Brodrribb, 2015). Despite these observations, there are many species that dwell in arid or Mediterranean climates and are very resistant to seasonal or prolonged drought, further suggesting physiological versatility (Liao *et al.*, 2008; Farrant *et al.*, 2009).

The understory wintergreen, or winter deciduous, Christmas fern (*Polystichum acrostichoides* (Michx, Schott) represents one of several understory herbaceous plants that dwell in low-light, mesic habitats in the Appalachian Mountains. Habitat preferences reflect the life-history strategies of this species, and the selective pressures in these environments

have produced a water-conservative, slow-growing life form. In addition, there is abundant evidence that trade-offs occur between drought and shade-tolerance, since the former requires an extensive investment in belowground biomass (roots) to obtain sufficient water whereas the latter requires greater investment in above-ground tissues such as leaves, often at the expense of root growth (Smith & Huston, 1989). Thus, shade-tolerant species are often considered intolerant of severe drought (Valladares & Pearcy, 2002). Compared to conifers and angiosperms, ferns generally respond to changes in frond water potential ( $\psi_f$ ) with a relatively large decline in stomatal conductance ( $g_s$ ) and a resultant decline in  $A$  (Pittermann *et al.*, 2015; Franks & Britton-Harper, 2016), and this response is more pronounced when water is abundant (Talbot *et al.*, 2003). In simple terms, ferns growing in mesic habitats willingly sacrifice carbon gain in order to conserve water while angiosperms and conifers have physiologies which allow them to maintain carbon gain under conditions of water stress (McDowell *et al.*, 2008).

Coupled with observations of habitat preference, the physiology of similar species suggests that Christmas fern may have stomata that respond conservatively to water stress in an effort to maintain a stable  $\psi_f$ , though these responses have not been studied in detail for this species (Damour *et al.*, 2010; Watkins *et al.*, 2010). However, the overwintering or “wintergreen” behavior observed in Christmas fern is associated with remarkably hardy foliage, and may influence these responses. Perennial ferns growing in Mediterranean climates often have high resistance to cavitation under moderate to severe drought conditions, and no observable decline in  $g_s$  under high xylem tensions (Burns *et al.*, 2017) which is in stark contrast to ferns growing in mesic habitats.

Presently, it is unclear where Christmas fern lands on this spectrum, given that it shares life history strategies with both groups. Furthermore, the habitat preference of Christmas fern is also driven by other factors in addition to water availability such as seasonal variations in light and heat load (Warren, 2008). The goal of this research is to elucidate the relative contribution of these abiotic factors and their potential roles in determining habitat preference for Christmas fern in the southern Appalachian Mountains.

Christmas fern is ubiquitous in woodlands of the eastern United States (USDA Plants Database, 2017). In the southern Appalachians on well-drained soils, it is often the dominant understory herbaceous species, though abundance surveys suggest that it prefers north-facing slopes and creek beds (Greer *et al.*, 1997; Tessier & Raynal, 2003; Warren, 2008, Patricia Cox personal communication 2015, this study – see Results section).

Preference for north-facing slopes is common among understory herbaceous species, and evidently driven by topography-induced differences in microclimate (Shanks & Norris, 1950; Hicks & Frank, 1984; Fekedulegn *et al.*, 2003; Desta *et al.*, 2004; Reudink *et al.*, 2005; Holst *et al.*, 2005, Horton *et al.*, 2009). In the Northern hemisphere, southern exposures receive a greater input of solar radiation, and are warmer and drier as a result. These differences affect understory plant distribution in two broad ways; first, they increase desiccation due to higher evaporative demand and lower soil moisture, and second, photoinhibition may be more severe during high-light, low-temperature conditions during winter (Fekedulegn *et al.*, 2003; Desta *et al.*, 2004; Horton *et al.*, 1996; Neufeld & Young 2014).

Warren (2008) found that the best predictive models of understory herbaceous plant distribution were winter-light and summer heat load, while Desta *et al.* (2004) suggested that

drought intolerance could best explain this distribution. High-light conditions on southern exposures, coupled with sub-freezing temperatures may cause damage to the photosynthetic apparatus during winter (Gu, *et al.*, 2008; Russell *et al.*, 2009; Tessier, 2014). Nonetheless, decreased canopy cover correlated with increased reproductive effort in Christmas fern, suggesting light and or heat intolerance may have long-term effects on fitness (Greer & McCarthy, 2000), especially if it adversely affects photophysiology.

The relative importance of temperature, light, and water relations to the distribution of Christmas fern has not been fully elucidated at this point. Given its prevalence on north-facing slopes and near creeks, I hypothesized that this species is extremely sensitive to water stress, and that water availability plays a key role in determining its habitat distribution. To test this, I deployed environmental monitoring stations on north and south-facing slopes and monitored light, temperature, and relative humidity at the leaf level for nearly a year. I hypothesized that south-facing sites would receive significantly greater solar input, especially after leaf fall in autumn, and consequently would be warmer and drier. To further characterize the drought sensitivity of this species, I performed a controlled dry-down experiment on potted plants in the University Greenhouse. I hypothesized that drought conditions would precipitate a decline in midday  $\psi_f$  followed by stomatal closure and reduced carbon assimilation. If this species is particularly sensitive to water stress, then stomatal conductance should be lowered at relatively mild levels of water stress, and this would provide evidence for why this species is restricted to relatively moist, shady habitats, such as on north-facing slopes.

In fall, after the first few hard frosts, the fronds of Christmas fern undergo freezing-induced leaf movements in which a specialized region of the stipe loses turgor allowing the

fronds to bend as if on a hinge yet maintaining xylem flow. These fronds reorient from an inclined to a prostrate position and remain so until they senesce in late spring (Figure 1). (Nooden & Wagner, 1997). In the Appalachian State University Biological Preserve, reorientation was observed after canopy senescence and leaf drop, and the majority of fronds remain exposed on the forest floor over most of the winter (except when self-shading occurs or when covered by forest debris).

Presently, it is unclear why fronds of Christmas fern reorient to a prostrate position. Adams *et al.* (2004) found that many overwintering evergreens cannot utilize radiation under freezing conditions due to the production of reactive oxygen species which damage the photosynthetic apparatus, particularly photosystem II (PSII). Russell *et al.* (2009) showed that overwintering leaves of *Rhododendron maxima* were photoinhibited when prevented from reorienting on cold, bright winter days. I hypothesized that fronds exposed to high light in winter conditions would exhibit signs of photoinhibition including decreased Fv/Fm, photosynthesis, and chlorophyll, coupled with visible leaf necrosis. I further hypothesized that the microclimate differs between inclined and prostrate fronds, and tested this by artificially reorienting prostrate fronds to an inclined position and measuring light and temperature at the leaf level, as well as gas exchange.

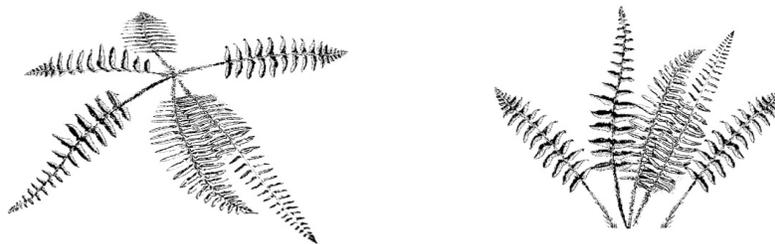


Figure 1: Themonastic leaf movements of Christmas fern (*Polystichum acrostichoides*). Reorientation from an inclined or upright position (left) to a prostrate or flat position (right) occurs after the first few hard frosts.

