Received: 19 January 2021 | Accepted: 25 May 2021

DOI: 10.1002/ajb2.1746

RESEARCH ARTICLE

Botany

Freshwater wetland plants respond nonlinearly to inundation over a sustained period

Junbin Zhao[1,2] | Sparkle L. Malone[1] | Christina L. Staudhammer[3] | Gregory Starr[3] | Henrik Hartmann[4] | Steven F. Oberbauer[1]

[1]Department of Biological Sciences and Southeast Environmental Research Center, Florida International University, Miami, FL, USA

[2]Department of Biogeochemistry and Soil Quality, Division of Environment and Natural Resources, Norwegian Institute of Bioeconomy Research, Ås, Norway

[3]Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, USA

[4]Max Planck Institute for Biogeochemistry, Hans-Knöll-Str 10, Jena 07745, Germany

Correspondence

Junbin Zhao, Department of Biological Sciences and Southeast Environmental Research Center, Florida International University, Miami, FL, USA; Department of Biogeochemistry and Soil Quality, Division of Environment and Natural Resources, Norwegian Institute of Bioeconomy Research, Ås, Norway.
Email: junbinzhao1985@gmail.com

Abstract

Premise: Wetland plants regularly experience physiological stresses resulting from inundation; however, plant responses to the interacting effects of water level and inundation duration are not fully understood.

Methods: We conducted a mesocosm experiment on two wetland species, sawgrass (*Cladium jamaicense*) and muhly grass (*Muhlenbergia filipes*), that co-dominate many freshwater wetlands in the Florida Everglades. We tracked photosynthesis, respiration, and growth at water levels of −10 (control), 10 (shallow), and 35 cm (deep) with reference to soil surface over 6 months.

Results: The response of photosynthesis to inundation was nonlinear. Specifically, photosynthetic capacity (A_{max}) declined by 25% in sawgrass and by 70% in muhly grass after 1–2 months of inundation. After 4 months, A_{max} of muhly grass in the deep-water treatment declined to near zero. Inundated sawgrass maintained similar leaf respiration and growth rates as the control, whereas inundated muhly grass suppressed both respiration and growth. At the end of the experiment, sawgrass had similar nonstructural carbohydrate pools in all treatments. By contrast, muhly grass in the deep-water treatment had largely depleted sugar reserves but maintained a similar starch pool as the control, which is critical for post-stress recovery.

Conclusions: Overall, the two species exhibited nonlinear and contrasting patterns of carbon uptake and use under inundation stress, which ultimately defines their strategies of surviving regularly flooded habitats. The results suggest that a future scenario with more intensive inundation, due to the water management and climate change, may weaken the dominance of muhly grass in many freshwater wetlands of the Everglades.

KEYWORDS

A-Ci curve, Cyperaceae, flooding, fructose, glucose, light curve, marsh, Poaceae, sucrose

Inundation substantially decreases the oxygen available to plant roots for metabolism and the carbon dioxide (CO_2) supply for photosynthesis in submerged leaves (Pezeshki, 2001; Mommer and Visser, 2005). Many wetland plants regularly experience different degrees of inundation stress depending on the duration of inundation and depth of water. As strategies to survive the anoxic soil conditions during inundation, multiple physiological changes may occur in these plants when submerged. As an initial reaction, stomata typically close as a result of decreased hydraulic conductivity under anoxic conditions (Pezeshki et al., 1996; Else et al., 2001; Jackson, 2002). Stomatal closure usually constrains photosynthesis, which leads to a reduction in plant carbon uptake (Pezeshki et al., 1996). In addition to the stomatal response, leaf Rubisco activity (Vu and Yelenosky, 1992; Liao and Lin, 1994; Pezeshki, 1994), chlorophyll fluorescence (Zhao et al., 2018; Chen et al., 2010) and nitrogen content (Zhao et al., 2018) are also reduced, indicating that nonstomatal processes

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *American Journal of Botany* published by Wiley Periodicals LLC on behalf of Botanical Society of America

(e.g., carboxylation, electron transport) also limit photosynthesis under inundation stress. While a combination of stomatal and nonstomatal limitations may interact in controlling photosynthesis, their contributions under different inundation stress levels need to be further assessed.

With reduced carbon uptake under inundation stress, plants may alter their strategies for using available carbon. Plants can decrease carbon consumption in metabolic activities and growth, resulting in a lower respiration rate (Else et al., 2001; Bragina et al., 2002; Islam and Macdonald, 2004). By contrast, some plants that use an "escape" strategy may invest more carbon in vertical leaf growth to avoid complete submergence and thus maintain the aerenchyma function to transport oxygen to roots (Akman et al., 2012; Voesenek and Bailey-Serres, 2015; Loreti et al., 2016). As a result of altered carbon balance, nonstructural carbohydrate (NSC) reserves also variably decline (Vu and Yelenosky, 1992; Pan et al., 2012; Qin et al., 2013). Given that NSC reserves are critical for plant survival under environmental stresses (Ram et al., 2002; Hartmann and Trumbore, 2016), further investigation of plant NSC storage under different levels of inundation stress could be very important for understanding different plant responses to inundation.

While the degree to which a plant reacts largely depends on the intensity of inundation stress, there is no explicit single measure of inundation intensity. Both increased water levels and duration of inundation have been used as indicators of enhanced inundation intensity, which result in elevated stresses on critical plant processes such as growth, biomass accumulation, and mortality (Ewing, 1996; Miller and Zedler, 2003; Troxler et al., 2014; Lan et al., 2019). Furthermore, since water level and inundation duration are widely monitored in long-term ecosystem observations (e.g., Zhao et al., 2019), knowledge of plant-level responses to changes in these variables could be directly used to inform ecosystem carbon process modeling of wetlands, which are an important carbon sink globally (Kayranli et al., 2010). Since oxygen availability generally decreases with increases in water depth (e.g., Rose and Crumpton, 1996), high water levels might intensify the anoxic stress to plants and induce earlier and greater physiological responses by plants. To investigate these relationships, one needs to measure changes in physiological processes over a substantial period of inundation at different water levels. Such measurements can reveal interactions between effects of water level and inundation duration, which are still poorly understood.

In this study, we studied the inundation responses of sawgrass (*Cladium jamaicense* Crantz, a C_3 sedge; Cyperaceae) and muhly grass (*Muhlenbergia filipes* M.A. Curtis, a C_4 grass; Poaceae) from freshwater wetlands of the Florida Everglades. Although sawgrass is generally distributed in areas with higher water levels and longer hydroperiods compared to muhly grass, habitats of the two species overlap in large areas of short-hydroperiod freshwater marshes and prairies in the Everglades (Todd et al., 2010). These two species are also abundant in coastal regions of the southeastern United States as well as the Caribbean. Sawgrass has flat or V-shaped leaves with sharp saw teeth at the edges and can develop large root systems (Richards and Olivas, 2020). Muhly grass has rolled leaf blades and fibrous roots. In a field study, we previously found that inundation caused greater stress on photosynthesis in muhly grass than in sawgrass (Zhao et al., 2018).

Here in a mesocosm experiment, we further investigated physiological changes in sawgrass and muhly grass when inundated with different water depths over a 6-month period. Our primary goal was to evaluate physiological responses to interactive effects of water level and inundation duration for the two species with different flood tolerances and elucidate their carbon-use strategies under different levels of inundation stress. We tested the following hypotheses: (1) higher water level induces a greater and earlier decline in photosynthesis; (2) photosynthesis is mainly limited by stomatal conductance under mild stress while nonstomatal limitation becomes more dominant as the stress intensifies; and (3) after a 6-month inundation, NSC pools are substantially reduced for the less inundation-tolerant species (i.e., muhly grass) but not for the more tolerant one (i.e., sawgrass).

MATERIALS AND METHODS

Plant transplantation and acclimation

On 15 December 2017, we collected 20 monoliths (plants with intact surrounding marl soil, 40 × 40 × 20 cm), 10 with at least one large individual of sawgrass and 10 with at least one large individual of muhly grass from a marl prairie outside of Everglades National Park. The monoliths were placed in opaque plastic containers (50 × 40 × 23 cm) and transported to the Florida International University (FIU) campus (25°45′33″N, 80°22′24″W, ~10 km away from the Everglades) within 2 h.

At the FIU campus, potting soil (Fafard 4 P Mix, Sun Gro Horticulture Ltd., Agawam, MA, USA) was added to gaps between the monolith soil and the walls of the containers (~20% of the container volume) to ensure that containers were adequately filled. To keep plants hydrated after transplantation, they were placed in the shade and watered to saturation.

After 2 weeks, we moved the containers to three outdoor unshaded mesocosms (cattle tanks, diameter 2 m, height 1.3 m) in the FIU mesocosm facility and exposed them to natural light conditions. To let water in the mesocosm flow into the container, small holes (diameter 1 cm) were drilled into the sides and bottoms of the containers before placing in the mesocosms. The mesocosms were filled with freshwater, and plant containers were placed on metal racks, the heights of which were adjusted so that the water level was 10 cm below the soil surface of each container (i.e., water level = −10 cm). This water level, keeping the soil saturated but not flooded, was maintained for ~5 months before the experiment to allow plants to recover from transplantation

and adapt to the mesocosm environment. The water level within mesocosms was regulated by a connected outlet hose with the opening set to the target water height, so that excess water, e.g., introduced by rainfall, was automatically discharged. At the same time, water was added manually as the water level dropped due to evaporation.

