THESIS

NOVEL WATER-USE STRATEGIES OF COLORADO WETLAND PLANTS: IMPLICATIONS FOR WETLAND WATER LOSS

Submitted by

Anna Wright

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Master's Committee:

Advisor: Troy Ocheltree
Co-advisor: Jeremy Sueltenfuss

Joe von Fischer

ABSTRACT

NOVEL WATER-USE STRATEGIES OF COLORADO WETLAND PLANTS: IMPLICATIONS FOR WETLAND WATER LOSS

In the arid west, there is a tension between wetland restoration and water rights. Wetlands, relied upon by humans and wildlife alike, also contribute significantly to evapotranspiration (ET) due to higher water tables and dense vegetation. It is therefore critical to understand how much water wetlands lose to evaporation and transpiration, and what affects wetland water loss. This paper quantifies the transpiration of five abundant wetland species and investigates physiological mechanisms that drive transpiration rates for each species. The focal species transpire significantly different amounts at the leaf-level and when scaled to ground area. Stomatal response to environmental stimuli differed from upland stomatal responses, which suggests that wetland plants prioritize carbon uptake over hydraulic safety and do not align with current paradigms for stomatal responses to vapor pressure deficit, leaf water potential, or turgor loss point. Understanding species' stomatal responses to extreme environmental conditions is key to managing this rare and critical ecosystem as the climate changes.

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Wetland restoration and water loss in the arid west

In the arid west, there is a tension between wetland conservation and water rights. Wetlands provide benefits for humans and wildlife; they mitigate floods, filter pollutants from water supplies, increase water storage, and provide critical habitat, food, water, and shelter. However, wetlands can also affect the overall water budget of streams, to the point that a bill to protect stream restoration projects from water rights litigation (SB 23-270) passed in the legislature in 2023. The legislation recognizes the essential function of stream and wetland restoration but only protects restoration projects conducted within the historical floodplain. It does not protect newly constructed wetlands, nor does it protect major stream work or any project that intentionally diverts water for other use.

Development projects near riparian areas frequently impact wetlands during the construction process. When this occurs, developers are required, by section 404 of the Clean Water Act, to pursue compensatory mitigation[1] for the impacted area. Practically, this requires developers to restore or construct a wetland near the impacted site (33 CFR § 230.93 (a)). The restored wetland must meet the following criteria:

- Restored project area equals or exceeds the impacted area

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[1] “Compensatory mitigation means the restoration (re-establishment or rehabilitation), establishment (creation), enhancement, and/or in certain circumstances preservation of aquatic resources for the purposes of offsetting unavoidable adverse impacts which remain after all appropriate and practicable avoidance and minimization has been achieved.” 33 CFR § 230.92

- Ecological performance standards must be developed, monitored, and met (different for each state, and subject to agency approval). In Colorado, standards include plant biodiversity and noxious weed management. Monitoring reports must be provided to the district engineer and local, state, and tribal, governments, and public upon request.

When wetlands are constructed or restored, previously unavailable water sources become “usable” via evapotranspiration (ET) - the sum of surface evaporation, evaporation from the soil, and plant transpiration. Restored wetlands bring the soil surface closer to the groundwater table, which increases evaporation from the soil, while newly seeded wetland plants increase transpiration. ET is considered water use by Colorado law, and projects that increase water use must allocate water rights.[2] If development projects increase systemwide water use, the developer must obtain the water rights for that use or pay to augment the water for downstream senior water rights holders. Quantifying wetland ET and understanding what drives ET variation is critical to ensuring the proper functioning of mitigated wetlands, while also providing clarity on whether water rights should be required for wetland mitigation.

Methods for quantifying wetland transpiration

Given the challenges in measuring full ET for small, restored wetlands, I focused this study on leaf-level transpiration (E). Measuring E and determining what environmental stimuli E responds to can help managers balance wetland water budgets. E is essentially a function of the conductance of water vapor through stomates from leaf to air and through the leaf boundary layer (g_{bw}), and vapor pressure deficit (VPD)

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[2] *Water Right Determination and Administration Act,* 1969

(Monteith 1965; Cowan and Farquhar 1977; Alam, Lamb, and Warwick 2021). Plants regulate stomatal conductance (g_{sw}) by controlling the aperture of leaf stomates. The smaller the opening, the less water and CO_2 are conducted and the lower overall *E*. The other major influence of *E* is VPD, which is a function of air temperature and relative humidity. Water transport through the plant occurs along a water vapor concentration gradient, from high concentration in soil to low concentration in air. The higher the VPD between air and leaf, the higher the *E*.

A few wetland ET studies have shown that (1) canopy transpiration can drive canopy ET rates and (2) species composition drives sitewide differences in ET (Hirano et al. 2016; Kabenge and Irmak 2012; Takagi, Tsuboya, and Takahashi 1998). Studying what drives variation in transpiration could reduce uncertainty and enable restoration managers to match restored wetland transpiration with mitigated sites. Transpiration can be measured directly at the leaf or plant level and scaled up to the canopy, using methods such as porometry, sap flux, or gas exchange (Table 1). Porometers use a mass balance of vapor formula to derive transpiration. They are relatively low-cost instruments capable of rapid measurements, enabling high repetitions and frequent measurements. They measure herbaceous species as well as they do woody. Scaling to a community or landscape requires leaf area index (LAI) and species cover. Other transpiration methods, including sap flux and gas exchange, were ill-suited for this study. Sap flux methods rely on thermal probes inserted into sapwood and are unsuitable for use on herbaceous plants (Granier 1985; Čermák, Deml, and Penka 1973). Gas exchange chambers connected to an infrared gas analyzer would be appropriate for measuring transpiration across all species but were disregarded for this

Table 1: Methods and limitations for measuring transpiration in wetlands.

Method/Instrument	**Limitations**
Gas exchange chamber or bag	Cannot fit into dense willow thickets Chamber alters VPD Difficult to make rapid measurements Expensive
Porometer	Difficult to scale to canopy
Sap flux probe	Only usable on woody species

study, as the willow thicket was too dense to place a chamber inside. A soft chamber constructed from a clear bag was briefly considered but was too difficult to install for rapid measurements.

This study quantified leaf-level transpiration of dominant wetland species from both woody and herbaceous functional groups, using a porometer (LI-600, LI-COR Environment, Lincoln, NE, USA). By measuring transpiration at the leaf level and scaling up to the canopy, I hoped to gain insight into the responses of individual plants to environmental conditions. I also hoped to determine whether wetland transpiration was simply a function of biomass and canopy leaf area, or if species composition and variation in plant water use could influence canopy transpiration.

To determine what drives transpiration rates in wetlands, I examined stomatal responses to several environmental stimuli; VPD, solar radiation, and leaf water potential (a metric of plant water stress). While much research exists on stomatal responses to these stimuli under drought conditions, observations of stomatal regulation in wetlands and mesic environments show that wetland plants behave differently than upland plants. Several recent studies have shown that plants in well-watered environments may not respond strongly to VPD or leaf water potential, instead prioritizing carbon uptake and conducting water through stomates despite highly negative leaf water potentials (Bucci et al. 2019; Jin et al. 2023). Stomatal regulation is critical in prevent catastrophic water loss to plants, but stomatal closure is viewed as a trade-off between survival and productivity as it reduces carbon uptake (Nolan et al. 2017; Pivovaroff, Cook, and Santiago 2018). Are wetland plants circumventing this tradeoff because they do not experience catastrophic water loss under natural conditions?

Investigating mechanisms for regulating transpiration and identifying functional thresholds to stomatal conductance improves our understanding of how species composition affects ET, and how wetlands might respond to climate change. Diurnal g_{sw} patterns have been shown to influence overall wetland ET (Takagi, Tsuboya, and Takahashi 1998), and species differ in their stomatal sensitivity to VPD, solar radiation, and Ψ_{leaf} (P. J. Franks and Farquhar 1999). If species vary in stomatal responses to environmental conditions, does species composition affect overall wetland ET? Additionally, if g_{sw} influences overall ET and is driven by environmental stimuli, then it is critical to understand how wetland plants will respond to climate change and increases in global VPD (Ficklin and Novick 2017).

Lastly, I developed a report to be delivered to the Colorado Department of Transportation (CDOT) which describes how this research can inform water-use budgeting in wetland restoration, included as Chapter 2 of this thesis. The report applies the above research to a restoration context. It details how leaf-level transpiration scales to a wetland canopy, and how species composition affects canopy transpiration.

CHAPTER 1: NOVEL WATER-USE STRATEGIES OF COLORADO WETLAND PLANTS AND IMPLICATIONS FOR WETLAND WATER LOSS

Introduction

In the arid west, there is a tension between wetland restoration and water rights, to the point that the Colorado legislature recently passed a bill protecting minor stream restoration projects from water rights litigation (SB 23-270). Wetlands are critical habitat, providing wildlife habitat and ecosystem services such as flood mitigation and pollutant filtration (Culver et al. 2013). Wetlands also contribute significantly to evapotranspiration (ET) due to higher water tables (Maxwell and Kollet 2008; Maxwell and Condon 2016), which is why they are a source of concern for water rights holders.

Measuring ET and understanding how each component of ET contributes to total water loss is an ongoing challenge (Stoy et al. 2019). Plant transpiration (*E*) is a critical component of ET because understanding *E*'s contribution to overall water loss, as well as sources of variation in *E*, improves water budgeting in wetlands. Identifying mechanisms plants utilize for regulating *E* is critical to understanding how wetlands may respond to forecasted increases in Vapor Pressure Deficit (VPD), especially in more arid regions like Colorado (Ficklin and Novick 2017).

Stomatal conductance to water vapor (g_{sw}) is the main mechanism by which plants regulate transpiration, yet stomatal regulation in wetland plants is poorly understood. We would expect wetland plants to have a similar stomatal regulation strategy as a group, due to similar moisture indexes and water availability (Lin et al. 2015). However, this is not supported in the few diurnal wetland studies that exist, where diurnal g_{sw} varied significantly across species (Dang et al. 1991; Takagi, Tsuboya, and Takahashi 1998; Opio et al. 2015). The diurnal g_{sw} patterns also diverged from the expected diurnal pattern of a midday depression in g_{sw}, which many attribute to negative midday leaf water potentials and water stress (Tim J. Brodribb and Holbrook 2003). Stomatal regulation in well-watered environments may be less sensitive to highly negative leaf water potentials, even in arid climates (Bucci et al. 2019; Jin et al. 2023). This contradicts the paradigm that stomates open to obtain CO_2 and close to prevent water stress, responding to high VPD and other water-related stimuli. Even so, maybe this shouldn't be surprising since wetland plants exist in a narrow range of conditions with fully saturated soils; most wetland plant species have a constant source of water and could thus keep stomates open without risk of catastrophic water loss. It remains unknown whether wetland plants reduce stomatal conductance under stressful in-field environmental conditions, or maintain high conductance over an entire day.

