FERTILIZATION OF WILLOW BIOENERGY CROPPING SYSTEMS IN SASKATCHEWAN, CANADA

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ABSTRACT

The detrimental effects of climate change and the threat of diminishing fossil fuel reserves is forcing society to search for renewable sources of energy. Energy can be derived from the biomass of plant material by co-fire combustion with coal or on its own for the production of electricity. Energy can also be created by converting the plant biomass into ethanol, a gasoline substitute. When converted into bioenergy, plant biomass from Short Rotation Woody Crop (SRWC) systems has the potential to offset the use of fossil fuels if the yields can be maintained at profitable levels. The effect of first year application of nitrogen (N) fertilizer on willow biomass production in a SRWC system is not well understood. Using field and growth chamber studies, the objectives of this study were to 1) determine biomass production in the growing seasons following a single application of N fertilizer in the year of planting, 2) determine the N recovery for five willow clones using a ^{15}N tracer, and 3) evaluate the effects of various types and rates of fertilizers on biomass production. Objectives 1 and 2 were addressed in a field fertilization study conducted on agricultural lands in the Moist Mixed Grassland ecozone and at tree nursery in the Boreal Transition ecozone. Willow cuttings were planted and fertilized with 100 kg N ha^{-1} of granular ammonium nitrate. Twelve trees were fertilized with 5 kg N ha^{-1} of double ^{15}N-labeled ammonium nitrate and 95 kg N ha^{-1} of granular ammonium nitrate. In the first growing season trees were browsed to a uniform height making biomass measurements unrepresentative of production potential. Annual shoot biomass production in the second year, however, was 0.39 to 2.0 Mg ha^{-1} and was not found to be significantly different between fertilizer treatments. Nitrogen recovery by entire trees ranged from 2.87 to 10.6 % in the first growing season and 0.39 to 2.95 % in the second growing season. Objective three was addressed in a growth chamber study. Willow cuttings were planted in pots and fertilized with 0, 50, 100 and 200 kg N ha^{-1} of granular ammonium nitrate and 100 kg N ha^{-1} of composted cattle manure. After a 90 day growth period shoot biomass production was significantly greater on the Prince Albert soil (1.28 to 5.34 g $tree^{-1}$) than on the Saskatoon soil (1.18 to 3.59 g $tree^{-1}$). No consistent trend between fertilizer treatments was observed. Further exploration into fertilization of willow SRWC systems should consider the application of multiple nutrient fertilizer blends, various rates and year of application to gain a better understanding of nutrient requirements of willow for the entire growth period.

DEDICATION

This work is dedicated in loving memory of my grandparents;

Dr. Arthur Alexander MacMillan
(October 19, 1921 - October 23, 2008)
&
Mrs. Helen MacMillan
(August 15, 1924 - August 16, 2009)

Both grandparents saw me begin this process but were unable to see me complete it. They gave me undying support in all my endeavours whether sports, dance, music or academics. I learned many priceless life lessons from each of them and they instilled in me a love for the outdoors and the pursuit of knowledge.

I would also like to dedicate this to my only living grandparent;

Mrs. Matilda Konecsni

When she was growing up, women did not have the opportunity to receive an education. Grandma's biggest desire was to finish her Grade 12 and go onto university. Unfortunately, she was never given the chance. Hopefully my pursuit of an education can somehow make up for her lost opportunity.

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My supervisory committee, Drs. Diane Knight and Jeff Schoenau have been constantly available to answer questions and ensure that I am on the right track. Their critiques of my work have moulded the ideas of a fresh Bachelor's grad into those of a some-what experienced junior researcher. Thank you to Dr. Vladimir Vujanovic for his role as my external examiner and for his comments and feedback.

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products. Another thank you goes to the many local conferences I was able to attend within Saskatchewan that gave me a good look at where our province is in its progress towards a bioenergy industry. All these conferences shed new light and gave me a new drive to work at completing my project.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

The interest in alternative energy sources has increased in recent years due to the realization of the negative consequences of climate change and the depletion of fossil fuel reserves. Renewable energy has the potential to replace petroleum and other fossil fuel energies. Plant biomass is a source of renewable energy and can be burned to produce heat or electricity, be converted into other bioproducts and provide environmental services and supply economic benefits (Adegbidi et al., 2003; Keoleian and Volk, 2005).

Agriculture, agroforestry and forestry crops and their residues can all be used as biomass for bioenergy. If crops are grown solely for the purpose of bioenergy production, they should be relatively straightforward to grow and high yielding. In November of 2005, the Premier of Saskatchewan announced his vision of planting 10 % of arable land in Saskatchewan to trees within the following 20 years as a way of creating a sustainable industry (Government, 2006). However, in the 2009 throne speech, the new government shifted their efforts to the investigation of a variety of energy options, including biomass (Government, 2009). Any plant material that can be used to produce bioenergy is classified as biomass (Lemus and Lal, 2005). These crops, whether conventional agricultural crops or fast growing trees, generally produce large volumes of biomass, have high energy potential and can be grown on marginal lands (Lemus and Lal, 2005).

Salix spp. (willow) are ideal for bioenergy because they are fast growing, they easily propagate from cuttings and have a large amount of exploitable genetic diversity that can be used in conventional breeding and molecular biotechnology (Dickmann, 2006). Since willows are native to Saskatchewan and are capable of being grown on marginal lands that are not suitable for agricultural crop production, they can enable Saskatchewan producers to utilize this land and diversify their operations. Plantations can be established and maintained over a 22-year life span with a harvestable economic product every three years. The development of large-scale willow bioenergy plantations will give Saskatchewan the reputation as a forerunner in agroforestry as well as the renewable energy production industry.

Breeding programs are well developed in Europe (Christersson, 1987; Christersson, 2006; Christersson and Sennerby-Forsse, 1994; Christersson et al., 1993;

Hytönen, 1987; Hytönen, 1995; Hytönen and Kaunisto, 1999) and New York State (Adegbidi and Briggs, 2003; Adegbidi et al., 2003; Adegbidi et al., 2001; Arevalo et al., 2005; Arevalo et al., 2007; Ballard et al., 2000; Volk et al., 2004) while breeding programs have just begun in Canada. The Agroforestry Division within the Saskatchewan Agri-Environment Services Branch has breeders at the Shelterbelt Centre in Indian Head, SK developing willow clones that are capable of withstanding Saskatchewan climates, insects and diseases. Researchers are currently looking into how foreign clones collected from Europe and the United States will perform under Saskatchewan conditions and how native Canadian species adjust to structured plantation systems.

Producing and maintaining yields in willow biomass crops requires an adequate supply of nutrients (Adegbidi et al., 2003). If the soil reserves cannot meet crop demands, these nutrients can be provided by synthetic fertilizers and/or organic residues such as green manure, animal manure and sewage sludge. Past research has looked at the effects of nitrogen (N) fertilizer application in the second year of growth (Ballard et al., 2000), while other studies have looked at the response of willow to annual N fertilizer application (Adegbidi and Briggs, 2003; Adegbidi et al., 2003) as well as fertilization after each harvest. However, no one has examined the effects of fertilization in the year of planting. This research study is designed to examine the effects of first year N fertilization on the biomass production of five willow clones in two different ecozones in Saskatchewan.

To accurately present the required knowledge for the promotion, establishment, production and development of a market for willow bioenergy, the fertility requirements for existing clones on Saskatchewan soils need to be examined. The main objective of this research was to determine the effects of first year fertilizer application on the growth of five willow clones in two Saskatchewan ecozones. The specific objectives were to 1) determine biomass production in growing seasons following the application of N fertilizer in the year of planting, 2) determine the N recovery for five willow clones using a ^{15}N tracer and, 3) evaluate the effects of various types and rates of fertilizers on biomass production.

This thesis is divided into five chapters which will address the research objectives using research plantations in the Boreal Transition and Moist Mixed Grassland ecozones of Saskatchewan as well as an indoor controlled environment growth chamber study. Chapter 2 is a literature review of the reasoning behind the interest in bioenergy and how Saskatchewan hopes to deal with the issue of climate change by implementing willow Short Rotation Woody Crops (SRWC). The review also covers cultural practices critical to the management and maintenance of willow SRWC systems. Chapter 3 illustrates a field N fertilization trial that aims to determine the effects of first year N fertilization on the biomass production, plant nutrition and N uptake capabilities of five willow clones. Chapter 4 describes a growth chamber study that evaluates the effects of fertilizer type and rate on the growth potential of willow clones. Finally, Chapter 5 summarizes the findings of the two research chapters and delivers recommendations for further development of willow SRWC systems in Saskatchewan for the purpose of bioenergy production.

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2. LITERATURE REVIEW

Beginning with the Industrial Revolution of the 18th century, humans in western cultures have abandoned manual labor, horse power and water power and replaced or enhanced it with machines (Friedman, 2008). The shift began with the replacement of wood with coal as the fuel source for steam engines and not long after the 19th century saw the implementation of crude oil as a fuel for lamps, heat and machines (Friedman, 2008). This increased consumption and technology led to humanity's dependence on fossil fuels.

The Earth's surface absorbs radiation from the Sun. The energy is then redistributed throughout the atmosphere, hydrosphere and lithosphere and then radiated back to space (IPCC, 2001). Incoming solar radiation is balanced with the outgoing terrestrial radiation and anything that alters either pathway or the redistribution within the Earth, will cause climate change (IPCC, 2001). Some disruption takes place in order to maintain the Earth's temperature at a hospitable degree. Because of their unique molecular structure, naturally occurring greenhouse gases (GHGs), such as carbon dioxide (CO_2), water vapor (H_2O), ozone (O_3) and methane (CH_4) trap the sun's radiation near the Earth's surface before the heat can escape back into space (Friedman, 2008). This process is called the greenhouse effect. When the concentrations of GHGs increase in the atmosphere, the outgoing terrestrial radiation is absorbed by the atmosphere and warms the lower atmosphere and the Earth's surface; less heat escapes the Earth and becomes the enhanced greenhouse effect (IPCC, 2001) which leads to climate change.

Fossil fuels are carbon-based fuels but emit more than just CO_2 during their combustion. For example, coal, oil and natural gas contain 80, 65 and 45 % carbon (C), respectively while the remainder is made up of hydrogen and environmental contaminants such as sulphur, heavy metals and carcinogens (Scott, 2007). As these fossil fuels are burned, CO_2 and other contaminants enter the atmosphere that can contribute to the enhanced greenhouse effect and thus climate change.

Planetary imbalance caused by climate change will result in many unpredictable occurrences, such as altered atmospheric circulation that changes intensity and frequency of tropical storms or of rainfall and cloud cover as well as accelerated melting of the polar ice caps and further increases in ocean levels (Scott, 2007). Today's CO_2 levels have not been exceeded in the past 420,000 years and likely not during the past 200 million years (IPCC, 2001). Prior to the Industrial Revolution, the atmospheric concentration of CO_2 was 280 ppm but has increased in the post World War II industry to 367 ppm in 1999 (IPCC, 2001). The concentration of CO_2 in the atmosphere continues to climb at a rate of 1.5 ppm yr^{-1} (IPCC, 2001) which has caused a 0.8 ^{o}C increase in global temperatures since 1750 with the most rapid increase occurring after 1970 (Friedman, 2008). The blame can lie predominantly on the current energy system, its energy consumption (Sari and Soytas, 2009) as well as deforestation and land use changes (Scott, 2007). Of the CO_2 emitted into the atmosphere, 75 % is derived from the burning of fossil fuels and the remainder is from deforestation (IPCC, 2001). If energy consumption decreases and the energy system changes, emissions and their negative effects should also decrease (Sari and Soytas, 2009). If the energy system does not change, models project atmospheric CO_2 concentrations of 540 to 970 ppm by 2100 (IPCC, 2001).

2.1 Renewable Energy

With the understanding of the detrimental effects of climate change and the threat of diminishing fossil fuel resources comes growing concerns surrounding energy security, GHG emissions, local and regional air and water pollution, natural resource sustainability, demand for sustainable and biodegradable products and a need to revitalize rural economies (Keoleian and Volk, 2005). Governments, organizations and small communities are looking into alternative energy sources especially after the Organization of the Petroleum Exporting Countries (OPEC) oil embargo of 1973 when the price of a barrel of oil increased four-fold between October 1973 and January 1974 (Dickmann, 2006). Hydro-, bio-, geothermal- and solar energies are among these alternative energy sources.

Renewable energies have lower CO_2 emissions released when compared to fossil fuel energies. Therefore, renewable energies are highly favorable for decreasing the conscious contribution of CO_2 and other GHGs to the atmosphere. The decision, then, on which renewable energy source to endorse is greatly dependent on the availability of resources in the area in question. Hydropower cannot be captured in an area without flowing water just as a desert or tundra will not be able to grow crops for bioenergy. An ideal energy system for a given community will need to be well thought through and may involve more than one renewable energy option.

The province of Saskatchewan is keen on implementing renewable energy into their energy system. In 2005 the Saskatchewan government had already put into place a wind energy operation capable of producing 172 MW of zero emission power that equals 5 % of Saskatchewan's energy use (Government, 2006). It had also become the first province to legislate ethanol-blended gasoline and has aimed to become a major producer of grain derived ethanol with the establishment of two new ethanol plants in Lloydminster and Weyburn (Government, 2006). The province had also funded research in the areas of biomass, biodiesel, hydrogen and solar energies (Government, 2006). After a change in government in November of 2007, the provincial leadership was still determined to explore all energy opportunities including gas turbines, cogeneration, clean coal, wind, hydro, solar, nuclear and biomass derived energy (Government, 2009). The goal is to meet energy needs in the most reliable, affordable and environmentally friendly manner possible (Government, 2009).

Biomass is any plant material that is used to produce bioenergy (Lemus and Lal, 2005). From the time humans discovered fire, biomass has been a primary fuel source. The use of agricultural and forestry products for large-scale industrial bioenergy was seriously considered following the 1970's energy crisis (Schneider and McCarl, 2003). Biomass can also be obtained from forest, agricultural and various other residue streams (Keoleian and Volk, 2005) or is grown for the sole purpose of bioenergy production. Species grown as bioenergy crops are chosen because of their adaptation to marginal lands, high energy potential and ability to produce large amounts of biomass (Lemus and Lal, 2005). Bioenergy crops can include herbaceous bunch-type grasses, such as switch grass (*Panicum virgatum*), elephant grass (*Pennissetum purpureum*) and tall fescue (*Fetusca arundinacea*) as well as woody perennials, termed Short Rotation Woody Crops (SRWC).

2.2 Short Rotation Woody Crops (SRWC)

SRWC are fast-growing woody plants. Some are highly adaptable and disease resistant while others are not. SRWC can include hardwoods, such as poplar (*Populus* spp.), willow (*Salix*, spp.), mesquite (*Prosopis* spp), cottonwood (*Populus fremontii*), sweet gum (*Liquidambar styracifus*), sycamore (*Plantanus occidentalis*), black locust (*Robinia pseudoacacia*), silver maple (*Acer saccharinum*) and *Eucalyptus* spp. (Lemus and Lal, 2005). SRWC are usually grown in short rotation intensive cultures (SRIC); silvicultural systems based on short clear-felling cycles, between one and fifteen years, that require intensive cultural management, such as fertilization, irrigation and weed control, and also utilize genetically superior planting material (Drew et al., 1987). SRWC are planted at densities up to 33,000 trees ha^{-1} and thus have high start-up costs due to the large amount of planting material required for initial plantation establishment (Dickmann, 2006).

SRWC are becoming an attractive practice because they are a sustainable system, they supply renewable feedstock for bioenergy and bioproducts while also providing a suite of environmental and rural benefits (Nordman et al., 2005). SRWC have the capability to increase site (Keoleian and Volk, 2005) and soil quality (Lemus and Lal, 2005). When compared to annual cropping systems, SRWC can increase soil porosity, infiltration, preferential flow and hydraulic conductivity in clayey soils (Mele et al., 2003). In order to satisfy plant nutritional requirements, deep perennial rooting systems of woody species are capable of absorbing cations and other trace elements that are out of reach for shallow annual root systems. The absorbed nutrients are returned to the soil surface through litterfall thereby enhancing the nutrient cycling of this silviculture system (Mele et al., 2003).

2.3 Genus *Salix*

Salix spp. (willow) are part of the Salicaceaeare family (Dickmann, 2006) and are widespread across Canada’s Boreal Forest and Aspen Parkland ecozones (Johnson et al., 1995). There are over 125 species of willow shrubs from the subgenus Caprisalix (Vertix) that are ideal for SRWC systems (Keoleian and Volk, 2005) because they are fast growing, can easily propagate from cuttings and have a large amount of easily exploitable genetic diversity that can be used in conventional breeding and molecular biotechnology (Dickmann, 2006). Willow have the ability to re-sprout after a disturbance, whether through natural breaking, grazing or human induced coppicing or pruning (Keoleian and Volk, 2005).

When used in SRWC systems, willows are capable of growing on marginal agricultural land (Labrecque and Teodorescu, 2005) and previously mined regions as a reclamation and land utilization tool (Gruenwald et al., 2007). A sustainable supply of fuel wood can be produced by willow on marginal land if adapted or tolerant clones are grown (Gruenwald et al., 2007). Willow plantations established on marginal lands can decrease fibre demands on existing natural forests and provide a means to recycle organic residues, such as sewage sludge and animal manures (Labrecque and Teodorescu, 2005). Bioenergy has raised the question of "food vs fuel?" Sugar cane, corn and grains have been used as biomass for bioenergy however; these crops have large roles in the agricultural food sector. Their use for energy has brought about debate over whether food or fuel is the more important commodity. Willows are not an agricultural food crop and thus are not a controversial source of bioenergy.

Willow SRWC systems can provide many benefits, the most obvious being monetary benefits. In order to accurately calculate the value of willow SRWC, it is necessary to take into consideration non-monetary benefits, such as increased biodiversity, C sequestration, quality of seepage water and aesthetic values (Gruenwald et al., 2007). Willow are capable of sequestering C in the soil at rates of 0.6 to 3.0 Mg C ha^{-1} yr^{-1}; however, net gains in C sequestration only occur if willow replaces annual row crops (Lemus and Lal, 2005). Aesthetically, willow have successfully been used as green wall structures to provide benefits such as improved urban acoustics and air quality (Vujanovic and Labrecque, 2008).

2.3.1 Site selection

In Canada, willows are naturally found in areas with readily available moisture. These preferential habitats include lakeshores, stream banks, moist clearings, floodplains, and the occasional active sand dune or sandy beach (Johnson et al., 1995). These regions are often characterized by calcium-rich, alkaline soils (Johnson et al., 1995) that are often unfavourable for traditional agriculture due to high soil moisture levels. The deep rooting system of willows may be able to control the soil water level while also utilizing agriculturally undesirable land. Thus, producers can increase economic benefits by capitalizing on land previously deemed unprofitable for traditional agricultural crops.

Schaff et al. (2003) determined optimal sites for willow bioenergy crop production. The survival study revealed that coarser textured soils (sands) were more conducive to the growth of willow bioenergy crops when compared to finer textured soils, such as silts and clays. For this reason, the study suggests that sites selected for willow SRWC have adequate soil moisture and be predominantly sandy or have sandy layers within the rooting zone (Schaff et al., 2003).

2.3.2 Management

In order to produce willow biomass it is necessary to combine a knowledge of forestry and agronomy (Keoleian and Volk, 2005). Throughout the growth cycle of willow SRWC systems, intensive management is required in site preparation, planting, weed and pest control, fertilization, coppicing, and harvesting (Table 2.1). Many of the management practices are agronomic techniques while some are more forestry focused.

Table 2.1 Timeline for the management of willow SRWC systems.

Year	Season	Activity
0	Fall†	Mow, contact herbicide, plough, disk, seed cover crop, cultivate
1	Spring‡	Disk, cultivate, plant, pre-emergent herbicide, mechanical and/or herbicide weed control
1	Winter	1st year coppice
2	Spring	Fertilize
3 / 4	Fall / Winter	1st harvest
5	Spring	Fertilize
6 / 7	Fall / Winter	2nd harvest
(8-22)		(Repeat 3 year cycle for 3rd – 7th harvests)
23	Spring/Summer	Elimination of willow stools

†Done only if the land is under a perennial cropping system.
‡Done if land is under an annual cropping system.
Modified from: (Heller et al., 2003; Keoleian and Volk, 2005).

