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PHYTOREMEDIATION OF NITROGEN IMPACTED SOIL AND GROUNDWATER AT A FERTILIZER FACILITY IN CENTRAL ALBERTA

by

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ABSTRACT

In-situ remediation techniques such as phytoremediation have shown promise as economical alternatives for reducing the risk of environmental contaminants at impacted sites. Research trials were initiated to determine the efficacy of phytoremediation for soil and groundwater contaminated with high levels of nitrogen fertilizer at a fertilizer plant in Alberta, Canada. Experimental trials were conducted in environmental growth chambers, and carried out for a growing degree day period equivalent to an average growing season. Initially, plant growth trials were conducted with soils artificially contaminated with varying levels of ammonium nitrate to determine the approximate upper limit of plant nitrogen tolerance. Historically contaminated soil and groundwater containing high levels of ammonium, nitrate, phosphate and sulfate fertilizers was then investigated using electromagnetic surveying, sampling and chemical analysis. Using this data, samples were collected and growth chamber experiments designed to determine if plants could assist in the remediation of naturally occurring soils and groundwater contaminated with excess fertilizer.

Results indicate that plants can take up excess soil nitrogen caused by fertilizer contamination. Phytoremediation is potentially effective under conditions where soils are contaminated by high concentrations of a variety of plant nutrients so long as conditions are not phytotoxic, as well as being economical, sustainable and aesthetically pleasing. The results of this research may be used to develop phytoremediation programs at western Canadian fertilizer facilities.

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1. INTRODUCTION

1.1 Effects of Nitrogen Contamination on Human and Ecosystem Health

Along with carbon and oxygen, nitrogen is the most critical and complex element essential for sustaining life (Keeney and Hatfield 2008). Nitrogen fertilizer is used largely in the agricultural industry for food production and an estimated 40 % of the per capita increase in food production in the past 50 years is due to its use (Mosier et al. 2001). Nitrification, denitrification, nitrous oxide formation, leaching of nitrate and volatilization of ammonia are all fates of fertilizer N when it is separated from carbon (Keeney and Hatfield 2008). Resulting nitrogen compounds such as nitrate and ammonium may be harmful to human and ecosystem health until returned to the atmosphere as N_2 or N_2O (McKean et al. 2005).

Excess nitrogen in surface and groundwater can be toxic to humans and cause water quality issues in natural aquifer systems (Keeney and Hatfield 2008). The United States Environmental Protection Agency has set the maximum contaminant level for NO_3^-N in drinking water at 10 mg/L (Polomski et al. 2009), while the Alberta Tier 1 Groundwater Remediation guideline value for NO_3^- is 13 mg/L for all water uses (Alberta Environment 2010). Williams et al. (1999) report that human exposure to high levels of nitrate are associated with diabetes, cancer, and methoglobinemia, a blood disorder most common in infants.

Runoff containing excess NO_3^- and other soluble nutrients such as reactive phosphates can reach surface waters and lead to accelerated eutrophication due to excessive algal and aquatic plant growth (Polomski et al. 2009). Nitrogen, in the form of NO_3^-, is the most common contaminant in aquifer systems (Burkart and Stoner 2008). Groundwater impacts are due to the high rate of mobilization of NO_3^- allowing for rapid infiltration through the vadose zone resulting in contamination of groundwater (McKeon et al. 2005).

1.2 Plants and the Nitrogen Cycle

Inputs of nitrogen into an ecosystem consist of fixation by symbiotic and nonsymbiotic bacteria and other organisms, atmospheric fixation by lightening, and that supplied by fertilization (Kozlowski and Pallardy 1997). For autotrophic growth to occur, the nitrogen required for the formation of plant cellular matter is met by the fixation of molecular nitrogen from the atmosphere or assimilation of nitrate or ammonium present in the surrounding soil or water (Heldt and Piechulla 2011). Nitrogen from the soil or groundwater enters the biomass primarily in the form of aqueous nitrate taken up by plants and microorganisms (Hopkins and Huner 2004), however plants can use nitrogen in other forms such as nitrites, ammonium salts and organic N compounds such as urea (Kozlowski and Pallardy 1997).

Nitrogen introduced into the soil via biological or industrial fixation is converted to ammonia, which if released into the soil, may accept an additional hydrogen ion to form ammonium (NH^{4+}), or evaporate back to the atmosphere (Campbell and Reece 2002). Although plants can use ammonium directly, the majority of ammonium in soil is used as an energy source for aerobic bacteria whose activity oxidizes ammonium to nitrite and then to nitrate. This nitrification allows for assimilation of nitrate by plants or conversion to organic forms of nitrogen such as assimilation into the soil microbial biomass. Ammonification, the decomposition of organic nitrogen back to ammonium by soil microorganisms, is responsible for additional cycling of nitrogen within the soil. Bacterial denitrification results in the conversion of nitrate to nitrogen gas which is then returned to the atmosphere (Campbell and Reece 2002).

Living plant roots alter the soil physical, hydrological, and chemical environment and associated biological processes important to nutrient cycling, plant productivity and ecosystem carbon balance (Gregory 2006). There are multiple and interacting effects of roots on soil carbon and nitrogen cycling and on soil water dynamics. Processes in the rhizosphere are primarily controlled by the interactions among roots, fauna and the microbiological community (Hawkes et al. 2007), and living roots can significantly alter soil nitrogen (and carbon) mineralization rates (Bird et al. 2011). It is extremely challenging, however, to

identify and quantify the primary mechanisms responsible for living root impacts on these dynamics. Bird et al. (2011) reported that living roots altered the soil microbial community composition compared with unplanted soils, this is likely an important factor in the alteration of available soil nitrogen.

1.3 Phytoremediation of Excess Nitrogen

Nitrogen is a critical component for plant health. It is essential for the production of proteins and nucleic acids, and is an integral part of chlorophyll required for photosynthetic activity (Havlin et al. 2005). Plant species with high nutrient requirements and/or tolerance can take up large amounts of nitrogen from impacted soils. Russelle et al. (2001) found 'Ineffective Agate' alfalfa (*Medicago sativa* L.) hay removed 972 kg N ha^{-1} at a fertilizer spill site in North Dakota over a three year period. McKeon et al. (2005) indicated that plants could remove nitrogen at a maximum rate of approximately 200 kg N yr^{-1} by incorporation into plant biomass. Studies on willows and poplars have reported that municipal and agricultural pretreated effluents containing large amounts of nitrogen constitute a source of nutrients for the plants (Hassegren 1998).

Plant roots also act as a sink for water within the soil. Root water uptake is vital for plant growth but can also play an important role in the management of soil and groundwater contaminants. Soil water extracted by plant roots is no longer free to carry soluble nitrogen away from the soil surface; thus preventing plume migration (Clothier and Green 1997). The movement of water past reactive soil surfaces transports dissolved chemicals to plants (Clothier et al. 1997). Since plants take up most nitrogen through their roots, the quality of impacted soil and groundwater can be improved by plants (Guidi and Labrecque 2010). McKeon et al. (2005) also found that irrigation of phytoremediation species induced a rapid initial rate of denitrification, leading to a decrease in soil nitrogen without resulting in leaching of nitrate away from the source area.

1.4 Nutrient Movement from Soil to Roots

For plant uptake of nutrients to occur, contact between the ion and the root surface is required. Mass flow, root interception and diffusion make up the general mechanisms by which nutrients reach the root surface (Havlin 2005). Mass flow is the most significant of these mechanisms in the movement of nitrogen through the soil, and makes up 99 percent of nitrogen movement to the root surface. Transpirational water uptake by the plant, water evaporation at the soil surface, and percolation of water within the soil profile result in the occurrence of mass flow leading to movement of nitrogen ions. Root interception accounts for only one percent of the movement of nitrogen from soil to plant roots (Havlin 2005). Root interception denotes the exchange of ions by way of physical contact between plant roots and mineral surfaces, and although roots usually occupy less than one percent of the soil volume, roots growing through soil pores with higher than average nutrient contents could come in contact with up to three percent of the available soil nutrients. In comparison to nitrogen, 27 percent of calcium required by plants is supplied via root interception (Havlin 2005). Diffusion occurs by way of concentration gradients, with ions in the soil solution moving from areas of high concentration to areas of low concentration. The percentage of nitrogen supplied to plants via diffusion is zero, however up to 94 percent of plant required phosphorus is transported by this mechanism (Havlin 2005). Nutrient concentration gradients are created due to the movement of ions along with the uptake of water by plant roots. As roots absorb nutrients from the surrounding soil a decreased concentration of nutrients at the root surface compared with the bulk soil solution is created.

1.5 Benefits and Limitations of Phytoremediation

Traditional methods for remediation of contaminated soil and groundwater, such as soil excavation and landfilling, pump and treat, and soil washing or flushing, are generally costly and harmful to soil properties (Komarek et al. 2008). Phytoremediation, a technology that uses green plants to remediate contaminated environmental media, has been successfully applied both *in situ* and *ex situ* to treat sites impacted by organics, metals and salts (Carman and

Crossman 2001). The first implemented and reported phytoremediation was as a cleanup technology for agricultural contaminants such as excess plant nutrients (fertilizers) and pesticides (Briggs et al. 1982).

1.5.1 Benefits of phytoremediation

Phytoremediation provides treatment with numerous advantages over chemical amendments and *ex situ* remediation. The most widely touted benefit of phytoremediation is its cost effectiveness due to capital and operating costs typically far lower than competing technologies (Glass 2000). There is on average a 50 percent decrease in cost per unit treated when compared to landfilling (Greenberg 2011). Low ongoing operation and maintenance costs make phytoremediation an attractive option for non-point source contamination, such as nitrates or pesticides in agricultural environments (Carman and Crossman 2001).

Further advantages to phytoremediation include an increase in soil quality (Greenberg 2011), due to increases in biomass and soil organic matter, and enhancement of microbial and fungal populations (Carman and Crossman 2001). In addition, the technology is driven by solar energy making it suitable to many regions and climates (Greenberg 2011). Phytoremediation is particularly applicable to the energy sector in Western Canada as it results in the preparation of a proper seedbed (Greenberg 2011). There is also positive public perception and acceptance as phytoremediation is an aesthetically pleasing and green technology (Glass 2000).

Phytoremediation is versatile and can be used to treat a range of soil types as well as surface and groundwater. In addition to remediation of soil within the root zone, contaminated water can be pumped and used to irrigate trees and grasses (Carman and Crossman 2001) where organic contaminants such as carbon and nitrogen compounds are used as fertigation for the plants (Zalesny and Bauer 2007). Hydraulic containment is a further application of phytoremediation, harnessing the ability of plants, especially phreatophytes, to root deeply and capture large quantities of groundwater or soil pore water (Carman and Crossman 2001). This reduction of water losses from the root zone can attenuate

migration of contaminant plumes with groundwater and allow for phytoextraction of contaminants from the soil matrix into plant tissue.

Phytoremediation can be used as a single treatment technology, or coupled with more aggressive conventional technologies. An example of such an application is contaminated soils from a site excavated and treated in engineered phytoremediation treatment units, rather than thermally treated or landfilled (Carman and Crossman 2001).

1.5.2 Limitations to phytoremediation

Although plants have adapted to grow in a variety of inhospitable conditions, phytoremediation will not be successful if soil conditions or contaminant concentrations and/or characteristics are phytotoxic (Carman and Crossman 2001). Phytoremediation may also be slower than some alternative technologies, and seasonally dependent (Glass 2000); active only during the growing season of the area being treated.

A specific soil condition that may affect the success of phytoremediation as an option for improvement of soil and groundwater quality is electrical conductivity (EC). Soils with EC values of generally greater than four decisiemens per meter ($dS\ m^{-1}$) are considered saline (Brady and Weil 2004). Saline soils contain relatively high concentrations of soluble salts (Marcar et al. 2010) and the source of these salts may vary from those transported by moving water to nitrogen or phosphorus salts introduced through the applications of fertilizers or manure.

1.6 Vegetation Selection for Phytoremediation

Plant species are selected for use in phytoremediation according to their ability to treat contaminants of concern and for their adaptability to site-specific factors such as local climate and soil type present. Preferred characteristics include: an ability to extract or degrade contaminants of concern to nontoxic or less toxic products, adaptability to local conditions, ease of planting and maintenance, fast growth rate and high water use. Plants with extensive root systems and high water and nutrient uptake have been successful in reducing leaching of nutrients

such as nitrogen and phosphorus from agricultural sites (USEPA 2001). Herbaceous species such as mustard, alfalfa and grasses can be used to remediate contaminants at the soil surface. Woody species having rapid growth rates, deep roots and high rates of transpiration are used to treat contamination at depth or in groundwater. Examples of species used include phreatophytic hybrid poplar, willow and cottonwood (USEPA 2001).

The preference for different nitrogen compounds by plants can also affect removal of nitrogen from soil and groundwater. Plants that can assimilate several forms of nitrogen will be more successful in nitrogen immobilization and removal than plants that are only able to take up nitrogen in one form (Neuschutz and Greger 2010).

1.7 Nitrate Fines Landfill – Project Background

Historical dumping of fertilizer fines and other wastes into a clay borrow pit at the Agrium Redwater, Alberta fertilizer facility adjacent to the North Saskatchewan River has resulted in significant ammonium, nitrate, phosphate and sulphate impacted soil and groundwater in the area. This contamination has also resulted in increases in nitrate in sediment pore water on the western bank of the river. Drilling tests completed by Agrium in 1993 indicate that the average nitrate concentration of the placed material was 0.48% (4800 mg/kg) and that a high proportion of the material in the landfill could be classified as waste (eg. wood, metal, cable) (Stantec 2001). Total volume of placed material is estimated to be approximately 40,000 m^3.

The nitrate fines landfill was capped in the fall of 1993 with a synthetic liner overlain by topsoil to prevent infiltration of surface water into the landfill cells and to minimize the mobilization of nitrogen compounds into the groundwater. A five to seven meter interceptor trench was constructed along the western boundary of the landfill in 1995 to intercept groundwater from the phosphogypsum stack and to lower the water table beneath the landfill. Despite the liner and interceptor trench, the groundwater table remains high and in some cases higher than the reported depth of the former landfill. Groundwater nitrogen concentrations up to

24,000 mg/L ammonium-N and 7000 mg/L nitrate-N have been observed and impacts to the North Saskatchewan River remain a concern. Options for remediation and improving soil and groundwater quality were investigated.

1.8 Research Objective and Hypothesis

1.8.1 Objectives

The main goal of this research was to determine the viability of using Okanese poplar ((*Populus deltoids x Populus xpetronslynna)* x *Populus xpetrowskyana)*, *Salix bebbiana* (beaked willow), *Medicago sativa var.* AC Nordica (alfalfa) and AC Saltlander grass *(Agropyron spicatum* x *Agropyron repens*) as species to treat nitrogen impacted soil and groundwater via phytoremediation. This was accomplished during a number of environmental growth chamber studies involving impacted soil and groundwater from the nitrate fines landfill at the Agrium fertilizer facility in Redwater, Alberta.

Specific research objectives were as follows:

- Evaluate which plant type is most effective in removal of excess nitrogen compounds from impacted soil and groundwater.
- Quantify the upper limit of plant nitrogen and EC tolerance.
- Determine the feasibility of using fertilizer impacted groundwater as an irrigation source.

