

Wetland and Terrestrial Phytoremediation of an End-of-Life Municipal Lagoon
Using Cattail (*Typha* spp.)

By

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ABSTRACT

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Spreading biosolids on farmland is a common biosolids management practice in western Canada. Wetland and terrestrial-based phytoremediation approaches may be viable options for remediating biosolids in end-of-life municipal lagoons. Water depth is regulated during wetland phytoremediation whereas there is no control of water regime during terrestrial phytoremediation.

Studies were conducted to quantify cattail (*Typha* spp.) biomass and nitrogen (N) and phosphorus (P) phytoextraction from biosolids in (i) a wetland constructed in the former primary cell and (ii) a dewatered secondary cell of an end-of-life municipal lagoon. Overall, the phytoextraction of N and P by cattail was lower with a single harvest than two harvests per year. The study also examined the effects of harvest timing (August, November, and April) on nutrient removal in the harvested cattail biomass. Compared to August, harvesting cattails in the wetland in November or April reduced N and P phytoextraction by 63-85%.

In the wetland study, nutrient phytoextraction was 6.2% of initial N content and 2.2% of initial P content while the terrestrial-based approach removed 5.8% and 2.3% of the initial N and P content, respectively. A greater fraction of P (~ 73%) taken up by cattail was sequestered in the rhizomes, which reduced its mobility and transport to surface waters.

A study examining nutrient availability using plant root simulator (PRS) probes during wetland-based phytoremediation showed that N supply rate increased with time after July whereas phosphate supply rate remained relatively unchanged. Cumulative nutrient supply rate was positively correlated with plant uptake.

The effects of flooding on P release during terrestrial phytoremediation in the secondary cell was investigated using biosolids cores. Dissolved reactive P (DRP) was the major fraction of P in floodwater. Flooding for more than 3 d resulted in the release of $>0.5 \text{ mg L}^{-1}$ DRP to floodwater. Our results suggest that biosolids pose a risk of P loss to surface water bodies receiving floodwater from the lagoon. Releasing floodwater closer to the start of the flooding event minimizes P release to floodwater.

Overall, this research shows that phytoremediation is a viable, low-cost option for managing biosolids from end-of-life municipal lagoons.

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FOREWORD

This thesis was prepared in manuscript format in accordance with the Department of Soil Science, University of Manitoba guidelines. The thesis consists of five chapters. Chapter 1 is the general introduction and chapter 2 to 5 are research chapters prepared as manuscripts. Chapter 2 focuses on biomass yield and N and P phytoextraction from biosolids in a wetland constructed in a lagoon cell previously used for primary treatment of municipal wastewater. Chapter 3 is based on the characterization of plant availability of nutrients and trace elements using PRS probes, and relating these to nutrient and trace element uptake by cattail during wetland-based phytoremediation. Chapter 4 reports on the characterization of cattail biomass yield and N and P phytoextraction under a terrestrial phytoremediation system designed to treat biosolids in a dewatered secondary cell of a municipal lagoon. Chapter 5 describes a study conducted to determine the effect of flooding depth and duration of flooding on the release of P from vegetated biosolids to pore water and surface floodwater. Chapter 6 is the overall synthesis of the findings reported in Chapters 2 to 5. Chapters 2 and 4 have been published in the Journal of Environmental Quality. Chapter 3 has been published in the Soil Society of America Journal and Chapter 5 has been published in Water, Air, & Soil Pollution.

Publication based on Chapter 2:

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TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
FOREWORD.....	v
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xii
1. GENERAL INTRODUCTION.....	1
1.1 Municipal Lagoons and Biosolids Production.....	1
1.2 Management Options for Municipal Biosolids.....	2
1.3 Phytoremediation.....	4
1.3.1 Constructed Wetlands for Phytoremediation.....	6
1.3.2 Nitrogen and Phosphorus Transformations in Submerged Sediments.....	7
1.3.3 Harvesting Emergent Wetland Plants for Nutrient Removal.....	10
1.3.4 Terrestrial Phytoremediation.....	12
1.4 Objectives.....	14
1.5 Thesis Outline.....	15
1.6 References.....	15
2. NITROGEN AND PHOSPHORUS PHYTOEXTRACTION BY CATTAIL (<i>TYPHA</i> SPP.) DURING WETLAND-BASED PHYTOREMEDIATION OF AN END-OF-LIFE MUNICIPAL LAGOON.....	23
2.1 Abstract.....	23
2.2 Introduction.....	24
2.3 Materials and Methods.....	27
2.3.1 Study Site.....	27
2.3.2 Experimental Setup.....	30
2.3.3 Plant Sampling.....	30
2.3.4 Laboratory Analysis.....	31
2.3.5 Statistical Analysis.....	32
2.4 Results and Discussion.....	32
2.4.1 Biosolids Properties.....	32
2.4.2 Biomass Yield.....	35

2.4.3 Nitrogen and Phosphorus Concentrations in the Biomass	39
2.4.4 Nitrogen and Phosphorus Phytoextraction.....	40
2.4.5 Seasonal Effects of Harvesting Cattail	43
2.4.6 Phosphorus and Nitrogen Concentrations in the Water Column	45
2.5 Conclusion	46
2.6 References.....	47
3. NUTRIENT SUPPLY RATES AND PHYTOEXTRACTION BY CATTAIL DURING CONSTRUCTED-WETLAND PHYTOREMEDIATION OF AN END-OF-LIFE MUNICIPAL LAGOON.....	52
3.1 Abstract.....	52
3.2 Introduction.....	53
3.3 Materials and Methods.....	56
3.3.1 Study Site.....	56
3.3.2 Plant Root Simulator Probes	56
3.3.3 Experimental Setup and Sampling.....	57
3.3.4 Laboratory Analysis.....	58
3.3.5 Statistical Analysis.....	59
3.4 Results and Discussion	60
3.4.1 Temporal Changes in Nutrient and Trace Element Supply Rates	60
3.4.2 Relationship Between PRS Supply Rates and Plant Uptake	66
3.4.3 Biomass Yield and Nutrient Uptake	68
3.5 Conclusion	73
3.6 References.....	73
4. A FIELD BIOASSAY OF NITROGEN AND PHOSPHORUS PHYTOEXTRACTION IN A SEASONALLY-FROZEN END-OF-LIFE MUNICIPAL LAGOON VEGETATED WITH CATTAIL	77
4.1 Abstract.....	77
4.2 Introduction.....	78
4.3 Materials and Methods.....	81
4.3.1 Study Site.....	81
4.3.2 Experimental Setup.....	83
4.3.3 Plants and Biosolids Sampling.....	84
4.3.4 Laboratory Analysis.....	84

4.3.5 Statistical Analysis.....	85
4.4 Results and Discussion	85
4.4.1 Initial Nutrient Concentrations in Biosolids	85
4.4.2 Biomass Yield.....	87
4.4.4 Plant Available Nitrogen and Phosphorus	94
4.5 Conclusion	97
4.6 References.....	98
5. FLOODING DEPTH AND TIMING EFFECTS ON PHOSPHORUS RELEASE FROM FLOODED BIOSOLIDS IN AN END-OF-LIFE MUNICIPAL LAGOON	103
5.1 Abstract.....	103
5.2 Introduction.....	104
5.3 Materials and Methods.....	107
5.3.1 Study Site.....	107
5.3.2 Experimental Setup.....	108
5.3.3 Sampling and Laboratory Analysis.....	109
5.3.4 Statistical Analysis.....	111
5.4 Results and Discussion	111
5.4.1 Biosolids Properties	111
5.4.2 Biosolids Redox Potential and pH.....	112
5.4.3 Dissolved Reactive P Concentration.....	115
5.4.4 Iron and Manganese Concentrations.....	117
5.4.5 Calcium and Magnesium Concentrations	120
5.4.6 Total Phosphorus Concentration in Floodwater	122
5.4.7 Phosphorus Fractions in Floodwater.....	123
5.4.8 Relationships Between Biosolids Phosphorus Fractions and Phosphorus Release to Pore Water and Floodwater	127
5.5 Conclusion	131
5.6 References.....	132
6. OVERALL SYNTHESIS	137
6.1 Relevant Findings and Implications of the Research.....	137
6.2 Recommendations.....	142
6.3 References.....	143

LIST OF TABLES

Table 2.1 Nutrient and trace element concentrations in biosolids from the primary and secondary cell and from the constructed wetland†.	34
Table 2.2 Cattail biomass yield and tissue nutrient concentration and phytoextraction as affected by harvest frequency and year of harvest.	36
Table 2.3 Cumulative biomass yield, nitrogen and phosphorus phytoextraction and phytoextraction rates after four years of harvesting cattail.	38
Table 2.4 Cattail biomass yield and nutrient concentration and phytoextraction (standing stock) as affected by timing of harvest and year of harvest.	44
Table 3.1 Cumulative nutrient supply (mean \pm standard error, $\mu\text{g cm}^{-2}$) calculated by summing the supply rates of successive 2-week burial periods.	64
Table 3.2 Beta sigmoid model parameters for biomass yield and nutrient uptake by cattail grown in biosolids sediment during constructed wetland phytoremediation of an end-of-life municipal lagoon.	69
Table 4.1 Initial nitrogen and phosphorus concentrations (mg kg^{-1}) in biosolids from the secondary cell of an end-of-life municipal lagoon.	86
Table 4.2 Biomass yield and nitrogen and phosphorus phytoextraction as affected by harvest frequency and year of harvest.	88
Table 4.3 Temporal variation in post-harvest available nitrogen and Olsen P concentrations in biosolids during the 5-year study.	95
Table 5.1 Initial phosphorus and metal concentrations in the biosolids.	114
Table 5.2 Changes in concentrations of dissolved reactive phosphorus (DRP), dissolved total phosphorus (DTP), particulate phosphorus (PP), and total phosphorus (TP), dissolved Fe, Mn, Ca, Mg, and in biosolids redox potential (Eh) and pH during flooding.	114
Table 5.3 Pearson correlation coefficients between pore water dissolved reactive P (DRP) and pore water dissolved Fe, Mn, Ca and Mg, and between floodwater DRP concentrations and pore water dissolved Fe, Mn, Ca and Mg. (n = 30).	118
Table 5.4 Changes in concentrations of P fractions in biosolids during flooding.	128
Table 5.5 Pearson correlation coefficients between pore water dissolved reactive P (DRP) and biosolids inorganic P (Pi) and organic P (Po) fractions (n = 29).	128

Table 5.6 Pearson correlation coefficients between pore water dissolved reactive P (DRP), floodwater DRP, floodwater total P (TP) and biosolids inorganic P (Pi) and organic P (Po) fractions. Flooding depths averaged across all sampling times. (N = 85). 130

LIST OF FIGURES

Figure 2.1 Satellite scans of the Niverville lagoon system during (a) operation and (b) during phytoremediation (b). Images courtesy of Google Earth.....	28
Figure 2.2 Primary cell (a) before and (b) during construction and (c) seeding of wetland system, and (d) one year (2014) after wetland establishment.....	30
Figure 2.3 Cattail biomass yield as affected by harvest frequency and year of harvest. Values for the control in a given year are cumulative since the start of the experiment. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.....	37
Figure 2.4 Cattail nitrogen phytoextraction as affected by harvest frequency and year of harvest. Values for the control in a given year are cumulative since the start of the experiment. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.....	40
Figure 2.5 Cattail phosphorus phytoextraction as affected by harvest frequency and year of harvest. Values for the control in a given year are cumulative since the start of the experiment. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.....	41
Figure 3.1 PRS TM probes retrieved from three plots from a transect, cleaned and combined to form a composite sample.	57
Figure 3.2 Temporal changes in nutrient supply rates in biosolids, measured by PRS probes, as a function of time. Vertical bars represent standard errors of the mean.....	61
Figure 3.3 Relationships between nutrient uptake by cattail and available nutrient supply rate as determined by PRS probes.....	67
Figure 3.4 Cattail biomass accumulation as described by a beta sigmoid model.....	70
Figure 3.5 Nutrient and trace element accumulation in cattail biomass as described by a beta sigmoid model.....	72
Figure 4.1 Monthly cumulative rainfall and average temperatures at St. Adolphe, Manitoba (10 km from study site) during the 5-yr study.	82
Figure 4.2 Mean monthly snow water equivalents (mm) estimated from snow depth at Kleefeld, Manitoba (17 km from study site).	83

Figure 4.3 Cattail biomass accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.	89
Figure 4.4 Cattail belowground biomass, nitrogen, and phosphorus accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.	90
Figure 4.5 (a) Harvesting of cattail in the secondary cell, 15 August 2017; (b) bales of the harvested cattail.	91
Figure 4.6 Cattail nitrogen accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.	92
Figure 4.7 Cattail phosphorus accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.	94
Figure 5.1 (a) 45.7-cm diam. by 60-cm ht. polyvinyl chloride (PVC) pipe; (b) extraction of a core using a backhoe; (c); gluing a polythene sheet to an extracted core; (d) cores placed near the edge of the lagoon cell.	109
Figure 5.2 Flooding depth (5, 15, and 25 cm) effects on temporal changes in biosolids (a) redox potential (Eh) and (b) pH during 42 d of flooding.	113
Figure 5.3 Flooding depth (5, 15, and 25 cm) effects on temporal changes in biosolids pore water dissolved reactive phosphorus (DRP) concentration during 42 d of flooding.	115
Figure 5.4 Flooding depth (5, 15, and 25 cm) effects on temporal changes in dissolved reactive P (DRP) concentrations in surface floodwater during 42 d of biosolids flooding.	117
Figure 5.5 Flooding depth (5, 15, and 25 cm) effects on temporal changes in dissolved Fe, Mn, Ca, and Mg concentrations in biosolids pore water during 42 d of biosolids flooding. Error bars represent standard errors of the means.	119
Figure 5.6 Flooding depth (5, 15, and 25 cm) effects on temporal changes in dissolved Fe, Mn, Ca, and Mg concentrations in surface floodwater during 42 d of biosolids flooding. Error bars represent standard errors of the means.	121
Figure 5.7 Flooding depth (5, 15 and 25 cm) effects on temporal changes in total phosphorus (TP) concentration in floodwater during 42 d of biosolids flooding. Error bars represent standard errors of the means.	123
Figure 5.8 Flooding depth (5, 15 and 25 cm) effects on temporal changes in dissolved reactive phosphorous (DRP), dissolved total phosphorus (DTP), and particulate P (PP) fractions (% of total P) during 42 d of biosolids flooding. Error bars represent standard errors of the means.	126

1. GENERAL INTRODUCTION

1.1 Municipal Lagoons and Biosolids Production

Municipal lagoons, also known as wastewater stabilization ponds or wastewater treatment ponds, are a common form of wastewater treatment in small and medium-sized communities in North America because of their lower construction, operational and maintenance costs and their ease of operation relative to municipal wastewater treatment plants (CCME, 2006; USEPA, 2011). There are more than 8000 municipal lagoons in the United States, representing >50% of wastewater facilities in the country (USEPA, 2011). In Canada, there were more than 1000 municipal lagoons in the late 1980s, accounting for approximately 50% of the wastewater treatment facilities, with most of them in western and northern Canada (Heinke et al., 1991; Smith and Emde, 1999). As of 2004, at least 350 communities in Manitoba, Canada, used municipal lagoons for wastewater treatment (Manitoba Land Initiative, 2004).

Wastewater treatment in municipal lagoons is a natural process that uses microorganisms and other physical factors likely settling and sorption to degrade organics and other constituents in the wastewater. Municipal lagoons reduce concentrations of nutrients (primarily nitrogen (N) and phosphorus (P)), biochemical oxygen demand, total suspended solids, and coliforms to meet effluent water quality standards. Nitrogen and P are removed from wastewater by incorporation into sludge (sedimentation, precipitation of P, sorption, and biomass assimilation) and loss of N to the atmosphere through ammonia volatilization and denitrification (Kayombo et al., 2010; USEPA, 2011). Sludge accumulates at the bottom of municipal lagoons from the settlement of wastewater solids and from surplus growth of microorganisms in the wastewater.

Sludge accumulation at the bottom of municipal lagoons gradually reduces wastewater treatment efficiency and will eventually require removal. Some municipalities desludge after

several years of sludge accumulation (5 to 20 yr) (National Research Council, 2004; Smith and Emde, 1999), while others never desludge until lagoons reach the end of their lifespan of 20-30 yr (Smith and Emde, 1999). Many municipal lagoons built in Canada in the 1960s and 1970s have reached their design capacity and require decommissioning. Before sludge is removed from the lagoons, it is typically stabilized and dewatered to reduce odour and pathogens. Biosolids are derived from the stabilization of sewage sludge and in municipal lagoons, this is achieved through long term storage of sewage sludge in municipal lagoons.

The disposal of biosolids during decommissioning of municipal lagoons is a challenge for many small rural communities. The research reported in this thesis evaluates in situ remediation of biosolids in an end-of-life lagoon from a small town with a population of 4,610 (Statistics Canada, 2017).

1.2 Management Options for Municipal Biosolids

Biosolids contain macronutrients such as N and P and micronutrients such as copper, iron and zinc which are essential for crop productivity. Agricultural field application of biosolids can therefore reduce the need for commercial fertilizers, thus providing an economic benefit to farmers. In addition, organic matter in the biosolids improves the bulk density, porosity and water holding capacity of receiving soils and stimulate soil microbial activity. Because of their nutrient value, biosolids are typically spread on farmland in many jurisdictions. In the USA, about 60% of biosolids produced annually are spread on farmland (NEBRA, 2007). Spreading biosolids on farmland is the most common practice in western Canada where it accounts for 80% of the biosolids generated, compared with about 25%-40% in eastern Canada (City of Winnipeg, 2013; LeBlanc et al., 2009). Where land spreading of biosolids is not economical or feasible, landfilling is a common biosolids disposal option.

Although land application is the most suitable option for managing biosolids in many jurisdictions, the spreading of biosolids on farmland can be expensive, restricted or infeasible. Regulations in some jurisdictions restrict land application of biosolids. For example, biosolids use on farmland is banned in some municipalities in Quebec, Canada (Jong and Oleszkiewicz, 2011). An increasing number of jurisdictions are introducing regulations on biosolids application rates based on crop P requirements (CMWC, 2015; Shober and Sims, 2003; Simpson, 1998), and this increases the land base required to apply biosolids. Limitations on nutrient application rates may limit land application of biosolids in Canada in the near future (Brydon and Langman, 2009). Manitoba is an example of a province in Canada with provincial regulations that may drive municipal biosolids off the farmland (Jong and Oleszkiewicz, 2011). In January 2011, changes to the Manitoba nutrient management regulations under the Water Protection Act reduced application rates of biosolids to farmland (City of Winnipeg, 2014a). The City of Winnipeg, Manitoba could not spread the biosolids on farmland at the reduced rates and the city began landfilling biosolids. In June 2011, Manitoba Sustainable Development amended the Water Protection Act of Manitoba to include a clause that requires the beneficial use of nutrients and organic matter in municipal biosolids (Manitoba Water Protection Act, 2018) and is therefore opposed to landfilling of biosolids. In addition, there is a proposed ban on landfilling of organic waste, including biosolids in Canada (LeBlanc et al., 2008; National Research Council, 2017), and in some jurisdictions, such as Nova Scotia, Prince Edward Island, landfilling of biosolids has already been banned (CCME, 2010). Landfilling of biosolids contributes to emissions of greenhouse gases, such as methane, due to organic matter decomposition in landfills. The City of Winnipeg, which currently landfills biosolids, is exploring other beneficial management options for biosolids, including thermal oxidation with energy recovery, composting, and pelletization (City of Winnipeg, 2014a).

In small communities in Manitoba and other jurisdictions, land spreading of biosolids is still the most common sustainable option. Any restriction on farmland application of biosolids will, therefore, greatly affect small communities, which do not have adequate funding to explore alternative options. The volume of biosolids from municipal lagoons can be high, ranging 8000-30,000 m³ (Brian, 2016; Gene, 2016; Keam and Whetter, 2008), and the agricultural land base required to accommodate such volumes can range from 200-300 ha.

In 2008, the Town of Niverville, Manitoba (population 2,500 in 2006) was faced with finding an option to dispose of biosolids from a 13-ha sewage lagoon. At that time, two options were available, landfilling at an estimated cost of ~\$2.64 million and spreading the biosolids on surrounding farmland at an estimated cost of at least \$1.45 million. Spreading biosolids on farmland was the cheaper option, but competition for spreading land with manure from the large number of livestock operations in the surrounding area made it challenging to find suitable land (~4800 acres) for spreading the biosolids. In Manitoba, land application of biosolids faces huge competition for land from hog manure production and it may be difficult to find suitable land within economic distances to spread the biosolids. Trucking of biosolids to landfills can be unaffordable for small communities with limited budgets. There is, therefore, a need for economical and sustainable options for managing biosolids from end-of-life lagoons in small communities. Phytoremediation may be a viable option for remediating biosolids in situ in end-of-life lagoons in small municipalities, but this has not been tested.

1.3 Phytoremediation

Phytoremediation is an emerging technology that uses plants to degrade, extract, contain, or immobilize contaminants from soil and water (Pilon-Smits, 2005). Use of plants and associated microorganisms to treat contaminants is solar driven, and therefore, phytoremediation is often

cheaper than engineering-based remediation approaches such as soil excavation, pump-and-treat, and soil washing. Phytoremediation involves many mechanisms, including phytoextraction, phytostabilization and phytodegradation. Removal of contaminants from contaminated sites is achieved by phytoextraction, i.e., the uptake of contaminants by plant roots and their translocation to aerial parts of the plant, along with harvesting of the plant biomass. Plants with high N and P requirements can take large amounts of these nutrients from impacted soils or wastewater. Nitrogen taken up by plants is incorporated into amino acids, proteins, nucleotides for DNA and RNA, nucleic acids, chlorophyll, and coenzymes. Phosphorus is used for synthesis of proteins, nucleic acids, coenzymes, and phospholipids, and for energy transfer in the form of adenosine triphosphate (ATP) (Reddy and DeLaune, 2008). While phytoremediation has been accepted as an economical and environmentally-friendly remediation technique (Mir et al., 2017; Pilon-Smits, 2005), studies on the efficacy of in situ phytoremediation of biosolids in end-of-life municipal lagoons are lacking.

Phytoremediation can be achieved using wetland or terrestrial approaches. Growing seeded crops or terrestrial plants during traditional terrestrial phytoremediation is about as reliable as farming. Terrestrial phytoremediation occurs under upland (i.e., not submerged or waterlogged) conditions while wetland phytoremediation involves the control of water depth. Wetlands are among the most productive ecosystems on earth (Mitsch and Gosselink, 2007; Reddy and DeLaune, 2008) and wetland conditions can support high biomass yields of emergent wetland plants. Therefore, wetland phytoremediation could be a more effective approach for treating contaminants compared to terrestrial phytoremediation. The manipulation of the physicochemical environment in the litter layer and fine sediments in wetlands may influence nutrient and contaminant dynamics that may be absent or minimal under terrestrial conditions. For example,

denitrification in cattail marshes increases as litter biomass increases (Bachand and Horne, 1999) and anoxic conditions in wetland sediments can stimulate P release from sediments or immobilize trace elements such as copper and zinc. Wu et al. (2014) reported that TP concentrations in cattail leaves, roots, and shoots were 12, 84, and 42%, respectively, greater in cattail which had established in sediments under waterlogged conditions than in cattail in aerated sediments.

1.3.1 Constructed Wetlands for Phytoremediation

Constructed and natural wetlands have been widely used as a reliable approach for removing nutrients, trace elements and other contaminants from wastewater from various sources, including municipal, domestic, agricultural, and industrial wastewater (Herath and Vithanage, 2015; Kadlec and Wallace, 2008; Vymazal, 2014). When wetlands are used for wastewater treatment, they receive wastewater and hold and recycle nutrients and other contaminants before relatively cleaner effluent is released to receiving waters. Nutrients and other contaminants are removed by various processes such as sedimentation, denitrification, sorption to sediments, and uptake by biota and wetland plants (Reddy and DeLaune, 2008; Vymazal, 2007). In wastewater treatment wetlands, designs which increase contaminant transfer to the sediment are desirable to provide removal of contaminants from wastewater. Sorption to sediments is a major pathway for removal of P and trace elements from wastewater (Reddy and DeLaune, 2008; Vymazal, 2007; Vymazal and Kröpfelová, 2008).

While harvesting plants from wastewater treatment wetlands may not be a significant removal pathway for elements such as P and trace elements from wastewater, it is an important pathway for removing contaminants during wetland phytoremediation of biosolids sediments. The conversion of municipal lagoons to engineered wetland systems and harvesting of wetland plants may be an acceptable remediation strategy during decommissioning of end-of-life lagoons. To

date, remediation of biosolids in end-of-life lagoons using wetland phytoremediation has not been evaluated. Therefore, the research reported in Chapter 2 of this thesis examined phytoremediation of N and P from biosolids in an end-of-life lagoon using a wetland-based approach.

1.3.2 Nitrogen and Phosphorus Transformations in Submerged Sediments

Nitrogen and P exist in both organic and inorganic forms in wetlands and the relative proportion of each form depends on the source of N and P in the wetlands (Reddy and DeLaune, 2008). Wetlands for wastewater treatment may frequently receive a fresh source of readily available N and P while in a closed system, such as wetland for remediating sediments, nutrient cycling is mainly driven by the biogeochemical changes in the sediment and water column.

Nitrogen transformation in wetland sediments is markedly different from that in well-drained, aerated soils in terrestrial systems (Buresh et al., 2008; De-Campos et al., 2012). Reduced conditions in wetlands affect the prevalent microbes and microbial processes, nutrient turnover and availability, and gaseous losses of N via denitrification. Nitrogen forms present in wetland sediments are generally similar to those in aerated terrestrial environments, but the relative proportions of the inorganic forms of N are distinctly affected by the oxidation status of the sediments. Ammonium N is the dominant form of inorganic N that accumulates in reduced wetland sediments, while $\text{NO}_3\text{-N}$ is the dominant form of inorganic N in aerated soils. Ammonium N accumulates due to the absence of oxygen, which is required to convert $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$. Wetland plants such as cattail are adapted to utilize ammonium N instead of nitrate N (Brix et al., 2002; Nakamura et al., 2010). The major N transformation processes in wetlands, as in terrestrial environments, are mineralization, immobilization, nitrification, denitrification, ammonia volatilization, and biological N_2 fixation (Kirk, 2004; Reddy and DeLaune, 2008).

Ammonification, the conversion of organic N to $\text{NH}_4\text{-N}$, supplies plant available N to wetland plants. The decomposition of organic matter in wetlands is achieved by relatively restricted anaerobes that operates at a lower energy level and are less efficient than a wide range of aerobes in aerated terrestrial systems (Buresh et al., 2008). The breakdown of organic matter and plant residues is slower in anaerobic sediments than in aerobic soils (Buresh et al., 2008; Villegas-Pangga et al., 2000); therefore, low N mineralization rates can be expected in wetland sediments. Low mineralization under anaerobic conditions may have important implications where the wetland sediments, e.g., biosolids, contain N mainly in the organic form and the wetland system does not receive fresh sources of readily soluble nutrients. Low mineralization under anaerobic conditions may result in low available N concentrations for plant uptake and therefore affect biomass yields and phytoextraction of N and other contaminants. Investigation of N availability from biosolids under wetland conditions is therefore warranted.

Phosphorus in wetlands is typically present in both organic and inorganic forms, and in dissolved and particulate forms (Reddy and DeLaune, 2008). The transformation of P within the water column and sediment/soil is regulated by both biotic and abiotic mechanisms. Biotic processes include uptake by vegetation, microorganisms, plankton, and periphyton, while abiotic processes include adsorption by sediments, precipitation, sedimentation, and exchange between the sediment and the water column (Reddy and DeLaune, 2008; Vymazal, 2007). The capacity of wetlands to remove P from the water column to the sediment and retain it in a form that is not readily released to the environment is an important attribute of wastewater treatment wetlands. While sorption and sedimentation are the main mechanisms for P removal in wastewater treatments (Reddy and DeLaune, 2008; Vymazal, 2007; Vymazal and Kröpfelová, 2008), harvesting macrophytes is an important pathway for P removal from sediments.

Redox potential in sediments influences nutrient availability for plant uptake. Plant availability of N decreases while that of P increases with decreasing redox potential (Dunne and Reddy, 2005; Kinsman-Costello et al., 2014). Phosphorus release from flooded sediments can be significant (Dunne et al., 2006; Kinsman-Costello et al., 2014), but P availability is further complicated by sediment characteristics that may affect P availability and other physiochemical reactions, such as adsorption to clay particles, changes in pH, changes in carbonate equilibrium, and precipitation of P with Ca and Mg (Dunne and Reddy, 2005). While a lot of studies have examined availability of N and P in biosolids applied to agricultural soils (Gil et al., 2011; Gilmour et al., 2003; Shaheen and Tsadilas, 2013), information on nutrient and trace element availability in biosolids under wetland conditions are lacking. This is partly because biosolids application has mostly been limited to upland situations such as agricultural land or reclamation sites. The difference in biogeochemistry between wetland and upland systems may result in differences in nutrient dynamics.

Ion exchange membranes such as plant root simulator (PRS) probes have been developed to measure nutrient availability under field conditions (Qian and Schoenau, 2002). These membranes adsorb ions at a rate dependent on ion activity and diffusion rate in solution and are considered to mimic uptake by plant roots (Qian and Schoenau, 2002) and can be used to examine temporal changes in nutrient supply throughout the growing season. While PRS probes have been widely used in agricultural fields (Qian and Schoenau, 2002; Shaheen and Tsadilas, 2013), it is not clear whether they could be equally useful in examining temporal changes in nutrient supply in biosolids sediments under wetland conditions (Nelson et al., 2007) or in submerged soils (Miller et al., 2017) and peatlands (Wood et al., 2016). Therefore, the study presented in Chapter 3 of this thesis was

conducted to investigate nutrient and trace element availability using PRS probes during wetland-based phytoremediation of biosolids vegetated with cattail.

