Appendix 5C-1: Evaluation of Inundation Depth and Duration Threshold for *Typha domingensis* (Cattail) Sustainability: Test Cell Study

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SUMMARY

Typha sp. (cattails) are the most abundant emergent aquatic vegetation (EAV) communities in the Everglades Stormwater Treatment Area (STAs). These EAV communities support STA performance by reducing particulate phosphorus (PP) in the water column and facilitating microbial phosphorus (P) cycling through production of litter. Previous field observations and studies indicated that water depths exceeding certain criteria and maintained over long periods resulted in physiological stress, reducing growth, biomass, density, and anchorage capacity of *Typha* in STA treatment cells. High flow events that occur during the wet season are a major factor resulting in these stressful water conditions that can last for weeks if not months. The high waters are exacerbated by *Typha* community architecture and vegetation density, which contributes to flow resistance resulting in increases of water depths at the inflow regions of treatment cells. This study measured several components over a 10-month period at various water level depths on *Typha* communities grown into 0.2-hectare test cells. The objective was to determine the depth and duration that could stress *Typha* communities and result in reduced performance in STAs.

Typha communities previously planted and grown in test cells located at the northern end of STA-1 West (STA-1W) were inundated on July 1, 2019. Five water level treatments were randomly assigned to the 15 test cells: control – 40 centimeters (cm), shallow – 61 cm, moderate – 84 cm, deep – 104 cm, and extremely deep – 124 cm. Three test cells each were assigned to each water level. For the first three months, measurements were made biweekly, followed by monthly measurements until April 2020. The biweekly and monthly measurements included *Typha* density (adult, juvenile, and dead), gas exchange rates (photosynthetic rates, stomatal conductance, and water use efficiency [WUE]), leaf area index (LAI), leaf elongation rate (LER), water depth, and outflow total phosphorus (TP) concentrations. Field observations included visible *Typha* damage and presence of floating *Typha* within the plots, presence of other emergent or floating aquatic plants within the plots, and photo documentation of each plot. Aboveground and belowground biomass and soils were sampled before and after the test. Statistical tests were considered significant at the probability (p) < 0.05 level.

Mean adult *Typha* densities declined over the 10-month inundation period by 23 and 26% in the deep and extremely deep water level treatments, respectively. This decline was significant in the first four months of the study (July–October) but not for the full 10 months compared to the control. Floating *Typha* mats were primarily observed in the deeper water level treatments over time, which is consistent with results from other studies. Mean juvenile densities in these deep and extremely deep conditions declined significantly by 37 and 57%, respectively. Lower adult densities in the deeper water level treatments could

be attributed to lower replacement over time by fewer juvenile *Typha* and the increased floating mats. Further analyses of the first 4 months of data is ongoing.

LER values were significantly higher in *Typha* plants from the deeper water level treatments, with the higher rates observed during the active growing period of the *Typha* population (July–October). LER values increased from 6.84 centimeters per day (cm/d) during the June baseline event to an average of 8.89 cm/d in the deep and extremely deep treatments after eight weeks of continuous flooding. In their effort to survive under deep water conditions, *T. domingensis* has the capacity to stimulate the elongation of leaves to escape deep water conditions to facilitate the exchange of gases to and from the roots and lower the reliance of external oxygen diffusion through the water and soil (Armstrong 1979, Brix 1993).

One of the primary effects of increasing water depths is increasing light limitation, which can severely reduce gas exchange between the plant and the environment. Plants in deeper waters have a decreasing proportion of their total biomass capable of photosynthetic activity, which can lead to oxygen and energy deficits due to restricted aerobic metabolisms (Crawford and Braendle 1996). However, an advantage of plants such as *Typha* is their ability grow taller under deep water conditions, which maintains effective carbon assimilation through aerial photosynthesis (Mommer et al. 2005). This may partially explain the limited effect of water level treatments on gas exchange rates. Photosynthetic rates across water level treatments during the entire monitoring period were significantly different only between the control and the extremely deep treatment (124 cm). Mean stomatal conductance (transpiration) and WUE values across water level treatments during the entire monitoring period were not significantly different. The limited effect of water level treatments on gas exchange rates measured in this study suggest that the *Typha* communities were not stressed to the point where these physiological parameters were significantly affected in deeper water level treatments. However, these parameters were highly variable by monthly events, suggesting a seasonal effect. In addition, outflow concentrations of TP, total nitrogen (TN), PP, and soluble reactive phosphorus (SRP) were very similar for all treatments over time, indicating little effect of water levels on P removal within the treatment cells.

This study shows that *T. domingensis* has strong morphological plasticity and effectively adapts to increased inundation to survive. These adjustments might include both changes in morphology (i.e. increase in plant height) and changes in biomass allocation (Grace 1989, Chen et al. 2010). As water depths increased, *Typha* height and weight significantly increased, i.e., fewer but significantly larger ramets were measured in the deeper treatments. Likewise, the average biomass weight per ramet was significantly higher in the deeper treatments with adult ramets from the extremely deep treatment being twice as heavy as adult ramets from the control plots. There were no significant differences in biomass allocation due to changes in water depth. However, there was a reduction in vegetative reproduction, as the juvenile density significantly decreased with increasing water depths.

Trade-off between plant density and plant weight in the *Typha* populations among the different water level treatments could in part explain the lack of significance of biomass change due to water depths in this study. Similarly, tissue nutrient concentrations were not significantly different with increases in water depth. The largest TP concentration among the different biomass components regardless of water level treatments was the shoot base. High nutrient concentrations in tissues associated with resource storage (i.e., shoot bases) indicate that *T. domingensis* stored nutrients even under deep water conditions. Likewise, similarities in shoot base biomass among the water level treatments indicates that *Typha* plants still allocated considerable amount of resources for shoot base growth, a strategy that improve post-flooding survivorship of *T. domingensis.*

Water depths negatively affected *Typha* density and their ability to reproduce vegetatively, especially in the deep and extremely deep water levels. However, most physiological factors examined indicated that flooding depths did not create an environment sufficiently stressful to limit these responses. *Typha* communities rapidly responded to the different water level treatments in this study, especially in the deep and extremely deep treatments, which allowed them to escape the deep water conditions and maintain a favorable gas exchange between above and below ground plant parts. However, during this process, the cell walls of stems and leaves weakened to allow cells to expand (Purves et al. 2004), which was more evident in *Typha* growing in the deep and extremely deep water depths treatments. While the treatment cells were flooded, the *Typha* were held upright due to their buoyancy in deeper water. In the post-treatment period, when water levels were lowered, *Typha* plants fell over, a process called lodging, because of the weak and elongated stems and leaves. This post study observations suggest a follow up to evaluate the duration of inundation threshold of water depths greater than 84 cm for shorter periods of time (specifically leaf elongation) to understand the potential effects when water levels return to shallower depths.

In this experiment, *Typha* communities were stressed at deep and extremely deep waters, which was evident in the increased tussock formation and lodging after water levels dropped at the end of the experiment. *Typha* compensated for these deep and extremely deep waters through increased LER, which allowed the *Typha* to maintain normal photosynthetic rates, transpiration, and WUE. While long periods of deep and extremely deep water caused reductions of the adult and juvenile *Typha* (particularly in the first 4 months), there was no effect on P removal. These results suggest that deep and extremely deep waters can be tolerated by *Typha* communities within the STAs if these deep conditions are regulated (less than 4 months), and after flooding, the waters can be slowly reduced to decrease lodging. This water level management can reduce replanting efforts and tussock removal.

INTRODUCTION

Emergent macrophytes are an essential component of wetlands constructed for nutrient reduction because of their ability to directly uptake nutrients (Vymazal 2007, Chen 2011, Bansal et al. 2019). *T. domingensis* and *T. latifolia* are the most common emergent macrophyte in the STAs managed by the South Florida Water Management District (SFWMD or District). *T. domingensis* is the most abundant species where EAV are found, while *T. latifolia* is found during the startup of new STAs persisting in shallow flooded areas (Toth and Galloway 2009). To provide continuous effective nutrient removal, it is important to understand the factors that impact vegetation health and maintain the stability of this EAV community.

Typha sp. are flood tolerant plants that can exist under a wide range of hydrological conditions (Newman et al. 1998, Redwine 2008). When grown in monocultures under controlled water depths, *Typha* sp. grow optimally at around 22-cm water depth (Grace 1989, Waters and Shay 1992, Redwine 2008, Miao and Zou 2012). Periods of deep water conditions in the STAs have been associated with *Typha* stress and increased mortality in EAV cells (Chen et al. 2010, Diaz and Vaughan 2019). However, the amplitude of fluctuating water levels can influence growth and productivity of emergent macrophytes (Harris and Marshall 1963, Edwards et al. 2003, Deegan et al. 2007). Harris and Marshall (1963) reported that *Typha* plants died after continuous flooding for 2 to 4 years at water depths ranging from 30 to 100 cm. Deegan et al. (2007) reported that *T. domingensis* declined when water levels fluctuated around an initial water depth of 60 cm. Biomass did not change in the static, 15-, and 30-cm amplitude treatments but decreased by 52% when the amplitude increased to 45 cm. In the Everglades STAs, the target depth in EAV cells is ~38 cm during no flow conditions; this depth is exceeded during flow events, reaching 120 cm or more during high flow events in the wet season. Diaz and Vaughan (2019) reported lower *Typha* densities in the deeper inflow region of STA-3/4 Cell 2A than in the shallower outflow region of the cell. *Typha* decline was attributed to the prolonged water depths during the 2016 and 2017 wet seasons particularly in the inflow region that experienced a total of 114 consecutive days of water depths ranging from 91 to 136 cm in Water Year 2017 (WY2017; May 1, 2016–April 30, 2017). Prolonged and repeated events of deep water conditions may result in decreased coverage and poor growth and produce ideal conditions for the formation of floating tussocks (Chimney et al. 2000, Chen et al. 2010, Diaz and Vaughan 2019).

Inundation exceeding the optimal depth and duration increases physiological stress of *Typha sp.* reducing their effectiveness to remove nutrients from the water column (Grace and Wetzel 1982). Several studies have documented that *T. domingensis* is stressed at water depths greater than 60 cm with an inundation duration ranging from a few weeks to more than a year (Grace 1989, Miao and Zou 2012). In a three-year study, *T. domingensis* density decreased at water depths greater than 58 cm, however, ramet height increased even at an inundation depth of 110 cm (Grace 1989). Similarly, mortality of *T. domingensis* increased 50% after one-year inundation of 60 cm water depth in a flow-through mesocosm study in STA-1W (Miao and Zou 2012). Chen et al. (2010) reported that increasing inundation depths of 40, 91, and 137 cm for six weeks in a mesocosm study resulted in significant decreases of shoot density, biomass, and photosynthesis in the deeper treatments. A decrease in the belowground biomass/leaf ratio also suggested that the roots and rhizomes of *T. domingensis* were impacted more substantially than shoots at the deeper water depths treatments. Increasing water depths also reduce the anchorage capacity of *Typha* plants, resulting in higher incidence of floating *Typha* mats as plants respond to deeper water by decreasing biomass allocation to roots and rhizomes and increasing allocation to shoots to physically escape the inundated environment (Grace 1989, Voesenek et al. 2003, Miao and Zou 2012).

Inundation effects on plant growth and survival have been evaluated previously using non-destructive methods from a group of aboveground plant features. For example, plant density and LAI are related to biomass production (Jensen et al. 1998, Jonckheere et al. 2004), while leaf elongation and gas exchange rates often convey overall plant health (Liao and Lin 2001, Kadlec and Wallace 2009). Low photosynthetic rates may indicate a stressed plant. Similarly, lower transpiration rates may indicate that the plant is under stress. These metrics are good indicators of inundation effects because prolonged inundation causes changes in soil hydro-edaphic properties that can limit plant growth (Ponnamperuma 1972, Li et al. 2010).

One of the operational challenges for STA management is maintaining optimal water levels. During high flow events, vegetation density, plant community architecture, and microtopography contribute to flow resistance, which in EAV cells with dense *Typha* stands can result in increased water depths at the inflow region of the cells (Lal 2017). The frequency of extreme rainfall events is expected to increase in Florida following projected climate change development (Raghavendra et al. 2018), which could increase the magnitude and duration of *Typha* inundation in the STAs or loading to flow equalization basins (FEBs). The effects may have profound implications for the management of water resources in Florida (Obeysekera et al. 2011). FEBs provide flexibility in water level management within the STAs temporarily storing stormwater runoff, reducing high flow to the STAs during the wet season, and providing a source of water during the dry season to decrease the frequency of dry out conditions.

The present study is designed to investigate inundation effects on *Typha* growth and overall health using the nondestructive methods described above and to provide further insights for STA operations and management. Following previous work (Diaz and Vaughan 2019), it is hypothesized that: (1) there is an inundation duration threshold for *Typha* sustainability at a specific inundation depth, in terms of survival, growth, and propagation, (2) the inundation period threshold is longer at relatively shallow inundation depth than at deeper inundation conditions, and (3) longer inundation durations than the threshold results in a decline in plant density, the ability to propagate, and biomass, thus affecting P removal efficiency in the STAs. To test these hypotheses, *Typha* growth characteristics, including *Typha* density, LER, LAI, and gas exchange rates, are evaluated along an inundation gradient in the STA-1W north test cells flooded continuously for 10 months.

METHODS

STUDY SITE

The Test Cell Study was conducted in the STA-1W north test cells (**Figure 1**; for the location of the test cells within STA-1W, see Figure 2 in Appendix 5B-1 of this volume). These test cells are fully lined (hydrologically isolated) shallow rectangular wetlands, approximately 0.2 hectares (ha) in size (Chimney et al. 2000), which allows for independent control of inflow, water levels and duration of inundation. High phosphorus, i.e., averaging above 100 micrograms per liter (µg/L), content water from the STA-1W inflow canal (G-303 structure) was delivered to the storage cell (**Figure 2**) to supply inflow water for this study. Canal water was first pumped into the storage cell, which was maintained at a stage several feet higher than the test cells. Water from the storage cell then flowed into a 30-inch pipe and distributed to each cell through 8-inch lateral pipes fitted with an inflow valve to regulate flow. Outflow from each test cell was controlled by an adjustable 90-degree v-notch weir, which allows control of water depths within the cells.

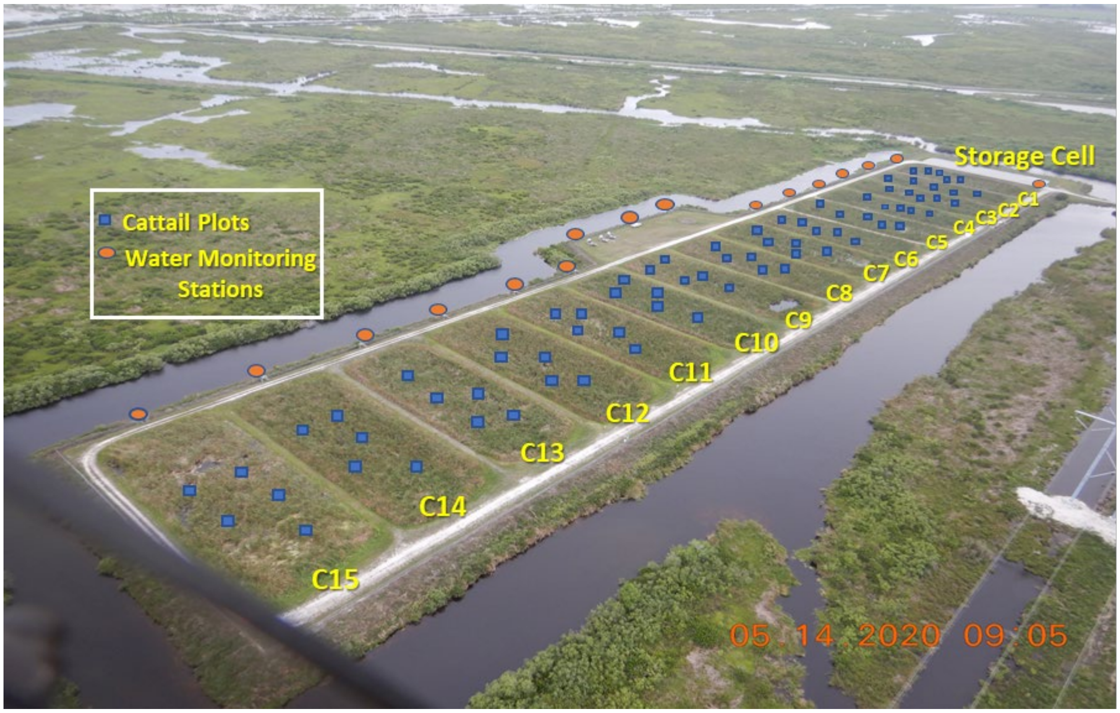

Figure 1. Photo of north test cells within STA-1W showing the locations of the *Typha* (cattail) plots, water quality monitoring stations, and the storage cell. (Photo by SFWMD.)

Figure 2. The storage cell that supplied inflow water to the 15 test cells. (Photo by SFWMD.)

ESTABLISHMENT OF *TYPHA* POPULATION

To establish *Typha* populations within the test cells, the cells first had to be refurbished. This refurbishment was completed by December 2016 by adding a new layer of soil from the STA-1W Expansion #1 site. The soil was from an old sugarcane farm with a very shallow organic soil layer. An outflow sump was installed in each cell to improve drainage (**Figure 3**).

Figure 3. An outflow sump installed in all test cells. (Photo by SFWMD.)

During 2017, all cells remained flooded to control unwanted terrestrial weeds inside the cells. During the first quarter of 2018, all test cells were prepared to begin seeding and planting of *Typha*. All cells were drained and seeded during the first week of May 2018 with *Typha domingensis* seeds collected from STA-3/4. In addition, approximately 2,100 *Typha* seedlings grown in a nursery at the S5A facility were transplanted from mid-May to mid-June 2018 at a density of 140 *Typha* plants per cell to improve uniformity of the *Typha* community in the cells (**Figure 4**). Water levels in the test cells during this period were maintained at soil saturation up to a few centimeters above soil surface to enhance *Typha* germination and facilitate growth of the transplanted young seedlings. Water level in the last half of 2018 and first half of 2019 were maintained at a depth ranging between 35 to 40 cm to encourage *Typha* growth and suppress terrestrial weed germination in the cells. *Typha* plants were mature when inundation depth treatments began on July 1, 2019.

Figure 4. *Typha* seedlings grown at the S5A nursery to be transplanted into the STA-1W north test cells for this study. (Photo by SFWMD.)

INUNDATION DEPTH TREATMENTS

The study consisted of two parts: inundation depth and measurement over time. There were five water level treatments: 40, 61, 84, 104, and 124 cm above soil surface, each replicated three times in the 15 test cells. Treatment 1 (40 cm) is the approximate target depth maintained in the STAs during normal operational no-flow periods (Goforth 2005), and it is considered the control treatment in this study. The 61- and 84-cm depth treatments represent shallow and moderate flooding and the 104- and 124-cm depth treatments represent deep and extremely deep flooding for STA management and operation, respectively. The inundation was maintained in all treatment cells for the 10 months of the study and measurements were conducted at regular intervals to evaluate changes over time.

