BIOMASS PRODUCTION AND NUTRIENT CYCLING IN SHORT-ROTATION COPPICE WILLOW (*SALIX* SPP.) BIOENERGY PLANTATIONS IN SASKATCHEWAN, CANADA

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By

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ABSTRACT

Biomass energy is currently the largest renewable contributor to global energy supply and there is increasing demand for bioenergy feedstock. Consequently, the production of purpose-grown woody bioenergy crops, such as short rotation coppice (SRC) willow, is expected to proliferate. Although the economic and environmental benefits associated with SRC willow production are well documented, systematic assessments of nutrient cycling within these plantations are rare. The objective of this study was to examine biomass production and biogeochemical cycling of nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg) during an initial four-year rotation of six willow varieties grown at four plantations along a 500 km north-south pedoclimatic gradient within Saskatchewan, Canada. Nutrient budgets were also calculated after quantifying various nutrient inputs (e.g., atmospheric deposition, soil mineral weathering, and organic matter mineralization), outputs (e.g., above- and below-ground biomass, leaching, and denitrification), and transfers (e.g., canopy exchange, leaf litter decomposition, and fine root turnover) affecting the plant available soil nutrient pool. Total stem, leaf litter, and below-ground (primarily fine roots) biomass production after four years averaged 19.0, 7.1, and 12.5 Mg ha^{-1}, respectively, with corresponding soil nutrient budget deficits of 17, 39, 112, 271, and 74 kg ha^{-1} of N, P, K, Ca, and Mg, respectively, but a soil S surplus of 60 kg ha^{-1}. Despite willow's relatively low nutrient-demanding nature, negligible leaching and denitrification losses, and substantial nutrient cycling from leaf litter, the nutrient export in harvested biomass over multiple rotations will require soil nutrient amendments, particularly N and P, to maintain plantation productivity. Given the apparent eventual need for supplemental fertility to support adequate willow growth over the 22-yr plantation life span, the fate of broadcast ^{15}N-labelled fertilizer was also examined. Though the willow accumulated less than ⅓ of the applied fertilizer N after one year, the majority of the residual fertilizer N (51%) remained available for willow uptake in subsequent years. Further research is needed to track the fate of applied fertilizers over multiple rotations to better understand fertilizer dynamics for optimizing SRC willow agronomy; thus helping to promote its viability as a biomass energy feedstock option.

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Although graduate students invariably endure difficult circumstances during their programs, I have experienced two severe trials that are atypical of most one might expect to encounter; the first near the beginning of my program and the second toward the end. The former taught me that one must be prepared to battle through unforeseen adversity to be successful in academia, while the latter taught me that life can literally change in a heartbeat and, therefore, academic success quickly becomes secondary to family survival. Suffice it to say, the only reason why I am still standing today is due to the Lord's assisting grace in every time of need. Countless times I have relied on the precious promises of The Bible, in particular the following two Scriptures, for support and strength to sustain me during the tough times:

"In the day of my trouble I shall call upon You, for You will answer me." (Psalm 86:7)

"For I am the Lord your God who takes hold of your right hand and says to you,
'Do not fear; I will help you.'" (Isaiah 41:13)

Above all, I give thanks daily that the Lord saw fit to allow the kids' Mom and my best friend to remain with us.

DEDICATION

I dedicate this work to my much better ¾. I have always said a family that 'weeds' together stays together, but arguably, the following two statements are much more profound: "*Truly, an excellent wife is worth far above jewels"* (Proverbs 31:10) and "*Behold, children are a gift from the Lord"* (Psalm 127:3). The assistance and patience afforded me by Lisa, Rosie, and Ray over the last several years have been second to none and only a graduate student's family can remotely empathize with the selfless sacrifice of time, energy, and finances these three have made in support of my academic endeavours. I love you three immeasurably and have been truly grateful for your help, although I did not always express it.

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LIST OF ABBREVIATIONS

A	Asymptote
ACC	Agriculture capability classification
AI	Aridity index
ANOVA	Analysis of variance
BD	Bulk deposition
CE	Canopy exchange
DD	Dry deposition
EC_e	Electrical conductivity of saturated paste extract
$EC_{1:2}$	Electrical conductivity of 1:2 (soil:water; on a weight basis) extraction
FC	Field capacity
FFD	Frost-free days
GHG	Greenhouse gas
GSL(A)	Growing season length based on mean daily air temperature
GSL(S)	Growing season length based on mean daily soil temperature
$k_{Biomass}$	Leaf litter decomposition rate constant
$k_{Nutrient}$	Leaf litter nutrient release rate constant
LSA	Leaf surface area
LSD	Least significant different
$LV_{Biomass}$	Leaf litter decomposition limit value
$LV_{Nutrient}$	Leaf litter nutrient release limit value
MAP	Mean annual precipitation
MAT	Mean annual temperature
MGSP	Mean growing season precipitation
MGST	Mean growing season temperature
MW_e	Megawatt of electricity
NER	Net energy ratio
NG	Natural gas
NTF	Net throughfall
NUE	Nutrient use efficiency
ρb_{WH}	Bulk density of the weathered horizon
PCA	Principal component analysis
PET	Potential evapotranspiration
PRS™	Plant Root Simulator™
r	Correlation coefficient
R^2	Coefficient of determination
RH	Relative humidity
SBA	Stem basal area
SE	Standard error
SF	Stemflow
SH	Stem height
SLA	Specific leaf area
SOC	Soil organic carbon
SOM	Soil organic matter
SRC	Short-rotation coppice

ST	Soil temperature
SUNY-ESF	State University of New York College of Environmental Science and Forestry
t	Time (in years)
TF	Throughfall
T_{WH}	Weathered horizon thickness
WS	Wind speed
X_i	Nutrient content remaining at collection time
X_{Input}	Cumulative input of nutrient element
X_0	Initial nutrient content within the leaf litter
X_{PM}	Percentage of element X in the parent material
X_{WH}	Percentage of element X in the weathered horizon
Zr_{PM}	Percentage of Zirconium in the parent material
Zr_{WH}	Percentage of Zirconium in the weathered horizon

1. INTRODUCTION

1.1 Renewable Energy Within the Global Energy Context

Worldwide concern regarding escalating atmospheric greenhouse gas (GHG) concentrations, energy security, rural and urban economies, and environmental stewardship has renewed interest in developing renewable energy sources to meet the increasing demands of a rapidly increasing global population. With an expected population of nine billion by 2050, the challenge of providing adequate food, feed, fibre, and facilities (i.e., infrastructure) is unprecedented (FAO, 2009; Valentine et al., 2012). Furthermore, a reliable energy supply is needed to support the escalating world energy consumption concomitant with widespread economic development (Demirbas, 2005). Consequently, these contemporary human welfare issues, within the context of economic, societal, and environmental benefits, have prompted many countries to initiate mandated ambitious renewable energy targets for reducing their dependence on conventional fossil energy carriers (e.g., oil, natural gas and coal). For example, the European Union is requiring its member states to achieve a renewable energy target of 20% of their total energy consumption by 2020, including a minimum of 10% fossil energy displacement within the transportation sector (European Commission, 2009). Likewise, the Unites States has mandated ambitious levels of renewable fuel production to be a blended component of primary liquid transport fuels by 2020 (United States Congress, 2007). Within Canada, renewable-energy policy and legislation have been implemented across the country, primarily driven by provincial initiatives, which helps support the national GHG emission reduction target of 17 per cent below 2005 levels by 2020 under the Copenhagen Accord (NRTEE, 2012).

Although Canada annually contributes approximately 2% of the global GHG emissions, because of its size, climate-induced energy demands, and resource-based economy, it ranks as one of the highest GHG-emitting countries with more than double the GHG emissions per person compared to the United States (Government of Canada, 2012). The three largest economic sectors contributing to Canada's GHG emissions are transportation, oil and natural gas, and electricity generation, which represent 24%, 22%, and 14% of the total emissions, respectively (Environment Canada, 2012). Canada has been a net energy exporter for some time and renewable energy currently contributes 18% of Canada's total primary energy supply, with biomass energy comprising 26% of total production (IEA, 2012a).

Biomass energy is unique among contemporary renewable energy sources (e.g., wind, solar, geothermal, and marine) in its capacity to contribute to both stationary (i.e., heat and electricity) and transport energy sector demands. Contemporary biofuels utilize annual agricultural food and feed commodities (e.g., sugar cane, corn, and canola). However, these so-called 'first-generation' biofuels may not persist, because of increasing realization among academics and the public that conventional biofuels cannot sustainably replace fossil fuels due to a number of inherent social, economic, and environmental shortcomings (Djomo and Ceulemans, 2012; Smith and Searchinger, 2012; Weiss et al., 2012). Conversely, 'second-generation' bioenergy systems, which utilize lignocellulosic feedstocks (e.g., agricultural and forestry residues, municipal solid waste and short-rotation woody crops), avoid these shortcomings and, therefore, are expected to displace conventional bioenergy crops in the near future (IEA, 2011; EIA, 2011; Englund et al., 2012).

Short-rotation woody crops possess a number of logistical, economic, and environmental advantages relative to other lignocellulosic energy crops, which are expected to increase establishment of purpose-grown woody crops in the future (Volk et al., 2011; Krasuska and Rosenqvist, 2012; Weih and Dimitriou, 2012). Hybrid poplar (*Populus* spp.) is the primary short-rotation woody crop in Canada, however, shrub willow (*Salix* spp.) is considered by many to be better suited for use as a dedicated biomass energy crop (Verwijst, 2001). The research and development of purpose-grown willow began decades ago in Europe and the U.S., resulting in the development of both commercial and environmental applications (Volk et al., 2004; Mirck et al., 2005; Kuzovkina and Volk, 2009; Abrahamson et al., 2012). After years of extensive research, short-rotation coppice (SRC) willow plantation protocols are well established (Abrahamson et al., 2002). Briefly, production begins with adequate site preparation prior to planting unrooted willow cuttings. The willow is allowed to grow for a year before the willow plants are cut down to approximately 2-4 cm above ground level, to encourage coppicing, which

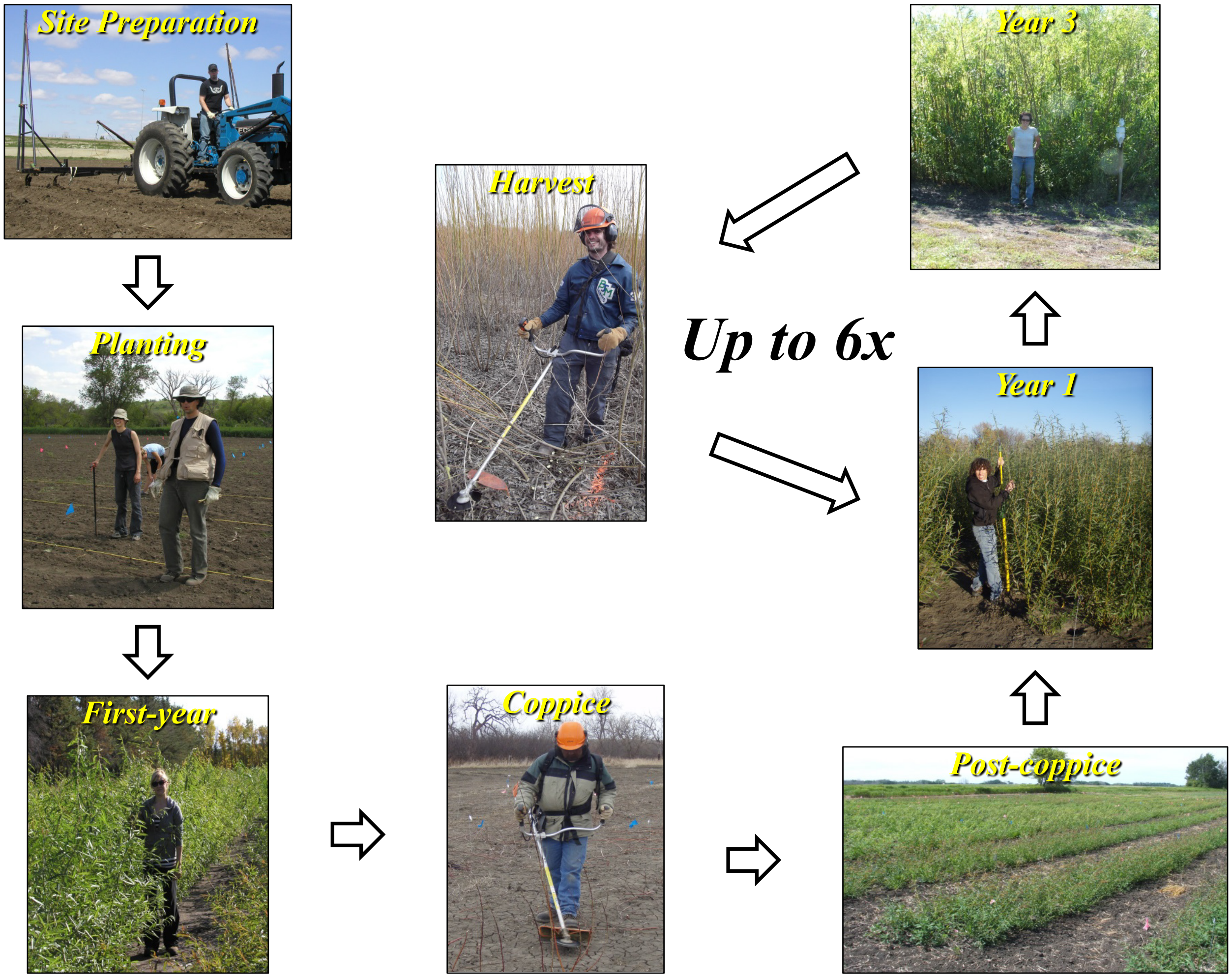

Fig. 1.1. Short-rotation willow production cycle during a 22-yr period.

is the production of a large number of shoots when a single stem is removed, but the established willow root system remains intact. Afterwards, the willow is allowed to grow for three years before harvesting and then this three-year growth cycle or rotation is repeated another six times for a total of seven rotations over a 22-year period (Fig. 1.1). After seven rotations, the viability of the root system to support adequate above-ground biomass production will began to wane, at which time, the plantation will need to be replanted. The availability of better willow varieties after the initial plantation life span will also mean that growers will want to re-plant using genetically superior varieties. Notwithstanding the potential benefits of growing SRC willow and its relative ease of propagation, an essential question remains; namely, how sustainable are multiple SRC willow rotations, considering its rapid growth and continued nutrient exports in harvested willow biomass over repeated rotations.

1.2 Ph.D. Research Justification

Fertilization has been historically used to manage plantation productivity, however, its efficacy has been inconsistent (Chapter 5). Given the importance of fertilizer use efficiency in controlling production costs, net energy ratio (NER), net GHG emissions, and non-point source pollution concerns associated with SRC willow (Djomo et al., 2011; McKenney et al., 2011; Schmidt-Walter and Lamersdorf, 2012; Caputo et al., 2013), a thorough understanding of soil-plant nutrient dynamics is required to accurately forecast plantation sustainability, along with optimizing the rate and timing of any fertilizer amendments required to maximize the economic, environmental, and social benefits associated with SRC willow production.

The general objective of this study was to quantify the nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg) budgets within SRC willow plantations, during the initial four-year rotation, to provide insight into the sustainability of these woody biomass energy production systems over multiple rotations in Saskatchewan. An experiment consisting of several commercial willow varieties was replicated at four different sites across a 500 km north-south pedoclimatic gradient, representative of the diverse soil types and climatic conditions in the province. Reasonable long-term soil productivity assessments for a variety of soil types were based on a comprehensive accounting of above- and below-ground nutrient pools, including: vectors of nutrient flux into (e.g., atmospheric deposition, soil mineral weathering, and SOM mineralization), out of (e.g., denitrification, above-ground biomass removal, fine- and coarse-root biomass, leaching, and litter fall), and transfers between (e.g., fine root turnover and leaf litter decomposition and) the plant available soil nutrient pool. Notwithstanding the excellent nutrient budget work previously reported for SRC willow plantations (Ericsson, 1984; Hytönen, 1996; Alriksson, 1997), to my knowledge, this is the first study to carry out a comprehensive examination of all nutrient vectors (i.e., input, output, and transfers), associated with the plant available nutrient pool, including S, across a large pedoclimatic gradient. This gradient allowed for the comparison of fertile and marginal agricultural soils and growing the same willow varieties established at the same time. I hypothesized that chronic removal of soil nutrients through harvested above-ground willow biomass would eventually necessitate nutrient amendments after several rotations, but the timing of fertilizer additions would differ depending on soil type and willow variety. With the million or more non-arable marginal hectares currently available for afforestation, an excellent opportunity exists to develop non-consumable woody crops as a bioenergy feedstock in Saskatchewan.

An important facet of sustainability for any production system is the recycling and efficient use of plant nutrients. By examining soil-plant nutrient dynamics for several essential plant nutrients and developing reliable nutrient budgets at different SRC willow plantations (involving several commercial willow varieties) along a 500 km north-south pedoclimatic gradient, a good understanding of overall nutrient biogeochemistry and cycling in for these woody crop production systems in Saskatchewan may be obtained. I believe that a tremendous opportunity exists to develop SRC willow as a biomass energy feedstock in Saskatchewan, especially if these plantations can be sustainably grown on the millions of hectares of marginal land considered unsuitable for annual crop production (Amichev et al., 2012).

1.3 Dissertation Arrangement

Following this introduction and subsequent literature review (Chapter 2), the research presented in this dissertation is a compilation of several manuscripts (Chapters 3-5 and Appendices A-D) detailing work that I have done examining different aspects of SRC willow production. Specifically, Chapter 3 details a three-year study that measured the rate of decomposition and nutrient release of willow leaf litter using litterbags. The objective of this study was to quantify the decomposition rate constants and decomposition limit values, and associated release rates and release limits of N, P, K, S, Ca, and Mg contained in leaf litter from several native and exotic willow varieties within SRC willow plantations during the initial four-year rotation, to provide insight into the relevance of leaf litter nutrient additions into the plant available soil nutrient pool. Quantifying SRC willow leaf litter dynamics should improve our understanding of carbon (C) sequestration and nutrient cycling potentials within these biomass energy plantations, along with providing valuable data for the development and validation of dynamic process-oriented biogeochemical models. Chapter 4 covers biomass production and associated biogeochemical cycling of N, P, K, S, Ca, and Mg during an initial four-year rotation of six willow varieties grown at four locations along a broad pedoclimatic gradient within Saskatchewan. The comprehensive nutrient budget described in Chapter 4 consisted of quantifying various nutrient inputs, outputs, and transfers (e.g., leaf litter nutrient turnover; Chapter 3) associated with the plant available soil nutrient pool. As available water and N are often considered to be a limitation to SRC willow production, a study investigating the fate of applied nitrogenous fertilizer is described in Chapter 5. Its objective was to determine the effect of irrigation on the fertilizer N uptake and use efficiency of ^{15}N-labelled fertilizer, by two willow varieties, within a three-year-old willow plantation. This is one of only a few studies that have used ^{15}N-labelled fertilizer to examine fertilizer N dynamics with SRC willow and is the first to investigate the effect of irrigation on fertilizer N uptake by willow. The final chapter (Chapter 6) integrates the research findings of the specific studies and draws conclusions and makes practical recommendations based on the results.

