Remediating Runoff and Creating Renewable Energy by Harvesting Invasive Plants from Illinois Tollway Detention Basins

Project: 9214

Final Report

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Preparing people to lead extraordinary lives

Executive Summary

This three year project investigated the utility of harvesting invasive plant biomass in Illinois Tollway (Tollway) detention basins and examined the benefits. Salt pollution of roadside environments is a common problem in areas where salt application is necessary to ensure driver safety in the winter. Excess salts can impact drinking water, degrade aquatic habitat, and cause heavy metals to mobilize, leading to deleterious effects on the environment. Invasive wetland plants tend to colonize roadside environments where their enhanced salt and nutrient tolerance allows them to outcompete native vegetation and produce copious aboveground biomass. In this project, a research team from the University of Connecticut (UConn) and Loyola University Chicago (LUC) worked closely with Tollway staff to identify 10 detention basins across the 294 mile Tollway system. Basins were selected for presence of invasive wetland plants (either cattail; *Typha* spp. or common reed; *Phragmites australis*), ease of access for a tracked wetland vegetation harvester (Loglogic Softrak Cut and Collect System), and ability to remove and transport biomass to an end user. Across three years, the research team evaluated how harvesting altered the chemical and physical properties of the basins by collecting soil, plant tissue, and biomass samples. The research team experimentally harvested five basins (with five being left as unmanipulated controls) over two consecutive years (2019 and 2020) and monitored the basins for changes in aboveground biomass, salt content (Cl, Na, Ca, and Mg), nutrient content (P), and heavy metal content (Zn, Cu, Mn, and Fe). All harvested biomass was removed from each basin and transported to end-users to make a compost product. Tollway Maintenance staff and equipment transferred and transported ~100 wet tons of material to the Metropolitan Water Reclamation District in 2019 to incorporate into compost as a part of their sludge drying program, and then Waste Management's Willow Creek composting facility in 2020.

Overall, harvesting showed benefits of reducing biomass and litter within basins, reducing invasive plant height, and physically removing salts and metals from basins. Harvesting two consecutive years reduced the subsequent year's average height of *Phragmites* stems by 101 cm (40 in) and *Typha* spp. stems by 65 cm (26 in). This reduction in plant height is of value in roadside environments where visibility is critical to driver safety. A full account of costs and benefits is included herein. Harvesting 14 acres over one season has the potential to remove 2,396 lbs of Cl, 1,073 lbs of Na, 2,009 lbs of Ca, and 343 lbs of Mg, 253 lbs of P, 11 lbs of Zn, 1.6 lbs of Cu, 80 lbs of Mn, and 156 lbs of Fe. Elemental removal values were lower after two consecutive years of harvest, because harvesting in year 1 reduced plant biomass in year 2. *Typha*-dominated plots tended to have higher salt content, while *Phragmites*-dominated plots tended to have higher metal contents. This complementary pattern of removal is advantageous in taking up common pollutants in roadside environments. Additionally, harvested biomass is a viable compost feedstock, though more work evaluating the salt and metal content of the compost, as well as the potential of this product to spread invasive seeds into new environments, is needed. Across the entire Tollway system, 89 basins (covering 250 acres) have good potential for harvest (calculated estimates of total potential elemental removal included herein). The research team recommends harvesting on a three year rotation to maximize benefits to the Tollway.

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1. Background

1.1 Introduction and literature review

Road managers in temperate regions combat ice and snow buildup using rock salts (typically sodium chloride, NaCl), resulting in excess chlorides (Cl^-) and minerals running off into roadside ditches, detention basins, and natural ecosystems. Road salt is increasingly contributing to the salinization of freshwater lakes and rivers throughout North America (Dugan et al. 2017; Kaushal et al. 2018). Salinization not only threatens drinking water quality (Stets et al. 2018), but can degrade aquatic ecosystems and is associated with reduced diversity of many aquatic species (Hintz et al. 2017; Wilcox 1986). Road salts can also mobilize heavy metals, which are particularly concentrated in urban roadside environments (Schuler and Relyea 2018). Furthermore, nutrient pollution (phosphorus (P) and nitrogen (N)) from agricultural and urban lands leads to soil acidification, biodiversity loss, eutrophication, and hypoxia of freshwater environments (Schindler and Fee 1974; Sobota et al. 2015; Vitousek et al. 1997). In the Chicago region, at least 235,890 tons (t) of Cl^- enters the environment via road salt annually (Kelly et al. 2010), 33,068 tons of which is retained in the region over the longer-term (Kelly et al 2012).

Mitigating downstream water quality issues by harvesting invasive plants in roadside detention basins is a novel approach with potential co-benefits. Cattails (*Typha* spp., hereafter *Typha*) and common reed (*Phragmites australis,* hereafter *Phragmites*) are large, ubiquitous invasive aquatic plants in roadside ditches and detention basins. Both species are adapted to degraded habitats with high salinity, and excess N and P (Farnsworth and Meyerson 2003; Tuchman et al. 2009). Invasion by these species results in reduced diversity of wetland plants, amphibians, invertebrates, and birds (Lawrence et al. 2016; Monfils et al. 2014; Rowe and Garcia 2014; Tuchman et al. 2009). Also, roadside ditches can act as invasion corridors through which invasive plants spread to uninvaded wetlands (Ahrens et al. 2014; Brisson et al. 2010). These plants are much taller and more productive than the species that they displace; their accumulating biomass (litter) acts as a slow-release reservoir for nutrients that contributes to eutrophication in aquatic habitats downstream. Within detention basins, *Typha* and *Phragmites* dominance degrades both visual aesthetics, by blocking views, as well as the proper functioning of detention basins by clogging orifices and reducing infiltration rates and water storage capacity.

Typha and *Phragmites* actively take up and store N, P, and Cl^- (and other salts) in their above-ground tissues (Carson et al. 2018; Monteau et al. 2014), therefore, **harvesting their biomass** directly removes these pollutants from aquatic environments. Also, harvesting biomass can increase a wetland's ability to sequester and process P (Shukla et. al 2017; Tanaka et al. 2016). There is potential for harvested biomass to be utilized for **bioenergy or agricultural use** (Carson et al. 2018). Biomass from waste materials and plants grown on degraded lands avoids many of the unintended environmental consequences of first-generation biofuels (i.e. crops grown specifically for fuel) and has greater greenhouse gas reduction benefits. Several highly productive plant species that are invasive in the Great Lakes region, including *Phragmites*, have been utilized as bioenergy feedstocks, yielding positive net energy balances (Hansson and Fredriksson 2004), potentially providing revenue to offset ecological restoration costs (Nackley et al. 2013; Quinn et al. 2014).

A series of studies, conducted at a range of scales (1 - >100 acres), have tested the efficacy of harvesting invasive plants from Great Lakes region wetlands and utilizing the biomass for various purposes. Several methods have been evaluated to utilize harvested biomass including for energy production (anaerobic digestion and biofuel pellets) and as an agricultural input (soil amendment, compost, and cattle bedding). This research has demonstrated the effectiveness of harvesting to increase native plant diversity and remove excess nutrients (Berke 2017; Keyport et al. 2018; Lishawa et al. 2015; Lishawa et al. 2017).

Here, a team of researchers from the University of Connecticut (UConn), Loyola University Chicago (LUC), supported by the Illinois Tollway (Tollway), and Technical Review Panel report on efforts from a 2019-2022 project to examine how repeated harvest of ~14 acres of *Typha* and *Phragmites*-dominated detention basins along the Tollway effected biomass height and production, removed excess salts (Na^+, Cl^-, Mg^+, Ca^+), metals (Zn, Cu, Mn, Fe) and nutrients (N, P), and altered water storage and infiltration. Further, the team examined opportunities to repurpose harvested biomass as a bioenergy product or soil amendment.

1.2 Research objectives

The research team examined if harvesting invasive species biomass improved the functioning of Tollway drainage features to handle stormwater, remove nutrients and salts, support biodiversity, and create a sustainable solution to invasive plant management by accomplishing all of the Tollway's four specified objectives (Research RFP #18-01R, Pg. 2) through the following interrelated tasks.

I. *Determine the pre-treatment chemical and ecological condition of a set of detention basins and quantify the effect of harvesting biomass on measured values (Obj. 1)*

In 2019, the research team collected soil and water samples from ten basins (five treatment and five control) and evaluated fertility, salinity, Cl^- concentration, and conducted total elemental analysis; to determine the chemical composition of the invasive vegetation the research team collected and analyzed plant tissues; quantified the standing biomass and plant community composition; and extrapolated results of the physical, chemical, and biological condition of each sampled basin. The research team assessed the potential of harvesting biomass to remediate nutrient and Cl^- loading by repeating all soil and plant chemical composition measures prior to harvest treatments in 2019 and after two years of harvesting in 2021. Biomass production was quantified in each of the three years (2019-2021) within all ten basins.

II. *Harvest wetland plant vegetation (Obj. 1, 2, & 3)*

In September and October of years 1 and 2 (2019 & 2020), the research team harvested the vegetation from five basins (approximately 14-acres) using the LUC-owned Loglogic Softrak wetland plant harvester. The remaining five unharvested basins served as unmanipulated controls throughout the course of the project.

III. *Analyze multiple utilization options for invasive plant biomass for energy and agricultural use, find end-users for all harvested biomass, and facilitate partnerships with Tollway (Obj. 1, 2, & 4)*

A goal was to develop a biomass utilization strategy across the Tollway region, allowing for the efficient use of harvested biomass. Utilizing biomass for energy can offset fossil fuel use, reduce greenhouse gas emissions, and provide a local and renewable energy source. Biomass can also be a cost-effective source of nutrients and organic matter for local farmers or for tree planting. The research team sought out partners from the energy, wastewater treatment, agriculture, and horticulture industries who are currently managing biomass and have the capacity to utilize additional biomass resources. The team leveraged an existing partnership between the Tollway and the Metropolitan Water Reclamation District (MWRD) of Chicago to deliver biomass to their sludge drying/composting operation in 2019, and then delivered biomass to Waste Management's Willow Creek composting facility in 2020.

IV. *Evaluate the costs and benefits of harvesting detention basins for multiple benefits (Obj. 1 & 2)*

The research team quantified all costs associated with biomass harvesting (e.g. labor, biomass harvest, equipment maintenance, etc.) and biomass utilization (e.g. biomass transport, tipping fees, etc.) and quantified the environmental benefits (Cl^-, N, and P removed from the watershed, etc.).

V. *Spatially analyze the Tollway detention basin system in light of research outcomes (Obj. 1,2 & 4)*

A detailed spatial analysis of the Tollway, the detention basins, and regional biomass utilization options were also conducted. Harvesting location within the Tollway region will inform local biomass utilization options. The team conducted a regional spatial analysis of the Tollway detention basin and drainage ditch network in the Tollway's asset management system, Cartegraph GIS program, allowing us to determine the potential of harvesting and biomass utilization system-wide to achieve multiple benefits.

1.3 Research tasks

1) Identify appropriate detention basins to conduct the project
2) Collect soil, plant, and water samples in 2019, 2020, and 2021
3) Harvest wetland plant biomass with LUC-owned Log logic SofTrak wetland tractor
4) Determine the best options for biomass utilization and will facilitate partnerships with end-users.
5) Analyze costs and benefits of harvesting detention basins.
6) Spatially analyze detention basin's potential for a sustainable harvesting program

2. Experimental methods

2.1 Basin selection

In 2019, the research team worked with Tollway to identify ~30 candidate detention basins based on dominance of invasive wetland plants (*Typha*, *Phragmites*), safe accessibility with the wetland vegetation harvester, size (greater than one acre), and location within the M-14 and M-8 Maintenance Districts. Basins typically contained two distinct "zones" of dominant wetland plants, one zone dominated by *Typha* and another dominated by *Phragmites.* The team visited basins to determine suitability in spring 2019 and selected 10 paired basins for future experimentation, with 5 basins set to be harvested in 2019 and 2020 and 5 basins left alone as unharvested "control" basins (Figure 1). Basins were grouped into 5 "pairs" on the basis of geographic area and vegetation, with paired basins tending to be located close together and containing similar assemblages of *Typha* and *Phragmites*.

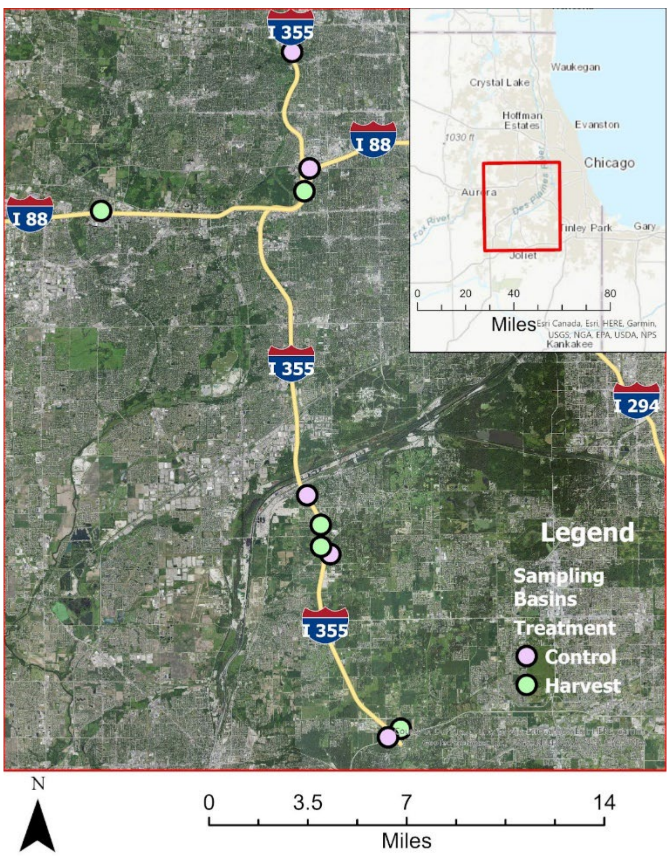

Figure 1 Locations of 10 focal detention basins in the Illinois Tollway system for evaluation of invasive species biomass harvesting.

2.2 Soil, plant and water sampling

To sample each basin, the research team established eight, one meter squared plots distributed across the two zones, with four plots in the *Typha* zone and four plots in the *Phragmites* zone.

Plots were established by generating random points within each zone in each basin in ArcMap. Additionally, the team established plots at each of the 10 basin outlets. All basins contained two zones and eight plots except for #237 which did not contain *Typha* and thus had only four *Phragmites* plots. The total number of plots sampled per year was 86: 9 plots per basin over nine basins containing both zones, plus five plots from #237.

In each plot, the team estimated plant and litter biomass in each year: 2019 (pre-harvesting), 2020 (one year post-harvest), and 2021 (after two consecutive years of harvest). The team estimated above ground biomass by counting and measuring height classes of all *Typha* and *Phragmites* stems within the one meter squared plot to the nearest five centimeters. Each year the team collected two stems from each plot, measured their heights, and dried the stems at 60° C for 48 hours to measure dry weight. The team calculated an allometric equation using the software R to convert stem height data to aboveground dry biomass. Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) model selection was deployed to determine appropriate factors for allometric equations of each species. The final *Typha* equation used height class, presence of an inflorescence, harvest treatment, and year, while the final *Phragmites* equation used height class and year (*Typha* Stem Dry Mass = height + inflorescence presence + harvest treatment + year; *Phragmites* Stem Dry Mass = height + year). Partial least squares regressions were then used to develop biomass predictions for each species at the basin level using all culm height data. In 2019 and 2021, the team also collected soil and plant tissue samples for chemical analysis.

2.3 Chemical analysis

2.31 Soils

Pretreatment (2019) and then after two consecutive years of harvest (2021), the research team collected 86 soil samples (one in each plot across the system) to 10-cm depth using a 3.6-cm radius bulb planter, dried samples for 24 hours at 40° C, separated out roots using a 2-mm sieve, and shipped soils to Kansas State University to be analyzed for P, K, Ca, Mg, Na, Zn, Cu, Fe, Mn, Cl, Pb, As, Cd, and Cr. N and C were analyzed at LUC using a Flash 2000 C:N analyzer. Secondly, the team investigated the effects of harvesting on plant-available nutrients by deploying Plant-Root Simulator (PRS) probes in all basins. PRS probes were left in soils for 14 days from late September to early October 2021 and sent to Western Ag (Saskatoon, CK, Canada) for analysis of plant-available NO_3, NH_4, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd.

2.32 Plant tissue

Pretreatment (2019) and then after two consecutive years of harvest (2021), the research team randomly chose two stems from each plot (stems of the dominant species of wetland plant; *Phragmites* stems in *Phragmites* plots and *Typha* stems in *Typha* plots), totaling 172 stems collected (two from each of 86 plots). The team also collected one litter sample from each of the 86 plots, dried the samples, ground them using a Thomas Wiley® mill (Figure 2), composited each plant stem sample at the plot level (keeping litter separate), and analyzed plant tissue for Na, Cl, K, P, Fe, Mn, Mg, S, Cu at Clemson University and N and C at LUC.

Figure 2 Loyola University Chicago's Thomas Wiley® Mill, used to grind biomass samples prior to chemical analyses.

2.33 Water loggers

Additionally, in 2021 the research team installed *In Situ* pressure transducers in six detention basins (3 control, 3 harvest) to monitor water level variation and drainage. Pressure transducers were deployed in March 2021 and removed in November to capture variation in water depths throughout the growing season.

2.4 Harvesting

Harvesting took place in late September and early October in 2019 and 2020. All harvesting occurred after soils and vegetation sampling in the "harvest" basins. The team harvested basins from the southernmost basin at the interchange between I-355 and I-80, and worked north. To harvest, the team used a Loglogic Softrak Cut and Collect System (Figure 3, A; Devon, UK), a low ground pressure tracked vehicle with a flail-style cutter that cuts and chops all aboveground biomass taller than ~30 cm off of the ground and loads it into a hopper, which was emptied at a designated biomass transfer site as determined by LUC and the Tollway. Harvesting proceeded at a rate of ~1 acre of wetland vegetation per day, with the smallest basin harvested in a single day and larger basins taking 3-5 days. Harvesting was faster in 2020 than 2019 due to the reduced litter after one previous harvest.

2.5 Biomass utilization

In order to remove and repurpose biomass, all harvested material was piled outside of harvested basins (Figure 3, B). As outlined in section 3.2, the research team pursued several options for biomass utilization and ended up composting the material by delivering it to Chicago's Metropolitan Water Reclamation District (MWRD) sludge drying program. In 2020, due to pandemic concerns, MWRD no longer accepted biomass for compost, so the team composted material at Waste Management's Willow Ranch Composting facility. All biomass was picked up by Tollway staff equipped with empty salt trucks and front-end loaders. Trucks repeatedly visited basins until all biomass was delivered, always within one week of biomass harvest.

Figure 3 A) Softrak wetland harvester equipped with the Cut and Collect system harvesting *Phragmites* in basin 184, September 2019. B) Biomass pile waiting to be transported to MWRD, September 2019.

3. Experimental results

3.1 Basin selection and harvesting

Of the 10 basins selected for this project (Figure 1, Table 1), five were identified for experimental harvest. These five basins were harvested with the Softrak wetland harvester late in the growing seasons (September-October) of 2019 and 2020, constituting a total area of 14.14 acres per year (Figure 1, 4, 5). Of this total, 6.92 acres were dominated by *Phragmites* and 7.22 acres were dominated by *Typha*. Time allocated to harvesting biomass from the detention basins also included maintenance of the Softrak. This included replacing a hydraulic pump in 2019 and other routine maintenance. Each individual basin had unique challenges associated with harvesting. Access was a major consideration: equipment and personnel needed to be transported into and out of basins safely and without interruption to traffic. Loyola's Softrak vehicle was able to handle wet basins and steep slopes to accomplish its harvesting.

Table 1 Selected basins for our project. "HARV" represents basins that were harvested in 2019 and 2020, "CTL" are unharvested control basins. "Pair" indicates our assigned group, basins were paired together on the basis of geography and vegetation to be compared.

Basin ID	Old ID	Harv/ctl	Pair	Route	Milepost	Direction	M Section	Type	Bottom El.	100-yr HWE	Construction Contract
355_000.10DET3	DET0184	HARV	a	I-355	0.10	N/A	M-14	Dry/Grass Bottom	684.00	689.29	CIP-93-706
355S000.10DET3	DET0183	CTL	a	I-355	0.10	N/A	M-14	Dry/Grass Bottom	685.20	690.91	CIP-93-706
355S007.50DET	DET0032	HARV	b	I-355	7.50	Southbound	M-14	Pond/Wet Bottom	663.50	668.39	I-05-7719
355N007.30DET	DET0028	CTL	b	I-355	7.30	Northbound	M-14	Pond/Wet Bottom	676.50	678.97	I-05-7719
355N008.25DET	DET0140	HARV	c	I-355	8.25	Northbound	M-14	Dry/Grass Bottom	698.20	708.49	I-05-7705
355N009.40DET	DET0141	CTL	c	I-355	9.40	Northbound	M-14	Dry/Grass Bottom	698.20	707.09	I-05-7705
088W125.30DET	DET0237	HARV	d	I-88	125.30	Westbound	M-8	Dry/Grass Bottom	689.00	693.19	CIP-91-465
355N026.95DET	DET0188	CTL	d	I-355	26.95	Northbound	M-14	Dry/Grass Bottom	689.00	696.00	CIP-618
355N021.60DET	DET0158	HARV	e	I-355	21.60	Northbound	M-14	Dry/Grass Bottom	678.85	688.36	CIP-616
355N021.80DET	DET0159	CTL	e	I-355	21.80	Northbound	M-14	Dry/Grass Bottom	684.00	695.00	

Another challenge was transporting harvested biomass from each basin to composting facilities with the MWRD (2019) or the Waste Management Willow Creek facility. Loyola coordinated closely with Tollway Maintenance staff to decide on placement of biomass harvest piles and timing of biomass pickup and delivery. Trucks were weighed upon delivery to both MWRD and Willow Creek facilities. In 2019, truck weight reports showed a total of ~45 wet tons of biomass were delivered to MWRD. The research team believes this result to be a severe underestimate of the delivered weight due to the trucks not being weighed or logged when making repeated daily deliveries. Our total from 2020 deliveries to the Willow Creek facility was ~100 wet tons. Since basins had higher overall biomass in 2019 than 2020, the team is confident that the 2019 total is an underestimate and suspect the real amount delivered was closer to ~150 wet tons.

