Phenotypic plasticity in linked plant hydraulic traits: an evaluation of a common-taxa experiment across a climatic gradient in the Western U.S.

Amelia Keyser-Gibson

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2024

Committee:

Soo-Hyung Kim

Claire Willing

Patrick Tobin

Program Authorized to Offer Degree:

School of Environmental and Forest Sciences

University of Washington

Abstract

Phenotypic plasticity in linked plant hydraulic traits: an evaluation of a common-taxa experiment across a climatic gradient in the Western U.S.

Amelia Keyser-Gibson

Chair of the Supervisory Committee:

Soo-Hyung Kim

School of Environmental and Forest Sciences

Abstract

Increasing drought conditions and variable water availability under climate change impact plant productivity, ecosystem function and the global carbon cycle, with many species-level responses remaining unknown. Variation in response and ability to acclimate to decreased water availability differs among plant species and across biomes. To assess the interactions between acclimation to climate and water availability across a growing season as well as taxa level plasticity, this project utilized a preexisting water deficit trial of horticultural taxa across sites in the Western U.S. Four focal taxa (*Cercis occidentalis, Cercis canadensis, Physocarpus* 'Diabolo' and *Physocarpus* 'Little Devil) shared across three locations were measured for physiological and morphological hydraulic traits in response to two irrigation deficit treatments.

Full gas exchange, specific leaf area, mean weighted vessel diameter, theoretical hydraulic conductance, stomatal conductance, and Φ_{PSII} were collected and water use efficiencies were calculated for each taxon at each location. Analysis of variance was used to test the effects of trait performance of species between sites as well as across sites, resulting in differing responses depending on species and site. Additionally, impacts of site, treatment, taxa and change across the growing season were analyzed using multivariate methods (RRPP and PERMANOVA). Results show distinctions in water use strategy by climatic location as well as variation between closely related species and cultivars, indicating plasticity to site and among taxa. Additionally, physiological and morphological measurements collected at multiple timepoints during the deficit period indicate measurable physiological acclimation across the growing season. These findings highlight the merits of common-taxa trials over multiple geographic locations to study and identify climate-ready plants. Our results provide novel examples of physiological shifts across the growing season, indicate the importance of experimental location on the ability of different plant taxa to acclimate to water stress, intraspecies level plasticity among horticulturally important species, and contribute to overall knowledge of plant drought responses, knowledge gaps that are crucial to address in the face of anthropogenic climate change.

Keywords: Plasticity, water-deficit, ecophysiology, horticultural taxa, climate-ready, hydraulic traits

Table of contents

Introduction

Increases in drought frequency and severity are predicted in terrestrial systems throughout much of the globe under climate change. Effects are already being felt across the Western U.S. due to warmer summer temperatures, changing precipitation patterns, decreasing snowpack and lowering soil moisture; these shifts in climate are projected to be more extreme by the mid to late 21st century (Cayan et al., 2010). In the Pacific Northwest region, the current rate of summertime warming is unprecedented, with recent climate models indicating a rapidly increasing risk of regular extreme summer temperatures (Heeter et al., 2023). A better understanding of how plants will function under increasing drought is crucial for predicting responses to climate change since the underlying mechanisms for plant response, and their capacity to adapt and persist, remain unknown (Ahanger et al., 2021). The field of plant ecophysiology provides important tools to investigate how plants respond to environmental shifts, including to conditions of water deficit. During photosynthesis, plants exchange water for CO_2 to synthesize into energy, a process that is highly linked to water availability through pores on the leaf surface called stomata. Stomata regulate gas exchange with the atmosphere during photosynthesis and transpiration, opening and closing based on environmental conditions and the need for carbon inside the leaf. In dry conditions it becomes no longer advantageous for stomata to open and lose water through transpiration, rendering them unable to fix carbon from the atmosphere for photosynthesis (Kim et al., 2020). Changing photosynthetic patterns under climate change impact the global carbon cycle through the trade-offs between limiting transpiration and fixing carbon. Improving our understanding of this interaction is necessary for more representative Earth systems models to better predict future conditions (Anderegg et al., 2016; Lemordant et al., 2018). Many questions remain about the underlying mechanisms controlling plant response to water availability and climate, as well as the variation in water use strategies which exist across the plant kingdom.

A key question in light of climate change is how shifting environmental conditions will affect plant capacity to adapt, acclimate, and persist outside their limits of environmental conditions (Armstrong et al., 2023). As climatic patterns shift towards the extremes, experiments across different climates can help infer future conditions and can help inform our understanding of plant response to water deficit. Under conditions of low water availability, plants can close stomata, decreasing stomatal conductance and the rate of carbon capture for photosynthesis. While the overall controls for stomatal opening and closing are understood, uncertainties remain in terms of the specifics of the response (Martin-StPaul et al., 2017). Decreased stomatal conductance changes plant water use efficiency and can be used as an indicator of reduced full plant photosynthetic productivity under water deficit conditions. Complications arise due to the coupling of photosynthesis and water-loss via transpiration when stomata open, and additional impacts result from the influences of temperature and humidity which increase or decrease rate of gas exchange, as well as other variables. The pathway of water movement from the soil to the leaf, known as the hydraulic feedback loop, is controlled by transpiration rate and the need to maintain a constant leaf water potential (Franks, 2004). Throughout the evolution of water transport, the prioritization of gas exchange efficiency has resulted in a variation of strategies among species (Brodribb & Holbrook, 2003; Franks, 2004; Tombesi et al., 2014).

Stomatal control allows plants to regulate their rate of water loss while maximizing carbon gain; however, there are several explanations for the underlying mechanisms of stomatal control. Stomatal closure has been hypothesized to be governed by responses to abscisic acid (ABA) increase in response to stress but has also been found to be correlated with hydraulic conductance via xylem and leaf turgor pressure (Fichot et al., 2009; Franks, 2004; Nardini & Salleo, 2000; Tombesi et al., 2014). Stomata have also been shown to respond to xylem tension as water travels from roots to leaves with xylem anatomy negatively correlated with plant water use efficiency (WUE). Smaller and more numerous vessels have been associated with increased WUE (Fichot et al., 2009; Panek, 1996). Xylem morphology is thought be controlled by a safety versus efficiency tradeoff, with narrower vessels enabling higher resistance to cavitation and freezing, but decreasing water transport efficiency, potentially imposing a limit on photosynthesis rate (Davis et al., 1999; Hacke et al., 2017; Wheeler et al., 2005). Variation in xylem vessel diameter has been found to be one of the most important variables when evaluating plant water relations, with trends responding to environmental constraints, especially aridity (Hacke et al., 2017; Sterck et al., 2008). Water deficits over the course of summer have been shown to cause xylem acclimating through a decrease and cell size and increase in vessel density (Fichot et al., 2009). In a study on *Populus deltides* x *P. nigra* hybrids vessel density was more plastic than vessel diameter which, under drought conditions, resulted in maintaining the vessel area and a limited decrease in specific hydraulic conductivity under conditions of water deficit (Fichot et al., 2009), and Sterck *et al.* (2008) observed a decrease in K_p due to dry soil. Research in aspen (*Populus tremuloides* Michx.) found that vessel diameters were sensitive to precipitation patterns on yearly scales, and the response was more pronounced in younger trees (Hillabrand et al., 2018). Hydraulic conductivity per xylem cross-sectional area is a proxy for water transport efficiency, which can be used as a comparison among species using the weighted mean vessel diameter and Hagen-Poiseuille equation, which calculates hydraulic efficiency as proportional to the fourth power of the vessel diameter (Hacke et al., 2017). Observed plasticity of xylem traits could indicate plants' ability to acclimate to and tolerate differing water availability.

As the majority of plant drought experiments focus on agricultural food crops, there remain important gaps in understanding how horticultural taxa respond to water deficit under climate change. Learning more about horticultural plant response to shifting water availability in general can help inform broader vegetation responses, as well as the large industry of nursery production and influence urban and residential landscapes (Sjöman et al., 2023). Up to 70% of residential water use is allocated to landscape plantings; thus, it is important to evaluate and encourage lower water-use taxa for horticultural planting, especially given changing water availability, primarily the projected decreases (Hayden et al., 2015). Recent work has highlighted the current lack of trait-based data related to water-availability and interestingly, found a variation in water-use among cultivars of the same species, with the wild-type preforming the most efficiently (Hirons et al., 2021; Sjöman et al., 2023; Tombesi et al., 2014). This brings into question if horticultural breeding selecting for appearance comes at the expense of drought tolerance; an avenue of research that requires more attention. Evaluating taxa for drought response now provides information through which to select individuals that will be most successful in future climatic conditions and should be prioritized in urban settings.

In this study, I used two water-deficit treatments in a coordinated horticultural plant research network to assess plasticity in trait response among taxa receiving the different treatments across three environmentally distinct field sites and the capacity for acclimation over a growing season. Horticultural plant response to drought is relatively understudied, and the use of this trial offered the opportunity to compare overlapping taxa under different climatic conditions and provide insight more broadly into plant drought stress response and acclimation.

Four target taxa were identified which were part of the preexisting Climate Ready Landscape Plants project (CRLP) shared across the University of Washington (UW), the North Willamette Research and Extension Center (OSU) and the Utah State University Greenville Research Farm (USU): *Cercis occidentalis, Cercis canadensis, Physocarpus* 'Diabolo', and *Physocarpus* 'Little Devil'. The selection of these taxa was intended to address a key research gap as a few previous studies focused on them, and allowed for comparisons among closely related species and cultivars, as well as treatment effects across sites. *Cercis occidentalis* and *C. canadensis* have been used in horticultural breeding efforts to create hybrids more adapted to drought conditions (Contreras & Hoskins, 2020; Sternberg, 2012). Previous work on a group of woody horticulture taxa found that *C. occidentalis* demonstrated significant variation in water-use between geographic locations, while the water use of many of the other species evaluated remained relatively consistent (Schuch & Burger, 1997). Both *C. occidentalis* and *C. canadensis* have also been found to be drought resistant but have differing responses to drought (Sternberg, 2012). *Physocarpus* 'Diabolo', *Physocarpus* 'Little Devil' are both cultivars of *Physocarpus opulifolius,* a popular landscape shrub. Even though many cultivars of *P. opulifolius* are commonly used in horticulture and have been bred for interesting vegetation (Lubell et al., 2011), few previous studies have used *P. opulifolius* cultivars as a focus taxon other than studies that examined the effect of pollution (Popek et al., 2018), and salt stress (Nan et al., 2018) and powdery mildew (Lubell et al., 2011) in urban landscapes. Thus, physiological analyses of these cultivars offer valuable information in terms of their performance under a water deficit, as well as crucial information for the horticultural industry.

Species-specific differences have been shown in the relationship between WUE and xylem hydraulics, demonstrating the need for species-level investigations (Fichot et al., 2009).

Cultivars of the same species have been shown to have differing relationships between stomatal conductance and xylem hydraulics (Tombesi et al., 2014). Additionally, hydraulic conductivity depends on water availability and has been shown to vary across a climatic gradient (Panek, 1996). Thus, the variation in coupling between hydraulic conductance, xylem anatomy and stomatal control results in plants with differing ability and strategies with which to cope with limited water. Measuring physiological and morphological traits in tandem allows for investigation into how these species vary in their approach to water use, and ability to acclimate. Additionally, this work occurred over the course of the growing season, allowing for the investigation of relatively unexplored temporal scale of physiological acclimation (Grossman, 2023).

Objectives and hypothesis

Through this study I investigated if closely related species and cultivars differ in xylem anatomy, stomatal conductance, and chlorophyll florescence; how these traits relate to photosynthetic rate and WUE; and response to irrigation deficit treatments across an environmental gradient.

Aim 1: Evaluate if site, taxa and irrigation deficit level contribute significant differences in the response of measured physiological and morphological traits at the end of the growing season. It was hypothesized that the measured suite of hydraulic traits will be coordinated under water deficit treatments but not consistently across taxa and/or site due to distinct climatic factors at each site affecting plant performance and each taxa exhibiting a different water use strategy that will be distinguishable at the species or genera level. *Cercis occidentalis* was hypothesized to perform distinctly across these western U.S. sites when compared to *C. canadensis* due to the former having a west coast distributional range while *C. canadensis* is predominately distributed in the eastern and central U.S. Additionally, the cultivars of *Physocarpus*, though closely related, will show distinguishable responses, and measured less water use efficiency than either *Cercis* species due to selection for appearance in the horticultural trade. Individual measured traits will respond to irrigation deficit treatments, and across all sites, and it was predicted that the lowest water treatment deficit (20% ET_0) will result in an increased WUE and exhibit a more conservative water use strategy, due to keeping stomata open less. Xylem anatomy was hypothesized to respond to the lower water treatment by producing smaller conduits due to the trade-offs between hydraulic efficiency and risk of cavitation. Additionally, due to the more variable weather conditions and more frequent occurrence of winter freezing temperatures, plants at the USU site would result in plants better acclimated to stress and in turn an increased WUE.

Aim 2: Evaluate physiological acclimation to the water irrigation treatments over the course of the growing season. By assessing traits measured at the beginning and end of the season, as well as additional traits measured at the UW site across multiple time points, we hypothesized that taxa would show acclimation in water use traits over the course of the growing season and in response to the irrigation treatment, resulting in shifting physiological traits. The temporal scale of acclimation is relatively underexplored yet is an intriguing application of this study system. Results from this exploration can inform future research directions and applications for related study systems.

Aim 3: Evaluate end of season physiological performance from additional traits collected from paired taxa after a growing season of irrigation deficit treatments at the UW site. Given some observed responses to the irrigation deficit treatment, additional physiological measurements were performed on plants at the UW site to investigate how shifts in performance were manifested in physiological responses. These measurements include Fv/Fm, which measures photosynthetic performance, and water potential as a metric for plant water status. It was hypothesized that distinctions in Fv/Fm and water potential at the treatment level would be observed, with low treatment plants exhibiting lower values of Fv/Fm and decreased values of water potential due to stress from the irrigation deficit.

Materials and Methods

Experimental design

This project harnessed existing field sites with overlapping taxa across the Western U.S. in areas with differing climatic conditions to investigate the role environment plays in plant function and response to irrigation deficit treatment. The Climate Ready Landscape Plants Project (CRLP) was established across six sites in 2021 (University of Washington (UW) Center for Urban Horticulture Seattle, WA, Oregon State University (OSU) North Willamette Research and Extension Center Aurora, OR (NWREC), University of California, Davis (UCD), Utah State University Greenville Research Farm, Logan UT (USU), University of Arizona (UA), and South Coast Research and Extension Center (SCREC) in Irvine, CA). A primary goal of CRLP is to evaluate horticultural taxa growth and aesthetics response to water deficit conditions across a climatic gradient, including some species that could be grown across all six sites (Fron *et al*. 2024, Fron *et al*. 2023, Oki, 2019). Field sites were planted in spring and summer of 2022 and the plants were allowed to establish for a year under normal irrigation (80% ET_0). Plots consisted of 2m spaced rows of plants in full sun. Eight plant replicates per treatment were planted, resulting in 24 per taxa; however, some mortality occurred over the establishment phase resulting in lower numbers in collected data (Fron *et al*. 2024, Fron *et al*. 2023). The preexisting setup of

CRLP provided the opportunity to collect physiological measurements on established taxa at receiving water deficit treatments as part of the broader study. This project narrowed in on shared taxa located at UW, OSU and USU for the 2023 field season.

Site characteristics

The three sites used in this study span a climatic range across the Western U.S. characterized by different growing conditions, historic climatic conditions and site-specific aspects such as soil type. The UW site and OSU site are only separated by one USDA hardiness zone in the 2023 update (9a to 8b), while USU is in zone 6a due to much colder winter temperatures (Fig. 1). The USDA hardiness zones provide climatic metrics commonly used in horticulture and agriculture for plant selection (Widrlechner et al., 2012).

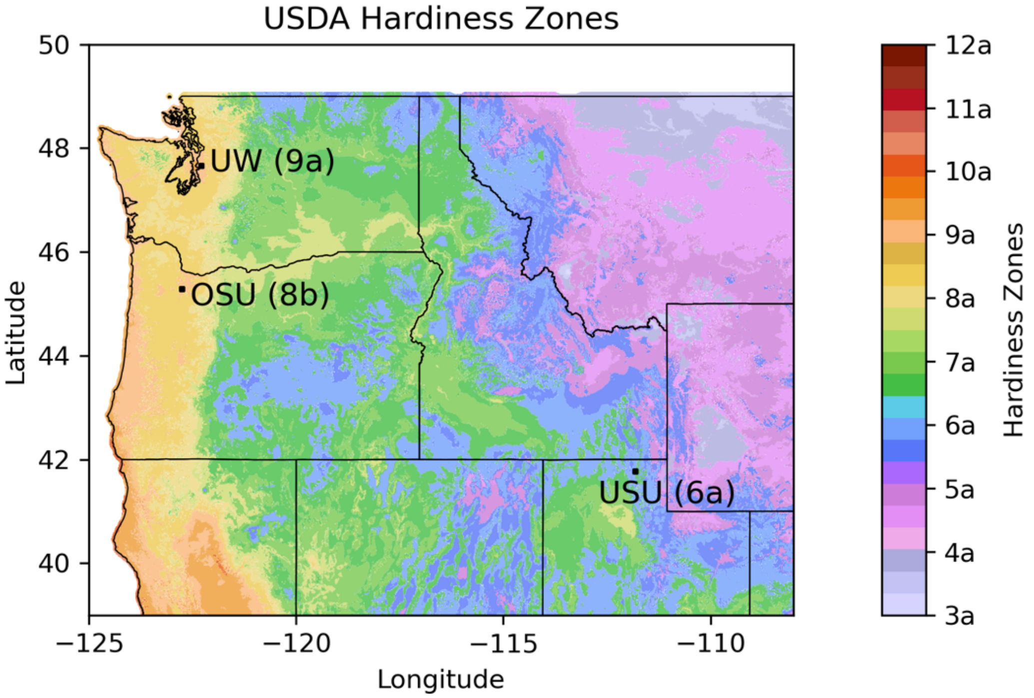

Figure 1. Map of the western U.S. with the USDA hardiness zones indicated and black dots to indicate the location of the three CRLP sites sampled in this study: UW Center for Urban Horticulture in zone 9a, OSU North Willamette Research and Extension center in zone 8b, and USU Logan Research Farm in zone 6a.