SOIL SAMPLING

Baseline soil samples were collected from every test cell to provide background information of selected physicochemical properties prior to experimental testing. Two intact cores 10 cm in diameter and 15 cm deep were collected from the inflow and outflow region of every treatment cell on January 9, 2019, using standard methods (SFWMD 2015). All cores were extruded in the field, transferred to pre-labeled plastic bags, and transported on ice to the S5A Laboratory where they were stored at 4 degrees Celsius (^{0}C) until processing. Samples were submitted to SFWMD's Chemistry Laboratory for analysis of soil moisture, ash content (ash free dry weight [AFDW], calculated), bulk density (BD, calculated), pH, TP, hydrochloric acid extracted-P (HCL-P, which represents calcium, magnesium, aluminum, and iron bound inorganic P), total carbon (TC), TN, and hydrochloric acid extracted calcium (HCL-Ca; SFWMD 2019a). At the end of the study on June 25, 2020, three intact sediment cores were collected from the inflow, mid-flow, and outflow region of every treatment cell. Sample collection and processing followed the same protocol as the samples collected at the beginning of the study.

WATER QUALITY MONITORING

Bi-weekly grab water samples were collected during the first three months of the study and monthly grab samples for the remainder of the study (SFWMD 2019e). Water samples were collected at the storage cell outlet (representing inflow water) and at the outflow from each test cell (**Figure 1**). Water samples were submitted to SFWMD's Chemistry Laboratory and analyzed for TP, total dissolved phosphorus (TDP), SRP, TN, total organic carbon, calcium, potassium, magnesium, sodium, hardness, ammonium (NH_4), and nitrate + nitrite (NOx). PP and dissolved organic P (DOP) were calculated from the measured parameters. PP was calculated as the difference between mean TP and mean TDP. DOP was calculated as the difference between TDP and SRP.

TYPHA MEASUREMENTS

Five 1-meter (m) x 1-m plots were randomly established within each cell in March 2019 for a total of 75 permanent plots (**Figure 1**). Two baseline monitoring events were completed in May and June 2019, prior to the initiation of the water level treatments on July 1, 2019. During the first three months of the study, *Typha* vegetation surveys and field measurements were conducted bi-weekly to capture any initial responses of *Typha* to the different water level treatments. These bi-weekly observations indicated that monthly field measurements were sufficient to capture the major phenological events due to the different water levels. The study parameters measured during each monitoring event included *Typha* density (adult and juvenile), gas exchange rates (photosynthetic rates, stomatal conductance, and WUE), LAI, LER, and water depth using a graduated polyvinyl chloride (PVC) pole. Field observations included visible *Typha* damage and presence of floating *Typha* in the plots, presence of other emergent or floating aquatic plants within the plots, and photo documentation of each plot.

Shoot Density

Typha shoot density was estimated as the number of *Typha* shoots per square meter (shoots/m^2). To facilitate accurate plant density assessments, a visual marker of the plot edges was established using a 1-square meter (m^2) quadrat made from four 1-m PVC poles (**Figure 5**). An additional PVC pole was placed approximately at the center of the plot, effectively dividing the plot in half to facilitate an accurate plant density count. The number of *Typha* plants within each plot was categorized into four groups: (1) live adults (> 1.5 m in height; robust leaf growth), (2) live juveniles (< 1.5 m in height; delicate leaf growth), (3) live reproductive adult plants (with flower or seed stalk), and (4) dead plants (SFWMD 2019c).

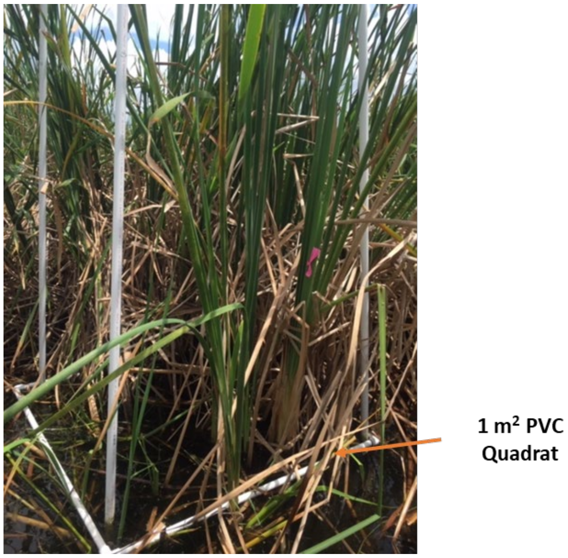

Figure 5. *Typha* plot set up during density measurements. (Photo by SFWMD.)

Leaf Elongation Rates

After determining *Typha* density, five representative adult plants were selected within each plot for LER measurements (SFWMD 2019d). A piece of bright flagging with an identification number (1 through 5) was tied around the shortest and youngest leaf from the inner culm on each plant so that it could be tracked for remeasurement. The leaf height from the base of the plant to the tip of the leaf was measured using a measuring pole graduated in cm. The first measurement was taken on the initial day of tagging the leaves, a second measurement was taken 7 to 11 days after the initial measurement. The LER was calculated by dividing the change in height by the number of days between measurements in cm/d.

Leaf Gas Exchange Rates

Leaf gas exchange rates were measured using a LI-6400XT Portable Photosynthesis System (Li-COR, Lincoln, Nebraska). The LI-6400XT is comprised of a main console unit connected to a handheld chamber that is used to gently clamp onto a leaf for photosynthetic readings. One mature, healthy leaf was selected for photosynthetic rate measurements from three of the five plants used in the LER measurements. Ideally, the leaf would show no signs of chlorosis (yellowing of leaf tissue due to a lack of chlorophyll), herbivory, or other malformations but depending on stressful conditions in the plot, these criteria could not always be met. In addition, *T. domingensis,* also known as "narrow-leaf *Typha*", commonly had leaves that were narrower than the width of the LI-6400XT chamber. The approximate percentage of the chamber width that each leaf filled (i.e., 100%, 90%, 75%, etc.) was recorded in the field. Since the instrument assumes that the selected leaf fills up the entire chamber, the percentage recorded in the field was multiplied by the leaf chamber area as a correction factor. The corrected area was subsequently used to calculate the gas exchange rates, ensuring an accurate representation of photosynthesis in the plant. The presence of any leaf anomalies (e.g., narrow, chlorosis, herbivory, malformation, etc.) was noted on the field data sheets. Once selected for measurement, the leaf was gently arced so that the LI-6400XT chamber could be clamped 6 to 18 inches from the tip of the leaf. The chamber was held in place to allow the gas exchange values to stabilize before collecting the reading. This process was repeated for each selected plant.

Leaf Area Index

LAI was measured using a LAI-2200C Plant Canopy Analyzer (Li-COR, Lincoln, Nebraska). The LAI-2200C measures light attenuation from above-canopy (A) and below-canopy (B) readings to calculate LAI (one-sided leaf surface area per ground surface area). Because the LAI-2200C is sensitive to all light blocking objects in its view, the term foliage area index is more appropriate. The LAI Plant Canopy Analyzer is composed of a control unit and an optical sensor (wand, ~18 inches in length). Light attenuation measurements were collected for each plot as follows. Four A readings were measured from a levee adjacent to the plot, well above the *Typha* canopy. Six B readings were collected starting along one edge of the plot (**Figure 6**). The first B reading was collected just inside the corner of the plot right along the edge. The wand was then moved toward the inside of the plot, approximately one wand length (18 inches) away from the edge of the second B reading. This process was repeated at the midpoint and at the corner along the same edge, for a total of six readings. A 180-degree lens cap was used for the B readings so that the fisheye lens would only capture the canopy in front of the unit and not behind it (i.e., excluding the effect of plants outside the plots). This allowed for readings to be collected along the outside edge of the plot, as well as the inside section of the plot. B readings were initially collected at 120 cm above the soil surface (i.e., during May monitoring event), following methods established in Chen and Vaughan (2014) and Diaz and Vaughan (2019). However, B reading collection height was amended to 125 cm following the final determination of water level treatments in June because the of treatment depths exceeded 120 cm. For consistency, B readings were always collected from the same edge of plots for the duration of the study. If sky conditions changed significantly between the A and B readings (e.g., a cloud that was covering the sun moved away and exposed full sunlight), all measurements were discarded, and the process was repeated from the beginning.

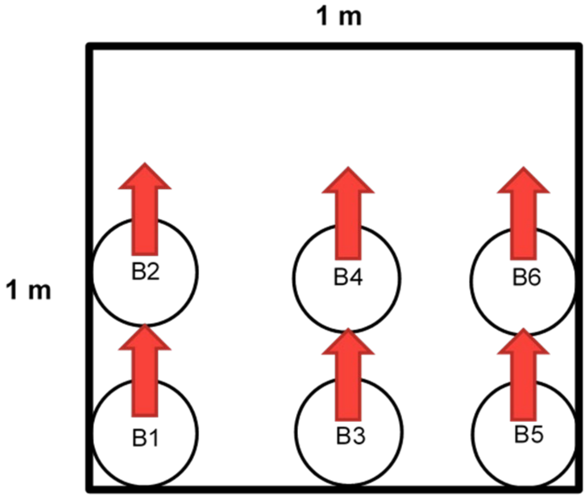

Figure 6. Leaf area index sampling diagram.

Biomass

Samples were collected for live and dead aboveground and belowground plant biomass in an area adjacent to the experimental plot to avoid destruction of the *Typha* community within the plot (SFWMD 2019b). During harvest, a PVC-constructed 0.16-m^2 (0.4 m x 0.4 m) quadrat with detachable sides was used to form a perimeter encompassing the quadrat area. Belowground biomass was removed using a long-bladed root pruning shovel, cutting along the perimeter formed by the 0.16-m^2 quadrat to a depth of about 30 cm. After cutting, the shovel was pushed underneath the soil and lifted to loosen all roots and rhizomes in the quadrat. Then, all the plants within the quadrat were slowly lifted, being careful to keep plants attached to rhizomes. Plants were thoroughly rinsed at the site with water from the cell and placed in pre-labeled burlap bags for transportation to the S5A Laboratory and stored at 4ºC until they were processed. Samples were processed within 7 days of harvesting. All plant materials were carefully washed to remove any soil using a garden hose with a high pressure nozzle. *Typha* biomass was defined as dry weight of live tissue from live ramets. Washed biomass material was separated into five different components: live leaves, live roots, live rhizomes, live shoot bases, and dead mass, then placed into paper bags to be dried (**Figure 7**). Live ramets from each quadrat were counted and the length of the longest leaf (plant height) from each *Typha* plant measured. Processed biomass samples were dried at 70ºC for a minimum of two weeks until a constant dry weight was obtained. A sub-sample of each biomass component was submitted to SFWMD's Chemistry Laboratory for analysis of TC, TN, and TP. Baseline biomass samples were collected April–May 2019. A final biomass sampling was collected in May–June 2020. Only biomass data from samples collected at the end of the study are presented in this report.

Figure 7. *Typha* biomass processing and separation into live leaves, roots, rhizomes, and shoot bases, and dead biomass. (Photos by SFWMD.)

DATA MANAGEMENT AND STATISTICAL ANALYSIS

The data were screened for outliers prior to analyses (Tukey 1977). Field notes were consulted for each outlier to determine if there was a valid reason to remove the value. For example, several LER outlier values were removed because the plant either died or experienced severe herbivory damage between the initial and final measurement. Conversely, some outliers were kept in the data set as they were representative of real events during the study. Additionally, several values that were not considered to be outliers were removed based on issues identified in the field (e.g., *Typha* floating upon LER remeasurement, not enough B readings collected for LAI, etc.).

Overall water level treatment effects on plant response were evaluated using one-way repeated-measures analysis of variance (ANOVA) followed by *post-hoc* mean separation test when appropriate. Prior to analyses, measurements were averaged within test cells for each sample event to avoid errors of pseudoreplication (Hulbert 1984). Data were standardized by sampling event using a Z-score standardization method (Gotelli and Ellison 2013). Test of model assumptions including normality and outlier assessment of standardized data were satisfactory. Mild violations of the sphericity assumption were offset using a linear mixed model approach (Wang and Goonewardene 2004). As such, standard parametric analyses were used to test plant response to each treatment. Dunett's *post-hoc* tests were used to compare plant responses to water level treatments (shallow – 61 cm to extremely deep – 124 cm) with the control. Pearson's correlation coefficients were also used to describe relationships between plant responses and water level treatments over the study period and within the period of active plant growth following initiation of water level regimes (i.e., July–October), where appropriate. Plant responses to duration of water levels treatments were evaluated using a variety of methods. Spearman's rank correlation coefficients (ρ) were calculated using mean control (40 cm) and treatment data from each sampling event throughout the study period to determine if water level treatment trends over time varied from the control, since seasonal trends were apparent in some plant responses and not others.

Most analyses were performed with R (R Core Team 2020) using functions in the base R packages. The "standardize" package for Z-score standardization (Eager 2017), the "rstatix" package to evaluate model assumptions (Kassambara 2020), the "Ime4" and "ImerTest" packages for model construction and significance test (Bates et al. 2015, Kuznetsova et al. 2017), and the "tidyverse" collection of packages for data import, formatting, and graphics (Wickham et al. 2019). Some analyses were performed with JMP® statistical software (Version 15.1.0, SAS Institute Inc., 2019, Cary, North Carolina). Data sets were tested for normality (Shapiro-Wilk test) and for equal variances before conducting any statistical analysis. Treatment means of parameters that met these assumptions were compared using one-way ANOVA to test that the means were significantly different across water level treatments. Parameters that did not meet these assumptions were compared using the Kruskal-Wallis Wilcoxon Rank Sum Test. For multiple comparisons, the parameters that met the assumptions for parametric analysis were compared using the Tukey-Kramer honest significant difference test. Parameters that did not meet the assumptions for parametric analysis were compared using the non-parametric multiple comparison Dunn All Pair for Joint Ranks test. Tests were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

SOIL PHYSICOCHEMICAL PROPERTIES

Although the added layer of soil used during refurbishment of the test cells came from the STA-1W Expansion #1 site, there were differences in some soil properties measured at the beginning of the study (**Table 1**). Baseline intact sediment cores collected before the study did not have a floc layer, an important layer usually found in STAs after several years of operation. The surface soil of all treatment cells was under flooded conditions for less than one year at the time the baseline soil samples were collected in early January 2019. Most of the differences found at the beginning of the study were also observed at the end of the study (**Table 2**).

Table 1. Mean ± standard error of physicochemical properties of soil samples collected at the beginning of the study. (Note: sample size is 6.) [a,b]

Water Level Treatment	Ash %	pH	BD g/cm^3	TC g/kg	TN g/kg	TP mg/kg	HCl-P mg/kg	HCL-Ca g/kg
Control – 40 cm	44.3 ±1.1 a	7.45 ± 0.04 a	0.416 ± 0.013 b	341 ± 8 a	17.6 ± 0.5 ab	544 ± 16 ab	205 ± 9 a	100.2 ± 7.2 ab
Shallow – 61 cm	37.4 ± 2.8 ab	7.42 ± 0.05 a	0.391 ± 0.022 b	369 ± 20 a	19.4 ± 1.3 a	501 ± 11 bc	246 ± 64 a	84.5 ± 6.6 ab
Moderate – 84 cm	43.5 ± 3.0 ab	7.38 ± 0.05 a	0.420 ± 0.022 b	353 ± 15 a	18.0 ± 0.9 ab	485 ± 12 bc	198 ± 14 a	91.8 ± 2.1 ab
Deep – 104 cm	34.0 ± 1.1 b	7.29 ± 0.05 a	0.347 ± 0.016 b	361 ± 32 a	20.9 ± 0.4 a	571 ± 17 a	189 ± 10 a	78.8 ± 2.6 b
Extremely Deep – 124 cm	53.4 ± 4.3 a	7.37 ± 0.07 a	0.529 ± 0.045 a	301 ± 23 a	14.6 ± 1.4 b	459 ± 18 c	203 ± 12 a	129.2 ± 12.9 a

a. Means with different letter in the same column are significantly different at the 0.05 probability level.

b. Key to units: g/cm^3 - grams per cubic centimeter; g/kg - grams per kilogram; and mg/kg - milligram per kilogram.

Table 2. Mean ± standard error of physicochemical properties of soil samples collected at the end of the study. (Note: sample size is 9.) [a,b]

Water Level Treatment	Ash %	pH	BD g/cm^3	TC g/kg	TN g/kg	TP mg/kg	HCl-P mg/kg	HCL-Ca g/kg
Control – 40 cm	45.5 ± 1.4 ab	7.17 ± 0.03 a	0.478 ± 0.033 a	333 ± 8 ab	18.1 ± 1.6 ab	516 ± 19 ab	213 ± 12 ab	96.7 ± 9.4 ab
Shallow – 61 cm	42.1 ± 2.9 ab	7.21 ± 0.03 a	0.419 ± 0.027 a	349 ± 17 ab	18.7 ± 3.2 ab	662 ± 67 a	341 ± 63 a	90.7 ± 5.5 ab
Moderate – 84 cm	44.8 ± 3.1 ab	7.13 ± 0.03 a	0.443 ± 0.029 a	338 ± 17 ab	17.9 ± 3.2 ab	499 ± 11 ab	221 ± 16 ab	96.4 ± 5.2 ab
Deep – 104 cm	35.8 ± 1.2 b	7.15 ± 0.03 a	0.422 ± 0.020 a	387 ± 6 a	21.3 ± 1.2 a	526 ± 11 a	188 ± 4 b	80.7 ± 4.7 b
Extremely Deep – 124 cm	54.3 ± 3.8 a	7.17 ± 0.02 a	0.504 ± 0.030 a	293 ± 20 b	14.8 ± 3.9 b	450 ± 12 b	204 ± 8 ab	128.6 ± 10.6 a

a. Means with different letter in the same column are significantly different at the 0.05 probability level.

b. Key to units: g/cm^3 - grams per cubic centimeter; g/kg - grams per kilogram; and mg/kg - milligram per kilogram.

Beginning of Study

Bulk Density

Average baseline bulk densities (BD) were higher for the extremely deep treatment than the other test cells. Baseline BD ranged from 0.347 to 0.529 grams per cubic centimeter (g/cm^3; **Table 1**), which is considerably higher than the BD values from the surface 10 cm (0.23 to 0.31 g/cm^3) reported from STA-2 (Zamorano et al. 2018) and resemble BD values from upland organic soils (Diaz et al. 1993). After the test cells were refurbished, they remained dry for more than six months due to an inflow pump malfunction.

This dry period may have allowed the surface soil layer to become compacted, increasing the BD values across all cells. In addition, the soil source used in the refurbishment of the cells had a lot of rock fragments from the underlying bedrock layer that were mixed with the organic layer during the scraping procedure of the area. All these factors may have influenced the high BD values measured at the beginning of the study.

Ash Content

Ash, inorganic residue remaining after the water and organic matter have been removed, was also significantly different among the different test cells, with values ranging from 34 to 53%. Ash content from the test cells was higher than the average values reported for the surface 10 cm of soils from STA-3/4 (30.7%; Scinto 2010) and STA-1W (21.2%; Ivanoff et al. 2012).