1.3.3 Harvesting Emergent Wetland Plants for Nutrient Removal

Harvesting macrophytes in wetlands has been shown to remove nutrients such as N and P from the wetland systems, but generally, the amount of nutrients removed has been considered insignificant (Toet et al., 2005; Vymazal, 2005; Vymazal, 2007). Most of these studies have been carried out in constructed wetlands receiving high nutrient loads ($122\text{-}4190\text{ g N m}^{-2}\text{ yr}^{-1}$ and $28\text{-}994\text{ g P m}^{-2}\text{ yr}^{-1}$) (Toet et al., 2005; Vymazal, 2005; Vymazal, 2007; Vymazal, 2014) and nutrient removal from harvesting plants was insignificant compared to the incoming wastewater load. For example, Toet et al. (2005) and Vymazal (2007) reported N and P removal rates of less than 10% from harvesting wetland plants and this was considered insignificant. The role of plants in these highly loaded treatment wetlands has been considered to be mainly physical, including reducing turbulence to aid sedimentation and providing surface area for bacteria involved in nutrient transformation. Nutrient removal from harvesting wetland plants can be significant in lightly loaded systems. Martin and Fernandez (1992), Koottaoe and Polprasert (1997), and Vymazal (2007) reported high removal rates (40-70% of total N and total P) from harvesting cattail in lightly loaded ($<100\text{-}260\text{ g N m}^{-2}\text{ yr}^{-1}$ and $<10\text{-}30\text{ g P m}^{-2}\text{ yr}^{-1}$) treatment wetlands. Harvesting wetland plants is also important to prevent nutrient loss back into the aquatic systems from decaying plant litter. Sharma et al. (2006) found that *T. angustifolia* released 4.6 g N m^{-2} and 0.8 g P m^{-2} from dead shoots into the water after its senescence. The nutrient loss represented 10% of total N (46.7 g m^{-2}) and 17% of total P (4.7 g m^{-2}) accumulated in the shoots. Harvesting wetland plants can be an important pathway to remove nutrients from sediments, especially in systems where there is no continuous supply of fresh sources of contaminants from incoming wastewater. Harvesting plants

established in biosolids sediments in end-of-life lagoons to phytoextract nutrients is therefore worthy of exploration.

Nutrient phytoextraction is a product of biomass yield and nutrient concentration. Annual harvesting of wetland plants has been demonstrated to maintain biomass yields and nutrient removal (Grosshans, 2014; Kadlec and Wallace, 2008; Toet et al., 2005). Some researchers suggested that total productivity of plants, hence nutrient removal, could be increased if plants were harvested repeatedly during the growth period (Jinadasa et al., 2008; Vymazal and Kröpfelová, 2005; Vymazal et al., 2010). Jinadasa et al. (2008) reported that a single harvest per year of *Typha angustifolia* and *Scirpus grossus* produced lower N ($1.16 \text{ kg m}^{-2} \text{ yr}^{-1}$) and P ($0.16 \text{ kg m}^{-2} \text{ yr}^{-1}$) removal than repeated harvesting (i.e., 4 times per year), which removed $4.63 \text{ kg N m}^{-2} \text{ yr}^{-1}$ and $0.64 \text{ kg P m}^{-2} \text{ yr}^{-1}$ in a tropical wetland treating wastewater, but aboveground biomass production declined significantly with four harvests per year. Harvesting *Typha* four times a year was sustainable in Mexican wetlands (Hall et al., 2008). If repeated harvesting of cattail in temperate regions sustains biomass yields and increases nutrient removal from biosolids sediments, it can effectively be used as a strategy to remediate biosolids in end-of-life lagoons.

Biomass yield and nutrient concentrations in tissues of perennial plants in temperate regions is affected by the timing of harvest. Typically, nutrients and carbohydrates are translocated to the aboveground biomass during periods of growth, but at the end of the growing season, nutrients and carbohydrates are translocated to belowground tissues for storage. Harvesting during peak biomass accumulation in summer maximizes nutrient removal (Grosshans, 2014). However, harvesting plants in wetland environments during summer offers serious challenges, such as operating harvesting equipment in wet environments and the high moisture content of plants (Cicek et al., 2006; Grosshans, 2014). Harvesting plants in fall or winter when the ground is frozen

can provide conducive conditions for operation of heavy equipment but can significantly decrease biomass and nutrient removal. The rate of translocation of nutrients and photoassimilates from leaves to rhizomes can be more than 50% and is influenced by plant species and environmental factors (Vymazal, 2007). Biomass decreases through a combination of resorption and litter fall and nutrients are translocated to rhizomes. In constructed wetland systems, water levels can be manipulated, e.g., by drawing down water to allow harvesting in summer. Specialized equipment has been developed to harvest plants in wetland environments (Grosshans, 2014; Grosshans and Greiger, 2013). Fall and winter harvests can also be alternative choices depending on how they affect phytoremediation timeframes. Whether to manipulate water levels and harvest in summer or to harvest in more conducive environments for harvesting equipment in fall or winter requires an evaluation of harvest timing effects on remediation timeframes for land managers to make informed choices.

1.3.4 Terrestrial Phytoremediation

Terrestrial phytoremediation has been used to phytoextract nutrients from enriched soils using agricultural crops and forages. Agricultural crops such as corn, wheat, and soybean, and forages such as switchgrass, elephant grass, and alfalfa have been used for the phytoextraction of P where repeated application of manure has resulted in elevated soil P concentrations (Fiorellino et al., 2017; Silveira et al., 2013). Phytoextraction of nutrients from soils in livestock manure lagoons after excavation of manure during decommissioning of lagoons has been demonstrated (Douglas-Mankin et al., 2010; Liphadzi et al., 2002; Zhu and Kirkham, 2003). A few studies have investigated the use of plants to remediate sewage sludge or biosolids in sewage or biosolids stockpiles (Laidlaw et al., 2012; Suchkova et al., 2015; Suchkova et al., 2014). None of these

studies were conducted directly in lagoons with the aim of developing a sustainable system for N and P extraction during decommission of end-of-life municipal lagoons.

Previous studies have examined phytoremediation of stockpiled biosolids removed from lagoons; however, biosolids excavation can be costly. In addition, municipal lagoons are underlain by an impermeable clay lining and may undergo periods of prolonged waterlogging from spring snowmelt or periods of heavy summer rainfall, which can influence the plant species that can be effectively used in lagoons. The frequency and duration of saturated conditions will determine which plants will grow or establish successfully on these sites. Suchkova et al. (2014) found that native plants established naturally in sewage sludge that was amended with soil and gravel to improve aeration and structure but not in sewage sludge alone, an observation which they attributed to poor aeration in the waterlogged unamended sludge (75-85% water content). Excess moisture conditions that can prevail in end-of-life lagoons can provide conditions which are suitable for establishment of plants such as cattails which are adapted to waterlogged conditions. After decommissioning of municipal lagoons, cattail plants can establish from the seedbank in the sludge if moisture is adequate. The investigation of native plants which self-establish at contaminated sites is the first starting point of plant selection for phytoremediation (USEPA, 2000). Currently, there is no information on the efficacy of harvesting cattail for nutrient removal from biosolids in seasonally-frozen end-of-life lagoons. Therefore, the study in Chapter 4 examined biomass yield and nutrient phytoextraction from harvesting cattail plants that established naturally in a dewatered secondary cell of a municipal lagoon.

Terrestrial phytoremediation in low-lying areas, such as end-of-life municipal lagoons, can be susceptible to flooding. End-of-life municipal lagoons undergoing terrestrial phytoremediation may require the release of floodwater following heavy rainfall or snowmelt. Municipal lagoons in

rural Canadian communities often release wastewater effluent into streams once or twice annually during operation (National Research Council, 2004), and land managers may consider releasing floodwater from end-of-life lagoons during terrestrial phytoremediation. Flooding of soils can result in P release to surface floodwater (Amarawansa et al., 2015; De-Campos et al., 2012; Kröger et al., 2012) and can contribute to eutrophication if the floodwater is released to surface water bodies. It is conceivable that flooding of municipal biosolids in end-of-life lagoons can stimulate the release of phosphorus (P) to floodwater and contribute to P enrichment of receiving waters if the floodwater is released. Flooding depth and duration of flooding may influence P release to the floodwater and P dynamics in the flooded biosolids; this was therefore the focus of the study reported in Chapter 5 of this thesis.

1.4 Objectives

The overall objective of this thesis was to evaluate and characterize phytoextraction of nitrogen and phosphorus from municipal biosolids in end-of-life lagoons using wetland and terrestrial phytoremediation approaches. Specific objectives were to (i) quantify biomass yield and N and P phytoextraction when cattail was harvested twice or once per growing season, and to compare effects of harvest timing on N and P removal in biosolids sediments in a wetland constructed within a lagoon cell previously used for primary treatment of municipal wastewater (**Chapter 2**); (ii) characterize the plant availability of nutrients and trace elements using PRS probes, and to relate these to nutrient and trace element uptake by cattail in a wetland system constructed to treat an end-of-life primary wastewater treatment cell (**Chapter 3**); (iii) characterize cattail biomass yield and N and P phytoextraction under a single harvest vs. two harvests per season under a terrestrial phytoremediation system designed to treat biosolids in a dewatered secondary cell of a municipal lagoon (**Chapter 4**); and (iv) determine the effect of flooding depth

and duration of flooding on the release of P from vegetated biosolids to pore water and surface floodwater in the secondary cell of the municipal lagoon; to examine P fractionation in surface floodwater under different flooding depths and durations of flooding, and to examine the relationship between concentrations of dissolved Ca, Mg, Fe, Mn, and P concentrations in pore water and floodwater (**Chapter 5**).

1.5 Thesis Outline

The general layout of this thesis follows the thesis guidelines of the Department of Soil Science, University of Manitoba. The individual thesis research chapters (Chapter 2 through 5) were prepared in manuscript format and are as follows:

Chapter 2: Nitrogen and phosphorus phytoextraction by cattail (*Typha* spp.) during wetland-based phytoremediation of an end-of-life municipal lagoon.

Chapter 3: Nutrient supply rates and phytoextraction by cattail during constructed- wetland phytoremediation of an end-of-life municipal lagoon.

Chapter 4: A field bioassay of nitrogen and phosphorous phytoextraction from biosolids in a seasonally-frozen end-of-life municipal lagoon vegetated with cattail.

Chapter 5: Flooding depth and timing effects on phosphorus release from flooded biosolids in an end-of-life municipal lagoon.

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2. NITROGEN AND PHOSPHORUS PHYTOEXTRACTION BY CATTAIL (*TYPHA* SPP.) DURING WETLAND-BASED PHYTOREMEDIATION OF AN END-OF-LIFE MUNICIPAL LAGOON

2.1 Abstract

Spreading biosolids on farmland can be an effective and beneficial option for managing end-of-life municipal lagoons. Where the spreading of biosolids on farmland is restricted or unavailable, in situ phytoremediation could be a sustainable alternative. This study examined nitrogen (N) and phosphorus (P) phytoextraction by cattail (*Typha* spp.) from biosolids in a wetland constructed within a lagoon cell previously used for primary treatment of municipal wastewater. The effect of harvesting season as well as harvest frequency on N and P removal were evaluated. Forty-eight 4-m² plots within the constructed wetland were used to determine the effect of cattail harvest frequency on plant N and P phytoextraction. Harvesting twice per season resulted in a 50–60% decrease in phytoextraction of N and P relative to a single harvest per season, which produced biomass yields of 0.58–0.6 kg m⁻² per year and accumulated 36.7 g N m⁻² and 5.6 g P m⁻² over the 4-yr period. Compared to August, harvesting cattails in November or April reduced N and P phytoextraction by 63–85%. These results demonstrate that phytoextraction of nutrients is more effective with a single harvest compared to two harvests per season. Additionally, we found that while harvesting in November and April is appealing logistically (since the wetland is frozen and provides easier access to harvest equipment), nutrient removal rates are significantly reduced.

2.2 Introduction

Small rural municipalities in North America use lagoons for low-cost wastewater treatment. Western and northern Canada are home to the majority of municipal lagoons built in Canada since the late 1980s (Heinke et al., 1991; Townsend and Knoll, 1987). In Manitoba, Canada, municipal lagoons accounted for 85% (127 lagoons) of the total wastewater treatment facilities in the late 1990's (Smith and Emde, 1999). Municipal lagoons have a typical life-span of ~30 years and many of the lagoons built in the late 1980s are due for decommissioning.

The management of large volumes of biosolids (residual solid matter) when lagoons cease operation is a challenge for small rural municipalities with limited funds or options for disposal of the biosolids. Spreading biosolids on farmland is the most common and sustainable option for managing biosolids in North America (Haynes et al., 2009; Lu et al., 2012). In some jurisdictions, such as in the province of Quebec, Canada and in Europe, land spreading of biosolids has been banned (Jong and Oleszkiewicz, 2011). Where land spreading is restricted, alternatives include landfilling (Roy et al., 2011), composting (City of Winnipeg, 2018), and incineration of biosolids (Kelessidis and Stasinakis, 2012). Issues associated with incineration have previously been highlighted (More et al., 2016).

Approximately 80% of biosolids produced in western Canada are spread on farmland (City of Winnipeg, 2014b). Increasingly stringent regulations on biosolids application on farmland, including reduced application rates in summer, the banning of land-spreading of biosolids in winter (City of Winnipeg, 2013), the increasing land base required for biosolids application based on P rates (Shober and Sims, 2003), competition with manure from livestock for farmland, and the high cost of transporting biosolids are some of the factors that can limit biosolids this practice. The future of biosolids landfilling in Canada is likely going to be limited due to the Canadian government's recent proposal to ban the landfilling of organic wastes to reduce greenhouse gas

emissions from landfills (Ouimet et al., 2015). There is therefore a need for economically sustainable alternatives for managing biosolids from end-of-life lagoons where the traditional disposal methods are limited or restricted. Constructed wetland-based phytoremediation of end-of-life lagoons provides a potentially viable option for managing biosolids during decommissioning of such lagoons.

Because of their simple operation and low implementation costs, constructed wetlands have been widely used as a sink and transformer for contaminants during treatment of wastewater from various sources, including municipal, domestic, agricultural, and industrial wastewater (Herath and Vithanage, 2015; Vymazal, 2014). To the best of our knowledge, constructed wetlands have not been tested for in situ remediation of biosolids in end-of-life municipal lagoons. Sorption to sediment is the major removal pathway for P and contaminants such as trace elements from wastewater treatment wetlands (Reddy and DeLaune, 2008; Vymazal, 2007; Vymazal and Kröpfelová, 2008). Harvesting of wetland plants is essential to remove nutrients such as N and P from sediments and prevent their recycling when plants die and decompose. Biomass from harvested plants can be used as a feedstock for bioenergy production (Cicek et al., 2006).

Harvesting wetland plants for nutrient removal from wastewater has been often considered insignificant because plants only take up a small fraction of nutrients passing through wetlands (Toet et al., 2005; Vymazal, 2007). Others argue that the benefits of nutrient capture by harvesting biomass depend on nutrient mass input into the wetland system. For example, some researchers reported that harvesting wetland plants removed less than 10% of total N and P and suggested that harvesting was insignificant in wastewater treatment wetlands (Newman et al., 1999; Toet et al., 2005; Vymazal, 2007). Liu et al. (2003) and Gottschall et al. (2007) reported $\leq 10\%$ removal of total N and total P inputs in the harvested biomass in their treatment systems as significant.

Substantial removal rates of between 15% and 80% of N and 24% and 96% of P in harvested biomass have been reported in lightly loaded systems (Greenaway and Woolley, 2001; Luederitz et al., 2001; Zheng et al., 2015). While other mechanisms such as sedimentation and sorption of P to sediments are more important in nutrient removal from wastewater compared to nutrient removal through harvesting plants (Vymazal, 2007), harvesting is an important pathway to export nutrients during clean-up of sediments. Harvesting of high biomass wetland plants such as cattails could be a useful management practice to remove nutrients from biosolids used as substrate for wetland creation.

Annual harvesting (Grosshans, 2014; Kadlec and Wallace, 2008; Toet et al., 2005) and multiple harvesting of plants per growing season (Jinadasa et al., 2008; Vymazal and Kröpfelová, 2008; Vymazal et al., 2010) have been suggested for phytoextraction of nutrients and contaminants in wastewater treatment wetlands. Multiple harvests per growing season have been reported to enhance nutrient and trace element removal relative to a single harvest per growing season (Amarakoon et al., 2017; Vymazal and Kröpfelová, 2005; Vymazal et al., 2010). Conversely, Jinadasa et al. (2008) reported that cattail did not sustain repeated harvesting and biomass yield decreased significantly following four consecutive harvests in a tropical wetland treating domestic wastewater. Harvesting cattail up to four times per year has been reported in Mexican wetlands (Hall et al., 2008). The successive regrowth of plants can exhibit marked differences due to climates, plant harvesting strategies and wetland characteristics in which the plants grow (Brisson and Chazarenc, 2009; Vymazal, 2007). Biomass accumulation and nutrient phytoextraction by cattail grown in municipal biosolids have been demonstrated in short growing cycles in growth room wetland microcosm studies (Amarakoon et al., 2017; Jeke et al., 2015), but have not been evaluated under field conditions. If repeated harvesting can sustain plant regrowth under field

conditions, multiple harvesting of above-ground biomass could be an effective strategy to enhance nutrient removal.

This study evaluated the effectiveness of phytoextraction for in situ remediation of biosolids in a wetland constructed in a former primary cell of an end-of-life municipal lagoon. The research compared biomass yield and N and P phytoextraction when cattail was harvested twice or once per growing season. The 4-yr study also examined the effects of harvest timing on nutrient removal in the harvested cattail biomass.

2.3 Materials and Methods

2.3.1 Study Site

The wetland was constructed on a former primary cell of an end-of-life municipal lagoon in Niverville, Manitoba, Canada (49°35'42.7"N, 97°02'50.3"W). The lagoon consisted of a primary cell (4.6 ha) which received raw domestic wastewater and a secondary cell (8.8 ha) into which effluent from the primary lagoon was transferred for further treatment and storage (Fig. 2.1). Wastewater was treated via microbial degradation and other physical factors like settling and sorption without any pre-treatment. The municipal lagoon operated for 37 years (1971 – 2008) and served a population of approximately 2500 people in 2006 (Statistics Canada, 2007). The volume of biosolids was estimated to be approximately 20,000 m³ in the primary cell and 28,000 m³ in the secondary cell when the lagoon ceased operation.

In the fall of 2012, biosolids in the primary cell and a portion of biosolids from the secondary cell were stripped down to the underlying clay liner and temporarily stockpiled in the centre channel of the primary cell (Fig. 2.2). The primary cell clay liner was excavated to a minimum depth of 15 cm followed by re-compaction to a minimum density of 95% standard proctor dry density. Soil embankments (~110 cm deep) were constructed by placing excavated clay in

maximum 15 mm lifts, followed by compaction to a minimum of 95% standard proctor dry density. The soil embankments served as benches for placement of biosolids. Biosolids were loosely placed on the constructed benches. The target biosolids depth after settling was 40 cm.

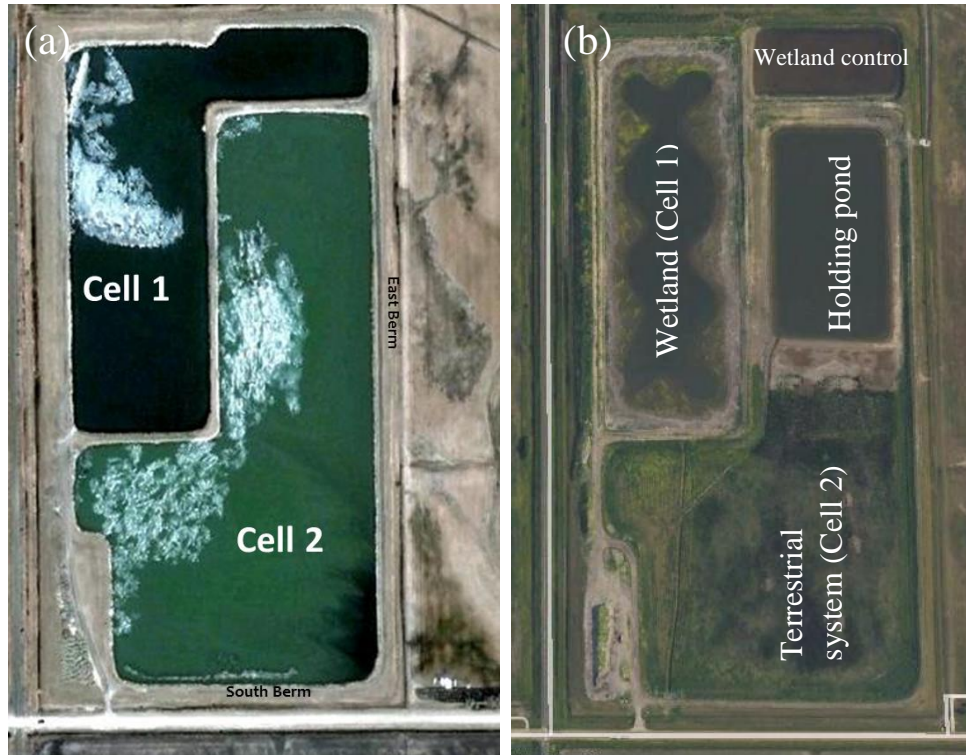


Figure 2.1 Satellite scans of the Niverville lagoon system during (a) operation and (b) during phytoremediation (b). Images courtesy of Google Earth

The wetland design included a water control system to maintain a water depth of 45 cm. Cattail rhizomes collected from surrounding ditches were planted stem side up in pre-dug divots on the biosolids benches in November 2012. Although our intention with cattail planting in the wetland would have been with *Typha latifolia* (as rhizomes), the hybrid cattail, *T. × glauca*, which established from the existing seed bank in the biosolids, was most widespread in the wetland. Part of the primary cell was isolated and undisturbed during construction of the wetland, and was left

unvegetated to serve as a control. During the first growing season (2013) following construction, the wetland was filled with water from spring melt and spring runoff from storm-water ponds within the Town of Niverville. The wetland was designed to control water depth at 45 cm, which is considered to be representative of average depths found in wetlands that support emergent plant growth (Kadlec and Wallace, 2008). In subsequent seasons, water depth in the wetland was maintained using water from an adjacent water holding pond which was replenished from the winter snowpack and spring runoff from an adjacent recreational park.

Initial biosolids samples were collected in 2011 from six locations in the primary cell. After construction of the wetland in 2013, biosolids and water samples were collected, using a sludge sampler, from each plot during harvesting of plants.



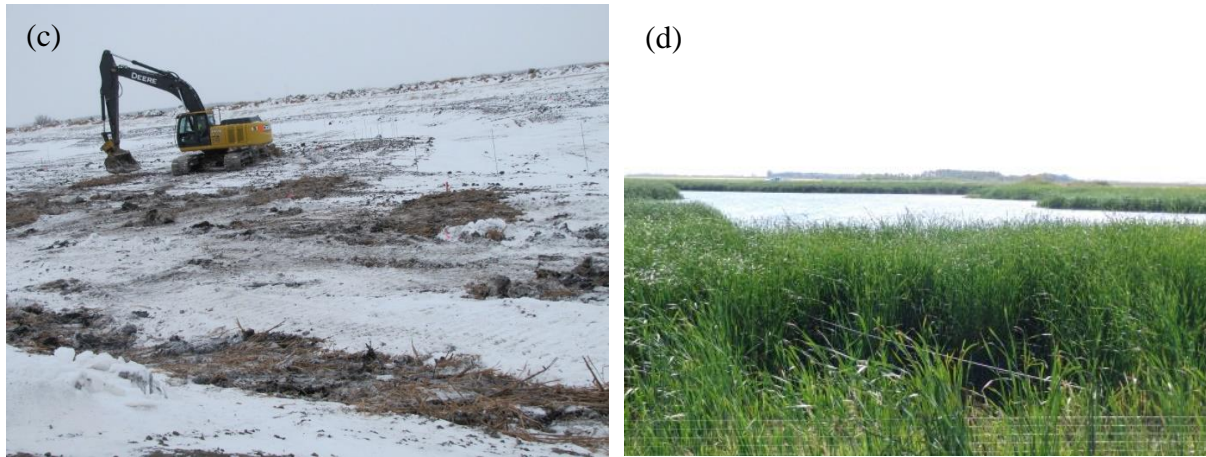


Figure 2.2 Primary cell (a) before and (b) during construction and (c) seeding of wetland system, and (d) one year (2014) after wetland establishment.

2.3.2 Experimental Setup

Eight vegetation transects ($17\text{ m} \times 2\text{ m}$) were delineated in the wetland during the spring of 2013. Six plots ($2\text{ m} \times 2\text{ m}$), with a 1 m separation between adjacent plots, were marked off in each transect. Harvest frequency treatments (one harvest and two harvests per season) were assigned to triplicate plots in each transect. The experimental layout was a randomized complete block design with a total of 48 experimental plots (2 harvest frequencies \times 3 replicates \times 8 transects), with transect as the blocking factor. Another set of nine plots ($2\text{ m} \times 2\text{ m}$) were set up in four transects that were delineated in the wetland in 2015. Three plots each were harvested in August, November, and April to determine the effects of harvest timing on cattail biomass yield and nutrient phytoextraction.

2.3.3 Plant Sampling

Cattail was harvested from a $1\text{ m} \times 1\text{ m}$ quadrat in each plot by cutting the plants at a height of $\sim 65\text{ cm}$ above the sediment layer to prevent flooding and drowning of the plants. Unharvested plot sections and adjacent sites were harvested after biomass was harvested from the quadrats

described above. The water depth was ~50–60 cm above the biosolids sediment layer during harvesting. During the first year, the plants established late and all plots were harvested once in August. In subsequent years, single-harvest plots were harvested in August. The first harvest in the two-harvest plots was in mid-July, followed by the second harvest in early September. Each year, plants in the unharvested control that had not been previously harvested were harvested to estimate cumulative biomass and nutrient accumulation.

2.3.4 Laboratory Analysis

Biosolids total Kjeldahl nitrogen (TKN) concentration was measured using a FIALab 2500 flow injection analyzer (FIALab Instruments, Bellevue, WA, USA) following digestion with sulfuric acid in a block digester (James 1993). Biosolids P and trace element (As, Cd, Cr, Cu, Pb, Hg, Ni, Se, and Zn) concentrations were determined following sample digestion in aqua regia (concentrated HNO₃/HCl) for 2 h at 90°C in a microprocessor-controlled digestion block. Phosphorus and trace element concentrations were measured in the digest with a Perkin Elmer SCIEX ELAN 6000 ICP-MS (Perkin-Elmer SCIEX Instruments, Concord, Ontario).

Biosolids samples were subjected to sequential chemical extraction to determine P fractionation using a modification of the procedure by Hedley et al. (1982). Extraction was performed sequentially using deionized water for the soluble P fraction, 0.5 M NaHCO₃ (pH 8.5) (loosely sorbed P), 0.1 M NaOH (P sorbed to Fe and Al oxides), and 1 M HCl (P associated with dissolved Ca and Mg). Inorganic P concentration in the filtrates was determined according to Murphy and Riley (1962) using an Ultrospec 3100 pro UV/visible spectrophotometer (Biochrom, Cambridge, UK) at a wavelength of 882 nm. Residual P concentration was measured after digestion of the biosolids residue with a sulfuric acid and hydrogen peroxide digestion mixture.

Plant samples were dried for 72 h at 60 °C to determine dry biomass yield, followed by grinding for tissue nutrient concentration analysis. Total N concentrations were measured by combustion using a Leco CNS 2000 analyzer (LECO Corporation, MI, USA). Phosphorus was measured using the ICP-MS described above following digestion of a 0.5-g sample in aqua regia for 2 h at 90°C using a microprocessor-controlled digestion block. Blanks and duplicates were analyzed to determine precision, and certified reference materials (GXR series) and USGS Standard Reference Materials (SRMs) to evaluate accuracy.

2.3.5 Statistical Analysis

Biomass yield and N and P concentrations and phytoextraction (product of biomass yield and nutrient concentration) data were analyzed using the GLIMMIX procedure for repeated measures in SAS version 9.4 (SAS Institute, 2015), with harvest frequency as a fixed effect and transect (block) as a random effect. Year of harvest was modelled as the repeated measure. Various covariance structures were compared and the best fit was chosen based on the lowest corrected Akaike information criterion (AICc). Treatment differences were deemed significant if $P < 0.05$ using the Tukey–Kramer adjustment for multiple comparisons.

2.4 Results and Discussion

2.4.1 Biosolids Properties

Total N and P concentrations in the biosolids (Table 2.1) were below the ranges of 13,700 – 27,600 mg TN kg⁻¹ and 3,860 – 11,000 mg TP kg⁻¹ reported for primary cell biosolids elsewhere in Manitoba (Brian, 2016; Sahulka D, 2013) but were comparable with TN (5,300 mg kg⁻¹) concentrations in lagoon biosolids from Melita, Manitoba (Gene, 2016). Trace element concentrations in the present study were low (Table 2.1) and only Zn (273 mg kg⁻¹), Cu (115 mg

kg⁻¹), and Cd (1.16 mg kg⁻¹) were above the threshold effect level (TEL) set by the Canadian Sediment Quality Guidelines (CCME, 2001).

Nitrogen in the biosolids was predominantly in the organic form, with plant available N (NO₃-N + NH₄-N) less than 50% of total N concentration (Table 2.1). Approximately 60–70% of the total P was in the inorganic form, which is common for biosolids due to the breakdown of P in organic and polymeric forms during wastewater treatment and the capture of orthophosphate by the biosolids phase (Hedley and McLaughlin, 2005). The labile P fraction (sum of H₂O- and NaHCO₃-extractable P) was 8–12% of total P. Labile P represents phosphate that is soluble or weakly adsorbed and is potentially available for phytoextraction. Most of the P in the biosolids was in the recalcitrant fractions.

Changes in nutrient concentrations of the biosolids were observed in the primary cell before and after construction of the wetland (Table 2.1). The results indicate a general decline in the mean concentrations of total N (8191 to 2921 mg kg⁻¹) and total P (2705 to 1215 mg kg⁻¹) concentrations in the wetland biosolids in 2013 compared with biosolids in the primary cell in 2011 (Table 2.1). This decline may be partly due to the dilution, during construction of the wetland in 2012, of primary cell biosolids with secondary cell biosolids, which had lower nutrient concentrations, to provide adequate biosolids for construction of the wetland benches.

Table 2.1 Nutrient and trace element concentrations in biosolids from the primary and secondary cell and from the constructed wetland[†].