(Illustration by Catherine Alexander).

## Materials and Methods

### *Distribution Surveys*

Distribution surveys were conducted on December 1st, 2014 in four locations within the Appalachian State University Biological Preserve, though we stopped collecting data from one site in 2016 due to its distance from the other field sites (Figure 2).

The topography of the preserve provides an ideal study site, with four east/west running ridges. Each ridge has a predominately north or south-facing slope, respectively, and all four have similar physical and vegetative characteristics.

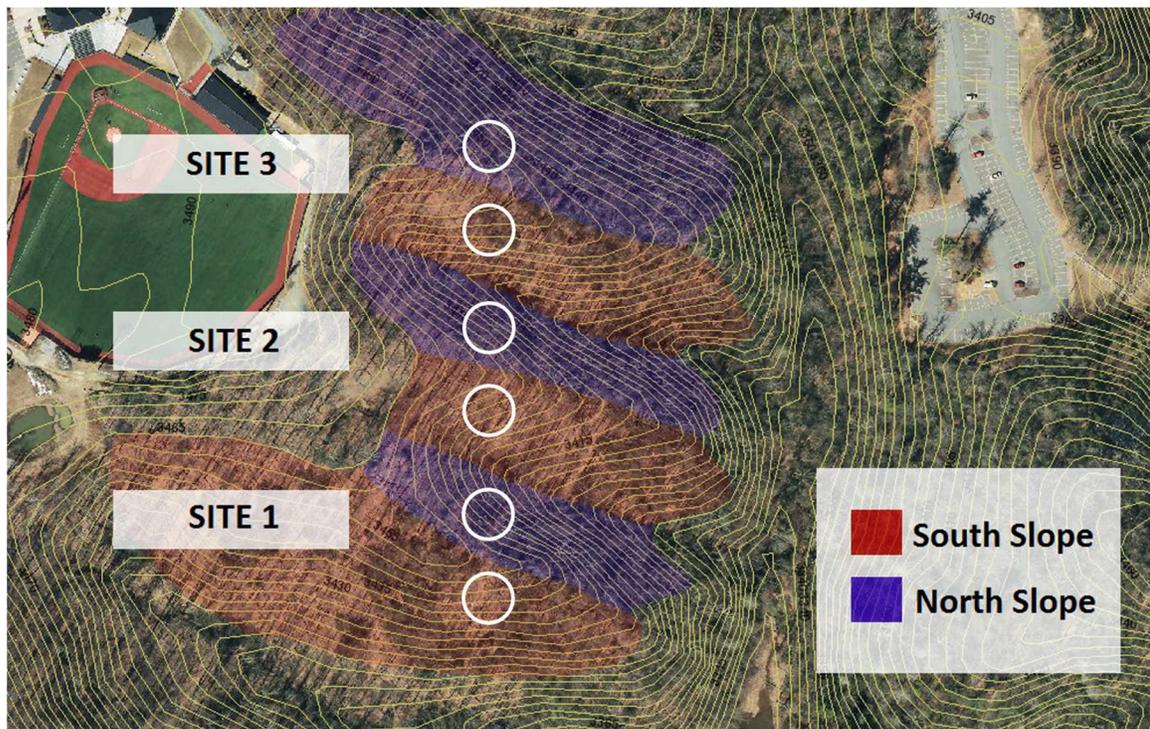


Figure 2: Map of field sites, Appalachian State University Biological Preserve.

Study sites were located within two kilometers of one another and at similar elevations. Sites were chosen as representative samples of the understory. Vegetation consisted of moderately mature mixed hardwood overstory and sparse understory. *Rhododendron maxima* thickets are present at some of the sites. At the time of the survey, Christmas fern represented ~100% of understory herbaceous vegetation at all locations.

At each location, on either side of the ridgeline (north-facing and south-facing), a 5 m x 5 m plot was designated 5-10 m downhill and the aspect, elevation, slope, and GPS coordinates were recorded at each location. The number of ferns within each plot was recorded.

#### *Microclimate*

Microclimate data were collected throughout winter beginning in September, 2016. Data collection stations were deployed on the north- and south-facing slopes of site 2. Weather data were retrieved from the stations and downloaded onto a laptop once or twice monthly. Campbell Scientific CR 1000 dataloggers equipped with quantum sensors (Apogee Instruments SQ-110, Logan, Utah) were deployed on a wood scaffolding or “surrogate fern” to monitor light levels at the average angle (60°) of inclined fronds (angles were measured in summer). On each “surrogate fern” light was measured in a north-facing direction and south-facing direction as well as a horizontal direction (Figure 3). These dataloggers also collected air temperature and humidity measurements (Campbell Scientific SDI-12 temp and RH probe), as well as soil temperature data from 15 cm below the surface using copper-constantan thermocouples.

Surface temperature of inclined and prostrate fronds was measured with a non-contact infrared thermometer (Omega OS425-LS, Norwalk, Connecticut). Volumetric water content of soil (%VWC) was measured at multiple locations on each north and south-facing plot (Campbell Scientific HydroSense II) equipped with 20 cm probes. These measurements were corroborated by determining the percent water content by mass at soil depths of 15 and 30 cm.

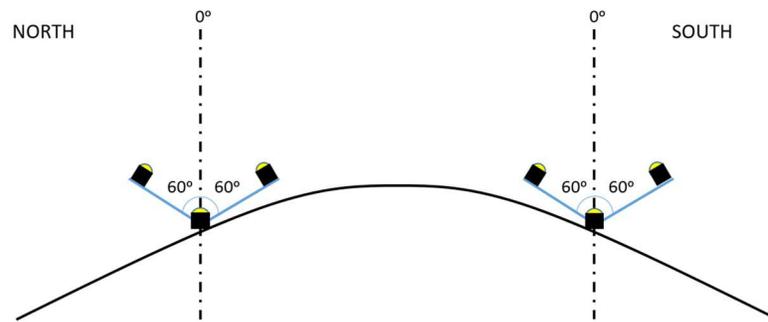


Figure 3: “Surrogate fern” equipped with quantum sensors were set on north- and south-facing slopes to provide a representation of the seasonal light environment experienced at the leaf level. The sensors were oriented North and South. Scale is grossly exaggerated to show detail.

### *Fronde Reorientation*

Before natural frond reorientation (October 1<sup>st</sup>, 2016), five individuals (4-6 fronds each) in each north-facing plot were fixed to an incline of approximately 60° using wooden dowels, wire mesh and floral wire.

### *Photoinhibition*

Quantum efficiency (Fv/Fm) measurements of chlorophyll fluorescence were made on inclined and prostrate fronds using a Handy PEA Meter (Hansatech Instruments, King’s Lynn, UK), and steady-state, light-saturated gas exchange was measured using the Li-6400

Portable Photosynthesis System in years 2014/15 (data not reported) and the Li-6800 in years 2016/17 (Li-Cor, Inc. Lincoln, Nebraska) equipped with extended reach cuvette. Cuvette conditions were: photosynthetically active radiation (PAR) = 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , ambient cuvette  $\text{CO}_2$  = 400 ppm, temperature = 25°C, relative humidity ~50%. Measurements were made on two mid-rachis pinnae from each plant, which averaged ~1.8  $\text{cm}^2$  each. Representative photographs were taken regularly and used to estimate the extent of and progression of visible necrosis during the treatment.

