DOES CANOPY COMPLEXITY INFLUENCE PHYSIOLOGICAL FUNCTION AND CONTRIBUTE TO ECOSYSTEM RESILIENCE OF OLD-GROWTH LONGLEAF PINE (*PINUS PALUSTRIS)?*

by

LAURA LEITH FOWLER

(Under the Direction of Doug Aubrey)

ABSTRACT

Old-growth longleaf pine ecosystems exhibit overall aesthetics due to canopy complexity of overstory individuals, with some exhibiting discrete canopies with branches concentrated at the top, while others retain more continuous lower branches comprised of additional leaf area. To investigate the influence of structural complexity on ecosystem resilience, we measured the physiological differences at two points along canopy height gradients. This research more specifically included quantifying the stand-level relative frequency of canopy types, vertical distribution of leaf area, and measurements of leaf water potential, photosynthetic assimilation, sap flow rates, and environmental variables. There were fewer discrete canopies, which exhibited more consistent physiological activity under varying environmental conditions, and more continuous canopies retaining the lower strata, which provided important contributions to physiological functions via different responses and proportional shifts from the upper strata. Therefore, greater structural complexity yielded greater resilience of longleaf pine to varying environmental conditions in this old-growth longleaf forest.

INDEX WORDS: ecophysiology, sap flow, water potential, gas exchange, restoration

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DEDICATION

To my precious Poppy, for being the serendipitous reminder of all the love, joy, and purpose in life when I need it the most.

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CHAPTER 1

INTRODUCTION

Canopy complexity is important, as the proportion of tree trunks, branches, and crowns are key to withstanding mechanical forces, intercepting light, and transporting water to foliage (King, 2011). The majority of previous studies on the relationship canopy structural complexity has with plant-water relations and physiological responses has focused on many of the much larger coniferous species found in the western United States. These include studies on Douglas-fir (*Pseudotsuga menziesii*) (Apple et al., 2000; Woodruff et al., 2004; Woodruff et al., 2007), giant sequoia (*Sequoiadendron giganteum*) (Williams et al., 2017), and coastal redwood (*Sequoia sempervirens*) (Sillett et al., 2018). The maximum height of a tree is dependent on a variety of factors including species, genotype, environment, as well as changes in morphology and physiology that impact the limitation of water transport (King, 2011). Trees also grow taller when they have abundant resources available, low stress levels, and are competing for light resources (Koch et al., 2004).

Leaf water stress due to gravity and path length resistance, which is based on tree height and xylem conductivity, can limit the expansion and photosynthesis of leaves for further growth, even when soil moisture is not limiting (Koch et al., 2004). Path length and conductivity of water through the xylem play a large role in the varying total resistance to water flow in trees, and paths with higher resistances require more tension, or lower water potential (ψ) in the xylem to move the water up the stem (Ryan and Yoder, 1997). Transpiration (E) maintains a variable gradient of water potential while driving water through the soil-plant-atmosphere continuum as a result of the vapor pressure gradient between leaf and atmosphere (Kupper, 2006). Using sap flow measurements, whole-tree E can be measured directly (Vose et al., 2003). As trees grow taller and water must travel through a longer path, the hydraulic resistance increases (Ryan and Yoder, 1997). Strong effects on water transport can result solely from the constant gravitational force of -0.01 MPa per meter increase in height impacting water potential (Williams et al., 2017).

There is a close inverse relationship between tree height and tree hydraulic conductance (K_L), and it has been found that trees on xeric sites have a higher average driving force for water flow as a result of their shorter stature (Addington et al., 2006). In addition, the increasing height of trees imposes several constraints on the tree physiological function, even at the leaf level (Woodruff et al., 2007). Canopy stomatal conductance (G_S) at a vapor pressure deficit (VPD) of one kPa comprises the reference canopy stomatal conductance (G_{Sref}), which was also shown to decline across both mesic and xeric sites with increases in tree height, implying that there is a consistent single relationship between these two factors (Addington et al., 2006).

An adequate level of water must be maintained by plants so that vital processes like photosynthesis will continue to occur, so the plant can survive and grow (Porporato et al., 2001). The gravitational component of plant water potential can impose physiological constraints on turgor that hinder both leaf expansion and branch extension growth in tall Douglas-fir trees (Woodruff et al., 2004). Xylem hydraulic capacity limits net photosynthesis, thus also limiting growth in older, taller trees, which is more extreme in the xylem of conifers since their sapwood anatomy is composed of only tracheids (Kupper, 2006). Tracheids are elongated water-conducting cells found in the xylem of coniferous tree species that are more susceptible to cell embolism with increasing water stress, which could eventually lead to cavitation and tree mortality (Woodruff et al., 2007). The net rate of photosynthesis for pine species drops to zero when water potential value is between -2.0 to -2.8 MPa (Teskey et al., 1994), so water potentials lower than these values would result in reduced carbon assimilation for tree growth due to respiration.

Trees display changes in their proportion and growth rates over time, and it has been proposed that the costs associated with mechanical support of growing trees influences height limitation and the general decline in growth rates of trees (King, 2011). The mechanisms associated with increasing tree height may also include compensatory adjustments that could contribute to the ability of trees to reduce such costs (Woodruff et al., 2007). A study measuring leaf hydraulic conductance (K_s) and stomatal conductance (g_s) with decreasing leaf level water potential (ψ_L) in Douglas-fir across a canopy height gradient found that stomatal closure was a vital compensatory method (Woodruff et al., 2007). Within tree canopies, stomata play a critical role in regulating plant water status by finding a balance between transpiration and the hydraulic system (Woodruff et al., 2007). This allows water to be supplied to the leaves as a means of reducing leaf embolism and death with increasing leaf water stress (Woodruff et al., 2007). Stomata close in response to lower water potentials as a means of conserving the tree water supply, but this also leads to reduced stomatal conductance (g_s) and photosynthesis, which results in lower levels of carbon assimilation for tree growth (Ryan and Yoder, 1997). For individuals of the same species growing on similar sites, mean g_s, as well as photosynthesis, will be lower in taller trees (Woodruff et al., 2007).

In addition to internal tree variables like canopy complexity and leaf area, a variety of external climatic variables, including but not limited to sun angle (Braghiere et al., 2020), vapor pressure deficit (Domec et al., 2009), soil moisture (Porporato et al., 2001; Vose et al., 2003), temperature, humidity, rainfall, and wind speed (Domec et al., 2009; O'Brien et al., 2004; Teskey et al., 1994) can influence tree physiological functions. The productivity of coniferous tree species may be affected by climate change through influencing needle morphology, such as size and surface characteristics, and needle function, like photosynthetic capacity (Apple et al., 2000). However, a study of Douglas-fir needles showed that there were no significant documented changes in the morphology and stomatal function when needles were exposed to the simulated climate change aspects of increased temperature and carbon dioxide levels (Apple et al., 2000). Similar results were found in a study on longleaf pine needle morphology showing that there was no change in the stomatal distribution or density found on needles following the simulation of elevated carbon dioxide levels (Pritchard et al., 1998). These studies provide a general indication that conifer needles are able to maintain similar function with changes in climate, but they do not provide context of how needles respond or differ throughout the canopy.

With a growing focus on trees and reforestation for climate change mitigation regarding more limited water availability during droughts, a better understanding of canopy complexity and its impacts on tree function must be obtained. Longleaf pines are tolerant of dry, well-drained sandhill soils (Gonzalez-Benecke et al., 2011). Therefore, they are generally considered to be more tolerant of drought stress than other native southern pine species, making them better suited to deal with the predicted increases in drought frequency in response to climate change (Samuelson et al., 2012). High or extremely high temperatures affect plant physiological processes, and these increased temperatures can lead to more frequent and longer periods without rainfall events (Samuelson et al., 2012). The ability of plants to assimilate carbon is highly dependent on total rainfalls, especially during the growing season, in addition to both the frequency and intensity of these rain events (Porporato et al., 2004).

All three of the previously mentioned western conifer species can reach maximum heights of about 100 to 120 meters, compared to longleaf pine which grows to be about 30 meters tall, yet they can still maintain water transport from the soil to the canopy for physiological processes to continue at the tree tops so that individuals continue to grow. Addington et al. (2006) found that maximum heights for longleaf pines were greater on mesic (moist) versus xeric (dry) sites, which were classified by soil drainage and woody plant composition. Mesic sites had higher overstory tree density, and the trees were significantly taller at given DBH with a higher sapwood-to-leaf area ratio than xeric sites, suggesting that shorter xeric site trees, which displayed greater belowground biomass, are hydraulically equipped for water stress (Addington et al., 2006). While these three western tree species are much taller than longleaf pine, mature overstory longleaf individuals can still maintain physiological functions across a canopy height gradient.

The natural range of longleaf pine is comprised of the majority of the Coastal Plains region of the southeastern United States, yet over 97 percent of the original land area once occupied by this species has been converted for other uses (Gonzalez-Benecke et al., 2011). As restoration of this species becomes increasingly important across the southeast, it will be equally important to have an understanding of how to manage for the restoration of the uneven-aged mosaic found in natural old-growth longleaf ecosystems (Brockway et al., 2005) if this canopy complexity does increase ecosystem resilience.

CHAPTER 2

CANOPY COMPLEXITY, PHYSIOLOGY, AND RESILIENCE OF OLD-GROWTH LONGLEAF PINE

Introduction

Canopy architectural complexity is important because it affects wind resistance, light interception, and water transport (King, 2011). Canopy architecture can vary widely across species and is generally interpreted as the size, shape, orientation, and distribution of spatially segmented aboveground plant material (Braghiere et al., 2020). Thus far, research studying the impacts of tree height and canopy architecture on the physiological responses of trees has been limited to the taller conifer species found in the western United States. These studies showed that canopy architecture affects physiology primarily through the impacts of gravity and xylem tension, causing a constant decline in water potentials with increasing height even without transpiration (Williams et al., 2017). Increasing height further causes greater friction from longer xylem element path lengths during transpiration (Woodruff et al., 2007), all of which reduce photosynthetic capacity (Woodruff et al., 2004).

Structural complexity and leaf area distribution may also contribute to physiological and functional differences that occur along canopy height gradients. In temperate regions, the simplification of stand-level canopy structure away from old-growth multilayered canopies (Mitchell et al., 2009), primarily via intensive even-aged management for higher short-term yields, has led to a decline in ecosystem functions (Peterson et al., 1998). This decline in function, including the biophysical processes that influence plant physiology and morphology, can lead to an increase in ecosystem vulnerability to disturbances across a wide range of spatial and temporal scales (Peterson et al., 1998). In the future, trees may have to overcome greater hydraulic limitations in regions where drought frequency and intensity increase (Granier et al., 2007; Domec et al., 2009). Plant physiological responses to acclimate and survive hot, dry periods vary with the intensity and duration of the drought (Pinheiro and Chaves, 2010). Therefore, trees must utilize conservative hydraulic architecture (Williams et al., 2017), achieved by stomatal regulation of plant water status based on soil, leaf, and atmospheric water availability (Bond and Kavanagh, 1999) to maintain physiological activity without cavitation under conditions of low water availability (Pinheiro and Chaves, 2010; Woodruff et al., 2007; Kupper, 2006; Porporato et al., 2001; Ryan and Yoder, 1997). Absolute hydraulic conductance increases with increasing leaf area due to more paths for water movement, but a compensatory response to improve diminished hydraulic conductance is to reduce leaf area relative to sapwood area (Becker et al., 2000; Kupper, 2006; Woodruff et al., 2007), with this reduction in canopy leaf area achieved by either inhibiting growth of new leaves or early senescence of older leaves (Pinheiro and Chaves, 2010). Also, differences in daily water use of co-occurring species results from differences in leaf area (Gonzalez-Benecke et al., 2011). This suggests that for individuals of similar size, those with more leaf area will have a greater ability to maintain physiological processes in comparison to those with less leaf area. Estimating whole-tree transpiration via xylem water flux is becoming increasingly important as a means of providing insights into physiological responses of forests, especially when water use is measured as multiple heights within a tree crown (Wullschleger et al., 1998). This estimation of water use at different canopy positions will indicate differences along the canopy height gradient based on the vertical distribution of leaf area.

There is a strong relationship between transpiration and photosynthesis (Lu et al., 2004), so differences in physiological functions, such as photosynthesis, are also similarly dependent on crown form and canopy position at the species and individual tree levels (Ishii et al., 2004). Recent comparative research on canopy architecture in other temperate forests has found that greater net primary productivity correlated with greater canopy structural complexity (Gough et al., 2019), and trees with less leaf area were less able to convert solar radiation to usable energy for growth (Pederson, 2010). Research on Douglas-fir showed marked changes in growth and morphological changes of leaves and branches, with the natural old-growth trees in a mixed conifer stand displaying a greater increase in leaf area along the vertical canopy height gradient when compared to a planted monoculture forest (Woodruff et al., 2004). This indicates that forests retaining greater stand-level structural complexity and leaf area are more physiologically active and productive overall. Expanding the current understanding of the existing relationship between physiological function and canopy complexity at both individual tree and stand levels can provide valuable insight to how this influences species and ecosystem resiliency to hydraulic limitations.

Canopy complexity may also be an important stand characteristic for determining the changing seasonal physiological capacity of evergreens in temperate systems under moderate winter climates, specifically if they maintain functions at similar or even higher rates across different canopy types and within the vertical stratification of canopies. In addition to endogenous tree variables like canopy complexity and leaf area, a variety of external climatic variables, including but not limited to vapor pressure deficit (Domec et al., 2009), soil moisture (Porporato et al., 2001; Vose et al., 2003), temperature, humidity, rainfall, and wind speed (Domec et al., 2009; O’Brien et al., 2004; Teskey et al., 1994) can influence tree physiological functions. Currently, most studies focus on photosynthesis measurements made on leaves receiving direct sunlight (Berry and Goldsmith, 2020), but sun angle could also have an influence (Braghiere et al., 2020). Studies are also conducted primarily during growing seasons, so winter is typically discounted as a dormant season with limited physiological activity. However, it has been shown that positive net photosynthesis can be measured during any season for pines located in warmer regions, but the duration of irradiance affects both diurnal and seasonal patterns (Teskey et al., 1994). Canopy reflectance and absorption efficiency of incident photosynthetically active radiation (PAR) are both dependent on the same factors, including vegetation clumping, leaf area, and solar angles (Bégué, 1993). Therefore, a greater understanding of photosynthesis under different light and seasonal conditions would be particularly relevant in the winter with changing solar zenith angles resulting in greater quantities of direct versus diffuse light and enhanced photosynthesis within the more typically light-limited lower strata of the canopy (Braghiere et al., 2020).

Fundamental differences in canopy architecture might have large impacts on tree physiological responses and resilience to drought. Old-growth longleaf pine (*Pinus palustris*) ecosystems have a structurally complex canopy architecture (Mitchell et al., 2009), with some trees exhibiting discrete canopies with branches concentrated at the tree top, while others retain many lower branches forming continuous crowns (Fig. 1). In this ecosystem, trees without a deep crown might experience greater water stress and embolization during extreme droughts than deep, complex crowns where lower partially shaded branches might still be able to photosynthesize. While longleaf pines reach maximum heights that are only about one third of the larger western conifer species, making them subsequently less affected by gravitational forces, they could still be sufficiently tall and exhibit enough canopy complexity to influence tree function, particularly since they are exposed to frequent fire every 1-5 years (Christensen, 1987). In the context of ecosystem resilience, longleaf pine restoration could prove to be important in the southeast because of this species' higher tolerance to drought conditions and poor soil quality in an uneven-aged, old-growth stand (Addington et al., 2006). They also have a greater ability to maintain physiological functions on the same site with limited access to water than other pine species planted and managed similarly with an even-aged approach (Samuelson et al., 2012), indicating that both species and structure are important aspects of resilience. Longleaf pine has this ability as an "isohydrodynamic" species (Gonzalez-Benecke et al., 2011), which allows them to grow across a range of xeric to mesic habitat types and adjust their structure (i.e. height and leaf area) and hydraulic architecture to maintain similar levels of physiological activity across these sites with varying water availability (Addington et al., 2006). The more southern portion of the longleaf pine range encompasses areas with moderate winter climates, which would allow these trees to maintain physiological activity year-round (Teskey et al., 1994). If canopy complexity does contribute to greater ecosystem resilience through physiology, then silviculture for restoration should include proactively managing for the structural differences found in natural old-growth longleaf stands, such as lower stand density, uneven size class distribution, and more complex spatial patterns (Noel et al., 1998), in addition to mere land area coverage (Mitchell et al., 2006). This approach will protect existing and promote future old-growth forests in the short-term, as well as a more long-term management approach that will ensure a reduction in vulnerability and increase in recovery of longleaf pine forests (Mitchell et al., 2009).