Property	Primary Cell	Secondary Cell	Wetland [‡]
TN, mg kg ⁻¹	8191	4279	2921
NH ₄ -N, mg kg ⁻¹	13	12	55
NO ₃ -N, mg kg ⁻¹	366	30	2
TP, mg kg ⁻¹	2705	1930	1215
Olsen P, mg kg ⁻¹	81	73	73
H ₂ O-P _i , mg kg ⁻¹ [†]	10.8	8	6.7
NaHCO ₃ -P _i , mg kg ⁻¹	284	165	141
NaOH-P _i , mg kg ⁻¹	802	82	171
HCl-P _i , mg kg ⁻¹	874	867	572
Residual P, mg kg ⁻¹	411	255	228
pH	7.3	7.9	7.8
As, mg kg ⁻¹	8	5.7	7
Cd, mg kg ⁻¹	1.2	0.4	0.6
Cr, mg kg ⁻¹	42	46	48
Cu, mg kg ⁻¹	115	40	62
Hg, mg kg ⁻¹	0.8	0.04	0.2
Ni, mg kg ⁻¹	33	42	43
Pb, mg kg ⁻¹	24	15	18
Se, mg kg ⁻¹	1.8	0.79	0.7
Zn, mg kg ⁻¹	273	109	162

[†]H₂O-P_i, water-extractable inorganic phosphorus; NaHCO₃-P_i, labile inorganic phosphorus; NaOH-P_i, Fe/Al-bound inorganic phosphorus; HCl-P_i, Ca/Mg-bound inorganic phosphorus

[‡] Mean concentrations in biosolids samples collected from the primary cell after redesigning and construction of the wetland in the primary cell. Some biosolids from the secondary cell were mixed with biosolids from the primary cell.

2.4.2 Biomass Yield

The effect of harvest frequency on cattail biomass yield varied with year of harvest, as indicated by the significant ($P < 0.0001$) year \times harvest interaction (Table 2.2). During the wetland establishment year (2013), the biomass yield was low (0.22 kg m^{-2}) (Fig.2.3). Plants were only harvested once in 2013 because there was insufficient regrowth during the establishment year to allow for a second harvest. Biomass yield more than doubled in 2014 relative to biomass yield in 2013 (Fig. 2.3). Biomass yield from harvesting cattail twice per season (0.54 kg m^{-2}) did not differ significantly from biomass yield from a single harvest per season (0.56 kg m^{-2}) in 2014, whereas two harvests per season decreased biomass yield by $>50\%$ in 2015 (0.22 kg m^{-2}) and 2016 (0.23 kg m^{-2}) compared to a single harvest in each of the two years. These results show that not only was there no benefit in harvesting cattail biomass twice per season, but, more importantly, that the two harvests had a negative effect on yield. Slow recovery of plants after the first harvest in a two-harvest frequency resulted in lower biomass yield in the second harvest compared to the first harvest. The second harvest of a two-harvest frequency yielded $\sim 80\%$ (0.5 kg m^{-2}) of the sum of the total biomass yield from the two harvests (0.6 kg m^{-2}) in 2014. On the other hand, the second harvest of a two-harvest frequency produced $\sim 20\%$ (0.04 kg m^{-2}) of the total biomass yield from the two harvests ($\sim 0.2 \text{ kg m}^{-2}$) in 2015. There was no second harvest in 2016 because the plants did not recover enough to produce a measurable second harvest. Plants from two-harvest plots exhibited poor regrowth after the first harvest. Biomass yield from harvesting once per season after the plant establishment year did not significantly change in 2014 (0.60 kg m^{-2}), 2015 (0.58 kg m^{-2}) and 2016 (0.58 kg m^{-2}), indicating that annual harvesting sustained cattail productivity over the four years.

Table 2.2 Cattail biomass yield and tissue nutrient concentration and phytoextraction as affected by harvest frequency and year of harvest.

Effect‡	Biomass kg DW m ⁻²	Nutrient concentration		Phytoextraction†	
		N g kg ⁻¹	P	N g m ⁻²	P
Year					
2013	0.21c§	22a	3.9a	5.2c	0.91b
2014	0.66a	22a	2.8c	15.3a	1.95a
2015	0.51b	19ab	2.2d	8.6b	0.99b
2016	0.53b	16b	3.3b	7.1bc	1.52a
Harvests					
Two harvests	0.33c	21a	3.0	8.2b	1.10b
Single	0.46b	20ab	3.2	8.4b	1.25b
Control	0.80a	17b	2.6	14.7a	2.16a
		P value			
Year (Y)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Harvests (H)	<0.0001	0.003	0.05	<0.0001	<0.0001
Y × H	<0.0001	0.69	0.73	0.01	<0.001

†Phytoextraction was calculated as the product of biomass and tissue nutrient concentration.

‡Significant interactions are presented in figures.

§ Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure

Biomass yield from plots harvested once per season and from previously unharvested control plots did not significantly differ in 2015 and 2016, suggesting that harvesting cattail once per season did not have a significant negative effect on cattail regrowth. Grosshans et al. (2014) also found similar peak cattail biomass accumulation between plants from unharvested control sites and plants that were harvested annually for four years from a natural wetland. Cumulative biomass yield in this study after four successive seasons of harvesting indicated that cumulative biomass yield when cattail was harvested once (1.99 kg m⁻²) or twice per season (1.2 kg m⁻²) was greater than the biomass from sites that were harvested once in the summer of 2016 (0.82 kg m⁻²) and had not been previously harvested (Table 2.3). This suggests that harvesting cattail stimulated more growth in new plants resulting in greater cumulative biomass yield compared with unharvested

plants. Annual harvesting of cattail can be used as a strategy to increase cumulative biomass yield rather than harvesting plants after years of cumulative growth without harvesting.

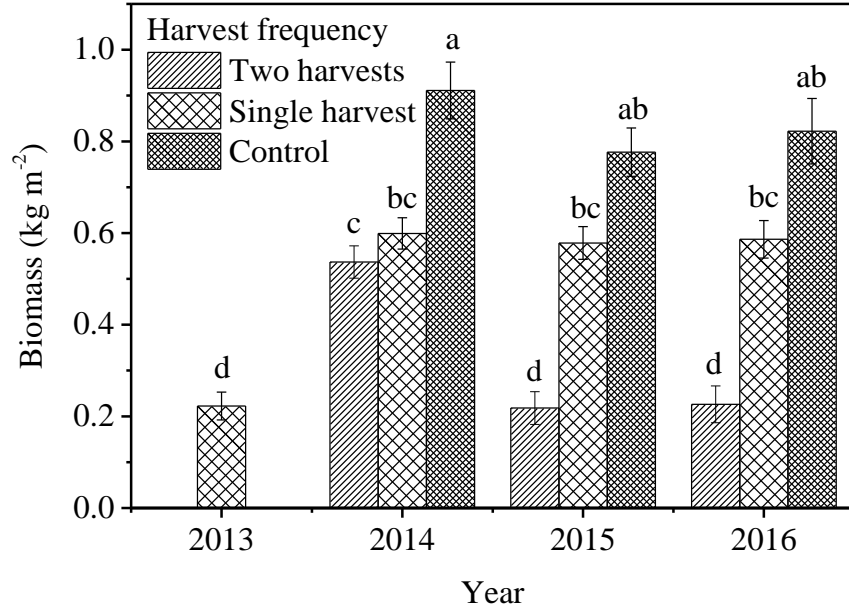


Figure 2.3 Cattail biomass yield as affected by harvest frequency and year of harvest. Values for the control in a given year are cumulative since the start of the experiment. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

Biomass yields from a single harvest per season in this study from 2014 to 2016 (0.58 – 0.6 kg m⁻²) are comparable to the 0.85 kg m⁻² yields reported in France (Salem et al., 2014) but lower than those reported by others in temperate regions. For example, Grosshans et al. (2014) reported cattail biomass yields of 1.0 to 1.5 kg m⁻² (dry wt.) over four growing seasons in a coastal wetland, while Wild et al. (2002) reported a yield of 1.45 kg m⁻² (dry wt.). Higher cattail biomass yields have been reported in other climates, including 5.65 kg m⁻² in Morocco (Ennabili et al., 1998) and 2.54 kg m⁻² in Estonia (Maddison et al., 2005). One reason for the lower biomass yields in our study compared with yields from other temperate regions was that plants were harvested at a height

of ~65 cm from the sediment to prevent flooding the plants, which left considerable biomass in the 65-cm stubble. Some researchers have cut cattails at 20-50 cm above the soil surface (Grosshans, 2014; Hall et al., 2008) while others harvested by cutting the plants at ground level (Ennabili et al., 1998; Maddison et al., 2005). Biomass yield of the stubble left behind in the 2016 harvest was 0.51 kg m⁻², which was approximately equal to the harvested biomass (0.56 kg m⁻²). Therefore, the total biomass above the biosolids sediment was 1.1 kg m⁻², which is consistent with the yields reported in the temperate region studies cited above. This indicates that harvestable biomass in the constructed wetland can be increased by drawing down water to lower water levels prior to harvesting. Reducing the stubble height to 45 cm would have increased biomass yield by 0.3 kg m⁻² to achieve a total biomass yield of 0.86 kg m⁻² (54% increase) compared to leaving a stubble height of 65 cm (0.56 kg m⁻²). It is also possible that yield was impacted because this was a newly established wetland and that after a few years of being established the biomass yields may be more similar to those from other wetlands with well-established emergent vegetation communities.

Table 2.3 Cumulative biomass yield, nitrogen and phosphorus phytoextraction and phytoextraction rates after four years of harvesting cattail.

	Biomass kg DW m ⁻²	Phytoextraction [†]		Phytoextraction rate [‡]	
		N	P	N	P
		g m ⁻²		%	
Harvests per year					
Control	0.82	11.2	2.5	1.9	1.0
One	1.99	36.7	5.6	6.2	2.2
Two	1.2	28.3	4.1	3.9	1.3

[†] Phytoextraction was calculated as the product of biomass and tissue nutrient concentration.

[‡] Phytoextraction rates were calculated by dividing nutrient phytoextraction by the initial amount of nutrient in the wetland biosolids

2.4.3 Nitrogen and Phosphorus Concentrations in the Biomass

Nitrogen concentration in the plant tissues was significantly affected by year ($P < 0.0001$) and harvest frequency ($P = 0.003$) (Table 2.2). Plant tissue N concentration, averaged across harvesting treatments, was similar in 2013 (22 g kg^{-1}) and 2014 (22 g kg^{-1}) and decreased thereafter to 19 g kg^{-1} in 2015 and 16 g kg^{-1} in 2016. Averaged across all years, N tissue concentration was significantly ($P = 0.003$) greater with two harvests per year (21 g kg^{-1}) than unharvested control sites (17 g kg^{-1}) (Table 2.2).

Phosphorus concentration in plant tissue varied significantly with year ($P < 0.0001$), decreasing in the order 2013 (3.9 g kg^{-1}) > 2014 (2.8 g kg^{-1}) > 2015 (2.2 g kg^{-1}) and then increasing to 3.3 g kg^{-1} in 2016. Overall, harvesting frequency had minimal effect on tissue N and P concentrations (within ~ 90 d growing cycle) but significantly affected cattail biomass yield. Therefore, nutrient phytoextraction by cattail depended on biomass yield more than it did on tissue nutrient concentration.

Cattail plants in our study contained higher N (16 – 22 g kg^{-1}) than the 14 – 17 g kg^{-1} reported in other studies (Cicek et al., 2006; Grosshans et al., 2012; McJannet et al., 1995). Phosphorus tissue concentrations (2.2 – 3.9 g kg^{-1}) in our study were within the range (2.1 – 3.2 g kg^{-1}) reported in previous studies (Cicek et al., 2006; Grosshans et al., 2012; McJannet et al., 1995). Cicek et al. (2006) measured higher tissue N and P concentrations than reported in the literature and attributed the higher concentrations to the availability of excess nutrients. Reddy and DeLaune (2008) argued that nutrient concentrations in high biomass plants may be lower because of dilution and distribution within plant tissue and it is important to take into account both biomass yields and nutrient concentrations (Vymazal, 2016).

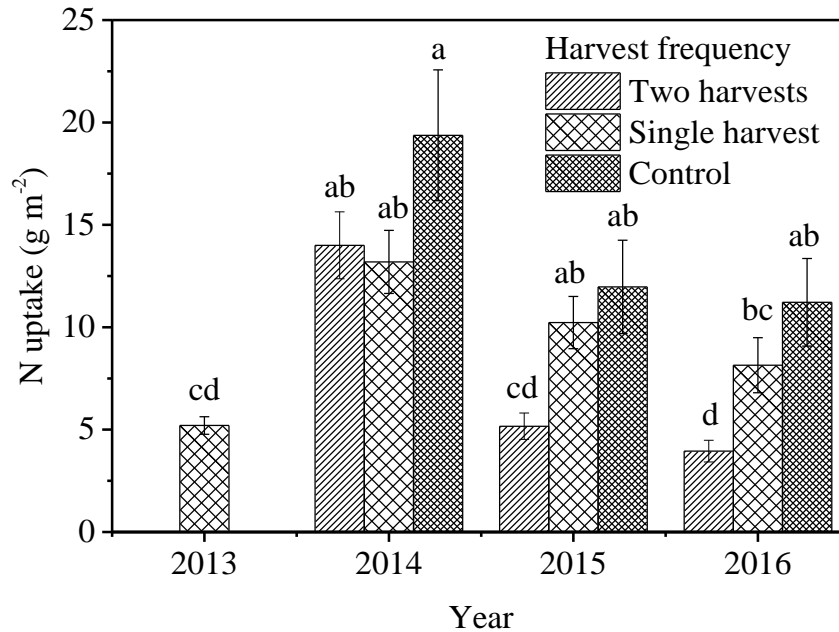


Figure 2.4 Cattail nitrogen phytoextraction as affected by harvest frequency and year of harvest. Values for the control in a given year are cumulative since the start of the experiment. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

2.4.4 Nitrogen and Phosphorus Phytoextraction

Nitrogen phytoextraction was significantly ($P < 0.0001$) affected by year and harvest frequency. Nitrogen phytoextraction in the establishment year (2013) was low (5.2 g m^{-2}) and increased by up to 4 times in 2014 (Fig. 2.4). Nitrogen phytoextraction did not differ significantly between two harvests per season (14 g m^{-2} for the two harvests) and one harvest per season (13.2 g m^{-2}) or the unharvested control (19.4 g m^{-2}) in 2014. Thereafter, harvesting cattail twice per season decreased N phytoextraction by ~50-60% compared to a single harvest and the unharvested control in 2015 and 2016. The decrease in N phytoextraction when cattail was harvested twice per season was likely due to the decrease in biomass yield when cattail was harvested twice in 2015 and 2016 (Fig. 2.4). Phosphorus phytoextraction was affected by year and harvest frequency ($P < 0.0001$ for both factors). Like N phytoextraction, P phytoextraction did not differ significantly

between two harvests per season (1.8 g m^{-2}) and one harvest per season (1.7 g m^{-2}) or compared with the unharvested control (2.4 g m^{-2}) in 2014 (Fig. 2.5). In 2015 and 2016, P phytoextraction from two harvests decreased by $\sim 60\%$ relative to the single harvest (Fig. 2.5).

Biomass yield from the wetland can be increased by harvesting cattail at a lower cutting height, as mentioned above. Although harvested biomass yield (0.56 kg m^{-2}) differed little from stubble biomass yield (0.51 kg m^{-2}), harvested biomass accumulated more N (8.1 g N m^{-2}) and P (1.74 g m^{-2}) than the stubble (1.7 g m^{-2} N and 0.72 g m^{-2} P). This was because N and P concentrations were greater in the harvested section of the plant than in the stubble biomass. Reducing the stubble height to 45 cm as suggested above would increase biomass yield by 54% and increase N and P phytoextraction by 13% (1.03 g m^{-2}) and 28% (0.48 g m^{-2}), respectively.

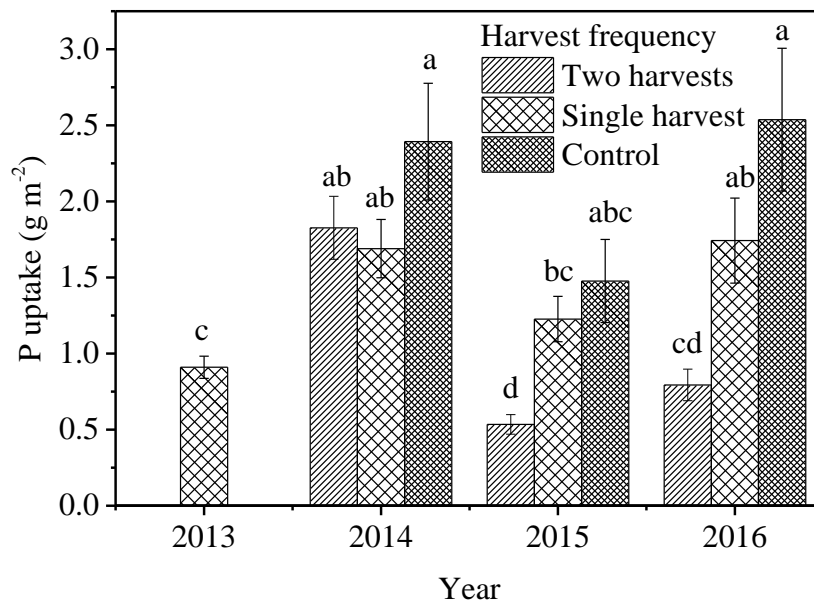


Figure 2.5 Cattail phosphorus phytoextraction as affected by harvest frequency and year of harvest. Values for the control in a given year are cumulative since the start of the experiment. Error bars represent standard errors of the means. Bars with the same letter represent means that are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

Cumulative N (36.7 g m^{-2}) and P (5.6 g m^{-2}) phytoextraction amounts from a single harvest per season over 4 yr were greater than N and P phytoextraction amounts from two harvests and from the unharvested control plots, which were harvested once in 2016 (Table 2.3). These results show the importance of annual harvesting on nutrient removal. Harvesting every year for 4 yr resulted in a three-fold increase in N phytoextraction and a two-fold increase in P removal relative to a single harvest at the end of the 4-yr period.

Nitrogen and P phytoextraction results from this study contradict those from a wetland microcosm study which showed that two cattail harvests per growth cycle ($\sim 90 \text{ d}$) increased N and P phytoextraction from biosolids by about 1.5 times compared with a single harvest in two of the three growth cycles evaluated (Amarakoon et al., 2017). This difference could be due to greater biomass yield when cattail was harvested twice per growth cycle compared with a single harvest in the wetland microcosm study, while in this field study two harvests did not produce greater biomass yields compared to a single harvest in the third and fourth years. This could also be due to optimal conditions encountered in the mesocosms situated in growth chambers relative to what plants experience under field conditions. Grosshans et al. (2012) reported N and P phytoextraction of 23.5 g m^{-2} and 3.58 g m^{-2} by cattail harvested at 28 sites in Netley-Libau Marsh, the largest coastal wetland on Lake Winnipeg, Manitoba, Canada. The greater cattail biomass yield (1.9 kg m^{-2}) in the coastal wetland relative to annual harvests from our study ($0.58 - 0.6 \text{ kg m}^{-2}$) explains the higher nutrient phytoextraction in the coastal wetland. The plants in the coastal wetland had been established for a long period while in this study, the plants were still developing and expanding. Maddison et al. (2005) reported cattail nutrient phytoextraction of $8-19.9 \text{ g N m}^{-2}$ and $1.1-3 \text{ g P m}^{-2}$ in three wastewater treatment wetlands in Estonia and nutrient accumulation depended on biomass yield.

Annual harvesting of cattail for 4 yr removed a total of 6.2% and 2.2% of the initial total N and P in the biosolids. Most of the P in the biosolids in our study was in the recalcitrant fractions (Table 2.1); P in the soluble or weakly adsorbed H₂O- and NaHCO₃-extractable fractions is considered potentially available for plant uptake and presents a threat to the environment.

2.4.5 Seasonal Effects of Harvesting Cattail

Cattail plants were harvested in August, November, and April in 2015 and 2016 to determine the effect of harvest timing on biomass and nutrient phytoextraction. Biomass yield was significantly ($P < 0.0001$) affected by time of harvest (Table 2.4). Averaged across all harvesting frequencies and years, biomass yield decreased in the order August (0.78 kg m^{-2}) > November (0.53 kg m^{-2}) > April (0.35 kg m^{-2}). Nitrogen and P standing stock in August (11.6 g N m^{-2} and 2 g P m^{-2}) were significantly reduced when cattail was harvested in November (4.3 g N m^{-2} and 0.53 g P m^{-2}) and April (2.3 g N m^{-2} and 0.3 g P m^{-2}). Lower N and P standing stock in November and April compared to a summer (August) harvest was partly due to the decrease in nutrient concentrations resulting from translocation of nutrients to rhizomes and the reduction in biomass (Table 2.4). Therefore, to maximize N and P phytoextraction, harvesting should occur in late August. However, summer harvesting presents challenges, such as operating harvesting equipment in wet environments and the high moisture content of plants, which is undesirable for bioenergy production (Cicek et al., 2006; Grosshans, 2014).

Table 2.4 Cattail biomass yield and nutrient concentration and phytoextraction (standing stock) as affected by timing of harvest and year of harvest.

Effect	Biomass kg DW m ⁻²	Nutrient content		Standing stock	
		N g kg ⁻¹	P g kg ⁻¹	N g m ⁻²	P g m ⁻²
Year					
2015	0.58	12.7a	1.5	7.8	0.93
2016	0.49	8.7b	1.3	5.0	0.90
Harvest					
August	0.78a†	14.9a	2.5a	11.6a	2.01a
November	0.53b	8.5b	1.0b	4.3b	0.53b
April	0.35c	7.4b	0.6b	2.3c	0.19c
			P value		
Year (Y)	0.39	<0.001	0.91	0.42	0.15
Harvests (H)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Y × H	0.22	0.01	0.06	0.62	0.28

† Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

Some researchers have suggested that harvesting plants in winter or early spring, when the wetland is frozen and can withstand harvesting equipment loads, can reduce the cost of drying the biomass and provide minimal ecological impacts (Cicek et al., 2006; Grosshans, 2014). However, from a phytoremediation perspective, as shown in this study, harvesting in April reduces N and P standing stock in harvested aboveground biomass by up to 85% compared with a summer harvest. Harvesting in the fall (i.e. between September and November) when the wetland can drawdown and dry might offer better conditions for harvesting equipment compared to wet summer conditions. Grosshans and Greiger (2013) demonstrated harvesting of cattail in September and October in ditches along highways and wetland areas on marginal lands in Manitoba using traditional agricultural equipment. Our results showed that harvesting in November reduced N and P standing stock by 63% and 74%, respectively, compared to a summer harvest. Grosshans and Greiger (2013) harvested in September and October, and P concentrations in cattail tissue was still

relatively high with a loss of up to 25% to 30% compared to summer plant P tissue concentrations, depending on time of harvest. In the study, cattail was harvested in November, which resulted in the greater decrease in extracted N and P. In this study, where water level control is possible within an engineered wetland system, water could be pumped from the wetland to the storage pond to facilitate harvesting in the summer; and specialized equipment developed for wetland environments can be used to harvest cattail in wet conditions in the summer (Grosshans, 2014; Grosshans and Greiger, 2013).

2.4.6 Phosphorus and Nitrogen Concentrations in the Water Column

Because the experiment was aimed primarily at phytoextraction of N and P from the primary source (that is, the biosolids sediment), our setup did not allow for isolation of treatment effects on nutrient concentrations in the water column. Nevertheless, water samples were periodically taken from the wetland and analyzed for N and P concentrations. In 2015, total P concentrations in the water column increased from $0.37 \pm 0.03 \text{ mg L}^{-1}$ in April to $0.71 \pm 0.04 \text{ mg L}^{-1}$ in June and remained fairly constant (1.0 ± 0.1 to $1.2 \pm 0.2 \text{ mg L}^{-1}$) in July through October. During the same year, $\text{NH}_4\text{-N}$ concentration ranged between 0.45 ± 0.16 and $0.58 \pm 0.23 \text{ mg L}^{-1}$ from April to May and ranged between <0.1 to $0.8 \pm 0.58 \text{ mg L}^{-1}$ from June to October. In 2016, TP concentration increased from $1.2 \pm 0.1 \text{ mg L}^{-1}$ in June to $2.0 \pm 0.2 \text{ mg L}^{-1}$ in September. Total N and $\text{NO}_3\text{-N}$ concentrations were below the detection limits of 0.025 and 0.1 mg L^{-1} , respectively, in all water samples analyzed.

The total P concentrations in July through October ($\geq 1 \text{ mg L}^{-1}$) exceeded the Manitoba Water Quality Guideline of 1 mg L^{-1} TP for wastewater effluent discharged into streams (Manitoba Water Stewardship, 2011). However, the guideline does not apply to our study case because the wetland water was not released to streams. Total P concentrations in floodwater samples collected from

drainage ditches near (< 0.5 km) the lagoon during flooding events ranged from 0.49 to 0.75 mg L⁻¹, with a mean of 0.63 mg L⁻¹. Therefore, releasing water from the wetland into the drainage ditches would increase P enrichment.

Although our setup did not allow for isolation of treatment effects on nutrient concentrations in the water column, we expect harvesting of cattail at a large scale in the wetland to reduce nutrient concentrations in the water column over time. Sharma et al. (2006) found that *T. angustifolia* released 4.6 g N m⁻² and 0.8 g P m⁻² from dead shoots into the water after senescence. The nutrient loss represented 10% of total N (46.7 g m⁻²) and 17% of total P (4.7 g m⁻²) accumulated in the shoots.

2.5 Conclusion

Harvesting cattail once per season in late August produced greater biomass yields and N and P phytoextraction than two harvests per season. Cumulative biomass yields and N and P phytoextraction from annual harvesting were greater than those from unharvested plots, indicating the benefits of annual harvesting. We suggest harvesting in late August to maximize nutrient phytoextraction. Harvesting in fall or spring reduced N and P standing stock by up to 85%, which in turn results in an increase in the time required to achieve a set remediation goal. These results indicate that in situ phytoremediation can effectively remove N and P from biosolids and may be a viable alternative where land spreading of biosolids is infeasible. Phytoremediation can potentially reduce decommissioning costs for small municipalities and can help minimize some of the water quality impacts associated with land application of biosolids. This is particularly relevant in the prairie agricultural systems where non-point source nutrient pollution is a concern. Findings from the study will inform policy makers and landowners on the effective strategies for harvesting cattail to optimize N and P phytoextraction from biosolids using wetland plants.

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3. NUTRIENT SUPPLY RATES AND PHYTOEXTRACTION BY CATTAIL DURING CONSTRUCTED-WETLAND PHYTOREMEDIATION OF AN END-OF-LIFE MUNICIPAL LAGOON

3.1 Abstract

In situ phytoremediation of municipal biosolids is a promising alternative to land spreading and landfilling during decommissioning of end-of-life municipal lagoons. Plant root simulator (PRS) probes can be used to examine nutrient availability during phytoremediation but their use under wetland conditions is limited. This study examined nutrient availability using PRS probes during wetland-based phytoremediation of biosolids vegetated with cattail. The probes were buried in the sediment for seven sequential 2-week burial periods beginning in June 2014. Plants were harvested to determine biomass yield and nutrient content. Nitrogen supply rate did not change significantly with sampling period in June and July ($4.5\text{-}5.9 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$) but increased thereafter to $11.8 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$. Phosphate supply rate ($20.5\text{-}24.2 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$) did not differ significantly among sampling times. Cumulative supply rates of the macronutrients N, P, K, Ca and Mg ($r = 0.77\text{-}0.92$) and the micronutrients B, Fe, and Mn ($r = 0.7\text{-}0.81$) were highly correlated with cattail uptake, while the correlation was weaker for Cu ($r = 0.42$) and Zn ($r = 0.40$). Maximum attainable biomass yield (0.87 kg m^{-2}) coincided with the period of maximum nutrient uptake, indicating that harvesting cattail between late August and early September maximizes nutrient removal. In situ burial of PRS probes appears to be an effective method of measuring availability of macronutrients but may have limited effectiveness for Cu and Zn.

3.2 Introduction

Phytoremediation is gaining worldwide attention as a cheaper and sustainable alternative to conventional methods of removing contaminants or making them harmless in contaminated lands (Mir et al., 2017; Pilon-Smits, 2005). Phytoremediation utilizes the ability of plants to take up contaminants. The plants are then harvested, resulting in removal of the contaminants in the harvestable plant tissues. Availability of nutrients and contaminants for plant uptake, the amounts of nutrients and contaminants that the plants absorb from the pool of available elements, and the plant harvesting stage are among the factors controlling the effectiveness of contaminant removal via harvesting of plants during phytoremediation (Ali et al., 2013).

In situ phytoremediation of municipal biosolids in end-of-life lagoons is a promising relatively inexpensive and sustainable biosolids management strategy where traditional methods of farmland application or landfilling are limited or restricted. Application of biosolids based on P rates (Shober and Sims, 2003), banning or restriction of spreading of biosolids on farmland in some jurisdictions (City of Winnipeg, 2013; Slaughter and Doverspike, 2008), shortage of suitable land within economic distances for transporting biosolids, and restrictions on or proposed banning of biosolids landfilling (Wang et al., 2008) dictate that alternative strategies are sought. Results from recent microcosm studies (Amarakoon et al., 2017; Jeke et al., 2015a) suggest that wetland-based phytoremediation of municipal biosolids may be a viable option for decommissioning of end-of-life lagoons in small rural municipalities.

Plant nutrient availability is essential to support healthy plants and therefore high biomass yields, which will in turn enhance contaminant removal during phytoremediation. On the other hand, the availability of non-essential elements such as Cd and Cr allows their phytoextraction from the contaminated media. Many studies have examined N and P availability in municipal biosolids incorporated into soil as an amendment (Gil et al., 2011; Shaheen and Tsadilas, 2013)

while a few studies have examined nutrient availability in biosolids alone (Corrêa et al., 2012; Jeke et al., 2015a). In contrast, no published study that we are aware of has examined nutrient and trace element availability in biosolids under a wetland system. This is partly because biosolids application has mostly been limited to upland situations such as agricultural land or reclamation sites. Availability of nutrients in biosolids-amended soil may not reflect availability in biosolids. For example, Corrêa et al. (2012) reported lower N mineralization rates in biosolids (0-2.5% of organic N) than in biosolids incorporated into soil (10-52% of organic N). Biosolids sediments in wetlands have low redox potential, which results in nutrient biogeochemistry different from that in upland systems (Reddy and DeLaune, 2008).