Species composition may influence canopy conductance (g_c) and canopy-level *E* because species differ in their stomatal sensitivity to VPD, solar radiation, and Ψ_{leaf} (Franks and Farquhar 1999). Several studies have shown that wetland plants are not an exception; stomatal responses to VPD, solar irradiance, and air temperature vary between species (Smith and Houpis 2004; Opio et al. 2015). While wetland plant gas exchange rates have been measured (Li, Yang, and Li 2007), little is known about diurnal patterns of gas exchange and how interspecific differences in stomatal sensitivity influences daily *E*. If interspecific differences do occur, then we would expect species composition to impact site ET and, therefore, site conditions should influence g_{sw} and *E* by determining which wetland species can establish. Hydrology and elevation are the conditions in wetlands which most strongly determine vegetation community (Silvertown, Araya, and Gowing 2015; Sueltenfuss, Ocheltree, and Cooper 2020). These conditions also affect soil water availability, VPD, and solar radiation – conditions known to alter wetland plant stomatal regulation (Kim et al. 2018; Linderson, Iritz, and Lindroth 2007)—which could create variation in g_{sw} and E within species.

This study had two main objectives: (1) to characterize stomatal regulation and transpiration across wetland plant species, and (2) to identify mechanisms that explain stomatal regulation of wetland plants. In this study, I measured g_{sw} and E alongside environmental conditions to test the following hypotheses: (1) Wetland plant species differ in daily transpiration; (2) transpiration rates are primarily driven by stomatal responses to VPD, temperature, turgor loss point (TLP), and leaf water potential (Ψ_{leaf}); (3) species differ in daily transpiration as a function of their different stomatal regulation strategies; and (4) stomatal conductance declines as species reached VPD, temperature, and Ψ_{leaf} thresholds, where TLP is threshold for Ψ_{leaf} as there is a theoretical loss of critical plant function at TLP.

Methods

Site selection

To test my hypotheses, I selected 5 natural and mitigated wetland sites to quantify species composition (from an original list of 13 sites) and 2 sites to make detailed water-use measurements. Sites were selected from a list of CDOT wetland restoration projects in the Front Range that received compensatory mitigation credits (Figure 1). These sites were within 2 hours driving of Fort Collins, between 4,500 and 6,000 feet in elevation, a minimum of 1 acre, and received or were scheduled to receive

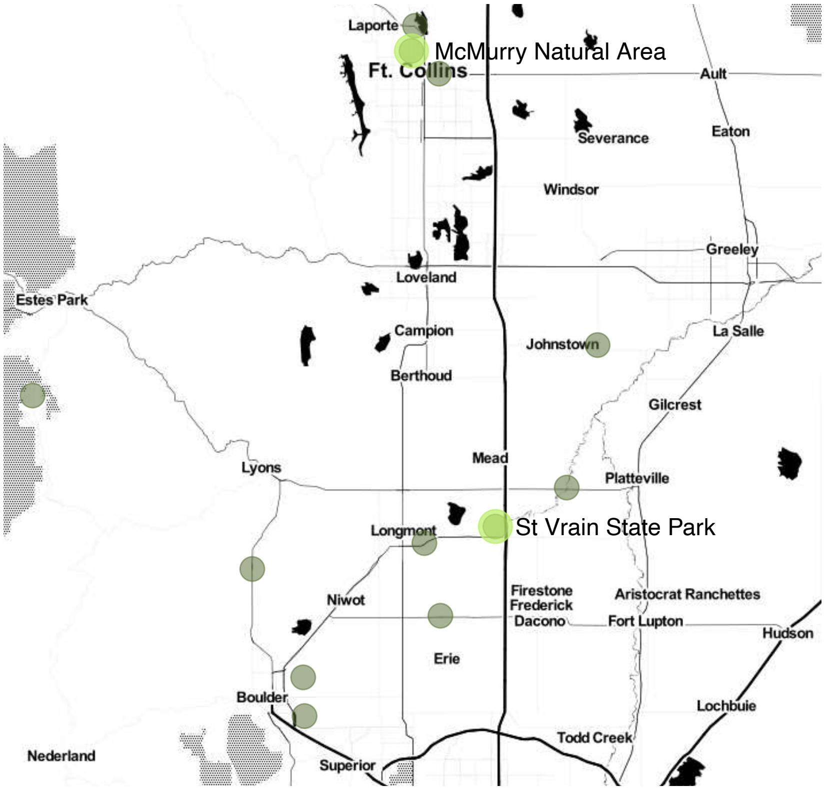

Figure 1: Site map. Green dots indicate visited wetlands. Light green dots indicate sites for plant water-use study.

mitigation credits. During the growing season of 2021, I first measured percent canopy cover for 13 selected sites. Of these, I excluded sites from study if: 1) 1-2 species comprised over 50% of the canopy, or 2) the presence of culverts, drains, dams, or concrete improvements did not meet current wetland mitigation criteria. Five sites met my criteria and were used to evaluate species composition and two sites were selected to test hypotheses about plant water-use strategies: McMurry Natural Area ("McMurry") and St. Vrain State Park Terrace site ("St. Vrain"). These two sites were selected due to similar species composition and establishment year.

McMurry is in northern Fort Collins, CO along the fringe of ponds created from gravel mining operations in the floodplain of the Cache la Poudre River. Initial mitigation of the 1.5 acres began in 2013 and was re-graded and re-planted in 2014 after destructive floods (Roth 2020). Four of the five mitigation locations border ponds, and one mitigation location is in a depression along an outflow stream connecting to the Cache la Poudre River. The wetlands contain willow thickets, young cottonwoods, wet meadow with some willow, and cattail/bulrush marshes. St. Vrain is in Firestone, CO adjacent to St. Vrain Creek. The 6-acre depressional wetland for this mitigation project was created through excavation in 2014 and officially completed in 2016 (Roth 2019). The wetland is separated from St. Vrain Creek by a berm, and contains willow thickets, wet meadows, and cattail marshes.

Species selection

Species percent cover data was recorded using Line-Point Intercept (LPI) methods as defined in the BLM draft AIM wetland protocol to obtain percent cover (Reynolds et al. 2021). Three transects were randomly placed within each wetland, with a pin dropped every half meter along the transect (n=50 per transect). At each pin drop, every species that the pin touched was recorded as a “hit.” If the canopy extended above the pin, any hits above the pin were estimated by eye and recorded. Surface (soil, rock, moss, water) were also recorded.

Five focal species were selected, representing the most abundant species in the study system (Figure 2). The focal species were selected from four functional groups: *Salix exigua* (Coyote Willow, woody), *Populus deltoides* (Plains Cottonwood, woody), *Typha latifolia.* (Cattails, monocot), *Phalaris arundinacea* (Reed Canarygrass, perennial grass), and *Carex emoryi* (Emory Sedge, sedge). Rush species, although abundant, were excluded because they are difficult to measure without crushing the stems, which sends a water pulse through the plant and invalidates water-use measurements.

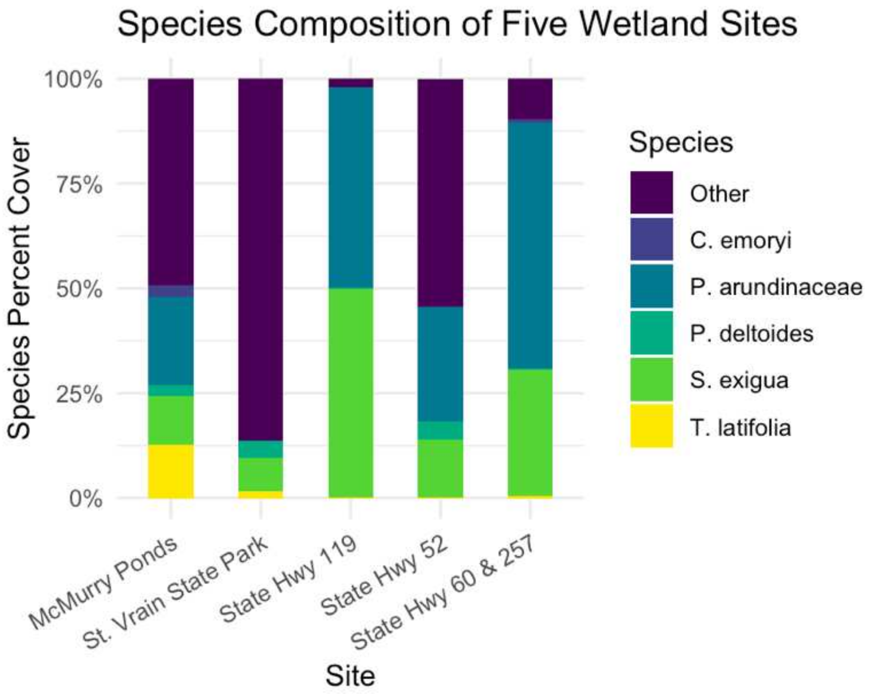

Figure 2: Species composition of wetland sites. "Other" includes all non-focal species. The focal species represented the most abundant species across all sites.

Diurnal Measurements

Physiology measurements were taken diurnally at several times through the growing season to control for seasonal plasticity of water-use traits on June 2, June 4, July 8, July 10, and August 8 and 9. Each site was visited once per month between June and August, within 4 days of one another, on days with full sun, and with no precipitation between measurement days. Diurnal stomatal conductance (g_{sw}, mol m^{-2} s^{-1}) was measured alongside multiple environmental variables: Ψ_{leaf}, VPD, and solar radiation. Fully-grown, healthy leaves were selected randomly from plants across the entire site area for each measurement.

The LI-600 porometer (LI-COR Environment, Lincoln, NE, USA) was used to measure transpiration and g_{sw}. The LI-600 measures transpiration (*E*, mmol m^{-2} s^{-1}) as a function of leaf area, air flow rate (μmol s^{-1}), and the water vapor concentration in reference (H_2O_r) and leaf (H_2O_s) (mmol H_2O mol air^{-1}):

$$E_{apparent} = \frac{(flow * 10^{-6})\,(H_2O_s * 10^{-3} - H_2O_r * 10^{-3})}{(leaf\ area * \ 10^{-4})\,(1 - H_2O_s * 10^{-3})}$$

where H_2O_x is a function of vapor pressure and atmospheric pressure.

g_{sw} is calculated as a function of boundary layer conductance to water vapor (g_{bw}, mol m^{-2} s^{-1}) and conductance to water vapor (g_{tw}, mol m^{-2} s^{-1}):

$$g_{sw} = \frac{1}{\frac{1}{g_{tw}} - \frac{1}{g_{bw}}}$$

Where g_{tw} is derived from $E_{apparent}$ and vapor pressure differences between the leaf and cuvette, and g_{bw} is calculated from flow rate and the measured leaf area (LI-COR Biosciences, 2022).

Because g_{sw} changes throughout the day, measurements were taken every 1.5 hours for each focal species (n=5 per species, randomly selected across the site at each time point) across a 14-hour period beginning just before dawn. Each measurement took 3 –5 seconds, enabling 5 repetitions per species per time point. VPD and leaf temperature were also recorded at each measurement interval.