UW and OSU are characterized by a Mediterranean climate, with dry summers and wet, temperate winters, whereas USU has a humid continental climate, with hot temperatures in the summer and colder winters with more precipitation in the form of snow. To further characterize climatic norms between the sites, data from the past decade (2011-2020) was acquired from ClimateNA (Wang et al., 2016). Yearly averages and two specific seasonal averages were compiled to correspond with the physiological data collected in this study, with growing season means corresponding to the time of year the irrigation deficit occurred (June-September), and winter means corresponding to the opposite four months (November-March). Climate data when visualized on a spider plot show strong overlaps between the UW and OSU site, with USU experiencing a higher number of chilling degree-days during the winter and higher rates of evapotranspiration during the growing season (Fig. 2).

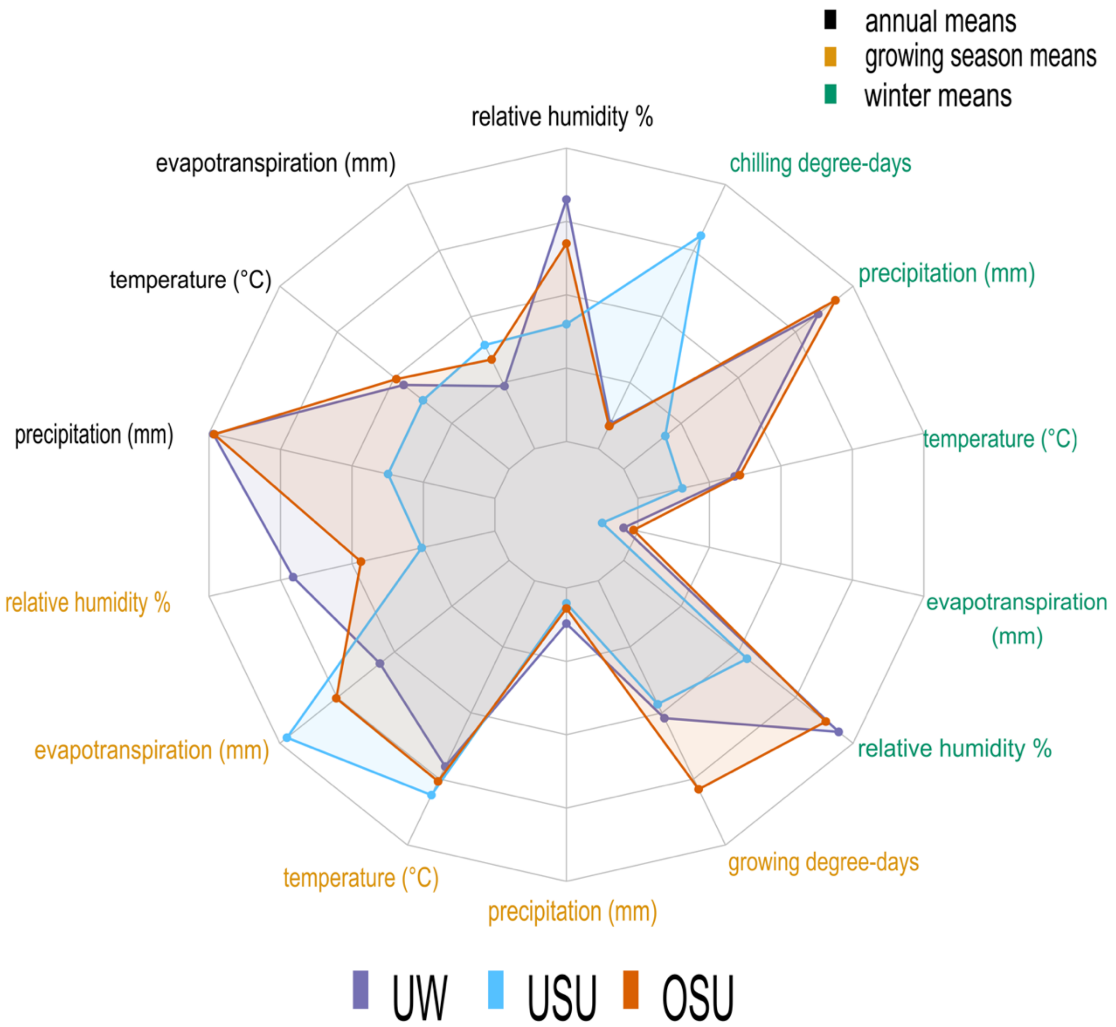

Figure 2. Climate norms for each site from the past decade (2011-2020), with black text indicating annual monthly means, yellow text indicating monthly means during the irrigation deficit/growing season months (June-September) and green text indicating monthly means during the opposite winter months (November-March). Data acquired from ClimateNA (Wang et al., 2016).

Plant taxa

I used two sets of paired taxa in this project: *Cercis occidentalis* and *Cercis canadensis* (Fabaceae), and *Physocarpus* 'Diabolo' and *Physocarpus* 'Little Devil' (Rosaceae). Both *Cercis* species are small deciduous trees which form pink flowers in spring before leaf out. *C. occidentalis,* commonly known as western redbud, has a range west of the Rocky Mountains and reniform leaves, while *C. canadensis* (eastern redbud), has a broader range across the eastern and central U.S. and cordate leaves (Hopkins, 1942) (Fig. 3A). The *Physocarpus* are both cultivars of *P. opulifolius* that have been bred to have dark purple to red foliage and are popular for horticultural use due to their ornamental qualities (Fig. 3B, C). *Physocarpus* Diabolo®, also called ‘Monlo’, was the first cultivar bred from the wildtype green foliage plants, discovered in a German nursery in 1968. More recently, ‘Monlo’ was crossed with a more compact green leaved cultivar ‘Nanus’ to create ‘Donna May’, or *Physocarpus* Little Devil™, which retains the purple foliage but has a more compact size and smaller leaves (Zlesak, 2012). Both cultivars of *Physocarpus* exhibit inflorescences of light pink flowers in spring.

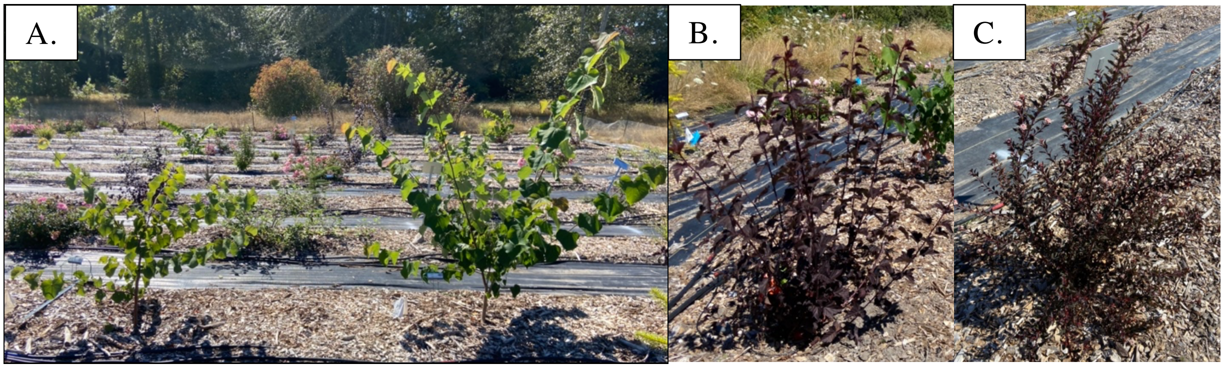

Figure 3. Target taxa in the UW 2023 CRLP plot. A. *Cercis occidentalis* (left) and *Cercis canadensis* (right), B. *Physocarpus* ‘Diabolo’, C. *Physocarpus* ‘Little Devil’.

Irrigation treatments

Irrigation deficit treatments occurred over the summer of 2023, and were initiated following the conclusion of spring rain (late-May for OSU, and mid-June for UW and USU). Fully randomized subsets of each taxon received two deficit irrigation treatments based on soil texture, recent precipitation rates and reference evapotranspiration (ET_0) calculated from weather station measurements at each site and administered at rates of 0.2 (low treatment) and 0.8 (high treatment) times ET_0 (Fron et al., 2024). Study sites vary in their amounts of annual precipitation, as well as environmental conditions during the deficit period (Table 1).

Table 1. Specifications for climatic conditions, soil, environmental conditions and irrigation events across the three sites.

Location	USDA Zone[1]	Annual precipitation (cm)[2]	Soil Type[3]	Available water storage (cm per 0-100)[3]	Cumulative precipitation (cm) during deficit[4,5,6]	Cumulative ET_0 (cm) during deficit[4,5,6]	Number of irrigations		Amount of total water applied (cm)	
							High	Low	High	Low
UW	9a	94.31	Loamy Sand (Ls)	7.3	1.07	31.01	12	2	21.39	4.06
OSU	8b	106.35	Willamette silt loam (88B)	20	0.05	41.22	10	2	46.91	9.38
USU	6a	47.19	Millville silt loam (MIB)	18.3	10.13	42.21	6	0	26.03	0

[1] 2023 USDA Plant Hardiness Zone Map https://planthardiness.ars.usda.gov/
[2] U.S. Climate Data https://www.usclimatedata.com/
[3] SoilWeb California Soil Resource Lab, University of California, Davis, CA https://casoilresource.lawr.ucdavis.edu/gmap/
[4] AgWeatherNet, Seattle Station https://weather.wsu.edu/
[5] Oregon AgriMet Weather Station, Aurora https://www.usbr.gov/pn/agrimet/agrimetmap/araoda.html
[6] Utah Climate Center, Greenville Farm, https://climate.usu.edu/mchd/

To verify the irrigation treatments Watermark 200SS sensors (Irrometer Company, Inc., Riverside, CA, USA) were installed at the UW site at depth of 15 cm to the center of the sensor at a subset of target plants throughout the plot ahead of end of the season physiology measurements. Soil water tension measurements show a distinction between the high and low treatments (Fig. 4).

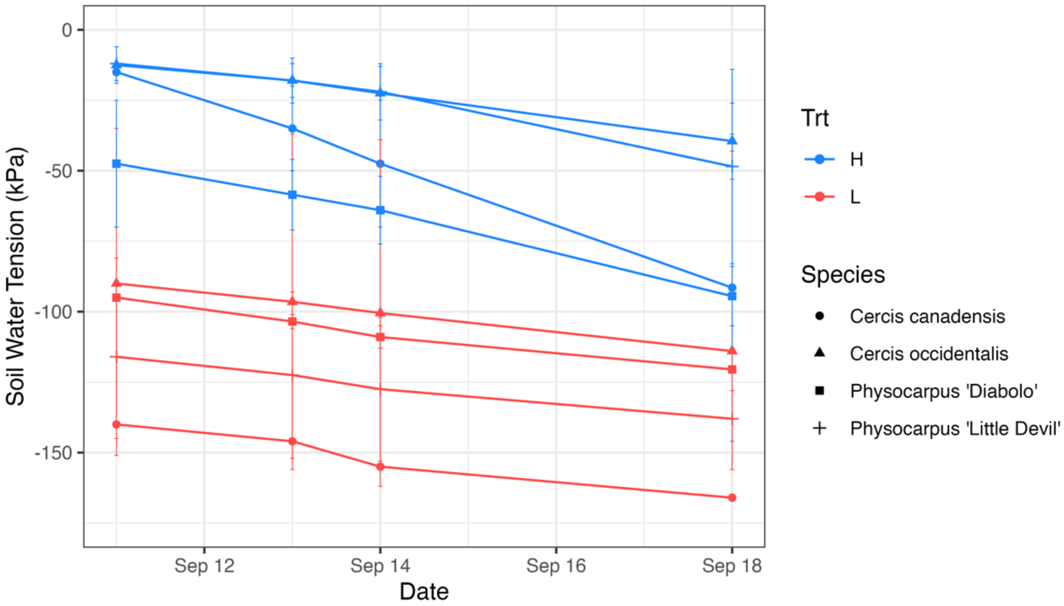

Figure 4. Watermark sensor data in kPa at the UW site ahead of end of the season physiological measurements with color indicating irrigation treatment, shape indicating taxa and error bars for standard error. The only irrigation event during this time period occurred on the high treatment on September 10, and end of season physiological measurements occurred on September 14.

Leaf physiology

End of season measurements of stomatal conductance to water vapor (g_{sw}, mol m^{-2} s^{-1}) and Φ_{PSII} were taken with a LI-600 Porometer/Fluorometer (LI-COR Biosciences, Lincoln, NE, USA) during midday (11:30-1:30pm, to reduce risk of variable weather influence) at the three sites. At the UW site, midday LI-600 measurements were also collected prior to the irrigation treatments, in the middle of the treatment period, as well as at predawn (4:30-6:00am) at the end of the growing season. The midday LI-600 configuration was set to automode with fast g_s, fluorescence and flowrate, with matching to ambient conditions set to every 10 minutes. The predawn measurement configuration included the addition of a 800 ms dark adapted 6000 µmol m^{-2} s^{-1} flash to measure Fv/Fm. Instantaneous gas exchange survey measurements were taken with a portable photosynthesis system (LI-6400XT at OSU and LI6800 at UW and USU; LI-COR Biosciences, Lincoln, NE, USA) with a 2 cm^2 chamber head and parameters set to 420 µmol mol^{-1} CO_2; flow rate 500 µmol s^{-1}, PAR at 2000 µmol m^{-2} s^{-1}. Since *Physocarpus* 'Little Devil' leaves were often too small to fully cover the chamber head, analysis in ImageJ was used to correct for leaf area and incorporated into the gas exchange data calculations. Full gas exchange survey measurements were used to calculate instantaneous water use efficiency (WUE; A/E) and intrinsic water use efficiency (iWUE; A/Cond). All leaf physiology measurements were conducted on full sun, undamaged, new but fully expanded leaves, with one representative leaf per plant selected for full gas exchange measurements, and four leaves measured for LI-600 measurements, of which the lowest value of g_{sw} was excluded and data were averaged to act as a per plant measurement. Gas exchange measurements were timed to occur prior to irrigation treatments to capture the effects of the deficit treatment.

Water potential

Leaf water potential was measured with a Model 1000 Pressure Chamber Instrument (PMS Instrument Company, Albany, OR, USA) at the end of the growing season at the UW site, and at two time points for diurnal collection: predawn (4:30-5:30 am) and midday (11:30am-12:30 pm). Fully expanded leaves were excised with a razor blade and placed in a plastic bag prior to analysis. The petiole was placed in the rubber gasket of the pressure chamber, and the pressure value recorded when droplets of water appeared on the cut surface.

Specific leaf area

Leaf punches of known areas were collected prior to the initiation of the irrigation deficits and at the end of the growing season at all three sites. Data was collected from new, fully expanded leaves at each time. Samples were dried in an oven overnight and weighed to calculate specific leaf area (SLA, cm^2/g). SLA is considered as an ideal trait for use as a proxy for plant growth, with lower values of SLA indicating thicker leaves and more investment into biomass production (Liu et al., 2017).

Xylem anatomy

Stem segments from the top 5 cm of actively growing branches were collected from target taxa at all three sites at the end of the growing season. Stem samples were stored frozen at -18 °C prior to being hand sectioned with a double-edge razor blade, stained with a 0.1% safranin solution for 40 seconds, cleared with DI water, and imaged under a microscope (E200 Eclipse, Nikon Instruments Inc., Melville, New York, USA) with a camera attachment (MU-1803 HS, AMScope, Irvine, California, USA). Image analysis was conducted using ImageJ-Fiji version:

2.14.0/1.54f (Schindelin, 2012). For images with good contract, vessel area was measured with an autothresholding function in ImageJ-Fiji, and for the rest, vessels were manually traced. A minimum of 50 vessels per individual were measured. Since vessels were not circular, diameter was estimated as the mean diameter of an ellipse: $D = (D_1 + D_2)/2$, with D_1 as the maximum vessel diameter and D_2 as the minimum vessel diameter. Vessel density (VD, $n\ \mu m^{-2}$) was calculated as number of vessels per set area from which mean vessel hydraulic diameter (D_{mh}; μm) was calculated as: $D_{mh} = (\sum D^4 / n)^{1/4}$ (Fontes et al., 2022). The theoretical specific conductivity (K_p; kg m $MPa^{-1}s^{-1}$ was calculated using the Hagen-Poiseuille equation: $K_p = \frac{\pi \rho_\omega}{128\eta} * VD * D_{mh}{}^4$ where ρ_ω is the density of water (998.23 kg/m^3) and η is the viscosity of water (1.002 $x10^{-9}$ MPa/s), both at 20°C (Santiago et al., 2004; Tyree & Ewers, 1991).

***Table 2**.* Explanation of all traits measured in this study, their abbreviations, and units. * indicates traits only measured at the UW site.

Abbreviation	**Trait**	**Unit**
gsw_MD or g_{sw}	Midday stomatal conductance (measured by LI600)	mol m^{-2} s^{-1}
PhiPS2 or Φ_{PSII}	Midday chlorophyll fluorescence - photosystem 2 efficiency	unitless
Fv/Fm	Dark adapted chlorophyll fluorescence *	unitless
g_{sw}_PD	Predawn stomatal conductance *	mol m^{-2} s^{-1}
SLA	Specific leaf area	cm^2/g
Photo (A)	Photosynthetic assimilation rate	μmol m^{-2} s^{-1}
Cond (g_s)	Stomatal conductance (measured by LI6400 or LI6800)	mol m^{-2} s^{-1}
Trmmol (E)	Transpiration rate	mmol m^{-2} s^{-1}
WUE (A/E)	Instantaneous water use efficiency	mmol mol^{-1}
iWUE (A/g_s)	Intrinsic water use efficiency	μmol mol^{-1}
D_{mh}	Mean vessel hydraulic diameter	μm
K_p	Theoretical specific conductivity	kg m $MPa^{-1}s^{-1}$
WP_PD	Predawn water potential *	MPa
WP_MD	Midday water potential *	MPa

Statistical analysis

All statistical analyses were performed in R version 4.3.1 (R Core Team, 2023). For individual traits measured at each site at each timepoint, analysis of variance (ANOVA) was used to assess the main effects of taxa and treatment, and their interaction, on each trait. ANOVAs were run in R using the *stats* package, or in the case of unequal sample size, type III sums of squares were used from Anova() from the package *car*. Tukey-Kramer HSD was used for post hoc pairwise comparisons, using the *emmeans* package for unbalanced pairwise comparisons (Fox & Weisberg, 2019, Lenth, 2024). If data did not fit the ANOVA assumption of normality, either log transformations were used, or if only one factor was significant and the residuals of transformed data were also not normal, a Kruskal Wallis test was with a Dunn post-hoc test from the package *FSA* (Ogle, 2023). Violin plots for use in data visualized were produced using *ggplot2* (version 3.5.0) (Wickham, 2016).