2.3.2.1 Site preparation

Conventional agricultural site preparation and weed control should begin in the fall prior to planting if a perennial herbaceous green cover is present, or the spring of planting if land is under an annual cropping system (Keoleian and Volk, 2005). Common site preparation techniques for both spring and fall include disking, cultivating along with mechanical and/or chemical weed control. Site preparation will create a favourable planting medium and minimize weed competition.

2.3.2.2 Planting

In a SRWC system, willows are grown from unrooted cuttings that have been harvested from one-year-old shoots during the dormant winter season (Keoleian and Volk, 2005). If the planting conditions are dry, cuttings are often soaked for 24 hours prior to planting. Cuttings are planted at the beginning of the growing season (May/June) and are always oriented vertically in the soil with the buds facing upwards. Generally the top 1 to 2 cm of the cuttings are left to protrude out of the soil surface.

The planting density of willow SRWC systems is greatly dependant on the entire production system because it affects management decisions, such as weed control and harvest efficiency (Keoleian and Volk, 2005). For willow SRWC systems, experimental planting densities of 15,300 plants ha^{-1} (Heller et al., 2004) and 10,000 to 20,000 plants ha^{-1} (Christersson et al., 1993) have been recommended. Higher densities tend to be more efficient at using resources earlier in the rotation, yet have higher establishment costs (Bullard et al., 2002) while lower densities have lower costs, but a delayed peak of mean annual increment (MAI) (Keoleian and Volk, 2005). A study carried out by Adegbidi et al. (2001) observed the effects of planting density on other components of the production system and found that planting densities, whether 107,600 ha^{-1}, 36,960 ha^{-1} and 15,000 ha^{-1}, had no significant effect on annual biomass production.

2.3.2.3 Weed control

The management of woody and herbaceous weed competition continues to be essential and can be effectively controlled with cultivation, herbicides or a combination of both (Dickmann, 2006) as long as weeds are not given time to fully establish. Different clones, species and sites have varied responses to herbicides (Dickmann, 2006) so it is necessary to be aware of the specific needs of the plantation in question. Herbicide applications can include pre- and post-emergent chemicals (Appendix A). Because willow SRWC systems are relatively new and still in experimental stages, herbicide resistance in clones has not been developed. For this reason many chemicals will be detrimental to the physiological functions of the willow. Avoidance of foliar application on the willow tissue is highly recommended. As a result of problematic herbicide applications, mechanical weed control is quite common. Cultivation can be carried out between the tree rows whenever weeds become unmanageable, although damage to root systems is likely to occur if tillage is deep.

2.3.2.4 Diseases and infestation

The overall productivity of willow SRWC systems can be drastically decreased if the plants become affected by disease, insects and/or other pests. Insects, such as willow leaf beetles (*Plagiodera versicolora* and *Disonycha alternate*), chrysomelid beetle (*Calligrapha multipunctata bigsbyana*), potato leaf hopper (*Empoasca fabae*), leaf aphid (*Chaitophorus populicola*), willow shoot sawfly (*Janus abbreviatus*) and giant willow aphid (*Tuberolachnus salignus*) (Labrecque and Teodorescu, 2005) as well as herbivores, such as deer (*Odocoileus virginianis*), moose (*Alces alces*) and gophers (various species of *Marmotini* tribe), can have detrimental affects on the health of willow plantations. These pests prey on the fleshy tissue of willow leaves and shoots. Fungal pathogens, such as *Melampsora* spp., have caused serious problems for SRWC systems (McCracken and Dawson, 1998) and have been found to become more frequent after a few growing seasons (Vujanovic and Labrecque, 2002). To reduce pest-induced damage, pesticide usage, operating cost and harmful environmental effects while still maintaining high biomass productivity, it is recommended that a variety of disease and insect resistant clones be planted in any one SRWC system (Nordman et al., 2005).

2.3.3 Fertilization

Willow have been chosen as an appropriate species for bioenergy production because they are fast growing (Dickmann, 2006) and thus produce high yields in relatively short harvest cycles. Because growth and nutrient uptake are closely linked (Ericsson, 1994), fast growing trees have high nutrient demands (Ballard et al., 2000).

Fast growing tree species require adequate amounts of nutrients to produce and maintain high yields (Adegbidi et al., 2003). Trees are capable of extending their roots deep into the soil profile to utilize both nutrients and water (Gruenwald et al., 2007). Available soil nutrients are then removed and incorporated into the biomass of the tree. At the end of a 3 to 4 year growth cycle (Keoleian and Volk, 2005), trees are harvested and the assimilated nutrients are permanently removed from the system. One study found that approximately 2.7 to 3.6 kg N tonne^{-1} of stem dry matter is removed at each harvest (Ericsson, 1994). Nutrient removal at harvest has negative effects on both the nutrient cycling and productivity of the system (Adegbidi et al., 2001). The fertility of the soil greatly affects the amount of biomass produced (Lemus and Lal, 2005) as well as negatively affecting root proliferation if nutrients are too low (Gruenwald et al., 2007). Adegbidi et al. (2001) and Keoleian and Volk (2005) suggest that nutrients lost during harvest should be replaced. This is not simply a tactic to maximize yields (Keoleian and Volk, 2005) but also to maintain the long-term soil fertility and sustainability of the bioenergy production system.

2.3.3.1 Fertilizer types

Depleted soil nutrient reserves can be replenished through the application of fertilizers whether synthetic or organic. These can include inorganic fertilizers, biosolids, green manure crops, municipal waste water and landfill leachates. Synthetic fertilizers, often referred to as inorganic or mineral, are undoubtedly one of the most effective means to increase crop productivity (Ericsson, 1994) although they require immense energy inputs for their production. Slow-release fertilizers are a type of synthetic fertilizer designed to degrade slowly, allowing the nutrients to be available over a longer period of time. These slow-release fertilizers can both minimize leaching losses and maximize fertilizer effects but they are often quite expensive (Adegbidi et al., 2003). Despite the accessibility and ease of use associated with synthetic fertilizers, immense amounts of energy are consumed in their production and therefore can account for 20 to 30 % of total bioenergy production costs (Hasselgren, 1998).

Inorganic fertilizers are an effective means by which to increase the productivity of willow SRWC systems (Ericsson, 1994). Inorganic fertilizers are made synthetically and come in various forms; a wide variety of which have been used on willow SRWC systems. Ammonium nitrate is a commonly used N fertilizer and can be broadcast (Adegbidi et al., 2001; Alriksson et al., 1997; Booth, 2008; Preston and Mead, 1994; Staples et al., 1999) or applied in liquid form using irrigation systems (Nilsson and Ericsson, 1986). Urea is another commonly used N fertilizer (Heller et al., 2003; Preston and Mead, 1994). In order to meet all plant demands, elements, such as phosphorus (P) and potassium (K) have been applied to willow solely or blended with N. The effects of triple-super phosphate, muriate of potash or potassium chloride (Adegbidi et al., 2001), N:P:K (Gruenewald et al., 2007; Hasselgren, 1998) and P:K fertilizers (Hytönen and Kaunisto, 1999) on willow production has been previously studied. Slow-release fertilizers are expensive but can minimize leaching losses to maximize returns and efficiency so researchers have also been observing the use of slow release N fertilizers (Adegbidi et al., 2003) and slow-release sulfur coated urea (Ballard et al., 2000).

Fertilization with biosolids, such as sewage sludge and animal manure, is favourable with bioenergy plantations because it is a non-food crop so there is a decreased risk of disease transmission to humans (Keoleian and Volk, 2005). Biosolids are an attractive fertilizer option because they are energy efficient, contain P and K that can also be utilized by willow and are not accompanied by the large energy costs associated with synthetic fertilizers (Heller et al., 2003). Their use also eliminates the need for landfill disposal (Adegbidi and Briggs, 2003). The application of organic residues to bioenergy plantations can significantly increase soil organic matter, pH, exchangeable cations and extractable P in the top 10 cm of soil profile (Adegbidi et al., 2003).

The application of biosolids as fertilizers can also raise environmental concerns (Adegbidi and Briggs, 2003). Application of biosolids in excess of the tree's N and P assimilation capability can lead to contamination of surface and ground water (Adegbidi and Briggs, 2003). Biosolids are higher in P than N so in order to avoid overloading the ecosystem with P, as well as calcium, magnesium, copper and zinc, they should only be applied to the P requirements for the given land base and not to the N recommendations (Hasselgren, 1998).

Ash byproduct from the burning of biomass during bioenergy conversion through gasification has also been used as a soil amendment to supply nutrients as well as disposal of gasification 'wastes' (Hytönen and Kaunisto, 1999). Nitrogen fixing green manure crops, such as Dutch white clover (*Trifolium repens*), have also been studied as an alternative nutrient source to replenish N reserves in the soil for bioenergy plantations, but results showed that the yield of above-ground biomass was more positively responsive to synthetic fertilizer and control treatments than Dutch white clover when it was used as a green manure crop (Arevalo et al., 2005).

Hasselgren (1998) examined the effects of using landfill leachates as a nutrient source. The leachates used were highly alkaline and contained sufficient ammonium and K levels; however, the P levels were so low that supplementary synthetic P was required. Landfill leachates were found to enhance the growth of willow and even though the leachates contained heavy metals and trace organics, they were not found to be phyto-toxic to the plants. Hasselgren (1998) also investigated the use of municipal waste water as a nutrient source for willow bioenergy plantations and found that the nutrient content of the willow was equal to the amount of N and P supplied by the waste water. There was also limited N and P leaching from the rooting zone because willow was able to remove 85 to 95 %, 95 to 96 % and 91 to 98 % of the N, P and biological oxygen demand, respectively. These results suggest that willow is a very successful species for the tertiary treatment of waste water (Hasselgren, 1998).

Comparisons have been carried out to determine which form of supplemental nutrients is most effective at increasing biomass yields, as well as maintaining ecological and economical sustainability. The above-ground biomass of willow was more responsive to inorganic N fertilizers than when Dutch white clover (*Trifolium repens*) was grown as a green manure crop (Arevalo et al., 2005). In New York State, the biomass production of willow was examined following the application of a slow release N fertilizer and organic residues; black plastic mulch, composted poultry manure and lime stabilized sludge (Adegbidi et al., 2003). The slow release fertilizer was applied at 100, 200 and 300 kg N ha^{-1} while 250 m^3 ha^{-1} of composted poultry manure and lime stabilized sludge was applied. During the first year, biomass production was greatest with slow release N fertilizer at 300 kg N ha^{-1}. In years two and three, the slow release fertilizer was out performed by the organic residues due to the dissipation of inorganic N after two years and the continuous decomposition of organic residues in years two and three. Although inorganic fertilizers are easily accessible, and expensive they are capable of providing adequate nutrients for willow plantations (Adegbidi et al., 2001). Organic fertilizers have low and often negative costs associated with them, but may require added costs, such as transportation and the purchase of new application equipment, that may cause the prices to be more than inorganic fertilizers (Adegbidi et al., 2003).

2.3.3.2 Fertilization effects

The application technique for N fertilizers will depend on the form of fertilizer used. Organic and inorganic fertilizers can be applied using different methods and equipment. The application method should coincide with the existing farming practices and technology in order to minimize production costs (Alriksson et al., 1997). Inorganic fertilizer is generally broadcast and incorporated or banded into the soil to a depth of 10 cm (Arevalo et al., 2005) while solid organic residues are broadcast and incorporated and liquid manures are injected into the soil to avoid volatilization. The rate and timing of fertilizer application should be adjusted to the nutrient uptake of the crop (Ericsson, 1994) and should decrease over time as the quantity of nutrients supplied by internal cycling increases (Alriksson et al., 1997). Poplar trees should be fertilized with N to maintain a foliar N concentration of 3 % according to one study (Hansen et al., 1988). Fertilizer carry over from year to year can account for 27 to 48 % of total N requirements in the third year of willow bioenergy systems (Alriksson et al., 1997). Based on a four-year, non-coppiced cycle, Alriksson et al. (1997) found that N fertilizer application at the beginning of the cutting cycle, years two and three, are most favorable for the growth of willows. Large amounts of N can be applied in the second year because willow can utilize at least 120 kg N ha^{-1}. Third year application should be less than the second year and there will be a low requirement for N in the fourth year. Hansen et al. (1988) found that N application in the fourth and fifth year in excess of 56 kg N ha^{-1} had no effect on foliar N and may have depressed the height growth of hybrid poplars. N fertilizer application depends on the crop's uptake and nitrogen use efficiency, accessibility to desired form or type of fertilizer, cost, application equipment available, weather conditions as well as biotic and abiotic soil characteristics.

2.3.3.3 Fertilization effects on biomass yields and cutting survival

Fertilizer applications, whether organic or inorganic, have the ability to significantly increase the biomass production of bioenergy cropping systems (Adegbidi et al., 2003; Adegbidi et al., 2001; Arevalo et al., 2005; Ballard et al., 2000; Christersson, 2006; Ferm et al., 1989; Gruenwald et al., 2007; Hytönen and Kaunisto, 1999). Fertilizers applied in the year after coppicing increased biomass production of willow plantations by 8 to 134 %, 7 to 75 % and 9 to 39 % over control treatments in years one, two and three, respectively in a study conducted in New York state (Adegbidi et al., 2003). Biomass production of poplar trees, also from the Saliceaceare family, has been observed to increase over the control treatment by 30 % after the application of 100 to 150 kg N ha^{-1} mineral, compost and mineral + compost fertilizers (Gruenwald et al., 2007) and 25 to 30 % as a result of NPK + micronutrient (100 kg N, 16 kg P and 65 kg K ha^{-1}) application (Christersson, 2006). A one-time application of ammonium nitrate at 90 kg N ha^{-1} resulted in a 24 % increase in total biomass of willow relative to the control treatment over a four-month field growth study (Arevalo et al., 2005). In the same study, Dutch white clover green manure increased foliar N content but did not have any effects on aboveground biomass. The application of P:K fertilizer at 575 kg ha^{-1} (8.6 % P, 16.6 % K and 0.03 % boron) to a coppiced mixed birch and willow stand increased biomass yields by 24 % over a coppiced unfertilized portion of the same stand (Hytönen and Kaunisto, 1999).

Fertilizer application has also increased the survival of cuttings during their first year of growth. At the end of the first growing season, unfertilized poplar cuttings had a survival of 50 % and by the end of the sixth growing season had 0 % survival (Ferm et al., 1989). With the application of 300 kg N ha^{-1} in year one and two, survival increased to 49 % after six growing season (Ferm et al., 1989). Poplar cuttings are similar to those of willow cuttings although the survival of poplar cuttings is much lower than willow cuttings because of slower root growth (Ferm et al., 1989).

2.3.3.4 Nitrogen uptake and use efficiency

Trees have the ability to store large amounts of nutrients temporarily in their woody components (Ericsson, 1994). Nutrient resources in trees include N compounds, starch, sugars, fats and hemicellulose (Bollmark et al., 1999). Nitrogen compounds play dominant roles in the regulation of growth processes (Ericsson, 1994) and therefore N is the element most likely to limit growth in SRWC systems (Hansen et al., 1988). Therefore, intensive N inputs to willow bioenergy plantations is required to achieve high yields (Keoleian and Volk, 2005). The annual uptake of N by actively growing willows in New York State was found to range between 18 and103 kg N ha^{-1} yr^{-1} for control and various fertilization treatments (Adegbidi et al., 2001). Nitrogen and P use efficiency for both fertilized and non-fertilized willow in New York State were 104 to 269 and 197 to 706 kg biomass kg^{-1} element on an annual basis, respectively (Adegbidi et al., 2001). These demands of N must be met in order to maintain optimal biomass production. No work has yet been done on N fertilization recovery by willows grown in SRWC systems.

2.3.3.5 Nitrogen cycling

Ericsson (1994) determined that decaying soil organic matter significantly contributed to soil nutrient supply in a willow SRWC system. Ericsson (1994) Approximately one-third of the total nutrient demands could be met by the mineralization of leaf litter in established bioenergy plantations. The nutrient supply released was found to depend on biotic and abiotic factors, such as pH, temperature, soil moisture, N to lignin ratio and microbial activity (Ericsson, 1994). Bollmark et al. (1999) observed that the amount of N lost from senescing leaves directly corresponded to the increase in N by perennial organs, such as roots and shoots, thus helping close the nutrient cycling loop once the leaves decompose and the nutrients become plant available. Internal cycling of nutrients within high yielding willow plantations can decrease the fertilizer requirements, thus reducing the production costs of the system (Ericsson, 1994).

Both organic and inorganic fertilizer N forms have the potential to be immobilized in the soil, absorbed by other vegetation, leached below the rooting zone and/or lost if they are converted to a gaseous form (Hansen et al., 1988). Excess applications of N will lead to leaching (Heller et al., 2003), although the perennial roots systems of bioenergy crops reduces leaching when compared to conventional agriculture crops (Ericsson, 1994). Ammonia (NH_3) volatilization occurs with fertilizer application if pH, soil and weather conditions are favorable. Losses can be 6 to 47 % of applied inorganic fertilizer and 50 % of ammonium (NH_4-N) applied as biosolids (Heller et al., 2003). Nitrogen can also be lost as nitrous oxide (N_2O), a byproduct of denitrification (Madigan et al., 2003). The formation of N_2O increases with fertilizer rate and is regulated by temperature, pH and soil moisture (Heller et al., 2003). Therefore, it is important to apply N only to the required amount and not to exceed the crop's ability to utilize the available nutrients.

2.3.4 Life cycle of willow SRWC systems

Willow bioenergy SRWC systems are generally harvested on a 3 to 4 year cycle (Table 2.1) and are managed using a coppice system (Keoleian and Volk, 2005). Coppicing refers to the harvesting or decapitation of certain plant parts in order to stimulate reinvigoration and accelerate growth toward the theoretical maximum (Sennerby-Forsse, 1995). Willows are coppiced (cut to < 5 cm tall) after the first growing season during the dormant season (winter) when carbohydrate reserves are at their maximum level in the tissues (Sennerby-Forsse, 1995). Coppicing has been found to double the density of willow SRWC systems by increasing the number of shoots by 3 to 4 fold (Hytönen, 1995). After 3 to 4 years of growth, harvesting takes place in the dormant season when nutrients are translocated to the roots and most leaf deposition has been contributed to soil organic C sequestration (Lemus and Lal, 2005). Since willow are capable of vigorous re-sprouting after each harvest, 7 to 10 harvests are possible from a single planting (Keoleian and Volk, 2005) resulting in a life span of more than 20 years (Anderson et al., 1983).

2.3.5 Energy production

The fate of willow SRWC will be determined by social, political and economic factors as well as by the price of oil (Dickmann, 2006). The energy conversion of biomass feedstock is material specific as well as dependant on the current energy system and the desired end product. Possible energy end products include fuel, heat and electricity. The production of fuel in the forms of ethanol, biodiesel, biomethane and biogas is done through enzymatic hydrolysis, cellulosic fermentation, pyrolysis, and acetic acid fermentation followed by hydrogenolysis. Heat and electricity can be created from biomass using gasification and combustion processes (Wu et al., 2008). Biomass gasification and power generation (BGPG) can be done using only biomass or using integrated gasification combined cycle (IGCC) systems which combines a fossil fuel source and supplementary firing with biomass. The overall efficiency of a 1 MW BGPG demonstration project in Fujian Province, China using only biomass was less than 20 % (Wu et al., 2008) which is less than desirable. Because of this low efficiency level, supplementary firing of biomass with a fossil fuel (IGCC) is a possible option to decrease the amount of fossil fuel used as well as to operate at an efficiency level above that of BGPG systems. Supplementary firing with coal and biomass produces syngas during the combined gasification of coal with biomass, and can increase the efficiency of the IGCC system by reheating low temperature gas turbine exhaust before it enters into the heat recovery steam generator (Gnanapragasam et al., 2009). A comparison of a coal IGCC system with different supplementary firing options (char, coal, and syngas) found that syngas from biomass was the best firing overall when considering net work output per unit mass of coal inputted and CO_2 emissions (Gnanapragasam et al., 2009).