TP

TP concentrations also were significantly different among the different test cells, with concentrations ranging from 459 to 571 milligrams per kilogram (mg/kg). These TP concentrations are slightly lower than the average surface TP concentrations (666 mg/kg) reported for STA-3/4 (Scinto 2010), and the overall surface soil TP concentration (650 mg/kg) reported for STA-1W during a WY2011 survey (Ivanoff et al. 2012).

End of Study

Measurements from the soil samples collected at the end of the study did not differ much from the initial measurements (**Table 2**). A visible floc layer was not observed in any of the intact cores collected at the end of the study.

Bulk Density

Bulk density values were not significantly different among the different water level treatments with average values ranging from 0.419 to 0.504 g/cm^3 for the shallow and extremely deep water level treatments, respectively (**Table 2**). Average BD from the extremely deep water level treatment was still higher than the rest of the cells but the difference was not significant. BD values at the end of the study were slightly higher than the initial BD values except for the average BD of the extremely deep water level treatment, which was slightly lower. These BD values are still higher than those reported from surface soils of well established STAs (Scinto 2010, Ivanoff et al. 2012).

Ash Content

Ash content was significantly different ($p < 0.05$) among the different water level treatments with values ranging from 36% in the soils from the deep water level treatment to 54% in the soils from the extremely deep treatment. Ash content at the end of the study was also slightly higher than measured at the beginning of the study, and considerably higher than the average values reported for surface soils from STA-3/4 and STA-1W (Scinto 2010, Ivanoff et al. 2012).

TP

TP values were significantly different among the different water level treatments with concentrations ranging from 450 mg/kg in the soils for the extremely deep water level treatment to 662 mg/kg in the soils from the shallow water level treatment.

WATER QUALITY MONITORING

Water quality measurements at the inflow were markedly different from the treatment outflows (**Table 3**). In most cases, the inflow measurements were significantly higher than the treatment outflow measurements (**Table 4**).

Table 3. Descriptive statistics of water quality parameters.

Water Level Treatment	Parameter	Unit of Measurement	Number of Samples	Minimum	Standard Deviation	Mean	Maximum
Inflow	PP	µg/L	12	21	11	33	59
	DOP	µg/L		9	3	13	20
	SRP	µg/L		2	66	75	224
	TP	µg/L		46	64	122	266
	NH_4	µg/L		48	106	215	382
	NO_x	µg/L		30	253	283	916
	TN	mg/L		1.83	0.60	2.54	3.41
Control 40 cm	PP	µg/L	36	6	7	17	31
	DOP	µg/L		9	2	12	18
	SRP	µg/L		2	19	22	80
	TP	µg/L		25	21	52	111
	NH_4	µg/L		27	43	73	203
	NO_x	µg/L		5	23	12	135
	TN	mg/L		1.34	0.44	1.97	2.76
Shallow 61 cm	PP	µg/L	36	5	8	18	35
	DOP	µg/L		7	3	11	17
	SRP	µg/L		2	7	7	34
	TP	µg/L		15	13	36	81
	NH_4	µg/L		16	77	72	454
	NO_x	µg/L		5	13	9	73
	TN	mg/L		1.44	0.42	1.98	2.80
Moderate 84 cm	PP	µg/L	36	6	8	16	33
	DOP	µg/L		5	2	9	13
	SRP	µg/L		2	4	4	21
	TP	µg/L		13	10	29	46
	NH_4	µg/L		20	34	55	141
	NO_x	µg/L		5	2	6	14
	TN	mg/L		1.38	0.44	1.95	2.72
Deep 104 cm	PP	µg/L	36	5	8	17	36
	DOP	µg/L		4	3	9	14
	SRP	µg/L		2	8	7	35
	TP	µg/L		12	14	33	66
	NH_4	µg/L		26	60	72	271
	NO_x	µg/L		5	6	7	35
	TN	mg/L		1.42	0.44	1.97	2.71
Extremely Deep 124 cm	PP	µg/L	36	7	11	20	45
	DOP	µg/L		4	3	9	15
	SRP	µg/L		2	3	3	11
	TP	µg/L		13	14	33	68
	NH_4	µg/L		25	122	110	585
	NO_x	µg/L		5	6	8	39
	TN	mg/L		1.40	0.46	2.01	2.82

Table 4. Mean ± standard error of water quality measurements. [a, b]

Water Level	NH_4 (µg/L)	NO_x (µg/L)	TN (mg/L)	SRP (µg/L)	TDP (µg/L)	TP (µg/L)	PP (µg/L)	DOP (µg/L)
Inflow	215 ± 31 a	283 ± 73 a	2.54 ± 0.17 a	75 ± 19.0 a	88 ± 19 a	122 ± 18.0 a	33 ± 3.0 a	13.1 ± 1.0 a
Control – 40 cm	73 ± 7 b	12 ± 4 b	1.97 ± 0.07 a	22 ± 3.1 ab	35 ± 3.3 ab	52 ± 3.4 ab	17 ± 1.1 b	12.4 ± 0.4 a
Shallow – 61 cm	72 ± 13 b	9 ± 2 b	1.98 ± 0.07 a	7 ± 1.1 c	21 ± 2.2 bc	36 ± 2.1 c	18 ± 1.4 b	10.8 ± 0.4 ab
Moderate – 84 cm	55 ± 6 b	6 ± 0 b	1.95 ± 0.07 a	4 ± 0.7 c	17 ± 2.1 c	29 ± 1.6 c	16 ± 1.3 b	9.3 ± 0.3 c
Deep – 104 cm	72 ± 10 b	7 ± 1 b	1.97 ± 0.07 a	7 ± 1.4 c	19 ± 2.4 c	33 ± 2.3 c	17 ± 1.4 b	9.0 ± 0.4 bc
Extremely Deep – 124 cm	110 ± 20 b	8 ± 1 b	2.01 ± 0.08 a	3 ± 0.4 c	16 ± 2.1 c	33 ± 2.4 c	20 ± 1.8 b	8.8 ± 0.5 c

a. Means with different letter in the same column are significantly different at the 0.05 probability level using the Wilcoxon on rank sum test and the non-parametric multiple comparison Dunn All Pairs or Joint Ranks.

b. Key to units: µg/L – micrograms per liter and mg/L – milligrams per liter.

TP Concentrations

TP concentrations at the inflow varied over time, with higher concentrations measured at the beginning of the study (July–September 2019), then decreasing during the remainder of the study (**Figure 8**). Inflow TP concentrations averaged 122 µg/L and ranged from 266 µg/L in late August 2019, to 46 µg/L in early April 2020 (**Table 3**). Outflow TP concentrations for the shallow to extremely deep treatments were significantly lower and less variable than inflow TP, with averages for the different water level treatments ranging from 52 µg/L in the control cells to 29 µg/L in the moderate treatment (**Table 3**). There was no significant difference in outflow TP concentration among the shallow to extremely deep treatments (**Table 4**). Outflow TP concentrations represented a TP reduction of the inflow TP that ranged from 57% in the control cells to 76% in the deeper water level treatments.

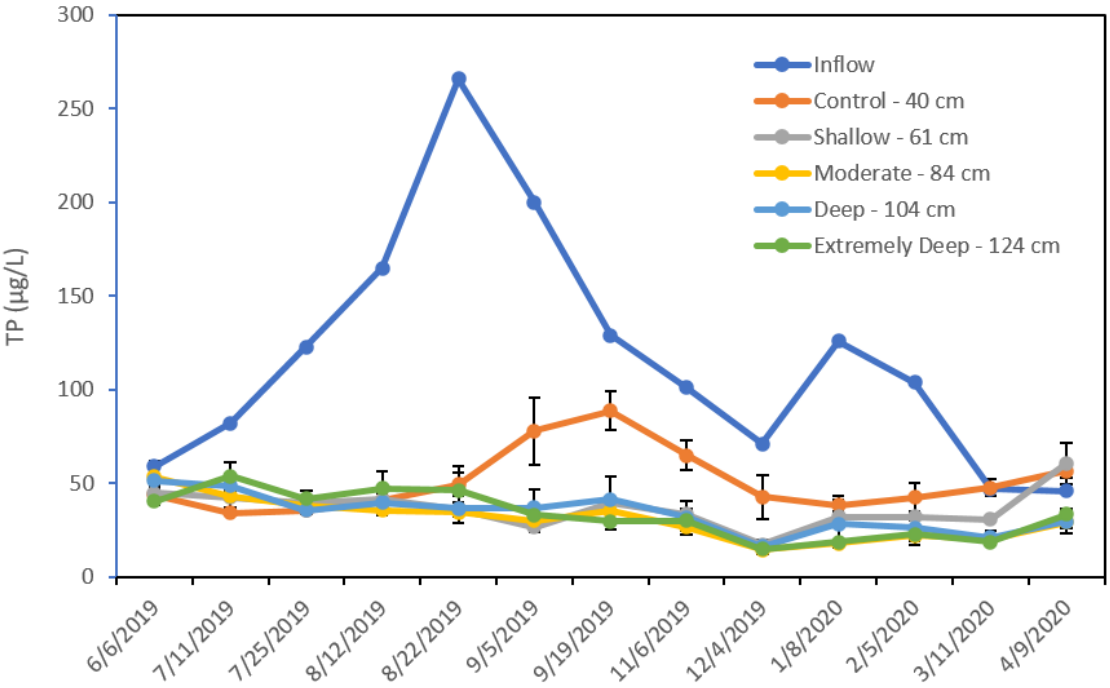

Figure 8. TP concentrations of monthly water samples collected at the inflow and outflow for each water level treatment.

SRP Concentrations

SRP at the inflow was the dominant P species throughout accounting for 61% of the total inflow TP to the treatment cells (**Figure 9**). Inflow SRP concentrations followed the same pattern as TP, with the higher concentrations measured during the first three months of the study (July–September 2019) then decreasing for the remainder of the monitoring period. The overall mean inflow SRP concentration was 75 µg/L, while concentrations ranged from 224 µg/L in late August 2019 to 2 µg/L at the end of the study in early April 2020 (**Table 3**). Average outflow SRP concentrations were significantly lower than inflow SRP at all water treatment levels except the control, with concentration ranging from 22 µg/L in the control cells to 3 µg/L in the extremely deep water level treatment cells (**Table 4**). There was no significant difference in outflow SRP concentration among the shallow to extremely deep treatments.

Outflow SRP concentrations were reduced by 70% in the control cells and > 90% in the deeper water level treatments cells, reflecting the efficiency of SRP removal as inflow waters flowed through the treatment cells. Villapando and King (2019) reported that SRP concentrations from STA-2 flow-way 1 significantly decreased as surface water traveled downstream from 27 µg/L close to the inflow structure to the method detection limit of 2 µg/L as water discharged from outflow structure.

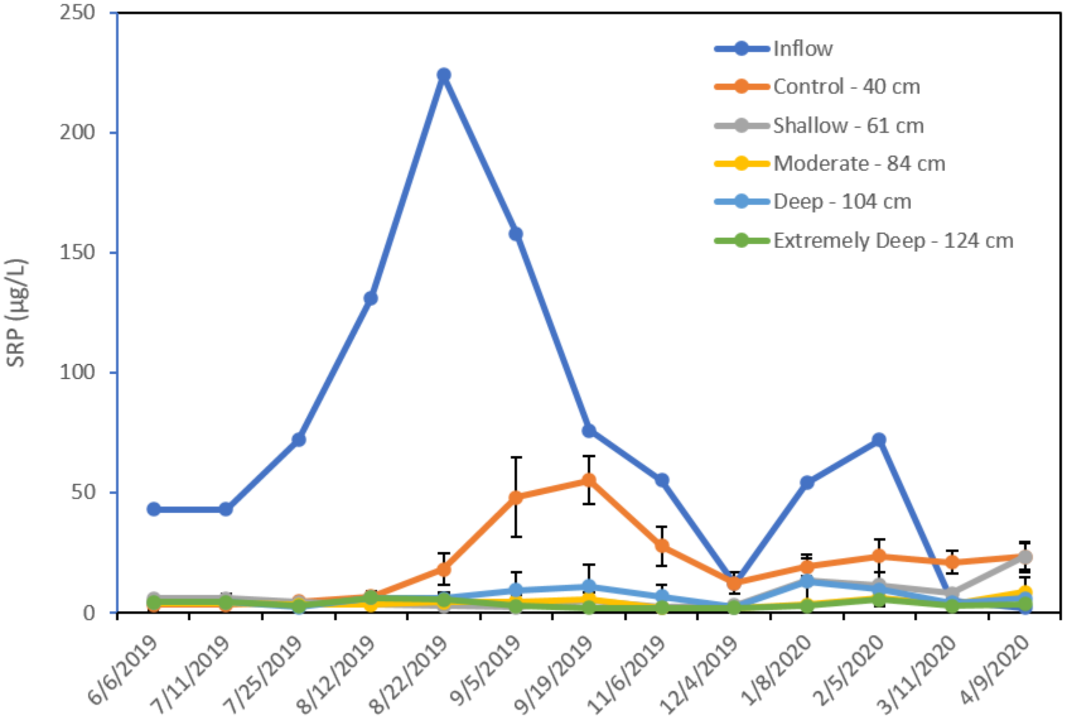

Figure 9. SRP concentrations of monthly water samples collected at the inflow and outflow of each water level treatment.

DOP Concentrations

DOP concentrations at the inflow were considerably lower than the other P species, accounting for only 11% of the inflow TP to the treatment cells. During the study, inflow DOP averaged 13.1 µg/L with concentrations ranging from 20 µg/L in late August 2019 to 9 µg/L at the end of the study in early April 2020 (**Table 3**). Average outflow DOP concentrations were significantly lower than inflow DOP only for the moderate, deep, and extremely deep treatments with no significant differences among the deeper treatments (**Table 4**). Average outflow DOP concentrations ranged from 12.4 µg/L in the control cells to 8.8 µg/L in the deepest treatment cells (**Table 3**).

PP Concentrations

PP concentrations at the inflow were variable accounting for 27% of the inflow TP to the treatment cells. During the study, PP concentrations averaged 33 µg/L and ranged from 59 µg/L in early January 2020 to 21 µg/L in early August 2019 (**Table 3** and **Figure 10**). Outflow PP concentrations were less variable than inflow concentrations, with average concentrations significantly lower than inflow concentrations at all water level treatments. There were no significant differences in outflow PP concentrations among the different water level treatments (**Table 4**). Average PP concentrations ranged from 17 ug/L in the control cells to 20 µg /L in the extremely deep treatment cells. Outflow PP concentrations were reduced by 48% in the control and by 51% in the deeper water level treatments.

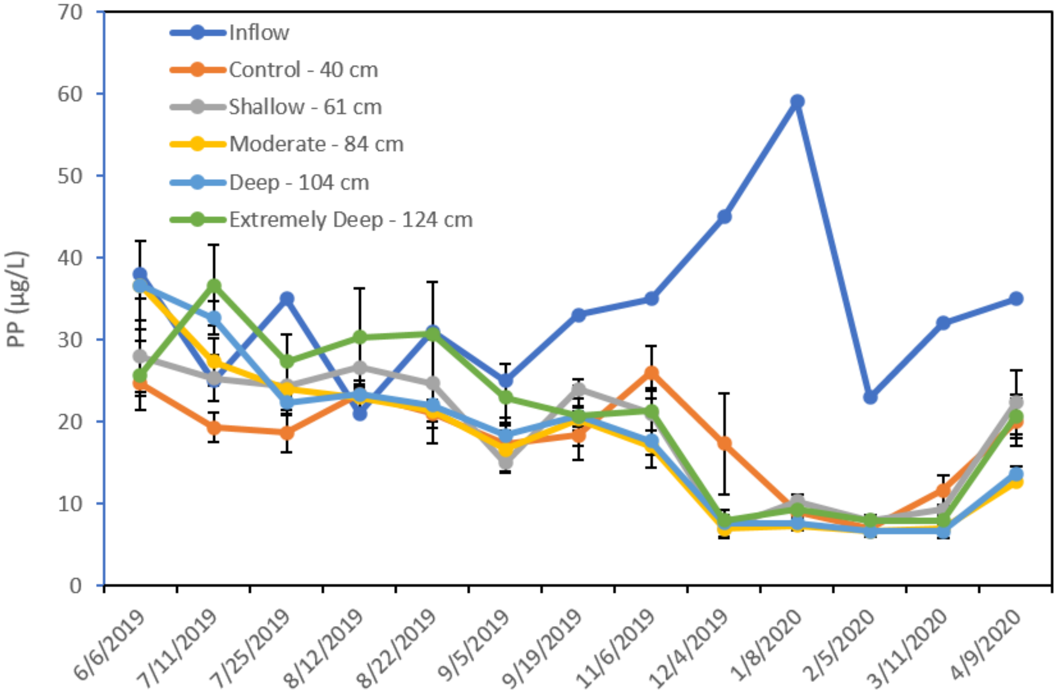

Figure 10. PP concentrations of monthly water samples collected at the inflow and outflow stations of each water level treatment.

Overall Phosphorus Species

All P species, except for DOP, declined significantly for the shallow to extremely deep treatments and these treatments were not significantly different from one another (**Table 3**). This result suggests that P removal rates in the treatment cells were not significantly affected by water depth. While TN did not change significantly in any of the treatment cells or inflow, there was a significant reduction of NOx-N and NH_4-N in the outflow concentrations. None of these treatments were significantly different from one another, suggesting that water levels did not affect inorganic nitrogen dynamics.

TN Concentrations

Inflow TN concentrations were slightly higher during the first three months (July–September 2019) of the study with concentrations steadily decreasing during the remainder of the study (**Figure 11**). Inflow TN averaged 2.54 milligrams per liter (mg/L) and ranged from 3.41 mg/L in early September 2019 to 1.83 mg/L in early February 2020 (**Table 3**). Outflow TN concentrations were lower than inflow TN during all but one sampling event, however the differences were not significant (**Table 4**). Average outflow TN concentrations ranged from 1.95 mg/L in the moderate water level treatment to 2.01 mg/L in the extremely deep water level treatment.

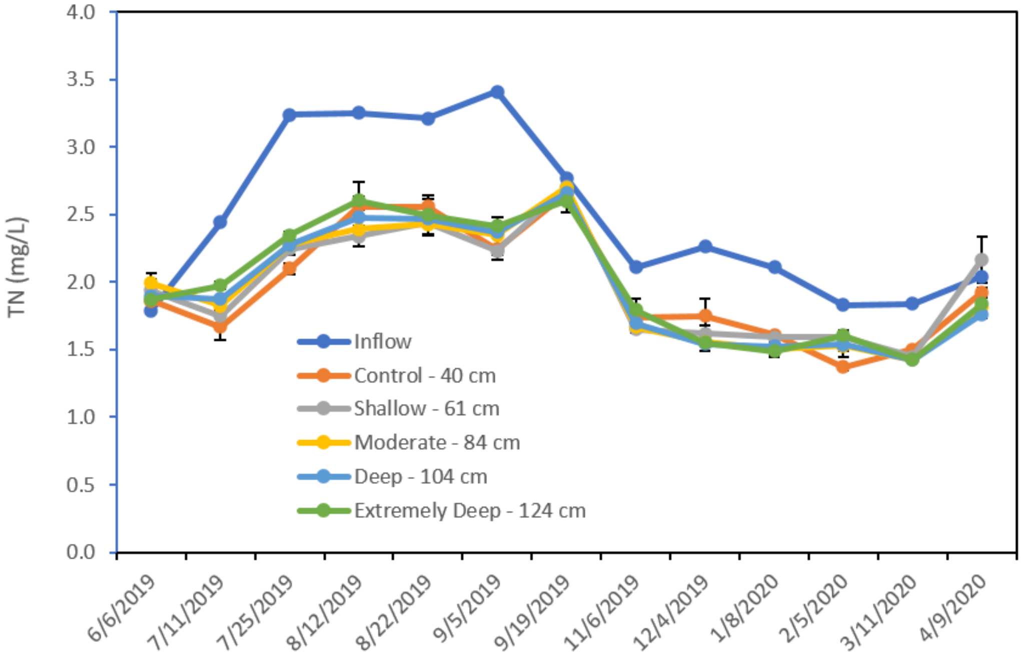

Figure 11. TN concentrations of monthly water samples collected at the inflow and outflow stations of each water level treatment.

