Remediation of Contaminated Water from a Livestock Farm Using a Floating Island Technology and Native Wetland Plants

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REMEDIATION OF CONTAMINATED WATER FROM A LIVESTOCK FARM USING A FLOATING ISLAND TECHNOLOGY AND NATIVE WETLAND PLANTS

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Executive Summary

Floating constructed wetlands ('floating islands') are increasingly used for phyto-remediation of nutrient rich waters. Nevertheless, there is a paucity of information regarding the potential effectiveness of different aquatic plant species with respect to nutrient removal in agricultural wastewater, particularly in a cold climate context. To address this issue, a study was conducted at Olds College, Alberta, to determine the effectiveness of seven different plant species, which were integrated into experimental floating islands, for removal of nutrients in feedlot effluent. Water use efficiency of each plant species was also assessed.

The greenhouse experiment consisted of floating islands in small tanks, planted with one of seven different wetland plant species, namely small-fruited bulrush, Baltic rush, wheat sedge, water sedge, cattail, mare's-tail, and smartweed. The control treatment consisted of floating islands that remained unplanted. Each treatment and control were done in triplicate (n = 24). Planted floating islands were grown for eight months to ensure plant roots were established prior to commencement of the experiment, in which floating island water was exchanged for feedlot effluent. Water, plant tissues (roots and shoots), and peat substrate were sampled on four occasions from mid-July until early November. Depending on sample type, the parameters that were measured included physical water quality parameters (pH, temperature, dissolved oxygen, electrical conductivity, and total dissolved solids), nutrients (nitrogen, phosphorus, potassium), sulfate, and metals. Water use and plant biomass for each tank were also assessed.

With respect to nutrient removal from feedlot effluent, all seven plant species effectively removed phosphorus from the water compared to the control tanks, with removal ranging from 77% to 84%. Likewise, removal of potassium from feedlot effluent was observed for all plant species except smartweed, and ranged from 27% to 45%. Nitrate concentrations in the effluent were below analytical detection limits. Curiously, concentrations of phosphorus and potassium in plant tissues did not correspond with removal of either of these nutrients from effluent. Although nutrient removal is generally associated with biomass production, we suspect this observation may be related to the exceptionally low concentration of nutrients in the effluent (especially nitrate) which may have limited plant growth and/or the advanced age of the plants when the trial commenced. Nonetheless, removal of biomass will be important to prevent the return of these nutrients back into the system. Understanding nutrient storage partitioning between above-mat and below-mat biomass is an important component to understanding how to optimize nutrient removal and to determine biomass harvesting and warrants further investigation.

In contrast to the concentration reductions seen in the effluent for nutrients, an increase in sulfate concentrations was seen for most plant species over the course of the experiment. It is possible this may be related to microbial transformation of the sulfur species present in the floating islands, as sulfate was the only sulfur species measured in the current study. Further investigation into the sulfur cycling dynamics are needed to understand the potential remediation of sulfate using floating island systems.

As for water use, the amount of evapotranspiration of the tanks differed according to plant species, with those that used more water also producing higher amounts of shoot biomass, consistent with the notion that plants which are actively growing typically use more water. Additionally, there was an interaction between the effect of month and species, indicating that water use differences between species was different, depending on the month. In general, more water was used in August than any other month. This represented a point at which plants generally reached maximum size and therefore maximum water requirements. Most of the plant species in this experiment removed water from their tanks at a higher rate than the controls, so if water removal from a

system is desired, the addition of these wetland plants would beneficial. Conversely, if conservation of water is desired, mare's-tail or smartweed would be a better option in terms of water use, but may give more limited results for nutrient removal at the same time.

Overall, this pilot study demonstrated proof-of-concept for the phyto-remediation of feedlot effluent using floating constructed wetlands with respect to nutrient removal. Future studies should focus on the application of this technology in an actual feedlot setting. Selecting species most appropriate for the environmental conditions will be of utmost importance to ensure robust growth for optimal nutrient removal and water conservation or increased water use (in cases where water is in excess). Additionally, it will be imperative to assess how nutrients might be stored or released from floating island biomass through a full seasonal cycle. This could help determine optimal timing of island deployment or retrieval, and whether biomass harvesting is required. Moreover, determining species that can endure a harsh northern climate would provide important information for applications in Alberta, and other similar climates.

1.0 Introduction

1.1 Eutrophication and Nutrient Loading

Contamination of water occurs through numerous anthropogenic activities across the globe, with the eutrophication of water bodies via high nutrient loads of particular concern in prairie lakes (Lewtas et al. 2015). High nutrient loads from anthropogenic runoff leads to increased primary producer biomass (algal blooms) and in turn, a reduction in water quality usually in the form of depleted oxygen levels and undesirable smells/aesthetics. One of the primary nutrients influencing primary production in water, and therefore water quality, is phosphorus, although nitrogen and other nutrients can also be of importance (Schindler 2006; Lewtas et al. 2015). Eutrophication can have immediate or long-term consequences that negatively effect water use for fisheries, recreation, industry, or human consumption due to algae blooms and depleted oxygen levels (Lewtas et al. 2015).

Runoff from golf courses or agricultural lands (e.g. cropland, pasture, feedlots) can lead to increased nutrient loading in nearby waterbodies (Kunimatsu et al. 1999; Miller et al. 2004; Kronvang et al. 2005). Other contaminants of concern from these sources could also potentially include metals, pesticides, or petrochemicals. Remediating these water sources before they are released or re-used is of importance to prevent accumulation of nutrients and other contaminants in the larger watershed.

1.2 Phyto-Remediation

The role of wetland ecosystems in water quality improvement and the importance of this “ecosystem service” is well documented (Zedler and Kercher 2005). Particularly, the remedial benefit of wetland plants to uptake and remove of nutrients and metals from contaminated water has been well explored for many different applications. Wetland plants have been found to facilitate the removal and accumulation of several nutrients, including: nitrogen (Tanner et al. 2011; Keizer-Vlek et al. 2014; Lewtas et al. 2015; Pavlineri et al. 2017; Solanki et al. 2017), phosphorus (Keizer-Vlek et al. 2014; Lewtas et al. 2015; Pavlineri et al. 2017; Solanki et al. 2017), and selenium (Tannas et al. 2017; Tannas et al. 2020). Although, less studied there is also some evidence to suggest potassium (Saidin et al. 2014) and sulfate (Zhao et al. 2012; Saidin et al. 2014) may be taken up by wetland plants, which may be useful for remediation applications.

Floating island systems integrate wetland plants as a means for water remediation, and allow plants to grow above the water and root into the water, allowing for increased surface area and treatment (Tanner et al. 2011; Solanki et al. 2017). These mats can displace algae, shade the water surface, and buffer water turbulence, in addition to facilitating nutrient removal from source waters (Tanner et al. 2011). The advantage of these floating island systems revolves around their ability to tolerate wide fluctuations in water depth. This allows them to easily be retrofitted on to existing stormwater facilities or placed on ponds, lakes, and waterways without concern for water depth changes that would otherwise kill emergent wetland plants (Tanner et al. 2011). This opens doors to treating water in a passive manner in locations with water that is either too deep or does not have shallow emergent zones (or enough area) to effectively allow for water to be remediated passively through plants.

While there has been a lot of research into engineered wetlands and floating islands for use in phyto-remediation of water there has been very little work done targeting the application of floating islands for the northern, cold weather climate experienced in Alberta. Increasing our understanding of wetland plant species

native to Western Canada within the context of floating island systems will inform the application of these systems to the unique environmental conditions experiences in this region.

1.3 Background Research

In 2019, a laboratory trial was conducted at Olds College (OC) to examine the use of native wetland plants in floating island systems for the phyto-remediation of water with excess nutrient concentrations (Trial 1). The goal of this trial was to determine the effectiveness of five different native wetland species, namely cattail, wheat sedge, water sedge, smartweed, and sweet flag, in removing nutrients from spiked water samples. The water usage and viability of the plants within a floating island system was also of interest. Key findings from this trial included:

- Cattails stored more nitrogen, phosphorus, potassium, and sulfur (in g/m^2) in their shoots than any other species, and this was possibly driven by their higher rate of growth, as evidenced by substantially higher biomass.
- Cattails had the lowest concentrations of phosphorus, potassium, and sulfate remaining in water than any other species and had the highest evapotranspiration rates.
- Evidence for other plant species having effective nutrient uptake was mixed and depended on the nutrient:
    - Nitrate concentrations remaining in plant-treated water did not differ from the control treatment for any species other than cattail.
    - Phosphate concentrations remaining in planted-treated water were higher than the control treatment for all species other than cattail. It is likely that algae, which was abundant specifically in the control treatment, utilized the available phosphate for growth, resulting in relatively low concentrations in the control treatment.
    - Potassium concentrations remaining in plant-treated water were lower than the control treatment for all species tested.
    - Sulfate concentrations remaining in plant-treated water were lower than the control treatment for all species tested, other than water sedge.

While Trial 1 yielded important information on the effectiveness of various wetland plants for phyto-remediation of water with excessive nutrient concentrations, several challenges related to experimental design were noted. The main recommendations for methodological refinements and future work that came from this lab trial included:

- Starting water quality sampling at day 1 after spiking with nutrients to allow a more accurate measure of starting concentrations. Water quality sampling was started 5-7 days after spiking with nutrients.
- Findings were confounded by large algal blooms in the control treatment, which may have consumed substantial amounts of nitrogen and phosphorus. Shading to prevent algae growth was recommended.
- Ammonia analysis should be included in the water quality testing.
- Future research should focus on real world applications, such as testing removal of nutrients from feedlot effluent. This will provide more information about the application of floating islands in practical situations.

1.4 Project Objectives

This current project focused on understanding how native plants can be used as a tool for improving water quality, with emphasis on nutrient impacts from agricultural activities typically found in Western Canada., namely, production in feedlots. Our objectives were to determine:

1) The effectiveness of seven different plant species in removing nutrients from water.
2) Determine the water use efficiency of each plant species.
3) Determine the potential of each species to be used in floating island systems.
4) Test constructed wetlands using feedlot effluent to determine the effectiveness of nutrient removal and water use.

2.0 Methods

2.1 Tanks and Island Configuration

This project used a batch mesocosm style tank (Tanner et al. 2011) with floating islands custom built by GP Restoration Solutions Inc. out of Cremona, Alberta (Appendix A). Floating islands were custom built out of plastic pipe and non-woven geotextile fabric with a plastic mesh to hold the fabric in place on the island. The growing area of each island was 46 cm by 71 cm with a total outside measurement of 65 cm by 89 cm. This configuration filled most of the available space in each tank, leaving only a small area accessible for testing the water. This was designed intentionally to limit the amount of water loss to surface water evaporation. The islands were filled with peat moss as a growing medium for the plants (approximately7.5 cm of peat) with five plugs planted per island. Twenty-four treatment tanks with 264 liters (L) capacity were set up in an environmentally controlled greenhouse at Olds College. Of these, 21 contained floating islands with planted species, and 3 served as control tanks with unplanted floating islands. Water in all tanks was aerated using bubblers that were active for 20 minutes every 4 hours.

Plants were placed in the islands as plugs (3 months old) on November 18, 2019 and grown in filtered tap water within the tanks until July 13, 2020 (8 months total), at which point the treatment was started. This was done to acclimatize the plants to the greenhouse environment and allow them to establish roots within the islands before beginning treatment. Treatment initiation was initially planned for the spring of 2020, but due to COVID-19, it was delayed. By the time the experimental treatments were started, the plants had root systems established within the island to the point of having contact with the water. Floating islands were created in triplicate for each of the selected species and controls (Table 1). Tanks were set up in five rows within the climate-controlled greenhouse with the tank placement randomly generated via computer and manually adjusted to ensure that all species were uniformly exposed to conditions along the outer wall of the greenhouse where climatic controls were the least effective. In addition, temperature monitoring occurred around the entire greenhouse to ensure temperatures were uniform.

Table 1: Common and scientific names of the plant species used in the trial.

Common Name	Scientific Name
Wheat sedge	*Carex atherodes*
Smartweed	*Persicaria amphibia var. emersa*
Common cattail	*Typha latifolia*
Mare's-tail	*Hippuris vulgaris*
Water sedge	*Carex aquatalis*
Baltic rush	*Juncus balticus*
Small-fruited bulrush	*Scirpus microcarpus*

2.2 Water Treatment

On July 13, 2020, the experimental water treatment began. Floating islands were lifted from the water and placed on the tank edges in order to drain the filtered tap water from the tanks. After the tanks were emptied, 250 L of OC feedlot runoff water was added into the tanks and then floating islands were returned to the tanks. The feedlot water was sourced from the OC feedlot holding pond located in on the south side of the college campus. Approximately 20 cattle were held in a pen near the holding pond, while 6-8 bulls grazed around the pond itself. The baseline nutrient concentration of the feedlot water was determined by testing the water the day after the water was added to the tanks from two sources:

1. On-site at the OC feedlot (3 sites)
2. From each treatment and control tanks within the experimental design

2.3 Sampling & Monitoring

2.3.1 Water Sampling

Water sampling in the lab was completed throughout the acclimatization period, the day after the feedlot water was added to the tanks (July 14, 2020), and at three occasions after the feedlot water was added in 4-to 6-week intervals (August 24, September 21, and November 2, 2020). Samples were sent to A&L Canada Labs, London, Ontario, Canada, an accredited laboratory (Standards Council of Canada and Canadian Association for Laboratory Accreditation), for testing nutrient, salt, and metal composition on each sample date, and for heavy metal analysis on the first and last sample date. Water samples were analyzed for phosphorus, potassium and sulfate using Inductively coupled plasma atomic emission spectroscopy (ICP-OES) [EPA Method 6010B], nitrates by Standard Methods 4500-NO3-F, and chloride by titration with AgNO3 [Standard Methods 4500-Cl] Argentometric Method. A 500 mL sample of water was taken from each treatment tank, the three control tanks, the filtered tap water used to top up the tanks, and the unfiltered tap water. In addition, 500 mL samples were taken from three locations at the OC feedlot and tested for nutrient concentration. These samples were taken on six occasions (October 16, 2019, and February 5, July 14, August 24, September 21, and November 2, 2020).

2.3.2 Tissue & Soil Sampling

2.3.2.1 Chemical Analyses

Plant tissue and peat samples were sent for nutrient and metal composition testing at A&L Canada Labs. Two types of samples were collected for nutrient, metal, and salts analyses before feedlot water was added to the tanks (July 6, 2020) and at the end of the experiment (November 2, 2020), and included:

- 500 mL each of above ground biomass (shoots) and below island biomass (roots) tissue were collected from plants in each tank, resulting in a total of 21 samples.
- 500 mL of peat (growing medium) samples were collected from each tank, including the three control tanks, resulting in a total of 24 samples.