Also included in this dissertation are several Appendices. Appendices A-D consist of four published companion studies. Appendix A covers a novel inexpensive root washing pre-treatment, using baking soda ($NaHCO_3$), for facilitating the separation of willow roots from a Vertisolic soil (70% clay). Details of a novel method of non-destructively measuring harvestable willow biomass that was developed as a research tool for this thesis work, involving measuring light attenuation through the willow canopy, is described in Appendix B and C, respectively. Finally, the four field research sites did not allow the opportunity for evaluation of willow performance under saline conditions, which are sometimes encountered on marginal soils in Saskatchewan. Consequently, Appendix D is a growth chamber study comparing the relative salt tolerance of 37 different native and exotic willow varieties grown on soils of varying salinity.

2. LITERATURE REVIEW

2.1 Biomass Energy Within the Renewable Energy Context

Like fossil energy, biomass energy is founded on organic carbon. Therefore, renewable biomass energy shares many characteristics with fossil energy and, therefore, can provide solid, liquid, and gaseous fuels through a variety of conversion technologies that allow storage and transportation within the existing energy supply infrastructure. This is an essential complimentary component within a renewable energy portfolio containing intermittent sources (e.g., wind, solar, and marine; Hall and Scrase, 1998). Biomass is the only renewable option capable of substituting for existing petrochemical feedstocks and products, thereby displacing the conventional petroleum refinery (Clark et al., 2012). Biorefineries would process the raw feedstock into a variety of high value-added platform chemicals and synthetic organic materials (e.g., plastics, paints, pharmaceuticals, solvents, adhesives, lubricants, etc.) currently produced by the petrochemical industry (Valentine et al., 2012; Weiss et al., 2012). The clear intention of the biorefinery model is to maximize the economic return by extracting the most valuable components from the raw biomass, with the residue used for lower value process heat and energy production.

Among the renewable alternatives to fossil energy, capitalizing on the myriad of end use options for biomass feedstock is the least expensive GHG mitigation strategy in the short to medium term (Rowe et al., 2009; Djomo et al., 2011). Perennial bioenergy crops have the further advantage of enabling carbon sequestration, increased biodiversity, and phytoremediation within the landscape (Lemus and Lal, 2005; Rowe et al., 2011; Abrahamson et al., 2012; Baum et al., 2012; Lockwell et al., 2012). Of the many drivers for promoting the use of biomass energy, perhaps its most appealing characteristic, especially to those seeking to revitalize rural economies, is that significantly more jobs are created along the biomass energy supply chain than any other alternative renewable energy source (European Commission, 1997; Volk et al., 2004; Valentine et al., 2012). As a result, although biomass energy currently supplies less than 15% of the world's energy, it remains the largest renewable contributor to global energy supply and is expected to provide the majority of renewable energy over the next several decades (Graham-Rowe, 2011; EIA, 2011; IPCC, 2012).

2.2 First Generation Biofuels

The biomass energy market currently is dominated by first-generation biofuels, which utilize conventional agricultural food and feed commodities such as sugar crops (e.g., sugar beet, sugar cane, sweet sorghum), starch crops (e.g., cassava, potato, wheat, barley, rice, and corn), and oil crops (e.g., rape/canola and palm tree oil, soybean, sunflower). The primary first generation biofuels produced commercially worldwide are bioethanol and biodiesel associated with well-established conversion technologies and mature markets within the transportation sector (Naik et al., 2010; Sims et al., 2010). Despite the progress observed in the bioenergy sector thus far, the future success of first-generation biofuels will be limited, because of increasingly widespread acceptance of their intrinsic inability to sustainability address the mandates that biomass energy development was predicated on. Specifically, some of the deleterious effects associated with conventional biofuels include: being indelibly linked with the moral conflict between food/feed availability and pricing within the context of an increasing world population, along with changing diets (associated with economic development) creating additional demand for food/feed production (Tilman et al., 2009; Pimentel et al., 2010; Fairley, 2011); large net GHG emissions and 'carbon debt' due to direct and/or indirect land use change and extensive nitrogenous fertilizer use (Crutzen et al., 2008; Fargione et al., 2008; Searchinger et al., 2008; Melillo et al., 2009); eutrophication, 'womb-to-tomb' GHG emissions, and stratospheric ozone depletion (Fargione et al., 2010; Brandão et al., 2011; Weiss et al., 2012); decreased biodiversity and ecosystem services (Groom et al., 2008; Searchinger et al., 2008; Fargione et al., 2010); unfavourable NER (i.e., energy output:fossil energy input; McKendry, 2002; Powlson et al., 2005; Hill et al., 2006); soil degradation (Brandão et al., 2011); and requiring substantial government aid to support economic viability with minimal opportunity for cost reductions (Solomon et al., 2007; Pimentel et al., 2010; Sims et al., 2010). Consequently, first generation biomass energy crops could be worse than the conventional fossil energy carriers they are replacing (Crutzen et al., 2008; Luque et al., 2008; Tilman et al., 2009; Djomo and Ceulemans, 2012; Smith et al., 2012; Smith and Searchinger, 2012; Weiss et al., 2012). Considering the widespread skepticism regarding the long-term viability of first-generation bioenergy systems, the expectation is that second-generation lignocellulosic biomass energy systems will rectify the aforementioned problems and, therefore, are predicted to supersede conventional bioenergy crops in the future (Fargione et al., 2010; IEA, 2011; EIA, 2011; Englund et al., 2012).

2.3 Second Generation Biofuels

Second generation bioenergy feedstock consists of lignocellulosic biomass sourced from a diversity of organic materials like agricultural and forestry residues (e.g., stover, straw, bagasse, black liquor, forest thinnings, slash, wood chips, saw dust, etc.), municipal solid waste (e.g., tree trimmings, yard debris, construction and demolition site residues, etc.), and animal manure. Notwithstanding the large quantities of potentially available non-food residual waste materials (Perlack et al., 2005; Junginger et al., 2006; Haberl et al., 2010), there are important logistical, economical, and environmental constraints limiting their availability as bioenergy feedstock (Walmsley et al., 2009; Williams et al., 2009; White, 2010). Consequently, in order to provide the reliable long-term lignocellulosic biomass quantities necessary to support commercial bioenergy production demand, dedicated purpose-grown perennial herbaceous (e.g., *Miscanthus* spp., switch grass, reed canary grass, etc.) and short-rotation woody (hybrid *Populus* spp. and shrub *Salix* spp.) crops are being developed worldwide to compliment the residue/waste streams (Hoogwijk et al., 2009; Sims et al., 2010; Langeveld et al., 2012; Valentine et al., 2012).

Short-rotation woody crops possess a number of advantages relative to other lignocellulosic energy crops, including: flexible harvest timing that avoids long-term storage costs and biomass decay; multiple end uses for feedstock; multi-year rotations that mitigate the risk of crop failure due to abiotic or biotic stresses (e.g., drought, disease, insects, etc.), but also time the harvest in response to market demand; higher energy density that reduces transportation costs; lower establishment costs with higher gross margin; lower ash content; fewer agrochemical requirements; improved GHG emission reductions; increased biodiversity; and potential for environmentally and economically favourable bioremediation applications (Rosenqvist and Dawson, 2005; Styles and Jones, 2008b; Rowe et al., 2009; Hinchee et al., 2010; Krasuska and Rosenqvist, 2012). Therefore, the establishment of dedicated woody biomass energy crops is expected to increase dramatically in the short-term, as more countries recognize their potential to improve the sustainability of the national energy system, environment, and economy (Volk et al., 2011; Weih and Dimitriou, 2012; Werner et al., 2012).

2.4 Purpose-Grown Short-Rotation Woody Crops

There are approximately 28,000 ha of short-rotation woody crops in Canada, consisting of primarily hybrid polar (Sylvain Masse, Canadian Forest Service, personal communication). However, compared with the genus *Populus*, *Salix* has a much broader genetic base (i.e., approximately ten times larger), more extensive geographical and physiognomic range, and offers a much greater variety of ecosystem services and environmental applications (Verwijst, 2001). Additionally shrub willow species possess numerous advantageous characteristics, such as: maximum production rates achieved relatively quickly with high yields obtained in 3-5 years; easily propagated vegetatively from dormant cuttings; tolerant of high planting density; extensive and diffuse fibrous root system; continues to re-sprout vigorously after multiple harvests; a broad underutilized genotypic variability (i.e., minimal domestication compared to arable crops) that is rapidly exploitable via a short breeding cycle, using either conventional or molecular breeding techniques; perennial nature with long vegetative season; high transpiration rates; tolerance of waterlogged conditions; limited insect and pest vulnerabilities; and, similar wood chemistry and energy density compared to commercial forestry species (Weih, 2004; Volk et al., 2006; Volk et al., 2011; Abrahamson et al., 2012). While the use of shrub willow is emerging in Canada, the research and development of purpose-grown willow began decades ago in the U.S. and Europe, which has not only led to the establishment of commercial plantations, but also an array of ecotechnology applications (e.g., biofiltration for wastewater treatment, phytoremediation, living snow fences, riparian buffers, erosion control, and alternative landfill covers) that directly benefit the environment (Volk et al., 2004; Mirck et al., 2005; Kuzovkina and Volk, 2009; Abrahamson et al., 2012).

2.5 Short-Rotation Coppice Willow

Numerous studies have identified social, economic, energetic, and environmental benefits attendant with establishing short-rotation coppice (SRC) willow plantations to help achieve renewable energy commitments. In particular, compared with conventional arable crop production (i.e., first-generation bioenergy crops), SRC willow plantations in the U.S. and

Europe have been found to: have a greater NER (Keoleian and Volk, 2005; Main et al., 2007; Rowe et al., 2009; Djomo et al., 2011; Don et al., 2012); superior net GHG emissions (Rowe et al., 2009; Djomo et al., 2011; Djomo and Ceulemans, 2012; Don et al., 2012; Langeveld et al., 2012); enhance soil quality (Baum et al., 2009; Rowe et al., 2009; Dimitriou et al., 2011; Dimitriou et al., 2012b; Jandl et al., 2012; Langeveld et al., 2012; Lockwell et al., 2012); reduce soil erosion (Rowe et al., 2009; Langeveld et al., 2012); improve water quality by decreasing nitrogen (N) and phosphorus (P) losses via leaching and surface runoff (Rowe et al., 2009; Börjesson and Tufvesson, 2011; Dimitriou et al., 2011; Dimitriou et al., 2012a; Langeveld et al., 2012; Schmidt-Walter and Lamersdorf, 2012); enhance plant, animal, invertebrate, amphibian, and reptile biodiversity (Rowe et al., 2009; Dimitriou et al., 2011; Rowe et al., 2011; Baum et al., 2012; Langeveld et al., 2012); require less maintenance and agrochemical inputs (Martin, 2011; Faasch and Patenaude, 2012; Krasuska and Rosenqvist, 2012; Valentine et al., 2012); higher biomass yield and greater potential for future production cost reductions (Main et al., 2007; Ericsson et al., 2009; Fischer et al., 2010; Sims et al., 2010); and, stimulate rural economic development (Volk et al., 2011; Abrahamson et al., 2012; Valentine et al., 2012). The purpose of such comparative studies and extensive life cycle analyses is to better understand the relative impacts of large-scale SRC production implementation on a variety of important land use functions, in order to develop necessary valuable criteria and indicators for regulating and maintaining sustainable biomass energy supply chains going forward (Englund et al., 2012; Langeveld et al., 2012). However, perhaps the most compelling reason to support the development of SRC biomass energy crops is the avoidance of the moral debate regarding growing biomass for food vs. fuel.

2.5.1 Suitability of growing short-rotation coppice willow on marginal land

With more than 99% of global food supply originating from terrestrial sources (Pimentel and Pimentel, 2000) and increased global population caloric consumption (Graham-Rowe, 2011), it is imperative that our finite agricultural soils sustainably produce adequate food, feed, and fibre to meet this demand, while accommodating the growing associated infrastructure requirements of a growing world population. Therefore, the growing consensus is to decouple biomass energy feedstock from arable lands by establishing inedible crops on anthropogenically degraded or abandoned lands, which are currently unsuitable for annual crop production, for encouraging the long-term viability of biomass energy systems (Tyndall et al., 2011; Erb et al., 2012; Valentine et al., 2012). Furthermore, growing dedicated energy crops with low nutrient demand (in particular N) on currently low productivity land, with inherently minimal carbon sequestration ability, will preclude any negative direct and/or indirect land use change effects on net GHG emissions (Graham-Rowe, 2011; Haberl et al., 2012; Smith et al., 2012; Smith and Searchinger, 2012).

Sustained willow plantations can enhance carbon sequestration, soil quality, and biodiversity within marginal landscapes (Labrecque and Teodorescu, 2003; Keoleian and Volk, 2005; Volk et al., 2006; Stolarski et al., 2011; Lockwell et al., 2012). Moreover, the agrochemical inputs and field maintenance operations required to successfully grow deep-rooted perennial willow are reported to be lower than annual crops (Zan et al., 2001; Williams et al., 2009; Sims et al., 2010; Scholz et al., 2011). A relative reduction in, along with very low opportunity costs of utilizing degraded and abandoned lands, could support more favourable net energy and net GHG emission balances of SRC willow, in addition to a positive economic return on investment for the farmer. It has been estimated that approximately 160 Mha of dedicated perennial crops will be required to supply the necessary feedstock to produce the expected levels of second-generation biofuels in 2050 (IEA, 2011a).

2.5.2 Areal extent of marginal land available for SRC willow plantations

The amount of degraded and abandoned agricultural lands suitable for establishing short-rotation woody crops globally is sizable, with approximately 147 Mha within North America alone (Hoogwijk et al., 2005; Lemus and Lal, 2005). Approximately 10% of this North American estimate is located within western Canada (Joss et al., 2008), 5.4 Mha of which are within 25 km of Canada's existing distributed network of forestry mills; representing a potential biomass feedstock supply of almost 1.5 Gm^3 over a 20-year period (assuming 13.6 $m^3\ ha^{-1}\ yr^{-1}$ growth rate; Brent Joss, personal communication, Canadian Forest Service). Saskatchewan is certainly no exception, with more than 2 Mha of available marginal land, which may be capable of supporting long-term SRC willow production, with a C sequestration potential capable of offsetting up to 80% of the annual anthropogenic GHG emissions of the province (Amichev et al., 2012). In addition to these degraded lands, there are approximately 4 Mha of abandoned salt-affected land across the Canadian prairies (roughly 1.6 Mha in Saskatchewan), which may be capable of supporting salt-tolerant willow varieties (Appendix D). With intensifying public unease over the displacement of arable land from food and feed production into bioenergy production, a tremendous opportunity exists to enhance the environmental quality and ecosystem services of degraded or abandoned lands.

2.5.3 Challenges to short-rotation coppice willow adoption

Notwithstanding the many reported benefits of SRC willow production, there are several important challenges to its widespread commercialization that need to be overcome, namely: absence of a well-developed market for willow biomass (Weih, 2004; Styles et al., 2008; Abrahamson et al., 2012; El Kasmioui and Ceulemans, 2012); its high initial establishment cost with delayed amortisation, due to multi-year rotations, that may delay profitability for several rotations (Ericsson et al., 2009; Abrahamson et al., 2012; Krasuska and Rosenqvist, 2012); lack of government policy and incentives to stimulate the industry (El Kasmioui and Ceulemans, 2012; Faasch and Patenaude, 2012; Stephen et al., 2012; Buchholz and Volk, 2013; Stephen et al., 2013); perceived financial risk by farmers relative to conventional annual crops (Faasch and Patenaude, 2012; Rosenqvist et al., 2013); farmers inexperience with growing willow (Weih, 2004; Dimitriou et al., 2011; Abrahamson et al., 2012; Faasch and Patenaude, 2012); immature second generation biochemical or thermo-chemical bioenergy conversion technologies (Hoogwijk et al., 2005; Naik et al., 2010; Sims et al., 2010); absence of infrastructure for collecting, transporting, and storing biomass (Tharakan et al., 2005b; White, 2010); potentially negative public perception of bioenergy being an old technology, inefficient, and inconvenient for use and/or uncertainty regarding reliable long-term feedstock supplies, due to the susceptibility of plants to disease, drought, fire, insects, frost damage, etc. (Rösch and Kaltschmitt, 1999; White, 2010; Abrahamson et al., 2012); and finally, not cost competitive with existing fossil fuels (Tharakan et al., 2005b; White, 2010; Abrahamson et al., 2012). None of these issues are insurmountable and are common for all contemporary second generation bioenergy feedstocks, but in the meantime, it is prudent to prepare for the possible large-scale adoption of SRC willow production by studying the long-term soil productivity of these purpose-grown woody crop plantations.

2.5.4 Long-term short-rotation coppice willow productivity

A Life Cycle Analysis is used to quantifying the net energy inputs, materials usage, and environmental impacts over the entire life cycle (i.e., from “womb-to-tomb”) of SRC willow production systems. Harvestable willow biomass productivity often is reported to be a primary controller of both net GHG emission and sustainability within SRC willow plantations (Scholz and Ellerbrock, 2002; Styles and Jones, 2008a; Buchholz and Volk, 2011; McKenney et al., 2011; Faasch and Patenaude, 2012; Caputo et al., 2013). Under ideal soil moisture conditions, willow productivity is predominantly controlled by soil nutrient availability; therefore, given its rapid growth rate and chronic nutrient exports via harvested willow stems, it is important to examine the long-term soil productivity within a multi-rotation SRC willow production system.

Inorganic fertilizers have been conventionally used to promote the successful establishment and growth of willow plantations, however, willow growth response to fertilizer addition has been inconsistent (Chapter 5). Optimizing fertilizer use efficiency is needed for improving NER and reducing net GHG emissions (Djomo et al., 2011), along with increasing the economic return for the farmer– as fertilization constitutes a large portion of SRC willow production costs (Heinsoo and Holm, 2010; McKenney et al., 2011). Additionally, there are a number of problems associated with excessive and non-timely fertilizer application, such as reduced plantation productivity due to fertilizer toxicity, stimulating non-crop species growth, and inducing soil nutrient imbalances (Nilsson and Ericsson, 1986; Mortensen et al., 1998; Balasus et al., 2012) or non-point source pollution events (Aronsson and Bergström, 2001; Dimitriou et al., 2012a). In order to promote sensible fertilizer use, an improved understanding of soil nutrient dynamics within SRC willow plantations, particularly the biogeochemical nutrient cycling of essential plant nutrients during the establishment phase, is required to reliably forecast long-term soil productivity and need for any supplemental nutrient amendments throughout the lifetime (i.e., 22 years) of this perennial woody crop.