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3. BIOMASS PRODUCTION AND NITROGEN FERTILIZER RECOVERY BY A WILLOW BIOENERGY PLANTATION

3.1 Introduction

Bioenergy and bioproducts have received attention in developed countries due to environmental concern and national energy security (Adegbidi et al., 2003). In 2006, the Government of Saskatchewan set goals to derive one-third of energy from renewable sources, develop biofuels and further implement agroforestry practices (Government, 2006). However, a shift in governing parties resulted in a change in perspectives. In 2009, the new government made a commitment to the people of Saskatchewan to look into possible renewable energy options including gas turbines, cogeneration, clean coal, wind, hydro, solar, nuclear and biomass in order to determine how best to meet the energy needs of the province well into the future (Government, 2009). Although the type of biomass was not specified, one option is the use of short rotation woody crops (SRWC). Willow SRWC systems are economically viable when used for renewable energy (Labrecque and Teodorescu, 2005), heat (Keoleian and Volk, 2005), pulp and paper (Labrecque and Teodorescu, 2005), and biofuels, such as ethanol (Schneider and McCarl, 2003). SRWC systems can also provide environmental benefits as an agroforestry system. Willows can decrease soil erosion by stabilizing the soil (Labrecque and Teodorescu, 2005), increase soil quality (Mele et al., 2003), sequester C both in the soil and biomass (Lemus and Lal, 2005), and be used for phytoremediation (Keoleian and Volk, 2005).

Research from Finland (Hytönen, 1987; Hytönen, 1995; Hytönen and Kaunisto, 1999), Sweden (Christersson, 1987; Christersson, 2006; Christersson and Sennerby-Forsse, 1994; Christersson et al., 1993), the United Kingdom (Kightley et al., 2008; McKenzie et al., 2008; Sugiura et al., 2008) and New York, USA (Adegbidi and Briggs, 2003; Adegbidi et al., 2003; Adegbidi et al., 2001; Arevalo et al., 2007; Ballard et al., 2000; Volk et al., 2004) have developed successful experimental and commercial bioenergy research trials using willow. For willow to become successful as a biomass crop in Saskatchewan, recommendations must be available to producers in regards to site selection, suitable clones, disease resistance, winter hardiness, and fertilizer application.

The major limiting factor to biomass production in willow SRWC systems in Poland was N availability (Kowalik and Randerson, 1994), therefore, fertilization is recommended to maintain growth rates of willow SRWC systems over many rotations (Lemus and Lal, 2005). However, the optimal time to apply fertilizer is still unknown for systems in North America. Previous research has examined the effects of fertilizer application after the first three-year harvest cycle (Christersson, 1987), for four consecutive years following planting (Alriksson et al., 1997), annually for up to nine years following planting (Adegbidi et al., 2001), prior to planting and six years following planting (Gruenewald et al., 2007), and most commonly, in the growing season following coppicing which is the second growing season (Adegbidi and Briggs, 2003; Adegbidi et al., 2003; Ballard et al., 2000; Hytönen and Kaunisto, 1999). Little work, however, has looked at the application of fertilizers in the year of planting.

The specific objectives of this study, therefore, were to: 1) determine biomass production in the growing seasons following the single application of N fertilizer in the year of planting, and 2) determine the N recovery for five willow clones using a ^{15}N tracer. The hypothesis for this study is that a single application of N fertilizer in the first year will have a positive effect on the growth of willow in a SRWC system.

3.2 Materials and Methods

3.2.1 Site description

Two willow fertilization trials were established in Saskatoon and near Prince Albert, Saskatchewan. Site characteristics are shown in Table 3.1. The Saskatoon site is located within the city limits at the University of Saskatchewan Horticulture Field Lab (106°36’28” W, 52°07’37” N) in the Moist Mixed Grassland ecozone. The site had previously been used for the production of barley and oats. The soil is mapped as the Sutherland Association and was classified as an Orthic Vertisol with a heavy clay texture (SCSR, 1978). The Prince Albert site was established in the Boreal Transition ecozone approximately 15 km north of Prince Albert, Saskatchewan at the Pacific Regeneration Technologies Inc. (PRT) nursery (105°46’26’ W, 53°21’18’ N). Since the nursery was

Table 3.1 Site characteristics of two willow fertilization trials in Saskatchewan, Canada.

	Soil Classification			Soil Properties†					Site Preparation	
Site	Association	Soil Type	Texture	pH	EC	TC	OC	Prior Crop	Mechanical	Chemical
				--	µS s^{-1}	---- % ----				
Saskatoon‡	Sutherland	Orthic Vertisol	Heavy clay	7.98	327	1.97	1.64	barley/oats	15 cm tillage	oxyfluorfen 4 L ha^{-1}
Prince Albert§	Pine	Orthic Eutric Brunisol	Sand to loamy sand	7.03	149	1.41	1.39	summer fallow/ white spruce seedlings	15 cm tillage	oxyfluorfen 4 L ha^{-1}

† Soil properties were measured for the top 30 cm of soil; EC – electrical conductivity, TC – total carbon and OC – organic carbon.

‡ (SCSR, 1978)

§ (SCSR, 1976)

taken over by the PRT in 1997 from the provincial government (Van Eerden, 2002) it has been used for growing conifer seedlings, specifically white spruce (*Picea glauca*). The soil at the site was mapped as an Orthic Eutric Brunisol of the Pine Association and the texture was sand to loamy sand (SCSR, 1976). The two sites were chosen because of their differing soil properties, land histories and ecozones to enable inferences on optimal site selection.

Planting material was collected from pre-established plantations in Saskatoon. Shoots were collected from one-year old willows in April of 2008 while trees were dormant and before flushing of leaves occurred. All shoots were cut into 15 cm cuttings and kept frozen at -4 ^{o}C until two days prior to planting when they were thawed for 24 hours followed by a 24 hour soak in water (Keoleian and Volk, 2005). The willow clones chosen for this study included Tully Champion, Marcy and Saratoga from the State University of New York – College of Environmental Science and Forestry (SUNY-ESF) and India from the Canadian Forest Service (CFS) (Table 3.2). The clones were chosen based on their performance at the previously established Saskatoon plantations. Tully Champion, Marcy and Saratoga were the tallest clones from the group of 30 SUNY-ESF cones at the Saskatoon site. India was chosen because it was the best performer of the seven clones from the CFS and because its growth form is dense and upright which may provide easier herbicide and tillage application between the rows. *Salix discolor* (Salix), a native Saskatchewan willow, was provided by the Agri-Environment Services Branch (AESB) at the Indian Head Shelterbelt Centre administrated by Agriculture and Agri-Food Canada. Due to material quantity limitations, *S. discolor* was only planted at the Saskatoon site.

Table 3.2 Nature of willow clones used in the current study.

Clone	Parentage	Gender	Origin†
Saratoga	*Salix purpurea* x *S. miyabeana*	Female	SUNY-ESF
Marcy	*S. sachalinensis* x *S. miyabeana*	Female	SUNY-ESF
Tully Champion	*S. viminalis* x *S. miyabeana*	Female	SUNY-ESF
India	SV1: *S. dasyclados*	Female	CFS
Salix	*S. discolor*	unknown	AESB

† The institution responsible for supplying the clones; SUNY-ESF (State University of New York – Environmental Science and Forestry), CFS (Canadian Forest Service) and AESB (Agri-Environment Services Branch).

3.2.2 Experimental design

In June of 2008, plots were set up at both sites according to the Swedish design which is organized in a three-double row orientation (Figure 3.1). Four days prior to planting, the research plots were roto-tilled with a Land Pride RTA1050 (Division of Great Plains Mfg., Inc., Salina, Kansas, USA.) attached to a Kubota tractor (BX2350, Kubota Corporation, Osaka, Japan) to a depth of 15 cm to ensure a satisfactory planting medium. The site was hand-planted using specially designed probes (9 June 2008 and 10 June 2008 for Saskatoon and Prince Albert, respectively). The probes were approximately 1 cm in diameter with a point at the end. The shaft had a foot rest 15 cm from the bottom of the probe. The planting probe was inserted into the ground to create a perfectly sized hole that only extended the depth of each willow cutting. The cuttings were then placed in the holes and the soil was compressed in around them by a simple kick of the planter's boot next to the cutting. Nine trees were planted in each row for a total of 54 trees in each plot. The outermost rows in each treatment were not used for

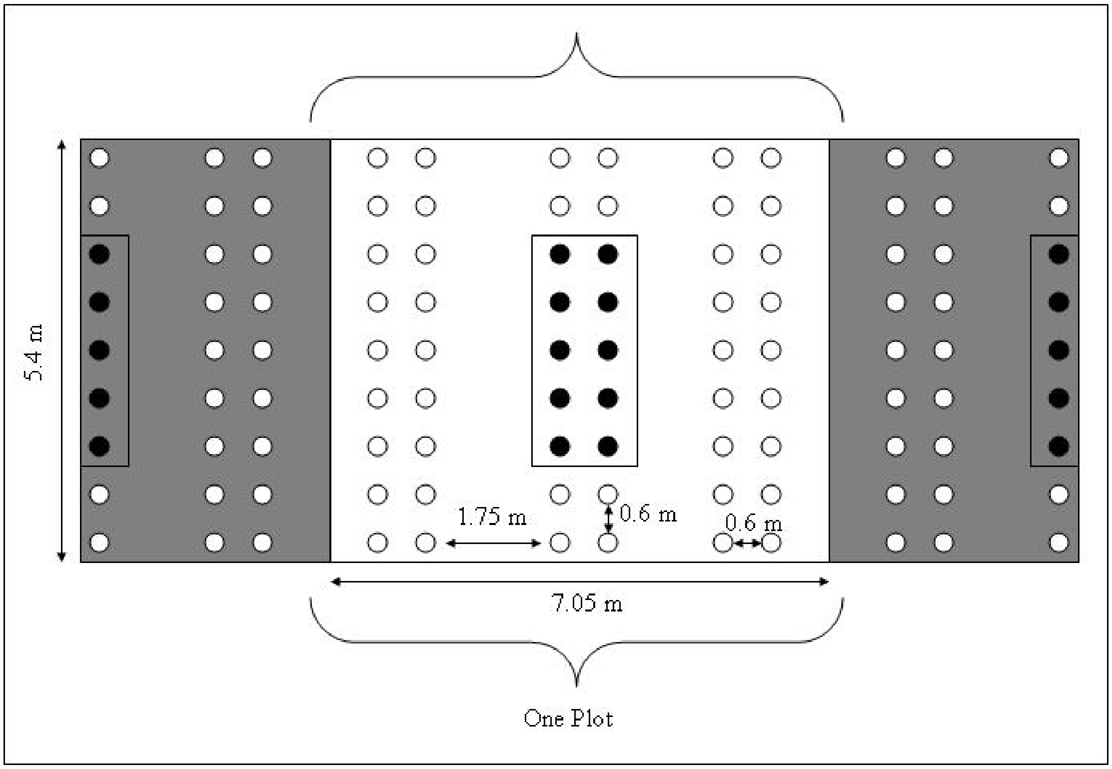


Figure 3.1 Plot design using the Swedish model with three-double rows and the highlighted circles representing the measurement trees.

measurements to avoid border effects. Inconsistent competition for nutrients and water may exist between two different clones which would provide an immeasurable variable. Thus only the ten middle trees were considered for measurements (Figure 3.1). Trees were easily planted at the Prince Albert site but the Saskatoon site had an underlying dense soil layer that did not get tilled and therefore made planting difficult. In order to control the weed species in the plantations, Goal 2XL (Dow AgroScience; active ingredient: oxyfluorfen) pre-emergent herbicide was applied at a rate of 4 L ha^{-1} 6 days after planting using a TPS300 sprayer (SprayTech Systems Ltd., Vonda, Saskatchewan) attached to the Kubota tractor.

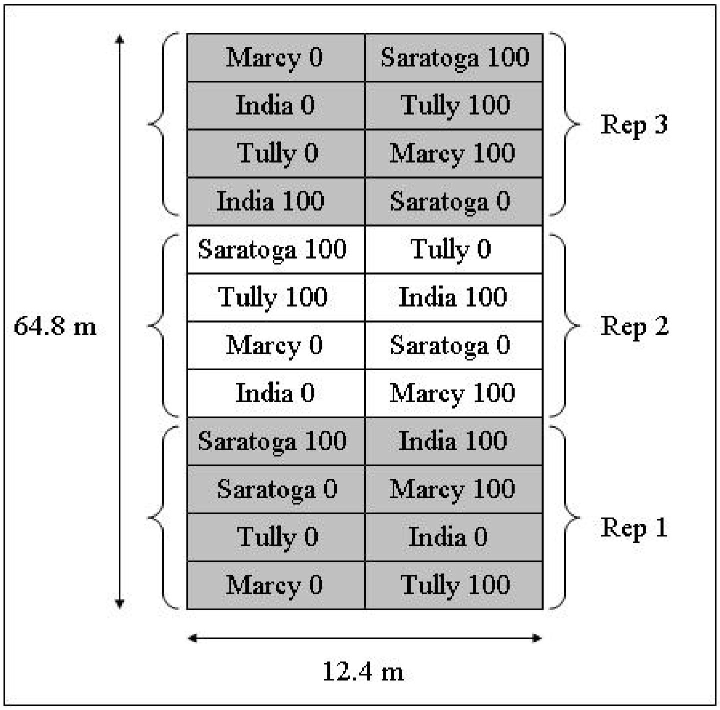
There was very poor establishment on the Saskatoon site. Survival ranged from 0 to 54 % per plot with average survival of 17 %. Many of the trees that did not establish were dug up and showed no evidence of root development. One study infers that first-year survival of less than 80 % is considered unsuccessful (Bergvist, 1996) Usint this value, the Saskatoon site was deemed unsuccessful and was not used for the remainder of the study.

3.2.3 Wildlife management

Grazing by wildlife had a detrimental effect on the growth, production and survival of the willow plantation. Throughout both growing seasons the willow trees were periodically browsed by white-tailed deer and in an attempt to minimize the damage caused by herbivory, deer repellants were applied. Plantskydd blood meal (Tree World Plant Care Products Inc., St. Joseph, Missouri, USA) was used to create a repelling buffer around the plantation and Tree Guard Deer Repellant (Becker Underwood, Inc.; active ingredient: Bitrex Benzyldiethyl, ammonium benzoate 0.20%) was applied directly to the measurement trees only because of a small quantity of chemical available. These products did not have any lasting effects as the plantation was browsed again after their application.

3.2.4 Fertilizer application

Once the trees at the Prince Albert research site had successfully established and reached a height of approximately 30 cm (15 July 2008), fertilizer treatments were applied. Two treatments (control and 100 kg N ha^{-1}) were replicated three times for each clone in a randomized complete block design (Figure 3.2). The N fertilizer was supplied in the form of granular ammonium nitrate which was hand broadcast over the tree rows.



Marcy 0	Saratoga 100	Rep 3
India 0	Tully 100	
Tully 0	Marcy 100	
India 100	Saratoga 0	
Saratoga 100	Tully 0	Rep 2
Tully 100	India 100	
Marcy 0	Saratoga 0	
India 0	Marcy 100	
Saratoga 100	India 100	Rep 1
Saratoga 0	Marcy 100	
Tully 0	India 0	
Marcy 0	Tully 100	

Figure 3.2 Research site layout at the Prince Albert PRT willow bioenergy plantation. Plot labels are clone names (*Tully*: Tully Champion, *India*, *Marcy* and *Saratoga*) are followed by fertilizer treatments (*0*: unfertilized control and *100*: fertilized with 100 kg N ha^{-1}).

3.2.5 ^{15}N tracer preparation and application

The application of ^{15}N was similar but not identical to the methods used in labeling poplar and spruce plantations (Booth, 2008; Staples et al., 1999). A stock solution of double de-ionized water and 10 % enriched double-labeled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$; Cambridge Isotope Laboratory, Inc., Andover, M.A.) was made in the laboratory prior to field application (Appendix B). Two non-measurement trees from the border rows of each fertilized plot (24 trees in total) were randomly selected in the field to be fertilized with labeled ammonium nitrate. Each tree was encased in a homemade 30 x 30 cm corrugated plastic box and covered with a lid to ensure the trees were not affected by the hand broadcasting of the granular fertilizer from Section 3.2.4. Labeled ^{15}N fertilizer was measured to supply 5 kg N ha^{-1} (45 mg N tree^{-1}) of the desired 100 kg N ha^{-1}. Ten mL of ^{15}N solution was used in the fertilizer application to each tree (see Appendix B for detailed ^{15}N calculations) applied to supply the required N fertilizer.

The labeled solution was applied on the same day as the granular fertilizer in Section 3.2.2. In the field, the 10 mL aliquot of the double-labeled ammonium nitrate stock solution was mixed into 500 mL of double de-ionized water and applied to the inner area of the box using a Haws watering can (Haws Elliott Ltd., West Midlands, England). Another 500 mL of double de-ionized water was used to rinse out the watering can and wash any residual label off the tree to minimize foliar uptake. The labeled solution accounted for 5 kg N ha^{-1}. To maintain consistency with the fertilization rate of 100 kg N ha^{-1} within the rest of the plot (Section 3.2.4), 95 kg N ha^{-1} (2.43 g per tree) of granular ammonium nitrate was hand broadcast over the soil surface encased inside the corrugated plastic box once the labeled solution had fully infiltrated the soil.

3.2.6 Plot management

The herbicide Goal 2XL was successful at controlling weed competition in the first growing season and thus hand weeding was not required. In the spring of 2009 trees were coppiced with brush saws (Stihl FS 110, Andreas Stihl AG & Co. KG, Waiblingen, Germany) at approximately 5 cm above the soil surface. Trees were coppiced prior to the second growing season to stimulate the growth of more shoots per tree. During the second growing season weeds were manually removed on a monthly basis.

3.2.7 Soil sampling and analysis

Preliminary soil samples were collected on the day of planting (10 June 2008). Samples were collected from three random locations within the measurement tree rows from the 0 - 30 cm depth layer using a JMC Backsaver Soil Sampler (Clements Associates Inc., Newton, I.A.). Soil samples were collected one week after fertilizer application to look at changes in soil N as well as at the end of each growing season (2008 and 2009) to look at the overall changes in soil nutrient concentrations. These samples were collected at depth increments of 0 - 10, 10 - 20, and 20 - 30 cm from three random locations within the measurement tree rows using the JMC Backsaver Soil Sampler. Soil samples were air dried, ground and sieved through a 2 mm mesh sieve.

Soil N (nitrate; NO_3^--N and ammonium; NH_4^+-N) and P (phosphate; PO_4^--P) were extracted using potassium chloride (KCl) (Keenley and Nelson, 1982) and modified Kelowna (Qian et al., 1994) extractions, respectively. Extractable NO_3^--N, NH_4^+-N and PO_4^--P were quantified colorimetrically using a Technicon Auto Analyzer (Pulse Insturmentation Ltd., Saskatoon, S.K.) Potassium, calcium (Ca), magnesium (Mg), and sodium (Na) were extracted using a 1 *M* ammonium acetate (NH_4OAc) solution (Simard, 1993) and analyzed on an atomic absorption spectrometer (SpectrAA-220, Varian Inc., Palo Alto, California, USA). Organic and inorganic C contents were analyzed by combustion using a LECO C632 Carbon Determinator (LECO Corporation, St. Joseph, M.I.). Soil electrical conductivity (EC) and pH were determined using a 2:1 water to soil suspension (Hendershot et al., 1993) and analyzed using a Horiba ES-12 Conductivity Meter (Horiba Ltd., Kyoto, Japan) and a Beckman 50 pH Meter (Beckman Coulter, Fullerton, C.A.), respectively.