Overall Nitrogen Species

The higher TN concentrations at the beginning of the study are also reflected in the higher NO_x measured during the first three months of the study. However, NO_x and NH_4 accounted for only 11 and 8.5% of the inflow TN, respectively, for the entire monitoring period. Inflow NO_x averaged 283 µg/L and ranged from 916 µg/L in late July 2019 to 30 µg/L in late September 2019 (**Table 3**). Outflow NO_x concentrations were significantly lower than inflow concentrations; however, there were no significant differences among the different water level treatments (**Table 4**). Inflow NH_4 averaged 215 µg/L and ranged from 382 µg/L in early December 2019 to 48 µg/L in in early January 2020 (**Table 3**). Outflow NH_4 concentrations were significantly lower that inflow concentrations ($p < 0.05$; **Table 4**); however, there were no significant differences among the different water level treatments. Outflow NH_4 concentrations ranged from 55 µg/L in the moderate water level treatment to 110 µg/L in the extremely deep water level treatment depth.

TYPHA MEASUREMENTS

Typha Shoot Densities

Typha density was broken down into three components for this study: (1) adult density, (2) juvenile density, and (3) dead density. Results for *Typha* density are displayed as (1) mean density trends by water level treatments for May 2019–April 2020 (**Figure 12**); (2) adult and juvenile *Typha* density values by water level treatments of monitoring data collected from July to October 2019 (**Figure 13**); (3) mean *Typha* densities values by water level treatments of monitoring data from July 2019 to April 2020 (**Figure 14**); (4) Spearman's rank correlation coefficients for each water level treatment (shallow to extremely deep) compared to the those of the control (**Table 5**); (5) results of the ANOVA models exploring the effects of different water level treatments on various *Typha* responses (**Table 6**); and (6) *Typha* density photos of various treatment plots at 12 weeks (September 2019 sampling event) of continuous inundation. Results discussions by density component follow the figures and tables.

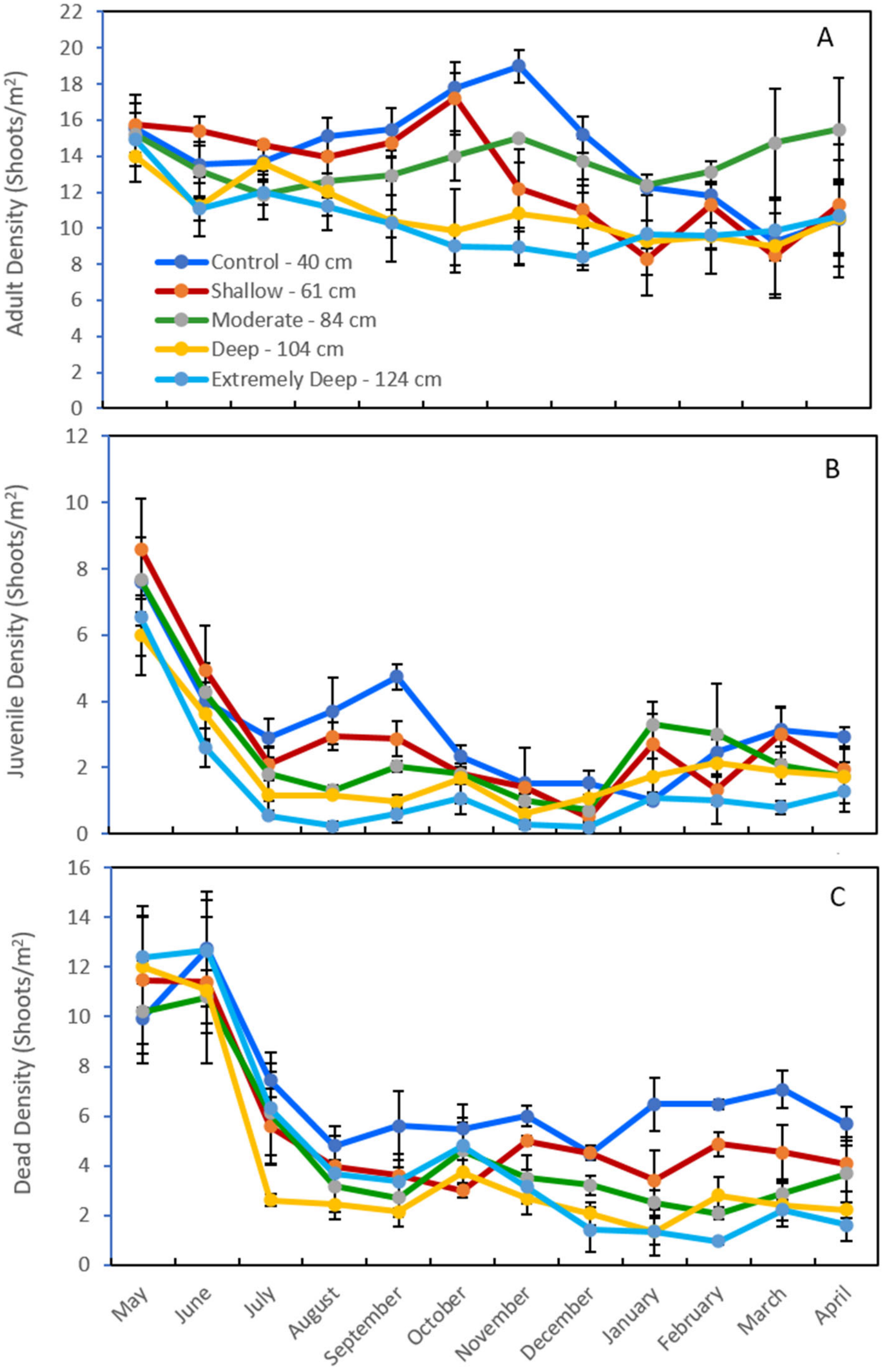

Figure 12. Mean density ± standard error for (A) adults, (B) juveniles, and (C) dead *Typha* for each water level treatment from May 2019 through April 2020.

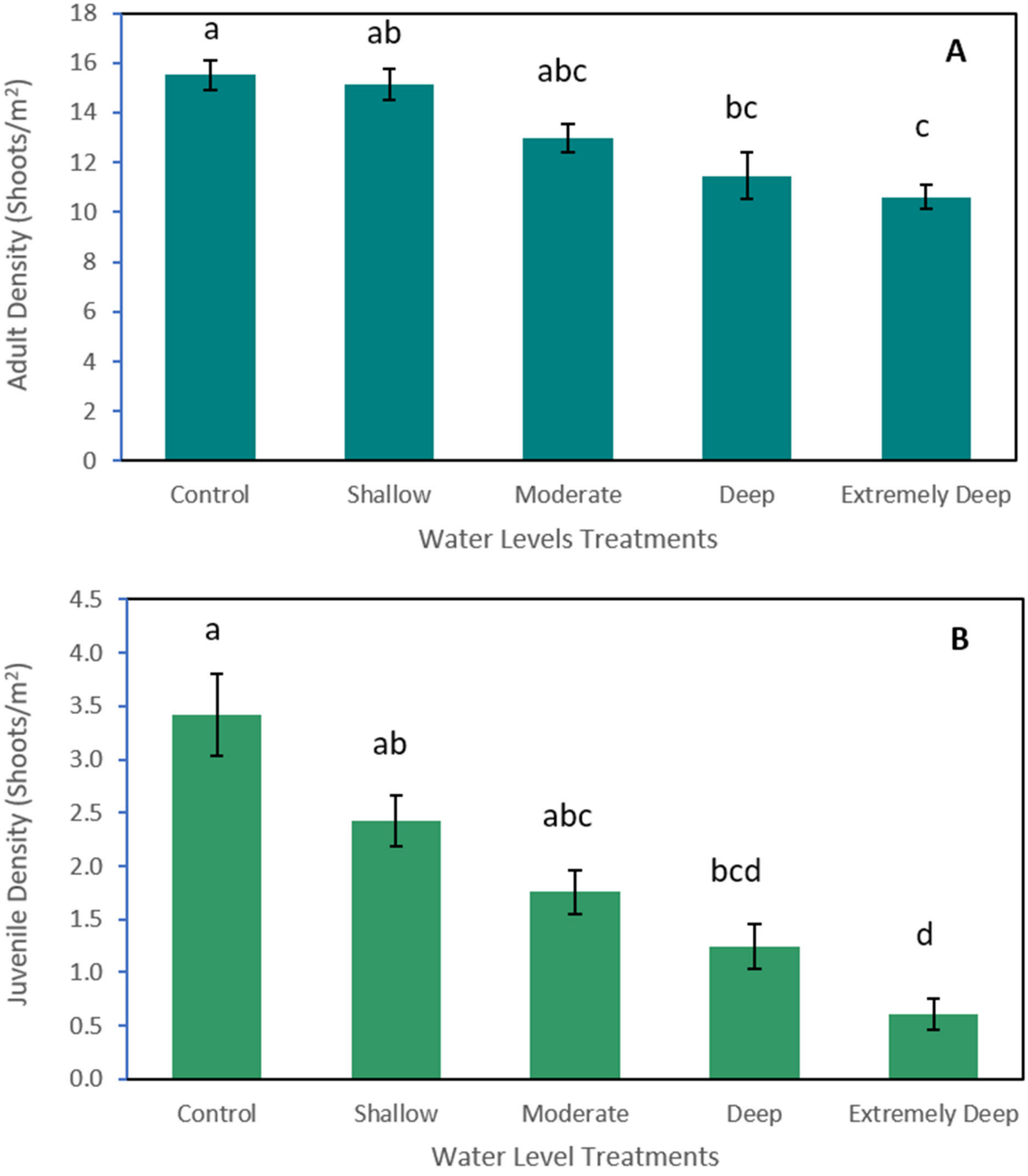

Figure 13. A) Adult and B) juvenile *Typha* density values for each water level treatment from monitoring data collected from July to October 2019 (mean ± standard error, sample size =12). Mean differences with the same letter are not significantly different at the 0.05 probability level using the non-parametric Dunn All Pair for Joint Ranks Test.

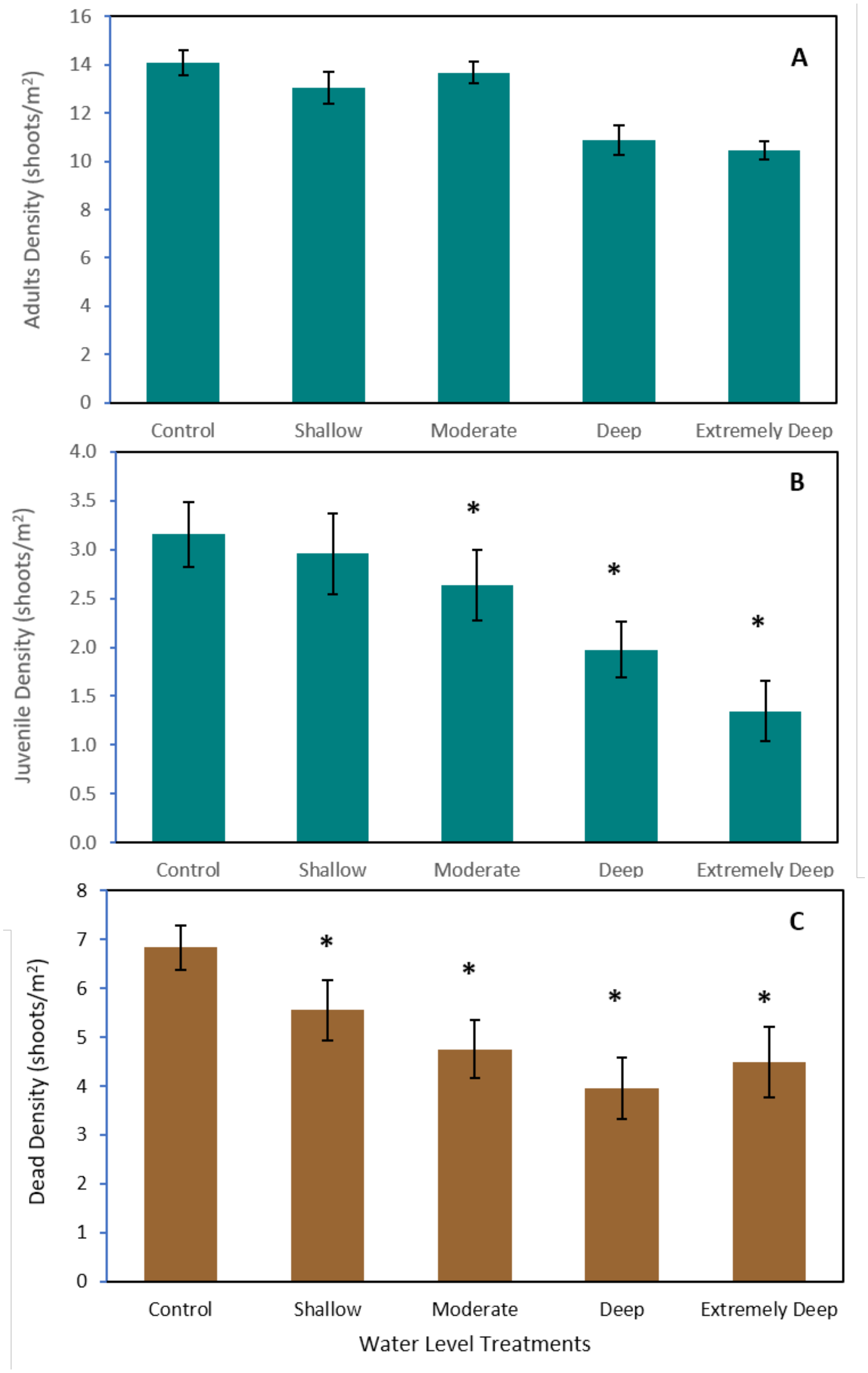

Figure 14. *Typha* A) adult, B) juvenile, and C) dead densities values for each water level treatment from monitoring data collected from July 2019 to April 2020 (mean ± standard error, sample size = 36). Stars above bars indicate treatments that are significantly different from the control.

Table 5. Spearman's rank correlation coefficients for *Typha* density comparisons. *Typha* densities for each water level treatment (shallow to extremely deep) were compared to those of the control. Spearman's rho (ρ) and associated p values are reported for each water level treatment comparison.

***Typha* Response**	**Correlation of Control (40 cm) to …**	**Spearman's ρ**	**p value**
Adult Density	Shallow – 61 cm	0.61	0.034 [a]
	Moderate – 84 cm	0.11	0.733
	Deep – 104 cm	0.42	0.177
	Extremely Deep – 124 cm	-0.17	0.604
Juvenile Density	Shallow – 61 cm	0.77	<0.01 [a]
	Moderate – 84 cm	0.42	0.170
	Deep – 104 cm	0.37	0.240
	Extremely Deep – 124 cm	0.35	0.270
Dead Density	Shallow – 61 cm	0.72	0.008 [a]
	Moderate – 84 cm	0.37	0.230
	Deep – 104 cm	0.50	0.100
	Extremely Deep – 124 cm	0.39	0.212

a. Significant probability of the relationship between treatment pairs.

Table 6. Results of the ANOVA models exploring the effects of different water level treatments on various *Typha* responses.

Variables	**F value**	**p-value**
Adult Density	1.93	0.182
Juvenile Density	4.17	0.030
Dead Density	8.37	0.003
Photosynthetic Rate	3.33	0.012
Stomatal Conductance	1.86	0.193
Water Use Efficiency	1.86	0.193
Leaf Elongation Rate	10.01	0.001
Leaf Area Index	4.16	0.031

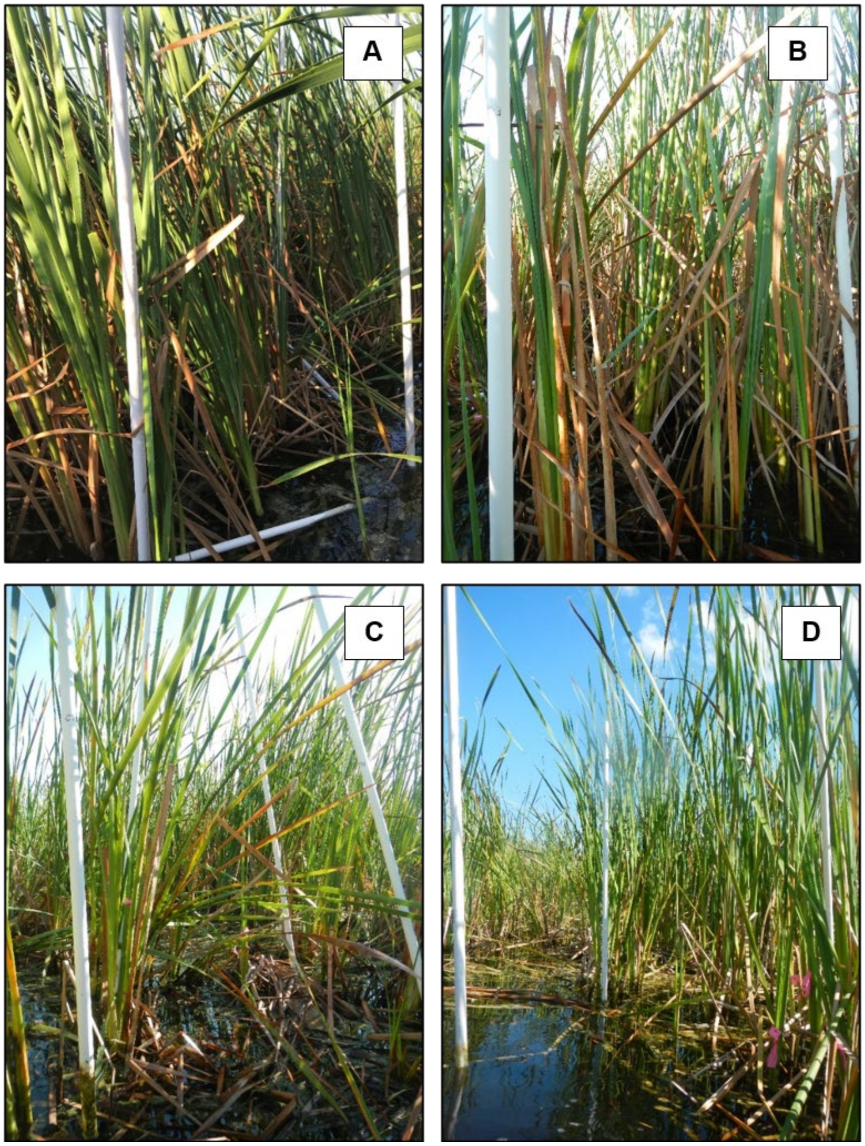

Figure 15. Photos of various treatment plots at 12 weeks (September 2019 sampling events) of continuous inundation: (A) 40 cm (control), dense *Typha*, 22 adults and 4 juveniles; (B) 84 cm (moderate treatment), sparse to moderate *Typha* density, 13 adults and 2 juveniles; (C) 104 cm (deep treatment), sparse *Typha* density, 2 adults and 2 juveniles; and (D) 124 cm (extremely deep treatment), sparse *Typha* density, 3 adults and 1 juveniles. Note the floating dead cattail in photos C and D. (Photos by SFWMD.)