Multivariate analysis was used to assess the effects of site, taxa and irrigation on measured traits via randomization of residuals in a permutation procedure (RRPP) from the package *RRPP* (version 2.0.0) (Collyer & Adams, 2018). RRPP is a multivariate technique with similar applications to permutational multivariate analysis of variance (PERMANOVA) both of which can be used to assess the effect of multiple variables on dataset variation by comparing groups via ANOVA, without data needing to fit assumptions of normality or variance homogeneity. RRPP differs from PERMANOVA by permuting the residuals after fitting the factors of interest and has recently been suggested as a statistical tool for studies interested in stress, since the impact of multiple traits can be tested simultaneously, and Euclidean distance matrices (best fit this time of quantitative data) can easily be used (Telemeco & Gangloff, 2020).

Additionally, RRPP reduces the chance of type-I error and the package *RRPP* has a function for post-hoc pairwise comparisons (Collyer & Adams, 2018, Telemeco & Gangloff, 2020).

RRPP was used to access the main effects site, taxa, and treatment, and all possible interactions for traits measured across all plant replicates at each site (10 shared traits measured at midday: stomatal conductance (g_{sw}), Φ_{PSII}, SLA, photosynthetic rate (Photo/A), conductance (Cond/g_s), transpiration (Trmmol/E), WUE and iWUE, and hydraulic traits (K_p and D_{mh}) across a total of 108 plants). Additionally, data from the UW site was analyzed separately to include 4 additional traits only measured at that site: predawn stomatal conductance, predawn Fv/Fm, predawn and midday water potential. All RRPPs were run using the lm.rrpp function from the package *RRPP,* with type 3 sums of squares, fixed effects and 10,0000 unrestricted permutations (Collyer & Adams, 2018). Type 3 sums of squares were selected since data were unbalanced and to consider the effects of all terms equally (Anderson *et al.,* 2008). RRPP results were used to create ANOVA tables across all sites and for the UW site with the additional measured traits. Pairwise contrasts were performed when terms were found to be significant.

Principle component analysis (PCA) was used to visual correlation between measured traits and taxa across all sites. PCAs were run using the function *princomp* and biplots were produced using the packages *ggbiplot* (version 0.6.2), *ggpubr* (version 0.6.0) and *ggplot2* (version 3.5.0) (Vu and Friendly, 2023, Kassambara, 2023, Wickham, 2016).

To assess the changes in plant response across the growing season, for SLA data collected at the beginning and the end of the growing season, given uneven sample sizes due to late leaf out, a linear mixed effect models using the function *lme* from the package *nlme* (version 3.1-162) with season as a variable and individual plant position as a random effect was used to determine the significance of season (Pinheiro et al., 2024, Pinheiro & Bates, 2000).

Additionally, measurements of stomatal conductance (g_{sw}) from the UW site at three time points across the season (baseline, mid and end) was assessed via two-way ANOVA for the impact of time of taxa and treatment on each time point, and then a paired t-test for the impact of time point on taxa. To assess the significance of time of season, measures of both g_{sw} and Φ_{PSII} from the same three time points were additionally analyzed in a PERMANOVA with a permutation control setting plant position as a blocking factor for 99999 permutations, using the *adonis2* function from the package *vegan* (version 2.6-4) (Anderson, 2003, Simpson, 2022, Oksanen, 2022). Visualizing via non-metric multidimensional scaling (NMDS) was performed using the *metaMDS* function from *vegan* with two dimensions, a maximum of 300 iterations, and minimum of 40 and a maximum of 100 random starts. Euclidean distance measured was used and dimensionality was determined due to the small size of the dataset. The final NMDS result was the product of 40 random starting locations, 2 iterations and the best run resulted in a stress of 0.485e-10. Visualizations were performed using the packages *ggplot2* (version 3.5.0) and *ggpubr* (version 0.6.0) (Wickham, 2016, Kassambara, 2023).

Results

Trait comparison within sites

In support of aim 1, individual physiological and morphological traits show variable trends between taxa and site, with most traits showing species level differences at all sites, with some traits from the UW site showing significance between irrigation treatment levels and one from USU showing significant interaction between treatment and taxa (Table 3). Between sites, all shared measured traits show significance of species level at UW and USU, and all traits except metrics of water use efficiency OSU. Treatment significance is observed across the majority of traits at UW, and no traits show treatment significance ($p < 0.05$) at OSU or USU, other than SLA at USU (Table 3).

Table 3. Two-way ANOVA or *Krustal-Wallis p-values for all traits measured at the end of the season across all four taxa at three sites (UW, OSU and USU) with bolding indicating significance at $p < 0.05$, and italics for values of $p < 0.1$. Trt indicates significance between high and low irrigation treatment factors, and int stands for the interaction between treatment and taxa. See Table S1 for full ANOVA tables including F statististics, degrees of freedom, etc.

	UW	**UW**		**OSU**			**USU**		
trait	**trt**	**taxa**	**int**	**trt**	**taxa**	**int**	**trt**	**taxa**	**int**
g_{sw}	**<0.001**	**<0.001**	0.19	0.61	**<0.001**	*0.08*	0.7	**<0.001**	0.72
ΦPSII	0.213	**0.002**	0.285	0.29	**<0.001**	0.84	0.2	**<0.001**	0.41
SLA	**0.011**	**0.005**	0.12	0.88	**0.004**	0.15	**0.01**	**<0.001**	**<0.001**
A/Photo	**0.028**	**0.002**	0.75	0.29	**<0.001**	0.8	0.18	**<0.001**	0.56
Cond/g_s	**0.002**	**<0.001**	0.362	0.24	**<0.001**	0.7	0.70	**0.02**	0.9
E/Trmmol	**0.002**	**<0.001**	0.54	*0.016*	**<0.001**	0.75	0.61	**0.05**	0.83
WUE	**<0.001**	**0.002**	0.98	0.89	*0.095*	0.93	0.47	**0.02**	0.98
iWUE	**<0.001**	**0.006**	0.997	0.998	0.17	0.74	0.48	**0.007**	0.99
Kp		**<0.001***		0.49	**0.004**	0.87	*0.09*	**0.038**	*0.07*
Dmh		**<0.001***		0.88	**<0.001**	0.18	*0.099*	**<0.001**	0.28

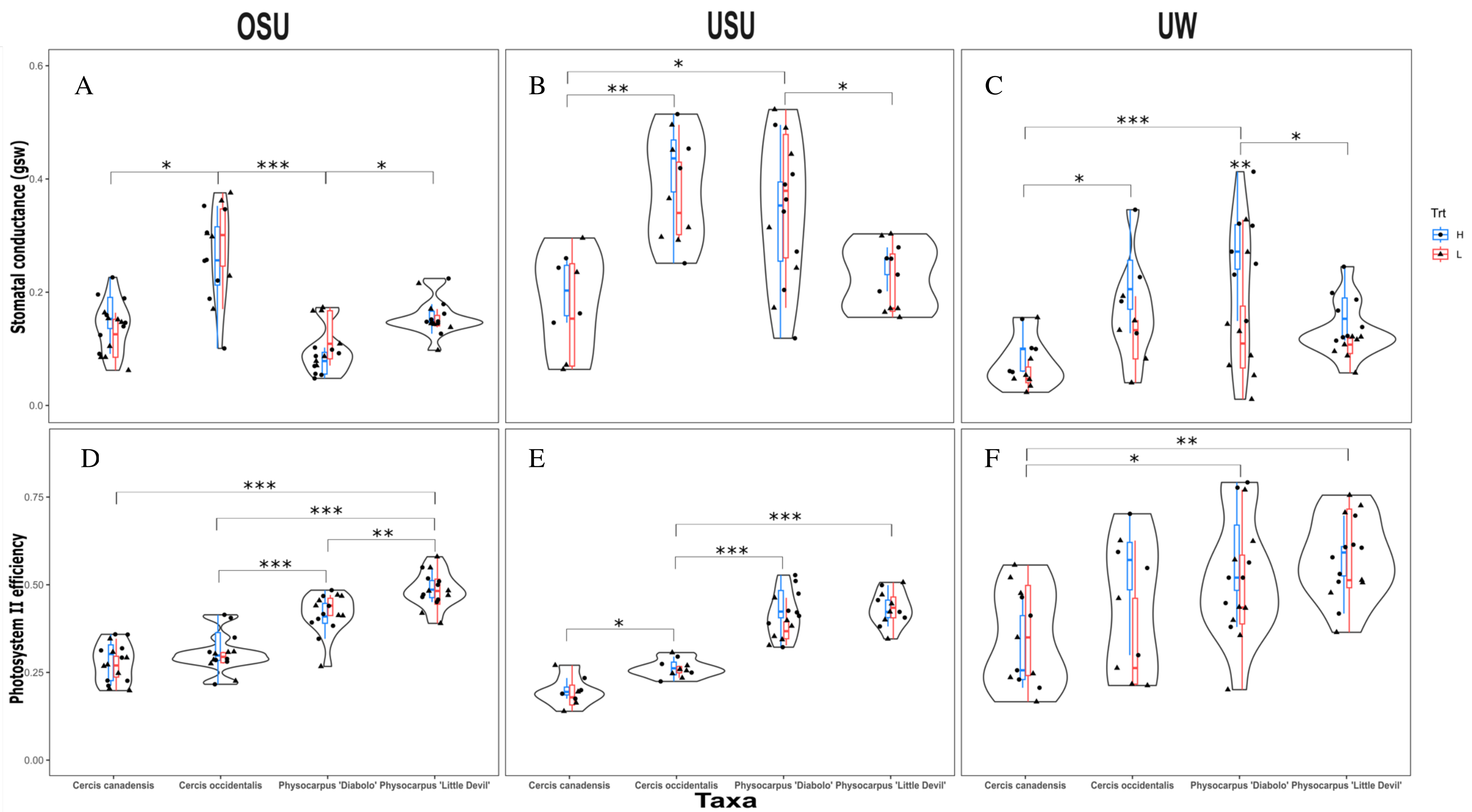

Figure 5. Stomatal conductance (g_{sw}, mol m^{-2} s^{-1}) and photosystem II efficiency (Φ_{PSII}) efficiency measured with a LI600 at the end of the season across all sites. Species ($p < 0.001$) significance is found across all sites for both traits, with *Physocarpus* 'Diabolo' showing treatment level differences in g_{sw}.

Gas exchange traits between species

Pairwise differences in trait response varied among taxa and across sites, though all taxa level comparisons were significantly different between at least two measured traits at each site. Stomatal conductance (g_{sw}) and photosystem II efficiency (Φ_{PSII}) show species level differences across all three sites, especially between genera (Fig. 5). Significantly higher g_{sw} was measured in *Physocarpus* 'Diabolo' ($p < 0.01$) receiving the high irrigation treatment (Fig. 5C, Table 3). General trends in g_{sw} and Φ_{PSII} are mostly consistent across sites in terms of the increase or decrease in taxa response, primarily with the species of *Cercis* having lower values of Φ_{PSII} than the cultivars of *Physocarpus.*

Full gas exchange measurements of net photosynthetic rate (A), stomatal conductance (g_s) and transpiration rate (E) also showed distinctions among taxa and between sites (Fig 6, Table 3). *C. occidentalis* measured higher values than *C. canadensis* across all three traits at USU and OSU, but no distinction between species of *Cercis* was measured at UW. *Physocarpus* cultivars show differences across g_s at UW and OSU, E at UW, and A at USU. *Physocarpus* 'Diabolo' has higher mean g_s than *Physocarpus* 'Little Devil' at UW, but at OSU the order was reversed, with *Physocarpus* 'Little Devil' measuring higher. No significant differences based on irrigation treatment are observed (*Physocarpus* 'Little Devil' at UW is close except for an outlier) (Fig. 6).

Calculations of water use efficiency from full gas exchange measurements resulted in different patterns than those observed from raw traits. Instantaneous water use efficiency (mmol $\mathrm{mol^{-1}}$) calculated as photosynthetic rate over transpiration rate (A/E) and intrinsic water use efficiency calculated as A/gs (μmol $\mathrm{mol^{-1}}$) show species significance in both metrics at UW and USU, but not OSU. Distinctions between both metrics of water use are observed between cultivars of *Physocarpus* at USU, and in WUE at UW, with higher values for *Physocarpus* 'Little Devil' across both sites. Species of *Cercis* from the UW site also displayed significant differences, with *Cercis canadensis* recording higher values than *C. occidentalis*. Irrigation treatment level significance is also observed at UW, but no individual species show treatment differences. Taxa at USU show consistently lower WUE than the other sites (Fig 7, Table 3).

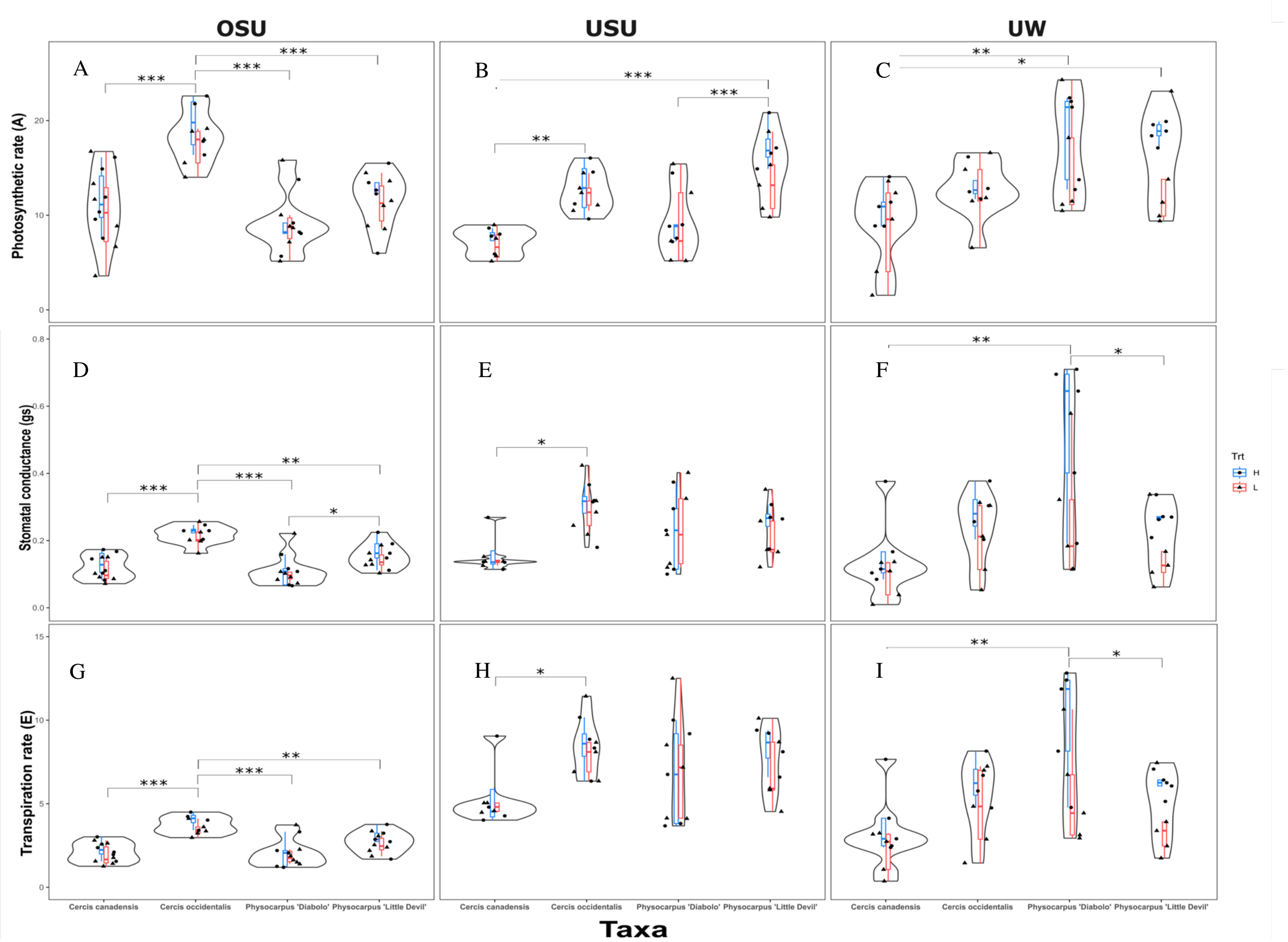

Figure 6. Full gas exchange measurements of net photosynthetic rate (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g$_s$, mol m^{-2} s^{-1}) as measured with a LI6400 or LI6800 at the end of the season across all sites with significance between taxa indicated (p<0.05) via brackets and stars.