3.2.8 Foliar sampling and analysis

Foliar samples were collected in September of 2008 and 2009 before leaf senescence. Five leaves were collected from the mid-stem region of each measurement tree per plot. The leaves collected from within the same plot were combined in a paper bag to produce a composite sample. The foliar samples were oven dried for two wk at 40 ^{o}C and then each bag of leaves was ground separately using a Hamilton Beach Custom Grind^{TM} Deluxe 15 Cup Hands-Free Coffee Grinder (Hamilton Beach Brands, Ltd., Washington, N.C.). Leaf samples, 1 per bag, were digested in sulfuric acid (Thomas et al., 1967) and analyzed for total N and P on a Technicon Auto Analyzer and K, Ca, Mg and Na concentrations were analyzed using atomic absorption spectroscopy.

3.2.9 ^{15}N labeled destructive tree sampling

At the end of each growing season, one ^{15}N-labeled tree from each plot was harvested. The trees were divided up into components: cutting, roots, shoots and leaves and placed in a paper bag. Using a garden trowel and a soils knife, the roots were carefully dug up to recover the entire rooting systems including roots that grew beyond the 30 x 30 cm labeled area. All tree harvesting activities were carried out with a new pair of latex gloves for each tree and the digging utensils were cleaned off with 99 %

ethanol (CH_3OH) to ensure no transfer of ^{15}N label from plant material and soil between trees. Ethanol is an ideal cleaning substance because it evaporates more quickly than water and is a topically non-toxic alcohol that is safe to handle without protective equipment. Plant samples were oven-dried for a week and a half to two weeks at 40 ^oC and then weighed. The plant material was coarsely ground using a Hamilton Beach coffee grinder and then finely ground on a rotating ball-bearing mill for two days. Between samples the grinders and mill were blown clean using compressed air and swabbed with 99 % ethanol to ensure no residual plant material remained in the equipment to contaminate the next sample. The samples were analyzed for % N and atom % ^{15}N excess on an isotopic ratio mass spectrometer (RoboPrep Sample Converter interfaced with a TracerMass Stable Isotope Detector, Europa Scientific, Crewe, England).

3.2.10 Soil sampling around ^{15}N labeled trees

Soil samples were collected from one location within the 30 x 30 cm area around the harvested ^{15}N labeled trees using a JMC Backsaver Soil Sampler. The location of sampling within the 30 x 30 cm area was randomly selected in the field. Between each sample, the probe was rinsed with water and then 99 % ethanol to maintain a clean sampling surface and avoid sample cross-contamination. Soil samples were collected in 2008 at 0 - 10, 10 - 20, and 20 - 30 cm depth increments. In 2009, samples were collected at depths of 0 - 10, 10 - 20, and 20 - 30, 30 - 40, 40 - 50, and 50 - 60 cm to monitor N leaching through the soil profile. All soil samples were air dried and sieved (2 mm). The sieve was swabbed with 99 % ethanol in between each sample. Soil samples were then finely ground on a rotating ball-bearing mill for two days and then analyzed for % N and atom % ^{15}N excess on the isotopic ratio mass spectrometer.

3.2.11 Tree measurements

Non-destructive measurements of trees were taken at the end of the first growing season and monthly throughout the second growing season. Non-destructive measurements included the height of the tallest shoot, the diameter of the tallest shoot at 30 cm above the soil surface along with the total number of shoots per tree.

Destructive biomass measurements were taken at the end of the 2009 growing season. One non-measurement (border affected) tree per plot was selected and leaves were removed shoots and the shoot were collected. Border affected trees were used in order to maintain the measurement trees for non-destructive measurements in future years. The two plant parts were bagged separately, oven dried at 40 ^{o}C and weighed. Biomass samples were not collected in 2008 due to the homogeneous height resulting from intensive browsing by deer.

3.2.12 Monitoring site specific environmental conditions

Environmental data at the site has been collected since the establishment of a previous willow plantation in June 2007. Campbell Scientific instrumentation (Campbell Scientific Inc., Logan, U.T.) was installed in 2007 to measure air temperature and rainfall (Appendix C).

3.2.13 Statistical analyses

Statistical analyses were carried out in the R environment (R Development Core Team, 2008). All variables were tested for normal distribution using the Shapiro-Wilk test. If required, transformations were performed on variables that were not normally distributed (Appendix D). Even after transformation, some variables were significantly different from a normal distribution. In these cases, if the transformation of a variable was more normal than the untransformed data, the log, square root or exponential transformation was used. If not, the untransformed variable was used for statistical analyses (Appendix D). A mixed model followed by an analysis of variance (ANOVA) was used to determine the effects of the explanatory variables on shoot biomass production as well as to test for the significance of block effects (Appendix E). To account for site quality variation in terms of soil nutrients, moisture, C, pH and EC, block was still used as a main effect variable. The post-hoc tests, Tukey's Honest Significant Difference test and t-tests, were used to compare means of values between fertilization treatments, clones and soils using a significance level of $P < 0.05$.

3.3 Results

3.3.1 Growth parameters

Coppicing significantly increased the number of shoots between 2008 and 2009 by 3.2 and 2 fold for unfertilized and fertilized Tully Champion clones, respectively and by 5.7 and 4.0 fold for unfertilized and fertilized treatments of Marcy clones, respectively (Table 3.3). Between 2008 and 2009 shoot numbers for India significantly increased by 11.6 fold for the fertilized treatment and 11.1 fold for the unfertilized treatment. The number of shoots per tree for the Saratoga clone significantly increased between 2008 and 2009 by 8.4 and 11.5 fold for the unfertilized and fertilized treatments, respectively. Coppicing was thus an effective method of increasing the number of shoots per tree. There were no significant differences in the number of shoots per tree when comparing

Table 3.3 Mean number of shoots per tree and the survival of willow clones in a SRWC system near Prince Albert, SK. in the years before and after coppicing.

Year	Mean Number of Shoots		Mean Tree Survival	
	Count		%	
	Unfertilized	Fertilized	Unfertilized	Fertilized
	------------------------------ Tully Champion ------------------------------			
2008	2.1b	2.1b	89a	97a
2009	6.7a	4.2a	87a	97a
	----------------------------------- Marcy -----------------------------------			
2008	0.8b	1.0b	67a	63a
2009	4.4a	4.1a	67a	63a
	----------------------------------- India -----------------------------------			
2008	1.2b	1.0b	93a	93a
2009	13.3a	11.6a	93a	93a
	-----------------------------------Satatoga -----------------------------------			
2008	1.0b	1.2b	60a	70a
2009	8.7a	13.5a	60a	77a

† Unfertilized and fertilized values in the same column for the same clones with the same letter are not significantly different ($P < 0.05$).

the two fertilization treatments in 2008 or 2009. However, there were differences between clones (data not shown). In 2008 Tully Champion trees had a significantly greater number of shoots per tree when compared to Marcy and Saratoga clones for both fertilization treatments. Under both fertilization treatments, Marcy clone had a significantly smaller number of shoots per tree than the other three clones in both the 2008 and 2009 growing seasons.

Generally, the survival of willow clones under the fertilizer treatments was similar between 2008 and 2009 (Table 3.3). The survival of measurement trees did not change between the two growing seasons for Tully Champion, India or Marcy. The survival of Saratoga trees, however, increased but not significantly (*P* > 0.05) from 70 to 77 % under fertilization but remained constant at 60 % without the application of fertilizer. It is not known why some clones did not grow in the first year but did in the second year.

Tree heights increased between September of the two growing seasons with mean heights of 37 and 77 cm in 2008 and 2009, respectively (Figure 3.3). In April of 2009

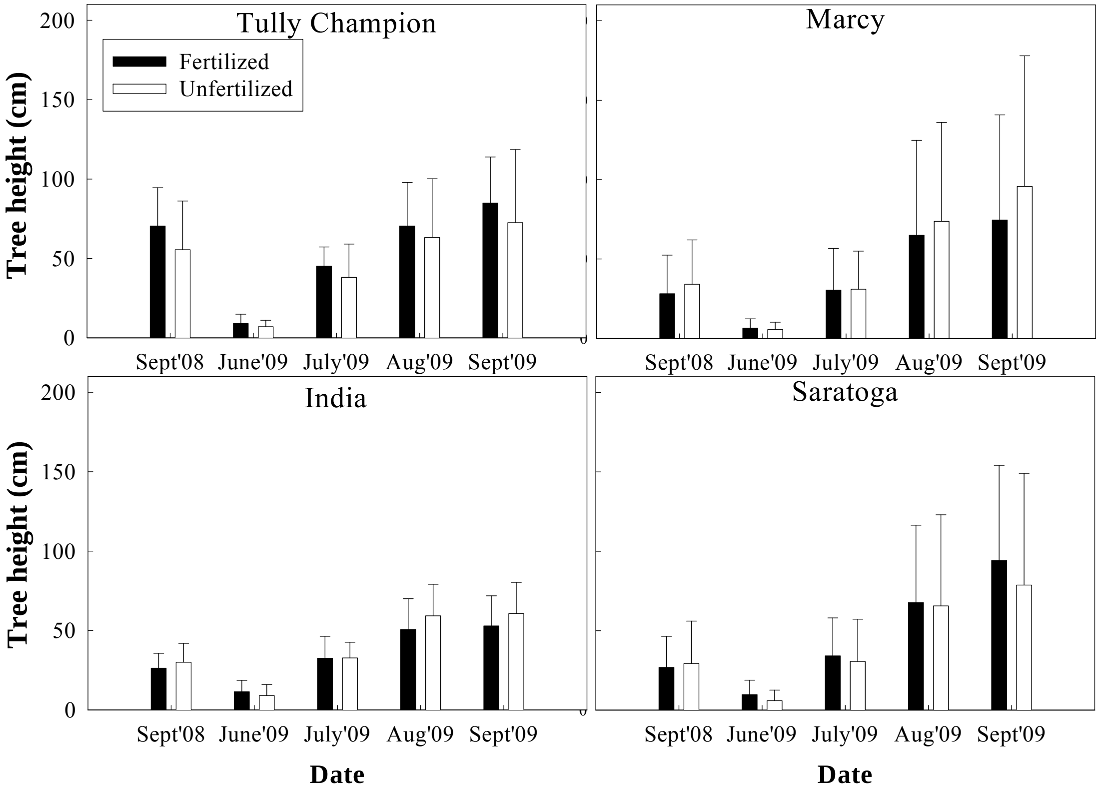


Figure 3.3 Mean tree heights of four willow clones grown in a SRWC system in Prince Albert, SK. over a two year growth period (error bars represent one standard deviation).

trees were coppiced to approximately 5 cm and were left to regenerate. The mean heights and shoot diameters of willow clones at the end of the 2008 and 2009 growing seasons are presented in Table 3.4. There were no significant differences in the tree height or shoot diameters between fertilization treatments for any clone in either growing season.

Averaged over fertilization treatments, in 2008 Tully Champion clones were significantly taller than the other three clones and in 2009; the Marcy and Saratoga clones were significantly taller than those of India (Table 3.4). In 2008 mean shoot diameters for Tully Champion were significantly greater than the other three clones and the India clones were significantly greater than the Saratoga clones. In 2009, Tully Champion and India clones had significantly larger shoot diameters than the Saratoga clones.

Table 3.4 Mean tree height and shoot diameter of willow clones grown for two year as a SRWC near Prince Albert, SK.

Clone	Mean Tree Height			Mean Shoot Diameter†		
	-------------- cm --------------			-------------- mm --------------		
	Unfertilized	Fertilized	Mean	Unfertilized	Fertilized	Mean
	---------------------------------- 2008 ----------------------------------					
Tully Champion	55.5a‡	70.4a	62.9A§	4.90a	6.36a	5.6A
Marcy	34.1a	28.1a	31.1B	3.22a	2.44a	2.8BC
India	30.1a	26.3a	28.2B	4.09a	3.68a	3.9B
Saratoga	29.2a	26.9a	28.1B	2.21a	2.16a	2.2C
	---------------------------------- 2009 ----------------------------------					
Tully Champion	72.5a	84.8a	78.7AB	5.39a	6.18a	5.8A
Marcy	95.7a	74.6a	85.2A	5.26a	4.71a	4.9AB
India	60.7a	52.9a	56.8B	5.96a	5.44a	5.7A
Saratoga	78.7a	94.2a	86.5A	6.50a	4.70a	4.1B

† Stem diameter is recorded for the tallest shoot at 30 cm above the soil surface.

‡ Unfertilized and fertilized values in the same row for the same property with the same lowercase letter are not significantly different ($P < 0.05$).

§ Mean values in the same column for the same year with the same capital letter are not significantly different ($P < 0.05$).

3.3.2 Biomass production

Leaf biomass ranged from 0.24 to 0.89 Mg ha^{-1} and accounted for 31 to 39 % of total tree biomass while shoot biomass ranged from 0.39 to 2.0 Mg ha^{-1} and accounted for 61 to 69 % of the total tree biomass (Figure 3.4). There were no significant differences in biomass production between the four clones or between the fertilization treatments.

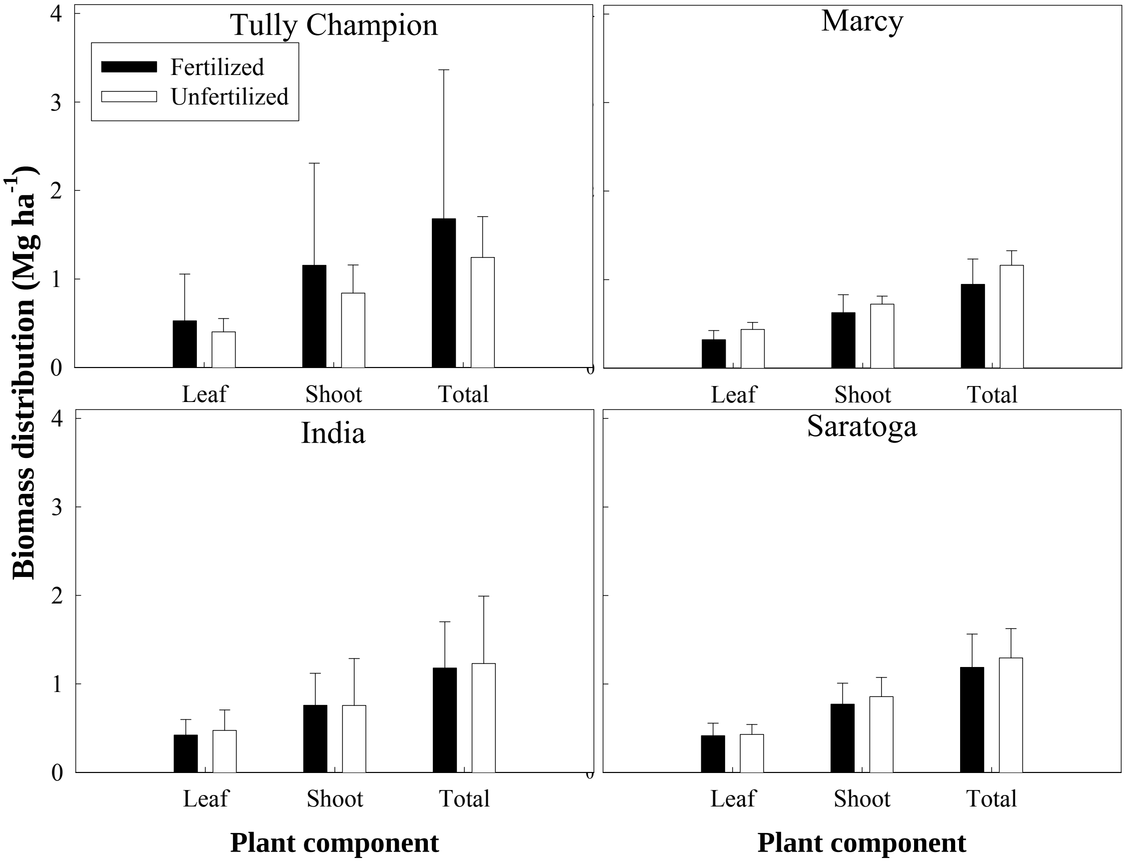


Figure 3.4 Mean biomass distribution for fertilized and unfertilized willow clones in the year following coppicing at a SRWC system in Prince Albert, SK. (error bars represent one standard deviation).

3.3.3 Foliar nutrient contents

Ammonium nitrate was applied in 2008 one month after planting. At the end of the 2008 growing season, the Marcy clone had significantly greater foliar N with fertilization compared to the unfertilized treatment (Table 3.5). No other differences were observed in 2008 or 2009 for either foliar N or P with the two fertilization treatments. Averaged over fertilization treatment Tully Champion and Saratoga clones had significantly greater foliar N concentrations than the India clones in 2008. In 2009, Tully Champion had significantly greater foliar N concentrations than the India clones. There were no significant differences in foliar P concentrations between any clones in either growing season.

Table 3.5 Mean foliar nutrient concentrations for fertilized and unfertilized willow clones grown in Prince Albert, SK. over two growing seasons.

Clone	Mean Total Foliar N			Mean Total Foliar P		
	mg g^{-1}					
	Unfertilized	Fertilized	Mean	Unfertilized	Fertilized	Mean
	2008					
Tully Champion	43.1a†	44.5a	43.8A‡	3.1a	2.7a	2.9A
Marcy	37.4b	45.4a	45.4AB	3.5a	2.4a	3.0A
India	35.8a	37.8a	36.8B	3.2a	2.6a	2.9A
Saratoga	45.4a	45.3a	45.4A	3.1a	3.1a	2.1A
	2009					
Tully Champion	21.5a	22.2a	21.8A	2.4a	2.5a	2.4A
Marcy	16.4a	20.3a	18.4AB	2.4a	3.2a	2.8A
India	16.4a	19.2a	17.8B	2.1a	2.3a	2.2A
Saratoga	16.9a	18.6a	17.8AB	2.5a	2.0a	2.2A

†Unfertilized and fertilized foliar nutrient values in a row for the same nutrient with the same lowercase letter are not significantly different ($P < 0.05$).

‡Mean foliar nutrient values in the same column for the same year with the same capital letter are not significantly different ($P < 0.05$).

No differences in N:P ratios were observed between unfertilized and fertilized treatments for any clone in either growing season (Table 3.6). In 2008 there were no differences in N:P ratios between clones, while in 2009 Tully Champion had significantly greater N:P ratios than the Marcy clones.

3.3.4 Soil nutrient availability

There were no differences in soil nutrient concentrations between the four clones for any of the sampling times and thus the soils data is presented as an average of all the clones (Table 3.7). Averaging the three sampling depths together, it was found that there were only significant differences between fertilization treatments for soil extractable

NO_3^--N and NH_4^+-N at the end of the 2008 growing season. Soil extractable PO_4^--P did not differ between fertilizer treatments at any sampling time during the study showing that levels were not affected by N fertilization.

Table 3.6 Mean N:P ratios for fertilized and unfertilized willow clones growing in a SRWC system in over two growing seasons in Prince Albert, SK.

Clone	Mean N:P Ratios		
	Unfertilized	Fertilized	Mean
	------------------------ 2008	------------------------	
Tully Champion	14.0a†	16.4a	15.2A‡
Marcy	10.6a	19.3a	15.4A
India	11.1a	14.6a	13.1A
Saratoga	14.8a	14.6a	15.3A
	------------------------ 2009	------------------------	
Tully Champion	8.9a	8.9a	8.9A
Marcy	6.8a	6.3a	6.8B
India	7.8a	8.4a	8.2AB
Saratoga	6.8a	9.3a	8.2AB

† Unfertilized and fertilized N:P ratios in the same row with the same lowercase letter are not significantly different ($P < 0.05$).
‡ Mean N:P ratios in a column for the same year with the same capital letter are not significantly different ($P < 0.05$).

Nutrient concentrations in the soil varied with sampling time under the two fertilization treatments. Surface soil samples (0 – 10 cm) had significantly more NO_3^--N than at deeper sampling depths for unfertilized plots in 2008 and fertilized plots one week after fertilization and in 2008. In the unfertilized plots in 2008, NH_4^+-N levels were significantly larger at depth (10 – 30 cm) than at the soil surface (0 – 10 cm), while the opposite trend was observed for fertilized plots in the same year. Unfertilized plots in 2008 and 2009 and fertilized plots in 2009 had significantly greater levels of PO_4^--P at 10 – 30 cm depth than at 0 – 10 cm depth.