For plant tissues, metals were tested using ICP-OES [EPA Method 6010B], nitrates were tested using cadmium reduction [Standard Methods 4500-NO3-F], total nitrogen was tested by combustion [Dumas Method], potassium and sulfate by acid digestion and ICP-OES [EPA 3050B / EPA 6010B], and chloride by K2SO4 extraction, then colourimetric [Standard Methods 4500-Cl] G Mercuric Thiocyanate Flow Injection Analysis.

2.3.3 Biomass Analyses

During tissue sampling, biomass samples were of taken for the above ground biomass. For each planted treatment island, all remaining above ground biomass was clipped at approximately 2.5 cm. Samples were weighed initially to get a wet weight and then placed in paper bags to dry. Samples were dried in a greenhouse for 15 days before the dry weight was taken. No samples were taken of below island biomass.

2.3.4 Water Use Monitoring

Water was topped up in the tanks throughout the experiment on every Thursday (except December 26 and January 2) using filtered tap water (Carbon Bed Filter). The amount of water added was recorded for each tank to provide information on evapotranspiration rates of each species and the evaporation from the control tanks. Evapotranspiration rates were calculated by summing the volume of water lost in a time period (roughly 1 month) and dividing by the area of a floating island (0.325 m^2). This number was then divided by the total number of days in each time period to standardize the rate as litres lost per meter squared per day.

2.3.5 Physical Water Quality Monitoring

In addition to assessing the water for nutrients, temperature, dissolved oxygen, electrical conductivity, total dissolved solids, and pH were measured. These parameters were measured for all tanks every Monday & Friday, and weekly for feedlot sites from November 18, 2019 to October 30, 2020. Water quality monitoring was completed using a YSI Pro Plus until September 28, 2020 and a YSI ProDSS for the remainder of the experiment due to equipment failure.

2.3.6 Greenhouse Environmental Monitoring

Environmental monitoring of greenhouse conditions was completed every Monday & Friday from November 18, 2019 to October 30, 2020. Parameters that were recorded in the greenhouse included: temperature, relative humidity, and barometric pressure. The temperature within the greenhouse ranged from 10°C and 50°C with a daily average of 18.7°C throughout the experiment and the relative humidity ranged from 16% to 85% with a daily average of 57%. The high maximum temperature in the greenhouse could be attributed to issues with the

air conditioner's compressor which had to be replaced during the experiment. The north end of the greenhouse tended to heat up more quickly due to its shared glass wall with another greenhouse unit.

2.3.7 Plant Growth Monitoring

Growth metrics for plants on floating islands were assessed every Monday & Friday from November 18, 2019 until the end of the experiment (October 20, 2020). Growth metrics measured included the plant height, change in plant height, number of new tillers, and total number of tillers. Tiller heights were assessed based on the tallest tiller from each Island.

2.4 Data Analysis

One-way Analysis of Variance (ANOVA) was used to test the data taken at one discrete time point in this experiment, which included four main questions:

1. Are the means of the physical water quality parameters the same between species?
2. Are the means of the nutrients in vegetation the same between species?
3. Are the means of the nutrients in roots the same between species?
4. Are the means of the nutrients in peat the same between species?

Two-way ANOVA was used to test the data taken over multiple time intervals in this experiment, which included two main questions:

1. Are the means of nutrients in water the same between species and over time, and is there an interaction effect between these two factors?
2. Is the mean water use of a tank different between species and over time, and is there an interaction effect between thee two factors?

The parameters of interest that were tested differed depending on the specific analysis and are summarized in Table 2. Models were checked for outliers, normality of the residuals, and homoscedasticity using data visualization techniques (boxplots, residuals vs fitted values plots, and normal qq plots). For certain parameters, a square root transformation was applied to meet the assumptions for the ANOVA test. If the effect of an independent variable was found to be statistically significant in the ANOVA, a post-hoc test was performed by calculating the Tukey adjusted comparisons of each factor. Statistical significance was defined as $p < 0.05$ for all tests. Statistical analysis was performed using R software version 4.0.2 (R Core Team 2017).

Table 2: Parameters of interest for which statistical analyses were conducted for each analysis type.

Analysis	Parameters of Interest
Physical water quality measures	Temperature (°C), Dissolved Oxygen (%), Dissolved Oxygen (mg/L), Electrical Conductivity (uS/cm at 25°C), Total Dissolved Solids (mg/L), & pH
Nutrients in vegetation/roots	Phosphorus (ug/g), Nitrogen (%),Potassium (ug/g), & Sulfur (ug/g)
Nutrients in peat	Phosphorus (ppm), Nitrate (ppm) Potassium (ppm), & Sulfate (ppm)
Nutrients in water	Phosphorus (µg/mL) Potassium (µg/mL), & Sulfate (ug/mL)
Water Use	L/m^2 per day

3.0 Results

3.1 Heavy Metals

The heavy metals tested in our experiment were all present in relatively low concentrations (in most cases below the detection limit), hence did not provide a meaningful analysis. Detailed results were thus not provided for these parameters.

3.2 Baseline Water Quality Values

Nutrient concentrations within the water from OC feedlot and the experimental tanks was compared after the feedlot water was added to each of the species' tanks, to ensure the nutrient concentrations had similar baseline water quality conditions. The mean concentrations for five nutrients of concern are outlined in Table 3. Nitrate concentrations were below detection limits at the start of the experiment. . All parameters, with the exception of sulfur, were detected at lower concentrations in the experimental tanks than in the feedlot water on July 14, 2020. This likely means that some uptake and assimilation of nutrients into plants had already occurred before the tanks began to be sampled.

Table 3: Initial mean values (± standard error of mean) of select parameters in OC feedlot wastewater. Samples were taken July 14, 2020, the day after the wastewater was added to experimental tanks.

Parameter	Concentration at OC Feedlot (n = 3; July 14, 2020)	Concentration in Tanks (n = 24; July 14, 2020)
Phosphorus ug/mL	9.00 ± 0.12	6.88 ± 0.20
Potassium ug/mL	321.63 ± 1.91	269.98 ± 3.84
Sulfur (as SO4) ug/mL	41.90 ± 0.49	44.70 ± 0.79
Chloride ug/mL	412.07 ± 14.54	361.24 ± 5.76
Nitrate-N ug/mL	Below Detection Limit	Below Detection Limit

3.3 Physical Water Quality Monitoring

Physical water quality parameters were recorded for each experimental tank throughout the experiment and are summarized in Table 4. Physical water quality parameters were tracked to identify if there were any inconsistencies between treatments and provide information about the environmental conditions the experiment was carried out in. Electrical conductivity was reported as specific conductance which was corrected to 25°C. For temperature and dissolved oxygen (% and mg/L) the mean values did not differ significantly between species treatment tanks, p-values were 0.059, 0.457, and 0.402, respectively. For electrical conductivity, total dissolved solids, and pH, the mean values differed significantly depending on the treatment ($p < 0.001$). For electrical conductivity and total dissolved solids, the plant species tanks had significantly lower mean values from the control. For pH, water sedge (7.55 ± 0.02), cattail (7.56 ± 0.07), and wheat sedge (7.56 ± 0.09), had a lower mean pH values than the control tank (7.66 ± 0.01).

Table 4: Summary of water quality parameters measured in experimental tanks throughout the experiment (n=24).

Water Quality Parameter	Minimum	Maximum	Mean (± standard error of mean)
Temperature (°C)	13.8	26.8	17.6 ± 0.1
Dissolved Oxygen (%)	4.0	101.4[2]	45.6 ± 1.0
Dissolved Oxygen (mg/L)	0.6	9.8	4.2 ± 0.1
Electrical Conductivity (uS/cm at 25°C)	359.1	2712.0	1876.3 ± 30.3[1]
Total Dissolved Solids (mg/L)	134.5	1644.5	1215.5 ± 19.9[1]
pH	7.0	8.4	7.7 ± 0.0[1]

[1]Mean values were significantly different between the different species treatment tanks.
[2]Dissolved oxygen values greater than 100% represent supersaturated conditions due to aeration and the presence of photosynthetic aquatic oxygen producers.

3.4 Biomass

Biomass samples of each species were taken and weighed on November 2, 2020 (Table 5). Small-fruited bulrush had the highest mean dry weight (83.7 ± 17.9 g) while smartweed had the lowest (5.6 ± 2.9 g). Mean wet weights did not necessarily reflect the dry mean weight, with mare's-tail having one of the lowest dry weights (32.7 ± 8.3 g) but one of the highest wet weights (261.9 ± 75.1 g).

Table 5: Mean wet and dry weights for each species type at the end of the experiment (November 2, 2020).

Species	Mean Wet Weight (g) (± standard error of mean)	Mean Dry Weight (g) (± standard error of mean)
Small-fruited bulrush	315.0 ± 48.2	83.7 ± 10.3
Baltic rush	248.8 ± 19.8	68.0 ± 7.3
Wheat sedge	190.7 ± 17.3	57.2 ± 8.3
Water sedge	166.3 ± 29.2	46.9 ± 8.4
Cattail	127.6 ± 28.9	44.9 ± 8.4
Mare's-tail	261.9 ± 43.4	32.7 ± 4.8
Smartweed	12.1 ± 3.0	5.6 ± 1.7

3.5 Plant Growth

Plant height and number of tillers were recorded over the course of the approximately 16-week experiment (n = 32). Cattail had the highest mean plant height (129.8 ± 6.4 cm) and mare's-tail had one of the lowest (34.8 ± 0.7 cm; Table 6). Plant height for each species treatment started to stabilize around late August to early September (5 to 8 weeks into the experiment; Figure 3-1). Baltic rush had the highest mean number of tillers per plant (221.7 ± 17.0). Cattail (11.1 ± 1.4), smartweed (14.6 ± 1.6), and small-fruited bulrush (46.3 ± 5.1), had the fewest number of tillers, and a slow increase in number of tillers throughout the length of the experiment. Baltic rush, mare's-tail, wheat sedge, and water sedge, had steady increases in the number of tillers throughout the course of the experiment (Figure 3-2).

Table 6: Mean plant height and number of tillers at the end of the experiment (October 30, 2020).

Species	Mean Plant Height (cm) (± standard error of mean)	Mean Number of Tillers (± standard error of mean)
Small-fruited bulrush	83.3 ± 2.0	46.3 ± 5.1
Baltic rush	95.9 ± 2.6	221.7 ± 17.0
Wheat sedge	75.4 ± 2.1	117.0 ± 10.5
Water sedge	97.4 ± 3.5	93.8 ± 9.7
Cattail	129.8 ± 6.4	11.1 ± 1.4
Mare's-tail	34.8 ± 0.7	147.6 ± 12.4
Smartweed	47.2 ± 2.2	14.6 ± 1.6

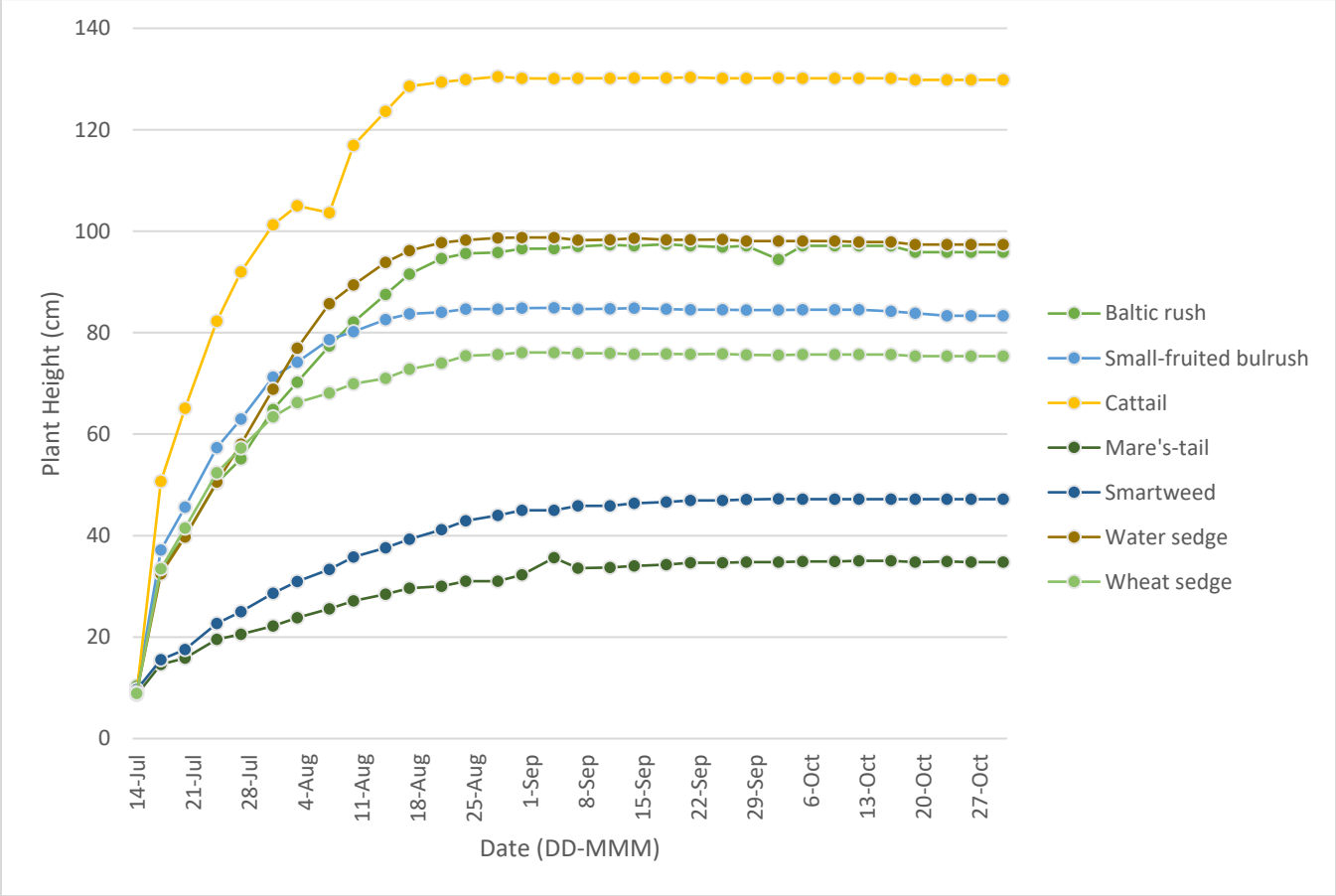

Figure 3-1: Mean plant height for each species treatment over the course of the experiment.