1.8.2 Hypothesis

General hypotheses regarding successful plant growth in nitrogen impacted soils and following irrigation with nitrogen impacted groundwater include the following:

- All plant types will show successful growth in soils with low level (100 ppm) nitrogen contamination.
- Saltlander grass will show the most successful growth and highest tissue biomass in soils with higher level nitrogen contamination due to its saline tolerance.

- Pots planted with alfalfa will show the greatest decrease in nitrogen levels compared to Saltlander and the woody species. This is due to the high nitrogen usage seen in alfalfa.
- Some decrease in nitrogen levels will be due to natural denitrification occurring within the system.
- Impacted groundwater will be a useful source for plant irrigation once diluted to avoid phytotoxicity.

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2. PHYTOREMEDIATION OF NITROGEN IMPACTED SOIL

2.1 Introduction

Because plants require nitrogen as an essential component for growth, phytoremediation; a technology using green plants to remediate impacted environmental media, may be a sustainable and economical option for the removal of excess nitrogen compounds from fertilizer-contaminated soils. However, concerns as to the feasibility of phytoremediation as an applicable remediation option for the nitrate impacted soil arise due to the increased soil electrical conductivity (EC) caused by the presence of nitrogen salts. High EC may result in a soil environment that is phytotoxic, reducing the growth and survival of plants and limiting phytoremediation potential.

Soil salinity, resulting from increased EC, limits plant growth by creating a water imbalance in the plant (physiological drought), as well as an ion imbalance that may result in increased energy consumption to maintain metabolic processes (Havlin et al. 2005). Salt stress, similar to other abiotic stresses, inhibits plant growth. A slower growth rate is an adaptive attribute for plant survival under conditions of stress as it allows plants to rely on multiple resources to combat stress (Zhu 2001).

Plant salt tolerance is influenced by plant factors, soil factors, and environmental factors. For many plant varieties, sensitivity to salt varies with growth stage, however most plants are sensitive to soil salinity at all stages of growth. Plants that grow well on nutrient deficient soils are generally more tolerant to salt than plants grown in soils with sufficient nutrients (Havlin et al. 2005). This tolerance is most likely due to lower growth rates and reduced water demand. The climatic effects of temperature and humidity on salt tolerance are also particularly important to plant health, as under cool and humid environmental conditions most plants are more salt tolerant than under hot, dry conditions (Havlin et al. 2005).

This chapter will outline and describe two environmental growth chamber experiments developed to examine the prospect of using phytoremediation as an option to treat soil impacted by nitrate fines from historical fertilizer production.

2.1.1 Research objectives and hypothesis

Historical disposal of nitrate fertilizer fines has resulted in average soil nitrate concentrations of 4800 mg/kg at the Agrium facility near Redwater, Alberta (Stantec 2001). These nitrogen compounds may be harmful to ecosystem health and impacts to the adjacent North Saskatchewan River are of concern.

The objective of this research was to determine the feasibility of using Okanese poplar (Walker x *P. xpetrowskyana)*, beaked willow (*Salix bebbiana)*, alfalfa (*Medicago sativa var.* AC Nordica) and AC Saltlander (*Agropyron spicatum* (bluebunch wheatgrass) x *Agropyron repens* (quackgrass)) as phytoremediation plants to treat fertilizer contaminated soil. The first study was conducted in an environmental growth chamber using soil artificially contaminated with various treatment levels of ammonium nitrate. A second growth chamber study was completed using soil excavated from the nitrate fines landfill. Research objectives were as follows:

- Characterize growth and survival for each plant type in nitrogen impacted soils.
- Identify an approximate upper limit of soil EC tolerance for each vegetation type.
- Investigate whether soil constituents other than nitrogen present in the landfill soil are affecting plant growth.
- Determine which plant types are most efficient in the removal of excess soil nitrogen.
- Quantify the nitrogen balance within the environmental growth chamber system.

Hypotheses regarding successful plant growth in nitrogen impacted soils, plant nitrogen tolerance and phytoremediation potential include the following:

- AC Saltlander grass will have maximum growth and biomass development at the highest nitrogen treatment level due to its saline tolerance.

- Okanese poplar will have successful growth in moderately to highly impacted soils due to its stress tolerance and hardness.
- All plant types will tolerate low to moderate levels of soil nitrogen and will exhibit high biomass development and survival rates.
- Plants with the highest growth rate in impacted soil will also exhibit the highest phytoremediation potential based on high tissue nitrogen content per unit of biomass indicating uptake of excess soil nitrogen.
- In the nitrate fines soil, the presence of other constituents such as sulfate-S will result in enhanced elevation of electrical conductivity compared to soils with only ammonium nitrate added, leading to increased plant stress.

2.2 Methodology

2.2.1 Preliminary sampling

Prior to setup of the environmental growth chamber experiments, preliminary sampling of the soil and groundwater in and around the nitrate fines landfill was completed to quantify existing levels of nitrogen and other parameters of interest.

2.2.1.1 Groundwater sampling

Groundwater samples were taken in spring 2010 from 13 wells within and surrounding the nitrate landfill. Samples were collected using a bailer and sent to Exova laboratories for analysis. Parameters measured include ammonium, orthophosphate, organic carbon, uranium, total dissolved solids, pH, electrical conductivity, chloride, fluoride, nitrate, nitrate and sulphate. At the time of sampling, five of the wells tested were dry and no samples were collected from these locations.

2.2.1.2 Geophysical investigation and soil sampling

A geophysical investigation of the nitrate fines landfill was conducted along with WorleyParsons in April 2010. Conductivity data was collected using Geonics EM31 and EM38 terrain conductivity meters (Daniels et al., 2008) and resistivity data was collected using Electrical Resistance Tomography (ERT; Allred et al.,

2008). Information gained from the geophysical investigation was used to prepare the soil sampling plan.

In June 2010, undisturbed soil cores were taken from 11 soil sampling locations within and around the nitrate landfill using a Geoprobe hydraulic soil corer under the supervision of the author and WorleyParsons. Geoprobe cores were completed to a maximum depth of eight meters below ground surface and samples were collected and screened at one metre intervals. Selected soil samples were collected directly from core sleeves and sent to a commercial lab for physical and chemical analysis. Samples were analyzed for the following parameters:

- benzene, toluene, ethylbenzene, and xylene (BTEX), petroleum hydrocarbon (PHC) fractions F1-F4:
- salinity;
- metals; and,
- landfill suitability (leachable metals and leachable nutrients)

2.2.2 Vegetation selection

Four vegetation types were used in the phytoremediation experiment. Selection was based on rate of water use, rapid growth capability and tolerance to salinity. Two deciduous trees were chosen including Okanese poplar ((*Populus deltoids x Populus spetrowskyana)* x *P. xpetrowskyana)*; a fast growing hybrid poplar which has demonstrated the ability to tolerate drought and resist stress (Treetime 2010). Poplar is known to be an excellent candidate for phytoremediation due to its highly efficient photosynthesis potential and its extensive root systems which facilitate the uptake of impacted groundwater (Yadav et al. 2010). The Poplar Council of Canada determined Okanese to be the most hardy hybrid poplar developed by Agriculture and Agri-Food Canada because it tolerates extreme climatic stresses and combines a fast growth rate with hardiness (AAFC-AESB 2009). *Salix bebbiana* (beaked willow) was also selected for the growth chamber experiment. Willow, also a phreatophyte, can extract large volumes of available soil water and can capture shallow groundwater similar to conventional pump and treat systems (Carman and Crossman 2001). This high rate of water

movement allows for the uptake and translocation of organics and inorganics from the soil to the plant roots. Alfalfa was selected for the experiment because of its high rate of nitrogen utilization and because alfalfa is a deep-rooted perennial forage that does not typically assimilate nitrogen slowly (Russelle et al. 2001). *Medicago sativa var.* AC Nordica, the specific variety of alfalfa used was selected due to availability of seed. AC Saltlander (Saltlander), a green wheatgrass based on Turkish selections of a natural cross between *Agropyron spicatum* (bluebunch wheatgrass) and *Agropyron repens* (quackgrass) was the fourth vegetation type chosen (Barker 2010). AC Saltlander has shown aggressive growth and saline tolerance in agricultural studies. Only perennial plant types were selected for the study as requested by Agrium, for the purpose of reduced maintenance requirements if the plants were to be used for future remediation on site.

2.2.3 Environmental growth chambers

The experiment was carried out in an environmental growth chamber located at Agrium, Redwater, Alberta. The chamber was set to mimic the natural environmental conditions at the site. Inside the growth chamber, the plants were exposed to 16 hours of light with a daytime temperature of 21°C, and 8 hours of dark with a night time temperature of 15°C per 24 hour growing period. Humidity in the chambers was not controlled but was measured periodically throughout the experiment with a Quest AQ5000 RH meter. Average humidity for the duration of the phytoremediation experiment was 46%. Light intensity in the growth chambers was measured with a Sper Scientific 840020 Light Meter and recorded as 212 µmol m^{-2} s^{-1}.

2.2.4 Experimental set up

2.2.4.1 Trial 1 - Controlled addition of NH_4NO_3 to loamy sand and growth chamber set up

For the first growth chamber experiment, clean loamy sand collected from a topsoil pile near the research site was artificially contaminated with ammonium nitrate at three treatment levels. Soil was sent to a commercial lab to test for

background salinity, available nutrients and cation exchange capacity prior to addition of ammonium nitrate (Table 2.1).

The bulk soil was screened and air-dried in soil drying trays for two weeks after collection. Once dry, 10 kilograms of soil was added to round treepots 20 cm in diameter and 40 cm high. Ammonium nitrate was dissolved in water and added to the pots for a total of 12 pots each of 100, 1000 and 4000 mg/kg (147, 1470 and 5880 kg N/ha) treatments. The soil was hand mixed to facilitate even distribution of the ammonium nitrate within each treepot. Twelve pots remained without ammonium nitrate to serve as experimental controls. 2000 ml of water was added to each pot to reach 20 percent by volume initial water content. This starting value was selected due to the sandy texture of the treated soil. A bulk density of 1.4 kg/m^3 was estimated based on the soil texture and packing. Water was added through a PVC tube inserted into the soil to the bottom of the pots for bottom-up watering to prevent leaching and accumulation of the ammonium nitrate at the base of the pots. There were no openings at the base of the pots therefore no water or solute was lost through drainage.

Following preparation of the treepots, 20 cm willow and Okanese poplar cuttings were inserted into the center of 24 pots with three centimeters of cutting protruding above the soil surface. Okanese cuttings were supplied by the Canadian Forest Service and stored at approximately -4 degrees Celsius prior to use. Prior to planting, these cuttings were removed from the freezer and soaked in water for 24 hours. Willow cuttings were collected from a shelter belt located on privately owned near the Agrium site and soaked in water for 24 hours before planting. Alfalfa and Saltlander seed were applied using a broadcast seeding method to the remaining 24 pots at a rate of 8 kg/ha (Alberta Agriculture 1981). Three replicates of each vegetation type – contaminant concentration combination were prepared for a total of 48 experiment units.

Each pot was weighed and placed randomly in the environmental growth chamber. The phytoremediation trial ran for 90 days; a time period equivalent to the average growing season in the Redwater area. To maintain 20 percent water content, the pots were weighed biweekly and watered with tap water as required to maintain initial weight. Treatment pots were given the same volume of water

as the controls. The pots were periodically rearranged to avoid possible edge effects.

2.2.4.2 Trial 2 – Collection of landfill soil and growth chamber set up

Soil was collected from the nitrate fills landfill at the Agrium site in September 2011 using a backhoe operated by Agrium personnel. Collection locations were based on results from the spring 2010 soil sampling. Soil was collected from two sampling locations where nitrogen levels were approximately 100 and 2500 mg/kg from nitrate and ammonium-N. Nitrogen levels were selected based on the results from the first growth chamber experiment (Trial 1). Vegetation and topsoil were stripped and the PVC liner pulled back to expose the contaminated soil (Plates 2.1 and 2.2). Soil was collected by hand shovelling from the backhoe bucket directly into large Rubbermaid bins. Clean soil was collected from above the PVC liner to serve as a control. Soil was then transported offsite for screening to remove course fragments and air-dried in soil drying trays for two weeks.

Plate 2.1 PVC liner exposed following stripping of vegetation and topsoil for collection of soil from nitrate fines landfill (September 15, 2010).

Plate 2.2 Removal of PVC liner and collection of nitrate impacted soil from below liner in nitrate fines landfill (September 15, 2010).

Treatment levels for the experiment included soil nitrogen concentrations of approximately 100, 1000 and 2500 mg/kg from nitrate and ammonium-N. To create the 1000 mg/kg treatment, landfill soil measuring 2500 mg/kg nitrogen soil was mixed with the control soil. Following drying and mixing, samples from each soil treatment level were sent to an analytical lab to confirm starting mineral nitrogen concentrations as well as available phosphorus and sulfate levels, electrical conductivity and soil pH (Table 2.2).

Once dry, 10 kilograms of soil was added to round treepots 20 cm in diameter and 40 cm high. Twelve pots were filled with soil from each treatment level for a total of 48 pots including controls. 2500 ml of water was added to each pot to reach 25 percent initial water content. Water was added through a PVC tube

inserted into the soil to the bottom of the pots for bottom-up watering to prevent leaching and accumulation of nutrients at the base of the pots.

Following preparation of the treepots, 20 cm willow and Okanese poplar cuttings were inserted into the center of 24 pots with three centimeters of cutting protruding above the soil surface. Alfalfa and Saltlander seed were applied using a broadcast seeding method to the remaining 24 pots. The cuttings and seeds were from the same sources as in Trial 1. Three replications of each vegetation type – contaminant concentration combination were created. Each pot was weighed and placed randomly in the environmental growth chamber. The phytoremediation ran for 90 days, a time period approximately equivalent to the average growing season in the Redwater area. To maintain 25 percent water content, the control pots were weighed biweekly and watered with tap water as required to maintain initial weight. Treatment pots were given the same volume of water as the controls. The pots were periodically rearranged to avoid possible edge effects.

2.2.5 Post trial sampling

2.2.5.1 Soil analysis

Following the 90 day growth periods, soil samples were taken from each of three 10 cm increments from each treepot. Increments included 0 – 10 cm, 10 – 20 cm, and 20 – 30 cm. Soil was collected by cutting and removing a panel from the side of each pot to expose the entire soil profile. The profile was separated into 10 cm increments and removed using a hand spade. Once screened to remove roots, a random grab sample of approximately 100 g of soil from each increment was placed in a large Ziploc bag and sealed. A total of 144 soil samples for Trial 1 and 108 soil samples for Trial 2 were taken to Exova laboratories the same day as collected and analyzed for available nitrate – N, nitrite-N, phosphorus, sulfate-S, and ammonium-N. Electrical conductivity and soil pH were also measured. In Trial 2, soil from the treepots originally planted with Okanese poplar was not analyzed as the cuttings proved not viable as there was no growth once soaked in water prior to planting, and no biomass development occurred.