Table 3.7 Extractable soil nutrients collected prior to fertilization and one and two years after fertilization from a willow SRWC system in Prince Albert, SK.

	Extractable soil nutrients ($\mu g\ g^{-1}$)							
Soil Depth	Pre – Fertilization		Post – Fertilization		2008		2009	
	UF†	F‡	UF	F	UF	F	UF	F
-- cm --	NO_3^- -N							
0 – 10	5.8§	5.6	12.9a*	101a	4.0a	34.9a	0.3a	ND††
10 – 20	-	-	11.7a	12.9ab	4.3ab	17.2b	0.6a	1.7a
20 – 30	-	-	7.2a	8.0b	3.0b	4.4c	0.9a	0.8a
Mean	5.8A‡‡	5.6A	10.6A	23.4A	3.7B	18.8A	0.6A	0.6A
	NH_4^+ -N							
0 – 10	6.0	6.2	3.9a	17.8a	3.5a	27.3a	5.7a	5.9a
10 – 20	-	-	3.7a	4.0a	3.7b	3.8b	4.6a	5.8a
20 – 30	-	-	3.4a	3.4a	3.1b	3.7b	4.8a	5.5a
Mean	6.0A	6.2A	3.7A	5.7A	3.4B	11.6A	5.0A	5.6A
	PO_4^- -P							
0 – 10	53.3	44.7	77.0a	75.6a	101b	96.8a	61.3b	64.0b
10 – 20	-	-	85.8a	77.2a	143a	118a	78.2a	75.7a
20 – 30	-	-	65.7a	62.7a	122ab	111a	83.0a	71.1ab
Mean	53.3A	44.7A	76.8A	70.7A	122.0A	108.6A	74.2A	70.3A

† Unfertilized treatment
‡ Fertilized treatment
§ Preliminary samples were collected as a composite sample from 0 – 30 cm.
* Nutrient values in the same column for the same nutrient with the same lowercase letter are not significantly different ($P < 0.05$).
†† Value was below detection limit.
‡‡ Mean nutrient values in the same row for the same sampling time with the same capital letter are not significantly different ($P < 0.05$).

Levels of extractable soil nutrients fluctuated throughout the experimental period. All sampling depths were taken into account when observing fluctuations in time. Both unfertilized and fertilized plots increased in NO_3^--N one week after fertilization took place and then decreased with time. Unfertilized plots were below initial NO_3^--N levels by the end of 2008 while fertilized plots did not fall below the pre-fertilized levels until the end of 2009. Unfertilized plots decreased in NH_4^+-N one week post fertilization and by the end of 2008. By 2009, unfertilized NH_4^+-N levels had increased but were, however, still below the initial levels. Fertilized plots increased in NH_4^+-N one week post fertilization and at the end of 2008 but only at 0 – 10 cm depth. By the end of 2009, NH_4^+-N levels on fertilized plots had decreased below initial values; however, they had increased at 10 – 30 cm depth from the levels of one week after fertilization and the end of 2008. Both fertilized and unfertilized plots increased in PO_4^--P from the initial sampling to the end of 2009 with the peak levels occurring at the end of 2008.

3.3.5 Nitrogen fertilizer recovery

The N recovery of the applied labeled ammonium nitrate by the entire tree ranged from 2.87 to 10.6 % in 2008 and from 0.39 to 2.95 % in 2009 (Figure 3.5). There were significant differences in N recovery by cuttings for all clones between the two growing seasons with the greater amounts recovered in 2008. The N recovery by the plant components varied significantly for certain clones in the two growing seasons. In 2008, N recovery by shoots of Tully Champion was significantly greater than that by Marcy and Saratoga shoots and Tully Champion cuttings recovered significantly more labeled N than Marcy cuttings. In 2009, Tully Champion shoots recovered significantly more N than Marcy shoots and cuttings of Tully Champion and India recovered significantly more N than Marcy cuttings. In the two growing seasons, the N recovery by clones was significantly different between the plant components. In 2008, Tully Champion recovered significantly more N in leaves and shoots than roots and India clones recovered significantly more N in the leaves than the three other plant components. In 2009, Tully Champion leaves recovered significantly more N than the roots, leaves of the Marcy clone recovered significantly more N in the leaves than all three other plant components, India leaves recovered significantly more than the two below ground components and Saratoga recovered significantly greater amounts in the leaves than the roots.

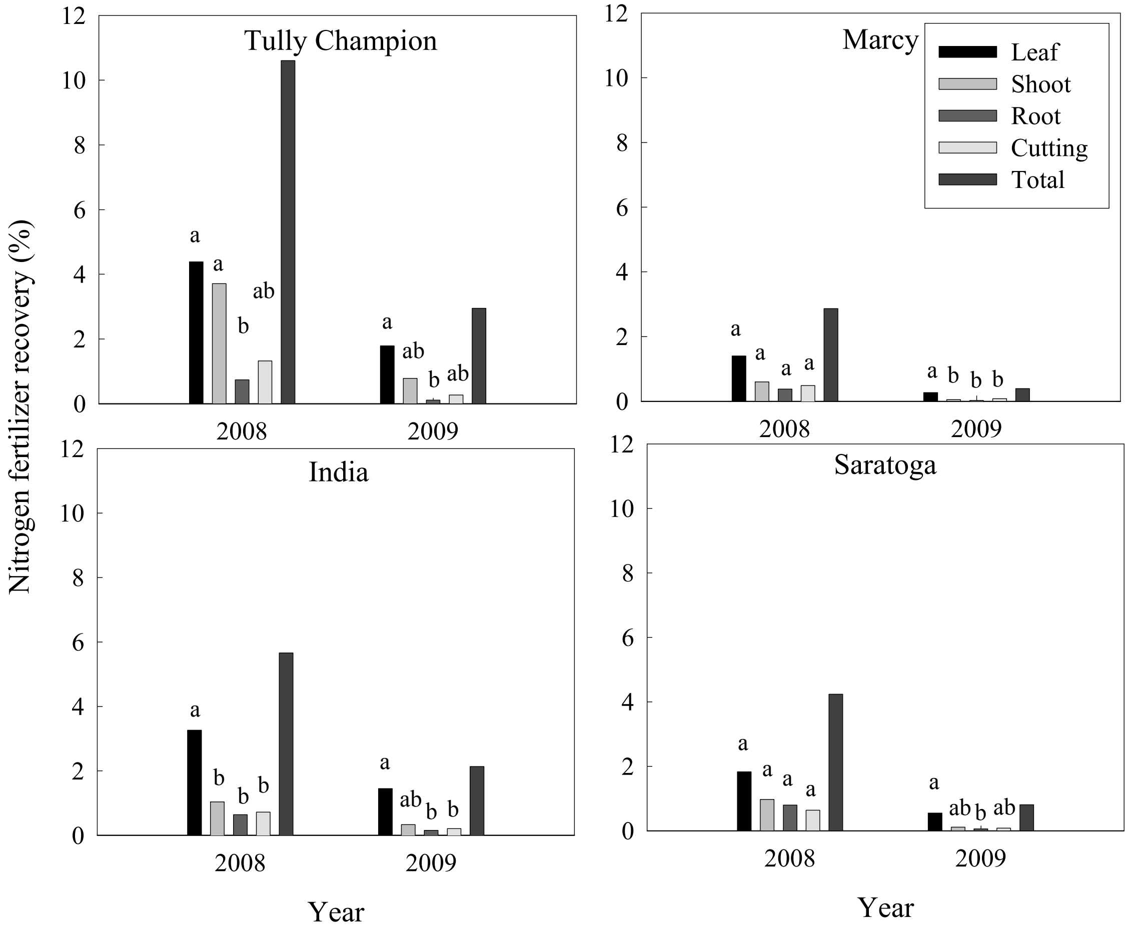


Figure 3.5 Mean percentage of applied nitrogen fertilizer recovery by willow clones in Prince Albert, SK for two growing seasons (vertical bars with the same letter in the same year and clone are not significantly different (*P* < 0.05)).

Atom %^{15}N excess refers to the amount of ^{15}N in the environment in excess of the natural background level known as natural abundance (0.3663). The amount of residual labeled N fertilizer in excess of the natural abundance of ^{15}N within the total N of the soil was very small (Figure 3.6). Regardless of the quantity remaining, differences in the accumulation of labeled N fertilizer at soil depths under the four clones still occurred. There were no significant differences between clones in 2008. In 2008, the surface soil (0 – 10 cm) had a significantly greater amount of the labeled fertilizer than the subsurface depths (10 – 30 cm). However, atom % ^{15}N excess in Saratoga plots differed significantly from both Marcy and India in 2009. The quantity of remaining labeled fertilizer accumulated in the soil varied between sampling depths. Because such small amounts of labeled N were found in the top 30 cm of the soil in 2008, sampling depths were increased to 60 cm in 2009. In 2009, there was a significantly greater amount of residual labeled fertilizer in the 40 – 60 cm depths than the 0 – 30 cm depths. As well, the 30 – 40 cm depth was significantly smaller than the 40 – 50 cm depth. These trends suggest a vertical translocation of labeled ^{15}N.

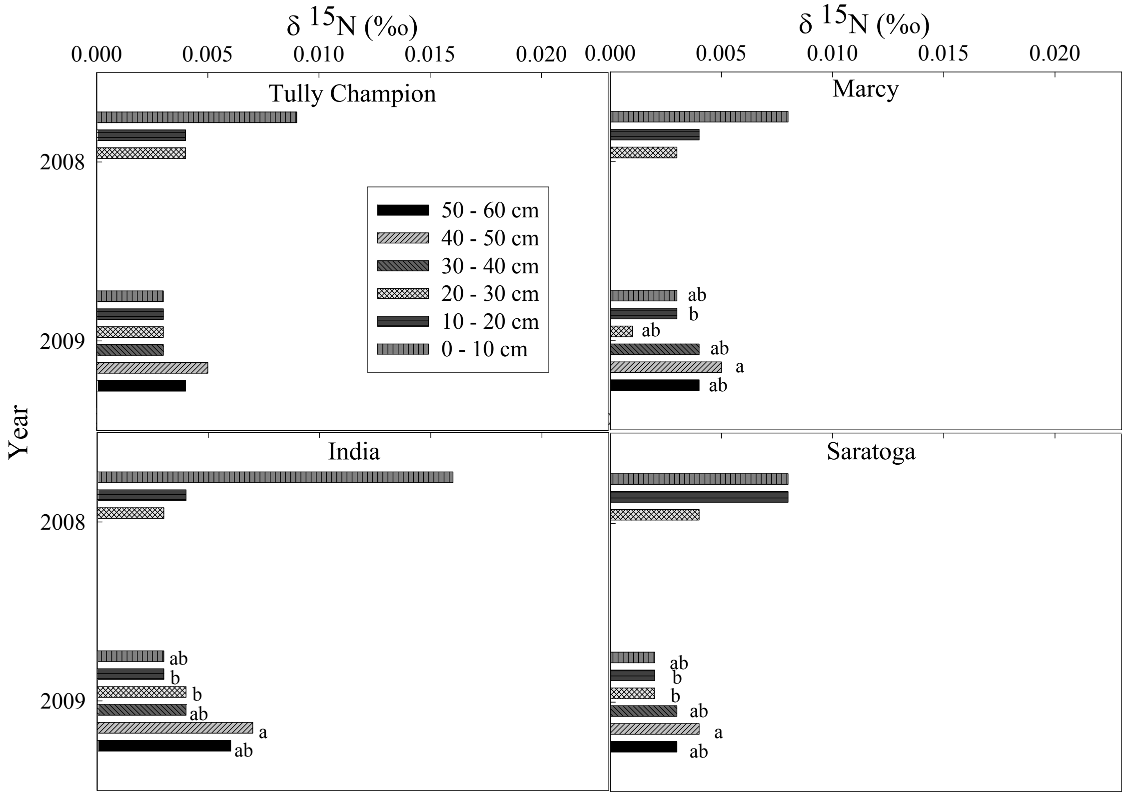


Figure 3.6 Mean atom ^{15}N % excess of the soil profile for two years under a willow short rotation woody cropping system in Prince Albert, SK (horizontal bars with the same letter within the same year and clone are not significantly different ($P < 0.05$)).

3.4 Discussion

One of the purposes of this study was to determine if fertilizer application to willow plantations in the year of planting had an effect on biomass production. When biomass was harvested in the year following coppicing it was found that fertilization did not increase biomass production. Unfertilized trees for all four clones ranged from 0.72 to 0.86 Mg ha^{-1} and fertilized trees ranged from 0.63 to 1.2 Mg ha^{-1}. In Sweden, irrigation with liquid ammonium nitrate resulted in biomass production of 13.3 Mg ha^{-1} in the year following the first three year harvest cycle (Nilsson and Ericsson, 1986). In New York, USA, annual application of a fertilizer blend (336 kg N ha^{-1}, 112 kg P ha^{-1} and 224 kg K ha^{-1}) led to yield increases of 6.1 to 12.5 Mg ha^{-1} yr^{-1} while unfertilized trees increased by 4.5 to 10.8 Mg ha^{-1} yr^{-1} (Adegbidi et al., 2001). Without any nutrient additions *S. viminalis* and *S. dasyclados* yielded 11.5 to 12.9 Mg ha^{-1}, respectively in a UK study in the year following coppicing (Bullard et al., 2002). The yields in the present study are much smaller than reported by other researchers however, they are larger than an older Swedish study that fertilized willows with liquid and solid ammonium nitrate at rates of 150 kg N ha^{-1} for both fertilizers (Christersson, 1987). In the year following the first harvest cycle, yields ranged from 7.0 x 10^{-4} to 1.4 x 10^{-3} Mg ha^{-1} and 7.0 x 10^{-4} to 1.3 x 10^{-3} Mg ha^{-1} with liquid and solid ammonium nitrate fertilizer, respectively (Christersson, 1987). Inorganic fertilizers applied in the second year of growth to a plantation of 15,200 plants ha^{-1} increased biomass production by 8 to 134 % after one year (Adegbidi et al., 2003).

Poor fertilizer recovery may have led to the lack of fertilizer response in the present study. Of the applied ^{15}N label 1.40 to 4.83 % was recovered by the foliar tissue in 2008 and 0.27 to 1.79 % in 2009. Booth (2008) found that in northern Saskatchewan, Canada the application of labeled N as ammonium nitrate (25 kg N ha^{-1}) in solution with another 75 kg N ha^{-1} supplied by granular ammonium nitrate in the year of planting, resulted in a recovery of only 0.8 to 2.5 % by poplar leaves. Fertilizer N recovery by the above-ground willow organis in this study were 2.00 to 8.54 % in 2008 and 0.32 to 2.57 in 2009. Staples et al. (1999) found that 6.45 % of the ^{15}N applied was incorporated into the above-ground biomass of white spruce seedlings after two years of growth in a reforested ecosystem in Saskatchewan. Total N recovery by lodgepole pine in British Columbia was 1.9 to 10.1 % of applied ^{15}N, while understory vegetation recovered 2.3 to 3.4 % (Preston and Mead, 1994). Soil sampling in search of the labeled fertilizer in this study did not result in the measurement of a large residual pool. Preston and Mead (1994) found 30.6 to 73.2 % of the applied ^{15}N under a lodgepole pine stand was found in the soil in organic forms. The label in the present study was barely evident above the natural background level of ^{15}N in the environment (0.3663). In 2009, the label was found in greater amounts at deeper soil depths suggesting leaching through the soil profile.

Coppicing is a practice that is carried out to increase the number of shoots per tree thus resulting in a greater shoot biomass yield. A 56 % increase in biomass production has been noted after the coppicing of a Swedish plantation (Nilsson and Ericsson, 1986). In this study, it is difficult to determine the potential of coppicing to increase biomass production of willows because of the continual uniform browsing of the plantation by white-tailed deer. The increase in number of shoots per tree, however, can still be quantified accurately since only the tips of shoots were browsed and not the entire shoots. Coppicing increased the number of shoots per tree by 2 to 11.6 fold between 2008 and 2009 which is larger than the suggested increase of 3 to 4 fold (Hytönen, 1995). Marcy, India and Saratoga were well above this projection while Tully Champion fell short with increases of only 2 to 3.2 fold. This small increase may be because Tully Champion had on average double the number of shoots in 2008 than the other clones. The increase in number of shoots per tree after coppicing is crucial to obtain the most shoots possible to reach maximum biomass production potential.

The importance of tree establishment was noted early on with the Saskatoon plantation. After one month, the establishment was 0 % and by the end of the first growing season, survival ranged from 0 to 54 % with an overall mean of 17 %. Planting material was made, treated the same and then randomly selected for planting at each site, so it is unlikely that the problem arose from the cutting quality. Probable causes of this poor establishment are environmental conditions at the time of planting and the quality of soil at the Saskatoon site. Poplar trees have been shown to have survival rates as low as 40 % because the conditions under which they were planted were unfavorable (Trnka et al., 2008); however, the mortality of poplars is generally larger than for willow (Ferm et al., 1989). Nonetheless, poplars suffer greatly from lack of moisture during the establishment stage (Trnka et al., 2008). In Saskatoon, in the five days prior to planting the site, approximately 6 mm of precipitation was received. In the five days following planting, approximately 18 mm was received (Appendix C). Although precipitation occurred, cuttings may still have experienced dessication due to the vertisolic properties of the soil.

Soils of the Vertisolic order are diagnosed by their shrink-swell characteristics (Group, 1998). During wetting and drying cycles, the soils swell and then crack, respectively leading to drying and hard, massive structures in the A horizon (Group, 1998). After the rainfall following planting, the soil would have first swelled as the water filled the soil pores. As the water was used and removed from the system the soil would have shrunk creating deep cracks which could have reduced contact between the soil and the cutting, and would have dried out the cuttings. Hard soil clods forming in the upper horizon would restrict root development. If soil moisture would have been maintained with adequate rainfall, these hard clods likely would not have formed and root growth would not have been restricted.

It has also been observed that willows prefer coarser textured soils over finer ones (Schaff et al., 2003). Survival of black willows in soils high in silt and clay was shown to be 25 % less than on coarse soils (Schaff et al., 2003). Schaff et al. (2003) found that clayey soils prevented rapid root elongation and thus decreased the health of the trees. Also, in fine textured soils, roots require a more negative internal potential in order to extract water from the surrounding soil (Schaff et al., 2003). A combination of the vertisolic shrink-swell characteristics of the soil, limited water availability and high content of clay likely led to the poor establishment at the Saskatoon site. It can be recommended that heavy clay soils are not favorable for the establishment of willow SRWC systems.

Tree survival in Prince Albert in the present study was greater than the suggested economically successful survival rate of 80 % (Bergvist, 1996). In both growing seasons under both fertilization treatments Tully Champion and India had survival rates of 87 to 93 %. The trees that survived in the first year were still alive in the second year; a trend also observed by Schaff et al. (2003). Because the survival rates of Marcy and Saratoga clones are less than the suggested 80 % (ranging from 60 to 77 %) they may not be the best suited clones for SRWC systems in the Boreal Transition ecozone of Prince Albert. These two clones may, however, have the potential to be better suited to other locations.

The application of fertilizer can change the chemistry of the soil. Irrigation with wastewater did not have a significant effect on the chemistry of the soil or ground water (Sugiura et al., 2008) whereas organic amendments significantly increased the concentrations of N and P in the top 10 cm of soil relative to a control (Adegbidi et al., 2003). In this study, fertilization increased NO_3^--N in the top 30 cm and NH_4^+-N in the top 10 cm by 18 and 3 fold, respectively compared to the control. The effects of fertilization on soil concentrations were no longer prevalent one year later. Interestingly, PO_4^--P increased in 2008 then decreased in 2009 under both fertilization treatments. The low values for soil nitrate on the day of planting may be due to sampling technique. The soil samples were collected as composite samples of 0 – 30 cm, therefore variations with depth was averaged across the sampling depth.