Experimental setup

On 29 May 2018, 18 (9 of each species) of the 20 containers were chosen and assigned to one of three water levels: −10 (soil saturated, as control), +10 (shallow water), and +35 cm (deep water) with reference to the soil surface, allowing for a balanced design for each species. In the deep-water treatment (+35 cm), approximately 50% and 70% of the leaf segments were submerged for sawgrass and muhly grass, respectively. The containers were redistributed into the three mesocosms with each mesocosm containing six containers and each container representing one of the six combinations of treatments and species (Appendix S1). Heights of the metal racks were then individually readjusted to achieve the target water level for each container. The water level treatments were maintained for 25 weeks, which allowed us to simulate a hydroperiod of approximately half a year. Since the majority (>85%) of the freshwater portion of the Everglades has a mean water level of ≤35 cm during inundation that lasts for ≤6 months per hydroperiod (Todd et al., 2010), our treatments covered a range that represents typical hydrological conditions for habitats of the two species. The number of replicates ($n = 3$) was chosen considering available mesocosms, project budget, and measurement feasibility given that the design included two species and three treatments. Nevertheless, the statistical power of the design was strengthened by repeated measurements over the experimental period.

Physiological measurements

We used a LI-6400XT Photosynthesis System (LI-COR, Inc., Lincoln, NE, USA) with a 2 × 3 cm leaf chamber to measure CO_2 and H_2O gas exchange on two emergent leaves (at approximately the midpoint of the emergent leaf section) per container between 10:00 and 14:00 hours every 2 weeks (see Appendix S2 for details). Light conditions within the chamber were controlled by the internal LED light and set to levels equivalent to photosynthetically active radiation (PAR) of 2000 and 0 μmol m^{-2} s^{-1} to measure net photosynthesis (A) and dark respiration (R_d) rates, respectively. Since photosynthesis of the two study species saturates below a PAR of 2000 μmol m^{-2} s^{-1} (Zhao et al., 2018), the measured net photosynthetic rate is referred to as the photosynthetic capacity (A_{max}). After switching PAR from 2000 to 0 μmol m^{-2} s^{-1}, we allowed R_d to stabilize for at least 30 s before the value was recorded. Since R_d was measured on sun-exposed plants, it represents mitochondrial respiration in the light (Kromer, 1995). In addition, corresponding transpiration, stomatal conductance (g_s) and leaf intercellular CO_2 concentration (C_i) were also computed as output variables by the LI-6400XT. The CO_2 concentration within the chamber (C_a) was set to 400 ppm and vapor pressure deficit (VPD) was controlled within 1.0–2.0 kPa with a mean of 1.7 kPa under the ambient temperature using a drierite scrubber to prevent water vapor saturation. Leaf temperatures within the chamber varied from 29 to 37°C with a mean of 33°C. After each measurement, we measured the leaf diameter of muhly grass or leaf width of sawgrass and the length of the leaf inside of the chamber. Based on these dimensions, the actual measured leaf surface area was estimated assuming a rectangular shape for sawgrass and cylinder for muhly grass. To ensure that the fluxes were comparable between the two studied species, we used the total leaf area (i.e., all sides of a leaf) for flux calculations. These leaf areas were then used to recompute A_{max}, R_d, and g_s. Water-use efficiency (WUE, μmol CO_2/mol H_2O) was computed as the ratio of A_{max} to g_s.

During week 19 of the experiment (October 2018), A/C_i curves of photosynthesis were estimated across a C_a sequence (400, 300, 200, 100, 0, 400, 800, and 1200 ppm) to determine the relationship between net photosynthetic rates (A) and C_i. This measurement was carried out on two leaves in each container and allowed us to determine maximum rate of carboxylation (V_{cmax}) and photosynthetic electron transport (J_{max}) (Farquhar et al., 1980). At the end of the experiment (week 24), we measured photosynthetic rates over a PAR gradient (2000, 1600, 1200, 800, 600, 400, 200, 100, 50, and 0 μmol m^{-2} s^{-1}) for one leaf per container to develop a light response curve for each species and thus determine the photosynthetic quantum efficiency and light compensation point.

Ambient air temperature during the physiological measurements was measured using a K-type thermocouple. However, due to its relatively small variance during the experiment (i.e., 27–35 °C, Appendix S3), which is typical for the wet season in the Everglades region (Schedlbauer et al., 2010), air temperature showed rather weak correlations with the plant physiological variables (A_{max}, R_d, and g_s, Appendix S4). Thus, temperature was not considered for further data analysis.

Leaf growth rates, allometric traits, and plant carbon reserves

For monitoring plant growth, four healthy leaves with lengths between 20 and 50 cm were selected in each container at the beginning of the experiment, and their lengths were measured every 4 weeks. In cases when the leaf turned completely brown, it was marked as dead, and another leaf of similar length was selected from the same container and measured to continue growth monitoring. Therefore, we measured four healthy leaves in each container throughout the experiment. For each measurement campaign, the growth rate was calculated as the increase in length per day,

and the proportion of leaf death (%) was also calculated for the 12 leaves in each species–treatment combination group.

To compare plant allometric traits among treatments, we randomly selected two leaves in each container at the end of the experiment (week 25) and weighed them before and after oven-drying at 68°C for 48 h. Leaf water content (WC, %) was then calculated as:

$$\mathrm{WC} = \frac{\text{Fresh mass} - \text{Dry mass}}{\text{Fresh mass}} \times 100\%. \quad (1)$$

We also measured the projected leaf areas using a LI-3000A Portable Leaf Area Meter (LI-COR) and calculated the specific leaf mass (SLM, g cm^{-2}) as:

$$\mathrm{SLM} = \frac{\text{Dry mass}}{\text{Projected leaf area}} \quad (2)$$

To determine plant carbon reserves, we collected leaf and root samples with two replicates from each container in week 25 for nonstructural carbohydrate (NSC) analysis. The samples were immediately freeze-dried for 72 h in a Scientific Freeze Dryer (Harvest Right, Inc., North Salt Lake, UT, USA) and were then ground into a fine powder using a ball mill (8000 M Mixer/Mill, SPEX CertiPrep, Inc., Metuchen, NJ, USA). Concentrations of NSCs for the samples were measured using the protocol outlined by Landhausser et al. (2018). Essentially, 80% ethanol solution was used to extract soluble sugars (glucose, sucrose and fructose) from samples, and the residue (pellet) was used for starch determination. Two enzymes, α-amylase and amyloglucosidase, were applied to convert starch to glucose hydrolysate. The NSC concentrations from all extracts (i.e., glucose, fructose, and sucrose in the ethanol extracts and the glucose hydrolysate in starch-digested solutions) were measured with high-pressure liquid chromatography–pulsed amperometric detection (HPLC-PAD) on a Dionex ICS3000 ion chromatography system at Max Planck Institute for Biogeochemistry (Jena, Germany). In each HPLC run, a series of duplicate samples were included to allow the coefficient of variation (CV) to be computed as a measure of analytical precision. Runs with CV > 10% were checked for potential sources of inconsistency and repeated. Internal standards (i.e., a homogenized mixture of tree sessile oak and European beech leaves and branches) were also measured with the test samples for long-term measurement stability.

Data processing and analysis

The gross light-saturated photosynthetic rate (P_{max}) was calculated as the sum of A_{max} and R_d. A decline in the ratio of P_{max} to C_i (P_{max}/C_i) was used as a measure of the increase in nonstomatal limitation on photosynthesis (Salmon et al., 2020).

The Farquhar-Berry-von Caemmerer model (Farquhar et al., 1980) was used to fit the relationship between A and C_i for the C_3 sawgrass, and the maximum rates of carboxylation (V_{cmax}) and electron transport (J_{max}) at 25°C were derived from the model (Bernacchi et al., 2001). The R package plantecophys was used to estimate these A/C_i curves (Duursma, 2015). For the C_4 muhly grass, we only compared patterns of A plotted against C_i among different treatments without fitting models because models for C_4 plants usually require other ancillary measurements and involve large uncertainties (von Caemmerer, 2000; Bellasio et al., 2016).

The rectangular hyperbolic model was used to fit A as a function of PAR (Thornley, 1998; Luo et al., 2000; e.g., Kyei-Boahen et al., 2003):

$$A = \frac{\Phi \times \mathrm{PAR} \times P_{max}}{\Phi \times \mathrm{PAR} + P_{max}} + R_d, \quad (3)$$

where the estimated parameters are Φ (quantum efficiency) and P_{max}. The light compensation point was derived from the fitted models as the value of PAR where A = 0 μmol CO_2 m^{-2} s^{-1}.

Mixed effects models were used to determine the effect of water level, inundation duration and species on A_{max}, R_d, g_s, P_{max}/C_i, WUE, and growth rates. Species, water level, and inundation duration and their two- and three-way interaction terms were included as independent variables. The measurement date and container ID nested within mesocosm ID were included as random effects to account for measurements taken repeatedly over time and sample groupings, respectively. Similar mixed effects models were also estimated for variables that were measured only once (i.e., V_{cmax}, J_{max}, Φ, light compensation point, WC, SLM, and soluble sugar and starch concentrations), without including the effect of inundation duration or time of measurement. Assumptions of normality and homoscedasticity were evaluated visually by plotting residuals. A post hoc Tukey honestly significant difference (HSD) test was used to further investigate the differences among different water-level treatments on each measurement occasion.

In addition, mixed effects models were also used to fit the relationship between P_{max}/C_i and g_s separately for sawgrass and muhly grass to investigate the correlation of nonstomatal and stomatal controls on photosynthesis. The water level, measurement date and container ID nested within mesocosm ID were included as random effects. Logarithmic transformations were applied to either P_{max}/C_i or g_s to meet the normality assumption for the residuals.

R 3.5.2 (R Core Team, 2020) was used for all data processing and analyses. The R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2016) were used to estimate mixed effects models, and the package emmeans was used to estimate marginal means and perform post hoc Tukey HSD tests (Lenth, 2019). All graphs were created with the package ggplot2 (Wickham, 2016). Graphical representations of model effects are shown with mixed model marginal means and SE, with all other effects in the model at their average values.