### *Pigment analysis*

Chlorophyll and carotenoid concentrations for inclined and prostrate fronds were periodically measured between October 2016 and March of 2017. At each field site, ~1.0  $\text{cm}^2$  of leaf was collected from the mid-rachis pinnae of five inclined individuals and five prostrate individuals and immediately submerged in 3 mL of *N,N* – dimethylformamide (DMF) and allowed to extract for ~48 hours at 5°C in the refrigerator. Absorbances were measured on a UV-1800 Shimadzu spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Chlorophyll and carotenoid concentrations were calculated using the equations described by Porra *et al.*, (1989) and Wellburn (1994).

### *Greenhouse Dry-down*

Before the first frost in the fall of 2016, 30 individual plants were harvested from a tract of University-owned land directly southwest of the Nature Preserve, with nearly identical habitat conditions as the field study sites. These individuals were established in pots (25 cm diameter, 30 cm deep) outside the University Greenhouse over winter. Rhizomes

were disturbed as little as possible and kept in native soil. The ferns were shaded at 30% full-sun (70% shade) watered regularly, and kept in a raised bed (still in pots) to avoid root frost damage. In February 2017, these individuals were transferred to the University Greenhouse. In May, after the development of mature new fronds, I subjected half of these plants to water deprivation and monitored their physiology over a period of 22 days. Baseline measurements were taken on the treatment group and control group after they had been well-watered on day 1. All measurements were made near midday (between 1:00 h and 14:00 h). Steady-state, light-saturated gas exchange was measured using the Li-6800 Portable Photosynthesis System equipped with the fluorescence cuvette. Cuvette conditions were: photosynthetically active radiation (PAR) = 2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , ambient cuvette  $\text{CO}_2$  = 400 ppm, temperature = 25°C, relative humidity ~50%. Measurements were made on two mid-rachis pinnae from each plant, which averaged ~1.8  $\text{cm}^2$  each. Water potential ( $\psi_f$ ) was measured on two mid-rachis pinnae from each plant using a PMS Model 600 pressure chamber equipped with a grass compression gland (PMS Instruments, Albany OR). Finally, soil moisture (%VWC) was measured using the Campbell Scientific HydroSense II.

### *Statistics*

For the protocols described above (with the exception of the data collected at the weather stations), the average of two or three samples was taken as the unit of replication. Two-tailed, paired *t*-tests were used to describe differences in inclined and prostrate fronds for the field experiment and two-tailed, two-sample *t*-tests were used to describe differences in the greenhouse dry-down with a significance level of  $p \leq 0.05$ . Analyses of variance (ANOVA) were used to compare the means between sites and across months.

## Results

### *Microclimate*

Field sites 1-3 were located at an elevation of approximately 1050 m (3450 ft) and were separated longitudinally by about 100-150 m (see Figure 2). All field sites were equidistant from the ridge line (8-10 m). The average aspect for north-facing slopes was  $38.7 \pm 4.7^\circ\text{N}$  by NE, and the average slope of these sites was  $0.40 \pm 0.04$  (rise/ run, unitless). The average aspect for south-facing slopes was  $173.3 \pm 6.7^\circ\text{S}$  by SW, and the average slope of these sites was  $0.30 \pm 0.007$  (see Figure 2). The average fern density was  $0.78 \pm 0.050$  ferns/m<sup>2</sup> and  $0.04 \pm 0.005$  ferns/m<sup>2</sup>;  $n = 3$ ,  $p = 0.003$  on north and south-facing slopes, respectively.

In January 2015, the average soil moisture at a depth of 15 cm (measured with the Campbell Hydrosense II) of north-facing field sites was  $27 \pm 1$  %VWC and  $26 \pm 1$  %VWC on south-facing slopes, and this difference was not significant ( $p = 0.63$ ,  $n = 20$ ) (Figure 4). In March 2017, after a period of little to no rainfall, the average soil moisture at a depth of 30 cm at the north-facing field sites was  $29 \pm 1$  % by mass and  $26 \pm 1$  % at south-facing field sites, and this difference was not significant ( $p = 0.24$ ,  $n = 15$ ). The average soil moisture at 15 cm was  $33 \pm 1$  % on north-facing slopes and  $28 \pm 1$  % on south-facing slopes, and this difference was significant ( $p = 0.002$ ,  $n = 9$ ). On north-facing slopes, the water content of the top 15 cm of soil was significantly greater than the water content from 15-30 cm ( $p = 0.04$ ), and this difference was not apparent on south-facing slopes ( $p = 0.37$ ).

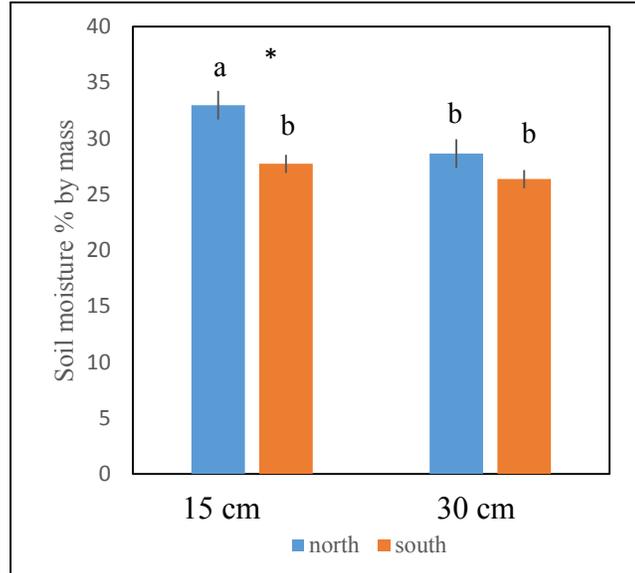


Figure 4: Soil moisture as % water by mass at two different depths on north- and south-facing slopes in March 2017. Asterisk indicates significant difference between slopes at 15 cm depth ( $p = 0.002$ ,  $n = 9$ ). Slopes not followed by the same letter indicate a significant difference between sampling depths ( $p = 0.040$  for north slopes,  $p = 0.370$  for south slopes). Bars are means  $\pm$  se.

### Light Environment on North- and South-Facing Slopes

The light environment was significantly different between north- and south-facing slopes and between light sensors oriented in north- and south-facing directions. South-facing slopes received significantly more PAR than north-facing slopes for all three sensor orientations (see Figure 3 “surrogate fern”). On the south-facing slope, the sensor facing south received the highest levels of PAR of all six sensors. Conversely, on the north-facing slope, the sensor facing north received the lowest levels of PAR. The sensor oriented horizontally (pointing straight up) was intermediate between the two, but this orientation still received more PAR on the south facing slope. Note on all light figures the dramatic rise in PAR in late October after canopy senescence.