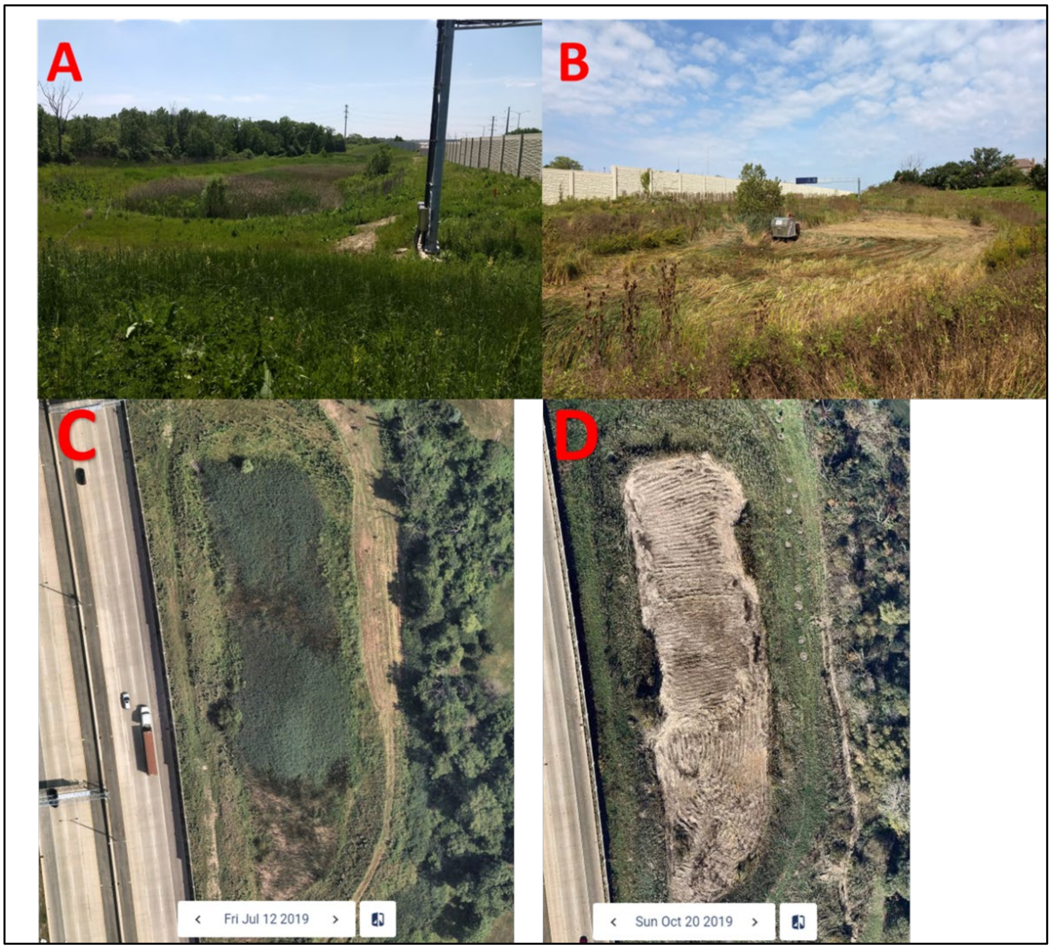

Figure 4 Tollway basin #140 was experimentally harvested in 2019 and 2020. A) Basin #140 prior to harvest in July 2019; B) Basin #120 during harvesting in 2019; C) Aerial image of Basin #140 prior to harvest on July 12, 2019; D) Aerial image of Basin #140 after biomass harvest on October 20, 2019. Photo credit: Drew Monks, LUC.

Figure 5 Tollway basin 158 was experimentally harvested in 2019 and 2020. A) Basin 158 prior to harvest in July 2019; B) Basin 158 during harvesting in 2019; C) Aerial image of Basin 158 prior to harvest on July 12, 2019; D) Aerial image of Basin 158 after biomass harvest on October 18, 2019. Photo credit: Drew Monks, LUC.

3.2 Biomass utilization

3.21 Potential to utilize harvested biomass to create compost

In consultation with Tollway staff, the research team pursued opportunities for harvested biomass utilization within the Chicagoland region. First, LUC contacted and coordinated with the Metropolitan Water Reclamation District (MWRD) of Greater Chicago's sludge drying operation at the Stickney plant (Cicero, IL) in 2019. They were interested in collaborating and receiving biomass harvested across the Tollway system for composting in their industrial scale facility. In early fall of 2019, ~45 wet tonnes of material were delivered using Tollway trucks and labor from the M-14 and M-8 Maintenance Sections. A front-end loader was used to scoop harvested material dumped in piles at the edges of Tollway detention basins during harvesting into Tollway trucks.

The research team visited and toured the MWRD sludge drying operation in November, 2019. Sludge is digested waste material that is centrifuged and then the solid fraction (aka "sludge cake") is extracted and contains about 25% moisture content. This product is delivered to farm fields directly and used as a "Class B biosolid" compost or it can be delivered via rail cars to a sludge drying facility adjacent to the Stickney plant (Figure 6).

Figure 6 The Illinois Tollway Deputy Chief with a "cake" pile at the MWRD Stickney plant in Cicero, IL. Photo credit: Drew Monks, LUC.

The MWRD has an industrial scale composting facility. Composting sludge cake converts "Class B" compost to "Class A" compost that can be used locally by individuals, municipalities, or businesses. Originally the compost was bagged to be sold, but it is now donated. During composting, cake is mixed in a 25:75 ratio of cake:biomass. Woodchips are the most available and commonly used biomass ingredient. Yard waste can be added to supplement the woodchips (25:50:25 ratio of cake:woodchips:yard waste). Harvested *Typha* and *Phragmites* biomass from the Tollway Right of Way (ROW) constitutes a yard waste substitute. During composting, piles need to reach ~110° F for three days and then are turned over by a giant auger (Figure 7); this process occurs five times before the compost can be cured. Composted product must cure for 16 weeks, then is screened to remove wood-bits, and finally is ready to use (Figure 8).

Figure 7 Auger used to turn compost piles at MWRD facility (above) and temperature monitor for active piles (below). Photo credit: Drew Monks, LUC.

Figure 8 Finished compost product (foreground) with active steaming piles of compost (background) at the MWRD composting facility. Photo credit: Drew Monks, LUC.

To test for the presence of invasive seeds, LUC collected subsamples of the compost that was amended with Tollway biomass in June 2020 and stored it in a fridge until further analysis. In early 2021, this compost was spread in trays in LUC's *EcoDome* greenhouse and watered regularly under ideal growth conditions and monitored for seed germination. Seedlings were identified at the earliest possible stage. Wood sorrel was the most common species emerging, but both *Typha* and *Phragmites* seedlings germinated from the compost, indicating compost may not be a viable end product for the biomass, as it could be a potential invasion vector (Figure 9).

Figure 9 Invasive *Phragmites australis* and *Typha* spp. seedlings emerging from composted biomass collected from Illinois Tollway detention basins.

In 2020, due to pandemic-related restrictions, MWRD stopped receiving vegetative biomass for their sludge drying program, so LUC coordinated with Waste Management to deliver harvested material for composting at the Willow Ranch compost facility. Tipping fees were charged at a rate of $42.10/ ton of material delivered. A total of ~100 wet tons were delivered, costing $5,045.46. The charges are in excess of the rate per ton because a minimum charge of $42.10 was assessed for loads less than 1 ton.

3.22 Potential to utilize harvested biomass for other value added products

LUC investigated other value-added products made from harvested invasive species biomass. Biochar, a soil amendment that improves soil organic matter and increases cation exchange capacity, can be produced from vegetative material. Evidence suggests that this material will improve plant growth by increasing organic carbon and enhance chloride and sodium uptake through cation exchange and complexation of anions to the porous biochar surface. The research team connected with several biochar producers and explored options for diverting this material, including Tom Marrero of Wakefield Biochar. Tom delivered a biochar Kiln to LUC's Retreat and Ecological Campus, where LUC has been producing biochar using wood waste, biosolids material from MWRD, and harvested *Typha* and *Phragmites*. Under direction of the Technical Review Panel, the team also connected with the Illinois State Geological Survey and introduced the idea of investigating the effects of biochar application in Tollway Bioswale TB7. In April, 2021, 280 lbs of biochar were added to the bioswale, along with several bags deployed at concrete check dams throughout the bioswale to investigate nutrient saturation of the biochar. The purpose was to investigate the potential nutrient retention benefits of biochar addition to Tollway assets. This is a topic for future research. It is anticipated that invasive plant-generated biochar may be valuable to Tollway nutrient retention and tree planting, and see it as a viable outlet for harvested biomass.

Harvested invasive plant biomass has many potential uses, including generating electricity through pelletization and direct combustion or anaerobic digestion and production of biogas. In order for energy generation to be feasible, green energy infrastructure must be present within a Tollway Facility or somewhere in the Chicagoland region. For instance, harvested invasive *Typha* biomass from wetlands around Horicon Marsh in central Wisconsin was delivered to an anaerobic digestion facility at the University of OshKosh: https://uwosh.edu/biogas/. Researchers found the material to be suitable, and produced ~94,000 cubic feet of biogas from 36 wet tons of material. Though the research team was unable to locate an anaerobic digester operator in the area, "The Plant" in Chicago currently has plans to build and open a facility, which may be potential stream for future biomass (https://www.plantchicago.org/post/anaerobic-digestion-at-plant-chicago-part-1). Further potential for utilizing invasive biomass for energy is discussed in the paper "Harvesting invasive plants to reduce nutrient loads and produce bioenergy" published in the journal *Ecosphere* and co-authored by the investigators associated with this project (Carson et al., 2018).

Harvested material can also be used as a fertilizer, or "green manure," as demonstrated in the paper "Wetland waterbird food resources increased by harvesting invasive cattails" (Lishawa et al, 2020). Care should be taken, however, to prevent the spread of these highly invasive species via seeds present in harvested materials (see section 3.23).

3.23 Challenges to using Illinois Tollway-harvested biomass

Our analyses of aboveground biomass tissues of *Typha* and *Phragmites* plants indicate that they are elevated in salts and heavy metals that are common in roadside environments (see section 3.32). While using harvested invasive species biomass can have some environmental benefits (closing the loop), when the biomass is laden with undesirable elements such as sodium, chlorides, zinc, and copper, transforming that biomass into usable products poses issues. Contaminant laden compost could pose an environmental and human health risk and further chemical analysis of compost amended with Tollway-harvested biomass is recommended prior to pursuing this option. The research team recommends chemical analysis of composted product prior to spreading compost. Further, the above described greenhouse study suggests that composted product contained seeds of both *Typha* and *Phragmites* (Figure 9), so distribution of the compost may risk further spread of these invasive plants.

3.3 Costs and benefits of harvesting detention basins

3.31 Costs

Harvesting at the basin scale requires a consideration of the labor required. It took an average of 6.5 hours to harvest one acre of Tollway basin habitat in 2020, with a range of 3.5 to 12.4 hours/acre depending on maintenance issues and characteristics such as water depth and slope, which influence the ease of operation. This estimate includes equipment maintenance (e.g., repairing harvester on-site, refueling, etc.), moving equipment between the M-14 storage garage and the basins, and transporting harvested material to designated dumping locations.

The Softrak harvester requires additional operation and maintenance costs. The harvester uses roughly 12 gallons of diesel fuel in a full day of operation, or 7 hours. While diesel price has fluctuated significantly over the past three years, a budget of $150 per week for fuel was sufficient. Additional necessary items include tractor grease, hydraulic fluid, engine coolant, and shop towels, for which we recommend a budget of $200 per two months of harvest. We also budgeted $500 per season for incidental repairs on the harvester, which covered necessary labor and replacement costs. This is a total of $3,100 for one season of operations.

Costs associated with the removal of the biomass from the basins varied year to year. In 2019, biomass was sent for composting to the Metropolitan Water Reclamation District for composting, who waived transport and tipping fees to support our research efforts. This program was changed in 2020 with the onset of the coronavirus pandemic, so biomass harvested in 2020 was sent to the Willow Ranch composting facility owned and operated by Waste Management, Inc. Tipping fees were charged at a rate of $42.10 per ton of material delivered. Roughly 100 wet tons were delivered for a total cost of $5,045.46. Excess charges of the per-ton rate are due to a minimum charge of $42.10 assessed for loads less than 1 ton. The transport was undertaken by the Tollway, who may have had additional costs associated with the use of hauling equipment and labor of the operators.

Finally, we accrued travel costs associated with driving the harvester to and from basins. We used a Ford F-150 to pull the harvester on a trailer and averaged 4.5 miles per gallon in transport. Total gas costs varied based on the distance between our storage facility and the respective basins, but we budgeted around $150 per week for gas needs.

3.32 Environmental benefits

Experimental harvesting of biomass over two consecutive years revealed several potential benefits including nutrient removal, improve basin function, and production of a value-added compost product.

3.321 Chemical benefits

To estimate potential biomass and elemental removal, the team utilized allometric equations of total aboveground biomass. These estimates represent potential removal instead of true removal, as our machine was only able to harvest stems down to 30 cm, leaving behind a layer of stubble. Throughout this experiment plots dominated by *Phragmites* contained more biomass than plots dominated by *Typha*, which was expected due to physiological differences between these species (Figure 10). Over the 14 acres harvested in 2019, the team calculated the total aboveground biomass of invasive plants (removal potential) as 336,749 lbs (168 dry tons).

About 45% of this biomass consisted of litter, which is dead material from prior years of growth and 55% of the material was green tissue that had grown in 2019. In 2020 and 2021, biomass harvest estimates were lower at 186,357 lbs (93 dry tons) and 205,186 lbs (103 dry tons), respectively (Figure 10). Lower harvest potential in years two and three are a result of reductions in litter, which made up closer to 40% of the overall biomass in 2021. The first harvest cleared out materials that had built up over many years, while the second harvest removed only recent growth. There was also a reduction in biomass production, which is a commonly documented response to harvest. These trends in biomass were used to make recommendations to harvesting as an annual or semi-annual tool to be used by Tollway (see section 5).

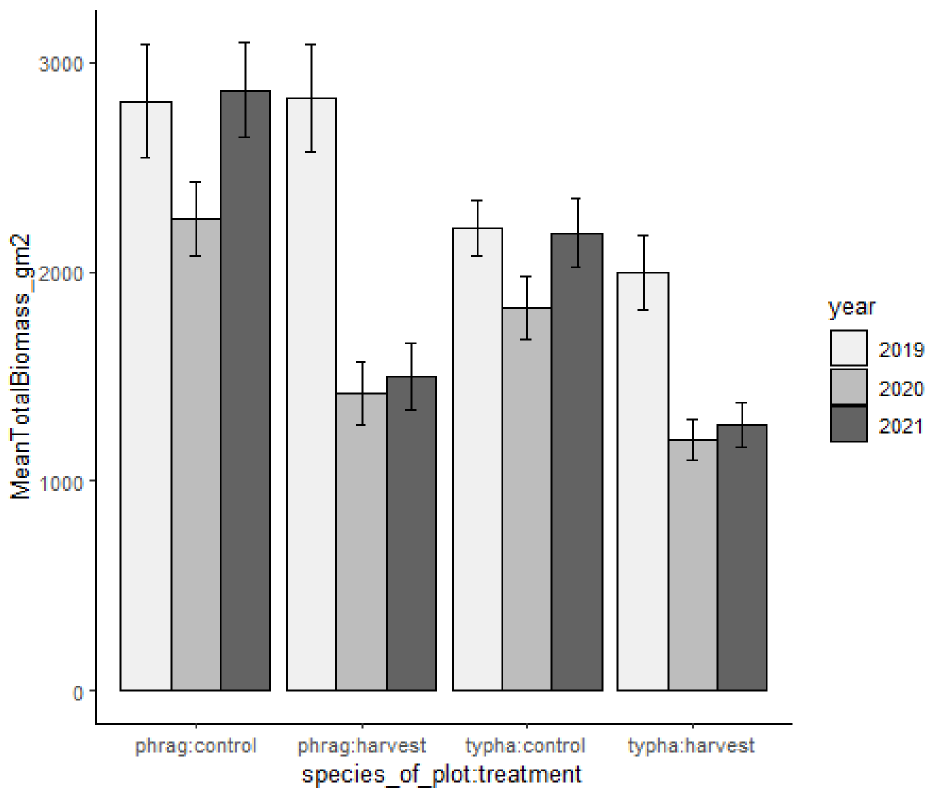

Figure 10 Total biomass (in grams/m^2) of sampled plots across three years, with sampling occurring before harvesting in 2019, one year after harvest in 2020, and after two consecutive years of harvest in 2021. “Species_of_plot” refers to the dominant species in the sampled plot, and “treatment” refers to control (unharvested) or harvest (experimentally harvested) plots. Means are presented with error bars showing standard error.

In order to calculate nutrient, salt, and metal removal potential of our harvest activities, the team incorporated plant tissue concentration values of various elements that were targeted for removal. In general, *Typha* tissue tended to contain more salts (Cl, Ma, Mg, Ca) than *Phragmites*, while *Phragmites* tissue tended to contain more metals (Zn, Cu, Mn, Fe; Table 2, Figure 10). Since the 14 acres of harvested basins were dominated by each plant at roughly a 50:50 ratio, harvest has great potential to remove both salts and metals from the system. In 2019, harvesting removed an estimated 2,396 lbs of Cl, 1,073 lbs of Na, 2,009 lbs of Ca, and 343 lbs of Mg. Phosphorus (P) is an essential plant nutrient that can lead to habitat degradation and eutrophication of aquatic environments, so its removal has a clear environmental benefit. In

2019, harvesting removed approximately 253 lbs of P. For metals, harvesting removed approximately 11 lbs of Zn, 1.6 lbs of Cu, 80 lbs of Mn, and 156 lbs of Fe. Overall removal rates were lower for all salts, metals, and nutrients in year two due to the reduced amount of biomass produced after consecutive harvests. Interestingly, metals tended to be more concentrated in litter tissue (especially Fe) while salts had higher concentrations in green tissue (Figure 11, 12). This finding influences recommendations on the timing of harvesting based on target pollutants (Section 4).

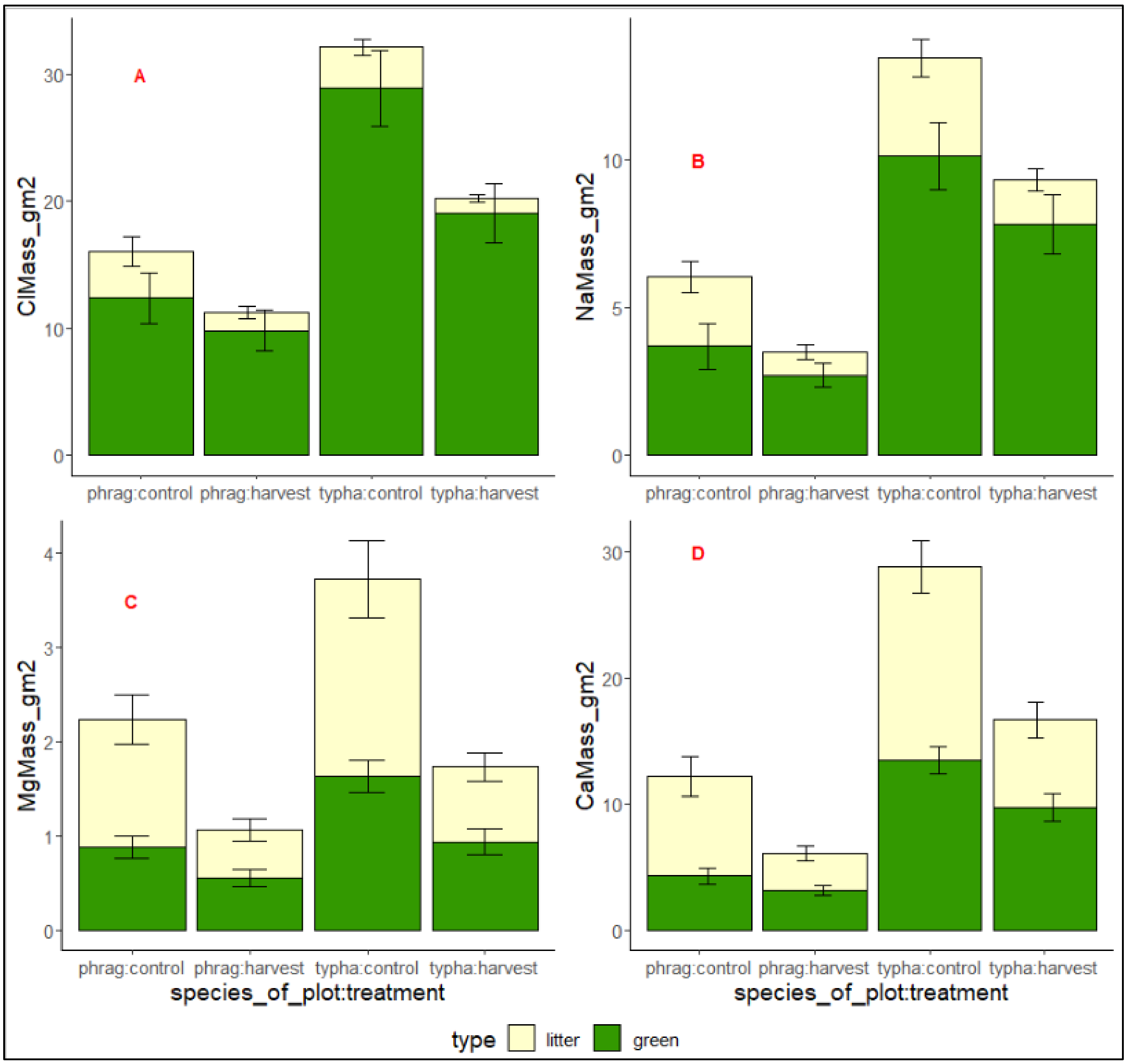

Figure 11 Salt content in aboveground biomass (grams per meter squared) in 2021 after two years of harvest in "harvest" treatment basins. "Type" refers to the tissue sample in the plot, "green" for living tissue and "litter" for dead, senesced tissue. "Species_of_plot" refers to the dominant species in the sampled plot, and "treatment" refers to control (unharvested) or harvest (experimentally harvested) plots. Means are presented with error bars showing standard error. A) Chloride (Cl), B) Sodium (Na), C) Magnesium (Mg), D) Calcium (Ca).