Baseline Conditions

Two baseline monitoring events were completed in May and June 2019. Water depths during the baseline events ranged from 41 to 48 cm in all test cells. In mid-June 2019, strong winds during a storm knocked down about 10% of the *Typha* population in most of the test cells. Most of these plants never recovered and eventually died. According to other SFWMD personnel, these microbursts are a common occurrence in the STAs during the rainy season.

Adult Densities

Adult *Typha* densities during the two baseline months, May and June 2019, averaged 15.1 and 12.9 shoots/m^2 (**Figure 12A**), respectively. Mean adult *Typha* density values measured from July 2019 to April 2020 decreased from 14.1 adults/m^2 in the control to 10.9 and 10.5 adults/m^2 in the deep and extremely deep water level treatments (**Figure 14A**), respectively; however, these differences were not significant (**Table 6**). These results represented a 23% and 26% decline in the adult *Typha* population in the two deepest water level treatments at the end of the study.

Changes in adult densities due to the water level treatments were best defined during the first four months of the study (July–October 2019), which coincide with the highest production period of *Typha* species in frost-free climates (Finlayson et al. 1983; **Figure 12A**). Mean adult *Typha* densities from this period were significantly lower in the deep (11.5 shoots/m^2) and extremely deep (10.6 shoots/m^2) water level treatments compared to the control (15.5 shoot/m^2, **Figure 13A**). These results show a 26% and 32% decline of the adult *Typha* population in the deep and extremely deep water level treatments, respectively, after four months of continuous inundation. Visual signs of decline due to water level treatments were observed as early as four weeks into the study, with the first two floating *Typha* reported during this monitoring event in one of the deep water level treatment plots. At 12 weeks *Typha* plants from the deeper water level treatments showed more chlorosis with a significant decline in their *Typha* population compared to the shallower water level treatments (**Figure 15**). Wildlife activities were also a problem in several plots with bird nesting’s contributing to *Typha* lodging and eventual loss of plants.

Adult densities from the control (40 cm) and shallow water level treatment (61 cm) were similar from July to October 2019 with densities increasing from 13.7 to 17.8 shoots/m^2 and from 14.6 to 17.2 shoots/m^2, respectively (**Figure 12A**). However, densities from these two water level treatments started to decline during the second half of the study (November 2019–April 2020), with monthly densities more variable than the trends observed from July to October 2019. This decline may have been a combination of stress after four months of continuous flooding and effect of numerous problems that disrupted the normal operations of the study. In contrast, densities from the deep and extremely deep water level treatments declined from 13.6 and 12.0 shoots/m^2 in July to 9.9 and 9.0 shoots/m^2 in October 2019, representing a decline of 27 and 25%, respectively. Densities from these two water level treatments remained low through the end of the study. Densities associated with the moderate water level treatment increased from July to November 2019 with densities remaining high through the end of the study. Toward the end of the study (i.e., February–April 2020), adult densities associated with shallow, deep, and extremely deep treatments were similar to the control while the moderate treatment densities increased above the control. The shallow water level treatment density trend was the only trend that was consistently similar to the control (**Table 5**).

Juvenile Densities

During the two baseline months (May–June 2019) juvenile densities averaged 7.3 and 3.9 shoots/m^2, respectively (**Figure 12B**). The baseline data showed that the *Typha* population in the test cells was actively growing with the juveniles accounting for 32.6% and 23.2% of the total *Typha* population during May and June 2019, respectively.

Juvenile densities declined across all water level treatments following the first baseline event in May 2019 and these densities continued to decline through July when they dropped to 1.7 juveniles/m^2 (**Figure 12B**). From July to October, differences between treatments and the control were most pronounced and sorted in descending order from the control (40-cm) to the extremely deep treatment, for a juvenile decline of 41% in the deep and 62% in the extremely deep treatment, compared to the juvenile population in the control in July 2019. After October 2019, juvenile densities remained low and the average number of juveniles did not exceed 3 juveniles/m^2 across all water level treatments through the remainder of the study.

Juvenile densities from the entire monitoring period were significantly influenced by the different water level treatments (**Table 6**) with densities significantly declined from 3.2 shoots/m^2 in the control to 2.0 and 1.4 juvenile shoots/m^2 in the deep and extremely deep water level treatments, respectively (**Figure 14B**). These results represented a 37 and 57% decline in the juvenile population in these two water level treatments, respectively.

Juvenile densities also were significantly lower after four months of continuous flooding, with densities declining in the deep (1.2 juveniles/m^2) and extremely deep (0.6 juveniles/m^2) water level treatments compared to the control (3.4 juveniles/m^2, **Figure 13B**). These results represented a 65 and 83%, respectively, decline in the juvenile population in these two water level treatments after four months of continuous treatment. The juvenile population growing in this study are mostly by asexual reproduction through rhizomes, where rhizome tips turn erect to produce upright new ramets (Grace and Harrison 1986). These new ramets probably had less difficulty growing through the water column in the control and shallow water level treatments (40 and 61 cm, respectively) than in the deeper treatments. Grace (1989) reported that prolonged inundation periods adversely affect growth and reproduction of *Typha* species. These findings agree with Diaz and Vaughan (2019) who reported that densities of the adult and juvenile *Typha* populations in STA-3/4 Cell 2A were significantly lower in the deeper inflow region of the cell.

Dead Typha Densities

During the baseline period, the number of dead *Typha* shoots averaged 11.2 and 12.0 dead shoots/m^2, for May and June, respectively (**Figure 12C**). After this period, the density of dead *Typha* declined from July to August, before averaging ~4 dead shoot/m^2 between September and December 2019 (**Figure 12C**). At this point, mean differences between densities of dead *Typha* associated with deeper water level treatments and those from the control became more pronounced.

Dead *Typha* were hard to find in the field, particularly in the deep and extremely deep water level treatments, because they are well below the water surface. The statistically significant differences between the control and other water level treatments is likely due to an undercount of dead *Typha* at the higher water level treatments (**Figure 14C**). Mean dead *Typha* densities for the entire monitoring period were significantly different (**Table 6**) and decreased from 6.8 dead shoots/m^2 in the control to 3.9 and 4.5 dead shoots/m^2 in the deep and extremely deep water level treatments, respectively. These results represented a 42% and 35%, respectively, decline in the dead *Typha* population in the two deepest water level treatments at the end of the study.

Leaf Elongation Rates

Plant survival in flooded environments is an equilibrium between the severity of oxygen limitation and the adaptations of the plant to overcome this oxygen shortage. It has been hypothesized that this equilibrium is generally tilted unfavorably to plants at higher water levels due to the reduced aboveground surface area to supply oxygen to the belowground parts of the plant (Kadlec and Wallace 2009). This hypothesis is supported by findings that some species respond to flooding by growing taller for a more favorable balance between emergent and submerged plant organs for a better gas exchange (Grace 1989, Voesenek et al. 2003). Baseline LER measurements during May and June 2019 averaged 7.30 and 6.84 cm/d, respectively with monthly water depths in the test cells ranging from 41 to 48 cm, an indication that the *Typha* populations in the test cells were actively growing. The *Typha* population rapidly responded to the different water level treatments, with the highest monthly LER averages measured after 8 weeks (August 2019) of continuous flooding (**Figure 16**).

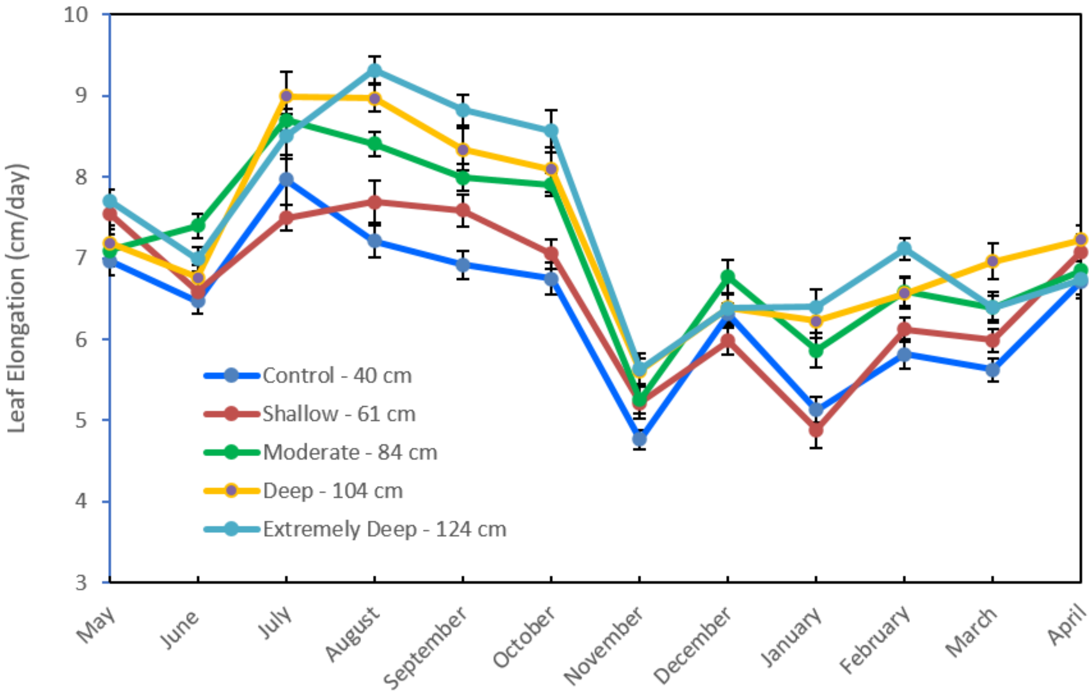

Figure 16. Monthly leaf elongation rates for each water level treatment from the May 2019–April 2020 study period.

Trends in LER varied throughout the study with all water level treatments tightly correlated to the control means (ρ ≥ 0.85, p < 0.01; **Table 7**). These responses, regardless of water treatment level, tended to increase following the initiation of water level treatments, especially among the moderate to extremely deep water level treatments. The *Typha* population rapidly responded to the different water level treatments, especially among the deeper treatments, which showed increased LER from an average of 6.84 cm/d in the June 2019 baseline event to an average of 8.60 cm/d in the deep and extremely deep treatments after four weeks of flooding (July 2019 event).

Table 7. Spearman's rank correlation coefficients for LER and LAI comparisons. Long-term trends in LER and LAI for each water level treatment (shallow to extremely deep) were compared to those of the control (40 cm). Spearman's rho (ρ) and associated p values are reported for each treatment comparison and for each *Typha* response.

***Typha* Response**	**Correlation of Control (40 cm) to …**	**Spearman's ρ**	**p value [a]**
Leaf Elongation Rate	Shallow – 61 cm	0.97	< 0.001
	Moderate – 84 cm	0.94	< 0.001
	Deep – 104 cm	0.87	< 0.001
	Extremely Deep – 124 cm	0.85	< 0.001
Leaf Area Index	Shallow – 61 cm	0.72	0.010
	Moderate – 84 cm	0.43	0.161
	Deep – 104 cm	0.56	0.063
	Extremely Deep – 124 cm	0.15	0.635

a. Significant p at the 0.05 confidence level.

LER responses remained stable from all treatments from July to October 2019. However, average LER values decreased considerably from 7.67 to 5.30 cm/d from October to November, respectively. During the November event (November 18—27, 2019), the first two cold fronts of the season occurred, the first on November 17 followed by a second front on November 25. The average temperature during those 10 days was 20.3 ºC with lows of 12 ºC (https://www.wunderground.com). The cool temperature during this event likely played a role in the low LER values. In the relatively mild climate of South Florida, *Typha* do not follow a common seasonal pattern as in more temperate climates (Duever et al. 1994, Weisner and Miao 2004). LERs bounced back to 6.37 cm/d in December before declining again in January 2020 and gradually increasing to near baseline rates by the end of the study (**Figure 16**).

Mean LER values were significantly affected by the different water level treatments ($p = 0.001$, **Table 6**), with rates increasing from 6.47 to 7.42 cm/d from the control to the deepest water level treatment. This increase was reflected in the treatment comparison to the control where every treatment LER was significantly greater than the control ($p < 0.001$, **Figure 17**). Like the adult *Typha* density, the effect of water level treatments on LER were better defined during the first four months of the study (July to October 2019), with values ranging from 7.20 cm/d in the control to 8.77 cm/d in the deepest treatment. The LER measurements for the moderate to extremely deep treatments were significantly higher ($p < 0.05$) than the control (**Table 8**) but were not significantly different from each other.

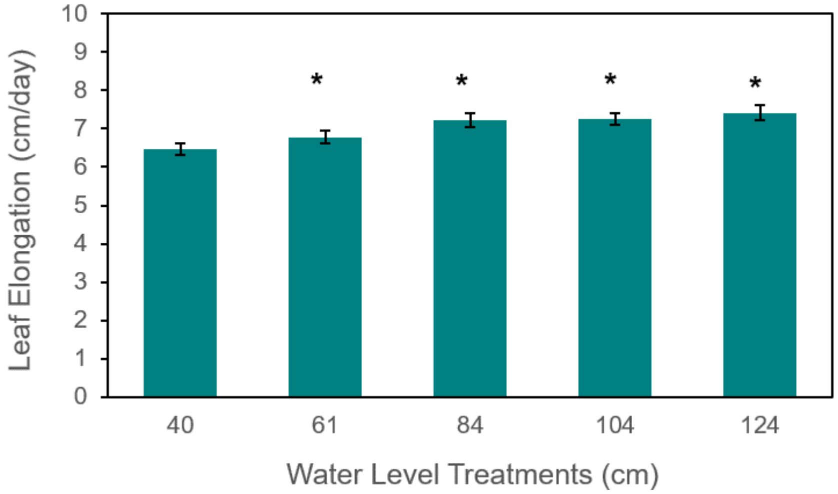

Figure 17. LER values for each water level treatment measured from July 2019 to April 2020 (mean ± standard error, sample size = 36). Stars above bars indicate treatments that are significantly different from the control.

Table 8. Leaf elongation rates (means ± standard error) from the first four and last six months of the study. Mean differences with the same letter were not significantly different at the 0.05 probability level using the Tukey-Kramer honestly significant difference test.

Water Level Treatment	LER July–October 2019 (cm/d)	LER November 2019–April 2020 (cm/d)
Control – 40 cm	7.20 ± 0.18 b	5.73 ± 0.17 b
Shallow – 61 cm	7.45 ± 0.12 b	5.98 ± 0.22 ab
Moderate – 84 cm	8.25 ± 0.13 a	6.35 ± 0.17 ab
Deep – 104 cm	8.59 ± 0.14 a	6.47 ± 0.15 a
Extremely Deep – 124 cm	8.77 ± 0.17 a	6.42 ± 0.13 a

Monthly LER values considerably decreased during the last six months of the study (November 2019–April 2020), with the lowest measured during the November 2019 event (**Figure 16**). Monthly LER values during this period ranged from 5.73 cm/d in the control to 6.47 cm/d in the deep water level treatment. LER from the deep and extremely deep water level treatments were significantly higher than those from the control (**Table 8**), while treatments other than the shallow treatment were not significantly different from each other during this period.

Chen and Vaughan (2014) reported that increasing water depths from 30 to 80 cm in a *Typha* community in STA-1 East adversely affected *Typha* growth rates. However, the results from this current study indicate that deeper water level treatments stimulated *Typha* growth rates of young leaves in an effort to restore gas exchange between the roots and the atmosphere and survive the deeper water levels (Bailey-Serres and Voesenek 2008). This is consistent with the findings from Diaz and Vaughan (2019) where *Typha* growing in the deeper inflow region of the STA-3/4 Cell 2A grew taller to escape deep water levels.

Leaf Area Index

Leaf area index (LAI), a key variable for plant growth, is defined as half the total green leaf area per unit ground surface (Chen and Black 1992). LAI is a dimensionless variable, but it can be defined as m^2 one-sided foliage/m^2 ground area. The LAI of vegetation depends on species composition, developmental stage of the plants, seasonality, and management practices. LAI is a dynamic parameter that can change from day to day or year to year especially during the spring and fall (Jonckheere et al. 2004). Baseline LAI values averaged 2.79 and 1.89 for May and June 2019, respectively (**Figure 18**). LAI responses across water level treatments tended to decline from those measured during the baseline period, then tended to follow similar trends as that observed in the adult *Typha* densities (**Figure 12A**). LAI values during the first four months (July–October 2019) were higher in the shallower treatment levels ranging from 1.72 in July (control) to 2.44 in October (moderate treatment). In contrast, LAI values in the deeper treatments during the same period ranged from 1.50 in the deep treatment to 1.76 in the extremely deep treatment. During the last six months of the study (November 2019–April 2020) monthly LAI values were more variable with the highest values measured in the shallow and moderate water level treatments. As with *Typha* responses, the shallow treatment LAI trends were most similar to the control ($\rho = 0.72$, $p = 0.01$; **Table 7**). All other treatment trends varied considerably from the control throughout the study period.

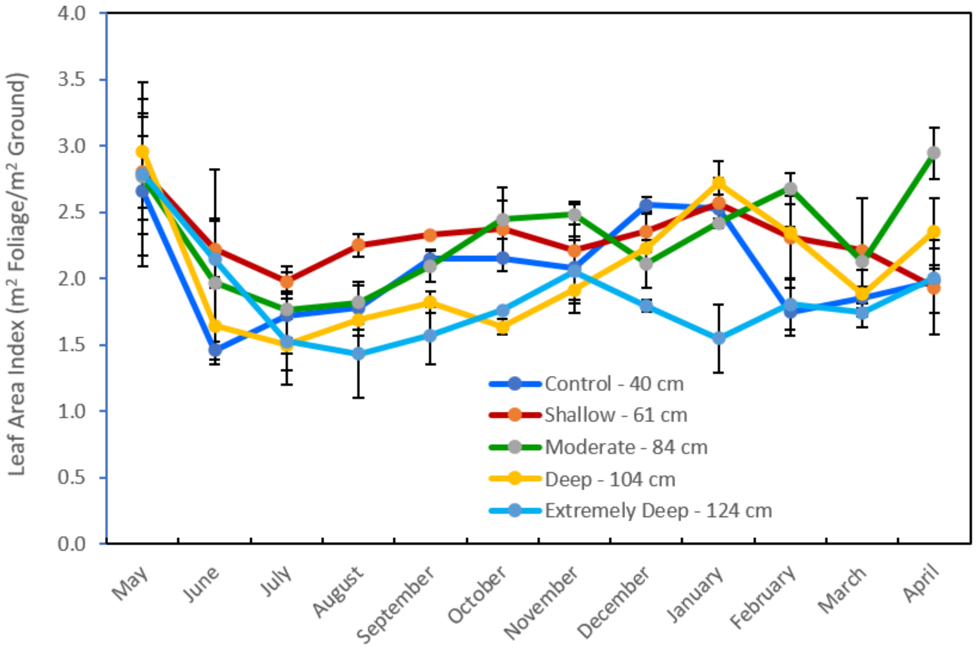

Figure 18. Monthly ± standard error LAI values for each water treatment level for monitoring data collected May 2019–April 2020.