2.2.5.2 Vegetation analysis

Above and below ground biomass was measured. Vegetation in each treepot was clipped to the soil surface using hand clippers and placed in paper bags. For the willow and poplar plants, foliage was separated from the branches and stems. Roots were collected by passing the dry soil from each treepot through a sieve to remove the bulk of the soil. The roots were then hand washed over a fine sieve to remove any remaining soil particles and placed into paper bags. Any material remaining from the original cuttings were discarded, as cuttings were not initially identical in size. Above and below ground plant material was oven dried for 48 hours at 60°C (Whalen et al. 2003) and weighed on an electronic scale to obtain biomass measurements. The root biomass values were used for comparison purposes only, and cannot be considered exact measurements. This is due to the possible loss of very fine roots during the washing process. Dried plant material was sent to Exova laboratories to be analyzed for total nitrogen content.

2.2.6 Statistical Analysis

2.2.6.1 Trial 1 analysis

Statistical analyses were conducted using R version 2.12.0 (R Development Core Team 2011). Plant biomass means and standard errors were calculated and graphed for exploratory purposes. A square root transformation was conducted on biomass data in an attempt to meet requirements of normality and homogeneity of variance. The Shapiro-Wilks test for normality was performed on residuals and Bartlett's test for equality of variance was completed. Test results deviated slightly from meeting assumptions of normality but homogeneity of variance was observed (Shapiro-Wilks $P = 0.003$, Bartlett's $P = 0.07$). Slightly non-normal results can likely be attributed to the small sample size within each treatment. Biomass data was subjected to analysis of variance (ANOVA) using procedures outlined in R. A least significant difference at $P \leq 0.05$ was used to determine significance among treatment means. A pair-wise comparison using the Tukey test was completed to determine which treatment levels were of significance.

Plant nitrogen uptake was calculated based on total tissue nitrogen per unit of biomass, and means and standard errors calculated and graphed. Plant biomass data did not meet assumptions of normality and equality of variance but was successfully transformed using an inverse transformation to meet assumptions of normality (Shapiro-Wilks P = 0.077, Bartlett`s P < 0.001). Although Bartlett's test indicated unequal variances, this can likely be attributed to smaller than ideal sample size. In addition, graphical exploration of the data set indicated relative equality of variance. An ANOVA was completed and plant nitrogen uptake data was subjected to a Tukey test for pair-wise comparison to determine which treatment levels were of significance. A least significant difference at P ≤ 0.05 was used to determine significance among treatment means.

2.2.6.2 Trial 2 analysis

Statistical analyses were conducted using R version 2.12.0 (R Development Core Team 2011). Plant biomass means and standard errors were calculated and graphed for exploratory purposes. The Shapiro-Wilks test for normality was performed on residuals and Bartlett's test for equality of variance was completed. Test results successfully met assumptions of normality and homogeneity of variance (Shapiro-Wilks P = 0.897, Bartlett's P = 0.07). Biomass data was subjected to analysis of variance (ANOVA) using procedures outlined in R. A least significant difference at P ≤ 0.05 was used to determine significance among treatment means. A pair-wise comparison using the Tukey test was completed to determine which treatment levels were of significance.

Plant nitrogen uptake was calculated based on total tissue nitrogen per unit of biomass, and means and standard errors calculated and graphed. Data did not meet assumptions of normality and equality of variance but was successfully transformed using an inverse transformation to meet assumptions of normality (Shapiro-Wilks P = 0.077, Bartlett`s P = < 0.001). Although Bartlett's test indicated unequal variances, this can likely be attributed to smaller than ideal sample size. In addition, graphical exploration of the data set indicated relative equality of variance. An ANOVA was completed and plant nitrogen uptake data was subjected to a Tukey test for pair-wise comparison to determine which

treatment levels were of significance. A least significant difference at $P \leq 0.05$ was used to determine significance among treatment means.

2.3 Results and Discussion

2.3.1 Preliminary sampling results

2.3.1.1 Groundwater sampling results

Nitrate and ammonium levels in the groundwater collected from within the landfill site ranged from 267 to 15,600 mg/L and 537 to 29,400 mg/L, respectively. Down gradient and south of the landfill nitrate levels ranged from 159 to 3950 mg/L, and ammonium levels from 371 to 3190 mg/L.

2.3.1.2 Geophysical investigation and soil sampling results

Elevated terrain conductivity values were discovered within the nitrate landfill, extending 100 to 150 m north of the landfill, along a topographic low north-northeast of the landfill, in the direct vicinity of two groundwater monitoring wells east of the landfill, and along the North Saskatchewan River (WorleyParsons 2010). Terrain conductivity data is presented in Figure 2.1.

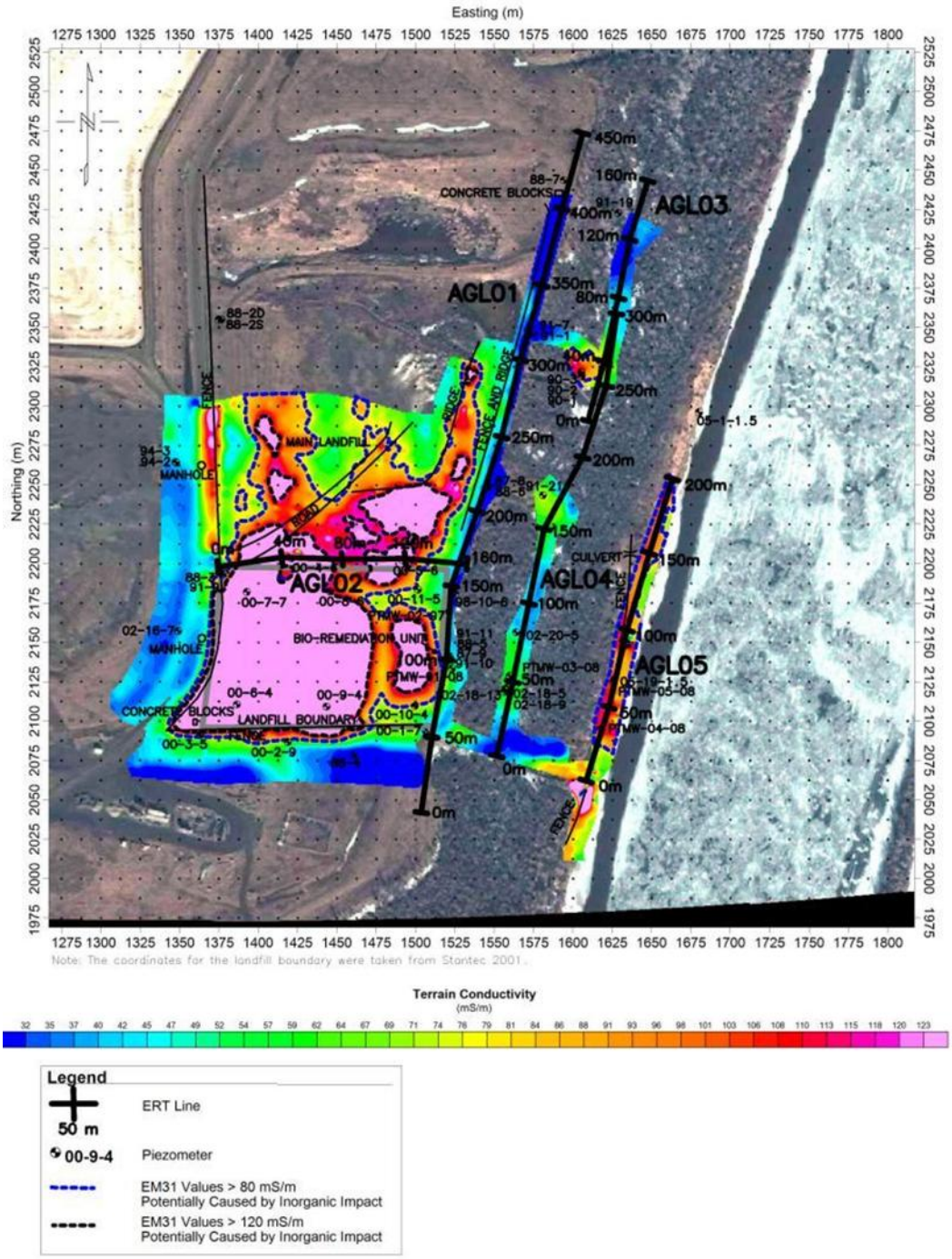

Figure 2.1 Terrain conductivity map of the nitrate fines landfill and surrounding area based on results from the electromagnetic survey by EM 31 and 38 and ERT.

Ammonium-N in the soil cores collected ranged from 17.6 to 72,900 mg/kg (0.12 to 510.3 kg N/ha) and nitrate-N ranged from 161 to 20,800 mg/kg (1.13 to 145.6 kg N/ha) with the highest values of both contaminants located 2.8 – 4.0 m below ground surface within the landfill (Worley Parsons 2010).

2.3.2 Pre-trial soil analysis

Table 2.1 illustrates results from the laboratory analysis of baseline soil characteristics for Trial 1. These conditions are representative of the soil prior to addition of the ammonium treatments, and the conditions in the control treepots. Pre-trial soil conditions for the soil excavated from the nitrate fines landfill for Trial 2 are presented in Table 2.2.

Table 2.1 Baseline soil characteristics of loamy sand collected from topsoil pile near research site prior to addition of ammonium nitrate treatments for first trial in phytoremediation study.

Analyte	Units	Results
Phosphorus (available)	kg/ha	117.6
Potassium (available)	kg/ha	336.0
Cation Exchange Capacity	meq/100g	18
pH	pH	6.7
Electrical Conductivity	dS/m	2.42
SAR		0.10
% Saturation	%	38
Calcium	kg/ha	932.4
Magnesium	kg/ha	92.4
Sodium	kg/ha	16.8
Potassium (soluble)	kg/ha	16.8
Chloride	kg/ha	42.0
Sulfate-S	kg/ha	84.0
Nitrate and Nitrite-N	kg/ha	23.4

Table 2.2 Baseline soil conditions of soil collected from nitrate fines landfill for second trial in phytoremediation study.

Analyte	Units	Results			
		High	Medium	Low	Control
Nitrate-N	kg/ha	7560.0	3108.0	336.0	29.4
Nitrite-N	kg/ha	1.68	4.2	5.88	3.78
Phosphorus (available)	kg/ha	197.4	336.0	176.4	29.4
Sulfate-S	kg/ha	3943.8	1365.0	2944.2	210.0
Ammonium-N	kg/ha	3645.6	1402.8	17.22	24.78
Electrical Conductivity	dS/m	20.4	8.41	5.67	1.11
pH	pH	8.0	7.9	8.4	8.0

2.3.3 Plant growth and survival

2.3.3.1 Trial 1 plant growth and survival

The growth chamber experiment commenced on August 4th, 2010. One week into the study, germination of alfalfa and Saltlander seed occurred in all pots. No visible difference in germination rate was apparent between the control pots and those treated with 100 and 1000 mg/kg of ammonium nitrate. In the pots treated with 4000 mg/kg of ammonium nitrate, only a small number of seeds germinated. Bud break occurred on all willow and Okanese cuttings with the exception of one willow in control soil.

After 21 days in the growth chamber, Saltlander pots treated with 4000 mg/kg ammonium nitrate showed a visible difference in biomass development compared to controls and lower ammonium nitrate treatments. Alfalfa planted in pots treated with 4000 mg/kg ammonium nitrate appeared stressed based on reduced biomass compared to other treatments and yellowing of leaves. Signs of stress in the form of yellowing and curling of young shoots and leaves were apparent in Okanese and willow at the highest treatment level.

Following the 90 day growth period, no Okanese or willow survived in the pots treated with 4000 mg/kg ammonium nitrate. In the pots treated with 1000 mg/kg of the contaminant, only one willow survived for the duration of the growth period, while two out of three Okanese persisted (Plates 2.3 and 2.4). Saltlander seeded in pots treated with 4000 mg/kg ammonium nitrate survived and appeared the

most vigorous when compared to the other plant types exposed to the high treatment level. Saltlander plants in the 4000 mg/kg treatment were stunted in comparison to Saltlander exposed to the 100 and 1000 mg/kg ammonium nitrate treatments (Plate 2.5). Alfalfa survival was minimal in pots treated with the highest level of ammonium nitrate, with less than three stunted plants remaining in each pot at the completion of the trial. The surviving plants were yellowish in color compared to alfalfa in the other treatment groups. Alfalfa exposed to soil treated with 1000 mg/kg ammonium nitrate exhibited growth and survival visually comparable to the controls and plants subjected to the lowest treatment level (Plate 2.6). All four plant types survived the 90 day period in soils treated with 100 mg/kg of ammonium nitrate. Plant growth and biomass development in pots with this treatment was visibly equal to, and in some instances higher than, that of the plants in the control pots.

Plate 2.3 Willow survival following 90 days in environmental growth chamber in soil treated with ammonium nitrate. Soil treatments from left to right: control, 100 mg/kg, 1000 mg/kg 4000 mg/kg (November 1, 2010).

Plate 2.4 Okanese poplar survival following 90 days in environmental growth chamber in soil treated with ammonium nitrate. Soil treatments from background to foreground: control, 100 mg/kg, 1000 mg/kg, 4000 mg/kg (November 1, 2010).

Plate 2.5 AC Saltlander survival following 90 days in environmental growth chamber in soil treated with ammonium nitrate. Soil treatments from left to right: control, 100 mg/kg, 1000 mg/kg, 4000 mg/kg (November 1, 2010).

Plate 2.6 Alfalfa survival following 90 days in environmental growth chamber in soil treated with ammonium nitrate. Soil treatments from left to right: control, 100 mg/kg, 1000 mg/kg, 4000 mg/kg (November 1, 2010).

2.3.3.2 Trial 2 plant growth and survival

The second growth chamber experiment commenced on January 24, 2011. One week into the study, germination of alfalfa and Saltlander seed occurred in all pots. No visible difference in germination rate was apparent between the control pots and those planted in the low and medium soil treatments. In the highest treatment, only a small number of alfalfa seeds germinated. Saltlander appeared to have similar germination rates as plants in the other soil treatments. Bud break occurred on all willow cuttings however none of the Okanese poplar cuttings showed any sign of bud break.

After 30 days in the growth chamber, Saltlander plants in the highest treatment soil showed a visible difference in biomass development compared to controls and lower soil treatments. Alfalfa planted in pots with highest treatment soil appeared stressed based on reduced biomass compared to other treatments and yellowing of leaves. Signs of stress in the form of yellowing and curling of young shoots and leaves were apparent in willow at the highest treatment level. Okanese cuttings were deemed nonviable as no growth occurred.

Following the 90 day growth period, only one of the willows planted in the highest treatment soil survived. In the pots containing the medium treatment soil, all willow survived for the duration of the growth period, with growth comparable to slightly lower than that in the low and control soils. Saltlander seeded in pots containing the highest treatment survived and appeared the most vigorous when compared to the other plant types exposed to the high treatment level. Saltlander plants in the high treatment were stunted in comparison to Saltlander exposed to the low and medium soil treatments. Alfalfa planted in the high treatments was minimal, with only three to five stunted plants remaining in each pot at the completion of the trial. The surviving plants were yellowish in color compared to alfalfa in the other treatment groups. Alfalfa exposed to the medium treatment soil exhibited growth and survival visually comparable to the controls and plants subjected to the lowest treatment level. All four plant types survived the 90 day period in soils with the lowest impact level. Plant growth and biomass development in pots with this treatment was visibly equal to, and in some

instances higher than, that of the plants in the control pots. Root development was comparable to above ground development.

