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Flooding tolerance of native and nonnative grasses: Variation in photosynthesis, transpiration, respiration, and carbon isotope discrimination

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FLOODING TOLERANCE OF NATIVE AND NONNATIVE GRASSES:

VARIATION IN PHOTOSYNTHESIS, TRANSPIRATION,

RESPIRATION, AND CARBON ISOTOPE

DISCRIMINATION

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ABSTRACT

Invasion by nonnative plants is particularly prevalent in wetlands. While the ecological patterns in wetland plants are well known, it is less well known how flooding-related soil conditions influence the physiological success of introduced species in wetlands. In chapter 1, effects of flooding were measured in invasive common reed (*Phragmites australis*), reed canarygrass (*Phalaris arundinacea*), johnsongrass (*Sorghum halepense*), and native prairie cordgrass (*Spartina pectinata*). The four species were kept at four levels of flooding (deep flooding, medium flooding, low flooding, and dry conditions), and their responses were measured after 7 and 28 days of treatment using by a Li-Cor LI-6400 photosynthesis and fluorescence system. Measurements included light harvesting abilities, CO_2 fixation rates, leaf carbon isotope ratios, and root anaerobic enzyme activities. CO_2 fixation and light harvesting abilities in *Phragmites* were maximized at deep flooding conditions whereas they were maximized in *Phalaris* at medium flooding conditions. Light harvesting abilities in *Sorghum* were maximized at deep flooding conditions after 7 days. However, at 28 days most of the *Sorghum* had died. Native *Spartina* had the lowest light harvesting and CO_2 fixation abilities after 7 days of flooding. After 28 days of flooding, light harvesting abilities of *Spartina* were maximized at deep flooding levels, but the rates were lower than *Phragmites*. In chapter 2, flooding-sensitive *Sorghum halepense* and flooding-tolerant *Phragmites australis* (n=5) were flooded to 8 cm depth or kept dry for 7 days. Transpiration, stomatal conductance, boundary layer conductance, and vapor conductance were measured for each. Transpiration was significantly higher in drained treatments compared to flooded treatments for *Sorghum*. However, transpiration was significantly higher in flooded

treatments compared to drained treatments for *Phragmites*. Thus, there was a significant species x treatment interaction in transpiration. A similar interaction was detected in both stomatal and vapor conductances. *Phragmites* had increased stomatal conductance when flooded, which indicated a high physiological tolerance to waterlogged soils. This allowed *Phragmites* to photosynthesize under waterlogged conditions and to be successful as wetland invaders. Further information on the conditions that maximize stomatal opening for *Phragmites* can help management efforts. By contrast, stomatal conductance in *Sorghum* was decreased under flooding, indicating a greater sensitivity to flooding. The sampled population of *Sorghum* is therefore not a threat to invade chronically flooded soils based on these results. Additional work will be needed to test the ability of *Sorghum* to acclimate to wet environments. Increased photosynthesis rates under flooded conditions, especially in short-term flooding, might help invasive grasses to invade wetland settings.

ACKNOWLEDGMENTS

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PREFACE

Chapter one is written in the style of <u>Environmental and Experimental Botany</u> and chapter two is written in the style of <u>Invasive Plant Science and Management</u>. These are the two journals to which each chapter will be submitted shortly after the defense of this thesis.

CHAPTER 1: PHOTOSYNTHETIC VARIATION AND CARBON ISOTOPE

DISCRIMINATION IN RESPONSE TO FLOODING IN NATIVE

AND INVASIVE GRASSES OF CENTRAL KANSAS

ABSTRACT

Leaf-level photosynthesis is necessary for ecological processes like plant growth
and reproduction. Moreover, the success of wetland invaders is dependent on
physiological processes like photosynthesis. Despite the link between physiological
tolerance and ecological success, effects of flooding on photosynthesis in wetland plants
is not well understood. In this study, invasive potential was compared to physiological
flooding tolerance in four wetland grasses. Experimental plants were flooded for 28 days,
including nonnative, invasive *Phragmites australis*, *Phalaris arundinacea*, *Sorghum
halepense*, and native *Spartina pectinata*. They were maintained at four levels of flooding
(deep flooding, medium flooding, low flooding, and dry conditions), and their responses
were measured at 7 and 28 days of treatment. Photosynthetic rates in *Sorghum* were
maximized at deep flooding conditions at 7 days, but at 28 days all deep flooded
Sorghum had died. Photosynthesis of *Phragmites* and *Spartina* were maximized at deep
flooding conditions whereas photosynthesis was maximized in *Phalaris* at medium
flooding at 28 days. Photosynthetic abilities of *Spartina* in medium and low flooding
were significantly lower than *Phragmites* and *Phalaris*. Analyses of leaf $\delta^{13}C$ supported
the gas exchange results. Flooding caused stomatal closure in the C_3 species *Phalaris*,

leading to an increase in $\delta^{13}C$. The opposite occurred in C_3 *Phragmites*. As flooding

increased, $\delta^{13}C$ decreased, indicating higher stomatal conductance under flooding. As

flooding increased in the C_4 species, $\delta^{13}C$ in *Spartina* decreased, but $\delta^{13}C$ remained

unchanged in *Sorghum*. Differences in photosynthetic abilities and leaf $\delta^{13}C$ suggested

Phragmites and *Spartina* were the most flood tolerant of the four species. *Phalaris* was

moderately flood tolerant and *Sorghum* was flood sensitive. Activities of the anaerobic

respiration enzyme alcohol dehydrogenase in roots also suggested invasive *Phragmites*

was more tolerant of flooding compared to the native *Spartina*. Invasion of grasses in

wetlands might be helped by increased photosynthesis in short-term flooding. Success at

the physiological level might be an important clue in determining community-level

processes in flooded environments.

INTRODUCTION

Nonnative plants are introduced in an area due to intentional or accidental methods (Pysek et al. 2004). Introduced plants can become invasive by reproducing in large numbers and dispersing large distances from the parent plant (Richardson et al. 2000). Invasive plants can decrease biodiversity and heterogeneity of an area (Kercher and Zedler 2004). Certain areas seem to be more susceptible to invasion than others (Mack et al. 2000). For example, disturbance can open an area to invasion (Lavergne and Molofsky 2004).

Invasion by nonnative plants is especially prevalent in wetlands. According to Zedler and Kercher (2004), 24% of the most invasive plants worldwide occur in wetlands, despite the small amount of land cover classified as wetlands (<6%). Wetlands are fragmented and fragile ecosystems (Keddy 2000). Zedler and Kercher (2004) summarized reasons for the prevalence of invasive plants in wetlands, including wetlands being landscape sinks and the easy dispersal of seeds or plant parts by water or flotation.

Grasses are common invaders due to their ability to alter ecosystems (Lavergne and Molofsky 2004). Wetlands of central Kansas have been invaded by many exotic plants including many grasses (Schweiger et al. 2002). *Phragmites australis* (Cav.) Trin. Ex Steud. (hereafter "*Phragmites*"), *Phalaris arundinacea* L. (hereafter "*Phalaris*"), and *Sorghum halepense* (L.) Pers. (hereafter "*Sorghum*") are three common invaders of waterlogged soils in Kansas. The ecology of *Phragmites* and *Phalaris* has been studied extensively to determine whether these species use flooding as a mechanism for invasion (Coops et al. 1996, Miller and Zedler 2003, Kercher and Zedler 2004, Fraser and

Karnezis 2005, Fraser and Miletti 2008, Jenkins et al. 2008). However, no study has identified the physiological advantages that allow grasses to be effective invaders. By understanding the physiological reactions to flooding, the processes through which *Phragmites*, *Phalaris*, and *Sorghum* successfully invade wetlands will be better understood.

Hydrological regime is considered the most important disturbance that influences composition of a wetland (Fraser and Karnezis 2005). Flooding is a prominent stress on plants because it causes the soil to become anoxic. Thus, submerged portions of the plant are unable to acquire oxygen from the soil. One common method for measuring responses of plants to flooding is an increase in alcohol dehydrogenase (ADH) activity in roots (Crawford and Braendle 1996, Maricle et al. 2006). ADH is an enzyme that catalyzes the final reaction in ethanol fermentation. An increase of ADH activity demonstrates an increased ability to respire anaerobically, so it is viewed as a measure of anoxia tolerance (Crawford 1967). However, a plant having a lower ADH activity in flooded conditions also might indicate it is not sensitive to flooding. A lower ADH activity could suggest the plant can move O_2 from leaves to roots to support aerobic respiration (Crawford 1967, Maricle et al. 2006). Therefore, ADH activities are valuable for studies on flooding, especially when combined with other measures.

Species that are better able to tolerate waterlogged soils will have an advantage as a wetland invader due to increased carbon gain and growing abilities. Flooding will often cause a plant to close its stomata, which causes a decrease in photosynthesis (Kozlowski 1997). Stomatal conductance (g_s) is a measure of stomatal frequency and the extent to

which individual stomata are open. g_s influences the rate that water vapor escapes from the plant as well as how much CO_2 can enter the leaf for photosynthesis. Studies have shown a decrease in photosynthesis and stomatal conductance in flooded sugarcane (Glaz et al. 2004). Stomatal closure is expected to decrease photosynthetic rates and decrease the internal (substomatal) CO_2 concentration.

Another measurement linking physiological responses to plant performance is carbon isotope discrimination. This measure was used to gauge average stomatal conductance, and was paired with CO_2 fixation rates to measure carbon used in photosynthesis. There are isotopic differences between C_3 and C_4 photosynthetic plants. C_3 plants tend to be lighter due to a preference of ^{12}C over ^{13}C (O'Leary 1988). The opposite is true for C_4 plants, which prefer ^{13}C for the initial reaction in photosynthesis (Maricle and Lee 2006).

Absorbed photon energy can support photochemistry, or it can be re-released as light or heat. Absorbed photon energy re-released as light is called chlorophyll fluorescence. It can be measured to determine the fate of absorbed light, and to assess changes to photosystem II (PSII). Combining the changes in photosynthetic rate due to flooding with changes in chlorophyll fluorescence can explain the fate of excess photon energy in stressed plants.

In the present study, native and invasive grasses were grown under flooding treatments in greenhouse studies. Native *Spartina pectinata* Bosc ex Link (hereafter "*Spartina*") was included for comparison with the introduced grasses. Changes in photosynthetic rates (A), stomatal conductance (g_s), and internal leaf (substomatal) CO_2

concentration (C_i) were expected among treatments in all species. By comparing the changes in A, g_s, and C_i among species and treatments, a relationship between physiological response to flooding and plant performance (e.g., growth, reproduction, and photosynthetic rates) can be explained. It was predicted that invasive grasses would tolerate flooding better than native grasses, and increased tolerance would be quantifiable in root ADH activity. Invasive species were also predicted to show increased rates of CO_2 fixation compared to natives.

METHODS

Plant collection and treatment conditions

All plants were collected in or near the Wilson Lake Wildlife Area (Sylvan Grove, KS). Sample tillers of *Sorghum* and *Phalaris* were collected in autumn 2008 and spring 2009. Sample tillers of *Phragmites* and *Spartina* were collected in spring 2008. Native populations of *Phalaris arundinacea* and *Phragmites australis* historically have been found in central Kansas (Lavergne and Molofsky 2006, Saltonstall 2002). Additionally, European genotypes of both species have been introduced to the United States (Lavergne and Molofsky 2004). Hybridization of native and nonnative *Phalaris arundinacea* and *Phragmites australis* has led to both species becoming very invasive in freshwater wetlands (Lavergne and Molofsky 2006, Saltonstall 2002).

All tillers were transplanted into potting soil. Each pot measured 11 cm x 11 cm, and contained one individual of a species. Transplanted tillers were grown 6-12 weeks before flooding treatments began. Greenhouse conditions included temperatures ranging from a low of 16 °C at night to a high of 41°C in daylight. Amount of light in greenhouse averaged 220 µmol quantum m^{-2} s^{-1} (PPFD) in daylight hours and peaked near 430 at midday. Relative humidity ranged from 20 to 30 percent during measurement times. Sample plants from each of the four species were randomly selected based on similar height and age. Large rubber tubs measuring 50 cm x 36 cm held 12 pots each. Three pots of each species were included in each tub, and each of the three pots was positioned to be at a different level of flooding. High flooding had water 6 cm above soil level, medium flooding had water saturation 4 cm below soil level, and low flooding had

water level even with the bottom of the pot. Water levels were checked daily and evaporated water was replaced in the tubs. Dry treatment samples were kept directly on the greenhouse bench so water could drain from them. Each treatment was replicated 8 times.

Three measurement cycles occurred, the first in December 2008 to January 2009 included four tubs and measured deep, medium, and low flooding. The second cycle of measurements was from July to August of 2009, which included four tubs and measured deep, medium, and low flooding. The third cycle included 8 samples from each species to measure dry conditions in October to November of 2009.