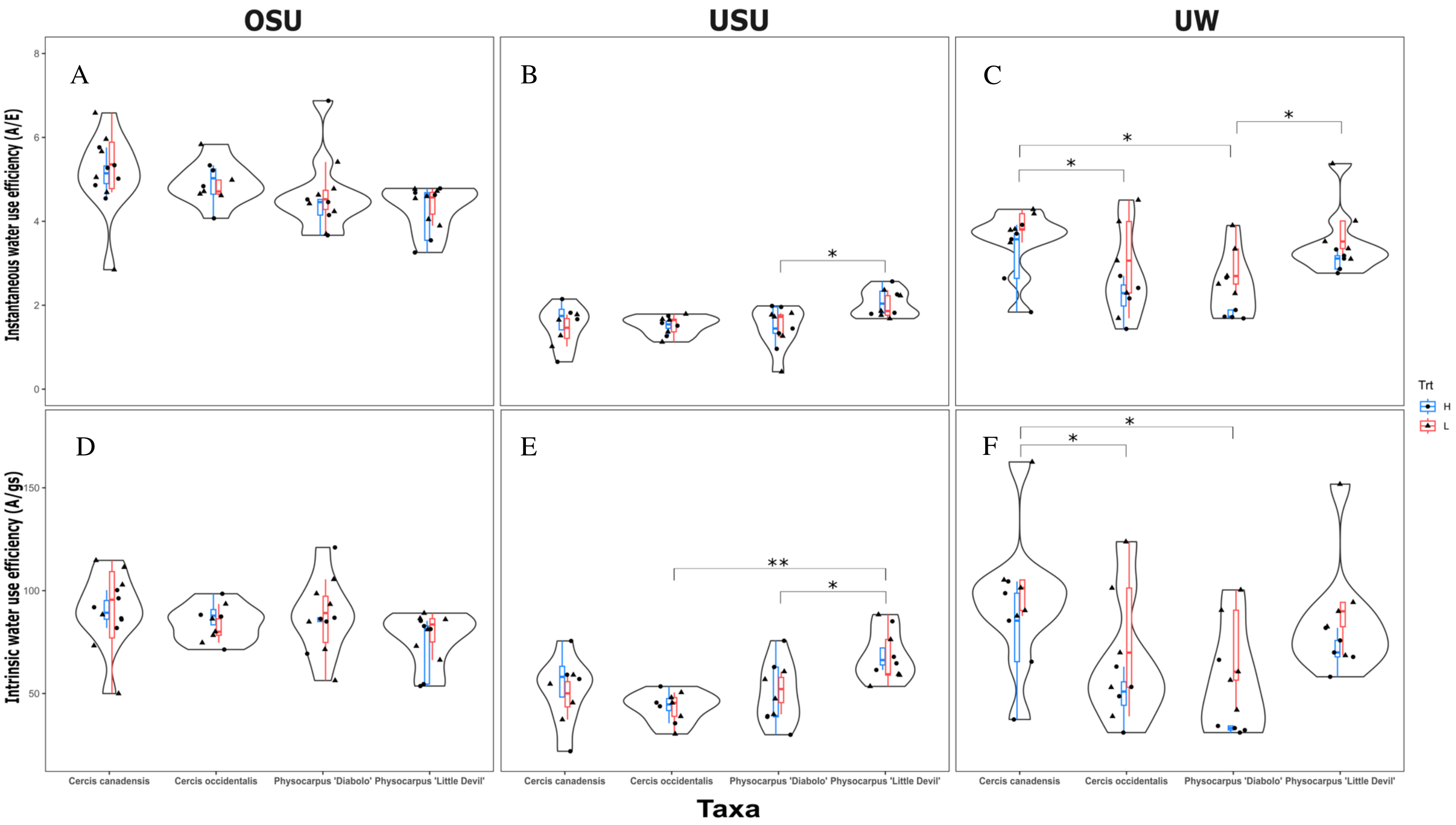

Figure 7. Instantaneous water use efficiency (WUE: A/E, mmol mol^{-1}) and intrinsic water use efficiency (iWUE: A/g_s, µmol mol^{-1}) calculated from full gas exchange measurements, with WUE (A/E), with significance between taxa at each site ($p<0.05$) indicated via brackets and stars.

Morphological traits between species

The three measured morphological traits in this study at the end of the growing season included two xylem traits: mean vessel hydraulic diameter (D_{mh}, μm) and theoretical specific conductivity (K_p, kg m $MPa^{-1}s^{-1}$), and one leaf trait: specific leaf area (SLA, cm^2/g). Morphological traits show species level significance across all sites, except for K_p at USU, with patterns between species differing across sites (Fig 8, Table 3). Significant treatment level differences are seen in *C. canadensis* SLA data at both the UW and USU site, with lower values of SLA for the low treatment indicating the formation of thicker leaves under the low irrigation deficit. Across all three sites, D_{mh} values for *Cercis* species are higher than those for both *Physocarpus* cultivars, with *Physocarpus* cultivar distinction seen at UW, with higher values of the xylem traits measured in *Physocarpus* 'Little Devil'. SLA displays strong species and cultivar significance at USU, while OSU plants show significance between all comparisons except the species of *Cercis*. Distinctions between species of *Cercis* are only observed between SLA at USU (Fig. 8H), with higher values for *C. canadensis* and at both USU and UW, SLA values for *C. canadensis* between high and low treatments are found to be significant ($p < 0.05$) (Fig. 8H, I). Across the three sites, at least one morphological trait shows distinctions between taxa pairs, *Physocarpus* cultivars showing differences in the xylem traits (K_p and D_{mh}) at UW (Fig. 8C , 7F) with higher values in *Physocarpus* 'Little Devil', SLA at USU with higher values in *Physocarpus* 'Diabolo' (Fig. 8H) and K_p and SLA at OSU, with higher values in *Physocarpus* 'Little Devil' (Fig. 8D and 8G).

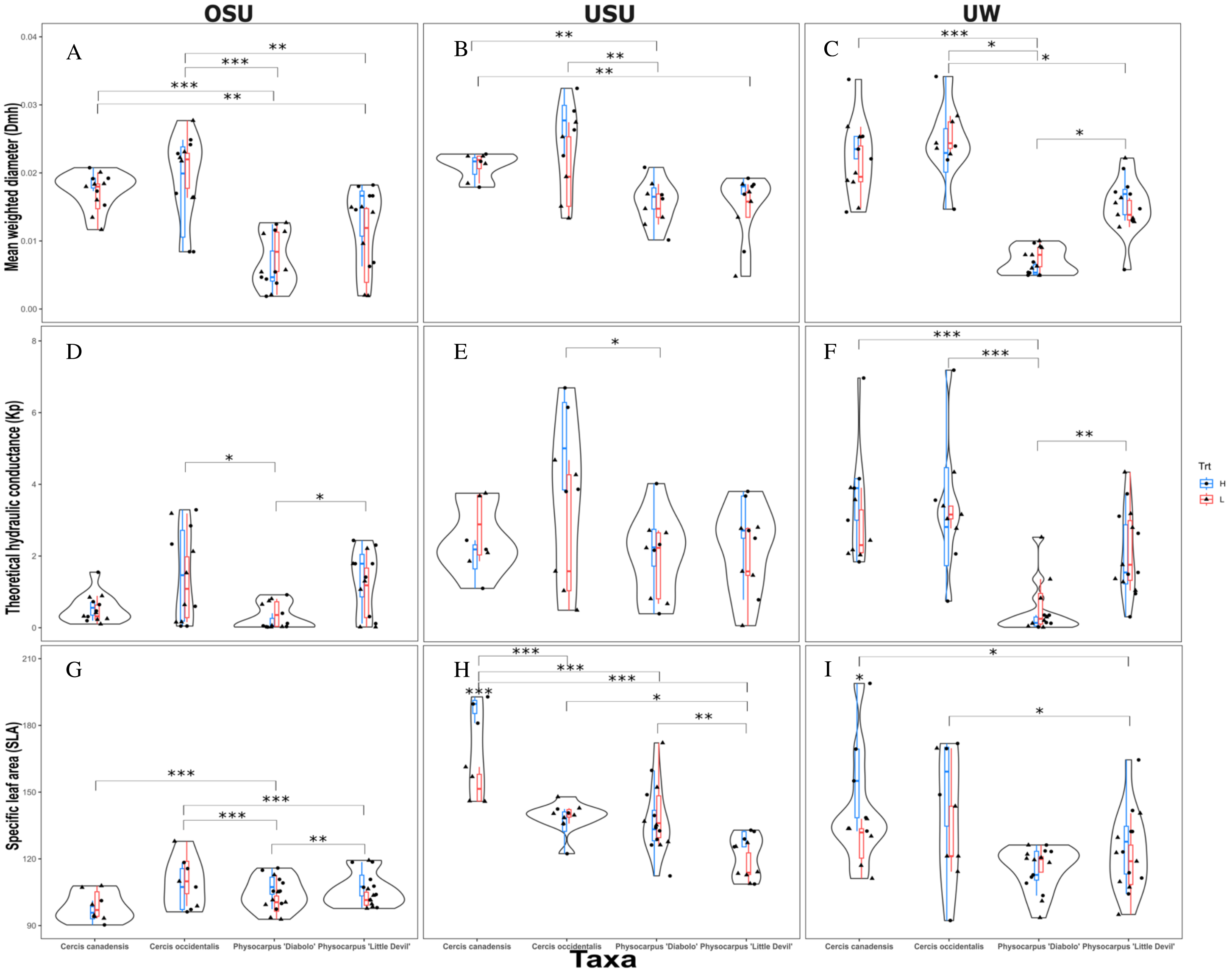

Figure 8. Mean weighted hydraulic diameter *(D_{mh}, μm) theoretical* hydraulic conductance ($K_{p,}$ $kg\ m\ MPa^{-1}s^{-1}$) and specific leaf area (SLA, cm^2/g) across all four taxa and three sites, with pairwise significance indicated between taxa, and between treatments ($p<0.05$).

Water use efficiency across sites

All taxa show significant differences in photosynthetic assimilation rate (A, µmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and instantaneous water use efficiency (WUE; A/E, mmol mol $^{-1}$) within sites, for most of the variables. Patterns of WUE are consistent across sites, with plants at USU consistently having lower values than those at UW and OSU, largely driven by higher transpiration rates at USU (Fig. 9). Both species of *Cercis* show different levels of WUE across all three sites (Fig. 9I, 9J), while both *Physocarpus* cultivars show similarity in WUE between two sites depending on cultivar, with *Physocarpus* 'Diabolo' preforming statistically similarly at OSU and UW, and *Physocarpus* 'Little Devil' performs similarly at OSU and UW (Fig 9K, 9L). *Physocarpus* 'Little Devil' shows no statistical difference in photosynthetic rate (A) across the sites. Across the sites, statistical differences in transpiration rate result in USU measuring significantly lower water use efficiency than OSU and UW in (Fig. 9I, J, K, L)

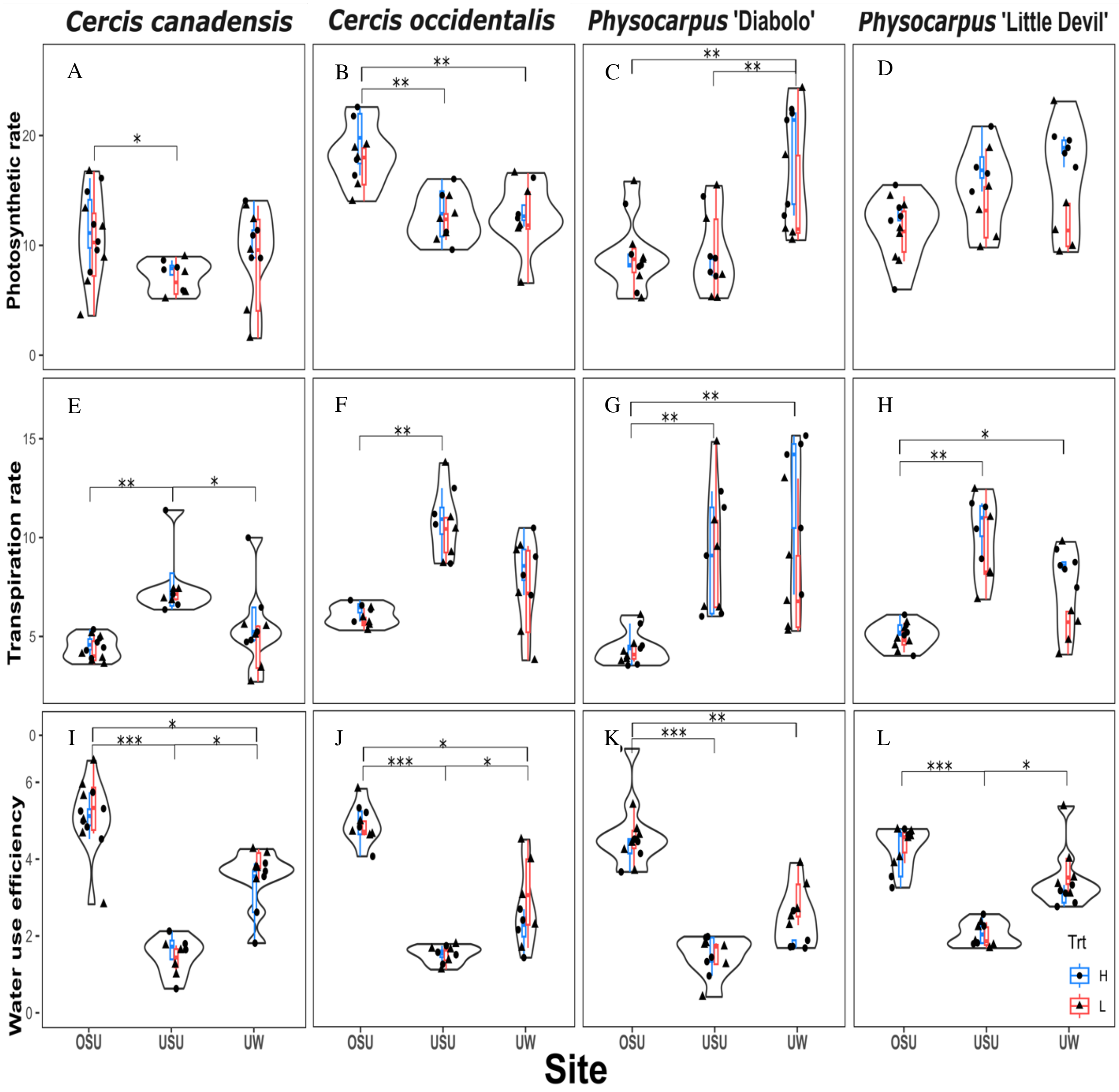

***Figure 9*.** **A, B, C, D.** Net photosynthetic assimilation (A, mmol m^{-2} s^{-1}), **E, F, G, H.** Transpiration rate (E, mmol m^{-2} s^{-1}), **I, J, K, L.** Instantaneous water use efficiency (A/E, mmol mol^{-1}) separated by taxa measured at each site. Brackets and * indicate level of statistical significance ($p<0.05$).

Effects of site, treatment and taxa on plant traits

Multivariate analyses were used to better understand the impact of site, as well as irrigation deficit and taxa on traits measured at all sites at the end of the growing season. Across all sites, all three main effects were found to be significant on all 10 traits measured at the end of the growing season: full gas exchange measurements (photosynthetic rate (Photo/A), conductance (Cond/g_s), transpiration (Trmmol/E), WUE and iWUE), stomatal conductance, Φ_{PSII}, SLA, and hydraulic traits (D_{mh} and K_p). The main effects of site ($p < 0.001$) accounted for 32.2% of the variation in the dataset, taxa ($p < 0.001$) accounted for 11.2% of variation, and irrigation treatment ($p = 0.004$), accounts for 2.4% of variation. The interactions between irrigation treatment and site ($p < 0.001$) and taxa and site ($p < 0.001$) were also significant, indicating that taxa significance is dependent on site location (Table 4). Pairwise contrasts showed significance between irrigation treatment at the UW site (p: 0.007) but note at OSU or USU. Taxa pairwise contrasts showd significant differences ($p < 0.05$) among all taxa at and within all sites, except for Physocarpus 'Little Devil' which is not significantly different across any sites.

Table 4. RRPP ANOVA results analyzing the impact of site, irrigation treatment (trt), taxa, and their interactions on the core traits (full gas exchange measurements (photosynthetic rate (A, μmol m^{-2} s^{-1}), conductance (Cond, mol m^{-2} s^{-1}), transpiration (Trmmol, mmol m^{-2} s^{-1}), WUE (mmol mol^{-1}), iWUE (μmol mol^{-1}), stomatal conductance (gsw, mol m^{-2} s^{-1}), Φ_{PSII}, SLA (cm^2/g), and hydraulic traits (D_{mh} (μm) and K_p (kg m $MPa^{-1}s^{-1}$)), measured across all sites at the end of the season. Bolding indicates statistical significance.

	Trt	Taxa	Site	Trt:taxa	Trt:site	Taxa:site	Trt:taxa:site
Rsq	0.024	0.112	0.322	0.014	0.063	0.148	0.031
p	**0.004**	**<0.001**	**<0.001**	0.323	**<0.001**	**<0.001**	0.220

Using a PCA for data reduction, the first two axes represented 67.3% of variation across two axes (PC1: 42.2%, PC2: 25.1%) and showed visual distinction among sites, with OSU and USU remaining fairly distinct from one another, and UW overlapping with both and over a broader area (Fig. 10A). Species level responses also differed across sites (Fig. 10B) indicating differing water use strategies by taxa across the sites represented in this study. PC1 is interpreted to be associated with water use efficiency and photosynthetic water traits (stomatal conductance and transpiration) while PC2 is interpreted to indicate morphological traits and growth, through the association with net photosynthetic rate. When loadings are separated out into site, distinction between and overlaps by taxa groups (species of *Cercis* and cultivars of *Physocarpus)* are observed, indicating more similar performance by phylogenetically similar taxa.

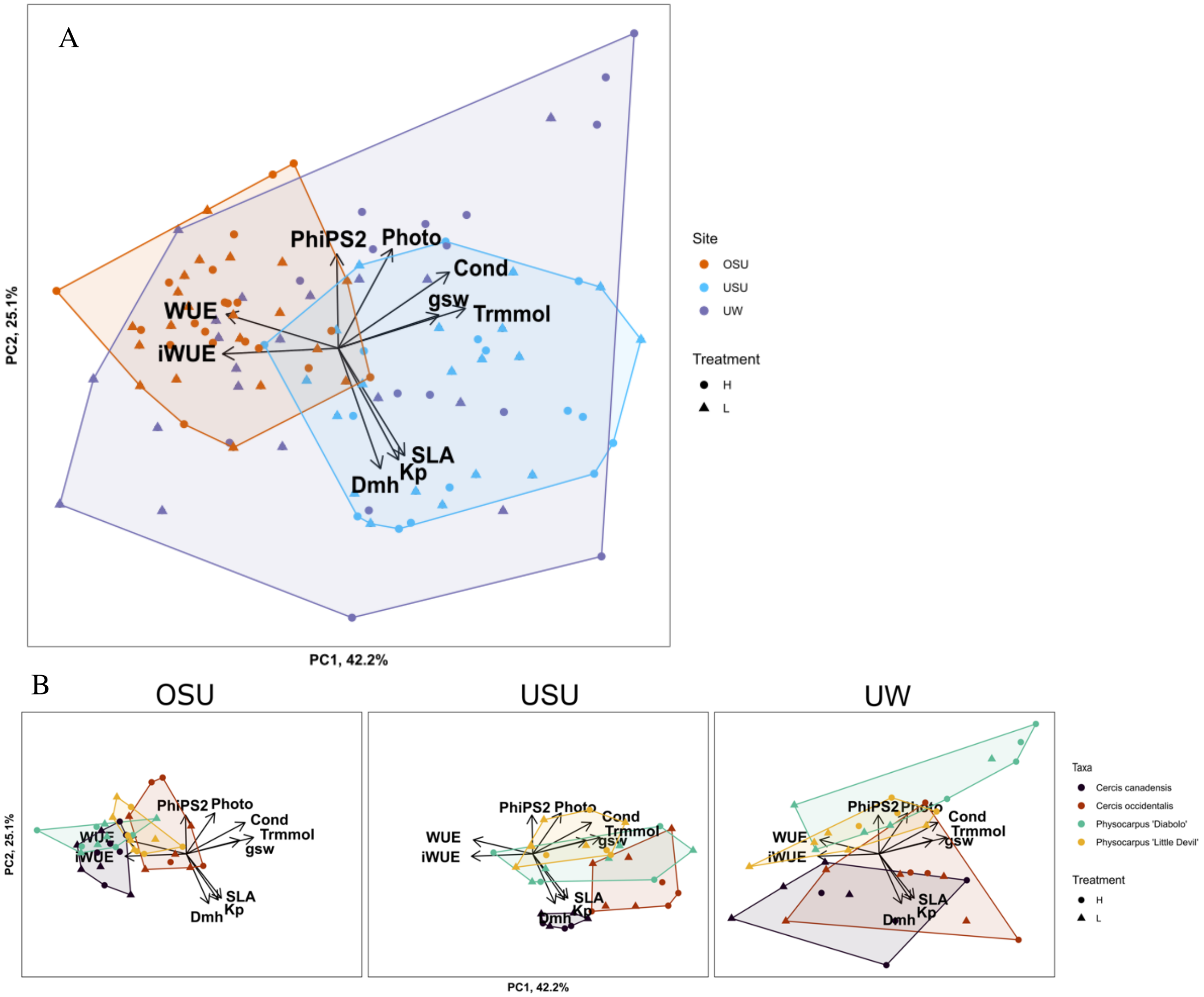

***Figure 10*.** **A**. Principal Component Analysis (PCA) of correlation between core traits measured at the end of the growing season. Hulls indicate differentiation between site identity. 67.3% of variation in the dataset is represented across two axes; PC1: 42.2%, PC2: 25.1%. **B.** The same PCA loadings separated out to each respective site, with hulls indicating individual taxa and their distribution by site.