3. Leaf Litter Decomposition and Nutrient Release Characteristics of Several Willow Varieties Within Short-Rotation Coppice Plantations in Saskatchewan, Canada

3.1 Preface

Leaf litter decomposition is a primary mechanism for C and nutrient cycling within terrestrial ecosystems. Although its essential role within short-rotation coppice (SRC) willow plantations has been previously recognized, there is a need to quantify the decomposition rate constants and limit values for mass loss and associated nutrient release for *Salix* spp. leaf litter. Assessing willow leaf litter dynamics will help to improve our understanding of C sequestration and nutrient cycling efficiency within SRC willow plantations (Chapter 4), provide valuable data for validating models, and help to optimize fertilization strategies (the largest variable input cost for farmers; Chapter 5) for supporting the long-term adoption of commercial SRC willow plantations in Saskatchewan (Chapter 6). This chapter was submitted to BioEnergy Research for publication and is currently being reviewed. The co-author contributions to this manuscript were greatly appreciated and consisted of: J.J. Schoenau (provided financial assistance, soil and tissue analyses, and manuscript editing); K.C.J. Van Rees (provided financial assistance, meteorological and soils data, and manuscript editing); N. Bélanger (provided principal component analyses guidance and manuscript editing); and, T. Volk (provided the willow planting material and manuscript editing).

3.2 Abstract

Quantifying short-rotation coppice (SRC) willow leaf litter dynamics will improve our understanding of carbon (C) sequestration and nutrient cycling potentials within these biomass energy plantations and provide valuable data for model validation. The objective of this study was to quantify the decomposition rate constants ($k_{Biomass}$) and decomposition limit values ($LV_{Biomass}$), along with associated release rates ($k_{Nutrient}$) and release limits ($LV_{Nutrient}$) of nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg) of leaf litter from several native and exotic willow varieties during an initial four-year rotation at four sites within Saskatchewan, Canada. The $k_{Biomass}$, $LV_{Biomass}$, $k_{Nutrient}$, and $LV_{Nutrient}$ values varied among the willow varieties, sites, and nutrients, with average values of 1.7 year^{-1}, 79%, 0.9 year^{-1}, and 83%, respectively. Tissue N had the smallest $k_{Nutrient}$ and $LV_{Nutrient}$ values, while tissue K and Mg had the largest $k_{Nutrient}$ and $LV_{Nutrient}$ values, respectively. The leaf litter production varied among willow varieties and sites with an average biomass accumulation of 7.1 Mg ha^{-1} after the four-year rotation and associated C sequestration rate of 0.2 Mg C ha^{-1} yr^{-1}. The average contribution of nutrients released from leaf litter decomposition during the four-year rotation to the plant available soil nutrient pool across varieties and sites was 22, 4, 47, 10, 112, and 18 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively. These findings support the contention that SRC willow leaf litter is capable of enhancing both soil organic C levels and supplementing soil nutrient availability over time.

3.3 Introduction

There is interest worldwide in developing renewable energy sources that can provide solid, liquid, and gaseous fuels through a variety of conversion technologies. Despite supplying less than 15% of the world’s primary energy, biomass energy is the largest renewable contributor to global primary energy supply and is expected to represent more than half of the near-term potential for expanding renewable energy (EIA, 2011; IPCC, 2012). The potential for biomass feedstock to substitute contemporary fossil fuel energy and its derivatives on many levels, ensures that biomass energy will have a progressively important and sustained role within the growing bioenergy sector and associated bioproduct markets in the foreseeable future (Don et al., 2012; Qin et al., 2012).

Numerous studies have quantified different social, economic, energetic, and environmental benefits attendant with establishing short-rotation coppice (SRC) willow biomass energy plantations to help achieve renewable energy commitments. For example, compared with first-generation bioenergy crops, SRC willow has been found to: enhance soil quality (Dimitriou et al., 2012b; Lockwell et al., 2012); reduce soil erosion (Rowe et al., 2009; Langeveld et al., 2012); improve water quality by decreasing nitrogen (N) and phosphorus (P) losses via leaching and surface runoff (Dimitriou et al., 2012a; Schmidt-Walter and Lamersdorf, 2012); improving plant, animal, invertebrate, amphibian, and reptile biodiversity (Rowe et al., 2011; Baum et al., 2012); require less maintenance and agrochemical inputs (Faasch and Patenaude, 2012; Krasuska and Rosenqvist, 2012); provide higher biomass yield and greater potential for future production cost reductions (Ericsson et al., 2009; Sims et al., 2010); and, stimulate rural economic development (Abrahamson et al., 2012; Valentine et al., 2012). Additionally, compared to both first-generation bioenergy crops and alternative second generation herbaceous bioenergy crops (e.g., giant reed grass, Miscanthus, switchgrass, etc.), SRC willow production is reported to have a greater net energy ratio (Djomo et al., 2011; Don et al., 2012) and lower net greenhouse gas emissions (Rowe et al., 2009; Don et al., 2012), which is primarily attributed to its relatively low fertilization requirement.

The relatively low nutrient-demanding nature of SRC willow is partly due to the efficient nutrient cycling within these plantations. For instance, substantial nutrients are contained within leaf litter biomass; thereby, providing a long-term supply of mineralizable nutrients that satisfies a substantial portion of its annual growth demand (Ericsson, 1994a). Considering the effect fertilization practices have on the economic (Heinsoo and Holm, 2010; McKenney et al., 2011), energetic (Rowe et al., 2009; Djomo et al., 2011), and environmental (Djomo et al., 2011; Balasus et al., 2012) facets of SRC willow production, it is important to quantify the decomposition rate and concomitant nutrient-release characteristics of willow leaf litter, in order to support the development of soil fertility management strategies that optimize fertilizer amendments needed to maximize biomass production (Šlapokas and Granhall, 1991a; 1991b). Moreover, increased concern over rising atmospheric CO_2 concentration has prompted efforts to increase terrestrial carbon (C) sinks and, therefore, the decomposition rate constant ($k_{Biomass}$) for willow leaf litter are needed for improving the calculated C sequestration potential of SRC willow plantations (Rytter, 2012; Caputo et al., 2013).

The well-established key abiotic and biotic factors affecting leaf litter decomposition rate are climate, leaf litter quality, inherent soil fertility, and the decomposer community composition and activity (Berg and McClaugherty, 2008). Strictly considering the decomposition rate, however, does not completely define the entire decay process, as C storage and nutrient release characteristics are ultimately governed by the maximum decomposition limit of the leaf litter, thus defining its decomposition limit value (i.e., % of leaf litter mass loss when decomposition ceases; $LV_{Biomass}$; Berg, 2000; Prescott, 2005). Estimating $LV_{Biomass}$ values for accumulated leaf litter mass loss during decomposition, using asymptotic functions, is a reliable indicator of the relatively stable fraction of residual organic matter that will cease to play a role in C dynamics and nutrient cycling under existing environmental conditions (Berg, 2000). The limit value principle is one of several methods (e.g., historic soil inventories, chronosequences, N-balance method, and dynamic process-oriented models) that can be used to estimate soil C accumulation over time (Mol Dijkstra et al., 2009). In order to quantify the leaf litter dynamics of several willow varieties, a litter bag experiment was replicated at four different sites across a 500 km north-south pedoclimatic gradient in Saskatchewan, Canada, covering a variety of soil types and climatic conditions. The objective of this study was to quantify the $k_{Biomass}$ and $LV_{Biomass}$ values, and associated release rates ($k_{Nutrient}$) and release limits ($LV_{Nutrient}$) of N, P, potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg) of leaf litter from several exotic willow varieties, along with a native willow variety for comparison, within SRC willow plantations during the initial four-year rotation, to provide insight into the relevance of leaf litter nutrient additions into the plant available soil nutrient pool. It was hypothesized that leaf litter mass loss and nutrient release characteristics would vary according to specific nutrient, willow variety, and site as related to the soil and environmental conditions. Although the essential role annual leaf litter additions play in augmenting soil organic C levels (Lockwell et al., 2012; Rytter, 2012) and nutrient cycling (Ericsson, 1984; Christersson, 1986) within SRC willow plantations has been previously recognized, to my knowledge, no $LV_{Biomass}$, $k_{Biomass}$, $k_{Nutrient}$, or $LV_{Nutrient}$ values have been developed for any *Salix* spp. leaf litter. Assessing willow leaf litter dynamics will help to improve our understanding of C sequestration and nutrient cycling efficiency within SRC willow plantations, in addition to providing valuable data for validating dynamic process-oriented biogeochemical models (Eckersten and Slapokas, 1990; Amichev et al., 2012).

3.4 Materials and Methods

3.4.1 Study sites and willow varieties

The data for this study were collected from four SRC willow variety trial plantations located along a 500 km north-south geoclimatic gradient within Saskatchewan, Canada, from the south-east corner of the province to the southern boundary of the boreal forest in the central area of the province. The selected sites represent many of the diverse soil types and climatic conditions existing in the province (Fig. 3.1 and Tables 3.1 and 3.2). At each of the four sites, a single pedon was excavated and a full soil taxonomic assignment given to classify the soils according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). In the spring of 2007, six willow varieties, developed by the State University of New York College of Environmental Science and Forestry (SUNY-ESF) breeding program, were planted at each site in a randomized complete block design (n = 4) adapted from the protocols of Abrahamson et al. (2002). The willow varieties used were: Allegany *(Salix purpurea)*, Canastota *(Salix sachalinensis × miyabeana),* Fish Creek *(Salix purpurea),* Sherburne *(Salix sachalinensis × miyabeana),* SX61 (*Salix sachalinensis*)*,* and SX64 (*Salix miyabeana*). Each varietal plot (6.3 × 7.8 m) consisted of 78 plants (three double-rows of 13 plants row^{-1}), with spacings of 1.5 m between the double-rows, 60 cm between rows within the double-row, and 60 cm between plants within the double-row; resulting in a planting density of approximately 15,873 plants ha^{-1}. In the spring of 2008, the willow plants were coppiced and grown for an additional three years before harvesting. Pre- and post-planting site preparation to control non-crop vegetation included both mechanical (deep tillage, light cultivation, tandem disc, mowing, and hand weeding) and chemical (Goal™ 2XL, 2 L ha^{-1}; Roundup WeatherMax®, 2 L ha^{-1}; Simazine 480, 4.7 L ha^{-1}; Pardner®, 0.5 L ha^{-1}) treatments. Stem counts, heights, and diameters (at 30 cm height) of the central 18 stools within each varietal plot were assessed after each growing season. Stem basal area was calculated on an individual stem basis and extrapolated to a stand level based on stem density measurements.

3.4.2 Measuring soil nutrient availability

After planting the willow at each site, three 60 cm depth soil cores were collected within each varietal plot using a JMC backsaver probe (Model PN001; Clements Assoc. Inc., Newton, IA, USA), separated into 10 cm depth increments, and composited by depth. All soil samples

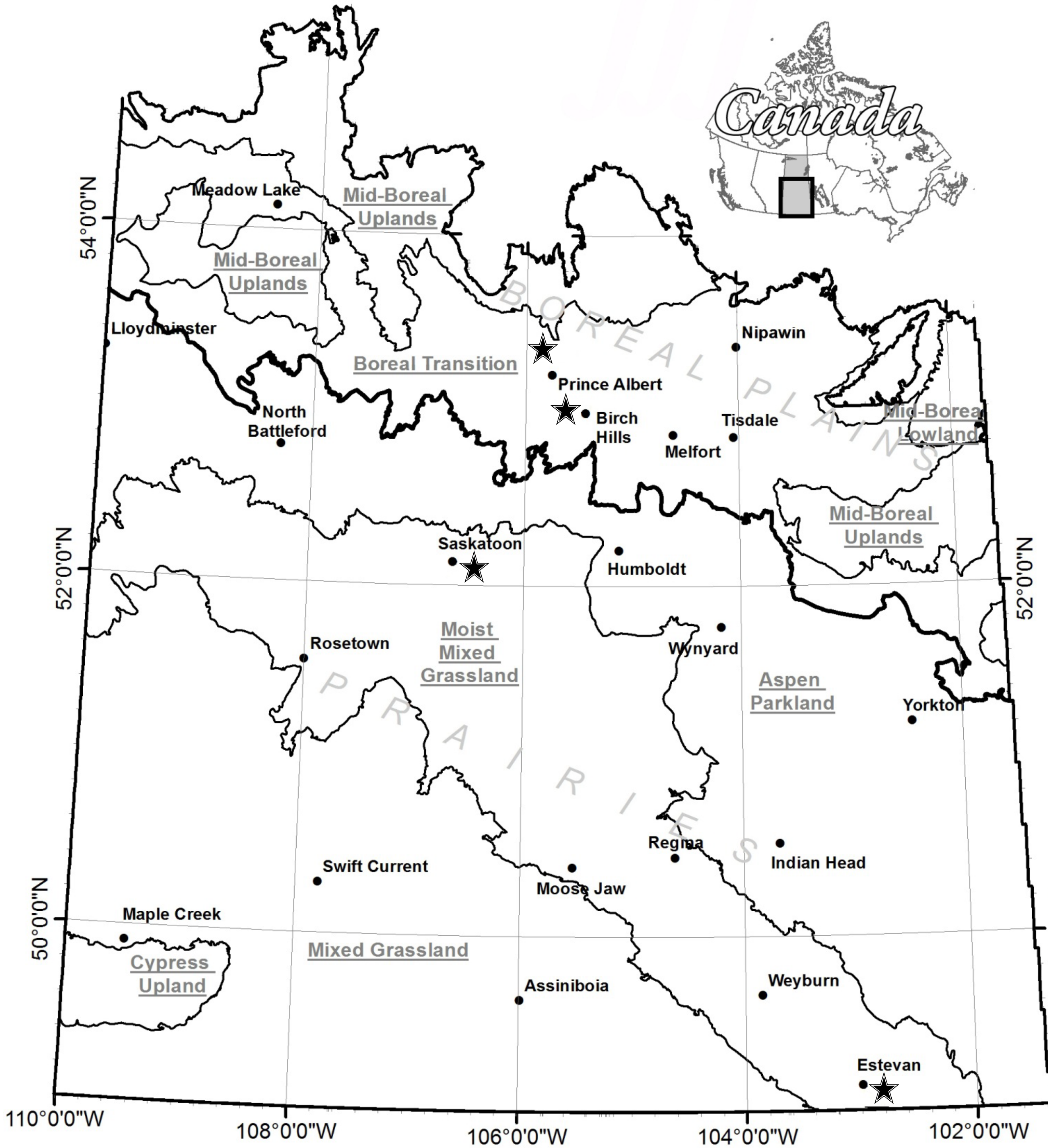

Fig. 3.1. Locations of four short-rotation coppice willow variety trial study sites in Saskatchewan, Canada. ArcGIS10 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) map courtesy of Dr. Beyhan Amichev.

Table 3.1. Selected site characteristics of different willow variety trial sites in Saskatchewan, Canada.

Site	UTM Co-ordinates	Prior crop	ACC[†]	MAP[‡] (mm)	MGSP[§] (mm)	MAT[¶] (°C)	MGST[#] (°C)	FFD[††] (#)	Water[‡‡] (m)
Prince Albert	13U 448501 5912029	fallow	5-6	450	295	1.2	14.2	85	1.5
Birch Hills	13U 467122 5872616	canola	1-2	420	277	1.3	14.3	90	1.2
Saskatoon	13U 389970 5776342	fallow	2-3	375	312	2.6	14.9	112	3.3
Estevan	13U 655043 5438201	fallow	3-4	430	341	3.3	15.4	124	4.3

[†] Agriculture capability classification (Class 1: no significant limitations; Class 2: moderate limitations; Class 3: moderately severe limitations; Class 4: severe limitations; Class 5: very severe limitations; Class 6: limited capability for arable agriculture)

[‡] Mean annual precipitation (snow + rainfall) during the rotation (SCSR 1976; 1978; 1989; and 1997, respectively)

[§] Mean growing season precipitation during the rotation; growing season length determined using 5 °C soil baseline

[¶] Mean annual air temperature during the rotation

[#] Mean growing season air temperature during the rotation; growing season length determined using 5 °C soil baseline

[††] Frost-free days (SCSR 1976; 1978; 1989; and 1997, respectively)

[‡‡] Average depth to groundwater; measured throughout the final growing season using an observation well installed at each site

Table 3.2. Mean (n = 24) selected soil characteristics of different willow variety trial sites in Saskatchewan, Canada[†].

Site	Soil type[‡]	Texture	BD	pH	$EC_{1:2}$[§]	Organic C	Organic C:N	MGSST[¶]	MGSSM[#]
		(% sand/clay)	(kg m^{-3})		(dS m^{-1})	(%)		(^{o}C)	(% v v^{-1})
Prince Albert	OBC (Meota)	sand to loamy-sand (91/2)	1588	6.6c[††]	0.16c	1.4c	15.5a	13.2	32
Birch Hills	OBC (Hoey)	silt-loam to clay-loam (29/28)	1002	7.0b	0.68a	3.2a	11.4b	13.2	54
Saskatoon	OV (Sutherland)	clay (13/70)	1422	7.1b	0.45b	2.3b	9.5c	13.3	48
Estevan	CHR (Alluvium)	silt-loam (33/23)	1238	8.0a	0.60ab	2.0b	12.7b	14.9	35

[†] 0-60 cm; average values of six 10 cm segments collected using a backsaver probe, except for extractable nutrient levels that are summed values of all segments

[‡] OBC (Orthic Black Chernozem), OV (Orthic Vertisol), and CHR (Cumulic Humic Regosol); taxonomy based on the Canadian System of Soil Classification (Soil Classification Working Group, 1998). Association name in brackets; for a complete description (e.g., map unit, parent material, stoniness, drainage, etc.) see SCSR 1976, 1989, 1978, and 1997, respectively.

[§] Electrical conductivity of a 1:2 (soil:water; on a weight basis) extraction

[¶] Mean growing season soil temperature (0-60 cm) during the rotation; growing season length determined using 5 ^{o}C soil baseline

[#] Mean growing season soil moisture (0-60 cm) during the rotation; growing season length determined using 5 ^{o}C soil baseline

[††] Means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

Table 3.2. continued.