Gas exchange measurements

The plants were flooded for 28 days. At 7 days and 28 days, photosynthetic light-harvesting rates and CO_2 fixation were measured using an LI-6400 photosynthesis and fluorescence system (Li-Cor Biosciences, Inc., Lincoln, NE). The youngest, fully-expanded leaves were used for gas exchange and fluorescence measurements. All leaves appeared healthy at the time of measurement. Two types of measurements were performed: fluorescence light curve and dark-adjusted maximum quantum yield of PSII (F_v/F_m). The fluorescence light curve measured changes in photosynthetic rate, stomatal conductance, and internal CO_2 concentrations at nine photosynthetic photon flux density (PPFD) levels (2000, 1500, 1000, 500, 200, 100, 50, 20, and 0 μmol m^{-2} s^{-1}). During each light response curve, chamber CO_2 was maintained at 385 ppm. Block temperature of the leaf chamber was 16-27ºC and the relative humidity was 0.16-0.25. Each measurement

was taken 3 to 10 minutes after the light had changed to allow photosynthesis to stabilize following the change in PPFD.

Data from the fluorescence light curve were used to calculate several parameters. The maximum rate of photosynthesis (P_{max}) was the highest rate of CO_2 fixation measured for each plant. The rate of oxygen evolution (J_{O_2}) is the rate of oxygen evolution from PSII, as calculated from fluorescence measurements. Fluorescence measurements give the electron transport rate per PSII (Genty et al. 1989). Electron transport rate was divided by 4 because there are four electrons transported per O_2 evolved (Krall and Edwards 1992). Quantum efficiency (qe) is a measurement of the efficiency with which absorbed photons are used in photosynthesis (Genty et al. 1989). qe is calculated as the initial slope of the light response curve, under limiting light. Net qe is a measure of the efficiency of CO_2 fixation and gross qe is a measure of the efficiency of O_2 evolution. Dark-adjusted fluorescence F_v/F_m ratios were measured prior to sunrise.

Stable isotope preparation

After measurements on the 7th and 28th day of treatment, the next-to-youngest leaf was collected for stable isotope analysis. The leaf was removed from the plant, by cutting just below the leaf collar. Leaf samples were dried at 45°C overnight. The youngest part of the leaf was used for measurement. The basal 1 mm of leaf was isolated, trimmed to 1.0 mg (±0.1 mg) of mass, and was packaged in tin capsules to be sent to Washington State University for leaf carbon isotope ratios ($\delta^{13}C$) analysis. $\delta^{13}C$ is reported on a per mil (‰) basis. $\delta^{13}C$ was calculated as:

$$\delta^{13}C\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

Where R is the ratio of $^{13}C/^{12}C$ in the sample and the $\delta^{13}C$ standard VPDB (Vienna Pee Dee Belemnite) (Ehleringer and Osmond 1991).

Enzyme extraction and assays

At 28 days of treatment, root samples were harvested from each plant. Roots were rinsed in tap water and immediately frozen in liquid nitrogen and then stored at -80ºC. Alcohol dehydrogenase (ADH) was extracted from the roots by using the methods of Maricle et al. (2006). The roots were ground in liquid nitrogen and cold extraction buffer was added at 5 mL g^{-1}. The extraction buffer was 50 mM HEPES (4-2-hydroxyethylpiperzine-1-ethane-sulfonic acid) at 8.0 pH, 5 mM $MgCl_2$, 2 mM cysteine hydrochloride, and 2% w/v PVP-40 (polyvinyl-pyrrolidone, MW $\approx$ 40,000) (John and Greenway 1976). The mixture was homogenized with mortar and pestle, and centrifuged at 10,000 x g for 10 minutes at room temperature. Samples were kept at 4ºC prior to centrifuging.

The supernatant was assayed spectrophotometrically at room temperature. The enzyme was assayed using 80 μM NADH and 10 mM acetaldehyde in 950 μL of a buffer solution of 40 mM bicine and 5 mM $MgCl_2$ (John and Greenway 1976). Activity of the enzyme was determined by oxidation rates of NADH, measured at 340 nm. The rate of oxidation in the presence of acetaldehyde was corrected for background rates of oxidation and converted to μmol per min per g fresh root weight.

Statistical analysis

All statistical analysis was performed using SPSS v.12 (SPSS Inc., Chicago, IL). Photosynthesis data, including P_{max}, J_{O_2}, and both qe, and fluorescence data were analyzed using repeated measures analysis of variance (ANOVA) for intra- and inter-specific comparisons. A Greenhouse-Geisser correction was applied to correct for lack of sphericity. Analyses of A, g_s, and C_i were performed using the values at PPFD of 1500 $\mu mol\ m^{-2}\ s^{-1}$. Differences in $\delta^{13}C$ and ADH activities due to species and treatment variation were analyzed with two-way ANOVA. ADH data were transformed using a square root transformation to make the data normally distributed.

RESULTS

Gas exchange comparisons within species

For native *Spartina*, mean photosynthetic rate (*A*) at a PPFD of 1500 μmol m^{-2} s^{-1} ranged from 11.6 μmol CO$_2$ m^{-2} s^{-1} in low flooding to 19.3 μmol CO$_2$ m^{-2} s^{-1} in deep flooding conditions (Figure 1*a-b*). *A* was not significantly different over time ($F_{1,3}$=3.443, p=0.076). There was a significant treatment x time interaction for *A* in *Spartina* ($F_{1,3}$=3.155, p=0.044). Mean stomatal conductance (*g$_s$*) ranged from 0.104 to 0.140 mol m^{-2} s^{-1} across treatments and sampling dates (Figure 1*c -d*). There was a significant interaction of treatments x time in *g$_s$* ($F_{1,3}$=3.416, p=0.034). Mean internal substomatal CO$_2$ concentration (*C$_i$*) ranged from 124 ppm in deep flooding to 208 ppm in dry conditions (Figure 1*e-f*). There was no significant interaction in *C$_i$* of treatment and time ($F_{1,3}$=1.630, p=0.210).

There was no significant interaction of time and treatment ($F_{1,3}$=0.189, p=0.903) in *A* between 7 day and 28 day measurements in *Phragmites* (Figures 2*a* and 2*b*). Mean *A* in *Phragmites* ranged from 6.2 to 18.0 μmol CO$_2$ m^{-2} s^{-1} at a PPFD of 1500 μmol m^{-2} s^{-1} across treatments and times. Mean *g$_s$* was also not significantly different in the interaction of treatment x time ($F_{1,3}$=0.419, p=0.741) for *Phragmites* (Figures 2*c* and 2*d*). Mean *C$_i$* ranged from 219 to 246 ppm (Figure 2*e* and 2*f*). There was no significant difference in *C$_i$* in time or treatments in *Phragmites* ($F_{1,3}$=0.333, p=0.801).

All the *Phalaris* in dry treatments were dying and had too little leaf area to perform photosynthetic measures at 7 and 28 days. At 7 days, mean *A* ranged from 9.9 to

12.5 μmol CO_2 m^{-2} s^{-1} (Figure 3a). After 28 days, three deep flooded *Phalaris* had died.

A in *Phalaris* was significantly different between 7 and 28 days (F$_{1,3}$= 5.949, p=0.029). *A*

ranged from 9.2 to 18.7 μmol CO_2 m^{-2} s^{-1} at 28 days. There was not a significant

interaction in time and treatment (F$_{1,3}$=0.171, p=0.844) (Figure 3b). Mean g_s of *Phalaris*

at 7 days ranged from 0.12 to 0.24 mol m^{-2} s^{-1}. There was no significant differences in g_s

due to time (F$_{1,3}$=2.547, p=0.133) or the interaction of time and treatment (F$_{1,3}$=0.212,

p=0.812) in *Phalaris* (Figures 3c and 3d). C_i of *Phalaris* ranged from 222 to 259 ppm

across treatments and times (Figure 3e-f). C_i decreased over time (F$_{1,3}$=6.080, p=0.027)

but there was no interaction in time and treatment (F$_{1,3}$=0.423, p=0.663) (Figure 3e and

3f).

In *Sorghum,* mean *A* ranged from 10.1 to 14.5 μmol CO_2 m^{-2} s^{-1} at 7 days (Figure

4a). By 28 days, all the deep flooded plants had died and three of the medium flooded

plants had died. Seven of the low flooded plants and three of the medium flooded plants

flowered between 7 days and 28 days. There was a significant decrease in *A* over time

(F$_{1,2}$=24.541, p<0.001), but there was no difference among treatments (F$_{1,2}$=0.613,

p=0.553). In surviving *Sorghum*, mean *A* ranged from 6.5 to 7.1 μmol CO_2 m^{-2} s^{-1}

(Figure 4b). g_s of *Sorghum* was similar to *A* in that g_s became lower over time

(F$_{1,2}$=19.637, p<0.001), but was not different among treatments (F$_{1,2}$=0.045, p=0.956)

(Figure 4c and 4d). At 7 days, mean g_s ranged from 0.077 to 0.097 mol m^{-2} s^{-1} and from

0.005 to 0.055 mol m^{-2} s^{-1} at 28 days. Mean C_i at 7 days ranged from 94 to 132 ppm and

140 to 174 ppm at 28 days. C_i in *Sorghum* became higher over time (F$_{1,2}$=9.492,

p=0.007), but was not different among treatments (F$_{1,2}$=2.106, p=0.154) (Figure 4e and

4f).

Comparisons among treatments and species

A was significantly different among species and treatments at a PPFD of 1500 μmol m^{-2} s^{-1}, and there was a significant interaction of time and species ($F_{1,3}$=5.648, p=0.001). Using a Tukey"s HSD post-hoc test, A in *Spartina* was greater than *Phragmites* and *Phalaris*, which were significantly greater than A in *Sorghum* (ANOVA, p$\leq$0.004). Repeated measures ANOVA did not detect significant differences in A over time ($F_{1,3}$=0.943, p=0.334,observed power=0.16), nor in the interaction of time and treatment ($F_{1,3}$=0.743, p=.530,observed power=0.20).

There was a significant time x species interaction in g_s at a PPFD of 1500 μmol m^{-2} s^{-1} ($F_{1,3}$=2.752, p=0.048). A Tukey"s HSD post-hoc test of g_s indicated *Phragmites* and *Phalaris* were significantly greater than *Spartina*, which in turn was greater than *Sorghum* (ANOVA, p$\leq$0.002). There were no significant differences in g_s over time ($F_{1,3}$=0.046, p=0.830, observed power=0.06), nor in the interaction of time and treatment ($F_{1,3}$=0.701, p=0.554, observed power=0.19) when measured across species. There was a significant time x species interaction in C_i at a PPFD of 1500 μmol m^{-2} s^{-1} ($F_{1,3}$=4.891, p=0.004). A Tukey"s HSD post-hoc test indicated C_i was highest in *Phalaris* and *Phragmites*, which were significantly greater than *Spartina*, which was in turn significantly greater than *Sorghum* (ANOVA, p $\leq$ 0.049). The repeated measures ANOVA did not detect significant differences over time ($F_{1,3}$=0.453, p=0.503, observed power=0.10), nor in the interaction of time and treatment in C_i ($F_{1,3}$=0.879, p=0.456, observed power=0.23).

F_v/F_m measurements ranged from 0.426 to 0.802 across species and treatments (Figure 5). F_v/F_m measurements for all species and treatments were significantly different over time ($F_{1,3}$=20.183, p<0.001), and there were significant interactions of time and treatments ($F_{1,3}$=4.486, p=0.005), time and species (p<0.001), and time, treatment, and species (p=0.003) (Figure 5). Changes in F_v/F_m indicate there was damage to PSII in some species as flooding persisted in certain treatments. Tukey's HSD indicated F_v/F_m was highest in *Phragmites*, followed by *Phalaris* and *Spartina*, which were significantly greater than *Sorghum* (Figure 5; ANOVA, p<0.001). Mean F_v/F_m of plants in medium flooding was the highest, followed by low flooding and deep flooding, which were significantly higher than dry treatments (Figure 5; ANOVA≤0.019).

Pmax data were transformed by using a square root transformation to normalize distribution. There was a significant time x species interaction ($F_{1,3}$=3.219, p=0.027) for *Pmax* (Figure 6). There was no significant effect of time ($F_{1,3}$=0.178, p=0.674, observed power=0.07), and the interaction of time and treatment was not significant ($F_{1,3}$=0.945, p=0.423, observed power=0.25). Tukey's HSD showed a significant difference in square root transformed means with *Spartina* being highest, followed by *Phalaris* and *Phragmites*, which were in turn higher than *Sorghum*.

J_{O_2} (gross rates of O_2 evolution) data at a PPFD of 1500 μmol m^{-2} s^{-1} were transformed by using a square root transformation. The repeated measures ANOVA showed no significant difference over time ($F_{1,3}$=3.476, p=0.066), nor in the interaction of time and species ($F_{1,3}$=1.787, p=0.156), or the interaction of time and treatment ($F_{1,3}$=1.602, p=0.195) (Figure 7).

Net *qe* data were transformed using a log transformation for normality. There was no effect of time ($F_{1,3}$=0.720, p=0.399, observed power=0.13), and the interactions of time x species ($F_{1,3}$=1.070, p=0.367, observed power=0.37), and the interaction of time x treatment ($F_{1,3}$=0.608, p=0.612, observed power=0.17) were not significant (Figure 8). Gross *qe* data were transformed using a square root transformation to normally distribute the data. The effect of time ($F_{1,3}$=6.256, p=0.014) was significantly different, and there was a significant interaction of time and species ($F_{1,3}$=5.958, p=0.001). Gross *qe* was highest in *Phragmites*, which was significantly greater than *Phalaris* and *Spartina*, which was in turn significantly greater than *Sorghum* (ANOVA, p≤0.008) (Figure 9).