Laboratory and field nutrient availability are commonly estimated using traditional soil-test procedures, which typically indicate nutrient concentrations at a point in time and may not adequately reflect temporal changes in nutrient supply throughout the growing season. Ion exchange membranes that can be used to measure nutrient availability under field conditions have been developed (Qian and Schoenau, 2002). Ion exchange membranes adsorb ions at a rate dependent on ion activity and diffusion rate in solution and are considered to mimic uptake by plant roots. Nutrient availability measurements from plant root simulator (PRS) probes (Western Ag Innovations, Saskatoon, SK, Canada) have been shown to correlate strongly with plant uptake (Nyiraneza et al., 2009; Qian and Schoenau, 2005; Sharifi et al., 2009). The probes have been used to provide a basis for fertilizer recommendations for various agricultural crops (Qian and Schoenau, 2002). Shaheen and Tsadilas (2013) reported that PRS probes were more reliable than the Olsen P test in predicting P availability for canola in biosolids-amended soils but failed to predict K availability for the crop. While PRS probes have been widely used in agricultural fields,

it is not clear whether they could be equally useful under wetland conditions (Nelson et al., 2007) or in submerged soils (Miller et al., 2017) and peatlands (Wood et al., 2016).

A study by Jeke et al. (2015b) showed that N mineralization in municipal biosolids was affected by moisture content and that Olsen P concentration decreased with incubation time regardless of moisture level. Anaerobic conditions under submerged or wetland conditions may alter such nutrient dynamics. Miller et al. (2017) reported that P absorption by anion probes was two to three times greater in submerged streambank soils than in exposed aerobic soils. Other studies that have used ion exchange membranes in saturated soils have shown that wetland conditions increase available P and reduce nitrate-N concentration (Nelson et al., 2007; Obour et al., 2011; Wood et al., 2016). Anaerobic conditions affect absorption of P and redox sensitive species such as NO₃, Fe, Mn, Cu, and S by PRS probes. The dynamics of nutrient and trace element availability in biosolids under wetland conditions will affect the effectiveness of phytoextraction of nutrients and, therefore, need to be investigated.

A better understanding of nutrient supply from biosolids sediments in wetland systems and uptake by cattail over the entire growing season is important since the timing of nutrient release, plant uptake, and harvesting of plants will affect the effectiveness of phytoextraction of contaminants. The objectives of this study were to characterize the plant availability of nutrients and trace elements using PRS probes, and to relate these to nutrient and trace element uptake by cattail in a wetland system constructed to treat an end-of-life primary wastewater treatment cell.

3.3 Materials and Methods

3.3.1 Study Site

The study was established in Niverville, Manitoba (49°35'42.7"N, 97°02'50.3"W), in the primary cell (4.6 ha) of an end-of-life municipal lagoon which had been converted to a constructed wetland for phytoremediation of the cell. The municipal lagoon had operated for 37 years (1971-2008) and treated raw domestic effluent via microbial degradation without any pre-treatment. In the fall of 2012, biosolids in the primary cell were stripped and temporarily stockpiled in the lagoon. The primary cell was redesigned and benches were constructed using the stockpiled biosolids supplemented with biosolids from the secondary cell. Cattail (*Typha latifolia* L.) rhizomes collected from surrounding ditches were planted into divots dug into the benches in November 2012. The constructed wetland was flooded in May 2013 with water from the winter snowpack and spring melt from storm water ponds (<0.025 mg L⁻¹ total N, <0.1–0.2 mg L⁻¹ NH₄-N and 0.31 mg L⁻¹ total P) within the Town of Niverville. In subsequent seasons, spring runoff channelled into a water holding pond was used to refill the constructed wetland. Wetland water depth was maintained at ~45 cm.

3.3.2 Plant Root Simulator Probes

The PRS probes (15 cm x 3 cm x 0.5 cm; Western Ag, Saskatoon, SK) used in this study consisted of anion- and cation-exchange membranes that were enclosed in plastic supports (Fig. 3.1). The anion-exchange membranes are positively charged and attract negatively charged ions while the negatively charged cation-exchange membranes attract and adsorb cations. The ion exchange membranes were pre-saturated with a counter-ion that was easily desorbed, allowing ready absorption of ions from the soil. Anion probes were saturated with HCO₃⁻ while cation probes were saturated with Na⁺. When buried, ions in the soil solution displace the counter-ions at

a rate that depends on their activity and diffusion rate in the soil solution. The anion probes measured supply rates of NO_3^- -N, SO_4^{2-} -S, H_2PO_4^- -P, and $\text{B}(\text{OH})_4^-$ -B, and the cation probes measured K^+ , Fe^{3+} , NH_4^+ -N, Ca^{2+} , Mn^{2+} , Al^{3+} , Mg^{2+} , Cu^{2+} , Pb^{2+} , and Zn^{2+} supply rates.



Figure 3.1 PRSTM probes retrieved from three plots from a transect, cleaned and combined to form a composite sample.

3.3.3 Experimental Setup and Sampling

The experiment was set up as a randomized complete block design with a total of 56 plots (4 transects \times 2 plots per transect per burial period \times 7 burial periods). Seven plots (2 m \times 2 m) were marked off in each of the four vegetation transects (14 m \times 2 m) that were set up in the wetland during the spring of 2014. Separate anion and cation probes were inserted in three randomly selected plots in each transect in June 2014. Each pair of probes was inserted in the 0-15 cm biosolids layer in PVC cylinders (10-cm diam. by 45-cm height) in which plant roots had been

removed to prevent root uptake of nutrients. The probes were buried for seven consecutive 2-wk periods. At the end of each 2-week period, the probes were retrieved and replaced with a new set of probes, which were carefully inserted into the same slot, with biosolids firmly pressed to ensure proper biosolids-membrane contact. Other studies in wetland systems have installed ion exchange membranes for 14 to 28 d (Kreiling et al., 2015; Lawrence et al., 2016; Obour et al., 2011; Wood et al., 2016). Cattail was harvested in 0.5 m × 0.5 m quadrats in two randomly-selected plots in each transect every 2 wk when PRS-probes were retrieved. The total number of pairs of probes deployed over the study period was 84 (4 transects × 3 pairs of PRS probes per transect × 7 burial intervals).

3.3.4 Laboratory Analysis

After retrieval, PRS probes were cleaned and rinsed with distilled water. Probes from three plots from each transect and each burial period were combined to form a composite sample (Fig. 3.1). The probes were shipped to the Western Ag laboratory in Saskatoon, SK for cation and anion analyses. Nitrate-N and NH₄-N concentrations were determined colorimetrically using an automated flow injection analysis while all the other ions were measured by inductively-coupled plasma (ICP) spectrometry.

Plant samples were oven-dried and weighed to determine biomass yield. Dry biomass subsamples were ground and analyzed for element concentrations. Total N concentration was measured by combustion using a Leco CNS 2000 analyzer (LECO Corp., MI, USA). Tissue P, S, Ca, Mg, K, Na, Fe, B, Cu, Mn, Zn, and Al concentrations were determined by ICP following digestion of a 0.5-g sample in aqua regia for 2 h at 90°C using a microprocessor-controlled digestion block. A Varian 735 ES ICP (Varian Inc.) was used to measure P while trace element

concentrations were measured with a Perkin-Elmer SCIEX ELAN 6000 ICP-MS (Perkin-Elmer SCIEX Instruments).

3.3.5 Statistical Analysis

Data for nutrient and trace element supply rates measured with PRS probes were analyzed using the GLIMMIX procedure for repeated measures in SAS 9.4 (SAS Institute, 2014) using a gamma distribution for each ion to determine if there were differences in supply rates among sampling periods. Burial period was modelled as the repeated factor using covariance structures selected based on the corrected Akaike Information Criteria (AICc). Nutrient and trace element uptake were calculated by multiplying element concentration by the corresponding biomass yields for every sampling date. The nutrient uptake calculated at each sampling date represented cumulative nutrient uptake. Cumulative biosolids nutrient supply was calculated as the sum of nutrients adsorbed onto the PRS probes during successive burial periods. Linear regressions were fitted using PROC REG in SAS to explore relationships between nutrient uptake by cattail and nutrient supply rates measured by PRS probes.

Six growth models - a beta function, a three-parameter logistic function, a four-parameter logistic function, the Gompertz function, the Richards model, and a modified Richards model (Archontoulis and Miguez, 2015) - were compared using PROC NLIN for their fit to biomass yield and nutrient uptake data. The beta sigmoid model (Eq. 1) provided the best fit ($P < 0.0001$) to the yield and uptake data based on the AICc. The beta sigmoid model is as follows:

$$y = y_{\max} [1 + (t_e - x)/(t_e - t_m)](x/t_e)^{t_e/(t_e - t_m)} \quad [1]$$

where y_{\max} is the maximum biomass yield or nutrient uptake, t_e is the time when biomass or nutrient uptake reaches the maximum value, and t_m is the inflection point at which growth or uptake rate is maximized.

3.4 Results and Discussion

3.4.1 Temporal Changes in Nutrient and Trace Element Supply Rates

Nitrate supply rate was low and ranged from 0.82–2.7 $\mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$, except for a spike (5.5 $\mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$) measured during the period August 4–19 (Fig. 3.2a; Table 3.2). The low nitrate supply rate can be attributed to the low nitrification potential or enhanced denitrification under saturated and anaerobic conditions (Reddy and DeLaune, 2008). Redox potential measured with a AgCl reference electrode in the wetland sediment in August averaged 33-90 mV, corrected to that of a standard hydrogen electrode, indicating anaerobic conditions (< 300 mV) (Reddy and DeLaune, 2008). Ammonium N concentration was low from June 4 to August 19 and supply rates ranged from 3.3–4.1 $\mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$, significantly increasing thereafter to 9.3 $\mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$ by the end of the sampling period on September 19 (Fig. 3.2b). Ammonium N was the dominant form of available N in the wetland, as expected under anaerobic conditions, and accounted for 57-86% of total available N supply. Wetland sediments accumulate high ammonium N levels because of low oxidation rates of ammonium and low N requirements of anaerobic microorganisms. Wetland plants such as cattail are adapted to utilize ammonium N instead of nitrate N (Brix et al., 2002; Nakamura et al., 2010).

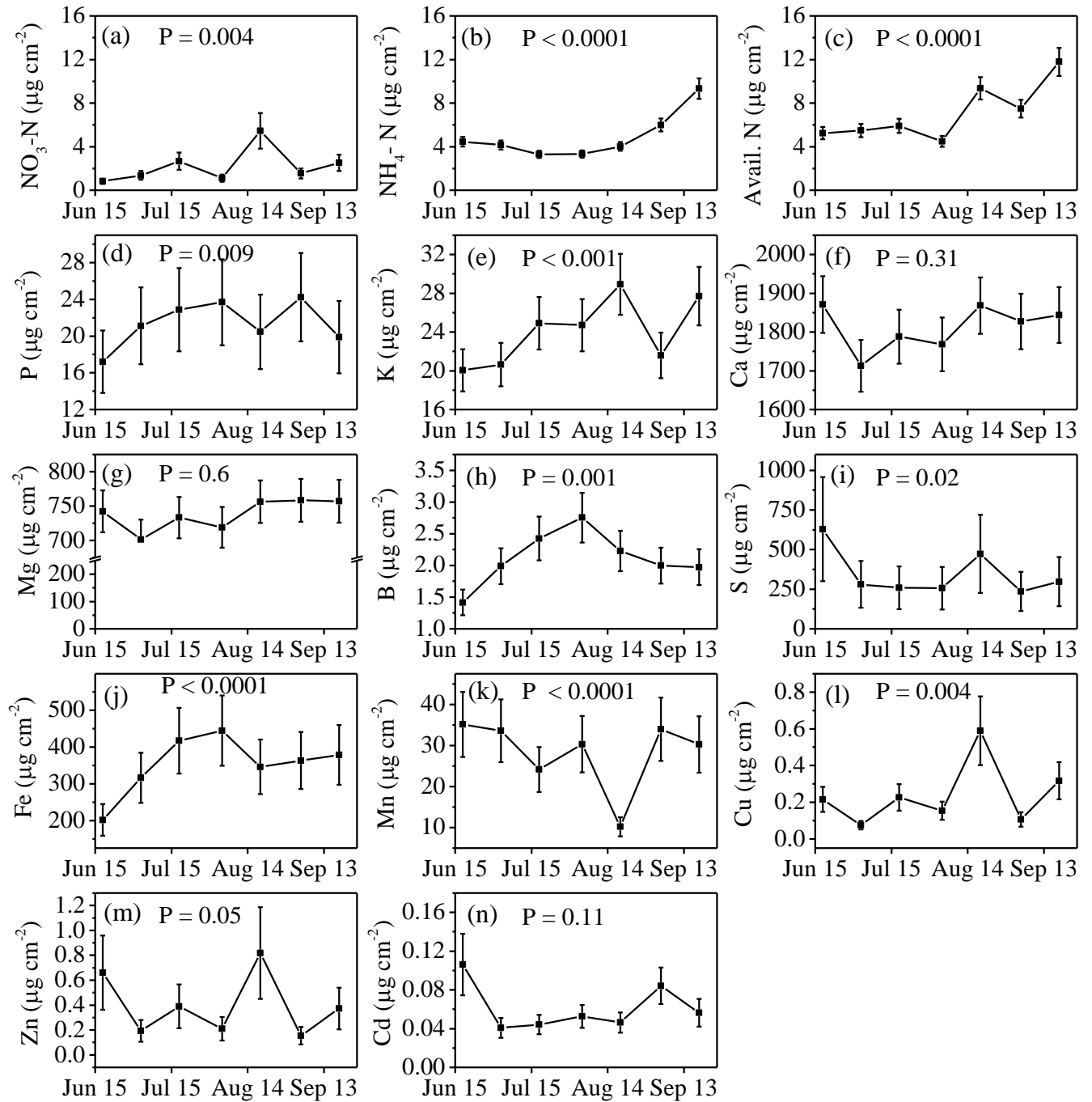


Figure 3.2 Temporal changes in nutrient supply rates in biosolids, measured by PRS probes, as a function of time. Vertical bars represent standard errors of the mean.

Available nitrogen ($\text{NH}_4 + \text{NO}_3\text{-N}$) supply rate (4.5 to $5.9 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) did not differ significantly ($P = 0.29$) among the first four sampling periods from June 4 to August 4 but increased significantly ($P < 0.0001$) thereafter to $11.8 \mu\text{g cm}^{-2}$ on September 19 (Fig. 3.2c). The increase in N supply rate coincided with cattail maximum biomass accumulation rate (Fig. 3.4) and an increase in plant N uptake (Fig. 3.5a). The increase in N supply rate in Aug. through Sep. might be a result of increased ammonification rate from the mineralization of organic N in the biosolids and/or from decomposing and senescing plant tissues.

No previous study has used PRS probes to measure nutrient availability in flooded biosolids sediments. Studies employing PRS probes under terrestrial systems showed higher N supply rates than those measured in our study. Quaye et al. (2015) reported higher rates of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ supply rates from an agricultural soil amended with biosolids compost at 150 kg N ha^{-1} and 200 kg N ha^{-1} on a field that had been previously used for agricultural purposes in Delhi, NY. Temporal NO_3 supply rate followed the order May ($\sim 120 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) > August ($\sim 40\text{-}50 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) > October ($\sim 30 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) while NH_4 supply rate followed the order August ($15\text{-}30 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) > May ($7 - 10 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) > October ($2\text{-}7 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$). Qian and Schoenau (2005) reported $\text{NO}_3\text{-N}$ supply rates of 200 to $>1100 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$ in agricultural soils in Saskatchewan, Canada. Lower N supply rates ($4.5 - 11.8 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) from biosolids in this study may be partly due to lower mineralizable N concentration in the biosolids and/or the wetland conditions, which likely reduced mineralization rates.

Available P supply rate ($20.5\text{-}24.2 \mu\text{g cm}^{-2} 2 \text{ wk}^{-1}$) did not differ with sampling time after the second sampling period (Fig. 3.2d). Phosphorus supply rate ($17.2 \mu\text{g cm}^{-2}$) during the first sampling period (June 4–June 18) was significantly lower than that at some of the other sampling

periods. Phosphate adsorption by resins showed considerable variability among replicates and this may have reduced the statistical power to detect significant differences or clear trends. Qian and Schoenau (2002) and Bair and Davenport (2013) also reported large variations in P supply rates measured by PRS probes among replicates and attributed this partly to microscale variations and root competition with PRS membranes.

We expected a general increase in P supply rate because, under anaerobic conditions, available P is released as ferric [Fe(III)] minerals of inorganic P are reduced to soluble ferrous [Fe(II)] forms by iron-reducing bacteria during anaerobic respiration (Reddy and DeLaune, 2008). Our results contradict studies that have shown increased P supply rate in saturated soils using ion exchange membranes (Nelson et al., 2007; Obour et al., 2011; Wood et al., 2016). High Ca supply rates (1700 to 1900 $\mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$) in our study may have immobilized P as insoluble calcium compounds. A modified Hedley P fractionation procedure of the biosolids showed that about 47% (572 mg kg^{-1}) of the total P (1215 mg kg^{-1}) was extracted by 1 M HCl, which extracts Ca/Mg-bound inorganic P (Jeke, unpublished, 2017). This showed that a large proportion of inorganic P was bound to Ca or Mg.

Table 3.1 Cumulative nutrient supply (mean \pm standard error, $\mu\text{g cm}^{-2}$) calculated by summing the supply rates of successive 2-week burial periods.

Nutrient	18 Jun	3 Jul	18 Jul	4 Aug	19 Aug	4 Sep	19 Sep	p-value
NO ₃ -N	0.7 \pm 0.2e†	2.0 \pm 0.5d	4.6 \pm 1.2c	5.9 \pm 1.6bc	11 \pm 3ab	13 \pm 3ab	15 \pm 4a	<0.0001
NH ₄ -N	4.4 \pm 0.3g	8.6 \pm 0.5f	12 \pm 0.8e	15 \pm 1d	19 \pm 1c	25 \pm 2b	35 \pm 2a	<0.0001
(NO ₃ ⁻ +NH ₄)-N	5.2 \pm 0.5e	11 \pm 1d	17 \pm 2c	21 \pm 2c	30 \pm 3b	38 \pm 4b	50 \pm 5a	<0.0001
H ₂ PO ₄ ⁻ -P	17 \pm 3f	38 \pm 8e	61 \pm 12d	85 \pm 17c	105 \pm 21b	130 \pm 26a	150 \pm 30a	<0.0001
K ⁺	20 \pm 2g	41 \pm 4f	65 \pm 6e	90 \pm 8d	119 \pm 11c	141 \pm 13b	169 \pm 16a	<0.0001
Ca ²⁺	1871 \pm 42g	3584 \pm 80f	5371 \pm 120e	7139 \pm 160d	9008 \pm 201c	10836 \pm 242b	12681 \pm 284a	<0.0001
Mg ²⁺	742 \pm 24g	1443 \pm 47f	2175 \pm 70e	2894 \pm 93d	3651 \pm 118c	4409 \pm 142b	5167 \pm 167a	<0.0001
B(OH) ₄ ⁻ -B	1.4 \pm 0.2f	3.4 \pm 0.4e	5.8 \pm 0.8d	8.6 \pm 1.1c	10.8 \pm 1.4b	12.8 \pm 1.7ab	14.8 \pm 1.9a	<0.0001
SO ₄ ²⁻ -S	581 \pm 224e	875 \pm 338d	1138 \pm 440cd	1407 \pm 543bc	1866 \pm 720ab	2127 \pm 821a	2449 \pm 946a	<0.0001
Fe ³⁺	202 \pm 39g	517 \pm 99f	934 \pm 179e	1378 \pm 264d	1724 \pm 331c	2089 \pm 401b	2468 \pm 474a	<0.0001
Mn ²⁺	35 \pm 7f	68 \pm 15e	93 \pm 20d	123 \pm 26c	133 \pm 28c	167 \pm 36b	198 \pm 42a	<0.0001
Cu ²⁺	0.2 \pm 0.04c	0.27 \pm 0.1c	0.5 \pm 0.1b	0.66 \pm 0.13b	1.3 \pm 0.3a	1.3 \pm 0.3a	1.7 \pm 0.3a	<0.0001
Zn ²⁺	0.6 \pm 0.2d	0.8 \pm 0.2cd	1.2 \pm 0.3bc	1.4 \pm 0.4b	2.3 \pm 0.6a	2.4 \pm 0.7a	2.8 \pm 0.8a	<0.0001

† Time periods that have the same letter in each row are not significantly different at P < 0.05.

The ratio of N:P supply rates ranged from 0.2:1 to 0.6:1 throughout the sampling period, suggesting that N rather than P may be a limiting nutrient for plant growth in the wetland system. Slama (2011) reported N:P ratios of 1.85:1, 8.22:1, and 37:1 measured by PRS probes buried for 3 days in a natural wetland, a wetland constructed using oil sands process water, and a wetland constructed using oil sands process sediments, respectively. Based on these ratios, Sharma suggested that P was not likely the reason for reduced submerged macrophyte growth observed in wetlands with N:P ratios less than 14:1. Nitrogen:phosphorus ratios below 14:1 indicate N limited systems (Koerselman and Meuleman, 1996; Verhoeven et al., 1996).

Potassium supply rates were generally comparable to those for P and increased significantly from July 3 ($20.6 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$) to August 19 ($28 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$) (Fig. 3.2e). The supply rate of Ca was the highest among all the ions and ranged from 1700 to 1900 $\mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$ with no significant temporal changes (Fig. 3.2f). High Ca supply rates may have affected P mobility, resulting in low P adsorption by anion probes. Supply rates of Ca, Mg, Zn, Pb, and Cd did not differ significantly among sampling periods. The supply rate of Fe significantly increased from $202 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$ (June 18) to a peak of $444 \mu\text{g cm}^{-2} \text{ 2 wk}^{-1}$ (Aug 4). The increase in Fe supply rate was likely due to the reductive dissolution of Fe compounds under the anaerobic wetland conditions. Supply rates of Mn and Cu (Fig. 3.2k-l) did not differ significantly among sampling dates, except for an increase in Cu supply rates or decrease in Mn supply rate on August 19. Copper, Zn, and Cd supply rates were low, likely due to the low concentrations of these elements in the biosolids (Table 2.1) and their low concentration in solution due to their strong affinity for the solid phase or association with organic compounds. Biosolids pH (7.8) was slightly alkaline, which may have reduced the availability of Cu, Zn, Mn and Fe via conversion to low-solubility compounds.

3.4.2 Relationship Between PRS Supply Rates and Plant Uptake

Uptake rates of the macronutrients N, P, K, Ca and Mg by cattail were highly correlated ($r^2 = 0.60\text{--}0.84$; $P < 0.0001$) with cumulative supply of these nutrients measured by PRS probes (Table 3.1; Figs. 3.3a-e). The primary nutrients N, P, and K are required in large amounts for growth and survival of plants and plants readily take up these nutrients. The relationship between S uptake and supply rate was weaker ($r^2 = 0.29$) (Fig. 3.3g) compared with the other macronutrients. This might be a result of low S uptake by cattail compared to the high S supply rates ($500\text{--}6000 \mu\text{g cm}^{-2} \text{ wk}^{-1}$) measured by PRS probes. The high supply rates of sulfate indicate S was not limiting.

Measured PRS supply rates and plant uptake were highly correlated ($r^2 = 0.49\text{--}0.67$; $P < 0.0001$) for the micronutrients B (Fig. 3.3f), Fe (Fig. 3.3h), and Mn (Fig. 3.3i) but weakly correlated for Cu ($r^2 = 0.18$) (Fig. 3.3j) and Zn ($r^2 = 0.16$) (Fig. 3.3k). The weaker correlations for Cu and Zn may be due to (1) cattail exclusion of metal ion uptake as a defense mechanism to protect aerial tissues from phytotoxic effects of Cu and Zn (Ali et al., 2013) and/or (2) low plant uptake due to strong binding of Cu and Zn by organic matter (Fageria et al., 2002), which is a primary component of biosolids. Copper and Zn accumulations in cattail have been shown to be restricted to belowground biomass, with minimal translocation to aboveground biomass (Jeke et al., 2015a). Jeke et al. (2015) reported that cattail grown in biosolids partitioned most of its N and P uptake to the aboveground biomass while Cu and Zn uptake values were low and preferentially partitioned to roots and rhizomes. Therefore, Cu and Zn in the present study might have been strongly correlated with the belowground biomass rather than the aboveground biomass; this needs to be investigated in future studies. Our results show that PRS probes supply rates can correlate strongly with cattail uptake of nutrients such as N, P, and K, which are readily translocated to

aboveground plant biomass, whereas correlation may be weaker for micronutrients such as Cu and Zn, which are restricted mostly to belowground biomass.

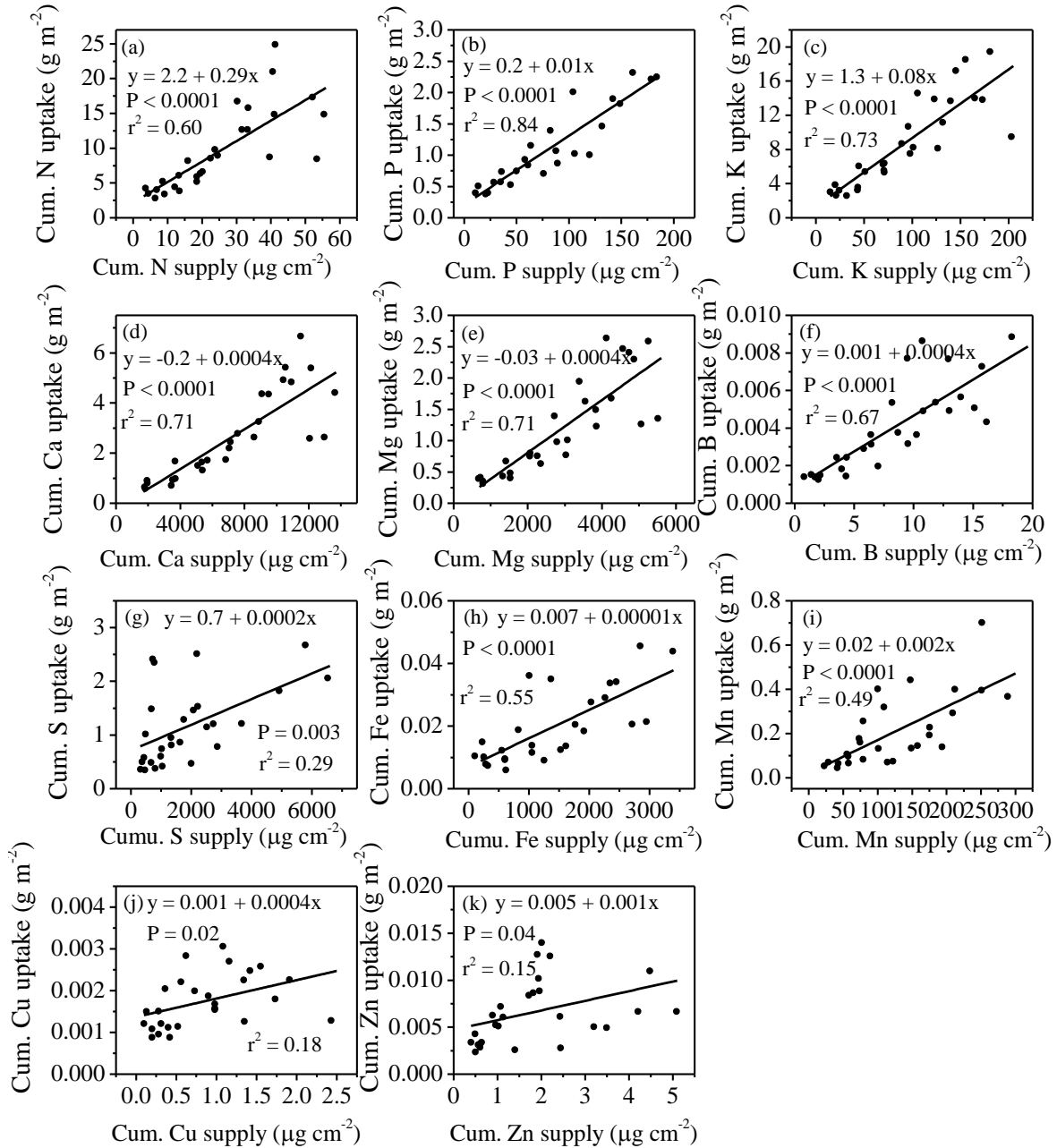


Figure 3.3 Relationships between nutrient uptake by cattail and available nutrient supply rate as determined by PRS probes.

3.4.3 Biomass Yield and Nutrient Uptake

Of all models tested, the beta sigmoid model provided the best fit to biomass yield data (Fig. 3.4). Maximum biomass accumulation rate (t_m) occurred on 16 August and corresponded to a cumulative biomass yield of 0.61 kg m^{-2} (Table 3.2; Fig. 3.4). Estimated maximum attainable biomass yield (y_{\max}) was 0.87 kg m^{-2} on 9 Sep (t_e), which corresponded to approximately 100 d after the start of cattail regrowth in early June. These results corroborate those from a recent temperate region study, which showed that maximum cattail biomass yields occurred in late August or early September (Grosshans, 2014). The maximum attainable cattail biomass yield in our study was lower than biomass yields ($1.0\text{-}1.5 \text{ kg m}^{-2}$) reported in other temperate region studies (Grosshans, 2014; Wild et al., 2002). Cattail in the present study was harvested by cutting the plants at a height of ~65 cm above the sediment to prevent flooding the plants. This cutting height left considerable biomass (~50% of total biomass) in the 65-cm high stubble unharvested. Higher biomass yield would have been attained with a lower cutting height.

Harvested cattail biomass can be used as animal bedding on livestock operations. Cattail biomass can also be used as a feedstock for generation of bioenergy, which can provide power to local communities.

Table 3.2 Beta sigmoid model parameters for biomass yield and nutrient uptake by cattail grown in biosolids sediment during constructed wetland phytoremediation of an end-of-life municipal lagoon.