Site	MAST[‡‡] (°C)	MASM[§§] (% v v^{-1})	Extractable nutrients: N (kg ha^{-1})	P	K	S	Ca	Mg
Prince Albert	7.1	30	55c	148a	715c	92b	14381b	1297d
Birch Hills	6.2	44	68b	16c	1297b	809a	20464a	4336b
Saskatoon	6.2	42	99a	64b	1963a	663a	19905a	9644a
Estevan	7.7	30	99a	36bc	1348b	764a	19404a	3134c

‡‡ Mean annual soil temperature (0-60 cm) during the rotation

§§ Mean annual soil moisture (0-60 cm) during the rotation; growing season length determined using 5 °C soil baseline were air-dried to a constant weight, ground with a rolling pin to break aggregates, mixed, sieved (< 2 mm fraction retained), and analyzed for extractable nutrient levels (N, P, K, S, Ca, and Mg), total and organic N, total P, organic and inorganic C, pH, and $EC_{1:2}$. Total inorganic N (NH_4^+-N and NO_3^--N) and inorganic P were determined using 2.0*M* KCl (Maynard et al., 2008) and modified Kelowna (Qian et al., 1994) extractions, respectively, with the extracts analyzed colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA). Extractable S was determined using 0.01*M* $CaCl_2$ (Hu et al., 2005) and analyzed using microwave plasma-atomic emission spectrometry (4100 MP-AES; Agilent technologies, Melbourne, Australia). Extractable K, Ca, and Mg were determined using 1.0*M* NH_4OAc (Hendershot et al., 2008) and analyzed using either atomic emission (K) or absorption (Ca and Mg) spectroscopy (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Total N was determined using a H_2SO_4/H_2O_2 digest (Thomas et al., 1967) and analyzed colorimetrically as well. Organic N was calculated from the difference between total N and inorganic N. Soil organic C (SOC) was measured using a LECO C632 Carbon Analyzer (LECO Corporation, St. Joseph, MI, USA; Wang and Anderson, 1998), following a 6% H_2SO_3 pre-treatment to remove the inorganic C (Skjemstad and Baldock, 2008). Soil pH and $EC_{1:2}$ (soil:water on a weight basis; Hendershot et al., 2008) were analyzed using a Beckman 50 pH Meter (Beckman Coulter, Fullerton, CA, USA) and an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA), respectively. Particle size distribution was determined using a Horiba LA-950 Particle Size Distribution Analyzer (Horiba Instruments Inc., Irving, CA, USA) after pre-treatment with bleach (sodium hypochlorite) to remove organic matter, followed by a 10% solution of sodium hexametaphosphate to breakdown clay aggregates.

3.4.3 Leaf litter production and nutrient content

Total leaf biomass for each willow variety was estimated annually at each site throughout the four-year rotation by collecting all of the leaves from three stems (representing the average size) within each plot in early September and extrapolating the leaf biomass to a stand level based on stem density measurements. Using this leaf biomass as a proxy is a more accurate estimation of stand level leaf litter biomass, compared to that estimated from litterfall traps placed randomly underneath the canopy, considering the assumption that absolute leaf fall is inevitable with deciduous species growing in temperate climates. In a companion study, fifty abscising leaves were collected throughout the canopy from each varietal plot every November for estimating the nutrient resorption efficiency (% of initial nutrients resorbed during leaf senescence; Yuan et al., 2005) prior to leaf abscission. The associated foliar nutrient mass loss during leaf senescence was used to correct the estimated stand level leaf biomass from September. Beyond mass loss due to nutrient retranslocation, the % initial mass loss during leaf senescence was assumed to be minor (Chapin et al., 1990). Estimates of accumulated leaf litter nutrients throughout the four-year rotation were then determined by multiplying the nutrient concentrations of abscising leaves collected in November by the corrected total leaf biomass estimates from September. The September and November leaves were dried at 65°C to a constant weight, thoroughly milled and homogenized prior to analyses, and their total N, P, K, Ca, and Mg concentrations were analytically measured following a H_2SO_4/H_2O_2 digest (Thomas et al., 1967) as previously described. Total S was measured using a TruSpec CNS analyzer (Leco Corporation, St. Joseph, MI, USA). Prior to drying the September leaves, the total leaf area was determined using a leaf surface area meter (LI3100; LI-COR Inc., Lincoln, NE, USA).

3.4.4 Leaf litter decomposition

Litterbags were used to measure the rate of decomposition and nutrient release of the willow leaf litter throughout the rotation. At the end of the establishment year (i.e., prior to coppicing), senesced and abscising leaves were collected in November from each varietal plot at every site. For comparison purposes, abscising leaves from a native willow species (*S. eriocephala*) collected from wetlands near Indian Head, SK, Canada (UTM coordinates:13U 593345 5596906) were also included at each site. The specific leaf area (SLA, $cm^2\ g^{-1}$; Tharakan et al., 2005a) of the November leaves used in the leaf litter bags was determined using a leaf surface area meter (LI-3100; LI-COR Inc., Lincoln, NE). All intact leaves were then dried at 65°C to a constant weight, and a 5 g subsample placed in a polyethylene screen bag (20 × 20 cm; 1 mm mesh) and stapled closed. Triplicate litterbags were placed on the soil surface within their respective varietal plots prior to snowfall, with a single randomly chosen litterbag removed from each plot every 12 months over the subsequent three years. All residual leaf litter was dried at 65°C to a constant weight, weighed to determine mass loss, and along with subsamples of the original leaf litter material (i.e., time = 0), were prepared and analyzed for their N, P, K, S, Ca, and Mg concentrations, as previously described, to estimate the nutrient release rates from the decomposing leaf litter throughout the rotation. For each willow variety, its $k_{Biomass}$ and $LV_{Biomass}$ values were determined at every site by fitting the proportion of litter remaining in the litter bag each year to the following asymptotic decomposition model (Eq. 3.1) using nonlinear modelling in JMP 10 (Version 10; SAS Institute, Cary, NC) following the protocols of Hobbie et al. (2012):

$$X = A + [1 - A]e^{-k_{Biomass}t} \qquad \text{(Eq 3.1)}$$

where X is the proportion of initial leaf litter mass remaining at time t and assumes that a recalcitrant fraction (A; asymptote) of the initial leaf litter biomass possesses a decomposition rate so slow that it is practically zero, while another fraction (1 - A) decomposes exponentially at rate $k_{Biomass}$. Although the model allows for complete litter decomposition (i.e., A = 0), a portion of leaf litter normally reaches a stage of relative stability, where further degradation of residual near-humus material is negligible under existing environmental conditions (Berg and McClaugherty, 2008), especially during the comparatively short time frame of a SRC willow production system (i.e., 22 years; seven three-year rotations). The $LV_{Biomass}$ value (%) is then calculated as $1 - A \times 100$. A similar approach was used to model nutrient release from decomposing leaf litter. However, due to inconsistent model convergence among nutrients using the asymptotic model, the single exponential model was used to estimate the nutrient release rate constant ($k_{Nutrient}$) values from the different varietal leaf litters at every site using Eq. 3.2, a simplified equation adapted from Olson (1963):

$$-k_{Nutrient} = \frac{\ln\left(\frac{X_i}{X_0}\right)}{t} \qquad \text{(Eq 3.2)}$$

where X_0 is initial nutrient content within the leaf litter and X_i is the nutrient content remaining at collection time (t) in years. This model assumes complete nutrient release over time, but given the observed asymptotic form of nutrient release after three years, which was consistent among willow varieties and sites (data not shown), I felt that it was acceptable to consider the proportion of initial nutrients released from the leaf litter after the incubation to represent the nutrient release limit value (i.e., $LV_{Nutrient}$;%), with the remaining nutrients essentially immobilized.

A well-documented shortcoming of litterbag studies is the contamination of decomposing leaf material with non-target organic or inorganic material (e.g., earthworm casts, fungal hyphae, weed litter, and soil) over time. In this study, the primary contaminant at each site was soil, which was easily removed by blowing the litter bags using pressured air. An exception to this, however, occurred after three years at Saskatoon where the heavy clay soil was intimately bound with the small leaf litter residue, which rendered the air treatment ineffective. Such difficulty with clay soil has been reported elsewhere and is typically accepted as unavoidable and not corrected for (Šlapokas and Granhall, 1991b). Soil contamination was corrected for by determining the proportion of residual mineral material following the H_2SO_4/H_2O_2 digest and multiplying its weight by the nutrient concentration of the plot-specific soil (0-10 cm) analyses to estimate the topsoil nutrient contribution to the contaminated leaf sample. For example, a leaf litter digest sample having 20 mg g^{-1} total N, but with 50% soil contamination (having a 3 mg g^{-1} soil N content), would require a -0.15% N correction (i.e., 0.5 × 0.003 × 100), resulting in only 1.85% N (93% of the total N in the sample) attributable to leaf litter N. The contribution of leaf litter nutrient release to the plant available soil nutrient pool was calculated using the estimated $k_{Nutrient}$ and accumulated leaf litter biomass over the four-year rotation. The leaf litter cohorts considered in the calculations for nutrient cycling during the rotation were three years of establishment year leaf litter (i.e., pre-coppice leaf biomass), two years of nutrient release from the first year post-coppice leaf litter, and one year of nutrient release from the second year post-coppice leaf litter. Given that the willow was harvested three years after coppicing, the remaining nutrient release contributions from the first to third year post-coppice leaf litter would be associated with the second rotation.

3.4.5 Meteorological conditions during incubation period

A Campbell Scientific CR10X (Campbell Scientific Inc., Edmonton, AB, Canada) was used at each site to monitor air temperature, rainfall, relative humidity, and wind speed throughout the study. Soil temperature (0-60 cm) was also assessed. Potential evapotranspiration (Thornthwaite and Mather, 1957) and Aridity Index (UNEP, 1992) were estimated annually for each site using the measured climate data. Accumulated snow depth was measured at each site annually in February. The beginning and ending of each growing season was determined using a 5 ^{o}C baseline mean daily temperature, sustained or unsustained for at least five consecutive days, respectively, using both air and soil temperatures for comparison. Growing season length was calculated annually in this manner.

3.4.6 Statistical Analyses

Means comparisons of measured variables were performed using least significant differences (LSD; Tukey-Kramer's method of multiple comparison) at a significance level of 0.05 using PROC MIXED in SAS (Littell et al., 2006; version 9.2; SAS Institute, Cary, NC, USA), with groupings performed with the pdmix800 SAS macro (Saxton, 1998). The effects of variety were considered fixed, while those of site and replicate (nested within site) were considered random. Normality of distributions (PROC UNIVARIATE) and homogeneity of variances (Bartlett's test) of all data sets were verified, and when required, the data were Log_{10} transformed prior to analysis. A principal component analysis (PCA) was performed using JMP 10 (Version 10; SAS Institute, Cary, NC, USA) to investigate the relationship between all estimated leaf litter decomposition and nutrient release variables and relevant soil and plant tissue characteristics measured at each site. An additional PCA was performed using the measured climatic data at each site and site averages of different willow canopy variables (e.g., total leaf surface area, average stem basal area, and average stem height), which were considered surrogate measures of microclimate effect, along with estimated leaf litter decomposition and nutrient release variables during the incubation period. Correlation strength among variables is indicated by the cosine of the angle between variable vectors and variable groupings were arbitrarily based on an angle of 30^o (i.e., r = 0.87). Vectors of directly and indirectly correlated variables point in the same or opposite direction, respectively, whereas uncorrelated variables have vectors at right angles to each other.

3.5 Results

3.5.1 Leaf litter production and nutrient content

The leaf litter production varied among the willow varieties and sites with an average accumulation of 7.1 Mg ha^{-1} after the initial four-year rotation (Table 3.3). Annual leaf litter biomass increased each year for all varieties and sites with 0.2, 1.5, 2.1, and 3.3 Mg ha^{-1} produced on average each year, respectively (data not shown). Although there were no significant differences ($P > 0.05$) in cumulative leaf litter biomass after four years among the six varieties, the average leaf litter production was lowest at Saskatoon compared to the other three sites (Table 3.3). There was a 50% greater variation in leaf litter biomass compared to nutrient concentration among varieties and sites (data not shown). There was a strong relationship (R^2 =

Table 3.3. Mean cumulative biomass and nutrient content of leaf litter after an initial four-year rotation for several exotic willow varieties at different plantations in Saskatchewan, Canada.

	Biomass	N	P	K	S	Ca	Mg
Variety (n =16)	(Mg ha^{-1})†	(kg ha^{-1})‡					
Allegany	7.4a§	100.8a	12.7a	90.2bc	30.6a	199.5bc	50.4a
Canastota	7.5a	72.8a	19.5a	157.6a	23.2ab	285.6ab	37.6a
Fish Creek	5.6b	80.8a	13.9a	63.8c	16.2b	187.2c	39.5a
Sherburne	7.0a	83.9a	12.0a	85.0bc	23.5a	239.0abc	41.3a
SX61	7.6a	75.7a	13.8a	129.3ab	26.2a	299.9a	37.7a
SX64	7.7a	70.7a	15.5a	122.6ab	27.5a	323.1a	40.8a
Site (n =24)							
Prince Albert	7.7a¶	74.2b	35.4a	149.3a	22.7ab	280.8b	24.7c
Birch Hills	7.2a	65.9b	10.4b	126.9a	28.8a	237.3b	40.7b
Saskatoon	4.6b	68.9b	6.4c	43.6b	13.5b	107.1c	32.5bc
Estevan	9.1a	114.1a	6.1c	112.5a	33.1a	397.7a	66.9a

† Estimated by collecting all of the leaves from three representative stems within each plot in early September and extrapolating the leaf biomass to a stand level based on stem density measurements. The estimated stand level leaf biomass was corrected for the foliar nutrient mass loss during leaf senescence

‡ Estimated by multiplying the nutrient concentrations of abscising leaves collected in November by the total leaf biomass estimates

§ Among the varieties, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

¶ Among the sites, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

0.64-1.00; P < 0.05; data not shown) between leaf litter biomass production throughout the rotation and leaf litter nutrient accumulation regardless of variety, site, or year. The average leaf litter nutrient content among willow varieties and sites was 81, 15, 108, 24, 256, and 41 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively (Table 3.3). The leaf litter N, P, and Mg contents were not significantly different (P > 0.05) among varieties. Generally, for the remaining nutrients and when comparing leaf litter nutrient content among sites, there was a trend of increasing nutrient content with increasing leaf litter biomass. For example, the Saskatoon site had significantly lower nutrient content because of its low biomass, whereas Estevan had significantly higher biomass and nutrient content (Table 3.3). There were strong relationships (R^2 = 0.64 to 1.00; P < 0.05; data not shown) between leaf litter biomass production throughout the rotation and leaf litter nutrient accumulation regardless of variety, site, or year. Exceptions to this were observed with the P and Mg contents of Estevan and Prince Albert leaf litter, respectively (Table 3.3). The average (SE) native leaf litter nutrient concentrations were 17.2 (0.7), 2.5 (0.1), 4.1 (0.1), 2.3 (0.3), 50.4 (8.3), and 20.5 (3.8) mg g^{-1} N, P, K, S, Ca, and Mg, respectively, compared to 13.1 (0.4), 1.5 (0.1), 12.1 (0.8), 3.3 (0.1), 66.9 (2.3), and 13.2 (1.0) mg g^{-1} N, P, K, S, Ca, and Mg, respectively, for the six exotic willow varieties (data not shown).

3.5.2 Leaf litter decomposition and nutrient release

The average leaf litter $k_{Biomass}$, $LV_{Biomass}$, and A values for the seven native and exotic willow varieties across the sites were 1.7 $year^{-1}$, 78.9%, and 0.21, respectively (Table 3.4). The estimated $k_{Biomass}$ and A were 28 and 40% greater at Prince Albert, respectively, but with an $LV_{Biomass}$ 11% smaller compared to the other sites. The average leaf litter mass loss was 62% after the first year across all varieties and sites (Fig. 3.2). The average (SE) $k_{Nutrient}$ values across all varieties and sites, were 0.45 (0.02), 0.69 (0.02), 1.13 (0.04), 0.91 (0.04), 1.00 (0.04), and 1.07 (0.04) for N, P, K, S, Ca, and Mg, respectively (Table 3.5). The $k_{Nutrient}$ values differed among the seven willow varieties examined, with Fish Creek and Sherburne consistently having the largest and smallest values (averaging 1.2 and 0.6 $year^{-1}$, respectively) across the different nutrients and sites (Table 3.5). Specifically, nutrients were released 56% faster from Fish Creek leaf litter and 40% slower from Sherburne leaf litter, compared to the other varieties. With the exception of K and Mg, the leaf litter at Estevan released its nutrients faster than the other sites; conversely, the decomposing leaf litter at Prince Albert had the slowest nutrient release rate,

Table 3.4. Mean leaf litter decomposition rate constant ($k_{Biomass}$), decomposition limit value ($LV_{Biomass}$), and asymptote (A) of native and exotic willow varieties, measured using leaf litter bag incubations at different plantations in Saskatchewan, Canada. The proportion of leaf litter biomass remaining after three years was fitted to a nonlinear asymptotic decomposition model.