Diurnal Ψ_{leaf} measurements paired with g_{sw} increases understanding of the plant's tolerance for xylem tension over an entire day, capturing minimum and maximum values. Ψ_{leaf} measurements were collected on each focal species (n=5 plants per species, randomly selected at each time point) using a Scholander-style pressure chamber (Model 1000, PMS Instruments, Corvallis, Oregon, USA). Two pressure chambers were brought into the field and were calibrated at the beginning and in the middle of the day by measuring the same leaf in both chambers and confirming Ψ_{leaf} values were within ±0.01 MPa. Because the times of maximum negative Ψ_{leaf} could have shifted seasonally, measurements were taken at regular 1.5 hour intervals across a 14-hour period simultaneously with g_{sw} measurements. Leaves for each sample were cut at the node with sharp hand pruners and placed in a sealed plastic bag for 1-3 minutes to allow equilibration before measurements.

Solar radiation data (W/m^2) was obtained from nearby weather stations in the CoAgMET system (https://coagmet.colostate.edu/). Data from the stations fct04 and lmt01 were downloaded as they are within 5 miles of McMurry and St Vrain, respectively. VPD_{leaf} values from the LI-600 were also cross-validated using VPD data from these stations.

Turgor loss point

TLP was measured on each focal species (n=5 per species) at the beginning of the season by creating a pressure-volume curve using a Scholander-style pressure chamber (Model 1000, PMS Instruments, Corvallis, USA), as described by Tyree and Hammel (1972). TLP was calculated again for each species using the same methodology, after maximum Ψ_{leaf} values in July were more negative than the species' turgor loss point (for TLP values, see Appendix, Table A1). 5 randomly sampled plants were removed with roots intact (herbaceous) or branches of at least 8” were cut (woody) from McMurry site at midday and rehydrated in the dark for 1 hour before measuring. Leaves for each species were cut at the base using a razor blade immediately before measurements began. All leaves were measured for Ψ_{leaf} and weighed (Discovery DV215CD analytical balance, Ohaus, 0.01 mg, Parsipanny, USA) concurrently and kept in sealed plastic bags between measurements. Measurements were entered into an excel workbook as work occurred, allowing for simultaneous graphing of $1/\Psi_{leaf}$ (y-axis) and the relative water content (x-axis). The shape of the pressure-volume curve is exponential while the leaf holds turgor, and linear once turgor is lost. Measurements were considered complete once five linear points were present on the graph.

Analysis

To determine if g_{sw} or transpiration rates changed significantly based on time of year, site, or species, a one-way ANOVA was conducted in R for diurnal and maximum g_{sw} and daily transpiration. Next, a two-way ANOVA was conducted with species and site, and species and month, to check for any interaction effect. Finally, a Tukey Honest Significant Difference (HSD) post-hoc comparison was performed on species with average diurnal g_{sw} values.

To identify which mechanisms influenced g_{sw}, linear regression and quadratic models were individually fit for Ψ_{leaf}, VPD, and solar radiation vs. g_{sw}. Linear and quadratic models were compared using the Akaike Information Criteria (AIC) to select the better-fitting model for each dependent variable. A multiple linear regression was not conducted, as most variables have multicollinearity and environmental effects were not experimentally controlled.

Results

Diurnal Measurements

In summer 2022, the maximum temperature ranged from 82°F in June to 97°F in July. Diurnally, transpiration (*E,* mmol $m^{-2}_{leaf\ area}$ s^{-1}) peaked midday then tapered in the evening, a pattern that remained consistent over the growing season. This was not the expected pattern, as I anticipated plants to reduce g_{sw} (mmol $m^{-2}_{leaf\ area}$ s^{-1}) and *E* once Ψ_{leaf} exceeded TLP, which occurred by midday each month. On average, willows had the highest rates of *E* and g_{sw} while cottonwoods had the lowest (Figure 3).

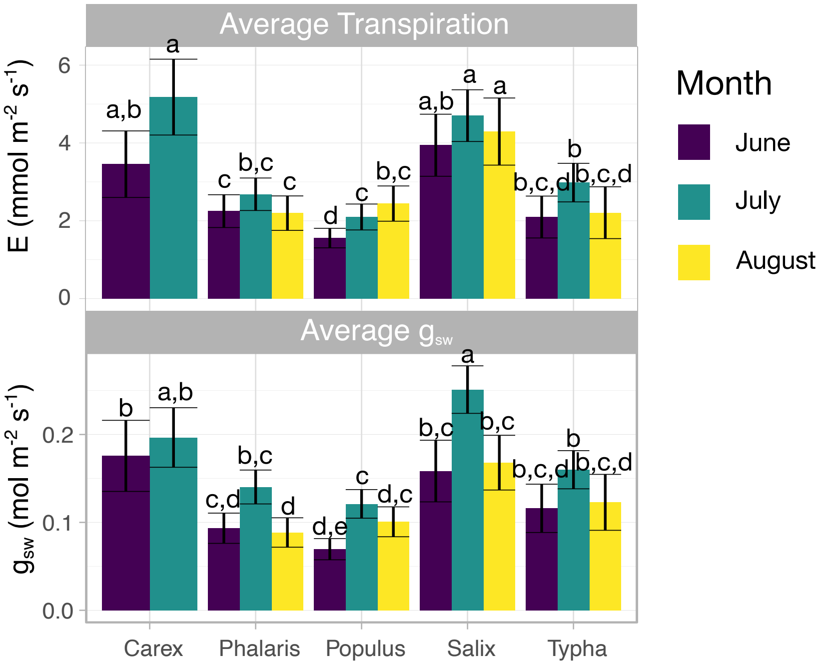

Figure 3: (top) Average transpiration by month and species. (bottom) Average g_{sw} by month and species. Similarities were identified with Tukey HSD and are indicated by letters.

E and g_{sw} were significantly different across species ($p < 0.001$) and months ($p < 0.001$), but not wetlands ($p = 0.745$) (Figure 3). The largest difference between species was between *S. exigua* and *P. deltoides*. *E* of *S. exigua* was higher than *P. deltoides* by

2.3 mmol m^{-2} s^{-1} on average. Months also differed significantly in *E*. In July, *E* rates were 0.677 mmol m^{-2} s^{-1} higher than June ($p < 0.001$), and 0.502 mmol m^{-2} s^{-1} higher than August ($p = 0.01$). *E* did not significantly differ across sites ($p=0.745$), and no interaction effect between species and site was detected with a two-way ANOVA. Importantly, there was no difference between wetlands in the model, suggesting that the physiology of these species that I measured is the same regardless of the exact conditions of the wetland and the method used for mitigation.

Mechanisms

E is a function of g_{sw}, boundary layer conductance (g_{bw}), and VPD. The LI-600 porometer created a constant g_{bw} (2.92 mmol m^{-2} s^{-1}) during measurement, while g_{sw} changed over the day in response to solar radiation, VPD, and Ψ_{leaf} (Figure 4). Species differed significantly in daily average g_{sw} ($p < 0.001$) and maximum daily g_{sw} ($p < 0.001$).

To understand variation in *E*, I analyzed the relationship between g_{sw} and these variables for each species. Solar radiation had the highest R^2 values of any independent variable and explained up to 41% of the variability in g_{sw}. I selected a quadratic model as the better fit for each explanatory variable, as AIC values were lower in 10/15 comparisons and adjusted R^2 values were higher than the linear model in 11/15 comparisons (Table 2).

Table 2: goodness of fit for a linear model (subscript lm) and a quadratic linear model (subscript quad). Most variables are significant for most species.
Significance levels for linear fit: 0 ^^^^ 0.001 ^^^ 0.01 ^^ 0.05 ^
*Significance levels for quadratic fit: 0 **** 0.001 *** 0.01 ** 0.05 **

Species	Variable	R^2_{lm}	R^2_{quad}	Adj R^2_{lm}	Adj R^2_{quad}	p_{lm}	p_{quad}	AIC_{lm}	AIC_{quad}
*C. emoryi^,**	Radiation	0.293	0.408	0.243	0.317	0.030	0.033	-33.61	-34.45
*P. arundinacea^,****	Radiation	0.105	0.257	0.086	0.225	0.023	0.001	-121.6	-128.8
*P.deltoides^^^^,******	Radiation	0.248	0.357	0.232	0.328	0.000	0.000	-133.6	-139.1

*S. exigua^^^^,*****	Radiation	0.306	0.408	0.291	0.382	0.000	0.000	-80.12	-85.73
*T. latifolia**	Radiation	0.072	0.188	0.047	0.143	0.099	0.024	-81.75	-84.97
*C. emoryi^,**	Ψ_{leaf}	0.293	0.365	0.243	0.268	0.030	0.052	-33.61	-33.33
*P. arundinacea^*	Ψ_{leaf}	0.105	0.112	0.086	0.074	0.023	0.065	-121.6	-120.0
*P. deltoides^^^^,****	Ψ_{leaf}	0.248	0.276	0.232	0.243	0.000	0.001	-133.6	-133.4
*S. exigua^^^^,*****	Ψ_{leaf}	0.306	0.419	0.291	0.394	0.000	0.000	-80.12	-86.67
T. latifolia	Ψ_{leaf}	0.072	0.092	0.047	0.042	0.099	0.175	-81.75	-80.62
*C. emoryi^*	VPD	0.327	0.360	0.279	0.261	0.021	0.055	-34.40	-33.19
*P. arundinacea^,***	VPD	0.082	0.212	0.063	0.178	0.046	0.004	-120.4	-125.9
*P. deltoides^,***	VPD	0.102	0.193	0.083	0.157	0.027	0.008	-125.1	-128.2
*S. exigua^^,***	VPD	0.174	0.235	0.156	0.201	0.003	0.002	-71.73	-73.46
*T. latifolia^^,***	VPD	0.205	0.221	0.184	0.179	0.003	0.010	-90.12	-88.93

A quadratic function best explained the relationship between g_{sw} and solar radiation, with g_{sw} increasing as solar radiation increases, then declining at high levels of solar radiation – except for *C. emoryi*, which showed a linear relationship (Figure 4, top left). Solar radiation vs. g_{sw} R^2 values ranged from 0.19 (*T. latifolia*) to 0.41 (*S. exigua, C. emoryi*).

VPD, a function of temperature and relative humidity, was significantly correlated with g_{sw} for all species and R^2 values ranged from 0.21 to 0.36 (Figure 4, top right). The VPD values at which g_{sw} declines differed across species, which contributes to differences in *E* when scaled to ground area. The highest VPD threshold was for *T. latifolia*, at 2.3 kPa. The lowest VPD threshold, 1.75 kPa, was seen in *P. deltoides*.