Parameter†	Biomass	N	P	K	Ca	Mg	B	S	Fe	Mn	Cu	Zn	Na
y_{max} (g m ⁻²)	865	17	2.1	15	5.1	2.2	0.007	2.1	0.04	0.47	0.002	0.01	3.4
t_e	9 Sep	5 Sep	5 Sep	7 Sep	6 Sep	9 Sep	15 Sep	7 Sep	7 Sep	10 Sep	27 Aug	8 Sep	8 Sep
t_m	16 Aug	12 Aug	10 Aug	10 Aug	15 Aug	13 Aug	13 Aug	12 Aug	16 Aug	21 Aug	7 Jul	5 Aug	17 Aug
RMSE (g m ⁻²)	125	3.2	0.34	2.3	0.72	0.32	0.001	0.32	0.01	0.09	0.0005	0.002	0.7

† y_{max} is the maximum y value (maximum biomass yield or nutrient uptake); t_e is the time (converted to actual date) when biomass or nutrient uptake reaches maximum value; t_m is the inflection point (converted to actual date) at which growth or uptake rate is maximized.

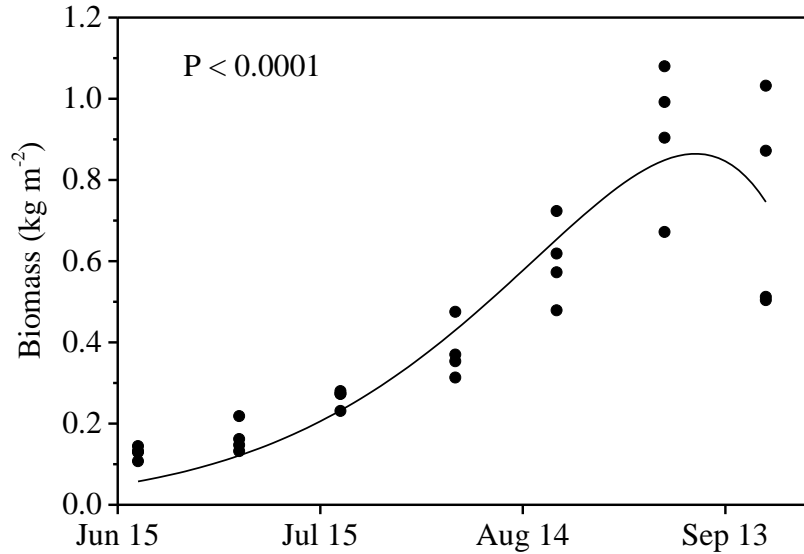


Figure 3.4 Cattail biomass accumulation as described by a beta sigmoid model.

Similar to biomass, the beta sigmoid model provided the best fit to uptake data. Maximum uptake of most nutrients and trace elements occurred during the first week of September (Table 3.2; Fig. 3.5). Maximum nutrient and trace element uptake (Table 3.2; Fig. 3.5) coincided with maximum biomass accumulation (Table 3.2), indicating that harvesting of cattail should occur between late August and early September. Peak accumulations of N ($17 \pm 1 \text{ g m}^{-2}$) and K ($15 \pm 0.9 \text{ g m}^{-2}$) in the cattail biomass were greater than those for Ca ($5.1 \pm 0.3 \text{ g m}^{-2}$), P ($2.1 \pm 0.1 \text{ g m}^{-2}$), and S ($2.1 \pm 0.1 \text{ g m}^{-2}$). Maximum uptake was very low for B ($0.007 \pm 0.0004 \text{ g m}^{-2}$), Fe ($0.04 \pm 0.004 \text{ g m}^{-2}$), Mn ($0.47 \pm 0.04 \text{ g m}^{-2}$), Cu ($0.002 \pm 0.0002 \text{ g m}^{-2}$), and Zn ($0.01 \pm 0.001 \text{ g m}^{-2}$), but high for Na ($3.4 \pm 0.3 \text{ g m}^{-2}$). These results show that, in addition to macronutrient removal, cattail has the capacity to effectively phytoextract the non-essential element Na. Grosshans (2014) examined average nutrient concentrations at 34 sites in Netley-Libau Marsh, Manitoba, and reported that cattail contained higher amounts of K (33.3 g m^{-2}), N (23.4 g m^{-2}), and Ca (17.2 g m^{-2}) compared with other nutrients tested.

Sulfur is required for protein synthesis and a balanced supply of N and S is essential for maximum crop growth. Optimum growth of various agricultural crops has been shown to require a N:S ratio of 9:1 to 16:1 in plant tissues (Hu and Sparks, 1992; Hu et al., 1991; Qian and Schoenau, 2007). The N:S ratio in cattail plant tissue in this study was between 7.2:1 and 9.1:1 throughout the sampling period, which suggests an adequate N:S ratio in the cattail, assuming N:S requirements of cattail are similar to agricultural crops. The ratio of available N: available S in biosolids as measured by PRS probes was very low (0.01:1 to 0.07:1), reflecting low N mineralization in the biosolids and a high sulphate supply of the biosolids. The biosolids available N:available S ratio determined by PRS probes did not reflect the relative supplies of available N and S as suggested by the N:S ratio in the cattail plant tissue. This contradicts results by Qian and Schoenau (2007), who reported that the soil available N:S ratio measured by PRS probes closely reflected the N:S ratio in plant tissues of canola and wheat. This is because the PRS probes measured greater supply rates of S relative to N in the biosolids while cattail uptake of N was greater than S uptake.

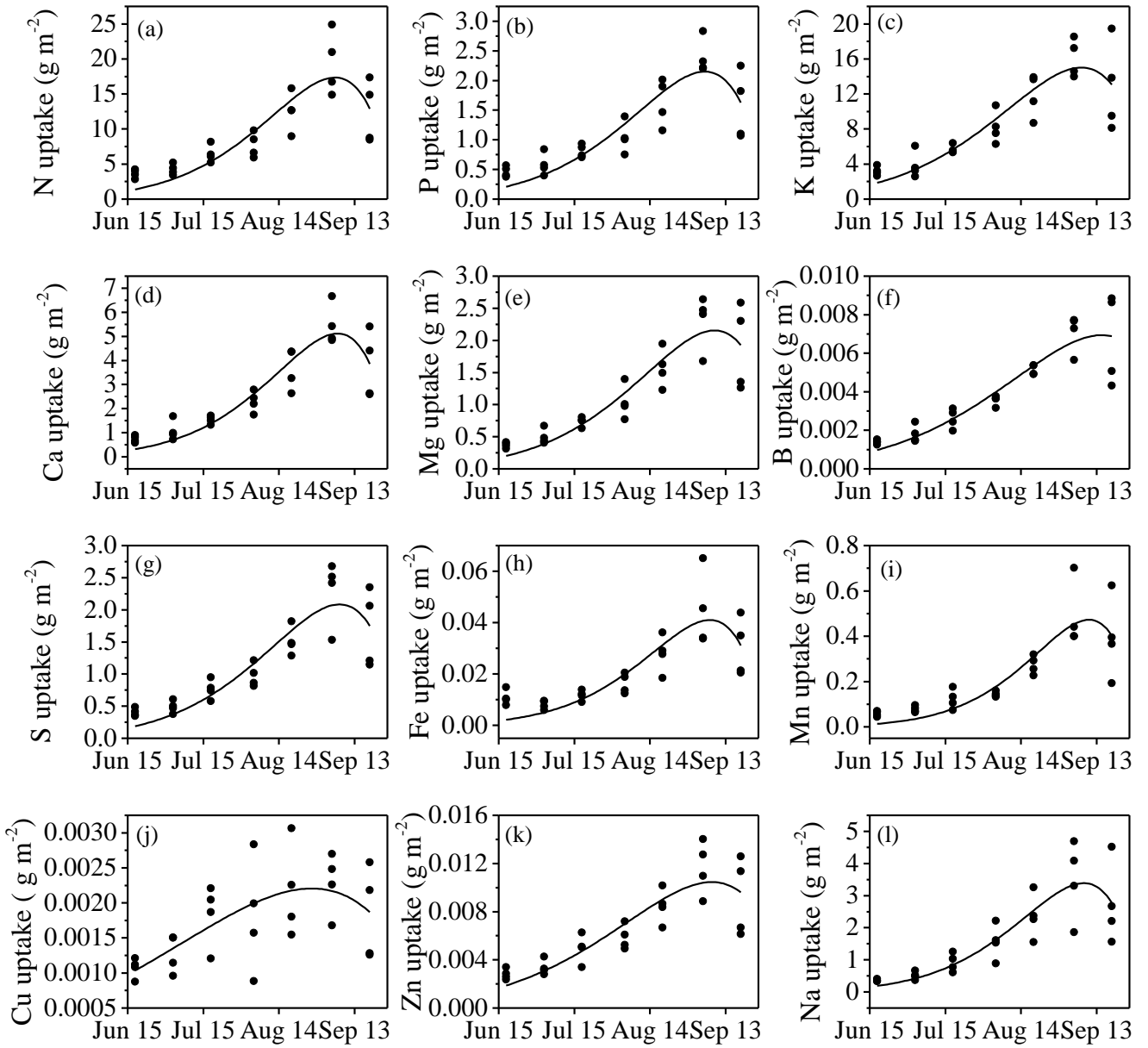


Figure 3.5 Nutrient and trace element accumulation in cattail biomass as described by a beta sigmoid model.

3.5 Conclusion

PRS probes were effective in monitoring the supply of available macronutrients N, P K, Ca, and Mg from biosolids sediment in the constructed wetland as nutrient supply rate correlated strongly with plant uptake. Correlations between measured PRS supply rates and plant uptake of the micronutrients Zn and Cu were significant but weaker than for other micronutrients, suggesting that PRS probes may have limited effectiveness in measuring Cu and Zn supply in situ. Copper and Zn supply rates may be strongly correlated with belowground biomass of cattail, but this needs to be examined. Macronutrient and micronutrient uptake coincided with maximum biomass accumulation. The results indicated that cattail should be harvested between late August and early September, or about 90 – 100 d after the start of cattail regrowth. The strong correlations of PRS supply rates of macronutrients with plant uptake suggest its potential use for in situ measurements during phytoremediation. Information from the study will inform wetland managers on the potential for using PRS probes to monitor seasonal nutrient variability and on the timing of cattail harvesting during wetland-based phytoremediation.

3.6 References

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4. A FIELD BIOASSAY OF NITROGEN AND PHOSPHORUS PHYTOEXTRACTION IN A SEASONALLY-FROZEN END-OF-LIFE MUNICIPAL LAGOON VEGETATED WITH CATTAIL

4.1 Abstract

Managing biosolids from end-of-life municipal lagoons is a major challenge for many small communities where landfilling or spreading of biosolids on farmland is restricted. Contaminant removal via phytoextraction maybe a viable remediation option for end-of-life lagoons in such communities. This study examined the effect of harvest frequency (once or twice per season) on cattail (*Typha latifolia* L.) biomass yield and N and P removal under a terrestrial phytoremediation system designed to treat the dewatered secondary cell of a municipal lagoon in Manitoba, Canada. Cattail was harvested once or twice per season from eight vegetation transects, each divided into two plots (2.5 m × 2.5 m) to accommodate the two harvest frequencies. Biomass yields were greater for the single harvest (5.7 Mg ha⁻¹ yr⁻¹) than for two harvests per season (4.8 Mg ha⁻¹ yr⁻¹). This was mirrored by N phytoextraction, which was also greater for the single harvest (71 kg ha⁻¹ yr⁻¹) than the two-harvest frequency (58 kg ha⁻¹ yr⁻¹). Phosphorus phytoextraction varied with year of harvest and ranged from 8–14 kg ha⁻¹ yr⁻¹. Cumulative N and P phytoextraction amounts during the 5 yr were 330 kg N ha⁻¹ and 57 kg P ha⁻¹. A greater fraction of N (51–91 kg ha⁻¹ yr⁻¹) and P (23–40 kg ha⁻¹ yr⁻¹) was sequestered in the belowground biomass (11–17 Mg ha⁻¹ yr⁻¹) and therefore was not removed by harvesting. These results show that phytoremediation using cattail is a viable option for managing N and P in end-life-lagoons.

4.2 Introduction

Municipal lagoons, also known as wastewater stabilization ponds, are widely used by small communities in North America for storage and treatment of wastewater because of their ease of construction and use and their low maintenance costs (CCME, 2006; USEPA, 2011). More than 1,000 waste stabilization ponds accounted for half of the wastewater treatment systems in Canada in the late 1980s, with the majority of them in western and northern Canada (Heinke et al., 1991). As of 2004, at least 350 communities in Manitoba, Canada, used lagoons for wastewater treatment (Manitoba Land Initiative, 2004). In the United States, there are over 8,000 stabilization ponds, accounting for >50% of wastewater treatment facilities (USEPA, 2011). Municipal lagoons have a finite life span, typically 20-30 yr, and must be closed when they reach their design capacity. Many lagoons that were built in the 1960s and 1970s are due for decommissioning. The disposal of large volumes of biosolids when lagoons close is a challenge for many small communities.

After lagoon closure, biosolids are typically spread on farmland or disposed of in landfills (CMWC, 2015; Laidlaw et al., 2012). Stringent regulations on farmland spreading of biosolids (Iranpour et al., 2004) and the proposed ban of organic waste landfilling in Canada (National Research Council, 2017) calls for alternative sustainable options. In situ phytoremediation of sewage sludge or biosolids in end-of-life municipal lagoons may provide a cost-effective and sustainable alternative in jurisdictions where farmland spreading and landfilling are restricted or infeasible.

Phytoextraction has been used as a strategy to remove excess nutrients from nutrient-enriched agricultural soils using high-yielding forage crops and/or legumes (Fiorellino et al., 2017; Ryan et al., 2016; Silveira et al., 2013). Phytoextraction has also been used in the reclamation of sewage sludge stockpiles or sewage sludge amended soils (Laidlaw et al., 2012; Suchkova et al., 2015; Suchkova et al., 2014) and the pre-treatment of dewatered sewage sludge before land application

(Wu et al., 2007; Xiaomel et al., 2005). While plants have been used for wastewater treatment in operational lagoons, removal of nutrients and other contaminants from biosolids during municipal lagoon decommissioning has been given little attention. Studies evaluating nutrient removal by cattails growing directly in biosolids or sludges in end-of-life municipal lagoons are lacking. Previous studies have examined phytoremediation in which plants were grown in pots in growth room experiments (Jeke et al., 2017; Jeke et al., 2015a; Jeke et al., 2016) or ex situ phytoremediation where plants were grown in stockpiled biosolids or biosolids deposited outside lagoons (Laidlaw et al., 2012; Suchkova et al., 2010; Suchkova et al., 2014). The growth room experiments provided restricted conditions because of the small volume of biosolids in pots and the absence of an ‘off-season’ break between growth cycles and may not accurately reflect field conditions. There is therefore a need to investigate in situ remediation, which precludes the costly removal of biosolids from lagoons. Moreover, municipal lagoons are underlain by an impermeable clay layer to prevent seepage to groundwater and as such lagoons are poorly drained. Ponding and saturation in end-life-lagoons is common during spring snow melt or periods of heavy rainfall, and this may influence plant species selection in favor of plants such as cattail that are better adapted to periodic waterlogging. Suchkova et al. (2014) reported that native plants established naturally in sewage sludge that was amended with soil and gravel to improve aeration and structure but not in sewage sludge alone, an observation which they attributed to poor aeration in the waterlogged unamended sludge (75-85% water content).

Cattail (*Typha* spp.) is a high biomass plant which is native to North America, where it typically grows in wetlands and ditches. Cattail can be used as a feedstock for bioenergy production (Grosshans, 2014) and as an alternative feed for livestock if harvested early (Lardy and Anderson, 2009). Cattail has been widely used for N and P phytoextraction during wastewater

treatment in wetlands (Grosshans, 2014; Maddison et al., 2005; Toet et al., 2005). Temporarily flooded or saturated conditions in end-of-life municipal lagoons can provide conducive environments for cattail establishment. There is a need to evaluate cattail biomass yield and nutrient phytoextraction potential in a terrestrial environment in dewatered end-of-life lagoons which are not permanently waterlogged.

Harvesting management practices, such as cutting frequency and time of harvesting, play an important role in determining biomass yield and nutrient removal during phytoremediation. Multiple harvesting of bioenergy crops such as switchgrass have been reported to increase biomass yield by about 15% compared with a single harvest (Fike et al., 2006). Nitrogen removal by bioenergy crops has been reported to be two to three times greater for two-harvests per season compared with one harvest per season (Fike et al., 2006; Lemus et al., 2008; Reynolds et al., 2000). Cattail has been shown to be resilient to repeated harvesting per year in wetlands (Hall et al., 2008; Jinadasa et al., 2008). Repeated harvesting during a growing season can restrict plants to immature phases which can have rapid growth and actively compete for more available nutrients, hence greater nutrient phytoextraction than mature plants. A growth room study by Jeke et al. (2017) showed that harvesting cattail and switchgrass twice during the first 90-day growth period significantly improved N and P phytoextraction compared with a single harvest. However, cattail biomass and phytoextraction decreased in the second growth cycle relative to the first cycle, ostensibly due to the restrictions imposed by the volume of the potted biosolids, which could no longer supply adequate available nutrients and/or the absence of an 'off-season' break between cycles. Research is needed to determine if cattail can sustain high biomass yields with multiple harvests under terrestrial conditions in end-of-life lagoons and if repeated harvesting of cattail can effectively remove N and P from biosolids.

Most published studies on nutrient removal by cattail and other emergent macrophytes have focused mostly on nutrient removal from wastewater before it is released from wetlands and operational municipal lagoons. ‘At the back end’ process, that is, when municipal lagoons cease operation and dewater through natural evaporation, the traditional practice has been to excavate biosolids for application to agricultural land or landfilling, and in situ phytoextraction of nutrients using cattail has not been given much consideration as an option used during decommissioning of municipal lagoons. This study examined the phytoremediation of biosolids in a seasonally-frozen secondary cell of an end-of-life municipal lagoon containing biosolids that had dewatered through natural evaporation. The overall objective was to characterize cattail biomass yield and N and P phytoextraction under a single harvest vs. or two harvests per season.

4.3 Materials and Methods

4.3.1 Study Site

This study was conducted in a secondary cell (8.8 ha) of a 13-ha municipal lagoon in Niverville, Manitoba, Canada (49°35'42.7"N, 97°02'50.3"W). The secondary cell received effluent from the primary cell for further treatment and storage. The municipal lagoon operated for 37 years (1971-2008) and treated wastewater via microbial degradation. At the onset of this study (2013), the biosolids had dewatered and dried naturally through evaporation. The biosolids were covered with a dense monoculture of cattail that established naturally under moist conditions which allowed cattail growth from the existing cattail seedbank in the biosolids.

Summer (June-August) average air temperature during the 5-yr study ranged between 18.9 and 19.4°C (Fig. 4.1), which is slightly above the long-term average of 18.4°C for the area (MAFRI, 2017). Cumulative summer rainfall during June through August decreased in the order

2014 (289 mm) > 2015 (282 mm) > 2016 (271 mm) > 2013 (226 mm) > (139 mm) (Fig. 1.4) (MAFRI, 2017).

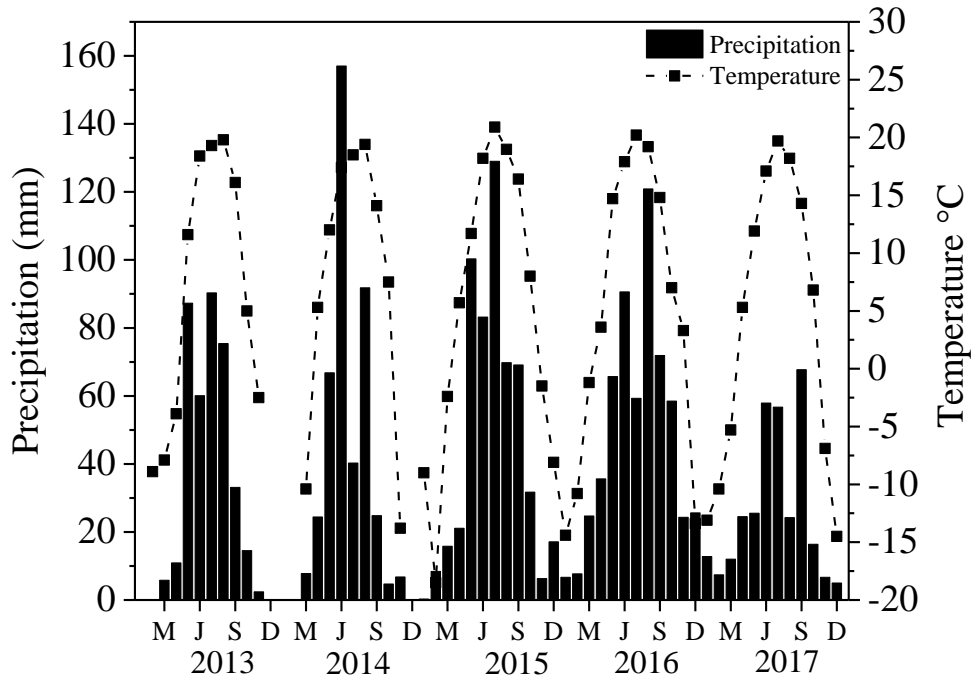


Figure 4.1 Monthly cumulative rainfall and average temperatures at St. Adolphe, Manitoba (10 km from study site) during the 5-yr study.

Snow water equivalent (SWE) was estimated from snow depth (h_s) measured at the Environment and Climate Change Canada weather station at Kleefeld, Manitoba (17 km from study site) using the following equation;

$$SWE = h_s \times (\rho_b / \rho_w) \tag{1}$$

where ρ_b is the local bulk density and ρ_w is the density of water (1 g cm^{-3}) (Sturm et al., 2010).

Local bulk density was modelled using snow depth, snow age, and a snow class defined by location using the following model:

$$\rho = \rho_0 + (\rho_{\max} - \rho_0) \times (1 - \exp(-k_1 \times h_s - k_2 \times \text{DOY}/100)) \tag{2}$$

where $\rho_0 = 0.2332$, $\rho_{\max} = 0.5940$, $k_1 = 0.0016$, and $k_2 = 0.0031$ are parameters for prairie snow class and DOY is day of the year, which runs from -92 on October 1 through -1 on December 31 and from 1 on January 1 through 181 on June 30 (Jonas and Marks, 2016; Sturm et al., 2010).

Based on these calculations, mean monthly SWE in December, January and February was greater in 2016 and 2017 than in 2015 (Fig. 4.2). Data for 2013 and 2014 were not available. Snow trapped in the lagoon cell provides moisture during spring snowmelt melt for the growth of cattail.

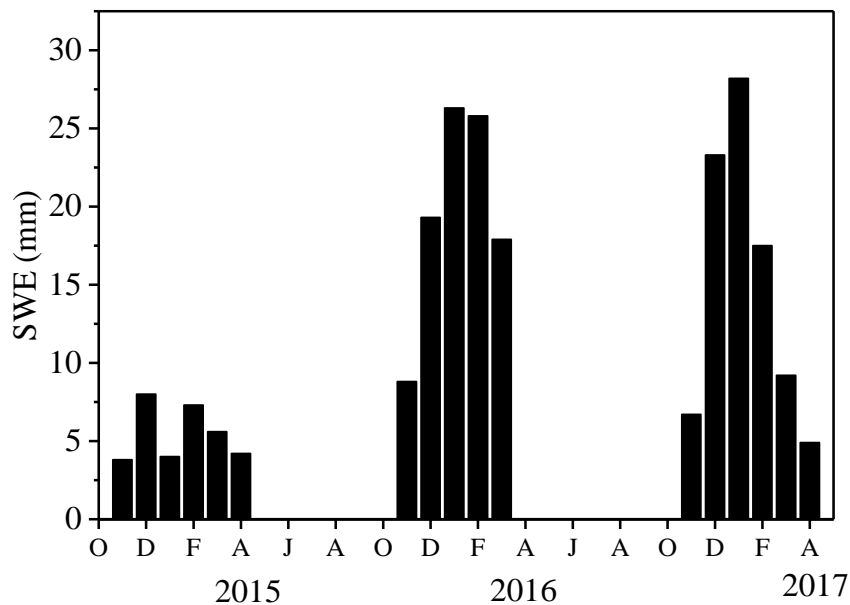


Figure 4.2 Mean monthly snow water equivalents (mm) estimated from snow depth at Kleefeld, Manitoba (17 km from study site).

4.3.2 Experimental Setup

The experiment was laid out as a randomized complete block design with a total of 16 experimental plots (two treatments \times eight transects (blocks)). Two plots (2.5 m \times 2.5 m) were marked off in each of the eight vegetation transects that were set up in the lagoon. Plants were

harvested twice (mid-July and early September) or once (late August) per season. The study was conducted from 2013 to 2017.

4.3.3 Plants and Biosolids Sampling

Cattail was harvested from a 1 m × 1 m quadrat at the centre of each plot by cutting the plants at ~ 10 cm above the biosolids surface. After harvesting from the quadrats, the rest of the remaining cattail in each plot and in the area adjacent to the plots was harvested. Belowground biomass (roots and rhizomes) was collected using root a 15-cm diameter corer. Biosolids samples were collected from the 0- to 15-cm layer before the start of the experiment and at the end of each growing season.

4.3.4 Laboratory Analysis

Total nitrogen (TN) concentration in the biosolids was measured by the combustion method using a LECO CNS-2000 analyzer (LECO Corporation, MI, USA). Nitrate and ammonium (salicylate method) concentrations were measured spectrophotometrically using a FIALab 2500 flow injection analyzer (FIALab Instruments, Bellevue, WA, USA). Olsen P was determined spectrophotometrically by the molybdenum blue method following extraction of 1 g soil with 20 ml 0.5 M NaHCO₃ at pH 8.5 (Olsen and Sommers, 1982). Total P concentration was measured following sample digestion in aqua regia (conc. HNO₃/HCl) for 2 h at 90°C in a microprocessor--controlled digestion block. Phosphorus concentration was measured in the digest using a PerkinElmer SCIEX ELAN 6000 ICP-MS (Perkin-Elmer SCIEX, Concord, Ontario, Canada).

A sequential extraction procedure based on the modified method of Hedley (Ajiboye et al., 2004) was employed to determine the fractionation of P in the biosolids. The biosolids were sequentially extracted using deionized water to remove soluble P, 0.5 M NaHCO₃ (pH 8.5) (loosely sorbed P), 0.1 M NaOH (P sorbed to Fe and Al oxides), and 1 M HCl (Ca and Mg associated P). Inorganic P concentration in the filtrates was determined by molybdate-blue colorimetry according

to Murphy and Riley (1962) using an Ultrospec 3100 pro UV/visible spectrophotometer (Biochrom, Cambridge, UK) at a wavelength of 882 nm. Residual P was measured after digestion of the biosolids residues with a H₂SO₄/H₂O₂ digestion mixture.

Plant samples were dried at 60°C to a constant weight to determine dry biomass yield. Subsamples were ground with a Wiley Mill grinder to pass a 1.6 mm screen. Total N concentration was measured by dry combustion using a Leco CNS 2000 analyzer (LECO Corporation, MI, USA). Phosphorus concentration was measured using the ICP-MS described above following a 2-h digestion of a 0.5-g sample with aqua regia at 90°C using a microprocessor-controlled digestion block. Nitrogen and P phytoextraction were calculated as the products of dry matter yield and nutrient concentration.

4.3.5 Statistical Analysis

Biomass yield and N and P phytoextraction data were analyzed with the GLIMMIX procedure for repeated measures in SAS version 9.4 (SAS Institute, 2014), with harvest frequency and year of harvest as fixed effects and transect and transect × harvest frequency as random effects. Year of harvest was modelled as the repeated measure. Various covariance structures were compared and the best fit was selected based on the lowest corrected Akaike information criterion (AICc). Treatment differences were assessed at $\alpha = 0.05$ using the Tukey–Kramer adjustment for multiple comparisons

4.4 Results and Discussion

4.4.1 Initial Nutrient Concentrations in Biosolids

Total N (4279 mg kg⁻¹) and total P (1930 mg kg⁻¹) concentrations were very high and the primary targets for phytoremediation (Table 4.1). Trace element concentrations (data not shown) were below Canadian Soil Quality Guidelines for the Protection of Environmental and Human

Health (CCME, 1999). Total N concentration in the biosolids was lower than typical N concentrations (10,000–30,000 mg kg⁻¹) in biosolids treated using lagoons with average residence time spanning several years (Brown and Henry, 2001; Cogger et al., 2004; Gilmour et al., 2003). Material in the lagoon had attributes similar to a combination of soil and biosolids as opposed to pure biosolids, and therefore, was not highly enriched in N, P or trace elements.

About 10% of the total N content was in the plant-available inorganic form (NO₃-N and NH₄-N) that is available for plant uptake. Plant available P (Olsen P) concentration (73 mg kg⁻¹), was sufficient for plant productivity. The sum of H₂O- and NaHCO₃-extractable inorganic P fractions (173 mg kg⁻¹ or 9% of TP) represents P that is soluble or weakly adsorbed and, therefore, potentially available for plant uptake. The low potential available P (< 10% of TP) is consistent with other studies examining biosolids (Ajiboye et al., 2004; Withers et al., 2001). About 1122 ± 74 mg kg⁻¹ (46-75%) of the TP (1930 ± 343 mg kg⁻¹) in the biosolids was in the inorganic fraction (Table 1). The difference between TP and other P fractions shown in Table 1 (553 mg kg⁻¹ or ~29% of TP) was most likely composed of organic P, which was not measured in this study. A study by Jeke et al. (2018a) using biosolids from the same lagoon showed that organic P in the biosolids was ~ 0% of TP.

Table 4.1 Initial nitrogen and phosphorus concentrations (mg kg⁻¹) in biosolids from the secondary cell of an end-of-life municipal lagoon.

TN	NH ₄ -N	NO ₃ -N	TP	OP [†]	H ₂ O-P _i [‡]	NaHCO ₃ -P _i	NaOH-P _i	HCl-P _i	Residual P	pH
mg kg ⁻¹										
4279	12	30	1930	73	8	165	82	867	255	7.9

[†]OP, Olsen P

[‡]H₂O-P_i is water-extractable inorganic phosphorus, NaHCO₃-P_i is labile inorganic phosphorus, NaOH-P_i is Fe/Al-bound inorganic phosphorus; and HCl-P_i is Ca/Mg-bound inorganic phosphorus.