2.3.4 Tissue biomass

2.3.4.1 Trial 1 biomass

The results of the ANOVA indicated that there were significant effects of treatment and of species (both $p < 0.001$). There was also a significant interaction between treatment and species ($p = 0.004$), as alfalfa and Saltlander had different responses to the ammonium nitrate treatment than did willow and Okanese.

Tissue biomass for all four plant types following 90 days in the environmental growth chamber varied among plant types and treatment levels (Figure 2.2). Total biomass was greater in soils treated with 100 mg/kg of ammonium nitrate than in control soils, although not significantly, for all plant types. This may indicate nitrogen limiting conditions in the control soil, whereas the slight increase in available nitrogen due to addition of ammonium nitrate at low concentrations allows for ideal plant growth conditions without creating a phytotoxic environment.

No significant difference in total tissue biomass occurred between soils treated with 1000 mg/kg of ammonium nitrate when compared to controls for each plant type. Results from the ANOVA indicate plant tissue biomass was significantly higher for Saltlander compared to willow ($P < 0.001$) and Okanese ($P = 0.002$), and for alfalfa compared to willow ($P = 0.027$) at the 1000 mg/kg treatment level.

A significant decrease in total tissue biomass was observed between soils treated with 4000 mg/kg of ammonium nitrate and control soils for alfalfa ($P = 0.007$), willow ($P = 0.035$) and Okanese ($P < 0.001$). No significant difference between the controls and 4000 mg/kg treatments was seen in Saltlander biomass. These results indicate that concentrations of nitrate at or around 4000 mg/kg are above the threshold of plant nitrogen tolerance for the alfalfa, Okanese and willow varieties examined. Alfalfa and AC Saltlander appear to be more robust over a wide range of treatment levels as there was little change in total

plant biomass between the controls, 100 and 1000 mg/kg treatments. Table 2.3 illustrates the ANOVA table for Trial 1 biomass analysis.

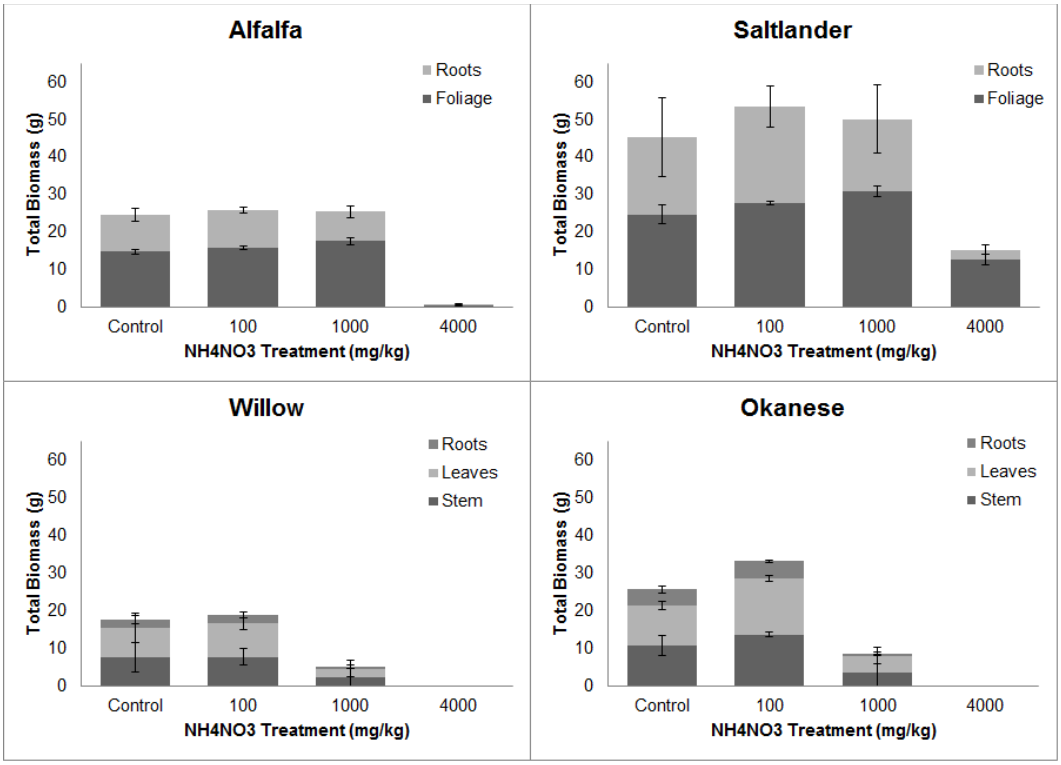

Figure 2.2 Plant tissue biomass for four plant types following 90 days in environmental growth chamber in soils treated with 100, 1000 and 4000 mg/kg of ammonium nitrate.

Table 2.3 ANOVA table for biomass analysis for Trial 1 of phytoremediation growth chamber study.

Source of Variation	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	2393.58	797.86	30.26	< 0.001
Species	3	1695.53	655.18	24.85	< 0.001
Treatment x Species	9	831.00	92.33	3.50	0.004
Residuals	32	843.72	26.37		

2.3.4.2 Trial 2 biomass

The results of the ANOVA indicated that there were significant effects of treatment and of species (both $p < 0.001$). There was also a significant interaction between treatment and species ($p < 0.001$), as alfalfa and Saltlander had different responses to the ammonium nitrate treatment than did willow and Okanese.

Tissue biomass for alfalfa, Saltlander and willow following 90 days in the environmental growth chamber planted in soil from the nitrate fines landfill varied among plant types and treatment levels (Figure 2.3). Total biomass for plants grown in the lowest soil treatment was greater than those grown in the control soil for all plant types, although not significantly.

Plants grown in the medium soil treatment exhibited significantly higher biomass than those grown in the control soil for all plant types (alfalfa $p = 0.01$, AC Saltlander $p < 0.001$, willow $p = 0.025$). Results of the ANOVA indicate plant tissue biomass was significantly higher for Saltlander compared to alfalfa ($p = 0.003$) and willow ($p < 0.001$) at this treatment level.

In the plants grown in soils from the highest treatment of landfill soil, biomass development in Saltlander plants was significantly higher than plants grown in the control soil ($p < 0.001$). Alfalfa plants also had higher biomass in this treatment than controls although not significantly. There was a decrease in biomass development in willows grown in the high treatment soil compared to controls, but this result was also not significant. Alfalfa and Saltlander appeared more robust over a wide range of treatment levels as there was little decrease in biomass development between controls and all three treatment levels. Although willow showed an increase in biomass in the low and medium treatments compared to controls, only two plants in the high treatment levels survived. This indicates that the concentration of nitrate salts and/or other solutes present in the landfill soil, and the resulting increased EC, created conditions that were phytotoxic to willow. Table 2.4 presents the ANOVA table for the Trial 2 biomass analysis.

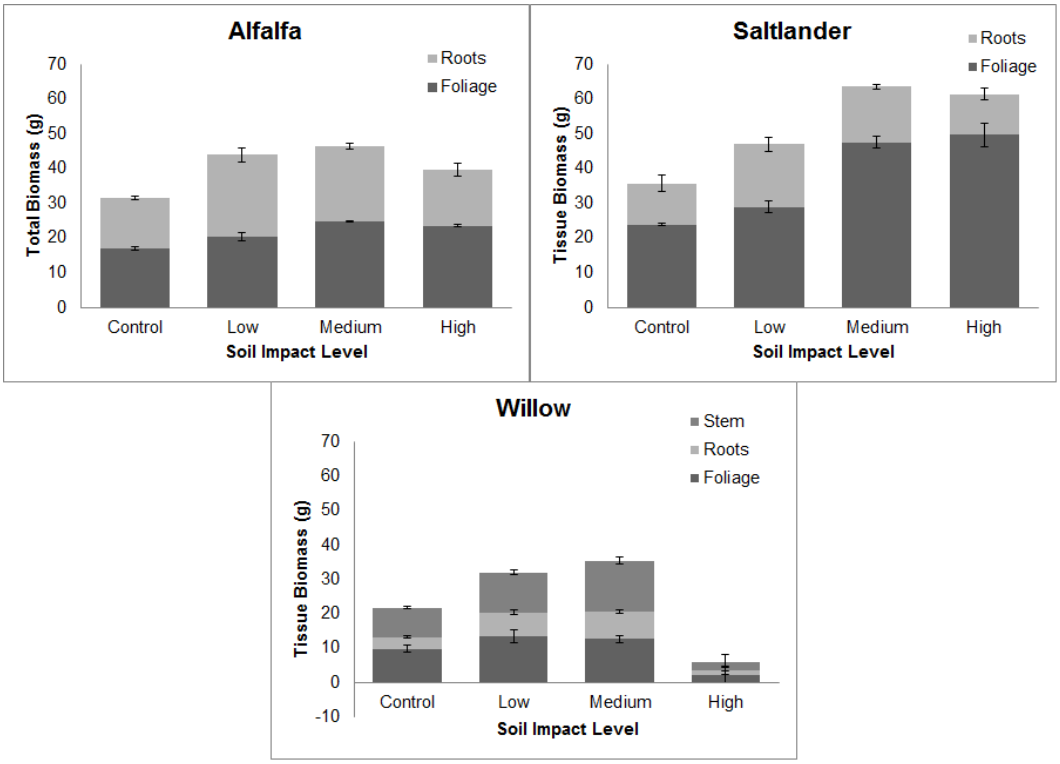

Figure 2.3 Plant tissue biomass for three plant types following 90 days in environmental growth chamber in nitrate impacted soil from nitrate fines landfill.

Table 2.4 ANOVA table for biomass analysis for Trial 2 of phytoremediation growth chamber study.

Source of Variation	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	1829.55	609.85	33.96	< 0.001
Species	2	2856.32	1428.16	79.52	< 0.001
Treatment x Species	6	711.36	118.56	6.60	< 0.001
Residuals	22	395.12	17.96		

2.3.4.3 Biomass discussion

Woody plants may adapt to salinity by osmotic adjustment (Kozlowski 1997). However, Shannon et al. (1994) indicate that while woody plants are generally relatively salt tolerant during seed germination, they become much more sensitive during the emergence and young seedling stages before becoming

progressively more tolerant with increasing age through the reproductive stage. This is consistent with the results of the growth chamber studies, as the Okanese and willow exhibited symptoms of stress and stunted biomass development while in these early stages of development.

The similar biomass development pattern seen in alfalfa and AC Saltlander may be due to similar mechanisms of salt tolerance inherent in these plant types. Alfalfa and many grass species use ion exclusion as a mechanism to cope with salinity toxicity (Scasta et al. 2012; Shannon 1997). Although previous research indicates that this mode of salinity tolerance is applicable to Na^+ ions, it is possible that the same mechanism was used by these plants in the growth chamber to prevent physiological drought due to excess nitrogen and other salts. Although alfalfa and wheatgrass varieties have shown moderate to high salinity tolerance (Mass and Hoffman 1977; Longenecker and Lyerly 1974), Rawlins (1979) reported that a 7% decrease in alfalfa yields can be expected with each dS/m increase in soil EC. This is consistent with the growth chamber results as increasing ammonium nitrate concentrations and the resulting increase in soil salinity caused decreased biomass in both alfalfa and AC Saltlander once salinity reached a certain level in Trial 1. Similarly, in Trial 2 alfalfa and Saltlander responded to the highest treatment with decreases in biomass development.

2.3.5 Plant uptake of excess soil nitrogen

2.3.5.1 Trial 1 nitrogen uptake

Total tissue nitrogen content and plant biomass values were used to calculate plant uptake of soil nitrogen (Figure 2.4). The results of the ANOVA indicated that there were significant effects of treatment and of species (both $p < 0.001$). There was also a significant interaction between treatment and species ($p < 0.001$), as alfalfa and Saltlander had different responses to the ammonium nitrate treatment than did willow and Okanese (Table 2.6).

In soils treated with 100 mg/kg of ammonium nitrate only Saltlander exhibited a significant increase in uptake of soil nitrogen when compared to the control for the same plant type ($P < 0.001$). Okanese, willow and alfalfa all showed

significantly higher tissue nitrogen content per gram of plant biomass than Saltlander at the lowest treatment level (all $P < 0.001$). Comparing controls for all plant types, Saltlander also had significantly lower uptake of soil nitrogen than Okanese, willow and alfalfa ($P < 0.001$ for all comparisons). This indicates that Saltlander has low requirements for nitrogen for biomass development in ideal soil conditions compared to Okanese, willow and alfalfa.

In soils treated with 1000 mg/kg of ammonium nitrate, there was a significant increase in soil nitrogen uptake by Saltlander when compared to the control ($P < 0.001$). Saltlander also had significantly higher nitrogen uptake than willow ($P < 0.001$) and Okanese ($P = 0.016$) in the 1000 mg/kg treatment. The significant difference found for willow and Okanese in soils treated with 4000 mg/kg ammonium nitrate compared to controls for the same plants and to alfalfa and Saltlander at this treatment level must be discounted due to the trees not surviving at all. Saltlander had a significant increase in soil nitrogen uptake at the highest treatment level compared to the control ($P < 0.001$). Soil lab analysis results following the 90 day growth period are presented in Table 2.5.

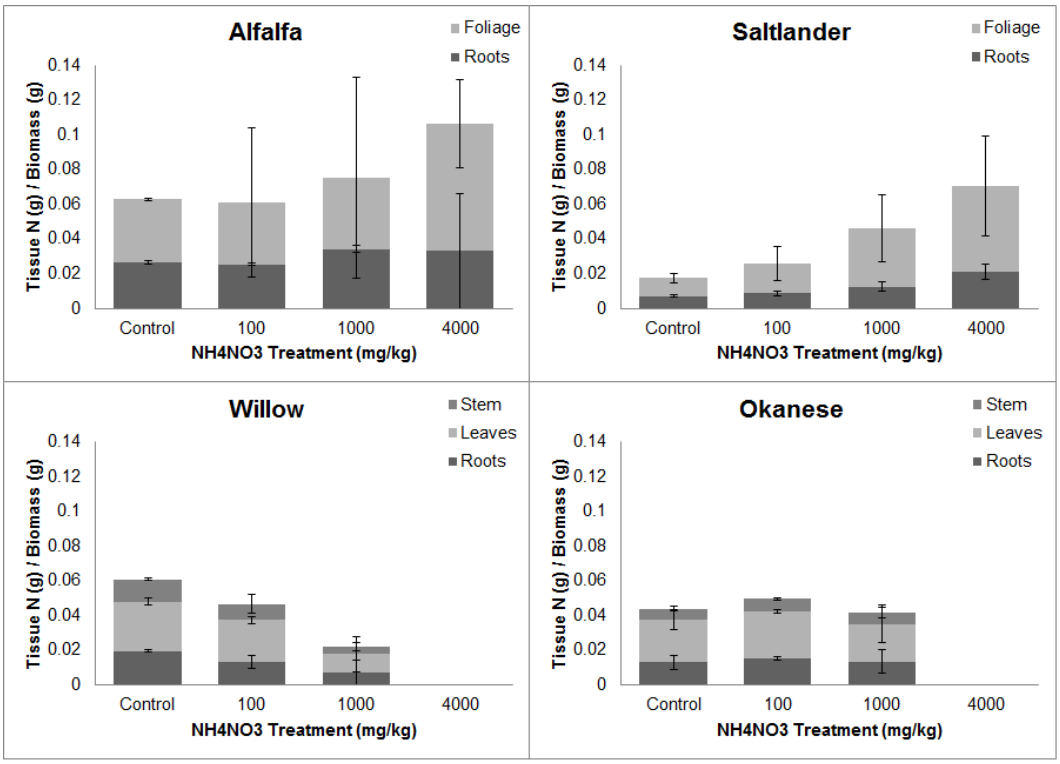

Figure 2.4 Plant uptake of soil nitrogen calculated as total tissue nitrogen per unit of tissue biomass for four plant following 90 days in environmental growth chamber in soils treated with 100, 1000 and 4000 mg/kg of ammonium nitrate.