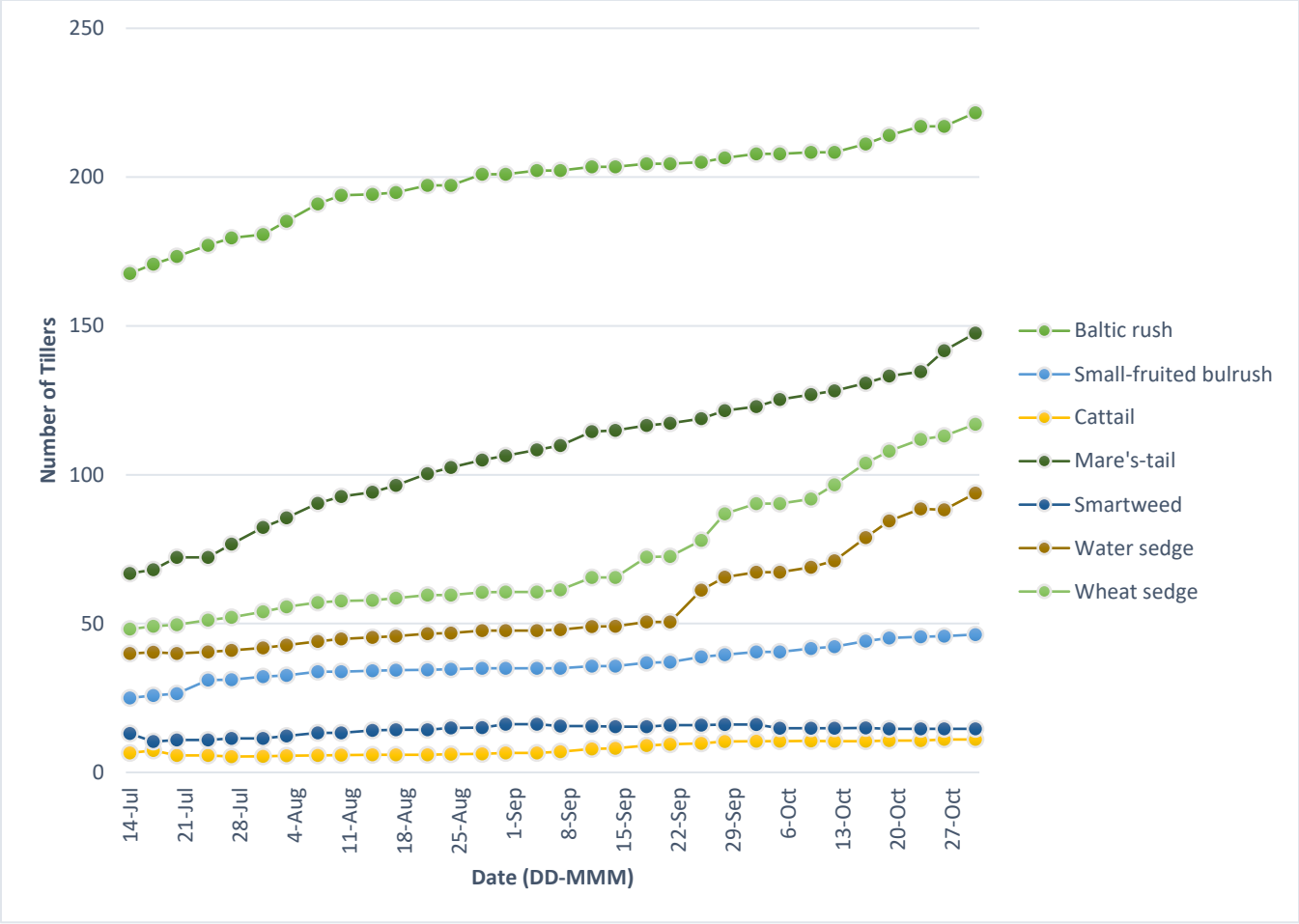

Figure 3-2: Mean number of tillers for each species treatment over the course of the experiment.

3.6 Phosphorus

Phosphorus concentration in the treatment tanks declined over the course of the experiment for each species (Figure 3-3). The control tanks spiked with feedlot effluent also saw a decrease in phosphorus.

The concentration of phosphorus in water was significantly affected by both plant species ($p < 0.001$) and date ($p < 0.001$). There was also a significant interaction between the two factors ($p = 0.013$), indicating that the uptake of phosphorus at each time point was affected differently by the species (or control treatment) of a tank. Because of this, the interpretation of the main effects should be done with caution and the plot of the results should be reviewed to determine how these factors interacted over time (Figure 3-3). The post hoc test on the plant species factor revealed there were significant differences in the uptake of phosphorus from the water both between species treatments and the control tanks. The mean concentration of phosphorus did not differ significantly between tanks containing mare's-tail (3.47 ± 2.70 µg/mL), water sedge (3.46 ± 2.56 µg/mL), wheat sedge (3.31 ± 2.58 µg/mL), Baltic rush (3.01 ± 2.00 µg/mL), cattail (3.16 ± 2.13 µg/mL), and small-fruited bulrush (2.90 ± 2.30 µg/mL), but was lower in these tanks overall than both the control tank (5.86 ± 0.76 µg/mL) and tanks containing smartweed (4.58 ± 1.76 µg/mL).

The mean concentration of phosphorus stored in above ground biomass (shoots) differed significantly depending on the plant species ($p < 0.001$; Figure 3-4). Wheat sedge (0.39 ± 0.12 µg/g) stored a higher concentration of phosphorus in its shoots compared to Baltic rush (0.22 ± 0.04 µg/g), mare's-tail (0.15 ± 0.01 µg/g), and cattail (0.10 ± 0.04 µg/g) but was not significantly differentiated from water sedge (0.27 ± 0.02 µg/g), smartweed (0.28 ± 0.07 µg/g), or small-fruited bulrush (0.25 ± 0.03 µg/g). Cattail, Baltic rush, mare's-tail, and small-fruited bulrush did not have significantly different levels of phosphorus from each other.

The mean concentration of phosphorus stored in plant below ground biomass (roots) differed significantly depending on the plant species ($p < 0.001$; Figure 3.3). Mare's-tail (0.68 ± 0.02 µg/g) stored a significantly higher concentration of phosphorus in its roots than all other species. Baltic rush (0.42 ± 0.09 µg/g) stored phosphorus at the next highest concentration in roots. Small-fruited bulrush (0.10 ± 0.01 µg/g) had a lower phosphorus concentration in the roots than any other species except wheat sedge. These differences in phosphorus concentrations in the roots and shoots do not correspond with differences in biomass between the species.

Phosphorus concentration in the peat soil substrate was not found to be significantly different ($p = 0.170$) among the different plant species (Figure 3-6).

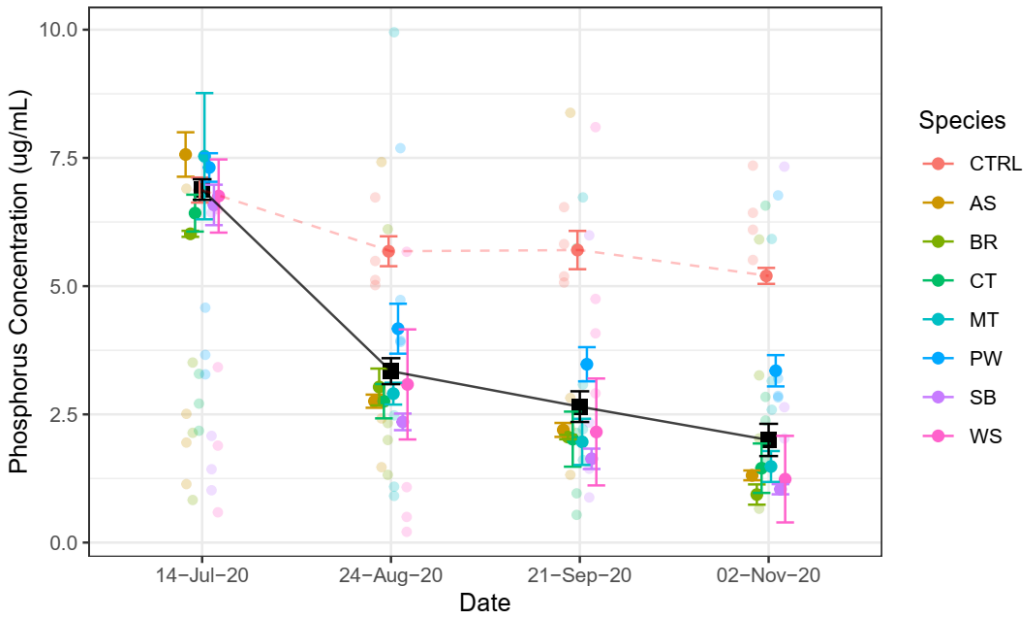

Figure 3-3. Changes in phosphorus concentration in the residual water for each species treatment and the control tanks. Black points and line represent the between group mean (across time points). Red points and dashed line correspond to the Control group measurements. Faded dots indicate individual sample values. Error bars represent the standard error of the mean. All groups have a sample size of n = 3 replicates. Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

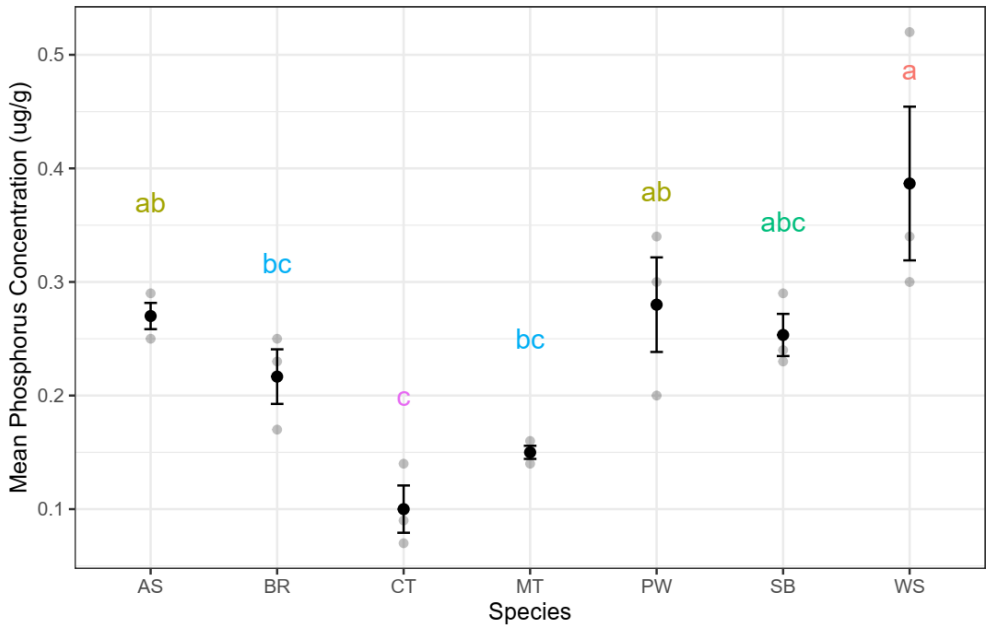

Figure 3-4. Mean phosphorus concentration in shoots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

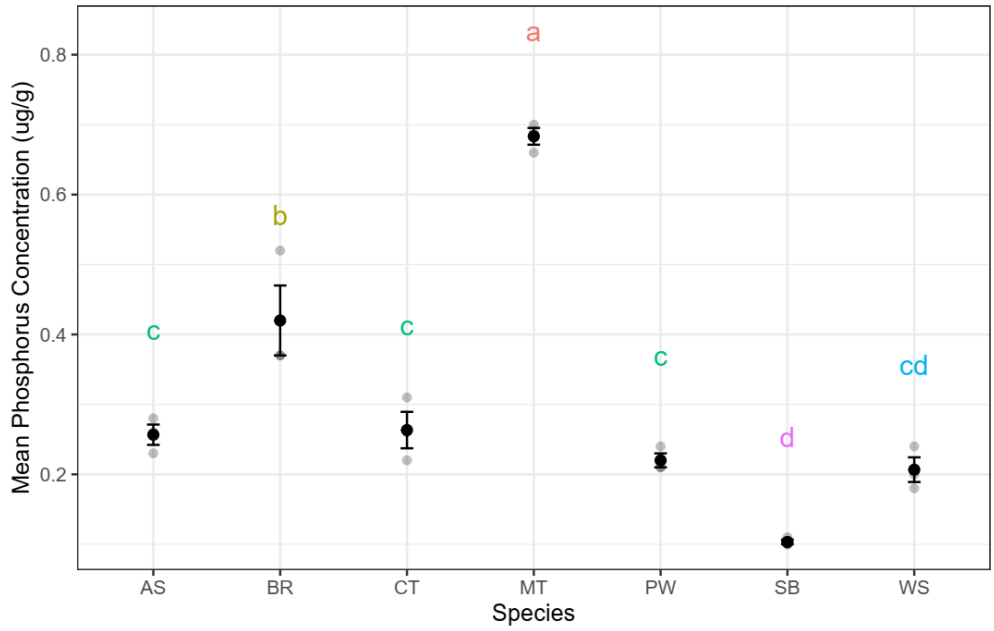

Figure 3-5. Mean phosphorus concentration in roots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

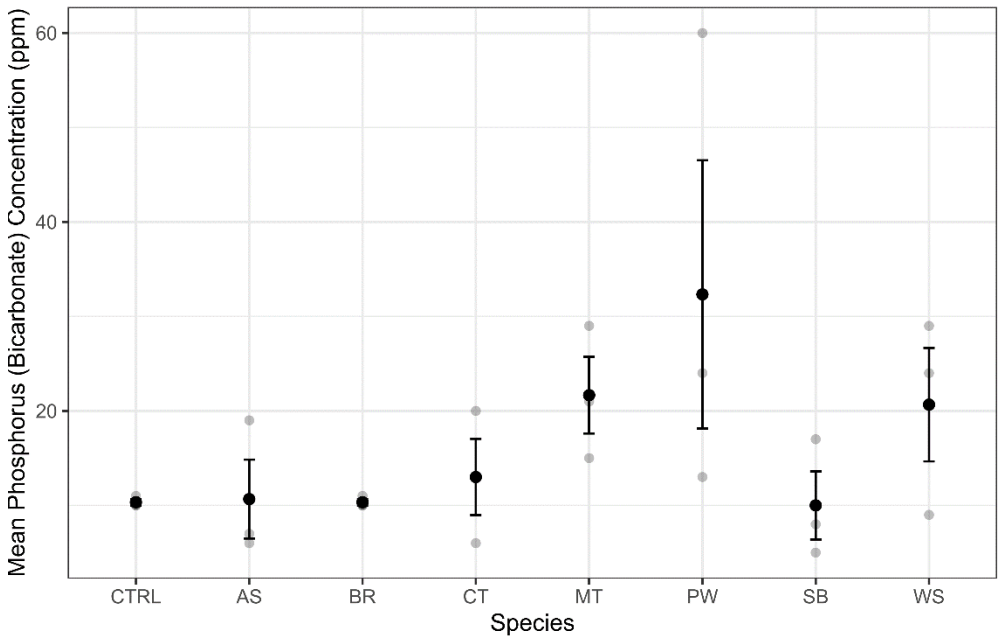

Figure 3-6. Mean phosphorus concentration in the peat soil substrate for each plant species and control tank at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Species was not found to be a significant factor affecting the amount of phosphorus in the soil (p = 0.17). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

3.7 Nitrogen/Nitrate

Water samples were analyzed for nitrate concentration, and this was the only form of nitrogen analyzed in the water samples. Almost all water samples analyzed were below the detection limit of 1.00 µg/mL.