RESULTS

Photosynthesis and dark respiration

Without inundation stress (week 0), sawgrass and muhly grass had similar photosynthetic capacities (A_{max}) of ~7 μmol m^{-2} s^{-1} (Figure 1A, B). Over the experimental period, the effect of inundation duration on A_{max} depended jointly on species and water level (3-way interaction, $P < 0.01$, Table 1, Figure 1A, B). Under inundation treatments, sawgrass A_{max} dropped to ~4.5 μmol m^{-2} s^{-1} in week 7 after inundation and remained significantly lower ($P < 0.05$) compared to the control until the end of the experiment (Figure 1A). However, A_{max} values in sawgrass were not different between the 10 and 35 cm treatments on all measurement occasions ($P < 0.05$). For muhly grass, the inundation treatments (both 10 and 35 cm water) decreased A_{max} by more than 70% to ~2 μmol m^{-2} s^{-1} during weeks 3 to 5 and remained significantly lower versus the control ($P < 0.01$) thereafter (Figure 1B). Further, A_{max} in the 35 cm treatment continued to decline and became significantly lower than that of the 10 cm treatment ($P < 0.01$) from week 17 on. At the end of the experiment (week 24), A_{max} in the 35 cm treatment was near zero (0.2 ± 0.2 SE μmol m^{-2} s^{-1}).

Dark respiration (R_d) was generally higher in the leaves of muhly grass than those of sawgrass (Figure 1C, D). The effect of inundation duration on R_d depended jointly on species and water level (3-way interaction, $P = 0.04$, Table 1). Sawgrass R_d averaged ~0.9 μmol m^{-2} s^{-1} and was not different between treatments and control over the entire period (Figure 1C). For muhly grass, R_d in the inundation treatments significantly declined below that of the controls ($P < 0.05$) from week 5 to the end of the experiment (but see exceptions in weeks 11 and 13 when R_d was similar across all water levels, $P > 0.05$) (Figure 1D). At the end of the experiment, R_d of muhly grass remained well above zero at 1.4 ± 0.2 SE and 0.7 ± 0.2 SE μmol m^{-2} s^{-1} in the 10 and 35 cm treatment, respectively.

Stomatal and nonstomatal responses

Values of g_s were generally lower for the leaves of muhly grass than those of sawgrass (Figure 2A, B). The effect of water level on g_s depended on both inundation duration (two-way interaction, $P < 0.01$) and species (two-way interaction, $P = 0.01$) (Table 1). Notably, g_s in sawgrass in the inundation treatments declined as early as the first week, as indicated by the significantly lower g_s in the 10 cm treatment compared to the control ($P < 0.01$, Figure 2A); in muhly grass, this effect was also observed but started later in the third week (Figure 2B). After that, g_s remained lower in the inundation treatments compared to that of the control for both species until the end of the experiment ($P < 0.05$), but values were similar between the 10 and 35 cm treatments on most occasions ($P > 0.05$).

P_{max}/C_i was significantly affected by the inundation duration, but its impact jointly depended on water level and species (3-way interaction, $P < 0.01$, Table 1). For sawgrass, P_{max}/C_i in the inundation treatments became ~20% lower than those in the control in week 7 (Figure 2C) and was maintained until the end of the experiment without further substantial declines. For muhly grass (Figure 2D), P_{max}/C_i in 10 and 35 cm treatments declined by >70% and became significantly lower than in the control starting week 3 and 5 onward, respectively.

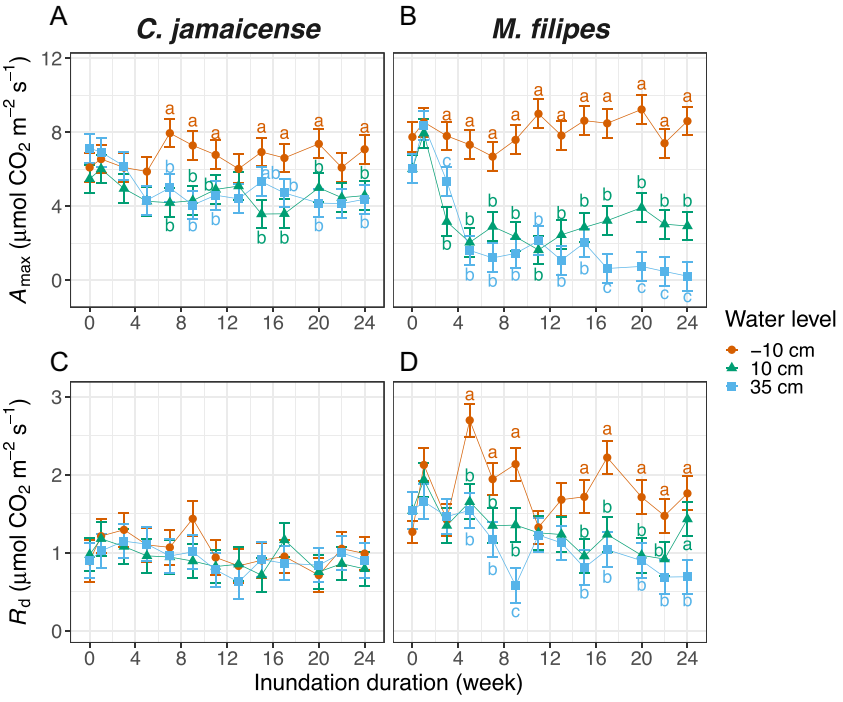


FIGURE 1 Marginal mean values (±1 SE) of photosynthetic capacity (A_{max}, A, B) and dark respiration (R_d, C, D) measured on leaves of sawgrass (*Cladium jamaicense*, A, C) and muhly grass (*Muhlenbergia filipes*, B, D) over time of inundation in different water level treatments. Different lowercase letters indicate significant differences among water level treatments for each measurement time ($P < 0.05$) based on post-hoc Tukey HSD tests on the corresponding mixed effects model. Days without significant differences are not marked

TABLE 1 Analysis of variance (ANOVA) of mixed effects models for photosynthetic capacity (A_{max}, μmol CO_2 m^{-2} s^{-1}), dark respiration (R_d, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), the ratio of gross light-saturated photosynthetic rate to intercellular CO_2 concentration (P_{max}/C_i, μmol CO_2 m^{-2} s^{-1}), water-use efficiency (WUE, μmol CO_2 mol^{-1} H_2O), maximum rate of carboxylation (V_{cmax}, μmol m^{-2} s^{-1}) and electron transport (J_{max}, μmol m^{-2} s^{-1}) for sawgrass, quantum efficiency (Φ, μmol CO_2 $quanta^{-1}$), light compensation point (LCP, μmol m^{-2} s^{-1}), growth rate (cm day^{-1}), leaf water content (WC, %) and specific leaf mass (SLM, g cm^{-2}), soluble sugar and starch concentrations in the leaves and roots (mg g^{-1})

Model	Effect	SS	NumDF	DenDF	*F*-value
A_{max}	Species	0.12	1	45	0.05
	Water level	71.63	2	45	13.88**
	Duration	19.43	1	13	7.53*
	Species × Water level	17.58	2	45	3.41*
	Species × Duration	28.76	1	441	11.15**
	Water level × Duration	180.78	2	441	35.04**
	Species × Water level × Duration	32.61	2	441	6.32**
R_d	Species	8.02	1	30	39.49**
	Water level	1.01	2	30	2.49
	Duration	2.30	1	11	11.34**
	Species × Water level	0.18	2	30	0.44
	Species × Duration	1.24	1	436	6.11**
	Water level × Duration	0.83	2	437	2.05
	Species × Water level × Duration	1.37	2	437	3.36*
g_s	Species	17.38×10^{-2}	1	23	268.31**
	Water level	1.44×10^{-2}	2	23	11.10**
	Duration	0.17×10^{-2}	1	11	2.61
	Species × Water level	0.73×10^{-2}	2	23	5.60**
	Species × Duration	0.08×10^{-2}	1	427	1.18
	Water level × Duration	1.48×10^{-2}	2	426	11.43**
	Species × Water level × Duration	0.09×10^{-2}	2	426	0.70
P_{max}/C_i	Species	15.72×10^{-3}	1	34	57.38**
	Water level	4.14×10^{-3}	2	34	7.55**
	Duration	2.07×10^{-3}	1	11	7.56*
	Species × Water level	2.53×10^{-3}	2	34	4.62*
	Species × Duration	4.68×10^{-3}	1	423	17.09**
	Water level × Duration	9.05×10^{-3}	2	423	16.51**
	Species × Water level × Duration	4.79×10^{-3}	2	423	8.74**
WUE	Species	106222	1	38	20.88**
	Water level	35967	2	38	3.54*
	Duration	3343	1	11	0.66
	Species × Water level	52113	2	38	5.12*
	Species × Duration	104356	1	428	20.51**
	Water level × Duration	68800	2	428	6.76**
	Species × Water level × Duration	16221	2	428	1.59
V_{cmax} (sawgrass)	Water level	156.90	2	12	6.00*

TABLE 1 (Continued)

Model	Effect	SS	NumDF	DenDF	*F*-value
J_{max} (sawgrass)	Water level	1203.20	2	6	6.54*
Φ	Species	6.90×10^{-5}	1	—	7.99*
	Water level	7.61×10^{-5}	2	—	4.41*
	Species × Water level	6.11×10^{-5}	2	—	3.54
LCP	Species	9703.70	1	—	11.54**
	Water level	19719.70	2	—	11.73**
	Species × Water level	22338.10	2	—	13.28**
Growth	Species	0.01	1	72	2.58
	Water level	0.07	2	72	9.15**
	Duration	0.27	5	360	13.97**
	Species × Water level	0.03	2	72	4.13*
	Species × Duration	0.03	5	360	1.40
	Water level × Duration	0.05	10	360	1.36
	Species × Water level × Duration	0.03	10	360	0.78
WC	Species	1612.96	1	10	95.13**
	Water level	303.13	2	10	8.94**
	Species × Water level	69.40	2	10	2.05
SLM	Species	8.16×10^{-3}	1	12	463.94**
	Water level	1.11×10^{-4}	2	12	3.15
	Species × Water level	8.65×10^{-5}	2	12	2.46
Sugar in leaves	Species	4732.50	1	36	241.85**
	Water level	37.60	2	36	0.96
	Species × Water level	466.40	2	36	11.92**
Sugar in roots	Species	2237.50	1	18	49.92**
	Water level	21.59	2	18	0.24
	Species × Water level	298.63	2	18	4.22*
Starch in leaves	Species	378.78	1	18	7.02*
	Water level	78.47	2	18	0.73
	Species × Water level	181.78	2	18	1.69
Starch in roots	Species	5.62	1	14	16.53**
	Water level	0.78	2	14	1.15
	Species × Water level	2.89	2	14	4.25*

Notes: Container ID nested within the mesocosm ID and the measurement date are included as random effects, except in models for Φ and LCP due to the limited replicates ($n = 3$). SS: type III sums of squares with Satterthwaite approximation for degrees of freedom. NumDF: numerator degrees of freedom. DenDF: denominator degrees of freedom. Significant levels:

*$P < 0.05$.