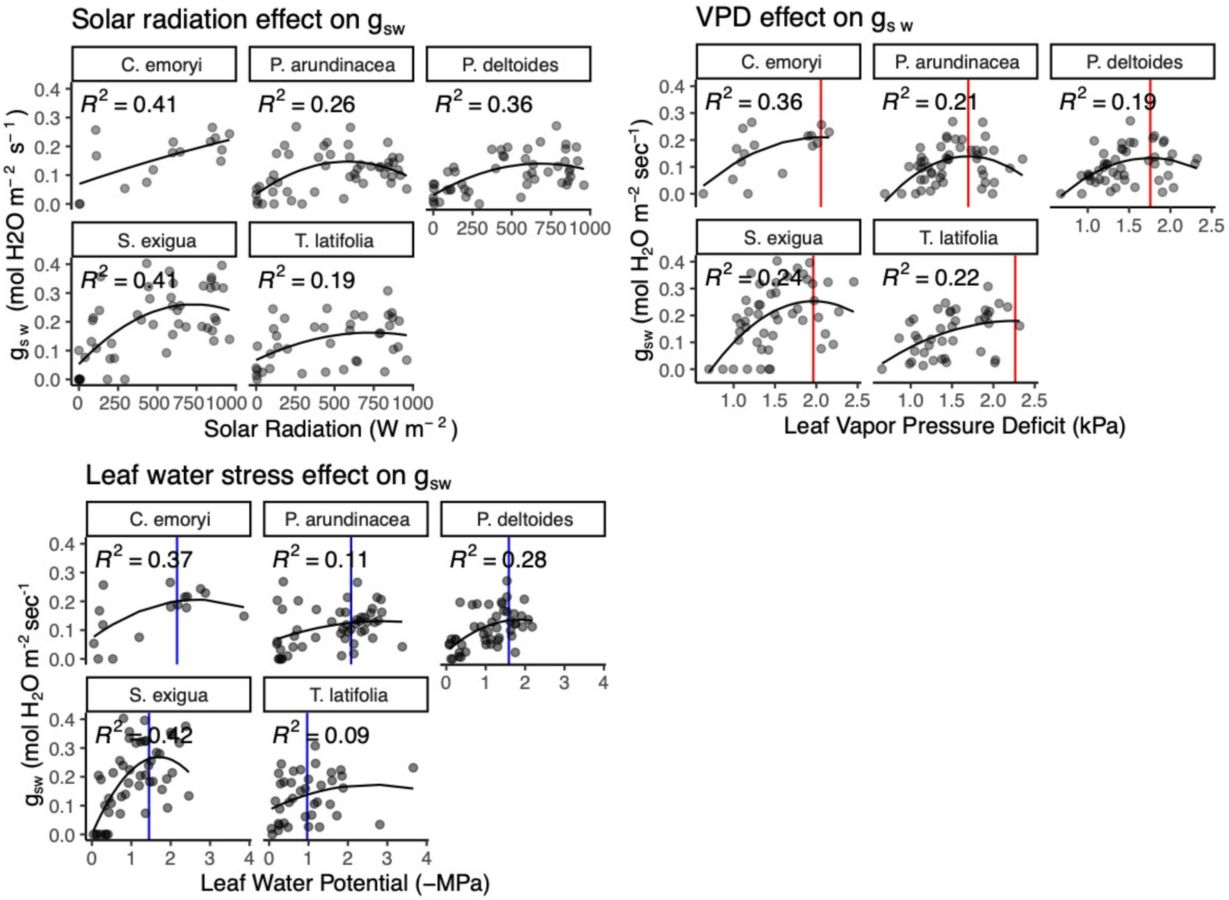

Figure 4:
(top left) g_{sw} response to solar radiation
(top right) g_{sw} response to VPD. The red line indicates the quadratic maximum for each species, after which g_{sw} declines.
(bottom) g_{sw} response to Ψ_{leaf}. The blue line indicates TLP for each species (see Appendix, Table X for TLP, modulus of elasticity, and osmotic potential values).

Diurnal Ψ_{leaf} values started around 0.0 to -0.1 MPa for each species on every measurement day. Ψ_{leaf} values were most negative at midday, then declined by sunset, though rarely returned to the Ψ_{leaf} value measured in the early morning of each day. R^2 values ranged from 0.09 (*Typha* spp.) to 0.42 (*S. exigua)* (Figure 4, bottom). Maximum Ψ_{leaf} exceeded TLP for all species. TLP is shown as the vertical blue line in Figure 4 (bottom). TLP did not change over the course of the season (Appendix, Table A1), nor did it change over shorter time periods during leaf rehydration for measurement (data not shown). TLP differed among species, but g_{sw} did not decline until Ψ_{leaf} far exceeded TLP.

Discussion

In this study I investigated the response of stomatal conductance to environmental conditions for five common wetland plants at two different mitigated wetlands. In general, the diurnal patterns of all species were remarkably similar and did not differ between the two sites, which had different hydrologic properties. Unlike previous wetland studies, g_{sw} was highest at midday rather than in the morning. This could be due to the higher frequency of measurements in this study. It is also possible that the different diurnal patterns observed was due to the wetland species included in this study, which were different from the other diurnal studies. Maximum rates of g_{sw} and daily transpiration differed between species.

Of all results, two stood out most: (1) g_{sw} did not consistently decrease with increasing VPD, and (2) g_{sw} rates remained high even when $\Psi_{leaf} <$ TLP. Both results contradict our current paradigm of what controls stomatal conductance and suggests that wetland plants may prioritize carbon uptake over hydraulic safety. These results, which include multiple plant genera and functional groups, also show that TLP is not always an appropriate proxy for hydraulic safety margins.

I measured g_{sw} throughout the day on five species to investigate interspecific differences in stomatal control and the environmental variables that drove changes in g_{sw}. g_{sw} exhibited non-linear responses to all measured variables: solar radiation, VPD, and water potential (Figure 4). The best-fit models for g_{sw} and each response variable were quadratic, suggesting that g_{sw} reaches a threshold for each variable. The following sections discuss each variable response in more detail. Some species had a better fit for a linear relationship for some environmental variables (*C. emoryi* for solar radiation, VPD; *P. deltoides* for solar radiation, g_{sw}), implying that if a threshold existed, it was not reached. This behavior supports the hypothesis that wetland plants regulate stomates in response to solar radiation, VPD, and water potential.

Solar radiation

Solar radiation and g_{sw} were well correlated (Figure 4). This was expected, as solar radiation is the main source of energy for photosynthesis and is known to trigger stomatal opening in the mornings (Hiyama et al. 2017; Matthews, Vialet-Chabrand, and Lawson 2018; 2020). The quadratic fit, where g_{sw} declines above certain solar radiation values (Figure 4), could indicate an upper limit to carbon assimilation or stomatal responses to other environmental conditions. At high solar radiation levels, photosystems are light-saturated and instead limited by carboxylation, which would cause a non-linear response. In addition, photodamage could be occurring, especially when air temperatures were highest, which could also trigger stomatal closure. However, we know stomata are responded to a suite of environmental conditions, and so it is likely that stomata are responding to stress caused by high VPD, T_{leaf}, or decreasing values of leaf water potential occurring at high solar radiation levels (Figure 4).

VPD Response

As expected, species responses to VPD varied (Figure 4). Unexpectedly, all species in this study showed an increase in g_{sw} as VPD increased in the morning (Figure 4). This relationship is surprising, as it is well-established that g_{sw} declines exponentially in response to VPD (Cowan and Farquhar 1977) and is remarkably consistant between species (Oren et al. 1999; Ocheltree, Nippert, and Prasad 2014). It is plausible that g_{sw} increases as a response to solar radiation below a critical VPD threshold, then declines as a response to VPD above that threshold (Figure 5).

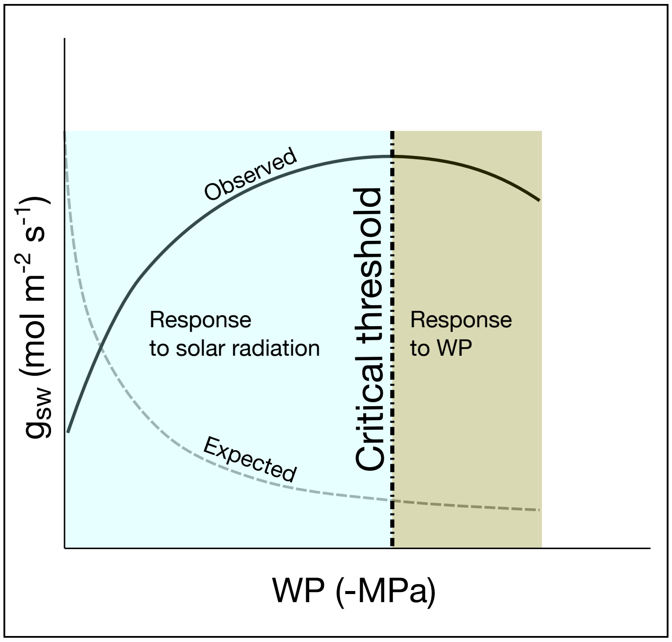

Figure 5: Theoretical and observed stomatal responses to VPD, Ψ_{leaf}, and solar radiation. When g_{sw} increases at low Ψ_{leaf}, it is likely maximizing carbon uptake and responding to solar radiation. When g_{sw} declines at high Ψ_{leaf}, the leaf response to stress is likely overriding its need for carbon uptake.

This behavior could be explained by the need for carbon overriding the relationship between g_{sw} and VPD, especially if plants in saturated soils respond differently to VPD than what previous upland studies have observed. Studies investigating stomatal responses to VPD are rare in wetland systems and are commonly performed in conjunction with precipitation manipulations (Oren et al. 1999; Ocheltree, Nippert, and Prasad 2014; Durand et al. 2019). If VPD and stomatal regulation are decoupled, I would expect to see a strong stomatal response to increases in solar radiation. Given the eventual decline of g_{sw} at high VPD, I propose that g_{sw} is maximizing carbon uptake up to a critical VPD threshold, at which point it does decline in response to high VPD (Figure 5). More research is needed on decoupling of VPD and stomatal regulation in well-watered soils to understand carbon assimilation mechanisms in wetlands, particularly in herbaceous plants.

The results stomatal responses to VPD measured in this study differ from previous work in two main ways; they include values below 1 kPa VPD and the data included diurnal measurements rather than VPD responses at consistently high solar radiation levels. I recorded and analyzed g_{sw} below 1 kPa VPD, where the majority of previous research focuses on VPD values >1. However, the positive relationship between g_{sw} and VPD extended to at least 1.75 kPa before reaching a quadratic maximum. This is not an artifact of measurements from LI-600, as LI-600 VPD_{leaf} values were cross-validated with VPD from a nearby weather station (CoAgMET). Ultimately, these results indicate that these wetland plants may not respond to VPD as strongly as plants in upland systems.

Ψ_{leaf} Response and TLP

The fact that 2022 was hot and dry, and g_{sw} remained high at very negative Ψ_{leaf} (Figure 4)[3] supports the hypothesis that species at the field sites are maximizing carbon uptake at the cost of safety. This may explain the similarity in diurnal g_{sw} patterns across

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[3] Ψ_{leaf} values were as low as to -6 MPa in *C. emoryi* and -3 MPa in other species, and air temperatures were regularly greater than 30°C with VPD above 2 kPa

study species because species maximizing carbon uptake would respond most strongly to solar radiation, with g_{sw} peaking when solar radiation is most intense. It is also possible that these wetland species do not have a strong response to negative Ψ_{leaf}, given that few establish outside a narrow range of specific water-availability and oxygen conditions (García-Baquero et al. 2016). Recently, several studies in mesic climates have observed species in which Ψ_{leaf} is more negative than TLP and that stomatal closure occurred at Ψ_{leaf} more negative than 80% loss of hydraulic conductivity due to embolism (Jin et al. 2023; Bucci et al. 2019). Jin et al. (2023) reviewed stomatal and hydraulic studies across an aridity gradient and found that plants in less arid climates prioritized carbon uptake over hydraulic safety. This could help explain why wetland plants – which exist in a moist microclimate – may be less sensitive to Ψ_{leaf} than upland plants.