Our goal was to discern how the structural complexity of old-growth longleaf pine canopies influences physiological function, and how this may relate to ecosystem resilience and inform restoration efforts. Initial observations suggested that continuous canopies would have more leaf area than discrete canopies. We hypothesized that any differences in whole-tree transpiration between canopy types and canopy strata would be largely explained by leaf area. However, we also hypothesized that continuous canopies would maintain higher rates of transpiration than discrete canopies during periods of low moisture availability and high VPD because lower canopy strata would maintain higher water potential simply due to height and gravity (i.e. not simply a leaf area effect). An alternative hypothesis is that retaining larger quantities of leaf area would lead to greater water stress for continuous canopies than discrete canopies. Moreover, we hypothesized that the relative difference in whole-tree transpiration between continuous and discrete canopies would increase during the winter because the greater solar zenith angle would favor transpiration in the lower continuous canopy strata relative to the upper strata. Support for our hypotheses would indicate that longleaf forests containing continuous canopies would be more resilient to hot, dry periods (i.e. expected future conditions across their range) than forests containing only discrete canopies.

Materials and Methods

Study Site

The study site is located on Greenwood Plantation, near Thomasville, Georgia, an area referred to as the Red Hills Region of southern Georgia. In total, Greenwood Plantation is comprised of over 2,000 ha, with more than 400 ha of old-growth longleaf pine forests. Our specific study site was located on a 182-hectare stand called the Plateau with an overstory dominated by old-growth longleaf pine. Individual longleaf pines on this site can exceed 300 years old. The old-growth longleaf pine landscape present on this site represents the historical accounts of an open, park-like woodlands maintained by frequent, low severity fires with an understory rich in biodiverse grasses and forbs (Peet, 2006).

Experimental Approach and Design

Mature overstory longleaf pines were categorized as either continuous or discrete according to canopy type (Fig. 1). Continuous canopy trees exhibited branches extending down roughly two thirds of the total tree height, whereas discrete canopy trees exhibited branches that only extended down about one third of the total tree height. Continuous canopies were experimentally divided into lower and upper strata to explore potential differences in physiological processes as a function of leaf area distribution.

Diameter, sapwood area, and a LIDAR-derived estimates of relative stand-level canopy type frequency and leaf volume were determined to explore differences in canopy characteristics and hydraulic architecture between canopy types and within continuous canopy strata. Transpiration was estimated via sap flow to determine absolute and relative differences in water use between canopy types and strata. Leaf water potential and gas exchange were measured seasonally to further explore physiological differences related to water use and water stress between canopy types and strata. Instantaneous water use efficiency (WUE) was calculated to determine how differing canopy type or canopy strata impacts tree physiological responses to water deficits. Integrated water stress of needles across time during the entire study period was also measured for individuals of differing canopy types, as well as for lower and upper strata within continuous canopies.

Measurements required accessing tree canopies with a boom lift, so all 24 experimental trees were within 5m of a narrow, mowed road. The sap flow portion of the study consisted of six randomly selected individuals from each of the two canopy types, which were spatially paired in blocks. Six additional trees of each canopy type that were not instrumented for sap flow were selected for the leaf water potential and gas exchange portions to avoid canopy damage to sap flow trees from repeated branch sampling. The need to measure trees using a boom lift constrained our ability to spatially pair these canopy types in blocks, so the leaf water potential and gas exchange portion of the study used a completely randomized design.

Tree Characteristics

Leaf area was estimated from terrestrial laser scanning (RIEGL VZ2000, Orlando, FL, USA) based on the methods of Rowell et al. (2020). This methodology uses reflectance to measure distance (Z), and Z is associated with a ground-level longitude (X) and latitude (Y) coordinate pair to generate a three-dimensional point cloud of an area which provides volumetric estimates at a fine scale (sub-cm^3) that are closely correlated to leaf area (Rowell et al., 2020). A 3D point cloud (Cloud Compare software v2.9.1) of the entire scanned study site was preserved, in addition to the individual point clouds for all 24 trees used in this study that were segmented from the final registered scan. Individual trees were later processed using the VoxR package (Lecigne et al., 2014) in R 3.5.1 (2018), which we used to bin leaf biomass from an individual tree into a 10 cm^3 voxelized grid. We used the number of occupied voxels for each tree as an estimate of the volume of leaf biomass and proxy for leaf area. A sum of the occupied voxels was then computed to determine the total volume for the two canopy types and the two strata of continuous canopies. While using this method precluded calculating an absolute volume of leaf area, we could infer a relative volume of leaf area from occupied voxel volumes based on the strong correlation of the two values (Rowell et al., 2020), and the volume allowed for accurate partitioning of leaf biomass within strata of continuous crowns. Analysis of the LIDAR scans using a minimum height threshold of 20m also allowed for the demographic quantification of mature overstory trees classified by canopy type for the study site.

Stem diameters were obtained at 1.4m (i.e., diameter breast height) and 20 meters to reflect the height placements of the thermal dissipation (Granier, 1985) sap flow sensors. Diameters were measured from the LIDAR scans and verified with a diameter tape. Incremental tree cores were taken at the conclusion of the year-long study period for the 12 sap flow trees at each sensor location to avoid damage to the xylem while measuring sap flow. The total area was calculated for the trees at both the breast height and mid-canopy positions using diameter measurements. Then the incremental tree cores were measured for sapwood width which was, along with 1cm to account for bark area, subtracted from total tree diameter, and the remaining diameter was used to calculate heartwood area. Sapwood, or functional xylem area was calculated as the difference between total area minus heartwood area.

Environmental Measurements

Soil volumetric water content (θ_V) was measured at 0.1 m depth within three of the six sap flow blocks and values were averaged and recorded every 15 minutes using battery powered dataloggers (CR10X Campbell Scientific Inc., Logan, UT, USA). Precipitation was collected using three rain gauges placed within 2m of the soil moisture probes. Temperature, humidity, solar flux, and wind speed were all taken at a weather station at Tall Timbers Research Station located ~20 miles south of the study site in Tallahassee, FL. Zenith angles were obtained for daily solar noon throughout the entire study period using a solar calculator based on the GPS coordinates of the study site (NOAA Solar Calculator, Earth System Research Laboratories Global Monitoring Laboratory).

In addition to measuring soil moisture values throughout the day, daily levels of hydraulic uptake from groundwater to the surface soil occurring at night with no transpiration could be calculated. The values of this hydraulic redistribution were then removed each day over prolonged periods of no rainfall to explore the impact of this water subsidy.

Sap Flow

Whole-tree transpiration was estimated via thermal dissipation sap flow (Granier, 1985), beginning on April 16, 2019, and ending on May 20, 2020. Sensors were fabricated following methods in Sun et al. (2010) and placed on the north and south sides of trees at breast height for both canopy types and at 20 m height for continuous canopies. The sensors located at breast height allow direct comparison of whole-tree water use by continuous and discrete canopies. Sap flow in the upper strata of continuous canopies was calculated directly from the sensors positioned at 20 m height and sap flow in the lower strata was calculated as the difference in sap flow between the breast height sensors and the sensors at 20 m height. Sensors were installed by drilling a small diameter hole with a #53 bit (1.5113mm), large enough to insert a 19-gauge hypodermic needle and connected to battery powered dataloggers (CR10X Campbell Scientific Inc., Logan, UT, USA) that measured sap flow every minute and logged 15-minute averages.

Water Stress

A pressure chamber unit (Model 1000, PMS Instruments, Corvallis, OR, USA) was used to measure ψ_L of a single fascicle comprised of three individual needles. Each sample was measured with the pressure chamber within three minutes of removal from the tree. For each canopy type and strata, three subsamples of needle fascicles were collected and averaged to account for within tree variation. Midday ψ_L measurements were conducted between 1300 to 1500 EST to calculate xylem tension under peak diurnal water stress. These diurnal measurements were conducted at the same canopy positions within the same 12 trees for 4 seasons. Seasonal averages were procured from the diurnal measurements to provide insight as to how tree xylem tension changes under varying environmental conditions for the differences between canopy types and continuous canopy strata.

Foliar Carbon isotope ($\delta^{13}C$) analysis was conducted on current-year needles once at the end of the year-long observational period for all 24 individuals used in the study. This provided an index of long-term integrated water stress for both canopy types and continuous canopy strata. Samples were collected, then dried and processed in a ball-mill grinder before being sent for chemical $\delta^{13}C$ analysis using mass flow spectrometry (University of Georgia Stable Isotope Lab, Athens, GA, USA).

Gas Exchange

Gas exchange measurements were collected in the morning from 0900 until 1200 EST prior to water potential measurements. Gas exchange was measured on 10 of the 12 trees used for the water potential measurements, with a sample size of five of each canopy position: LC, MC, and MD. Three leaves from each canopy type and strata were measured. Values for A_{max} were generated at 1200 PAR (photosynthetically active radiation) using a LI-6400XT portable photosynthesis system equipped with a LI-6400-02B LED light source (Li-Cor Instruments, Lincoln, NE, USA). Other parameters were held constant, with a reference CO_2 of 400 μmol/mol, leaf temperature of 20°C, and flow rate of 500 μm. Instantaneous WUE was calculated as the ratio between leaf-level CO_2 assimilation (A_{max}) and transpiration (E) using gas exchange measurements obtained with the LI-6400XT.

Since the needles of pines do not completely fill the cuvette area of the light source, the needles used for each measurement were collected so that subsample photosynthetic rates could be adjusted for leaf area and included in post-processing of data. Leaf surface area length was a standard 3cm per the length of the LI-6400XT cuvette, and the width for each subsample was measured at mm scale using a mini comparator. Gas exchange measurements were also collected seasonally (Table 6), but due to time constraints and equipment issues during the boom rental period, we were unable to collect discrete canopy samples in the Summer, so a canopy type comparison could not be done for this season.

Data Analysis

The six blocked pairs of canopy types (continuous and discrete) used for measurements of sap flow were first processed to calculate mean daily volumes (l day^{-1}) of water use by canopy types and strata. From these daily values, the cumulative sum of the volume of water use (L) over the year-long study period was calculated and analyzed using ANOVA with an alpha of 0.05 and means separation using Fisher’s LSD. This analysis determined the relative differences in whole-tree water use of continuous versus discrete canopy types and water use by the lower and upper continuous canopy strata. Canopy type was treated as a fixed factor with two levels (continuous and discrete), and canopy strata were similarly treated as a fixed factor with two levels (lower and upper). For both canopy type and canopy strata comparisons, experimental units (individual trees) were treated as random factors

The environmental variables were analyzed using a principal component analysis (PCA) in R 4.0.2 (2020). A PCA can interpret correlated data sets that are orthogonal, and thus independent of each other, while retaining much of the information about the original variables. There are multiple external environmental variables that interact with each other, the internal tree factors, as well as the varying scales of leaves, crowns, and forest canopies to drive transpiration (O'Brien et al., 2004). Using this method of analysis allowed for the integration of external environmental variables with whole-tree water use by canopy type, and the comparative volume of water use between lower and upper strata within the continuous canopy height gradient. For the PCA, a factor analysis of weather data was conducted first to determine the subset of variables that explained variations in tree water use. This method analyzes factors and their contributions independently of each other to eliminate redundancies due to their interactions. Following the original factor analysis, a varimax rotation was tested, but only added nominal improvement to the explanation of variation, so the factors obtained from the original analysis were used instead of those from the rotation. Once the significant factors (PC1 and PC2) were established, they were regressed against sap flow responses to determine the relationship between these principal components and tree water use as a function of differences in canopy structural complexity.

A univariate repeated measure ANOVA with an alpha of 0.05 and means separation using Fisher's LSD was used to compare seasonal water potential and gas exchange between canopy types and continuous canopy strata. Canopy type was treated as a fixed factor with two levels (continuous and discrete), and experimental unit (individual tree) was treated as a random factor. Similarly, canopy strata were treated as fixed factors (lower and upper), and the experimental unit (individual tree) was treated as a random factor. For this analysis, gas exchange values consist of maximum photosynthesis (A_{max}) at 1200 PAR. All ANOVAs were performed using the lme4 package (Bates et al., 2015) in R 4.0.2 (2020).

Results

Tree Characteristics

Continuous canopies were more common than discrete canopies in this old-growth longleaf stand. The 5.6 ha site scanned using LIDAR was comprised of 76% continuous canopies and 24% discrete canopies, with an average of 30.9 continuous canopies per hectare and 9.6 discrete canopies per hectare.

Diameter at breast height (DBH) was similar between canopy types for the trees used for water potential and gas exchange measurements (Table 1, p=0.4730) and for trees used for sap flow measurements (Table 2, p=0.6840). Functional sapwood area at breast height was similar between canopy types (Table 1, p=0.5250). There was discernible taper from lower to upper strata of continuous canopy trees, with diameter at breast height that was 40% higher than diameter at mid-crown (Table 3, p=0.0008). Sapwood area was 55% higher at breast height than at mid-crown for continuous canopy trees (Table 3, p=0.0016).

The quantity of occupied voxels, which was used as a proxy for leaf area, was 43% higher for continuous than discrete canopies (Table 1, p= 0.0392). Occupied voxel quantity was similar between lower and upper canopy strata (Table 4, p=0.2770), suggesting leaf area was somewhat proportionally distributed throughout continuous canopies.

Cumulative Sap Flow

Cumulative water use (l) differed by canopy type ($p<0.0001$; Fig. 2), with continuous canopies using 40.8% more water than discrete canopies across the year-long study period. While overall cumulative water use (l) was similar among continuous canopy strata ($p=0.6650$), proportional shifts occurred where greater volumes of water were allocated to the different strata at different times across the study period, most notably the greater rate of increasing water allocation to the lower strata in the winter (Fig. 3).

Environmental Variables

The first two PCA axes explained 67% of the total variance in the environmental variables, with the first principal component (PC1) axis explaining 41.4% and the second principal component (PC2) axis explaining an additional 25.5% (Fig. 4). Temperatures (maximum, average, and minimum) and solar zenith angle were most strongly correlated with PC1, at 47%, and 38% respectively (Table 5), so the first axis could be categorized as seasonally sunny and hot. PC1 was positively correlated with solar zenith angle, VPD, and soil moisture, while negatively correlated with maximum, mean, and minimum temperatures (Table 5). PC2 was most strongly correlated with irradiance at 52%, relative humidity at 57%, and VPD at 48% (Table 5), so the second axis could be categorized as sunny and dry. PC2 was positively correlated with solar irradiance and VPD, but negatively correlated with humidity, soil moisture, and precipitation (Table 5). Overall, there were strong negative correlations of temperatures with wind speed, zenith angle, VPD, and soil moisture (Table 6). There were also strong negative correlations of relative humidity with irradiance, VPD, and soil moisture (Table 6).

Irradiance was positively correlated with VPD, but negatively correlated with both zenith angle and soil moisture (Table 6).

Daily Sap Flow

When analyzed in relation to the environmental variables in the PCA, the absolute volume of whole-tree water use (l day^{-1}) for continuous canopies was not influenced ($p=0.6330$), but discrete canopies had a negative relationship ($p<0.0001$) between water use and PC1 (Fig. 5). Both continuous ($p<0.0001$) and discrete ($p=0.0043$) canopies had similar negative relationships between water use and zenith angle (Fig. 6). Continuous canopy water use was not related ($p=0.1240$) to temperature, but discrete canopies had a positive relationship ($p<0.0001$) between water use and temperature (Fig. 7).