**$P < 0.01$.

Moreover, P_{max}/C_i in the 35 cm treatment became even lower than in the 10 cm treatment in week 13, 20 and 24 ($P < 0.05$) and dropped to near zero (i.e., 0.003 ± 0.001 SE μmol m^{-2} s^{-1}) at the end of the experiment.

In addition, there were significant relationships between P_{max}/C_i and g_s across the three water level treatments for both sawgrass ($P < 0.01$, Figure 3A) and muhly grass ($P < 0.01$, Figure 3B). However, the relationship

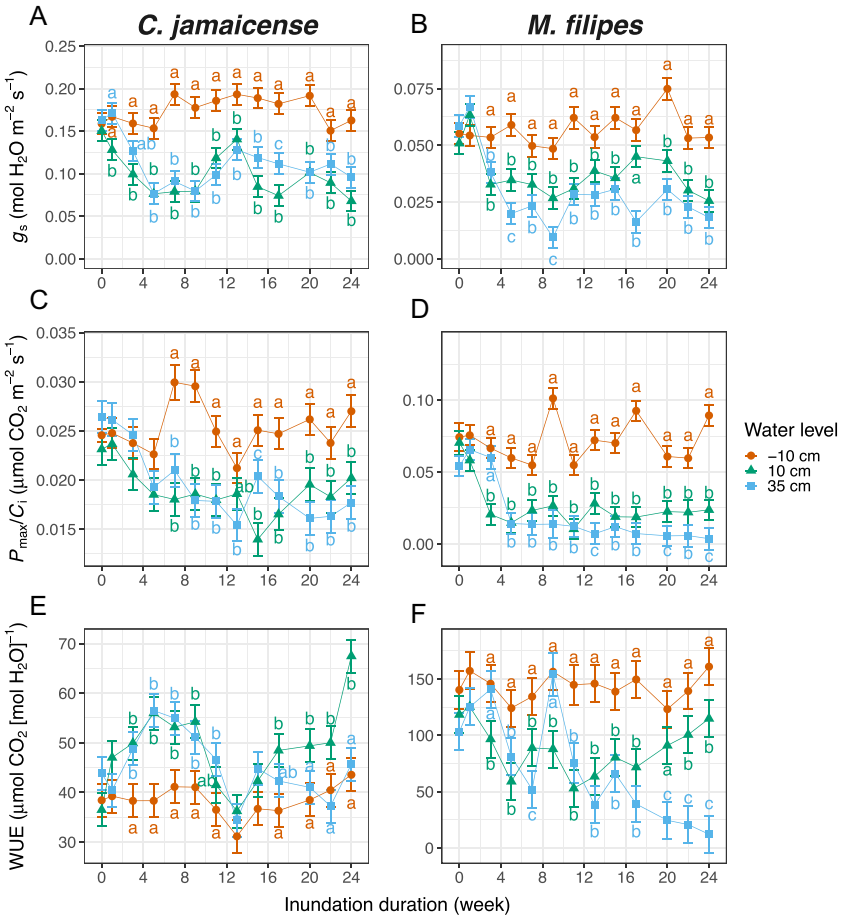


FIGURE 2 Marginal mean values (±1 SE) of stomatal conductance (g_s, A, B), the ratio of gross light-saturated photosynthetic rate to intercellular CO_2 concentration (P_{max}/C_i, C, D), and water-use efficiency (WUE, E, F) for leaves of sawgrass (*Cladium jamaicense*, A, C, E) and muhly grass (*Muhlenbergia filipes*, B, D, F) over time of inundation in different water level treatments. Different lowercase letters indicate significant differences among water level treatments for each measurement time ($P < 0.05$) based on post-hoc Tukey HSD tests on the corresponding mixed effects model. Times without significant differences are not marked. Note that different scales are used for the two species

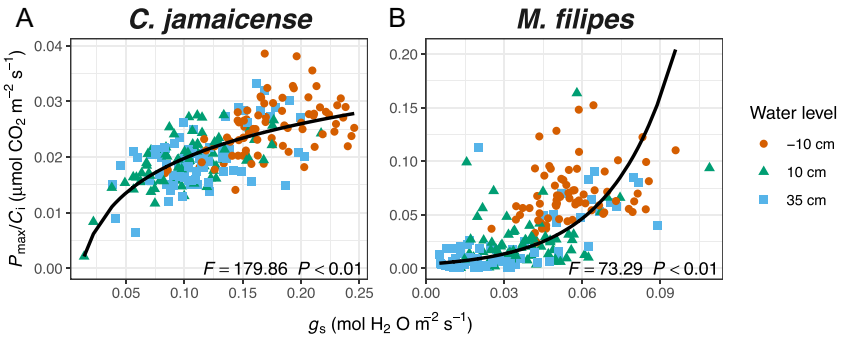


FIGURE 3 Ratio of gross light-saturated photosynthetic rate to intercellular CO_2 concentration (P_{max}/C_i) as a function of stomatal conductance (g_s) for sawgrass (*Cladium jamaicense*, A) and muhly grass (*Muhlenbergia filipes*, B). The curves were estimated using mixed-effects models with the measurement date, water level and container ID nested within the mesocosm ID as random effects. Note that different axis scales are used for the two species

tended to be logarithmic for sawgrass and exponential for muhly grass.

The WUE of sawgrass was significantly lower than that of muhly grass, and for each species, the effect was significantly different by water level and inundation duration (two-way interactions, $P < 0.01$, Table 1, Figure 2E, F). Sawgrass in both the 10 and 35 cm treatment exhibited higher WUE than the control ($P < 0.05$) from week 3 to week 9 (Figure 2C). This high WUE was present again in the 10 cm treatment from week 17 and lasted until the end of the experiment. By contrast, muhly grass reduced WUE in 10 and 35 cm treatments from week 3 and 5 on, respectively, and became significantly lower than the control on most occasions ($P < 0.05$, Figure 2D). Particularly in the 35 cm treatment, muhly grass WUE decreased to near zero (9.8 ± 6.2 SE µmol CO_2 mol^{-1} H_2O) by the end of the experiment (week 24).

Derived from A/Ci curves, sawgrass V_{cmax} and J_{max} were significantly affected by water levels ($P < 0.05$) (Table 1). V_{cmax} in the 35 cm treatment was ~35% lower than that of

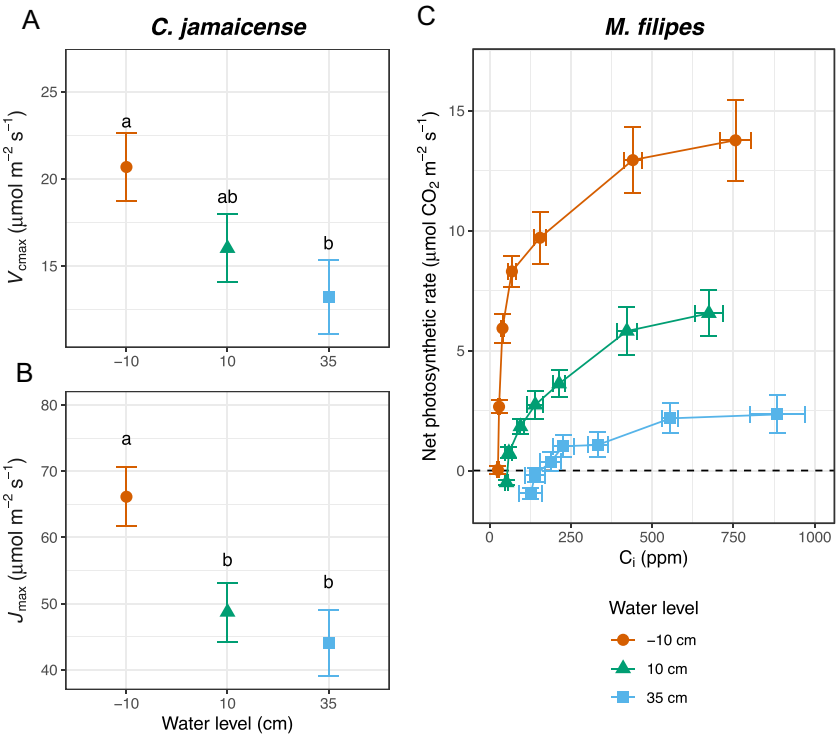


FIGURE 4 Marginal mean values (±1 SE) of maximum rate of carboxylation (V_{cmax}, A) and electron transport (J_{max}, B) for leaves of sawgrass (*Cladium jamaicense*). For muhly grass (*Muhlenbergia filipes*, C), leaf net photosynthetic rates (±1 SE) are plotted against intercellular CO_2 concentration (C_i) in different water level treatments. The measurements were conducted in week 19. Different lowercase letters in (A) and (B) indicate significant differences among water level treatments ($P < 0.05$) based on post-hoc Tukey HSD tests on the corresponding mixed effects model. The points in (C) represent measurements across a CO_2 concentration gradient of 0, 100, 200, 300, 400, 800, 1200 ppm in the air