SLA across the growing season between sites

Shifts in specific leaf area (SLA) from the beginning and end of the growing season result in at least one species at each site showing significant change between the time points, indicating morphologic shifts since the initiation of the irrigation treatments. Time of season was found to be significant ($p < 0.001$) and significant decreases in SLA between time points was observed at OSU with both *C. canadensis* and *Physocarpus* 'Diabolo' ($p < 0.001$), at USU with *C. occidentalis* ($p < 0.05$), and at UW with *Physocarpus* 'Diabolo' ($p < 0.001$) (Fig. 11). Significance between irrigation treatments at the end of the season are observed for *Cercis canadensis* at both the OSU and UW site; individuals that received the low irrigation treatment had lower values of SLA than those receiving high irrigation treatment (Fig. 11E, I). Due to late leaf out, a limited number of *C. canadensis* were collected at USU at the beginning of the season ($n = 4$ compared to $n = 12$ from UW and $n = 10$ from OSU)

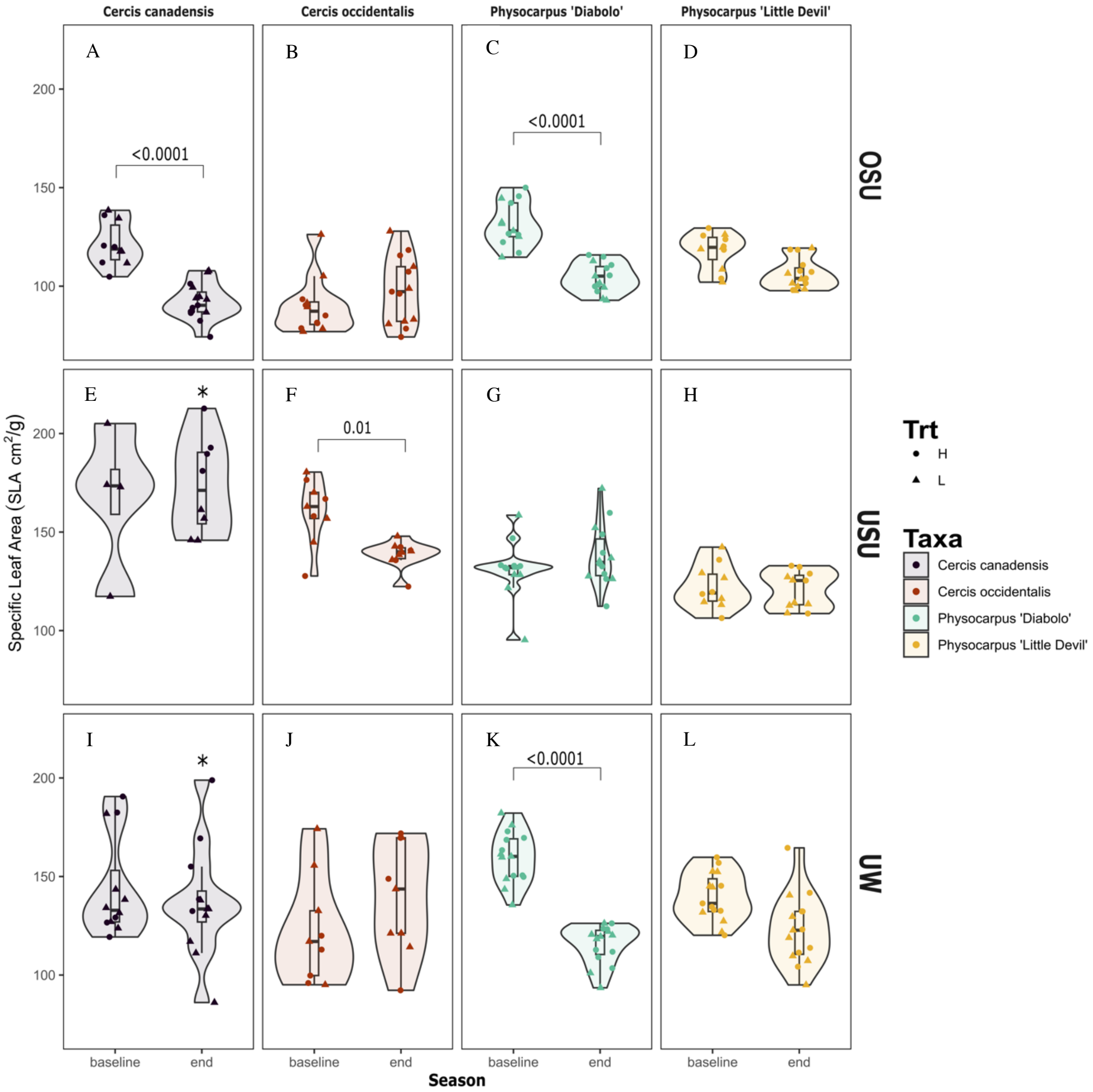

Figure 11. Specific leaf area (cm^2/g) changes across the growing season at all three sites for all 4 taxa. Recent, fully expanded leaves were collected at each time point. Significant change across time points is indicated with brackets and p-value, and treatment level significance at the end of the growing season is indicated with *.

UW physiology across the growing season

In order to assess physiological acclimation across the growing season, measurements of midday stomatal conductance (g_{sw}) and Φ_{PSII} were collected at three time points at the UW site, beginning (prior to initiation of the irrigation deficit treatment), middle (midway through the growing season) and at the end of the growing season. g_{sw} data show significant shifts between time point of measurement ($p < 0.002$). Baseline measurements of stomatal conductance (g_{sw}) show significant differences between taxa ($p < 0.001$) but not irrigation treatments. *Cercis occidentalis* measured irrigation treatment level differences at the mid-season measurement ($p < 0.05$) and *Physocarpus* 'Diabolo' shows treatment level differences at both the middle and end of the season timepoints ($p < 0.05$) (Fig. 12). In all cases, significant irrigation treatment level responses results in a decrease in g_{sw} under the low irrigation treatment. Across seasonal measurement points, *C. canadensis* decreases g_{sw} between the middle and end of the growing season ($p < 0.05$, Fig. 12A), and *Physocarpus* 'Little Devil' decreases g_{sw} between each of the measurement points ($p < 0.05$, Fig. 12D).

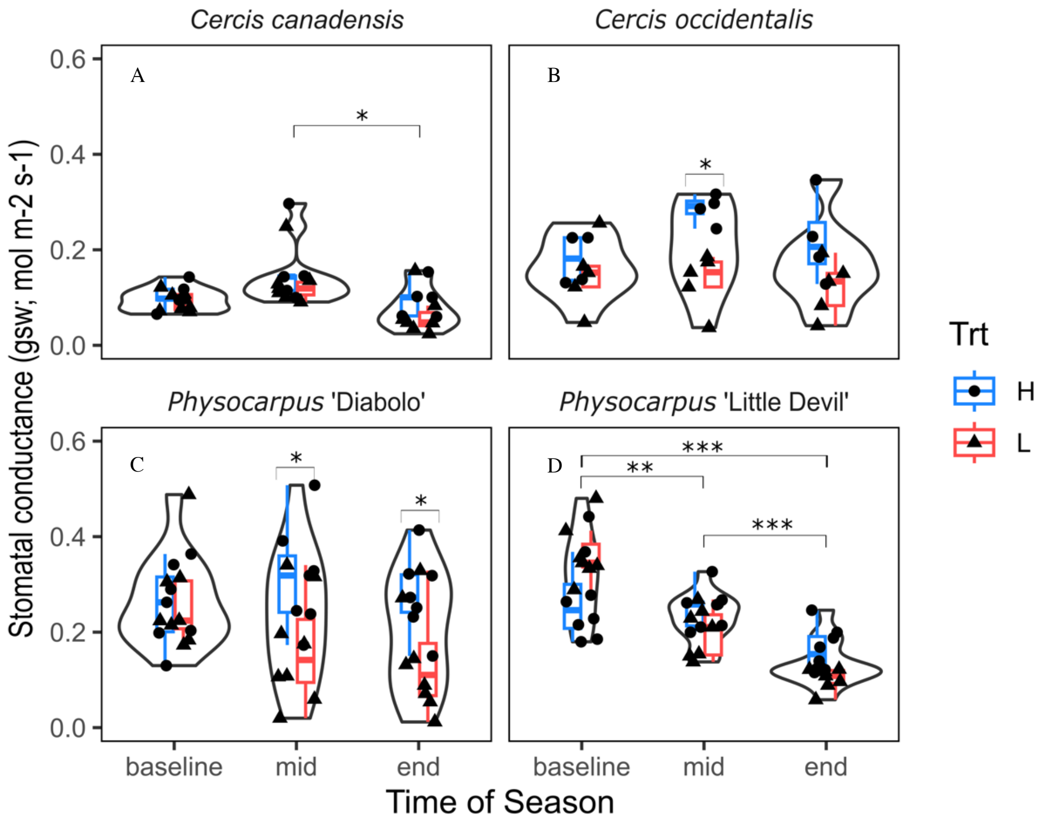

Figure 12. Measurements of stomatal conductance at three time points across the growing season. Baseline: prior to initiation of growing season, mid: mid-season, and end: end of the growing season. Brackets and * above violins indicate significance between irrigation, brackets and * between violins indicate significance level between timepoints.

Results from the PERMANOVA on stomatal conductance and Φ_{PSII} with permutations restricted with individual plant position as a blocking term reveal a significant impact of season ($p < 0.001$), as well as the interaction between season and taxa ($p = 0.003$) on the measured traits. After accounting for the variation explained by position, season accounted for 12.4% of the variation in this dataset (Table 5). A two-dimensional NMDS showed both overlap as well as distinction between points when colored by season, as well as the placement of the data cloud being driven by irrigation treatment (Fig. 13). Points associated with the baseline measurements before initiation of the irrigation treatment overlap almost completely with those measured in the middle of the season, with the bulk of the variation observed between the middle of the growing season and then end of the growing season (Fig. 13).

Table 5. PERMANOVA table from traits measured at three separate time points across the growing season, with plant position as a blocking term in order to assess the impact of time on the measurements, showing R^2 and p-values for the impact of individual position, time of season, and their interaction.

	position	season	interaction
R^2	0.62	0.124	0.04
p	**< 0.0001**	**< 0.0001**	**0.003**

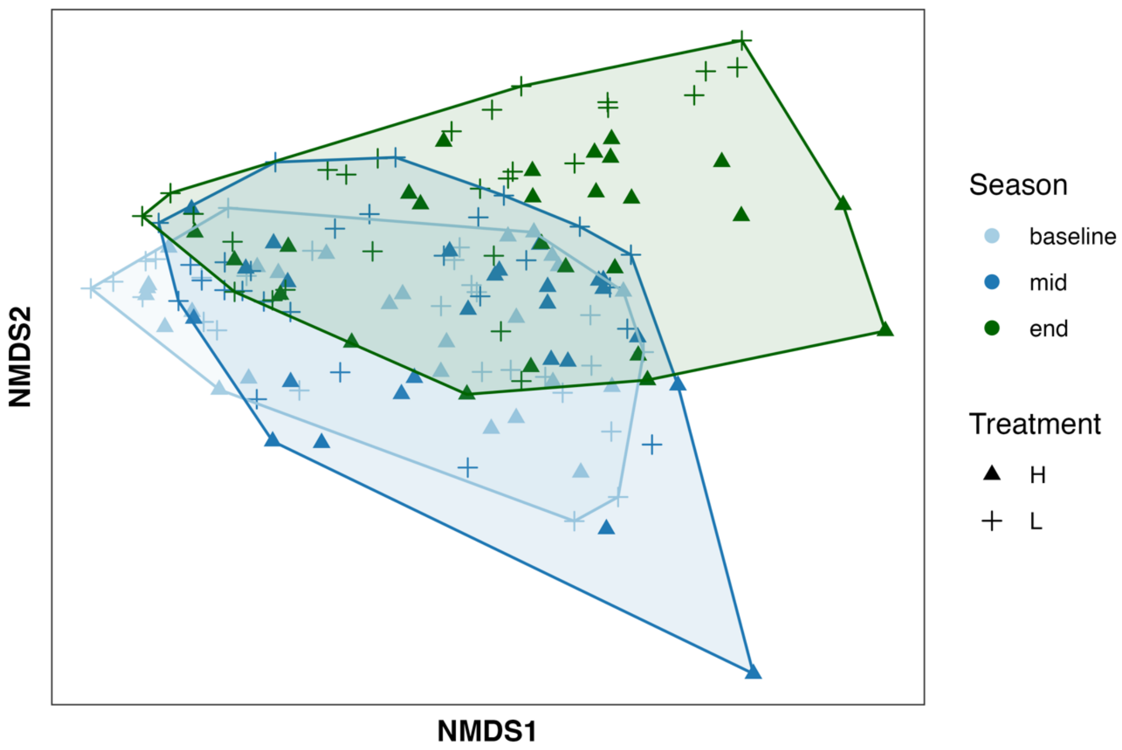

Figure 13. Two-dimensional NMDS orientation visualizing grouping of physiological traits (stomatal conductance and Φ_{PSII}) for UW plants across three time points in the growing season. Hulls represent season, and symbols represent treatment. Stress = 0*.485e-10.*

UW taxa physiology

Results of a multivariate ANOVA (via RRPP) on the UW data with additional traits (predawn stomatal conductance (gsw_PD), predawn Fv/Fm (Fv/Fm), and predawn (WP_PD) and midday water potential (WP_MD)) showed a significance both of irrigation treatment (p = 0.009) and taxa (p = 0.002), accounting for 10.3% and 21.2% of the variation in the dataset respectively (Table 6). Species pairwise comparisons showed significant differences between species of *Cercis* (p = 0.049), cultivars of *Physocarpus* (p = 0.038), and all individual species comparisons, except *C. occidentalis* and *Physocarpus* 'Diabolo' (p = 0.06). Pairwise comparisons at the genera level show that *Physocarpus* cultivars differ significantly from species of *Cercis* (p = 0.001) (Fig. 14).

Table 6. RRPP ANOVA results analyzing the impact of treatment, taxa, and their interactions on the expanded traits measured at the UW site at the end of season, with type III sums of squares. Bolding indicates statistical significance.

	Treatment	Taxa	Interaction
Rsq	0.103	0.212	0.036
p	**0.009**	**0.002**	0.673

When visualized on a PCA, 68.3% of the variation in the dataset was represented across two axes (PC1: 45.4, PC2: 22.9%), with PC1 interpreted to show physiological traits, primarily gas exchange, while PC2 shows morphological traits. Fluorescence traits (Fv/Fm and ΦPSII) show close correlation with one another, and negative correlation with morphological traits (SLA, Dmh, Kp) and midday water potential (WP_MD), while measures of water use efficiency (iWUE and WUE) show negative correlation with measurements of gas exchange. Clear distinctions between taxa groups (species of *Cercis* and cultivars of *Physocarpus)* appear via PCA (Fig. 14).