The mean content (%) of nitrate stored in the shoots differed significantly depending on the plant species ($p < 0.001$; Figure 3-7). Baltic rush (1.73 ± 0.28%) had a higher content of nitrate in its shoots but was not significant differentiated from water sedge (1.48 ± 0.12%), mare's-tail (1.48 ± 0.06%), and wheat sedge (1.38 ± 0.20%). Cattail (0.60 ± 0.10%) and smartweed (0.68 ± 0.06%) had significantly lower nitrate contents in the shoots compared to all other species.

The mean content of nitrate stored in roots differed significantly depending on the plant species ($p < 0.001$; Figure 3-8). Mare's-tail (2.47 ± 0.30%) stored a significantly higher content of nitrate in its roots than all other species. Smartweed (1.80 ± 0.06%) stored nitrate at the next highest content in roots. Water sedge (0.88 ± 0.16%), Baltic rush (0.79 ± 0.12%), cattail (1.03 ± 0.05%), and wheat sedge (1.02 ± 0.09%) had even lower nitrate contents stored in the roots. Small-fruited bulrush (0.58 ± 0.06%) had a lower nitrate content in the roots than any other species but could not be statistically differentiated from water sedge and Baltic rush. These differences in nitrate contents in the roots and shoots do not account for the differences in biomass between the species.

Nitrate concentration in the peat soil substrate did not differ significantly according to plant species ($p = 0.139$; Figure 3-9).

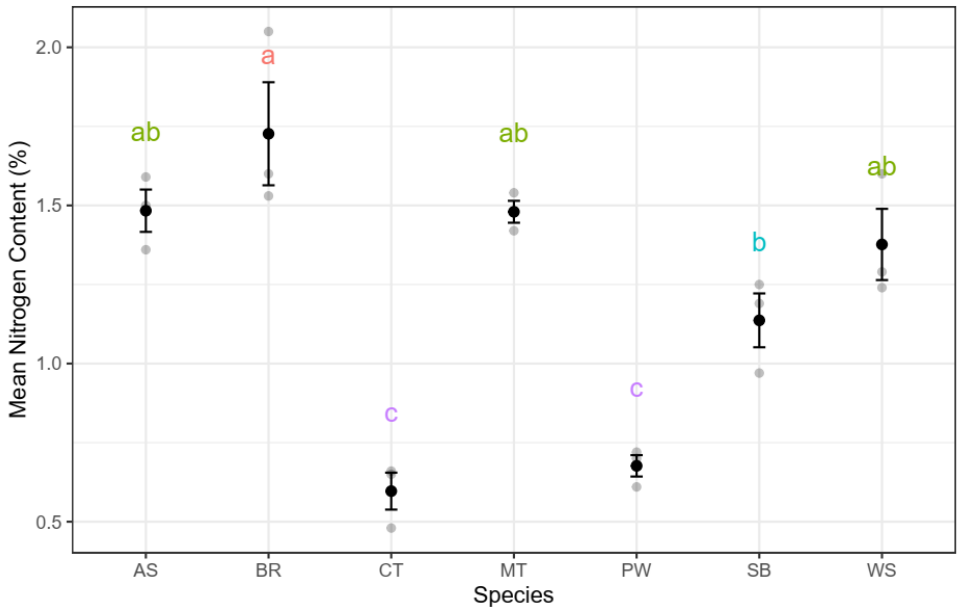

Figure 3-7. Mean nitrate content (%) in shoots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

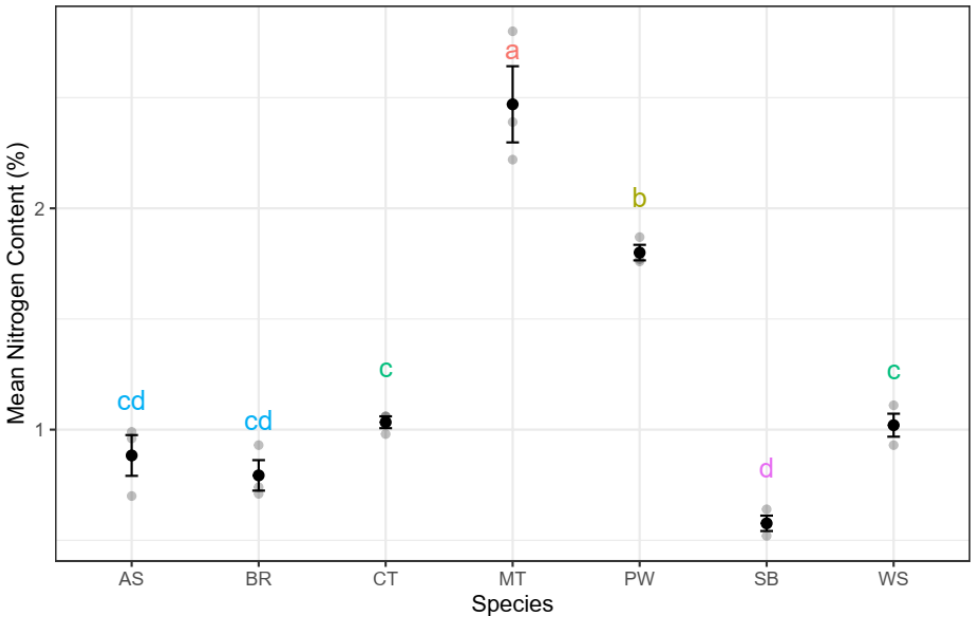

Figure 3-8. Mean nitrate content (%) in roots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

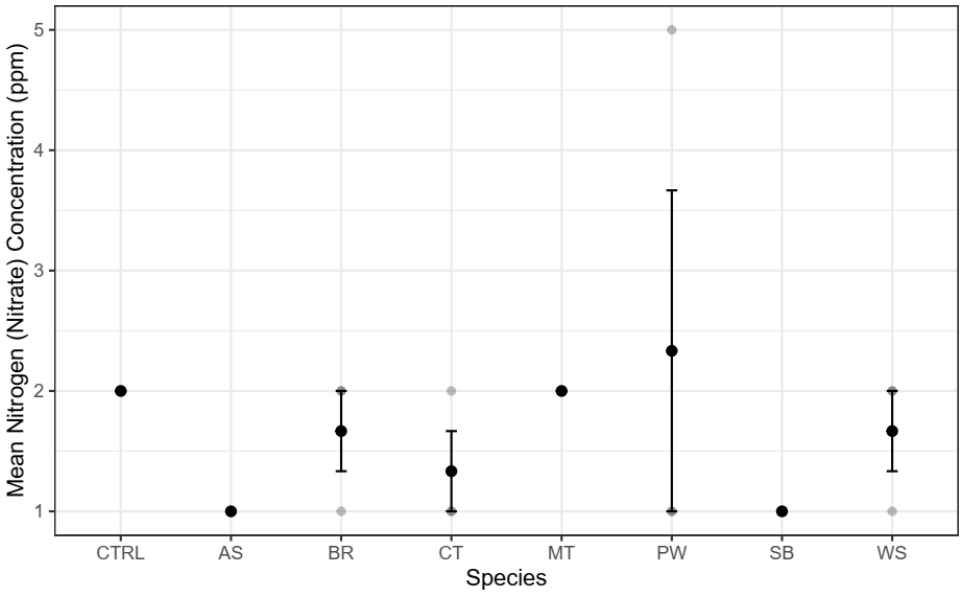

Figure 3-9. Mean nitrate concentration in the peat soil substrate for each plant species and control tank at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Data were not normally distributed and were analyzed using a Kruskal Wallis Test. Species was not found to be a significant factor affecting the amount of nitrate in the soil (p = 0.139). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

3.8 Potassium

Potassium concentration in the treatment tanks declined over the course of the experiment for each species (Figure 3-10). The control tanks spiked with feedlot effluent also saw a decrease in potassium. The concentration of potassium in water was significantly affected by the two factors, plant species ($p < 0.001$) and date ($p < 0.001$). The interaction between the two factors was not significant ($p = 0.641$). The post hoc test on the plant species factor revealed there were significant differences in the uptake of potassium from the water both among species treatments and the control tanks. The mean concentration of phosphorus did not differ significantly between tanks containing cattail (188.05 ± 50.09 µg/mL), mare's-tail (198.52 ± 36.48 µg/mL), small-fruited bulrush (202.04 ± 46.00 µg/mL), and Baltic rush (207.17 ± 36.08 µg/mL), but was lower in these tanks overall than both the control tank (251.14 ± 24.96 µg/mL) and tanks containing smartweed (232.87 ± 36.13 µg/mL).

The mean concentration of potassium stored in the shoots differed significantly depending on the plant species ($p < 0.001$; Figure 3-11). Cattail (5.42 ± 1.16 µg/g) stored a higher concentration of potassium in the shoots but did not differ from mare's-tail (4.73 ± 0.50 µg/g), or small-fruited bulrush (4.10 ± 0.33 µg/g). Baltic rush (2.89 ± 0.21 µg/g) and wheat sedge (2.85 ± 0.42 µg/g) had a lower concentrations of potassium in the shoots but were not significantly differentiated from water sedge (3.80 ± 0.54 µg/g), smartweed (3.61 ± 0.37 µg/g), or small-fruited bulrush.

The mean concentration of potassium stored in the roots differed significantly depending on the plant species ($p < 0.001$; Figure 3-12). Cattail (4.94 ± 0.67 µg/g) stored a significantly higher concentration of potassium in the roots than all other species. Mare's-tail (2.98 ± 0.20 µg/g) stored potassium at the next highest concentration in

the roots. Water sedge (1.44 ± 0.25 µg/g), Baltic rush (1.54 ± 0.23 µg/g), smartweed (1.40 ± 0.05 µg/g), small-fruited bulrush (12 ± 3.00 µg/g), and wheat sedge (1.75 ± 0.17 µg/g) had a lower phosphorus concentration in the roots than the other species. These differences in potassium concentrations in the roots and shoots do not correspond with differences in biomass between the species.

Potassium concentration in the peat soil substrate was found to be significantly different depending on the plant species growing in the soil (p = 0.002; Figure 3-13). Potassium concentration was significantly higher in the soil of the control tanks compared to the wheat sedge (637.33 ± 128.22 ppm), water sedge (561.67 ± 107.58 ppm), Baltic rush (539.33 ± 103.40 ppm), and small-fruited bulrush (488.00 ± 57.42 ppm). These species did not have significantly lower soil potassium concentrations compared to mare's-tail (761.67 ± 154.42 ppm), smartweed (756.00 ± 171.04 ppm), or cattail (691.67 ± 168.63 ppm).

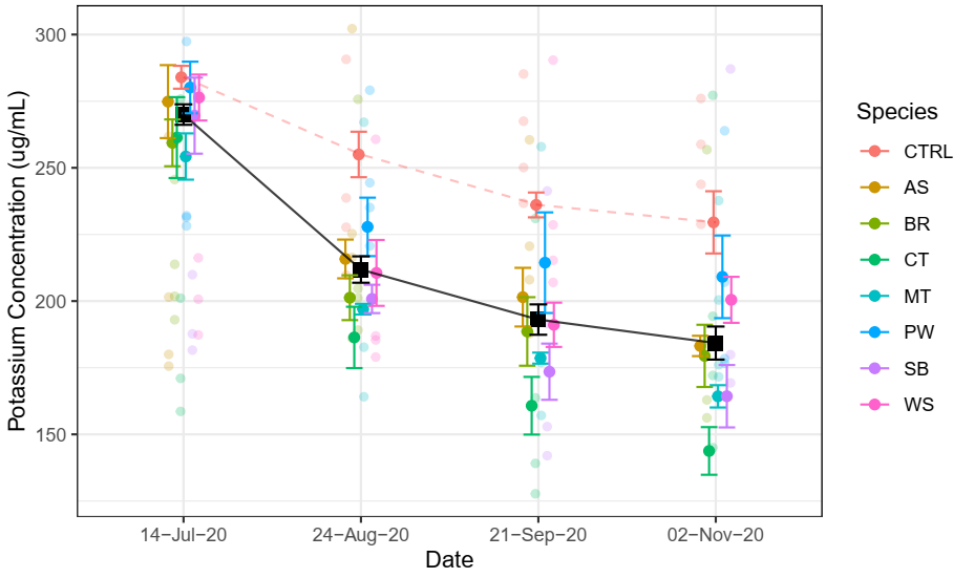

Figure 3-10. Changes in potassium concentration in the residual water for each species treatment and the control tanks. Black points and line represent the between group mean (across time points). Red points and dashed line correspond to the Control group measurements. Faded dots indicate individual sample values. Error bars represent the standard error of the mean. All groups have a sample size of n = 3 replicates. Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

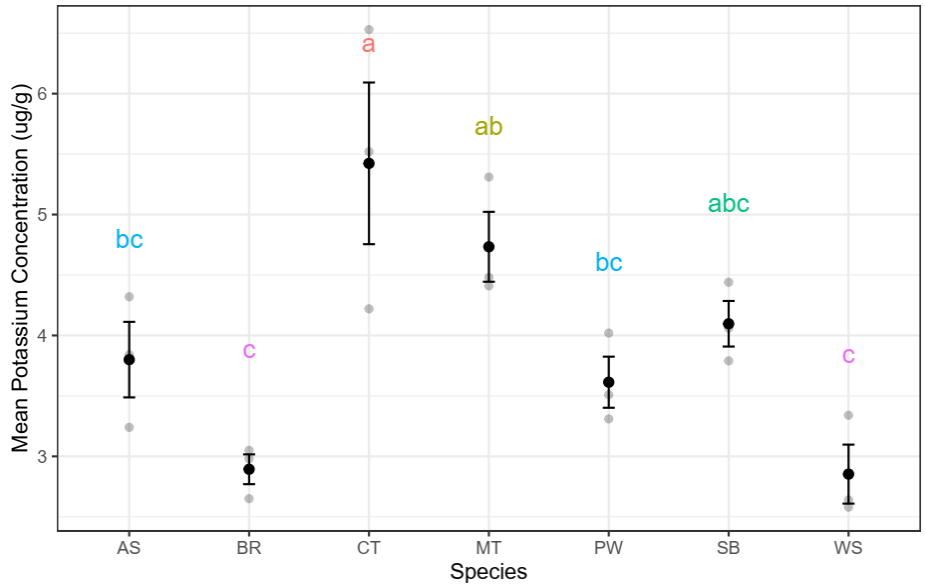

Figure 3-11. Mean potassium concentration in shoots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