the control ($P < 0.01$, Figure 4A), while V_{cmax} in the 10 cm treatment was not significantly lower than the control ($P = 0.10$). J_{max} was ~30% lower in both inundation treatments versus the control ($P < 0.01$, Figure 4B). For muhly grass (Figure 4C), the photosynthetic rate reached 13.8 ± 1.7 SE μmol m^{-2} s^{-1} under the highest air CO_2 concentration (i.e., 1200 ppm) in the control. In the 10 and 35 cm treatments, the maximum rate (corresponding to the 1200 ppm air CO_2 concentration) was ~50% (6.6 ± 1.0 SE μmol m^{-2} s^{-1}) and ~80% (2.3 ± 0.8 SE μmol m^{-2} s^{-1}) lower, respectively, than the control, indicating a lower J_{max} in the inundation treatments. Furthermore, given slower initial increases of photosynthetic rate corresponding to the air CO_2 concentration from 0 to 400 ppm, V_{cmax} also appeared to be lower in the inundation treatments than in the control.

For the variables derived from the photosynthetic light response curves, quantum efficiency (φ) differed between species ($P = 0.02$) and among water levels ($P = 0.04$), but there was no significant interaction ($P = 0.07$, Table 1). Across the water levels, φ was not significantly different ($P > 0.05$, Figure 5A) for sawgrass, but was significantly lower in the 35 cm treatment than in the control ($P = 0.02$) for muhly grass (Figure 5B).

The effect of water level on the light compensation point (LCP) was significant and differed by species ($P < 0.01$, Table 1). Generally, LCP varied between 30 and 80 μmol m^{-2} s^{-1} in the control for both sawgrass and muhly grass and was similar across the water levels for sawgrass ($P > 0.05$, Figure 5C). For muhly grass, LCP was much higher in the 35 cm water treatment (296 μmol m^{-2} s^{-1}) versus that of the control and 10 cm treatment (<100 μmol m^{-2} s^{-1}) ($P < 0.01$, Figure 5D).

Growth and death rates

Growth rates were significantly affected by water level, but the effect depended on species (two-way interaction, $P = 0.02$, Table 1). Overall, sawgrass in the inundation treatments had similar growth rates to the control ($P > 0.05$, Figure 6A). By contrast, muhly grass exhibited lower growth rates in the inundation treatments compared to the control ($P < 0.05$) at weeks 4, 12, and 25 (Figure 6B), while growth rates between 10 and 35 cm treatment were similar over the entire inundation period ($P > 0.05$).

Leaf death proportion of sawgrass in the control remained below 10% over the entire period, but it reached 18 and 33% for the sawgrass in the 10 and 35 cm treatments, respectively (Figure 6C). For muhly grass, the maximum leaf death proportion in the control was 18% but reached 42 and 68% for those in the 10 and 35 cm water, respectively (Figure 6D).

Leaf traits

Leaf water content was significantly affected by water levels and species ($P < 0.01$, Table 1) and was lower in the inundation treatments than in the control ($P < 0.05$) for muhly grass (by ~21%) but not for sawgrass ($P > 0.05$) (Figure 7A, B). The difference between the 10 and 35 cm treatments was not significant in either species ($P > 0.05$).

Specific leaf mass was different between species ($P < 0.01$) but not among different water levels ($P = 0.08$, Table 1). However, a higher specific leaf mass was present in muhly grass under the inundation treatments compared to that of the control ($P < 0.05$, Figure 7D).

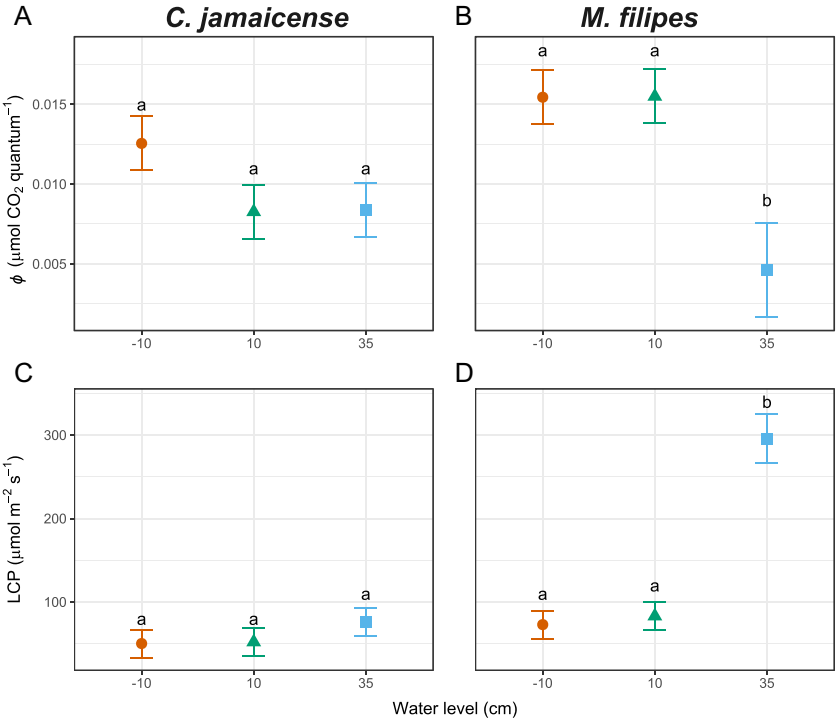


FIGURE 5 Marginal mean values (±1 SE) of quantum efficiency (Φ, A, B) and light compensation point (C, D) estimated from light response curves of photosynthetic rates for sawgrass (*Cladium jamaicense*, A, C) and muhly grass (*Muhlenbergia filipes*, B, D) in different water level treatments measured at the end of the experiment (week 24). Different lowercase letters indicate significant differences among water level treatments ($P < 0.05$) based on post hoc Tukey HSD tests on the corresponding mixed effects model

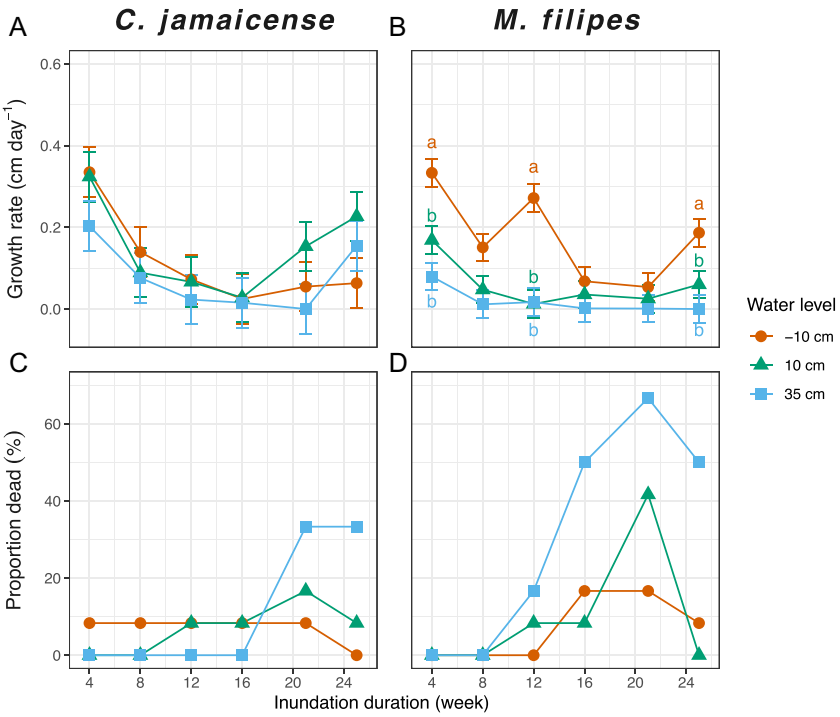


FIGURE 6 Marginal mean values (±1 SE) of leaf growth rate (A, B) and measured proportion of leaf death (C, D) for sawgrass (*Cladium jamaicense*, A, C, E) and muhly grass (*Muhlenbergia filipes*, B, D, F) over time of inundation with different water levels. For the growth rate, different lowercase letters indicate significant differences among water level treatments for each measurement ($P < 0.05$) based on post hoc Tukey HSD tests on the corresponding mixed effects model. Times without significant differences are not marked. The proportion of leaf death was calculated for 12 leaves per species-treatment group for each measurement occasion

Nonstructural carbohydrates

Both the sugar concentrations in leaves and roots were affected by water level, but this effect differed by species (two-way interaction, $P < 0.05$, Table 1). Sawgrass in the inundation treatments had similar soluble sugar concentrations as the control in both leaves and roots ($P > 0.05$, Figure 8A, C). For muhly grass, sugar concentrations in both leaves and roots remained the same as the control in the 10 cm treatment ($P > 0.05$) but were significantly lower in the 35 cm treatment compared to the control ($P < 0.05$, Figure 8B, D). In particular, the sugar concentration in the roots was almost depleted (1.6 ± 0.5 (SE) mg g^{-1}).