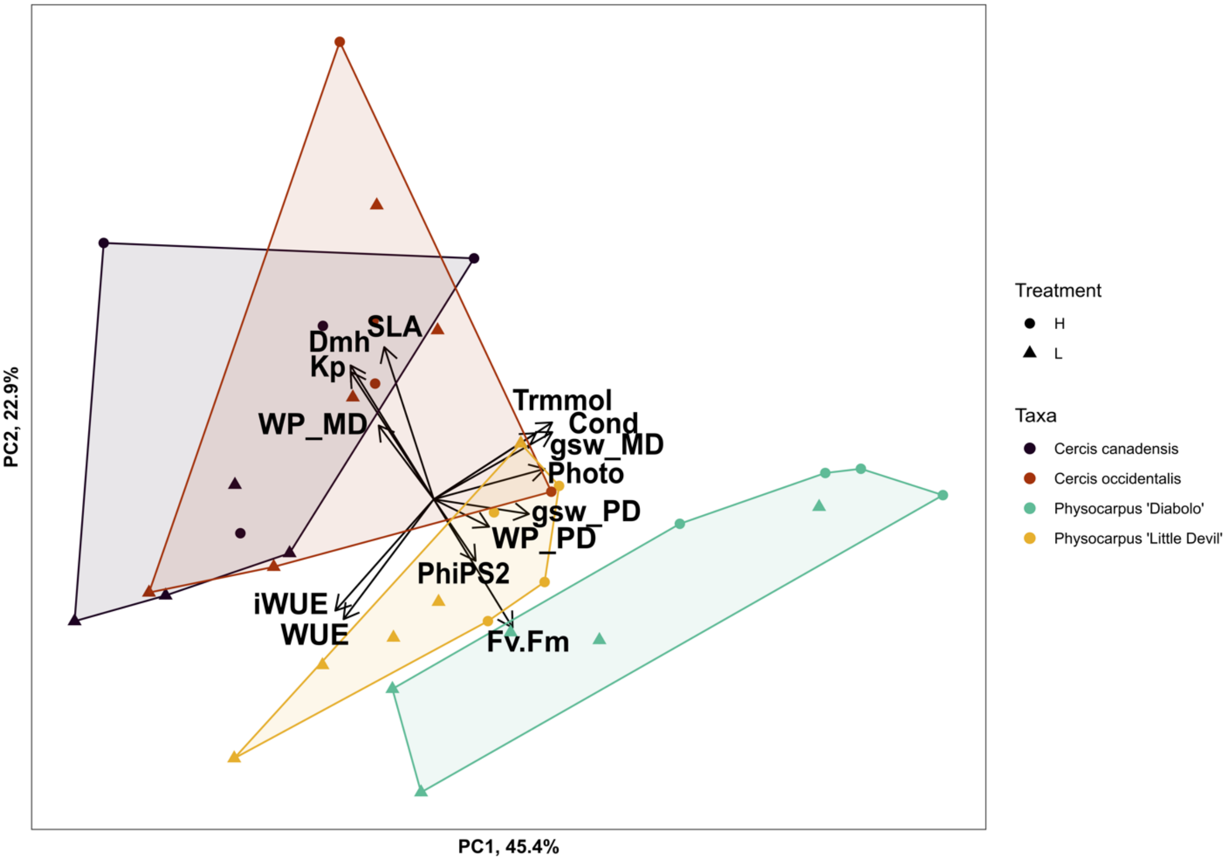

Figure 14. Principle component analysis of traits measured at UW at the end of the growing season, with 68.3.1% of the variation represented across two axes (PC1: 45.4, PC2: 22.9). Hulls color represents the four taxa represented in the dataset, and indicates clear distinction between genera.

Fv/Fm and diurnal water potential results were analyzed univariately for the effects of taxa and irrigation treatment. For Fv/Fm, there was a strong species level significance ($p < 0.001$), but no effect of irrigation treatment impact. Pairwise comparisons showed significant distinctions between each species, with the following species means: *C. canadensis* = 0.747*, C. occidentalis* = 0.771, *Physocarpus* 'Diabolo' = 0.845, *Physocarpus* 'Little Devil = 0.828 (Fig. 15).

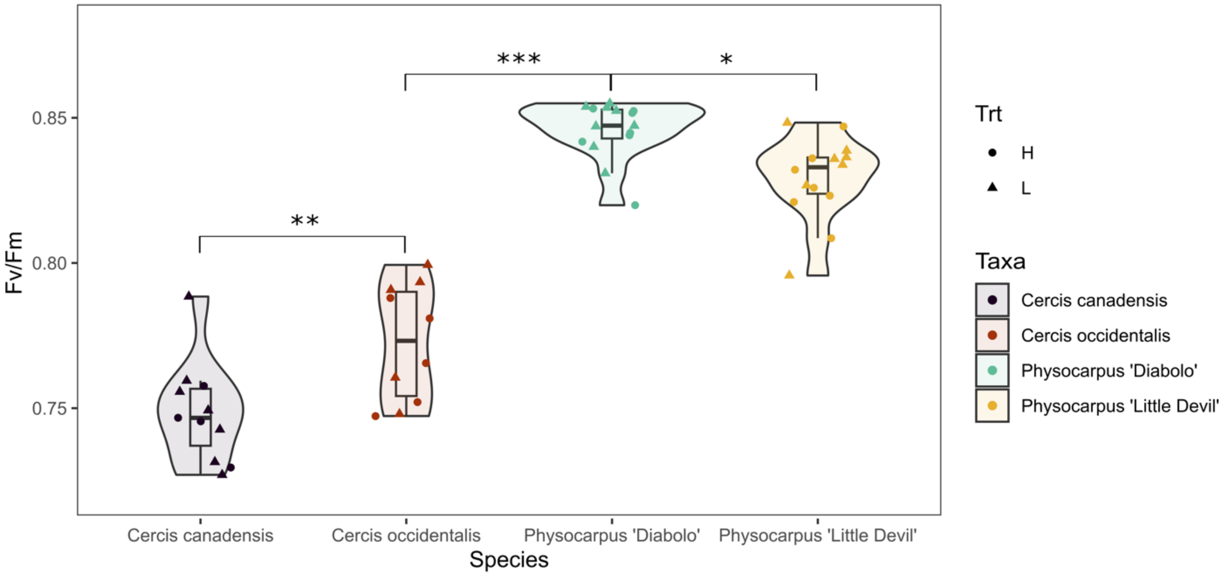

Figure 15. End of season measurements of Fv/Fm across the four species at UW, showing significant differences between taxa, and no significance of treatment level.

There was no effect of irrigation treatment on diurnal water potential at the UW site, but both time points show taxa level significance (predawn: p = 0.03, midday: p < 0.001; Fig. 15). The predawn time point showed no significant differences between species, while at midday pairwise *Physocarpus* ‘Diabolo’ had significantly lower water potentials than the other three taxa (Fig. 16).

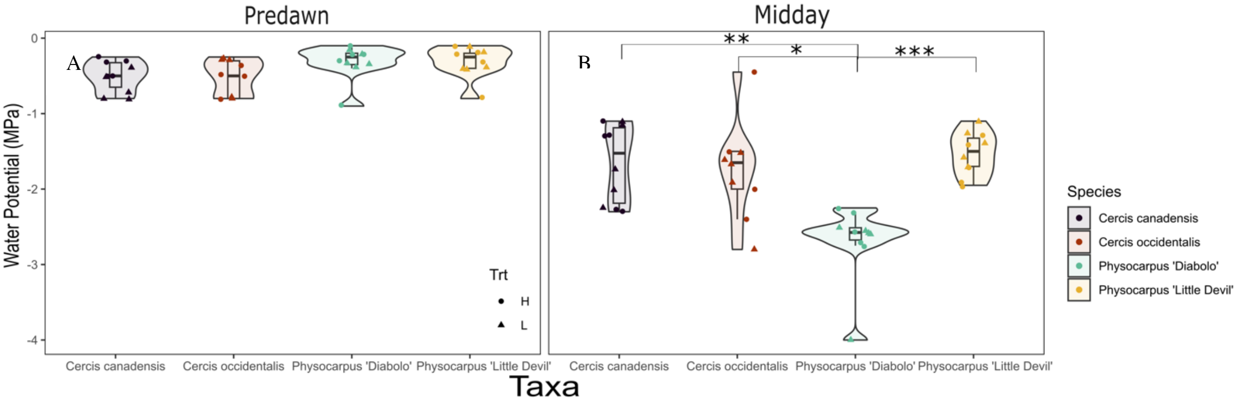

Figure 16. **A**. Predawn and B. midday water potential measurements at the end of the growing season at UW. Predawn values show no significant pairwise differences between taxa, while at midday, Physocarpus ‘Diabolo’ is significantly different from the other three species (p<0.002).

Discussion

In this study I evaluated distinctions in plant performance for the same taxa across three distinct sites (UW, OSU and USU), reported measurable differences between pairs of closely related taxa, and recorded an observable shift in physiological performance over the course of the growing season. Taxa identity was a primary source of the variation in the dataset at a site dependent level, with some shifts in response to irrigation deficit treatment also observed. While previous research as documented shifts in plant ecophysiological shifts across climates, many of those use common garden approaches where the same taxa from multiple locations are grown in a shared location, or measurements in native environments (Berend et al., 2019; Ebeling et al., 2011; Sáenz-Romero et al., 2017). This study demonstrates the utility of a shared-taxa study across different climatic sites and evaluates the impact of controlled irrigation treatments. Additionally, this work explores the less clear ability of individual plants to demonstrate plastic responses in these traits across acute shifts in environmental conditions. By measuring plant traits on individual plants through the course of a growing season across three sites with distinct climate and edaphic characteristics, we demonstrate that traits such as SLA, stomatal conductance (g_{sw}) and Φ_{PSII} are plastic across short timescales.

In regard to aim 1, individual taxa responded differently across the suite of measured traits depending on site. Distinctions between the closely related species of *Cercis* and cultivars of *Physocarpus* were observed across all sites from end of season g_{sw} measurements (Fig. 4A, B, C), and inconsistently across other traits between the sites (Figs. 4, 5, 6, 7). Strong trends in WUE between sites showed lower values at the USU site (Figs. 7, 8) which could support the hypothesis that plants at that site would be better acclimated to withstand drought conditions due to previously experiencing significant freezing temperatures the winter prior to irrigation deficit.

However, USU also experienced the highest amount of precipitation and cumulative ET_0 during the deficit period which could influence the recorded metrics for water use efficiency (Table 1). It was hypothesized that the cultivars of *Physocarpus* would show lower values of WUE than the species of *Cercis* due to horticultural selection; however, no significant trend was observed across any sites (Fig. 6). Kaproth et al., 2023 reported that winter conditions affect SLA; however, in this study OSU, which has a more temperate climate than USU, had the lowest values of SLA (Fig. 7). Lower values of SLA at OSU could also indicate higher water use efficiency, which is supported by higher values of WUE per gas exchanges measurements (Fig. 6).

Contrary to my hypotheses that the low irrigation deficit treatment would result in an increased WUE and more conservative water use strategy, few irrigation level distinctions were observed from individual measured traits. Treatment level differences that exist come from measurements of g_{sw} and SLA. Stomata have previously been shown to be one of the main sites of physiological regulation on short timescales due to their role in water transport regulation. Thus, distinction in g_{sw} could indicate differing safety margins by each taxon in the face of decreased water availability and summertime conditions (Martin-StPaul et al., 2017, Martínez-Vilalta et al., 2014). Lower values of SLA have been previously associated with lower water availability or amount of precipitation (Gouveia & Freitas, 2009; Harrison & LaForgia, 2019; Kaproth et al., 2023; Liu et al., 2017). In this study *C. canadensis* decreases SLA under the low treatment at USU and UW (Fig. 7H, I). Despite significant shifts in SLA for *C. canadensis* indicating a faster growth rate and adjustment to leaf light capture potential, there is no measured adjustment in photosynthetic variables (Fig. 5), indicating plasticity to the water deficit to maintain normal performance (Gratani, 2014).

Investigations into xylem morphology were undertaken to see if the observed decrease in stomatal conductance due to irrigation treatments would manifest in xylem morphological adjustments. Contrary to my hypotheses, no significant effect of the irrigation treatment was observed across any taxa or site; however, trends in closely related taxa were observed (Fig. 7). Previous research in hydraulic function presents a range of results in terms of relationship between and plasticity of xylem anatomy and water availability, including recent work across climatic gradients and irrigation treatments which also fail to find a relationship between water availability and xylem morphology (Ramesha et al., 2022; Yang et al., 2020; Y. Zhang et al., 2023). Work in oak species across a climatic gradient concluded that wood anatomic traits show limited plasticity and mainly respond to environmental conditions (Fontes et al., 2022), supporting the results here that a growing season of irrigation deficit does not result in shifts in xylem morphology by treatment, and that plants are adjusting other traits in order to survive water deficit. Previous work has also shown species from more humid areas to have a broader range of D_{mh} values, which were observed in our data at the UW and USU sites (Fig 7B) (Hacke et al., 2017).

Based on the clear site influence on univariate traits, considering all overlapping traits together via multivariate analyses, PCA results shows OSU and USU to be mostly distinct from one another, while UW overlaps some with USU, some with OSU and is unique from both of them (Fig. 9A). Overlap between UW and OSU is logical due to similar precipitation patterns and climatic conditions (Fig. 1), yet USU is characterized by significantly more freezing temperatures, and both OSU and USU have warmer summers than UW (Table 1, Fig. 2). Outside of climatic factors, differences between site identity can be seen in soil type and soil water storage capacity with USU and OSU both having soil of silt loam and similar available water storage, while the loamy sand soil at the UW results in a large reduction in available water storage decreases (Table 1), which is supported by research in on these soil types (Shwetha & Varija, 2015). Lower water storage capacity has been shown to result in lower nutrient content and soil nutrient availability has also been shown to impact hydraulic traits (Gadermaier et al., 2024; Ramesha et al., 2022). Additional site level considerations result from site installation differences: the UW site was a grass field prior to plot installation while OSU and USU sites were installed at preestablished research stations, resulting in higher quality and less variable plot conditions. Some variation in trait response across sites could also driven by temperature, since acclimation rates of photosynthetic rate and transpiration can differ depending on temperature (Campbell et al., 2007). The distinct site responses point to the validity of a cross-site comparison to better understand plant response and plasticity across environmental zones. Significance in deficit treatment levels was only observed at UW and USU, despite all three sites having the same set up for irrigation management including plot spacing and irrigation drip rings. OSU elected to not top dress their plot with mulch while the other sites did; characteristics of the study design which should be considered in the future.

In response to aim 2, this study presents interesting results in terms of observing over the course of three-month growing season, shifts in plant performance and response. SLA measurements across all species at all sites at the beginning and end of the growing season result in decreased SLA across the season by each taxon at a minimum of one of the sites, except for *Physocarpus* ‘Little Devil’ (Fig. 10). Similarly, by measuring both g_{sw} and Φ_{PSII} at multiple points across the growing season at UW (beginning, middle and end), we likely observed plasticity to summer hot and dry conditions, since the response was driven by both irrigation deficit treatments. Unlike SLA however, *Physocarpus* ‘Little Devil’ shows the largest decreases in g_{sw}

and significance between time points (Fig. 11), indicating the taxon is likely adapted to maintain leaf size but control stomatal conductance; a strategy adapted for occasional water deficit. When visualized via NMDS, the most separation in response occurs between the middle of the season measurement and the end of the season measurement (Fig. 12). An approach such as this can couple response to both decreasing water availability and warmer temperatures projected under climate change. Some previous work on thermotolerance acclimation over the course of the growing season exhibits a bell curve with highest tolerance at the midpoint, while drought stress tolerance in deciduous plants peaks at the end of the growing season (Grossman, 2023). Our results, with the end of the season being the more distinct time point from baseline, better match with the water deficit tolerance patterns despite a lack of irrigation treatment level driving the shift in traits. A better understanding of the temporal scale of plasticity and acclimation can inform plant tolerance levels to hotter and dried conditions, with application to cut down on water use in agricultural, horticultural and other settings. While this aim is limited by the small number of traits collected, Φ_{PSII} is considered a good trait to measure for change over time (Maxwell & Johnson, 2000), and the LI-600 allows for easy and rapid collection of this trait in a field-based setting when compared to full gas exchange measurements. Considering acclimation to stressors has additionally been shown to be important for more accurate modeling photosynthetic response to climatic change, and has not been well studied (Fang et al., 2023), this temporal scale of acclimation presents a knowledge gap in ecophysiology. While we were limited by only collecting a few time points, these results demonstrate the utility of study systems such as this to fill this existing knowledge gap (Grossman, 2023). Future work should evaluate an increased number of plant traits across the growing season, or if possible, across multiple growing seasons, to better understand temporal acclimation. Measuring the degree of plastic response across multiple seasons of water deficit treatment could ascertain if acclimation occurred and is maintained across the winter season, which could allow plants to better respond to variable environmental conditions. Investigating if trends in water use are distinguishable between levels of irrigation treatments and taxa could inform plant capacity to acclimate to more variable water availability under climate change.

Additional traits measured at the UW site help better characterize the four taxa in this study, in response to aim 3. Multivariate analyses across the expanded suite of traits from the UW site better clarify distinctions between taxa. Not only is a clear distinction observed between genera, with observed traits of *C. occidentalis* and *C. canadensis* being distinct from those of *Physocarpus* ‘Diabolo’ and *Physocarpus* ‘Little Devil’, but additional distinction between species and cultivars can be observed (Fig. 13). This is an especially pertinent result for the *Physocarpus* cultivars since they are genetically identical to the species level, but supports other recent work that closely related cultivars can exhibit measurable differences in water use. Sjöman et al., (2023) demonstrated different rates of turgor loss point (a metric for full plant drought tolerance) among different cultivars of *Spirea*, suggesting that selections for horticultural aesthetical appeal could result in decreased water use efficiency. Additional distinctions among cultivars in terms of stomatal control has also been reported by Tombesi et al., 2014. A future study could compare additionally among wildtype *P. opulifolius* and cultivars. Additionally, this work presents distinct performance between species of *Cercis,* supporting previous work by Sternberg (2012) which found differing but not consistent water-use strategies across taxa. In the PCA for the UW site, both species of *Cercis* were associated with measures of water use efficiency. When looking only at WUE across sites (Fig. 7), the WUE and iWUE of the two species of *Cercis* are similar, except for at the UW site, and both *Physocarpus* present statistically different WUE across the sites (Fig. 8). These findings are in opposition to our hypothesis that *C. occidentalis* would have higher WUE given the taxon has a native distributional range west of the Rocky Mountains, and is considered adapted to the dry, Mediterranean summers that characterize the West.

Additional traits collected at the end of the season at the UW site help to better characterize the individual species and their responses to the irrigation deficit. Plants have evolved differing levels of regulation under water deficit and leaf water potential is tied to stomatal regulation (Kaproth et al., 2023; Knipfer et al., 2020). *Physocarpus* 'Diabolo' shows very low midday leaf water potential compared to the other species and also lower stomatal conductance, meaning it exhibits anisohydry, while the other three taxa have a lower midday leaf water potential and are more isohydric (Knipfer et al., 2020; Sade et al., 2012). Isohydric species are considered less responsive to environmental change (Martínez-Vilalta et al., 2014); however, plants with broader ranges of water potential have been shown to have a more plastic response to modifying leaf hydraulic parameters (Johnson et al., 2018). *Physocarpus* 'Diabolo' also has lower values of K_p and D_{mh}, which has been used as a metric for hydraulic safety-efficiency tradeoff, and an indicator of increased drought tolerance (Fontes et al., 2022). Similarly, measurements of Fv/Fm were not affected by irrigation deficit, which would be expected had the treatments resulted in altered photosynthetic efficiency. The lower values of Fv/Fm for the species of *Cercis* likely indicate a degree of stress and photoinhibitory damage due to site conditions or summer climate, as opposed to irrigation treatments (Fig. 14).