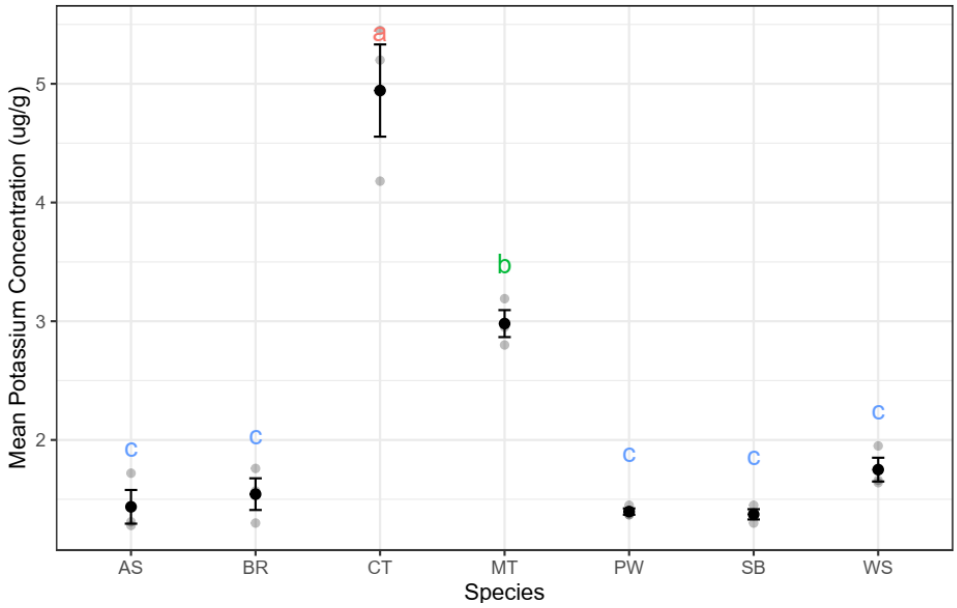

Figure 3-12. Mean potassium concentration in roots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter a are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

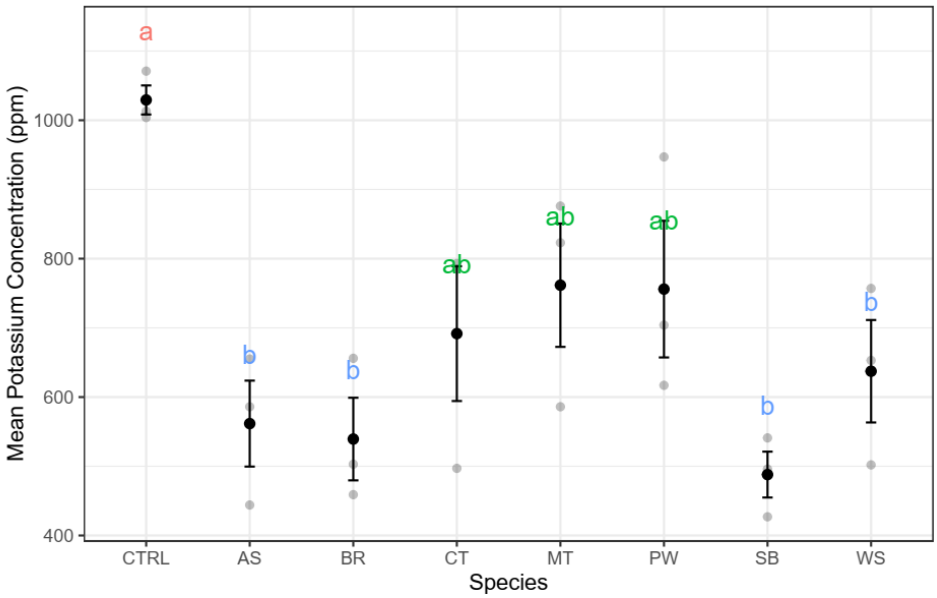

Figure 3-13. Mean potassium concentration in the peat soil substrate for each plant species and control tank at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

3.9 Sulfate

Sulfate concentrations increased over the course of the experiment for most plant species (Figure 3-14). The control tanks spiked with feedlot effluent also saw an increase in sulfate concentrations. The concentration of sulfate in the water was significantly affected by the two factors, plant species ($p < 0.001$) and date ($p < 0.001$). The interaction between the two factors was also significant ($p = 0.002$). The post hoc test on the plant species factor revealed there were significant differences in the uptake of sulfur from the water both between species treatments and the control tanks. The mean concentration of sulfur did not differ significantly between tanks containing mare's-tail (43.63 ± 1.46 µg/mL), small-fruited bulrush (41.71 ± 1.50 µg/mL), and the control tank (45.42 ± 3.84 µg/mL), but was lower in these tanks overall than the tanks containing water sedge (56.84 ± 3.54 µg/mL), Baltic rush (64.73 ±4.98 µg/mL) and cattail (54.19 ± 3.00 µg/mL).

The mean concentration of sulfur stored in the shoots differed significantly depending on the plant species ($p < 0.001$; Figure 3-15). Mare's-tail (2.16 ± 0.15 µg/g) stored the highest concentration of sulfur in the shoots, followed by small-fruited bulrush (1.03 ± 0.06 µg/g). Sulfate concentrations among other plant species did not differ significantly.

The mean concentration of sulfur stored in the roots differed significantly depending on the plant species ($p = 0.008$; Figure 3-16). Mare's-tail (0.31 ± 0.02 µg/g) and smartweed (0.27 ± 0.01 µg/g), stored the highest concentration of sulfur in the roots, and were significantly higher compared to small-fruited bulrush (0.16 ± 0.01 µg/g), and water sedge (0.14 ± 0.01 µg/g). These differences in sulfur concentrations in the roots and shoots did not correspond with the differences in biomass between the species.

Sulfate concentration in the peat soil substrate was found to be significantly different depending on the plant species growing in the soil ($p = 0.043$; Figure 3-17). Sulfate concentration was significantly higher in the soil of Mare's-tail (94.33 ± 30.94 ppm) compared to the control tank (16.67 ± 1.76 ppm), but could not be statistically differentiated from any of the other plant species treatments.

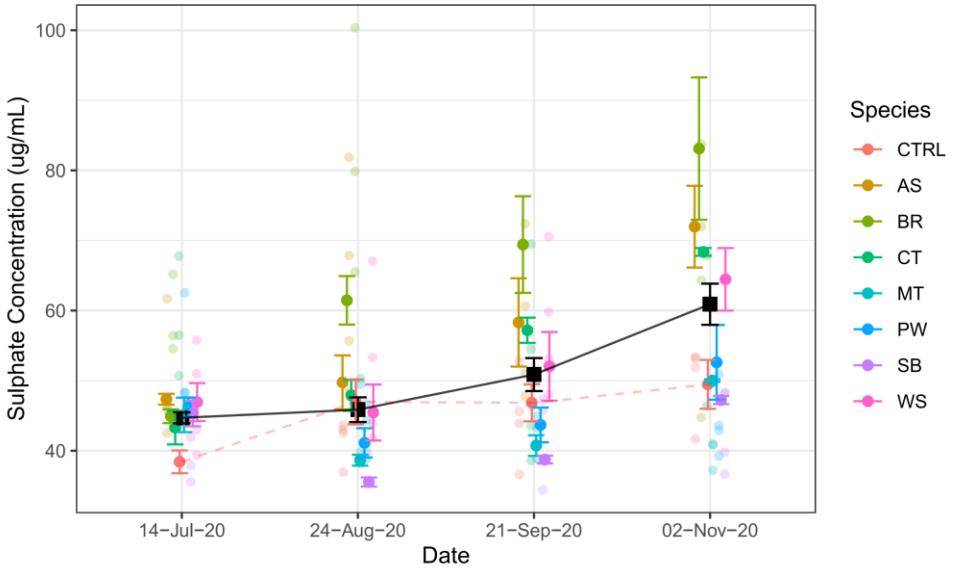

Figure 3-14. Changes in sulfate concentration in the residual water for each species treatment and the control tanks. Black points and line represent the between group mean (across time points). Red points and dashed line correspond to the Control group measurements. Faded dots indicate individual sample values. Error bars represent the standard error of the mean. All groups have a sample size of n = 3 replicates. Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

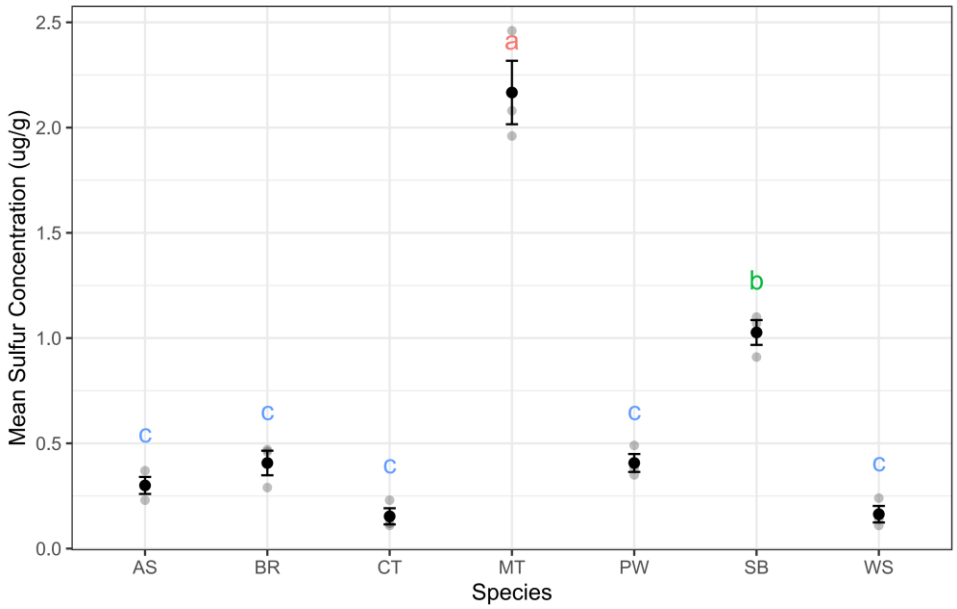

Figure 3-15. Mean sulfur concentration in shoots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

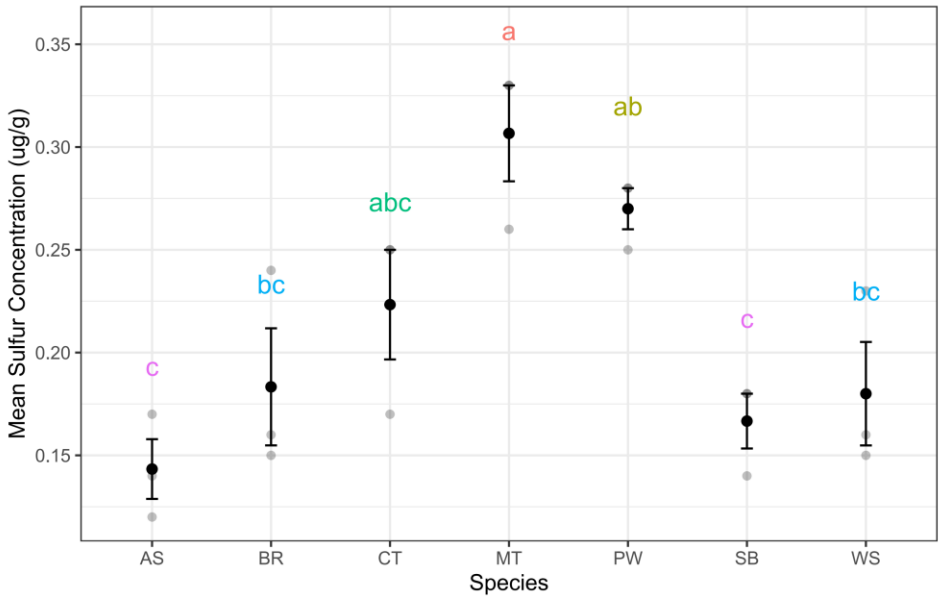

Figure 3-16. Mean sulfur concentration in roots for each plant species at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

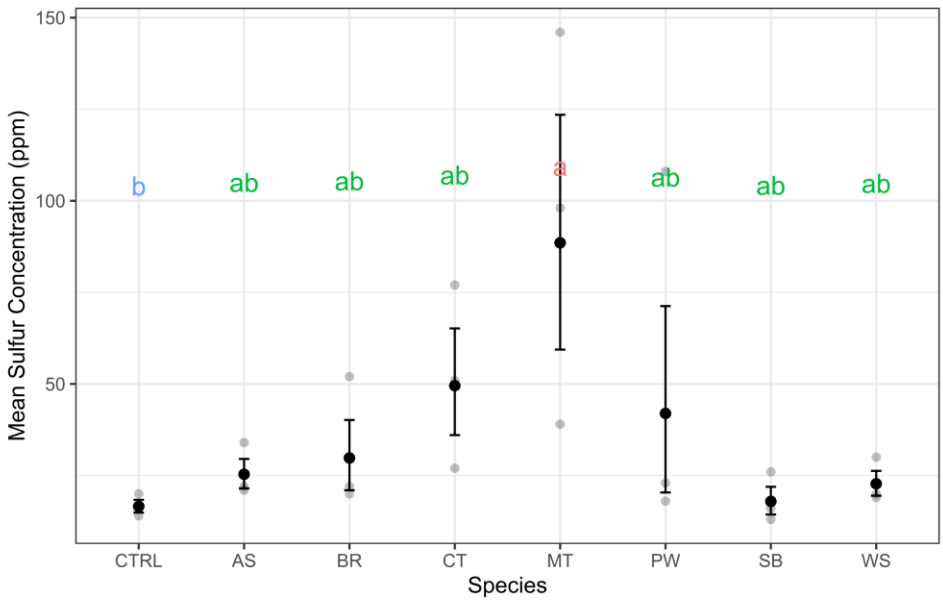

Figure 3-17: Mean sulfur concentration in the peat soil substrate for each plant species and control tank at the conclusion of the trial. Confidence intervals are based on the standard error of the mean. All groups have a sample size of n = 3 replicates. Means sharing a grouping letter are not significantly different (Tukey-adjusted comparisons). Species: SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

3.10 Water Use

Water use in each tank was significantly affected by both the species ($p < 0.001$) and month ($p < 0.001$). There was also a significant interaction between the species and month ($p = 0.003$), meaning that the water use between species differed depending on the month (Figure 3-18). The post hoc test on the plant species factor revealed there were significant differences in water use both among species treatments and the control tanks. The tanks of Baltic rush (8.92 ± 1.32 L/m^2), wheat sedge (8.43 ± 1.41 L/m^2), cattail (8.34 ± 2.27 L/m^2), small-fruited bulrush (8.23 ± 1.39 L/m^2), and water sedge (7.82 ± 1.20 L/m^2) species had a greater water use than mare's-tail (6.02 ± 1.25 L/m^2), smartweed (5.91 ± 1.27 L/m^2) and the control (5.36 ± 1.43 L/m^2).