Foliar N and P concentrations were not significantly affected by fertilization treatments which does not agree with the findings of Arevalo et al. (2005) who observed significant increases in foliar N of *S. sachalinensis* and *S. discolor* when fertilized with 90 kg N ha^{-1} ammonium nitrate in New York, USA. Total foliar contents go through significant seasonal fluctuations (Bollmark et al., 1999). During mid-season, foliar N of willow was quantified as 3.2 to 3.4 % and had decreased to 1.6 to 1.7 % by the end of the growing season (Christersson, 2006). In this study, foliage was collected in September and foliar N concentrations ranged from 3.6 to 4.5 % in 2008 and 1.6 to 2.2 % in 2009. Unfertilized willows in Germany in July and August were recorded to have total foliar N concentrations of 2.6 to 3.4 % and increased by 1 to 3 % when fertilized with 50 and 100 kg N ha^{-1} yr^{-1} (Jug et al., 1999). In one study, the optimal level of total foliar N is suggested to be 3.0 % for willow leaves collected in July and August (Jug et al., 1999). In 2008, the total foliar N concentrations of willows in this study for both fertilized and unfertilized were above this level. In 2009, the foliar N concentrations were well below 3.0 % inferring N deficiency or less than optimal biomass yields. However, the 3 % level is only recommended by one study.

Decreases in foliar P concentrations have been shown in willows and poplars following fertilization (Nilsson and Ericsson, 1986; van den Driessche, 1999). Fertilized and unfertilized poplar trees were observed to have foliar P concentration of 0.2 % and 0.14 %, respectively (van den Driessche, 1999). Willows and poplars are in the same family, Salicaceae (Dickmann, 2006) but differ in nutritional behavior and growth reactions (Jug et al., 1999). However, nutritional findings for poplar can be used as an approximation for willow just not an exact comparison. The foliar P concentrations were greater than the range recommended by van den Driessche (1999).

To better understand these concentrations the N:P ratio can be used. Although there were no significant differences between the two years and treatments, N:P ratios decreased by 30 to 67 % from 2008 to 2009. For poplars, one Canadian study infers that ratios of greater than 9.5 represent P deficiencies while ratios less than 9.5 infer N deficiencies (Zabek, 2001). In 2008, all N:P ratios were above 9.5 but were below 9.5 in 2009 in the current study. Both total foliar N and P decreased significantly between the two growing seasons. Foliar N fell below the suggested critical level of 3.0 % (Jug et al., 1999) in 2009 while foliar P remained above the levels observed by van den Driessche (1999) in 2009. Reduced N availability in 2009, as shown in the foliar tissue, likely caused the decrease in foliar N in 2009 and was the cause of the lowered N:P ratio.

In 2009, the N foliar tissue levels were below the optimal value of 3.0 % N, suggesting that to obtain optimal yields fertilizer may need to be applied in the second growing season. The lower N:P ratios in 2009 also suggest that fertilization may be more beneficial in the second year of growth rather than in year one at the time of planting. Previous studies exploring the optimal timing of fertilizer application also found that application in the second and third years of growth will result in maximal biomass yields (Alriksson et al., 1997). Another study in New York, USA recommends N applications annually to maintain yields in the long run (Adegbidi et al., 2003). By waiting to apply fertilizer in the second year, plants are given a chance to establish more expansive root systems which will be better capable of capturing fertilizer before it is lost through the soil profile.

3.5 Conclusion

The single application of N fertilizer in the first year did not have any positive or negative effects on the growth of willows in this SRWC system. There were no differences in biomass production, tree height, number of shoots per tree or total foliar P concentrations between the fertilized and unfertilized treatments. However, there were only single isolated cases in 2008 when foliar N and shoot diameter differed significantly between fertilizer treatments. The majority of N recovered accumulated in the leaf components, although recovery of the fertilizer was very low among all clones. By the end of 2009, the effects of N fertilizer were no longer evident in soil inorganic N concentration, and would therefore no longer benefit the system as seen. Foliar P concentrations were above the critical level suggested by Zabek (2001) in both years however foliar N concentrations fell below the critical level for poplar in 2009 inferring a N deficiency. Since N fertilization had no effect on biomass production in either year and the biomass produced in the first year was lost through coppicing, fertilization in the first year may not be ideal. It is possible that the fertilizer would have resulted in increased biomass production if it had remained in the soil for a longer period of time or had been applied later in the growth cycle once the trees were more established. Therefore, it can be recommended that in the year of planting, efforts should be made to ensure high survival rates while in the second year focus can be directed at achieving optimal biomass yields through the application of N fertilizer. Since the single application of N fertilizer in the first year did not have a positive effect on the growth of willows in this SRWC system, the hypothesis was rejected.

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4. THE EFFECTS OF FERTILIZER RATES AND TYPES ON BIOMASS PRODUCTION OF SALIX SPP.

4.1 Introduction

It has been suggested that the 21st century will become the "age of biology" as renewable biomass resources, such as willow SRWC systems replace petroleum and other fossil fuels as energy sources and industrial products (Keoleian and Volk, 2005). Although willow has only become a topic of intensive research in the past few years, its use can be dated back to the Roman Empire when willow was used for baskets, medicine, fences and as framing for shields (Keoleian and Volk, 2005). In England, willow basket production can be traced back to at least 100 B.C. (Keoleian and Volk, 2005). Currently in Saskatchewan, there exists a small demonstration of willow research trials.

Intensive management of SRWC systems will increase production and therefore increase the rate of nutrient removal at the time of harvest (Adegbidi et al., 2001). Adegbidi et al. (2001) however, stated that producers must maintain a balance of nutrients in the soil to ensure long-term sustainable production of the system. Nutrients can be supplied to willow SRWC systems in many forms. Inorganic fertilizer is most often considered because they are effective and accessible in developed countries. Research, however, has been looking into using local organic wastes as nutrient sources to fertilize SRWC systems. These have included biosolids (Heller et al., 2003), green manure (Arevalo et al., 2005) anaerobically digested sewage sludge, composted poultry manure, composted sewage sludge (Adegbidi and Briggs, 2003), waste water and landfill leachates (Hasselgren, 1998).

Researchers have found many benefits to using organic fertilizers. Because fertilizers add to the cost of production (Adegbidi et al., 2001), organic wastes provide nutrients to the SRWC system at low or negative costs (Adegbidi et al., 2003) thus lowering production costs and eliminating the need for landfill disposal of the wastes (Adegbidi et al., 2001). Both fertilizer types have demonstrated the ability to increase biomass production. Organic fertilizers increased biomass by 30 to 38 % (Adegbidi et al., 2003) compared to unfertilized control treatments. Inorganic fertilizers increased biomass production by 25 to 30 % by the first harvest (Adegbidi et al., 2003). The organic fraction of nutrients supplied by organic wastes will be released at a slower rate making their benefits available for longer periods of time (Heller et al., 2003). Inorganic fertilizers were effective nutrient suppliers initially but after 3 to 4 yr their effects began to decline (Christersson, 2006). Organic fertilizers also had positive effects on soil organic matter, exchangeable cations and extractable P (Adegbidi et al., 2003).

The objective of this study, therefore, was to evaluate the effects of two soil types as well as various types and rates of fertilizers in willow biomass production. The hypothesis of this study was that willow clones would show a greater growth response to granular ammonium nitrate and composted cattle manure over an unfertilized control.

4.2 Materials and Methods

4.2.1 Soil preparation

Surface soil was collected from the PRT nursery site north of Prince Albert, SK. and from the willow plantation in Saskatoon, SK. (Table 4.1). The soil was air dried, ground, and passed through a 2 mm sieve. The plant pots used were 15 cm diameter at the top with a total volume of 3 L. These were filled with 3 kg of soil. A coffee filter was used to line the bottom inside each pot to restrict soil from seeping out the drainage holes.

4.2.2 Material preparation

In January 2009, planting material was collected from pre-established plantations in Saskatoon. Three clones from the field fertilization trial were chosen for this study based on their heights during the first year of the field trial. In 2008, the average shoot height per clone were ranked as Tully Champion > Marcy > Saratoga > India (Table 3.4). Because the three SUNY-ESF clones all include *S. miyabeana* (Table 3.2), India was chosen over Saratoga to enhance the biodiversity of the study and not because it was a top performer. Material from one-year old Tully Champion, Marcy and India were collected in the field. In the lab, the shoots were cut into 15 cm cuttings using hand held clippers and stored between -2 and -4 ^{o}C. The cuttings were thawed at room temperature for 24 hours and soaked for 24 hours immediately before planting.

Table 4.1 Site characteristics of two willow fertilization trials in Saskatchewan, Canada.

Site	Soil Classification			Soil Properties†				Prior Crop
	Association	Soil Type	Texture	pH --	EC µS s^{-1}	TC ---- % ----	OC	
Saskatoon‡	Sutherland	Orthic Vertisol	heavy clay	7.98	327	1.97	1.64	barley/oats
Prince Albert§	Pine	Orthic Eutric Brunisol	Sand to loamy sand	7.03	149	1.41	1.39	summer fallow/ white spruce seedlings

† Soil properties were measured for the top 30 cm of soil; EC – electrical conductivity, TC – total carbon and OC – organic carbon.

‡ (SCSR, 1978)

§ (SCSR, 1976)

4.2.3 Planting, treatments and watering

A single cutting was planted directly into the centre of each pot of dried, ground soil. Five fertilization treatments were immediately applied to the three clones following planting. Inorganic fertilizer at three rates; 50, 100 and 200 kg N ha^{-1} in the form of granular ammonium nitrate was hand broadcasted on the soil surface. Composted cattle manure at 100 kg N ha^{-1} was incorporated into the top 5 cm of soil. An unfertilized control was used for comparison. The composted cattle manure was collected from a producer in Dixon, S.K. The water extractable NH_4^+-N and PO_4^--P in the manure were 3 μg g^{-1} and 193 μg g^{-1}, respectively, while the total N and total P concentration was 2997 μg g^{-1} and 2518 μg g^{-1}, respectively. The five fertilizer treatments (control, 50, 100, 200 kg N ha^{-1} as ammonium nitrate, and 100 kg total N ha^{-1} manure) were replicated four times for each of the three clones (Tully Champion, Marcy and India) on the two soils (Saskatoon and Prince Albert). The 120 pots were randomly organized in a Conviron controlled environment chamber (Controlled Environments Inc., Pembina, N.D.) at the University of Saskatchewan in the College of Agriculture and Bioresources. Willows were grown under an 18 hour light:6 hour dark photoperiod with day:night air temperatures of 22:18 ^{o}C.

Plants were initially watered to 70 % field capacity (Hangs et al., 2002) immediately after the application of the fertilizer. The Saskatoon soil required about 640 g of water while the Prince Albert soil required about 360 g of water. Every other day there after, five random pots of each soil were weighed to determine watering requirements to reach 3.1 kg and 3.7 kg for Prince Albert and Saskatoon, respectively. The average was taken and that amount of water was added to the Saskatoon and Prince Albert pots. The trees grew for 90 days and every 30 days the pots were re-randomized in the chamber.

4.2.4 Survival and infestation management

After 45 days, the cuttings planted in the Saskatoon soil had an establishment rate of 0 %. The cuttings were removed and water was added to the pots until it began to slowly drip from the bottom of the pot. This was done to ensure consistent water distribution throughout the pot. It was unknown as to how much fertilizer would be lost during this re-wetting, but the application of more fertilizer would have also led to unknown levels of the fertilizer. Water was added slowly to minimize the amount of leaching through the pot. Once the soil was thoroughly moistened, the Saskatoon pots were replanted with new willow cuttings and left to grow for 90 days.

The growth chamber became infested with black fruit flies part way through the experiment. The problem persisted so the chamber was periodically sprayed with Raid Max (House and Garden; active ingredients: pyrethrins 0.25 % and piperonyl butoxide 1.0 %).

4.2.5 Measurements and tree harvesting

On the day of planting the diameter of each cutting in each pot was measured and recorded with its corresponding fertilizer treatment. Non-destructive measurements were made on days 20, 28, 37, 40, 47, 55, 64, 72, 81 and 90 after planting to record the number of shoots, height of the tallest shoot and the presence of disease for each pot. On day 90, the trees were harvested and the leaves were removed and the shoots were clipped off at approximately 2 cm above the soil surface. The leaves and shoots were bagged separately, weighed and oven dried at 40 ^{o}C for approximately one week and weighed. The entire root system and soil from each pot was bagged and stored at 4 ^{o}C pending root washing. The cutting and attached roots were manually shaken loose from the soil. A handful of soil was collected from each pot and bagged for future procedures. The remaining soil was placed in a 1 mm mesh window screen and rinsed with water to capture the roots. Once washed, the cutting was removed from the rooting system. The biomass production of the three willow clones included leaves, shoots and roots. The cutting biomass was not included as a variable because the biomass would not have differed greatly from time of planting. The cutting biomass was, however, included in as a component of the total tree biomass.

4.2.6 Plant and soil analysis

All plant samples were oven dried for approximately one week at 40 ^{o}C and then weighed. The leaves were ground using a Hamilton Beach Custom Grind Deluxe 15 Cup Hands-Free Coffee Grinder (Hamilton Beach Brands, Ltd., Washington, N.C.). Leaves were digested in sulfuric acid (Thomas et al., 1967) and analyzed on a Technicon Auto Analyzer (Pulse Instrumentation Ltd., Saskatoon, SK.) to quantify total N and P contents.

The subsample of soil from each pot was air dried, then ground and passed through a 2 mm sieve. A KCl extraction (Keenley and Nelson, 1982) was used to extract soil NO_3^--N and NH_4^+-N. The soil PO_4^--P was extracted with a modified Kelowna extraction (Qian et al., 1994).

Both extracts were analyzed on a Technicon Auto Analyzer to quantify NO_3^--N, NH_4^+-N and PO_4^--P.

4.2.7 Statistical analyses

Statistical analyses were carried out in the R environment (R Development Core Team, 2008) using the measured variables and a mixed model followed by an ANOVA (Appendix F). Prior to analyses, variables were checked for normality of distribution using the Shapiro-Wilk test. For variables without normal distributions, log, exponential and square-root transformations were carried out to achieve normality or as close to normality as possible. No variable in this study achieved normality after transformations. Heights, number of shoots per tree, leaf, shoot, cutting and total biomass did not require transformations to reach normality. The transformations closest to normality were log transformation for soil NO_3^--N, foliar P content and foliar N:P ratios, exponential for PO_4^--P, square root transformation for foliar N contents and root biomass. Soil NH_4^+-N was closest to normality when no transformations were used. Comparisons of property means between treatments, clones and soils were performed using post hoc tests (Tukey's Honest Significant Difference and t tests) and a significance level of $P < 0.05$.

4.3 Results

4.3.1 Growth curves

Tree height was monitored throughout the experiment to observe the growth trends of willows under optimal growing conditions. On both Saskatoon and Prince Albert soils (Figure 4.1), all trees became infected with disease on approximately day 60. Tree heights had increased exponentially until this point and after day 60 the tree heights decreased. On the Saskatoon soil, only the control treatment for the Tully Champion clones exceeded the height the trees had obtained before infection set in. On the Prince Albert soil, most clones under most treatments were able to surmount the height at time of infection.

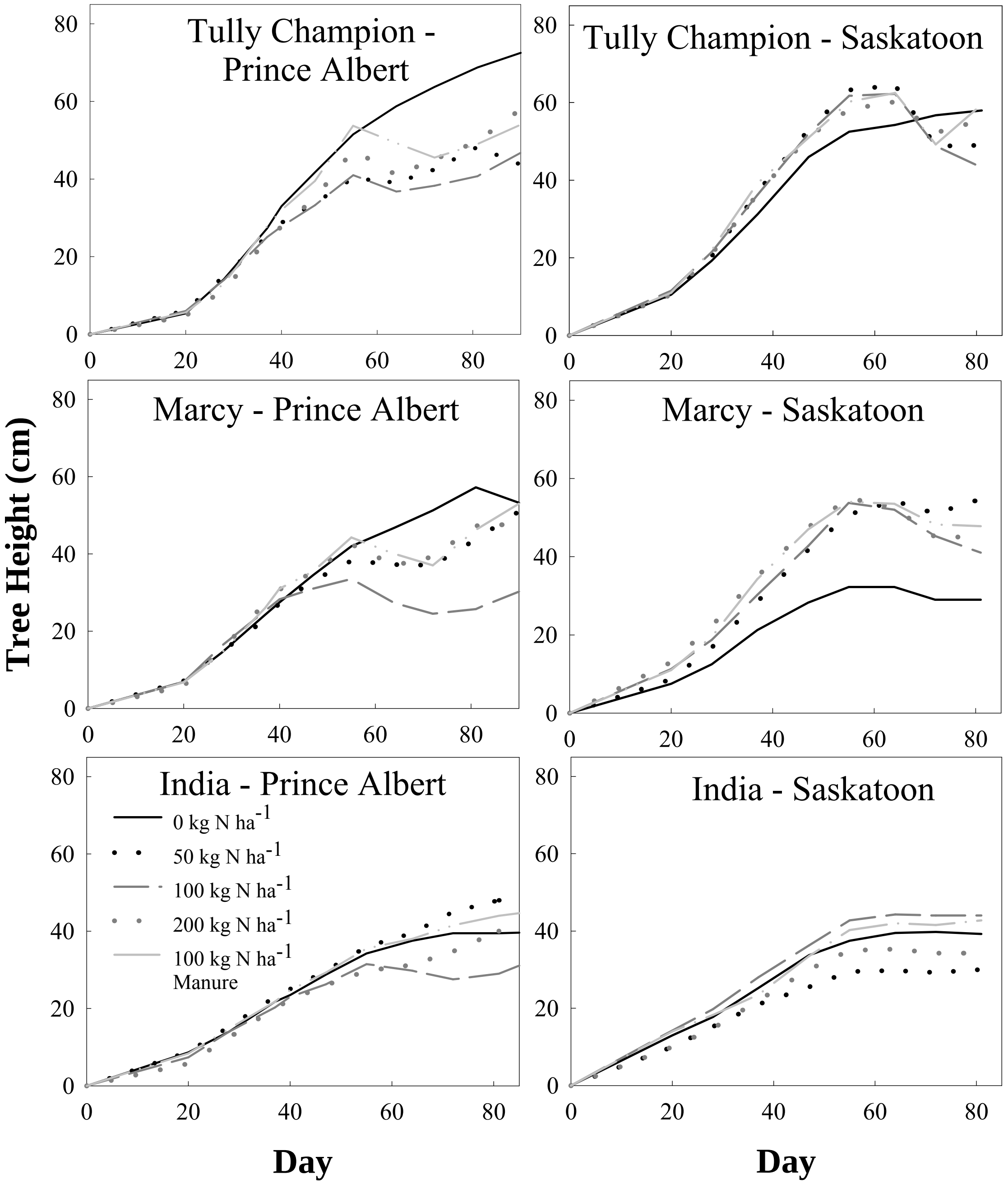

Figure 4.1 Growth curves of mean tree height for three clones grown on two soils and fertilized with five fertilization treatments. On day 60 disease infestation of the willows was observed.

The disease appeared to have many visual forms. The disease was first noted on the trees as a slight shrivelling of the leaves followed by complete drying out of the leaves and a loss of rich green pigmentation. On some leaves, the disease presented itself as a black line reaching from one edge of the leaf across the main vein and to the other leaf edge. This black line acted as a boundary for the disease symptoms previously seen to affect whole leaves. From the black line to the tip of the leaf, the tissue shrivelled and dried up, while the tissue towards the shoot of the tree still appeared healthy. The disease is suggested to be *Glomerella* spp. but no testing was carried out to support this.

4.3.2 Growth parameters

In order to nullify the effects of disease, statistics on tree heights were carried out using the heights on day 55 before disease had set in as well as day 90 at the end of the study. There were no significant differences between fertilization treatments for any of the three clones on either the Prince Albert or Saskatoon soil (Table 4.2). Because there were no significant differences between fertilization treatments for any clone, the tree heights on day 55 for all treatments were averaged and compared between clones. On both soils, there were significant differences in heights on day 55 among all three clones and were thus ranked as Tully Champion > Marcy > India. Tully Champion and Marcy clones were significantly taller on day 55 on the Saskatoon soil than the Prince Albert soil. India clones did not show differences between the two soils. There were significantly more shoots per tree for India clones on the Prince Albert soil than the Saskatoon soil, while the two other clones did not show any differences. There were no significant differences observed in the number of shoots per tree between clones.