A two-way ANOVA was used to analyze leaf carbon isotope ratios ($\delta^{13}C$). There was a significant difference in $\delta^{13}C$ among treatments (F_3=9.632, p<0.001). Mean $\delta^{13}C$ across species was highest in deep flooding, which was significantly higher than medium flooding and low flooding, which were in turn significantly higher than dry flooding (Tukey"s HSD, p<0.001). There were significant differences between C_3 and C_4 species. $\delta^{13}C$ ranged from -25.0 to -29.2‰ in C_3 species *Phragmites* and *Phalaris* and from -12.2 to -13.5‰ in C_4 species *Spartina* and *Sorghum* (Figure 10). There were significant differences in the two-way ANOVA among species (F_3=1120.208, p<0.001). Mean $\delta^{13}C$ of *Sorghum* and *Spartina* were significantly higher than means of *Phragmites* and *Phalaris* (p<0.001).

Data for root ADH activities were transformed by using a square root transformation so the data were normally distributed for a two-way ANOVA. There was a significant difference among species (F_3=11.683, p<0.001) and a significant interaction

of species and treatment (F_9=2.144, p=0.032) (Figure 11). Root ADH activity ranged

from -0.047 to 1.799 μmol gfw^{-1} min^{-1}. Root ADH activity was highest in *Spartina*,

which was significantly greater than *Phalaris*, *Phragmites*, and *Sorghum* (ANOVA,

p<0.001).

DISCUSSION

Gas Exchange Measurements

A, g_s, and C_i are closely related. A is the rate of CO_2 fixation in the leaf. g_s is a measure of stomatal density and the extent to which individual stomata are open (Kozlowski 1997). More stomata open correlates with higher g_s, and more CO_2 is able to enter the leaf (Lawson et al. 2008). Therefore, increases in g_s correlate with a higher A due to a higher availability of CO_2 in the leaf for fixation (Mojzes and Kalapos 2008). C_i is the intercellular (substomatal) concentration of CO_2 in the leaf. C_i is influenced by the ease that CO_2 can enter the leaf (g_s) as well as the rate CO_2 is used in chloroplasts (A) (Farquhar and Sharkey 1982). Higher A correlates with lower C_i, but lower g_s often correlates with lower C_i. As CO_2 is used for photosynthesis, concentration of CO_2 within the leaf decreases (Farquhar and Sharkey 1982). Therefore, changes in C_i can be used to link the relationship between A and g_s (Stuart et al. 1985). Since g_s and A are both influenced by flooding, changes in A, g_s, and C_i are a good indication of how well a species can tolerate flooding. Individual species can be compared to test if flooding tolerance is a mechanism for invasion.

Mean A of *Spartina* decreased in dry, low, and medium treatments between 7 and 28 days. However, A increased in deep flooding between 7 and 28 days (Figures 1*a* and 1*b*). Increase in A among treatments suggests *Spartina* performed better with increased flooding. Values of g_s in *Spartina* were similar to the results of Maricle et al. (2007) in many *Spartina* species, including *Spartina pectinata*. In the present study, g_s decreased in all treatments from 7 to 28 days while C_i increased over time and began to vary more

among treatments (Figures 1*e* and 1*f*). The decrease in g_s with an increase in A further supports that *Spartina* performed best in deeper flooded conditions. Since g_s decreased, but A increased, this indicates *Spartina* used CO_2 more efficiently as flooding increased. *Spartina* opened fewer stomata as flooding increased. This indicated that *Spartina* had an increased carboxylation capacity which allowed *Spartina* to not limited by CO_2.

A and g_s were highest at 7 days in the dry treatment. *Spartina* is considered an indicator of a wetland based on its facultative wetland indicator status (U.S. Fish and Wildlife Service 1988). This means that if *Spartina* occurs in a field setting the field has a 67-99% likelihood of being a wetland. As such, *Spartina* was expected to increase A and g_s as flooding increased. High A in dry treatments at 28 day measurements could indicate that the effects of flooding take more than 7 days to manifest in *Spartina*. Flooding increased photosynthetic measures like A and g_s between 7 and 28 days. Flooding might take more than 7 days to benefit *Spartina*.

A of invasive *Phragmites* did not change between 7 and 28 days (Figures 2*a* and 2*b*). *Phragmites* had equally high A and g_s in all flooding treatments. Significantly lower photosynthesis rates in dry treatments compared to any of the flooded treatments support the idea that *Phragmites* performs optimally in flooded conditions.

Variability in C_i decreased in *Phragmites* as the flooding continued. However, g_s varied more among treatments at 28 days.   The similar level of C_i in all treatments at 28 days indicated stomatal closure was not limiting for photosynthesis in any treatment. Deep flooding had the highest g_s as well as the highest A at 28 days. Increased g_s allowed

for higher turnover of CO_2, which led to a higher A without changing the C_i within the leaf.

$Phalaris$ is regarded as a drought tolerant species (Kim et al. 2005). However, all dry $Phalaris$ were too unhealthy for gas exchange measurements or had died by 7 days in the present study. Comparisons within the flooded $Phalaris$ plants show a strong preference for low or medium flooded conditions (Figures $3a$ – $3f$). Although at 7 days there was little difference in A or g_s in any treatment, at 28 days A and g_s were significantly higher in low and medium flooding compared to deep flooding. These results agree with those of Coops et al. (1996) and Jenkins et al. (2008) that $Phalaris$ performs strongly in saturated soils, but if the plant is submerged, the performance drastically decreases and can lead to death. Similarly, death can result from growth in soil conditions that are too dry.

There were significant decreases of A and g_s in $Sorghum$ over time (Figure 4). g_s in $Sorghum$ was similar to the ranges of $Sorghum halepense$ in field conditions reported by Stuart et al. (1985), but A was lower in this study than values reported by Stuart et al. (1985). Ranges of g_s and A were similar to those reported for $Sorghum halepense$ in greenhouse conditions by Mojzes and Kalapos (2008). In the present study, there was a significant increase in C_i in $Sorghum$ over time. Increase in C_i was related to the decrease in A and g_s. C_i increased because lowering A led to less CO_2 being fixed. Stomatal closure then resulted from the increase in C_i. At 7 days, the treatment with the highest A was medium flooding, where A averaged 14.5 µmol CO_2 m^{-2} s^{-1}. At 28 days, the maximum A of medium flooding averaged 6.5 µmol CO_2 m^{-2} s^{-1}. The significant

decrease in A of *Sorghum* indicates *Sorghum* from the sampled population is not adapted to survive in deeply-flooded environments.

Inter-specific comparisons were performed by comparing A, g_s, and C_i at a PPFD of 1500 μmol m^{-2} s^{-1}. Ranges of A and g_s in all species were similar to measurements by Pagter et al. (2005) for *Phragmites australis* in field settings. By contrast, ranges for C_i were lower in Pagter et al. (2005) compared to those measured in this study. A across treatments was significantly higher in native *Spartina* compared to invasive *Phragmites* and *Phalaris*. However, when comparing treatments and species, *Spartina* had the highest A and g_s in deep flooded treatments, but both *Phragmites* and *Phalaris* had higher A and g_s in medium and low flooded treatments (Figures 1-3).

Increased A was correlated with an increase in g_s across species in all treatments. Decreases in g_s can lead to a decrease in A because there is less CO_2 available for photosynthesis when stomata are closed (Farquhar and Sharkey 1982). Increases in C_i also can lead to decreased g_s at leaf level (Lawson et al. 2008). In the present study, g_s was expected to be different among the native and invasive grasses. Both *Phragmites* and *Phalaris* are C$_3$ photosynthetic plants. Their similar g_s could possibly be attributed to this (Scheidegger et al. 2000). C$_4$ plants have lower g_s compared with C$_3$ plants, as C$_4$ plants concentrate CO_2 within bundle sheath cells (Mojzes and Kalapos 2008). As a result, C$_4$ plants are able to saturate photosynthesis with ambient (or lower) levels of CO_2 (Taiz and Zeiger 2006). Thus, lower g_s in *Spartina* and *Sorghum* are due to their photosynthetic pathway.

Significant differences in A, g_s, C_i, and the interaction of time and species can be used along with differences in P_{max}, J_{O_2}, and net and gross qe to determine which species was best adapted physiologically for flooding. P_{max} is the maximum photosynthetic rate measured in the light response curve. Comparing P_{max} among species shows the maximum performance each is reaching in different treatments. P_{max} of *Spartina*, *Phragmites*, and *Phalaris* was higher than P_{max} reported by Krauss et al. (2006) in greenhouse studies of neotropical mangroves. While P_{max} was significantly different over time among species, there were no significant differences in J_{O_2} among species (Figure 7). J_{O_2} is the rate of oxygen evolution from photosystem II (PSII), as calculated from fluorescence measurements (Crofts and Horton 1991). Differences in maximum J_{O_2} among treatments would indicate damage to PSII. Due to significant differences in P_{max} among species over time, but not maximum J_{O_2}, the results suggest limitations to any photosynthetic process are occurring in CO_2 fixation, and not PSII.

Net qe is a measurement of the efficiency of carboxylation (Genty et al. 1989). Decreases in net qe among treatments and species can show how well adapted a species is to flooding. Limitation in CO_2 fixation due to stress of flooding in the C_4 species *Spartina* and *Sorghum* could be evident in the net qe. For example, leakage of CO_2 from bundle sheath cells in C_4 plants is detectable by decreases in net qe, since extra light energy is needed to recycle leaked CO_2 (Farquhar 1983). Because the net qe was not significantly different among treatments or species over time, there were no increases in bundle sheath leakage as a result of flooding.

Increases in gross *qe* indicate an increase in efficiency of O_2 production (Crofts

and Horton 1991). Much like J_{O_2}, a low gross *qe* indicates decreased efficiency of PSII.

Differences in gross *qe* among treatments can be used to explain how well adapted each

species is to flooding. Gross *qe* of *Spartina* and *Phragmites* increased over time in

flooding treatments (Figure 7). With increased photosynthetic rates, *Spartina* and

Phragmites are best adapted photosynthetically for flooded conditions compared to the

other species in this study. Gross *qe* decreased over time in all flooding treatments in

Phalaris. This could be an indication that longer flooding is detrimental to PSII in

Phalaris.

There was no detected effect of time or the interaction of time x treatments in *A*,

g_s, C_i, P_{max}, and net *qe* in inter-specific comparisons of all species. Although they were

not significantly different, there was a low observed power in the analysis (observed

power ≤ 0.23). This means these results could only explain 23% or less of the variation in

values. Because of this, it cannot be definitively stated that the different flooding

treatments had no effect on parameters like *A*, g_s, C_i, P_{max}, and net *qe*. In the future, an

increased sample size could overcome the issues with observed power in those

measurements.

Fluorescence measurements

Photon energy absorbed by PSII can have three possible fates. First, the absorbed

light energy can be used in photochemistry. If the light energy is not used for

photochemistry, it can be released as heat or lost as chlorophyll fluorescence.

Chlorophyll fluorescence is absorbed light energy re-released by the plant as light (Baker

2008). The three energy dissipation processes occur in competition with one another

(Maxwell and Johnson 2000). If one process decreases, it can be assumed the other two

processes have increased. Measuring the maximum quantum yield of PSII (F_v/F_m) will

indicate the efficiency at which PSII is operating. Dark adapted F_v/F_m is maximized at

0.83 in most plants (Björkman and Demmig 1987, Baker 2008). Decreases in F_v/F_m

indicate damage to PSII caused by abiotic or biotic stress (Baker 2008). Moreover,

measurements of fluorescence can be used to estimate A in C_4 plants (Edwards and Baker

1993).

In the present study, F_v/F_m measurements were mostly in the range of 0.75-0.83,

similar to results reported by Mauchamp and Méthy (2004) for flooded *Phragmites* and

Phalaris. There was little variation in F_v/F_m over time and treatments in *Phragmites* and

Spartina (Figure 5). This indicates there was no damage to PSII from flooding in those

species. The increase in A and g_s, and the decrease in C_i in *Phragmites* and *Spartina*,

coupled with high F_v/F_m measures, suggests photosynthetic abilities increased as flooding

increased. This was further supported by the lack of significant change in J_{O_2} over time or

among species. J_{O_2} and F_v/F_m are closely linked to CO_2 fixation in maize leaves

(Edwards and Baker 1993). Due to the lack of significant change in J_{O_2} and F_v/F_m, the

benefits of flooding to *Phragmites* and *Spartina* were not due to changes in PSII.

F_v/F_m values for *Phalaris* increased over time in low and medium flooding, but

decreased in deep flooding. Decrease in deep flooding F_v/F_m, along with increased

mortality and decreased A, suggests *Phalaris* has decreased photosynthetic capabilities

when flooded above the soil surface. This is particularly true for extended flooding.

Decreased F_v/F_m in *Phalaris* could be a result of damage to PSII. F_v/F_m decreased in all treatments in *Sorghum* between 7 days and 28 days (Figure 5). Differences among treatments and species support that *Sorghum* is not adapted to short-term flooding compared to the other species included in this study.

Stable Isotope Analysis

There are two stable isotopes of carbon: „light" carbon-12 (98.9% abundance) and „heavy" carbon-13 (1.1% abundance) (O"Leary 1988). Much information can be learned from the ratio of heavy to light carbon in organisms, and how this differs between product and substrate of a biochemical reaction or physical processes. In most biochemical reactions (including the carbon reactions of photosynthesis), the light isotope of an element reacts more quickly than the heavier isotope (Choi et al. 2005). The difference in isotope values between substrate and product is termed discrimination.