The mean water use was higher in August (9.10 ± 2.02 L/m^2) than any other month, while July (7.28 ± 1.14 L/m^2) was higher than October (6.43 ± 1.62 L/m^2). Mean water use in September (6.71 ± 1.68 L/m^2) was not significantly different from in July or October. With respect to how the water use of various species changed in each month, it appears that water use was relatively similar in July, then from August onwards the water use of the control, mare's-tail, and smartweed tanks was lower than the other species.

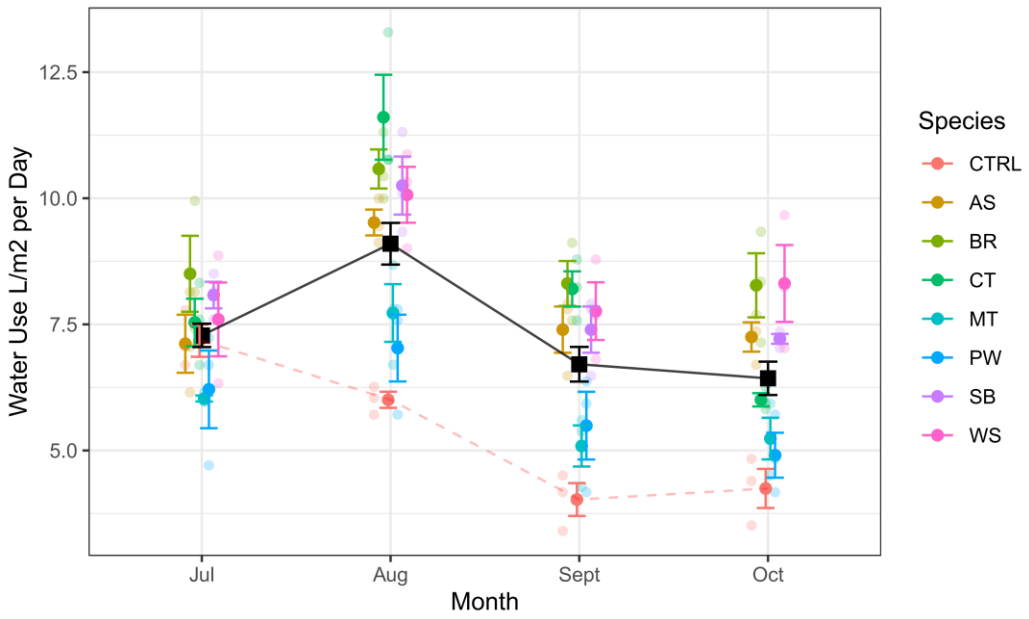

Figure 3-18. Changes in water use for each species treatment and the control tanks. Black points and line represent the between group mean (across time points). Red points and dashed line correspond to the Control group measurements. Faded dots indicate individual sample values. Error bars represent the standard error of the mean. All groups have a sample size of n = 3 replicates. SB = Small-fruited bulrush, BR = Baltic rush, WS = Wheat sedge, AS = Water sedge, CT = Cattail, MT = Mare's-tail, PW = Smartweed.

4.0 Discussion

This experiment used a baseline nutrient treatment that had lower levels of nutrients than previously reported for feedlot runoff in similar regions. Miller et al. (2004) and Rahman et al. (2013) investigated levels of select nutrients in cattle feedlot runoff water in southern Alberta and North Dakota respectively, finding mean total phosphorus levels between 25.41 – 105.36 ug/mL, mean nitrate between 0.52 – 3.4 ug/mL, mean ammonia/ammonium levels between 13.54 – 33.0 ug/mL, mean potassium levels between 465 - 515 ug/mL, mean chloride levels of 604 ug/mL, and mean sulfate levels of 217 ug/mL. Even the minimum range of these nutrients is higher than the feedlot baseline levels used in this study. The low level of nutrients in this study is most likely due to the small size of the feedlot used for the source water (approximately 28 cattle). Most notably, in the current study, nitrate concentrations were below detection limits. Previous studies have also tended to report nitrogen levels in the form of ammonia/ammonium or Total Kjeldahl Nitrogen (TKN) which were not included in our results.

Garcia Chance et al. (2019) found that for certain species such as Pickerelweed (*Pontederia cordata*), nutrient removal rates increased with higher initial concentrations of nutrients (total phosphorus and total nitrogen). With the low starting concentration of certain nutrients studied in this experiment, this may be a reason why our plants had lower removal rates for certain parameters than other simliar studies. Because nutrients were only added at the beginning of our experiment, this could also be why we see a reduction in the rate of removal as time went on. Additionally, the general trend of nutrient reduction slowing down into August and September could be due to plants nearing the end of their growth cycle and entering senescence (Garcia Chance et al. 2019).

4.1 Water Quality Monitoring

Tracking physical water quality parameters is important for understanding the environmental conditions that could affect plant growth, and provides additional context for potential real-world applications (e.g., quality of effluent). For this experiment, it was expected that temperature and dissolved oxygen would not differ significantly between treatment tanks, as the tanks were all stored in the same location in the greenhouse, and every tank was aerated. The reduction in electrical conductivity and total dissolved solids in all species tanks is consistent with the observations of Farid et al. (2014), in their study of the phyto-remediation potential of wetland plants in municipal wastewater. A 33.7 % reduction in electrical conductivity was reported by Farid et al. (2014) in their wetland systems, and a similar result was observed for total dissolved solids. The pH values recorded through out our experiment ranged from 7.25 to 7.90, with is within the range of natural variation seen in Alberta wetlands, but more typical of marshes or rich fens (Nicholson 1995; Roy et al. 2019).

4.2 Plant Growth

Increased nutrient uptake with plant growth in aquatic macrophytes is a consistent observation in phyto-remediation studies (i.e., Edwards et al. 2006; White and Cousins 2013; Garcia Chance et al. 2019). Compared with Trial 1, which had greater nutrient loading for most parameters including nitrate, phosphorus, potassium, and sulfate, plant growth was greatly reduced. Nutrient deficiencies may have limited growth, especially since nitrate levels were below detection limits in the OC Feedlot water. Garcia Chance et al. (2019), tested two different nutrient treatments (high and low) and observed greater vegetative growth associated with greater nutrient loading, and consequently greater nutrient uptake from the treatment water. For the three species that were observed in Trial 1 (cattails, water sedge, and wheat sedge), plant height and number of tillers was nearly double that observed in this experiment. We hypothesize that nutrient availability limited plant growth and the timing of Trial 2 later in the growing season may have also impacted plant growth. Plant plugs were established in November of the previous year for both experiments, but Trial 1 was initiated in February 2019 and ran until August 2019. Delays due to COVID-19 restrictions in 2020, pushed the initiation of Trial 2 to July 2020, and the experiment ran until the end of October 2020, past the end of the growing season in Alberta. Garcia Chance et al. (2019) observed two plant species, an evergreen *Juncus* species and a deciduous *Pontederia* species in flowing treatment wetlands. They found that biomass accumulation and nutrient uptake decreased with senescence at the end of the growing season, but this trend was less pronounced in the deciduous species. These results emphasize the importance of understanding the nutrient composition of the source water to optimize nutrient removals in floating island systems, and the influence of seasonality on nutrient removals.

4.3 Phosphorus

Over the course of the experiment, all plant treatments effectively removed phosphorus from the feedlot spiked water compared to the control tanks. In our experiment, the rate of phosphorus removal ranged from 77% to 84%, which is higher than the average of 49% reported by Pavlineri et al. (2017), who performed a meta-analysis of fifteen similar floating islands studies. Nutrient removal rates based on percent reductions should proceed with caution however, as these values correspond with loading rates (Keizer-Vlek et al. 2014). Algae did not appear to interfere with observed phosphorus removal to the same extent as documented in Trial 1 in 2019.

When looking at phosphorus uptake in the plant tissues, phosphorus storage trends differed between the above ground and below island biomass. Wheat sedge, water sedge, smartweed, or small-fruited bulrush had the greatest concentrations of phosphorus stored in their shoots. For root tissue, mare's-tail had the greatest

concentration of phosphorus followed by Baltic rush. Understanding nutrient storage partitioning between above-mat and below-mat biomass is an important component to understanding how to optimize nutrient removal and to determine biomass harvesting (Garcia Chance et al. 2019). Chance et al. (2019) found small root-to-shoot ratios for nitrogen and phosphorus storage throughout their study, with greater nutrient accumulation accounted for by plant shoots.

When comparing plant species, the higher concentrations of phosphorus in plant tissue was not mirrored with higher removals of phosphorus in water. Characterizing nutrient removal on a per area basis is an important measure when comparing nutrient accumulation, and warrants further investigation. Zhu et al. (2011) and Keizer-Vlek et al. (2014) found that there was a positive, linear relationship between nitrogen and phosphorus accumulation and plant biomass. Accordingly, plant species with greater biomass accumulations will likely remove greater amounts of nutrients from source water.

4.4 Nitrogen/Nitrate

A key benefit of floating treatment wetlands is the removal of nitrate from the water. In a meta-analysis on floating wetlands experiments by Pavlineri et al. (2017), similar studies had an average nitrogen removal from water of 58%. In the current study, most water samples from the treatments had nitrates at levels below the detection limit of the laboratory. Consequently, nitrogen removal from the source water was not able to be characterized in this experiment. Forms of nitrogen often examined in feedlot effluent include ammonium, nitrate, and total Kjeldahl nitrogen (TKN) which includes organic nitrogen plus ammonia and ammonium nitrogen (Rahman et al. 2013; Wall 2013). Ammonium and TKN were found to be much higher in feedlot water than nitrate (Rahman et al. 2013), and are commonly examined nitrogen components used in similar floating island studies (Stewart et al. 2008; Zhang et al. 2014; Pavlineri et al. 2017). In the current study, it is possible that nitrogen in the feedlot effluent existed in these alternate nitrogen forms, which would account for the low nitrate concentrations that were observed.

With respect to aquatic vegetation, the species with the highest nitrogen content in the shoots were Baltic rush, water sedge, mare's-tail, and wheat sedge, with a mean content ranging from 1.38 – 1.73 %. In the roots, mare's-tail and smartweed had the highest nitrogen content, with a mean content ranging from 1.80 - 2.47 %. While nitrogen removals from the water could not be characterized, Garcia Chance et al. (2019) found that nitrogen removals and accumulation in plant tissue increased in species with greater biomass. In our experiment, small-fruited bulrush, Baltic rush, and wheat sedge had the greatest biomass by weight, and likely would have larger overall nitrogen removals, as similarly found with phosphorus.

We also examined oxygenation as it can affect nitrogen removal in aquatic systems (Stewart et al. 2008; Tao and Wang 2009; Cao and Zhang 2014). As denitrification is an anaerobic process, nitrogen removal can be facilitated in water with low dissolved oxygen levels (Stewart et al. 2008; Cao and Zhang 2014). Aeration can impede nitrogen removal through maintaining an oxidized environment under the floating islands (Stewart et al. 2008; Tao and Wang 2009). During the course of the experiment, dissolved oxygen levels were elevated in all the tanks due to aeration. Water can also become supersaturated (oxygen levels above 100%) because of the presence of photosynthetic aquatic oxygen producers (Prasad et al. 2014). Future work should take this into consideration.

4.5 Potassium

Over the course of the experiment, cattail, mare's-tail, small-fruited bulrush, Baltic rush, water sedge, and wheat sedge removed more potassium from the water relative to the control tank overall, with removal rates ranging

from 27% to 45%, and only smartweed not having a lower concentration of potassium in its water than the control tanks. Overall, the concentration of potassium in water declined throughout the experiment, with concentrations highest in July, second highest in August, and lowest in September and November. The concentration of potassium in water was relatively similar between the species treatment tanks and control in July, and began to be differentiated in August through November. Saidin et al. (2014) found that potassium reduction in wastewater samples increased by 77% for water hyacinth (*Eichhornia crassipies*), while conversely decreasing by 90% for caladium (*Colocasia esculenta*). This discrepancy is thought to be due to the degradation of plant material releasing potassium back into the water specifically for hyacinth. This may explain the removal differences seen between smartweed and the rest of the plant species, as smartweed degraded earlier than the other species and had completely dead tissue by October 30th, while the other species were at earlier stages of senescence.

When looking at potassium uptake in the plant tissues, potassium storage trends were similar between the above ground and below island biomass. Cattails and mare's-tail stored higher concentrations of potassium in their shoots compared to Baltic rush and wheat sedge, while small-fruited bulrush water sedge, and smartweed stored intermediate levels of potassium. Similarly, cattails and mare's-tail stored higher concentrations of potassium in their roots than any other species, with cattail storing the highest concentrations. The concentration of potassium stored in shoot versus root tissue was quite similar, with mean concentrations in shoots ranging from 2.85 – 5.42 µg/mL and mean concentrations in the roots ranging from 1.37 – 4.94 µg/mL. Higher concentrations of potassium in tissue did not correspond with removal of potassium from water.

4.6 Sulfate

In contrast to the concentration reductions seen in the water with the previously described nutrients, an increase in sulfate concentrations was seen for most species over the course of the experiment. This result is in contrast to sulfate reductions observed by Saidin et al. (2014) in wastewater phyto-remediation for three aquatic plant species. Although, the initial concentration of sulfate in the wastewater effluent used by Saidin et al. (2014) was nearly three times less than the OC Feedlot water. The decomposition of organic matter can be a source of sulfur/sulfate in the environment (Edwards 1998), and likely a contributing factor to the increases in sulfate observed in our experiment. Additionally, the filtered tap water that was used to top up all treatment tanks contained a mean sulfate concentration of 35.88 ± 2.26 µg/mL, which is only slightly less than mean concentration in the OC Feedlot water (41.90 ± 0.49 µg/mL). We hypothesize that the continual addition of sulfate in the top up water and potential decomposition of organic matter may have accounted for the increases of sulfate observed in the water.

When examining the sulfur uptake in the plant tissues, sulfur storage trends were similar between the above ground and below island biomass. Mare's-tail stored higher concentrations of sulfur in its tissue than most other species. Mare's-tail also had a significantly lower concertation of sulfate in the water than Baltic rush, water sedge, cattails, and wheat sedge, but was not significantly different than the control. Mare's-tail was the only species where the peat substrate in the tanks had a higher concentration of sulfur than the control tanks, which mirrors the results of the tissue analysis. Further investigation into the sulfur/sulfate dynamics are needed to understand the potential removal opportunities of floating island systems.