4.4.2 Biomass Yield

Biomass yield varied significantly ($P = 0.003$) with year of harvest (Table 4.2). Averaged across harvest frequencies, biomass yield was significantly lower in 2015 (0.37 kg m^{-2}) than in 2016 (0.63 kg m^{-2}) and 2017 (0.62 kg m^{-2}). It is not clear what caused the significant decrease in biomass yield in 2015. The differences in annual biomass yields did not appear to reflect precipitation and temperature (Fig. 4.1) data from the respective seasons. For example, cattail biomass yield was greater in 2016 and 2017, which had lower cumulative summer (June -August) rainfall (271 and 139 mm, respectively) than 2014 (289 mm) and 2015 (282 mm). Moreover, cumulative (January to May) rainfall in 2015 (146 mm), the year with the lowest biomass yield, was greater than or comparable to cumulative rainfall in 2014 (99 mm), 2016 (141 mm), and 2017 (82 mm) (Fig.4.1). Snow accumulation, hence moisture availability during spring snowmelt, likely affected biomass yield. Mean monthly snow water equivalent estimated from snow depth for January through March was greater in 2016 (18 to 26 mm) and 2017 (18 to 28 mm) than in 2015 (4 to 7 mm) (Fig. 4.2), which likely explains the greater biomass yield in 2016 and 2017 compared with 2015 (Table 4.2; Fig. 4.3). The lagoon cell receives snowmelt in spring and remains flooded during at least the early part of the growing season. The larger snowpack in 2016 and 2017 provided wetter conditions during the early part of the season, which might have resulted in higher biomass yields compared with 2015. Growth of cattail may have been closely related to the duration of flooding or period of saturation, which would have been a key parameter to monitor in the lagoon.

Table 4.2 Biomass yield and nitrogen and phosphorus phytoextraction as affected by harvest frequency and year of harvest.

Effect	Biomass kg DW m ⁻²	N g m ⁻²	P
Year			
2013	0.54ab†	7.5	1.4a
2014	0.48ab	7.7	1.3a
2015	0.37b	5.6	0.8b
2016	0.63a	5.9	1.2ab
2017	0.62a	5.8	1.2ab
Harvest frequency (yr ⁻¹)			
Single	0.57a	7.1a	1.1
Two	0.48b	5.8b	1.2
		P-value	
Year (Y)	0.003	0.1	0.01
Harvests (H)	0.03	0.04	0.56
Y × H	0.28	0.17	0.25

†Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

Two harvests per season produced significantly ($P = 0.03$) lower biomass yield (0.48 kg m^{-2}) than a single harvest per season (0.57 kg m^{-2}) (Table 4.2). Thus, there was no benefit in harvesting cattail twice per season. A growth room study by Jeke et al. (2017) also showed that there was no significant benefit in harvesting cattail twice per growing cycle, but there was no significant difference between one and two harvests per growth cycle. On average, 83% of the total annual biomass yield of 0.48 kg m^{-2} in a two-harvest system in the present study came from the first harvest, with the second harvest contributing a mere 17% (0.08 kg m^{-2}) of the total annual yield. The low biomass yield from the second harvest could be a result of the dry conditions that prevailed during much of the summer or the physiology, growth and regenerative characteristics of cattail, which likely prevented robust regrowth of the cattail. Plants from the second harvest appeared weaker and thinner compared to those from the first harvest. Periodic drought during the growing season has been shown to reduce cattail biomass production compared to continuous flooding (Li

et al., 2004). In each season, biomass yield did not differ between the two-harvest and the one-harvest systems (Fig. 4.3), indicating that most of the biomass was produced in June and July.

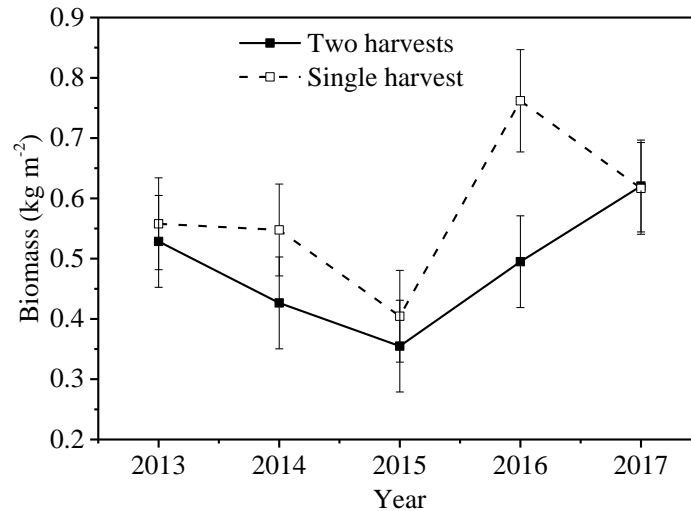


Figure 4.3 Cattail biomass accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.

Biomass yield from the single harvest per season averaged 0.57 kg m^{-2} (Table 4.2; Fig. 4.3) over the 5 yr of repeated harvesting. The study showed that cattail under a terrestrial system can be repeatedly harvested over many years without reducing biomass yields. Cattail biomass yields of 1.0 to 2.2 kg m^{-2} have been reported in prolonged flooding conditions which enhanced cattail growth in ditches, lakes and wetlands in Manitoba (Grosshans, 2014; Grosshans and Grieger, 2013) while lower biomass yields of 0.82 kg m^{-2} have also been reported in marshes in Manitoba (Peterson, 2015). Asamoah and Bork (2010) and Li et al. (2004) found that cattails exposed to continuous flooding and field capacity conditions maintained high biomass yields, while periodic drought significantly reduced biomass yields. The lower biomass yields in our study may be largely due to the dry conditions which prevailed during much of each growing season. In comparison, harvestable cattail biomass yield averaged 1.1 kg m^{-2} in the primary cell of the same

lagoon, which was under continuous flooding conditions (~50 cm water depth) during the growing season, which favored cattail growth (Jeke et al. 2018c).

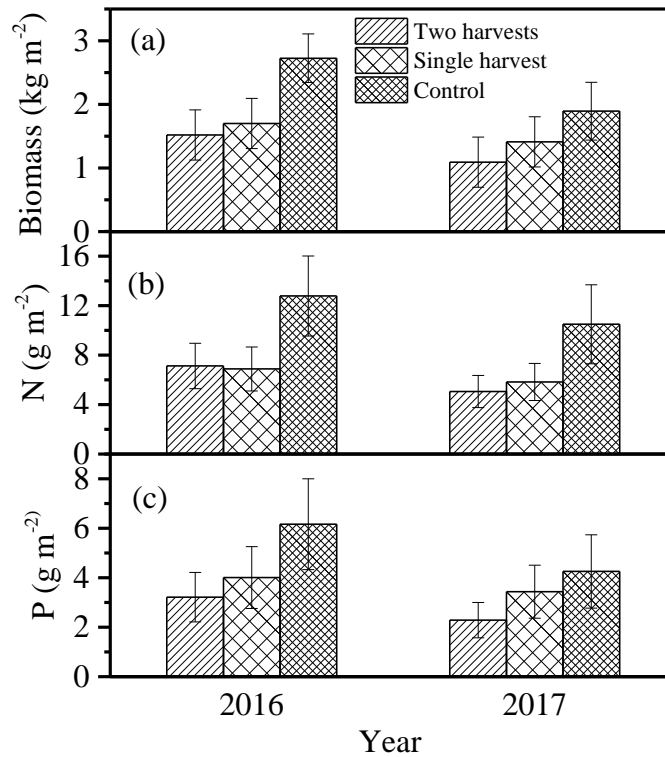


Figure 4.4 Cattail belowground biomass, nitrogen, and phosphorus accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.

Belowground biomass (BGB) yields assessed in 2016 and 2017 (Fig. 4.4) reflected the effects of four and five years, respectively, of aboveground biomass (AGB) harvesting. There were no significant ($P = 0.7$) differences in BGB biomass between a single harvest per season (overall mean = 1.6 kg m^{-2}), two harvests per season (1.3 kg m^{-2}), and no harvest in previous years (unharvested control) (2.3 kg m^{-2}). A larger fraction (~65-75%) of the cattail biomass was in the BGB (Fig. 4.3; Fig. 4.4).

Cattail biomass was harvested from the entire secondary cell in August 2017 and baled (Fig. 4.5), which is a critical step in the removal of nutrients from the biosolids. Use of cattail biomass from the lagoon is still being explored but identified potential avenues for the biomass include use as a feedstock for bioenergy to power a local farmer's flax-heat drying system, heat energy for a college in Manitoba, and the use of the cattail on the local farmer's livestock farm as feed or bedding.



Figure 4.5 (a) Harvesting of cattail in the secondary cell, 15 August 2017; (b) bales of the harvested cattail.

4.4.3 Nitrogen and Phosphorus Phytoextraction

Nitrogen phytoextraction differed significantly ($P = 0.04$) between harvesting frequencies (Table 4.2). Averaged across all years, N phytoextraction was greater when cattail was harvested once (7.1 g m^{-2}) compared with two harvests (5.8 g m^{-2}) per season. The lower N phytoextraction in the two-harvest system was a result of the lower biomass yield compared with a single harvest. There was no significant ($P = 0.17$) harvest frequency \times year interaction for N phytoextraction (Table 4.2; Fig. 4.6).

Phosphorus phytoextraction varied significantly ($P = 0.01$) with year of harvest (Table 4.2; Fig. 4.7). Phosphorus phytoextraction was significantly lower in 2015 (0.8 g m^{-2}) than in 2013 (1.4 g m^{-2}) and 2014 (1.3 g m^{-2}), likely due to the relatively lower biomass yield in 2015. Phosphorus phytoextraction did not differ significantly between two harvests (1.2 g m^{-2}) and a single harvest (1.1 g m^{-2}) per season.

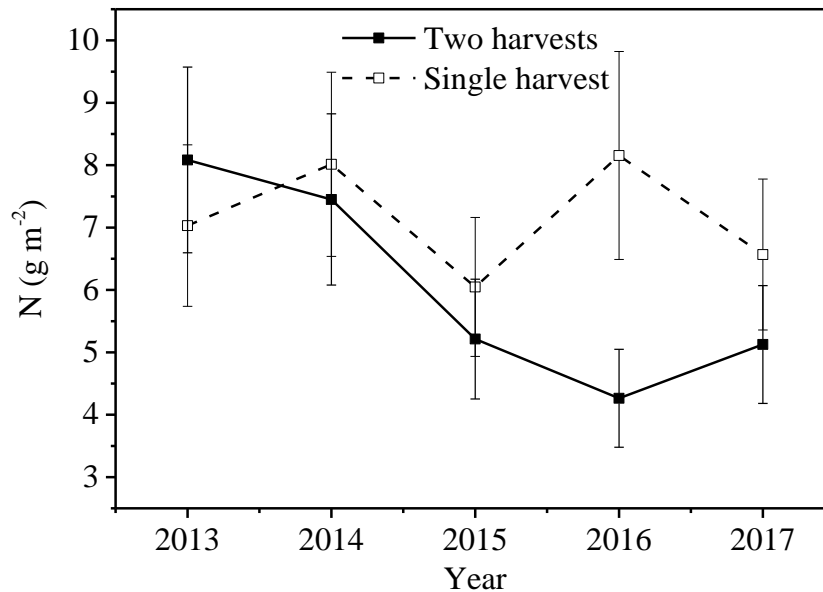


Figure 4.6 Cattail nitrogen accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.

This study showed no significant improvement in nutrient phytoextraction from harvesting cattail twice under a terrestrial system, which corroborates results from a recent terrestrial microcosm study (Jeke et al., 2017), but contradicts results from a related wetland-based growth room experiment (Amarakoon et al., 2018). Amarakoon et al. (2018) reported that harvesting cattail under a wetland-based phytoremediation of biosolids increased N and P phytoextraction by 47% and 41% relative to a single harvest per growing cycle.

Nitrogen ($5.6 - 7.7 \text{ g m}^{-2}$) and P ($0.8 - 1.4 \text{ g m}^{-2}$) phytoextraction values for cattail in our study were lower than those observed in wetlands in Manitoba (Grosshans, 2014; Peterson, 2015). Grosshans (2014) reported 23.5 g N m^{-2} and 3.6 g P m^{-2} while Peterson (2015) reported $8.6 - 11.2 \text{ g N m}^{-2}$ and $1.2 - 1.8 \text{ g P m}^{-2}$. Mean tissue N (15 g kg^{-1}) and P (2.3 g kg^{-1}) concentrations in our study were within the range of tissue N ($8.3 - 14 \text{ g kg}^{-1}$) and P ($1.5 - 2.6 \text{ g kg}^{-1}$) concentrations reported by Grosshans (2014) and Peterson (2015). Nutrient phytoextraction is calculated as the product of plant tissue nutrient concentration and dry biomass yield. Nutrient concentrations in our study were within the range of nutrient concentrations reported in other studies in Manitoba, but our nutrient phytoextraction rates were lower because of the lower biomass yield in our study. This suggests that biomass production was the primary determinant of nutrient phytoextraction and therefore the lower biomass yields observed in the present study, explains the lower nutrient removals. Because cattail is adapted to wetland conditions, periodic summer droughts in our study at least partly explain the lower biomass yields relative to potential biomass yields under wetland conditions. Nevertheless, the N and P phytoextraction values show that cattail can be used to phytoextract nutrients from lagoon biosolids under upland conditions.

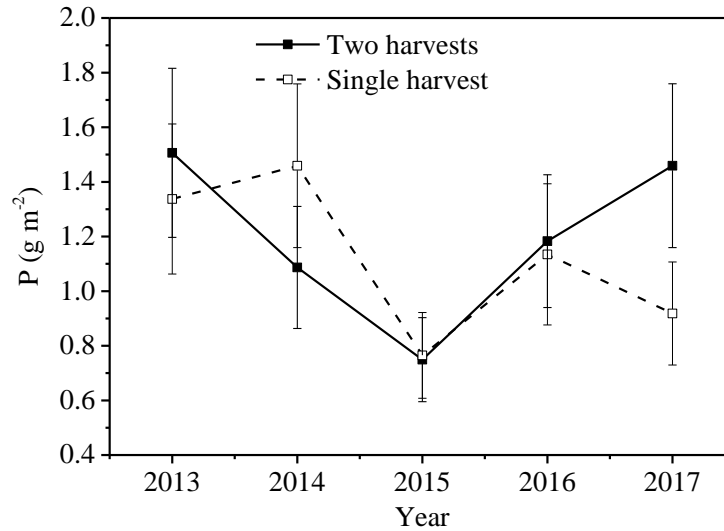


Figure 4.7 Cattail phosphorus accumulation as affected by harvest frequency and year of harvest. Error bars represent standard errors of the means.

Aboveground biomass harvesting frequency did not significantly ($P > 0.05$) affect belowground N and P accumulation in 2016 and 2017 (Fig. 4.4). Importantly, BGB accounted for approximately 50% of total N (Fig. 4.4; Fig. 4.6) and 73% of total P phytoextraction by the plant (Fig. 4.4; Fig. 4.7). Therefore, a greater fraction of P taken up by cattail remained belowground in the rhizomes and was not removed when the aboveground biomass was harvested. Although the P in the BGB was not removed with harvesting, it remains sequestered in the living BGB, thereby reducing its mobility and transport to surface waters.

4.4.4 Plant Available Nitrogen and Phosphorus

Available N concentration in the biosolids at the end of each growing season varied significantly ($P < 0.05$) with year (Table 4.3). Most (67- 93%) of the plant available N (PAN) was in the $\text{NH}_4\text{-N}$ form. Plant available N concentration at the end of the growing season decreased significantly from 56 mg kg^{-1} (81 kg ha^{-1}) in 2013 to $27\text{--}29 \text{ mg kg}^{-1}$ ($39\text{--}42 \text{ kg ha}^{-1}$) in 2015 and 2016 (Table 4.3). The decrease in PAN corresponded to a decrease in N phytoextraction from 7.5 g m^{-2} (75 kg ha^{-1}) in 2013 to $5.6\text{--}5.9 \text{ g m}^{-2}$ ($56\text{--}59 \text{ kg ha}^{-1}$) in 2015 and 2016 (Table 4.2). The

decrease in available N concentration with time may adversely affect cattail biomass yields in the long term. A previous laboratory incubation study using biosolids from the secondary cell showed that organic N mineralization rates in the biosolids were low (Jeke et al., 2015b). Therefore, the biosolids will likely provide inadequate available N to maintain high biomass yields in the long-term, with a concomitant reduction in the P phytoextraction effectiveness.

Olsen P concentration (93–101 mg kg⁻¹) in the biosolids at the end of each season did not vary significantly during the 5-yr study (Table 4.3). In addition to P phytoextraction by plants, Olsen P concentration is affected by dynamic processes such as mineralization, sorption/desorption, and dissolution of inorganic P fractions.

Table 4.3 Temporal variation in post-harvest available nitrogen and Olsen P concentrations in biosolids during the 5-year study.

Year	NO ₃ -N	NH ₄ -N	PAN†	Olsen P
	mg kg ⁻¹			
2013	11.8ab‡	44a	56a	101
2014	14.5a	28b	42b	98
2015	4.2bc	23b	27c	93
2016	1.3c	27b	29bc	98

†PAN, plant available N (sum of NO₃-N and NH₄-N)

‡Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

Snowmelt in the lagoon can result in nutrient leaching from the biosolids layer to the underlying clay layer. An analysis of a composite sample (from six subsamples) of the clay below the biosolids layer indicated low concentrations of NO₃-N (5.9 mg kg⁻¹), NH₄-N (15.5 mg kg⁻¹) and Olsen P (40.8 mg kg⁻¹). This suggests that NO₃-N leaching was minimal because of the dense clay layer which restricted water infiltration. The concern with nutrient transport was on the

potential nutrient loss to surrounding surface water if floodwater from snowmelt was released from the lagoon. A study investigating the effect of flooding biosolids in the lagoon showed that P concentration in the floodwater increased with flooding duration (Jeke and Zvomuya, 2018a), indicating a risk of P enrichment of receiving surface waters if the floodwater from snowmelt or periods of heavy rainfall was released from the lagoon.

Snowmelt can also play an important role in the mineralization of nutrients in the lagoon. The decomposition of organic matter in anaerobic flooded conditions is achieved by a relatively restricted anaerobe population that operates at a lower energy level and is less efficient than in aerated terrestrial systems with a wide range of aerobes (Buresh et al., 2008). Available N ($\text{NH}_4 + \text{NO}_3\text{-N}$) supply rate in biosolids in the primary cell of the municipal lagoon that were permanently flooded (~ 50 cm) showed that mineralization rates were lower (4.5 to $5.9 \mu\text{g cm}^{-2} \text{ wk}^{-1}$) throughout the growing season (June to September) (Jeke and Zvomuya, 2018b) compared to rates reported for agricultural field soils (200 to $>1100 \mu\text{g cm}^{-2} \text{ wk}^{-1}$) in Saskatchewan, Canada (Qian and Schoenau, 2005) and biosolids-amended soils (~ 30 - $120 \mu\text{g cm}^{-2} \text{ wk}^{-1}$) in Delhi, New York (Quaye et al. 2015). Therefore, N mineralization in our study may be low during spring snowmelt partly due to lower mineralizable N concentration in the biosolids (Jeke et. al 2015) and/or submerged conditions, which can reduce mineralization rates.

Initial TN ($5.6 \pm 0.4 \text{ t ha}^{-1}$) and TP ($2.5 \pm 0.4 \text{ t ha}^{-1}$) loading in the lagoon were calculated from nutrient concentrations in the biosolids (Table 1) and bulk density of the biosolids ($0.87 \pm 0.11 \text{ Mg m}^{-3}$). Bulk density was measured from sixteen biosolids cores (10-cm diam. by 20-cm height) to a depth of ~15-cm and adjusted to exclude roots by taking into account the volume and weight of the root biomass in each core. Mean annual annual N and P phytoextraction rates in the AGB of cattail ranged from 1.0-1.3% of TN concentration and 0.3-0.6% of TP concentration in

the biosolids at the start of the experiment, while BGB accumulated 0.9–1.3% of the TN and 0.9–1.6% of the TP. Cumulative N (32.5 g m^{-2} or 325 kg ha^{-1}) and P (5.9 g m^{-2} or 59 kg ha^{-1}) removal in harvested AGB over the 5 yr represented 5.8% of TN and 2.3% of TP concentration in the biosolids at the start of the experiment. Cattails have been widely used in wastewater treatment in wetlands and nutrient phytoextraction rates from harvesting cattail vary considerably depending on the nutrient load of the incoming wastewater. For example, Toet et al. (2005), Gottschall (2005) and Newman et al. (2000) reported annual phytoextraction rates of 0.7–11% and 4.5–9.2% for TN and TP, respectively, from harvesting cattails in wastewater treatment wetlands.

4.5 Conclusion

In situ phytoremediation of N and P by cattail in end-of-life lagoons is a viable option for N and P removal from biosolids. Annual N and P phytoextraction ranged from 58 to 77 kg N ha^{-1} and 8 to 14 P kg ha^{-1} P, representing annual phytoextraction rates of 1.0–1.3% for initial TN and 0.3–0.6% for TP. Two harvests per season significantly decreased biomass yield and N phytoextraction compared with a single harvest, whereas P phytoextraction was not significantly affected by harvesting frequency. Harvesting cattail over 5 yr removed 5.8% and 2.3% of initial TN and TP, respectively. In addition to P removal in the harvested aboveground biomass, a large fraction of P taken up by cattail was sequestered in the rhizomes (~75%), a phytoremediation mechanism that renders the nutrient unavailable for transport to surface water systems. Cattail requires relatively high moisture or ponded environments; therefore, further studies should explore P removal potential of other perennial plants, such as switchgrass, which are better adapted to low moisture conditions or other annual crops and forages which can grow in end-of-life municipal lagoons that are not permanently saturated.

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5. FLOODING DEPTH AND TIMING EFFECTS ON PHOSPHORUS RELEASE FROM FLOODED BIOSOLIDS IN AN END-OF-LIFE MUNICIPAL LAGOON

5.1 Abstract

Municipal biosolids in end-of-life lagoons can release phosphorus (P) to floodwater and contribute to P enrichment of receiving waters if the floodwater is released. Phosphorus release to floodwater is well-documented in agricultural and wetland soils, but information on flooding depth and timing effects on P release from flooded biosolids in end-of life municipal lagoons is currently lacking. This 42-d experiment utilized intact, cattail- (*Typha latifolia* L.) vegetated biosolids cores (45.7-cm diam. by 60-cm height) to investigate the effects of flooding depth (5, 15, and 25 cm) on P release from biosolids and on P fractionation in pore water, floodwater, and biosolids upon flooding of municipal biosolids. Averaged across flooding depths, TP rapidly increased from the onset of flooding (0.45 mg L^{-1}) to day 14 (1.8 mg L^{-1}) and remained relatively constant thereafter ($1.8 - 1.9 \text{ mg L}^{-1}$). Dissolved reactive P was the major fraction of P in pore water and floodwater. Flooding for more than 3 d resulted in the release of $> 0.5 \text{ mg L}^{-1}$ dissolved reactive P (DRP) to floodwater. Phosphorus release was positively correlated with Fe and Mn concentrations in pore water and with water extractable inorganic P, labile inorganic P and Fe/Al-bound organic P concentrations in biosolids. Results indicate that P release to floodwater, hence risk to receiving water bodies, is minimal during the first 3 d of flooding. This suggests that release of floodwater from the lagoon presents minimal adverse impact to receiving surface waters if done during the early stages ($< 3 \text{ d}$) of flooding.

5.2 Introduction

In situ phytoremediation of municipal biosolids offers great potential as a less expensive and sustainable alternative to landfilling and the spreading of biosolids on agricultural land during decommissioning of end-of-life municipal lagoons (Jeke et al., 2017). Studies have shown that flooding of soils can result in P release to surface floodwater (Amarawansa et al., 2015; De-Campos et al., 2012; Kröger et al., 2012). Therefore, it is conceivable that flooding of biosolids in end-of-life municipal lagoons could also stimulate the release of P to surface floodwater, thus posing a risk of P enrichment of the receiving surface waters if the floodwater is released. However, information on the effects of flooding depth and duration on P release from flooded biosolids in end-of-life municipal lagoons is currently lacking.

Numerous laboratory and field studies have examined P release in flooded or reduced [low oxidation-reduction (redox) potential, Eh] agricultural soils amended with manure (Ajmone-Marsan et al., 2006; Amarawansa et al., 2015; Pant et al., 2002) or biosolids (Elliott et al., 2005; Penn and Sims, 2002; Shober and Sims, 2009) and in soils from natural wetlands (Dunne et al., 2010; Kinsman-Costello et al., 2014) and agricultural lands converted to wetlands (Pant and Reddy, 2003; Smith and Jacinthe, 2014; Wong et al., 2011). In most of these and other studies (Hoffman et al., 2009; Kröger et al., 2012; Scalenghe et al., 2012), dissolved reactive P in floodwater increased with the onset of flooding or reducing conditions while others (Amer et al., 1991; Shober and Sims, 2009; Vadas and Sims, 1998) reported a decrease in dissolved P concentration with the onset of reducing conditions. Although numerous studies have examined the release of P from flooded or reduced soils, little is known about the effect of flooding on P release from municipal biosolids to the surface floodwater. This is partly because biosolids have largely been confined to dry sites, for example, where biosolids are applied as an agricultural soil amendment or soil conditioner in land reclamation projects.

In agricultural fields, P in biosolids has been reported to be less susceptible to runoff than other P sources, such as inorganic fertilizers and manures (Penn and Sims, 2002; Withers et al., 2001). Biosolids type and the interaction with soil influences the potential for P release to solution (Maguire et al., 2001; Penn and Sims, 2002). Sharpley et al. (1997) and Penn and Sims (2002) , reported decreasing runoff P concentration with time from soil amended with biosolids and poultry litter, a result which they attributed to P release being affected more by soil properties than soil amendment characteristics as the amendment equilibrated with soil. Recent studies have demonstrated the effectiveness of phytoremediation as an approach for removing N and P from municipal biosolids (Jeke et al., 2017). Jeke et al. (2017) reported that biosolids supported greater cattail biomass yield than biosolids mixed with soil during the phytoremediation of municipal biosolids in end-of-life lagoons. Flooding from heavy snowfalls or heavy rainfall episodes may result in flooding of a lagoon during phytoremediation. This underlies the importance of characterizing P release from biosolids under different scenarios of floodwater depth and duration of flooding in the absence of the physico-chemical influence of soil.

Many of the previous laboratory and simulated runoff studies on the effect of flooding or reducing conditions on biosolids-amended soils may not accurately reflect P dynamics in biosolids flooded under natural field conditions. For example, P released from shaking soil suspensions (Pant et al., 2002; Shober and Sims, 2009) does not reflect the oxidized-water interface, which is typical of field condition and has a significant impact on P dynamics under flooded conditions. In runoff simulation studies (Elliott et al., 2005; Penn and Sims, 2002; Withers et al., 2001), the continuous flow systems do not reflect static conditions of flooded soils; additionally, such setups may not produce reduced conditions in soils and may result in the removal of products of reducing

conditions, such as dissolved P, dissolved Fe, and microbial metabolites from the system (Scalenghe et al., 2014).

Changes in Eh and pH associated with flooding play an important role in controlling P sorption and release in submerged soils. Reductive dissolution of Fe and Mn phosphates influences P release in acidic to neutral soils (Kröger et al., 2012; Scalenghe et al., 2012) and in alkaline soils (Amarawansa et al., 2015; Scalenghe et al., 2012). Different flooding depths may influence changes in Eh, which can significantly influence P dynamics in the sediment and surface floodwater. Hydrologic regimes in wetlands, such as dry, moist, or flooded sediments, influence P forms, transformations, and release into solution (Aldous et al., 2005; Dunne et al., 2006; Reddy and DeLaune, 2008). Lui et al. (2012) reported that higher flooding depth could result in TP accumulation in surface soil in a river delta marsh soil, but the relationship between water level and TP concentration in the marsh soil were unclear. Tong et al. (2010) reported significant positive correlations between TP concentration in sediment soil and water levels in a river estuary. Although Tong et al. (2010) and Liu et al. (2012) did not measure P concentrations in the water column, water depth may have influenced P concentration in the surface floodwater. Flooded biosolids in vegetated lagoons may temporarily behave like wetland soils if saturated for a prolonged period. The depth and duration of flooding of biosolids in lagoons may influence P dynamics and, therefore, warrant investigation.

Lagoons in rural Canadian communities often release wastewater effluent into small streams once or twice annually during operation (National Research Council, 2004). End-of-life municipal lagoons undergoing terrestrial phytoremediation may require the release of floodwater following heavy rainfall or snowmelt. Furthermore, the conversion of end-of-life municipal lagoons to wetlands, hence flooding of the lagoons to facilitate wetland-based phytoremediation (Jeke et al.,

2017), and the use of biosolids under flooded conditions during mine tailing remediation (Jia et al., 2015) have been proposed. Such flooding could stimulate dissolution of P and increase P concentration in the surface floodwater. Therefore, land managers need to understand the dynamics of P in flooded biosolids and the timing of P release.

The objectives of this study were to (i) determine the effect of flooding depth and duration of flooding on the release of P from vegetated biosolids to pore water and surface floodwater, (ii) examine P fractionation in surface floodwater under different flooding depths and durations of flooding, and (iii) examine the relationship between concentrations of dissolved Ca, Mg, Fe, and Mn and P concentrations in pore water and floodwater.

5.3 Materials and Methods

5.3.1 Study Site

The study was conducted in the secondary cell of an end-of-life municipal lagoon system in Niverville, Manitoba, Canada (49°35'42.7"N, 97°02'50.3"W), using intact biosolids cores that were extracted from the cell. The lagoon had operated for 37 yr (1971–2008), treating domestic wastewater by natural microbial degradation without any pretreatment. During its operation, the secondary cell (8.8 ha) received effluent from the primary cell (4.6 ha) for storage and further microbial treatment. The lagoon has no record of any desludging during its life-span. During the present study, the secondary cell was undergoing terrestrial phytoremediation using cattail (*Typha latifolia* L.) (Jeke et al., 2016). Inundation occurs periodically in the secondary cell of the lagoon system and flooding depths ranging from a few centimeters to about 30 cm have been observed during spring snow melt and following heavy summer rains.