4.3.3 Biomass distribution

Biomass production of the three plant organs (leaves, shoots and roots) varied between fertilizer treatments (Figure 4.2). On the Prince Albert soil, Tully Champion clones had significantly greater leaf biomass produced with 200 kg N ha^{-1} treatment than in the absence of fertilizer. The Marcy clones on the Prince Albert soil produced significantly great shoot biomass with the 200 kg N ha^{-1} treatment than the 100 kg N ha^{-1} treatment. On the Saskatoon soil, the India clones produced a significantly greater amount of shoot biomass when fertilized with manure than with 50 kg N ha^{-1} of the granular ammonium nitrate fertilizer.

Table 4.2 Growth parameters for three willow clones grown on two soils and under five fertilization treatments after 55 and 90 days in an indoor growth chamber.

Treatment	Mean Tree Height				Mean Number of Shoots	
	---------- cm ----------				------ # tree^{-1} ------	
	Day 55 (before disease)		Day 90		Day 90	
	P.A. †	Saskatoon	P.A.	Saskatoon	P.A.	Saskatoon
kg N ha^{-1}‡	---------- Tully Champion ----------					
0	51.5a§	52.5a	60.5a	51.3a	2.5a	2.3a
50	40.3a	63.3a	62.3a	46.0a	2.8a	2.5a
100	41.0a	61.8a	41.5a	43.0a	3.8a	2.3a
200	47.5a	58.3a	49.3a	44.5a	2.8a	2.3a
Manure	53.8a	60.3a	68.8a	54.8a	2.3a	2.0a
Mean	46.8B*	59.2A	56.2A	47.9A	2.8A	2.3A
	---------- Marcy ----------					
0	42.0a	32.3a	47.5a	48.5a	2.3a	2.5a
50	38.3a	50.8a	56.8a	44.8a	2.3a	1.3a
100	33.5a	53.8a	45.5a	40.5a	2.8a	1.8a
200	42.5a	55.0a	32.0a	32.3a	2.8a	2.3a
Manure	44.3a	54.0a	49.5a	42.3a	2.3a	2.0a
Mean	40.1B	49.2A	46.3A	42.3A	2.5A	2.0A
	---------- India ----------					
0	34.3a	37.5a	44.0a	43.8a	2.5a	2.3a
50	36.0a	29.5a	42.8a	31.8a	2.8a	2.0a
100	31.5a	42.8a	40.3a	37.8a	2.8a	1.5a
200	29.8a	35.0a	44.8a	40.0a	2.3a	2.3a
Manure	35.5a	40.3a	43.3a	37.5a	2.8a	1.8a
Mean	33.4A	37.0A	43.0A	38.2A	2.8A	2.0B

† Prince Albert

‡ Treatments are granular ammonium nitrate unless specified as Manure (100 kg N ha^{-1}).

§ Height and shoot values in the same column for the same clone with the same lowercase letter are not significantly different ($P < 0.05$).

* Mean values in a row for the same clone, property and day with the same capital letter are not significantly different ($P < 0.05$).

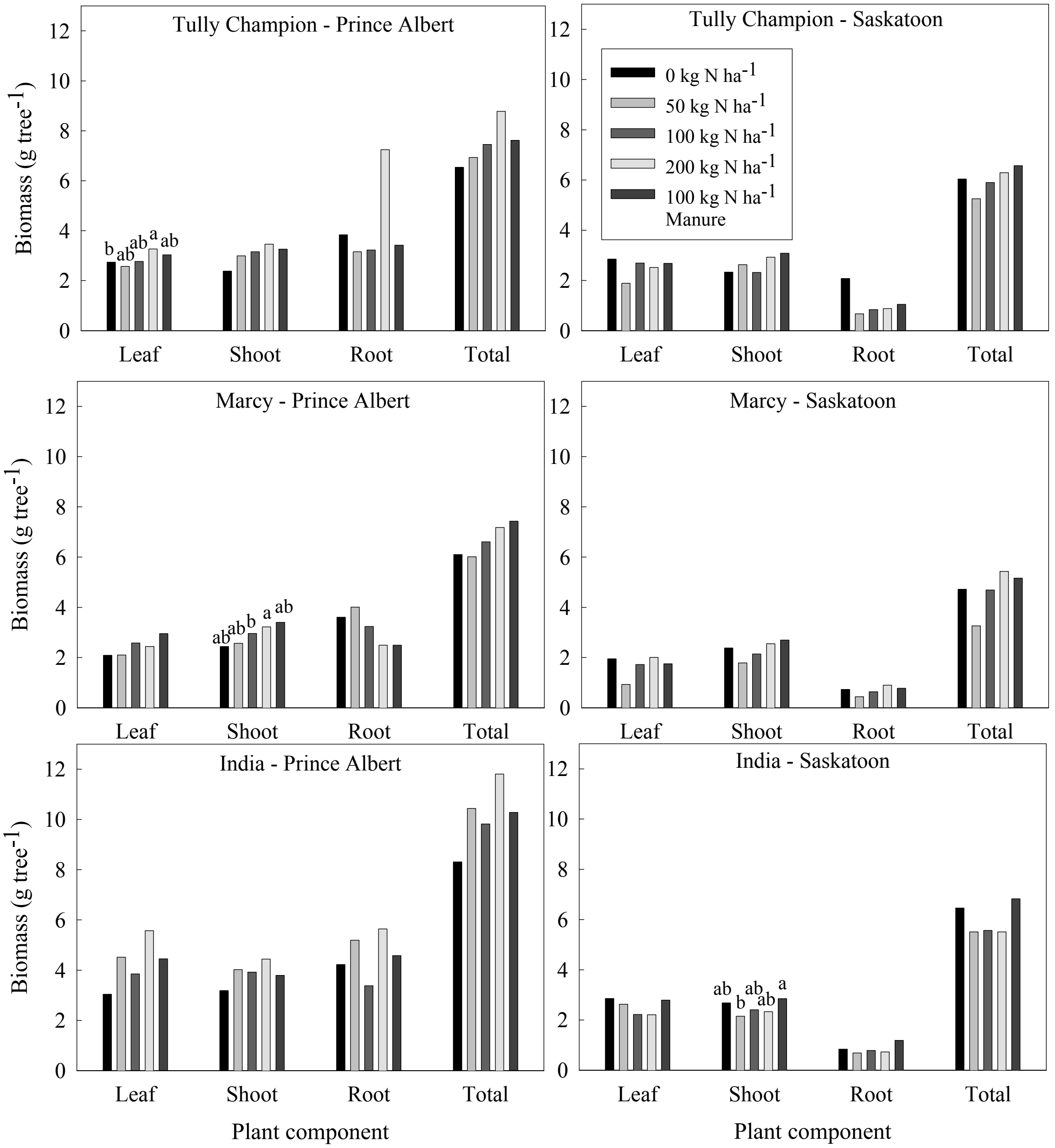

Figure 4.2 Mean biomass distribution of three willow clones grown in two soils under the effects of five fertilization treatments after a 90 day growth period in an indoor growth chamber (vertical bars for the same clone, plant component and soil with the same letter are not significantly different ($P < 0.05$)).

When averaging together the biomass production of each component for all clones it was found that clones produced more leaf, shoot and root biomass on the Prince Albert soil than the

Saskatoon soil except in the case of Tully Champion shoot biomass where no significant differences were noted between the two soils. On the Prince Albert soil, across all treatments, Tully Champion and Marcy clones produced significantly more leaf and shoot biomass than India clones while India clones produced a significantly greater amount of root biomass than the Marcy clones. On the Saskatoon soil, Tully Champion and Marcy clones produced significantly more leaf biomass than India clones. No clonal differences were noted for shoot and root biomass on the Saskatoon soil.

4.3.4 Soil nutrients

Extractable soil nutrients available at the end of the 90 day growth period varied significantly between some treatments (Table 4.3). On the Prince Albert soil, a significantly greater amount of NO_3^--N was measured under the 200 kg N ha^{-1} fertilizer treatment for both Tully Champion and Marcy clones and the lowest levels were observed for Tully Champion manure treatments and for Marcy manure and unfertilized treatments. A similar trend was noted for Tully Champion clones on the Saskatoon soil as the Prince Albert soil. Extractable NH_4^+-N was significantly greater without fertilizer under India clones and smallest for the 50 kg N ha^{-1} fertilizer treatment. On the Saskatoon soil, NH_4^+-N was significantly greater for India clones fertilized with 50 kg N ha^{-1} and smallest for the 200 kg N ha^{-1} treatment. Extractable PO_4^--P was significantly greater after the application of manure for Tully Champion and Marcy clones on both soils and India clones on only the Prince Albert soil.

Averaging the extractable soil nutrients levels across all fertilization treatments, the mean varied between soils for most clones and nutrients. Soil NO_3^--N was significantly greater in the Saskatoon soil for all clones. Soil NH_4^+-N was significantly greater in the Saskatoon soil for the Marcy clones and greater in the Prince Albert soil for the India clones. Soil PO_4^--P was significantly greater in the Prince Albert soil for all clones.

4.3.5 Foliar nutrients

Foliar N and P varied between treatments for Tully Champion and India clones on both soils (Table 4.4). On the Prince Albert soil foliar N for the Tully Champion clones was significantly greater for manure and 200 kg N ha^{-1} treatments compared to the control. On the Saskatoon soil Tully Champion clones of the 100 and 200 kg N ha^{-1} treatments had significantly greater foliar N compared to the control. Foliar P was significantly greater for

Table 4.3 Soil nutrient concentrations of willow clones grown in two soils under five fertilization treatments after a 90 day growth period in an indoor growth chamber.

Treatment	Extractable soil nutrients (μg g^{-1})					
	Tully		Marcy		India	
	Prince Albert	Saskatoon	Prince Albert	Saskatoon	Prince Albert	Saskatoon
- kg N ha^{-1}† -	-------------------------------- NO_3^- -N --------------------------------					
0	1.2ab‡	21.5b	1.3c	32.7a	1.5a	36.1a
50	0.9ab	34.6ab	3.2bc	26.2a	3.3a	35.2a
100	1.2ab	31.3ab	5.0ab	44.0a	1.4a	32.7a
200	3.0a	85.1a	9.6a	39.5a	5.2a	36.5a
Manure	0.8b	22.9b	1.4c	38.5a	2.5a	34.7a
Mean	1.4B§	39.1A	4.1B	36.2A	2.8B	35.0A
	-------------------------------- NH_4^+ -N--------------------------------					
0	0.4a	0.4a	0.4a	0.4a	0.5a	0.4ab
50	0.5a	0.7a	0.3a	0.5a	0.3b	0.5a
100	0.3a	0.4a	0.4a	0.5a	0.4ab	0.3ab
200	0.3a	0.4a	0.4a	0.6a	0.4ab	0.1b
Manure	0.4a	0.4a	0.3a	0.5a	0.3ab	ND*
Mean	0.4A	0.5A	0.4B	0.5A	0.4A	0.3B
	-------------------------------- PO_4^- -P--------------------------------					
0	1.5ab	0.6ab	1.3ab	0.5b	1.4b	0.7a
50	1.4bc	0.5b	1.4ab	0.5b	1.5b	0.5a
100	1.2c	0.6b	1.4ab	0.5b	1.3b	0.5a
200	1.3bc	0.5b	1.3b	0.5b	1.4b	0.5a
Manure	1.7a	0.7a	1.6a	0.8a	1.7a	0.8a
Mean	1.4A	0.6B	1.4A	0.6B	1.5A	0.6B

† Treatments are granular ammonium nitrate unless specified as Manure (100 kg N ha^{-1}).

‡ Nutrient values within a column for the same nutrient followed by the same lowercase letter are not significantly different ($P < 0.05$).

§ Mean values in the same row for the same clone with the same capital letter are not significantly different ($P < 0.05$).

* Not detected - below detection limit.

Table 4.4 Mean foliar nutrient concentrations for three willow clones grown on two soils under five fertilization treatments after a 90 day growth period in an indoor growth chamber.

Treatment	Mean Foliar Nutrient Concentrations (mg g^{-1})			
	Total N		Total P	
	Prince Albert	Saskatoon	Prince Albert	Saskatoon
-- kg N ha^{-1} †--	------------------ Tully ------------------			
0	28.9b‡	8.0b	2.0a	3.4a
50	34.2ab	12.5ab	2.2a	2.7ab
100	37.6ab	13.0a	2.1a	2.4b
200	42.8a	11.2a	2.0a	2.1b
Manure	43.2a	11.2ab	2.1a	2.5ab
Mean	37.3 A§	11.2B	2.1B	2.6A
	------------------ Marcy ------------------			
0	19.4a	6.9a	2.0a	3.5a
50	17.5a	14.9a	1.7a	2.4a
100	17.7a	13.6a	1.9a	3.5a
200	17.3a	10.4a	1.6a	1.9a
Manure	19.0a	10.9a	2.0a	2.4a
Mean	18.2A	11.3B	1.9B	2.8A
	------------------ India ------------------			
0	14.3b	7.4a	1.5a	2.0a
50	15.3ab	8.9a	1.4ab	1.8a
100	17.1ab	11.2a	1.0b	2.1a
200	18.3a	10.6a	1.1ab	1.6a
Manure	15.4ab	7.5a	0.9b	1.5a
Mean	16.1A	9.1B	1.2B	1.8A

† Treatments are granular ammonium nitrate unless specified as Manure (100 kg N ha^{-1}).

‡ Mean foliar concentrations in the same column for the same column with the same lowercase letter are not significantly different ($P < 0.05$).

§ Mean values in the same row for the same foliar nutrient with the same capital letter are not significantly difference ($P < 0.05$).

unfertilized Tully Champion clones on the Saskatoon soil. No differences were noted for Tully Champion clones grown on the Prince Albert soil. However, on the Prince Albert soil foliar N of India clones was significantly greater for the 200 kg N ha^{-1} treatment compared to the control and foliar P was significantly greater for the control compared to the 100 kg N ha^{-1} and manure treatments.

Foliar N and P were significantly different between the two soils. For all three clones foliar N was significantly greater on the Prince Albert soil compared to the Saskatoon soil. Foliar P was found to be significantly greater on the Saskatoon soil for all clones compared to the Prince Albert soil.

Foliar N and P also varied significantly between clones (data not shown). When taking all treatments into account, Tully Champion trees had significantly greater foliar N than the two other clones on the Prince Albert soil. Foliar P was significantly greater for the Tully Champion and the Marcy clones than the India clones on both soils.

Foliar N:P ratios were significantly different between treatments on the Prince Albert soil (Table 4.5). Both Tully Champion and India clones had significantly greater N:P ratios for 100 kg N ha^{-1}, 200 kg N ha^{-1} and manure treatments on the Prince Albert soil. On the Saskatoon soil, Tully Champion had significantly greater N:P ratios for 10 and 200 kg N ha^{-1} treatments and India clones had significantly greater N:P ratios for 200 kg N ha^{-1} treatments than all others. N:P ratios were significantly greater on the Prince Albert soil than the Saskatoon soil for all clones. Significant differences in N:P ratios between clones were only noted on one soil (data not shown). On the Prince Albert soil, Tully Champion had significantly greater N:P ratios than the other two clones and India had greater ratios than Marcy clones when taking all treatments into account. There were no significant differences between clones on the Saskatoon soil.

Table 4.5 N:P ratios for three willow clones grown on two soils under five fertilization treatments after a 90 day growth period in an indoor growth chamber.

Treatment	N:P Ratios	
	Prince Albert	Saskatoon
------- kg N ha^{-1} †-------	------------------- Tully Champion -------------------	
0	14.5b‡	2.4c
50	15.7b	4.1b
100	17.8a	5.3ab
200	21.6a	6.2a
Manure	20.3a	4.4b
Mean	18.0A§	4.5B
	------------------------- Marcy -------------------------	
0	10.1	2.2
50	10.2	4.6
100	9.7	4.9
200	11.0	7.2
Manure	9.6	4.5
Mean	10.1A	4.7B
	------------------------ India ------------------------	
0	9.7b	3.8c
50	10.9b	5.1b
100	17.5a	5.4b
200	16.8a	6.6a
Manure	16.9a	5.1b
Mean	14.4A	5.2B

† Treatments are granular ammonium nitrate unless specified as Manure (100 kg N ha^{-1}).

‡ N:P ratios in the same column for the same clone with the same lowercase letter are not significantly different ($P < 0.05$).

§ Mean N:P ratios in the same row with the same capital letter are not significantly different ($P < 0.05$).

4.4 Discussion

To reach optimal production, it is suggested that most trees need fertilization and irrigation (Christersson, 2006). The purpose of this study was to evaluate the response of willow to different fertilizers and rates on two soils to determine if trees perform better under the influence of fertilizers. When the trees were harvested on day 90 it was unclear as to which treatment resulted in the best growth response. Leaf and shoot biomass only differed between fertilization treatments in isolated cases; not enough basis to make a generalization or recommendation. Tree heights and the number of shoots per tree also did not differ significantly between treatments. It is possible that the length of the experiment was not long enough for the nutrient benefits of the fertilizer treatments to be utilized and affect the plant growth to any varying degree. Although the onset of disease around day 60 eliminated any advantages from earlier on in the study, the statistics on day 55 heights did not show any differences in treatments. Due to a lack of indoor growth chamber studies using willow, biomass and tree heights cannot be compared with any published values. Organic fertilizers have been shown to speed up the growth of willows and increase biomass over unfertilized controls (Adegbidi et al., 2003). In this study, the composted cattle manure treatment did not outperform the other fertilizer treatments or the unfertilized control. The amount of time required for the break down of organic fertilizers to a plant available form is specific to both the material and experimental conditions (Adegbidi and Briggs, 2003). Composted manures, such as the one used here, have a much smaller mass loss and thus nutrients released when compared to uncomposted manures (Adegbidi and Briggs, 2003). Thirty-six weeks after a top dressing of organic fertilizers it was found that N concentrations in the soil only increased by 3 and 8 g kg^{-1} for composted poultry manure and sewage sludge compost, respectively, resulting in a gross N mineralization rate (N mass released expressed as % of applied N) of 12 % (Adegbidi and Briggs, 2003). Similarly, composted poultry and dairy composts showed gross mineralization rates of 11 to 29 % after 32 weeks at 30 ^{o}C (Hadras and Portnoy, 1994). Adegbidi et al. (2003) suggests that after 3 years or more, organic fertilizers will outperform inorganic fertilizers because of their long-lasting effects due to slow degradation. If the current study was to be carried out for a longer period of time, the manure treatment may have shown some significantly greater increases in biomass production as greater amounts of N were released from the manure. If the manure had been incorporated deeper into the soil profile, it may have degraded more quickly; however it would not have mimicked manure application practices. Also, P from manure may not have been accessible to willow roots, as manure was added to the surface and incorporated to a shallow depth.

Trees on some sites may not be responsive to fertilization (Dickmann, 2006). The willow trees on the Prince Albert soil in the current study had significantly greater biomass production of shoots than on the Saskatoon soil. Extractable NO_3^- -N and PO_4^- -P were significantly different between the two soils at the end of the study; Prince Albert had 93 % less NO_3^- -N and 59 % more PO_4^- -P than the Saskatoon soil. The Saskatoon soil had been rewetted after the poor survival of the initial planted cuttings and it is plausible that N may have been lost from the pots and have led to the poor production of biomass; however, the greater concentration of NO_3^- -N in the Saskatoon soil does not support this.

The foliar N of Tully Champion clones grown on both soils and for India clones on the Prince Albert soil increased in foliar N under fertilized treatments when compared to the unfertilized control which is a trend observed in both Swedish and American studies (Arevalo et al., 2005; Ericsson, 1981; Nilsson and Ericsson, 1986). Foliar N concentrations varied between clones and soils. In Sweden, trees fertilized with 150 kg N ha^{-1} and irrigated with 330 mm had foliar N concentrations of 3 to 4 % while the unfertilized non-irrigated trees had foliar N concentrations of less than 3 % (Christersson, 1987). Tully Champion clones on the Prince Albert soil was the only combination to meet the range (3 to 4 %) suggested by Christersson (1987) for fertilized and irrigated trees; all others were well below the suggested range which according to Christersson (1987) is indicative of unfertilized and non-irrigated trees. Clones grown on the Saskatoon soil had significantly less foliar N than those on the Prince Albert soil. Significantly smaller root systems of clones found on the Saskatoon soil suggests that even after soil rewetting, N remained available in the soil but the root systems of the trees were not able to utilize the available N.