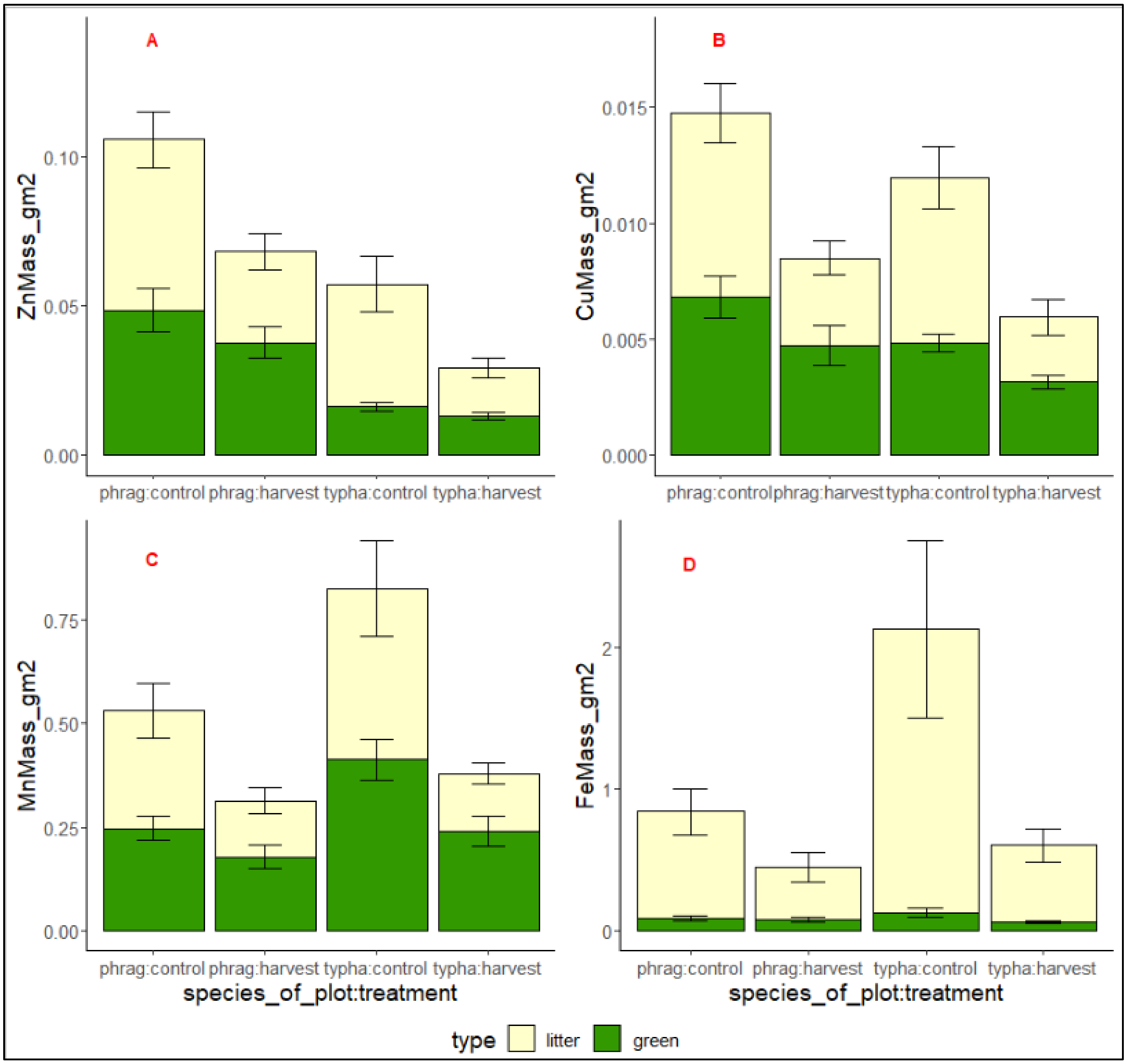

Figure 12 Metal content in aboveground biomass (grams per meter squared) in 2021 after two years of harvest in "harvest" treatment basins. "Type" refers to the tissue sample in the plot, "green" for living tissue and "litter" for dead, senesced tissue. "Species_of_plot" refers to the dominant species in the sampled plot, and "treatment" refers to control (unharvested) or harvest (experimentally harvested) plots. Means are presented with error bars showing standard error. A) Zinc (Zn), B) Copper (Cu), C) Manganese (Mn), D) Iron (Fe).

The research team observed some physical changes to basins after two consecutive years of harvest as well. Not only did the amount of biomass and litter decrease following harvest, but mean plant heights decreased as well each year following harvest (Figure 14). A single harvest reduced the subsequent year's average height of *Phragmites* stems by 40 cm and *Typha* stems by 34 cm, while harvesting two consecutive years reduced the subsequent year's average height of *Phragmites* stems by 101.3 cm and *Typha* stems by 65.3 cm. This reduction in plant height is of value in roadside environments where visibility is critical to driver safety. The team also observed an improvement in basin habitat quality following consecutive years of harvest. At basin #158, which is at the center of the Tollway system where I-88 and I-355 come together, after two consecutive years of harvest, invasive *Typha* and *Phragmites* populations were giving way to native *Solidago* spp. plants which provide higher habitat value for pollinators. The team also noticed use by ducks, geese, and even deer in the basin in 2021. The team examined drainage patterns in harvested and unharvested basins, but variability in basin design and vegetation did not allow the team to draw any conclusions on how harvest affects drainage. Future studies on basin drainage using multiple water depth monitors over particularly wet seasons are merited.

Table 2 Estimated potential removal of target salts (Cl, Na, Ca, Mg), nutrients (P), and metals (Zn, Cu, Mn, Fe) associated with biomass harvest in 2019 (no previous harvest) and 2021 (after two consecutive harvests) across the five harvested basins.

Element	2019 Removal (lbs)	2021 removal (lbs)
Cl	2396.25	1920.50
Na	1072.79	768.67
Ca	2008.64	1364.80
Mg	343.24	172.40
P	253.15	144.07
Zn	11.10	6.42
Cu	1.62	0.93
Mn	79.96	43.29
Fe	155.99	65.31

3.232 Basin function benefits

The research team sought to evaluate if harvesting affected two metrics of basin function: water drainage rate following rain events and visual line of sight as represented by plant height.

The team assessed basin drainage function by using near continuously (every 15 minutes) measured water level data from six tollway basins (3 harvested and 3 controls) collected from April 22 to November 16, 2021. Researchers identified 10 of the biggest rainfall events through this period, and calculated two measures for each basin: time for water to return to yearly mean; time for water to return to 0 cm depth (Figure 13). Contrary to expectations, both rates of drainage were significantly slower in the harvested basins as compared to the control basins. Despite the differences, it is not clear that harvesting caused the observed differences. The team did not collect pre-treatment data in the harvest basins, so it is possible that these basins had slower drainage than the chosen control basins regardless of vegetation harvest. When assigning control or harvest treatments to basins, the Technical Review Panel assisted in determining the feasibility and ease of harvesting. Alternatively, while the team used low pressure equipment, it is possible that repeated harvest compacted sediment and reduced drainage rates. In the future, the team recommends collecting pre-harvest and post-harvest drainage data, in order to more definitively isolate harvest effects on rates of basin drainage.

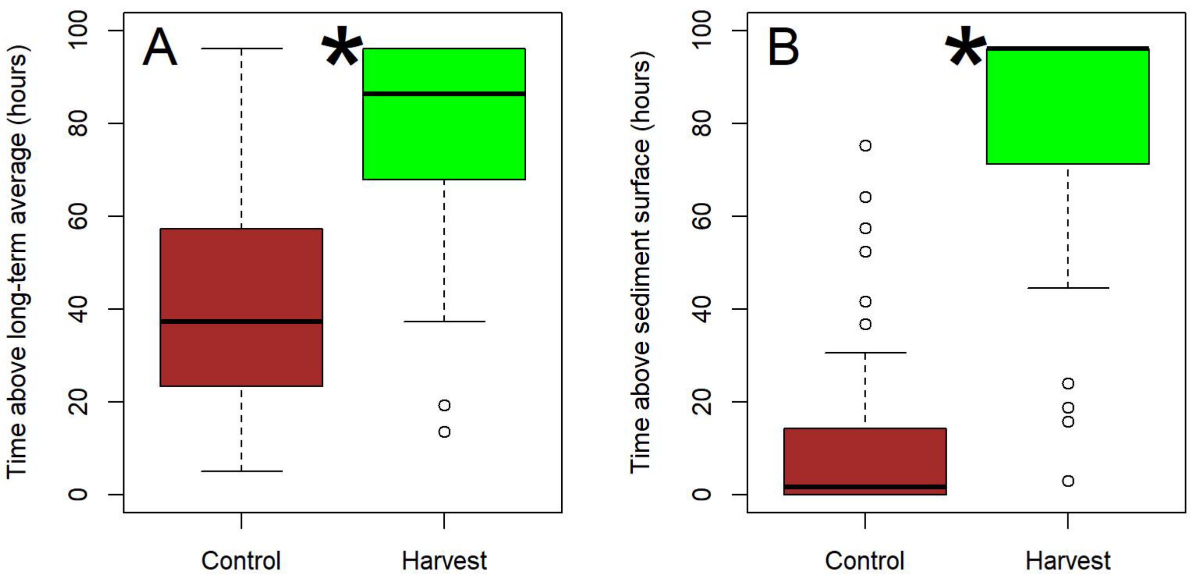

Figure 13 Rates of water level drainage from three control and three harvested basins following the 10 largest rainfall events of 2021, A) time above the annual long-term basin water level following each rainfall event, and B) time above the basin sediment surface following each rainfall event. Asterisk indicates a significant difference (Analysis of Variance; $p < 0.05$) between basin-types.

The team calculated the average height of vegetation within each vegetation type (*Phragmites*, *Typha*) and harvest treatment (control, harvest) from one-year following initial harvest and from one-year following the second consecutive harvest. Following a single harvest, average *Phragmites* heights were significantly reduced (control *Phragmites* 2.72 ± 0.11 m, harvest *Phragmites* 2.06 ± 0.11 m; $p < 0.001$), whereas there was a marginal difference between *Typha* treatment plots (control *Typha* 2.32 ± 0.07 m, harvest *Typha* 1.98 ± 0.07 m; $p = 0.053$). Following a second consecutive harvest, however, average *Phragmites* heights were reduced by approximately 1 m (control *Phragmites* 2.78 ± 0.11 m, harvest *Phragmites* 1.77 ± 0.08 m; $p < 0.001$), and average *Typha* heights were reduced by 0.66 m (control *Typha* 2.38 ± 0.07 m, harvest *Typha* 1.73 ± 0.07 m; $p < 0.001$; Fig. 13).

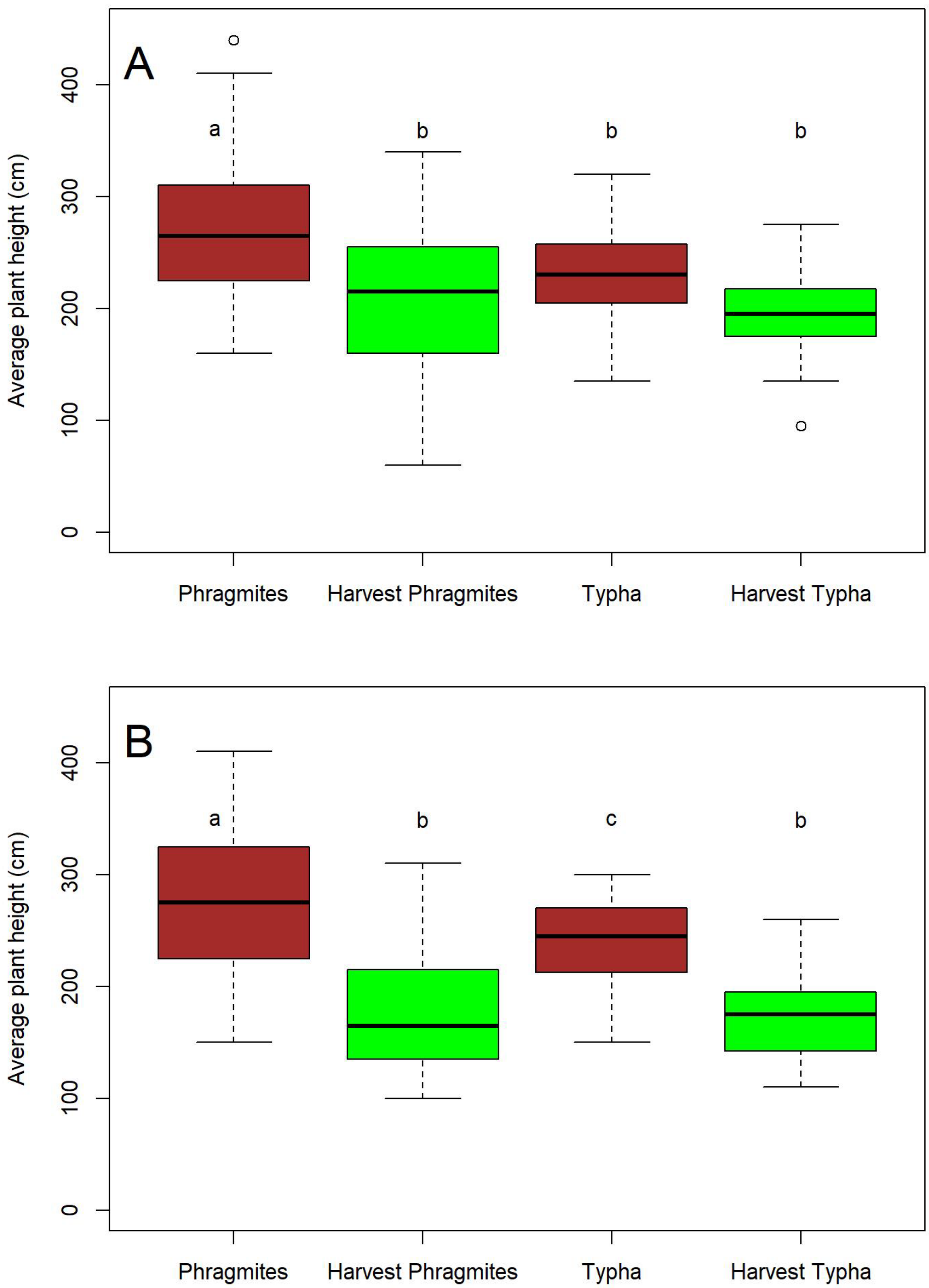

Figure 14 Average heights of *Phragmites* and *Typha* spp. in control plots (*Phragmites* and *Typha*) and in harvested plots, A) one year following a single biomass harvest, and B) one year following two consecutive years of biomass harvest. Significant differences between plots indicated by non-overlapping letters above boxplots.

3.4 Potential for biomass harvest to remediate pollutants across Tollway system

3.41 Basin suitability

The research team sought to evaluate the potential for harvest treatments to remediate pollution across the full Illinois Tollway system. The first step was to determine which detention basins were suitable for this type of management based upon basin size and basin vegetation composition. In total there are 350 Tollway basins covering 447.6 acres. Basins less than 1-acre in size were not suitable for harvesting, as smaller basins are generally poor candidates for the harvester. Of the 350 tollway basins, 141 meet the greater than 1 acre size criteria. Second, suitable basins were limited to those with appropriate vegetation cover and harvester accessibility by visually inspecting the remaining 141 basins using aerial imagery from the Tollway Cartegraph and Nearmap systems (Figure 15). Basins that contained ponds were eliminated as the harvester does not operate in deep water, and basins without targeted wetland plant species (*Typha* and/or *Phragmites*) were also removed. This pared down the system to 89 basins, which were most appropriate sites for future plant harvest. These 89 basins account for 250 harvestable acres. The team created a decision tree to illustrate the selection process for appropriate basins for future harvesting operations (Figure 16): Node A represents all basins in the Tollway system; Node B filters out those that were smaller than 1 acre; Node C represents the selected basins. A complete list of the harvestable basins that were selected based on this process is provided in Appendix A.

Figure 15 A) Imagery from the nearmap system showing basin 355N021.60DET (Old ID DET0158) containing harvestable *Typha* and *Phragmites*. This basin was successfully harvested in 2019 and 2020. B) Basin 355N011.87DET (Old ID: DET0143) with low potential for successful harvest. This basin contains some wetland vegetation but also has a wet bottom/ pond and contains many trees and shrubs, making it unsuitable for harvesting.

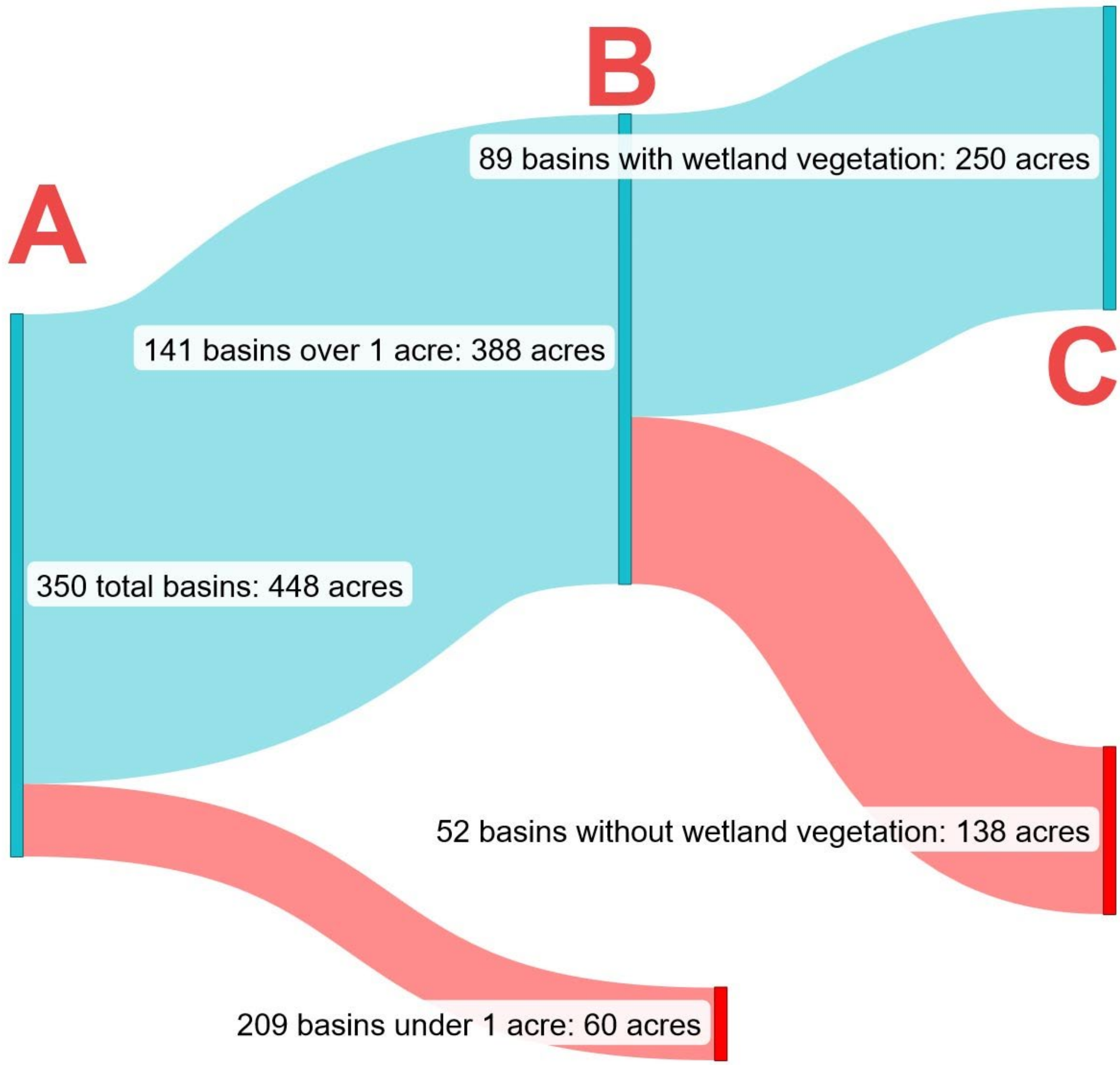

Figure 16 Decision tree for selecting Illinois Tollway detention basins that are suitable for harvesting.