	$k_{Biomass}$	$LV_{Biomass}$ †	A ‡
Variety (n =16)	($year^{-1}$)	(%)	
Native	1.6 (0.1)§	62.8 (2.2)	0.37 (0.02)
Allegany	1.7 (0.1)	80.1 (1.8)	0.20 (0.02)
Canastota	1.5 (0.1)	80.8 (1.5)	0.19 (0.02)
Fish Creek	1.9 (0.1)	82.8 (1.6)	0.17 (0.02)
Sherburne	1.8 (0.3)	84.7 (1.5)	0.15 (0.02)
SX61	1.8 (0.1)	78.6 (2.1)	0.21 (0.02)
SX64	1.5 (0.1)	82.0 (1.3)	0.18 (0.01)
Site (n =28)			
Prince Albert	2.0 (0.1)	72.7 (1.4)	0.27 (0.01)
Birch Hills	1.4 (0.1)	81.8 (1.7)	0.18 (0.02)
Saskatoon	1.6 (0.1)	79.5 (2.4)	0.21 (0.02)
Estevan	1.7 (0.1)	81.4 (1.1)	0.19 (0.01)

† (1 – A) × 100

‡ The recalcitrant proportion of initial leaf litter mass with a practical decomposition rate of zero

§ Varietal and site mean (standard error) values are reported due to significant ($P < 0.05$) variety × site effect

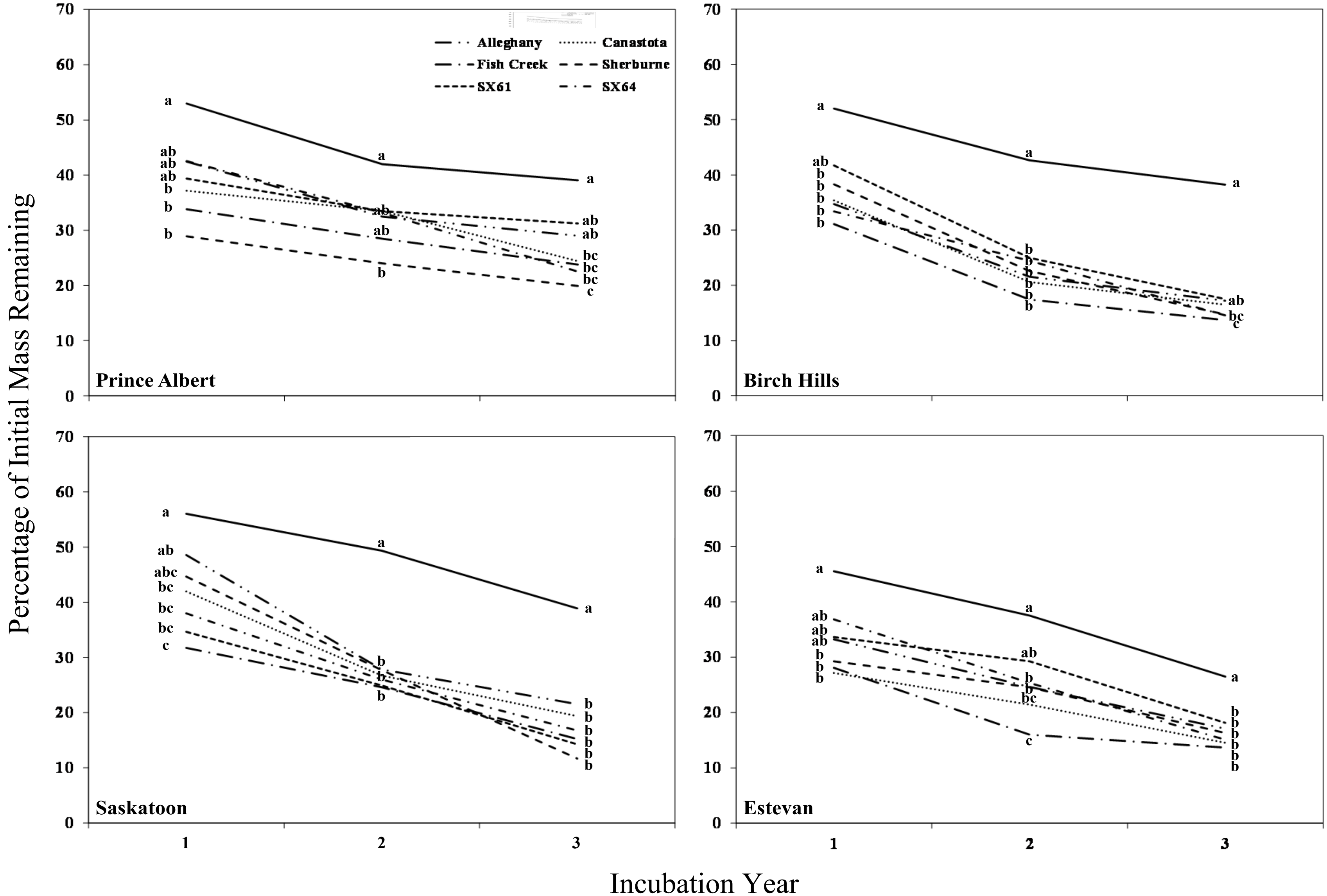

Fig. 3.2. Mean (n = 4) percent of initial leaf litter mass remaining of native and exotic willow varieties, measured using a three-year leaf litter bag incubation at different plantations in Saskatchewan, Canada. For each year, means with the same letter are not significantly different (*P* > 0.05) using LSD.

Table 3.5. Mean leaf litter nutrient release rate constant ($k_{Nutrient}$) of native and exotic willow varieties, measured using a three-year leaf litter bag incubation at different plantations in Saskatchewan, Canada. The proportion of initial leaf litter nutrient content remaining after three years was fitted to a single exponential model.

	N	P	K	S	Ca	Mg
Variety (n = 16)	($year^{-1}$)					
Native	0.39b†	0.64bc	1.29ab	1.1ab	1.2ab	1.00c
Allegany	0.44b	0.70b	0.94c	0.7d	1.0bc	1.07bc
Canastota	0.43b	0.66b	1.41a	1.0bc	0.9cd	0.94c
Fish Creek	0.64a	0.98a	1.39a	1.4a	1.5a	1.54a
Sherburne	0.41b	0.47d	0.76d	0.4e	0.4e	0.66d
SX61	0.45b	0.55cd	0.98c	1.0bc	1.2ab	1.23ab
SX64	0.41b	0.50d	1.11b	0.8cd	0.8d	1.07bc
Site (n = 28)						
Prince Albert	0.33c‡	0.52c	1.64a	0.76b	0.85b	1.29a
Birch Hills	0.43b	0.71b	1.14b	0.86b	1.01ab	1.16a
Saskatoon	0.45b	0.70b	0.78d	0.83b	1.08ab	0.97b
Estevan	0.61a	0.87a	0.95c	1.21a	1.10a	0.87b

† Among the varieties, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

‡ Among the sites, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

except for K and Mg. Relative to the initial leaf litter nutrient contents, the average (SE) release of N, P, K, S, Ca, and Mg after the first year across all varieties and sites was 36 (2), 51 (2), 69 (1), 61 (2), 63 (2), and 61 (2)%, respectively (Fig. 3.3). The trend in $k_{Nutrient}$ values among varieties and sites was similar to the $LV_{Nutrient}$ values observed, resulting in a strong relationship (R^2 = 0.82; $P < 0.02$; data not shown) between the two variables. The $LV_{Nutrient}$ values differed among varieties, sites, and nutrients during the rotation with average values of 75, 83, 86, 82, 84, and 89% for N, P, K, S, Ca, and Mg, respectively, across varieties and sites (Table 3.6). The $LV_{Nutrient}$ values were similar among all six exotic varieties (i.e., CV < 5%), but 20% smaller for the native variety leaf litter over the incubation period. The $LV_{Nutrient}$ values at Prince Albert were 35, 20, and 14% smaller for N, P, and S, respectively, compared to the other three sites, but had the largest $LV_{Nutrient}$ values for K and Mg (Table 3.6). The k_N and LV_N values were 53 and 12% smaller, respectively, compared to the average values of other nutrients (Tables 3.5 and 3.6). Using the estimated $k_{Nutrient}$ and leaf litter biomass accumulation values, the average contribution of nutrients released from leaf litter decomposition during the four-year rotation to the plant available soil nutrient pool across varieties and sites was 22, 4, 47, 10, 112, and 19 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively, with the Estevan soil receiving 52% more nutrients from decomposing leaf litter during the rotation than the other sites (Table 3.7).

3.5.3 Principle Component Analysis

The PCA ordination identified several distinct groupings among the measured soil, plant tissue, climatic, and willow canopy properties associated with willow leaf litter decomposition and nutrient release dynamics (Figs. 3.4 and 3.5). Specifically, variable clustering clearly indicated: SLA and soil C:N were the primary variables directly related to $k_{Biomass}$; initial leaf litter nutrient concentrations (i.e., litter quality) were directly linked with the $k_{Nutrient}$ values; and soil nutrient availability was directly correlated to the $LV_{Biomass}$ and $LV_{Nutrient}$ values (Fig. 3.4). Additionally, a second PCA incorporating climate and canopy data revealed: the first year climate (e.g., annual and growing season rainfall and snowfall, relative humidity, and growing season length) and willow canopy variables (e.g., stem height, leaf surface area, and stem basal area) were more closely related to $k_{Biomass}$ than second and third year conditions; climatic variables indicating less moisture availability at the soil surface (e.g., annual air temperature, aridity index, wind speed, and potential evapotranspiration) were indirectly related to $k_{Biomass}$;

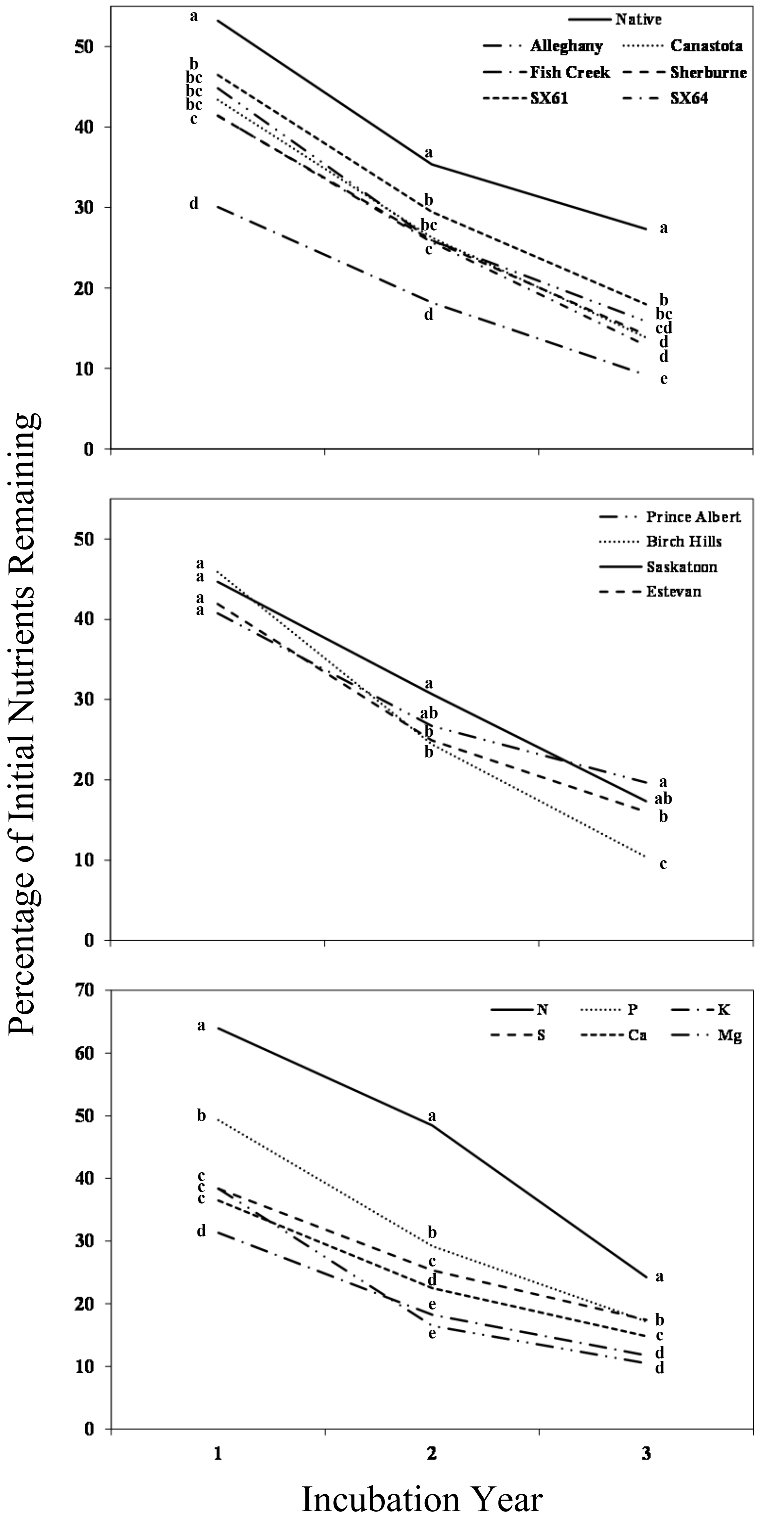

Fig. 3.3. Percentage of initial leaf litter nutrients remaining of native and exotic willow varieties, measured using a three-year leaf litter bag incubation at different plantations in Saskatchewan, Canada. For each year, means with the same letter are not significantly different (*P* > 0.05) using LSD. Note: n = 16, 28, and 112 for comparisons among varieties, cites, and nutrients, respectively.

Table 3.6. Mean nutrient release limit value ($LV_{Nutrient}$) of initial nutrients contained within the leaf litter of native and exotic willow varieties after three years, measured using a leaf litter bag incubation at different plantations in Saskatchewan, Canada.

	N	P	K	S	Ca	Mg
Variety (n = 16)			(%)			
Native	61.5c†	82.5b	75.2c	63.6d	55.6b	74.8c
Allegany	78.2ab	83.2b	82.0b	80.3c	85.9a	90.2ab
Canastota	75.5bc	82.5b	90.4a	84.2abc	87.0a	89.3ab
Fish Creek	86.0a	89.1a	90.0a	89.6a	92.6a	94.9a
Sherburne	76.1ab	81.0b	86.4ab	85.1abc	91.0a	92.5ab
SX61	70.5bc	75.7c	87.9ab	82.1bc	81.9a	89.6ab
SX64	76.7ab	84.9ab	90.6a	87.1ab	91.1a	89.3b
Site (n = 28)						
Prince Albert	59.2b‡	72.1c	90.6a	74.0b	77.0a	91.0a
Birch Hills	82.1a	89.5a	93.3a	82.8a	86.3a	93.7a
Saskatoon	80.5a	84.1b	75.7c	85.9a	86.4a	83.5b
Estevan	77.7a	84.9b	84.5b	83.8a	84.4a	86.2b

† Among the varieties, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

‡ Among the sites, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

Table 3.7. Mean nutrients released from leaf litter decomposition during an initial four-year rotation for several exotic willow varieties, measured using leaf litter bag incubations at different plantations in Saskatchewan, Canada.[†]

	N	P	K	S	Ca	Mg
Variety (n = 16)	(kg ha^{-1})					
Allegany	25.9 (5.6)[‡]	3.4 (0.4)	29.6 (5.6)	11.2 (2.2)	81.1 (14.6)	21.4 (3.4)
Canastota	17.0 (2.1)	5.9 (1.8)	72.6 (15.3)	10.1 (1.5)	112.1 (22.1)	15.1 (1.7)
Fish Creek	24.6 (4.3)	3.7 (0.8)	24.2 (5.2)	7.6 (1.2)	91.5 (18.7)	21.9 (3.6)
Sherburne	23.0 (3.2)	3.2 (0.6)	35.0 (7.7)	9.9 (1.4)	97.6 (14.4)	19.3 (1.6)
SX61	21.8 (3.3)	3.1 (0.7)	69.6 (21.5)	12.8 (2.5)	142.0 (36.4)	18.6 (2.6)
SX64	15.6 (1.9)	4.1 (0.9)	53.9 (10.2)	11.2 (1.5)	139.1 (25.2)	15.0 (1.7)
Site (n = 24)						
Prince Albert	18.5 (2.0)	8.4 (1.1)	100.7 (13.2)	11.9 (1.4)	158.1 (20.3)	16.1 (1.9)
Birch Hills	16.8 (1.4)	3.0 (0.2)	32.1 (3.7)	11.8 (1.3)	79.1 (6.1)	21.2 (1.9)
Saskatoon	13.1 (1.2)	1.6 (0.1)	10.8 (0.9)	3.6 (0.3)	25.3 (1.9)	11.3 (1.0)
Estevan	37.4 (3.7)	2.5 (0.2)	45.4 (4.0)	14.6 (1.0)	185.6 (14.5)	25.3 (2.2)

[†] The decomposing leaf litter cohorts considered were three years of pre-coppice leaf litter, two years of first year post-coppice leaf litter, and one year of nutrient release from second year post-coppice leaf litter

[‡] For each nutrient, varietal and site mean (standard error) values are reported due to significant ($P < 0.05$) variety × site effect

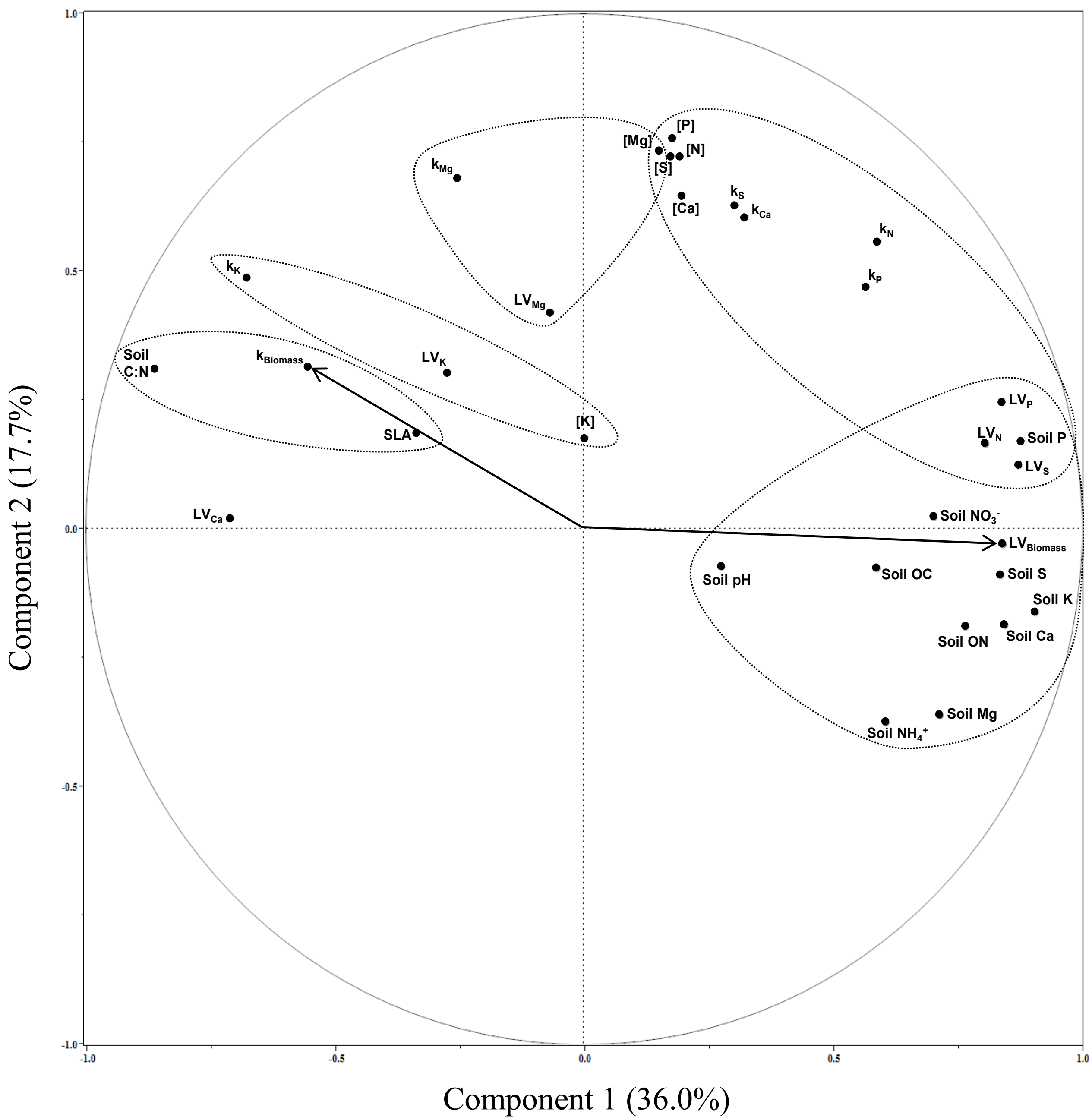

Fig. 3.4. Principle component analysis of plant tissue and soil properties associated with leaf litter decomposition and nutrient release variables of several native and exotic willow varieties, measured using leaf litter bag incubations at different plantations in Saskatchewan, Canada. Variables analyzed were: leaf litter decomposition rate constant ($k_{Biomass}$) and limit value ($LV_{Biomass}$); leaf litter nutrient release rate constant ($k_{Nutrient}$) and limit value ($LV_{Nutrient}$) for N, P, K, S, Ca, and Mg; specific leaf area (SLA); leaf litter nutrient concentration ([ ]) for N, P, K, S, Ca; and soil (Soil) pH, organic C:N, along with initial extractable levels of NH_4^+-N + NO_3^--N, P, K, S, Ca, and Mg.