Mean LAI displayed a unimodal-type response across water level treatments with values increasing from 2.05 in the control to 2.30 in the moderate water level treatment and declining to 2.06 and 1.86 in the deep and extremely deep water level treatments, respectively (**Figure 19**). The deepest water level treatment had significantly lower LAI ($p = 0.03$, **Table 6** and **Figure 19**) than the control. Comparing the LAI values during the highest production period of *Typha* plants in this study (July–October 2019, **Figure 20**), they followed the same pattern observed in the adult *Typha* density from the same period (**Figure 13A**). Mean LAI values were significantly higher ($p < 0.05$) in the shallow treatment (2.23), decreasing to 1.66 and 1.58 in the deep and extremely deep treatments, respectively. These results agree with the adult densities that significantly decreased from 15.13 shoots/m^2 in the shallow water level treatment to 11.47 and 10.62 in the deep and extremely deep treatments, respectively. These results show that LAI values change during the season and follow developmental stages and treatments affecting the *Typha* population. Mean LAI values were significantly affected by the deeper water level treatments in the same manner that adult density significantly declined with water level treatments.

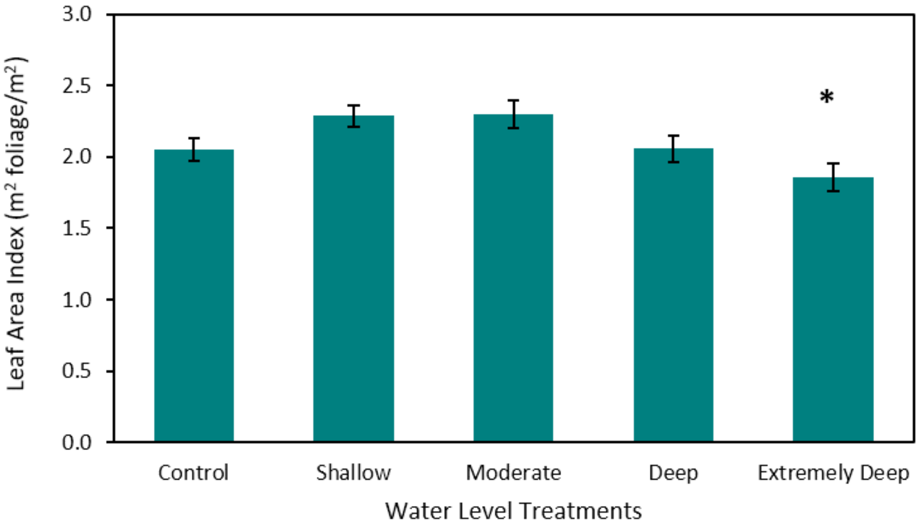

Figure 19. LAI measurements for each water level treatment collected July 2019–April 2020 (mean ± standard error, sample size= 36). Stars above bars indicate treatments that are significantly different from the control.

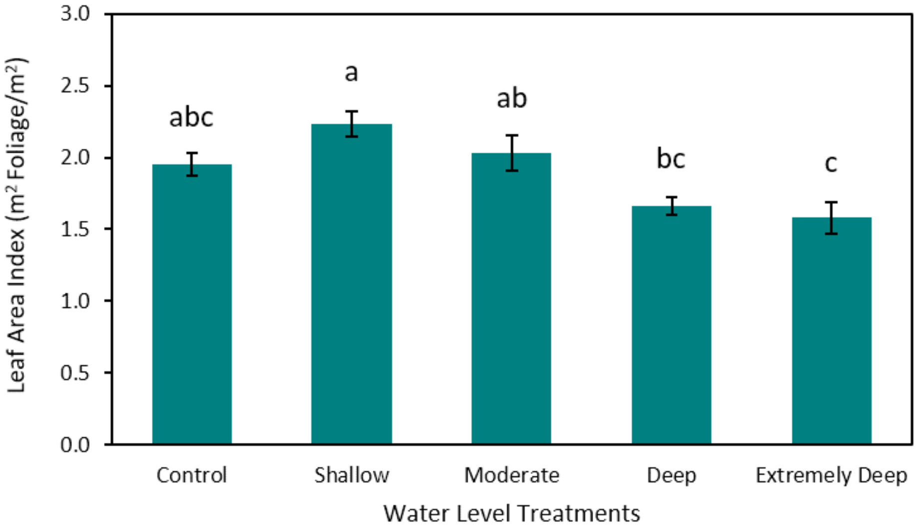

Figure 20. LAI values from the first four months of the study (July–October 2019) for each water level treatment (mean ± standard error, sample size = 12). Mean differences with the same letter were not significantly different at the 0.05 probability level using the non-parametric Dunn All Pair for Joint Rank tests.

Gas Exchange Rates

This analysis focused on three physiological parameters that are good indicators of how healthy or stressed a plant is: net photosynthetic rate, transpiration (i.e., stomatal conductance), and water use efficiency (WUE) (Kadlec and Wallace 2009, Liao and Lin 2001).

Timing of Peak Rates

Trends among gas exchange rates were highly variable among the different water level treatments but were similar in terms of the timing of peak rates (**Figure 21**). In all cases peak values were observed between September and October. However, like other responses, there were subtle differences among the gas exchange rate responses. Photosynthetic rates and stomatal conductance were tightly clustered during the baseline period, averaging 24.6 micromoles carbon dioxide per square meter per second (μmol $CO_2/m^2/s$) and 0.53 moles water per square meter per second (mol $H_2O/m^2/s$), respectively. Both values declined between June and August before rising to October peak values, which were similarly stratified by water level treatments (**Figures 21A** and **21B**). The highest values for both responses were observed among individuals associated with the deep and extremely deep water level treatments, which were 34.5 μmol $CO_2/m^2/s$ and 0.73 mol $H_2O/m^2/s$ for photosynthetic rate and stomatal conductance, respectively. The responses among the control and moderate water level treatments varied, however photosynthetic rates from the control and shallow treatment still peaked in October (~27.9 μmol $CO_2/m^2/s$) to a lesser degree compared to those observed in the deeper treatments. Control stomata conductance also peaked at ~0.60 mol $H_2O/m^2/s$ while values declined to 0.48 and 0.45 mol $H_2O/m^2/s$ in the shallow and moderate water level treatments, respectively. This stratification of differences between treatments in stomatal conductance values effectively reversed in November and was highly variable thereafter (**Figure 21B**). Photosynthetic rates became tightly clustered but were similarly variable thereafter (**Figure 21A**). None of the trends in both responses among water level treatments were correlated to those of the control ($p > 0.05$; **Table 9**). However, trends in WUE responses for the shallow and extremely deep treatments were significantly correlated to those of the control ($\rho \geq 0.71$ and $p < 0.01$; **Table 9**). Like the other gas exchange rates, WUE peaked in October with the highest value measured in the shallow water level treatment (59.4 μmol CO_2/mol H_2O, **Figure 21C**). Trends thereafter were variable, declining from October to December, then gradually increased throughout the remainder of the study. WUE among all water level treatments was surprisingly similar at the conclusion of the study at around 51 μmol CO_2/mol H_2O.

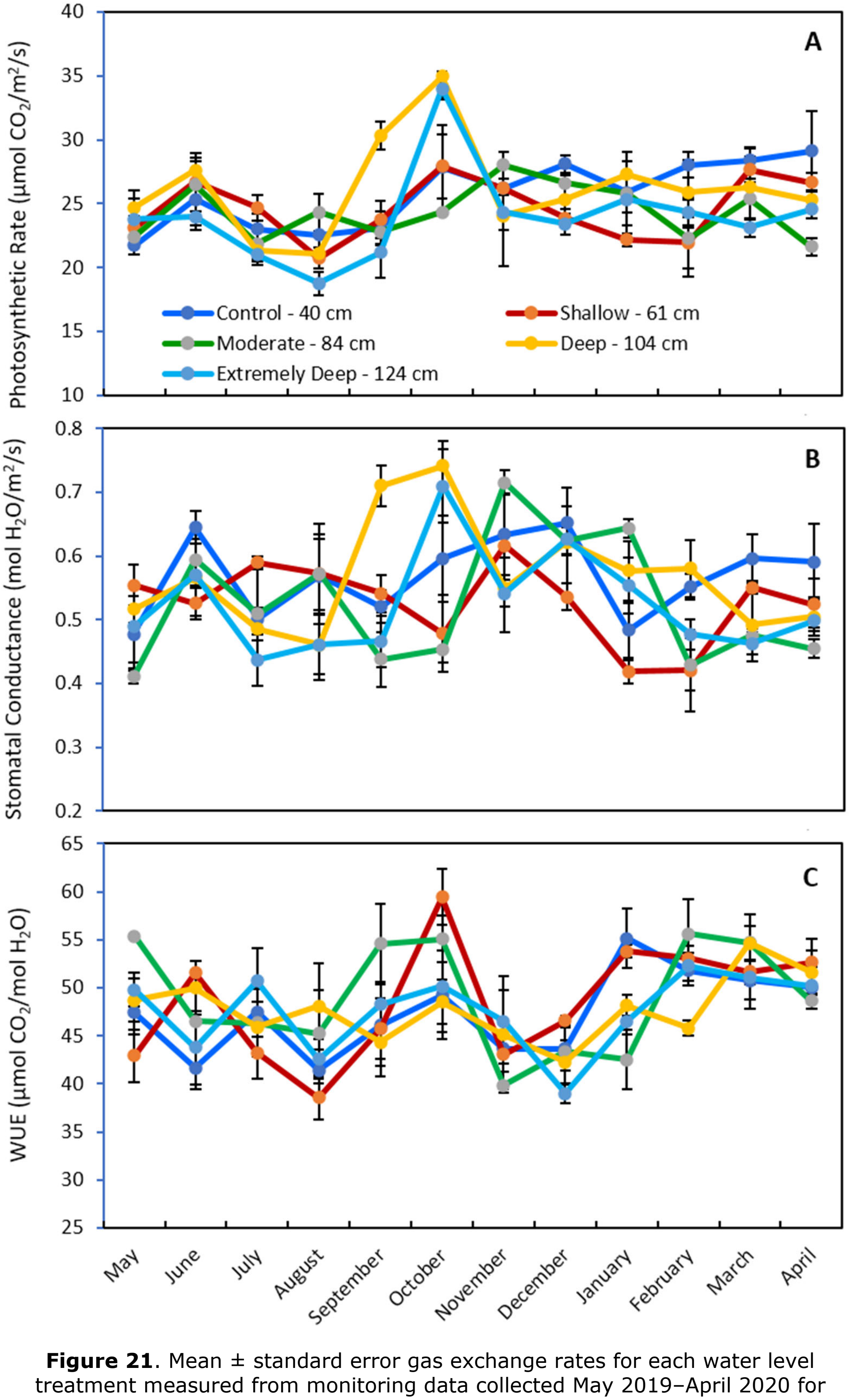

Figure 21. Mean ± standard error gas exchange rates for each water level treatment measured from monitoring data collected May 2019–April 2020 for (A) photosynthetic rate, (B) stomatal conductance, and (C) WUE. (Note: µmol CO^2/mol H_2O – micromole carbon dioxide per mole water.)

Table 9. Spearman's rank correlation coefficients for gas exchange rates comparisons. Study long trends in photosynthetic rates, stomatal conductance, and WUE for each water level treatment (shallow to extremely deep) were compared to those of the control (40 cm). Spearman's rho (ρ) and associated p values are reported for each comparison and for each gas exchange rate.

***Typha* Response**	**Correlation of Control (40 cm) to …**	**Spearman's ρ**	**p value [a]**
Photosynthetic Rate	Shallow – 61 cm	0.47	0.128
	Moderate – 84 cm	0.11	0.733
	Deep – 104 cm	0.29	0.366
	Extremely Deep – 124 cm	0.45	0.140
Stomatal Conductance	Shallow – 61 cm	0.01	0.991
	Moderate – 84 cm	0.45	0.140
	Deep – 104 cm	0.22	0.485
	Extremely Deep – 124 cm	0.53	0.079
Water Use Efficiency	Shallow – 61 cm	0.71	0.012 [a]
	Moderate – 84 cm	0.36	0.246
	Deep – 104 cm	0.31	0.331
	Extremely Deep – 124 cm	0.76	0.006 [a]

a. Significant p values at the 0.05 confidence level.

Photosynthetic Rate

Photosynthetic rate is the rate at which the plant can fix carbon for growth. Low photosynthetic rates can indicate that a plant is stressed, while higher values show that the plant is functioning under more favorable conditions. Mean photosynthetic rates across water level treatments during the entire monitoring period varied by less than 3 µmol $CO_2/m^2/s$, ranging between 26.2 and 24.0 µmol $CO_2/m^2/s$ in the deep and extremely deep treatments, respectively. However, water level effects were significant for photosynthetic rate ($p = 0.01$; **Table 6**), which is attributed to the significant difference between the control and the extremely deep water level treatment based on the means test (**Figure 22A**). In contrast, Chen et al. (2010) and Chen and Vaughan (2014) reported significant decreases in photosynthetic rates of *T. domingensis* growing at water depths higher than 80 cm. When comparing across sampling events, photosynthetic rates tended to increase toward the end of the growing season (i.e., October; **Figure 21A**), which correspond to a spike in adult *Typha* density (**Figure 12A**) during that month, which may indicate that slightly cooler temperatures provide less stressful conditions for growth. Photosynthetic rates from the deep (34.9 µmol $CO_2/m^2/s$) and extremely deep treatment (34.0 µmol $CO_2/m^2/s$) during the month of October were significantly higher than rates from the moderate treatment (24.3 µmol $CO_2/m^2/s$) and higher than the shallow treatment and the control, respectively. Similar higher photosynthetic rates were observed toward the end of the growing season in the *Typha* community of the in-situ study in STA-3/4 Cell 2A (Diaz and Vaughan 2019).

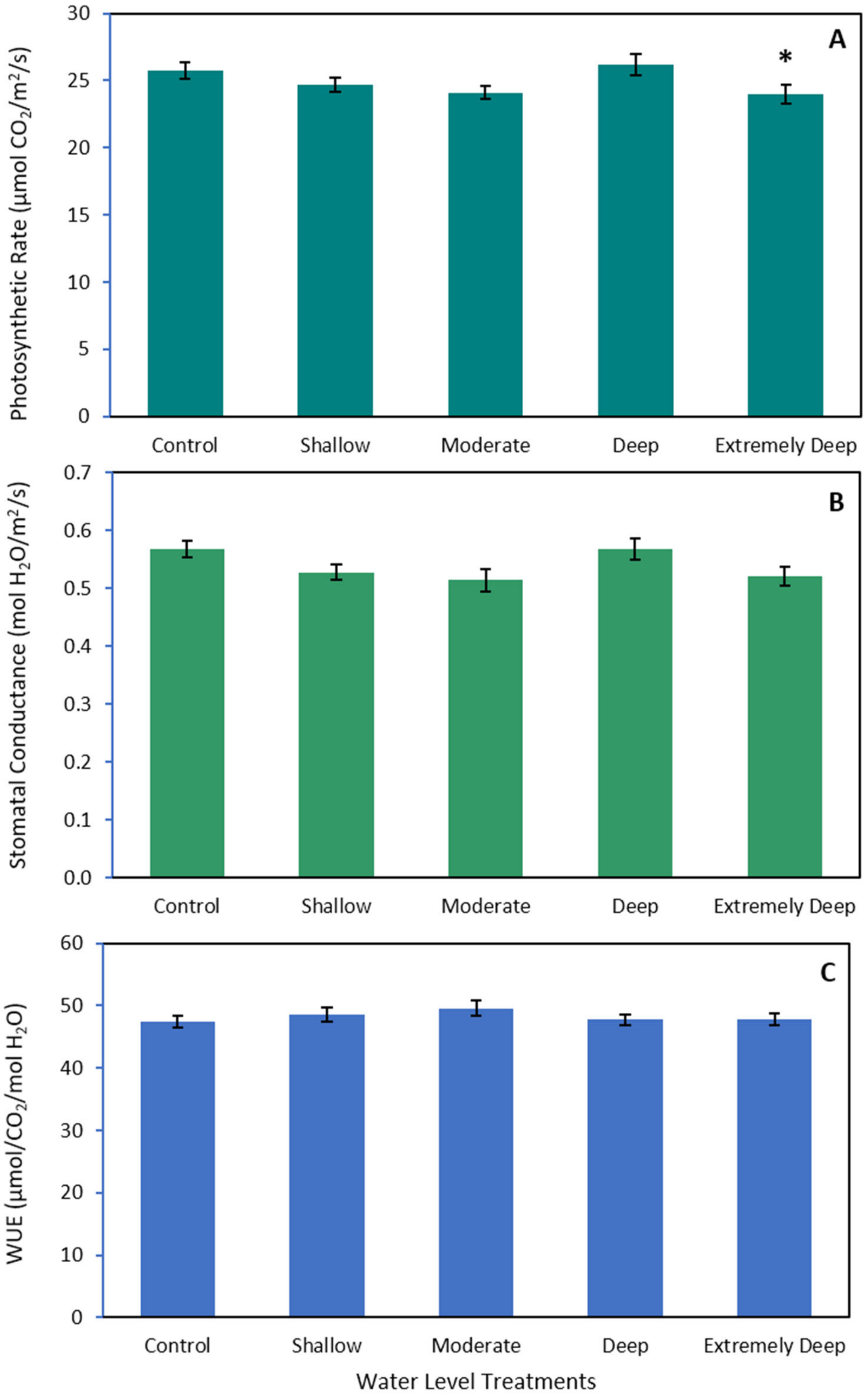

Figure 22. Gas exchange rates for each water level treatment from monitoring data collected July 2019–April 2020 for (A) photosynthetic rate, (B) stomatal conductance, and (C) WUE (mean ± standard error, sample size = 36). Stars above bars indicate treatments that are significantly different from the control. (Note: µmol CO_2/mol H_2O – micromole carbon dioxide per mole water.)