I expected that g_{sw} would not exceed turgor loss point, based on safety strategies which have been observed under drought in a variety of ecosystems (Novick, Miniat, and Vose 2016; Jacob et al. 2022; Choat et al. 2012). Instead, the highest g_{sw} rates in this study occurred when Ψ_{leaf} was more negative than TLP (Figure 4). This was surprising, as a decline in g_{sw} when Ψ_{leaf} approaches TLP has been well-documented in systems including wetlands. This is attributed to stomatal response to low Ψ_{leaf} and high VPD (Otieno et al. 2012; Bucci et al. 2019; T. J. Brodribb and Holbrook 2004), and TLP is considered a robust indicator of stomatal response to water deficit (Bartlett et al. 2014; Meinzer et al. 2016). Even though some species have been observed maintaining high g_{sw} past TLP (Farrell, Szota, and Arndt 2017; Guyot, Scoffoni, & Sack 2012; Scholz et al. 2014), I am unaware of any studies that have observed this across functional groups.

These results raise the question of what happens to leaves in well-watered systems when Ψ_{leaf} exceeds TLP thresholds, and they could mean that TLP is an inappropriate proxy for embolism or hydraulic damage in wetland plants. In this study, g_{sw} did not decline until Ψ_{leaf} was far more negative than TLP (Figure 4). In upland plants, it is generally accepted that this would indicate catastrophic water loss and result in likely irreversible embolism (Choat et al. 2019). It does not appear that these species experienced catastrophic embolism during times of highly negative Ψ_{leaf}, as I would have expected to see (1) Ψ_{leaf} below -0.1 MPa in the morning, and (2) evidence of mass leaf mortality at the sites. Neither were observed in these sites and species during the experiment.

Current evidence suggests that TLP can adjust quickly in leaves (Bartlett et al. 2014; Johnson et al. 2018), but I am confident this does not explain the patterns discussed above. In this experiment, Ψ_{leaf} < TLP was not a function of instrument error nor of adjustments to TLP or Ψ_{leaf} dis-equilibrium. Ψ_{leaf} instruments were calibrated before beginning diurnal measurements. TLP was measured twice in the growing season, and values estimated later in the growing season (July) did not change significantly from those measured in early June (Appendix, Table A1).

As some hydraulic failure studies note a disequilibrium between Ψ_{leaf} and Ψ_{stem} due to hydraulic segmentation (Zimmerman 1978, Pivovaroff, Sack, and Santiago 2014), I tested whether Ψ_{leaf} measurements were dis-equilibrated. I conducted a sensitivity analysis on *T. latifolia*, the species which Ψ_{leaf} most exceeded TLP (Figure 4).

In late August, I randomly selected *T. latifolia* leaves (n=18), cut them to 1/3 of the leaf, placed them in individually sealed plastic bags, then measured Ψ_{leaf} without replacement every 10 minutes for an hour (n=3 per interval). Ψ_{leaf} did not become less negative after sitting in a sealed plastic bag; rather, Ψ_{leaf} became slightly more negative over the hour (Appendix, Figure A1). I found no evidence of disequilibrium, so this phenomenon cannot explain midday Ψ_{leaf} being more negative than TLP. Ultimately, these results challenge our existing understanding of what can be inferred about leaf function through bulk TLP, and what occurs to photosynthetic mechanisms past TLP, particularly in water-abundant ecosystems.

Conclusion

This study demonstrates that wetland plants respond differently than upland to environmental conditions, which affects transpiration rates and wetland water loss. Within the wetland system, plants provide well-established benefits, such as water quality improvements, habitat, and flood mitigation. Many wetland plant species only exist in a narrow range of water and oxygen conditions (García-Baquero et al. 2016). Understanding species' stomatal responses to high VPD, high solar radiation, and extremely negative Ψ_{leaf} is key to managing this critical ecosystem as the climate changes.

CHAPTER 2: MANAGEMENT APPLICATIONS: WETLAND PLANT SPECIES COMPOSITION INFLUENCES SITE WATER-USE

Introduction

Wetlands and riparian corridors provide outsized benefits in terms of wildlife habitat and flood mitigation (Wohl et al. 2021); restoring impaired wetland landscapes thus increases the benefits they provide. However, in the arid west there is a tension between wetland restoration and water rights, to the point where Colorado legislature passed a bill in 2023 protecting minor stream restoration projects from water rights litigation (SB 23-270). SB 270 is a consequence of the potential increase in water loss due to high wetland evapotranspiration (ET) rates, which are due to higher water tables (Maxwell and Kollet 2008; Maxwell and Condon 2016). Measuring ET and understanding how each component of evapotranspiration contributes to total water loss is an ongoing challenge, and current methodologies do not allow for accurate total ET measurements in small wetlands adjacent to open water (Stoy et al. 2019). Thus, partitioning ET into evaporation and transpiration is the best way to understand system water-use. Quantifying transpiration of wetland plant communities is the critical step in accurately allocating water rights for restoration projects. Plant communities can often be designed and managed, enabling measurement and control of wetland transpiration.

The primary objective of this study is to investigate whether wetland species composition affects wetland water consumption. Evaluating which wetland community types use the most water can help identify plant communities to prioritize when water loss must be minimized.

Study Site Description

The Front Range is a semi-arid grassland in the South Platte River basin and is characterized by rapid population growth. Wetlands are a rare and critical ecosystem in the Front Range; while only 2% of its land area is wetland, over 80% of wildlife in the area rely on wetlands for habitat, food, and nesting (Culver et al. 2013). Due to the regional aridity, wetlands primarily establish adjacent to bodies of water, including rivers and lakes. The wetland types most common in this region are marshes, existing as either small depressional features or along the banks of lakes and reservoirs, and riparian wetlands.

I selected 5 restored wetland sites to quantify species composition and 2 sites to make detailed water-use measurements. Sites were selected from a list of CDOT wetland restoration projects in the Front Range that received compensatory mitigation credits (Figure 6). These sites were within 2 hours driving of Fort Collins, between 4,500 and 6,000 feet in elevation, a minimum of 1 acre, and were restored between 2007 and 2015. During the growing season of 2021, percent vegetative canopy cover was estimated for each site.

Two of these sites were also selected to test hypotheses about plant water-use strategies: McMurry Natural Area (“McMurry”) and St. Vrain State Park Terrace site (“St. Vrain”). These sites were selected due to similar wetland types, species composition and establishment year. McMurry is in northern Fort Collins, CO along the fringe of ponds created from gravel mining operations in the floodplain of the Cache la Poudre

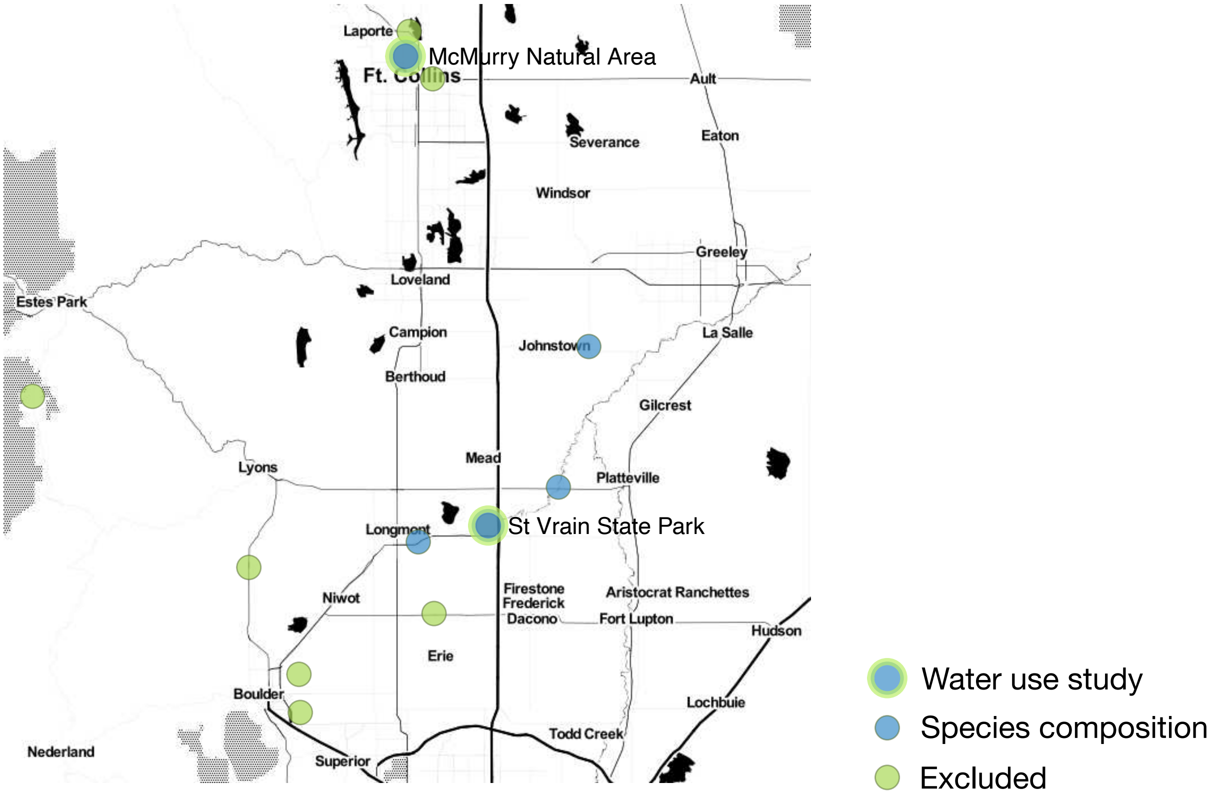

Figure 6: Map of study sites. Species composition was recorded from five sites, which were similar in elevation, appeared to have natural hydrology, and were biodiverse enough to contain more than two dominant plant species.

River. Initial mitigation of the 1.5 acres began in 2013 and was re-graded and re-planted in 2014 after destructive floods (Roth 2020). The restored wetlands at McMurry are a pond fringe and a depression along an outflow stream connecting to the Cache la Poudre River. The wetlands contain willow thickets, young cottonwoods, wet meadow with some willow, and cattail/bulrush marshes. St. Vrain is in Firestone, CO adjacent to St. Vrain Creek. The 6 acre depressional wetland for this mitigation project was created through excavation in 2014 and officially completed in 2016 (Roth 2019). The wetland is separated from St. Vrain Creek by a berm, and contains willow thickets, wet meadows, and cattail marshes.

Methods

Species selection

Species percent cover data was estimated using Line-Point Intercept (LPI) methods as defined in the BLM draft AIM wetland protocol to obtain percent cover (Reynolds et al. 2021). Three transects were randomly placed within each wetland, with a pin dropped every half meter along the transect (n=50 per transect). At each pin drop, every species that the pin touched was recorded as a "hit." If the canopy extended above the pin, any hits above the pin were estimated by eye and recorded. Ground cover (soil, rock, moss, water) was also recorded.