Table 2.5 Lab analysis results for soil following the first trial of 90 day environmental growth chamber phytoremediation studies.

	NH_4NO_3 Treatment (mg/kg)			
Alfalfa	Control	100	1000	4000
Nitrate-N (kg/ha)	9.80	43.9	1255.8	4965.3
Nitrite-N (kg/ha)	1.59	1.12	0.93	0.98
Ammonium-N (kg/ha)	3.97	4.34	1517.3	1517.5
EC (dS/m)	2.26	2.25	6.30	19.37
pH	6.6	6.5	5.6	5.2
Saltlander				
Nitrate-N (kg/ha)	9.80	14.0	1118.1	1689.3
Nitrite-N (kg/ha)	2.70	2.01	1.68	2.05
Ammonium-N (kg/ha)	0.50	3.22	24.22	290.7
EC (dS/m)	2.38	2.18	7.31	7.85
pH	6.4	6.48	5.4	4.8
Okanese				
Nitrate-N (kg/ha)	13.7	85.4	1675.3	5460.0
Nitrite-N (kg/ha)	1.54	1.35	1.82	0.89
Ammonium-N (kg/ha)	4.71	4.57	6.72	2304.4
EC (dS/m)	2.33	2.47	7.60	20.2
pH	6.6	6.5	5.4	5.1
Willow				
Nitrate-N (kg/ha)	29.4	56.5	877.3	2772.0
Nitrite-N (kg/ha)	1.82	0.84	1.68	1.49
Ammonium-N (kg/ha)	2.29	2.52	3.45	835.3
EC (dS/m)	2.95	2.36	4.97	11.6
pH	6.3	6.5	5.3	4.8

Table 2.6 ANOVA table for plant uptake of soil nitrogen for Trial 1 of phytoremediation growth chamber study.

Source of Variation	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	1829.55	609.85	33.96	< 0.001
Species	2	2856.32	1428.16	79.52	< 0.001
Treatment x Species	6	711.36	118.56	6.60	< 0.001
Residuals	22	395.12	17.96		

2.3.5.2 Trial 2 nitrogen uptake

Total tissue nitrogen content and plant biomass values were used to calculate plant uptake of soil nitrogen for Trial 2 (Figure 2.5). The results of the ANOVA indicated that there were significant effects of treatment and of species (both $p < 0.001$). There was also a significant interaction between treatment and species ($p < 0.001$), as alfalfa and Saltlander had different responses to the ammonium nitrate treatment than did willow and Okanese.

Of plants in the low treatment soil, Saltlander and willow showed a significant increase in uptake of soil nitrogen when compared to the control for the same plant type ($p = 0.014$ and 0.001, respectively). Alfalfa planted in the low treatment soils had significantly higher uptake of nitrogen than Saltlander ($p < 0.001$) and willow ($p = 0.002$). Comparing controls for all three plant types, alfalfa had significantly higher uptake of nitrogen than Saltlander and willow (both $p < 0.001$). Similar to the first trial, Saltlander had less uptake of soil nitrogen in control soils than the other plants. This is indicative of low nitrogen requirements under non stress conditions.

Saltlander and willow plants in the medium treatment soil had significantly higher soil nitrogen uptake when compared to controls (both $p < 0.001$). There were no significant differences between plant types at this treatment level. In the high treatment soils, there was a significant increase in nitrogen uptake between Saltlander ($p < 0.001$) and alfalfa (0.005) and the controls for the same plant. Willow showed a decrease in nitrogen uptake at this treatment level but this result was not significant. Across plant types, no significant differences were seen at the high soil treatment level. Soil lab analysis results following the 90 day growth period are presented in Table 2.7. Table 2.8 is the ANOVA table for Trial 2 nitrogen uptake analysis.

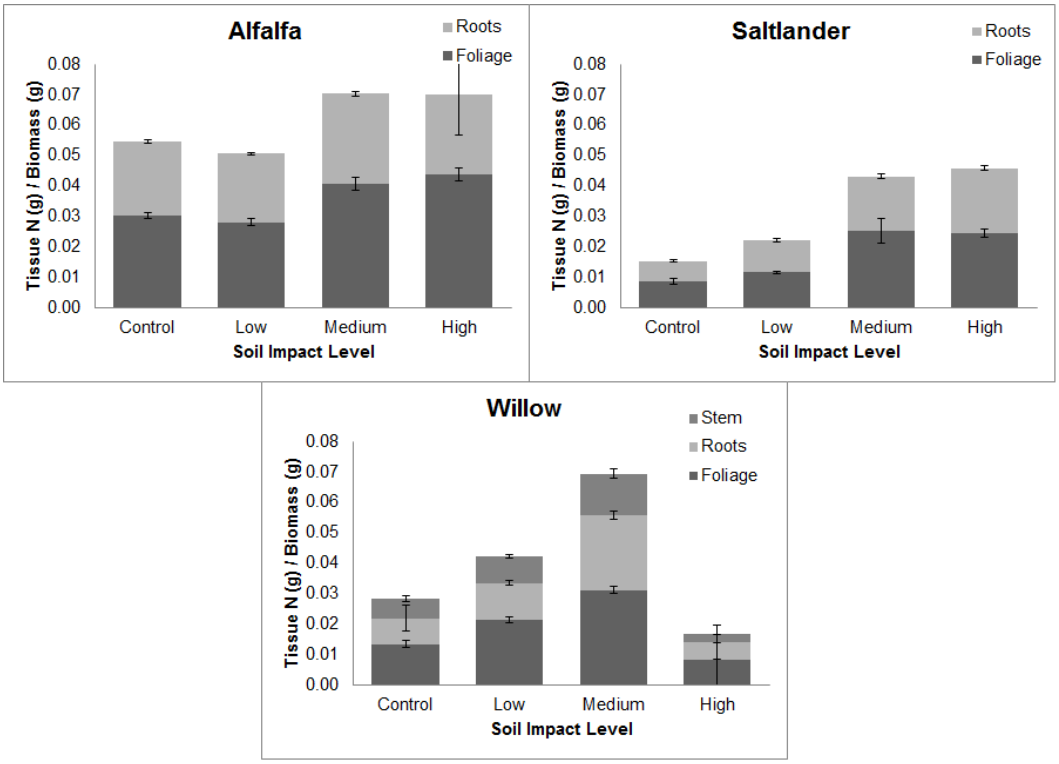

Figure 2.5 Plant uptake of soil nitrogen calculated as total tissue nitrogen per unit of biomass for three plants following 90 days in environmental growth chamber in nitrate impacted soils from nitrate fines landfill.

Table 2.7 Lab analysis results for soil from nitrate fines landfill following the second trial of 90 day environmental growth chamber studies.

	Treatment			
Alfalfa	Control	Low	Medium	High
Nitrate-N (kg/ha)	7.93	64.40	2892.3	4340.0
Nitrite-N (kg/ha)	2.38	2.47	1.82	2.33
Ammonium-N (kg/ha)	2.43	0.42	2.75	0.32
EC (dS/m)	0.93	3.80	8.08	10.55
Saltlander				
Nitrate-N (kg/ha)	7.93	4.20	3752.0	6766.7
Nitrite-N (kg/ha)	0.42	0.52	0.43	0.50
Ammonium-N (kg/ha)	0.13	0.00	1.00	0.37
EC (dS/m)	1.56	5.17	10.14	16.19
Willow				
Nitrate-N (kg/ha)	5.13	93.3	1918.0	5138.0
Nitrite-N (kg/ha)	2.71	2.99	2.29	2.52
Ammonium-N (kg/ha)	2.47	0.51	1.26	5141.5
EC (dS/m)	1.06	3.27	6.77	12.45

Table 2.8 ANOVA table for plant uptake of soil nitrogen for Trial 2 of phytoremediation growth chamber study.

Source of Variation	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	18681.5	6227.2	50.78	< 0.001
Species	2	17739.7	8869.9	72.34	< 0.001
Treatment x Species	6	7428.2	1238.0	10.10	< 0.001
Residuals	22	2697.7	122.6		

2.3.5.3 Plant uptake of soil nitrogen discussion

When considered in conjunction with the plant tissue biomass data, these results indicated that Saltlander and alfalfa are more tolerant to increases in salinity than the woody species in the early stages of growth. Both biomass and soil nitrogen uptake decrease with increases in soil nitrogen for willow and Okanese. It is apparent that the juvenile forms of these woody species are not capable of adapting well to saline conditions and are affected by physiological drought. This is consistent to findings by Papadopoulus and Rendig (1983), where total water and nitrogen uptake in tomato plants was decreased due to increases in soil salinity. As the salts present in the soil due to the addition of ammonium nitrate prevent the movement of water into plant roots, so too do they prevent the uptake of nitrogen. This response was not observed for alfalfa and Saltlander, as in both trials as soil solute concentration increased, so too did plant uptake of soil nitrogen.

There was no apparent trend in the post-trial nitrogen profile with depth from soil surface within the treepots following graphical exploration. Although soil samples were taken from each of three depth increments, a composite averaged value over the entire soil profile of post-trial total nitrogen concentrations was used in analysis.

Results from Trials 1 and 2 presented similar trends in biomass development and soil nitrogen uptake. However, in Trial 2 biomass development and nitrogen were overall higher than in Trial 1. This is likely due to the increased clay content in the Trial 2 soil in comparison to the loamy sand used in Trial 1. As soils with higher

clay contents generally possess higher cation exchange capacity (CEC) and higher buffering capabilities, it is likely that some of the excess soil solutes present in the landfill soil, such as positively charged ammonium ions, were adsorbed to the clay particles. This would result in a decrease in the ionic concentration of the soil solution allowing a more hospitable environment for plant growth. With some of the excess solutes held by clay particles, the plants are able to take up water and nitrate.

It is possible that non-nitrogen nutrient deficiencies were present in the soil used in Trial 1. This would have led to non-ideal growth conditions for the plant types used. It is likely that the increase in other nutrients such as phosphorus and sulfate-S in the landfill soil may have provided a more ideal environment for plant growth resulting in an increase in nitrogen uptake. In addition, the highest treatment in Trial 2 had an approximately concentration of initial nitrogen of 2500 mg/kg. This is below the starting nitrogen concentration for the highest treatment in Trial 1 (4000 mg/kg). As there was much greater biomass development for alfalfa and Saltlander in the highest treatments in Trial 2 than in Trial 1, it is evident that this concentration is below the threshold for nitrogen tolerance of these plant types.

2.3.6 Soil EC and pH

Two noticeable trends occurred in the post-trial soils data following the 90 day growth chamber experiments. As expected, soil EC increased proportionally with increasing concentrations of available soil nitrogen (Figure 2.6). Plant available nutrients are present in the soil in the form of salts, and these salts carry an ionic charge. As ions in solution conduct electricity, it is expected that with an increase in the concentration of nitrogen salts an increase in soil EC would result. This was apparent for both Trial 1 and Trial 2 of the growth chamber experiments.

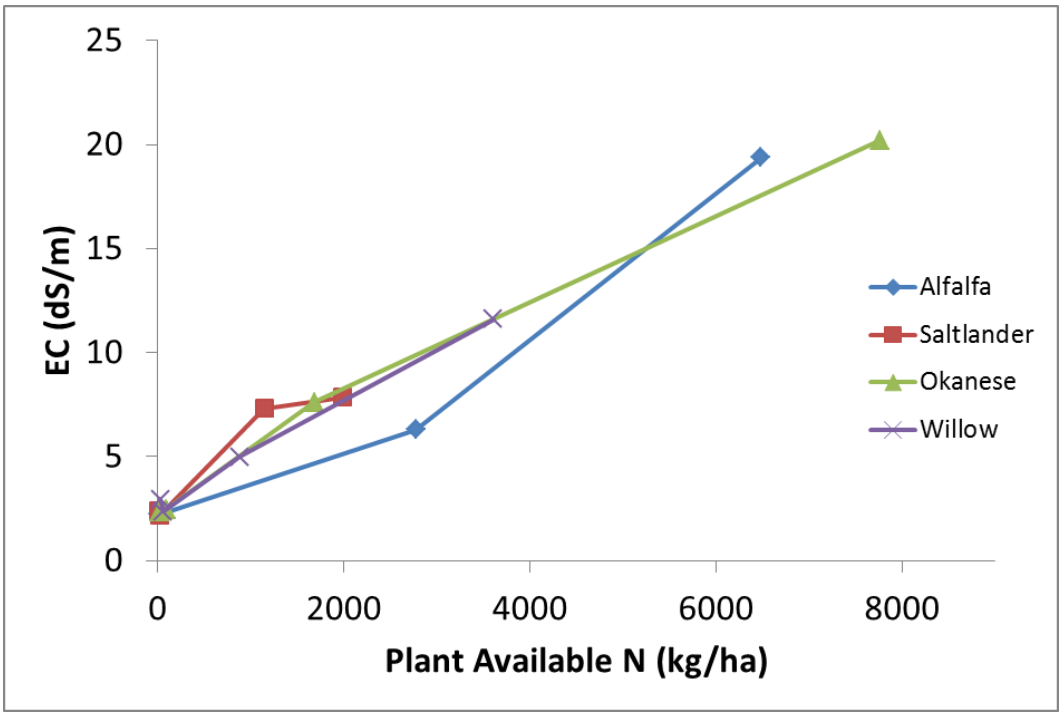

Figure 2.6 Change in soil EC with increases in plant available nitrogen in the soil following 90 days in an environmental growth chamber for four plant types (Trial 1).

There was also a trend in soil pH following the 90 day growth period for Trial 1 (Figure 2.7). pH was not sampled post-trial for Trial 2. With increases in available nitrogen, a subsequent decrease in soil pH was observed. As nitrogen fertilizer concentration increases in the soil, nitrification occurs involving the conversion of ammonium ions to nitrate and hydrogen ions. The presence of these excess hydrogen ions results in soil acidification. It is likely that this increase in soil acidity with increases in available nitrogen concentration added to the decrease in plant survival and biomass development in the higher treatment soils.