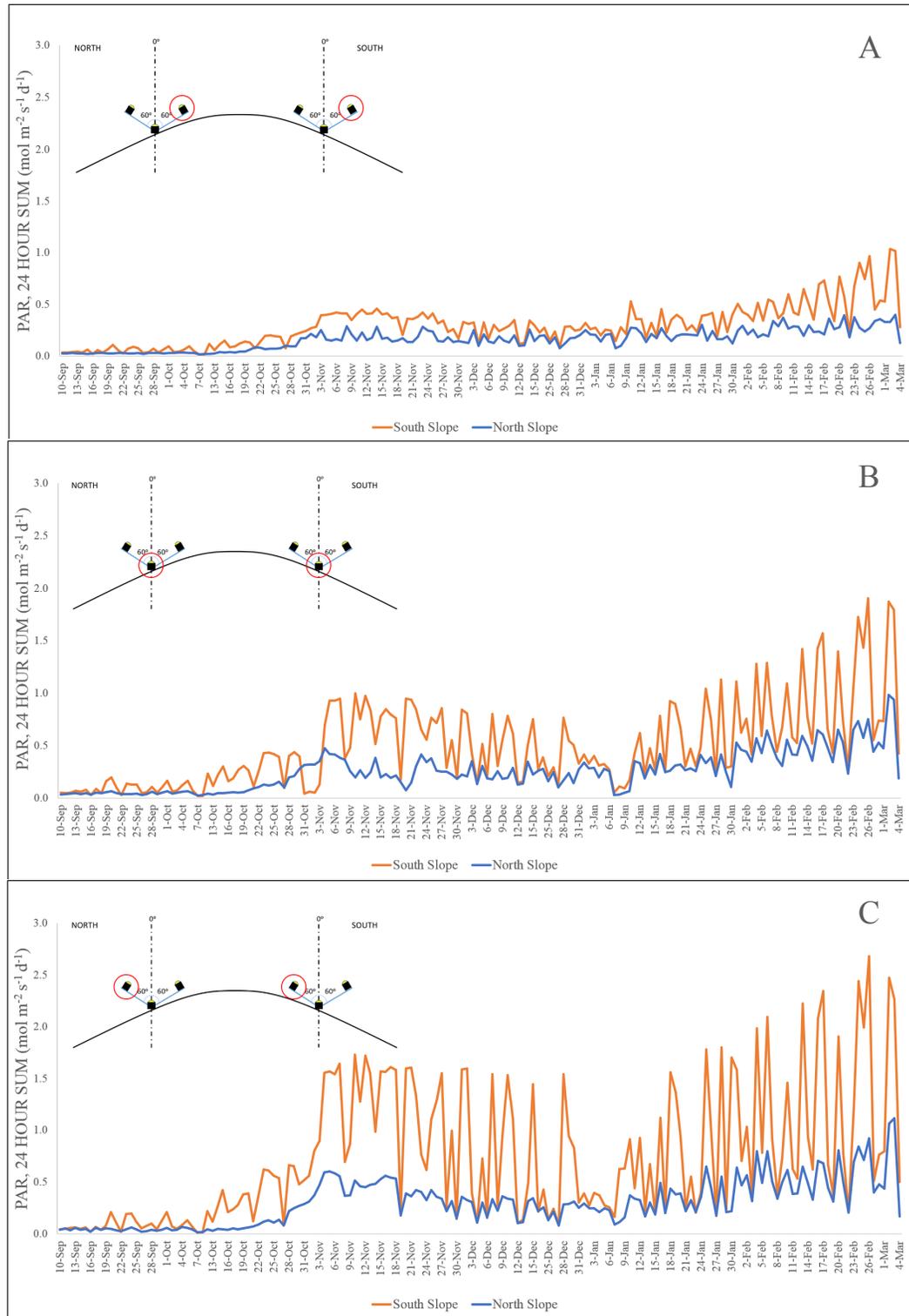


Figure 5: Total PAR reaching each sensor over 24-hour intervals (sensor orientation depicted in the upper-left corner of each figure). Note that the only difference between the two sensors was their position (north slope vs. south slope) the elevation, distance from the ridge, aspect, and angle relative to the vertical were identical.

The average daily minimum and daily maximum air temperature at 30 cm above the forest floor was not different between north- and south-facing slopes (Figure 6), but the light environment was (Figure 5). On the north-facing slope, there were 28 days during which the average daytime temperature did not rise above freezing (0.0°C). Of these cold days, there were 16 days in which the maximum light reaching the sensor oriented south (see Figure 5C) exceeded 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the average daily maximum light was 1131.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Conversely, the sensor oriented north experienced 1 day in which the maximum light exceeded 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and an average daily maximum light of 539.1  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

*Table 1: Summary of light environment of “surrogate” ferns during days below freezing. Data were collected for 166 days total.*

<b>Slope</b>	<b>Sensor</b>	<b># of days <math>\leq 0^\circ\text{C}</math></b>	<b># of days in which temp <math>\leq 0^\circ\text{C}</math> and light <math>&gt; 500 \mu\text{mol m}^{-2} \text{s}^{-1}</math></b>	<b>Average maximum light of days <math>\leq 0^\circ\text{C}</math> (<math>\mu\text{mol m}^{-2} \text{s}^{-1}</math>) <math>\pm se</math></b>
South	South Facing	28	22	1502 $\pm$ 109
South	North Facing	28	7	690 $\pm$ 48
North	South Facing	28	16	1131 $\pm$ 72
North	North Facing	28	1	539

On the south-facing slope, there were 28 days at or below freezing, and of those days, there were 22 in which the maximum light reaching the sensor oriented south (see Figure 5A) exceeded 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the average maximum light (the average of the highest light level recorded for each day below freezing) was 1501.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The sensor oriented north experienced 7 days in which the maximum light exceeded 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and the average maximum light was 690.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

Table 2: Maximum light experienced by surrogate ferns. Note that on the north-facing slope, the highest light is experienced in late winter.

Sensor location	Maximum light reading ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Corresponding temperature ( $^{\circ}\text{C}$ )	Date	Time
<b>North Slope</b>				
South Sensor	1674	1.3	3/3/2017	12:01:00 PM
Perpendicular Sensor	1499	1.3	3/3/2017	11:55:40 AM
North Sensor	539	1.3	3/3/2017	10:46:50 AM
<b>South Slope</b>				
South Sensor	2063	17.3	2/23/2017	12:32:00 PM
Perpendicular Sensor	1577	16.5	9/20/2016	1:15:40 PM
North Sensor	908	3.7	1/10/2017	12:14:40 PM