3.42 System-wide estimates

After estimating the total harvestable basin acreage, the team estimated the total potential Tollway-wide harvestable biomass using the allometric equations generated in section 2.2 (soil, plant, and water sampling). There are approximately 2.41 million pounds of dry litter biomass and 2.91 million pounds green biomass (dry weight), or a total of 5.32 million pounds of biomass (dry weight), in harvestable basins. The team estimated total salt, metal, and nutrient removal potentials by multiplying the tissue chemistry concentrations (salts, metals, and nutrients) for each harvestable tissue assessed (*Typha*, *Phragmites*, standing dead litter) by the estimated biomass across the Tollway ROW (Table 3). Overall, considerable amounts of metals, salts, and nutrients are available in invasive plant above ground biomass across the Tollway system.

Harvest of these available basins represents a major opportunity to remove pollutants while generating biomass that can be redirected to composting or other beneficial use.

Table 3 Total removal potential across all basins that meet harvest selection criteria. Estimates for each element were determined by averaging concentrations in litter and green biomass separately and scaling them against the respective litter biomass estimates for the total harvestable basin area in the Tollway system.

Element	***Total removal potential (lbs)***
Biomass (dry weight)	2,910,000
Litter (dry weight)	2,410,000
Chloride (Cl)	45,898
Sodium (Na)	19,870
Calcium (Ca)	40,593
Phosphorus (P)	4,273
Magnesium (Mg)	6,567
Zinc (Zn)	180.7
Copper (Cu)	29.0
Manganese (Mn)	1,430
Iron (Fe)	2,805

4. Recommendations

4.1 Harvesting feasibility

This three year demonstration project has shown that harvesting invasive *Typha* and *Phragmites* not only is feasible in Tollway detention basins from a logistical and safety standpoint, but that harvesting is a viable strategy for reducing salts, nutrients, and heavy metal pollution downstream while generating a potentially useful compost product.

With access to proper equipment, such as a Softrak Cut and Collect system, the Tollway could potentially harvest several basins per year. In this study, harvesting led to a reduction in overall biomass and plant height in basins, especially after consecutive years. While this biomass reduction should benefit overall basin filtration and function and reduced vegetation heights improve visibility, the amount of biomass removed from basins harvested in consecutive years is reduced. Therefore, in order to maximize benefits, the research team recommend harvesting basins on a multi-year rotation. A viable strategy would involve harvesting every one of the 89 identified feasible basins over a three year rotation. This would require harvesting ~30 basins and ~80 acres per year. Treatment efficacy can be maximized by harvesting basins during months where *Typha* and *Phragmites* above ground biomass is at its peak, but before green biomass starts to senesce in the fall. This gives Tollway a window from Mid-July to early October, ~14 weeks, to harvest. Given that our team of two employees was able to harvest 14 acres over ~4 weeks using a single harvester, we feel that this is a realistic management technique to be employed by Tollway.

One of the biggest logistical challenges faced by this project is collecting and transporting harvested biomass to end users. Tollway staff played a crucial role in this throughout the project by using their equipment (empty salt trucks and front end loaders) and staff to load and transport harvested materials. A partnership between Tollway and MWRD was crucial to the success of this project in 2019, but progress was hampered when MWRD stopped accepting herbaceous material for its biosolids composting program. Partnering with Waste Management is a viable alternative, but the tipping fee charged ($42/ wet ton) increases the cost of this method. While composting Tollway-harvested biomass is feasible, further chemical analysis of the compost prior to widespread implementation and spreading is warranted, as salts and metals accumulated in harvest plant tissues likely persist in the compost. Production of biochar or green energy through anaerobic digestion are viable options for Tollway to consider, and may produce more value than compost. Overall, this project represents a sustainable and feasible management tool that provides many benefits to the environment and the function of Tollway assets.

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Appendices

Appendix A: Harvestable basins in the Illinois Tollway system

ID	Type	Route	Area During 100 Year Storm (ac)	Old ID	Condition Group
355S021.45DET	Dry/Grass Bottom	I-355	1.76	DET0083	Detention Basin-Dry
355S000.10DET1	Dry/Grass Bottom	I-355	2.33	DET0178	Detention Basin-Dry
355N000.95DET	Dry/Grass Bottom	I-355	2.2	DET0166	Detention Basin-Dry
355S012.70DET	Dry/Grass Bottom	I-355	4.33	DET0148	Detention Basin-Dry
355S000.45DET	Dry/Grass Bottom	I-355	1.93	DET0165	Detention Basin-Dry
390W012.25DET	Dry/Grass Bottom	IL 390	1.15	DET0059	Detention Basin-Dry
355S003.45DET	Dry/Grass Bottom	I-355	1.66	DET0173	Detention Basin-Dry
355S003.20DET	Dry/Grass Bottom	I-355	1.88	DET0172	Detention Basin-Dry
090E012.50DET	Dry/Grass Bottom	I-90	1.43	DET0208	Detention Basin-Dry
355_000.10DET1	Dry/Grass Bottom	I-355	8.71	DET0179	Detention Basin-Dry
390W012.45DET3	Dry/Grass Bottom	IL 390	2.41	DET0064	Detention Basin-Dry
355N029.90DET1	Dry/Grass Bottom	I-355	2.05	DET0138	Detention Basin-Dry
294N014.20DET	Dry/Grass Bottom	I-294	3.47	DET0216	Detention Basin-Dry
088W125.10DET	Dry/Grass Bottom	I-88	2.05	DET0236	Detention Basin-Dry
355_000.10DET3	Dry/Grass Bottom	I-355	20.66	DET0184	Detention Basin-Dry
390W012.10DET	Dry/Grass Bottom	IL 390	1.81	DET0057	Detention Basin-Dry
088W085.70DET	Dry/Grass Bottom	I-88	2.02	DET0233	Detention Basin-Dry
090W029.10DET	Dry/Grass Bottom	I-90	2.66	DET0212	Detention Basin-Dry
355N008.25DET	Dry/Grass Bottom	I-355	2.29	DET0140	Detention Basin-Dry
355S026.25DET	Dry/Grass Bottom	I-355	1.41	DET0185	Detention Basin-Dry
088W130.00DET	Dry/Grass Bottom	I-88	1.9	DET0230	Detention Basin-Dry
294S007.75DET	Dry/Grass Bottom	I-294	2.02	DET0123	Detention Basin-Dry
390E012.30DET	Dry/Grass Bottom	IL 390	2.33	DET0060	Detention Basin-Dry
355S000.10DET3	Dry/Grass Bottom	I-355	3.13	DET0183	Detention Basin-Dry
294S047.80DET	Dry/Grass Bottom	I-294	1.17	DET0200	Detention Basin-Dry
355N021.80DET	Dry/Grass Bottom	I-355	1.78	DET0159	Detention Basin-Dry
355_000.10DET2	Dry/Grass Bottom	I-355	5.35	DET0181	Detention Basin-Dry
294N022.00DET	Dry/Grass Bottom	I-294	1.39	DET0201	Detention Basin-Dry
094W013.75DET	Dry/Grass Bottom	I-94	1.23	DET0122	Detention Basin-Dry
088E123.40DET	Dry/Grass Bottom	I-88	2.29	DET0229	Detention Basin-Dry
355N012.60DET	Dry/Grass Bottom	I-355	5.93	DET0147	Detention Basin-Dry
355S012.43DET	Dry/Grass Bottom	I-355	4.47	DET0146	Detention Basin-Dry
294S006.30DET	Dry/Grass Bottom	I-294	1.36	DET0215	Detention Basin-Dry
355N024.80DET	Dry/Grass Bottom	I-355	1.44	DET0324	Detention Basin-Dry
355N021.60DET	Dry/Grass Bottom	I-355	8.03	DET0158	Detention Basin-Dry
355S004.75DET1	Dry/Grass Bottom	I-355	1.47	DET0161	Detention Basin-Dry
355S000.10DET2	Dry/Grass Bottom	I-355	1.77	DET0180	Detention Basin-Dry
355S017.10DET	Dry/Grass Bottom	I-355	1.39	DET0152	Detention Basin-Dry

ID	Type	Route	Area During 100 Year Storm (ac)	Old ID	Condition Group
390E014.35DET	Dry/Grass Bottom	IL 390	2.28	DET0070	Detention Basin-Dry
355S002.75DET	Dry/Grass Bottom	I-355	2.29	DET0175	Detention Basin-Dry
390W012.45DET1	Dry/Grass Bottom	IL 390	2.29	DET0062	Detention Basin-Dry
390E015.65DET	Dry/Grass Bottom	IL 390	3.08	DET0072	Detention Basin-Dry
355N009.40DET	Dry/Grass Bottom	I-355	2.22	DET0141	Detention Basin-Dry
094W008.55DET	Dry/Grass Bottom	I-94	1.09	DET0272	Detention Basin-Dry
355S025.75DET	Dry/Grass Bottom	I-355	1.8	DET0187	Detention Basin-Dry
088E086.50DET	Dry/Grass Bottom	I-88	6.32	DET0232	Detention Basin-Dry
390E013.80DET	Dry/Grass Bottom	IL 390	1	DET0069	Detention Basin-Dry
355N000.10DET	Dry/Grass Bottom	I-355	3.61	DET0182	Detention Basin-Dry
088E085.70DET	Dry/Grass Bottom	I-88	2.84	DET0231	Detention Basin-Dry
355N026.95DET	Dry/Grass Bottom	I-355	4.97	DET0188	Detention Basin-Dry
294N044.30DET	Dry/Grass Bottom	I-294	1.19	DET0193	Detention Basin-Dry
294S007.55DET	Dry/Grass Bottom	I-294	5.71	DET0125	Detention Basin-Dry
294N007.60DET	Dry/Grass Bottom	I-294	1.56	DET0124	Detention Basin-Dry
355N029.80DET1	Dry/Grass Bottom	I-355	3.17	DET0130	Detention Basin-Dry
294S044.30DET	Dry/Grass Bottom	I-294	1.54	DET0194	Detention Basin-Dry
355S014.50DET	Dry/Grass Bottom	I-355	1.44	DET0151	Detention Basin-Dry
090E073.70DET	Dry/Grass Bottom	I-90	2.07	DET0310	Detention Basin-Dry
088W125.30DET	Dry/Grass Bottom	I-88	3.92	DET0237	Detention Basin-Dry
088E135.05DET	Dry/Grass Bottom	I-88	2.74	DET0227	Detention Basin-Dry
355S015.80DET	Pond/Wet Bottom	I-355	3.22	DET0066	Detention Basin-Wet
355N007.30DET	Pond/Wet Bottom	I-355	1.49	DET0028	Detention Basin-Wet
088E114.60DET	Pond/Wet Bottom	I-88	1.01	DET0107	Detention Basin-Wet
355S007.50DET	Pond/Wet Bottom	I-355	2.59	DET0032	Detention Basin-Wet
294S038.25DET	Pond/Wet Bottom	I-294	1.1	DET0120	Detention Basin-Wet
094W025.10DET	Pond/Wet Bottom	I-94	8.11	DET0116	Detention Basin-Wet
090W046.25DET	Pond/Wet Bottom	I-90	2.5	DET0094	Detention Basin-Wet
090W032.90DET	Pond/Wet Bottom	I-90	1.12	DET0092	Detention Basin-Wet
088E056.40DET	Pond/Wet Bottom	I-88	1.49	DET0235	Detention Basin-Wet
090W032.30DET	Pond/Wet Bottom	I-90	1.25	DET0091	Detention Basin-Wet
294S032.30DET	Pond/Wet Bottom	I-294	2.15	DET0119	Detention Basin-Wet
094E021.80DET	Pond/Wet Bottom	I-94	1.07	DET0113	Detention Basin-Wet
090W027.15DET	Pond/Wet Bottom	I-90	2.08	DET0088	Detention Basin-Wet
390W008.55DET	Pond/Wet Bottom	IL 390	1.66	DET0047	Detention Basin-Wet
355N018.95DET	Pond/Wet Bottom	I-355	3.14	DET0155	Detention Basin-Wet
090W030.60DET	Pond/Wet Bottom	I-90	2.04	DET0089	Detention Basin-Wet
355S028.65DET	Pond/Wet Bottom	I-355	1.85	DET0085	Detention Basin-Wet
355N019.45DET	Pond/Wet Bottom	I-355	5.59	DET0156	Detention Basin-Wet
294N029.60DET	Pond/Wet Bottom	I-294	1.1	DET0118	Detention Basin-Wet
355S017.40DET	Pond/Wet Bottom	I-355	3.33	DET0153	Detention Basin-Wet

ID	Type	Route	Area During 100 Year Storm (ac)	Old ID	Condition Group
090W041.85DET	Pond/Wet Bottom	I-90	1.26	DET0104	Detention Basin-Wet
090W047.55DET	Pond/Wet Bottom	I-90	1.32	DET0097	Detention Basin-Wet
090W058.60DET	Pond/Wet Bottom	I-90	1.22	DET0312	Detention Basin-Wet
355S018.05DET	Pond/Wet Bottom	I-355	2.69	DET0154	Detention Basin-Wet
090E046.35DET	Pond/Wet Bottom	I-90	3.34	DET0096	Detention Basin-Wet
390W011.00DET	Wetland Bottom	IL 390	4.29	DET0052	Detention Basin-Wet
090E058.20DET	Wetland Bottom	I-90	3.9	DET0283	Detention Basin-Wet
090W059.80DET2	Wetland Bottom	I-90	3.81	DET0288	Detention Basin-Wet
390W008.70DET	Wetland Bottom	IL 390	2.82	DET0049	Detention Basin-Wet
090E056.30DET	Wetland Bottom	I-90	2.97	DET0295	Detention Basin-Wet
Total Area			249.3		

Appendix B: Influence of biochar, Phragmites australis, and Typha × glauca on salinity in simulated wetland systems.

Influence of biochar, *Phragmites australis* and *Typha* × *glauca* on salinity in simulated wetland systems

by

Samuel Schurkamp

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
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Abstract

Salinization is an emerging threat towards the function and quality of freshwater wetlands due to its deleterious effects on aquatic biota and plant life. Phytoremediation is a proposed solution, but different salt tolerance strategies among halophytes result in variable estimates of salt uptake. Salt-accumulating species in particular may have a significant effect on the salinity of their surrounding environment due to salt uptake. Biochar, a high-carbon soil amendment made from the partial combustion of organic material, has been proposed as a potential solution to mitigate salt stress. However, little is known about its effects on wetland soils generally and in particular how it interacts with salt ions in soil and water. This thesis describes the results of a fully factorial greenhouse experiment investigating the uptake potential of two salt-tolerant species, *Phragmites australis* and *Typha* × *glauca*, and explores whether biochar addition at three rates (0%, 2.5%, 5% wt/wt) improves uptake potential or mitigates salt pollution through other plant-independent mechanisms. After 88 days, *T.* × *glauca* biomass had a significantly higher concentration of Na^+ ($P < 0.001$) and Cl^- ($P < 0.001$) in aboveground tissue than *P. australis* and significantly lowered soil Na^+ ($P < 0.05$) concentration. *T.* × *glauca* and *P. australis* both significantly decreased Cl^- leachate relative to unvegetated controls ($P < 0.01$), while 2.5% biochar addition increased leachate chloride concentration ($P < 0.001$). Neither the 2.5% nor 5% biochar additions increased plant growth nor salt uptake, but the 5.0% wt/wt application did increase Na^+ tissue concentration in *T.* × *glauca* ($P < 0.05$). My results indicate that (1) plant uptake may be a viable means of mitigating wetland salinization, (2) amending wetland soils with biochar application may facilitate salt leaching in plant species-specific contexts, and (3) biochar may provide additional benefits in the uptake of salt ions by salt-accumulating plant species. I suggest future research explores the potential impacts to wetland salinity of field-scale biochar application and the harvest of salt-accumulating plant species.

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Acknowledgments

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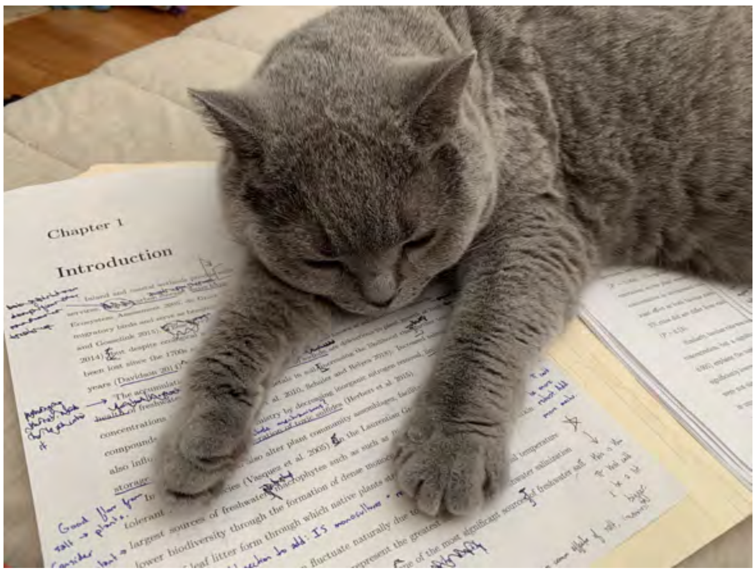
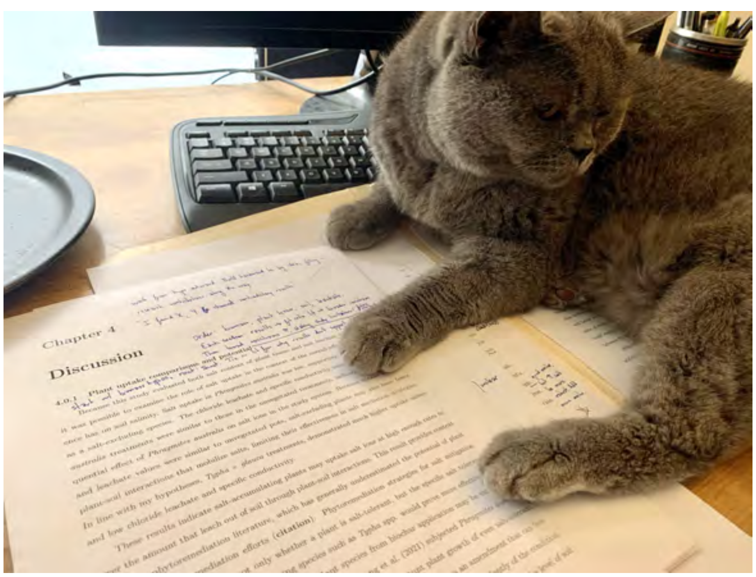
"If you have ever gone to the woods with me, I must love you very much."
–Mary Oliver

Dedication

To all the plants and animals of the world– but especially my two lovely cats, who spent almost as much time on my thesis as I did.





Chapter 1

Introduction

1.1 Introduction

Inland and coastal wetlands provide essential habitat for migratory birds and serve as breeding grounds for rare fish, reptile, and amphibian species (Mitsch and Gosselink 2015). Wetlands also provide carbon storage, water filtration, nutrient cycling, and many other globally significant ecosystem services that benefit humanity and biodiversity (Millennium Ecosystem Assessment 2005, de Groot et al. 2012). These services are valued at $26.4—36.2 trillion/yr globally (Costanza et al. 2014), but global wetland acreage is projected to decline by half over the next 140 years due to drainage for agriculture and urban development (Davidson 2014). In the Laurentian Great Lakes, estimates suggest 30-50% of wetlands have been lost (Albert 2003, Wolter et al. 2006). Additionally, climate change is likely to diminish wetland area and quality through changes to precipitation and air temperature (Mitsch and Hernandez 2013).

An emerging threat to the function of inland and coastal wetlands is the accumulation of soluble salts above natural levels, also known as salinization. Although salinity levels fluctuate naturally, anthropogenic sources represent the greatest contribution to freshwater salinization in the present day (Herbert et al. 2015). The most significant source of freshwater salt pollution in the United States is the application of de-icing road salts, where northern states apply between 175,000–1,000,000 metric tons of salt solids and 5-45 million metric liters of salt brines each year (Hintz et al. 2021). Although only applied during the winter, road salts persist in high concentrations throughout the year and remain deleterious to aquatic organisms, plant growth, and macroinvertebrate development months after their application (Findlay and Kelly 2011). For example, Corsi et al. (2010) found that chloride concentration exceeded chronic water quality criteria at between 55-100% of monitored wetlands and inland lakes throughout the year in the lower Wisconsin region. Yearly increases in road salt usage are deemed necessary to keep roadways safe (Kelly et al. 2005, Lubowski et al. 2006), limiting the options available to mitigate the resulting increase in salt runoff.

Sodium (Na^+) and chloride (Cl^-) are deleterious to plant growth at high concentrations and can mobilize heavy metals in soil by altering ion exchange and soil chemistry, increasing the volume of toxic compounds entering waterways (Corsi et al. 2010, Schuler and Relyea 2018). Increased salinization also influences wetland biogeochemistry by decreasing inorganic nitrogen removal, limiting carbon storage, and increasing the generation of phytotoxic sulfides (Herbert et al. 2015). Increased salinity can also facilitate the spread of salt-tolerant invasive species such as the common reed *Phragmites australis* (Vasquez et al. 2005). *Phragmites* and *Typha* spp. (cattail) are dominant throughout the Laurentian Great Lakes basin, and their presence has far-reaching consequences for wetland structure and function (Zedler and Kercher 2004). These invasive plants form dense monocultures that displace native plant communities, homogenize habitats, and alter the nutrient composition of soil (Tuchman et al. 2009, DeRoy and MacIsaac 2020). Particularly, the dense layer of leaf litter that accumulates in mature stands of both species perpetuates changes to soil temperature, light penetration, and nutrient dynamics, creating unfavorable conditions for native plant growth (Holdredge and Bertness 2011, Larkin et al. 2012). The aggressive nature of these plants complicates restoration efforts, as multiple years of management are typically required to reduce their competitive advantages (Bonello and Judd 2020).