Both continuous ($p<0.0001$) and discrete ($p<0.0001$) canopies had similar positive relationships between water use and PC2 (Fig. 8). Continuous ($p<0.0001$) and discrete ($p<0.0001$) canopies also had similar positive relationships between water use and irradiance (Fig. 9). Continuous ($p<0.0001$) and discrete ($p<0.0001$) canopies had similar negative relationships between water use and relative humidity (Fig. 10). Additionally, both continuous ($p<0.0001$) and discrete ($p<0.0001$) canopies had similar positive relationships between water use and VPD (Fig. 11).

The volume of water use by continuous canopy strata had a positive relationship between lower strata ($p<0.0001$) and PC1, but there was no relationship between the upper strata ($p=0.0509$) and PC1 (Fig. 12). Water use by the lower continuous canopy strata ($p=0.8140$) was not influenced by zenith angle, but the upper strata ($p<0.0001$) had a negative relationship between water use and zenith angle (Fig. 13). The lower canopy strata ($p<0.0001$) had a negative relationship between water use and temperature, but the upper canopy strata ($p<0.0001$) had a positive relationship between water use and temperature (Fig. 14).

Both the lower ($p<0.0001$) and upper ($p=0.0437$) continuous canopy strata had similar positive relationships between water use and PC2 (Fig. 15). Lower ($p<0.0001$) and upper ($p=0.0491$) canopy strata both had similar positive relationships between water use and irradiance (Fig. 16). Lower ($p<0.0001$) and upper ($p<0.0001$) continuous canopy strata both had similar negative relationships between water use and relative humidity (Fig. 17). The lower ($p<0.0001$) and upper ($p<0.0001$) strata both had positive relationships between water use and VPD (Fig. 18).

Water Stress

ψ_L was influenced by canopy type and season, but these effects were not independent (i.e., canopy type × season interaction; $p=0.0282$) For example, ψ_L was 30% and 41% lower in continuous compared to discrete canopies in fall and spring, respectively, but similar between canopy types in summer and winter (Fig. 19). ψ_L was 64% and 33% lower in the fall than in the spring for continuous and discrete canopies, respectively (Fig. 19). ψ_L was also 34% and 44% lower for continuous and discrete canopies, respectively, in the fall than in the spring (Fig. 19). Continuous canopies had 58% and 45% lower ψ_L in the summer and spring, respectively, than in the winter, and discrete canopies had 40% lower ψ_L in the summer than spring (Fig. 19).

ψ_L of continuous canopies was influenced by canopy strata ($p=0.0125$) and season ($p<0.0001$). The lower strata of continuous canopies exhibited higher ψ_L than the upper strata (Fig. 20) by 16% in the summer, 13% in the fall, 19% in the winter, and 25% in the spring.

Seasonally, ψ_L was highest in the winter, intermediate in the spring, and lowest in the summer and fall (Fig. 21).

Analyses of carbon isotope ($\delta^{13}C$) results showed no difference by canopy type (p=0.6300) or by canopy strata (p=0.0752), indicating no differences in integrated water stress over the entire study period (data not presented).

Gas Exchange

A_{max} was influenced by canopy type and season, but these effects were not independent (i.e., canopy type × season interaction; p=0.0033). Continuous canopies had a 26% lower A_{max} than discrete canopies in the fall, but continuous canopies had a 38% and 29% higher A_{max} in the winter and spring, respectively, than discrete canopies (Fig. 22). Continuous canopies had a 51% higher A_{max} in the winter than in the fall, and spring A_{max} was similar to both fall and winter (Fig. 22). Discrete canopies had a similar A_{max} for fall, winter, and spring (Fig. 22).

A_{max} of continuous canopy strata was influenced by season (p<0.0001), but the lower and upper canopy strata were similar for all seasons. A_{max} was highest in the winter, intermediate in the spring, and lowest in the summer and fall (Fig. 23). Winter A_{max} was 58% higher than summer, 54% higher than fall, and 37% higher than spring.

Instantaneous water use efficiency (WUE) was statistically similar for seasonal comparisons of both canopy types (p=0.3403) and continuous canopy strata (p=0.3455).

Discussion

Old-growth longleaf stands are characterized by complex canopy structure (Mitchell et al., 2009; Johnson et al., 2018). In this study, we confirmed that crown morphology was diverse, with a greater proportion of continuous canopy individuals relative to discrete canopy individuals in the stand. Continuous canopies in old-growth forests and their greater abundance in this study will have important impacts on physiological function relative to other crown classes, especially with regard to stand level carbon and water use. Leaf area is one of the most important characteristics for analyzing canopy structure, which influences transpiration, photosynthesis, and respiration (Meng et al., 2019). Continuous crowns contained greater quantities of evenly distributed leaf area relative to discrete canopies, which was the mechanism driving the relationship between canopy complexity, physiological functions, and ultimately ecosystem resilience to variation in climate. Contrary to our prediction that lower branches within continuous canopies would maintain higher rates of physiological processes (i.e., transpiration and photosynthesis) during periods of low soil moisture availability and high VPD during the summer months, we found that the main benefit of lower branches occurred during the winter months. This was related to the vertical distribution of leaf area and the decreased solar elevation, which increased transpiration in the lower canopy strata. The lower strata also displayed decreased transpiration while the upper strata exhibited increased transpiration with increasing temperatures, further reinforcing that lower branches are more beneficial physiologically during the winter.

Canopy transpiration is driven by evaporative demand (VPD), but soil water availability can limit transpiration during periods of high soil water deficits because stomata close under conditions of low soil moisture (Ford et al., 2005), which can further limit forest productivity (Goode et al., 2019). Mature overstory longleaf pines occupying xeric sites have been documented to have shorter heights, lower A_L:A_S, and greater stomatal sensitivity to increasing VPD and decreasing soil moisture than individuals of similar diameters on mesic sites, allowing the xeric site trees to shift their hydraulic architecture to continue extracting and using water to maintain physiological functions at similar levels (Addington et al., 2006; Gonzalez-Benecke et al., 2011). In addition to using compensations of aboveground biomass with decreasing height and $A_L:A_S$ to maintain physiological functions, with xeric site trees doing so by inhibiting elongation of new needles as opposed to mesic site trees shedding older needles (Wright et al., 2013), trees can utilize belowground biomass to compensate for diminishing soil water availability. Older, larger longleaf pines on xeric sites also display greater allocation to the production of belowground biomass to create larger root systems (Addington et al., 2006; Goode et al., 2019), so these trees are equipped for withstanding periods of limited water availability.

Trees that are long-lived must utilize these adaptations to overcome the challenges presented by immense changes in their size and external environmental factors (Day and Greenwood, 2011). For example, large trees in older forests tend to exhibit greater water use, which can be attributed to hydraulic lift from groundwater creating enhanced reservoirs of water to be transpired in the upper layers of the soil, making these large trees less sensitive to environmental stress than smaller trees on the same site that are only able to access soil water at the surface (Dawson, 1996). Through measurements of soil volumetric water content and calculations of hydraulic redistribution occurring at night in the absence of transpiration, it was possible to indicate the occurrence of hydraulic lift (Caldwell and Richards, 1989; Burgess et al., 2001) that was providing additional water for transpiration through two hot, dry periods during our study. The first occurred mid to late summer and the second occurred mid fall, so both of which took place during times of the year when longleaf has been shown to be more sensitive to droughts (Goode et al., 2019). During the summer 2019 drought period, both canopy types were able to maintain similar levels of water use with declining soil moisture, while the lower continuous canopy strata proportionally used more water than the upper strata (Fig. 24). However, the fall 2019 drought period was more prolonged and showed that both canopy types maintained similar levels of water use until a marked decline after soil moisture reached zero because hydraulic lift was no longer providing additional water towards the latter portion of this period (Fig. 25). This also shows the lag between changes in transpiration at the canopy and sap flow at the tree base (Meinzer et al., 2004) with changing environmental conditions. Initially, there was a proportional shift with greater water use by the upper continuous canopy strata, until both strata began to decline at similar rates (Fig. 25).

Even with variability due to unavoidable heterogeneity of age, structure, and productivity of individuals (Sillet et al., 2018), which is further driven by diurnal changes in atmospheric conditions (Clinton et al., 2011), continuous crowns overall transpired more water than discrete crowns during our study. While this can be explained largely by differences in leaf area (Vose et al., 2003; Meng et al., 2019) between canopy types, as documented by the similar percentages in greater leaf area (43%) and greater cumulative water use (40.8%) for continuous versus discrete canopies. The dominant position of the canopy is also an important factor (Kelliher et al., 1992), and the different responses of transpiration to environmental factors in the upper and lower strata of continuous canopies were also influential. Transpiration of continuous and discrete canopies increased in a similar response to solar radiation and VPD, and there was a similar response of both canopy types exhibiting decreased transpiration with increasing solar zenith angle and relative humidity, with the magnitude of difference in transpiration again being attributed to the differences in leaf area between the canopy types. The response to temperature differed by canopy type and can be attributed to different responses of transpiration in upper and lower continuous canopy strata. Due to the environmental gradients occurring within forest canopies, branches of tall trees located at various crown positions are exposed to differing conditions and thus acclimate physiologically to best utilize their existing microclimate (Kupper, 2006; Kelliher et al., 1992), which we saw in these varying responses of the lower and upper continuous canopy strata. Another example was the stronger relationship between transpiration in the lower canopy strata and VPD in comparison to that of the upper strata. Additionally, the lower strata transpiration response remained relatively constant across a wide range of solar zenith angles, but transpiration in the upper strata decreased with increasing solar zenith angle. Differences in how transpiration in upper and lower canopy strata respond to environmental factors result in different seasonal patterns in transpiration (driven largely by solar zenith angle) between the upper and lower strata. The upper strata exhibited a decrease in transpiration during the winter months, whereas the lower strata exhibited increased transpiration during the winter. The different responses of transpiration in the upper and lower canopy coalesce to reveal a relatively consistent linear cumulative transpiration volume across an annual period. This consistent linear response is in contrast to the cumulative annual transpiration volume in the discrete canopy which exhibited a much smaller rate of increase during the winter, which is similar to what we observed in the upper strata of the continuous canopy.

That the lower strata of continuous canopies responded more strongly to VPD than the upper strata provided partial support for our hypothesis that retention of lower branches influences physiological function; however, the lower water potential of the upper continuous canopy strata compared to the discrete canopy suggests that discrete canopies may experience relatively lower levels of water stress in the summer and fall. Differences in water potential between upper and lower continuous canopy strata (-0.18 ± 0.13 MPa; Fig. 16) were directly related to the theoretical gravitational component of water potential (i.e., -0.1 MPa per 10m increase in height) and agree with observations seen in longleaf pine (Addington et al., 2006), as well as other species exhibiting complex canopies, like *Sequoiadendron giganteum* (Williams et al., 2017) and *Pseudotsuga menziesii* (Woodruff et al., 2007). Regardless of differences in water potential, A_{max} remained similar between continuous and discrete canopies in the fall and spring, suggesting that carbon assimilation was similar at the leaf-level, and therefore higher in continuous canopies because of the increased leaf area.

While sap flow was measured over a year-long study period with water potential and gas exchange measured in each of the four seasons within this year, there is the limitation in seasonal sampling frequency with only one week of each season serving as a representation of the environmental variables and trees' responses to these conditions. Accessing canopies was also difficult and limited by boom lift height and proximity of trees to the road, which limited the selection of individuals that could be used for sampling to those along the road with additional light opening. However, sap flow does act as a whole-tree integration of physiological activity (Kupper, 2006; McDowell et al., 2002), so we can assume that these patterns would extend across times that were not measured. Additionally, if sample trees are representative of the mature overstory individuals in the stand, then these patterns would extend across the representative canopy types and strata.

Transpiration remained relatively constant throughout the year in continuous canopies, largely due the vertical distribution of leaf area and the decreased solar elevation; however, few studies have acknowledged or demonstrated similar seasonal dynamics. Even with evergreen species in temperate climates and mild winters documented to maintain physiological functions (Teskey et al., 1994), we often refer to winter as a dormant period, but our results suggest that winter may be a critical period for forest carbon assimilation. For example, water potential and net photosynthetic rates were higher in the winter for a single sampling period than during any other sampling period, while whole-tree transpiration in continuous canopies was maintained at a relatively constant level throughout the year.

Gas exchange was influenced by the same seasonal changes in environmental conditions during sampling periods as ψ_L. However, the lack of a difference in seasonal instantaneous WUE by canopy type or strata suggests that trees adjust water use and photosynthetic assimilation accordingly to changing quantities of water availability. The seasonal values of A_{max} by canopy type (Fig. 22) reflect the seasonal changes in ψ_L (Fig. 19), with higher ψ_L corresponding with higher rates of A_{max}, and lower ψ_L with lower A_{max}. This indicates that greater water stress resulted in a lessened capacity for photosynthetic assimilation (Teskey et al., 1994) across canopy types, and since photosynthetic capacity is an indicator of resource allocation, this suggests that as water becomes more limiting, less is allocated to assimilatory systems for photosynthesis (Paul and Foyer, 2001). This pattern could have also been related to stomatal closure to reduce water loss and manage water stress under drought conditions, which also reduces photosynthetic assimilation (Becker et al., 2000). Continuous canopies had lower rates of A_{max} in the fall and spring, but the highest rate of A_{max} in the winter (Fig. 23), with minimal rainfall during the fall and spring and larger quantities of rainfall during the winter (Tables 7 & 8). This suggests that continuous canopy individuals are more sensitive to increases in water stress but maximize photosynthetic assimilation under more optimal, well-watered conditions as photosynthesis is dependent on larger, more frequent rainfall events (Porporato et al., 2004). Discrete canopies had more consistent rates of A_{max} in fall, winter, and spring (Fig. 20), suggesting that the photosynthetic capacity of these individuals is less affected by changes in environmental conditions and ψ_L. However, this result also indicates that photosynthesis of longleaf pine in a region with milder winter climates is not to be discounted as dormant (White and Harley, 2016; Rother et al., 2018), as these individuals also maintain their photosynthetic capacity year round despite not investing in new xylem (Rother et al., 2018; Teskey et al., 1994). Moreover, longleaf pine showed linear increases in growth rates with light availability up to full sunlight (Pecot et al., 2007), and increasing daylength during winter months at lower latitudes would appear to be a critical factor in predicting longleaf pine growth across its range.

Estimating whole-tree water use and extrapolating from the individual tree to a stand-level is becoming increasingly important as it provides invaluable insight to whole-tree physiology, especially when made at multiple positions along the canopy height gradient (Wullschleger et al., 1998). However, measuring sap flow through different parts of a tree canopy is relatively rare, but we found that such an approach provided critical information about canopy complexity that would have gone unnoticed had we only measured whole-tree transpiration. Though whole-tree transpiration would have demonstrated differences among canopy types, we would not have been able to attribute the seasonal dynamics related to lower solar elevation to particular canopy strata. Studies quantifying sap flow in regards to canopy complexity found that sensors installed in the outer sapwood near the base of the tree stem better characterized the total water supply from the fine roots located near the soil surface, while sap flow measured in the outer sapwood at the base of the live crown (BLC) indicated the water supply to the tree top and outer crown layers (Čermák and Nadezhdina, 2011). In a younger monoculture longleaf stand, whole-tree sap flow was measured at 1.3m (DBH) with additional sensors placed at 5m (just below BLC) as a means of quantifying transpiration per unit leaf area to document the corresponding decline as a result of canopy scorch from prescribed fire (Clinton et al., 2011), but even with sensors somewhat vertically distributed, the relative differences are not shown for lower versus upper strata. Sap flow was measured to quantify whole-tree water use at 2.5m on a 1.4m diameter and 58m tall old-growth Douglas-fir during the growing season, with another set of sensors installed later in the study for a five-day period at both the base of the tree and also for branches at heights of 54m to measure simultaneous sap flow to estimate crown transpiration (Woodruff et al., 2007), but again this did not quantify relative differences of strata, and did not estimate leaf area of the crowns.