There is a difference in carbon isotope discrimination between C_3 and C_4 plants (Farquhar et al. 1989). C_3 photosynthetic plants favor light carbon because of a strong fractionation by the initial carbon fixation enzyme, ribulose-1,5, bisphosphate carboxylase/oxygenase (Rubisco) (Ehleringer and Osmond 1991). In contrast, the initial carbon fixation enzyme in C_4 plants, Phosphoenolpyruvate carboxylase (PEPCase), does not favor the light carbon, causing C_4 plants to be heavier (higher leaf carbon isotope ratios ($\delta^{13}C$)) (Brooks et al. 2002, O"Leary 1988). C_3 plants are therefore „lighter" (lower $\delta^{13}C$) than C_4 plants, giving a measurable difference between the two types of photosynthetic plants (O"Leary et al. 1992). Changes in carbon fractionation are also more sensitive to changing environmental conditions in C_3 plants compared to C_4 plants,

so effects of flooding would be expected to be much greater for C_3 plants (Hanba et al. 2010). Greater environmental stress in a C_3 plant is usually accompanied by closure of stomata, which results in an increase in $\delta^{13}C$ (O'Leary et al. 1992). The opposite is true of C_4 plants, which often become lighter with stomatal closure due to environmental stress (e.g., Maricle and Lee 2006).

Leaf $\delta^{13}C$ of the C_3 species, *Phalaris* and *Phragmites* ranged from -26.6 to -29.0‰, which is lower than shoot values of *Phragmites* reported by Choi et al. (2005) but similar to $\delta^{13}C$ values in *Festuca rubra*, *Potentilla aurea*, and *Achillea millefolium* as reported by Scheidegger et al. (2000). As flooding levels increased, $\delta^{13}C$ in *Phragmites* became lower (Figure 10). This indicates *Phragmites* increased stomatal conductance as flooding levels increased. This agrees with the changes in g_s in photosynthetic measures of *Phragmites*. The opposite occurred in *Phalaris*. As flooding increased in *Phalaris*, $\delta^{13}C$ increased. *Phalaris* had increased mortality and decreased photosynthetic performance as flooding increased. The clear correlation between decreased photosynthetic performance and higher $\delta^{13}C$ indicates stable isotope data could be used to assess flooding tolerance in other C_3 species.

Leaf $\delta^{13}C$ in the C_4 species, *Sorghum* and *Spartina* ranged from -12.2 to -13.5‰. This is similar to the results of Maricle and Lee (2006) in *Spartina* grasses. Differences in $\delta^{13}C$ in C_4 species could result from changes in C_i/C_a or leakage of CO_2 from bundle sheath cells (Farquar 1983). A difference in net *qe* would indicate an increase in bundle sheath leakage in C_4 species (Farquar 1983). The lack of difference in net *qe,* coupled with no significant difference in $\delta^{13}C$, means there was no increase in bundle sheath

leakage in C_4 species. C_i/C_a ranged from 0.32 to 0.54 in *Spartina* and 0.36 to 0.45 in *Sorghum*. The lack of differences in $\delta^{13}C$ in C_4 species are explained by no changes in C_i/C_a. This is consistent with the lack of difference in net *qe* and $\delta^{13}C$ between treatments in *Sorghum* and *Spartina*.

ADH activity

Alcohol dehydrogenase (ADH) is needed for glycolysis to continue in cells under anoxic and hypoxic soil conditions. Fermentation replenishes NAD^+ for glycolysis. ADH is the enzyme that catalyzes the reduction of acetaldehyde to ethanol, and NADH is oxidized to NAD^+ in the process (Crawford 1967). In the present study, root ADH activity ranged from -0.05 to 1.80 $\mu mol\ gfw^{-1}min^{-1}$ in all species and treatments. The range of ADH is similar to the ranges of ADH reported by McManmon and Crawford (1971) for *Senecio aquatics, Caltha palustris*, and *Ranunculus flammula* in lab conditions and by Maricle et al. (2006) for *Spartina* grasses in greenhouse conditions. It was hypothesized that ADH activity would increase as flooding levels increased. This hypothesis was supported in *Spartina* and *Phalaris* (Figure 11). Both species increased ADH activity to acclimate to saturated soils. Increased ADH activity indicates oxygen deficiency. In *Sorghum*, ADH activity only increased in low flooded plants. Increased ADH activity was a response to flooding that could be correlated with an effort to prolong life while flowering. Low activities of ADH were measured in the other flooding treatments, because the below ground tissue was likely dead. Dry *Sorghum* had low ADH because there was no oxygen deficiency, and therefore no reason for the individuals to produce ADH.

Phragmites had low activities of ADH in all treatments. ADH activities decreased in many cases as flooding increased. *Phragmites* can survive without oxygen in soil for more than 28 days (Crawford and Braendle 1996). This is because *Phragmites* can use internal gas transport to move oxygen from leaves to roots (Armstrong et al. 1992, Colmer and Flowers 2008). As a result, there is no need for *Phragmites* to undergo fermentation when it can continue aerobic respiration. Because of different methods used to tolerate flooded soils in *Phragmites* and *Spartina*, it is difficult to say which is more tolerant of saturated soils. Further research is needed on the two species in field conditions to see which tolerates soil saturation better. Differences in this study support the hypothesis that ADH activity would increase as flooding increases. However, there did not seem to be an advantage as far as ADH activity in the invasive species versus the native species.

Flooding tolerance and invasiveness

Phragmites was the most flood tolerant of the invasive species. *Phragmites* is the best adapted to invaded frequently flooded areas. *Phragmites* had the highest A, g_s, and P_{max} in flooded conditions. Low root ADH activity because of abundant internal O_2 transport allows *Phragmites* to tolerate flooding longer than many other wetland species (Crawford and Braendle 1996). Correlations between decreasing $\delta^{13}C$ and g_s suggested *Phragmites* kept its stomata open more when flooded compared to dry conditions. This indicates that *Phragmites* is very well adapted for flooding. Possibly *Phragmites* could use its physiological advantages in flooded conditions as a mechanism to invade. At the

least, the ability of *Phragmites* to withstand flooding could allow it to outlive native species in the flooded area and *Phragmites* could spread farther when the water recedes.

Photosynthetic abilities of native *Spartina* were comparable with *Phragmites*. *Spartina* had the highest A and g_s in deep flooding as well as increased ADH activity to tolerate saturated soils. However, A and g_s of *Spartina* were lower than *Phragmites* in low and medium flooding. *Spartina* is physiologically adapted to compete with *Phragmites* in deep flooded conditions, but as water levels decrease *Spartina* could be more susceptible to invasion by *Phragmites* and other wetland invaders. Field studies would be needed in the future to verify these greenhouse results and to assess the invasibility of *Spartina* by *Phragmites* in wetland environments.

Physiological processes of *Phalaris* are moderately flood tolerant. *Phalaris* was the best adapted species in medium or low flooding, owing to high A and g_s. *Phalaris* was not suited for deep flooding in which the plant was partially submerged, similar to Miller and Zedler (2003). This indicates that *Phalaris* could be the best adapted to invaded areas that are not constantly flooded. $\delta^{13}C$ became heavier as flooding increased. *Phalaris* closed its stomata as flooding increased, which decreased its overall photosynthetic performance. F_v/F_m of *Phalaris* decreased in deep flooding over time, whereas F_v/F_m values in low and medium flooding were not changed over time. Decreases in F_v/F_m in deep flooding indicate damage to PSII, which provides more evidence that *Phalaris* was moderately tolerant to flooding.

In this study, *Sorghum* was the most sensitive species to flooding. Ability to tolerate flooding is not part of the invasive capabilities of this species. Many *Sorghum*

samples died in flooded conditions. Additionally, F_v/F_m in flooded *Sorghum* decreased over time. This could be correlated with *Sorghum* individuals that flowered. Energy and nutrients were reallocated to flowering and taken away from photosynthetic upkeep. There were also significant decreases in all photosynthetic process due to flooding in *Sorghum*. The population of *Sorghum* used in this study was not flooding tolerant since all deep flooded plants as well as a number of medium flooded plants died before 28 days. *Phragmites* and *Phalaris* performed better than native *Spartina* in some flooding treatments as hypothesized. Poor performance by *Sorghum* in flooded conditions causes a rejection of the hypothesis that this invasive species would perform better under flooded conditions compared to the native species.

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Figure 1. Light response curve (*A* and *B*), stomatal conductance (*C* and *D*), and internal CO_2 concentration (*E* and *F*) in *Spartina pectinata*. Panels *A*, *C*, and *E* are 7 day measurements. Panels *B*, *D*, and *F* are 28 day measurements. The black circle is dry conditions, the white circle is low flooded conditions, the black triangle is medium flooded conditions and the white triangle is deep flooded conditions. The points are the means of 6-8 individuals ± SE.

Figure 2. Light response curve (*A* and *B*), stomatal conductance (*C* and *D*), and internal CO_2 concentration (*E* and *F*) in *Phragmites australis*. Panels *A*, *C*, and *E* are 7 day measurements. Panels *B*, *D*, and *F* are 28 day measurements. The black circle is dry conditions, the white circle is low flooded conditions, the black triangle is medium flooded conditions and the white triangle is deep flooded conditions. Points are the means of 6-8 individuals ± SE.
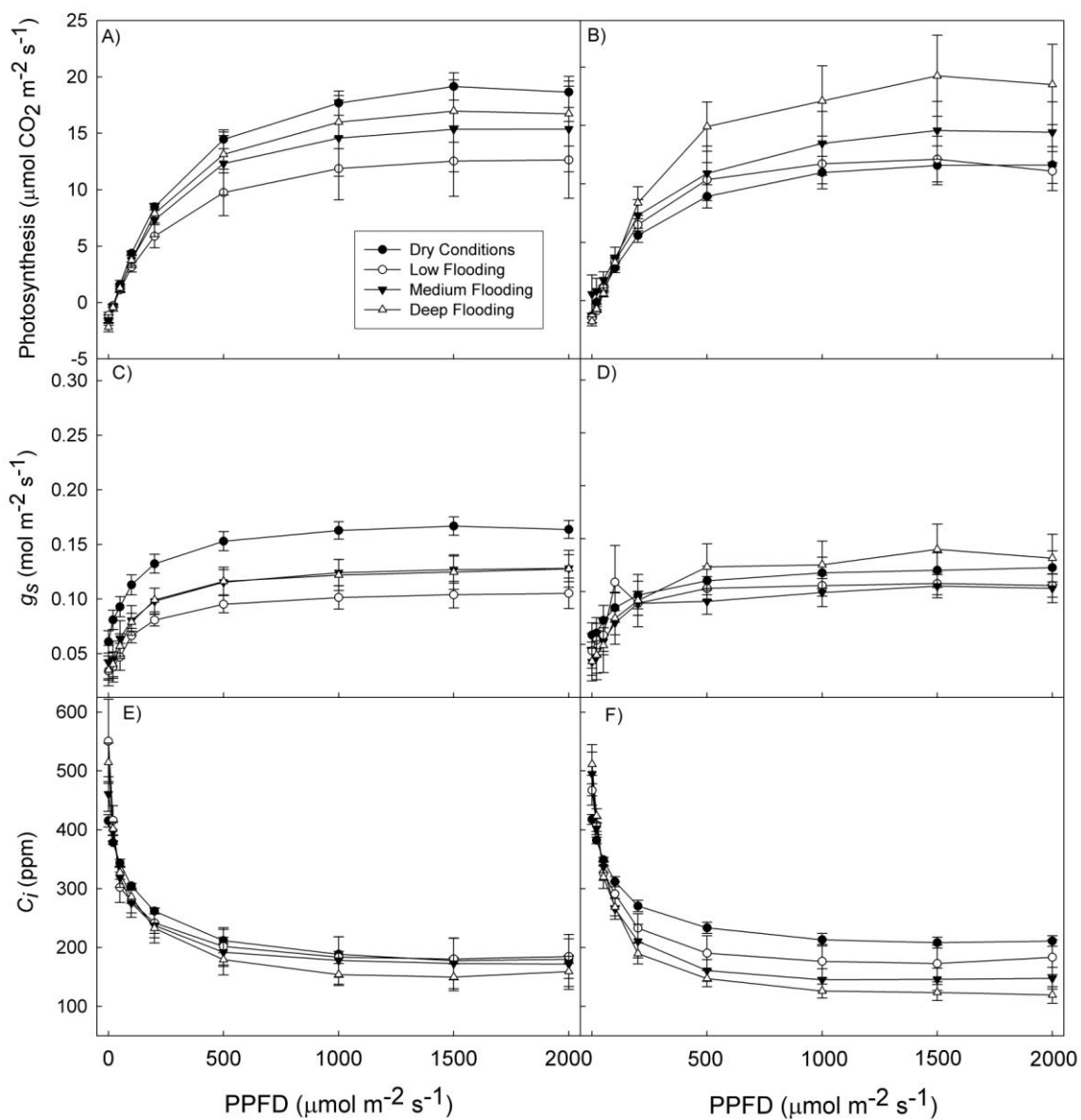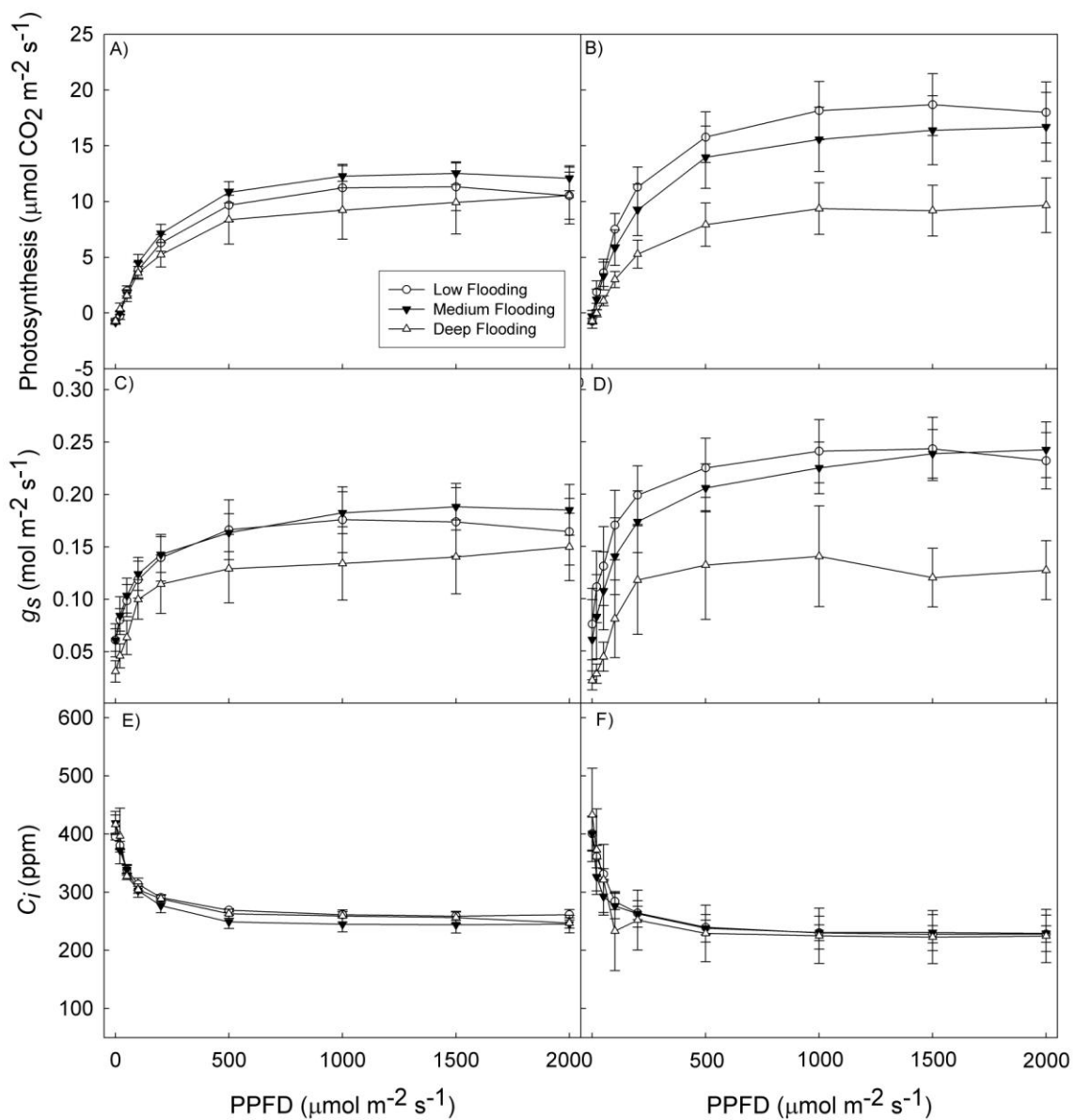
Figure 3. Light response curve (*A* and *B*), stomatal conductance (*C* and *D*), and internal CO_2 concentration (*E* and *F*) in *Phalaris arundinacea*. Panels *A*, *C*, and *E* are 7 day measurements. Panels *B*, *D*, and *F* are 28 day measurements. Panels *A*, *C*, and *E* are 7 day measurements. Panels *B, D,* and *F* are 28 day measurements. The black circle is dry conditions, the white circle is low flooded conditions, the black triangle is medium flooded conditions and the white triangle is deep flooded conditions. Points are the means of 6-8 individuals ± SE.