The most widely-utilized method to reduce the spread of invasive *Phragmites australis* and *Typha* spp. is herbicide (Hazelton et al. 2014). While generally effective, this technique may reduce native plant diversity and increase porewater nutrient concentrations, creating conditions in which reinvasion is likely (Lawrence et al. 2016). Furthermore, herbicide application does not prevent the buildup of leaf litter. Harvest and removal of invasive macrophytes offers unique advantages without the drawbacks associated with herbicide application. Physical removal of *Phragmites* and *Typha* spp. promotes plant diversity in coastal wetlands (Lishawa et al. 2015), improves waterbird food resources (Lishawa et al. 2020), and increases property values (Isely et al. 2017). Harvesting may also influence chemical dynamics at a landscape scale. For example, Carson et al. (2018) found that large-scale harvest of invasive *Phragmites*, *Typha* spp. and *Phalaris arundinacea*, another invasive graminoid, has the potential to reduce nutrient loads to Great Lakes Coastal Wetlands (GLCWs). These large plants uptake a significant amount of phosphorus and nitrogen, which can be removed from the system via harvest rather than retained in the litter after plants senesce.

This harvest-removal mechanism has potential to decrease additional pollutants. For example, *Typha* spp. and *Phragmites* harvest has been studied for heavy metal mitigation potential (Sasmaz et al. 2008, Kumari and Tripathi 2015, Hejna et al. 2020). In the context of salt mitigation, phytoremediation generally minimizes the potential of salt uptake as a mitigation strategy. The primary mechanism of salt removal is the plant-soil interactions that move Na^+ ions from cation exchange sites on soil aggregates to the soil solution, or leachate, which then flows out of the system (Qadir et al. 2001; 2005). However, differences in experimental design and a focus on crop species suggest the overall effect of uptake may be greater than accepted (Rabhi et al. 2009, Jesus et al. 2015).

Salt-tolerant species have different strategies to overcome elevated salt levels, varying the degree to which different species uptake salt ions: "excluder" species prevent salt uptake into plant tissue, while "accumulator" and "conductor" species uptake high levels of salts and offset this uptake through osmotic adjustment or evapotranspiration (Yensen and Biel 2008). Plant species is therefore crucial to understand the salt removal potential of a given system, as excluder-type plants or agricultural crops which are not salt-tolerant will likely uptake negligible levels of salt ions relative to accumulator species. Some evidence already exists that plants uptake salt ions at a large scale: bioswales, constructed channels designed to filter stormwater runoff through vegetated areas, have been shown to reduce downstream salt levels (Mazer et al. 2001, Anderson et al. 2016). Miner et al. (2016) found that bioswales installed along the I-294 highway in northeastern Illinois reduced Cl^- in runoff by up to 44% through a combination of plant uptake and soil interactions.

Salt uptake and invasive plant harvest may be effective when used in conjunction with more common methods of salt mitigation, which typically focus on leaching salt out of the system. Chemical amendments such as phosphogypsum lower salt levels through cation exchange, as Ca^{2+} ions replace more detrimental Na^+ ions which then leach out into the soil solution (Qadir et al. 2001, Jesus et al. 2015). However, these methods are resource-intensive and context-dependent, limiting viability outside of agricultural settings (Laudicina et al. 2009).

Biochar addition to soil may provide similar benefits with some additional advantages. Biochar is a high-carbon soil amendment created by combusting organic material at high temperatures in a low-oxygen environment (Lehmann and Joseph 2015), and it has high potential to combat deleterious soil conditions due to its high porosity, cation exchange capacity, and pH (Smith 2016, Palansooriya et al. 2019). Biochar has the potential to decrease plant salt stress (Chen et al. 2018a), but the mechanisms by which plants are freed from salt stress are unclear. Xiao and Meng (2020) suggested biochar application alleviates salt stress by leaching Na^+ ions through replacement on cation exchange sites, but few other studies exist to corroborate such findings. Currently, it is unclear whether biochar lowers baseline soil salinization, or whether this effect is due to an increase in plant growth or uptake, or changes to soil properties which facilitate the leaching of detrimental salt ions. Understanding how biochar releases plants from salt stress would provide necessary information on its potential as a tool in salt mitigation.

1.2 Hypotheses

1) I hypothesized that biochar application would increase the growth of both *Typha* and *Phragmites* but not significantly change plant tissue chemistry nor root:shoot ratio relative to unvegetated controls. Biochar has been shown to increase growth, but changes to tissue concentrations of salt ions are rarely reported.

2) I hypothesized that *Phragmites* tissue would have lower concentrations of salt ions (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}) than *Typha*. While both plants are salt-tolerant, *Phragmites* likely excludes salts from its tissue through osmotic adjustment while *Typha* may accumulate salt ions and offsets these gains through water accumulation.

3) I hypothesized that biochar would decrease soil Na^+ concentration compared to controls. I also hypothesized that leachate would have higher specific conductivity and Cl^- levels with biochar addition compared to control. There is evidence that biochar relieves plant salt stress by promoting salt leaching into the soil solution, which occurs as Ca^{2+} and Mg^{2+} ions displace Na^+ ions on cation exchange sites. Increased soil cation exchange due to biochar addition may therefore increase Na^+ leaching from the soil into the leachate.

4) I hypothesized that plant presence would decrease specific conductivity and Cl^- leachate compared to unvegetated controls. The effect of plant uptake on salt remediation is likely underestimated, while the plant-soil interactions that promote salt leaching are understood. Lowered specific conductivity and Cl^- leachate in planted treatments would indicate biological uptake.

Chapter 2

Materials and Methods

2.1 Overview

I devised a fully factorial greenhouse experiment to explore 1) the effect of wood-derived biochar on biomass and tissue chemistry of *Phragmites australis* (hereafter *Phragmites*) and *Typha* × *glauca* (hereafter *Typha*), and 2) the cumulative effects of wood-derived biochar, *Phragmites* and *Typha* on the movement of salt ions in simulated wetland microcosms. The experimental factors were: (3 rates of biochar (0, 2.5, and 5% wt/wt) × 3 plant treatments (unvegetated, *Typha* × *glauca*, and *Phragmites australis*) × 7 replicates = 63 microcosms). The experiment ran for 88 days from August 11, 2021 to November 5, 2021 in the EcoDome greenhouse at Loyola University Chicago's School of Environmental Science. Average temperature of the greenhouse was 24°C and average humidity was 44%. Supplemental light was provided on a 14-hour cycle using LumiGrow Pro 325 LED lights (Emeryville, CA, USA) to match the available light of the peak growing season. A PAR Quantum Light Meter from Sun System (Vancouver, WA, USA) was used to verify light was evenly distributed across all microcosms.

2.2 Materials

2.2.1 Microcosm design

I constructed sixty-three microcosms out of 6" diameter polyvinyl chloride (PVC) with a height of 12" (Figure 2.1). The base was made from an 8" x 8" square of 3/4" plywood topped with 1/8" Wal-Tuf paneling (Kutztown, PA, USA). I used a router with a circular guided jig to cut an insert for the microcosm, which was glued in place. I installed a water sampling port six cm above the base of the microcosm and sealed it with plumber's tape. All seams were sealed with 100% silicone sealant and all microcosms were filled with deionized water for 72 hours to test for leakage.

Figure 2.1: Wetland microcosm setup in the EcoDome greenhouse.

2.2.2 Soil preparation

I obtained soil from a drainage basin in the Illinois Tollway system located at (41°49'17.70" N, 88°01'38.17"W) on August 1, 2021 (Figure 2.2). The basin is located at the intersection of two major highways and the base of a road salt storage facility adjacent to the Illinois Tollway system. Soil samples collected from this site in 2019 for an ongoing study provided baseline chemical properties (Table 2.1). All soil was air-dried and passed through a 0.3 cm screen to homogenize.

Figure 2.2: An eye-level view of Basin 159, located at the intersection of I-88 and I-355 of the Illinois Tollway system. Dense strands of *Phragmites* and *Typha* spp. inhibit navigability and drainage. Soils for the greenhouse microcosm experiment were collected near the base of the culvert in the foreground.

Table 2.1: Baseline soil characteristics (mean ± standard error) from Tollway Basin 159 taken in 2019. Eight replicates were analyzed. These data were preliminary measures not initially taken for this greenhouse experiment (Monks et al., 2022 in preparation).

pH	**Na^+** *ppm*	**Cl^-** *ppm*	**K^+** *ppm*	**Ca^{2+}** *ppm*	**Mg^{2+}** *ppm*
7.28 ± 0.11	1033 ± 248	1506 ± 418	90.83 ± 6.31	3138 ± 277	257 ± 34

2.2.3 Biochar preparation

Wood-derived biochar was purchased from Chip Energy, Inc. (Goodfield, IL, USA) for use in this study. The biochar was made from recycled wood pallets heated between 430 and 650°C for 6–8 hours. The biochar was crushed by hand and passed through a 1 cm screen before it was mixed into soil.

2.2.4 Plant preparation

Phragmites and *Typha* rhizomes were collected from drainage basins located on the campus of Oakton Community College in Skokie, IL (42°01'18.56" N, 87°44'54.51" W) on August 11, 2021 (Figure 2.3). The full aboveground plant and a long attached section of rhizome were collected for each sample of both species. To remove *Phragmites* samples from the drainage basin, a trench shovel was used to dig a 20cm x 20cm x 20cm cube of soil around each rhizome with a living stem. Rhizomes were then separated from the soil in a water bath. *Typha* samples were collected by manually pulling up long sections of rhizomes connected to living aboveground stems and clipping with garden shears. Once harvested, plants were transported to the greenhouse, where all rhizomes of both species were immediately washed and cut into 15cm segments, each with a 12cm stalk of living green tissue above the soil surface (Figure 2.4). Stem and rhizome diameter were measured to the nearest millimeter to account for variability among samples.

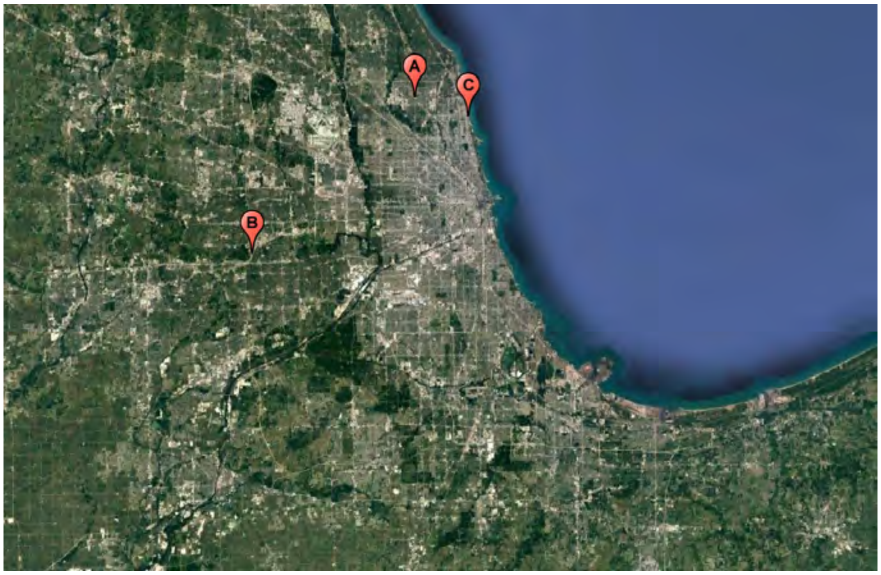


Figure 2.3: An aerial view of the sites in the Chicagoland region where *Phragmites* and *Typha* were harvested (A), soil was collected (B), and the experiment was conducted (C).

Figure 2.4: Three randomly selected *Typha* rhizomes from the Oakton Community College retention basin after processing from collected samples. Rhizomes where cut to standard lengths of aboveground (left section of stem in photograph) and belowground (right section) tissue.

2.3 Setup

Microcosms were filled with 7 cm of pea gravel once constructed and tested for leaks. Soil and biochar were mixed at one of three rates (0, 2.5%, and 5% biochar rate/wet soil rate) by weighing each in separate buckets using a hanging scale to the nearest 0.01kg, then mixing together in a "common garden" third bucket before filling each microcosm with 20 cm of one of the three treatments. The 2.5% biochar rate represents a high level of a field-applicable application rate, while the 5% is a higher rate than what is recommended for soil treatments to test whether changes to salinization or plant physiology occur at especially high biochar rates. *Typha* and *Phragmites* rhizomes were added to the microcosms and watered until soil was saturated and the water level was roughly 2 cm above the soil surface. All microcosms were then randomly placed in the greenhouse and re-randomized every two weeks. After two weeks, all microcosms with plants were inspected for signs of growth. In total, three *Typha* (one at each biochar rate) and three *Phragmites* (two 0% and one 2.5% biochar rate) did not exhibit signs of growth. These plants were replaced with rhizomes harvested following identical protocol. All microcosms were watered at least once daily to maintain a standing water depth of 2 cm.

2.4 Data collection

2.4.1 Leachate chemistry sampling

I collected 60 mL of water from each microcosm via the sampling port a total of five times (every two weeks beginning September 11, 2021). Samples were immediately analyzed for chloride (Cl^-), pH, and specific conductivity using YSI multiparameter instruments (Xylem, Yellow Springs, OH, USA).

2.4.2 Plant biomass and tissue chemistry

Plant height and number of new shoots were recorded for all *Typha* and *Phragmites* treatments after 88 days. Aboveground and belowground biomass were separated at the soil surface and all biomass for vegetated microcosms was dried at 60°C for 48 hours. Dried above and belowground samples were weighed to the nearest 0.1 g and ground using a Wiley mill in preparation for chemical analysis.

Aboveground and belowground tissue samples were prepared for ionic analyses with HCl extraction following a protocol modified from Cataldi et al. (2003). Salt cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were quantified with ion chromatography (IC) in Loyola's analytical chemistry lab. Chloride was extracted with calcium nitrate and quantified using inductively coupled plasma (ICP) mass spectrometry at the K-State Research and Extention Soil Testing Lab (Manhattan, KS, USA).

2.4.3 Soil properties

Three cups of soil by volume were collected from each microcosm for analysis after removal of all plant material. Soil pH and electrical conductivity were determined following protocols outlined in Gliessman (2015). Soil subsamples were dried at 105 °C for a minimum 48 hours and pulverized by hand with a mortar and pestle. Subsamples were then suspended in a 1:1 solution with deionized water and stirred thoroughly. After 30 minutes, electrical conductivity and pH were measured using a YSI multiparameter instrument. Remaining samples were stored at 4°C for further analyses.

The remaining soil analyses were conducted at the K-State Soil Testing Lab following protocol modified from the University of Missouri Agricultural Experiment Station (1998). Soil exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) were extracted with 1M ammonium acetate. These cations were quantified using ICP spectrometry. Cation exchange capacity (CEC) was determined via the displacement method by saturating ammonium acetate and determining the $NH_4{}^+$ released with the colorimetric assay used for KCl extracts. Chloride was extracted with calcium nitrate and analyzed with the mercury thiocyanate colorimetric method.

2.5 Statistical analyses

All statistical analyses were conducted using R version 4.1.2 and R Studio version 2021.09.1 (R Core Team 2018, RStudio Team 2020). Shapiro-Wilk, Bartlett (one-way ANOVAs), and Levene tests (two-way ANOVAs, LMEs) were used to assess residual normality and homogeneity of variance before each statistical test. Transformations were performed when necessary in order to meet these assumptions. Raw data is presented in figures and tables. Two microcosms were excluded from analyses due to leaks that compromised water retention: one *Typha* at the 5% biochar rate and one unvegetated microcosm at the 5% biochar rate (Table 2.2). A *Phragmites* microcosm with 0% biochar was also eliminated, as no aboveground biomass ever grew.

Table 2.2: Statistical replicates of the fully factorial experimental design within the study. Differences in replication are due to either lack of plant growth or faulty microcosms and were excluded from all analyses.

Plant	**Biochar rate (% wt/wt)**	**Replication**
Typha × glauca	0%	7
	2.5%	7
	5%	6
Phragmites australis	0%	6
	2.5%	7
	5%	7
unvegetated	0%	7
	2.5%	7
	5%	6

Leachate chemistry data were analyzed with linear mixed-effects (LME) models using the lme4 package (Bates et al. 2015). Biochar rate, plant type, date since experiment start, and an interaction term between plant type and biochar rate were used as fixed effects, while individual microcosm identity was included as a random effect. LME models were used to address repeated sampling measures. P-values are reported for each LME but non-significant values with large overall effects were also considered in the results due to inconsistencies in the interpretation of significance calculations within the lme4 package (Luke 2017).

Analyses of covariance (ANCOVAs) were initially explored to account for initial stem diameter as a covariate, though no influence was detected. Therefore, one-way ANOVAs were ultimately used to examine the effect of biochar rates on aboveground biomass, total biomass, and root:shoot ratio. Significant differences were evaluated with post-hoc Tukey HSD tests.

Type II and III ANOVAs were prioritized over type I to account for the unbalanced factorial design. Type III two-way ANOVAs with biochar rate and plant species as factors were used to examine tissue chemistry concentrations and aboveground uptake of salt cations and chloride. If the interaction between factors was insignificant, a type II ANOVA was run. Soil data were analyzed with type III two-way ANOVAs using biochar rate and plant species as factors. Interactions were dropped and the model was rerun as a type II ANOVA if the interaction was not statistically significant (minimum significance threshold of $P < 0.05$).

Chapter 3

Results

3.1 Influence of biochar on biomass growth

One-way ANOVAs indicated biochar at 2.5% and 5% wt/wt had no significant effects on either *Typha* or *Phragmites* growth relative to a 0% biochar control. No significant differences were detected in *Phragmites* total biomass ($P = 0.45$), aboveground biomass ($P = 0.14$), belowground biomass ($P = 0.80$), and root:shoot ratio ($P = 0.48$) between controls and either application rate (Table 3.1). Similarly, p-values for *Typha* total biomass ($P = 0.30$), aboveground biomass ($P = 0.63$), belowground biomass ($P = 0.35$), and root:shoot ratio ($P = 0.79$) were all insignificant (Table 3.2).

Table 3.1: Means ± SE of dried biomass metrics of *Phragmites*.

Biochar rate *(% wt/wt)*	**Total biomass** *(g)*	**Aboveground** *(g)*	**Belowground** *(g)*	**Root:shoot**
0%	34.03 ± 8.38	14.07 ± 3.06	19.97 ± 6.08	1.44 ± 0.26
2.5%	38.90 ± 4.00	16.06 ± 1.92	22.84 ± 3.10	1.55 ± 0.25
5%	45.64 ± 6.48	21.00 ± 2.18	24.64 ± 5.21	1.17 ± 0.19

3.2 Tissue chemistry

3.2.1 Sodium

The concentration (ppm) of Na^+ in aboveground tissue was significantly affected by biochar rate differently in the two plant species (interaction $P < 0.01$), see Figure 3.1. The

Table 3.2: Means ± SE of dried biomass metrics of *Typha*. No significance was found between either biochar rates and control.

Biochar rate *(% wt/wt)*	**Total biomass (g)** *(g)*	**Aboveground** *(g)*	**Belowground** *(g)*	**Root:shoot**
0	51.53 ± 6.04	10.60 ± 0.87	40.93 ± 6.07	4.05 ± 0.65
2.5%	55.92 ± 5.72	10.77 ± 0.81	45.16 ± 5.83	4.36 ± 0.68
5%	43.08 ± 4.52	9.38 ± 1.50	33.70 ± 3.13	3.77 ± 0.27

2.5% and 5% biochar rates did notsignificantly differ from controls in *Phragmites* treatments ($P > 0.05$). However, Na^+ ppm was significantly greater in 5% *Typha* aboveground tissue relative to a control ($P = 0.03$). A positive correlation was detected between the control aboveground tissue and the 2.5% application ($P = 0.07$). The 2.5% and 5% rates did not differ ($P > 0.05$).

A significant difference was detected for the effect of plant species on belowground tissue Na^+ concentration ($P = 0.001$). Post-hoc Tukey HSD tests indicated Na^+ concentration (ppm) in belowground tissue was significantly lower ($P < 0.001$) in *Phragmites* (91.47 ppm ±2.06) than *Typha* (128.78 ppm ±4.54), but biochar application did not significantly impact belowground Na^+ concentration at either rate relative to control ($P > 0.05$). Na^+ concentration in belowground tissue was significantly lower than the aboveground concentrations for both *Phragmites* (2015.37 ppm ±80.06, $P < 0.001$) and *Typha* (3811.19 ppm ±159.04, $P < 0.001$).

The root:shoot ratio of Na^+ ppm was significantly affected by biochar rate differently in the two plant species (interaction $P < 0.01$) see Figure 3.2. However, post-hoc Tukey testing of the model revealed no significant changes in Na^+ concentration within the context of each individual plant species at any biochar levels relative to control ($P > 0.05$).

A significant difference in Na^+ uptake was found between plant species ($P = 0.04$). Post-hoc Tukey testing indicated the total uptake of Na^+ was significantly higher ($P = 0.05$) when comparing *Typha* treatments (0.044g ± 0.003) to *Phragmites* treatments (0.036g ± 0.003).