4.7 Water Use

In this experiment, both the plant species type and the month affected the amount of evapotranspiration of the tanks. Additionally, there was an interaction between the effect of month and species, indicating that water use differences between species was different, depending on the month. The tanks of Baltic rush, wheat sedge, cattail, small-fruited bulrush, and water sedge species all used more water than tanks with mare's-tail, smartweed, or the control. All the species with a higher water use also had higher amounts of shoot biomass than the species with the lower water use, indicating that there could be a correlation between these two factors (i.e. plants with more shoots use more water). This is supported by Taylor et al. (1983) who showed that the transpiration rate of a plant is proportional to their biomass.

In the previous experiment, Trial 1, cattails used significantly more water than any other species (almost double), a finding that was not replicated in this experiment. The reasons for this discrepancy are unknown, but could be due to an unidentified environmental factor (i.e., treatments may have been subject to warmer temperatures in lab trial 1) or a difference between the plants used with respect to age, size, or health. In this regard, cattails are particularly nutrient hungry. We speculate that the lower nutrient concentrations in this trial as compared to Trail 1, may have limited growth of the cattails, and thus could be responsible for the lower water use.

The mean water use also varied depending on the month, with tanks using more water in August than any other month. Water use in July appeared to be relatively similar between the different species and control tank, but from August onwards, there was more differentiation between the species, with mare's-tail, smartweed, and the control tanks using less water than the other species tanks. One hypothesis for the difference between months is the growing stage of the plant. August would be near the end of the growing season with plants likely reaching their maximum size (and therefore maximum water requirements) and by September plants would be starting senescence, potentially slowing down water/growth requirements.

Water use appears to be connected with the plant's potential to uptake nutrients from the water. In this experiment, smartweed (a low water-use species) appeared to be less effective in removing phosphorus and potassium from water than any other species (as suggested by higher mean concentrations of these nutrients in the tank water), indicating that its low water use could be related to removing less phosphorus and potassium from the water. Previous experiments have found that there is a positive relationship between nitrogen and phosphorus accumulation and plant biomass (Zhu et al. 2011; Keizer-Vlek et al. 2014). Because plant biomass is proportional to water use, this may also indicate a similar relationship between nutrient accumulation and water use.

Most of the plant species in this experiment removed water from their tanks at a higher rate than the controls, so if water removal from a system is desired, the addition of these wetland plants would beneficial. If conservation of water is desired, mare's-tail or smartweed would be a better option in terms of water use, but may give more limited results for nutrient removal at the same time.

5.0 Recommendations

Out of this experiment, we have a number of recommendations both for future research as well as for implementation of phyto-remediation using floating island systems.

5.1 Future Research

The experimental design allowed for effective assessment of the potential uptake of nutrients by plants. Recommendations and future research are highlighted below to improve experimental design, and explore related research questions:

- **Assess potential effects of overwintering in cold climate:** So far most of our knowledge is based on plant nutrient uptake during the growing months, but little is known about how nutrients might be stored or released from floating island biomass through a full seasonal cycle. This could help determine optimal timing of island deployment or retrieval, and whether biomass harvesting is required. In addition, determining species that can endure a harsh northern climate would provide important information for species selection for applications in Alberta, and other similar climates.
- **Test for ammonia/ammonium, TKN, nitrate, total nitrogen:** Because nitrogen can exist in an aquatic environment in many forms that are highly influenced by oxygen levels, multiple forms of nitrogen should be tested for during any future analysis.
- **Account for biomass in nutrient accumulation testing:** Accounting for plant biomass in nutrient accumulation testing will allow for a standard nutrient storing comparison between species and experiments, specifically representing nutrient accumulation in g/m^2 would allow for comparison among similar studies.
- **Increased or repeated nutrient loading:** This experiment had a lower initial nutrient loading than most local feedlot sources found in literature. A lower initial nutrient loading can result in a lower rate of plant uptake. In order to examine the maximum nutrient uptake potential of these species, a higher or repeated nutrient loading should be considered.
- **Real world application:** An experimental design involving the use of islands on existing in-situ nutrient high water sources would allow us to examine if lab results translate to real life situations, and explore if there are practical constraints to real world applications.
- **Baseline allocation of nutrients in plant tissue:** Baseline nutrient concentrations in shoot and root tissue should be analyzed to allow a comparison to values at the end of the experiment.

5.2 Recommendations for Implementation

There were a number of important results observed, and relevant related experimental results that have larger applications implications. However, careful assessment of each application is required to ensure the right recommendations are implemented.

1. **Nutrient removal and biomass:** Nutrient removal appears to be highly related to biomass production. Many species with larger above ground biomass production have increased nutrient storage in their shoots. Removal of biomass will be important to prevent the return of these back into the system. Potassium removal was facilitated by all the species observed in this experiment.
2. **Water Use:** Water use varied between the species over time, with higher water use observed in species with greater biomass production, and in turn greater nutrient removal.
3. **Species Selection:** Selecting species most appropriate for the environmental conditions will be of utmost importance to ensure robust growth for optimal nutrient removal.

6.0 Conclusion

Overall, nutrient removal from source water was found to be successful with the seven plants used in the experiment. Nutrient concentration in plant tissues was not overly indicative of overall nutrient removal from the water. When comparing with similar floating island studies, nutrient removal was associated with biomass production, with species with greater biomass production accounting for increased nutrient accumulations. This was a finding also supported by the Trial 1 results, where cattails removed large quantities of nutrients from the water and tended to store more nutrient in their shoots than the other species. In our Trial 2 experiment, cattails did not outperform the other species in the trial for nutrient removal from the water. We hypothesize that this difference could be due to the lower nutrient levels of the source water compared to the supersaturated nutrient formula used in Trial 1. Cattail has rapid growth in systems with increased nutrient inputs (Li et al. 2010; Bansal et al. 2019). None of species examined displayed any signs of nutrient deficiency. Small-fruited bulrush, Baltic rush, wheat sedge were the top three species with the most biomass accumulation, and anecdotally were found to be thriving. This highlights the importance of understanding nutrient loading and environmental conditions when selecting appropriate species for floating treatment island applications.

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Appendix A

Photo Log

Photo 1: Wheat Sedge (*Carex atherodes*) at the beginning of the experiment (July 13, 2020).

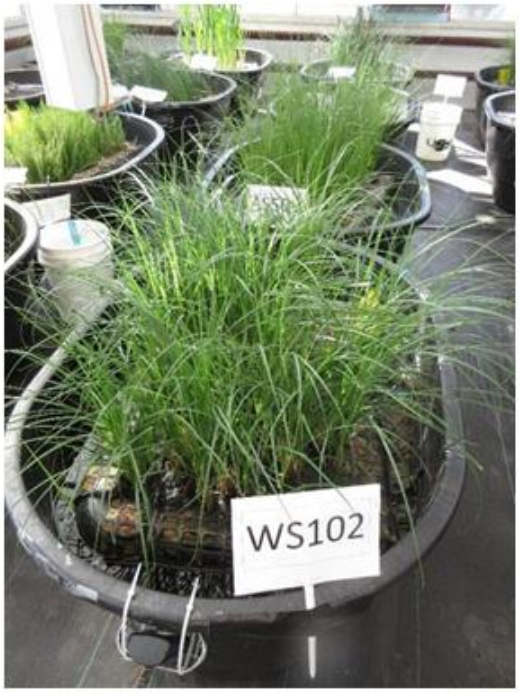

Photo 2: Wheat Sedge (*Carex atherodes*) mid experiment (August 24, 2020).

Photo 3: Wheat Sedge (*Carex atherodes*) near the end of the experiment (October 30, 2020).

Photo 4: Wheat Sedge (*Carex atherodes*) roots at the end of the experiment (November 2020).

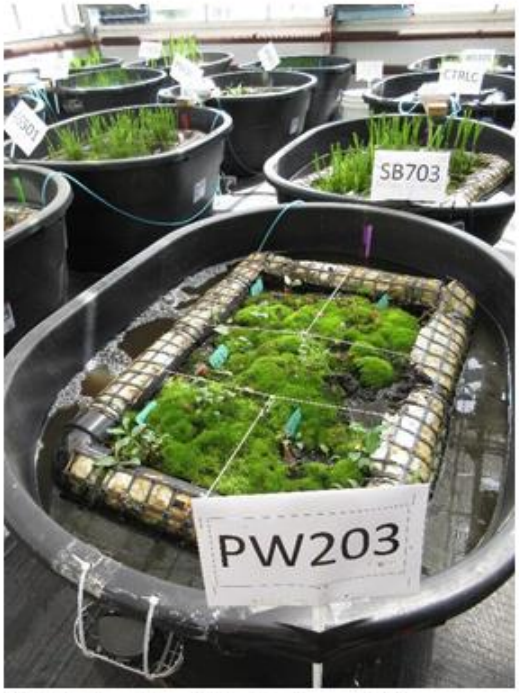

Photo 5: Smartweed (*Persicaria amphibia var. emersa*) at the beginning of the experiment (July 13, 2020).

Photo 6: Smartweed (*Persicaria amphibia var. emersa*) mid experiment (August 24, 2020).

Photo 7: Smartweed (*Persicaria amphibia var. emersa*) mid-experiment (September 21, 2020).

Photo 8: Smartweed (*Persicaria amphibia var. emersa*) near the end of the experiment (October 30, 2020).

Photo 9: Cattail (*Typha latifolia*) at the beginning of the experiment (July 13, 2020).

Photo 10: Cattail (*Typha latifolia*) mid-experiment (August 24, 2020).

Photo 11: Cattail (*Typha latifolia*) near the end of the experiment (October 30, 2020).

Photo 12: Cattail (*Typha latifolia*) roots at the end of the experiment (November 2020).

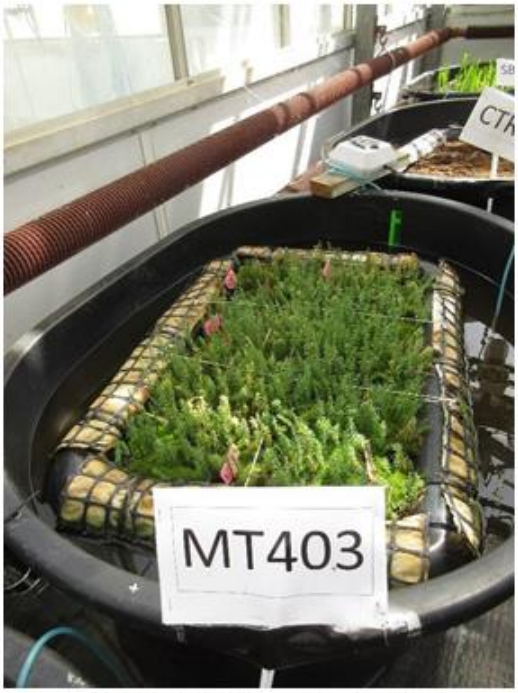

Photo 13: Mare's Tail (*Hippuris vulgaris*) at the beginning of the experiment (July 13, 2020).

Photo 14: Mare's Tail (*Hippuris vulgaris*) mid-experiment (August 24, 2020).

Photo 15: Mare's Tail (*Hippuris vulgaris*) near the end of the experiment (October 30, 2020).

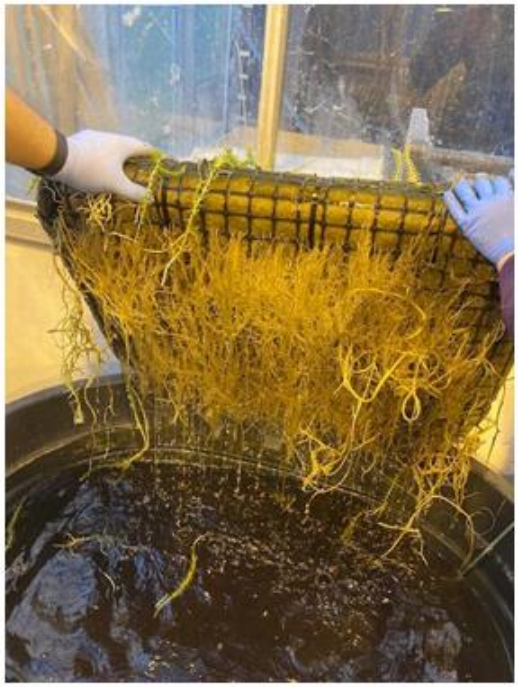

Photo 16: Mare's Tail (*Hippuris vulgaris*) roots at the end of the experiment (November 2020).

Photo 17: Water Sedge (*Carex aquatalis*) at the beginning of the experiment (July 13, 2020).

Photo 18: Water Sedge (*Carex aquatalis*) mid-experiment (August 24, 2020).

Photo 19: Water Sedge (*Carex aquatalis*) near the end of the experiment (October 30, 2020).

Photo 20: Water Sedge (*Carex aquatalis*) roots at the end of the experiment (November, 2020).

Photo 21: Baltic Rush (*Juncus balticus*) at the beginning of the experiment (July 13, 2020).

Photo 22: Baltic Rush (*Juncus balticus*) mid- experiment (August 24, 2020).

Photo 23: Baltic Rush (*Juncus balticus*) near the end of the experiment (October 30, 2020).

Photo 24: Baltic Rush (*Juncus balticus*) roots at the end of the experiment (November 2020).

Photo 25: Smallfruit Bullrush (*Scirpus microcarpus*) at the beginning of the experiment (July 13, 2020).

Photo 26: Smallfruit Bullrush (*Scirpus microcarpus*) mid-experiment (August 24, 2020).

Photo 27: Smallfruit Bullrush (*Scirpus microcarpus*) mid-experiment (September 21, 2020).

Photo 28: Smallfruit Bullrush (*Scirpus microcarpus*) near the end of the experiment (October 30, 2020).

Photo 29: Control tank at the beginning of the experiment (July 13, 2020).

Photo 30: Control tank near the end of the experiment (October 30, 2020).

Photo 31: Overview of research setup in a greenhouse (July 17, 2020).