Our approach to estimating transpiration using thermal dissipation sap flow sensors provides robust comparisons between canopy types and canopy strata, but likely does not reflect actual transpiration volumes. For example, the accuracy of thermal dissipation sensors requires calibration (Steppe et al., 2010; Sun et al., 2012; Bush et al., 2010), but multiple factors, including the size of our measurement trees and landowner's desire to minimize the impact of our study, precluded our own calibration. Likewise, sap flow tends to decrease radially through the xylem (Ford et al., 2004), but we only measured at 1.0 cm depth and scaled those measurements across the entire amount of conducting sapwood, which likely overestimated transpiration. Despite these limitations to accurately estimating transpiration, we feel confident that our approach was suitable for comparing the response of transpiration to environmental factors and comparing sap flow volumes since our observations were restricted to similar sized trees of the same species growing at the same site (Dix and Aubrey, 2021).

Our results suggest that canopy complexity in longleaf pine forests influences tree physiology and ecosystem processes, which are linked to ecosystem resilience. These results have direct implications on efforts to restore longleaf pine ecosystems that rely largely on establishing high-density plantations of seedlings that self-prune lower branches through stand development and result in relatively simple, discrete canopy trees when mature. These plantation-grown trees with sun exposure limited to primarily overhead light typically attain their maximum heights at younger ages and are spatially limited with smaller crowded crowns that limit light interception and photosynthetic assimilation (King, 2011). Conversely, open-grown trees tend to bear much broader and longer crowns than the plantation grown trees of similar heights (King, 2011), which would allow these trees with more leaf area and less crowding to better intercept light throughout the canopy. Given the increased physiological function of individuals with continuous canopies that results not only from increased leaf area, but also from an ability of the lower canopy and whole tree to respond to increased VPD and the capacity to maintain relatively consistent transpiration (and presumably, carbon assimilation) year-round, best practices for longleaf restoration should incorporate thinning at earlier stages to promote retention of lower branches. Doing so will ultimately result in an old-growth longleaf stand reflective of natural regeneration that occurs in canopy gaps (Mitchell et al., 2009; Brockway et al., 2005; Jose et al., 2003; Noel et al., 1998) and provides greater resilience (Goode et al., 2019). Future research should aim to determine the frequency and importance of canopy complexity in other old-growth longleaf stands since our current understanding is limited to a single site.

These results for old-growth longleaf pine forests may have implications for other open canopied coniferous forests. For example, ponderosa pine (*Pinus ponderosa*) stands are often restored or regenerated in a similar way as longleaf pine with high-density stocking, while mature ponderosa stands exhibit canopy complexity similar to that of old-growth longleaf with retention of lower branches and broad vertical distribution of leaf area (Franklin and Pelt, 2004). However, winters are less mild where ponderosa pine occurs, which limits photosynthesis (Bassman, 2005), and ultimately, the benefit of vertical leaf area distribution of continuous canopies maintaining transpiration as we observed in longleaf pine. Still, lower branches may help maintain higher transpiration in ponderosa as VPD increases and temperature decreases as we observed in longleaf pine. In fact, this particular benefit of canopy complexity may be even more important for ponderosa pine than longleaf pine because of its cooler, arid climate. How these more arid climates would interact with seasonally lower temperatures and moisture to affect tree and forest stand carbon assimilation patterns may impact their resilience to projected hotter and drier conditions under climate change. Future research should explore canopy complexity and physiological function in other woodland forest ecosystems to see if restoration, management, and ecosystem resilience can be improved and enhanced by considering the interaction of canopy form and function, though this relationship may also depend on the severity of the regional winter climate.

CHAPTER 3

CONCLUSIONS

Our study found that, in this old-growth longleaf stand, retaining the lower strata of continuous canopy individuals provided important contributions to the overall physiological functions and ecosystem processes as it responded differently than the upper strata under varying environmental conditions. These proportional shifts between lower and upper canopy strata allowed continuous canopies to maintain consistent levels of physiological activity even while supporting abundant additional leaf area in comparison to discrete canopies. Also, the relative frequency of continuous canopies compared to discrete canopies in this stand provides additional evidence supporting the importance of the lower canopy strata. Managing longleaf pine in an uneven-aged approach would promote greater canopy complexity similar to that found in this natural old-growth stand, which encompasses both continuous and discrete canopy types with some individuals retaining these lower canopy strata. By managing for a longleaf pine forest that can maintain physiological functions under a more diverse range of environmental conditions, the forest will inherently be more resilient to extremities it my face in the wake of a changing climate predicted to bring about an increasing number of more severe droughts to its native southeast region.

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APPENDIX A

TABLES

Table 1: Comparison of the characteristics of all 24 continuous and discrete canopy individuals used in this study. Mean ± standard error for DBH, sapwood area, and sum of occupied voxels.

Canopy Type	**DBH (cm)**	**Sapwood Area (cm^2)**	**Occupied Voxels**
Continuous	44.7 ± 3.2	1057.6^ ± 113.2	49283.2 ± 8274.7
Discrete	47.2 ± 1.4	954.6^ ± 72.1	28028.8 ± 5047.9

^ values were only from 12 sap flow trees

Table 2: Comparison of the characteristics of the 12 continuous and discrete canopy individuals used for the sap flow portion of this study. Mean ± standard error for DBH, sapwood area, and sum of occupied voxels.

Canopy Type	**DBH (cm)**	**Sapwood Area (cm^2)**	**Occupied Voxels**
Continuous	45 ± 2.8	1057.6 ± 113.2	42667.5 ± 3642.7
Discrete	47.08 ± 2.4	954.6 ± 72.1	25237 ± 4687.3

Table 3: Comparison of the characteristics of the lower and upper canopy positions within the 6 continuous canopy individuals used for the sap flow portion this study. Mean ± standard error for diameter, sapwood area, and sum of occupied voxels.

Crown Strata	**Diameter (cm)**	**Sapwood Area (cm^2)**	**Occupied Voxels**
Lower	45 ± 2.8	1057.56 ± 113.2	17891 ± 2305.4
Upper	27.07 ± 1.1	471.81 ± 42.8	24776.5 ± 2219.5

Table 4: Comparison of the characteristics of the lower and upper canopy positions within the continuous canopy individuals used in this study. Mean ± standard error for diameter, sapwood area, and sum of occupied voxels.

Crown Strata	**Diameter (cm)**	**Sapwood Area (cm^2)**	**Occupied Voxels**
Lower	44.7* ± 3.2	1057.6^ ± 113.2	21199.9 ± 3543.1
Upper	27.1^ ± 1.1	471.8^ ± 42.8	28083.3 ± 5057.4

*DBH for all 12 continuous canopies

^ values were only from 6 continuous sap flow trees

Table 5: Correlations of external environmental variables and principal components, with correlations less than ±0.2 denoted with an x. TMax is max temperature (◦C), TAvg is mean temperature (◦C), and TMin is minimum temperature (◦C). RHAvg is average relative humidity (%), Wind is wind speed (kph), Precip. is total precipitation (mm), Irrad. is solar irradiance (W m^{-2}), zenith is solar zenith angle (◦), VPD is vapor pressure deficit (kPa), and SVWC is soil volumetric water content (%).

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**
TMax	-0.47	x	x	x	x	x	-0.29	0.64	-0.33	0.38
TAvg	-0.48	x	x	x	x	x	-0.31	x	x	-0.78
TMin	-0.47	x	x	0.20	x	x	-0.26	-0.73	x	0.35
RHAvg	x	-0.57	x	x	x	0.21	0.40	x	-0.61	-0.25
Wind	x	x	x	0.92	0.28	x	x	x	x	x
Precip.	x	-0.21	-0.85	x	0.47	x	x	x	x	x
Irrad.	x	0.52	x	x	x	0.70	0.39	x	x	x
Zenith	0.38	x	0.28	-0.20	0.32	0.58	-0.52	x	x	x
VPD	0.28	0.48	x	x	x	-0.23	-0.25	x	-0.68	-0.25
SVWC	0.22	-0.27	-0.37	0.21	-0.76	x	-0.29	x	x	x

Table 6: Correlations of the external environmental variables used in the PCA, with correlations less than ±0.2 denoted with an x. TMax is max temperature (◦C), TAvg is mean temperature (◦C), and TMin is minimum temperature (◦C). RHAvg is average relative humidity (%), Wind is wind speed (kph), Precip. is total precipitation (mm), Irrad. is solar irradiance (W m^{-2}), zenith is solar zenith angle (◦), VPD is vapor pressure deficit (kPa), and SVWC is soil volumetric water content (%).

	TMax	**TAvg**	**TMin**	**RHAvg**	**Wind**	**Precip.**	**Irrad.**	**Zenith**	**VPD**	**SVWC**
TMax	1.00	0.97	0.87	x	-0.35	x	0.38	-0.73	-0.39	-0.48
TAvg		1.00	0.96	x	-0.27	x	0.25	-0.74	-0.50	-0.43
TMin			1.00	0.33	-0.22	x	x	-0.69	-0.61	-0.34
RHAvg				1.00	x	0.21	-0.67	x	-0.92	0.24
Wind					1.00	x	x	x	0.26	x
Precip						1.00	x	x	x	x
Irrad.							1.00	-0.39	0.49	-0.35
Zenith								1.00	x	0.22
VPD									1.00	x
SVWC										1.00

Table 7: Weather data from the study period, with information on temperature, wind speed, humidity, and solar radiation obtained from the weather station at Tall Timbers Research Station in Tallahassee, FL. Precipitation values were from on-site rain gauges, and soil volumetric water content (θ_V) was from on-site soil moisture sensors. Vapor pressure deficit (VPD) was calculated using average temperature and humidity during daytime period.

Month & Year	**Temperature (°C)**			**Precipitation (mm)**	θ_V	**Relative Humidity (%)**	**VPD (kpa)**	**Wind Speed (kph)**	**Zenith Angle (◦)**	**Solar Radiation (Wm^{-2})**
	Max	*Mean*	*Min*	*Sum*	*Mean*	*Mean*	*Mean*	*Mean*	*Solar Noon*	*Max*
5/2019*	36.2	29.1	22.2	0	0.027	68.2	0.125	4.5	8.8	829
6/2019	32.5	26.7	22.3	111.25	0.079	81.1	0.079	4.0	7.4	704
7/2019	32.8	26.8	22.5	140.46	0.047	84.2	0.066	3.3	9.6	756
8/2019	32.7	27.1	23.1	156.72	0.044	86.9	0.054	3.3	17.3	744
9/2019	33.4	27.0	21.5	0	0.015	75.1	0.104	3.9	28.2	1006
10/2019	28.8	23.0	18.0	77.74	0.043	81.0	0.090	4.2	39.8	707
11/2019	20.4	13.8	8.3	69.09	0.074	81.4	0.120	4.0	49.1	634
12/2019	19.0	13.7	9.4	37.59	0.073	82.4	0.118	5.0	53.5	488
1/2020	19.0	13.3	8.6	146.05	0.077	77.7	0.154	4.8	51.2	562
2/2020	19.5	13.8	8.7	94.74	0.089	81.6	0.123	4.8	43.1	600
3/2020	27.0	20.3	14.9	98.30	0.051	78.5	0.116	4.3	31.9	850
4/2020	26.5	20.2	14.4	164.34	0.059	72.1	0.145	4.8	20.4	942
5/2020^	28.0	21.1	14.0	25.40	0.049	63.0	0.183	4.9	13.2	1115

*data begins 5/26/2019

^data ends 5/15/2020

Table 8: The environmental conditions during the seasonal sampling periods for gas exchange and water potential. Information on temperature, wind speed, humidity, and solar radiation obtained from a nearby weather station at Tall Timbers Research Station in Tallahassee, FL. Soil volumetric water content (θ_V) was measured at 0.1 m depth within three of the six sap flow blocks. VPD (vapor pressure deficit) was calculated using temperature and humidity.

Season	**Temperature (°C)**			θ_V	**Wind Speed (kph)**	**Relative Humidity**	**VPD (kpa)**	**Zenith Angle (◦)**	**Solar Radiation (Wm^{-2})**
	Max	*Mean*	*Min*	*Mean*	*Mean*	*Mean*	*Mean*	*Solar Noon*	*Max*
Summer 2019	36.1	28.8	22.1	0.016	4.4	67.7	0.128	8.3	779
Fall 2019	37.8	27.3	21.0	0.0	3.7	72.0	0.116	34.6	949
Winter 2019	18.9	15.1	12.6	0.04	5.8	89.5	0.068	53.5	327
Spring 2020	28.8	21.1	12.7	0.023	5.2	54.1	0.210	12.2	1140

APPENDIX B

FIGURES

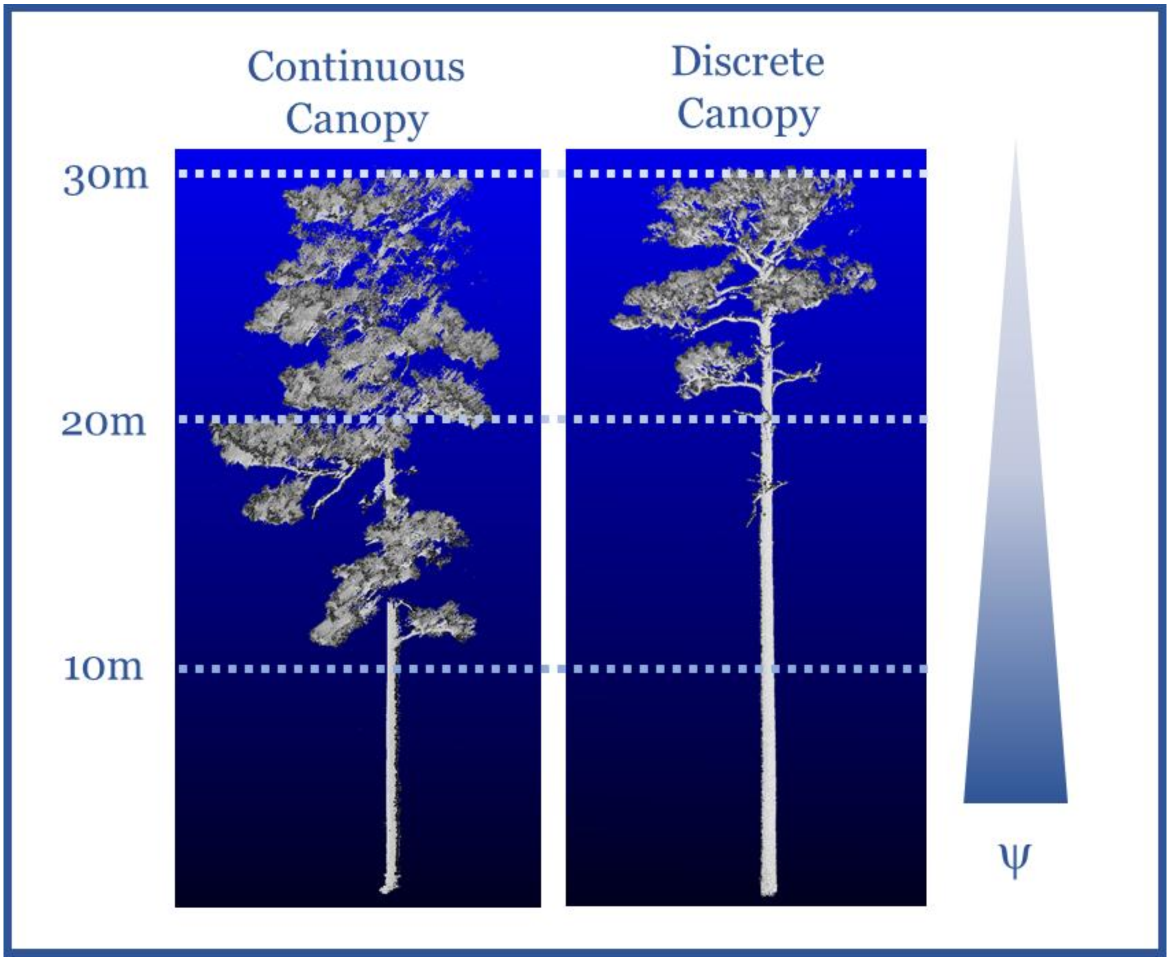

Figure 1: The two types of old-growth longleaf pine canopy complexity that will be studied depicting the heights of strata at which measurements will be obtained as well as the gradient of high soil water potential to low atmospheric water potential as water moves upward against gravity.