Multivariate analyses when visualized via PCA for data reduction explore interesting patterns in terms of trait response and plasticity (Figs. 9, 13). Traits that contribute to photosynthetic rate drive a lot of the variation observed among sites. Based on the results from

PC1 across all sites (Fig. 9) and UW (Fig. 13) the measured traits such as stomatal conductance and transpiration that contribute to both photosynthetic rate and measures of water use efficiency drive a large percentage of the variation in measured traits. When considering sites, the measures of WUE drive a lot of the variation in data from OSU, while SLA and transpiration rate drive USU data. The trends for UW are less clear, with points encompassing most measured traits except for SLA (Fig. 9B). Based on the PCA results, a negative correlation is observed between morphological traits (SLA, K_p, D_{mh}) and measurements of chlorophyll fluorescence (both Φ_{PSII} and Fv/Fm). This pattern is seen in PCAs across all sites and UW alone (Fig. 9, Fig. 13.) where the vectors indicate negative correlation between these traits along PC2. Lower values of SLA indicate thicker leaves, so this indicates correlation in the dataset between leaf thickness and photosynthetic productivity. SLA has previously been shown to respond to environmental variables at regional scales, and additionally but less strongly with responses to nutrient levels, meaning the association seen at UW and USU but not OSU could reflect soil nutrient level, likely nitrogen (Liu et al., 2017). SLA has also been shown to respond on the genera level prompting future investigation into taxa level responses, and if patterns can be seen across sites. Higher values of SLA are considered associated with less drought tolerant, or faster growing plants, two possible explanations for the association with the USU site (Harrison & LaForgia, 2019). Surprisingly, among the traits found to be significant in a univariate analysis of the effect of irrigation deficit level, g_{sw} and SLA were uncorrelated (perpendicular) to one another. The measured traits for chlorophyll fluorescence point to rate of photosynthetic productivity. Φ_{PSII} is a measure of the rate of electron transfer, and a measure of overall photosynthetic rate, whereas Fv/Fm is a measure of altered photosynthetic efficiency, and a measure of plant stress (Maxwell & Johnson, 2000). Leaf thickness has been hypothesized to be one of the factors determining chlorophyll content, and the negative correlation observed through this study supports that hypothesis (H. Zhang et al., 2022). These results also indicate that SLA could be an indicator of water use efficiency, which is supported by previous research where lower SLA is expected to enhance water use efficiency and increased photosynthetic capacity (Gratani, 2014; Harrison & LaForgia, 2019). Future work could further investigate if this correlation between leaf morphology, measures of chlorophyll content and WUE persists across taxa and geographic locations.

The cultivar of *Physocarpus* 'Little Devil' presents interesting results in terms of plasticity throughout this study. When accessed across all sites, the cultivar does not show distinction in performance at the site level. WUE metrics also show *Physocarpus* 'Little Devil' to be more similar across the sites than the other taxa (Fig. 8), and SLA does not shift across the growing season (Fig. 10), supporting other recent findings that horticultural selection for aesthetics decrease plasticity and water use efficiency (Sjöman et al., 2023). However, in the across the growing season analysis, *Physocarpus* 'Little Devil' shows the most significant shifts between time points, indicating a significant degree of plasticity across the growing season. This taxon has the smallest leaves of the four studied here, thus potentially able to more easily respond to environmental shifts due to less investment in producing each individual leaf. Given the lack of difference in physiological and morphological performance across the climatic gradient studied here, *Physocarpus* 'Little Devil' could have a higher capacity to adapt changing climatic conditions, despite being selected for horticultural appeal.

Given the response of stomatal conductance to the irrigation deficit, but the lack of correlation between measured xylem traits and the treatments, future studies could instead focus additional morphological traits such as vein density and leaf hydraulic conductance (K_{leaf}) which has been shown to be highly correlated with stomatal conductance and photosynthetic rate (Brodribb & Jordan, 2011; Tabassum et al., 2016; Zhong et al., 2020). An additional future consideration could be vessel length, since this study only considered the effect of vessel diameter on hydraulic function, though vessel length has been found to be correlated with vessel diameter for some species (Pan et al., 2015), as well as the presence of vasicentric tracheids, which has been shown to be important for drought tolerance (Fontes et al., 2022;). This study also targeted recently produced growth, which may be responding to the irrigation treatments, and did not take into account stem length, which has been shown to correlate with vessel diameter (Olson et al., 2020). However, only sampling from the tip of actively growing branches limited observing variation which can be found between different plant locations (Y. Zhang et al., 2023).

Conclusion

Results from this study not only emphasize the importance of site and geographic location when assessing impacts of decreasing water availability due to climate change, but also contribute knowledge on understudied horticultural taxa. Through measuring taxa planted across distinct climatic locations, site identity was found to be the driving factor in measured physiological and morphological responses, indication an acclimation to site conditions that was observed through the collected traits. Lack of clear trends between taxa across sites indicate a high degree phenotypic plasticity, but also a high degree of intraspecific variation which limits predictions for potential taxa level responses to changing climatic conditions (Cavender-Bares & Ramírez-Valiente, 2017; Gratani, 2014; Henn et al., 2018; Hulshof & Swenson, 2010; Kerr et al., 2022; Liancourt et al., 2015). Understanding patterns of plant plasticity and the effect of site is becoming more pertinent under climate change for clarifying plant species' ability to persist under new and changing environments, as well as producing more accurate models (Sáenz-Romero et al., 2017). Few traits responded to the irrigation deficit treatments over the course of the season, indicating a resiliency to variable conditions. A better understanding of plant response to varying water availability across climates can assist with broader scale expectations for plant response under future conditions. These results point to the need for climate and site level specificity in global modeling due to the observed plasticity and acclimation to location presented. CRLP is a multiyear study, providing the opportunity to continue to test these patterns across geographic locations and a range of taxa in coming iterations of the project to further flush out these relationships.

Acknowledgements

I would like to extend a big thank you to my advisor, Soo-Hyung Kim, for his guidance, support and feedback throughout the thesis process. I greatly appreciated the balance between allowing me to grow as a scientist and figure things out on my own, while providing advice and direction when needed. Additional thanks to my master's supervisory committee: Claire Willing and Patrick Tobin, who took the time to serve on my committee and provided valuable guidance and recommendations throughout the process.

This work would not have been possible without the help of current and former members of the Plant Ecophysiology and Modeling Lab. Thank you to the graduate students and postdocs working on the project when I joined the lab: Allison Fron, Miro Stuke, Dr. Arthur Hsin-Wu Hsu, and Dr. Darshi Banan, who helped orient me to the project and answered countless questions. Other lab members contributed to data collection for this project, including: Matt

Hendrickson, Dr. Sun Woo Chung, Shubroto Sarkar, Carter Corcoro, Jane Callagan and Leah Valentine, and ESRM499 students who assisted with data analysis: Fiona Clayton, Kayce Hsueh, Julia Robbins, Dahlia Gietka and Abby Lehn. Additional thanks for collaborators on the Climate Ready Landscapes Plants project, especially Zirui Wang of Utah State University for assistance in data collection. Within UW SEFS, Jon Bakker additionally provided help with statistical methods, primarily multivariable techniques.

This research was supported by the California Department of Food and Agriculture (CDFA), the Northwest Horticulture Society, and funding for my graduate studies via the UW School of Environmental and Forest Sciences, and the USDA National Institute of Food and Agriculture National Needs Graduate and Postgraduate Fellowship Program (NNF) through the Bridging the Gap Fellowship.

References

Ahanger, M. A., Siddique, K. H. M., & Ahmad, P. (2021). Understanding drought tolerance in plants. *Physiologia Plantarum*, *172*(2), 286–288. https://doi.org/10.1111/PPL.13442

Anderegg, W. R. L., Klein, T., Bartlett, M., Sack, L., Pellegrini, A. F. A., Choat, B., & Jansen, S. (2016). *Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe*. *113*(18), 5024–5029. https://doi.org/10.5061/dryad.116j2

Anderson, M.J., Gorley, R.N., and Clarke, K.R.. 2008. *PERMANOVA+ for PRIMER: guide to software and statistical methods*. PRIMER-E Ltd, Plymouth Marine Laboratory, Plymouth, UK. 214 p.

Anderson, M.J., and ter Braak, C.J.F.. 2003. Permutation tests for multi-factorial analysis of variance. *Journal of Statistical Computation and Simulation* 73:85-113.

Armstrong, E. M., Larson, E. R., Harper, H., Webb, C. R., Dohleman, F., Araya, Y., Meade, C., Feng, X., Mujoye, B., Levin, M. J., Lacombe, B., Bakirbas, B., Cardoso, A. A., Fleury, D., Gessler, A., Jaiswal, D., Onkokesung, N., Pathare, V. S., Phartyal, S. S., … Grierson, C. S. (2023). One hundred important questions facing plant science: an international perspective. *New Phytologist*, *238*, 470–481. https://doi.org/10.1111/nph.18771

Berend, K., Haynes, K., & MacKenzie, C. M. D. (2019). Common garden experiments as a dynamic tool for ecological studies of alpine plants and communities in northeastern North America. In *Rhodora* (Vol. 121, Issue 987, pp. 174–212). The New England Botanical Club,Inc. https://doi.org/10.3119/18-16

Brodribb, T. J., & Holbrook, N. M. (2003). Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology*, *132*(4), 2166–2173. https://doi.org/10.1104/pp.103.023879

Brodribb, T. J., & Jordan, G. J. (2011). Water supply and demand remain balanced during leaf acclimation of Nothofagus cunninghamii trees. *New Phytologist*, *192*(2), 437–448. https://doi.org/10.1111/j.1469-8137.2011.03795.x

Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O., & Hurry, V. (2007). Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. *New Phytologist*, *176*(2), 375–389. https://doi.org/10.1111/j.1469-8137.2007.02183.x

Cavender-Bares, J., & Ramírez-Valiente, J. A. (2017). *Physiological Evidence from Common Garden Experiments for Local Adaptation and Adaptive Plasticity to Climate in American Live Oaks (Quercus Section Virentes): Implications for Conservation Under Global Change* (pp. 107–135). https://doi.org/10.1007/978-3-319-69099-5_4

Cayan, D. R., Das, T., Pierce, D. W., Barnett, T. P., Tyree, M., & Gershunova, A. (2010). Future dryness in the Southwest US and the hydrology of the early 21st century drought. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(50), 21271–21276. https://doi.org/10.1073/PNAS.0912391107/-/DCSUPPLEMENTAL/PNAS.0912391107_SI.PDF

Collyer, M. L., & Adams, D. C. (2018). RRPP: An r package for fitting linear models to high-dimensional data using residual randomization. *Methods in Ecology and Evolution*, *9*(7), 1772–1779. https://doi.org/10.1111/2041-210X.13029

Contreras, R., & Hoskins, T. (2020). Creating your own path: Inducing novel traits using mutagenesis. *Acta Horticulturae*, *1291*, 67–72. https://doi.org/10.17660/ActaHortic.2020.1291.9

Davis, S. D., Sperry, J. S., & Hacke, U. G. (1999). The relationship between xylem conduit diameter and cavitation caused by freezing. *American Journal of Botany*, *86*(10), 1367–1372. https://doi.org/10.2307/2656919

Fang, L., Martre, P., Jin, K., Du, X., van der Putten, P. E. L., Yin, X., & Struik, P. C. (2023). Neglecting acclimation of photosynthesis under drought can cause significant errors in predicting leaf photosynthesis in wheat. *Global Change Biology*, *29*(2), 505–521. https://doi.org/10.1111/gcb.16488

Fichot, R., Laurans, F., Monclus, R., Moreau, A., Pilate, G., & Brignolas, F. (2009). Xylem anatomy correlates with gas exchange, water-use efficiency and growth performance under contrasting water regimes: Evidence from Populus deltoides × Populus nigra hybrids. *Tree Physiology*, *29*(12), 1537–1549. https://doi.org/10.1093/treephys/tpp087

Fontes, C. G., Pinto-Ledezma, J., Jacobsen, A. L., Pratt, R. B., & Cavender-Bares, J. (2022). Adaptive variation among oaks in wood anatomical properties is shaped by climate of origin and shows limited plasticity across environments. *Functional Ecology*, *36*(2), 326–340. https://doi.org/10.1111/1365-2435.13964

Fox J, Weisberg S (2019). *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Franks, P. (2004). Stomatal control and hydraulic conductance, with special reference to tall trees. *Tree Physiology*, *24*, 865–878.

Fron, A., et al (2023). Climate Ready Landscape Plants: Aesthetic Qualities and Physiology in Landscape Plants in Water Deficit Conditions Across the Western U.S. *Manuscript in preparation*.

Fron A, Stuke M, Hsu H-W, Kim S-H (2023) *Climate-ready landscape plants: 2021-2022 University of Washington trial report*. Seattle, WA: University of Washington Botanic Gardens, p. 52 (https://doi.org/10.5281/zenodo.11226963)

Gadermaier, J., Vospernik, S., Grabner, M., Wächter, E., Keßler, D., Kessler, M., Lehner, F., Klebinder, K., & Katzensteiner, K. (2024). Soil water storage capacity and soil nutrients drive tree ring growth of six European tree species across a steep environmental gradient. *Forest Ecology and Management*, *554*. https://doi.org/10.1016/j.foreco.2023.121599

Gouveia, A. C., & Freitas, H. (2009). Modulation of leaf attributes and water use efficiency in Quercus suber along a rainfall gradient. *Trees - Structure and Function*, *23*(2), 267–275. https://doi.org/10.1007/s00468-008-0274-z

Gratani, L. (2014). Plant Phenotypic Plasticity in Response to Environmental Factors. *Advances in Botany*, *2014*, 1–17. https://doi.org/10.1155/2014/208747

Grossman, J. J. (2023). Phenological physiology: seasonal patterns of plant stress tolerance in a changing climate. In *New Phytologist* (Vol. 237, Issue 5, pp. 1508–1524). John Wiley and Sons Inc. https://doi.org/10.1111/nph.18617

Hacke, U. G., Spicer, R., Schreiber, S. G., & Plavcová, L. (2017). An ecophysiological and developmental perspective on variation in vessel diameter. In *Plant Cell and Environment* (Vol. 40, Issue 6, pp. 831–845). Blackwell Publishing Ltd. https://doi.org/10.1111/pce.12777

Harrison, S., & LaForgia, M. (2019). Seedling traits predict drought-induced mortality linked to diversity loss. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(12), 5576–5581. https://doi.org/10.1073/pnas.1818543116

Hayden, L., Cadenasso, M. L., Haver, D., & Oki, L. R. (2015). Residential landscape aesthetics and water conservation best management practices: Homeowner perceptions and preferences. *Landscape and Urban Planning*, *144*, 1–9. https://doi.org/10.1016/J.LANDURBPLAN.2015.08.003

Heeter, K. J., Harley, G. L., Abatzoglou, J. T., Anchukaitis, K. J., Cook, E. R., Coulthard, B. L., Dye, L. A., & Homfeld, I. K. (2023). Unprecedented 21st century heat across the Pacific Northwest of North America. *Npj Climate and Atmospheric Science*, *5*. https://doi.org/10.1038/s41612-023-00340-3

Henn, J. J., Buzzard, V., Enquist, B. J., Halbritter, A. H., Klanderud, K., Maitner, B. S., Michaletz, S. T., Pötsch, C., Seltzer, L., Telford, R. J., Yang, Y., Zhang, L., & Vandvik, V. (2018). Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Frontiers in Plant Science*, *871*. https://doi.org/10.3389/fpls.2018.01548

Hillabrand, R. M., Lieffers, V. J., Hogg, E. H., Martínez-Sancho, E., Menzel, A., & Hacke, U. G. (2018). Functional xylem anatomy of aspen exhibits greater change due to insect defoliation than to drought. *Tree Physiology*, *39*(1), 45–54. https://doi.org/10.1093/treephys/tpy075

Hirons, A. D., Watkins, J. H. R., Baxter, T. J., Miesbauer, J. W., Male-Muñoz, A., Martin, K. W. E., Bassuk, N. L., & Sjöman, H. (2021). Using botanic gardens and arboreta to help identify urban trees for the future. *Plants People Planet*, *3*(2), 182–193. https://doi.org/10.1002/ppp3.10162

Hopkins, M (1942). Cercis in North America. *Rhodora, 44, 193-211.*

Hulshof, C. M., & Swenson, N. G. (2010). Variation in leaf functional trait values within and across individuals and species: An example from a Costa Rican dry forest. *Functional Ecology*, *24*(1), 217–223. https://doi.org/10.1111/j.1365-2435.2009.01614.x

Johnson, D. M., Berry, Z. C., Baker, K. V., Smith, D. D., McCulloh, K. A., & Domec, J. C. (2018). Leaf hydraulic parameters are more plastic in species that experience a wider range of leaf water potentials. *Functional Ecology*, *32*(4), 894–903. https://doi.org/10.1111/1365-2435.13049

Kaproth, M. A., Fredericksen, B. W., González-Rodríguez, A., Hipp, A. L., & Cavender-Bares, J. (2023). Drought response strategies are coupled with leaf habit in 35 evergreen and deciduous oak (Quercus) species across a climatic gradient in the Americas. *New Phytologist*, *239*(3), 888–904. https://doi.org/10.1111/nph.19019

Kassambara A (2023). *ggpubr: 'ggplot2' Based Publication Ready Plots*. R package version 0.6.0, https://rpkgs.datanovia.com/ggpubr/.

Kerr, K. L., Anderegg, L. D. L., Zenes, N., & Anderegg, W. R. L. (2022). Quantifying within-species trait variation in space and time reveals limits to trait-mediated drought response. *Functional Ecology*, *36*(9), 2399–2411. https://doi.org/10.1111/1365-2435.14112

Kim, S-H., *et al*. (2020). Advances in crop modelling for a sustainable agriculture. *Burleigh Dodds Science Publishing*. http://dx.doi.org/10.19103/AS.2019.0061.01

Knipfer, T., Bambach, N., Isabel Hernandez, M., Bartlett, M. K., Sinclair, G., Duong, F., Kluepfel, D. A., & McElrone, A. J. (2020). Predicting stomatal closure and turgor loss in woody plants using predawn and midday water potential. *Plant Physiology*, *184*(2), 881–894. https://doi.org/10.1104/pp.20.00500

Lemordant, L., Gentine, P., Swann, A. S., Cook, B. I., & Scheff, J. (2018). Critical impact of vegetation physiology on the continental hydrologic cycle in response to increasing CO2. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(16), 4093–4098. https://doi.org/10.1073/PNAS.1720712115/-/DCSUPPLEMENTAL

Lenth R (2024). _emmeans: Estimated Marginal Means, aka Least-Squares Means_. R package version 1.10.0, <https://CRAN.R-project.org/package=emmeans>.