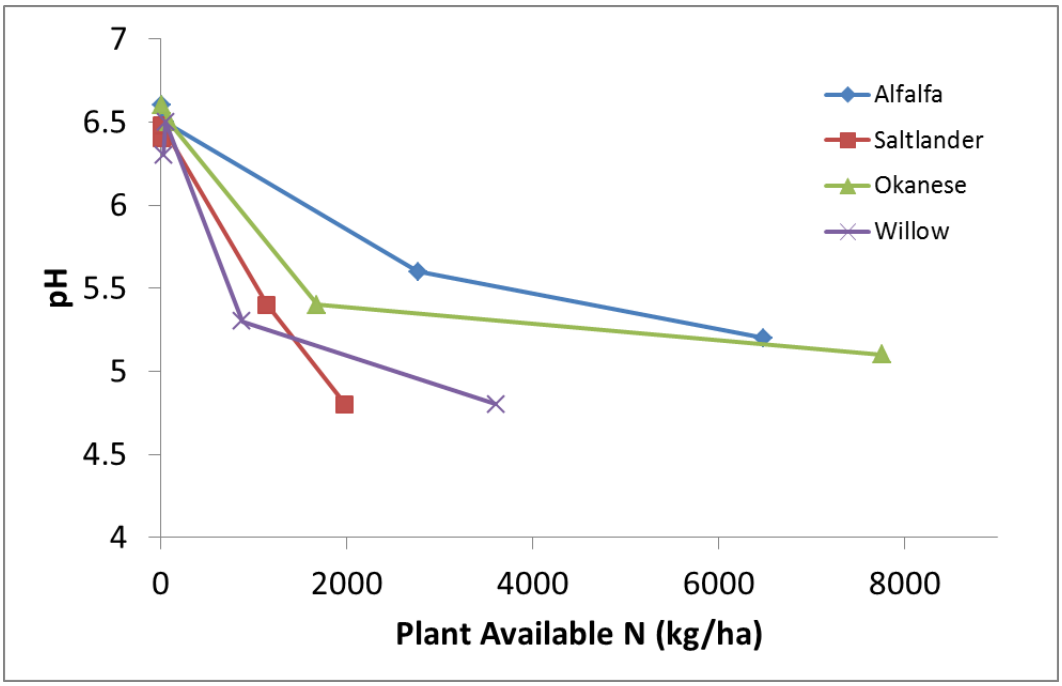

Figure 2.7 Change in soil pH with increases in plant available nitrogen in the soil following 90 days in an environmental growth chamber for four plant types (Trial 1).

2.3.7 Nitrogen balance

Total nitrogen supply is generally distributed between three major pools: the atmosphere, the soil (and associated groundwater) and nitrogen contained within the biomass (Hopkins and Huner 2004). In the growth chamber environment, soil nitrogen levels resulted from inputs due to fertilizer addition and N fixation by alfalfa, and outputs due to plant uptake and gaseous loses. Mineralization of organic nitrogen within the soil also resulted in changes in measurable total mineral nitrogen. Quantification of the nitrogen balance within the environmental growth chamber system is as follows:

$\Delta N_a = N_f - N_i = NH_4NO_3$ addition + mineralization – plant removal – other losses

where ΔN_a is the change in plant available N (kg/ha), N_f is available N after harvest (kg/ha), and N_i is the initial available N.

All components within the system are known with the exception of mineralization and other losses (i.e. denitrification, immobilization) which can be grouped together as such:

ΔN_a = [NH_4NO_3 addition – plant removal] + [mineralization - other losses]

Rearranging the above equation results in the following relationship:

[mineralization - other losses] = ΔN_a – [NH_4NO_3 addition – plant removal]

If [mineralization - other losses] is a negative value, the losses (denitrification or immobilization) were greater than mineralization. It is important to note that losses are from the available nitrogen pool and not necessarily from the soil system. Gaseous losses due to denitrification do result in losses from the soil system, however immobilization losses deem N present but unavailable for plant use. These loses could not be quantified for the growth chamber system as organic nitrogen and gaseous loses were not measured.

2.3.7.1 Trial 1 nitrogen balance

Nitrogen balance data for all plant types for Trial 1 is presented in Table 2.9. Completion of a correlation matrix (Table 2.10) results in the following noteworthy correlations:

- Ammonium nitrate additions leading to increased soil salinity are positively correlated with plant nitrogen uptake for alfalfa and Saltlander, but negatively correlated for Okanese and willow, suggesting that alfalfa and Saltlander may have an increase in salt tolerance in the presence of excess nitrogen. Therefore, alfalfa and Saltlander are potentially good candidates for remediation under extreme saline conditions. The ANOVA previously discussed provides defensible support of this correlation. Papadopoulos and Rendig (1983) observed similar effects in Bermuda grass (*Cynodon dactylon* L) and clover (*Trifolium alexandrinum*) where an apparent increase in salt tolerance was noted when nitrogen levels supplied under saline conditions exceeded those that were optimum under nonsaline conditions.

- Ammonium nitrate additions are negatively correlated to [mineralization – other losses] for all plant varieties indicating that the greater the addition of ammonium nitrate the greater the unaccountable nitrogen losses.
- Plant uptake is negatively correlated to [mineralization – other losses] for alfalfa and Saltlander, but positively correlated for Okanese and willow. This indicates that the greater plant nitrogen uptake by alfalfa and Saltlander are somewhat able to offset nitrogen losses.

Table 2.9 Nitrogen balance in environmental growth chamber following 90 day phytoremediation Trial 1.

Variety	Treatment	$\Delta N = N_f - N_i$ (kg/ha)	NH_4NO_3 Addition (kg/ha)	Plant Uptake (kg/ha)	[NH_4NO_3 Addition - Plant Uptake] (kg/ha)	[Mineralization - Other Losses] (kg/ha)
Alfalfa	Control	-8.0	0.0	10.3	-10.3	2.3
	100 mg/kg	-121.1	147.0	10.1	136.9	-258.0
	1000 mg/kg	-541.3	1470.0	12.5	1457.5	-1998.8
	4000 mg/kg	580.3	5880.0	23.8	5856.2	-5275.9
Saltlander	Control	-12.4	0.0	2.8	-2.8	-9.7
	100 mg/kg	-156.0	147.0	4.1	142.9	-298.9
	1000 mg/kg	-635.4	1470.0	8.0	1462.0	-2097.4
	4000 mg/kg	-3921.3	5880.0	14.4	5865.6	-9786.9
Okanese	Control	-3.5	0.0	8.6	-8.6	5.1
	100 mg/kg	-101.9	147.0	10.1	136.9	-238.8
	1000 mg/kg	50.5	1470.0	8.6	1461.4	-1411.0
	4000 mg/kg	-79.4	5880.0	0.0	5880.0	-5959.4
Willow	Control	1.8	0.0	12.1	-12.1	13.8
	100 mg/kg	-125.5	147.0	9.8	137.2	-262.7
	1000 mg/kg	-831.5	1470.0	4.4	1465.6	-2297.1
	4000 mg/kg	-2294.6	5880.0	0.0	5880.0	-8174.6

Table 2.10 Correlation matrix of nitrogen balance data for four plant types following Trial 1 of a phytoremediation study in an environmental growth chamber.

Alfalfa	$\Delta N = N_f - N_i$	NH_4NO_3 Additions	Plant Uptake	[NH_4NO_3 Addition - Plant Uptake]	[Mineralization - Other Losses]
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	0.7246	1			
Plant Uptake	0.7776	0.9965	1		
[NH_4NO_3 Addition - Plant Uptake]	0.7245	1.0000	0.9965	1	
[Mineralization - Other Losses]	-0.6277	-0.9913	-0.9773	-0.9914	1
Saltlander	$\Delta N = N_f - N_i$	NH_4NO_3 Additions	Plant Uptake	[NH_4NO_3 Addition - Plant Uptake]	[Mineralization - Other Losses]
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	-0.9949	1			
Plant Uptake	-0.9562	0.9790	1		
[NH_4NO_3 Addition - Plant Uptake]	-0.9950	1.0000	0.9789	1	
[Mineralization - Other Losses]	0.9982	-0.9992	-0.9710	-0.9992	1
Okanese	$\Delta N = N_f - N_i$	NH_4NO_3 Additions	Plant Uptake	[NH_4NO_3 Addition - Plant Uptake]	[Mineralization - Other Losses]
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	-0.2698	1			
Plant Uptake	0.3019	-0.9755	1		
[NH_4NO_3 Addition - Plant Uptake]	-0.2698	1.0000	-0.9756	1	
[Mineralization - Other Losses]	0.2932	-0.9997	0.9763	-0.9997	1
Willow	$\Delta N = N_f - N_i$	NH_4NO_3 Additions	Plant Uptake	[NH_4NO_3 Addition - Plant Uptake]	[Mineralization - Other Losses]
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	-0.9936	1			
Plant Uptake	0.9597	-0.9224	1		
[NH_4NO_3 Addition - Plant Uptake]	-0.9936	1.0000	-0.9226	1	
[Mineralization - Other Losses]	0.9967	-0.9995	0.9341	-0.9995	1

2.3.7.2 Trial 2 nitrogen balance

Nitrogen balance data for the three plant types for Trial 2 is presented in Table 2.11. Completion of a correlation matrix (Table 2.12) reveals similar correlations as observed in Trial 1:

- As in Trial 1, increases in soil solute concentration (nitrogen from nitrate fines and other solutes) leading to increased soil salinity is positively correlated with plant nitrogen uptake for alfalfa and Saltlander, but negatively correlated for Okanese and willow, suggesting that alfalfa and Saltlander may have an increase in salt tolerance in the presence of excess nitrogen. Therefore, alfalfa and Saltlander are potentially good candidates for remediation under extreme saline conditions. Though present, these correlations are not as strong as in Trial 1.
- Soil solute concentration is negatively correlated to [mineralization – other losses] for all plant varieties indicating that the greater the concentration of nitrate and other solutes the greater the unaccountable nitrogen losses. This correlation is not as strong as the ammonium nitrate additions versus final available nitrogen correlation in Trial 1.
- Plant uptake is negatively correlated to [mineralization – other losses] for alfalfa and Saltlander, but positively correlated for Okanese and willow. This indicates that the greater plant nitrogen uptake by alfalfa and Saltlander somewhat offsets nitrogen losses.

Table 2.11 Nitrogen balance in environmental growth chamber following 90 day phytoremediation Trial 2.

Variety	Treatment	$\Delta N = N_f - N_i$ (kg/ha)	N from Nitrate Fines (kg/ha)	Plant Uptake (kg/ha)	[NH_4NO_3 Addition - Plant Uptake] (kg/ha)	[Mineralization - Other Losses] (kg/ha)
Alfalfa	Control	-45.2	57.96	274.9	-217.0	171.8
	Low	-291.8	359.1	350.4	8.74	-300.5
	Medium	-1918.1	4515.0	524.1	3990.9	-5909.0
	High	-6864.6	11207.3	447.4	10759.8	-17625.5
Saltlander	Control	-47.7	58.0	89.7	-31.8	-15.9
	Low	-352.7	359.1	165.5	193.6	-546.3
	Medium	-757.0	4515.0	469.0	4046.0	-4803.0
	High	-4438.1	11207.3	466.8	10740.5	-15178.6
Willow	Control	-47.6	57.96	52.5	5.4	-53.1
	Low	-262.3	359.1	119.3	239.8	-502.1
	Medium	-2593.5	4515.0	190.4	4324.6	-6918.0
	High	-925.3	11207.3	24.9	11182.4	-12107.7

Table 2.12 Correlation matrix of nitrogen balance data for three plant types following Trial 2 of phytoremediation study in environmental growth chamber.

Alfalfa	$\Delta N = N_f - N_i$	*N from Nitrate Fines*	*Plant Uptake*	*[NH_4NO_3 Addition - Plant Uptake]*	*[Mineralization - Other Losses]*
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	-0.9905	1			
Plant Uptake	-0.5309	0.6344	1		
[NH_4NO_3 Addition - Plant Uptake]	-0.9924	0.9999	0.6216	1	
[Mineralization - Other Losses]	0.9971	-0.9981	-0.5880	-0.9989	1
Saltlander	$\Delta N = N_f - N_i$	*N from Nitrate Fines*	*Plant Uptake*	*[NH_4NO_3 Addition - Plant Uptake]*	*[Mineralization - Other Losses]*
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	-0.9624	1			
Plant Uptake	-0.6742	0.8413	1		
[NH_4NO_3 Addition - Plant Uptake]	-0.9676	0.9998	0.8295	1	
[Mineralization - Other Losses]	0.9835	-0.9956	-0.7899	-0.9973	1
Willow	$\Delta N = N_f - N_i$	*N from Nitrate Fines*	*Plant Uptake*	*[NH_4NO_3 Addition - Plant Uptake]*	*[Mineralization - Other Losses]*
$\Delta N = N_f - N_i$	1				
NH_4NO_3 Addition	-0.3743	1			
Plant Uptake	-0.7041	-0.3276	1		
[NH_4NO_3 Addition - Plant Uptake]	-0.3626	0.9999	-0.3403	1	
[Mineralization - Other Losses]	0.5306	-0.9846	0.1680	-0.9823	1

2.3.7.3 Nitrogen balance discussion

According to the nitrogen balance results, the nitrogen loss was always an order of magnitude or more greater than plant nitrogen uptake. Much of this loss is likely due to denitrification. As rates of denitrification are known to be higher at higher soil moisture contents (Machefert and Dise 2004), this may explain some of the increased nitrogen losses with increasing ammonium nitrate concentrations. Soil water tends to moderate oxygen diffusion in the soil and denitrification generally only occurs at soil moisture contents of greater than 60% water-filled pore space (Mosier et al. 2002). In the control treepots for Trial 1, 20% water content and an assumed bulk density of 1.4 kg/m^3 calculates to a volumetric water content of 28%. Porosity of the soil (calculated as $1 – \rho_b/\rho_p$) is 47% and the resulting water filled pore space is approximately 60%. With increasing ammonium nitrate additions, reduction in plant biomass occurred resulting in there being less plant uptake of soil water and increases in water filled pore space. As all treatments were watered equally, pots with higher concentrations of ammonium nitrate were consistently moister than lower level treatments between watering periods. This was particularly evident during the second half of the growth chamber trial when biomass in the lower level treatments was significantly higher than in high level treatments and plant water uptake was high. The same can be said for Trial 2, particularly in the willow and alfalfa treepots with the high treatment soils.

In addition to denitrification, it is likely that some of the nitrogen loss in the treepots was likely due to immobilization. Although this is not a loss from the soil system, it is a decrease in the available nitrogen pool and because levels of organic nitrogen were not analyzed, rates of immobilization are unknown. It is likely though that microbial biomass was increased due to the presence of excess nitrogen and carbon excreted via plant roots. Because root development was less in plants treated with high levels of ammonium nitrate, immobilization is assumed to have been a less significant mechanism for reductions in available soil nitrogen than denitrification.

2.4 Conclusions

Environmental growth chamber experiments were designed to test the feasibility of using Okanese poplar, *Salix bebbiana* (beaked willow), *Medicago sativa var.* AC Nordica (alfalfa) and AC Saltlander grass as phytoremediation varieties to treat soils impacted with excess nitrogen salts. Results indicate that all plant types can survive in soils with some degree of excess nitrogen, but that 4000 mg/kg of ammonium nitrate is above the tolerance levels of all plant types except Saltlander grass. Results indicate that the coping mechanisms of alfalfa and AC Saltlander against salinity may lead to higher survival rates and biomass development than those of the woody plants investigated, as alfalfa and AC Saltlander were more robust over a wider range of nitrogen concentrations.

Phytoremediation may be more applicable to soils with higher clay contents due to adsorbtion of excess ions to clay surfaces resulting in a soil solution more hospitable for plant growth. This allows for more likely uptake of water and soil nitrogen. It is evident that nitrogen loss, likely due to atmospheric denitrification or immobilization, occurs at rates greater than plant uptake of nitrogen.