Five focal species were selected for evaluating plant-water use strategies, representing the most abundant species in the study system (Figure 7), including *Salix exigua* (Coyote Willow), *Populus deltoides* (Plains Cottonwood), *Typha latifolia.* (Cattail), *Phalaris arundinacea* (Reed Canarygrass), and *Carex emoryi* (Emory Sedge). *S. exigua* has one of the largest ranges of any North American willow and is abundant in riparian areas and wetlands across Colorado. *P. deltoides* is a common tree species in riparian areas, mostly occurring in the plains. Both species provide critical habitat for wildlife. *T. latifolia* is a widespread plant that grows in slow-moving or still water and tolerates poor soil and low oxygen conditions. It provides food for many species, from waterfowl to ungulates. *P. arundinacea* is a vigorous grower which will outcompete many other herbaceous species and is broadly considered invasive, as its invasion fundamentally alters community composition and results in negative effects for species from multiple taxa (Lavergne and Molofsky 2004; Annen, Kirsch, and Tyser 2008;

Spyreas et al. 2010). *C. emoryi* is a native sedge. It is common in riparian areas and lake or pond fringes and provides nesting cover for waterfowl and rodents.

Water-use Measurements

Daily water-use of the five species was measured monthly through the growing season on June 2, June 4, July 8, July 10, and August 8 and 9. The LI-600 porometer (LI-COR Environment, Lincoln, NE, USA) was used to measure transpiration (mmol m^{-2} s^{-1}) and stomatal conductance (mol m^{-2} s^{-1}). The LI-600 measures transpiration as a function of leaf area, air flow rate (μmol s^{-1}), and the water vapor concentration in the air and in the leaf (mmol H_2O mol_{air}^{-1}).

Stomatal conductance was measured on fully-grown, healthy leaves randomly selected across the entire site area. Because stomatal conductance changes throughout the day, measurements were taken every 1.5 hours for each focal species (n=5 per species) across a 14-hour period beginning just before dawn. Each measurement took 3 –5 seconds, enabling 5 repetitions per species per time point.

Estimating Wetland Water Loss

To estimate community-level transpiration, I combined estimates of daily water-use and leaf area index (LAI) of each species. Daily transpiration of each species was calculated for each species as the area under the diurnal transpiration curve from each day using a trapezoidal integration method (see Kabenge and Irmak 2012). Each daily transpiration estimate (mmol m^{-2} d^{-1}) was scaled to ground area by multiplying daily transpiration by the species' LAI.

LAI was calculated using light intensity measurements taken in August at McMurry Natural Area. LAI measurements were taken with a pyranometer and light meter (LI-200R and LI-250A, LI-COR Environment, Lincoln, NE, USA) above and below the canopy of each study species (n=10 per canopy). These measurements were then used in the Beer-Lambert law, solved for L, to calculate LAI:

$$L = \frac{ln\left(\frac{I}{I_o}\right)}{-k}$$

where I is the incident radiation below the canopy of interest, I_o is the incident radiation above the canopy of interest, k is an extinction coefficient pulled from estimates in literature, and L is leaf area index.

K-coefficients were pulled from literature from a search of studies that directly measured LAI (Nagler 2004; Williams et al. 2017). K-coefficients were not available for all species in this study, and so proxy species of similar function and form were used as necessary. Table 3 shows the study species, the proxy species (as necessary), and k-coefficients. LAI estimates for each species were multiplied by daily transpiration to scale to ground area. I then used the August LAI of each species to convert leaf-level transpiration estimates to transpiration per unit ground area using species-specific LAI. LAI values ranged from 1.13 (*T. latifolia*) to 3.15 (*P. arundinacea)*. *P. deltoides* and *S. exigua* k-coefficients were taken from a study with similar species in a semi-arid riparian environment.

Table 3: k-coefficients and proxy species (as necessary), and LAI values.

Study species	**Species in literature**	**K**	**LAI**
P. arundinacea	*P. arundinacea*	0.75	3.15
S. exigua	*Salix gooddingii*	0.6	2.68
P. deltoides	*Populus fremontii*	1.25	1.71
C. emoryi	*Carex atherodes*	0.8	1.54
T. latifolia	*T. latifolia*	0.83	1.13

Results

Species composition differs across sites

Across sites, the five most abundant species were *S. exigua*, *P. deltoides*, *T. latifolia*, *P. arundinacea*, and *C. emoryi*. Because fifty-nine unique species were identified, not all are included in the abundance figure. A list of all identified species and their abundance at each site can be found in the Appendix (table A2). For the purpose of visualization, species are grouped into the following categories in Figure 7:

- Cattail (*Typha* species);
- Grass (including *P. arundinacea, Polypogon monspeliensis,* and *Poa palustris*);
- Herbaceous (all dicot forbs, including but not limited to *Cirsium arvensis*, *Verbena hastata,* and *Lycopus asper*);
- Rush (including *Juncus balticus, J. gerardii,* and *J. torreyi*);
- Sedge (including *C. emoryi, C. scoparia,* and *Eleocharis palustris*);
- Tree (*Populus deltoides* and *Gleditsia triacanthos*); and
- Willow (*S. exigua, S. fragilis, and S. ligulifolia*).

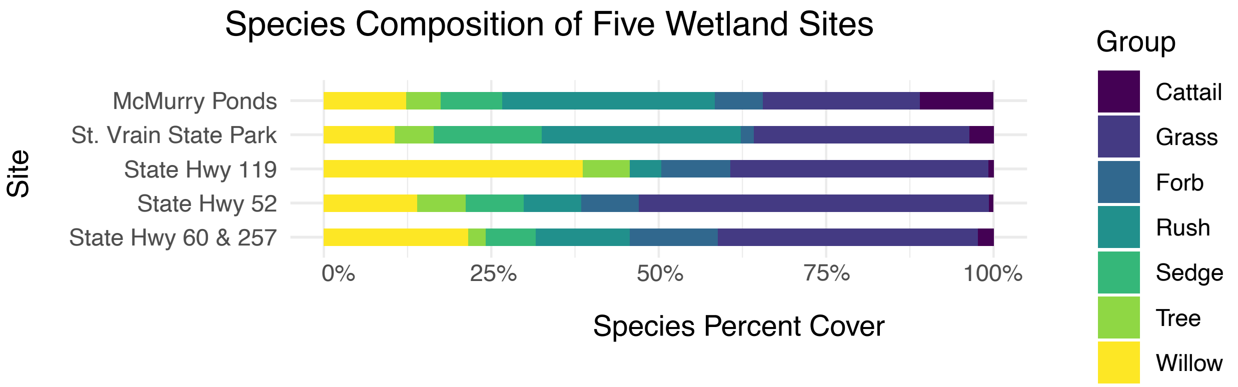

Figure 7: Species composition of wetland sites by functional group. Most sites are dominated by grass and rush species and have more willow canopy than tree (non-willow woody species) cover. For complete species composition data, see Appendix (table A2).

Together, grasses, rushes, and sedges comprised over 50% of sites. The site with the most grass species was State Highway 52, a riparian site with little tree and shrub cover. Willows were common at most sites, though their presence ranged from 10% cover at St. Vrain State Park to 33% cover at State Highway 119 (Figure 7).

P. arundinacea and *S. exigua* were the most abundant species across all sites. *S. exigua* is a shrubby willow native to the Colorado plains and generally considered beneficial for habitat, food, and bank stabilization. *P. arundinacea* is a perennial grass which dominates wetlands across North America and is broadly considered invasive and has been found to negatively impact plant communities by reducing biodiversity (Werner and Zedler 2002; R. D. Foster and Wetzel 2005).

The sites at State Highway 119 and State Highway 60 & 257 were both heavily dominated by *P. arundinacea* and *S. exigua*. Both sites were riparian. The dominance of *P. arundinacea* at these sites is not surprising, as I observed large sediment deposits in spring 2021 and 2022, and *P. arundinacea* commonly outcompetes other plant communities in sediment deposits (Maurer et al. 2003). *S. exigua* also readily establishes in riparian zones, regardless of whether it was intentionally planted. Given the similarity in species composition between the two riparian sites, I would have also expected State Highway 52 to have similar species composition. Instead, it had a diverse wet meadow community on the north side of the site between a berm and an upland. Water level differences may have played a role in species differences.

The sites with a more diverse species list were McMurry Natural Area, St. Vrain State Park, and State Highway 52. These sites do not share many common characteristics in terms of hydrology or construction; McMurry is a pond fringe site with

5 separate wetland areas ranging from 0.02 acres – 0.76 acres in size, and predominantly features wet meadow and willow. Its soils are a mix of sand, clay, and cobble. McMurry received significant sediment deposition after flooding in 2012 and was re-graded and re-planted. The site’s topography is mostly homogenous and gently slopes up from the ponds. St. Vrain is a 6 acre depressional wetland that is separated from St. Vrain Creek by a berm, and predominantly features wet meadow, cattail marsh, and willow. Its soils are predominantly clay. Microtopography was a key feature during its construction, with approximately 6” troughs installed across the depression to provide niches to different species. The depression features a low-lying cattail marsh, surrounded by slightly dryer wet meadows and willow thickets. Lastly, State Highway 52 is a narrow riparian wetland abutting Boulder Creek totaling 0.51 acres. Boulder Creek floods the wetland seasonally when snow runoff peaks. Its soils are predominantly sand and cobble, with pockets of clay in depressions. The topography of this site varies the most of the three due to its narrow area; it is comprised of low river fringe, wet depressions, and a sloping transition from wetland to upland. Given their differences in restoration type and site history, little can be concluded about why these three sites supported more biodiversity than the others.

Transpiration differs between species

In this study, the focal species transpired different amounts of water. *S. exigua* lost the most water through transpiration – 325,000 gallons per acre per month –while *T. latifolia* and *P. deltoides* lost the least (Figure 8). Water losses from *P. arundinacea* and *C. emoryi* were between these. This study was conducted across two sites, and species lost similar amounts of water at both sites despite differences in soil type and groundwater flow.

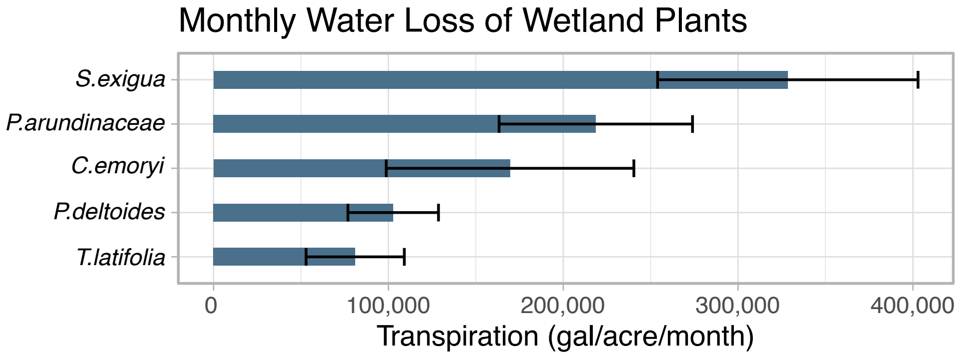

Figure 8: Transpiration for each species in gallons per acre and scaled to one month using measurements from June through August. Confidence intervals indicate that S. exigua is significantly higher in transpiration than C. emoryi, P. deltoides, and T. latifolia.