5.3.2 Experimental Setup

Intact, cattail-vegetated biosolids cores were extracted in September 2016 by pushing polyvinyl chloride pipes (45.7-cm diam. by 60-cm ht.) with a backhoe into the biosolids (0-20 cm) and clay (20-30 cm) layers in the secondary cell (Fig. 5.1). Three cores were extracted from each of four randomly selected blocks in the secondary cell, which was undergoing phytoremediation using cattail. The ~20-cm thick biosolids layer in the cell was underlain by a compacted layer of clay, which served as a lining for the lagoon to prevent leaching. The bottom of each core unit was sealed by gluing polythene sheets to the PVC pipe to prevent water leakage. The cores were placed near the edge of the lagoon cell, where they remained for the duration of the experiment. Borehole water was added to the three core assemblies from each block to attain flooding depths of 5, 15, and 25 cm above the biosolids surface. The experimental units (core assemblies) were laid out in a randomized complete block design with the three flooding depths randomized within each of the four blocks.

The borehole water used to flood the core units contained 0.03 mg L⁻¹ dissolved reactive P (DRP), 0.09 mg L⁻¹ dissolved total P (DTP), and 0.17 mg L⁻¹ total P. Total Ca, Mg, Fe, and Mn concentrations in the borehole water were 24.1, 11.6, 0.04 and < 0.01 mg L⁻¹, respectively. At 21 d after the start of flooding, borehole water was added to the cores soon after sampling to replace water lost via evapotranspiration and sampling. The water replenishment was a desirable artifact because it mimicked the addition of precipitation to floodwater that occurs under natural conditions during summer months.

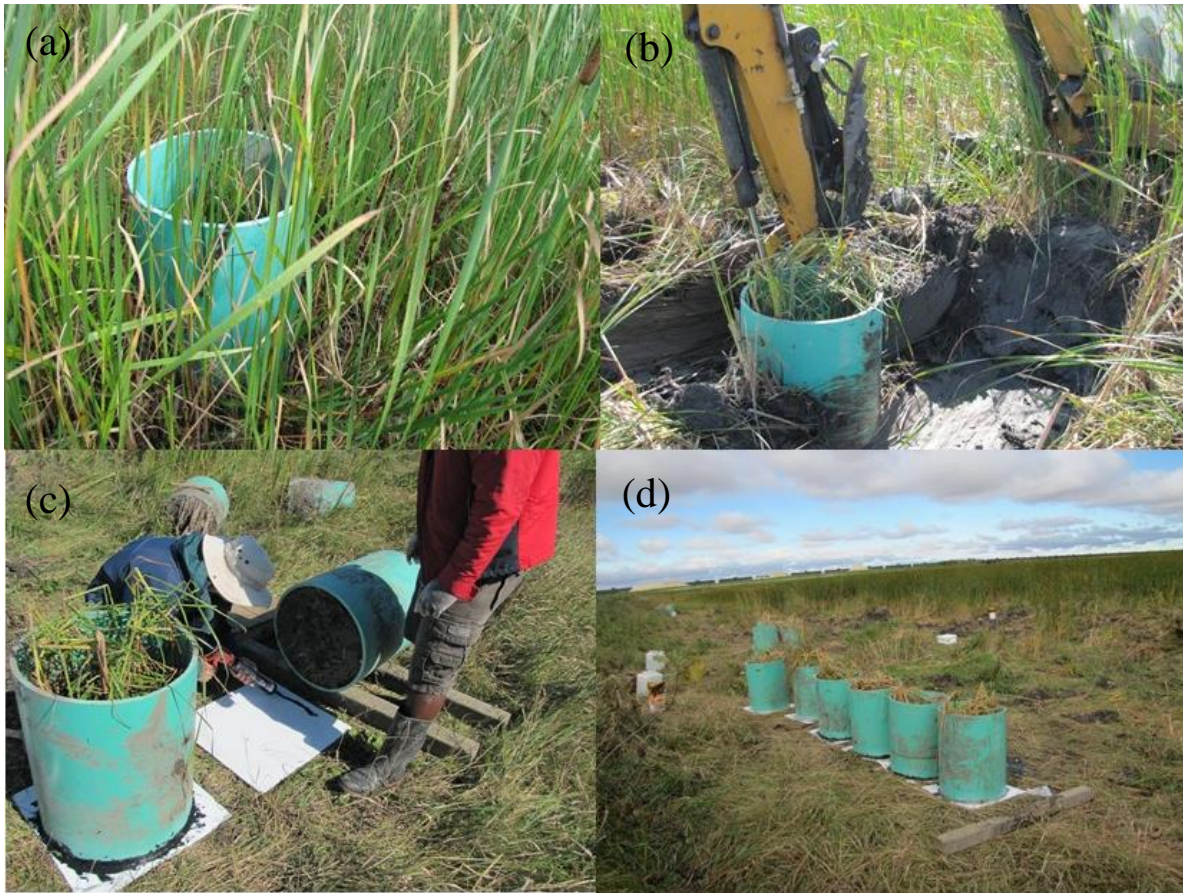


Figure 5.1 (a) 45.7-cm diam. by 60-cm ht. polyvinyl chloride (PVC) pipe; (b) extraction of a core using a backhoe; (c); gluing a polythene sheet to an extracted core; (d) cores placed near the edge of the lagoon cell

5.3.3 Sampling and Laboratory Analysis

Biosolids samples were collected from each core before the start of flooding. Subsamples (0.5 g biosolids) were digested in aqua regia (concentrated HNO_3/HCl) for 2 h at 90 °C in a microprocessor-controlled digestion block for determination of initial TP, Fe, Mn, Ca, and Mg concentrations. Total P concentration in the digest was measured using a Varian 735 ES inductively coupled plasma mass spectrometer (ICP-MS) (Varian Inc., Palo Alto, CA) while metal concentrations were measured with a Perkin Elmer SCIEX ELAN 6000 ICP-MS (Perkin-Elmer SCIEX Instruments, Concord, Ontario).

Phosphorus fractions in the biosolids were determined according to the modified Headley fractionation procedure (Ajiboye et al., 2004). Briefly, a 0.5 g (oven-dry basis) biosolids sample was sequentially extracted with 30 mL of each extraction solution. The extractants were distilled water for water extractable inorganic P, 0.5 M NaHCO₃ for moderately labile P, 0.1 M NaOH for Fe/Al-bound P, and 1 M HCl for Ca/Mg-bound inorganic P (Pi). Inorganic P concentration in extracts was measured colorimetrically using the molybdate-blue method (Murphy and Riley, 1962) while TP was measured by ICP-MS (Varian Inc., Palo Alto, CA). Biosolids pH and EC were measured in a 1:2 biosolids:distilled water suspension using a Fisher Accumet AB15 pH meter and an AB30 conductivity meter (Fisher Scientific Ltd, Ottawa, Canada), respectively.

Water samples were collected on days 0, 1, 2, 7, 14, 28, 35, and 42 after flooding. Surface floodwater samples (100 ml) were collected using a syringe from the center of each core unit. Pore water samples (50 ml) were collected using 2.5 mm diam. Rhizon Flex solution samplers (Rhizosphere Research Products, Wageningen, The Netherlands) that had been vertically installed to a depth of 10 cm in the biosolids layer before flooding. Redox potential was determined at the same depth using a platinum electrode referenced to a Ag/AgCl electrode. The measured Eh values were corrected to the standard hydrogen electrode by adding 200 mV (Schlesinger and Bernhardt, 2013). Biosolids samples were collected on each sampling day and sequentially extracted as previously described above.

Pore water and surface floodwater samples (50 ml) were filtered through 0.45 µm membrane filters and analyzed for DRP concentration within 24 h of sampling using the molybdate blue method (Murphy and Riley, 1962). Dissolved TP, Ca, Mg, Fe, and Mn concentrations were measured in the filtrates by ICP-MS. Dissolved organic P was estimated from the difference between DTP and DRP. Total P, Ca, Mg, Al, Fe, and Mn concentrations in the surface floodwater

were measured by inductively coupled plasma atomic-emission spectroscopy following persulfate digestion of unfiltered subsamples (Pierzynski, 2000). Particulate P fraction in the floodwater was estimated from the difference between TP and DTP concentrations.

5.3.4 Statistical Analysis

Data for DRP, DTP, TP, Ca, Mg, Fe, and Mn concentrations in water samples were analyzed using the GLIMMIX procedure for repeated measures in SAS 9.4 (SAS Institute, 2015). Flooding depth and duration (days after flooding) were modelled as fixed effects while block and the block \times flooding depth interaction were analyzed as random effects. The spatial power covariance structure was selected based on the Akaike Information Criterion to model the repeated effect (flooding duration). Treatment means were compared using the Tukey multiple comparison procedure. Treatment differences were considered significant at $\alpha = 0.05$. Correlations between DRP concentration and concentrations of dissolved Fe, Mn, Ca, and Mg were tested using PROC CORR in SAS 9.4.

5.4 Results and Discussion

5.4.1 Biosolids Properties

Most (83%) of the TP in the biosolids was in the inorganic fraction (Table 5.1), with only 17% in the organic form. The low organic P fraction is a result of the mineralization of organic P during wastewater treatment and the capture of orthophosphate by the biosolids phase (Hedley and McLaughlin, 2005). Most of the inorganic P (62% of TP) in the biosolids was recovered in the HCl fraction, with a smaller fraction recovered in the NaOH (12 %), NaHCO₃ (10%) and H₂O (0.5%) fractions (Table 5.1). The lower H₂O- + NaHCO₃-extractable P fractions (11%) measured in this study are consistent with those reported in other studies (Ajiboye et al., 2004; Ajiboye et al., 2007; Withers et al., 2001).

Inorganic P concentration measured in the HCl fraction (954 mg kg^{-1}) by the molybdate-blue method was slightly greater than TP in the HCl fraction measured by ICP (943 mg kg^{-1}). This anomaly may have been due to sample heterogeneity, overestimation of inorganic P due to hydrolysis of some organic P during HCl extraction (Turner and Leytem, 2004), or underestimation of TP in the HCl extract by ICP caused by reprecipitation of orthophosphate as calcium phosphate (Ajiboye et al., 2007). Inclusion of direct organic P measurements by enzyme hydrolysis in the Headley fractionation method (He et al., 2010) instead of the commonly used approach of estimating organic P from the difference between TP and inorganic P concentration may provide a more accurate quantification of organic P in biosolids.

The biosolids had an unusually high Fe concentration (22 g kg^{-1}) for biosolids produced without the addition of Fe salts. This suggests a source of Fe to the municipal lagoon during its operation, probably from rusting pipes and/or sediments washed into drains. Withers et al. (2001) also reported high Fe concentration (10.6 g kg^{-1}) in biosolids that were not treated with Fe; they attributed this to an industrial source or drinking water treatment plant Fe residuals added to wastewater.

5.4.2 Biosolids Redox Potential and pH

Redox potential was significantly ($P = 0.01$) affected by flooding duration regardless of flooding depth (Table 5.2; Fig. 5.2a). The Eh increased from 125 mV at the start of flooding to 172 mV on day 21 and then decreased thereafter to 128 mV by the end of the flooding period. This result contradicts previous studies which showed a rapid decrease in Eh following flooding (Amarawansa et al., 2015; De-Campos et al., 2012). However, other studies have shown that flooding does not always produce intense anaerobiosis (Dunne et al., 2006; Vadas and Sims, 1998), particularly in soils with low organic C and low microbial activity. After 60 d of incubation, Dunne

at al. (2006) reported Eh values of 114 mV in soils flooded with a 25-cm column of water, 132 mV in surface saturated soil, and 152 mV in soil with water drawn 5 cm below the soil surface. They concluded that the soils had low reduction intensity. The range of Eh (+100 to +180 mV) in our study is within the range of moderately reduced (100 to 300 mV) flooded soils, where facultative reducing microbes are active (Reddy and DeLaune, 2008). Biosolids pH ranged between 7.6 and 7.7 on the first day of flooding and fluctuated by 0.2 pH units, except on day 14 at the 25-cm flooding depth when a greater decrease was observed (Fig. 5.2b).

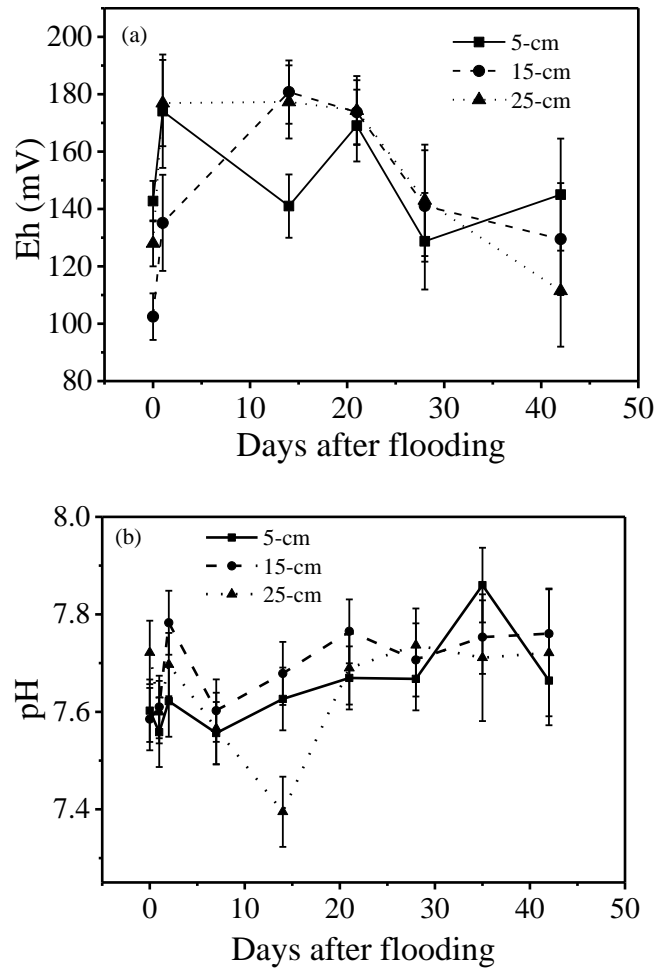


Figure 5.2 Flooding depth (5, 15, and 25 cm) effects on temporal changes in biosolids (a) redox potential (Eh) and (b) pH during 42 d of flooding.

Table 5.1 Initial phosphorus and metal concentrations in the biosolids.

TP	H ₂ O-P _i †	NaHCO ₃ -P _i	NaOH-P _i	HCl-P _i	H ₂ O-P _o	NaHCO ₃ -P _o	NaOH-P _o	HCl-P _o	Fe	Mn	Ca	Mg
mg kg ⁻¹					g kg ⁻¹							
1500	6.6	139	128	974	1.1	15	59	-31	22	0.5	54	22

†H₂O-P_i and H₂O-P_o are water-extractable inorganic and organic phosphorus, NaHCO₃-P_i and NaHCO₃-P_o are labile inorganic and organic phosphorus, NaOH-P_i and NaOH-P_o are Fe/Al-bound inorganic and organic phosphorus; and HCl-P_i and HCl-P_o is Ca/Mg-bound inorganic and organic phosphorus.

Table 5.2 Changes in concentrations of dissolved reactive phosphorus (DRP), dissolved total phosphorus (DTP), particulate phosphorus (PP), and total phosphorus (TP), dissolved Fe, Mn, Ca, Mg, and in biosolids redox potential (Eh) and pH during flooding.

	Pore water						Floodwater									
	DRP	DTP	Fe	Mn	Ca	Mg	DRP	DTP	PP	TP	Fe	Mn	Ca	Mg	Eh	pH
	mg L ⁻¹						mV									
Flooding depth																
5 cm	0.55	0.45	0.03	0.30	167	114	0.77	0.74	0.29	1.24	0.01	0.02	93a‡	52a	147	7.6
15 cm	0.49	0.44	0.02	0.38	159	121	0.74	0.72	0.26	1.16	0.01	0.01	79ab	42ab	150	7.7
25 cm	1.54	0.7	0.12	0.52	131	75	0.68	0.72	0.21	1.75	0.03	0.06	65b	31b	156	7.6
	P-value															
Flooding depth (D)	0.2	0.54	0.08	0.53	0.43	0.27	0.97	0.99	0.43	0.61	0.2	0.21	0.03	0.01	0.72	0.1
Time (T)†	0.02	<0.001	0.07	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	0.14	0.07	<0.001	<0.001	0.01	0.02
D × T	0.01	0.26	0.12	0.16	0.99	0.94	0.04	0.04	0.14	0.66	0.49	0.99	0.46	0.37	0.17	0.58

†Time effects are presented in figures. ‡Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) according to the Tukey multiple comparison procedure.

5.4.3 Dissolved Reactive P Concentration

The effect of flooding depth on DRP concentration in biosolids pore water varied with flooding duration, as indicated by the significant ($P = 0.01$) flooding depth \times time interaction (Table 5.2). Pore water DRP concentrations at the 25-cm flooding depth on days 14 (2.7 mg L^{-1}) and 21 (2.6 mg L^{-1}) were five- to six-fold greater than pore water DRP concentrations on these days at the 5- and 15-cm flooding depths, but depth differences at other sampling dates were not significant (Fig. 5.3). Over the 42-d flooding period, pore water DRP concentration increased only slightly ($\sim 0.1 \text{ mg L}^{-1}$) at the 15-cm flooding depth while pore water DRP concentration at the 5-cm depth increased twofold from 0.4 mg L^{-1} at the start of flooding to 0.8 mg L^{-1} by the end of the experiment. In comparison, pore water DRP concentration at the 25-cm flooding depth increased to a peak of 2.7 mg L^{-1} on day 14 and decreased thereafter to 1.8 mg L^{-1} by the end of the flooding period. The results indicated greater pore water DRP concentrations at the higher flooding depth than at the lower flooding depths.

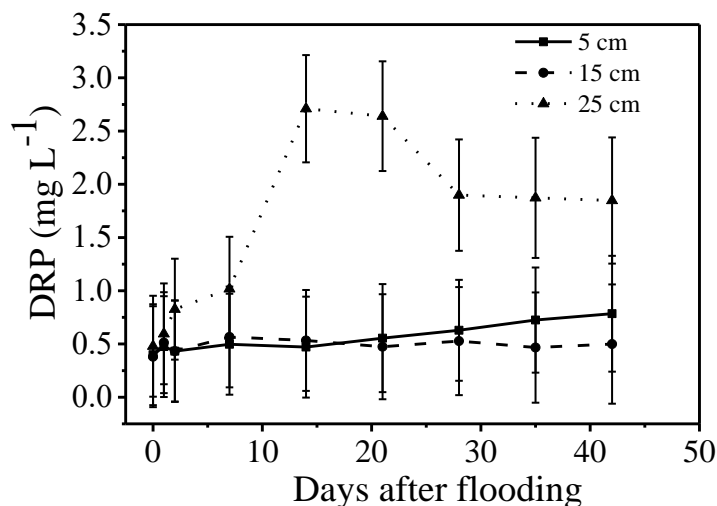


Figure 5.3 Flooding depth (5, 15, and 25 cm) effects on temporal changes in biosolids pore water dissolved reactive phosphorus (DRP) concentration during 42 d of flooding.

There was a significant flooding depth \times time ($P = 0.04$) interaction for DRP concentration in the surface floodwater (Table 5.2). However, multiple comparisons of floodwater DRP concentrations among the three flooding depths on each sampling day showed no significant differences. The significant interaction was a result of differences in temporal changes in DRP among flooding depths, comparisons which were not of interest. Generally, surface floodwater DRP concentration increased at all flooding depths, peaking at 1.3, 1.4, and 1.6 mg L⁻¹ at the 5-, 15-, and 25-cm flooding depths, respectively, on Day 21, and decreased thereafter (Fig. 5.4). The decrease in DRP concentration in soil solution has been reported by others, who attributed this to resorption of released P (Amarawansa et al., 2015; Chacon et al., 2006; Shober and Sims, 2009). Pore water and floodwater DRP concentrations were highly positively correlated at the 5-cm ($r = 0.84$, $P < 0.0001$), 15-cm ($r = 0.59$, $P < 0.0001$), and 25-cm ($r = 0.95$, $P < 0.0001$) flooding depths, indicating effective diffusion of DRP from pore water to the floodwater. High positive correlation between pore water and surface floodwater DRP concentrations has been reported to indicate efficient diffusion of P from pore water to floodwater (Amarawansa et al., 2015), whereas poor correlation indicates low or no mobilization of P to floodwater (Amarawansa et al., 2015; Young and Ross, 2001), likely because of adsorption and reprecipitation of P in the oxidized zone at the soil-floodwater interface (Young and Ross, 2001).

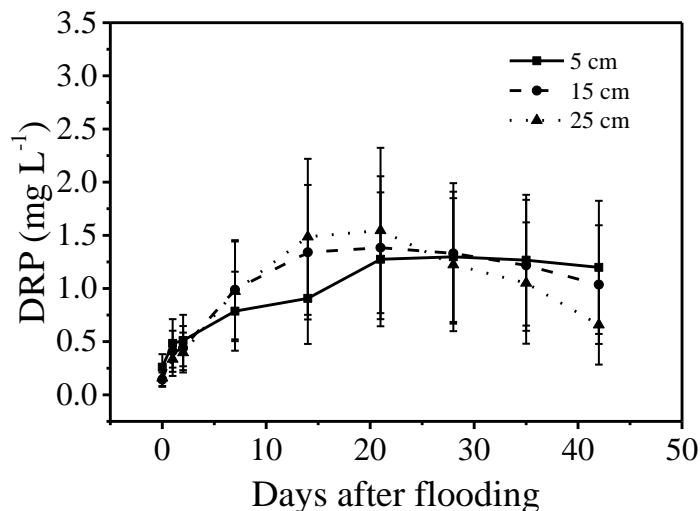


Figure 5.4 Flooding depth (5, 15, and 25 cm) effects on temporal changes in dissolved reactive P (DRP) concentrations in surface floodwater during 42 d of biosolids flooding.

5.4.4 Iron and Manganese Concentrations

Dissolved Fe concentration in pore water at the 5- and 15-cm flooding depths was below the detection limit ($< 0.01 \text{ mg L}^{-1}$) during the first 14 d of flooding but became detectable at low concentrations (0.01 mg L^{-1}) in samples taken on day 21 and thereafter (Table 5.2; Fig. 5.5). The critical Eh for Fe (III) reduction is between 300 and 100 mV at pH 7 and -100 mV at pH 8 (Gotoh and Patrick, 1974; Patrick and Henderson, 1981). Therefore, at the pH (7.6-7.9) and Eh (100 to 180 mV) ranges in this study, very low dissolved Fe concentrations are expected because of the low capacity of iron oxides to undergo reductive dissolution. Dissolved Fe concentration was not detected during the first 14 d at the 5- and 15-cm flooding depth, probably because Eh was increasing (from 125 to 172 mV) (Fig. 5.2); thereafter, very low Fe concentrations ($\sim 0.01 \text{ mg L}^{-1}$) were detected, which was likely a result of the decreasing Eh (from 172 to 128 mV). In contrast, Fe was detected earlier (on day 2) in pore water under the 25-cm flooding depth, but concentrations were generally low ($0.03 \text{ to } 0.3 \text{ mg L}^{-1}$) (Fig. 5.5). It is not clear why dissolved Fe concentration was detected earlier and at greater concentrations at the 25-cm flooding depth compared to

concentrations observed at the 5- and 15-cm flooding depth, despite similar Eh and pH range. Dissolved Mn concentration in the pore water generally increased from the onset of flooding at all flooding depths, with mean concentrations ranging from 0.04 to 2.6 mg L⁻¹. Manganese concentrations in pore water were greater than Fe concentrations at all flooding depths across the entire flooding period (Fig. 5.5). This occurs as the manganese threshold (300 to 100 mV) is above that of iron. Dissolved Fe and Mn concentrations in floodwater were very low (< 0.1 mg L⁻¹) at all flooding depths (Fig. 5.6).

Table 5.3 Pearson correlation coefficients between pore water dissolved reactive P (DRP) and pore water dissolved Fe, Mn, Ca and Mg, and between floodwater DRP concentrations and pore water dissolved Fe, Mn, Ca and Mg. (n = 30).

	Pore water DRP				Floodwater DRP			
	Fe	Mn	Ca	Mg	Fe	Mn	Ca	Mg
Flooding depth								
5 cm	0.47*	0.38*	0.07ns	0.01ns	0.58**	0.50*	0.43*	0.37ns
15 cm	-0.19ns†	0.40*	0.06ns	0.01ns	0.21ns	0.67***	0.25ns	0.34ns
25 cm	0.86***	0.85***	0.07ns	0.09ns	0.86***	0.93***	0.13ns	0.10ns

†ns, not significant at 0.05 probability level

*, **, and ***, significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Highly significant positive correlations ($P < 0.0001$) were observed between pore water concentrations of DRP and dissolved Fe ($r = 0.86$) and, similarly, between pore water concentrations of DRP and dissolved Mn ($r = 0.85$) under the 25-cm flooding depth (Table 5.3). The positive correlations were likely due to reductive dissolution of ferric and manganese oxides, which released soluble Fe, Mn, and bound P (Aldous et al., 2005; Scalenghe et al., 2012; Shahandeh et al., 2003). Correlations between DRP concentration and concentrations of Fe and Mn in pore water were weaker under the 5- and 15-cm flooding depths (Table 5.3).

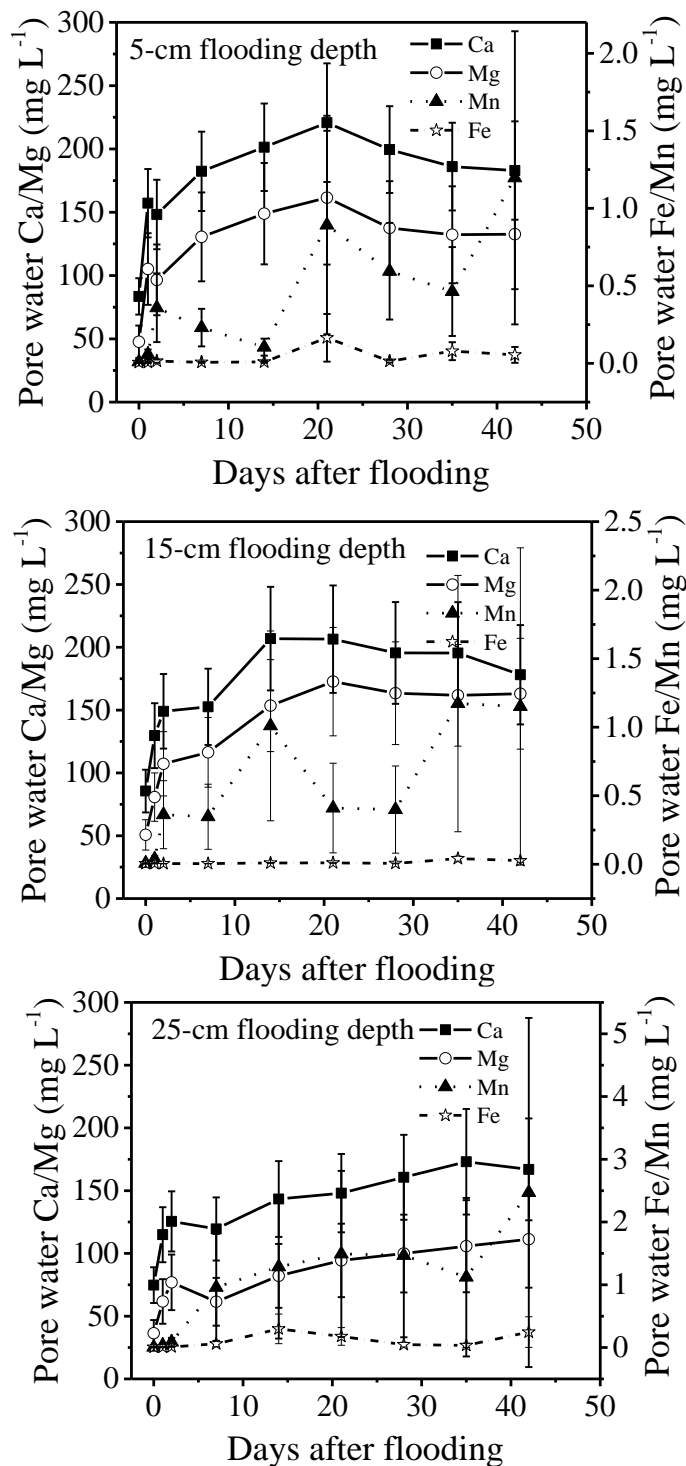


Figure 5.5 Flooding depth (5, 15, and 25 cm) effects on temporal changes in dissolved Fe, Mn, Ca, and Mg concentrations in biosolids pore water during 42 d of biosolids flooding. Error bars represent standard errors of the means.

5.4.5 Calcium and Magnesium Concentrations

Concentrations of Ca and Mg in pore water increased rapidly during the first day following the onset of flooding but changed little ($P < 0.05$) after day 1 (Table 5.2; Fig. 5.5). The initial increase in pore water Ca and Mg concentrations might have been a result of dissolution of Ca and Mg compounds and the release of readily exchangeable Ca and Mg following the onset of flooding.

There were no significant correlations between DRP concentration in pore water or floodwater and pore water concentrations of Ca and Mg, except under the 5-cm flooding depth when floodwater DRP was significantly and positively correlated with pore water Ca concentration ($r = 0.43$, $P < 0.05$; Table 5.3). These results suggest that the release of dissolved P in pore water and floodwater was not dependent on pore water Ca and Mg dissolution. Concentrations of Ca and Mg were lower in surface floodwater than in pore water. Surface floodwater Ca and Mg concentrations increased significantly ($P < 0.005$) during the first 7 d of flooding under the 5-cm flooding depth and during the first 28 d under the 15- and 25-cm flooding depths, with the concentrations changing insignificantly thereafter (Fig. 5.6).