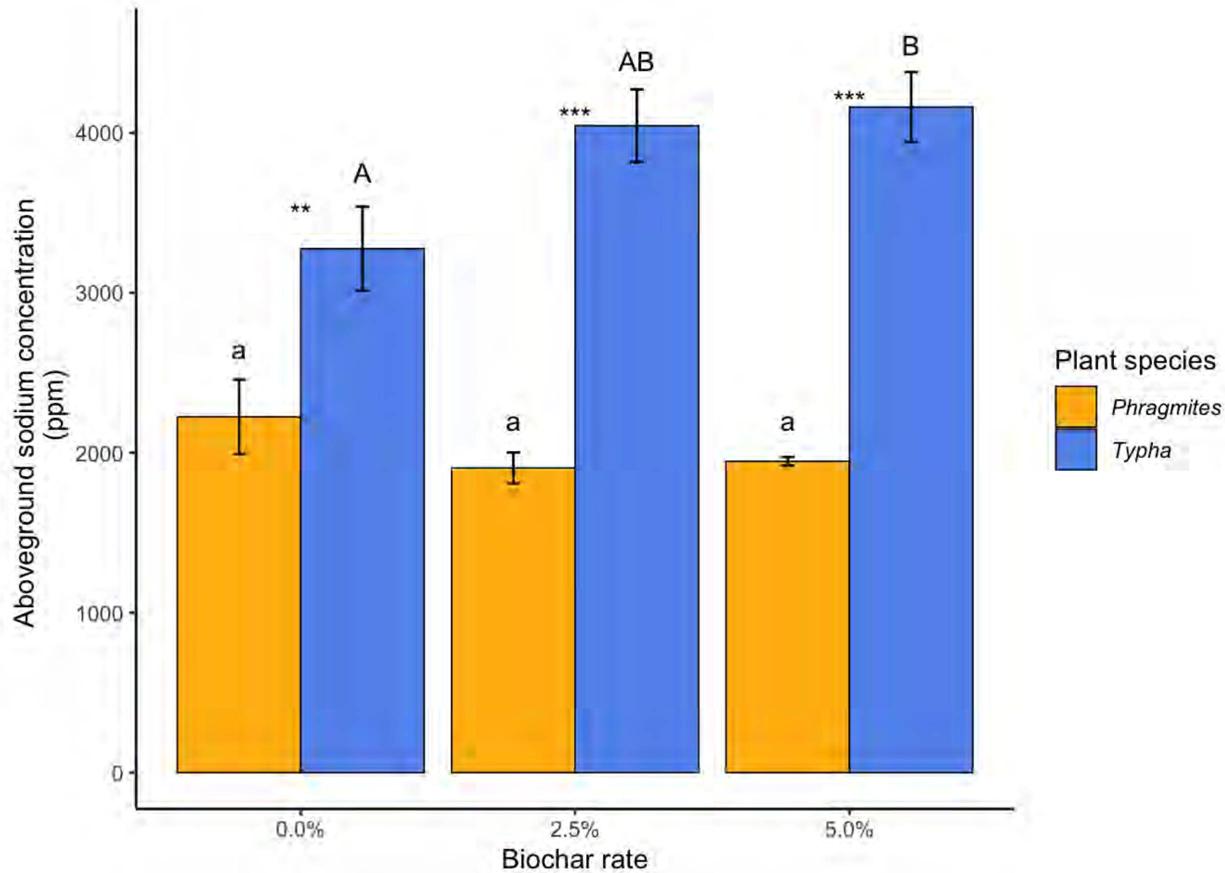


Figure 3.1: Total sodium concentration in aboveground tissue of each plant species. Non-overlapping lower case letters denote significant differences between *Phragmites* values at each biochar rate, non-overlapping capital letters denote significant differences between *Typha* values at each biochar rate, and the asterisks indicate significant differences between plant species within each biochar rate (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Error bars denote standard error.

Neither the 2.5% nor 5% biochar applications resulted in significantly different uptake, and they were not significantly different from each other ($P > 0.05$).

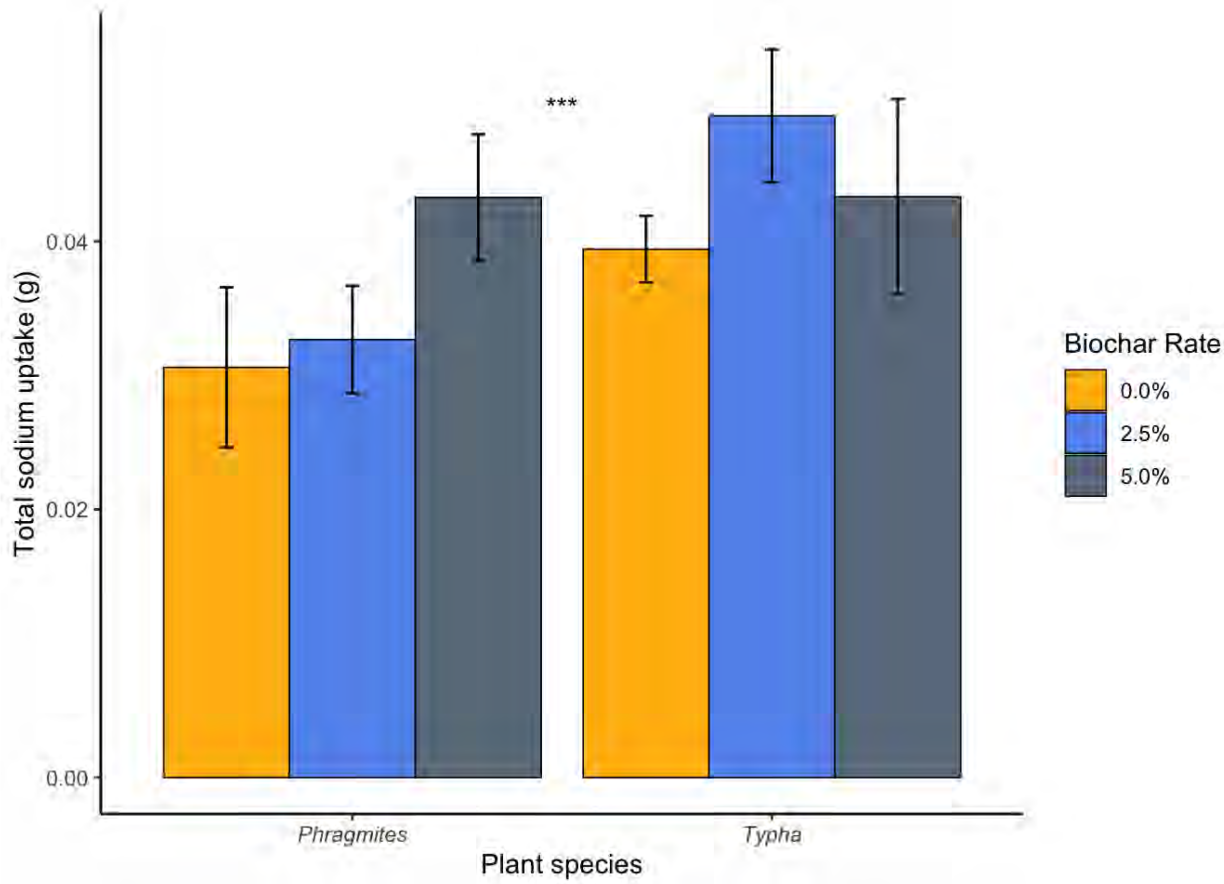


Figure 3.2: Total sodium uptake (aboveground and belowground) in each plant species. Significance denotes comparisons between plant species. No significant differences were detected with biochar application. Error bars denote standard error (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

3.2.2 Chloride

Tissue concentration (ppm) of Cl^- was affected by biochar rate differently in the two plant species (interaction $P = 0.02$), see Figure 3.3. However, no significant trends were detected for 2.5% or 5% biochar rates relative to a control in the context of either *Phragmites* or *Typha* ($P > 0.05$).

Cl^- percent concentration in belowground tissue was significantly different between *Phragmites* (0.47% $\pm$ 0.04) and *Typha* (0.50% $\pm$ 0.04, $P = 0.03$), but a Tukey HSD test revealed biochar application did not significantly impact belowground Cl^- concentration at either rate relative to control ($P > 0.05$). Cl^- concentration in belowground tissue was sig-

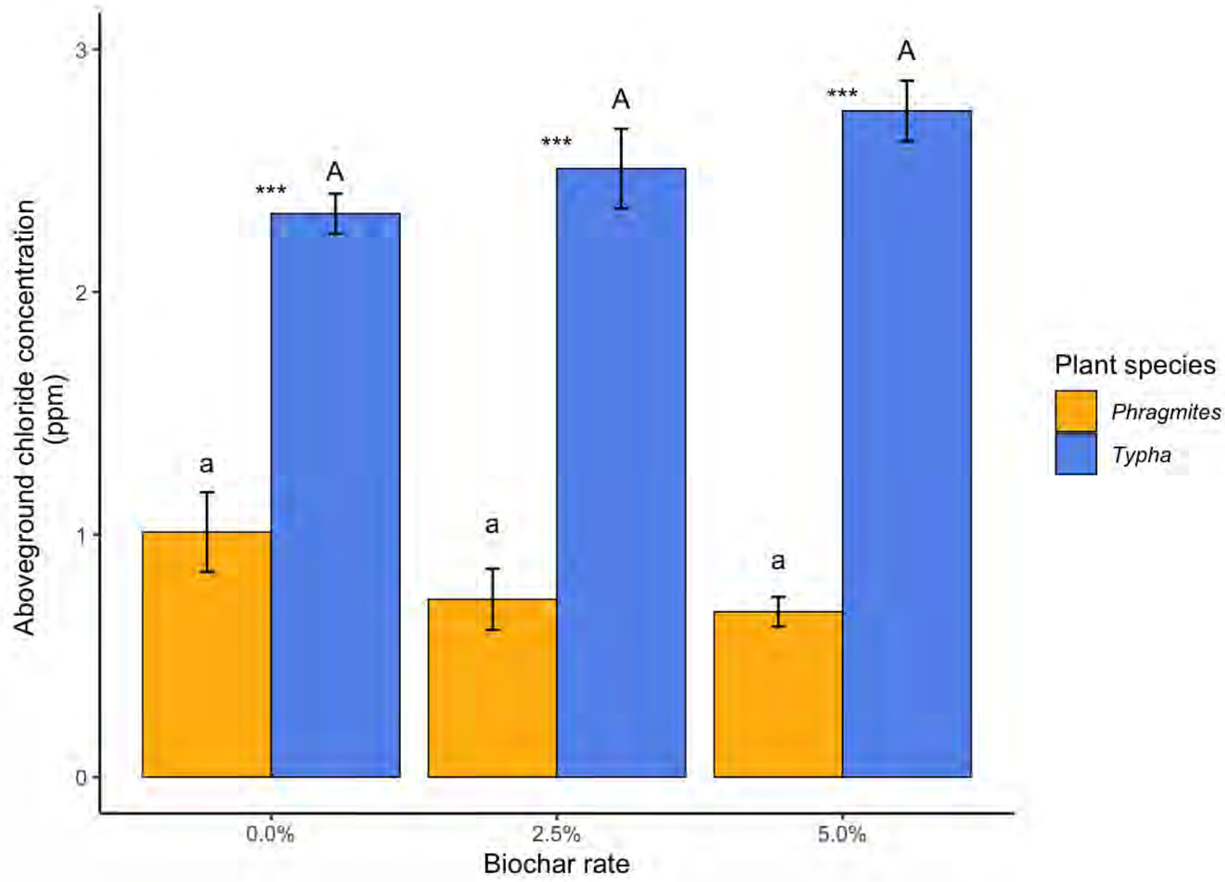


Figure 3.3: Total chloride concentration in aboveground tissue of each plant species. Non-overlapping lower case letters denote significant differences between *Phragmites* values at each biochar rate, non-overlapping capital letters denote significant differences between *Typha* values at each biochar rate, and the asterisks indicate significant differences between plant species within each biochar rate (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Error bars denote standard error.

nificantly lower than the aboveground concentrations for both *Phragmites* ($0.80\% \pm 0.07$, $P < 0.001$) and *Typha* ($2.52\% \pm 0.08$, $P < 0.001$).

Cl^- root:shoot ratio was significantly affected by biochar rate ($P = 0.01$), while the effect of plant species was insignificant ($P = 0.09$). A post-hoc Tukey HSD test indicated significantly higher root:shoot ratio in *Phragmites* at the 2.5% biochar rate relative to a control ($P = 0.04$), although the 5% application rate was not significantly different from either the 2.5% application or the control ($P > 0.05$). *Typha* root:shoot ratio was not significantly different at either biochar rate relative to control ($P > 0.05$).

The total uptake of Cl^- was significantly higher ($P < 0.001$) in *Typha* (0.441g ± 0.016) than *Phragmites* (0.246g ± 0.025), see Figure 3.4. Post-hoc Tukey testing indicated neither the 2.5% nor 5% biochar applications resulted in significantly different uptake compared to control, and they were not significantly different from each other ($P > 0.05$).

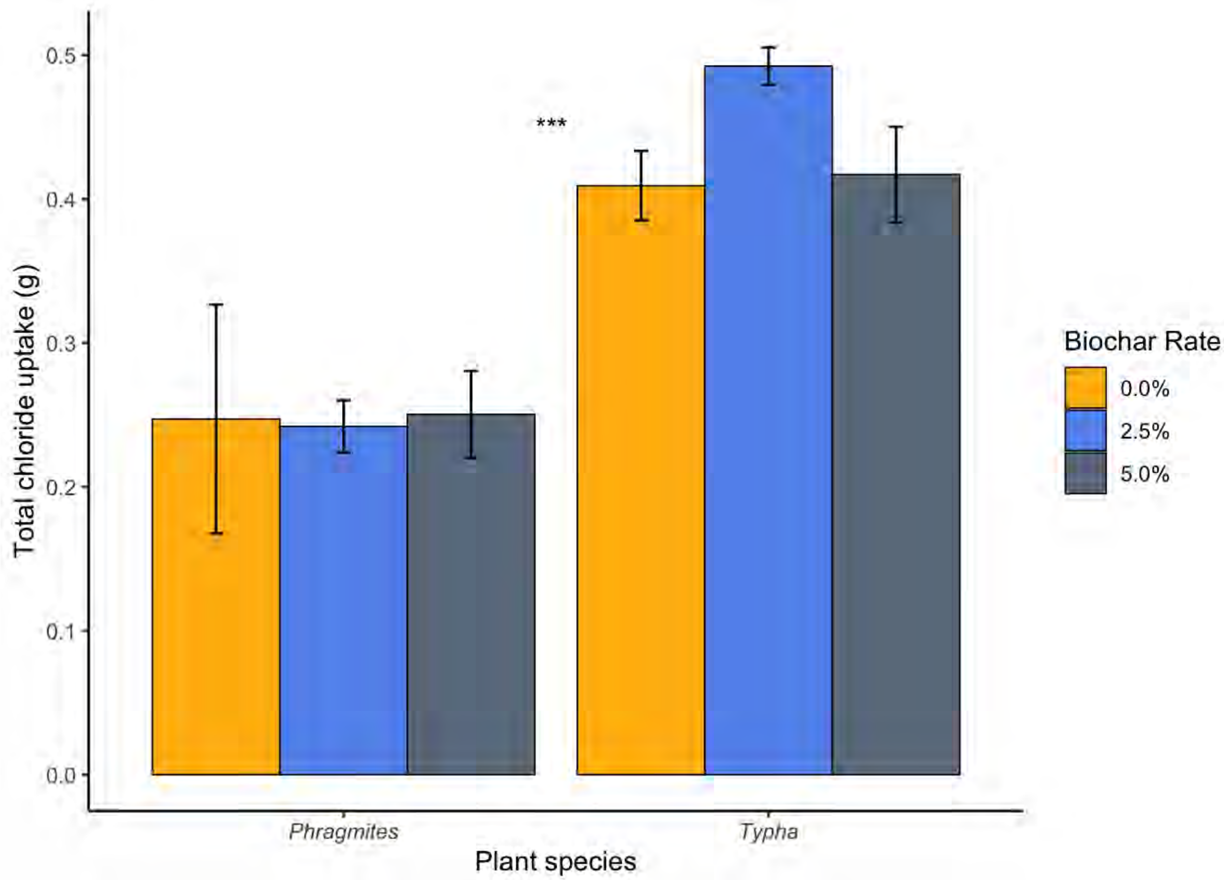


Figure 3.4: Total chloride uptake in each plant species. Significance denotes main effect of plant species (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). No significant differences were detected with biochar application. Error bars denote standard error.

Table 3.3: Tissue concentrations of salt cations and chloride in aboveground plant tissue. **P** = *Phragmites*, **T** = *Typha*. Subscript denotes biochar rate (% wt/wt). Means are reported with standard errors. Concentrations given in parts per million (ppm) or percent (%).

Treatment	**Na^+** *ppm*	**Cl^-** %	**K^+** *ppm*	**Ca^{2+}** *ppm*	**Mg^{2+}** *ppm*
P_0	2225 ± 233	1.01 ± 0.16	219 ± 61	132 ± 7	154 ± 12
$P_{2.5}$	1905 ± 97	0.73 ± 0.13	152 ± 14	133 ± 2	151 ± 1
P_5	1947 ± 27	0.68 ± 0.06	138 ± 9	125 ± 5	150 ± 3
T_0	3276 ± 263	2.32 ± 0.08	157 ± 9	193 ± 4	182 ± 8
$T_{2.5}$	4045 ± 227	2.51 ± 0.16	162 ± 9	188 ± 7	182 ± 4
T_5	4162 ± 219	2.75 ± 0.12	163 ± 5	204 ± 9	194 ± 7

3.2.3 K^+:Na^+ ratio

The K^+:Na^+ ratio of aboveground plant tissue was significantly higher ($P < 0.001$) in *Phragmites* (0.085 ± 0.010) than *Typha* (0.044 ± 0.002) (3.5). A post-hoc Tukey HSD test indicated neither the 2.5% nor 5% biochar applications resulted in significantly different K^+:Na^+ ratios compared to control, and they were not significantly different from each other ($P > 0.05$) (Table 3.3).

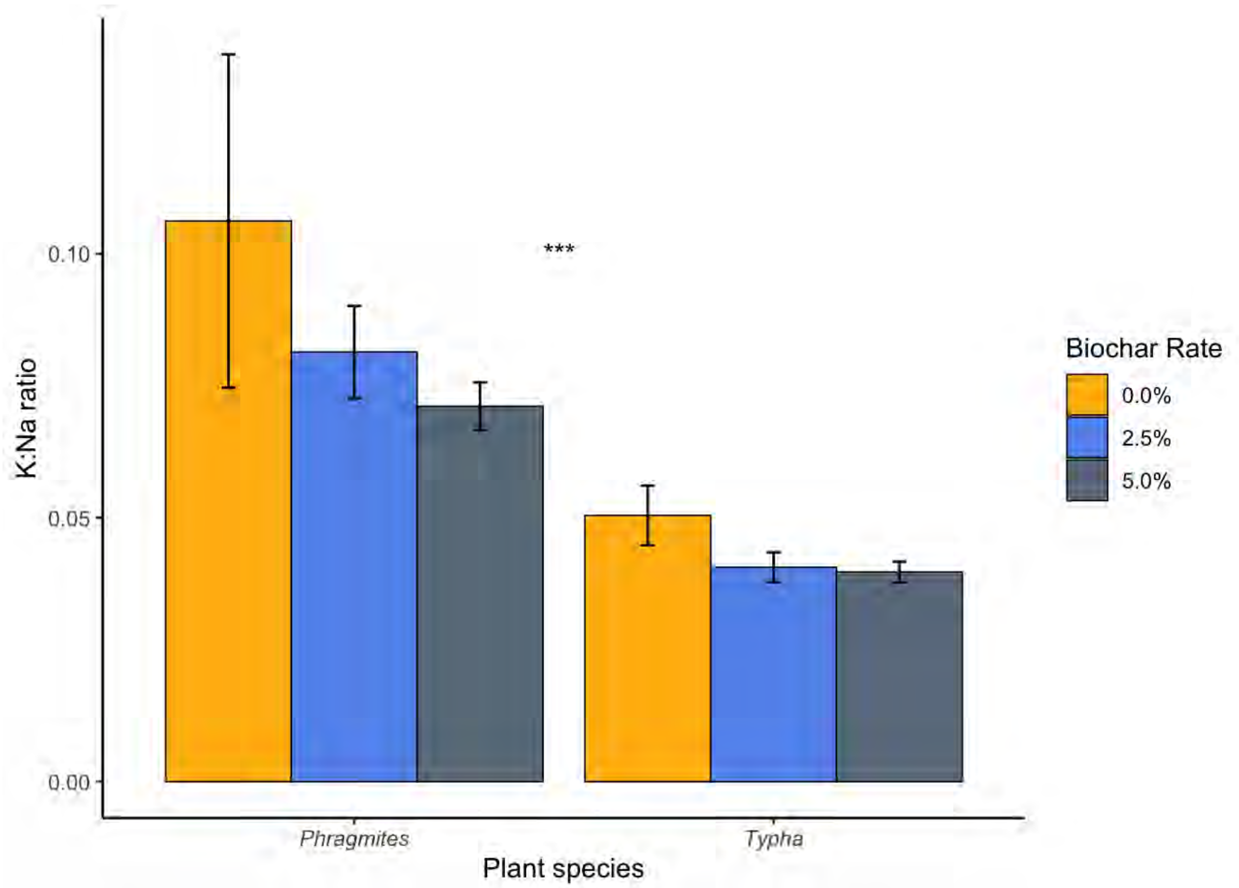


Figure 3.5: K^+:Na^+ ratio of each plant species. Significance denotes main effect of plant species (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). No significant differences were detected with biochar application. Error bars denote standard error.