Water level did not affect starch concentrations in leaves of either species ($P > 0.05$, Table 1, Figure 8E, F). By contrast, starch in the roots was significantly affected by the

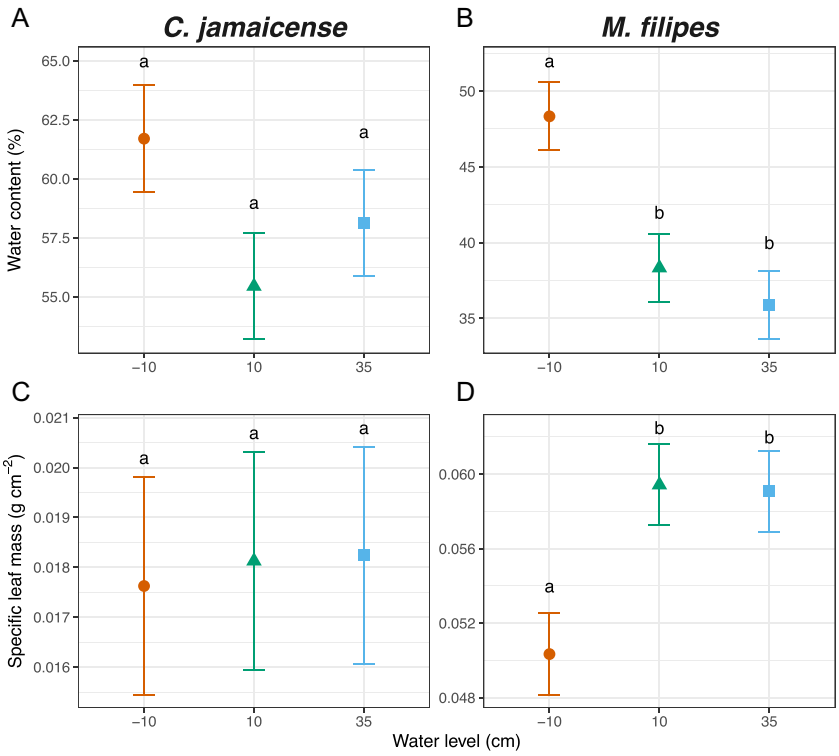


FIGURE 7 Marginal mean values (±1 SE) of leaf water content (A, B) and specific leaf mass (C, D) for sawgrass (*Cladium jamaicense*, A, C) and muhly grass (*Muhlenbergia filipes*, B, D) in different water levels measured in week 22. Different lowercase letters indicate significant differences among water level treatments ($P < 0.05$) based on post hoc Tukey HSD tests on the corresponding mixed effect model. Note that different scales are used for the two species

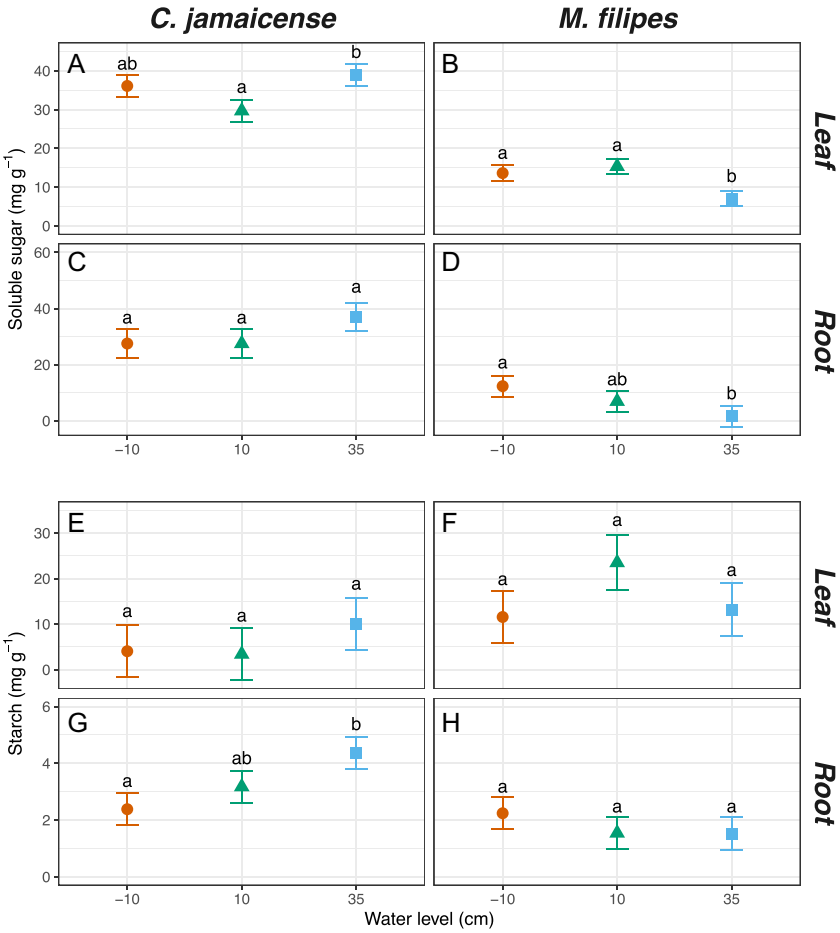


FIGURE 8 Marginal mean values (±1 SE) of total soluble sugar (A–D) and starch concentration (E–H) in leaves (A, B, E, F) and roots (C, D, G, H) for sawgrass (*Cladium jamaicense*, A, C, E, G) and muhly grass (*Muhlenbergia filipes*, B, D, F, H) in different water levels measured at the end of the experiment (week 24 and 25). Different lowercase letters indicate significant differences among water level treatments ($P < 0.05$) based on post hoc Tukey HSD tests on the corresponding mixed effect model

interaction between water level and species ($P = 0.03$, Table 1). Notably, roots of the sawgrass in the 10 cm treatment contained a similar amount of starch as the control ($P = 0.59$, Figure 8G), but those in the 35 cm treatment retained almost twice the amount of starch as in the control ($P = 0.04$). For muhly grass, root starch concentrations were similar across the three water levels ($P > 0.05$, Figure 8H).

DISCUSSION

Wetland plants include a large group of species that have a wide range of tolerances to inundation (Pezeshki, 2001). Compared to species that increase photosynthesis when being inundated (e.g., Jones et al., 2018), the two species in this study generally decreased photosynthesis to different degrees, representing species that are negatively impacted by inundation. The results highlight the significant interactive effects of water level and inundation duration on physiological processes, which, to our knowledge, have not been explicitly addressed before. Particularly important were nonlinear responses of photosynthesis over the course of inundation with a sharp drop in photosynthetic rates in the first 2 months that was sustained over an extended period. With a weak inundation tolerance, muhly grass had a second phase of photosynthetic decline in the deep-water treatment (35 cm) after 4 months of inundation that most likely resulted in a carbon deficit for the plant. Given the linkage between carbon acquisition and plant survival (Ram et al., 2002) and ecosystem sustainability (Chapin et al., 1996), these relationships need to be considered in wetland models at the plant and the ecosystem scales.

Responses in photosynthetic carbon uptake

In line with our first hypothesis, the two species had significantly different photosynthetic responses to inundation duration (Table 1, Figure 1A, B); A_{max} was inhibited almost 1 month earlier and with a greater decline for muhly grass than for sawgrass. However, the deep-water treatment (35 cm) did not cause an earlier or greater inhibition than the shallow-water treatment (10 cm) for either of the species, which refutes our hypothesis. For wetland plants, oxygen supply for submerged leaves and roots is maintained by aerenchyma tissues that transport oxygen from the emergent leaves (Blom and Voesenek, 1996; Jackson and Armstrong, 1999; Voesenek and Bailey-Serres, 2015; Loreti et al., 2016). In theory, deeper water leads to a longer transport pathway for oxygen to reach roots through the aerenchyma. In addition, higher water levels also submerge more leaf area that is photosynthetically active, which further constrains carbon uptake and oxygen production. Our results suggest that deeper water does not impose greater stress to the plants in the early stage of inundation. However, as the inundation persists, with less available oxygen and accumulated carbon, plants in the deeper water experience an enhanced stress, as observed for muhly grass with a further drop in A_{max} after 4 months of inundation (Figure 1B). Therefore, we suggest that inundation stress is initially driven by inundation duration, while a higher water level enhances the stress only when the inundation period is considerably long.

The restrained A_{max} can be partially explained by declines in g_s (Pezeshki, 1993), which were found in both species. At the same time, we also observed simultaneous declines in P_{max}/C_i that were closely correlated with g_s for both species (Figures 2C, D, 3). The decline of P_{max}/C_i can be used as an indication of an increase in nonstomatal limitation on photosynthesis (Salmon et al., 2020). The relationships between P_{max}/C_i and g_s also tended to be nonlinear, suggesting that the importance of stomatal and nonstomatal limitations varied under different stress levels. In sawgrass, P_{max}/C_i and g_s had a logarithmic relationship, suggesting that g_s responds more promptly than nonstomatal processes under mild stress, while nonstomatal limitations dominated only when g_s reduced below ~0.07 mol m^{-2} s^{-1}, which rarely occurred. These responses found in the sawgrass are in line with our second hypothesis. Similar relationships were also documented for some other species when environmental stresses (e.g., drought) were imposed (Salmon et al., 2020). By contrast, for muhly grass, this relationship was better described by an exponential function with a greater initial decline in nonstomatal processes (P_{max}/C_i) than g_s as the stress was imposed, which contradicts to our second hypothesis. As a C_4 plant, muhly grass has a different photosynthetic pathway from the C_3 sawgrass. C_4 plants are known to respond differently than C_3 plants to environmental stresses (e.g., drought) in terms of nonstomatal processes (Ghannoum, 2009). However, the responses we found in muhly grass may not represent all C_4 species. Therefore, more C_4 plant studies are needed to conclude whether this unique nonstomatal response of the muhly grass to inundation is related to the C_4 photosynthetic pathway.