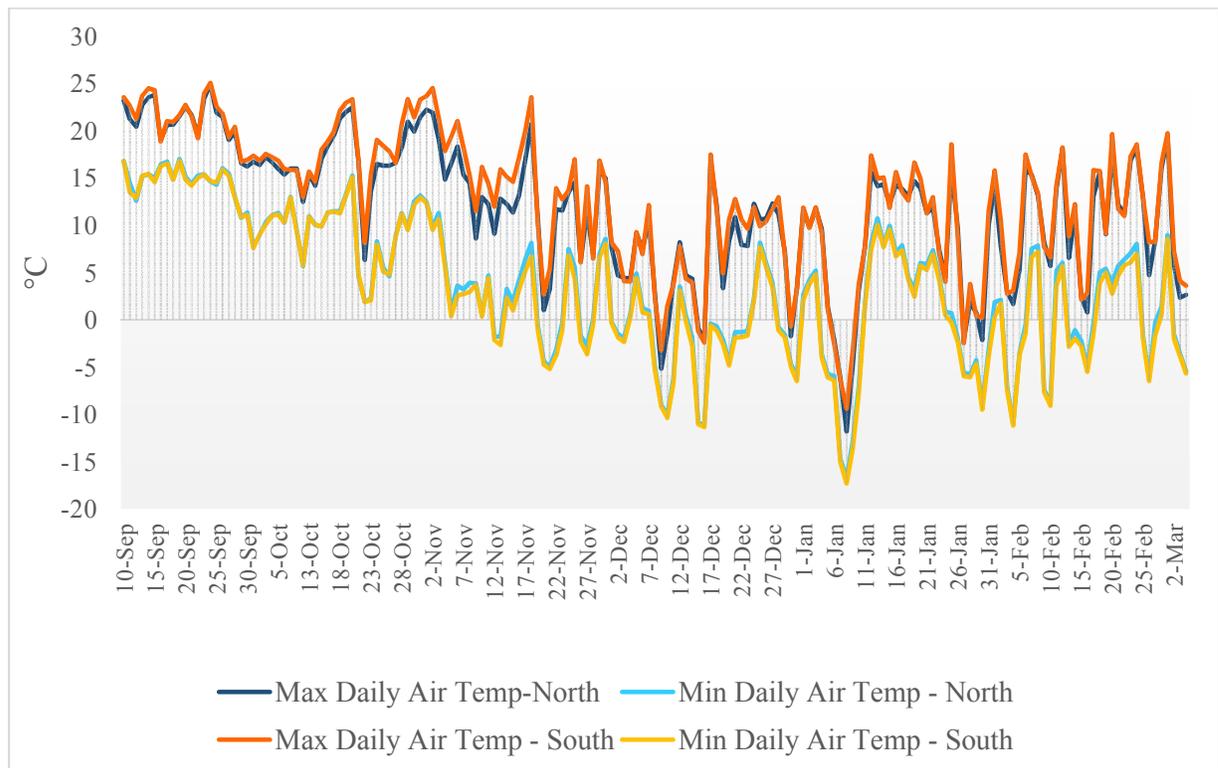


Figure 6: Maximum and minimum daily air temperatures on north- and south-facing slopes (measured 30 cm above the forest floor).

### *Physiological Measurements of Artificially Reoriented Fronds*

Initial measurements taken during the first week of October, 2016 of artificially-reoriented (inclined) and prostrate fronds (allowed to reorient naturally) revealed no significant differences between Fv/Fm or chlorophyll concentration (Figure 8). On November 22<sup>nd</sup>, 2016, there were no significant differences in gas exchange between treatments. By the first week of December, 2016, inclined fronds showed significant decline in Fv/Fm (inclined  $0.693 \pm 0.01$ , prostrate  $0.765 \pm 0.01$   $p \leq 0.0001$ ,  $n = 15$ ), but no significant declines in total chlorophyll concentration (inclined =  $30.6 \pm 1.2 \mu\text{g}/\text{cm}^2$ , prostrate =  $31.8 \pm 1.2 \mu\text{g}/\text{cm}^2$ ,  $p = 0.522$ ,  $n = 15$ ). Gas exchange was not measured at this time. There were no detectable differences in visible leaf necrosis between groups at this time, with all individuals bearing dark green, unblemished foliage. On February 10<sup>th</sup> 2017, inclined fronds exhibited further diminished Fv/Fm, while prostrate fronds showed little to no declines (inclined  $0.600 \pm 0.03$ , prostrate  $0.762 \pm 0.01$   $p \leq 0.0001$ ,  $n = 15$ ). Conversely, chlorophyll concentration did not show significant declines in February (inclined =  $27.72 \pm 1.8 \mu\text{g}/\text{cm}^2$ , prostrate =  $31.2 \pm 1.2 \mu\text{g}/\text{cm}^2$ ,  $p = 0.143$ ,  $n = 15$ ). By March 25<sup>th</sup>, 2017, nearly 100% of the foliage of inclined fronds was necrotic or discolored, while the foliage of the control group was still dark green. Fv/Fm declined further in both inclined and prostrate fronds (inclined  $0.482 \pm 0.02$ , prostrate  $0.666 \pm 0.02$   $p \leq 0.0001$ ,  $n = 15$ ). At this time total chlorophyll declined in inclined fronds, and was significantly lower than prostrate fronds (inclined =  $23.25 \pm 1.2 \mu\text{g}/\text{cm}^2$ , prostrate =  $31.2 \pm 1.2 \mu\text{g}/\text{cm}^2$ ,  $p = 0.007$ ,  $n = 15$ ). Inclined fronds had significantly lower rates of photosynthesis than prostrate fronds (inclined =  $0.523 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ , prostrate =  $2.79 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $p < 0.0001$ ,  $n = 15$ ).

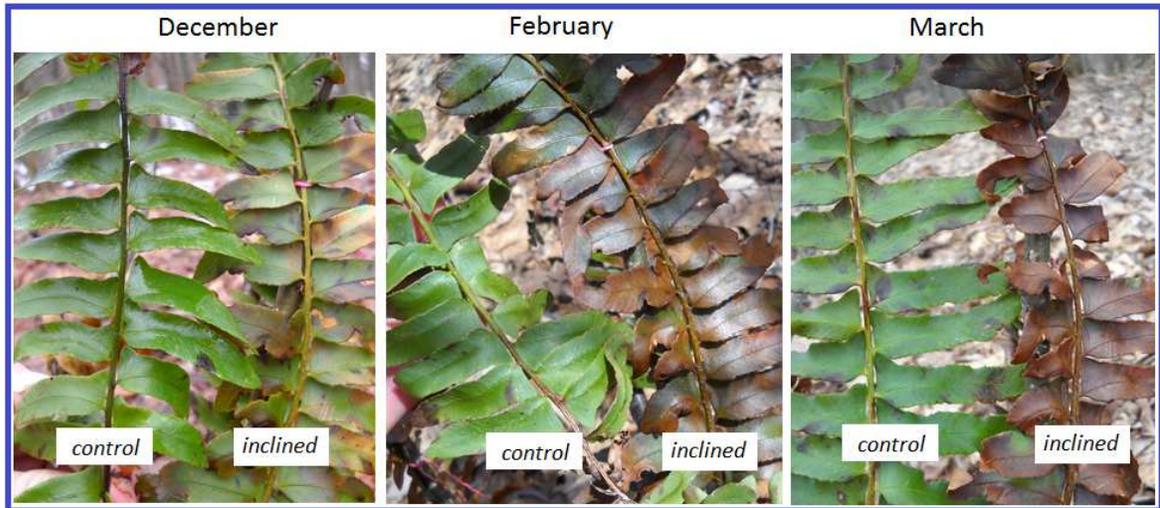


Figure 7: Representative photographs of inclined and prostrate ferns over winter. The control group (prostrate) is depicted in the left of each frame and the treatment group (inclined) is depicted in the right of each frame.

Point measurements of surface temperature indicated that inclined fronds were significantly warmer than prostrate fronds (inclined =  $5.86 \pm 0.75$  °C vs. prostrate =  $2.96 \pm 0.76$  °C,  $p = 0.0007$ ,  $n = 15$ ). Ambient temperature at the time of measurement was  $-3.6$  °C and the slope was in full sun (11:30 h).

Visible leaf necrosis presented in the treatment group by late December and progressed into spring, with some fronds bearing an estimated 90-100% necrotic foliage, while the control group showed no such necrosis and continued to bear dark green foliage well into March (Figure 7).