3.2.4 Water content

The water content of aboveground plant tissue was significantly higher ($P < 0.001$) in *Typha* (77.27% ± 0.21) than *Phragmites* (63.90% ± 1.26), see Figure 3.5. However, biochar rate did not significantly influence water content ($P > 0.05$).

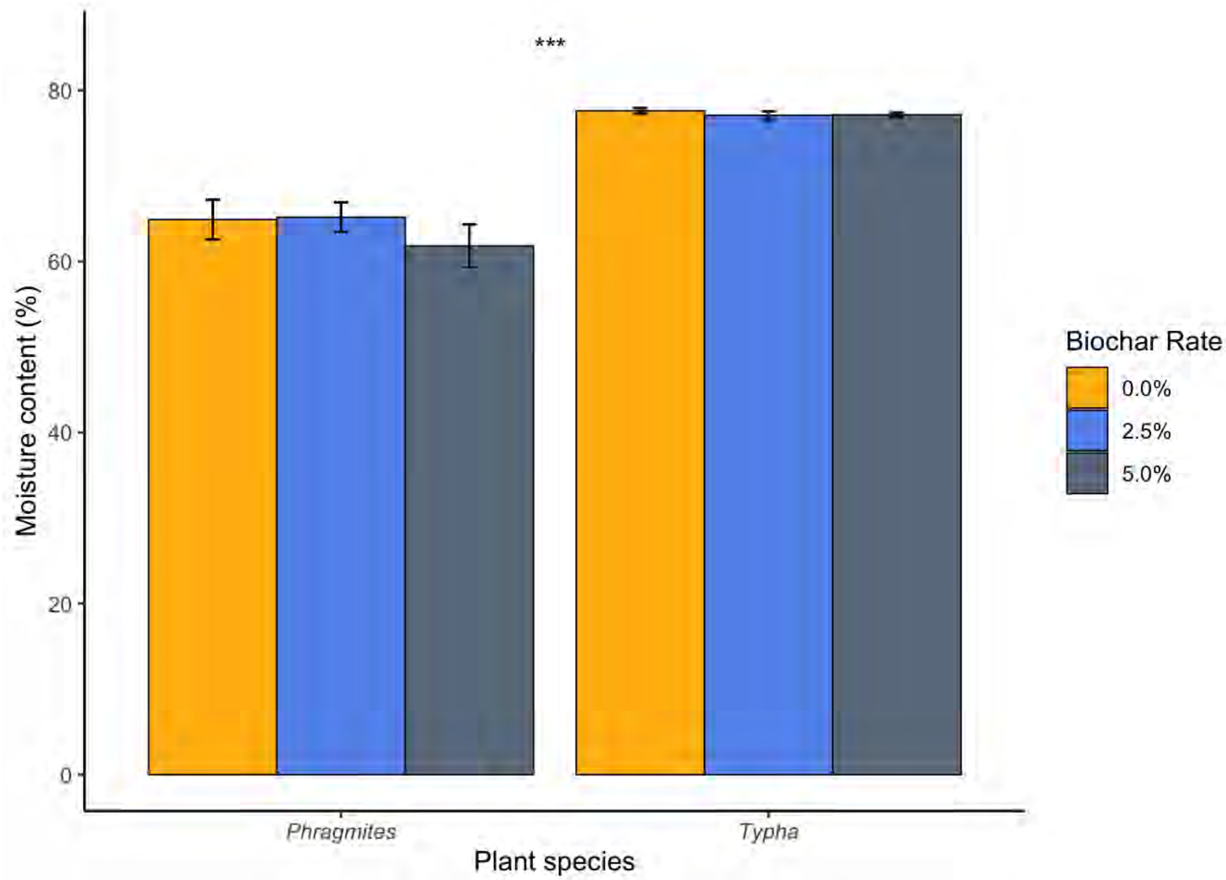


Figure 3.6: Aboveground water content in each plant species. Significance denotes main effect of plant species. No significant differences were detected with biochar application (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Error bars denote standard error.

3.3 Soil characteristics

3.3.1 Sodium

I observed differences in soil Na^+ between biochar rates (P = 0.006) (Figure 3.7). Significantly higher concentrations of Na^+ were found in the 2.5% biochar application (209 ppm ±10) relative to both the 5% application (180 ppm ±9, $P = 0.006$) and the control (172 ppm ±7, $P = 0.04$), which did not differ from each other ($P = 0.76$). Microcosms with *Typha* had significantly lower sodium (169 ppm ±7) than unvegetated microcosms (197 ppm ±7, $P = 0.05$) and trended lower than microcosms with *Phragmites* (196 ppm ±12, $P = 0.056$). Unvegetated and *Phragmites* microcosms were not significantly different ($P = 0.99$) from each other.

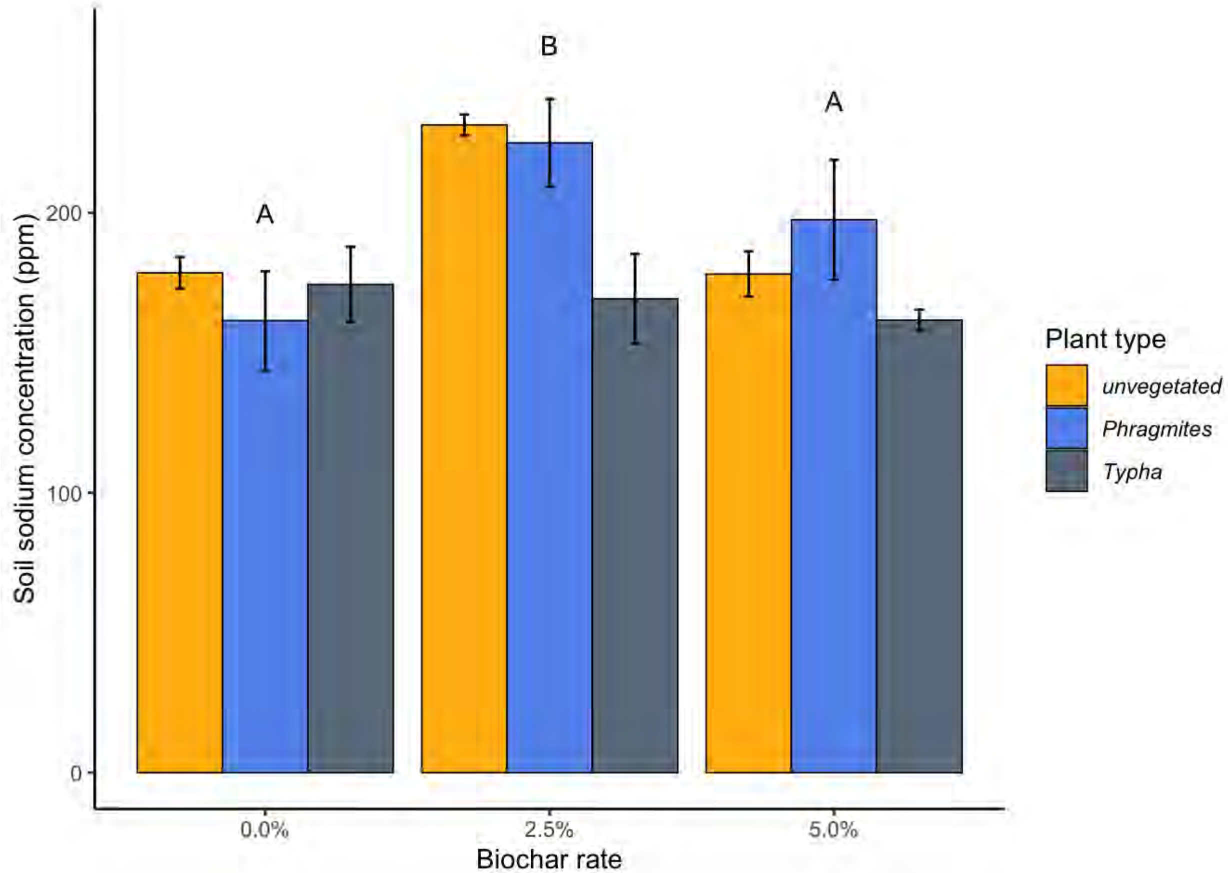


Figure 3.7: Concentration of Na^+ in soil. Non-overlapping capital letters denote significance of the main effect of biochar rate (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Error bars denote standard error.

3.3.2 Chloride

Significant differences were found in soil Cl^- between biochar rates ($P < 0.001$) (Figure 3.8). Significantly higher concentrations of Cl^- were found in the 2.5% biochar application (37 ppm ±3) relative to both the 5% application (29 ppm ±3, $P = 0.02$) and the control (28 ppm ±3, $P = 0.002$), which were not significantly different ($P = 0.77$). Significantly higher Cl^- concentrations were found in unvegetated microcosms (46 ppm ±1) than *Phragmites* (23 ppm ±2, $P < 0.001$) and *Typha* (25 ppm ±2, $P < 0.001$). No significance difference was found between plant types ($P = 0.46$).

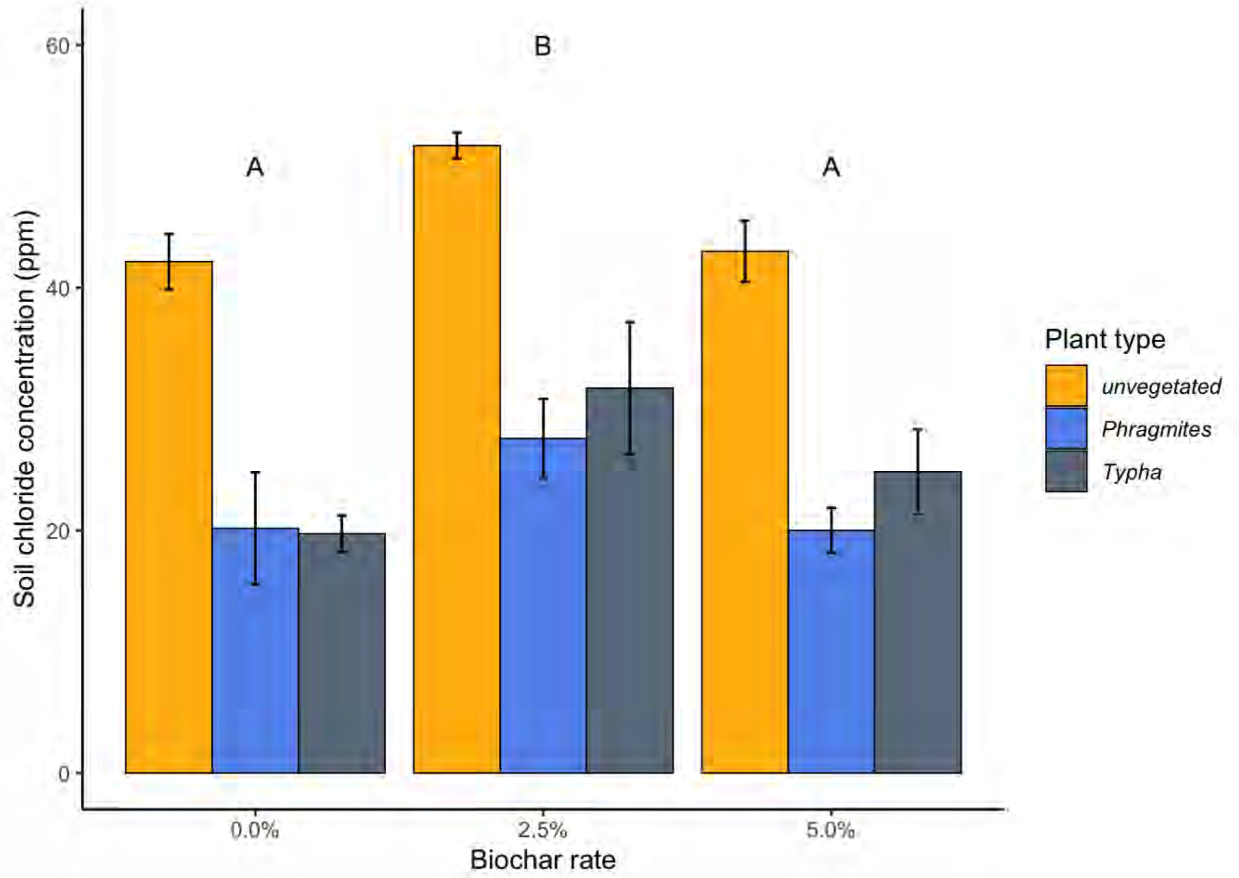


Figure 3.8: Concentration of Cl^- in soil. Non-overlapping capital letters denote significance of the main effect of biochar rate (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Error bars denote standard error.

3.3.3 Potassium

A significant interaction was found between plant type and biochar rate on soil K^+ concentration ($P < 0.001$). No significant differences were found in K^+ concentrations by biochar rate in *Typha* ($P = 0.29$) or unvegetated microcosms ($P = 0.85$). However, within *Phragmites* microcosms, K^+concentrations were significantly higher at the 0% biochar rate (246 ppm ±6) than the 2.5% (220 ppm ±6, $P = 0.05$) and 5% (204 ppm ±8, $P =< 0.001$), which did not significantly differ ($P = 0.51$).

3.3.4 Magnesium

A significant interaction was found between plant type and biochar rate on soil Mg^{2+} concentration ($P < 0.001$). Within the context of unvegetated microcosms, no significant differences were found between any biochar rates and the control ($P = 0.62$). In *Phragmites* microcosms, the 0% biochar rate contained significantly higher K^+ concentrations (780 ppm ±5) than the 2.5% (736 ppm ±11, $P = 0.01$) and 5% (714 ppm ±6, $P < 0.001$) rates, which were not significantly different ($P = 0.58$). In *Typha* microcosms, the 2.5% biochar application contained significantly lower K^+ concentrations (677 ppm ±10) than the 5% rate (721 ppm ±8, $P = 0.01$) but was not significantly different from the control (696 ppm ±6, $P = 0.74$). The control and 5% rates did not significantly differ ($P = 0.48$).

3.3.5 Calcium

A significant interaction was found between plant type and biochar rate on soil Ca^{2+} concentration ($P < 0.001$). In unvegetated microcosms, Ca^{2+} concentrations at the 5% biochar rate (2967 ppm ±30) were significantly lower from the control (3171 ppm ±36, $P < 0.001$) and 2.5% rate (3157 ppm ±17, $P = 0.002$), which were not significantly different ($P = 0.99$). Similarly, Ca^{2+} concentrations at the 5% biochar rate in *Phragmites* microcosms (2924 ppm ±20) were significantly lower than the control (3186 ppm ±26, $P < 0.001$) and 2.5% (3064 ppm ±25, $P = 0.04$) concentrations, which did not significantly differ ($P = 0.13$). However, in *Typha* microcosms, K^+ concentrations at the control biochar rate (2951 ppm ±25) were not significantly different than those at the 5% rate (2949 ppm ±37, $P = 0.99$) while concentrations at the 2.5% rate (2807 ppm ±44) were significantly lower than both the control ($P = 0.03$) and 5% rate ($P = 0.05$).

3.3.6 pH

Significant differences were found in soil pH among biochar rates ($P = 0.007$). Post-hoc Tukey testing indicated that both the 2.5% (6.82 ± 0.02) and 5% (6.85 ± 0.02) biochar applications significantly increased soil pH relative to control (6.75 ± 0.03, $P = 0.007$). There was no significant difference between the 2.5% and 5% rates ($P = 0.76$), and plant type did not significantly affect soil pH ($P = 0.26$).

3.3.7 Cation exchange capacity

A significant interaction was found plant type and biochar rate on soil cation exchange capacity ($P < 0.001$). In unvegetated microcosms, cation exchange capacity at the 5% biochar rate (22.5 meq/100g ± 0.2) was significantly lower from the control (23.6 meq/100g ± 0.2, $P = 0.03$) and 2.5% rate (23.7 meq/100g ± 0.1, $P = 0.008$), which were not significantly different ($P = 0.99$). Cation exchange capacity was also significantly lower at the 5% biochar rate in *Phragmites* microcosms (21.9 meq/100g ± 0.2) than the control (23.8 meq/100g ± 0.2, $P < 0.001$) and 2.5% (23.0 meq/100g ± 0.2, $P = 0.04$) concentrations, which did not significantly differ ($P = 0.39$). In *Typha* microcosms, however, cation exchange capacity at the control biochar application (21.9 ± 0.2 meq/100g) was not significantly different than at the 2.5% rate (20.9 meq/100g ± 0.3, $P = 0.1$) or the 5% rate (22.0 meq/100g ± 0.3, $P = 0.99$), which did not significantly differ ($P = 0.07$).

3.4 Leachate characteristics

3.4.1 Chloride

The LME for chloride showed significant effects of both plant types on chloride concentration of leachate (Figure 3.9). *Phragmites* decreased chloride values by 61.7 mg/L $\pm$ 20.3 when holding all other effects constant ($P < 0.001$), and the effect in *Typha* was even greater (decrease of 165.2 mg/L ± 19.3, $P < 0.001$). 2.5% biochar application increased chloride by 102.2 mg/L ± 19.3 ($P < 0.001$), while the 5% application decreased chloride levels by 26.6 mg/L ± 20.1 but was not statistically significant. Interactions between plant type and biochar rate demonstrated significance as well. An increase in chloride relative to controls was noted with *Phragmites* at both 2.5% (44.5 mg/L ± 28.0) and 5% (90.1 mg/L ± 28.5) biochar rate, but only the 5% value was statistically significant compared to control ($P < 0.01$). Similarly, the effect of *Typha* on chloride levels was lower in the context of biochar interaction. *Typha* decreased chloride levels by 89.1 mg/L ± 27.4 in the presence of 2.5% biochar ($P < 0.01$) and increased chloride by 12.5 mg/L ± 28.5 with 5% biochar, although the effect was insignificant. The random effect of microcosm ID explained 24.9% of the variation in the model (Table 3.4).

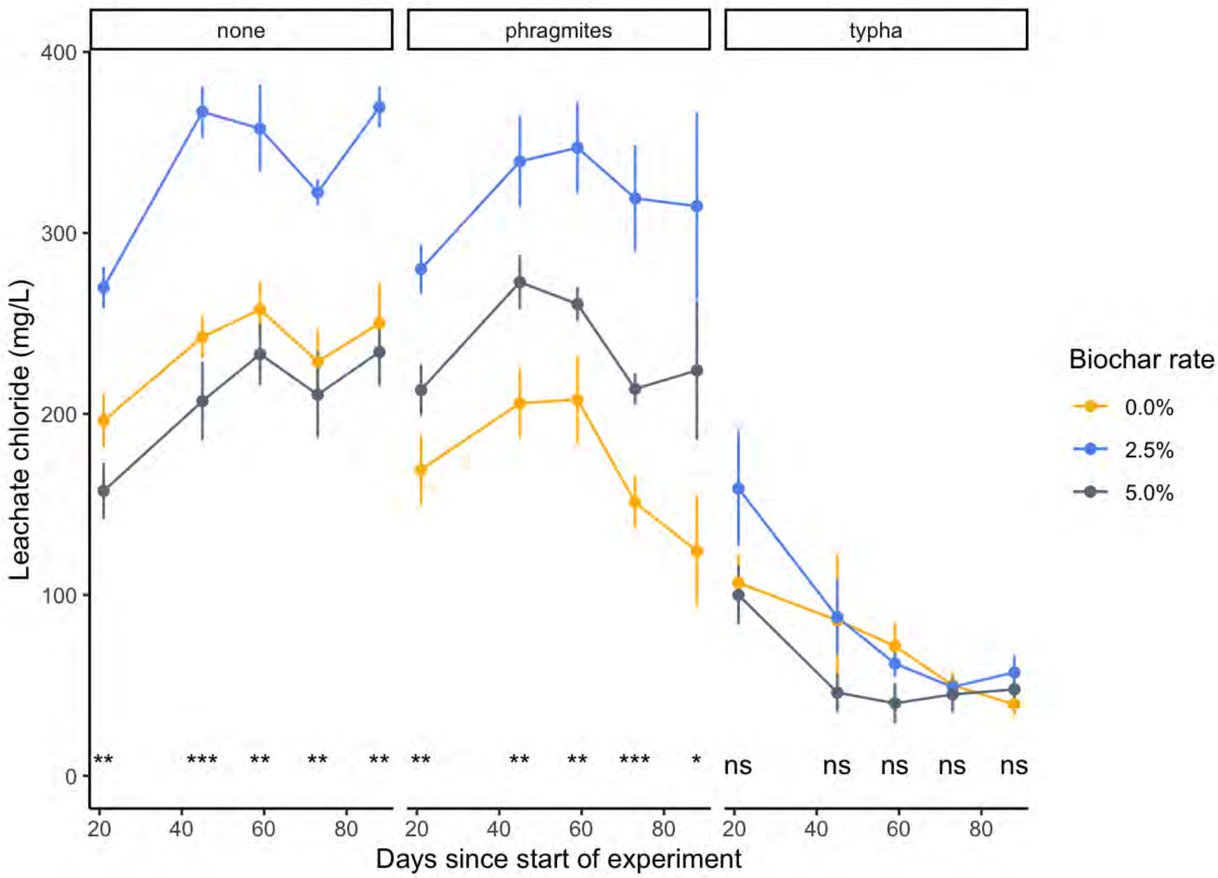


Figure 3.9: Chloride concentration of leachate over the duration of the experiment. Error bars denote standard error. Asterisks represent significant differences between biochar rates at each sampling date (ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

3.4.2 Specific conductivity

Specific conductivity significantly increased over time ($P < 0.001$), although the sampling date effect represented only a 0.005 mS/cm ± 0.001 addition to controls (Figure 3.10). The different biochar rates had contrasting effects: when accounting for other effects, 2.5% biochar increased specific conductivity by 0.222 mS/cm ± 0.114 relative to control ($P > 0.05$) while the 5% rate decreased the value by 0.238 mS/cm ± 0.119 ($P < 0.05$). Both plant species were associated with lower specific conductivity, but *Phragmites* significantly increased specific conductivity by 0.371 mS/cm ± 0.165 and 0.515 mS/cm ± 0.168 in the presence of biochar applied at the 2.5% ($P < 0.01$) and 5% ($P < 0.05$) rates respectively. *Typha* was associated with a larger change in specific conductivity than any other factors, decreasing the value by 0.295 mS/cm ± 0.115 ($P < 0.05$) when accounting for other effects. Values remained lower than control when *Typha* interacted with 2.5% and 5% biochar rates as well, although the interaction effect was smaller than the main effect of *Typha* ($P > 0.05$). The random effect of microcosm ID explained 29.5% of the variation in the model (Table 3.4).