Nonstomatal limitations include the diffusion of CO_2 into chloroplast (i.e., mesophyll conductance) and a series of biochemical processes within chloroplast (Grassi and Magnani, 2005; Kaiser et al., 2015). While it is known that mesophyll conductance could decrease under inundation stress (Moldau, 1973), our results also show evidence of downregulations in biochemical processes including V_{cmax} and J_{max}. These declines in V_{cmax} and J_{max} were likely more substantial for muhly grass than for sawgrass, especially those in the 35 cm treatment (Figure 4). These biochemical limitations in muhly grass were also reflected in its low quantum efficiency in the 35 cm water level (Figure 5B). These larger downregulations of biochemical processes in muhly grass are linked to its stronger nonstomatal limitation in photosynthesis than sawgrass. Moreover, this strong nonstomatal limitation also largely explains the second phase of A_{max} decline after 4 months of inundation for the muhly grass in the 35 cm water level (Figure 1B).

Overall, with an earlier and greater decline in photosynthesis, muhly grass appears to have a more extreme

strategy than sawgrass to cope with inundation stress. By contrast, the initial photosynthetic inhibition of sawgrass even showed signs of enhanced WUE (Figure 2E), suggesting that sawgrass has a physiological advantage over muhly grass in an inundated environment. While muhly grass could survive under mild inundation, it could be threatened in deep water over an extended period by the constrained carbon uptake due to severe metabolic impairment.

Carbon-use strategies

Despite limited carbon input via photosynthesis under inundation, sawgrass maintained its carbon metabolism via leaf growth and respiration at the same levels as the control (Figures 1C, 6A). No reduction was observed in their NSC reserves (Figure 8). These well-maintained patterns seen in both carbon metabolism and storage provide evidence that the inundation stress was mild for the sawgrass, even in the deep-water treatment. This may be largely attributed to the extensive aerenchyma system that is developed throughout the leaves and roots of sawgrass (Appendix S5; Kludze and DeLaune, 1996), allowing sufficient oxygen transport to the roots (Jackson and Armstrong, 1999). In particular, sawgrass had more unsubmerged leaf area above water compared to the muhly grass under the same water level treatment, and these leaves could potentially produce more oxygen through photosynthesis and facilitate oxygen transportation to roots. It is worth noting that sawgrass in the 35 cm water level treatment accumulated even more starch in roots compared to the control (Figure 8G). This result differs from previous studies that reported decreased starch concentrations in plant roots under inundation (e.g., Wample and Davis, 1983; Vu and Yelenosky, 1992). Starch, the primary form of NSC storage in plants, can be converted into sugar for metabolic consumption (Hartmann and Trumbore, 2016). With increased starch storage, our result implies a strategy the plants take under mild stress to prepare for more severe conditions. Given that the photosynthetic carbon input was lower in the 35 cm water level treatment than in the control, the excess starch that accumulated in roots might be a result of reduced root metabolism or growth (Pezeshki et al., 1991), which has as yet to be determined for sawgrass.

In contrast to sawgrass, muhly grass suppressed leaf respiration and growth under inundation to cope with the reduced photosynthesis, representing a typical "quiescence" strategy (Loreti et al., 2016). As the primary energy source for metabolism, sugars were significantly depleted, particularly in roots, for the plants in the 35 cm water level compared to the control (Figure 8B, D), which is consistent with our third hypothesis. Interestingly, of the measured sugars, sucrose in leaves was significantly reduced in the 35 cm water level, while glucose remained unchanged compared to the control (Appendix S6). This result may indicate that, with limited carbon input, plants tended to keep photosynthates as glucose to maintain metabolism in the leaves, while carbon translocation to other portions of the plant in the form of sucrose was significantly reduced. Starch pools in leaves and roots were unchanged for muhly grass under inundation compared to the control (Figure 8F, H). This result differs from many previous inundation studies that found reductions in starch reserves (Vu and Yelenosky, 1992; Pan et al., 2012; Qin et al., 2013). Our results indicate that, even under severe physiological stress, muhly grass managed to maintain a starch pool level that is essential for post-stress recovery (Das et al., 2005) and thus survival (Ram et al., 2002). These carbon-use strategies enable muhly grass, with a relatively weak tolerance to inundation, to survive and dominate in areas that are regularly flooded.

Implications for changes in plant communities

A plant community that consists of plants with different inundation tolerances can be vulnerable to shifts in hydraulic conditions. For example, the Comprehensive Everglades Restoration Plan aims to increase freshwater flow in the Everglades to restore the landscape and alleviate some of the effects of saltwater intrusion (Perry, 2004). With an elevated water flow, higher water levels and longer hydroperiods are expected in many areas of the Everglades, which potentially may lead to changes in the plant communities (Armentano et al., 2006). The study by Armentano et al. (2006) showed that sawgrass is mostly distributed in areas with a mean water level <40 cm, but sawgrass can be present in areas with water up to 100 cm deep and year-round inundation (Todd et al., 2010). By contrast, muhly grass mainly dominates areas with water levels within 15 cm and inundation up to 6 months. Our results provide physiological evidence that reveals the inundation tolerance of the two species, which is in line with their distribution patterns. The results suggest that, under a more intensive inundation scenario, the survival of muhly grass would be threatened due to carbon deficiency, while sawgrass, with a well-maintained carbon balance, may still thrive in most areas of the Everglades. Future studies that track the recovery of plant carbon balance after being treated with different levels of inundation should provide more insights into the sustainability of a plant community.

ACKNOWLEDGMENTS

This study was supported in part by funds from the Department of Energy's National Institute for Climate Change Research grant (07-SC-NICCR-1059) and the National Science Foundation Division of Atmospheric & Geospace Sciences Atmospheric Chemistry Program awards (1561139, 1233006, and 1807533). We thank Dr. Jennifer Richards for help with leaf anatomy observations, Dr. Diego Salazar Amoretti for providing freeze-drying equipment, and Ms. Savoyane Lambert for analyzing the nonstructural carbohydrates. We are grateful to Dr. Paulo C. Olivas for advice on experimental design and Mr. Matthew J. Simon for

assistance with the experiment. We also thank the anonymous reviewers for constructive comments on the original manuscript.

AUTHOR CONTRIBUTIONS

J.Z. conceived the idea and designed the experiment with contributions from S.M., C.S., G.S. and S.O. J.Z., G.S., and S.O. collected the plant monolith samples. J.Z. conducted the experiment. H.H. was responsible for NSC analysis. Data were analyzed by J.Z. with help from C.S. J.Z. led the manuscript writing with critical contributions from all authors.

DATA AVAILABILITY STATEMENT

All data in the study are available at Zenodo (doi: 10.5281/zenodo.4781709).

ORCID

Junbin Zhao http://orcid.org/0000-0001-5142-4901
Sparkle L. Malone https://orcid.org/0000-0001-9034-1076
Gregory Starr https://orcid.org/0000-0002-7918-242X
Henrik Hartmann https://orcid.org/0000-0002-9926-5484

REFERENCES

Akman, M., A. V. Bhikharie, E. H. McLean, A. Boonman, E. J. W. Visser, M. E. Schranz, and P. H. Tienderen. 2012. Wait or escape? Contrasting submergence tolerance strategies of Rorippa amphibia, Rorippa sylvestris and their hybrid. *Annals of Botany* 109: 1263–1275.

Armentano, T. V., J. P. Sah, M. S. Ross, D. T. Jones, H. C. Cooley, and C. S. Smith. 2006. Rapid responses of vegetation to hydrological changes in Taylor Slough, Everglades National Park, Florida, USA. *Hydrobiologia* 569: 293–309.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.

Bellasio, C., D. J. Beerling, and H. Griffiths. 2016. Deriving C_4 photosynthetic parameters from combined gas exchange and chlorophyll fluorescence using an Excel tool: theory and practice. *Plant Cell and Environment* 39: 1164–1179.

Bernacchi, C. J., E. L. Singsaas, C. Pimentel, A. R. Portis, and S. P. Long. 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant Cell and Environment* 24: 253–259.

Blom, C. W. P. M., and L. A. C. J. Voesenek. 1996. Flooding: the survival strategies of plants. *Trends in Ecology & Evolution* 11: 290–295.

Bragina, T., I. Drozdova, Y. V. Ponomareva, V. Alekhin, and G. Grineva. 2002. Photosynthesis, respiration, and transpiration in maize seedlings under hypoxia induced by complete flooding. *Doklady Biological Sciences* 384: 274–277.

Chapin, F. S., M. S. Torn, and M. Tateno. 1996. Principles of ecosystem sustainability. *American Naturalist* 148: 1016-1037.

Chen, H. J., M. F. Zamorano, and D. Ivanoff. 2010. Effect of flooding depth on growth, biomass, photosynthesis, and chlorophyll fluorescence of Typha domingensis. *Wetlands* 30: 957–965.

von Caemmerer, S. 2000. Biochemical models of leaf photosynthesis. Techniques in plant sciences no. 2. CSIRO Publishing, Collingwood, Australia.

Das, K. K., R. K. Sarkar, and A. M. Ismail. 2005. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. *Plant Science* 168: 131–136.

Duursma, R. A. 2015. Plantecophys–An R package for analysing and modelling leaf gas exchange data. *PLoS One* 10: 1–13.

Else, M. A., D. Coupland, L. Dutton, and M. B. Jackson. 2001. Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (Ricinus communis) despite diminished delivery of ABA from the roots to shoots in xylem sap. *Physiologia Plantarum* 111: 46–54.

Ewing, K. 1996. Tolerance of four wetland plant species to flooding and sediment deposition. *Environmental and Experimental Botany* 36: 131–146.

Farquhar, G. D., S. V. Caemmerer, and J. A. Berry. 1980. A biochemical-model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149: 78–90.

Ghannoum, O. 2009. C_4 photosynthesis and water stress. *Annals of Botany* 103: 635–644.

Grassi, G., and F. Magnani. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell and Environment* 28: 834–849.