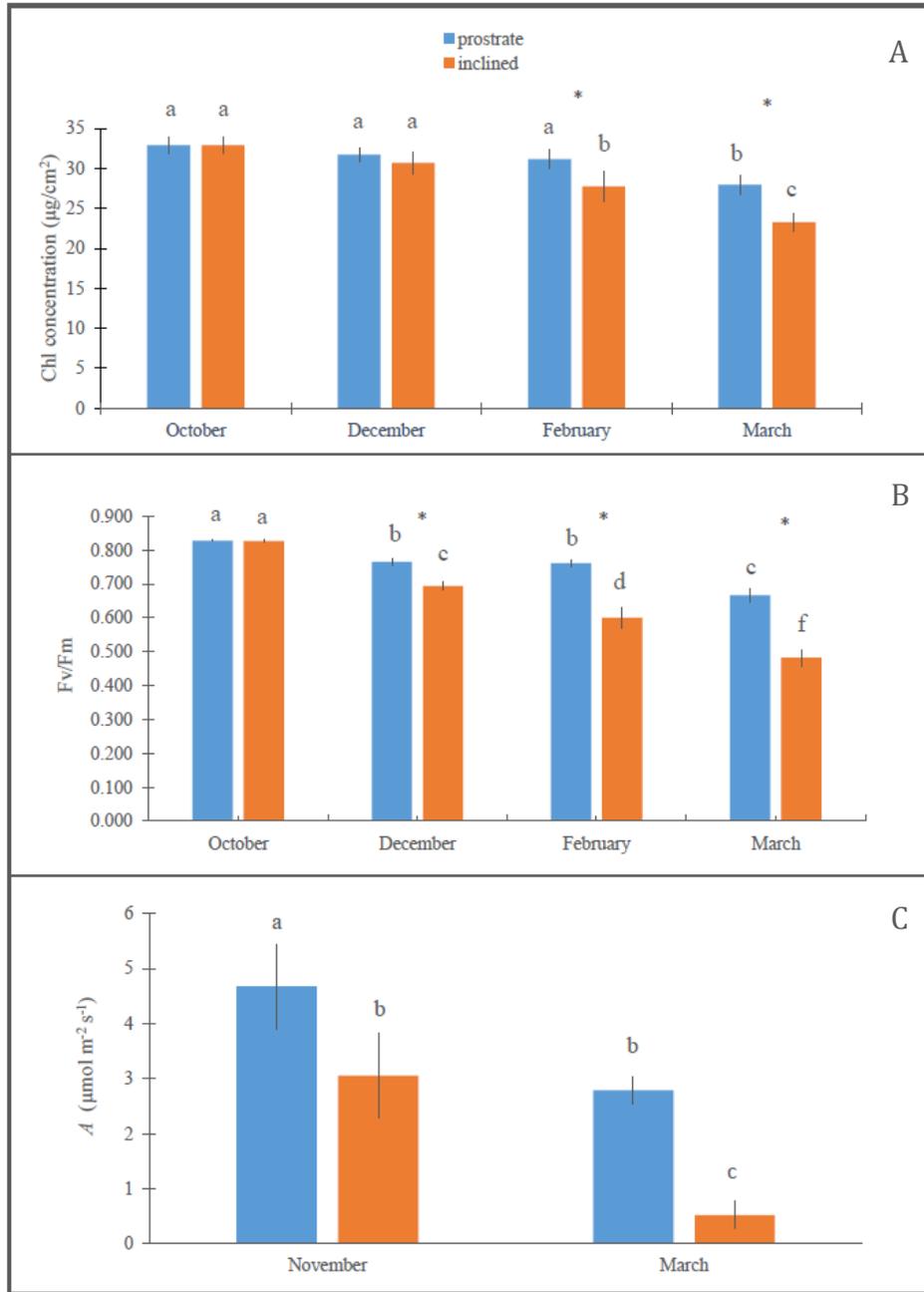


Figure 8A: Total chlorophyll concentration over winter. Measurements taken October 9<sup>th</sup>, 2016; December 17<sup>th</sup>, 2016; February 10<sup>th</sup>, 2017; March 5<sup>th</sup>, 2017. Figure 8B: Chlorophyll fluorescence (Fv/Fm) of prostrate (control) fronds and inclined fronds over winter. Measurements taken October 1<sup>st</sup>, 2016; December 8<sup>th</sup>, 2016; February 10<sup>th</sup>, 2017; March 25<sup>th</sup>, 2017. Figure 8C: Photosynthesis of prostrate (control) fronds and inclined fronds over winter. Measurements were made on November 22<sup>nd</sup>, 2016 and March 25<sup>th</sup>, 2017. Asterisks indicate significant difference ( $p \leq 0.05$ ,  $n = 15$ ). Bars not followed by the same letter indicate a significant difference between sampling dates ( $p = 0.24$  for prostrate fronds,  $p < 0.00001$  for inclined fronds). Bars are means  $\pm$  se.

### Greenhouse Dry-down

Soil moisture (%VWC) of potted plants declined monotonically before approaching zero around 20 days after watering was withheld. Significant differences in soil moisture became apparent by day 3 of the experiment ( $p = 0.02$ ) and continued to diverge (Figure 10A). The mean soil moisture of the control group (watered daily) was 31.2 % for the duration of the experiment.

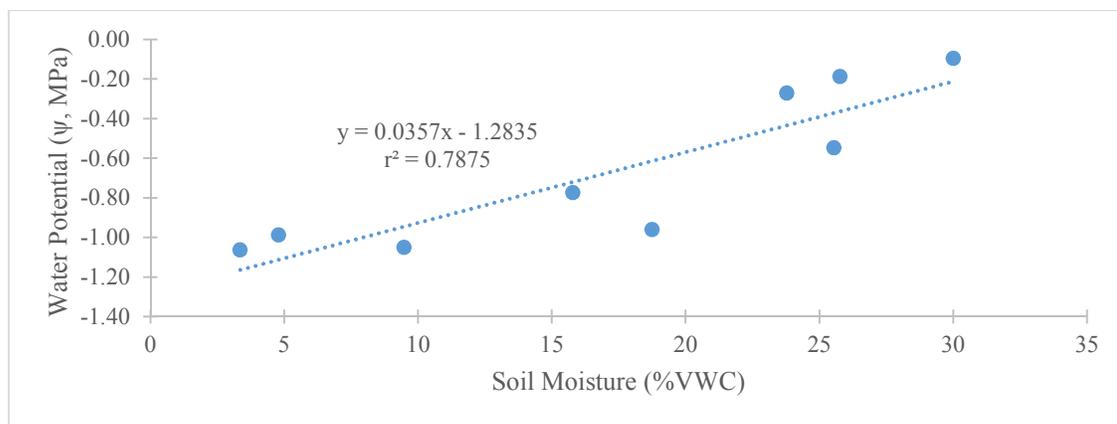


Figure 9: Water potential ( $\psi_f$ ) as a function of soil moisture.

Midday frond water potential ( $\psi_f$ ) varied with environmental conditions inside the greenhouse, and did not follow a monotonic trend during the dry-down, but significant differences in the treatment and control group manifested after 4 days of water deprivation ( $p < 0.0001$ ). Once  $\psi_f$  reached about -1.0 MPa, they plateaued and did not become anymore negative. Well-watered fronds continued to maintain about 0.4 MPa higher  $\psi_f$  over this interval (Figure 10B). A regression analysis showed that water potential ( $\psi_f$ ) linearly tracked soil moisture, with declines in soil moisture corresponding with proportional declines in water potential ( $r^2 = 0.79$ ) (Figure 9).