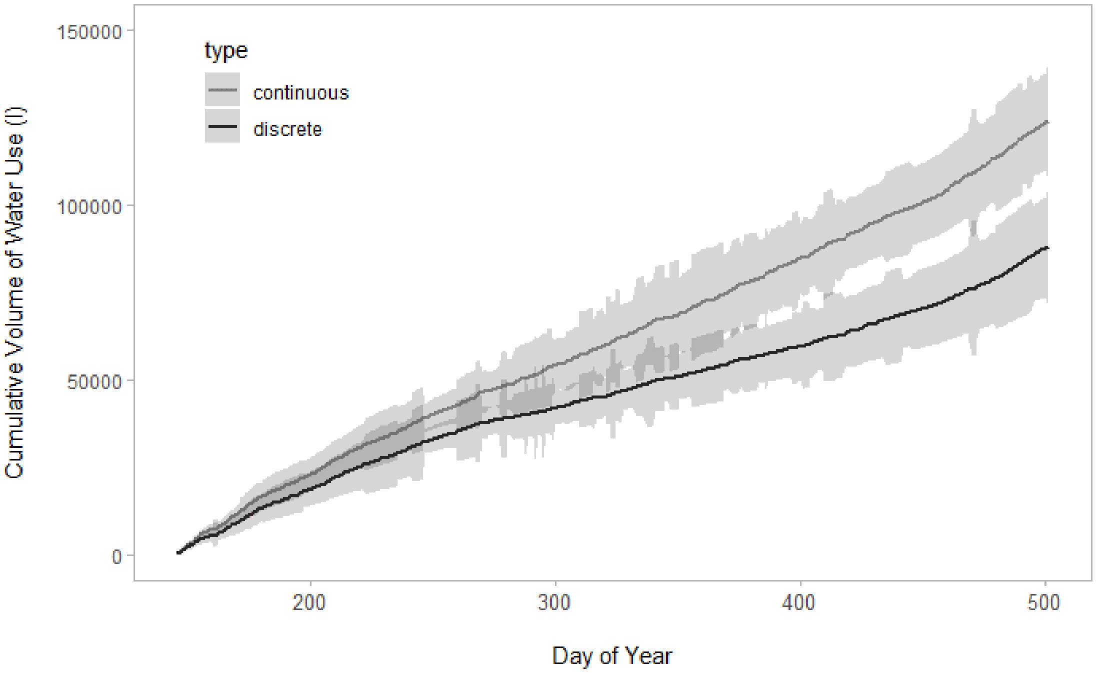

Figure 2: Cumulative volume of water use (l) across the entire study period from May 2019 to May 2020 for continuous and discrete canopies.

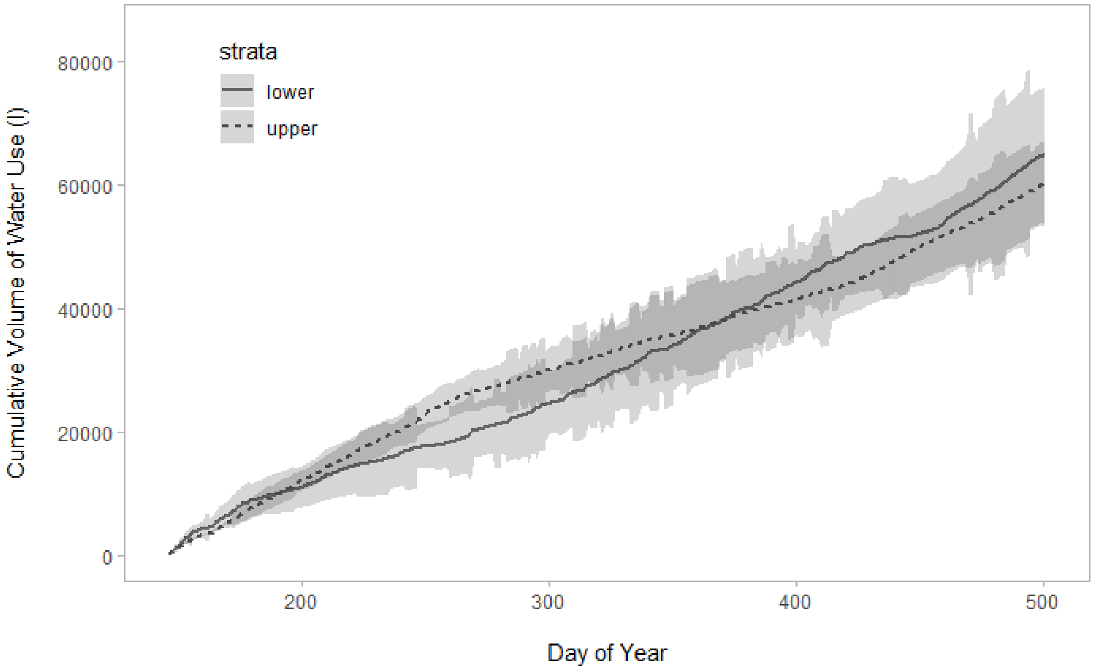

Figure 3: Cumulative volume of water use (l) across the entire study period from May 2019 to May 2020 for lower and upper continuous canopy strata.

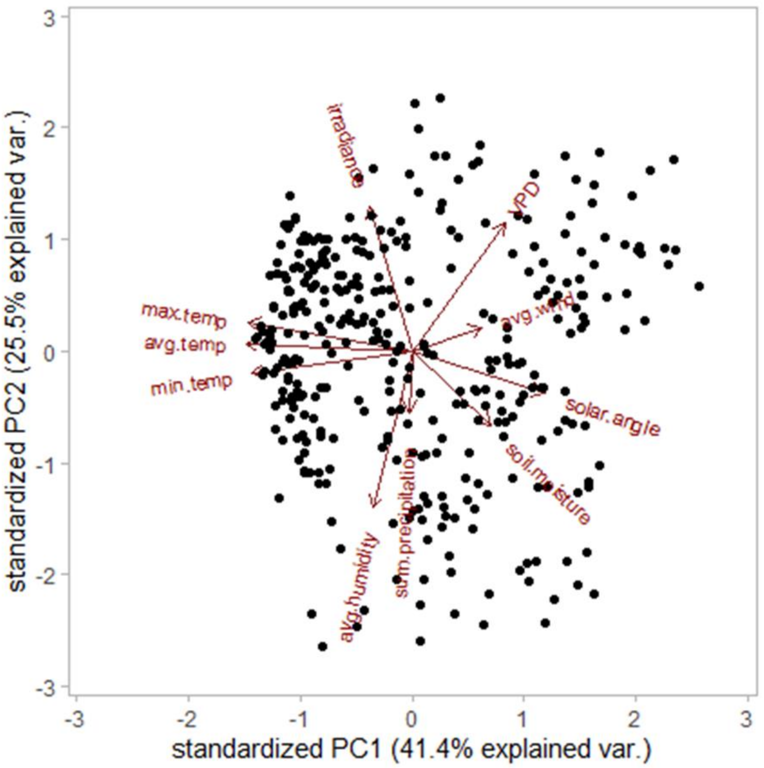

Figure 4: Biplot of external environmental variables in relation to standardized principal components axes 1 (PC1) and 2 (PC2).

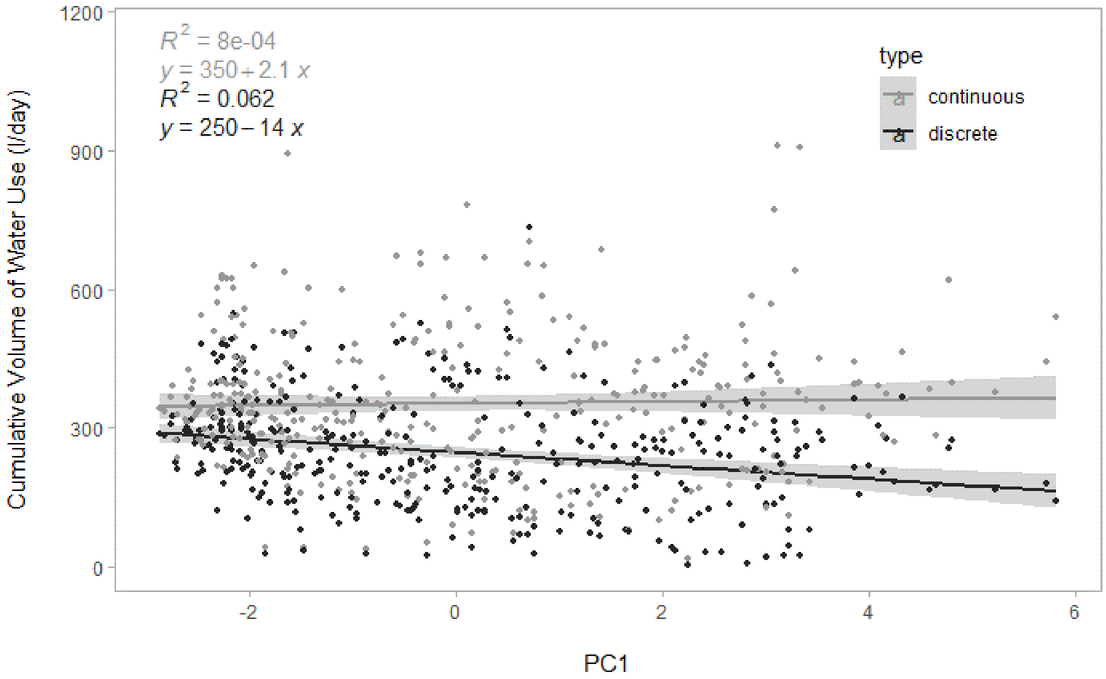

Figure 5: Relationship between PC1, characterized as seasonally sunny and hot, and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

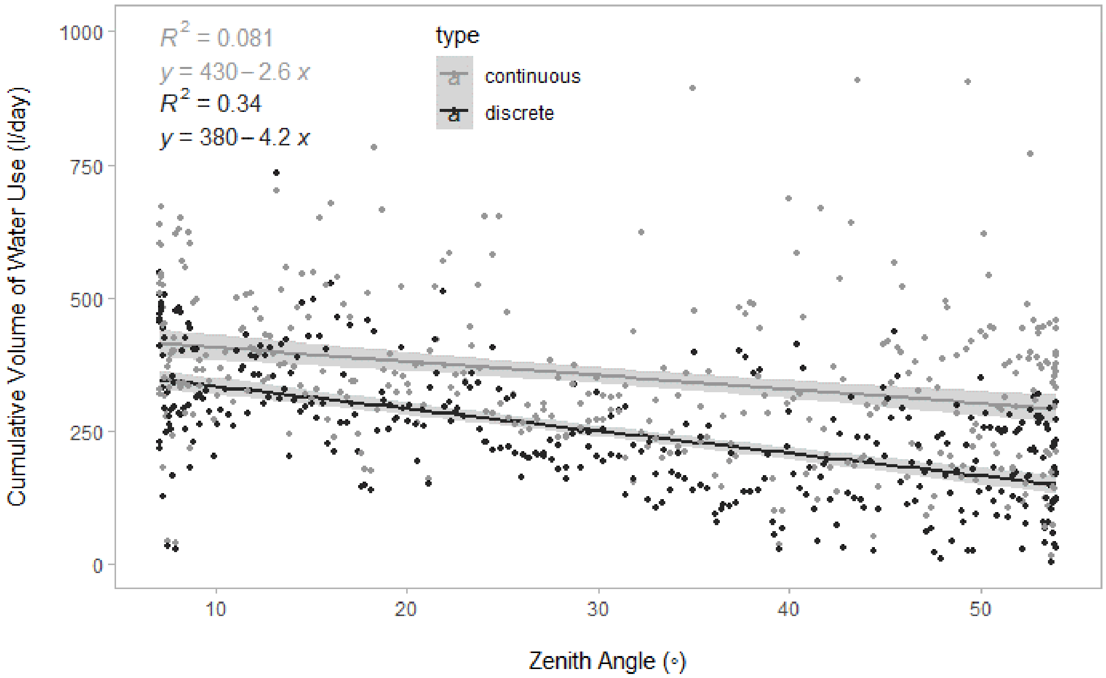

Figure 6: Relationship between solar zenith angle and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

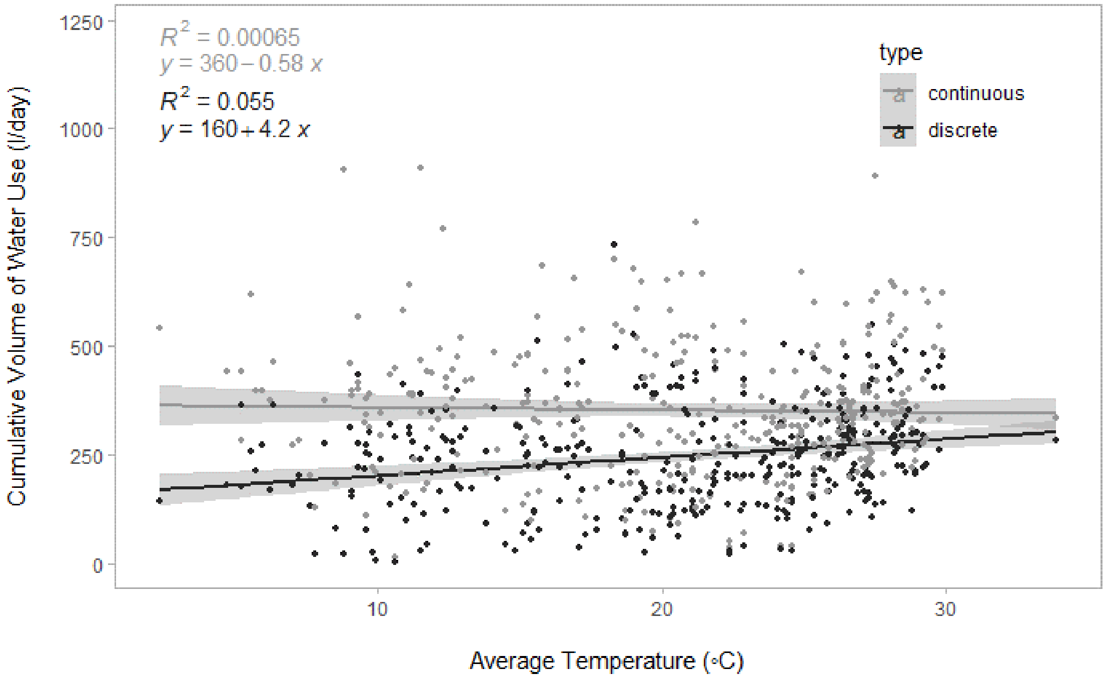

Figure 7: Relationship between average temperature and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