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3. PHYTOREMEDIATION OF NITROGEN IMPACTED GROUNDWATER

3.1 Introduction

Saline water is used to irrigate croplands in many parts of the world, particularly in regions with poor water quantity and quality (Huang et al. 2011). Concerns related to the use of salt-affected water as an irrigation source include the accumulation of salts in the soil and the effect on soil properties. The concentration of ions in soil layers can affect the dispersion of clay particles (Shainberg and Letey 1983), soil hydraulic conductivity and soil aggregate stability (Huang et al. 2011). The results of salt accumulation in the soil due to irrigation with saline water may lead to vegetation stress, desertification and environmental degradation (Wang and Cui 2004). However, the negative impacts of saline irrigation water can be mitigated by implementing appropriate management of saline water and soil (Huang et al. 2011).

The successful use of low quality water requires the selection of salt tolerant crops, the application of a suitable water management strategy and the choice of the most appropriate irrigation system (Malash et al. 2008). Water management, soil type and salinity distribution can all affect vegetation growth and crop productivity. According to Malash et al. 2008, irrigation water can be used as a mixture of saline water with fresh water, and the use of saline water for irrigation has environmental advantages as it reduces the fresh-water requirements for salt-tolerant species and decreases the volume of impacted water requiring disposal or treatment.

The majority of residual inorganic nitrogen, especially in the form of nitrates, is water soluble (De Jong et al. 2009). Therefore, the risk of nitrate leaching from soil to groundwater is high and nitrogen-laden groundwater is a well-documented risk to human and ecosystem health in rural areas of North America (Russelle et al. 2001). Nutrient removal and transformation processes in subsurface water include microbial conversion, decomposition, plant uptake, sedimentation, volatilization and adsorption-fixation reactions (Tchobanoglous 1993). The contribution of plants in removing nutrients varies with the nature of the impacted medium and the surrounding environment (Huett et al. 2005). Studies by Tanner

et al. (1995) and Hunter et al. (2001) indicated that improved nutrient removal was reported where plants were present in comparison to water treatment systems composed of gravel substrates alone.

This chapter will outline and describe the environmental growth chamber experiment developed to examine the prospect of using phytoremediation as an option to treat groundwater impacted by nitrate fines from historical fertilizer production.

3.1.1 Research objectives and hypothesis

Historical dumping of nitrate fertilizer fines has resulted groundwater nitrogen concentrations up to 24,000 mg/L ammonium-N and 7000 mg/L nitrate-N at the Agrium facility near Redwater, Alberta (Stantec 2001). These nitrogen compounds may be harmful to ecosystem health and impacts to the adjacent North Saskatchewan River are of concern.

The objective of this research was to determine the viability of using four plant varieties to treat nitrogen fertilizer impacted groundwater via phytoremediation. This was accomplished through an environmental growth chamber study examining the growth of Okanese poplar (Walker x *P. xpetrowskyana)*, beaked willow (*Salix bebbiana)*, alfalfa (*Medicago sativa var.* AC Nordica) and AC Saltlander (*Agropyron spicatum* (bluebunch wheatgrass) x *Agropyron repens* (quackgrass)) grass in, and the subsequent removal of nitrogen compounds from impacted groundwater located beneath the nitrate fines landfill at the Agrium fertilizer facility in Redwater, Alberta. Specific research objectives were as follows:

- Characterize growth and survival for each plant type in soil irrigated with nitrate impacted groundwater.
- Determine which plant type is most tolerant to increases in soil electrical conductivity due to the addition of saline groundwater.
- Determine whether plants can offset the increase in soil electrical conductivity due to saline irrigation water through root uptake of nitrogen salts.

Hypotheses regarding successful plant growth in nitrogen impacted soils, plant nitrogen tolerance and phytoremediation potential include the following:

- AC Saltlander grass will have the most successful growth and highest biomass development when irrigated with nitrate impacted groundwater due to its previously documented saline tolerance.
- All plant types will tolerate irrigation with groundwater having low concentrations of nitrate and will exhibit good biomass development and survival rates at such levels.
- Okanese poplar and willow will best offset the increase in soil electrical conductivity in soils irrigated with low to moderately concentrated groundwater due to their high rate of water use. As water is taken up by the roots, the excess nitrogen salts will follow and therefore not remain in the soil.

3.2 Methodology

3.2.1 Preliminary sampling

In the spring of 2010 and 2011 prior to initiation of the environmental growth chamber study, two groundwater sampling events occurred in and around the nitrate fines landfill. Groundwater samples were taken from 13 wells and sent to a commercial lab for analysis of the following parameters: ammonium, orthophosphate, organic carbon, uranium, TDS, pH, EC, chloride, fluoride, nitrate, nitrite and sulphate. Results from the preliminary groundwater sampling are presented in Table 1. A geophysical investigation including EM31, EM38 and ERT was completed of the landfill and surrounding area. A comprehensive description of the preliminary groundwater sampling and subsequent soil sampling of the landfill area can be found in Chapter 2.

3.2.2 Vegetation selection and environmental growth chambers

Based on the rate of water use, rapid growth capability and tolerance to salinity, four vegetation types were selected for the growth chamber study. Plants selected for the phytoremediation experiment included Okanese poplar ((*Populus deltoids x Populus xpetrowskyana*) x *P. xpetrowskyana)*, *Salix bebbiana* (beaked willow), *Medicago sativa var.* AC Nordica (alfalfa) and AC Saltlander grass

(*Agropyron spicatum* (bluebunch wheatgrass) x *Agropyron repens* (quackgrass)). Details of each selection and applicability of each plant type as potential phytoremediation candidates is available in Chapter 2.

The 90 day experimental trial was conducted in an environmental growth chamber located at the Agrium fertilizer facility in Redwater, Alberta. Conditions within the chamber were set to mimic natural environmental conditions at the landfill site. Trial duration was similar to an average growing season in the Redwater area. A detailed description of the growth chamber environment for the duration of the trial can be found in Chapter 2.

3.2.3 Experimental design and irrigation treatments

Loamy sand soil was collected from a topsoil pile near the research site and sent to a commercial lab to test for background salinity, available nutrients and cation exchange capacity. Results of the background soil analysis are available in Chapter 2 - Table 2.1. The bulk soil was screened and air-dried in soil drying trays for two weeks following collection. Once dry, 10 kilograms of soil was added to 20 by 40 cm round treepots. 2000 ml of tapwater was added to each pot to reach 20 percent initial water content. This starting value was selected due to the sandy texture of the treated soil. A bulk density of 1.4 g/cm^3 was assumed based on the texture of the soil.

Following soil preparation, 20 cm willow and Okanese poplar cuttings were inserted into the center of 24 pots. Alfalfa and Saltlander seed were applied using a broadcast seeding method to the remaining 24 pots. For detailed information on cutting preparation and seeding rate see Chapter 2. Three repetitions of each vegetation type – contaminant concentration combination were prepared for a total of 48 experiment units. The prepared pots were weighed and placed in a random arrangement in the environmental growth chamber on July 4, 1011. To maintain 20 percent water content, the control pots were weighed biweekly and all pots were watered as required to maintain initial weight. For the first three weeks of the growth chamber trial, only regular tap water was applied to the pots as required to allow for seedling emergence and establishment of all plant types prior to treatment with impacted groundwater.

One monitoring well from within the nitrate fines landfill site was bailed twice weekly to purge stagnant water using Waterra tubing. Approximately three litres of groundwater was then pumped into clean five gallon pails by the same method and transported to the growth chamber area within the Agrium facility. Water was collected from the same well for the entire trial duration. Monitoring well 00-9-4 was selected as the source for irrigation water based on results from the previous spring groundwater monitoring events. A sample of the collected groundwater was sent to Exova for analysis and recording of preliminary groundwater parameters. The collected groundwater was mixed with tap water at three different dilution rates to create irrigation treatments. Treatments included 1:1 (high), 3:1 (medium) and 9:1 (low) groundwater to tap water combinations. Tap water only was used as a control treatment for the irrigation study. Biweekly weighing of the treepots to maintain initial water content was continued for the remainder of the 90 day phytoremediation trial. The pots were periodically rearranged to avoid possible growth chamber effects.

3.2.4 Electrical conductivity monitoring

Soil electrical conductivity (EC) was calculated throughout the irrigation trial by measuring conductivity of the soil using an YSI Model 33 S-C-T time domain reflectrometer (TDR). The TDR was first calibrated for use on the soil in the treepots. This was accomplished by preparing a 2:1 saturated paste of soil and deionized water and treating it with measured amounts of 150 g/L calcium chloride solution. The calcium chloride solution was added to five soil columns containing the saturated paste at rates equivalent to 0, 5, 10, 15 and 20 dS/m. The columns were covered and after 24 hours, EC readings were recorded and a calibration curve created. Plotting EC against the inverse of conductivity for the treepot soil resulted in the following relationship:

$$y = 451.23x + 6.6859$$

where x is the inverse of soil resistivity measured in 1/ohm and y is soil EC measured in dS/m.

Soil conductivity was measured by inserting the TDR probes horizontally into the soil profile through predrilled holes in the treepots. Two conductivity

measurements were taken following each irrigation event, one within the upper 15 centimetres of the profile and one from 15 to 30 centimetres below soil surface. A 10 minute lag time following irrigation prior to conductivity measurement occurred to allow for infiltration of the irrigation water throughout the entire soil column within the treepots. Using the above equation, soil EC was monitored for the duration of the irrigation study.

3.2.5 Post trial sampling

3.2.5.1 Soil sampling

Following the 90 day growth period, one composite soil sample was taken from each treepot. Soil was collected by cutting and removing a panel from the side of each pot to expose the entire soil profile. Once screened to remove roots, a grab sample of approximately 100 g of soil was placed in a large Ziploc bag and sealed. Soil samples were taken to a commercial lab and analyzed for various salinity parameters, notably nitrate and nitrite-N and EC, as well as the available nutrients phosphorus and ammonium-N.

3.2.5.2 Vegetation sampling

Above and below ground biomass was measured. Live vegetation in each treepot was clipped to ground level using hand clippers and placed in paper bags. In the case of the willows and Okanese poplar, foliage was separated from the branches and stems. Roots were collected by passing the dry soil from each treepot through a sieve to remove the bulk of the soil. The roots were then hand washed over a fine sieve to remove any remaining soil particles and placed into paper bags. Any material remaining from the original cuttings were disregarded, as cuttings were not identical in size initially. Above and below ground plant material were oven dried for 48 hours at 60°C (Whalen et al. 2003) and weighed on an electronic scale to acquire measurements of biomass. The root biomass values were used for comparison purposes only, and cannot be considered exact measurements. This is due to the possible loss of very fine roots during the washing process. Dried plant material was sent to a commercial lab to be analyzed for nitrogen content. Dead plant material was not retained and analyzed.

3.3. Results and Discussion

3.3.1 Preliminary groundwater sampling results

Laboratory results from water samples collected from wells within the nitrate fines landfill are presented in Table 3.13.

Table 3.13 Analytical results of parameters measured from groundwater sampled from monitoring well within nitrate fines landfill prior to dilution for growth chamber irrigation treatments.

Analyte		Units	Results
Ammonia-N		mg/L	19,900
Kjeldahl Nitrogen	Total	mg/L	22,900
Nitrogen	Total	mg/L	32,200
Organic Nitrogen	Total	mg/L	3,000
Orthophosphate-P	Dissolved	mg/L	5,360
Organic Carbon	Total Nonpurgeable	mg/L	42.5
pH			6.92
Temperature		°C	22.4
Electrical Conductivity		µS/cm	111,000
Calcium	Dissolved	mg/L	<40
Magnesium	Dissolved	mg/L	<40
Sodium	Dissolved	mg/L	1,500
Potassium	Dissolved	mg/L	1,500
Iron	Dissolved	mg/L	2.4
Manganese	Dissolved	mg/L	<1
Chloride	Dissolved	mg/L	1,600
Nitrate-N		mg/L	9,300
Nitrite-N		mg/L	<1
Nitrate and Nitrite-N		mg/L	9,300
Sulfate (SO_4)	Dissolved	mg/L	22,600
Hydroxide		mg/L	<5
Carbonate		mg/L	<6
Bicarbonate		mg/L	13,300
P-Alkalinity	As $CaCO_3$	mg/L	<5
T-Alkalinity	As $CaCO_3$	mg/L	10,900
TDS	Calculated	mg/L	59,000
Hardness	Dissolved as $CaCO_3$	mg/L	<300
Ionic Balance	Dissolved	%	109

3.3.2 Plant growth and survival

Irrigation was first applied on July 4, 2011. Within three days of treatment application, signs of stress were visible in the alfalfa, Okanese and willow pots. In the treepots treated with the medium (3:1) and high (1:1) irrigation treatments, leaves on the woody plants were slightly brown in color and flaccid. The alfalfa plants were also drooping. In the pots irrigated with the low (9:1) treatment, brown spots were visible of the Okanese and willow leaves while alfalfa was comparable to the control plants. No visible differences in plant growth were observed between treatments in the AC Saltlander pots.

Following 26 days in the environmental growth chamber with biweekly irrigation events, no willow, Okanese or alfalfa plants subjected to the high concentration treatment remained alive. AC Saltlander exposed to the high treatment displayed stunted growth compared to plants in all other treatments with some minor yellowing of tissue. In the pots irrigated with the 3:1 treatment, only a few green leaves remained on the Okanese and willow plants and two out of three alfalfa treepots exhibited severe necrosis. AC Saltlander in the 3:1 treatment were comparable to both low and control pots. In the low treatment, the willow and Okanese plants were similar in size to the controls with some brown spotting observed on leaves. Alfalfa subjected to the low treatment was comparable to the control in color although growth was slightly stunted.

Forty days after initial irrigation all vegetation subjected to the medium and high irrigation treatments was dead with the exception of the AC Saltlander grass which exhibited little remaining green tissue in the high treatments and stunted growth compared to the control in the medium treatments. Alfalfa irrigated with the low concentration water was stunted compared to the control although tissue remained green. Okanese in the low treatments pots showed signs of severe stress as evident by flaccid and yellowing leaves, many spotted black and brown. At the conclusion of the growth chamber experiment, following the 90 day growth period and subjected to irrigation with impacted groundwater from the nitrate fines landfill, tissue samples were only collected the control and low treatment pots for alfalfa, willow and Okanese. All other plant tissue was dead. AC Saltlander tissue was also collected for pots irrigated with the medium

concentration treatment. In the low treatment willow plants, only upper leaves remained and exhibited extreme blackening of the petioles. Only AC Saltlander grass had tissue biomass samples comparable to controls.

3.3.3 Soil electrical conductivity monitoring and post irrigation soil analysis

The soil electrical conductivity profile over time was similar for all plant types (Tables 3.6 - 3.9). The electrical conductivity of soils irrigated with the control irrigation treatment remained between 7 and 9 dS/m for the duration of the trial. There was a gradual increase in soil EC for plants irrigated with the lowest irrigation treatment, with all plant types showing similar rates of increase. While soil EC measurements were higher for all plant types in the early stages of the trial, EC was higher at depth upon final measurements. Electrical conductivities ranged from 14 dS/m to 39 dS/m over time in the upper 15 cm, and from 11 dS/m to 35 dS/m in the 15 to 30 cm depth range.