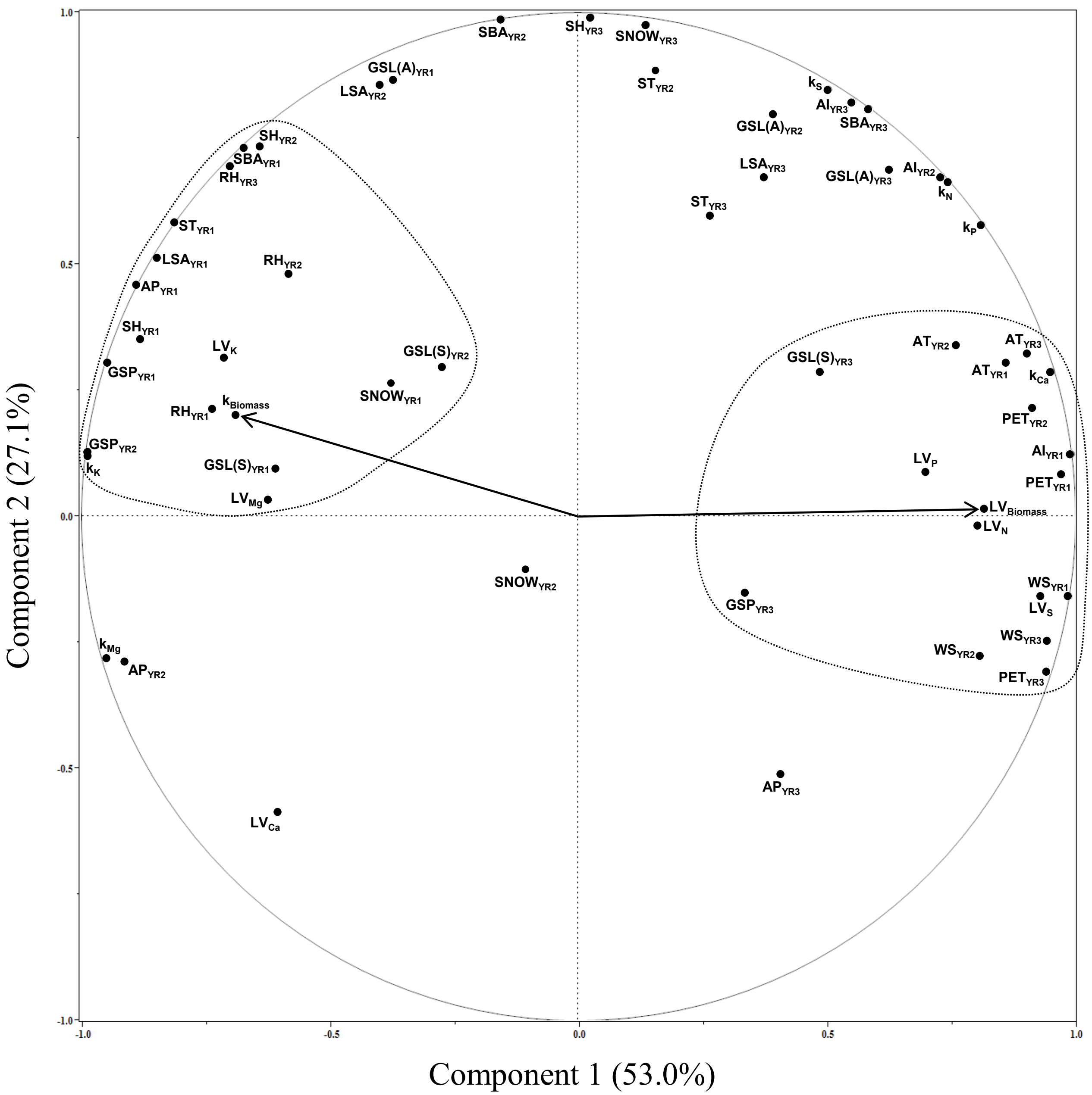

Fig. 3.5. Principle component analysis of meteorological properties and canopy variables associated with leaf litter decomposition and nutrient release variables of several native and exotic willow varieties, measured using leaf litter bag incubations at different plantations in Saskatchewan, Canada. Variables analyzed were: leaf litter decomposition rate constant ($k_{Biomass}$) and limit value ($LV_{Biomass}$); leaf litter nutrient release rate constant ($k_{Nutrient}$) and limit value ($LV_{Nutrient}$) for N, P, K, S, Ca, and Mg. Also included were the yearly (YR1, YR2, and YR3): annual (AP) and growing season (GSP) precipitation; relative humidity (RH); potential evapotranspiration (PET); aridity index (AI); mean daily air temperature (AT), wind speed (WS), soil temperature (ST; 0-10 cm); growing season length based on either mean daily air temperatures (GSL(A)) or 0-60 cm soil temperatures (GSL(S)); average stem height (SH); stem basal area (SBA); leaf surface area (LSA).

increased moisture conditions were associated with increased $k_{Nutrient}$ values of K and Mg; and, there was a stronger relationship between growing season length based on soil temperature data and $k_{Biomass}$ compared to growing season length derived from air temperature data and $k_{Biomass}$ (Fig. 3.5).

3.6 Discussion

3.6.1 Leaf litter production and nutrient content

The estimated leaf litter biomass accumulated during the initial four-year rotation and its nutrient concentrations are within the range reported in the literature (Kahle et al., 2010; Rytter, 2012) and (Šlapokas and Granhall, 1991a; 1991b), respectively. The greater variation in leaf litter biomass compared to nutrient concentration among varieties and sites indicates that varying biomass production was primarily responsible for the observed differences in leaf litter nutrient content and helps to explain the expected strong direct relationship between leaf litter biomass production and leaf litter nutrient accumulation. For example, the smallest leaf litter nutrient sink at Saskatoon was consistent with its reduced leaf production compared to the other sites. Notable exceptions to this trend were the leaf litter P content at Estevan and leaf litter Mg content at Prince Albert (Table 3.3). These observed deviations are explained by differences in foliar nutrient concentrations, due to the contrasting soil P and Mg availability at these two sites (Table 3.2). Specifically, Estevan and Prince Albert had the lowest measured soil P and Mg levels, respectively, while repeated fertilizer P applications at Prince Albert prior to plantation establishment lead to high residual soil P, resulting in apparent enhanced P uptake by the willow varieties growing at Prince Albert and resultant larger leaf litter P sink over time.

3.6.2 Leaf litter decomposition

The estimated $k_{Biomass}$ and $LV_{Biomass}$ values of the different willow variety leaf litter across the four sites were within the range reported for deciduous species (Berg and McClaugherty, 2008). Varietal differences in leaf litter decomposition as observed in this study have also been reported for *Salix* spp. elsewhere (Šlapokas and Granhall, 1991a; 1991b). The strong inverse relationship between $k_{Biomass}$ and $LV_{Biomass}$ observed for all varieties and sites is consistent with the well-established understanding of leaf litter decomposition dynamics (Berg and Ekbohm, 1991). For example, the leaf litter at Prince Albert had the largest $k_{Biomass}$ value, but the largest proportion of recalcitrant leaf litter (i.e., A value) and attendant lowest $LV_{Biomass}$ value at the end of the incubation period compared to the other sites (Table 3.4). Increased leaf litter quality (i.e., high initial nutrient concentrations, especially N and P) typically supports an initially high decomposition rate over the short-term, but leads to a larger fraction of recalcitrant biomass remaining (i.e., decreased $LV_{Biomass}$). Consequently, there is often a negative correlation between initial leaf litter N concentrations with $LV_{Biomass}$ (Berg and McClaugherty, 2008) and this helps to explain the difference in $LV_{Biomass}$ values between the native *S. eriocephala* leaf litter and exotic *Salix* varieties (Table 3.4 and Fig. 3.2).

During the early-stage of decomposition (< 1 year), the primary regulator of $k_{Biomass}$ often is litter quality (i.e., macronutrient concentration; Berg and McClaugherty, 2008), but as decomposition proceeds and biomass is lost, the relative concentration of lignin increases and typically becomes the key factor controlling subsequent decomposition (Šlapokas and Granhall, 1991a; Berg, 2000). Furthermore, the relative enrichment of low-molecular weight N compounds within decomposing leaf litter can also inhibit decomposition by not only reacting with lignin to create more recalcitrant aromatic compounds, but also repressing ligninolytic enzymes production by soil fungi (Berg, 2000). Similar leaf litter N enrichment was evident in this study after three-years. Namely, the leaf litter N content decreased less over time among the varieties and sites compared to other nutrients (Fig. 3.3c) while leaf litter N concentration among the varieties increased 58, 14, 11, and 25% at Prince Albert, Birch Hills, Saskatoon, and Estevan, respectively. The remaining nutrient concentrations decreased over time (data not shown), which is in agreement with other studies (Ingestad and Ågren, 1984; Šlapokas and Granhall, 1991a). This marked increase in $k_{Biomass}$, coupled with leaf litter N enrichment (i.e., immobilization) observed at Prince Albert, is presumably due to a greater soil microbial response to the added leaf litter C source within the sandy soil having inherently poor fertility and less SOC (Table 3.2; Šlapokas and Granhall, 1991b). The strong negative relationship between SOC level and $k_{Biomass}$ across the four sites ($k_{Biomass} = -0.3244(SOC) + 2.3969$; $R^2 = 0.95$; $P < 0.05$) appears to support this assertion. Additionally, the widest C:N ratio of the coarse-textured Prince Albert soil helps to explain the enhanced leaf litter N enrichment, as the decomposer population would immobilize more N, resulting in 26% less N released from the leaf litter after three years compared to the other sites (Table 3.6). The recalcitrant portion of annual leaf litter production is anticipated to accumulate over time, thus representing one mechanism whereby SRC willow plantations are capable of sequestering SOC. The smaller $LV_{Biomass}$ value at Prince Albert implies enhanced leaf litter accumulation compared to the other sites, which will benefit this sandy soil. Undoubtedly, this is one of the mechanisms underlying the measured increase in SOC level following the introduction of SRC willow on sandy former agricultural soil that has been reported elsewhere (Jug et al., 1999b; Kahle et al., 2010).

Simply multiplying the varietal leaf litter production throughout the rotation by its corresponding A value (Tables 3.3 and 3.4), yields an estimated average rate of SOC sequestration from leaf litter of 0.21 Mg C ha^{-1} year^{-1} (assuming a C fraction of 0.5) and is in the midst of recently reported estimates (Kahle et al., 2010; Rytter, 2012; 0.28 and 0.15 Mg C ha^{-1} year-1, respectively). Contrary to contemporary alternative bioenergy crops, which experience chronic removals of all above-ground biomass, the SRC willow plantations in this study cycled more than seven tonnes per hectare of leaf litter biomass on average prior to harvest (Table 3.3), which will play an important role not only in augmenting SOC levels, but also long-term nutrient cycling. Notwithstanding the substantial leaf litter nutrient additions to the soil surface, it is important to recognize that these nutrients are principally bound in the organic matter and consequently, are not readily available for plant uptake until mineralized.

3.6.3 Leaf litter nutrient release

The bulk release of leaf litter nutrients other than N (only approximately 1/3 released after the first year; Fig. 3.3c), concurs with previous studies (Granhall and Slapokas, 1984; Šlapokas and Granhall, 1991a). The markedly smaller k_N value at Prince Albert is probably a function of greater leaf litter N immobilization by the decomposer community of the relatively poor soil (Table 3.2). Inorganic N from either leaf litter mineralization or fertilizer N has been shown to become rapidly immobilized within the stable soil organic N pool, especially in sandy soils, representing long-term N retention within SRC willow plantations (Šlapokas, 1991b; Aronsson, 2001). Although some N, P, and S can be partially leached immediately after litter fall (Berg and McClaugherty, 2008), observed differences in k_P and k_S values are likely due to greater microbial immobilization as well, while differences in k_K, k_{Ca}, and k_{Mg} values are typically associated with leaf litter leaching of these base cations, with less dependence on microbial-mediated release (Potter, 1991; Šlapokas and Granhall, 1991b).

The observed differences in nutrient release among willow varieties and sites (Figs. 3.3a and 3.3b), along with differing CV of $k_{Nutrient}$ and $LV_{Nutrient}$ values (ranging from 9-38% depending on the nutrient; Tables 3.5 and 3.6) are attributed primarily to the effect of differing soil nutrient availability on leaf litter quality (CV ranging from 31-74% depending on the nutrient; data not shown) and the degree of nutrient immobilization during decomposition. Tissue nutrient concentration is a principal determinant of litter quality and a strong factor controlling litter decomposition rates and nutrient cycling (Weih and Nordh, 2002); although relative differences in lignin or tannin content are also important (Šlapokas and Granhall, 1991a; Schofield et al., 1998).

Knowledge of leaf litter nutrient release characteristics is useful when selecting varieties (e.g., Sherburne; Fig. 3.3a and Table 3.6) for use in environmentally sensitive areas (e.g., riparian zones) where enhanced nutrient immobilization (especially N and P) within leaf litter would be advantageous. Conversely, when the objective is to reduce fertilizer requirement for SRC willow production, using varieties (e.g., Fish Creek; Fig. 3.3a and Table 3.6) with leaf litter possessing quicker nutrient release characteristics would be beneficial, in order to satisfy a larger portion of the immediate nutritional demand naturally and more economically. Improving the synchrony between nutrient release from leaf litter and subsequent plant nutrient demand will nutrient uptake efficiency by the willow while increasing nutrient retention in the system (Myers et al., 1994). It is important to note that less than half of the nutrients immobilized within leaf litter were released during the four-year rotation (Tables 3.3 and 3.7). Consequently, the majority of leaf litter nutrients will be available for willow uptake during the second rotation and such capacity of leaf litter nutrient cycling to support the nutritional requirements of subsequent rotations has been reported elsewhere (Ingestad and Ågren, 1984; Christersson, 1986; Ericsson, 1994a).

3.6.4 Principal component analyses

Principle component analysis of soil and plant tissue properties associated with willow leaf litter decomposition and nutrient release variables (Fig. 3.4) visually supports the aforementioned inter-relationships, namely: i) the direct relationship between $LV_{Biomass}$ and soil nutrient availability (along with an indirect relationship with $k_{Biomass}$), with less influence of litter quality; ii) $k_{Nutrient}$ values were primarily controlled by litter quality, with relatively little influence of soil nutrient availability; iii) $k_{Nutrient}$ is directly related to $LV_{Nutrient}$, with the exception of Ca; iv) $LV_{Nutrient}$ values for N, P, and S are chiefly controlled by the availability of these soil nutrients, while $LV_{Nutrient}$ values for K and Mg was mainly dependant on initial litter contents; and, v) the direct relationship between SLA and $k_{Biomass}$. Strong positive correlations between SLA and $k_{Biomass}$ have been acknowledged previously, with smaller SLA values indicative of increased leaf thickness and density, which is associated with a physically tougher foliar structure and increased concentration of recalcitrant chemical constituents, such as lignin (Huang et al., 2007). In this study, the native willow variety SLA was about half (89.6 cm^2 g^{-1}; SE 2.9) the average value for the six exotic willow varieties (134.8 cm^2 g^{-1}; SE 1.0), which helps to explain the measured differences in leaf litter dynamics observed in mass loss and nutrient release characteristics. Although strong relationships among these variables were identifiable with PCA, only 53.7% of the variability was accounted for in the two principal axes (Fig. 3.4), thus, indicating the need to include additional factors affecting leaf litter decomposition, particularly climatic variables.

Moore et al. (1999) examined the three-year decomposition dynamics of 11 litter types across 18 sites throughout Canada and found annual precipitation to be strongly related to litter mass remaining. Likewise in our study, the relationship between climate and leaf litter decomposition was explicit, with 80.1% of the variability explained in the two principal axes (Fig. 3.5). Specifically, the strong direct relationship between first-year precipitation (growing season and annual rainfall and snowfall) and relative humidity with leaf litter mass loss and nutrient (e.g., base cations) release, contrasted with the indirect relationship between climate variables attendant with less moisture availability at the soil surface (e.g., annual average air temperature, aridity index, wind speed, and potential evapotranspiration), indicates the prominent role climate (i.e., moisture availability) plays in controlling willow leaf litter decomposition and nutrient release dynamics in semi-arid Saskatchewan. Additionally, the closer relationship between first year climate conditions and $k_{Biomass}$ and $k_{Nutrient}$ agrees with the majority loss of leaf litter mass and nutrients during the first year (Fig. 3.3). Trofymow et al. (2002) suggests that the first-year loss of soluble compounds (e.g., carbohydrates, phenolics, and tannins) largely control leaf litter mass loss during the first year and might be related to accumulated winter precipitation following leaf fall and proportional leaf litter leaching during snow melt in the subsequent spring. Their proposed role of subsequent snowfall following litterfall was corroborated in this study with the strong relationship between first-year snowfall and $k_{Biomass}$ and $k_{Nutrient}$ (Fig. 3.5). Although the effect of climate on leaf litter dynamics is often referred to, the degree of canopy cover can also regulate the understory microclimate (Berg and McClaugherty, 2008), which was manifested in the strong relationship between the first-year surrogate measures of microclimate (e.g., leaf surface area, average stem height, and total basal area) and $k_{Biomass}$ (Fig. 3.5).