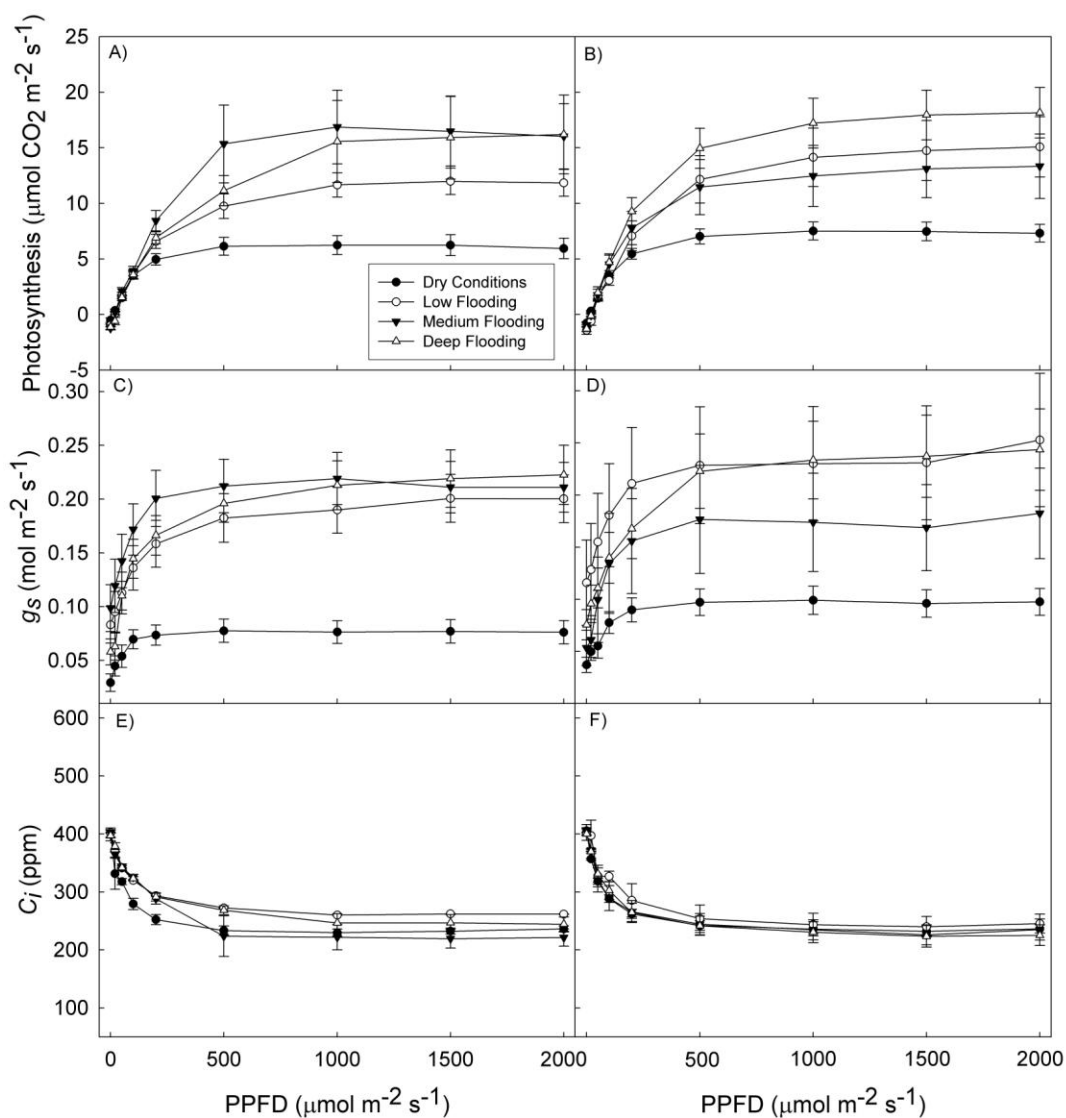Figure 4. Light response curve (*A* and *B*), stomatal conductance (*C* and *D*), and internal CO₂ concentration (*E* and *F*) in *Sorghum halepense*. Panels *A*, *C*, and *E* are 7 day measurements. Panels *B*, *D*, and *F* are 28 day measurements. Panels *A*, *C*, and *E* are 7 day measurements. Panels *B*, *D*, and *F* are 28 day measurements. The black circle is dry conditions, the white circle is low flooded conditions, the black triangle is medium flooded conditions and the white triangle is deep flooded conditions. Points are the means of 6-8 individuals ± SE.
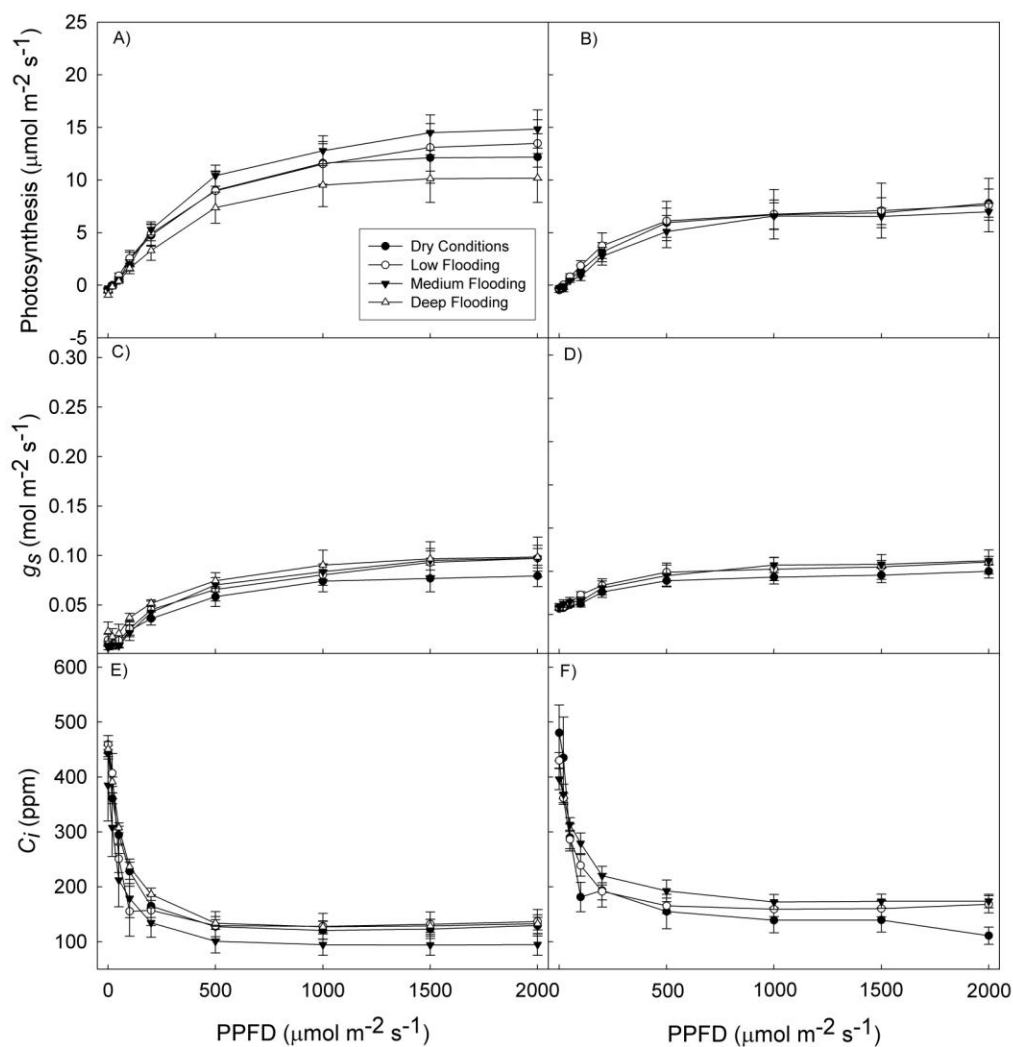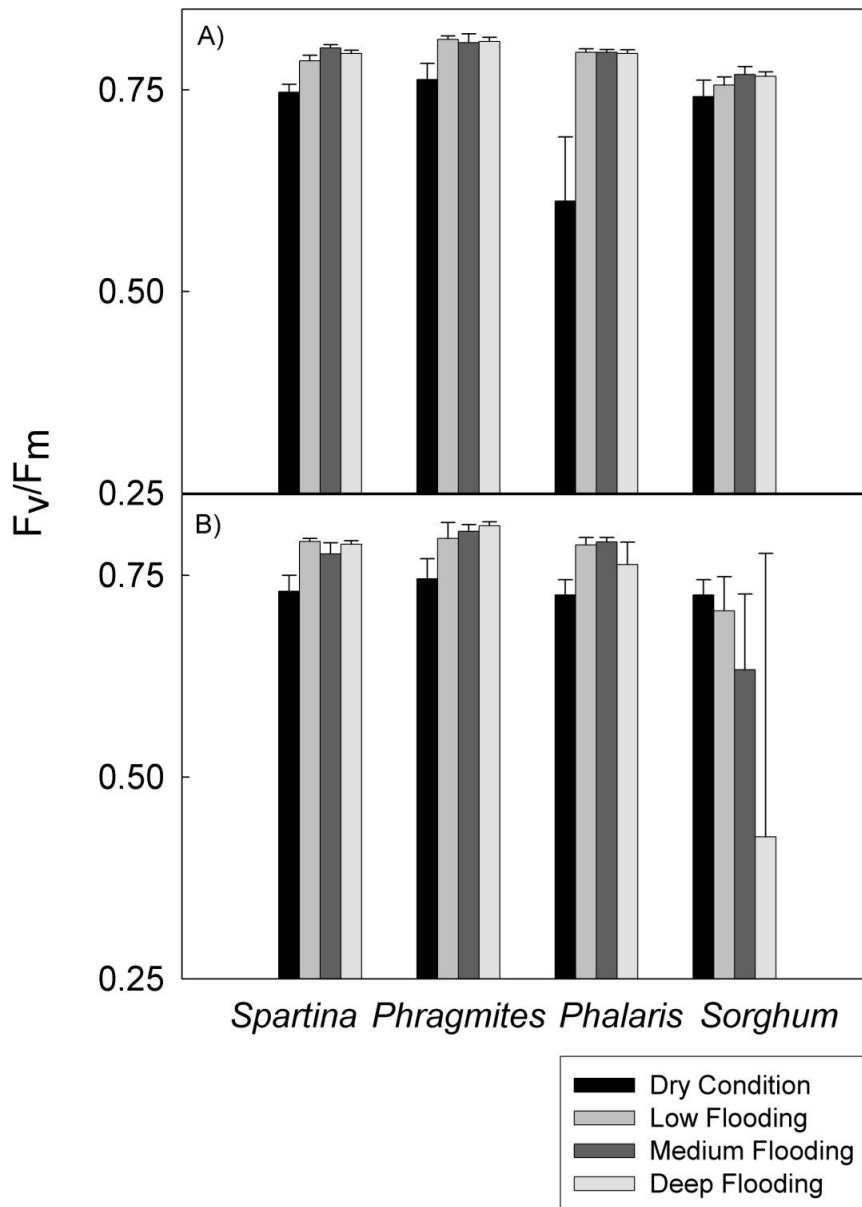
Figure 5: Maximum quantum efficiency of PSII (F_v/F_m) for plants at 7 days (*A*) and at 28 days (*B*) for all treatments. Bars are means of 6-8 individuals ± SE, except for deep flooded *Phalaris* which was 5 individuals and deep flooded *Sorghum* was 3 individuals. Four species are represented. The black bars are dry conditions, the light gray bars are low flooded conditions, the dark gray bars are medium flooded conditions, and the lightest bars are deep flooded conditions.
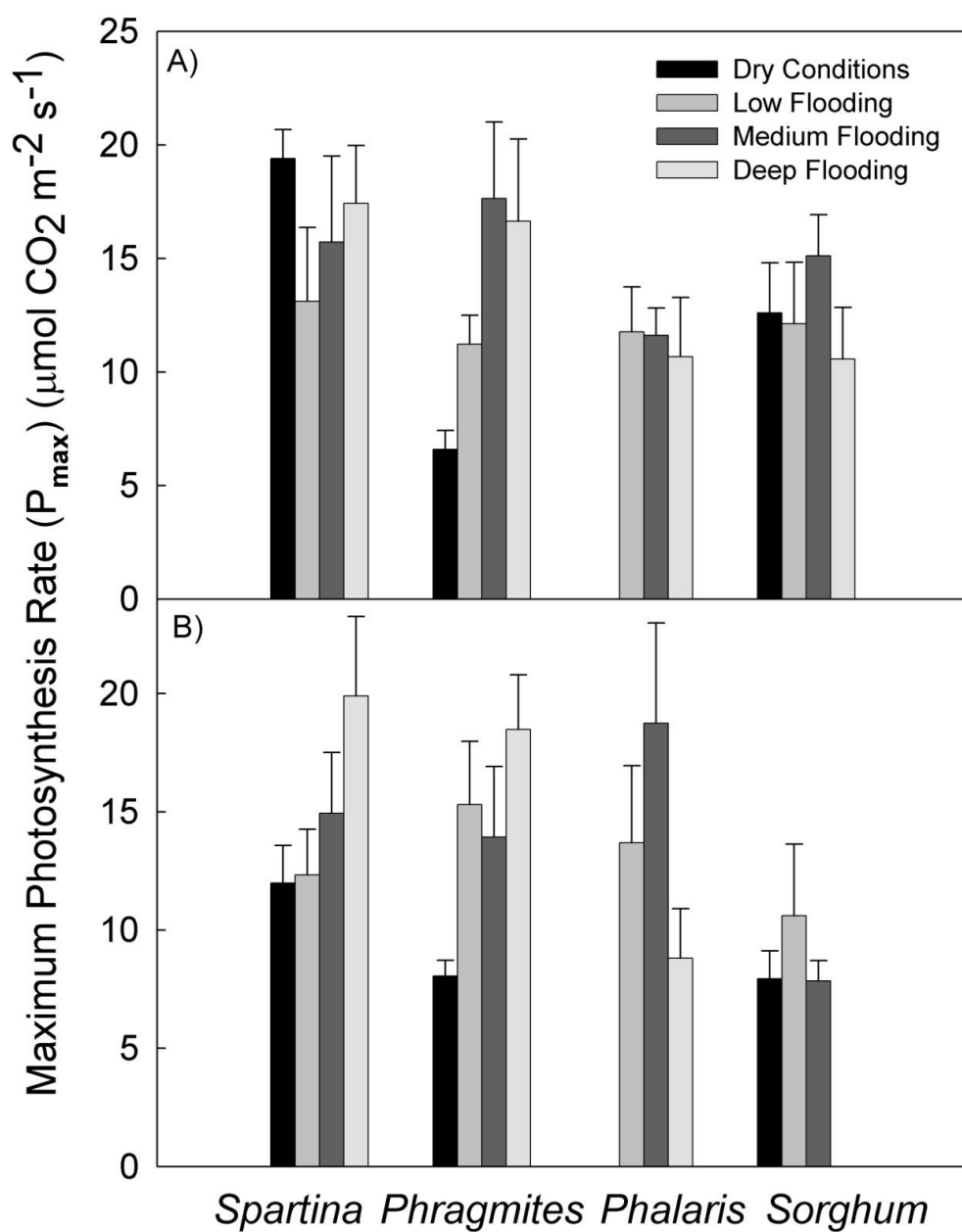