A similar gradual increase in soil EC was recorded for plants irrigated with the medium treatment. As well, EC measurements were always higher at the surface early on in the trial but higher at depth upon trial completion. This is indicative of the downward movement of solutes with water percolation being more prevalent than any upward movement due to plant root activity and evaporation. Electrical conductivities ranged from 14 dS/m to 39 dS/m over time in the upper profile, and from 14 dS/m to 46 dS/m at depth.

Where the highest irrigation treatment was applied, a more drastic increase in soil electrical conductivity was observed over time for all plant types. In the upper 15 cm, conductivities measurements ranged over time from 26 dS/m to 89 dS/m, while a range of 13 dS/m to 71 dS/m was observed from 15-30 cm. The lower rate of increase in EC in the low and medium irrigation treatments may be due to some uptake of solutes by the plants throughout their growing period. In the case of the plants irrigated with the highest treatment, the concentration of solutes present in the water resulted in a phytotoxic environment leading to plant death and therefore no chance for nutrient uptake. As there were some Saltlander

plants survived at this treatment level at the end of the trial, it is apparent that this plant type has higher salt tolerance than willow, Okanese poplar and alfalfa.

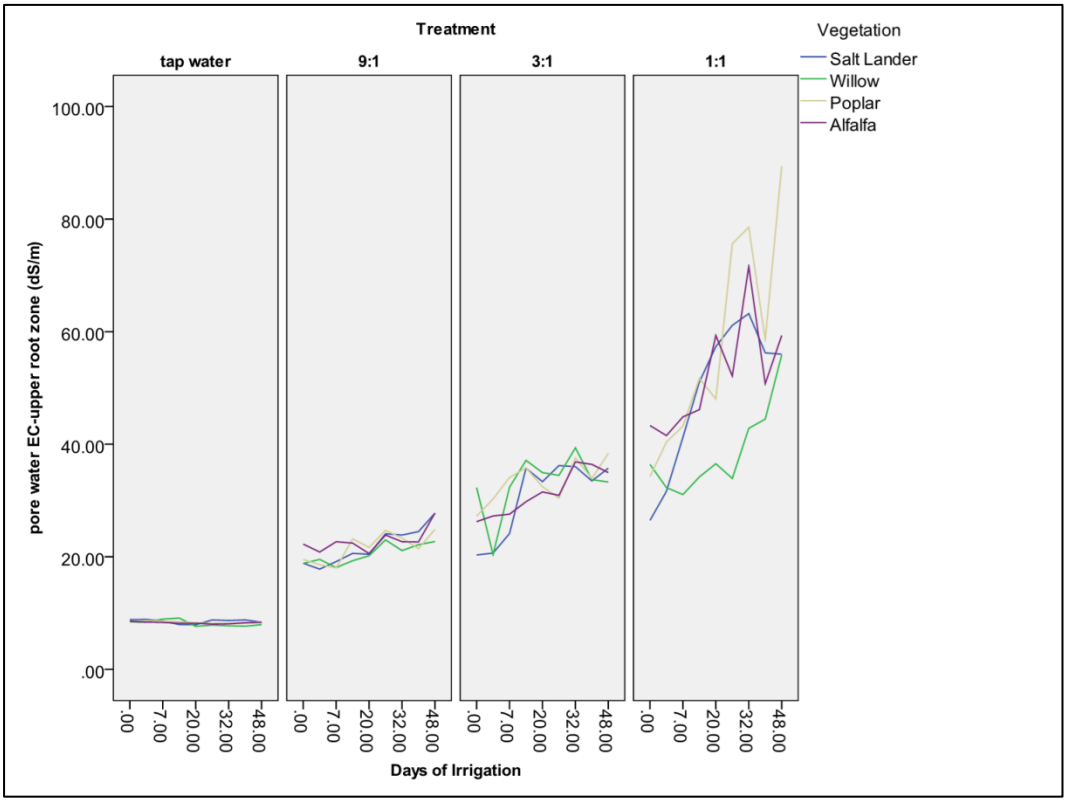

Figure 3.8 Change in soil electrical conductivity in upper root zone over time in environmental growth chamber for four plant types irrigated with nitrate impacted groundwater.

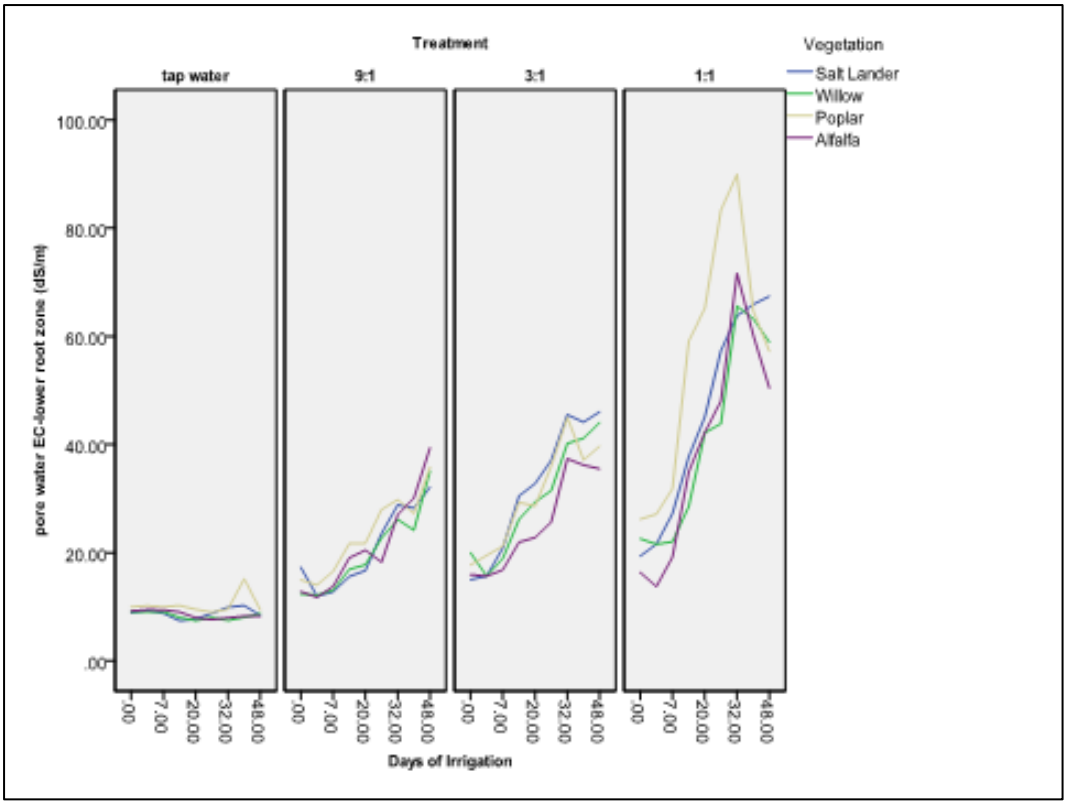

Figure 3.9 Change in soil electrical conductivity in lower root zone over time in environmental growth chamber for four plant types irrigated with nitrate impacted groundwater.

The results of the soil analysis following the irrigation trial are presented in Table 3.14. Along with high concentrations of ammonium and nitrate-N, phosphorus and sulfate levels are substantial and extreme EC values were recorded.

Table 3.14 Analytical results of soil following 90 days in environmental growth chamber with four plant types irrigated with nitrate impacted groundwater.

	Treatment			
Alfalfa	Control	9:1	3:1	1:1
Phosphorus (kg/ha) (available)	107.8	1442.0	2982.0	5320.0
Ammonium-N (kg/ha)	18.8	4018.0	7812.0	16800.0
pH	29.4	22.5	22.3	24.9
EC (dS/m)	5.7	53.6	112.4	157.1
SAR	1.8	5.5	12.0	28.1
% Saturation	165.2	159.6	151.2	165.2
Calcium (kg/ha)	506.9	1276.8	1148.0	550.5

Magnesium (kg/ha)	47.2	119.8	117.0	71.5
Sodium(kg/ha)	42.0	208.6	446.6	704.2
Potassium (kg/ha	5.3	200.2	417.2	673.4
Chloride (kg/ha)	14.0	197.4	483.0	849.8
Sulfate-S (kg/ha)	423.5	1082.2	2553.6	4039.0
Nitrate and Nitrite-N (kg/ha)	8.4	2214.0	3488.8	5152.0
Saltlander				
Phosphorus (kg/ha)	102.2	2338.0	3696.0	6580.0
Ammonium-N (kg/ha)	15.5	5964.0	10430.0	18816.0
pH	29.8	22.3	24.6	27.0
EC (dS/m)	6.4	67.5	114.2	175.1
SAR	1.7	6.6	12.0	34.4
% Saturation	190.4	170.8	170.8	152.6
Calcium (kg/ha)	633.4	176.3	1471.4	392.3
Magnesium (kg/ha)	79.7	156.1	218.7	35.8
Sodium(kg/ha)	54.6	316.4	544.6	715.4
Potassium (kg/ha	9.8	256.2	483.0	690.2
Chloride (kg/ha)	19.6	305.2	547.4	778.4
Sulfate-S (kg/ha)	550.6	1610.0	2766.4	4956.0
Nitrate and Nitrite-N (kg/ha)	12.5	1639.4	4813.2	5544.0
Okanese				
Phosphorus (kg/ha)	21.0	1848.0	3262.0	4200.0
Ammonium-N (kg/ha)	16.1	4646.6	8414.0	13972.0
pH	29.0	22.3	22.3	24.5
EC (dS/m)	4.3	59.1	112.1	165.9
SAR	2.9	5.9	11.6	19.0
% Saturation	159.6	182.0	158.2	159.6
Calcium (kg/ha)	273.4	1338.4	1248.8	1015.0
Magnesium (kg/ha)	28.7	120.5	135.9	153.4
Sodium(kg/ha)	57.4	273.0	483.0	725.2
Potassium (kg/ha	7.0	268.8	448.0	686.0
Chloride (kg/ha)	32.2	288.4	526.4	925.4
Sulfate-S (kg/ha)	191.2	1811.6	2874.2	4897.2
Nitrate and Nitrite-N (kg/ha)	28.6	1979.6	3676.4	5390.0
Willow				
Phosphorus (kg/ha)	96.6	1498	3108	2870
Ammonium-N (kg/ha)	3.5	4634	9492	11284
pH	30.38	22.12	22.96	23.1
EC (dS/m)	5.88	57.26	121.52	119.84
SAR	2.38	5.6	14.14	13.72
% Saturation	177.8	175	159.6	166.635
Calcium (kg/ha)	543.2	1468.6	1096.2	1121.4
Magnesium (kg/ha)	61.6	145.18	111.44	123.76
Sodium(kg/ha)	58.8	264.6	547.4	530.6

Potassium (kg/ha	7	245	494.2	481.6
Chloride (kg/ha)	25.2	260.4	603.4	660.8
Sulfate-S (kg/ha)	455.84	1668.8	3215.8	3255
Nitrate and Nitrite-N (kg/ha)	9.38	1939	4145.4	4172

3.4 Conclusions

An environmental growth chamber experiment was designed to test the feasibility of irrigating Okanese poplar, *Salix bebbiana* (beaked willow), *Medicago sativa var.* AC Nordica (alfalfa) and AC Saltlander grass with nitrate impacted groundwater. The objective of this was to find a use for the impacted groundwater and remove excess salts via plant uptake. Based on the poor survival rates for willow, alfalfa and Okanese poplar, and the reduced growth of AC Saltlander, irrigation with groundwater from below the nitrate fines landfill was unsuccessful at the levels applied. It is evident that the solute concentration and electrical conductivity of the groundwater, even at diluted rates, resulted in an environment that was toxic to plant survival and therefor prevented the opportunity for groundwater remediation via plant nutrient uptake.

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4. RESEARCH SUMMARY AND APPLICATIONS

4.1 Research Summary

Historical dumping of fertilizer fines and other wastes into a clay borrow pit at the Agrium Redwater fertilizer facility adjacent to the North Saskatchewan River has resulted in significant ammonium, nitrate, phosphate and sulphate impacted soil and groundwater in the area. Environmental growth chamber experiments were designed to test of feasibility of using phytoremediation, a technology that uses green plants to remediate contaminated environmental media.

This research was designed to address two main objectives. The first being to determine the viability of using Okanese poplar (Walker x *P. xpetrowskyana)*, *Salix bebbiana* (beaked willow), *Medicago sativa var.* AC Nordica (alfalfa) and AC Saltlander grass *(Agropyron spicatum* x *Agropyron repens*) as plants to treat nitrogen impacted soil via phytoremediation. The second objective was to investigate the possibility of using nitrate impacted groundwater as an irrigation source for these plants.

Results of this research indicate that the physiological mechanisms of alfalfa and AC Saltlander grass against salinity in early growth stages are better suited than those of the woody plants investigated. Phytoremediation may be more applicable to soils with higher clay contents due to adsorbtion of excess ions to clay surfaces resulting in a soil solution more hospitable for plant growth. This allows for more likely uptake of water and soil nitrogen. Nitrogen loss, likely due to atmospheric denitrification or immobilization, occurs at rates greater than plant uptake of nitrogen.

4.2 Application to Industry

This research demonstrates that there are plant species that have the potential able to grow on fertilizer impacted soil however solute concentrations leading to high electrical conductivities must be monitored to avoid conditions detrimental to plant survival. In the fertilizer industry, in locations where excess or unwanted product release occurs, selecting plants such as alfalfa or AC Saltlander grass

will allow for revegetation of barren areas provided that the levels of impact are below the levels discussed in this paper. This is beneficial if aesthetics are of concern, or if issues such as soil erosion due to poor vegetation cover are present.

This research also suggests that nitrate impacted soils need to be exposed to sources of carbon and the atmosphere to allow for the mechanisms of denitrification and immobilization to occur. It is likely possible that soils with excess nitrogen only require aeration and exposure to a high carbon substrate to allow for nitrogen reduction.

As it is possible that AC Saltlander may be considered invasive, this variety may not be an ideal plant for use in native areas. However, on industrial sites where restrictions for revegetation using only native species are unlikely, Saltlander could be an ideal selection.

4.3 Research Limitations and Future Research

Limits to the results and conclusiveness of this research could possibly have been reduced based on a number of alterations to field work and project establishment. Additional research is required to fully understand the potential of using plants to remediate nutrient impacted soil and groundwater.

- A better understanding of the nitrogen balance within the growth chamber system could have been reached with the measurement of organic soil nitrogen and the amount of atmospheric release.
- Planting in treepots prevented unrestricted root development and possibly prevented plant nitrogen uptake or other rhizophere effects on nitrogen fates from reaching full potential.
- The growth chamber environment was controlled at all times and moisture levels were maintained at close to optimal throughout the study. It is possible that *in situ* treatments in the outside environment would have different effects on plant growth and nitrogen uptake.
- AC Saltlander appeared tolerant to high levels of nitrogen salts and soil EC, it is possible that longer term growth of this grass on soils containing

high levels of nitrogen would result in a gradual, more substantial uptake of excess nitrogen.

- The young willow and Okanese poplar trees that were established from cuttings proved not tolerant to higher levels of salts. Future studies using larger more mature trees may see different results as tolerance will likely be higher.
- The addition of a high carbon substrate to the soil may have increased microbial immobilization of nitrogen in the root zone leading to a less phytotoxic environment allowing for increased uptake of remaining soil nitrogen by the plants.