Leaf litter lignin concentration data may have improved the PCA results, given its consistent control on decomposition across regional scales (Moore et al., 1999; Trofymow et al., 2002). Additionally, considering the importance of soil flora and fauna populations within SRC willow plantations (Püttsepp et al., 2004; Baum et al., 2009), including soil biota community structure and activity data among varieties and sites may have also enhanced the PCA results, given their vital relationship with leaf litter dynamics (Prescott, 2005). Although soil biota were not assessed in this study, their importance may be indicated indirectly by the closer relationship between calculated growing season length based on soil temperature (instead of air temperature) and $k_{Biomass}$ (Fig. 3.5). Fluctuating air temperature would have less influence on soil biota, due to the ability of soil to buffer large diurnal changes in air temperature throughout the year. For example, the CV of measured air, soil (0-10 cm), and soil (0-60 cm) temperature across the four sites throughout the three-year incubation ranged from 428-1755, 105-144, and 108-129%, respectively. Consequently, soil temperature appears to be a reliable variable for modelling willow leaf litter dynamics within temperate climates like Saskatchewan, presumably due to its close association with soil biota abundance and activity.

3.6.5 Leaf litter nutrient cycling, long-term soil nutrient availability, and SRC willow plantation sustainability

The estimates of leaf litter nutrient cycling in this study are at the low end of available literature values (Table 3.6; e.g., Ericsson, 1994b). These results are a function of not only the relatively low leaf production at the plantations (Table 3.3), but also these study values are estimates of actual nutrient additions to the plant available soil nutrient pools, due to mineralization during the four-year rotation, as opposed to leaf litter nutrients presumed to be entirely released eventually. Under Saskatchewan conditions, however, assuming complete nutrient release would result in an overestimate of leaf litter nutrient release from 5-44% depending on willow variety, nutrient, and site (Table 3.6). Also, a considerable portion of nutrients bound in the accumulated leaf litter during the four-year rotation will not be released until the second rotation and perhaps beyond; therefore, these nutrients were not included in these estimates (Tables 3.3 and 3.7). Regardless, these findings support the contention that decomposing leaf litter is an important nutrient cycling mechanism helping to satisfy the long-term nutritional demands of SRC willow plantations (Ingestad and Ågren, 1984; Ericsson, 1994b). The significant ($P < 0.05$) variety × site interaction effect on leaf litter nutrient additions was influenced more by the differences in accumulated leaf litter biomass during the rotation, across the exotic varieties and sites, instead of variation in their leaf litter nutrient concentrations (CVs of 38 and 22%, respectively; data not shown). For example, the Saskatoon soil received 65% less nutrient contributions from leaf litter compared to the other sites (Table 3.7) and is primarily a function of differences in biomass allocation (i.e., root growth favoured over leaf production) under the drier growing season conditions at Saskatoon observed throughout the rotation.

3.7 Conclusion

Litterfall decomposition is a primary mechanism for C and nutrient cycling within most terrestrial ecosystems and SRC willow plantations are certainly no exception. The estimated leaf litter decomposition and nutrient release variables presented herein are the first reported values for *Salix* spp. Modelling efforts aimed at estimating the climate change mitigation potential and long-term sustainability of SRC willow plantations are highly dependent on reliable input parameters; in particular, leaf litter decomposition data for predicting the magnitude of C sequestration and nutrient release to forecast the potential need of supplemental nutrient amendments. Contrary to contemporary alternative bioenergy crops (e.g., giant reed grass, Miscanthus, switchgrass, etc.), which experience chronic removals of all above-ground biomass, the SRC willow plantations in this study cycled more than seven tonnes of leaf litter biomass during the initial four-year rotation. This accumulated leaf litter will play an important role not only in augmenting SOC levels, but also in long-term nutrient cycling, especially in a sandy soil (e.g., Prince Albert). Less than half of the nutrients immobilized within leaf litter were released during the rotation, with the remainder available for willow uptake during the second rotation. Knowledge of leaf litter nutrient release characteristics is useful for selecting appropriate varieties (e.g., Sherburne) for use in environmentally sensitive areas where enhanced nutrient immobilization within leaf litter would be advantageous for minimizing the risk of contaminating adjacent water bodies. Conversely, selecting varieties (e.g., Fish Creek) having quicker leaf litter nutrient release characteristics for use in SRC willow production would help to satisfy a larger portion of nutritional demand naturally and more economically. Principle component analysis identified numerous key relationships between the measured soil, plant tissue, climate and microclimate variables and observed willow leaf litter decomposition and nutrient release characteristics, namely: i) $LV_{Biomass}$ was influenced more by soil nutrient availability than litter quality; ii) $LV_{Biomass}$ was indirectly related to $k_{Biomass}$; iii) $k_{Nutrient}$ was primarily controlled by litter quality, with relatively little influence of soil nutrient availability; iv) $k_{Nutrient}$ is directly related to $LV_{Nutrient}$ v) $LV_{N,\ P,\ and\ S}$ are chiefly controlled by soil nutrient availability, while $LV_{K\ and\ Mg}$ were mainly dependant on litter quality; vi) SLA strongly influenced $k_{Biomass}$; vii) first-year precipitation (total and growing season rainfall and snowfall) played a prominent role in controlling willow leaf litter decomposition and nutrient release dynamics in semi-arid Saskatchewan; and, viii) surface soil (0-10 cm) temperature measurements is a reliable variable for modelling willow leaf litter dynamics within temperate climates like Saskatchewan, presumably due to its close association with soil biota abundance and activity. Further research is needed to quantify the relative importance of leaf litter nutrient cycling within the context of N, P, K, S, Ca, and Mg biogeochemical cycling within SRC willow plantations, to provide insight into the long-term sustainability and productivity of these woody biomass energy production systems grown on a variety of soil types in Saskatchewan over multiple rotations.

4. First Rotation Biomass Production and Nutrient Cycling Within Short-Rotation Coppice Willow Plantations

4.1 Preface

Under conditions of adequate soil moisture, short-rotation coppice (SRC) willow productivity is considered to be principally controlled by soil nutrient availability. Willow has a rapid growth rate and continued nutrient export off-site over seven rotations may substantially deplete soil fertility and reduce plantation productivity. Therefore, in order to support sustainable levels of adequate willow growth throughout a 22-yr plantation lifespan, it is important to thoroughly examine the soil nutrient budgets of essential plant nutrients during the establishment phase to accurately forecast the long-term soil productivity of this production system and the potential need of supplemental fertilization (Chapter 5). Nutritional amendments, which promote the sustainability of these purpose-grown woody plantations, will support SRC willow as a viable bioenergy alternative in Saskatchewan (Chapter 6). This is the first study to carry out a comprehensive examination of all nutrient vectors (i.e., input, output, and transfers) within different sites across a large pedoclimatic gradient. Insight into the nutrient contributions of leaf litter decomposition (Chapter 3), along with other inputs, outputs, and transfers examined in this chapter, are used to build nutrient budgets. In addition to N, P, K, Ca, and Mg, this study also examines S, an element sometimes deficient for food and fibre crops in western Canada, but which has received minimal consideration in the SRC willow literature. This chapter was submitted to BioEnergy Research for publication and is currently being reviewed. The co-author contributions to this manuscript were greatly appreciated and consisted of: J.J. Schoenau (provided financial assistance, methodological guidance, soil and tissue analyses, and manuscript editing); K.C.J. Van Rees (provided financial assistance, methodological guidance, meteorological and soils data, and manuscript editing); N. Bélanger (provided methodological guidance and manuscript editing); T. Volk (provided the willow planting material and manuscript editing); and, T. Jensen (provided financial assistance and manuscript editing).

4.2 Abstract

Although numerous studies have quantified different social, economic, energetic, and environmental benefits associated with short-rotation coppice (SRC) willow plantations, comprehensive assessments of nutrient cycling are lacking. The objective of this study was to examine the biomass production and associated biogeochemical cycling of nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg) during an initial four-year rotation of six willow varieties grown at four locations along a 500 km north-south geoclimatic gradient within Saskatchewan. Nutrient budgets consisted of quantifying various nutrient inputs (e.g., atmospheric deposition and soil mineral weathering), outputs (e.g., fine and coarse root biomass, leaf biomass, harvested biomass, leaching, and denitrification), and transfers (e.g., soil organic matter mineralization, canopy exchange, leaf litter decomposition, and fine root turnover) associated with the plant available soil nutrient pool. Total production during the rotation averaged 19.0, 7.1, and 12.5 Mg ha^{-1} for stem, leaf, and below-ground (primarily fine roots) biomass, respectively, with corresponding calculated soil nutrient budget deficits of 17, 39, 112, 271, and 74 kg ha^{-1} of N, P, K, Ca, and Mg, respectively, averaged across the four sites, but a soil S surplus of 60 kg ha^{-1}. While nutrient budget deficits varied among sites, there were no significant differences ($P > 0.05$) among willow varieties. Despite the relatively low nutrient-demanding nature of willow and negligible leaching or denitrification losses, nutrient export in harvested biomass over multiple rotations will require soil nutrient amendments to maintain SRC willow productivity, particularly N and P, albeit a fraction of the amount required for annual agronomic crops.

4.3 Introduction

Within western Canada, there are roughly 15 Mha of land suitable for establishing short-rotation woody crops (Joss et al., 2008), 5.4 Mha of which are in close proximity (i.e., within 25 km) to Canada's existing network of forestry mills and represent a potential biomass feedstock supply of almost 1.5 Bm^3 over a 20-year period (assuming 13.6 m^3 ha^{-1} yr^{-1} growth rate; Brent Joss, personal communication, Canadian Forest Service). The global potential area of degraded and abandoned lands available for establishing second-generation biomass energy crops is substantial, with approximately 147 Mha within North America alone (Hoogwijk et al., 2005; Lemus and Lal, 2005). Saskatchewan is certainly no exception, with more than 2 Mha of available degraded land, which could support long-term SRC willow production, with a C sequestration potential capable of offsetting up to 80% of the annual anthropogenic GHG emissions in Saskatchewan (Amichev et al., 2012). Along with this marginal land, there are an estimated 4 Mha of salt-affected abandoned land across the Canadian prairies (approximately 1.6 Mha in Saskatchewan alone), which is unsuitable for arable crop production, but could support SRC production of salt-tolerant willow varieties (Appendix D).

Sensitivity analyses are a common component of a life cycle analysis and often identify biomass productivity as a primary controller of both economic viability (Buchholz and Volk, 2011; McKenney et al., 2011; Faasch and Patenaude, 2012) along with net energetic (McKendry, 2002; Scholz and Ellerbrock, 2002; Hinchee et al., 2010) and net GHG emission sustainability within SRC willow plantations (Börjesson, 1996; Scholz and Ellerbrock, 2002; Styles and Jones, 2008a). Under conditions of adequate soil moisture, willow productivity will be primarily controlled by soil nutrient availability. A fundamental question concerning sustainable SRC willow yields, therefore, is whether long-term soil productivity is maintained within a multi-rotation production system, given the rapid growth rate and nutrient exports offsite when harvesting the willow biomass after repeated short rotations. Fertilization traditionally has been used as a management tool to support the establishment and growth of willow plantations, however, its efficacy has been inconsistent (Chapter 5), which is disconcerting given that it constitutes a large portion of SRC willow production costs (Heinsoo and Holm, 2010; McKenney et al., 2011). Furthermore, after examining all published SRC willow life cycle analyses, Djomo et al. (2011) reported that minimizing fertilizer additions was key to optimizing the net energy ratio (i.e., energy output:fossil energy input) and reducing net GHG emissions of willow biomass energy production. Additionally, superfluous fertilizer application has been linked to decreased plantation productivity either directly due to fertilizer toxicity (Mortensen et al., 1998) or indirectly by stimulating weed species growth (Weih and Nordh, 2005; Balasus et al., 2012) or inducing soil nutrient imbalances (Nilsson and Ericsson, 1986; Kopp et al., 1996). Non-point source pollution associated with excessive and non-timely fertilizer application has also been reported (Aronsson and Bergström, 2001; Dimitriou et al., 2012a; Schmidt-Walter and Lamersdorf, 2012). A clear understanding of soil nutrient dynamics, particularly the soil nutrient budgets of essential plant nutrients during the establishment phase, is required to accurately forecast the sustainability of the production system and the necessity of nutritional amendments.

Influential factors affecting long-term site productivity include the inherent soil fertility at a given site (Mitchell, 1995; Quaye et al., 2011), genotypic variability in nutrient requirements, uptake capacity, and/or utilization efficiency (Adegbidi et al., 2001; Weih and Nordh, 2005), and genotype × environment interactions (Hofmann-Schielle et al., 1999; Ballard et al., 2000). In order to account for these effects when developing reliable nutrient budgets for SRC willow production in Saskatchewan, an experiment consisting of several commercial willow varieties was replicated at different sites across a 500 km north-south gradient, which covered a range of soil type and climatic conditions. The objective of this study was to quantify the biogeochemical cycling of nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), and magnesium (Mg), within SRC willow plantations during the initial four-year rotation, to provide insight into the long-term sustainability and productivity of these woody biomass energy production systems grown on a variety of soil types in Saskatchewan over multiple rotations. A prerequisite for providing a reasonable estimate of long-term sustainability across a variety of soil types is an accurate accounting of above- and below-ground nutrient pools, including: vectors of nutrient flux into (e.g., soil mineral weathering and atmospheric deposition), out of (e.g., fine and coarse root biomass, leaf biomass, harvested biomass, leaching, and denitrification), and transfers between (e.g., soil organic matter mineralization, litter decomposition, and fine root turnover) the plant available nutrient pool. Although some excellent nutrient budget work has been done within SRC willow plantations (Ericsson, 1984; Hytönen, 1996; Alriksson, 1997), to my knowledge, this is the first study to carry out a comprehensive examination of all nutrient vectors (i.e., input, output, and transfers), within different sites across a large geoclimatic gradient involving both fertile and marginal agricultural soil used to grow the same willow varieties established at the same time. The study also examines sulfur, an element sometimes deficient for annual grain crops in western Canada, but which has received little attention in SRC willow production systems. It was hypothesized that inputs and outputs of nutrients and the degree of depletion or accumulation in the various soil and plant pools would vary according to specific nutrient, willow variety, and from site to site as related to the soil and environmental conditions and stand productivity. A tremendous opportunity exists to develop SRC willow as a bioenergy feedstock in Saskatchewan, especially if they can be sustainably grown on millions of hectares of marginal land that is deemed unsuitable for annual crop production.

4.4 Materials and Methods

4.4.1 Study sites, experimental design, willow varieties, and site maintenance

The data for this study were collected from four SRC willow variety trial plantations located along a 500 km north-south geoclimatic gradient within Saskatchewan, Canada, from the south-east corner of the province to the southern boundary of the boreal forest in the central area of the province, which were selected to represent the diverse soil types and climatic conditions existing in the province (Fig. 3.1 and Tables 4.1 and 4.2). At each of the four sites, a single pedon was excavated and a full soil taxonomic assignment given to classify the soils according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). In the spring of 2007, six willow varieties, developed by the State University of New York College of Environmental Science and Forestry (SUNY-ESF) breeding program, were planted at each location in a randomized complete block design (n = 4) adapted from the protocols of Abrahamson et al. (2002). The six willow varieties used were: Allegany *(Salix purpurea)*, Canastota *(Salix sachalinensis × miyabeana),* Fish Creek *(Salix purpurea),* Sherburne *(Salix sachalinensis × miyabeana),* SX61 (*Salix sachalinensis*), and SX64 (*Salix miyabeana*). Each varietal plot (6.3 × 7.8 m) consisted of 78 plants (three double-rows of 13 plants row^{-1}), with spacings of 1.5 m between the double-rows, 60 cm between rows within the double-row, and 60 cm between plants within the double-row; resulting in a planting density of approximately 15,873 plants ha^{-1}. In the spring of 2008, the willow plants were coppiced and grown for an additional three years before harvesting. In order to prevent edge effects, the central 18 plants constituted the measurement plot and were used for sample collection and biomass measurements (Fig. 4.1). Pre- and post-planting site preparation to control non-crop vegetation included both mechanical (deep tillage, light cultivation, tandem disc, mowing, and hand weeding) and chemical (Goal™ 2XL, 2 L ha^{-1}; Roundup WeatherMax®, 2 L ha^{-1}; Simazine 480, 4.7 L ha^{-1}; Pardner®, 0.5 L ha^{-1}) treatments.

4.4.2 Baseline plant available soil nutrient pools and nutrient supplying power

After planting the willow varieties at each site, three 60 cm soil cores were collected within each varietal plot using a JMC backsaver probe (Model PN001; Clements Assoc. Inc., Newton, IA, USA), separated into 10 cm depth increments, and composited. A 60 cm depth is believed to represent the effective rooting depth (i.e., contain the vast majority of roots) for most

Table 4.1. Selected site characteristics of different willow variety trial sites in Saskatchewan, Canada.

Site	UTM Co-ordinates	Prior crop	ACC[†]	MAP[‡] (mm)	MGSP[§] (mm)	MAT[¶] (°C)	MGST[#] (°C)	FFD[††] (#)	Water[‡‡] (m)
Prince Albert	13U 448501 5912029	fallow	5-6	450	295	1.2	14.2	85	1.5
Birch Hills	13U 467122 5872616	canola	1-2	420	277	1.3	14.3	90	1.2
Saskatoon	13U 389970 5776342	fallow	2-3	375	312	2.6	14.9	112	3.3
Estevan	13U 655043 5438201	fallow	3-4	430	341	3.3	15.4	124	4.3

[†] Agriculture capability classification (Class 1: no significant limitations; Class 2: moderate limitations; Class 3: moderately severe limitations; Class 4: severe limitations; Class 5: very severe limitations; Class 6: limited capability for arable agriculture)
[‡] Mean annual precipitation (snow + rainfall) during the rotation (SCSR 1976; 1978; 1989; and 1997, respectively)
[§] Mean growing season precipitation during the rotation; growing season length determined using 5 °C soil baseline
[¶] Mean annual air temperature during the rotation
[#] Mean growing season air temperature during the rotation; growing season length determined using 5 °C soil baseline
[††] Frost-free days (SCSR 1976; 1978; 1989; and 1997, respectively)
[‡‡] Average depth to groundwater; measured throughout the final growing season using an observation well installed at each site

Table 4.2. Mean (n = 24) selected soil characteristics of different willow variety trial sites in Saskatchewan, Canada[†].