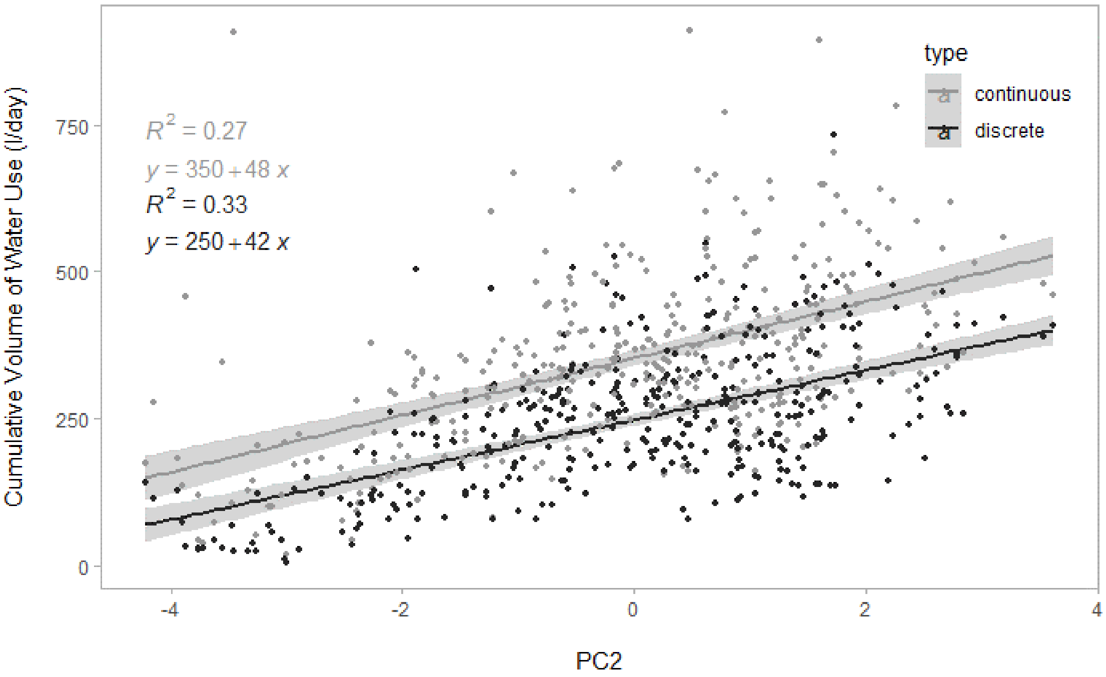

Figure 8: Relationship between PC2, characterized as sunny and dry, and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

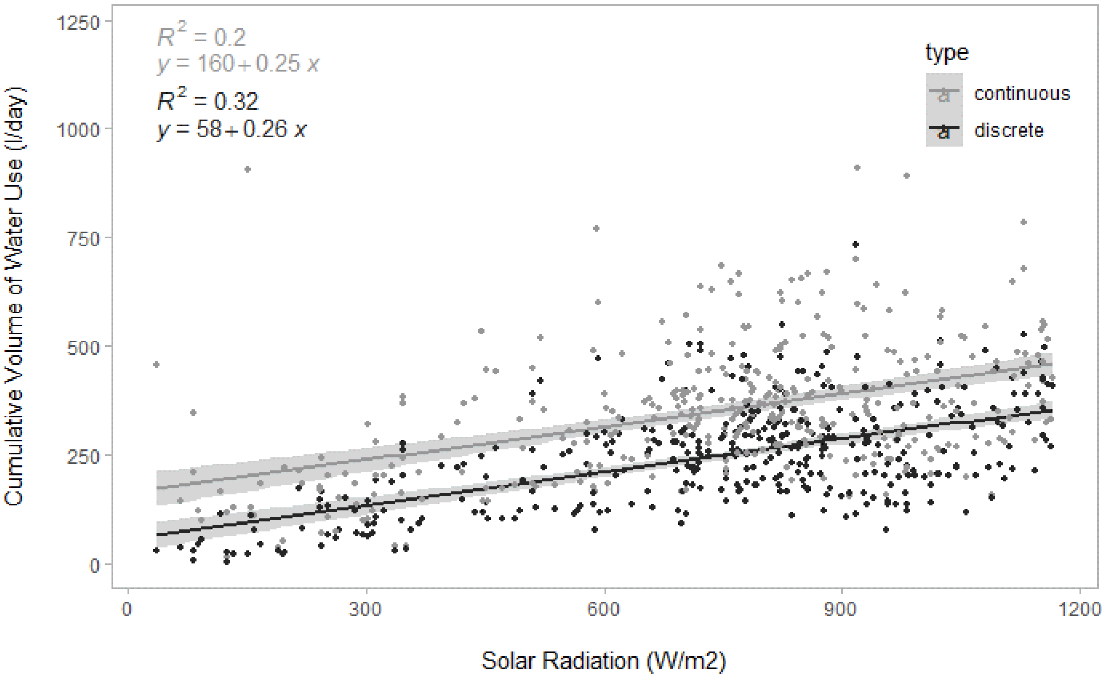

Figure 9: Relationship between solar irradiance and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

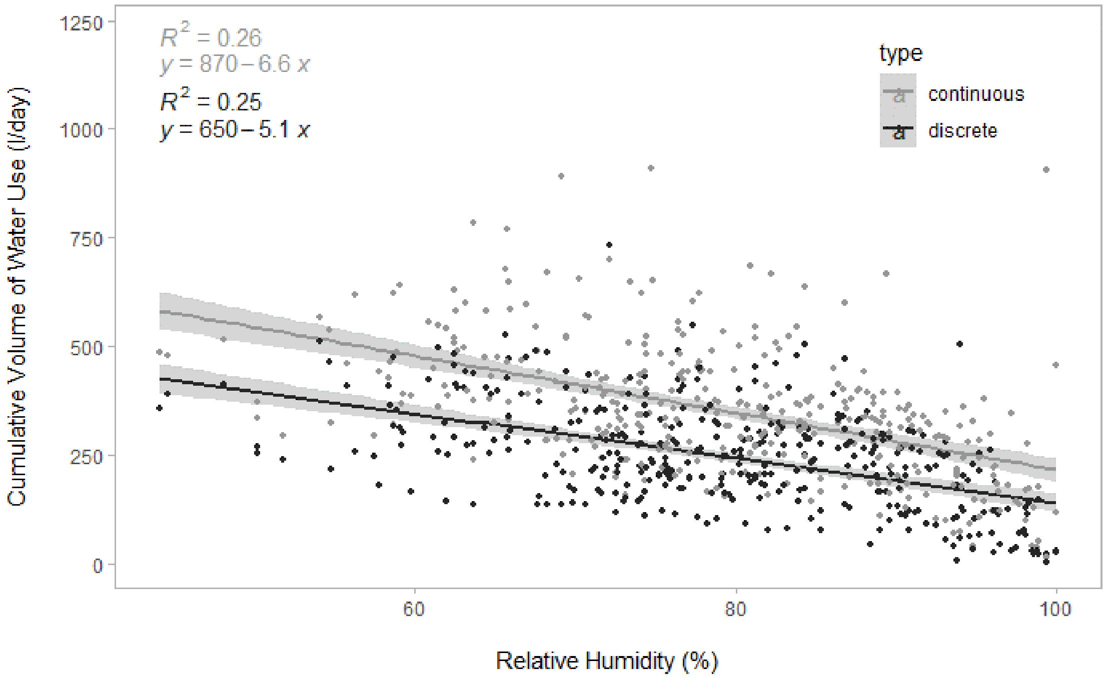

Figure 10: Relationship between relative humidity and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

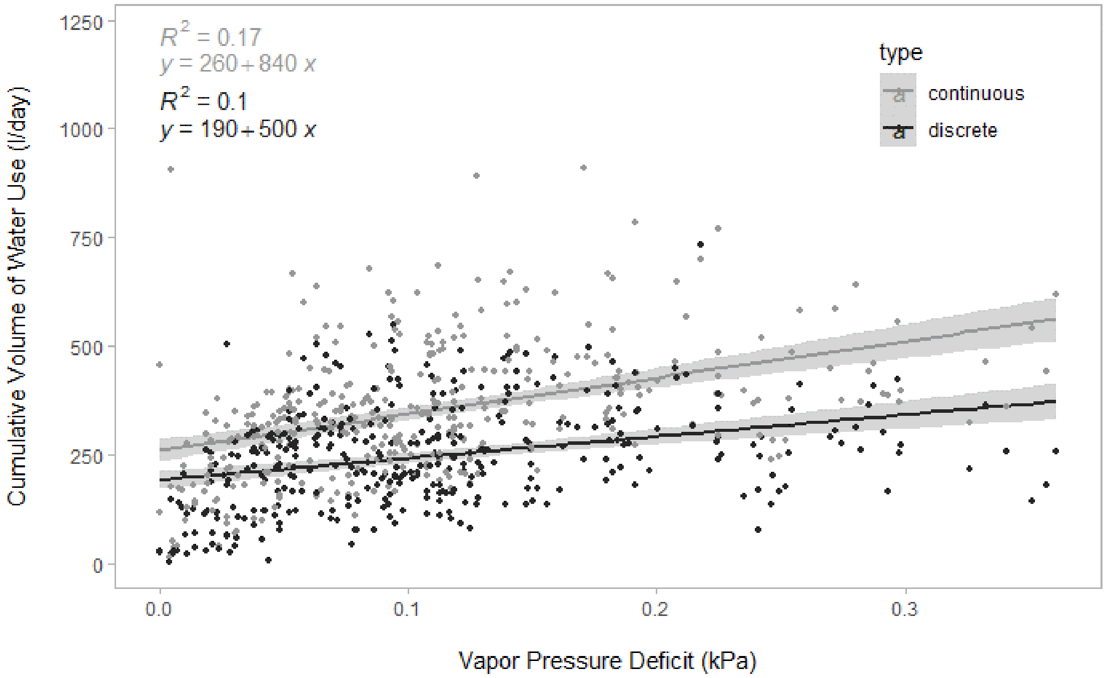

Figure 11: Relationship between vapor pressure deficit and cumulative daily sap flow (l day^{-1}) for continuous and discrete canopies.

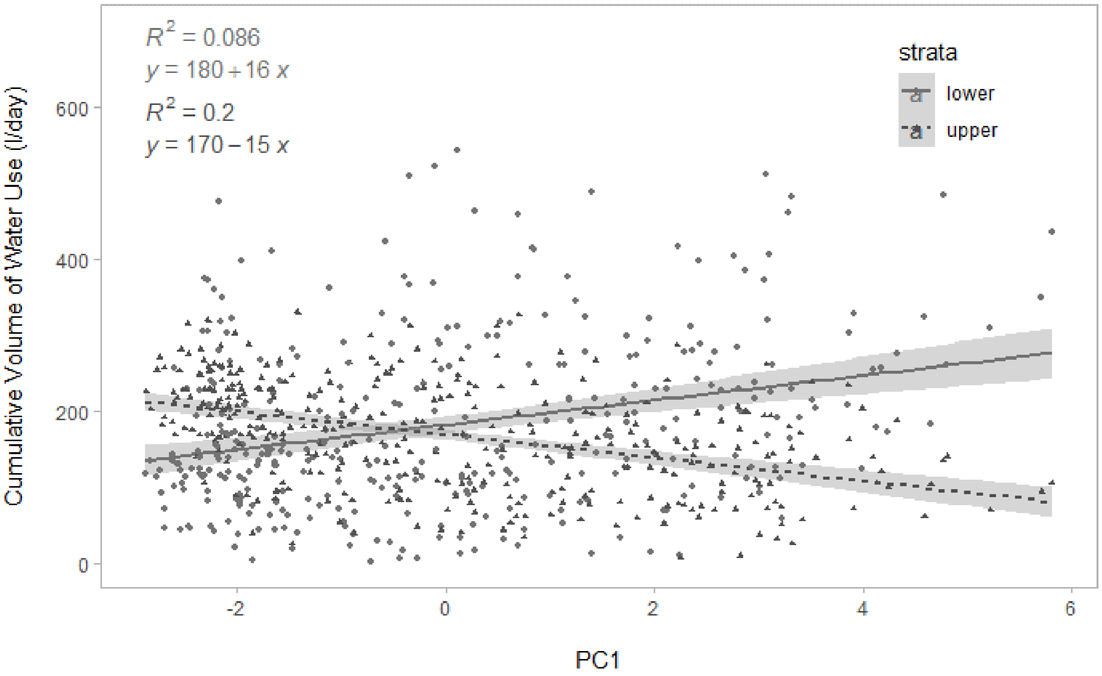

Figure 12: Relationship between PC1, characterized as seasonally sunny and hot, and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

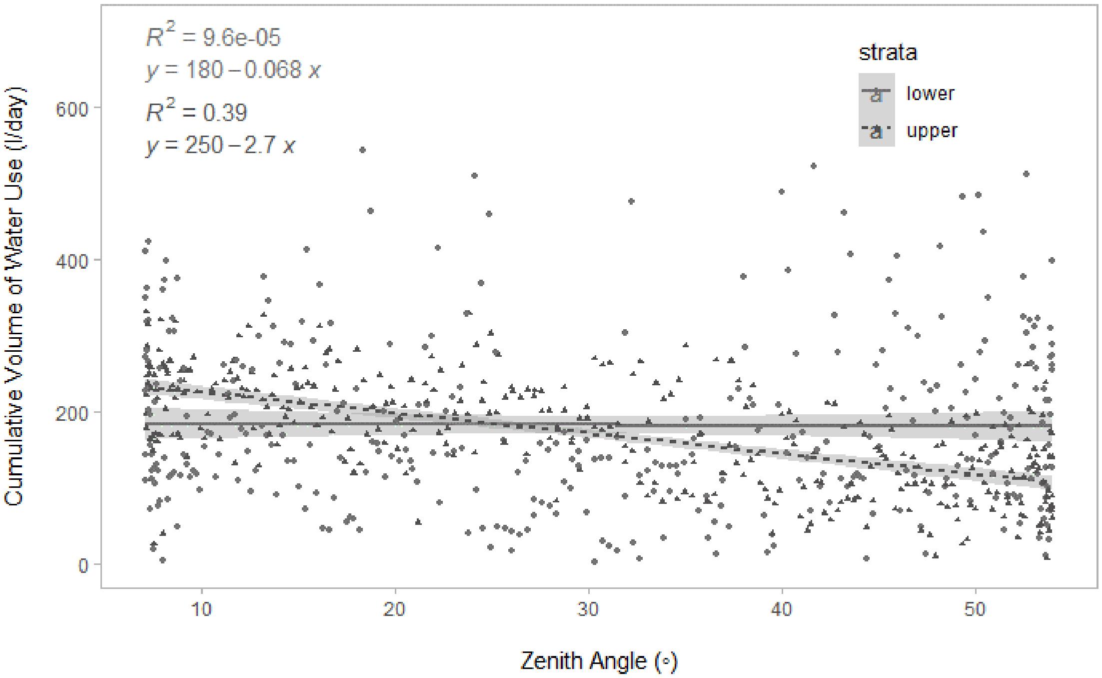

Figure 13: Relationship between solar zenith angle and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

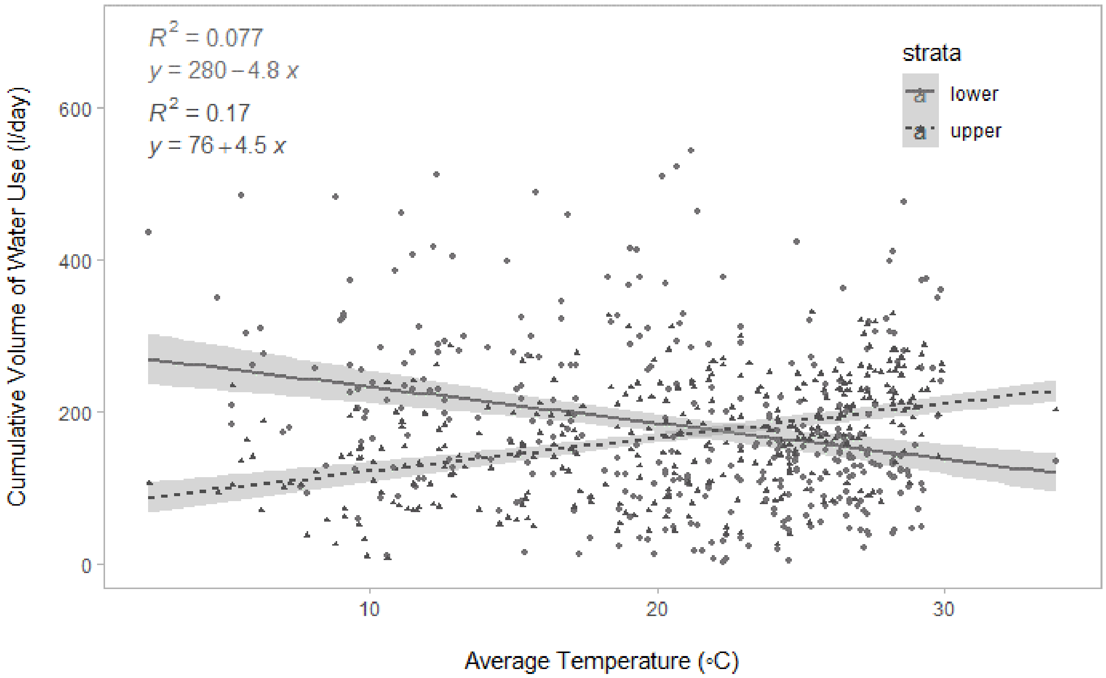

Figure 14: Relationship between temperature and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