S. exigua transpired the most at the leaf level AND at the site level, despite having less total leaf area than *P. arundinacea* (Table 3). *P. arundinacea* was the second highest in water loss, which can be attributed to its high total leaf area. *C. emoryi* had mid-level water loss, with higher variability in water loss over time than other species. This is consistent with other *C. emoryi* measurements, which also had significant variability between individuals. *P. deltoides* and *T. latifolia* were very similar in terms of low water loss through transpiration. While old *P. deltoides* has a reputation for high water loss, young *P. deltoides* had a low total leaf area (Table 3), contributing to its low water loss in this study. *T. latifolia* also had less total leaf area than other species.

These data can be scaled to theoretical water loss for wetlands with different species composition (Figure 9). Sites with more *S. exigua* and *P. arundinacea* lose more water through transpiration than other sites, while marshes of *T. latifolia* lose less water. Figure 9 demonstrates how species with high water-use, like *P. arundinacea* and *S. exigua*, drive overall wetland transpiration. Communities with an even balance of species have a median transpiration, meaning that more biodiverse wetlands lose less water to transpiration than wetlands than communities solely comprised of plants with high water-use rates. Wet meadows, partly by nature of having less leaf mass, tend to use less water than willow sites or sites with all study species. Considering that it is difficult to establish wet meadows in wetlands dominated by *P. arundinacea* (Green and

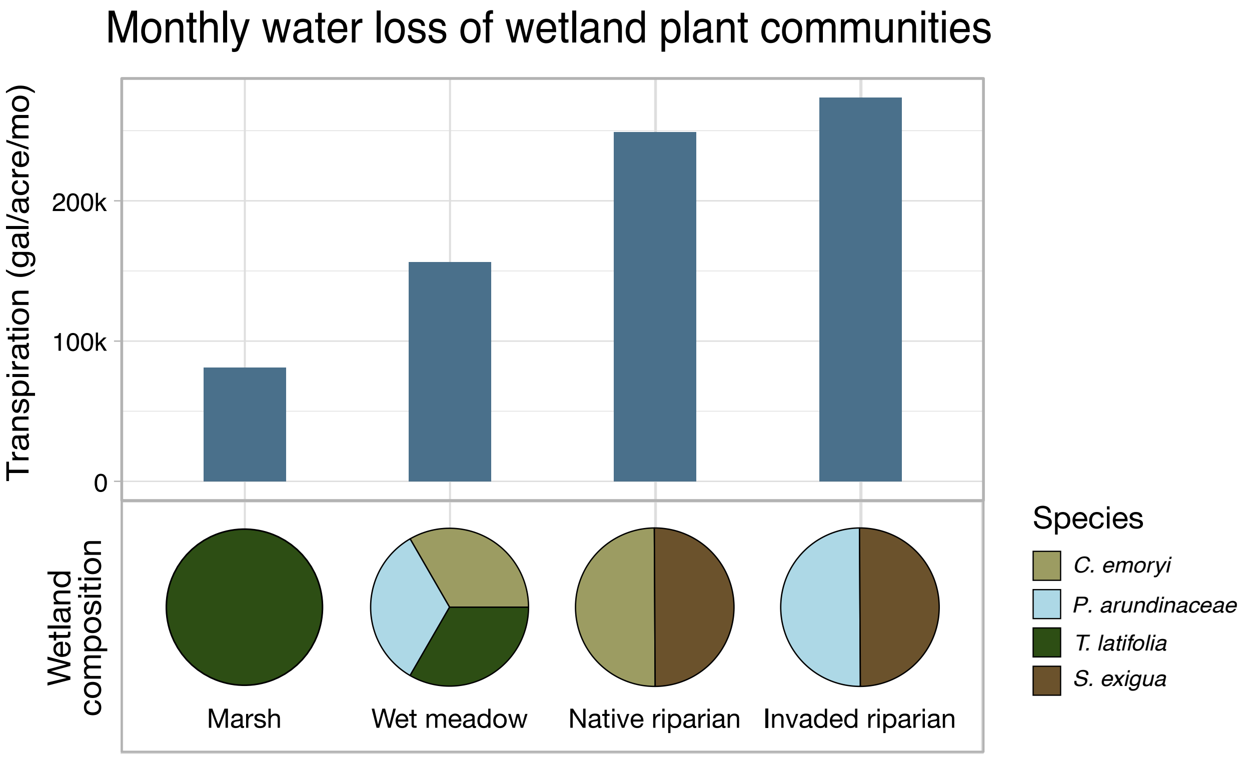

Figure 9: transpiration for different wetland communities in gallons per acre (using average values across the summer) and scaled to one month. Wetland plant community composition is shown with the pie charts along the x axis for the following communities: cattail marsh, wet meadow, native riparian, and invaded riparian.

Galatowitsch 2001; Werner and Zedler 2002), minimizing *P. arundinacea*'s presence is important to minimizing site water use. *P. arundinacea* has much higher water use than native wet meadow species like *C. emoryi*, and once *P. arundinacea* is present, it is difficult to mitigate its spread. Invasion by high water-use plants can significantly increase wetland plant water-use.

Water-use implications for restoring in-kind and with a watershed approach

"In-kind" restoration was first introduced in federal wetlands restoration policy in the Water Resources Development Act of 2000 for bottomland hardwood forests (Ungaro, BenDor, and Riggsbee 2022). In the context of wetland mitigation, in-kind restoration restores or creates wetlands that are as similar as possible to wetlands that are drained, filled or otherwise affected by development and construction ("impacted wetlands"). In terms of plant water loss, in-kind restoration would enable matching the water loss profiles from impacted site to restored site. These results demonstrate that species transpire similar amounts of water regardless of site or location. Thus, transpiration between impacted and restored sites will be similar so long as the species composition is also similar.

However, watershed approaches to restoration are increasing in popularity as they prioritize enhancing the ecological benefits of restoration projects. The principle behind a watershed approach is to consider watershed needs and characteristics for restoration projects and to restore a wetland of high value for the watershed. In Colorado, a watershed approach has been implemented in the Colorado Water Plan of 2015 and the Colorado Wetland Program for 2020-2024. The Colorado Water Plan (2015) states a purpose to "protect and restore watersheds critical to water infrastructure, environmental or recreational areas” and “work on creating resilient watersheds to protect, restore, and enhance water quality in the face of climate change (Colorado Water Conservation Board 2015). The Colorado Wetland Program for 2020-2024, which was developed across many partner restoration organizations, prioritizes watershed-scale planning and restoration for stream and wetland work (Marshall and Lemly 2020).

If applying a watershed approach to wetland restoration, there is a possibility of increasing overall water loss through transpiration, which should be weighed against the many ecological benefits of a watershed approach. For example, cattails transpired the least of all species in this study. If a cattail marsh is impacted and requires compensatory mitigation, a watershed approach might encourage restoring a wetland with higher ecological value such as a wet meadow or riparian willow shrubland. This approach would provide many ecological benefits but would likely increase watershed water-use. Understanding the water-use profile of different wetland plant communities is therefore an important decision-making tool for restoration managers.

Conclusion and Recommendations to Managers

When water-use is an important restoration project consideration, the following recommendations will improve budgeting for plant transpiration and water-use:

Recommendation 1: To ensure plant community transpiration is not higher at restored wetlands, balance species composition between impacted and restored sites.

In this study, species had similar water-use rates at two different wetlands, showing that location alone does not affect plant water-use rates. Thus, a simple method to balance plant water-use budgets between sites is to also balance species composition. For example, if the two wetlands have similar percentages of cattails, grasses, willow shrubs, and trees, the plant water-use would likely be similar for both wetlands. If using this approach, it is not necessary to measure the water-use of every species, as these results suggest that water-use rates of wetland species across functional groups do not differ across sites.

Recommendation 2: When employing a watershed approach to restoration, consider the water-use impact of planting species with high water-use rates.

Watershed approaches to restoration are intended to improve the health and quality of the entire watershed. High water-use plants may provide significant ecological value but should be carefully considered in watershed restoration plans if water conservation is a project concern. Planting managers could theoretically reduce the water-use impact of restoration by substituting high water-use species with lower water-use species that fill the same functional niche.

Recommendation 3: Maximize planting biodiversity to lessen the impact of plants transpiring at high rates.

Mathematically, high biodiversity ensures a wetland plant community with a wide range of water-use. Even among the five species in this study, a site with all five wetland plants had median water-use, which was 125,000 gallons/acre/month less than a site of all *S. exigua* or a mix of *S. exigua* and *P. arundinacea*. Maximizing the biodiversity of a wetland restoration site results in the median theoretical water-use, in effect mitigating for the high water-use of certain species.

The two highest water-use species were *S. exigua* and *P. arundinacea. S. exigua* is a native shrub providing benefits to multiple taxa, including food and habitat for wildlife and pollutant uptake for humans (C. G. Franks, Pearce, and Rood 2019). It is also frequently used in restoration for bank stabilization, critical to enabling the establishment of other desired plant communities (Laub, Detlor, and Keller 2020). Its high water-use should not determine its inclusion in restoration plantings, though if overall wetland water loss is of high concern, other functionally similar shrubs could be included to reduce the impact of *S. exigua*. On the other hand, invasions by *P. arundinacea* should be avoided if possible. *P. arundinacea* will increase the water-use of a site due to its rapid growth and ability to outcompete native wet meadow species. When seeking to reduce wetland evapotranspiration, *P. arundinacea* presence should be minimized.

Recommendation 4: Invest in future water-use studies.

Considering that this research showed a wide range of water-use among species, it will be important to consider additional species, particularly other dominant wetland species. Future research should test variation across additional sites to determine whether intraspecific variation exists across ranges and elevations.

This study only addressed the "T" of ET and did not address evaporation. More research is needed on how hydrology affects ET, and how constructed hydrology may increase or decrease evaporative wetland water loss. Because current ET methodology cannot reliably and accurately measure small, stream-adjacent wetlands as a whole (Drexler et al. 2004; Kool et al. 2014; Ellsäßer et al. 2020), studying evaporation and transpiration separately is the best path forward to quantifying ET in restored wetlands.

CONCLUSION

This study demonstrated that wetland plants respond differently than upland to environmental conditions, which affects transpiration rates and wetland water loss. Understanding species' stomatal responses to high VPD, high solar radiation, and extremely negative Ψ_{leaf} is key to managing this critical ecosystem as the climate changes, as wetland plants provide ecosystem services for humans and habitat for wildlife.

For restoration projects where plant water-use is a concern, this study suggests four key recommendations to managers: (1) Balance species composition between impacted and restored sites to ensure plant community transpiration is not higher at restored wetlands; (2) Consider the water-use impact of planting species with high water-use rates when employing a watershed approach to restoration; (3) Maximize planting biodiversity to lessen the impact of plants transpiring at high rates; and (4) Invest in future water-use studies.

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APPENDIX

Table A1: Turgor loss point, osmotic potential (Π_O), and bulk modulus of elasticity (ε) of focal species. TLP did not increase significantly in any species from June to July. Π_O increased significantly in only T. latifolia. ε increased significantly in S. exigua, T. latifolia, and P. arundinanacea.