Foliar P decreased as a result of fertilization for Tully Champion clones on the Saskatoon soil and India clones on the Prince Albert soil. Both poplars and willows have demonstrated decreases in foliar P following N fertilization (Nilsson and Ericsson, 1986; van den Driessche, 1999). The foliar P of willows in this study ranged from 0.9 for fertilized trees to 3.4 mg g^{-1} for unfertilized trees (or 0.09 and 0.34 %) compared to 0.2 % and 0.14 % observed for fertilized and unfertilized poplars (van den Driessche, 1999). Foliar P values were slightly lower for the fertilized treatment but larger for the unfertilized trees when compared to the findings of van den Driessche (1999). Even though willow are in the same family as poplars (Dickmann, 2006), the tree physiology in terms of nutrition and growth may not be the same (Jug et al., 1999) and thus the findings of van den Driessche (1999) must not be viewed as critical values for willows but as approximations against which willow values can be compared.

Ratios of N:P in foliar tissue can assist with the understanding of plant nutrition. A study carried out on poplar trees suggested 9.5 as a critical N:P ratio where ratios below 9.5 indicate N deficiency below and ratios above 9.5 indicate P deficiency (Zabek, 2001). The N:P ratios in the current study were significantly greater on the Prince Albert soil where values were above 9.5 suggesting a possible P deficiency when using the critical poplar N:P ratio found by Zabek (2001). Nitrogen fertilization using ammonium can decrease the pH of the soil (Gahoonia et al., 1992) and under lower pH conditions the solubility of phosphates (Hinsinger, 2001)can decrease resulting in unavailable stores of P which may have occurred in the Prince Albert soil. Clones grown on the Saskatoon soil had N:P ratios below 9.5 suggesting N deficiency: however, large amounts of NO_3^- -N still remained in the soil at the end of the 90 day experiment. This supports the idea that the smaller root system on the Saskatoon soil could not capture the soil N that was available in order to utilize it as part of the biomass and foliar nutrient status.

4.5 Conclusion

The application of five different fertilization treatments did not show any consistent response trend in terms of biomass production. Tree heights and the number of shoots per tree were not significantly different between treatments and only some significant differences were noted between foliar N and P concentrations. The length of the study was perhaps too short in order for the effects of the fertilizer treatments to be made evident. It is likely that the manure treatment, in particular, did not have enough time for the organic bound nutrients to be made plant available. The onset of disease more than half way through the study also would have restricted tree response to treatments.

Soil type played an interesting role and resulted in more significant differences than fertilizer treatments when observing the response of willow to fertilizer application. Tree heights (Tully Champion and Marcy only), soil NO_3^- and foliar P were all found to be significantly greater on the Saskatoon soil for most clones and treatments while the number of shoots per tree

(India only), biomass, soil PO_4^- and foliar N and N:P ratios were significantly greater on the Prince Albert soil. Shoot biomass is the economically profitable component of willow and was significantly greater on the Prince Albert soil. The application of fertilizers in this study did not apparently supply enough N to reach a desired critical foliar N concentration (except for Tully Champion clones on the Prince Albert soil) but it does demonstrate a potential for further biomass production potential if plant N requirements were met. The willows did not show a greater growth response to granular ammonium nitrate and composted cattle manure over the unfertilized control, therefore the hypothesis was rejected.

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5. GENERAL DISCUSSION AND CONCLUSIONS

Since 1750, atmospheric CO_2 concentrations have increased by 31 % (IPCC, 2001). Much of this increase has been due to the burning of fossil fuels for energy, heat and transportation. Depleting fossil fuel reserves, increasing prices of fossil fuel commodities, increasing atmospheric CO_2 concentrations and growing awareness of environmental issues has led to a search for alternative and renewable energy sources and technologies. Growing trees on agricultural landscapes, whether for energy or forest products, has attracted interest from researchers, governments and communities around the world. Willow can be grown on agricultural land as an agroforestry crop for the purpose of energy conversion. Energy from biomass requires large amounts of agricultural land which are available in Saskatchewan. Biomass energy crops can provide producers with crop diversification, soil stabilization and increased biodiversity; however, many issues need to be resolved before producers will adapt this practice.

The success of willow SRWC systems for biomass energy depends on factors important in the establishment and growth of the crops as well as factors determining the end utilization of the product. Costs involved in the initial establishment of SRWC systems can be high when including planting material, site preparation, planting, post-emergent herbicides, maintenance equipment and harvesting equipment. In order for the practice of producing willow SRWC for biomass energy to be adapted in Saskatchewan, a market must be established so producers can be assured of a buyer for their product. A market, however, cannot develop unless some critical production questions are answered.

The first decision of willow SRWC establishment is site selection. Sites should be chosen based on soil texture and nutrient content, water availability and protection from browsing. Sites should be capable of supplying nutrients to the fast growing tree species for a long period of time as the plantations will grow for up to 22 years. Fertilizers, whether organic or inorganic, can be used to supply nutrients and their costs can be greatly lowered if nutrients can be supplied partially from the soil. Sites should be located in close proximity to the final destination of the product, whether a bioenergy converter or a biofuel production facility, to keep transportation costs to a minimum.

According to one study, survival should be above 80 % by the end of the first growing season in order to consider the SRWC system a success (Bergvist, 1996). In the field study (Chapter 3), coarse textured soils (Prince Albert) were more conducive to willow establishment when paired with an adequate supply of moisture. Clones grown on the heavy clay soil of Saskatoon site did not receive enough moisture and the vertisolic properties of the soil (deep cracking and clod formation) restricted the establishment of the SRWC system. In the growth chamber study (Chapter 4), clones grown on the Prince Albert soil had significantly greater biomass production compared to the Saskatoon soil.

Constant management of the SWRC systems is required to maintain optimal biomass yields. To eliminate competition for light, nutrients and moisture, herbicides, intensive mechanical and manual weeding need to be used. There are no herbicides that are registered for use on willow in Canada; however, recommendations have resulted from research in New York, USA to use Goal 2XL as a pre-emergent herbicide. In the field study in Chapter 3, this chemical was successful in controlling weed populations in the first growing season but manual and mechanical weeding was needed in the second growing season. As pesticides are not registered yet for use with willow, non-chemical techniques will need to be done to minimize damage from disease and insects. Including a selection of clones in a willow SRWC system will assist with managing disease and insect infestations; however, in Chapter 4 the unidentified disease affected all three clones in the growth chamber. The trees were able to recuperate after infection but growth was greatly reduced by the disease.

A continuous supply of nutrients to meet the demands of the fast growing willow is crucial in reaching and maintaining the optimal production yields. Literature does not suggest a recommended level of soil nutrients to meet plant demands but does present a wide range of recommendations of fertilizer rates, blends and timing which are specific to soils and environmental conditions of the study region. Fertilizers were applied in both the field and growth chamber studies in hopes of stimulating a growth response. In Chapter 3, N fertilizer recovery was low ranging from 2.87 to 10.6 % in 2008 and 0.39 to 2.95 % in 2009, respectively. In both years, this amount was not enough to have resulted in a significant increase in biomass. Understanding N fertilizer recovery will decrease fertilizer costs as application is correlated with plant recovery. Fertilizer applied in 2008 was evident through the soil profile until the end of the 2008 growing season but was no longer present at the end of the 2009 growing season. To reach optimal yields, fertilizer application must coincide with the requirements of the trees. Foliar N was above the 3 % recommended level (Christersson, 1987) in 2008 and well below in 2009, suggesting that fertilizer may have played a more important role in the growth and production of willows in the second year.

Fertilizer application rates and forms are important considerations for producers. Fertilization in the growth chamber study (Chapter 4) led to significant differences between treatments in only three isolated cases, but no definite trends were evident after the 90 day growth period. The manure did not lead to increased biomass as suggested by the literature (Adegbidi and Briggs, 2003; Adegbidi et al., 2001) likely because the experimental period was not long enough for degradation of the nutrient to a plant available form but this does not explain why the granular ammonium nitrate did not result in any differentiation between fertilizer treatments.

Foliar nutrients can assist with the interpretation of the fertilizer response of willows. A critical N:P level of 9.5 has been established for hybrid poplar trees (Zabek, 2001); however, no work has been done to discover a similar value for willow. The N:P ratios in the second growing season of Chapter 3 and on the Prince Albert soil in Chapter 4 suggested a P deficiency. Trees grown on the Prince Albert soil may require P fertilization along with N despite the high background levels of the nutrient. Clones on the Saskatoon soil in Chapter 4 showed N:P ratios lower than 9.5 suggesting that this soil requires more N. Fertilization will be a site specific management practice with recommendations arising from further research and soil testing.

For willow SRWC systems to be a practice adopted by Saskatchewan producers, resources need to be made available to help them make difficult decisions. Support systems are available for producers of agricultural crops. Since willow SRWC systems are purely experimental in Saskatchewan there is no current support network for prospective producers. The findings of these studies are not concrete enough to be directly forwarded to producers to assist them with the decision making involved in the establishment and maintenance of willow SRWC systems in Saskatchewan. They can, however, assist researchers, industry, and governments to determine the next direction to take this alternative energy option. These studies were preliminary in bringing the well respected Swedish practice of willow biomass energy to

Saskatchewan. The issues with implementing the technology here are far from solved and have perhaps raised further questions surrounding the production of willow SRWC systems for the purpose of bioenergy. Further research into willow SRWC system fertilization should be carried out to examine the effects of fertilizer blends, rates and application times on willow growth. In order to create a thorough support systems, further research needs to be carried out in terms of site selection, clone selection, clone breeding for disease resistance, in situ fertilizer application recommendations, and irrigation. Long term studies will be required to better understand of how these systems will survive and perform in the long term here in Saskatchewan. The answers to these questions will be critical information for prospective producers to successfully establish willow SRWC systems in Saskatchewan.

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APPENDICIES

APPENDIX A: Herbicide Listing for Willow SRWC Systems

Herbicides registered for use on willow SRWC systems

Company	Active Ingredient	Product Name	Herbicide Type
Chematura Canada Co.	Dichlobenil	Casoron G-4	Pre-emergent
Monsanto Canada Inc.	Glyphosate	Vision Silvicultural	Post-emergent
Cheminova Canada	Glyphosate	Forza Silvicultural	Post-emergent
Cheminova Canada	Glyphosate	Cheminova Glyphosate	Post-emergent
Dow AgroSciences Canada Inc.	Glyphosate	Vantage Forestry	Post-emergent
Monsanto Canada Inc.	Glyphosate	Vision Max Silvicultural	Post-emergent

Source: IPC Report 2008 by the Pesticide Working Group – Poplar Council of Canada. Last updated 10 March 2010

APPENDIX B: ^{15}N Calculations – Chapter 3

^{15}N Label → $^{15}NH_4{}^{15}NO_3$
→ Double Labelled
→ 10 atom % excess

In order to supply 45 mg N tree^{-1} in the 10 mL of solution, 1.35 g of double labeled ammonium nitrate was required to make the 300 mL of stock solution. However, this calculation assumes 100 kg ammonium nitrate ha^{-1} and not the 100 kg N ha^{-1} that is desired. To account for this, the molecular weight of the labeled ammonium nitrate must be taken into account. Ammonium nitrate includes two N atoms, four hydrogen (H) atoms and three oxygen (O) atoms. The H and O have a weight of 52.03 g but the N must take into account the percent enrichment of the product used. Since the product used was 10 % enrichment of ^{15}N the remaining 90 % of N is ^{14}N. The ^{15}N weight (2*15) is multiplied by 10 % enrichment (0.1) plus the natural background level of ^{15}N (0.3663 atom % or 0.00366) which is 0.103663 making the weight of ^{15}N 3.11g. The remaining 90 % of N is then ^{14}N so the weight was calculated (2*14) and multiplied by 100 % minus the content of ^{15}N (1.0 – 0.103663). The weight of ^{14}N was 25.1 g which makes the final weight of N to be 28.21 g. The amount of N in the solution was calculated (weight N/total weight) and was 0.352 or 35.2 %. In previous calculations it was found that 1.35 g of $^{15}NH_4{}^{15}NO_3$ was required for 300 mL of solution assuming that it was 100 % ^{14}N. With the understanding of the proportions of the two N isotopes, 1.35 g divided by the amount of ^{15}N present means that 1.92 g of double labeled $^{15}NH_4{}^{15}NO_3$ is required for 300 mL of solution (Appendix 1 for detailed calculations).

- **TREES**
  - 2 trees / fertilized plot
    - *Prince Albert*
      = (2 trees/fertilized plots) * (12 fertilized plots)
      = 24 trees

- **FERTILIZER PER TREE**

  \- 100kg N/ha fertilizer rate of NH_4NO_3

  - *Tree Spacing*

    = 0.3m * 0.3m

    = $0.09m^2$ * (1ha / $10{,}000m^2$)

    = 0.000009ha

  - *Fertilizer*

    \- put 95kg N/ha on as UN-labelled NH_4NO_3

    \- put 5kg N/ha on as labelled $^{15}NH_4{}^{15}NO_3$

    = (5kg N/ha) * (0.000009ha/tree)

    = 4.5 x 10^{-5}kg N/tree

    = 45mg N/tree

- **STOCK SOLUTION**

  \- if I want to add 10mL aliquots to each tree….

  - *Total Solution*

    = 54 trees * 10mL

    = 540mL

    ~Round up to **600mL** for simplification and extra!!

- Do in 2 sets (one per site) so each should be 300mL

  - *^{15}N Solution*

    $$= \frac{(45\text{mg N/tree})}{(10\text{mL solution/ tree})} * \frac{(X)}{(600\text{mL solution})}$$

    $$X = \frac{(45\text{mg N/tree})*(600\text{mL solution})}{(10\text{mL solution/tree})}$$

    = 2700mg

    = 2.7g labelled $^{15}NH_4{}^{15}NO_3$ / 600mL water

This assumes 100kg **$^{15}NH_4{}^{15}NO_3$** NOT 100kg **N**/ha.

- *Molecular Weight of* $^{15}NH_4{}^{15}NO_3$

  2 N =

  4 H = 4(1.0079) = 4.03

  3 O = $\underline{3(16) = 48}$

  52.03g

  ^{15}N = 2(15)*(0.103663)

  = 3.11g

  ^{14}N = 2(14)*(1.0-0.103663)

  = 25.10g

  $^{15}N + {}^{14}N$ = 3.11g + 25.10g

  = 28.21g

- *%N in* $^{15}NH_4{}^{15}NO_3$

  $$= \frac{^{15}N + {}^{14}N}{^{15}N + {}^{14}N + H + O} * 100\%$$

  $$= \frac{28.21g}{28.21 + 52.03g} * 100\%$$

  = 0.352

- $^{15}NH_4{}^{15}NO_3$ *in solution*

  $^{15}NH_4{}^{15}NO_3$ is 35.2% N…Therefore...

  $$= \frac{2.7g\ {}^{15}NH_4{}^{15}NO_3}{0.352}$$

  = 7.67g $^{15}NH_4{}^{15}NO_3$

  $$= \frac{7.67g\ {}^{15}NH_4{}^{15}NO_3}{2}$$

  =$\underline{\mathbf{3.835g\ {}^{15}NH_4{}^{15}NO_3}}$

  300mL on per site basis

APPENDIX C: Environmental data of Prince Albert and Saskatoon willow research sites – Chapter 3

Average monthly rainfall for the Prince Albert and Saskatoon willow research sites during the experimental periods of 2008 and 2009.

Month	Average monthly rainfall			
	Prince Albert		Saskatoon	
	2008	2009	2008	2009
	---------------------------------- mm ----------------------------------			
March	2.1	12.2	1.1	1.3
April	15.9	5.3	16.0	2.4
May	9.8	44.4	3.4	12.1
June	30.7	59.8	56.7	51.5
July	48.0	69.1	66.0	54.2
August	25.4	54.1	29.0	83.7
September	11.1	-	13.7	-
October	18.5	-	47.3	-

Average monthly air temperature for the Prince Albert and Saskatoon willow research sites during the experimental periods of 2008 and 2009.

Month	Average air temperature			
	Prince Albert		Saskatoon	
	2008	2009	2008	2009
	---------------------------------- ^{o}C ----------------------------------			
January	-17.3	-15.2	-14.4	-15.8
February	-18.3	-	-15.3	-14.2
March	-8.8	-10.5	-5.2	-9.5
April	0.5	2.0	2.0	3.3
May	9.9	7.6	11.7	9.7
June	15.3	14.2	16.0	16.2
July	17.3	15.5	18.4	16.8
August	16.9	14.6	18.6	16.5
September	7.9	-	11.8	-
October	4.2	-	6.1	-
November	-4.5	-	-1.7	-
December	-21.8	-	-18.9	-

APPENDIX D: Transformations of Variables to Normality – Chapter 3

Variable	Transformations	Normal
Number of Shoots	square root	close†
Survival	none	close
Height	none	close
Shoot Diameter	none	close
Leaf Biomass	log	yes
Shoot Biomass	log	yes
Total Biomass	log	yes
Foliar N	log	close
Foliar P	log	close
N:P Ratios	log	yes
NO_3^- -N	square root	close
NH_4^+ -N	square root	close
PO_4^- -P	square root	close
N Fertilizer Recovery	exponential	close
Atom ^{15}N % Excess	log	close

† Specifies that transformation resulted in a distribution significantly different from normal but was the closest to normality out of all transformation possibilities.

APPENDIX E: Mixed Model Output – Chapter 3

Mixed model table for shoot biomass yields following the 2009 growing season.

Effect	numDF†	denDF‡	F value§	*P* value††
Intercept	1	7	27980.755	<0.0001
Clone	3	7	92.872	<0.0001
Treatment	1	7	0.001	0.9760
Block	1	1	32.754	0.1101
Total foliar N 2008	1	7	294.793	<0.0001
Total foliar N 2009	1	7	151.351	<0.0001
Total foliar P 2008	1	7	203.942	<0.0001
Total foliar P 2009	1	7	98.468	<0.0001
Tree height	1	7	957.368	<0.0001
Diameter	1	7	85.976	<0.0001
Number of shoots	1	7	159.570	<0.0001
Leaf biomass	1	7	232.355	<0.0001
Tree Height:Diameter	1	7	78.384	<0.0001
Total foliar N 2008:Diameter	1	7	0.666	0.4414

† Numerator degrees of freedom
‡ Denominator degrees of freedom
§ F ratio
†† Least significance difference (LSD) used was $P < 0.05$.

APPENDIX F: Mixed Model Output – Chapter 4

Mixed model table for shoot biomass yields of willow following a 90 day growth period in an indoor growth chamber.

Effect	df†	SS‡	MS§	F ratio	*p*††
Soil	1	19.1944	19.1944	84.2474	4.951×10^{-15}
Clone	2	6.3773	3.1887	13.9955	4.199×10^{-6}
Fertilization treatment	4	7.1856	1.7964	7.8847	1.386×10^{-5}
Diameter of cutting	1	0.0131	0.0131	0.0577	0.81068
Number of shoots	1	0.4109	0.4109	1.8037	0.18221
Tree height	1	0.6237	0.6237	2.7373	0.10107
Leaf biomass	1	25.3562	25.3562	111.2924	$<2.2 \times 10^{-16}$
Root biomass	1	1.3660	1.3660	5.9957	0.01603
Total foliar N	1	0.0003	0.0003	0.0014	0.97043
Total foliar P	1	0.5261	0.5261	2.3093	0.13167
Replicate	1	0.9183	0.9183	4.0304	0.04731
Residuals		23.4669	0.2278		

† Degrees of freedom
‡ Sum of Squares
§ Mean Squares
†† Least significance difference (LSD) used was $p<0.05$.