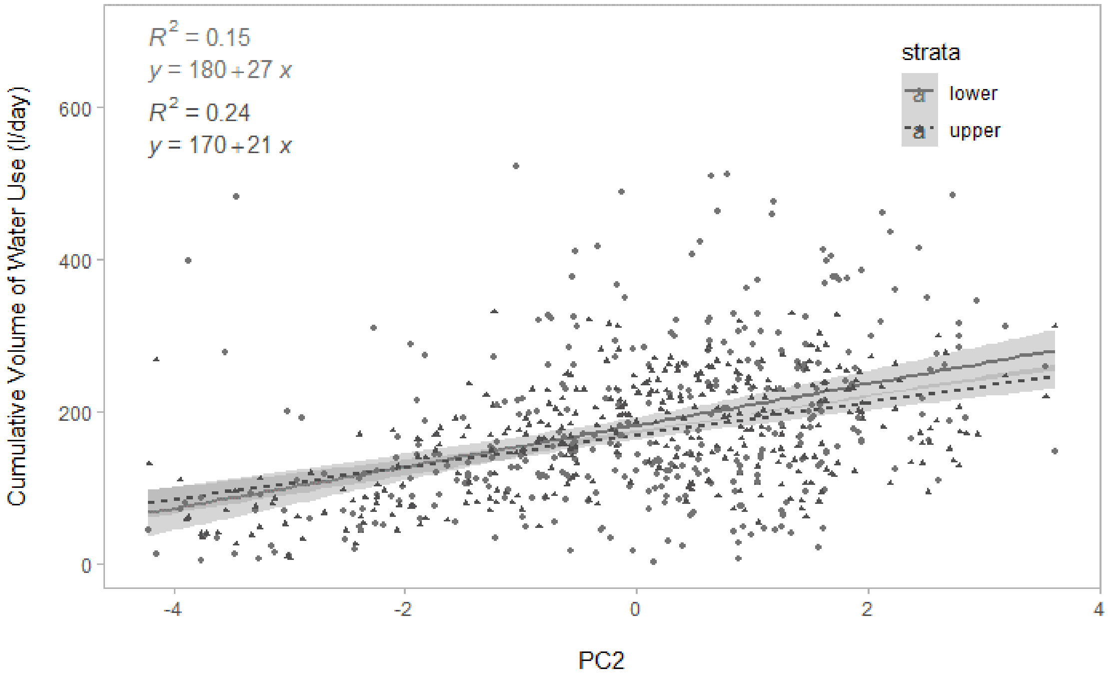

Figure 15: Relationship between PC2, characterized as sunny and dry, and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

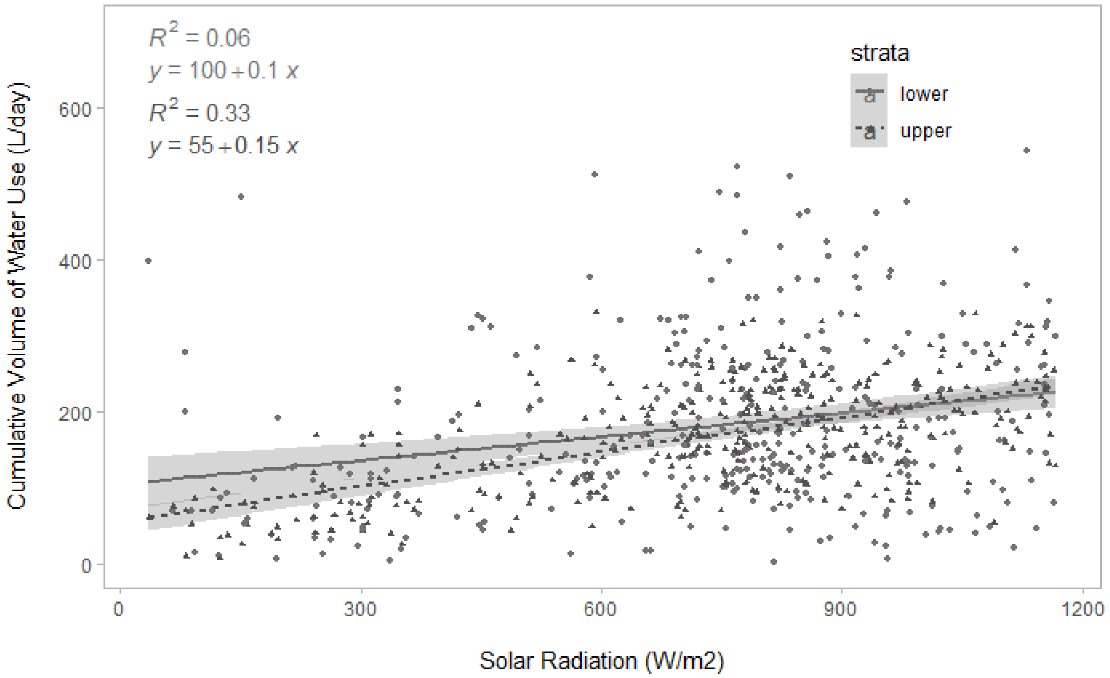

Figure 16: Relationship between solar irradiance and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

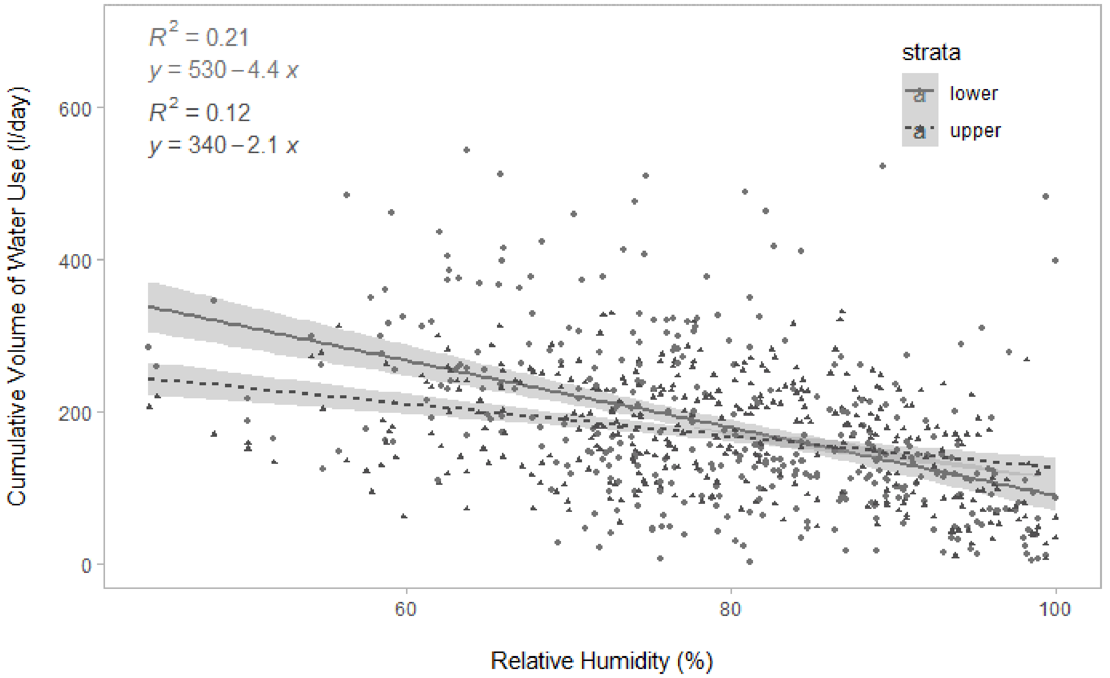

Figure 17: Relationship between relative humidity and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

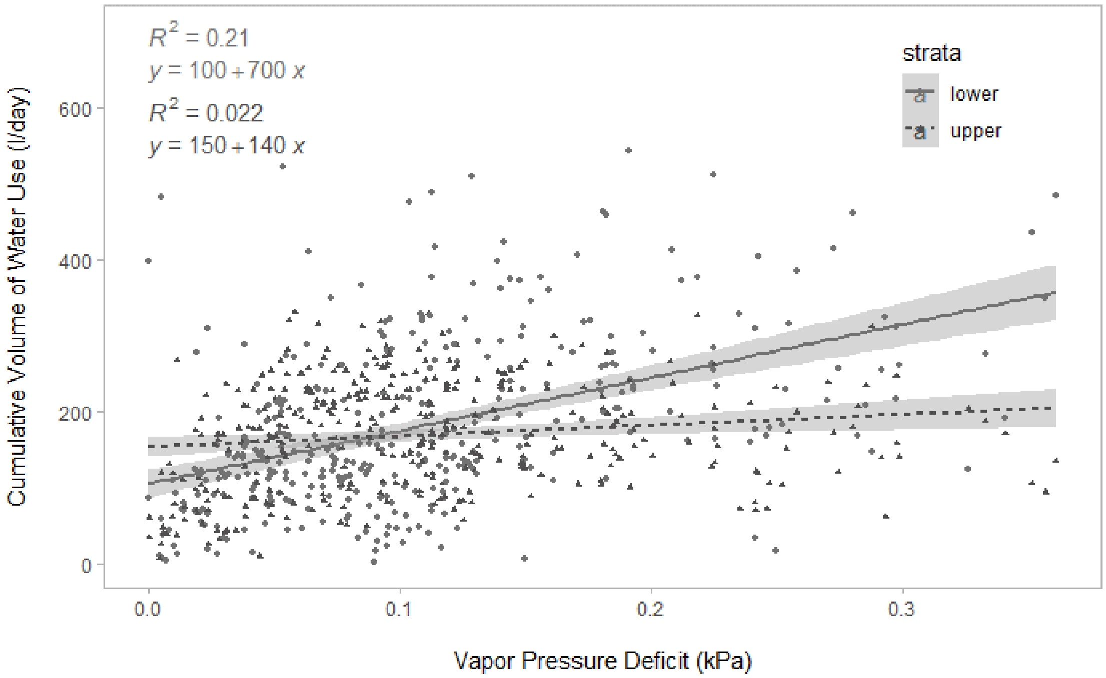

Figure 18: Relationship between vapor pressure deficit and cumulative daily sap flow (l day^{-1}) for lower and upper strata of continuous canopies.

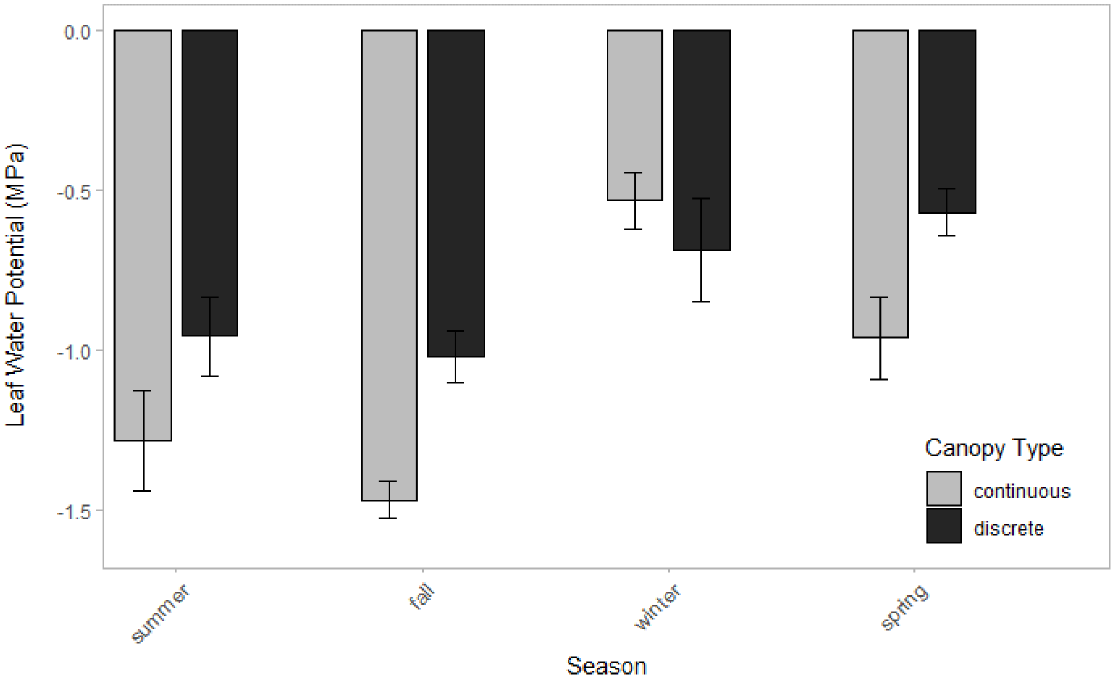

Figure 19: Seasonal leaf water potential by canopy type, and dates for seasonal data collection are May 28 to June 5, 2019 for Summer, September 30 to October 4, 2019 for Fall, December 9 through 12, 2019 for Winter, and May 11 through 13, 2020 for Spring.

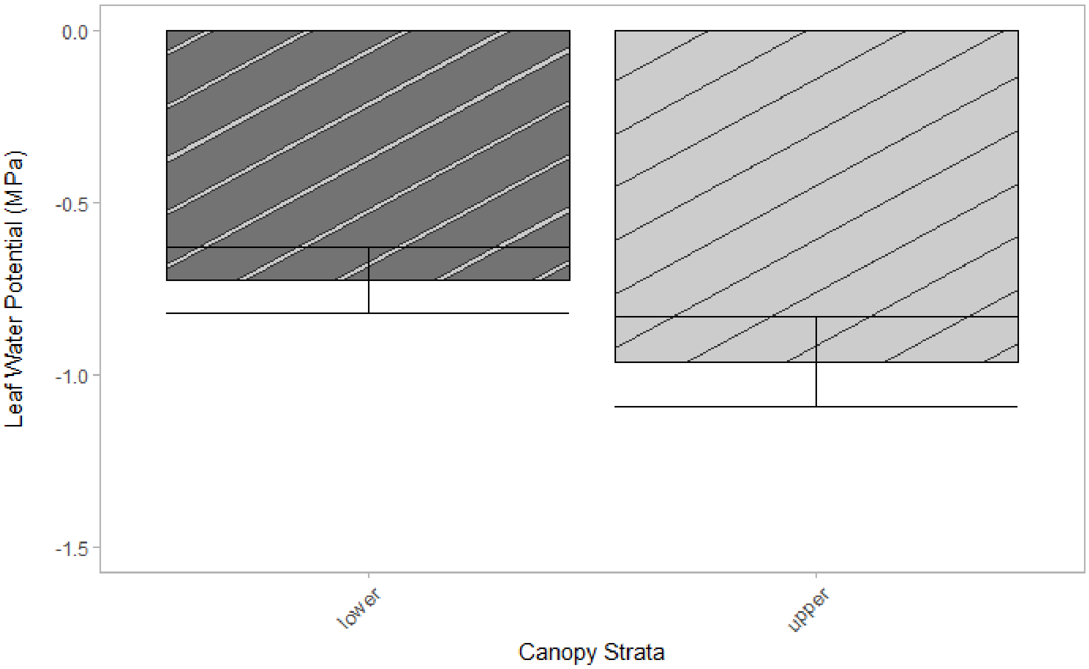

Figure 20: Leaf water potential of the lower and upper continuous canopy strata averaged across all 4 seasons.