Figure 6. Maximum measured photosynthetic rate (P_{max}) at 7 days (*A*) and at 28 days (*B*). Bars are means of 5-8 individuals ± SE. The species and treatments are labeled as in Figure 5. There are no dry *Phalaris* samples because all samples were dead or too unhealthy at 7 days. All deep *Sorghum* died between 7 and 28 days.
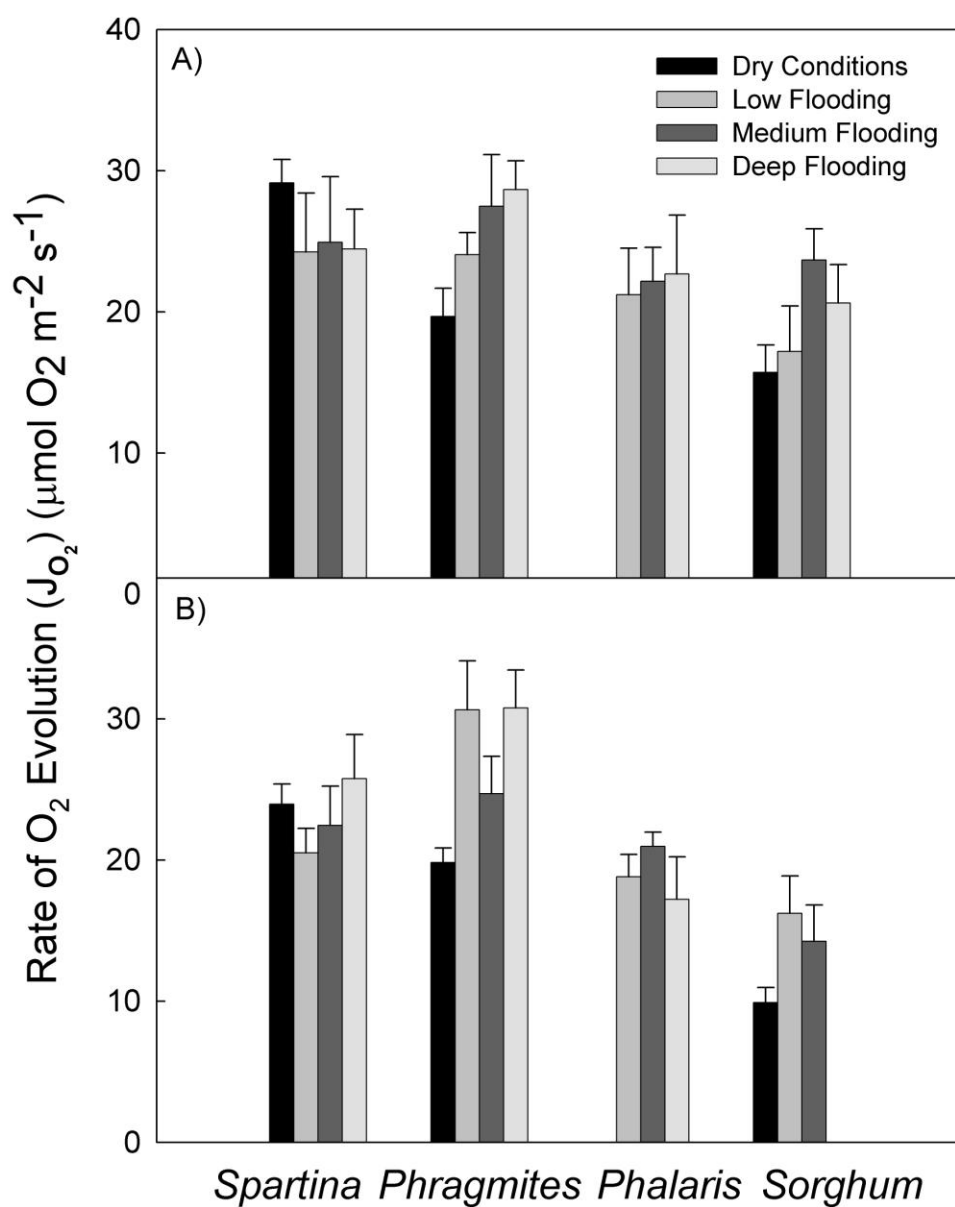
Figure 7. Rate of O_2 evolution from PSII (J_{0_2}), measured at 7 days (*A*) and measured at 28 days (*B*). Bars are means of 5-8 individuals ± SE. Species and treatments are labeled as in Figure 5.
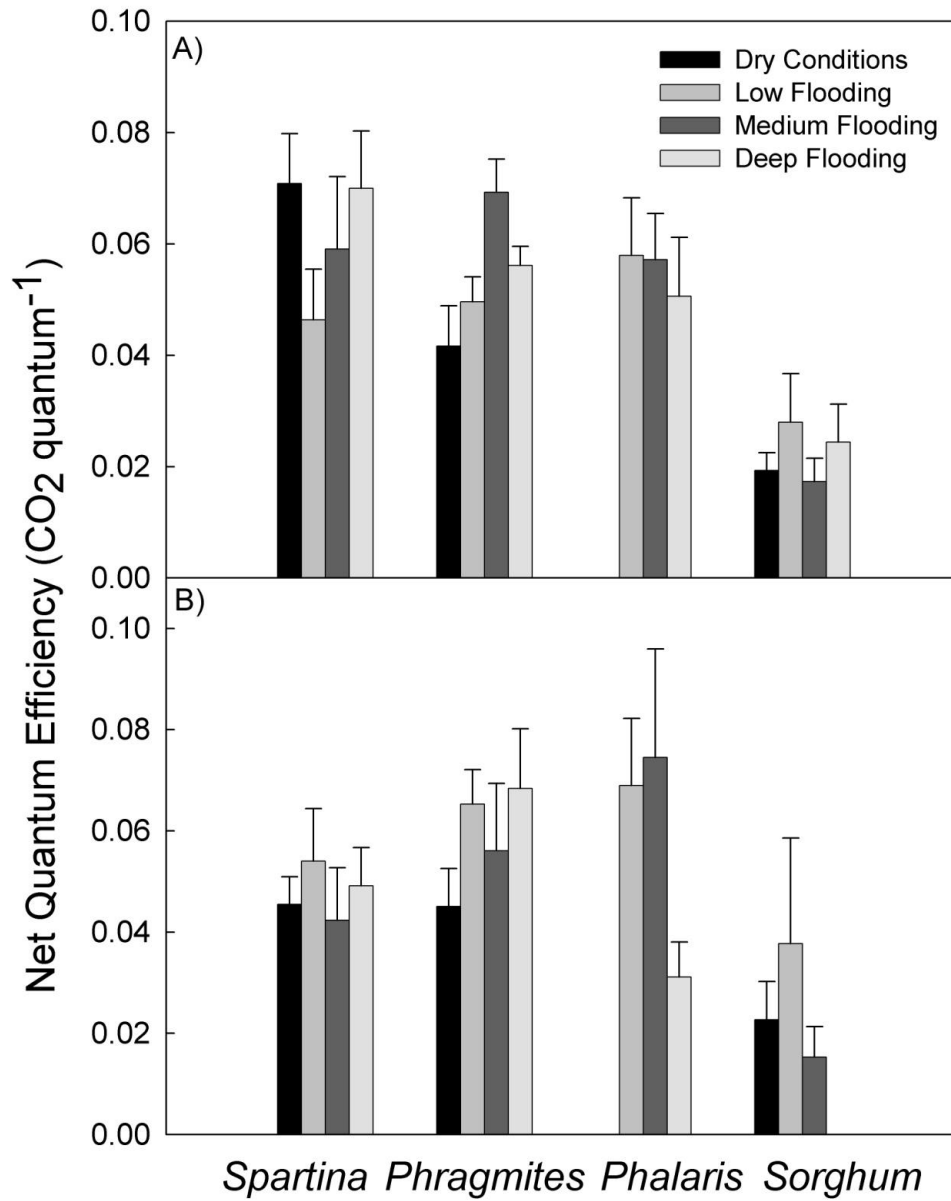
Figure 8. Net *qe* during photosynthesis measured at 7 days (*A*) and measured at 28 days (*B*). Bars are means of 5-8 individuals ± SE. Species and treatments are labeled as in Figure 5.
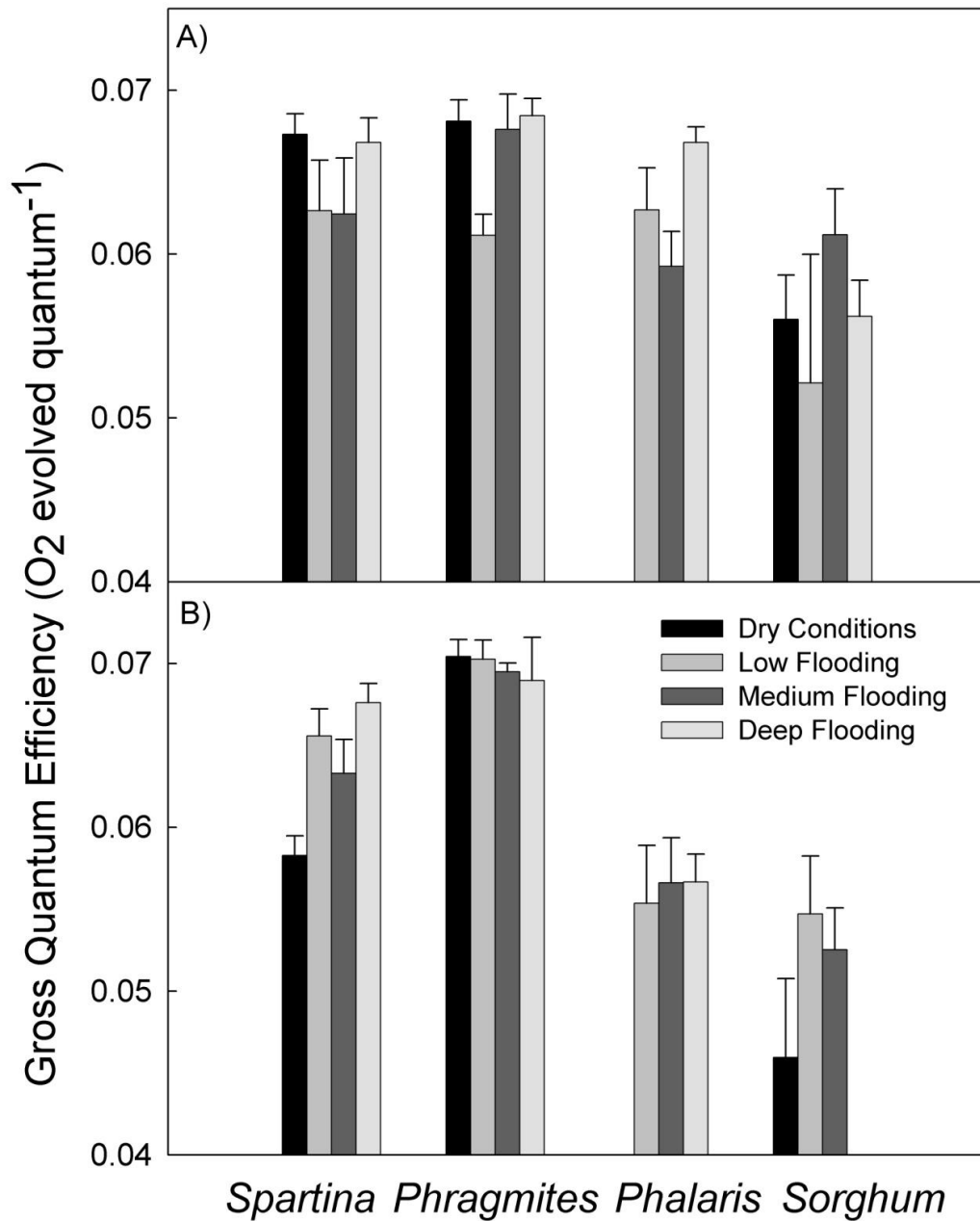
Figure 9: Gross *qe* of photosynthesis measured at 7 days (*A*) and measured at 28 days (*B*). Bars are means of 5-8 individuals ± SE. Species and treatments are labeled as in Figure 5.
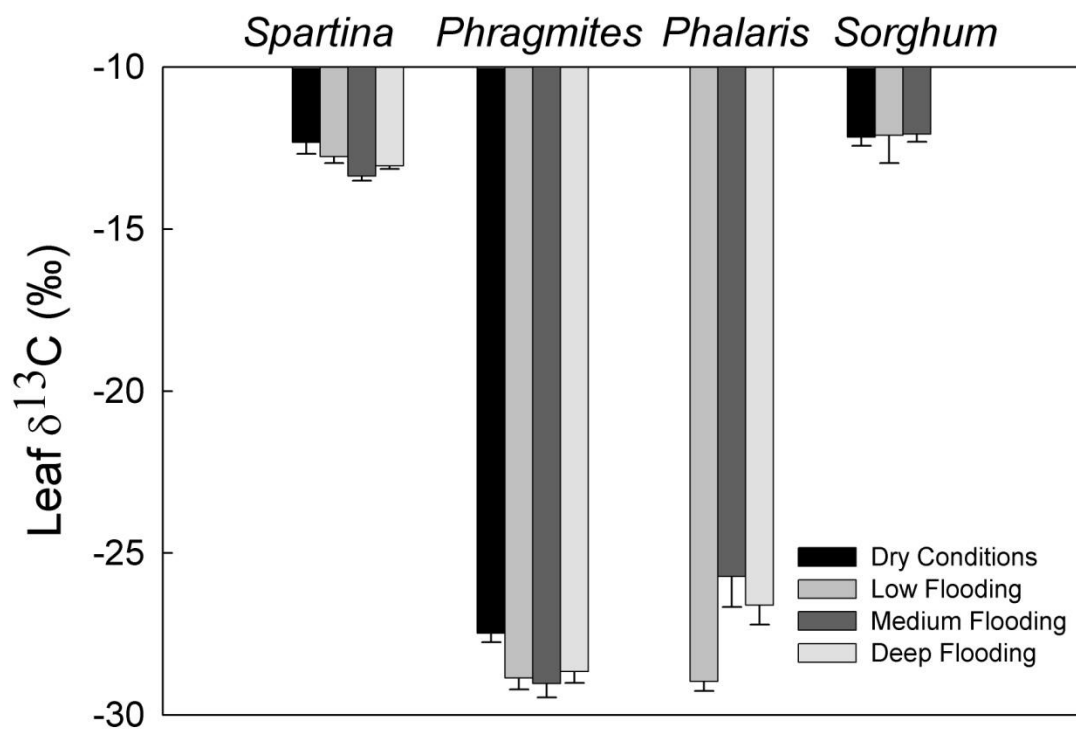
Figure 10: Leaf carbon isotope values ($\delta^{13}C$) among treatments and species. Bars are means of 6-8 individuals ± SE. Species and treatments are labeled as in Figure 5.