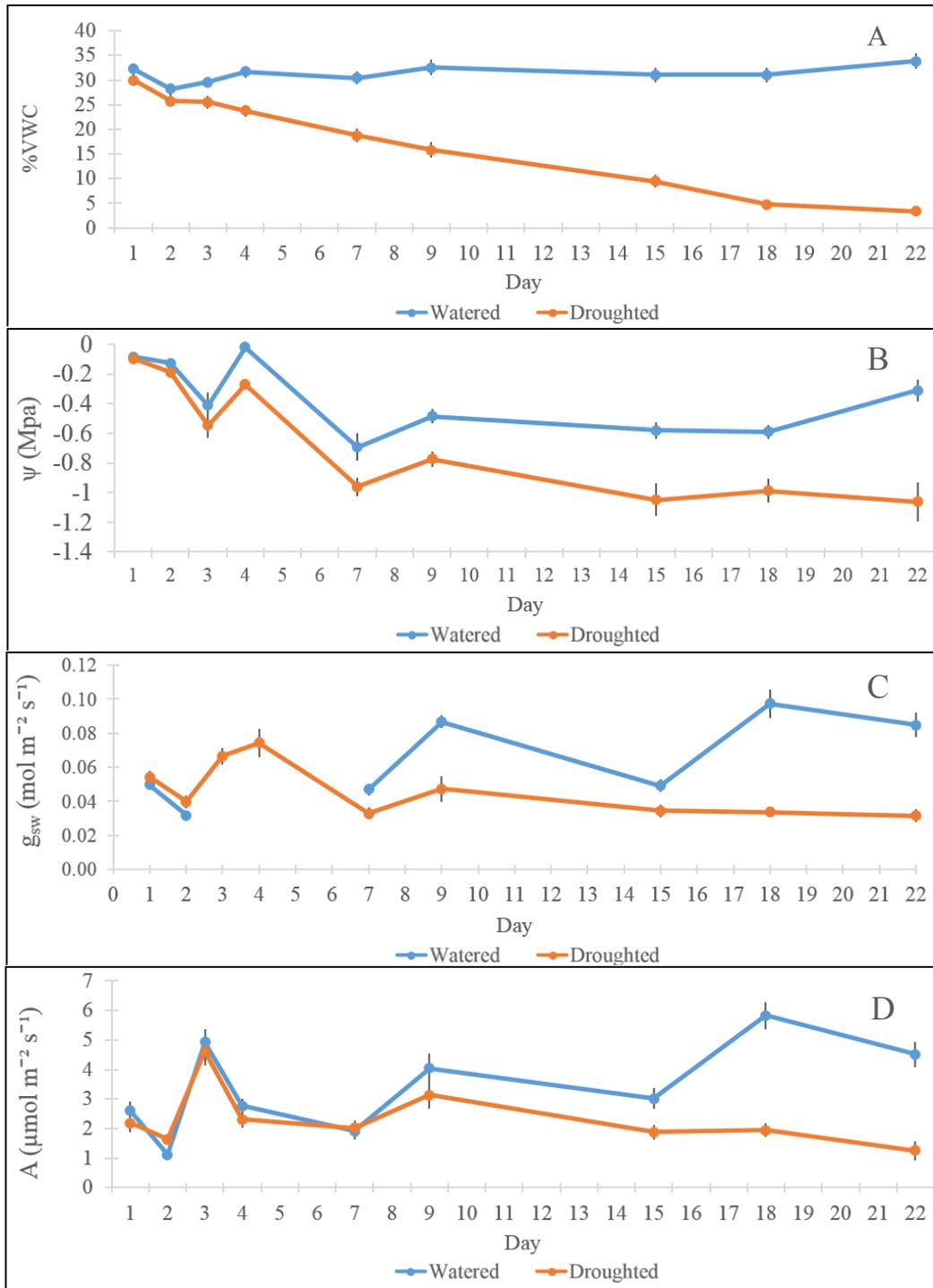


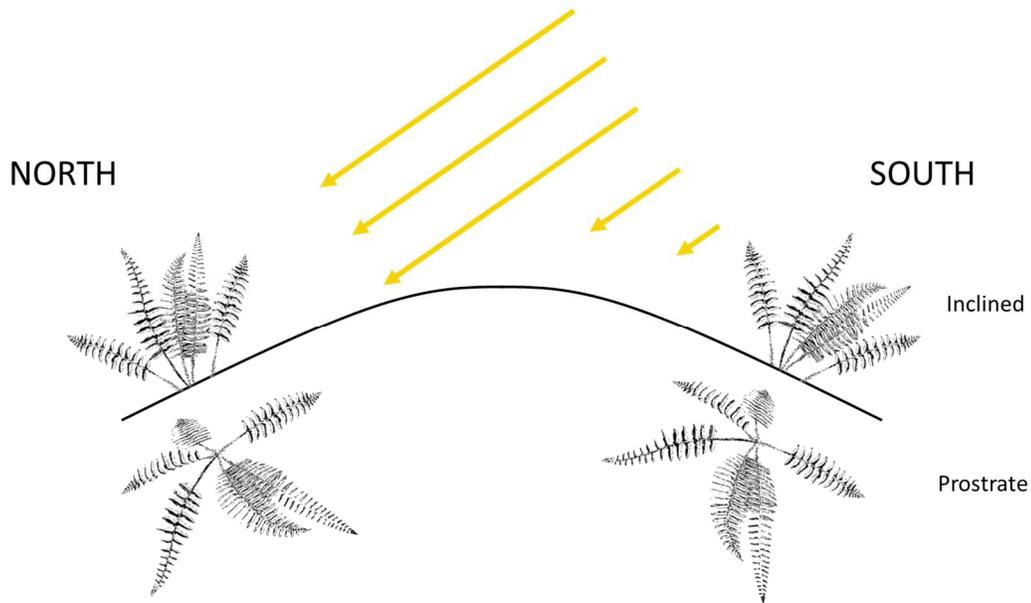
Figure 10: Soil moisture (%VWC) of potted plants declined linearly before approaching zero around 20 days after watering was withheld. Significant differences in soil moisture became apparent by day 3 of the experiment ( $p = 0.02$ ,  $n = 10$ ) and continued to diverge. Error bars = standard error. Midday frond water potential ( $\psi_f$ ). ( $p < 0.0001$ ,  $n = 10$ ) and continued to diverge. Bars are means  $\pm$  se. Stomatal conductance ( $g_s$ ) of watered and droughted plants. ( $p \leq 0.05$ ,  $n = 10$ ). Markers are means  $\pm$  se. Photosynthesis ( $A$ ). ( $p \leq 0.05$ ,  $n = 10$ ). Bars are means  $\pm$  se.

The stomatal conductances ( $g_s$ ) data for days 3 and 4 for the watered group were discarded because the leaflets were wet during measurements. Significant differences between watered and drying ferns manifested on day 7, though the magnitude of this difference was variable over time. Such low conductances made determining drought impacts difficult as the rates were near the lowest levels able to be measured by the Li-6800 (Figure 10C and 10D).

## Discussion

Microclimate data indicated that the most significant difference between north- and south-facing slopes was the amount of winter light received, with south-facing slopes receiving much more light than north-facing slopes, while temperature and water availability were comparable. These results generally agree with other studies of aspect-induced differences in microclimate (Werling & Tajchman, 1984; Desta, *et al.*, 2004; Holst *et al.*, 2005; Warren, 2008; Pauli *et al.*, 2013; Neufeld & Young, 2014). The surrogate fern scaffolding revealed that the light environment is different for fronds at different orientations and that these differences are modified by being on either a north- or south-facing slope. Inclined fronds facing south received much more incident light than fronds facing north and fronds facing straight up, particularly in winter. These data indicate that a prostrate frond on a north-facing slope (parallel to the slope) would receive less light, and that frond reorientation dramatically changes the light environment. This difference in microclimate is important because artificially-reoriented (inclined) fronds suffered significant physiological declines during winter, and these declines are likely due to photoinhibition caused by high light and low temperatures observed during winter (Raven, 1989; Long *et al.*, 1994; Adams *et al.*, 2004; Tessier, 2014). Since declines were relatively slight in the control group, the data support the hypothesis that frond reorientation in Christmas fern confers the advantage of photoprotection by decreasing the angle of incident light during cold, bright days in winter when foliage is vulnerable to photoinhibition. Conceptually, reorientation would not confer

this advantage on south-facing slopes, as it would likely increase the incident angle (Figure 18). However, as Givnish (1982, 1986) and Neufeld and Young (2014) note, the temperature of prostrate fronds on north-facing slopes can be up to 6°C colder in the morning than inclined fronds, while they can be 6°C warmer in the afternoon. Presently, it is unclear how much protection this thermal boundary layer affords on sunny winter days, and frond reorientation is likely driven by a combination of factors in addition to photoprotection.