Stomatal Conductance

Transpiration, as measured by taking readings of stomatal conductance, is the rate of water vapor lost through stomata as a function of atmospheric conditions and stomata density in the leaves (Cronk and Fennessy 2001). Mean stomatal conductance across water level treatments during the entire monitoring period were not statistically significant (**Figure 22B**). Stomatal conductance varied by only 0.054 mol $H_2O/m^2/s$ from the control (0.568 mol $H_2O/m^2/s$) to the moderate treatment (0.514 mol $H_2O/m^2/s$). This indicates that there was no physiological reduction in transpiration despite the deeper water depths and more difficult growing conditions in the deeper treatments. When comparing stomatal conductance across sampling events, October was the only event where the stomatal conductance from the deep treatment (0.742 mol $H_2O/m^2/s$) was significantly higher than that of the shallow treatment (0.478 mol $H_2O/m^2/s$) and moderate treatment (0.453 mol $H_2O/m^2/s$). In general, stomatal conductance tends to increase toward the end of the growing season (October—November) and decrease during the winter months and early spring (January—April) (Mann and Wetzel 1999). Higher transpiration rates are generally measured during the rapid growth period of the plant in the summer months and early fall coupled with higher temperatures during this period, while lower transpiration rates indicate that the water demand for the plant is declining usually toward the end of the growing season (Cronk and Fennessy 2001). These findings are consistent with results reported by Diaz and Vaughan (2019) from the *Typha* population of the in-situ study in STA--3/4 Cell 2A.

Water Use Efficiency

WUE is defined as the ratio of photosynthetic rate to stomatal conductance. In general, higher WUE indicates greater stress within a plant, while lower WUE indicates more optimal growing conditions. Mean WUE across water level treatments during the entire monitoring period were not statistically significant (**Figure 22C**), where WUE values varied by only 2.26 micromole carbon dioxide per mole water (µmol CO_2/mol H_2O) from the moderate treatment (49.6 µmol CO_2/mol H_2O) to the control (47.4 µmol CO_2/mol H_2O), respectively. When comparing WUE values across monitoring events, February was the only sampling event where the mean WUE value from the moderate treatment (55.6 µmol CO_2/mol H_2O) was significantly higher than the mean value from the deep treatment (45.7 µmol CO_2/mol H_2O). Although there was no significant differences over time, WUE values peaked in October (shallow treatment 59.4 µmol CO_2/mol H_2O), then dropped in November and December (**Figure 21C**), indicating that the growing conditions at the end of the of the year may be less physiologically stressful compared to earlier part of the growing season.

Typha Biomass

Effect of Depth Treatments on Biomass at End of Study

Total *Typha* biomass consisted of live aboveground and belowground biomass at the different water level treatments. Biomass of the different plant components (leaves, roots, rhizomes, and shoot bases) varied with water level treatment; however, the differences were not significant at the end of the study (**Table 10**). Shoot base biomass was the only belowground biomass component with values higher in the control plots; however, the differences were still not significant. Shoot base biomass ranged from 787 grams per square meter (g/m^2) in the control treatment to 603 g/m^2 in the deep water treatment level. Although, shoot base biomass was lower in the deeper treatments compared to the control at the end of the study, the proportion of shoot base biomass in the deeper treatments indicates that the stressed *Typha* plants were still allocating resources to the belowground parts that will be beneficial in post-flooding recovery of the plant. Similar results were observed in the *Typha* in-situ study where total live biomass and biomass of the different plant components between the deeper inflow and shallower outflow regions in STA-3/4 Cell 2A were not significantly different ($p > 0.05$, Diaz and Vaughan 2019). Likewise, Chen and Vaughan (2014) also reported that leaf, belowground, and total biomass from experimental plots in STA-1 East also were not significantly affected by different water levels.

Table 10. Mean ± standard error *Typha* biomass by water level treatments at the end of the study.

Water Level Treatment	Live Biomass Components (g/m^2) Leaves	Roots	Rhizomes	Shoot Base	Belowground Biomass (g/m^2)	Aboveground Biomass (g/m^2)	Total Live Biomass (g/m^2)	Belowground/ Leaf Ratio
Control – 40 cm	2601 ± 211	363 ± 26	348 ± 24	787 ± 91 [a]	1498 ± 126	2640 ± 214	4138 ± 309	0.61 ± 0.05
Shallow – 61 cm	2363 ± 281	306 ± 15	346 ± 21	578 ± 40	1229 ± 64	2387 ± 283	3616 ± 334	0.65 ± 0.09
Moderate – 84 cm	2906 ± 291	351 ± 16	372 ± 28	615 ± 67	1338 ± 92	3047 ± 305	4385 ± 377	0.50 ± 0.04
Deep – 104 cm	2480 ± 338	343 ± 36	348 ± 32	603 ± 76	1295 ± 131	2543 ± 360	3838 ± 476	0.59 ± 0.06
Extremely Deep – 124 cm	2863 ± 386	364 ± 22	384 ± 30	633 ± 49	1381 ± 87	2943 ± 400	4324 ± 480	0.59 ± 0.07

a. Means of different biomass components were not significantly different at the 0.05 probability level using the Tukey-Kramer honestly significant difference test.

Effects of Depth Treatments on Population at the End of Study

Although there were no differences in total biomass due to water level treatments, there were considerable differences in the *Typha* populations harvested at the end of the study. Adult density during the final biomass harvest was not significantly affected by water level treatments, with densities ranging from 17.9 to 25.4 adult shoots/m^2 in the deep and moderate treatments, respectively (**Figure 23A**). However, the juvenile *Typha* population was significantly larger in the control (10.8 juvenile/m^2) compared to those in the deepest treatments, with the lowest density measured in the deep treatment (0.42 juveniles/m^2, **Figure 23B**). These results agree with juvenile densities reported during the study, with juvenile densities declining across all water level treatments. In the control and shallow water level treatment the adult *Typha* population accounted for 68 and 79% of the total *Typha* density, respectively, while in the in the deeper water level treatments, the adult population accounted for > 92% of the total *Typha* density. In contrast, the juveniles were a significant component of the total *Typha* population in the shallower water level treatments, accounting for 32 and 21% of the total density in the control and shallow treatments, respectively, compared to < 8% in the deeper treatments.

Effects of Depth Treatments on Plant Height and Weight at End of Study

Typha density at the end of the study, especially the juvenile population, decreased significantly with water level treatments (**Figure 23**); however, plant height and weight increased significantly with increasing water levels (**Figures 24** and **25**), i.e., fewer but significantly larger ramets were found in the deeper water level treatment ($p < 0.05$, **Figure 24**). Adult ramets gradually increased in size as water depth increased, with ramet height averaging 343 and 374 cm in the deep and extremely deep water level treatments, respectively, compared to 285 and 312 cm in the control and shallow treatment depths, respectively. Likewise, the average total biomass per ramet also was significantly higher in the deeper treatments with adult ramets from the extremely deep water treatment level weighing two times (256 grams) than adult ramets in the control treatment plots (123 grams) (**Figure 25**).

Effects of Depth on Aboveground and Belowground Biomass

Aboveground biomass values were slightly higher in the deeper water level treatments; however, the differences were not significant (**Table 10**). Belowground biomass was higher in the control than in the deeper water level treatments, however, the differences also were not significant. Although belowground biomass values were not significantly affected by the different water level treatments, the consistent lower biomass values in the deeper water level treatments agree with other studies that increasing water depths decreases the biomass allocation to rhizome and roots reducing the anchorage capacity of *Typha* plants (Grace and Wetzel 1982, Miao and Zou 2012). *Typha* plants from the deeper treatments were more susceptible to be uprooted during the study (**Figure 15D**).

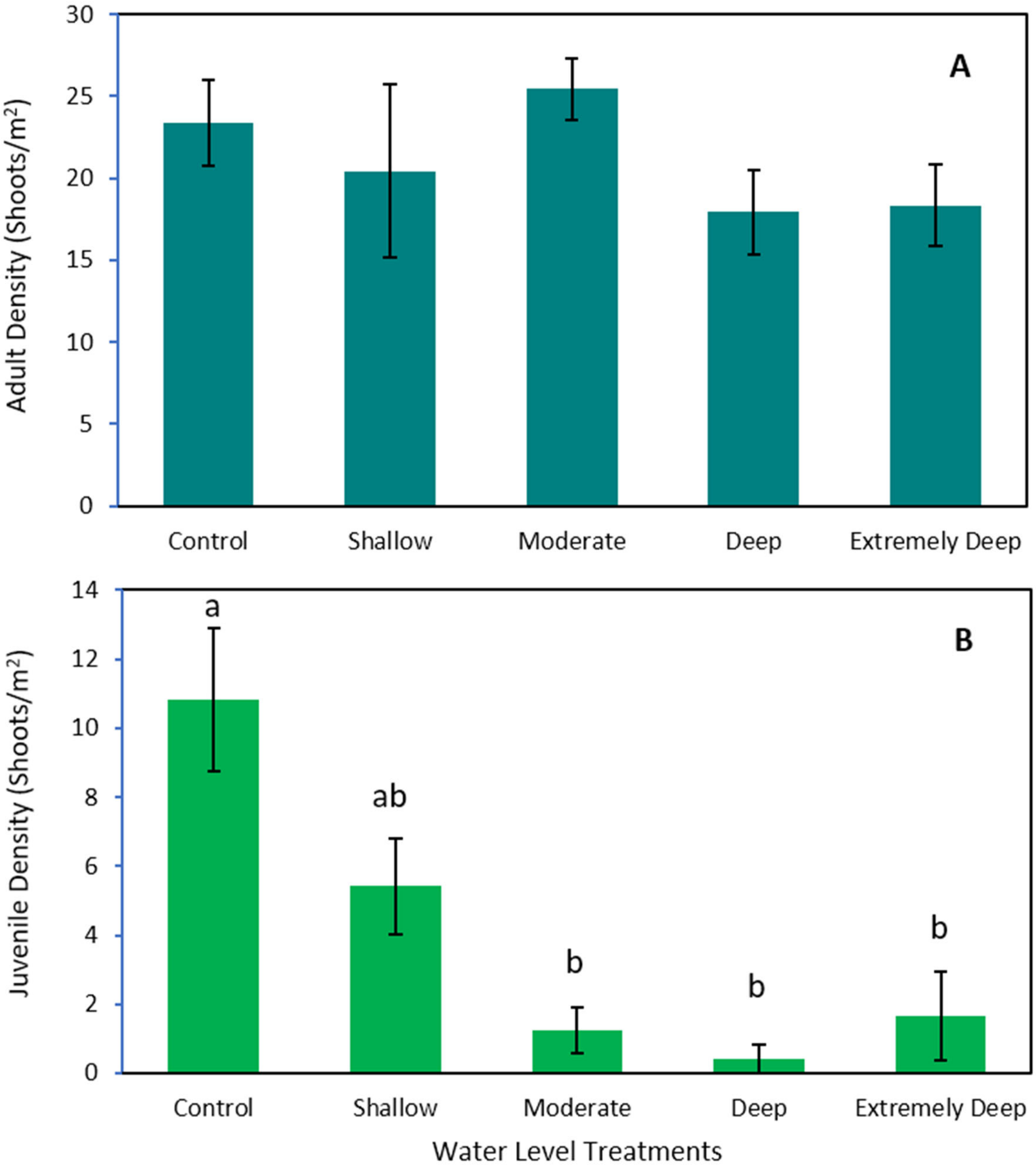

Figure 23. (A) Adult and (B) juvenile *Typha* densities measured during the biomass harvest at the end of the study (mean ± standard error, sample size = 15). Mean differences with the same letter are not significantly different at the 0.05 probability level using the Tukey-Kramer honestly significant difference test.

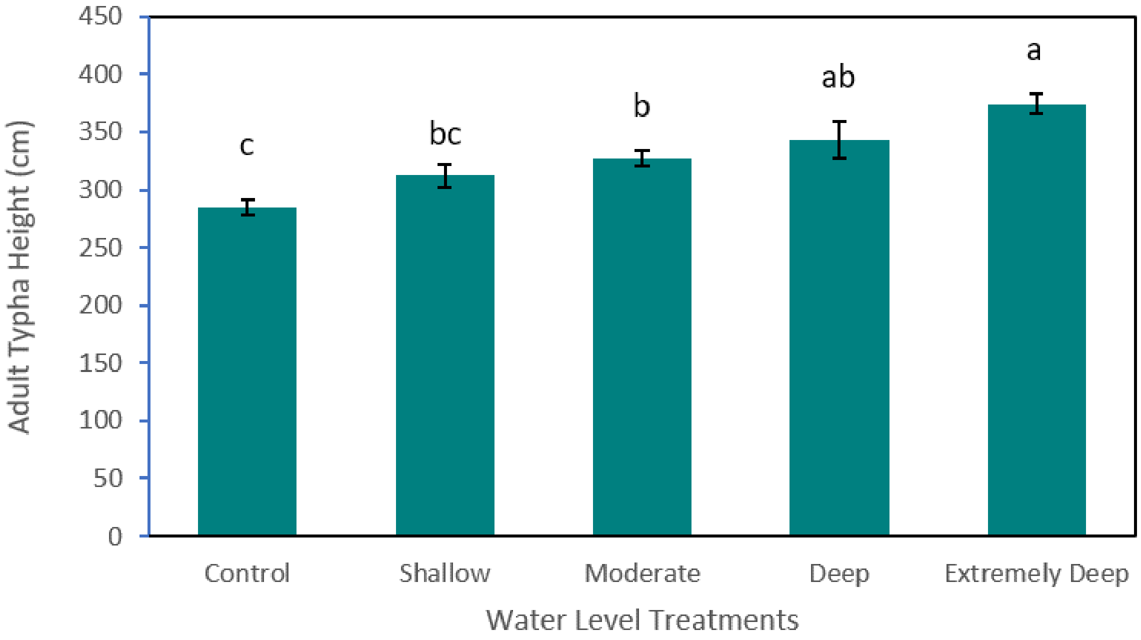

Figure 24. Changes in adult *Typha* height as a function of water depths at the end of the study (mean ± standard error, sample size = 15). Mean differences with the same letter are not significantly different at the 0.05 probability level using the Tukey-Kramer honestly significant difference test.

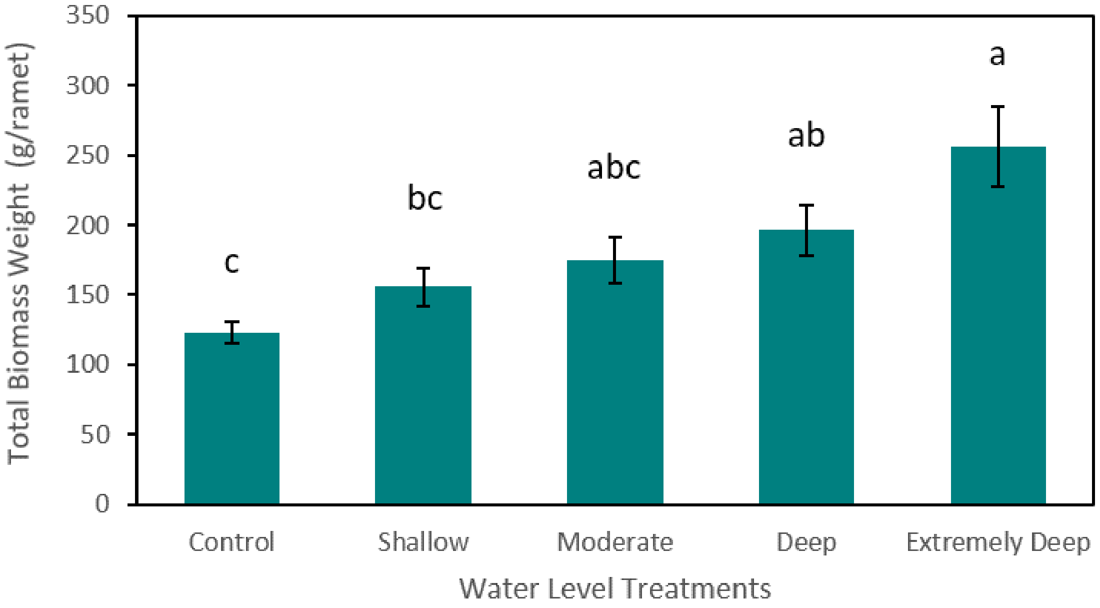

Figure 25. Total *Typha* biomass weight per ramet at the end of the study (mean ± standard error, sample size = 15). Biomass differences with the same letter are not significantly different at the 0.05 probability level using the non-parametric Dunn All Pair for Joint Ranks test.

Belowground biomass:leaf ratios between water depths were not significant (**Table 10**). Some studies have suggested that a decrease in the belowground biomass:leaf ratio is a sign that roots and rhizomes have been stressed more than shoots under deeper water conditions (Chen et al. 2010, Diaz and Vaughan 2019). Although belowground biomass:leaf ratios were not significantly different, *Typha* plants from deeper treatments showed more signs of stress than *Typha* in the shallower water level treatments. At four weeks of continuous flooding *Typha* plants from the deep and extremely deep treatments started to show signs of chlorosis and the first floating *Typha* were reported from the deep water level treatment. After 12 weeks of continuous flooding, *Typha* density from some plots in the deep and extremely deep treatments had declined significantly with higher number of floating *Typha* observed throughout the entire cell (**Figures 15C** and **15D**). At the end of the study, the overall health of the *Typha* plants declined especially in the deeper water level treatments.

Biomass Allocation

Biomass allocation was calculated as the biomass of a specific plant tissue divided by the total biomass of all plants in each quadrat. Overall, biomass allocation to leaves and belowground tissues were not significantly different among the different water level treatments (**Figure 26**). *Typha* plants from all treatment cells allocated approximately 64% of their total biomass to leaves and 36% to belowground tissues (roots, rhizomes, and shoot bases). Similar biomass allocation was observed in the *Typha* population from STA-3/4 during the third year (2017) of the in-situ study (Diaz and Vaughan 2019). Likewise, Miao and Sklar (1998) also reported that *Typha* growing along a nutrient gradient in Water Conservation Area 2A allocated approximately 60% of their total biomass to leaves and 40% to belowground tissues. *Typha* plants growing in the control treatment plots showed higher shoot base allocation (18.9%) and lower leaf allocation (62.8%) than those growing in the moderate treatment plots (14.5% and 67.4%, respectively).