Figure 11: Root alcohol dehydrogenase (ADH) activity among treatments and species. Bars are means of 6-8 individuals ± SE. Species and treatments are labeled as in Figure 5.

CHAPTER 2: EFFECTS OF FLOODING ON TRANSPIRATION IN JOHNSONGRASS

(*SORGHUM HALEPENSE*) AND COMMON REED (*PHRAGMITES AUSTRALIS*)

ABSTRACT

Adaptation to flooding can indicate the ability of a species to invade wetlands.

Numerous adaptations exist, but effects of flooding on stomatal conductance are
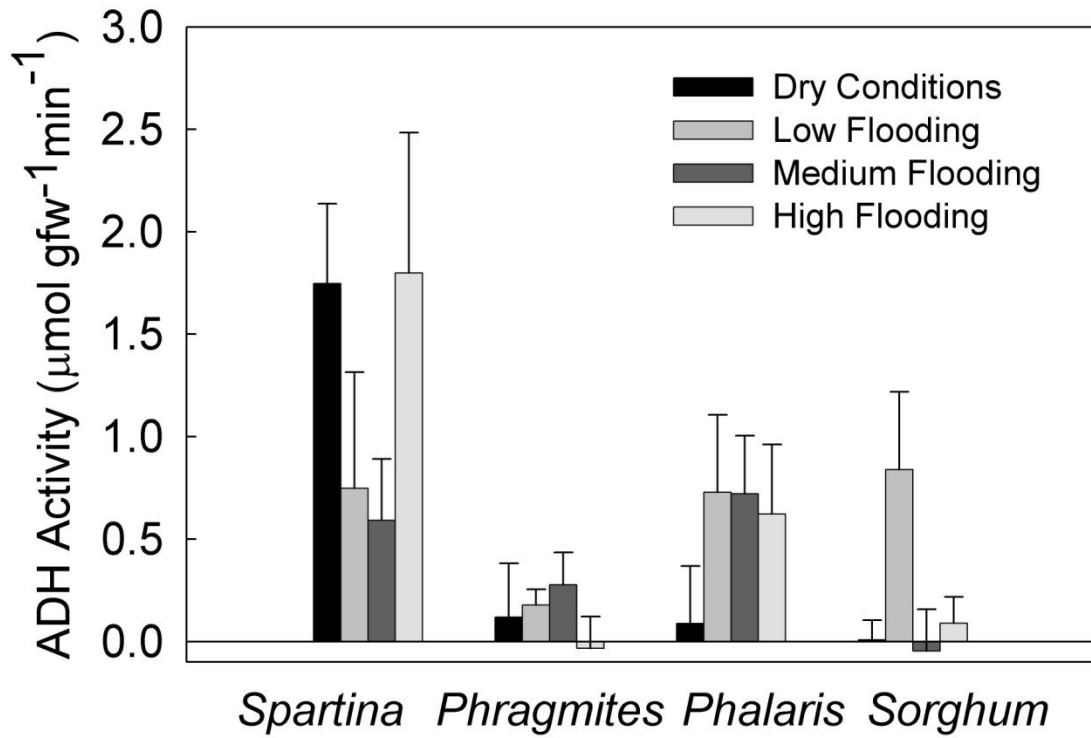
especially notable. Measurements of stomatal conductance and transpiration can provide

information on plant carbon gain and water loss. In this study, flooding-sensitive

Sorghum halepense and flooding-tolerant *Phragmites australis* (n=5) were flooded to 8

cm depth or kept dry for 7 days. Transpiration, stomatal conductance, boundary layer

conductance, and vapor conductance were measured for each. Transpiration was

significantly higher in drained treatments compared to flooded for *Sorghum*. However,

transpiration was significantly higher in flooded treatments compared to drained for

Phragmites. Boundary layer conductances were not significantly different between

species or treatments. *Phragmites* had increased stomatal conductance when flooded,

which indicates a high physiological tolerance to waterlogged soils. This allows

Phragmites to photosynthesize under waterlogged conditions and to be successful as a

wetland invader. By contrast, stomatal conductance in *Sorghum* was decreased under

flooding, indicating a greater sensitivity to flooding. The sampled population of *Sorghum*

is therefore not a threat to invade chronically flooded soils based on these results. Further

information on the conditions that maximize stomatal opening for *Phragmites* can help

management efforts.

Nomenclature: *Phragmites australis* (Cav.) Trin. ex Steud.; Common Reed, *Sorghum halepense* (L.) Pers.; Johnsongrass

Keywords: environmental physics, invasive species, stomatal conductance, wetland

INTRODUCTION

Invasion by exotic plants is especially prevalent in wetlands, because wetlands are landscape sinks and are prone to disturbance (Zedler and Kercher 2004). Both common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) and johnsongrass (*Sorghum halepense* (L.) Pers.) are highly invasive plants (Gries et al. 1990, Taylor and Smith 2005). Common reed is a frequent invader in wetland settings whereas johnsongrass more frequently inhabits upland areas (e.g., according to the wetland indicator status of each; U.S. Fish and Wildlife Service 1988). Many plants cannot survive in regularly-flooded soils (Armstrong et al. 1994), which becomes important when considering invasiveness of plants in wetlands. To understand plant growth and potential invasions in wetlands, one must consider O_2 availability.

Flooding is the most prominent stress on wetland plants (Mitsch and Gosselink 2007). This stress comes from a displacement of oxygen from soil spaces, and the limited solubility of oxygen in a dissolved state. These conditions quickly lead to anoxia (Armstrong et al. 1994). Species that are better able to tolerate anoxia will have a clear advantage as invaders in a waterlogged environment.