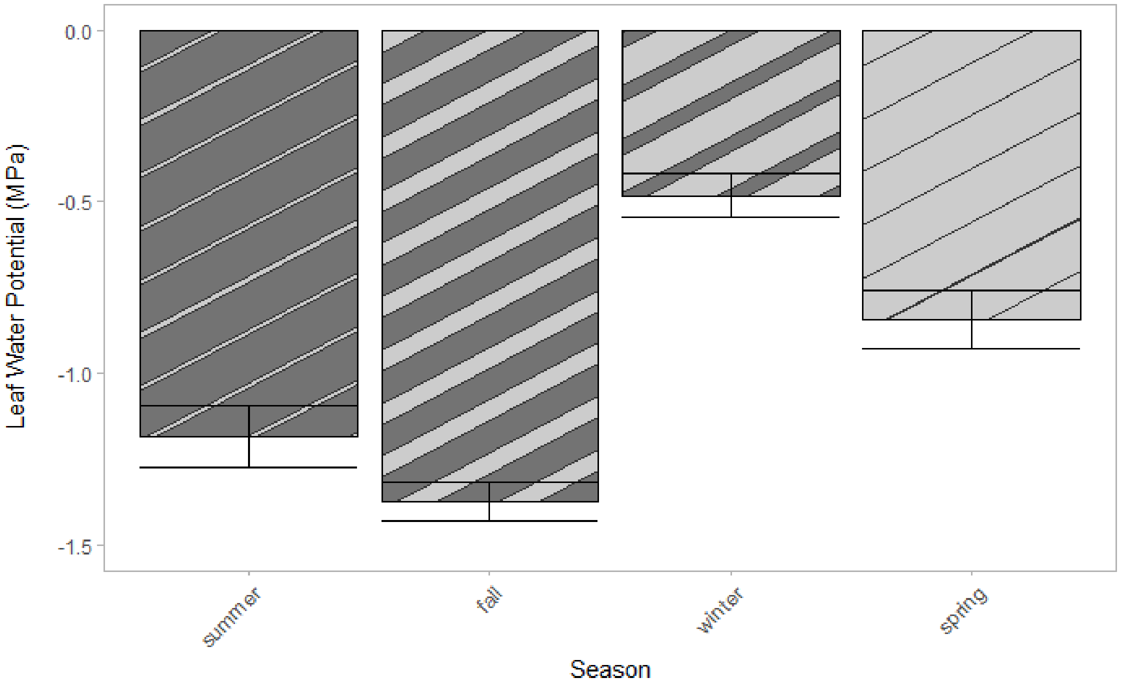

Figure 21: Seasonal leaf water potential of continuous canopies averaged across lower and upper strata, and dates for seasonal data collection were May 28 to June 5, 2019 for summer, September 30 to October 4, 2019 for fall, December 9 through 12, 2019 for winter, and May 11 through 13, 2020 for spring.

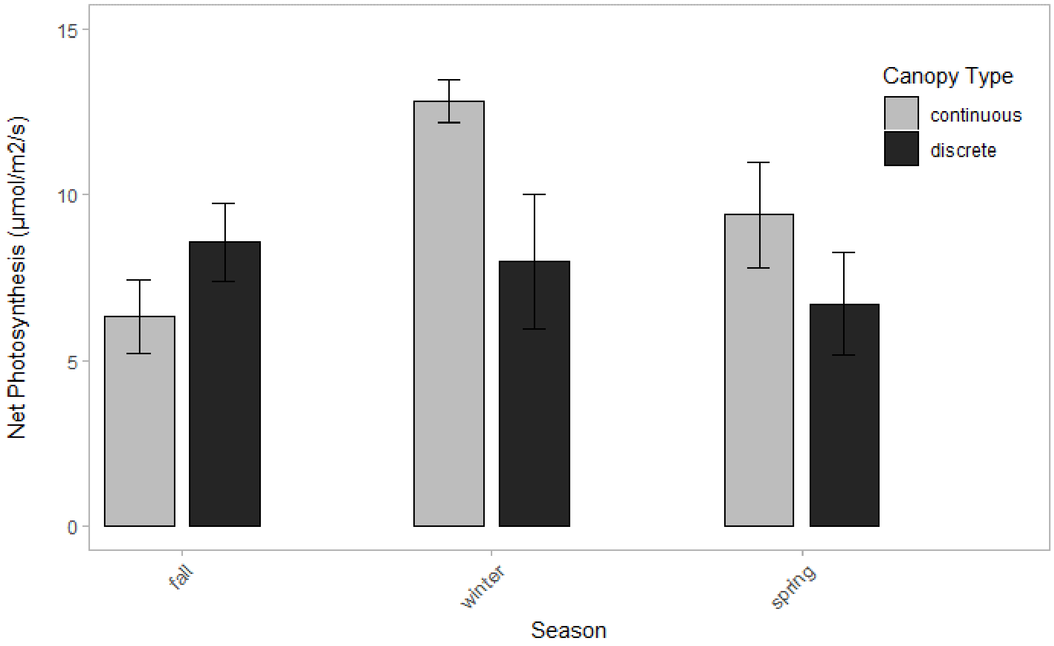

Figure 22: Seasonal leaf level comparison of continuous canopy versus discrete canopy rates of net photosynthesis, and dates of data collection were September 30 to October 4, 2019 for fall, December 9 through 12, 2019 for winter, and May 11 through 13, 2020 for spring.

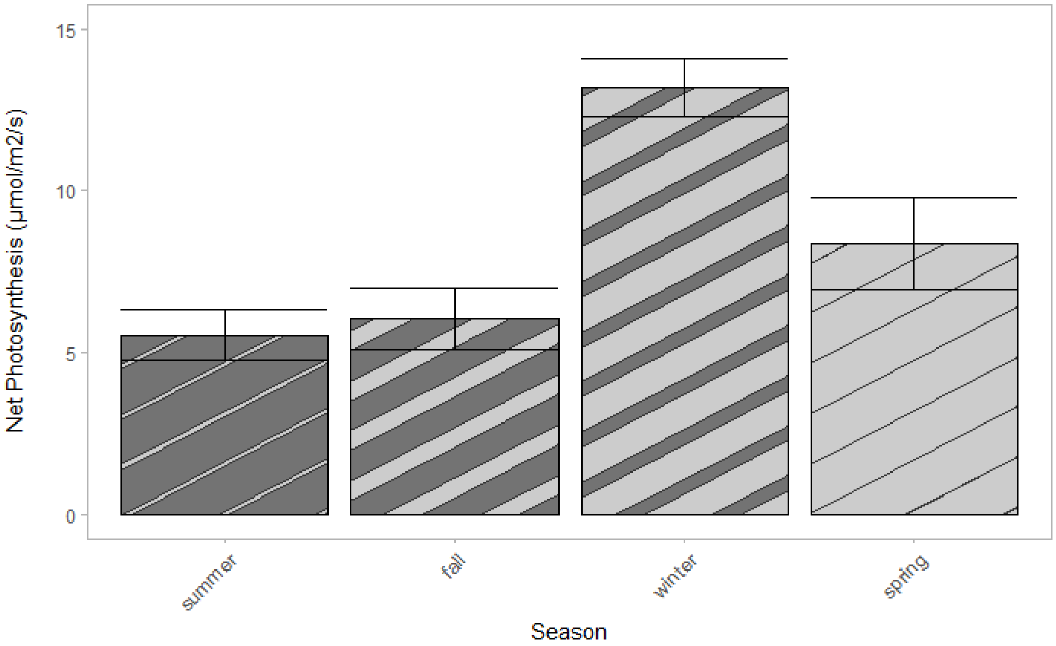

Figure 23: Seasonal leaf level comparison of continuous canopy rates of net photosynthesis averaged across lower and upper strata, and dates of seasonal data collection were May 28 to June 5, 2019 for summer, September 30 to October 4, 2019 for fall, December 9 through 12, 2019 for winter, and May 11 through 13, 2020 for spring.

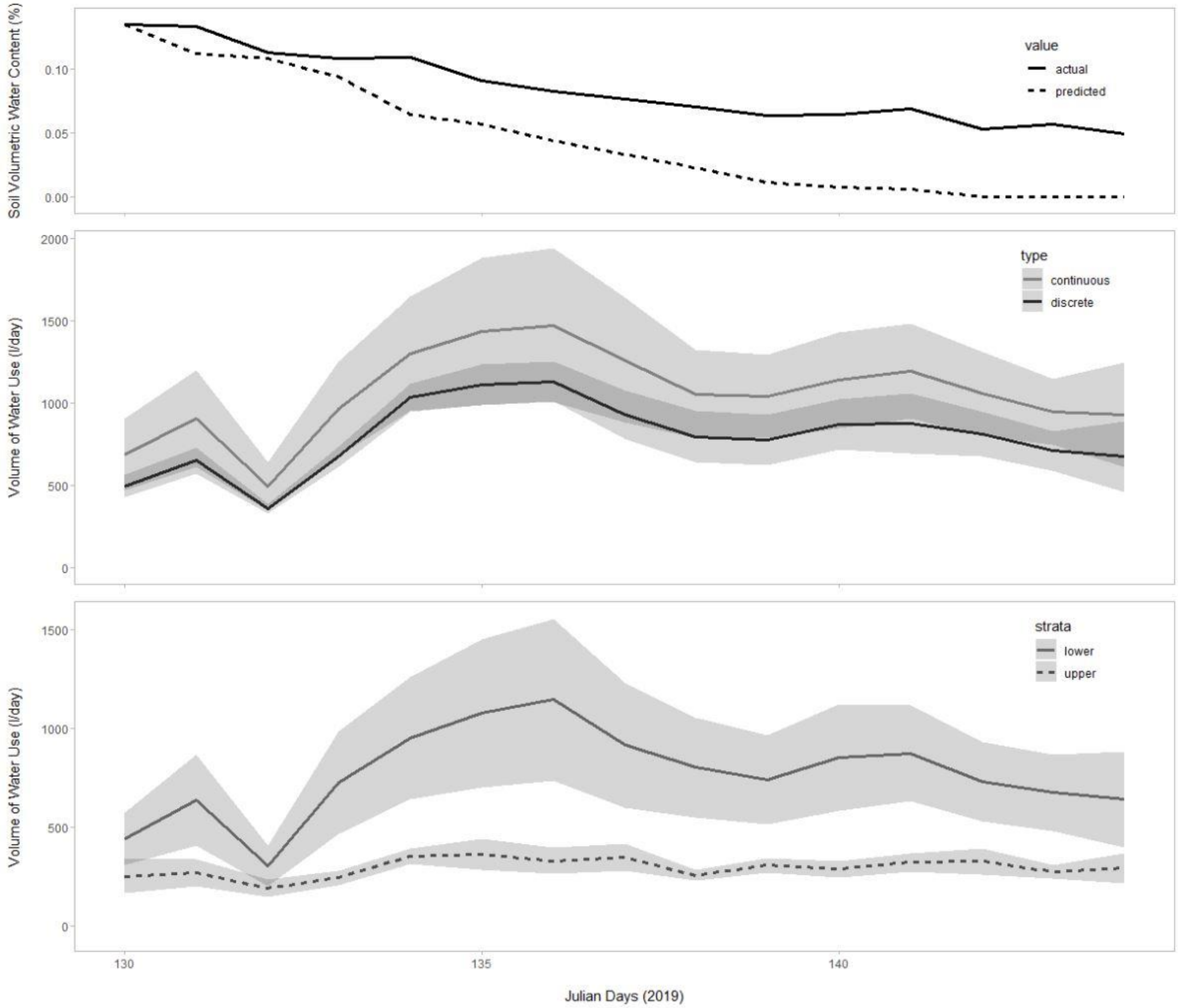

Figure 24: Soil moisture at levels with hydraulic redistribution taking place to provide additional soil moisture availability and predicted if hydraulic redistribution did not occur in relation to coinciding sap flow responses of continuous and discrete canopy types as well as the lower and upper continuous canopy strata during the summer 2019 drought period.

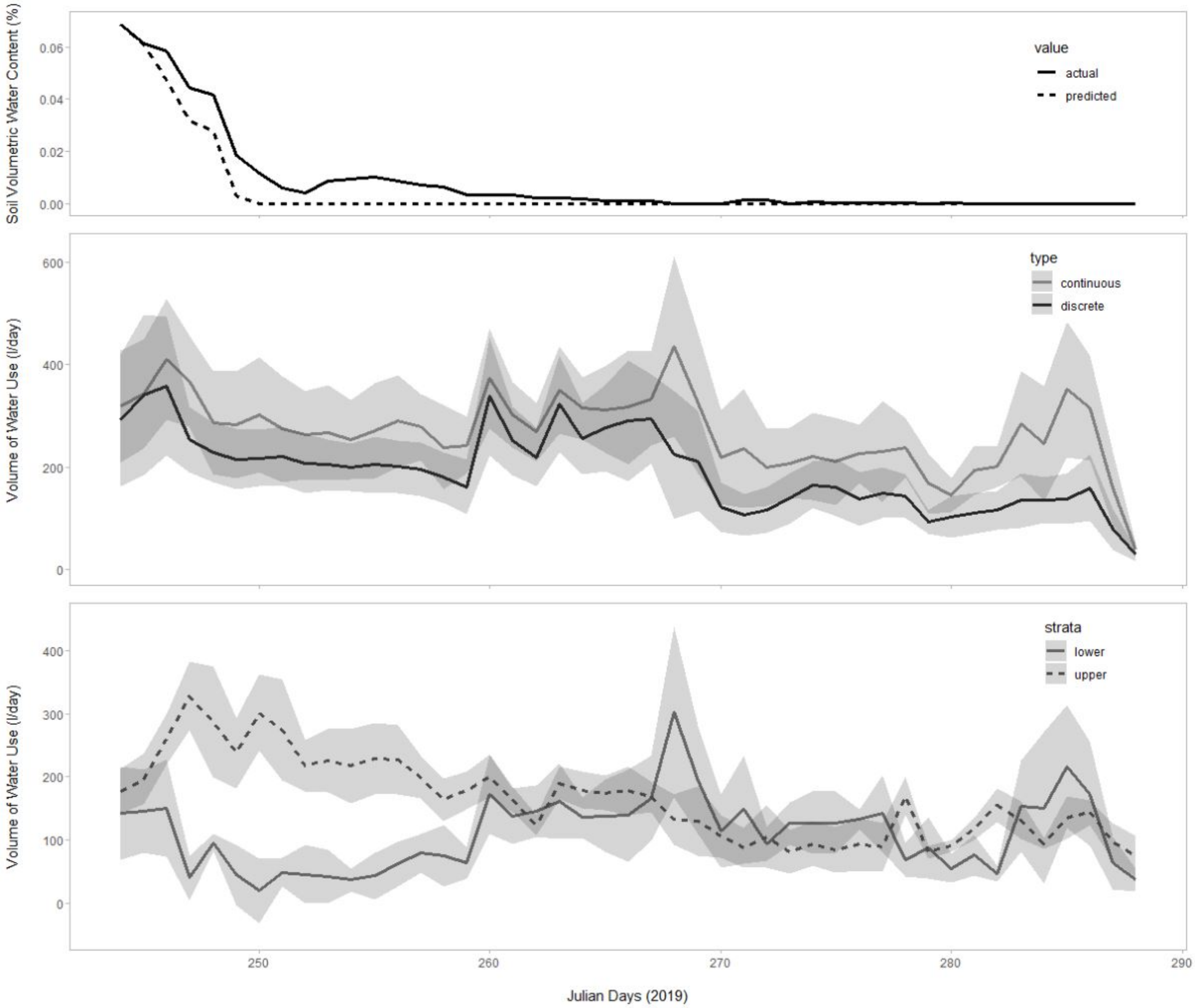

Figure 25: Soil moisture at levels with hydraulic redistribution taking place to provide additional soil moisture availability and predicted if hydraulic redistribution did not occur in relation to coinciding sap flow responses of continuous and discrete canopy types as well as the lower and upper continuous canopy strata during the fall 2019 drought period.