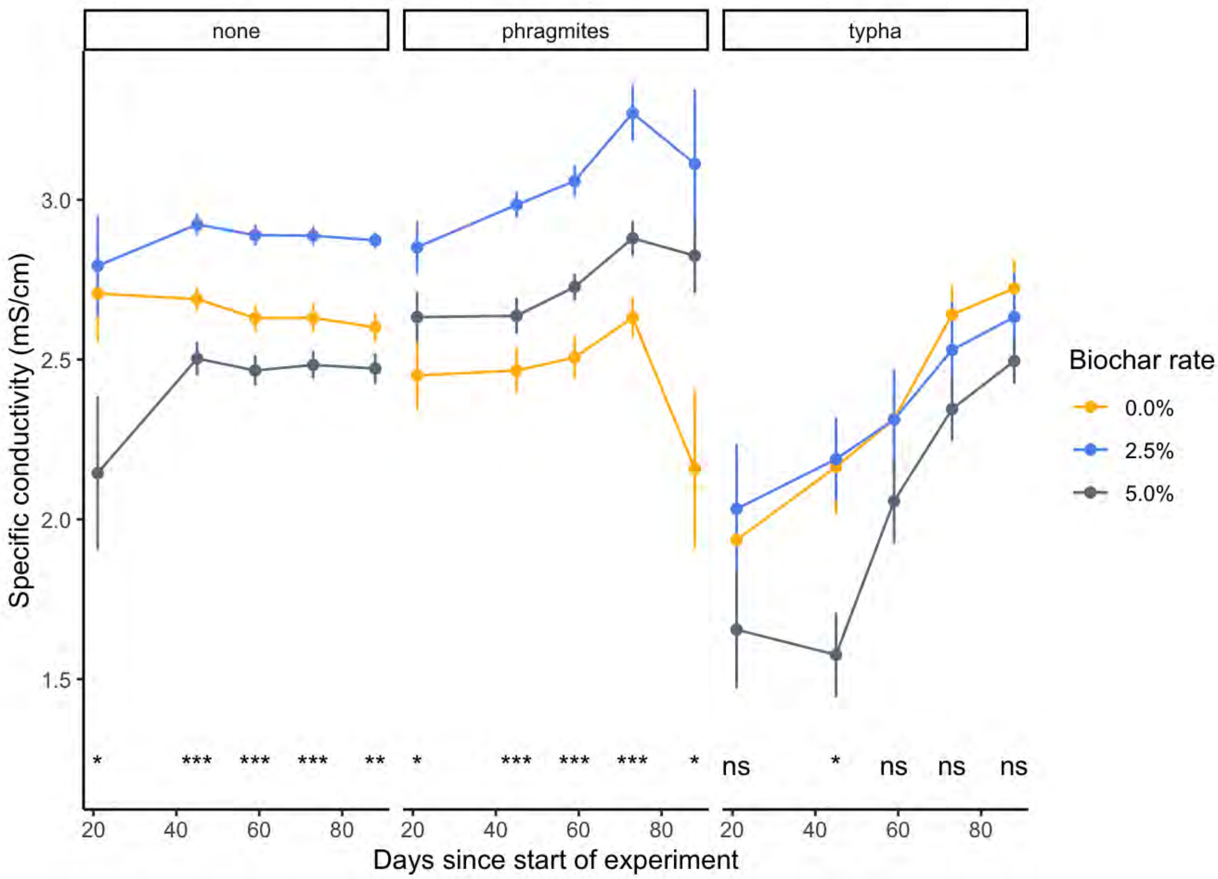


Figure 3.10: Specific conductivity of leachate over the duration of the experiment. Error bars denote standard error. Asterisks represent significant differences between biochar rates at each sampling date (ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

3.4.3 pH

Modest changes to pH over time were found with both plant type and biochar rate. Sampling date was a statistically significant variable in the model and pH increased over time ($P < 0.001$), although the effect was small: pH ranged from 6.84 – 7.53 in the dataset, with 95% of all values ranging from 7.0 – 7.25. Sampling date was the only variable associated with statistical significance. *Phragmites* (0.003 ± 0.021), *Typha* (-0.004 ± 0.02), 2.5% biochar (0.034 ± 0.02), and 5% biochar (0.027 ± 0.021) were associated with only modest increases in pH changes relative to the model controls. Similarly, no interaction term between any biochar rates and plant types had an effect greater than a 0.05 change in pH when holding all other factors constant. 3.1% of the variation in the model was explained by the random effect of microcosm ID.

Table 3.4: Model output of linear mixed effects models of collected leachate data. Values in parentheses are standard errors. The intercept represents conditions with 0% biochar and the unvegetated controls. Coefficient estimates represent the expected deviation from the model intercept.

	Dependent variable		
	chloride *(mg/L)*	**specific conductivity** *(mS/cm)*	**pH**
Intercept	240.509***	2.350***	7.044***
	(15.419)	(0.090)	(0.016)
Phragmites	−61.651**	−0.188	0.003
	(20.265)	(0.119)	(0.021)
Typha	−165.231***	−0.295*	−0.004
	(19.377)	(0.115)	(0.020)
2.5% biochar	102.173***	0.222	0.034
	(19.317)	(0.114)	(0.020)
5% biochar	−26.592	−0.238*	0.027
	(20.106)	(0.119)	(0.021)
Sampling date	−0.095	0.005***	0.002***
	(0.125)	(0.001)	(0.0001)
Phragmites:2.5% biochar	44.523	0.371*	−0.024
	(27.997)	(0.165)	(0.029)
Typha:2.5% biochar	−89.061**	−0.239	0.020
	(27.361)	(0.162)	(0.029)
Phragmites:5% biochar	90.094**	0.515**	−0.046
	(28.547)	(0.168)	(0.030)
Typha:5% biochar	12.511	−0.093	−0.052
	(28.475)	(0.168)	(0.030)
Observations	297	298	298
Marginal/Conditional R^2	0.753/0.815	0.495/0.644	0.402/0.548
Note:			*p<0.05; **p<0.01; ***p<0.001

Chapter 4

Discussion

4.1 Leachate

The decreases in specific conductivity and chloride content of leachate associated with plant presence in this study indicate potential for plant uptake as a means of salinity mitigation. *Typha* in particular was the strongest factor in the Cl^- model, reducing leachate chloride content by over 50% when accounting for other factors. This effect is roughly 2.7 times stronger than the effect of *Phragmites* presence, although *Phragmites* was still associated with a significant Cl^- reduction. Similarly, both plants reduced leachate specific conductivity, but the effect of *Typha* was almost twice as strong. The pronounced difference between plant species provides additional context in validating the salt-accumulating nature of *Typha* versus the tendency of *Phragmites* to exclude salt uptake. These results provide evidence that the effect of uptake is consequential, and provides further evidence that phytoremediation literature has undervalued its potential for salt removal (Rabhi et al. 2009, Jesus et al. 2015). Although a large body of evidence exists that plant-soil interactions promote leaching (Qadir et al. 2001; 2005), the net loss of leachate Cl^- ions and reduction in specific conductivity indicate uptake may even be stronger than such interactions.

The effect of plant presence on leachate Cl^- and specific conductivity is complicated by biochar addition. Generally, both 2.5% and 5% biochar addition increased leachate Cl^- and specific conductivity relative to control when accounting for other factors, but the strength of addition depended on plant species present. Overall Cl^- in leachate increased with 5% biochar addition combined with either plant species, while the 2.5% application was associated with increases in both Cl^- and specific conductivity levels relative to control, while the 5% application was associated with lower levels of both variables. This discrepancy may be due to the significant decrease in cation exchange capacity associated with the 5% application rate, as lower cation exchange would mean fewer available sites for the exchange of salt compounds. Although little research exists to verify this claim, this mechanism would be consistent with results found in Na^+ leaching in Xiao and Meng (2020). However, the degree of increase in leachate salinity also depended on plant species present. Cl^- increased with 5% biochar addition in the context of plant species but the 2.5% application resulted in increased Cl^- in the context of *Phragmites* but lower Cl^- with *Typha* present. The interactions between the biochar rates and plant species complicate clear interpretations of the results, as the effect of the 2.5% biochar rate on Cl^- leachate is greater than that of the 5% rate when controlling for other factors but not when interpreting interactions with either plant species. As such, further study may be required to elucidate the effect the interactions between plant roots and biochar in the soil and how these interactions influence leachate salinity. It is possible that these effects depend on feedstock: Awan et al. (2021) found that wheat straw-based biochar increased sodium adsorption rate (SAR) and replaced Na^+ on exchange sites with Ca^{2+} and Mg^{2+}. Conversely, hemp feedstock lowered SAR and was not associated with changes to salt ion concentrations. Based on these results, biochar has potential to aid in the removal of salt ions from soil, but further research is needed to understand which physical and chemical traits best contribute to such outcomes and which feedstocks best result in such traits.

4.2 Plant tissue chemistry

The higher concentration of Na^+ and Cl^- in *Typha* tissue relative to *Phragmites* indicates differences in salt tolerance strategies between plant species. *Typha* accumulated roughly double the concentration of Na^+ and Cl^- in its aboveground tissue and exhibited greater uptake of both elements despite lower biomass values. These results are consistent with the characterization of *Typha* as a salt accumulating plant, as it has been found to uptake greater quantities of salts than other macrophytes (Guesdon et al. 2016). Salt accumulation partially occurs through the distribution of Na^+ and Cl^- ions at the intracellular level to prevent toxic concentrations of these ions within the cytoplasm, increasing its natural tolerance to higher salt levels (Munns and Tester 2008). Increased water accumulation and retention may also aid in salt uptake and storage (Glenn and O'Leary 1984), a mechanism supported by the high water content of *Typha* relative to *Phragmites* I observed.

In contrast, *Phragmites* had minimal uptake of Na^+ and Cl^- ions, a result also observed in other studies (Vasquez et al. 2005). Exclusion of Na^+ by the root or shoot may occur through the retention of K^+ ions in the cytoplasm, which maintains cell pressure against higher soil Na^+ concentrations (Wang et al. 2002, Munns and Tester 2008, Chen et al. 2018b). This study provides evidence of this exclusion mechanism: the K^+:Na^+ ratio was higher in *Phragmites* than the salt-accumulating *Typha*, indicating osmotic adjustment in cells (Zivcak and Brestic 2016). Osmotic adjustment may similarly aid in the exclusion of Cl^-, although this mechanism has not been explored in detail and may differ across salt-excluding species (Wu and Li 2019).

Although Na^+ and Cl^- tissue concentrations were different between *Phragmites* and *Typha*, the impact of biochar on salt content of both species is less clear. Although biochar application increased Na^+ concentration in *Typha*, this difference may not be biologically meaningful. No significant differences in Na^+ uptake were found between any biochar rates within *Typha* treatments, likely due to the lack of growth response. There is some evidence in other studies that biochar has potential to influence tissue concentrations and uptake of nutrients generally (Gunarathne et al. 2020), and *Typha latifolia* had greater concentrations of carbon, nitrogen, and phosphorus with increasing biochar rates in one study (Kasak et al. 2018). However, there is an apparent gap in the literature addressing the potential change to salt ion accumulation induced by biochar addition. It is possible that the lack of differences in uptake between biochar addition is an artifact of the study length, as the 88-day greenhouse study was not long enough for either species to grow to the sizes typically seen in a growing season. Although biochar addition did increase Na^+ concentration in *Typha*, it may take a longer time scale for the difference in uptake to be observable.

4.3 Soil chemistry

The impact of plant presence on soil salinity levels provides further evidence of the variable salt tolerance strategies employed by *Typha* and *Phragmites*. *Typha* pots had significantly lower soil Na^+ than both *Phragmites* and unvegetated pots, indicating greater salt uptake and/or leaching. This result was expected, as *Typha* spp. have been found to uptake significant salts and nutrients in other studies (Morteau et al. 2015, Guesdon et al. 2016). Soil Na^+ levels were not significantly different between *Phragmites* and unvegetated pots, however, indicating the salt exclusion strategy. This result demonstrates a smaller effect of plant presence on soil salinity than what literature suggests: although the effect of uptake is regarded as minimal in many studies, Na^+ leaching is expected with plant presence due to plant-soil interactions and partial pressure of CO_2 in the root zone (Qadir et al. 2001; 2005). Salt-excluding species may therefore have not only minimal salt uptake potential, but also a limited capacity to alter soil Na^+ values. However, soil Cl^- levels were significantly lower in both *Phragmites* and *Typha* pots than unvegetated pots and the plant species were not significantly different from each other. Some potential for salinization mitigation may therefore be possible with both salt-excluding and accumulating species, although Cl^- is less biologically harmful than Na^+ (Munns and Tester 2008, Wu and Li 2019).

While plant presence had a significant and expected effect on soil salinity levels, results from biochar addition were contrary to my hypotheses. Similar to Xiao and Meng (2020), I hypothesized that increasing biochar additions would increase cation exchange capacity, which in turn would result in greater amounts of Na^+ replacement by Ca^{2+} on exchange sites, thus lowering soil Na^+ values. In this study, soil Na^+ was significantly greater in the 2.5% biochar application relative to control, while the 5% rate was not different. Furthermore, Ca^{2+} concentrations were lower than control with biochar application, although the application rate at which significance was found varied within plant treatments. These greater soil salinity values may be a result of the decrease in cation exchange capacity associated with biochar in this study. Contrary to my expectations, biochar in this study significantly lowered cation exchange at the 5% application relative to control, while the 2.5% application was not significantly different from the control. Biochar feedstock, cooking temperature, and time are all variables that can influence its chemical and physical properties (Lehmann and Joseph 2015), and it is possible that the biochar used in this study had relatively low porosity or surface area which could lower its potential for cation exchange. However, wood-based material is a frequently used feedstock in biochar studies and the temperature and time of biochar creation were within common ranges (Yargicoglu et al. 2015). It is also possible that the increase in cation exchange capacity associated with biochar is overstated, as methodological differences across studies lead to a wide range of reported values (Munera-Echeverri et al. 2018). Indeed, Da Silva Mendes et al. (2021) found a decrease in cation exchange associated with biochar, although smaller biochar quantities resulted in greater decreases than larger application rates. Regardless, this study provided evidence that cation exchange capacity and salt leaching and plant uptake are closely related phenomena, and amendments improving cation exchange may also result in greater exchange of salt ions.

While an increase in cation exchange capacity with biochar addition is broadly accepted, it is unlikely that cation exchange is the sole driver of change within the systems to which it is added. Liang et al. (2021) documented up to a 35% increase in *Phragmites australis* biomass with biochar and biochar-compost additions under salt stress, but lower electrical conductivity values in the soil indicated a decrease in soil salinity as a possible driver of growth. This reported *Phragmites* growth could be attributed to a release from salt stress rather than a growth response under less deleterious conditions, as plants were subjected to acute salt stress rather than the chronic levels used in my study. Biochar-compost treatments outperformed control, biochar-only, and compost-only treatments in other studies as well, indicating the addition of nutrient-rich amendments may provide further advantages for plant growth and nutrient uptake (Agegnehu et al. 2016, Liu et al. 2021). Absent of nutrient addition, particularly high biochar rates may starve the system of nutrients or disrupt beneficial microbial communities without additional benefits provided by compost (Ohsowski 2015). Coupling the physical and structural benefits associated with biochar with nutrient-rich amendments may prevent such disruptions.

4.4 Plant biomass

No evidence was found in this study that biochar improves the growth of *Phragmites* or *Typha*. Biochar has generally been found to increase the growth of many plant species, from tree species (Thomas and Gale 2015, Wang et al. 2020) to agricultural crops (Yu et al. 2019). Biomass increases associated with biochar application are particularly pronounced in soils with high salinity, although a wide variance in methodology complicates efforts to reliably replicate soil conditions (Ali et al. 2017). The lack of growth response in either plant species from biochar application in my study may be explained through the experimental design. Other studies subjected plants to acute levels of salinization at rates which are known to stunt the growth of even salt-tolerant species (Thomas et al. 2013, Ali et al. 2017). Liang et al. (2021), for example, applied salt treatments of up to 15 parts per thousand to pots containing *Phragmites*, much higher than the chronic salinity levels found in this study. As such, it may be more accurate to view the role of biochar as an amendment that can free a plant from acute salt stress rather than one that promotes growth independently of the condition of the soil. It is possible that growth effects were not observed in this study because the level of soil salinity, although within chronic salt stress ranges (Hintz et al. 2021), was not high enough to be deleterious to these salt-tolerant species. As such, there were no constraints from which *Typha* nor *Phragmites* could be freed which would have otherwise stunted growth.

4.5 Management implications

Concerning plant uptake of salt ions, the conclusion that *Typha* takes up high levels of sodium and chloride regardless of biochar is strong evidence for invasive plant harvest as a means of reducing soil and freshwater salinization. While *Phragmites* did not greatly impact soil salinity likely due to its salt-excluding strategy, *Phragmites* and *Typha* spp. often occur in similar areas and harvesting either species leads to similar improvements in native plant populations and wildlife diversity (Breen et al. 2014). Phytoremediation strategies for salt mitigation should therefore consider not only whether a plant is salt-tolerant, but the specific salt tolerance strategy it deploys. Unless the goal of a management project is exclusively to remediate soil or freshwater salinization, harvesting *Phragmites* spp. when it is found near *Typha* spp. monocultures would lead to similar environmental and economic benefits. Furthermore, the aboveground Na^+ and Cl^- levels in both plant species were much higher than those in belowground tissue, indicating the majority of salt ions these plants uptake are harvestable.

My results also highlight the context-dependent nature of biochar addition. Although this study provides evidence that salt-accumulating species such as *Typha* meaningfully reduce salt levels through uptake and plant-root interactions, biochar did not improve uptake of salts nor growth. The increase in salt measurements of the leachate is promising in certain contexts, such as soil salinity remediation, where management goals would not be concerned with higher salt content in leachate. In other systems such as freshwater wetlands, however, increasing water salinity could exacerbate deleterious effects on biota if the effect is observably strong at a larger application scale. While this study did not directly explore how biochar impacts the mobilization of heavy metals via soil salinization, the observed increase in salt leaching is compatible with the growing body of literature demonstrating the capacity of biochar to mitigate heavy metals through immobilization and complexation (Reddy et al. 2014, Al-Wabel et al. 2015). It is possible that biochar could further improve heavy metal remediation efforts which already utilize *Phragmites* and *Typha* spp. in constructed wetlands (Kumari and Tripathi 2015).

Outside the context of salt mitigation, there remains strong evidence for biochar as a tool in restoration. Biochar application represents a semi-permanent storage of carbon in the soil and can be used in carbon accounting and offsetting greenhouse gas emissions (Lehmann 2007). Structural soil benefits associated with biochar, such as higher SOM, carbon storage, water filtration, and plant growth (Lehmann and Joseph 2015), may outweigh the costs of greater salt in leachate even in systems where freshwater desalinization is a priority. One of the goals of this study was to determine if deleterious effects begin to appear in plants when biochar is applied at very high rates, and it appears on these results that plants did not exhibit any negative conditions as a result of high biochar levels.

4.6 Further research

Additional research assessing fundamental physical and chemical properties of biochar is necessary. The mechanisms through which biochar facilitates salt leaching are not well understood, although this study indicates that cation exchange is not the sole driver of change. Additional research on optimal biochar feedstocks and plant species utilized would improve management outcomes (Awan et al. 2021). Field scale biochar research is uncommon (Saifullah et al. 2018), but assessments of the impact of biochar in conditions that closely mimic the real-world systems in which it is used would validate claims so far only seen in exploratory greenhouse studies.

Furthermore, the implementation of large-scale harvesting would provide novel opportunities for research. Harvesting generates a massive volume of invasive plant biomass which can be expensive and hazardous to transport (Carson et al. 2018). This biomass could be combusted in situ to create biochar, creating a much higher-value product that could be used to further advance restoration goals. However, more research is needed to understand the chemical components of invasive plant-derived biochar and biochar derived from phytoremediation efforts. At present, it is not known if biochar derived from plants used in phytoremediation would contain higher levels of pollutants than biochar created through more common methods. A life-cycle analysis of pollutants in this phytoremediation-biochar creation system would validate such large-scale efforts and provide insight on the chemical composition of biochar from varied feedstocks. There is significant potential for biochar amendments in combination with harvesting to provide solutions to salinization and the spread of invasive plants, and further research on the efficacy and viability of these management approaches would provide necessary insight into the restoration and preservation of wetlands.

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