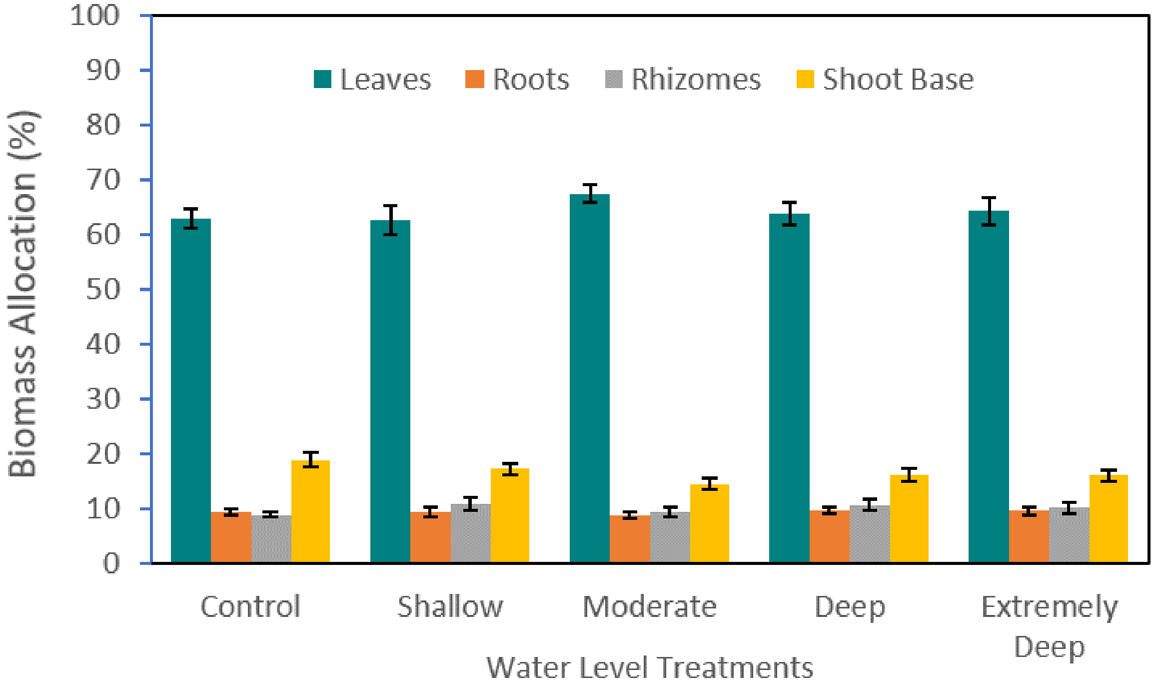

Figure 26. Proportional biomass allocation to leaves, roots, rhizomes, and shoot bases for *Typha* plants growing under each water level treatment (mean ± standard error, sample size = 15). Means were not significantly different at the 0.05 probability level.

Tissue Nutrient Concentration and Allocation

TP concentrations in the different tissue biomass components were not significantly different among the different water level treatments (**Figure 27A**). Leaves and shoot bases were the only biomass components where TP concentrations tended to decrease with water depth, with leaf TP decreasing from 1,234 mg/kg in the control plots to 1,163 mg/kg in the extremely deep water level treatment. Shoot base TP decreased from 2,978 in the shallow treatment to 2,621 mg/kg in the extremely deep treatment; however, differences were not significant. The greatest TP concentration among the four biomass components regardless of water level treatment was shoot base. Shoot base TP (overall average from all treatments) was 235% higher than leaf TP, 190% higher than rhizome TP, and 240% higher than roots TP. Rhizomes showed the second highest TP concentrations and roots showed the least. Similar higher shoot base TP concentrations were observed in *Typha* biomass from the inflow region of STA-3/4 Cell 2A during the 2015 and 2016 monitoring of the in-situ study (Diaz and Vaughan 2019).

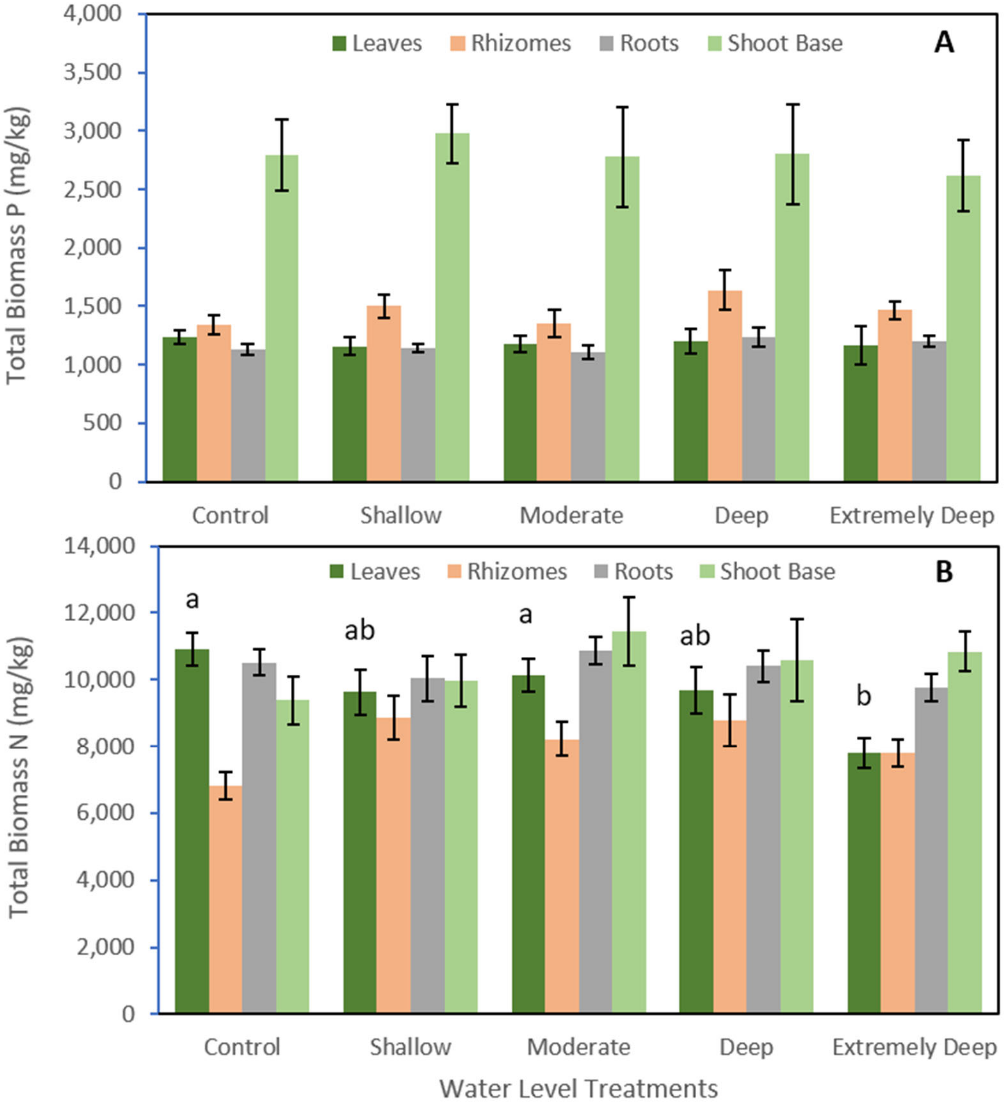

Figure 27. (A) TP and (B) TN tissue concentrations of *Typha* plants grown under each water treatment level at the end of the study (mean ± standard error, sample size = 15). Mean difference with the same letter are not significantly different at the 0.05 probability level.

TN behaved differently with concentrations more evenly distributed among the different plant components. Leaf TN was the only tissue part that was significantly different due to water level treatment, with concentrations from the extremely deep treatment plots (7,792 mg/kg) significantly lower ($p < 0.05$) than concentration from the control plots (10,900 mg/kg) (**Figure 27B**). Differences in biomass TN from the other components were not significant ($p > 0.05$) and did not show an obvious trend among the treatments. The highest TN concentrations occurred in shoot bases and roots (overall average from all treatments), while rhizomes showed the least. Similar results were observed in the 2017 biomass TN concentrations of the in-situ study, with shoot bases and roots from the inflow region showing the highest concentrations and rhizomes from outflow region showing the least (Diaz and Vaughan 2019).

Nutrient allocations were calculated as the nutrient contents of a specific tissue divided by the total nutrient contents of all plants growing in each quadrat. Therefore, TP and TN allocation were affected by both tissue concentration and tissue biomass. Differences in allocations among the different *Typha* tissues due to water level treatment were not significant ($p > 0.05$, **Figure 28**). Overall, TN allocation was like biomass allocation, with *Typha* plants allocating approximately 63.5% of their TN to leaves and 36.5% to belowground tissues (roots, rhizomes, and shoot bases, **Figure 28B**). Shoot base showed the highest TN allocation among the belowground tissues (17.6%), while rhizomes showed the least (8.6%). TP allocation among the different *Typha* tissues was different with plants allocating ~52.4% to leaves and 47.6% to belowground tissues (**Figure 28A**). Shoot base showed the highest TP allocation among the belowground tissues (29.7%), while roots showed the least (7.7%). Shoot base TP allocation tended to decrease with water depth, with allocations ranging from 33.9% in the control plots to 25.3% in the moderate treatment.

None of the water level treatments resulted in the total collapse of the *Typha* communities at the end of the 10-month inundation period as reported in a shorter mesocosm study (Chen et al. 2010). However, treatments had a significant effect on several of the measured plant responses discussed in the previous sections that may have limited the sustainability of affected *Typha* stands. At the end of the study, water levels from all cells were lowered to facilitate the final biomass harvest of aboveground and belowground biomass components. *Typha* populations from the control and shallow water level treatment did not show any lodging (*Typha* lying flat on the ground) after lowering the water levels (**Figure 29A**). *Typha* plants from the moderate water level treatment, showed about 10 to 15% lodging of the *Typha* population after lowering the water levels (**Figure 29B**). In the *Typha* population from the deep (**Figure 29 C**) and extremely deep (**Figure 29D**) water level treatments there was 50 to 60% lodging of the *Typha* community after lowering the water levels.

Results from LER measurements showed that the *Typha* population rapidly responded to the different water level treatments, especially at deep and extremely deep water levels. LER increased from 6.84 cm/d at the June baseline event to an average of 8.89 cm/d in the deep and extremely deep treatments after eight weeks of flooding (**Figure 16**, August event). In their efforts to survive under deep flooding conditions, *Typha* plants grow taller in order to restore gas exchange between aboveground and belowground plant parts (Grace 1989). During submergence-induced leaf elongation, no new cells are formed, indicating that the increase in leaf elongation is completely attributed to cell expansion (Voesenek et al. 2003), a process where the cell walls from stems and leaves get weaker to allow the cells to expand (Purves et al. 2004). The weakening of the stems was more evident in the *Typha* plants growing in the deep and extremely deep water level treatments at the end of the study. During the 10 months of inundation during this study, the deep water level treatments provided enough buoyancy to maintain the *Typha* plants erect. However, after the water levels were lowered to facilitate biomass harvest, the *Typha* plants lost the buoyancy of the water column and the week stems were not able to keep most of the plants upright causing a significant lodging of the *Typha* population in these two treatments. This has important implications for STAs management. At the control and shallow water level treatments (**Figures 29A** and **29B**), *Typha* plants did not show any major problems during the study. For the moderate water level treatment, *Typha* plants thrived for the most part, however, for deep and extremely deep treatments, a significant part of the *Typha* population collapsed after lowering the water levels (**Figures 29C** and **29D**).

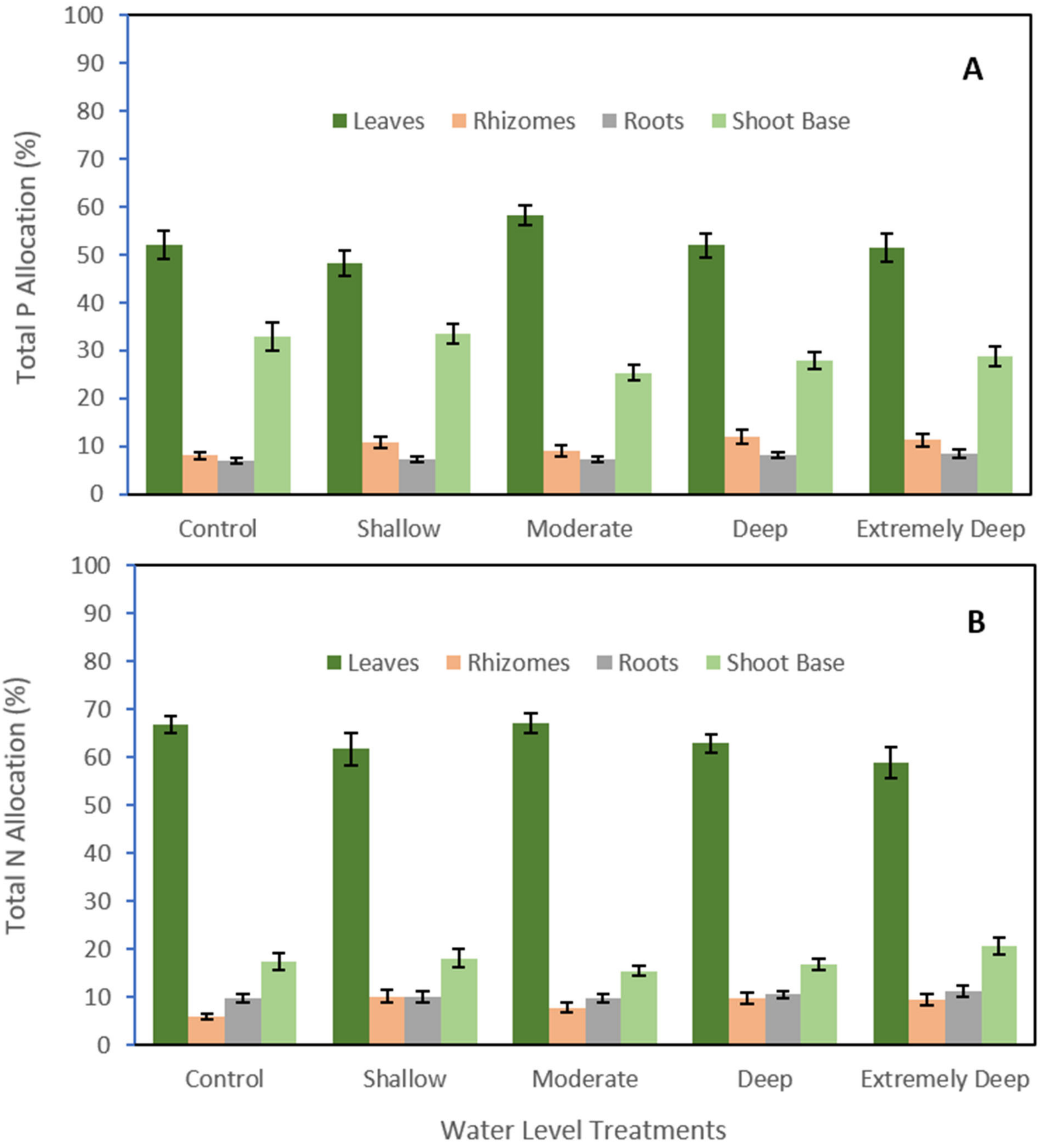

Figure 28. Proportional tissue TP and TN allocation to leaves, rhizomes, roots, and shoot bases of *Typha* plants growing at each water level treatment (mean ± standard error, sample size = 15). Mean were not significantly different at the 0.05 probability level.

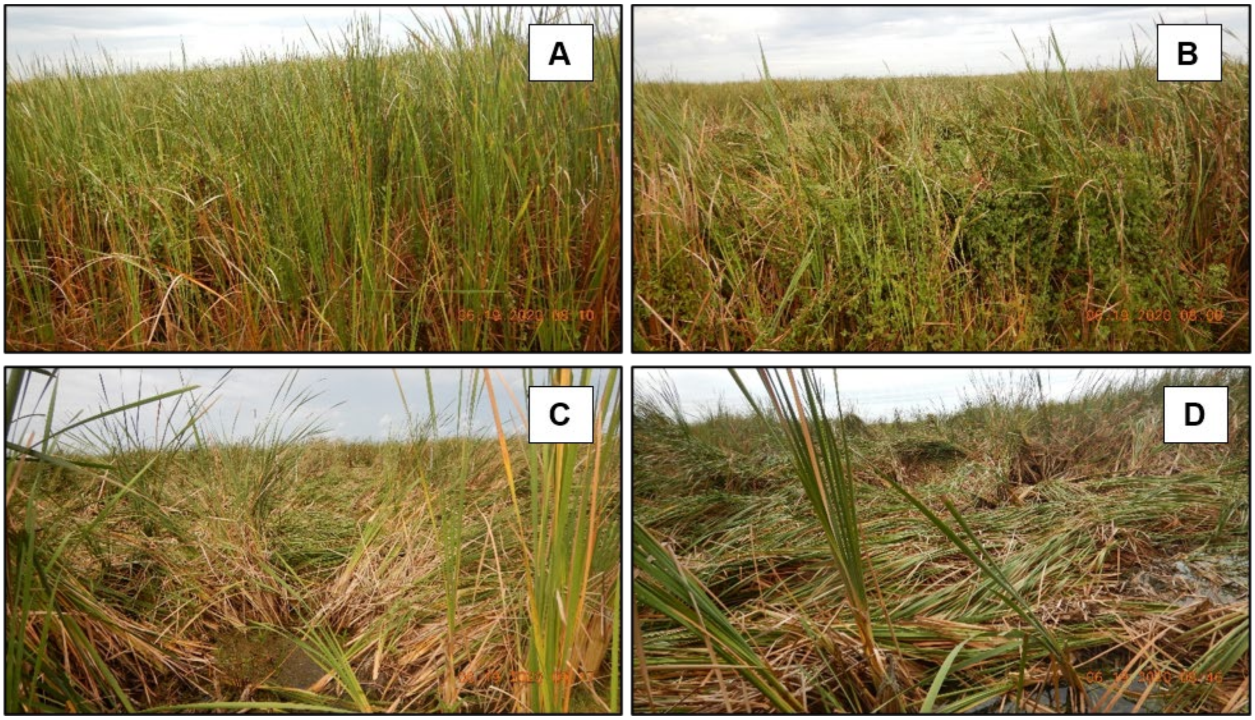

Figure 29. Photos taken after the final biomass harvest at end of the study. Water levels were lowered below 20 cm to harvest aboveground and belowground biomass. Photos are of a (A) control cell, (B) 84-cm (moderate) water level treatment cell, (C) 104-cm (deep) water level treatment cell, and (D) 124-cm (extremely deep) water level treatment cell. (Photos by SFWMD.)

CONCLUSIONS

When *Typha* are stressed by high water, it is expected that densities will decline, litter and floating plants will increase, and P removal will be reduced. In this study, it was hypothesized that water depths and duration of inundation would negatively affect *Typha* sustainability. *Typha* did show signs of stress, as observed in the decline of adult and juvenile densities (**Figure 12**) and increased in floating *Typha* specially under deep and extremely deep water level treatments (104 and 124 cm, respectively). Despite this stress, there was no apparent reduction in P removal (**Figure 8**). For the full 10 months of the experiment, only juvenile densities were significantly lower in the deeper water level treatments. Lower adult densities in the deeper water level treatments could be attributed to a reduction in the number of juveniles that could develop to adults and increase of floating plants. *Typha* mitigated deep water stress through increased in LER and biomass allocation of individual ramets. These two factors led to taller *Typha* plants that allowed them to escape the deep water conditions and maintain a favorable gas exchange between above and submerged plant parts. Gas exchange parameters were only minimally affected by water level treatments. Unfortunately, increased LER in these deep water conditions resulted in less structural support, producing significant lodging when the water levels were lowered to ~20 cm at the end of the experiment. Overall, while *Typha* demonstrated tolerance for deep (104 cm) and extremely deep (124 cm) constant water levels for 10 months, the stress based on reduced recruitment (i.e., fewer juveniles and adults) and increased floating plants were observed much sooner and should be considered in the overall water management of these systems. Further analyses of measurements made in the first four months of the study is ongoing and may provide a more specific time period of inundation that do not affect *Typha*. This information will support STA management decisions regarding water discharged to and from the STAs that will maintain healthy *Typha* communities and support STA P removal performance into the future.

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