Liancourt, P., Boldgiv, B., Song, D. S., Spence, L. A., Helliker, B. R., Petraitis, P. S., & Casper, B. B. (2015). Leaf-trait plasticity and species vulnerability to climate change in a Mongolian steppe. *Global Change Biology*, *21*(9), 3489–3498. https://doi.org/10.1111/gcb.12934

Liu, M., Wang, Z., Li, S., Lü, X., Wang, X., & Han, X. (2017). Changes in specific leaf area of dominant plants in temperate grasslands along a 2500-km transect in northern China. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-11133-z

Lubell, J. D., Brand, M. H., & Lehrer, J. M. (2011). Susceptibility of Eastern Ninebark (Physocarpus opulifolius (L.) Maxim.) Cultivars to Powdery Mildew. *Journal of Environmental Horticulture*, *29*(3), 105–107. https://doi.org/10.24266/0738-2898-29.3.105

Martínez-Vilalta, J., Poyatos, R., Aguadé, D., Retana, J., & Mencuccini, M. (2014). A new look at water transport regulation in plants. *New Phytologist*, *204*(1), 105–115. https://doi.org/10.1111/nph.12912

Martin-StPaul, N., Delzon, S., & Cochard, H. (2017). Plant resistance to drought depends on timely stomatal closure. *Ecology Letters*, *20*(11), 1437–1447. https://doi.org/10.1111/ELE.12851

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany*, *51*(345), 659–668.

Nan, X., Huihui, Z., Haixiu, Z., Yining, W., Jinbo, L., Li, X., Zepeng, Y., Wenxu, Z., Yi, Q., & Guangyu, S. (2018). The response of photosynthetic functions of F1 cutting seedlings from physocarpus amurensis maxim (♀) × physocarpus opulifolius "diabolo"(♂) and the parental seedlings to salt stress. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.00714

Nardini, A., & Salleo, S. (2000). Limitation of stomatal conductance by hydraulic traits: Sensing or preventing xylem cavitation? *Trees - Structure and Function*, *15*(1), 14–24. https://doi.org/10.1007/s004680000071

Oki, L. R. (2019). Climate Ready Landscape Plants – 2019 Specialty Crop Multi-State Program. *University of California, Davis, CA*. Climate Ready Landscape Plants-Project Narrative_final.pdf.

Ogle DH, Doll JC, Wheeler AP, Dinno A (2023). *FSA: Simple Fisheries Stock Assessment Methods*. R package version 0.9.5, https://CRAN.R-project.org/package=FSA.

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022). _vegan: Community Ecology Package_. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.

Olson, M., Rosell, J. A., Martínez-Pérez, C., León-Gómez, C., Fajardo, A., Isnard, S., Cervantes-Alcayde, M. A., Echeverría, A., Figueroa-Abundiz, V. A., Segovia-Rivas, A., Trueba, S., & Vázquez-Segovia, K. (2020). Xylem vessel-diameter–shoot-length scaling: ecological significance of porosity types and other traits. *Ecological Monographs*, *90*(3). https://doi.org/10.1002/ecm.1410

Pan, R., Geng, J., Cai, J., & Tyree, M. T. (2015). A comparison of two methods for measuring vessel length in woody plants. *Plant Cell and Environment*, *38*(12), 2519–2526. https://doi.org/10.1111/pce.12566

Panek, J. (1996). Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. *Tree Physiology*, *16*, 747–755.

Pinheiro JC, Bates DM (2000). *Mixed-Effects Models in S and S-PLUS*. Springer, New York. doi:10.1007/b98882.

Pinheiro J, Bates D, R Core Team (2024). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-166, https://CRAN.R-project.org/package=nlme.

Popek, R., Łukowski, A., & Grabowski, M. (2018). Influence of particulate matter accumulation on photosynthetic apparatus of Physocarpus opulifolius and Sorbaria sorbifolia. *Polish Journal of Environmental Studies*, *27*(5), 2391–2396. https://doi.org/10.15244/pjoes/78626

Ramesha, M. N., Link, R. M., Paligi, S. S., Hertel, D., Röll, A., Hölscher, D., & Schuldt, B. (2022). Variability in growth-determining hydraulic wood and leaf traits in Melia dubia across a steep water availability gradient in southern India. *Forest Ecology and Management*, *505*. https://doi.org/10.1016/j.foreco.2021.119875

Sade, N., Gebremedhin, A., & Moshelion, M. (2012). Risk-taking plants. *Plant Signaling & Behavior*, *7*(7), 767–770. https://doi.org/10.4161/psb.20505

Sáenz-Romero, C., Lamy, J. B., Ducousso, A., Musch, B., Ehrenmann, F., Delzon, S., Cavers, S., Chałupka, W., Dağdaş, S., Hansen, J. K., Lee, S. J., Liesebach, M., Rau, H. M., Psomas, A., Schneck, V., Steiner, W., Zimmermann, N. E., & Kremer, A. (2017). Adaptive and plastic responses of Quercus petraea populations to climate across Europe. *Global Change Biology*, *23*(7), 2831–2847. https://doi.org/10.1111/gcb.13576

Santiago, L. S., Goldstein, G., Meinzer, F. C., Fisher, J. B., Machado, K., Woodruff, D., & Jones, T. (2004). Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia*, *140*(4), 543–550. https://doi.org/10.1007/s00442-004-1624-1

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. doi:10.1038/nmeth.2019

Schuch, U. K., & Burger, D. W. (1997). Water Use and Crop Coefficients of Woody Ornamentals in Containers. *Journal of the American Society of Horticultural Ccience*, *122*(5), 727–734.

Shwetha, P., & Varija, K. (2015). Soil Water Retention Curve from Saturated Hydraulic Conductivity for Sandy Loam and Loamy Sand Textured Soils. *Aquatic Procedia*, *4*, 1142–1149. https://doi.org/10.1016/j.aqpro.2015.02.145

Simpson, G.L. 2022. Restricted permutations; using the permute package. https://cran.r-project.org/web/packages/permute/vignettes/permutations.html

Sjöman, H., Ignell, S., & Hirons, A. (2023). Selection of Shrubs for Urban Environments—An Evaluation of Drought Tolerance of 120 Species and Cultivars. *HortScience*, *58*(5), 573–579. https://doi.org/10.21273/HORTSCI17063-22

Sterck, F. J., Zweifel, R., Sass-Klassen, U., & Chowdhury, Q. (2008). Persisting soil drought reduces leaf specific conductivity in Scots pine (Pinus sylvestris) and pubescent oak (Quercus pubescens). *Tree Physiology*, *28*, 529–536.

Sternberg, P. (2012). *Physiological and Morphological Basis for Differences in Growth, Water Use and Drought Resistance among Cercis L. taxa* [Dissertation]. The Ohio State University.

Tabassum, M. A., Zhu, G., Hafeez, A., Wahid, M. A., Shaban, M., & Li, Y. (2016). Influence of leaf vein density and thickness on hydraulic conductance and photosynthesis in rice (Oryza sativa L.) during water stress. *Scientific Reports*, *6*. https://doi.org/10.1038/srep36894

Telemeco, R. S., & Gangloff, E. J. (2020). Analyzing stress as a multivariate phenotype. *Integrative and Comparative Biology*, *60*(1), 70–78. https://doi.org/10.1093/icb/icaa005

Tombesi, S., Nardini, A., Farinelli, D., & Palliotti, A. (2014). Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of Vitis vinifera. *Physiologia Plantarum*, *152*(3), 453–464. https://doi.org/10.1111/ppl.12180

Tyree, M. T., & Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants. *New Phytologist*, *119*(3), 345–360. https://doi.org/10.1111/j.1469-8137.1991.tb00035.x

USDA Plant Hardiness Zone Map, 2023. Agricultural Research Service, U.S. Department of Agriculture. Accessed from https://planthardiness.ars.usda.gov/ and https://prism.oregonstate.edu/projects/plant_hardiness_zones.php.

Vu VQ, Friendly M (2023). *ggbiplot: A Grammar of Graphics Implementation of Biplots*. https://github.com/friendly/ggbiplot, https://friendly.github.io/ggbiplot/.

Wang, T., Hamann, A., Spittlehouse, D., & Carroll, C. (2016). Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS ONE*, *11*(6). https://doi.org/10.1371/journal.pone.0156720

Wheeler, J. K., Sperry, J. S., Hacke, U. G., & Hoang, N. (2005). Inter-vessel pitting and cavitation in woody Rosaceae and other vessel led plants: A basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell and Environment*, *28*(6), 800–812. https://doi.org/10.1111/j.1365-3040.2005.01330.x

Wickham H (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Widrlechner, M. P., Daly, C., Keller, M., & Kaplan, K. (2012). Horticultural Applications of a Newly Revised USDA Plant Hardiness Zone Map. *HortTechnology*, *22*(1), 6–19. https://doi.org/10.21273/HORTTECH.22.1.6

Yang, D., Wang, A. Y., Zhang, J. L., Bradshaw, C. J. A., & Hao, G. Y. (2020). Variation in stem xylem traits is related to differentiation of upper limits of tree species along an elevational gradient. *Forests*, *11*(3). https://doi.org/10.3390/f11030349

Zhang, H., Ge, Y., Xie, X., Atefi, A., Wijewardane, N. K., & Thapa, S. (2022). High throughput analysis of leaf chlorophyll content in sorghum using RGB, hyperspectral, and fluorescence imaging and sensor fusion. *Plant Methods*, *18*(1). https://doi.org/10.1186/s13007-022-00892-0

Zhang, Y., Ding, C., Liu, Y., Li, S., Li, X., Xi, B., & Duan, J. (2023). Xylem anatomical and hydraulic traits vary within crown but not respond to water and nitrogen addition in Populus tomentosa. *Agricultural Water Management*, *278*. https://doi.org/10.1016/j.agwat.2023.108169

Zhong, M., Cerabolini, B. E. L., Castro-Díez, P., Puyravaud, J. P., & Cornelissen, J. H. C. (2020). Allometric co-variation of xylem and stomata across diverse woody seedlings. *Plant Cell and Environment*, *43*(9), 2301–2310. https://doi.org/10.1111/pce.13826

Zlesak, D. C. (2012). Physocarpus opulifolius (L.) Maxim "Donna May": A New Compact, Purple-leafed Landscape Shrub. In *HORTSCIENCE* (Vol. 47, Issue 9). http://planthardiness.ars.

Supplemental material

Table S1. Full two-way ANOVA table for all variables measured across all sites at the end of the growing season (long version of Table 3) by site, showing the impact of species, irrigation treatment, their interaction and residuals. * indicates traits assessed via Kruskal-Wallis due to not fitting the assumtions for ANOVA.

Site	Variable		Sum Sq	DF	F value	p-value
UW	gsw_MD	Treatment	0.08	1	18.1	0.0001
		Species	0.12	3	8.4	0.0002
		Species:Treatment	0.02	3	1.66	0.191
		Residuals	0.2	43		
UW	PhiPS2_MD	Treatment	0.036	1	1.6	0.21
		Species	0.4	3	5.95	0.002
		Species:Treatment	0.09	3	1.3	0.285

		Residuals	0.97	43		
UW	SLA	Treatment	2702	1	7.07	0.011
		Species	5693	3	4.96	0.005
		Species:Treatment	2349	3	2.05	0.12
		Residuals	16443	43		
UW	Photo	Treatment	97.8	1	5.35	0.28
		Species	334.4	3	6.1	0.002
		Species:Treatment	22	3	0.4	0.75
		Residuals	566.8	31		
UW	Cond	Treatment	0.19	1	10.5	0.003
		Species	0.37	3	7.03	0.001
		Species:Treatment	0.056	3	1.06	0.38
		Residuals	0.55	31		
UW	Trmmol	Treatment	65.02	1	11.9	0.002
		Species	115.8	3	7.09	0.0009
		Species:Treatment	12	3	0.73	0.54
		Residuals	168.9	31		
UW	WUE	Treatment	7.27	1	14.4	0.0007
		Species	9.1	3	6.0	0.002
		Species:Treatment	0.08	3	0.05	0.98
		Residuals	15.7	31		
UW	iWUE	Treatment	7996	1	12.5	0.001
		Species	9742	3	5.08	0.006
		Species:Treatment	31	3	0.02	0.990
		Residuals	19831	31		
UW	Kp*	Treatment				
		Species	26.6	3		7.1E-06
		Species:Treatment				
		Residuals				
UW	Dmh*	Treatment				
		Species	0.06	3		9.70E-08
		Species:Treatment				
		Residuals				
OSU	SLA	Treatment	24.8	1	0.18	0.67
		Species	11556.7	3	28.01	9.93E-10
		Species:Treatment	431.3	3	1.045	0.38
		Residuals	5225.9	38		
OSU	gsw_MD	Treatment	0.0007	1	0.26	0.61
		Species	0.232	3	30.0	2.18E-11
		Species:Treatment	0.018	3	2.37	0.081

		Residuals	0.134	52		
OSU	PhiPS2_MD	Treatment	0.003	1	1.15	0.2884
		Species	0.43	3	47.4	6.67E-15
		Species:Treatment	0.003	3	0.28	0.84
		Residuals	0.159	52		
OSU	SLA	Treatment	3	1	0.05	0.88
		Species	1726	3	5.11	0.004
		Species:Treatment	633	3	1.87	0.146
		Residuals	5625	50		
OSU	Photo	Treatment	13.1	1	1.17	0.29
		Species	464.1	3	13.74	4.40E-06
		Species:Treatment	11.1	3	0.33	0.8
		Residuals	393.9	35		
OSU	Cond	Treatment	0.002	1	1.45	0.24
		Species	0.07	3	16.3	8.48E-07
		Species:Treatment	0.002	3	0.49	0.69
		Residuals	0.05	35		
OSU	Trmmol	Treatment	0.9	1	2.03	0.16
		Species	18.5	3	13.89	3.99E-06
		Species:Treatment	0.54	3	0.40	0.75
		Residuals	15.55	35		
OSU	WUE	Treatment	0.01	1	0.02	0.9
		Species	4.31	3	2.29	0.095
		Species:Treatment	0.29	3	0.16	0.93
		Residuals	21.96	35		
OSU	iWUE	Treatment	0	1	0	1
		Species	1291	3	1.77	0.17
		Species:Treatment	304	3	0.42	0.74
		Residuals	8492	35		
OSU	Kp	Treatment	0.35	1	0.49	0.49
		Species	11	3	5.2	0.004
		Species:Treatment	0.52	3	0.24	0.87
		Residuals	30.3	43		
OSU	Dmh	Treatment	0	1	0.02	0.88
		Species	0.001	3	16.85	2.20E-07
		Species:Treatment	0.0001	3	1.71	0.18
		Residuals	0.001	43		
USU	gsw_MD	Treatment	0.003	1	0.32	0.57
		Species	0.26	3	8.63	2.00E-04
		Species:Treatment	0.014	3	0.45	0.72

		Residuals	0.36	35		
USU	PhiPS2_MD	Treatment	0.0023	1	1.1	0.3
		Species	0.38	3	52.97	5.60E-13
		Species:Treatment	0.007	3	0.98	0.41
		Residuals	0.09	35		
USU	SLA	Treatment	925	1	6.8	0.01
		Species	12728	3	31.4	5.00E-10
		Species:Treatment	3390	3	8.36	0.00025
		Residuals	4731	35		
USU	Photo	Treatment	16.5	1	1.92	0.18
		Species	342.1	3	13.3	1.40E-05
		Species:Treatment	17.8	3	0.69	0.56
		Residuals	240.1	28		
USU	Cond	Treatment	0.001	1	0.15	0.7
		Species	0.09	3	3.82	0.02
		Species:Treatment	0.005	3	0.19	0.90
		Residuals	0.22	28		
USU	Trmmol	Treatment	1.43	1	0.27	0.61
		Species	47.3	3	2.94	0.05
		Species:Treatment	4.75	3	0.3	0.83
		Residuals	149.8	28		
USU	WUE	Treatment	0.097	1	0.55	0.47
		Species	2.03	3	3.83	0.02
		Species:Treatment	0.029	3	0.05	0.98
		Residuals	4.95	28		
USU	iWUE	Treatment	115	1	0.51	0.48
		Species	3367	3	4.97	0.007
		Species:Treatment	21	3	0.03	0.99
		Residuals	6327	28		
USU	Kp	Treatment	5.26	1	3.01	0.09
		Species	16.87	3	3.22	0.38
		Species:Treatment	13.6	3	2.6	0.073
		Residuals	47.2	27		
USU	Dmh	Treatment	0.00005	1	2.92	0.099
		Species	0.0005	3	8.77	3.00E-04
		Species:Treatment	0.00008	3	1.34	0.28
		Residuals	0.0005	27		

Table S2. Full RRPP ANOVA table for all sites (long version of Table 4) and the impact of treatment, taxa, site and all possible interactions

	DF	SS	MS	Rsq	F	Z	p
treatment	1	3584	3582	0.024	6.04	2.50	**0.004**
taxa	3	17015	5671	0.113	9.57	5.58	**1e-05**
site	2	48698	24349	0.322	41.07	7.15	**1e-05**
treatment:taxa	3	2119	706	0.014	1.19	0.51	0.310
treatment:site	2	9481	4741	0.063	8.0	3.79	**3e-05**
taxa:site	6	22415	3736	0.148	6.30	5.73	**1e-05**
treatment:taxa:site	6	4667	778	0.031	1.31	0.79	0.212
residuals	85	50400	593	0.333			
total	108	151182					

Table S3. Full PERMANOVA table for UW across the growing season (long version of Table 5), indicating the impact of position, season and their interaction.

	DF	SS	R^2	F	p
position	50	3.52	0.62	5.57	**1e-05**
season	2	0.70	0.124	27.1	**1e-05**
season:taxa	6	0.24	0.04	3.14	**0.003**
residual	94	1.22	0.21		
total	152	5.69	1.00		

Table S4. Full RRPP ANOVA table for UW expanded end of season traits (long version of Table 6).

	DF	SS	MS	Rsq	F	Z	p
treatment	1	6265	6265	0.103	5.79	2.30	**0.009**
taxa	3	12827	4276	0.212	3.95	2.90	**0.002**
interaction	3	2183	727.8	0.036	0.67	-0.45	0.673
residual	27	29224	1082.4	0.483			
total	34	60541					