*Figure 11: Conceptual illustration of frond reorientation as a photoprotective strategy on north-facing slopes, reducing incident angle of radiation. On a south-facing slope, the angle of incidence is more direct, so reorientation would actually increase PAR reaching the plant.*

I predicted that southern exposures would be warmer, brighter, and drier than north-facing slopes. After collecting microclimate data for nearly a year, I found that southern exposures received significantly more light throughout the day, especially during the winter months. Surprisingly, the greater solar input did not correspond with a significant difference in the maximum or minimum daily air temperature at 30 cm above the forest floor, meaning

that on a typical day, a fern on the north slope would experience a similar temperature regime as it would on a south slope, yet significantly less light. I chose to look at daily extremes (maximum and minimum) rather than averages because they are the distribution-limiting factor. Extreme events are important because a lethal environment only needs to arise periodically to limit distribution (Asada, 1999; Melis, 1999; Anderegg *et al.*, 2012). The data support the hypothesis that the microclimate between slopes varies with respect to light, temperature, and soil moisture, and winter light is the most significant difference.

I hypothesized that Christmas fern is highly sensitive to drought stress, and its preference for north-facing slopes may be explained by differences in water availability or evaporative demand between north- and south-facing slopes. While microclimate data established that soil water availability was lower on south-facing slopes, at least at 15 cm depth, analogous conditions produced during the greenhouse dry-down experiment did not correspond to markers of physiological stress in Christmas fern. The results of the dry-down suggest that, at least in the short-term, Christmas fern during the fall, winter and spring months, would not be limited by water availability on south-facing slopes. It remains to be seen if soils become significantly drier on south-facing slopes during the hottest portion of the year in July and August.

The surprising drought tolerance of Christmas fern may be a result of its life history strategy, particularly the wintergreen nature of its foliage. Similar patterns of drought tolerance in other ferns have been found in dry-down studies (Zhang *et al.*, 2009) and these authors also noted complete stomatal closure after drought became severe, which would coincide with my results and the finding that water potentials did not decrease further after reaching about -1.0 MPa. Similar results were found by Holmlund *et al.* (2016) in the field

during the historic California drought between 2012 and 2016. There, evergreen species adapted to either riparian or chaparral habitats maintained their foliar water potentials above -1.3 MPa, similar to the level that my Christmas ferns did in the drought experiment.

McAdam and Brodribb (2013) also noted that stomata in most fern species are sensitive to drought, possibly because they rely more on hydropassive signals for closure rather than hydroactive via the hormone ABA, but more recent evidence indicates that fern stomatal responses may be more complex than first thought (Horak *et al.*, 2017). Pittermann *et al.* (2011) and Brodersen *et al.* (2012, 2015) found that an examination of xylem structure alone could not sufficiently explain the resistance to cavitation in some species of perennial ferns which experience seasonal drought. Similarly, one species aptly named “resurrection fern” (*Pleopeltis polypodioides*) capable of withstanding severe drought by completely closing stomata (John & Hasenstein 2017), due in part to peltate scales on the pinnae (John & Hasenstein 2017) and can tolerate losing up to 95% of their water content (John & Hasenstein 2017). The presence of high light when desiccated has been found to contribute to photoinhibition in this species (Muslin & Homann, 1992), which suggests that a combination of drought and high light, such as could occur during the winter on south-facing slopes, could be detrimental to Christmas ferns also. However, in a drought study of tree ferns, with two species differing in shade tolerance, there was little impact of light level on drought tolerance (Volkova *et al.*, 2010). Furthermore, Baer, *et al.*, (2016) have suggested that perennial ferns may have “capacitance” or the ability to store water in the rhizomes and slowly release it to the leaves during drought. As Christmas fern overwinters, it may experience drought-like conditions due to increased evaporative demand on warm winter days, or to desiccation on cold, low humidity days, especially when the soils are frozen and the plant cannot take up

water from the soil or transport it to the fronds from the rhizome. This would be especially so for inclined fronds because they would be exposed to higher light and wind and this may be one reason this species re-orient its fronds to lie prostrate on the forest floor. This may also help restore water content to desiccated leaves in the winter, because prostrate fronds would be more likely to be moist by lying in the boundary layer of the forest floor, and to retain water on their pinnae after rains or conditions that produce dew, and under such conditions, they can absorb water directly through their leaf surfaces (Limm *et al.*, 2009, Schwerbrock & Leuschner 2017).

## Conclusion

In summary, the goal of this research was to disentangle the abiotic factors driving the asymmetrical distribution of Christmas fern on north- and south-facing slopes, and to elucidate the benefits, if any, of frond reorientation during winter. I conclude that frond reorientation confers the advantage of photoprotection during winter, though there may be other selective pressures driving this behavior. Similarly, this behavior would not offer refuge from high light on south-facing slopes, and the vulnerability of this species to high light may be a principle determinant of its distribution on the landscape. Christmas fern showed surprising tolerance to drought during a controlled dry-down. At this point, I conclude that winter light plays a major role in driving both frond reorientation and the distribution of Christmas fern on the landscape in the southern Appalachian Mountains. Drought, and in particular, summer drought, may further contribute to the asymmetric distribution of this species on slopes in the southern Appalachian Mountains. Future research should further investigate Christmas fern water relations and especially those in the summer when drought conditions may be maximized so as to firmly establish its drought sensitivity and the role of water relations in driving its habitat distribution.

Forecasted climate change in our region is expected to decrease water availability in the understory due to reduced rainfall and increased evaporative demand on forests (IPCC Working Group, 2013, Wiens, 2016). Coupled with higher temperatures, the distribution of Christmas fern may shift toward more mesic habitats such as stream and riverbanks.

Although ferns are highly adaptable, and Christmas fern does exceptionally well in a spectrum of habitats along the east coast of North America, it is not clear if phenotypic plasticity can produce enough local adaptation to outpace shifts in climate. Christmas fern may persist (Agrawal *et al.*, 2004; Flinn, 2006; Colautti & Barrett, 2013) in a future high CO<sub>2</sub>, warmer and drier world, but the necessity of a cold period in Christmas fern phenology, or increases in canopy density and a shrinking vernal window may offset these gains (Allen & Breshears, 1998; Bernhardt-Römermann *et al.*, 2015).

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## **Vita**

David Christian Nielsen was born in Princeton, New Jersey, to David and Jennifer Nielsen. He graduated from East Rutherford High School in Bostic, North Carolina in June 2009. That autumn, he entered Appalachian State University to study Biology, and in December 2013 he was awarded the Bachelor of Science degree. In the spring of 2014, he began study toward a Master of Science degree. The M.S. culminated in summer 2017 with the submission of his Master's Thesis.

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