Hartmann, H., and S. Trumbore. 2016. Understanding the roles of nonstructural carbohydrates in forest trees–from what we can measure to what we want to know. *New Phytologist* 211: 386–403.

Islam, M. A., and S. E. Macdonald. 2004. Ecophysiological adaptations of black spruce (Picea mariana) and tamarack (Larix laricina) seedlings to flooding. *Trees-Structure and Function* 18: 35–42.

Jackson, M. B. 2002. Long-distance signalling from roots to shoots assessed: the flooding story. *Journal of Experimental Botany* 53: 175–181.

Jackson, M. B., and W. Armstrong. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology* 1: 274–287.

Jones, S. F., C. L. Stagg, K. W. Krauss, and M. W. Hester. 2018. Flooding alters plant-mediated carbon cycling independently of elevated atmospheric CO_2 concentrations. *Journal of Geophysical Research: Biogeosciences* 123: 1976–1987.

Kaiser, E., A. Morales, J. Harbinson, J. Kromdijk, E. Heuvelink, and L. F. M. Marcelis. 2015. Dynamic photosynthesis in different environmental conditions. *Journal of Experimental Botany* 66: 2415–2426.

Kayranli, B., M. Scholz, A. Mustafa, and Å. Hedmark. 2010. Carbon storage and fluxes within freshwater wetlands: a critical review. *Wetlands* 30: 111–124.

Kludze, H. K., and R. D. DeLaune. 1996. Soil redox intensity effects on oxygen exchange and growth of cattail and sawgrass. *Soil Science Society of America Journal* 60: 616–621.

Kromer, S. 1995. Respiration during photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 46: 45–70.

Kuznetsova, A., P. Brockhoff, and R. Christensen. 2016. lmerTest: tests in linear mixed effects models. R package version 2.0-30. Website: https://CRAN.R-project.org/package=lmerTest

Kyei-Boahen, S., R. Lada, T. Astatkie, R. Gordon, and C. Caldwell. 2003. Photosynthetic response of carrots to varying irradiances. *Photosynthetica* 41: 301–305.

Lan, Z. C., H. Huang, Y. S. Chen, J. S. Liu, J. G. Chen, L. Li, L. Li, et al. 2019. Testing mechanisms underlying responses of plant functional traits to flooding duration gradient in a lakeshore meadow. *Journal of Freshwater Ecology* 34: 481–495.

Landhausser, S. M., P. S. Chow, L. T. Dickman, M. E. Furze, I. Kuhlman, S. Schmid, J. Wiesenbauer, et al. 2018. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. *Tree Physiology* 38: 1764–1778.

Lenth, R. 2019. emmeans: estimated marginal means, aka least-squares means: R package version 1.3.4. Website: https://CRAN.R-project.org

Liao, C. T., and C. H. Lin. 1994. Effect of flooding stress on photosynthetic activities of Momordica charantia. *Plant Physiology and Biochemistry* 32: 479–485.

Loreti, E., H. van Veen, and P. Perata. 2016. Plant responses to flooding stress. *Current Opinion in Plant Biology* 33: 64–71.

Luo, Y. Q., D. F. Hui, W. X. Cheng, J. S. Coleman, D. W. Johnson, and D. A. Sims. 2000. Canopy quantum yield in a mesocosm study. *Agricultural and Forest Meteorology* 100: 35–48.

Miller, R. C., and J. B. Zedler. 2003. Responses of native and invasive wetland plants to hydroperiod and water depth. *Plant Ecology* 167: 57–69.

Moldau, H. 1973. Effects of various water regimes on stomatal and mesophyll conductances of bean leaves. *Photosynthetica* 7: 1-7.

Mommer, L., and E. J. W. Visser. 2005. Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. *Annals of Botany* 96: 581–589.

Pan, Y., Y. H. Xie, X. S. Chen, and F. Li. 2012. Effects of flooding and sedimentation on the growth and physiology of two emergent macrophytes from Dongting Lake wetlands. *Aquatic Botany* 100: 35–40.

Perry, W. 2004. Elements of south Florida's comprehensive Everglades restoration plan. *Ecotoxicology* 13: 185–193.

Pezeshki, S. R. 1993. Differences in patterns of photosynthetic responses to hypoxia in flood-tolerant and flood-sensitive tree species. *Photosynthetica* 28: 423–430.

Pezeshki, S. R. 1994. Responses of baldcypress (Taxodium distichum) seedlings to hypoxia-leaf protein-content, ribulose-1,5-bisphosphate carboxylase oxygenase activity and photosynthesis. *Photosynthetica* 30: 59–68.

Pezeshki, S. R. 2001. Wetland plant responses to soil flooding. *Environmental and Experimental Botany* 46: 299–312.

Pezeshki, S. R., S. W. Matthews, and R. D. Delaune. 1991. Root cortex structure and metabolic responses of Spartina patens to soil redox conditions. *Environmental and Experimental Botany* 31: 91–97.

Pezeshki, S. R., R. D. DeLaune, H. K. Kludze, and H. S. Choi. 1996. Photosynthetic and growth responses of cattail (Typha domingensis) and sawgrass (Cladium jamaicense) to soil redox conditions. *Aquatic Botany* 54: 25–35.

Qin, X. Y., F. Li, X. S. Chen, and Y. H. Xie. 2013. Growth responses and non-structural carbohydrates in three wetland macrophyte species following submergence and de-submergence. *Acta Physiologiae Plantarum* 35: 2069–2074.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: http://www.R-project.org/

Ram, P. C., B. B. Singh, A. K. Singh, P. Ram, P. N. Singh, H. P. Singh, I. Boamfa, et al. 2002. Submergence tolerance in rainfed lowland rice: physiological basis and prospects for cultivar improvement through marker-aided breeding. *Field Crops Research* 76: 131–152.

Richards, J. H., and P. C. Olivas. 2020. A common-mesocosm experiment recreates sawgrass (Cladium jamaicense) phenotypes from Everglades marl prairies and peat marshes. *American Journal of Botany* 107: 56–65.

Rose, C., and W. G. Crumpton. 1996. Effects of emergent macrophytes on dissolved oxygen dynamics in a prairie pothole wetland. *Wetlands* 16: 495–502.

Salmon, Y., A. Lintunen, A. Dayet, T. Chan, R. Dewar, T. Vesala, and T. Hooltta. 2020. Leaf carbon and water status control stomatal and nonstomatal limitations of photosynthesis in trees. *New Phytologist* 266: 690–703.

Schedlbauer, J. L., S. F. Oberbauer, G. Starr, and K. L. Jimenez. 2010. Seasonal differences in the CO_2 exchange of a short-hydroperiod Florida Everglades marsh. *Agricultural and Forest Meteorology* 150: 994–1006.

Thornley, J. H. M. 1998. Dynamic model of leaf photosynthesis with acclimation to light and nitrogen. *Annals of Botany* 81: 421–430.

Todd, M. J., R. Muneepeerakul, D. Pumo, S. Azaele, F. Miralles-Wilhelm, A. Rinaldo, and I. Rodriguez-Iturbe. 2010. Hydrological drivers of wetland vegetation community distribution within Everglades National Park, Florida. *Advances in Water Resources* 33: 1279–1289.

Troxler, T. G., D. L. Childers, and C. J. Madden. 2014. Drivers of decadal-scale change in southern everglades wetland macrophyte communities of the coastal ecotone. *Wetlands* 34: S81–S90.

Voesenek, L. A. C. J., and J. Bailey-Serres. 2015. Flood adaptive traits and processes: an overview. *New Phytologist* 206: 57–73.

Vu, J. C. V., and G. Yelenosky. 1992. Photosynthetic responses of rough lemon and sour orange to soil flooding, chilling, and short-term temperature fluctuations during growth. *Environmental and Experimental Botany* 32: 471–477.

Wample, R. L., and R. W. Davis. 1983. Effect of flooding on starch accumulation in chloroplasts of sunflower (Helianthus annuus L). *Plant Physiology* 73: 195–198.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag, NY, NY, USA.

Zhao, J., S. L. Malone, S. F. Oberbauer, P. C. Olivas, J. L. Schedlbauer, C. L. Staudhammer, and G. Starr. 2019. Intensified inundation shifts a freshwater wetland from a CO_2 sink to a source. *Global Change Biology* 25: 3319–3333.

Zhao, J., S. F. Oberbauer, P. C. Olivas, J. L. Schedlbauer, J. L. May, J. G. Moser, S. L. Malone, et al. 2018. Contrasting photosynthetic responses of two dominant macrophyte species to seasonal inundation in an Everglades freshwater prairie. *Wetlands* 38: 893–903.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix S1. Design of the inundation experiment.

Appendix S2. Dates of measurements.

Appendix S3. The air temperature (red) and mesocosm water temperature (blue) on each measurement time.

Appendix S4. Spearman correlation coefficients between the gas exchange variables (A_{max}, R_d, and g_s) and air temperature over the entire period of the experiment.

Appendix S5. Leaf cross-sectional structure for (A) sawgrass (*C. jamaicense*), and (B) muhly grass (*M. filipes*) in the 10 cm water level treatment at the end of the experiment (Week 25).

Appendix S6. Least square mean values (±1 SE) of the content of different types of soluble sugar (glucose, sucrose, and fructose) in leaves and roots of sawgrass (*C. jamaicense*) and muhly grass (*M. filipes*) in different water level treatments at the end of the experiment (weeks 24 and 25).

How to cite this article: Zhao, J., S. L. Malone, C. L. Staudhammer, G. Starr, H. Hartmann, and S. F. Oberbauer. 2021. Freshwater wetland plants respond nonlinearly to inundation over a sustained period. *American Journal of Botany* 108(10): 1917–1931. https://doi.org/10.1002/ajb2.1746