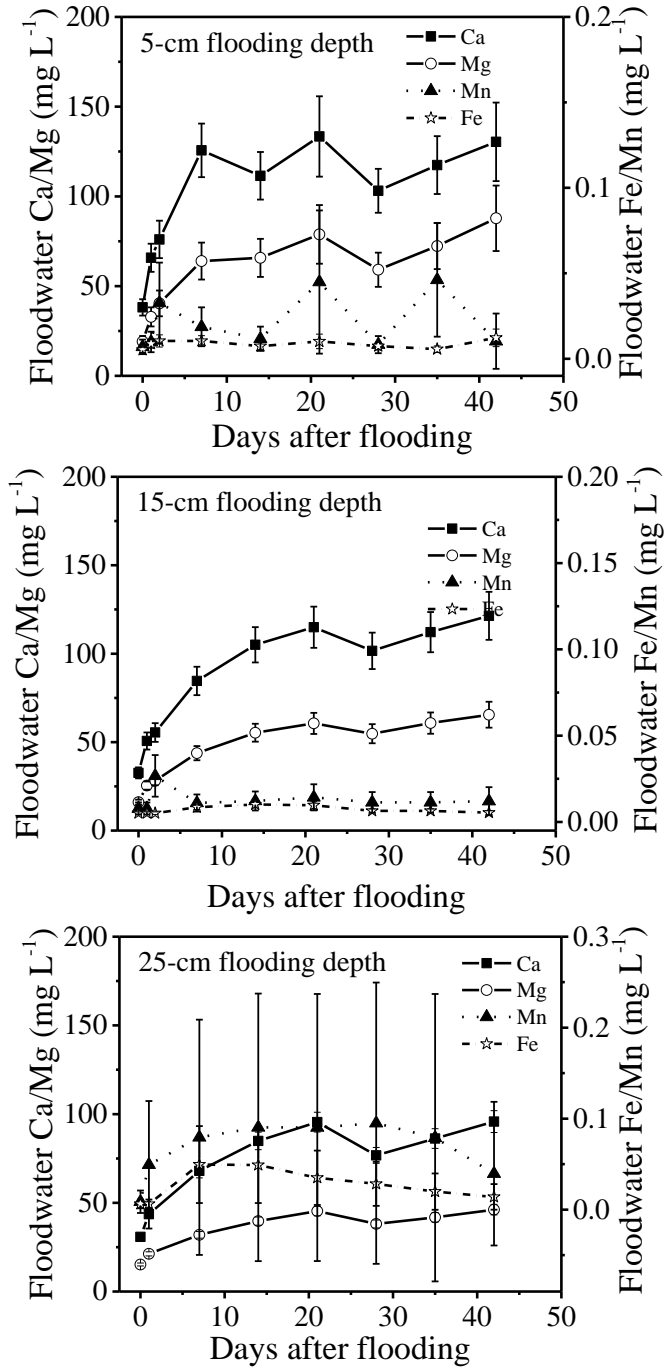


Figure 5.6 Flooding depth (5, 15, and 25 cm) effects on temporal changes in dissolved Fe, Mn, Ca, and Mg concentrations in surface floodwater during 42 d of biosolids flooding. Error bars represent standard errors of the means.

5.5.6 Total Phosphorus Concentration in Floodwater

Total P concentration in the surface floodwater varied significantly ($P < 0.001$) with flooding duration but did not differ significantly ($P = 0.66$) among flooding depths (Table 5.2). Averaged across flooding depths, TP increased rapidly from the onset of flooding (0.45 mg L^{-1}) to day 14 (1.8 mg L^{-1}) and remained relatively constant ($1.8\text{--}1.9 \text{ mg L}^{-1}$) thereafter. Manitoba Water Quality Guidelines require that TP concentrations in wastewater effluent discharged into streams should not exceed 1 mg L^{-1} TP (Manitoba Water Stewardship, 2011). Averaged across flooding depths, surface floodwater concentrations exceeding 1 mg L^{-1} were observed with flooding durations greater than 5 d. Total P concentrations in floodwater samples collected from drainage ditches near ($< 0.5 \text{ km}$) the lagoon during three flooding events in June and July ranged from 0.49 to 0.75 mg L^{-1} , with a mean of 0.63 mg L^{-1} . The floodwater in the ditches drained from soybean and canola fields. Although not statistically different, TP concentrations were greater under the 25-cm flooding depth from day 3 onwards compared to TP concentrations under the 5 and 15-cm flooding depths (Fig. 5.7).

Our results suggest that releasing floodwater from the lagoon will negatively impact receiving surface water quality, depending on the flooding duration preceding the release. Reducing the residence time of floodwater, hence minimizing the duration of floodwater-biosolids contact, may help reduce P loss to receiving waters upon release of the floodwater from the lagoon.

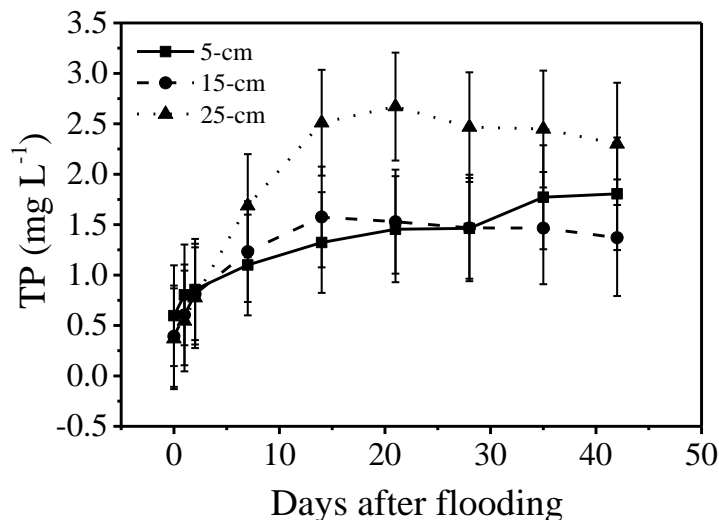


Figure 5.7 Flooding depth (5, 15 and 25 cm) effects on temporal changes in total phosphorus (TP) concentration in floodwater during 42 d of biosolids flooding. Error bars represent standard errors of the means.

5.5.7 Phosphorus Fractions in Floodwater

Averaged across flooding depths, the percentage of floodwater TP that was in the DRP form increased with flooding duration from 39% on day 0 to 83% on day 28, followed by a decrease to 67% on day 42 (Fig. 5.8). The DRP fraction did not differ significantly ($P = 0.99$) among flooding depths across the entire flooding period. Overall, the results indicated that most of the TP in the surface floodwater was in the orthophosphate form, which is most readily available for biological uptake.

While the proportion of TP that was in the DTP form did not differ significantly among flooding depths ($P = 0.98$), it was significantly affected by flooding duration ($P = 0.01$). Dissolved TP fraction followed the same increasing temporal trend as observed for the DRP fraction during the first 28 d of flooding. The percentage of floodwater TP that was in the DTP form increased with flooding duration from 29% on day 0 to 79% on day 28; however, unlike DRP fraction, which decreased thereafter, DTP fraction increased to 90% on day 42 (Fig. 5.8). The increase in DTP

fraction coincided with a decrease in the DRP fraction, which suggests an increase in dissolved organic P (DOP). Dissolved TP is made of inorganic and organic components. More than 90% of DTP from day 0 to day 35 was in the DRP fraction, indicating that DOP calculated as the difference between DTP and DRP concentrations was very low. Dissolved organic P was less than 3% of TP from day 0 to 35 and increased to about 16% of TP on day 42. Smith and Jacinthe (2014) reported that the contribution of DOP to DTP in floodwater from wetlands constructed on former agricultural lands increased with flooding duration, from 53% on day 2 to 92% on day 36. The low DOP concentrations in our study were likely due to low organic P concentration in biosolids as most of the P was in the inorganic form (Table 5.1).

The proportion of TP that was in the particulate form (PP, the fraction of P that is associated with particulate, colloidal, inorganic and organic material that can be captured by a 0.45 μm filter) varied significantly with flooding duration ($P < 0.001$). Particulate P was the major fraction of TP in the surface floodwater on day 0 (71%) and rapidly decreased during the first 14 days followed by a gradual decrease to 10% of TP by day 42. The decrease in the PP fraction was likely a result of settling of particulates with increasing flooding duration. The high percentage of the PP fraction on day 0 (71% of TP) is consistent with previous studies that showed a greater percentage of the PP fraction relative to dissolved P in runoff from biosolids-amended soils following heavy rains (Millier and Hooda, 2011; Withers et al., 2001). In a closed system, such as in the present study, PP settles with time, with the result that DRP was the major P fraction in the floodwater. Our results are in contrast with P inputs (to surface waters) from agricultural runoff, where a major fraction of the P is PP, which is less bioavailable than DRP and is more likely to settle in ditches or channel beds. Overall, the P fraction results indicate that if floodwater were released

immediately after flooding, P release to receiving waters would be low, with PP being the major P fraction, whereas prolonged flooding would increase the DRP fraction, which is bioavailable.

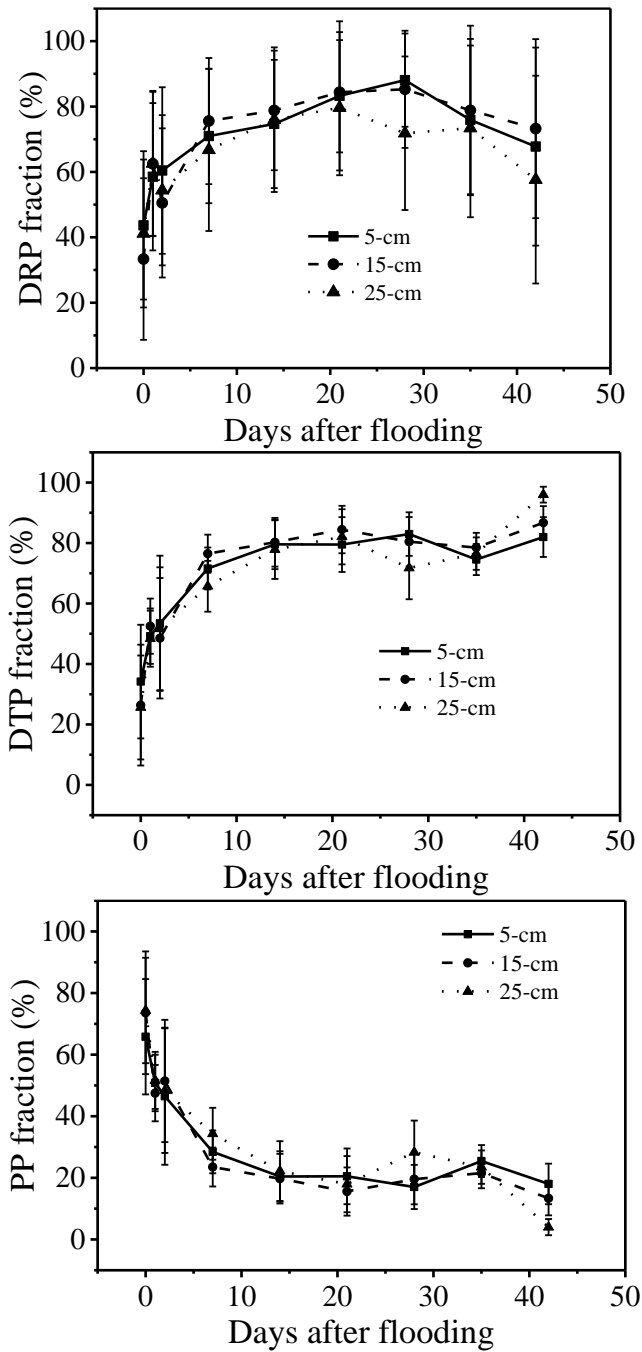


Figure 5.8 Flooding depth (5, 15 and 25 cm) effects on temporal changes in dissolved reactive phosphorous (DRP), dissolved total phosphorus (DTP), and particulate P (PP) fractions (% of total P) during 42 d of biosolids flooding. Error bars represent standard errors of the means.

5.4.8 Relationships Between Biosolids Phosphorus Fractions and Phosphorus Release to Pore Water and Floodwater

Averaged across flooding depths, water-extractable inorganic P (WEP P_i) ($P < 0.0001$), labile organic P (P_o) ($P < 0.0001$), and Fe/Al-bound P_o ($P < 0.02$) fractions varied significantly with flooding duration, but no particular trends were observed (Table 5.4). There were highly positive correlations between pore water DRP concentration and concentrations of WEP P_i ($r = 0.8$, $P < 0.0001$), labile P_i ($r = 0.64$, $p < 0.001$), and Fe/Al-bound P_o ($r = 0.64$, $P < 0.001$) under the 25-cm flooding depth (Table 5.5). The positive correlation between pore water DRP concentration and biosolids WEP and labile P concentrations may explain the greater concentration of DRP in pore water under the 25-cm flooding depth than under the 15- and 25-cm flooding depths since WEP and labile P would be flushed easily from biosolids with flooding. Except for biosolids under the 25-cm flooding depth, P fractions in biosolids were not significantly correlated with DRP concentration in floodwater (data not shown). Floodwater DRP was strongly and positively correlated with WEP P_i ($r = 0.68$, $P < 0.001$), labile P_i ($r = 0.68$, $P < 0.001$), and Fe/Al-bound P_o ($r = 0.71$, $P < 0.0001$) under the 25-cm flooding depth. These results suggest that WEP P_i , labile P_i , and Fe/Al bound P_o were the main contributors to P release in the biosolids and the main sources of P released to the floodwater.

Table 5.4 Changes in concentrations of P fractions in biosolids during flooding.

	H ₂ O-P _i ^a	NaHCO ₃ -P _i	NaOH-P _i	HCl-P _i	H ₂ O-P _o	NaHCO ₃ -P _o	NaOH-P _o	HCl-P _o
	mg kg ⁻¹							
Flooding depth								
5 cm	11.7	145	110	823	1.17	12.5	58	41
15 cm	10.7	136	96	846	1.22	11.9	54	-15
25 cm	9.4	127	116	791	1.27	12.7	64	-2.6
P-value								
Flooding depth (D)	0.7	0.65	0.29	0.72	0.5	0.96	0.53	0.07
Time (T)	< 0.001	0.35	0.08	0.44	0.008	< 0.001	0.02	0.11
D × T	0.5	0.64	0.29	0.37	0.3	0.86	0.03	0.74

^aH₂O-P_i and H₂O-P_o are water-extractable inorganic and organic phosphorus, NaHCO₃-P_i and NaHCO₃-P_o are labile inorganic and organic phosphorus, NaOH-P_i and NaOH-P_o are Fe/Al-bound inorganic and organic phosphorus; and HCl-P_i and HCl-P_o is Ca/Mg-bound inorganic and organic phosphorus.

Table 5.5 Pearson correlation coefficients between pore water dissolved reactive P (DRP) and biosolids inorganic P (Pi) and organic P (Po) fractions (n = 29).

	H ₂ O-Pi	NaHCO ₃ -Pi	NaOH-Pi	HCl-Pi	H ₂ O-Po	NaHCO ₃ -Po	NaOH-Po	HCl-Po
Flooding depth								
5 cm	0.38ns†	0.30ns	-0.15ns	-0.14ns	-0.05ns	-0.09ns	0.02ns	-0.27ns
15 cm	0.37ns	0.26ns	0.41*	-0.61**	0.07ns	-0.25ns	0.40*	0.34ns
25 cm	0.8***	0.64**	0.09ns	0.08ns	-0.06ns	0.08ns	0.64**	0.04ns

†ns, not significant at 0.05 probability level. *, **, and ***, significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Averaged across flooding depths, WEP P_i , labile P_i and Fe/Al-bound P_o were significantly correlated with concentrations of DRP in pore water, DRP in floodwater, and TP in floodwater (Table 5.6). Although the correlation coefficients were relatively low ($r = 0.29-0.46$; Table 5.6), the results show that these fractions may have been the main contributors to P release. Water extractable P_i and labile P_i were the most strongly correlated ($r = 0.74$, $P < 0.0001$) of the biosolids P fractions, suggesting that the increase in WEP P_i was caused by labile P_i . Highly significant correlations were also detected between WEP P_i and Fe/Al-bound P_o ($r = 0.55$, $P < 0.0001$) and between labile P_i and Fe/Al-bound P_o ($r = 0.61$, $P < 0.0001$), suggesting that the origin of WEP P_i and labile P_i was the same as the origin of Fe/Al-bound P, or the decomposition of Fe/Al-bound P_o contributed to WEP P_i and labile P_i .

Table 5.6 Pearson correlation coefficients between pore water dissolved reactive P (DRP), floodwater DRP, floodwater total P (TP) and biosolids inorganic P (Pi) and organic P (Po) fractions. Flooding depths averaged across all sampling times. (N = 85).

	H ₂ O-Pi	NaHCO ₃ -Pi	NaOH-Pi	HCl-Pi	H ₂ O-Po	NaHCO ₃ -Po	NaOH-Po	HCl-Po
Pore water DRP	0.29*	0.32*	0.08ns†	-0.04ns	-0.08ns	0.05ns	0.44***	-0.02ns
Floodwater DRP	0.35*	0.41***	0.06ns	-0.03ns	0.03ns	-0.15ns	0.46***	-0.06ns
Floodwater TP	0.49***	0.46***	0.08ns	-0.05ns	0.03ns	-0.26*	0.30*	-0.08ns

†ns not significant at 0.05 probability level

*, **, and ***, significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Flooding can stimulate the release of P, and high biomass plants, such as cattails, adapted to flooded conditions can effectively take up released P during phytoremediation in submerged sediments or wetlands. Wu et al. (2004) reported that TP concentrations in cattail leaves, roots, and shoots were 12%, 84%, and 42%, respectively, greater in cattail which had established in sediments under waterlogged conditions than in cattail in aerated sediments. Although we did not measure P uptake by cattail in the intact biosolids cores, plant uptake may have reduced P concentration in the surface floodwater. Since we used intact cores extracted from a lagoon cell in which cattail established naturally, we did not have unvegetated control cores to allow for an investigation of the effect of plants on P dynamics.

Our study was conducted during September through October when cattail was senescing. There is a need to study P release at different plant growth stages and to evaluate the effect of the presence or absence of plants on P release. Preliminary results from a wetland microcosm study using the same biosolids showed that DRP concentration in pore water was greater in non-vegetated microcosms compared with microcosms vegetated with cattails, but DRP concentration in surface floodwater was greater in vegetated pails than in non-vegetated units (Wang, unpublished data, 2017). These results suggest that vegetation can enhance P migration from pore water to floodwater, which increases the risk of P impacts on receiving water bodies if floodwater is released during phytoremediation.

5.5 Conclusion

Regardless of flooding depth, TP release to floodwater increased rapidly during the first two weeks and remained relatively unchanged thereafter. Dissolved reactive P was the major P fraction in the surface floodwater. Our results, therefore, suggest that biosolids undergoing terrestrial phytoremediation pose a risk of P loss to surface water bodies receiving floodwater from

the lagoon. Flooding duration should be given consideration if floodwater is to be released from the lagoon, particularly since our results indicate that the risk of P loss increases with residence time of the floodwater. These results have important implications on the management of floodwater during in situ phytoremediation of end-of-life municipal lagoons. Managers should monitor P water quality during flooding and release floodwater closer to the start of the flooding event to minimize P release to surface floodwater.

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6. OVERALL SYNTHESIS

6.1 Relevant Findings and Implications of the Research

Spreading of biosolids on farmland is a common practice for managing biosolids in many jurisdictions in North America (LeBlanc et al., 2009; NEBRA, 2007). Limitations on nutrient application rates, competition with animal manure for farmland for spreading of the biosolids, and shortage of suitable land within economic distances are some of the challenges that may limit or restrict land spreading of biosolids. Any restrictions on spreading biosolids on farmland will greatly affect small municipalities with limited budgets and force them to explore alternative economic and environmentally sustainable options for managing biosolids when municipal lagoons reach their lifespan. Finding alternative economic and environmentally sustainable solutions for managing biosolids from end-of-life lagoons is, therefore, of great importance for these small rural municipalities. The main purpose of this research was to investigate in situ wetland- and terrestrial-based phytoremediation of nitrogen (N) and phosphorus (P) in biosolids in an end-of-life municipal lagoon.

Nutrient phytoextraction is a product of biomass yield and tissue nutrient concentration. Some researchers suggest that total productivity of plants, hence nutrient removal, could be increased by repeated harvesting of plant biomass during the growth period (Jinadasa et al., 2008; Vymazal, 2005; Vymazal et al., 2010). The effect of harvest frequency on plant biomass yield and N and P phytoextraction from biosolids was reported in Chapters 2 and 4. Chapter 2 focused on nutrient phytoextraction in a wetland constructed in a former primary cell of a municipal lagoon and the study in Chapter 4 describes phytoextraction in a dewatered secondary cell of the lagoon. In both the wetland (Chapter 2) and terrestrial-based (Chapter 4) phytoremediation studies, harvesting cattail twice per season reduced biomass yield and N and P phytoextraction relative to a single

harvest per season. These results demonstrate that phytoextraction of nutrients is more effective with a single harvest compared to two harvests per season under the climate (prairies) and environmental conditions (biosolids) tested.

Annual harvesting of cattail for 4 yr in the constructed wetland removed 6.2% of initial TN and 2.2% of initial TP (19% of labile P, i.e., sum of H₂O-P and NaHCO₃-P) in the biosolids. In the dewatered secondary cell of the lagoon, annual harvesting of the cattail biomass removed 5.8% of initial TN and 2.3% of initial TP concentration (24% of labile P) over the 5-yr period. Nitrogen and P phytoextraction in the constructed wetland could be increased by 10% and 30%, respectively, if the water level was lowered to allow cutting of plants at a height of 45 cm instead of 65 cm above the biosolids sediment layer during harvesting. Annual removal rates, via cattail harvesting, of biomass, N and P were greater under a wetland-based system (5.9 t biomass ha⁻¹ yr⁻¹, 105 kg N ha⁻¹ yr⁻¹, and 16 kg P ha⁻¹ yr⁻¹; Chapter 2) than the terrestrial-based phytoremediation system (5.7 t biomass ha⁻¹ yr⁻¹), 71 kg N ha⁻¹ yr⁻¹ and 11 kg P ha⁻¹ yr⁻¹; Chapter 4). Nutrient phytoextraction was greater in the wetland system regardless of the 65-cm stubble height in the wetland system compared with the 15-cm stubble height in the terrestrial system, indicating the superior performance of the wetland phytoremediation system.

There are several factors municipalities may consider in choosing between a terrestrial and a wetland-based phytoremediation approach. Constructing wetlands in lagoons and pumping water to supply the wetland make wetland phytoremediation more expensive than a terrestrial phytoremediation approach. In addition, harvesting in a wetland system offers serious logistic challenges while harvesting in the terrestrial based approach is simpler and uses readily available traditional agricultural harvesting equipment. Harvesting plants in the wetland during winter or spring when the wetland is frozen may appeal logistically as the conditions can provide easier

access to harvest equipment, reduce the cost of drying the biomass and provide minimal ecological impacts (Cicek et al., 2006; Grosshans, 2014). However, harvesting cattail in November reduced N and P phytoextraction by 63% and 74%, respectively, while an April harvest reduced phytoextraction by up to 85% relative to an August harvest. Where harvesting in wet summer environments is a challenge, harvesting in fall or spring when the ground is frozen may be a compromise, but nutrient phytoextraction will be significantly reduced. In constructed wetlands where water can be drawn down, a summer harvest could be a better option to maximize nutrient phytoextraction. This study is the first to examine in situ phytoremediation of nutrients in biosolids in an end-of-life municipal lagoon. Findings from the study will inform policy makers and landowners on the effective strategies for harvesting cattail to optimize N and P phytoextraction from biosolids using wetland plants.

Phytoremediation with cattail, amounts of nutrient removed and relationship to in situ measurements of nutrient availability under conditions as they exist on-site (Chapter 3) were examined using plant root simulator (PRS) probes (Western Ag, Saskatoon, SK). Nitrogen supply rate was low and increased with time after July. Nitrogen supply rates from the biosolids were lower than N supply rates from other studies in agricultural soils amended with biosolids, which can be attributed to lower mineralizable N concentration in the biosolids and/or the wetland conditions, which likely reduced mineralization rates. Phosphorus supply rates did not significantly change with time, thus contradicting previous studies that showed increased P supply rate in saturated soils using ion exchange membranes (Nelson et al., 2007; Wood et al., 2016). High Ca supply rates measured in our study may have immobilized P via precipitation as calcium phosphates. Cumulative supply rates of the nutrients were highly correlated with cattail uptake, indicating that PRS probes are effective in measuring availability of nutrients in biosolids

sediments. Cattail biomass yield and uptake of nutrients were best described by a beta sigmoid model which indicated that maximum attainable biomass coincided with maximum nutrient uptake, approximately 90-100 d after the start of cattail regrowth.

Biosolids have been mostly restricted to dryland conditions, such as application to agricultural land as a source of nutrients, but there is a potential for end-of-life municipal lagoons to get flooded during terrestrial phytoremediation and for the use of biosolids under flooded conditions to facilitate wetland-based phytoremediation (Chapters 2 and 3). Wetland-based remediation has also been proposed for mine tailings (Jia et al. 2015). The flooding experiment reported in Chapter 5 showed that dissolved reactive P (DRP) concentration was the major fraction of P in both biosolids pore water and floodwater 3 d after flooding and thereafter. Flooding biosolids for more than 3 d resulted in floodwater DRP concentrations $>0.5 \text{ mg L}^{-1}$, indicating that releasing floodwater within the first 3 d of flooding minimizes adverse impacts on receiving surface water. During these first 3 d, a large fraction of P was in the particulate form (71% on Day 0 and 49% on Day 3) and if the floodwater were released, the particulate P would most likely settle in ditches or channel beds. Overall, prolonged water retention in end-of-life lagoons enhances P release from biosolids sediments. Therefore, land managers should monitor P concentration during flooding if the floodwater is to be released.

In situ phytoremediation of biosolids in end-of-life municipal lagoons has previously not been tested in Canada or elsewhere. Thus, remediation targets for N and P concentrations in end-of-life lagoons currently do not exist. In the absence of set guidelines, site specific remediation goals or a risk assessment approach can be considered. Biosolids TN and TP concentrations in both the wetland and terrestrial-based phytoremediation studies were lower than TN (6347 mg kg^{-1}) and TP (3684 mg kg^{-1}) concentrations measured in a natural wetland within 1 km of the study lagoon.

Nitrate and Olsen P concentrations in the biosolids were below the agro-environmental thresholds set by the Nutrient Management Regulation under Manitoba's Water Protection Act. Nitrate levels in this research were below the Manitoba residual soil limits of 157 kg ha⁻¹ and Olsen P levels were below the threshold for P application (Olsen P <120 mg kg⁻¹) where additional P could still be applied on agricultural land at two times the crop removal rate. Results from the flooding experiment indicated a potential risk of P enrichment of receiving surface waters if the floodwater from the lagoon were released. Phosphorus concentration in the lagoon floodwater was also greater than P concentration in floodwater from nearby (< 0.5 km) drainage ditches during flooding events. However, the floodwater in this study was not released from the lagoon and there was low risk of nutrient runoff, hence low risk of P loss to surface water.

The lagoon site is a closed system so there is no environmental threat from runoff and leaching and nutrient levels are below agro-environmental thresholds. This study showed that annual harvesting of cattail in the lagoon for nutrient phytoextraction and biomass for bioenergy production can be sustainable. Phytoremediation takes several years and any potential uses of land during the phytoremediation process is appealing. The Town of Niverville's long-term goal was to develop the site as an interpretive centre and integrate it with an adjacent community park to allow public access for interpretive and educational purposes, wildlife viewing and biomass harvesting. Overall, excluding research costs, the phytoremediation approach for the lagoon was estimated to cost only 30-40% of the costs associated with the traditional methods of lagoon decommissioning by landfilling or spreading biosolids on farmland. This research indicates that in situ phytoremediation can be an environmentally sustainable and economically viable option for lagoon decommissioning and will make a significant contribution to how small municipalities decommission their sewage lagoons.

6.2 Recommendations

This research is the first step towards providing information on N and P removal from end-of-life lagoons using cattails. The economics of harvesting cattail as a biomass feedstock for bioenergy and nutrient capture needs to be explored. Although potential uses of the biomass in this study, which include using the biomass as a feedstock for bioenergy to power a local farmer's flax-heat drying system, or produce heat energy for a college in Manitoba, have been identified, future studies need to evaluate the economic benefits and efficiency of these avenues.

The wet-dry cycle during terrestrial phytoremediation may have reduced cattail productivity and nutrient uptake. Periodic drought during the growing season has been shown to reduce cattail biomass production compared to continuous flooding (Li et al., 2004). Future studies should explore upland plants such as switchgrass that are better adapted to dry conditions. We showed in a growth chamber study that switchgrass performs better than cattail under terrestrial environments (~70% water-filled porosity) (Jeke et al., 2017) but this has not been tested directly in a municipal lagoon. In this field study, we attempted to establish switchgrass in the secondary cell of the lagoon but switchgrass seedlings were choked by the dense monoculture of cattail that self-established in the lagoon. Therefore, our study shifted focus and we evaluated the phytoextraction potential of cattail alone. There is a need to evaluate other species which can perform better in the wet-dry cycle of municipal lagoons. This will allow managers to make informed decisions on whether to invest in eradicating cattails that establish naturally in lagoons in favor of species that will enhance biomass yield and nutrient phytoextraction.

Our study on the effect of flooding on P release was conducted during September through October when cattail was senescing. There is a need to study P release at different plant growth stages. Different plant growth stages and timing of flood events may affect P release. Processes such as plant uptake, rhizosphere processes, and microbial activity, which may determine P that

will eventually be released to surface floodwater, may differ with plant growth stage. This study also evaluated P release when biosolids were flooded for one continuous flooding cycle (42 d). These results reflect permanent flooding conditions in wetlands but may not reflect periodic flooding cycles that may occur in terrestrial environments. Future studies should investigate the effects of the duration and frequency of draw-down and reflooding cycles on P release from flooded biosolids. The duration and frequency of flooding can affect P dynamics. Ajmone-Marsan et al. (2006) reported that redox cycles resulted in soil P shifting towards more labile forms that were easily released on subsequent flooding and reduction. Scalenghe et al. (2012) reported that more P is released under repeated wet-dry cycles. Penn and Simms (2002) and Sharpley (1997) found that runoff P concentration in biosolids and poultry litter decreased with successive rainfall events. Future studies should also investigate P dynamics in floodwater during simulated release of floodwater and natural drawdown by evaporation during repeated flooding cycles. This will provide information on whether releasing floodwater from the lagoon during flooding cycles will continuously result in elevated P levels in the floodwater relative to situations in which floodwater is not released from the lagoon and recedes by evaporation between flooding cycles.

6.3 References

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