Species	**Month**	**TLP**	**Π_O**	**ε**	**n**
Carex emoryi	June	-2.12 ± 0.09	-1.46 ± 0.062	12.99 ± 2.64	5
	July	-2.20 ± 0.11	-1.31 ± 0.12	10.06 ± 1.46	5
Phalaris arundinacea	June	-2.19 ± 0.19	-1.61 ± 0.16	5.67 ± 1.92	4
	July	-1.99 ± 0.09	-1.69 ± 0.082	11.37 ± 1.74	5
Populus deltoides	June	-1.64 ± 0.04	-1.09 ± 0.042	11.75 ± 0.574	5
	July	-1.53 ± 0.11	-1.10 ± 0.11	13.91 ± 1.16	5
Salix exigua	June	-1.38 ± 0.09	-0.95 ± 0.067	7.10 ± 1.06	5
	July	-1.52 ± 0.06	-1.08 ± 0.011	10.36 ± 1.43	5
Typha latifolia	June	-0.87 ± 0.05	-0.37 ± 0.038	1.70 ± 0.418	4
	July	-1.04 ± 0.15	-0.60 ± 0.054	5.62 ± 1.15	5

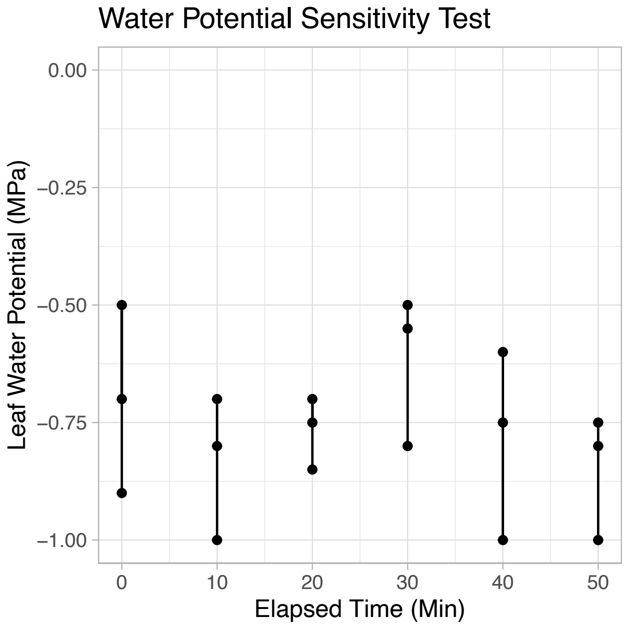

Figure A1: No evidence of disequilibrium was found when conducting a sensitivity analysis. Leaves sampled at the same time then placed in a plastic bag for increasing durations did not show any positive adjustment to Ψ_{leaf} over time.

Table A2: Species Composition from Five Wetland Sites

Site	**Name**	**Percent**
McMurry Ponds	*Astragalus anisus*	0.002
McMurry Ponds	*Asclepius incarnata*	0.002
McMurry Ponds	*Carex atherodes*	0.002
McMurry Ponds	*Carex emoryi*	0.051
McMurry Ponds	*Carex nebrascensis*	0.009
McMurry Ponds	*Carex scoparia*	0.022
McMurry Ponds	*Carex vulpinoidea*	0.004
McMurry Ponds	*Cirsium arvensis*	0.011
McMurry Ponds	*Cymopterus acaulis*	0.007
McMurry Ponds	*Eleocharis palustris*	0.009
McMurry Ponds	*Unknown perennial graminoid*	0.002
McMurry Ponds	*Juncus ensifolius*	0.002
McMurry Ponds	*Juncus balticus*	0.136
McMurry Ponds	*Juncus gerardii*	0.007
McMurry Ponds	*Juncus torreyi*	0.007
McMurry Ponds	*Lycopus asper*	0.009
McMurry Ponds	*Mentha arvensis*	0.007
McMurry Ponds	*Muhlenbergia asperifolia*	0.013
McMurry Ponds	*Pascopyrum smithi*	0.009
McMurry Ponds	*Panicum virgatum*	0.007
McMurry Ponds	*Unknown perennial grass*	0.007
McMurry Ponds	*Unknown perennial grass*	0.002
McMurry Ponds	*Phalaris arundinacea*	0.141
McMurry Ponds	*Populus deltoides*	0.049
McMurry Ponds	*Polygonum lapathifolium*	0.002
McMurry Ponds	*Poa leptocoma*	0.002
McMurry Ponds	*Polypogon monspeliensis*	0.036
McMurry Ponds	*Poa palustris*	0.013
McMurry Ponds	*Polygonum ramosissium*	0.004
McMurry Ponds	*Ribes inerme*	0.002
McMurry Ponds	*Salix amygdaloides*	0.002
McMurry Ponds	*Salix exigua*	0.105
McMurry Ponds	*Salix fragilis*	0.011
McMurry Ponds	*Salix ligulifolia*	0.004
McMurry Ponds	*Schoenoplectus pungens*	0.002
McMurry Ponds	*Schoenoplectus tabernamontanii*	0.157
McMurry Ponds	*Stipa pinetorum*	0.009
McMurry Ponds	*Thlaspi arvense*	0.002
McMurry Ponds	*Typha ssp.*	0.110
McMurry Ponds	*Verbena hastata*	0.018
St. Vrain State Park	*Carex atherodes*	0.114
St. Vrain State Park	*Cirsium arvensis*	0.008
St. Vrain State Park	*Clover*	0.003
St. Vrain State Park	*Distichlis spicata*	0.072
St. Vrain State Park	*Eleocharis palustris*	0.053
St. Vrain State Park	*Hordeum jubatum*	0.006
St. Vrain State Park	*Juncus balticus*	0.178

St. Vrain State Park	*Juncus gerardii*	0.036
St. Vrain State Park	*Juncus torreyi*	0.022
St. Vrain State Park	*Muhlenbergia asperifolia*	0.036
St. Vrain State Park	*Panicum virgatum*	0.069
St. Vrain State Park	*Unknown dicot forb*	0.008
St. Vrain State Park	*Phalaris arundinacea*	0.006
St. Vrain State Park	*Populus deltoides*	0.058
St. Vrain State Park	*Poa leptocoma*	0.003
St. Vrain State Park	*Polypogon monspeliensis*	0.100
St. Vrain State Park	*Poa palustris*	0.031
St. Vrain State Park	*Salix exigua*	0.083
St. Vrain State Park	*Salix fragilis*	0.022
St. Vrain State Park	*Schoenoplectus pungens*	0.047
St. Vrain State Park	*Schoenoplectus tabernamontanii*	0.008
St. Vrain State Park	*Typha ssp.*	0.036
State Hwy 119	*Agrostis idahoensis*	0.016
State Hwy 119	*Carduus nutans*	0.008
State Hwy 119	*Cirsium arvensis*	0.016
State Hwy 119	*Eleocharis palustris*	0.039
State Hwy 119	*Gleditsia triacanthos*	0.031
State Hwy 119	*Juncus balticus*	0.008
State Hwy 119	*Gleditsia triacanthos*	0.008
State Hwy 119	*Unknown perennial grass*	0.008
State Hwy 119	*Unknown perennial grass*	0.008
State Hwy 119	*Phalaris arundinacea*	0.362
State Hwy 119	*Populus deltoides*	0.024
State Hwy 119	*Polygonum lapathifolium*	0.039
State Hwy 119	*Poa palustris*	0.008
State Hwy 119	*Ribes inerme*	0.016
State Hwy 119	*Salix exigua*	0.370
State Hwy 119	*Salix ligulifolia*	0.016
State Hwy 119	*Sagittaria cuneata*	0.016
State Hwy 119	*Typha ssp.*	0.008
State Hwy 52	*Carex emoryi*	0.013
State Hwy 52	*Eleocharis palustris*	0.026
State Hwy 52	*Juncus balticus*	0.013
State Hwy 52	*Juncus torreyi*	0.033
State Hwy 52	*Mentha arvensis*	0.046
State Hwy 52	*Muhlenbergia asperifolia*	0.053
State Hwy 52	*Panicum virgatum*	0.033
State Hwy 52	*Phalaris arundinacea*	0.185
State Hwy 52	*Populus deltoides*	0.073
State Hwy 52	*Polygonum lapathifolium*	0.026
State Hwy 52	*Polypogon monspeliensis*	0.232
State Hwy 52	*Poa palustris*	0.020
State Hwy 52	*Rumex crispus*	0.007
State Hwy 52	*Salix exigua*	0.132
State Hwy 52	*Salix fragilis*	0.007
State Hwy 52	*Schoenoplectus pungens*	0.073

State Hwy 52	*Schoenoplectus tabernamontanii*	0.013
State Hwy 52	*Typha ssp.*	0.007
State Hwy 52	*Verbena hastata*	0.007
State Hwy 60 & 257	*Astragalus anisus*	0.030
State Hwy 60 & 257	*Carex atherodes*	0.014
State Hwy 60 & 257	*Carex emoryi*	0.033
State Hwy 60 & 257	*Carex nebrascensis*	0.016
State Hwy 60 & 257	*Carex scoparia*	0.002
State Hwy 60 & 257	*Carex vulpinoidea*	0.007
State Hwy 60 & 257	*Cirsium arvensis*	0.052
State Hwy 60 & 257	*Cymopterus acaulis*	0.005
State Hwy 60 & 257	*Eleocharis elongata*	0.005
State Hwy 60 & 257	*Eleocharis palustris*	0.042
State Hwy 60 & 257	*Helianthus nuttalli*	0.007
State Hwy 60 & 257	*Juncus balticus*	0.070
State Hwy 60 & 257	*Juncus torreyi*	0.002
State Hwy 60 & 257	*Unknown forb (legume?)*	0.005
State Hwy 60 & 257	*Unknown dicot forb*	0.007
State Hwy 60 & 257	*Mentha arvensis*	0.005
State Hwy 60 & 257	*Mentha officinalis*	0.002
State Hwy 60 & 257	*Muhlenbergia asperifolia*	0.014
State Hwy 60 & 257	*Pascopyrum smithi*	0.016
State Hwy 60 & 257	*Panicum virgatum*	0.019
State Hwy 60 & 257	*Phalaris arundinacea*	0.290
State Hwy 60 & 257	*Populus deltoides*	0.019
State Hwy 60 & 257	*Polypogon monspeliensis*	0.035
State Hwy 60 & 257	*Poa palustris*	0.014
State Hwy 60 & 257	*Rumex crispus*	0.016
State Hwy 60 & 257	*Salix exigua*	0.208
State Hwy 60 & 257	*Salix fragilis*	0.002
State Hwy 60 & 257	*Salix ligulifolia*	0.005
State Hwy 60 & 257	*Schoenoplectus pungens*	0.002
State Hwy 60 & 257	*Schoenoplectus tabernamontanii*	0.021
State Hwy 60 & 257	*Symphoricarpos albus*	0.007
State Hwy 60 & 257	*Typha ssp.*	0.023
State Hwy 60 & 257	*Verbena hastata*	0.002