Flooding-induced anoxia at the roots can influence stomata in leaves. Stomatal conductance is a measure of stomatal density, and the extent to which individual stomata are open. Stomatal conductance influences the rate that water vapor escapes from the plant, as well as the rate CO_2 can enter the leaf for photosynthesis. The lack of O_2 obtained through the roots causes the plant to close stomata (Keddy 2000), producing a response similar to drought stress. For example, Glaz et al. (2004) showed a decrease of

photosynthesis and stomatal conductance in flooded sugarcane. Such measures are useful when working with stressed plants, as it describes basic leaf physiology and general responses to the environment.

Physiological tolerance to flooding can become relevant ecologically. For example, common reed can move oxygen internally from leaves to roots (Colmer 2003, Colmer and Flowers 2008). This allows the plant to keep stomata open. Indeed, common reed has been suggested to use flooding as a mechanism for invasion (e.g., Fraser and Karnezis 2005, Gries et al. 1990). By contrast, physiological responses of johnsongrass to flooding have not been studied previously. Knowledge on how johnsongrass and common reed respond to different environmental conditions can be used in future restoration and management plans in waterlogged soils.

In the present study, common reed and johnsongrass were subjected to flooding in greenhouse treatments. Leaf-level physiological responses were measured to determine stomatal activity during flooding. It was hypothesized that the high flooding tolerance of common reed would manifest itself as an increase in transpiration, stomatal conductance, and vapor conductance under flooding. By contrast, the higher sensitivity of johnsongrass to flooding would be measureable as a decrease in transpiration, stomatal conductance, and vapor conductance under flooding.

METHODS AND MATERIALS

Data Collection

Ten individuals each of common reed and johnsongrass were selected randomly from plants of similar age and height. Each individual was planted with potting soil in a pot that measured 11 cm x 11 cm x 10 cm. Five individuals of each species were placed in a tub measuring 50 cm x 36 cm and flooded to a depth of 8 cm, enough to submerge the soil completely. The other five individuals of each species were kept in dry conditions; these plants were watered once per week, and excess water was allowed to drain from pots. After seven days, data were collected using the youngest, fully-expanded leaf on each plant. Measured leaves appeared healthy on all plants. All data were collected during midday hours on 12 November 2009 in the Fort Hays State University greenhouse (Hays, KS). Conditions in the greenhouse included natural lighting with a mean PPFD of 202 μmol quanta m^{-2} s^{-1} during measurements, mean relative humidity was 0.50, and air temperature was 26 ºC. There was an average of 10 hours of daylight during the 7 days of treatment. Wind speed (u) (m s^{-1}) was measured with an AM-4204 hot wire anemometer (Lutron Electronic Enterprise Co., Ltd.; Taipei, Taiwan). Dry bulb (T_a) and wet bulb (T_w) temperatures (ºC) in the greenhouse were measured using a digital psychrometer (Extech Instruments RH300; Waltham, MA, USA). Lastly, a SC-1 Leaf Porometer (Decagon Devices, Inc.; Pullman, WA, USA) was used to measure stomatal conductance (g_{vs}) (mmol m^{-2} s^{-1}) on abaxial surfaces of sample leaves.

Data analysis

Vapor pressure (e_a, kPa) in the greenhouse was calculated after Campbell and Norman (1998) as

$$e_a = e_s(T_w) - \gamma \times p_a(T_a - T_w) \tag{1}$$

where the vapor pressure (e_a) is a function of the saturated vapor pressure (e_s, kPa) at the wet bulb temperature (T_w), γ is the psychrometer constant (6.66×10^{-4} °C^{-1}), and p_a is atmospheric pressure (94.05 kPa at the study location).

The dominant type of convection was determined according to Campbell and Norman (1998) as

$$Gr/\text{Re} \tag{2}$$

which is a ratio of the Grashof number (Gr) to the Reynold number (Re). The Grashof number is calculated as:

$$Gr = \frac{gd^3\delta T}{Tv^2} \tag{3}$$

The Grashof number (Gr) uses the gravitational constant ($g = 9.81$ m s^{-2}), the characteristic dimension of the leaf ($d = 0.72 \times$ leaf width, m), the temperature difference between leaf and air (δT), the Kelvin air temperature (**T**), and the kinematic viscosity of air ($v = 1.55 \times 10^{-5}$ m^2 s^{-1} at 25°C). The Reynold number is calculated as:

$$\text{Re} = \frac{ud}{v} \tag{4}$$

The Reynolds number is dependent on wind speed (u), characteristic dimension of the leaf ($d = 0.72$ x width of leaf), and the kinematic viscosity of air ($v = 1.55$ x $10^{-5} m^2 s^{-1}$). Since the ratio Gr/Re^2 was $<< 1.0$, convection was determined to be forced (Campbell and Norman 1998).

Water vapor conductance of the boundary layer (g_{va}, mol m^{-2} s^{-1}) around the leaf was determined after Campbell and Norman (1998). For forced convection, g_{va} was calculated. The equation for forced convection is as follows:

$$g_{va} = 0.147 \sqrt{\frac{u}{d}}$$

[5]

where u is wind speed. Total conductance to water vapor (g_v, mol m^{-2} s^{-1}) for the leaf was found by the following equation:

$$g_v = \frac{1}{\frac{1}{g_{va}} + \frac{1}{g_{vs}}}$$

[6]

where g_{vs} was determined with the porometer. Transpiration rate (E, mol m^{-2} s^{-1}) was determined after Campbell and Norman (1998) as

$$E = \frac{g_v \left[e_s(T_L) - e_a \right]}{p_a}$$

[7]

where $e_s(T_L)$ is saturated vapor pressure at leaf temperature, and p_a is atmospheric pressure (94.05 kPa).

Statistical analysis was performed using SPSS v. 12 (2003, SPSS Inc. Chicago, IL). Two-way analysis of variances (ANOVA) were used to test for a difference among species and treatments ($\alpha=0.05$).

RESULTS AND DISCUSSION

Both johnsongrass (*Sorghum halepense*) and common reed (*Phragmites australis*) are introduced species that invade waterlogged areas (Gries et al. 1990, Taylor and Smith 2005). A species that is better able to tolerate flooded conditions has a clear advantage when invading a wetland area. One way to measure responses to physiological stress (including flooding) is to measure stomatal conductance of leaves. Leaf stomatal conductance is easy to measure with hand-held porometers, and g_{vs} is fairly sensitive to environmental changes (e.g., Taiz and Zeiger 2006). Measuring g_{vs} and E of both species in dry and flooded conditions can give information on carbon gain and growth potential. The results of this project could be used to explain how the species use physiological advantages while invading. This information can then be used in management and restoration efforts to control common reed and johnsongrass.

Leaf stomatal conductance to water vapor (g_{vs}) ranged from 11 to 84 mmol m^{-2} s^{-1} across species and treatments (Figure 1*b*). Similar results were reported by Pagtar et al. (2005) for common reed (*Phragmites australis*) in native European populations. These results are somewhat lower than field measures of g_{vs} for plants in wetland settings reported by Oue (2001) and Teal and Kanwisher (1970). Low light levels in the greenhouse in the present study most likely resulted in g_{vs} and E values lower than one would expect under full sunlight. There was a significant interaction among species and treatment (p<0.001) in g_{vs}, but there was no difference between species (p=0.158) or treatments (p=0.053). The significant interaction of species and treatment supports the

hypothesis that g_{vs} would increase in flooded conditions for common reed, but decrease in flooded conditions for johnsongrass.

Leaf boundary layer conductance to water vapor (g_{va}) ranged from 447 to 1000 mmol m^{-2} s^{-1} across species and treatments (Figure 1a). These figures are far greater than stomatal conductances (g_{vs}; see below), indicating there was sufficient air flow in the greenhouse to allow adequate mixing, and g_{vs} was the main influence on total leaf conductance (g_v) and transpiration (E). There was no significant difference in g_{va} between treatments (p=0.272), species (p=0.414), or their interaction (p= 0.627). Equation 5 demonstrates that g_{va} is dependent on wind speed and the characteristic dimension of the leaf. Wind speed in the greenhouse was due to fans in the environmental control system and remained constant. Leaf width in common reed averaged 8.3 mm, whereas johnsongrass leaves averaged 11.8 mm (data not shown). The small difference in leaf width was not enough to influence g_{va}.

Total leaf conductance to water vapor (g_v) ranged from 11 to 78 mmol m^{-2} s^{-1} across species and treatments (Figure 1c). These numbers are similar to greenhouse measures of g_v by Maricle et al. (2007) in salt marsh grasses. The analysis of species (p=0.156) and of treatments (p=0.055) were not significant. A significant interaction of species and treatment (p<0.001) in g_v also supports the hypothesis that g_v would increase in flooded conditions for common reed, but decrease in flooded conditions for johnsongrass.

Transpiration rates from leaves (E) ranged from 0.17 to 1.41 mmol m^{-2} s^{-1} across species and treatments (Figure 1d). These numbers are lower than field measures of E in

a salt marsh (Teal and Kanwisher 1970) or a prairie wetland (Jacobs et al. 2002). A lower

value of E in greenhouse studies compared to field studies illustrates the influence of

light conditions on stomatal conductance and transpiration. In the present study, E was

significantly different among treatments (p<0.001) and in the interaction of treatment and

species (p=0.013), but E was not different between species (p=0.573). The hypothesis

that flooding would increase stomatal conductance and transpiration in common reed was

supported. Similarly, the hypothesis that flooding would decrease the stomatal

conductance and transpiration in flooded johnsongrass was also supported (Figure 1d).

The g_{vs}, g_v, and E were not different dry johnsongrass and flooded common reed.

However, when considering differences in species as well as treatments, the different

adaptations to flooded conditions of johnsongrass and common reed become apparent in

a significant interaction.

Increase of transpiration was correlated with an increase in stomatal conductance.

Higher stomatal conductance means the plant is taking in more CO_2, which allows the

plant to produce more triose-phosphates and ATP in photosynthesis (Farquhar and

Sharkey 1982). The increase in stomatal conductance could be an explanation as to why

common reed is able to reproduce quickly and colonize wetlands with rapid rhizomatous

growth. Common reed had an increase in E and g_{vs} under flooding, which is related to

tolerance of waterlogged soils in field settings. Tolerance or preference to waterlogged

soils could be an important factor to the invasive potential and actions of common reed.

Decreased E and g_{vs} under flooded conditions in johnsongrass indicate decreased growth

potential. The inability of the plants to remove water from the soil caused them to act water

stressed. This stress would ultimately lead to lower amounts of CO_2 intake and lower

photosynthetic products. Individuals from the population of johnsongrass used in this study are

therefore not an invasion treat to chronically waterlogged environments. However, future

research is needed to determine if this population could acclimate to a wetter environment, or if

there is variation in adaptations to waterlogged soils among populations of johnsongrass.

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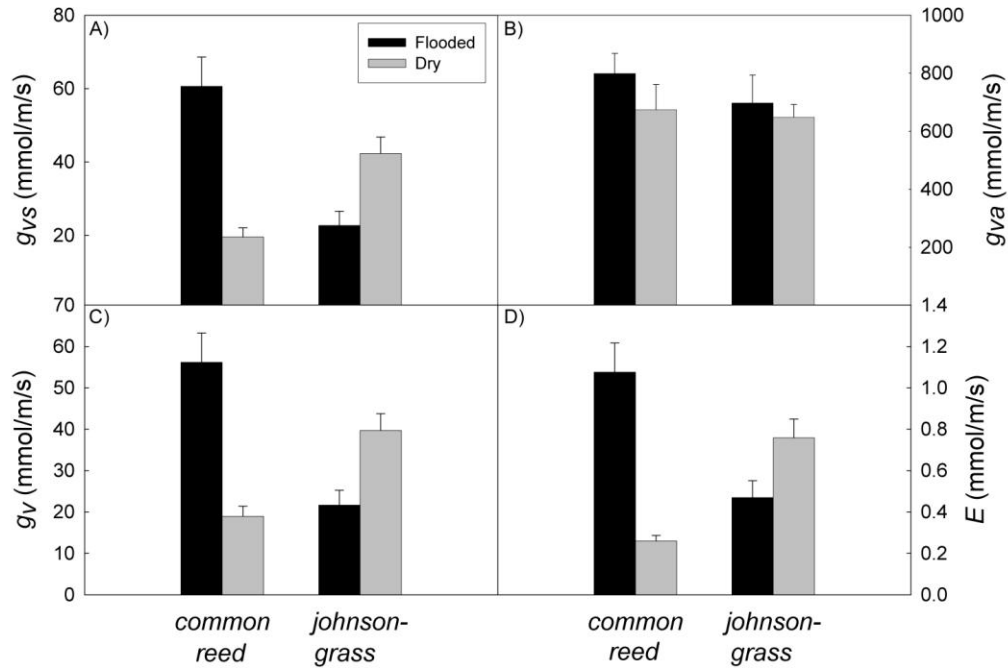
Science. 23:431-452.

Figure 1. Effects of flooding on transpiration and vapor conductances in common reed and johnsongrass. (A) stomatal conductance to water vapor, g_{vs}, (B) boundary layer conductance to water vapor, g_{va}, (C) total conductance to water vapor, g_v, and (D) transpiration, E. Bars show the mean of 5 replicates ± standard error. Black bars indicate flooded plants and gray bars indicate dry plants.