Appendix B

Summary Statistics

1. Vegetation - Leaf Analysis

Mean nitrogen per species (%)

Species	mean	sd	se	min	max	n
AS	1.48	0.12	0.07	1.36	1.59	3
BR	1.73	0.28	0.16	1.53	2.05	3
CT	0.60	0.10	0.06	0.48	0.66	3
MT	1.48	0.06	0.03	1.42	1.54	3
PW	0.68	0.06	0.03	0.61	0.72	3
SB	1.14	0.15	0.09	0.97	1.25	3
WS	1.38	0.20	0.11	1.24	1.60	3

Mean sulfur per species (ug/g)

Species	mean	sd	se	min	max	n
AS	0.30	0.07	0.04	0.23	0.37	3
BR	0.41	0.10	0.06	0.29	0.47	3
CT	0.15	0.07	0.04	0.11	0.23	3
MT	2.17	0.26	0.15	1.96	2.46	3
PW	0.41	0.07	0.04	0.35	0.49	3
SB	1.03	0.10	0.06	0.91	1.10	3
WS	0.16	0.07	0.04	0.11	0.24	3

Mean phosphorous per species (ug/g)

Species	mean	sd	se	min	max	n
AS	0.27	0.02	0.01	0.25	0.29	3
BR	0.22	0.04	0.02	0.17	0.25	3
CT	0.10	0.04	0.02	0.07	0.14	3
MT	0.15	0.01	0.01	0.14	0.16	3
PW	0.28	0.07	0.04	0.20	0.34	3
SB	0.25	0.03	0.02	0.23	0.29	3
WS	0.39	0.12	0.07	0.30	0.52	3

Mean potassium per species (ug/g)

Species	mean	sd	se	min	max	n
AS	3.80	0.54	0.31	3.24	4.32	3
BR	2.89	0.21	0.12	2.65	3.05	3
CT	5.42	1.16	0.67	4.22	6.53	3
MT	4.73	0.50	0.29	4.41	5.31	3
PW	3.61	0.37	0.21	3.31	4.02	3
SB	4.10	0.33	0.19	3.79	4.44	3
WS	2.85	0.42	0.24	2.58	3.34	3

2. Vegetation - Root Analysis

Mean nitrogen per species (%)

Species	mean	sd	se	min	max	N
AS	0.88	0.16	0.09	0.70	0.99	3
BR	0.79	0.12	0.07	0.71	0.93	3
CT	1.03	0.05	0.03	0.98	1.06	3
MT	2.47	0.30	0.17	2.22	2.80	3
PW	1.80	0.06	0.04	1.76	1.87	3
SB	0.58	0.06	0.03	0.52	0.64	3
WS	1.02	0.09	0.05	0.93	1.11	3

Mean sulfur per species (ug/g)

Species	mean	sd	se	min	max	n
AS	0.14	0.03	0.01	0.12	0.17	3
BR	0.18	0.05	0.03	0.15	0.24	3
CT	0.22	0.05	0.03	0.17	0.25	3
MT	0.31	0.04	0.02	0.26	0.33	3
PW	0.27	0.02	0.01	0.25	0.28	3
SB	0.17	0.02	0.01	0.14	0.18	3
WS	0.18	0.04	0.03	0.15	0.23	3

Mean phosphorous per species (ug/g)

Species	mean	sd	se	min	max	n
AS	0.26	0.03	0.01	0.23	0.28	3
BR	0.42	0.09	0.05	0.37	0.52	3
CT	0.26	0.05	0.03	0.22	0.31	3
MT	0.68	0.02	0.01	0.66	0.70	3
PW	0.22	0.02	0.01	0.21	0.24	3
SB	0.10	0.01	0.00	0.10	0.11	3
WS	0.21	0.03	0.02	0.18	0.24	3

Mean potassium per species (ug/g)

Species	mean	sd	se	min	max	n
AS	1.44	0.25	0.14	1.28	1.72	3
BR	1.54	0.23	0.13	1.30	1.76	3
CT	4.94	0.67	0.39	4.18	5.45	3
MT	2.98	0.20	0.11	2.80	3.19	3
PW	1.40	0.05	0.03	1.37	1.45	3
SB	1.37	0.08	0.04	1.30	1.45	3
WS	1.75	0.17	0.10	1.64	1.95	3

3. Water Nutrient Analysis

Mean sulphur as sulphate per species (ug/ml)

Species	mean	sd	se	min	max	N
AS	56.84	12.25	3.54	42.51	81.87	12
BR	64.73	17.24	4.98	43.32	100.35	12
CT	54.19	10.40	3.00	38.58	69.48	12
CTRL	45.42	6.04	1.74	36.63	53.40	12
CTRLT	42.64	13.29	3.84	26.48	66.45	12
FILT	35.88	4.52	2.26	30.21	41.19	4
MT	43.63	5.06	1.46	37.20	50.31	12
PW	45.91	6.49	1.87	38.85	62.55	12
SB	41.71	5.21	1.50	34.41	48.27	12
TAP	32.30	4.17	2.09	28.37	37.41	4
WS	52.23	9.88	2.85	39.42	70.56	12

Mean sulphur per species (ug/ml)

Species	mean	sd	se	min	max	N
AS	18.95	4.08	1.18	14.17	27.29	12
BR	21.58	5.75	1.66	14.44	33.45	12
CT	18.06	3.47	1.00	12.86	23.16	12
CTRL	15.14	2.01	0.58	12.21	17.80	12
CTRLT	14.21	4.43	1.28	8.83	22.15	12
FILT	11.96	1.51	0.75	10.07	13.73	4
MT	14.54	1.69	0.49	12.40	16.77	12
PW	15.30	2.16	0.62	12.95	20.85	12
SB	13.90	1.74	0.50	11.47	16.09	12
TAP	10.77	1.39	0.70	9.46	12.47	4
WS	17.41	3.29	0.95	13.14	23.52	12

Mean phosphorous per species (ug/ml)

Species	mean	sd	se	min	max	N
AS	3.46	2.56	0.74	1.14	8.38	12
BR	3.01	2.00	0.58	0.66	6.11	12
CT	3.16	2.13	0.61	0.54	6.96	12
CTRL	5.86	0.76	0.22	5.02	7.35	12
CTRLT	-	-	-	-	-	-
FILT	-	-	-	-	-	-
MT	3.47	2.70	0.78	0.91	9.95	12
PW	4.58	1.76	0.51	2.83	7.69	12
SB	2.90	2.30	0.66	0.88	7.33	12
TAP	-	-	-	-	-	-
WS	3.31	2.58	0.75	0.21	8.10	12

Mean potassium per species (ug/ml)

Species	mean	sd	se	min	max	N
AS	218.82	38.63	11.15	175.60	302.20	12
BR	207.17	36.08	10.41	156.20	275.70	12
CT	188.05	50.09	14.46	127.70	277.20	12
CTRL	251.14	24.96	7.21	209.70	290.70	12
CTRLT	4.14	0.99	0.29	2.86	5.50	12
FILT	2.70	0.44	0.22	2.40	3.35	4
MT	198.52	36.48	10.53	157.10	267.10	12
PW	232.87	36.13	10.43	176.70	297.40	12
SB	202.04	46.00	13.28	142.00	287.10	12
TAP	2.33	0.73	0.37	1.72	3.37	4
WS	219.62	37.74	10.90	179.00	290.40	12

Percent change from start sample date to end sample date:

Percent change for phosphorous

	Species	02-Nov-20	14-Jul-20	21-Sep-20	24-Aug-20	PChange
1	AS	1.31	7.57	2.20	2.76	-82.69
5	BR	0.94	6.02	2.06	3.03	-84.44
9	CT	1.45	6.42	2.02	2.76	-77.43
13	CTRL	5.20	6.87	5.70	5.68	-24.35
17	CTRLT	-	-	-	-	-
21	FILT	-	-	-	-	-
25	MT	1.48	7.53	1.96	2.90	-80.31
29	PW	3.35	7.31	3.48	4.17	-54.19
33	SB	1.04	6.58	1.63	2.35	-84.20
37	TAP	-	-	-	-	-
41	WS	1.24	6.76	2.16	3.08	-81.70

Percent change for potassium

	Species	02-Nov-20	14-Jul-20	21-Sep-20	24-Aug-20	PChange
1	AS	183.17	274.83	201.47	215.80	-33.35
5	BR	179.43	259.37	188.60	201.27	-30.82
9	CT	143.77	261.33	160.73	186.37	-44.99
13	CTRL	229.53	283.97	236.07	255.00	-19.17
17	CTRLT	5.27	3.28	4.74	3.28	60.51
21	FILT	2.40	3.35	2.61	2.45	-28.36
25	MT	164.27	254.23	178.60	196.97	-35.39
29	PW	209.10	280.13	214.40	227.83	-25.36
33	SB	164.30	269.57	173.50	200.80	-39.05
37	TAP	1.95	3.37	2.29	1.72	-42.14
41	WS	200.47	276.37	191.10	210.57	-27.46

Percent change for sulphate

	Species	02-Nov-20	14-Jul-20	21-Sep-20	24-Aug-20	PChange
1	AS	71.97	47.35	58.31	49.75	52.00
5	BR	83.11	44.93	69.42	61.46	84.98
9	CT	68.37	43.30	57.19	47.90	57.90
13	CTRL	49.47	38.42	46.84	46.97	28.76
17	CTRLT	60.87	26.76	44.13	38.79	127.43
21	FILT	41.19	30.21	36.75	35.37	36.35
25	MT	50.01	45.11	40.74	38.65	10.86
29	PW	52.61	46.22	43.68	41.13	13.83
33	SB	47.23	45.33	38.74	35.54	4.19
37	TAP	37.41	29.47	33.93	28.37	26.96
41	WS	64.46	46.94	52.04	45.46	37.32

Percent change for sulphur

	Species	02-Nov-20	14-Jul-20	21-Sep-20	24-Aug-20	PChange
1	AS	23.99	15.78	19.44	16.58	52.00
5	BR	27.70	14.98	23.14	20.49	84.98
9	CT	22.79	14.43	19.06	15.97	57.90
13	CTRL	16.49	12.81	15.61	15.66	28.76
17	CTRLT	20.29	8.92	14.71	12.93	127.38
21	FILT	13.73	10.07	12.25	11.79	36.35
25	MT	16.67	15.04	13.58	12.88	10.86
29	PW	17.54	15.41	14.56	13.71	13.83
33	SB	15.74	15.11	12.91	11.85	4.19
37	TAP	12.47	9.82	11.31	9.46	26.99
41	WS	21.49	15.65	17.35	15.15	37.32

4. Soil Analysis

Mean Phosphorus Bicarb per species (ppm)

Species	mean	sd	se	min	max	n
AS	10.67	7.23	4.18	6	19	3
BR	10.33	0.58	0.33	10	11	3
CT	13.00	7.00	4.04	6	20	3
CTRL	10.33	0.58	0.33	10	11	3
MT	21.67	7.02	4.06	15	29	3
PW	32.33	24.58	14.19	13	60	3
SB	10.00	6.24	3.61	5	17	3
WS	20.67	10.41	6.01	9	29	3

Mean sulphur per species (ppm)

Species	mean	sd	se	min	max	n
AS	25.67	7.23	4.18	21	34	3
BR	31.33	17.93	10.35	20	52	3
CT	51.67	25.01	14.44	27	77	3
CTRL	16.67	3.06	1.76	14	20	3
MT	94.33	53.59	30.94	39	146	3
PW	49.67	50.58	29.20	18	108	3
SB	18.33	6.81	3.93	13	26	3
WS	23.00	6.08	3.51	19	30	3

Mean nitrate nitrogen per species (ppm)

Species	mean	sd	se	min	max	n
AS	1.00	0.00	0.00	1	1	3
BR	1.67	0.58	0.33	1	2	3
CT	1.33	0.58	0.33	1	2	3
CTRL	2.00	0.00	0.00	2	2	3
MT	2.00	0.00	0.00	2	2	3
PW	2.33	2.31	1.33	1	5	3
SB	1.00	0.00	0.00	1	1	3
WS	1.67	0.58	0.33	1	2	3

Mean potassium per species (ppm)

Species	mean	sd	se	min	max	n
AS	561.67	107.58	62.11	444	655	3
BR	539.33	103.40	59.70	459	656	3
CT	691.67	168.63	97.36	497	793	3
CTRL	1029.33	36.36	20.99	1004	1071	3
MT	761.67	154.42	89.16	586	876	3
PW	756.00	171.04	98.75	617	947	3
SB	488.00	57.42	33.15	427	541	3
WS	637.33	128.22	74.03	502	757	3

5. Water Use Analysis

Mean water use per species (L/m²) per day

Species	mean	sd	se	min	max	n
CTRL	5.36	1.43	0.41	3.40	7.78	12
AS	7.82	1.20	0.35	6.15	10.00	12
BR	8.92	1.32	0.38	7.14	11.31	12
CT	8.34	2.27	0.66	5.82	13.29	12
MT	6.02	1.25	0.36	4.28	8.68	12
PW	5.91	1.27	0.37	4.17	7.80	12
SB	8.23	1.39	0.40	6.48	11.31	12
WS	8.43	1.41	0.41	6.33	10.87	12

Mean water use per month (L/m²) per day

Month	mean	sd	se	min	max	n
Jul	7.28	1.14	0.23	4.70	9.95	24
Aug	9.10	2.02	0.41	5.71	13.29	24
Sep	6.71	1.68	0.34	3.40	9.12	24
Oct	6.43	1.62	0.33	3.51	9.67	24

Mean water use per species and month (L/m²) per day

Month	Species	mean	sd	se	min	max	N
Jul	CTRL	7.18	0.55	0.32	6.69	7.78	3
Jul	AS	7.12	1.00	0.58	6.15	8.14	3
Jul	BR	8.50	1.30	0.75	7.42	9.95	3
Jul	CT	7.54	0.82	0.47	6.69	8.32	3
Jul	MT	6.03	0.10	0.06	5.97	6.15	3
Jul	PW	6.21	1.33	0.77	4.70	7.24	3
Jul	SB	8.08	0.46	0.26	7.60	8.50	3
Jul	WS	7.60	1.27	0.73	6.33	8.86	3
Aug	CTRL	6.00	0.28	0.16	5.71	6.26	3
Aug	AS	9.52	0.44	0.26	9.12	10.00	3
Aug	BR	10.58	0.67	0.39	10.00	11.31	3
Aug	CT	11.61	1.46	0.84	10.76	13.29	3
Aug	MT	7.73	0.99	0.57	6.70	8.68	3
Aug	PW	7.03	1.15	0.66	5.71	7.80	3
Aug	SB	10.25	1.00	0.58	9.34	11.31	3
Aug	WS	10.07	0.96	0.55	9.01	10.87	3
Sep	CTRL	4.03	0.56	0.33	3.40	4.50	3
Sep	AS	7.40	0.79	0.46	6.48	7.91	3
Sep	BR	8.31	0.77	0.45	7.58	9.12	3
Sep	CT	8.20	0.60	0.35	7.58	8.79	3
Sep	MT	5.09	0.71	0.41	4.28	5.60	3
Sep	PW	5.49	1.16	0.67	4.17	6.37	3
Sep	SB	7.40	0.79	0.46	6.48	7.91	3
Sep	WS	7.76	0.99	0.57	6.81	8.79	3
Oct	CTRL	4.25	0.67	0.39	3.51	4.83	3

Month	Species	mean	sd	se	min	max	N
Oct	AS	7.25	0.50	0.29	6.70	7.69	3
Oct	BR	8.27	1.10	0.64	7.14	9.34	3
Oct	CT	6.00	0.23	0.13	5.82	6.26	3
Oct	MT	5.24	0.71	0.41	4.50	5.93	3
Oct	PW	4.91	0.77	0.45	4.17	5.71	3
Oct	SB	7.21	0.17	0.10	7.03	7.36	3
Oct	WS	8.31	1.32	0.76	7.03	9.67	3