Site	Soil type[‡]	Texture (% sand/clay)	BD (kg m^{-3})	pH	$EC_{1:2}$[§] (dS m^{-1})	Organic C (%)	Organic C:N	MGSST[¶] (^{o}C)	MGSSM[#] (% v v^{-1})
Prince Albert	OBC	sand to loamy-sand (91/2)	1588	6.6c[††]	0.16c	1.4c	15.5a	13.2	32
Birch Hills	OBC	silt-loam to clay-loam (29/28)	1002	7.0b	0.68a	3.2a	11.4b	13.2	54
Saskatoon	OV	clay (13/70)	1422	7.1b	0.45b	2.3b	9.5c	13.3	48
Estevan	CHR	silt-loam (33/23)	1238	8.0a	0.60ab	2.0b	12.7b	14.9	35

[†] 0-60 cm; average values of six 10 cm segments collected using a backsaver probe, except for extractable nutrient levels that are summed values of all segments

[‡] OBC (Orthic Black Chernozem), OV (Orthic Vertisol), and CHR (Cumulic Humic Regosol); taxonomy based on the Canadian System of Soil Classification (Soil Classification Working Group, 1998)

[§] Electrical conductivity of a 1:2 soil (soil:water; on a weight basis)

[¶] Mean growing season soil temperature (0-60 cm) during the rotation; growing season length determined using 5 ^{o}C soil baseline

[#] Mean growing season soil moisture (0-60 cm) during the rotation; growing season length determined using 5 ^{o}C soil baseline

[††] Means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

Table 4.2. continued.

Site	MAST‡‡ (°C)	MASM§§ (% v v^{-1})	Extractable nutrients N (kg ha^{-1})	P	K	S	Ca	Mg
Prince Albert	7.1	30	55c	148a	715c	92b	14381b	1297d
Birch Hills	6.2	44	68b	16c	1297b	809a	20464a	4336b
Saskatoon	6.2	42	99a	64b	1963a	663a	19905a	9644a
Estevan	7.7	30	99a	36bc	1348b	764a	19404a	3134c

‡‡ Mean annual soil temperature (0-60 cm) during the rotation

§§ Mean annual soil moisture (0-60 cm) during the rotation; growing season length determined using 5 °C soil baseline

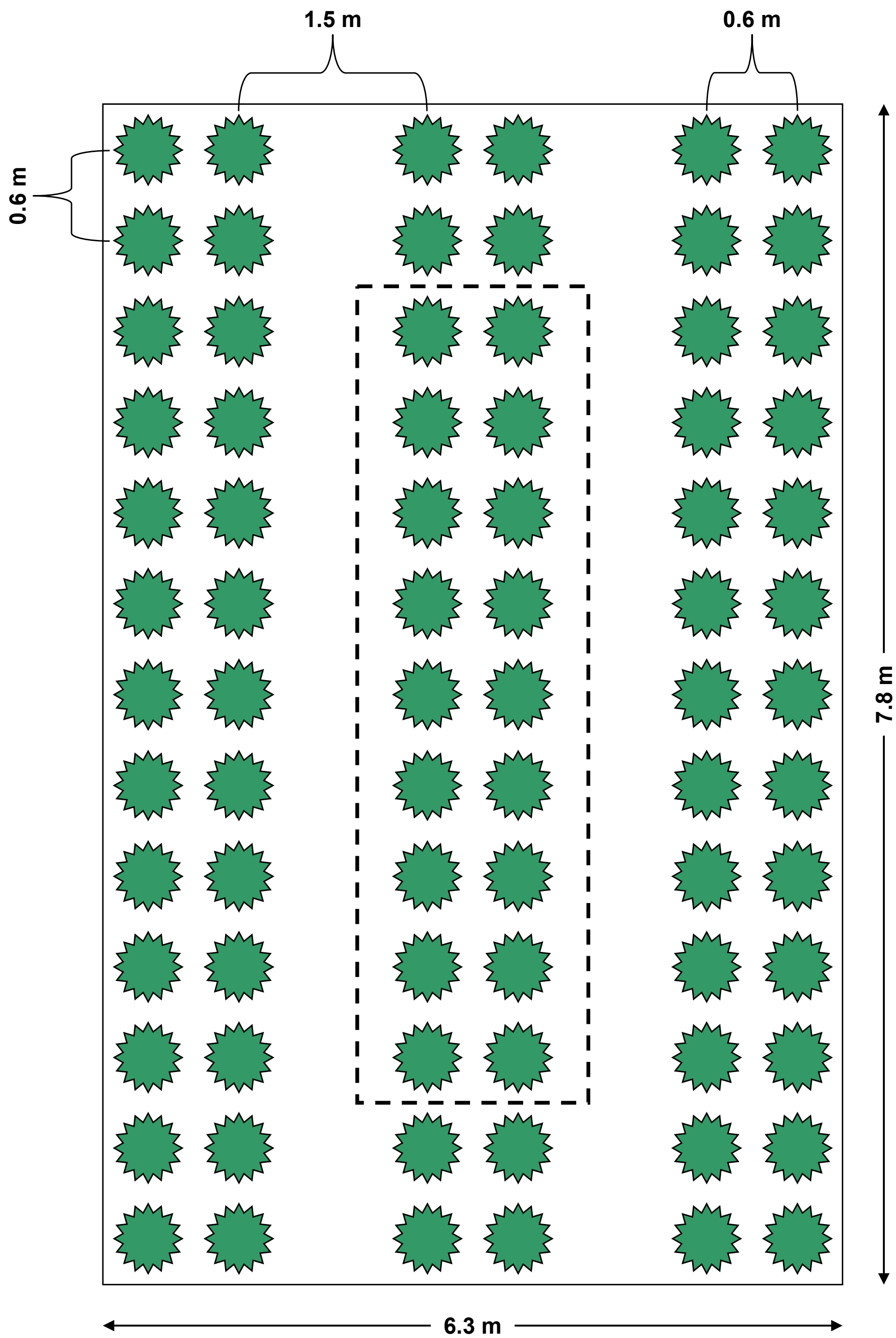

Fig. 4.1. Plot layout of short-rotation coppice willow variety trial study sites in Saskatchewan, Canada. Note: the dashed line indicates the measurement plot for sample collection and biomass measurements.

willow varieties (Mirck and Volk, 2010b; Schmidt-Walter and Lamersdorf, 2012). Additionally, bulk density cores (100 cm^3) were collected at each depth and these values were used to convert extractable soil nutrient concentrations to kg ha^{-1}. All soil samples were air-dried to a constant weight, ground with a rolling pin to break aggregates, mixed, sieved (< 2 mm fraction retained), and analyzed for extractable nutrient levels (N, P, K, S, Ca, and Mg), total and organic N, total P, organic and inorganic C, pH, and EC. Total inorganic N (NH_4^+-N and NO_3^--N) and inorganic P were determined using 2.0*M* KCl (Maynard et al., 2008) and modified Kelowna (Qian et al., 1994) extractions, respectively, with the extracts analyzed colorimetrically (Technicon AutoAnalyzer; Technicon Industrial Systems, Tarrytown, NY, USA). Extractable S was determined using 0.01*M* $CaCl_2$ (Hu et al., 2005) and analyzed using microwave plasma-atomic emission spectrometry (4100 MP-AES; Agilent technologies, Melbourne, Australia). Extractable K, Ca, and Mg were determined using 1.0*M* NH_4OAc (Hendershot et al., 2008) and analyzed using either atomic emission (K) or absorption (Ca and Mg) spectroscopy (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Total N and P were determined using a H_2SO_4/H_2O_2 digest (Thomas et al., 1967) and analyzed colourimetrically as well. Organic N was calculated from the difference between total N and inorganic N. Total soil carbon (C) was measured using a LECO C632 Carbon Analyzer (LECO Corporation, St. Joseph, MI, USA). Soil organic C (SOC) was likewise measured (Wang and Anderson, 1998), but following a 6% H_2SO_3 pre-treatment to remove the inorganic C (Skjemstad and Baldock, 2008). Soil pH and EC (soil:water on a weight basis; Hendershot et al., 2008) were analyzed using a Beckman 50 pH Meter (Beckman Coulter, Fullerton, CA, USA) and an Accumet AP85 pH/EC meter (Accumet, Hudson, MA, USA), respectively. Particle size distribution was determined using a Horiba LA-950 Particle Size Distribution Analyzer (Horiba Instruments Inc., Irving, CA, USA) after pre-treatment with bleach (sodium hypochlorite) to remove organic matter, followed by a 10% solution of sodium hexametaphosphate to breakdown clay aggregates.

Plant Root Simulator (PRS)™-probes (Western Ag Innovations Inc., Saskatoon, SK, Canada) were used to measure nutrient supply rates *in situ* and have been successfully used in SRC willow plantations previously (Quaye, 2011; Moukoumi et al., 2012). At each of the four sites, one pair (i.e., one cation-exchange and one anion-exchange) of PRS™-probes were placed within each varietal plot, for a total of 48 PRS™-probes per burial period at each site (6 varieties

× 4 reps × 2 PRS™-probes). The PRS™-probes were inserted vertically into the Ap horizon; thereby having the ion-exchange membrane effectively measure soil nutrient supply rates in the zone having the largest concentration of willow roots (Rytter, 1999; Labrecque and Teodorescu, 2001). The PRS™-probes were installed shortly after plantation establishment, left in the soil for four weeks, and then replaced with fresh PRS™-probes twice during the growing season for a total of 12 weeks. Replacing fresh PRS™-probes in the same soil slot provides an accurate *in situ* measure of nutrient availability, yielding a reliable index of soil nutrient supplying power over time (Qian and Schoenau, 2002). The analysis and regeneration of the PRS™-probes followed the protocol of Hangs et al. (2004). Briefly, after removal, the PRS™-probes were washed free of residual soil using deionized water and then eluted with 0.5 mol dm^{-3} HCl, with the eluate analyzed for NH_4^+-N and NO_3^--N colorimetrically and P, K, S, Ca, and Mg measured using ICP (IRIS Intrepid II XSP, Thermo Fisher Scientific Inc. Waltham, MA, USA). Prior to reuse, the PRS™-probes were regenerated by being shaken three times in 0.5 mol dm^{-3} $NaHCO_3$ for 4 h. The anion-exchange PRS™-probes were further shaken in 0.01 mol dm^{-3} EDTA for 4 h.

4.4.3 Nutrient inputs through mineral weathering and atmospheric deposition

Phosphorus, K, Ca, Mg are the macroelements for which mineral weathering is expected to make a substantial contribution to plant available pools over the life of SRC willow plantation, and these inputs were estimated at every site using the elemental depletion method (Melkerud et al., 2003). The technique is based on the increased enrichment of immobile and recalcitrant $ZrSiO_4$ since the last glaciation and is an accurate surrogate for the weathering losses of other nutrient-bearing minerals (i.e., inputs into the plant available soil nutrient pool). At every site, a soil sample was collected from each soil horizon within the excavated classification pedon and prepared as previously stated and then additionally ground to a fine powder using a rotating ball-bearing mill. Sub-samples of the pulverized samples were sent to the Department of Earth Sciences' X-ray Laboratory at the University of Ottawa for X-ray fluorescence spectroscopic analysis (Philips PW2400; PANalytical, Almelo, The Netherlands). The measured concentrations of P, K, Ca, Mg, and Zr within each weathered horizon, along with their associated concentrations within the unweathered parent material, were used to calculate the cumulative input of each nutrient element (X_{Input}; kg ha^{-1}) into the plant available soil nutrient pool using Eq. 4.1, a simplified equation adapted from Melkerud et al. (2003):

$$X_{Input} = \left[\left(\frac{Zr_{WH}}{Zr_{PM}} \times X_{PM}\right) - X_{WH}\right] \times T_{WH} \times \rho b_{WH} \times 100 \qquad \text{(Eq 4.1)}$$

where Zr_{WH} and Zr_{PM} are the percentage of Zr in the weathered horizon and parent material, respectively; X_{PM} is the percentage of element X in the parent material; X_{WH} is the percentage of element X in the weathered horizon; T_{WH} is the weathered horizon thickness (m); ρb_{WH} is the bulk density of the weathered horizon (kg m^{-3}), and 100 is a unit conversion factor. The historic annual nutrient supply rate from mineral weathering was estimated by dividing the calculated cumulative input by the soil age (i.e., years since deglaciation; Christiansen, 1979), although this is not necessarily either the current or future weathering rates.

The contribution of total atmospheric deposition to plant available soil nutrient pools through either precipitation-borne nutrients (i.e., wet deposition) or air-borne nutrients (i.e., dry deposition; DD) was estimated by measuring bulk deposition (BD) and throughfall (TF) water nutrient contents at each site during the three post-coppiced growing seasons (May to October) until harvest. Bulk deposition water samples were collected using open-ended 2 L polypropylene containers initially painted black and then white (capped with countersinked 100 cm^2 polypropylene funnels) and placed at the four corners of each replicated block, but adequately separated from the willow to avoid canopy interference. A polyethylene screen (2 mm mesh) was placed over the funnel to prevent contamination from coarse debris. With the countersunk design of the collectors, it was assumed that the BD samples strictly represented wet deposition, although this may be valid only for N, P, and S (Staelens et al., 2008). Identical containers were placed within the four SX64 variety replicate plots to collect TF water samples under the willow canopy. The variety SX64 was chosen due to its proven reliability within North American SRC plantations (Labrecque and Teodorescu, 2005; Tharakan et al., 2005a). Collecting TF water samples under each willow variety canopy for comparison was cost-prohibitive and, therefore, it was assumed that SX64 was representative of all varieties. The volume of collected BD and TF samples was measured monthly, sub-sampled and refrigerated during transport back to the lab, filtered (0.05 μm; Millipore Filter Corporation Bedford, MA, USA), and then frozen until analyzed. The water samples were analyzed for their NH_4^+-N, NO_3^--N, P, K, S, Ca, and Mg concentrations in the same manner as the soil extraction samples, with the Na concentration measured using atomic emission spectroscopy (Varian Spectra 220 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA).

Growing season average compositions of precipitation and throughfall were calculated based on volume-weighted mean concentrations. Neither bulk deposition nor throughfall samples were collected during the establishment year. The contribution of BD during the establishment year was estimated using the volume of rainfall received and the average concentration of BD samples collected during the three post-coppice years. Considering the relatively small willow leaf surface area during the establishment year, any canopy exchange contributions to the nutrient budget (measured via throughfall) were assumed to be negligible. Given the circuitous nature of TF, the resultant water chemistry is complex and includes DD nutrients washed off foliage and branches. Additionally, TF samples are altered by canopy exchange (CE) processes, which can be either a nutrient source (i.e., foliar leaching) or sink (i.e., foliar absorption) as precipitation passes through the willow canopy. The relative effects of DD and CE on the net throughfall (NTF; $NTF_{Nutrient} = TF_{Nutrient} - BD_{Nutrient}$) contribution of NH_4^+, NO_3^-, P, K, S, Ca, and Mg to the plant available nutrient pool at each site, was estimated indirectly from collected BD and TF samples using the canopy exchange model (Ulrich, 1983). This model has been widely used to successfully estimate DD and CE fluxes within a variety of canopies, based on the inert tracer Na^+ DD correction factor (Staelens et al., 2008). Specifically, the DD contribution for a given nutrient ($DD_{Nutrient}$; kg ha^{-1}) is calculated using Eq. 4.2:

$$DD_{Nutrient} = \left(\frac{TF_{Na^+} + SF_{Na^+} - BD_{Na^+}}{BD_{Na^+}}\right) \times BD_{Nutrient} \qquad \text{(Eq 4.2)}$$

where TF_{Na+} and SF_{Na+} are the TF and stemflow sample Na^+ content (kg ha^{-1}), respectively; BD_{Na+} is the BD sample Na^+ content (kg ha^{-1}); and $BD_{Nutrient}$ is the BD sample nutrient content (kg ha^{-1}). Considering the technical difficulty associated with quantifying the cumulative stemflow nutrient flux of numerous small-diameter willow stems, along with stemflow accounting for only 2% of gross precipitation within SRC willow plantations (Martin and Stephens, 2006a), the contribution of stemflow was assumed to be negligible (i.e., 0) when calculating $DD_{Nutrient}$ values using Equation 4.2. For each nutrient except N, the relative contribution of CE ($CE_{Nutrient}$) was calculated by subtracting $DD_{Nutrient}$ from $NTF_{Nutrient}$ ($CE_{Nutrient} = NTF_{Nutrient} - DD_{Nutrient}$; Bélanger et al., 2002). A positive $CE_{Nutrient}$ value indicates the willow canopy is contributing to $NTF_{Nutrient}$ flux through foliar leaching, while a negative $CE_{Nutrient}$ value points to a reduced $NTF_{Nutrient}$ flux due to foliar absorption of precipitation-borne nutrients. The canopy budget model is considered inadequate for estimating DD_N values, given the variety of atmospheric N compounds (e.g., dissolved, gaseous, and particulate), therefore DD_N was assumed to be zero, resulting in $CE_N = NTF_N$ (Bélanger et al., 2002).

4.4.4 Nutrient output through the export of above-ground willow biomass, along with immobilization within leaf litter, stool, and root tissue

Biomass removal from each site during coppicing after the first growing season and again during harvesting at the end of the rotation (three years post-coppice) was quantified by bundling the cut stems (including branches) within the measurement area of each varietal plot and recording their fresh weight. Numerous stem subsamples from each bundle were then dried at 65^{o}C to a constant weight and weighed to determine moisture content. The moisture contents were used to determine the bundle oven-dry weight, in order to extrapolate the measurement plot data to a stand level (i.e., total oven-dry tonnes of biomass per hectare). The subsamples were then thoroughly milled and homogenized prior to analyzing for N, P, K, S, Ca, and Mg contents of the biomass removed from the site. Total N, P, K, Ca, and Mg contents were analytically measured following a H_2SO_4/H_2O_2 digest (Thomas et al., 1967) as previously described. Total S content was measured using a TruSpec CNS analyzer (Leco Corporation, St. Joseph, MI, USA). Stem tissue nutrient contents for each willow variety were also monitored throughout the rotation at each site. At the end of each growing season, seven stems (representing the diameter range within each willow variety plot) were destructively sampled for developing allometric equations, by calibrating measured stem diameter (at 30 cm height) with harvested leafless biomass (Arevalo et al., 2007). These plot-specific allometric models ($R^2 > 0.98$; $P < 0.001$), were applied to the diameter and stem density measurements from each plot to estimate above-ground biomass. The seven stems were then prepared and analyzed in the same manner as the coppiced/harvested stem samples to determine the total N, P, K, S, Ca, and Mg content in the harvestable biomass within each varietal plot and was extrapolated to a stand level.

Total leaf biomass for each willow variety was estimated annually at each site by collecting all of the leaves from three representative stems within each plot in early September and extrapolating the leaf biomass to a stand level based on stem density measurements. Arguably, this is a more accurate estimation of stand level leaf litter biomass, compared to litterfall traps placed randomly underneath the canopy, considering the assumption of absolute leaf fall is inevitable with deciduous species growing in temperate climates. Estimates of annual nutrient output from the plant available soil nutrient pool through accumulated leaf nutrients were determined by multiplying the nutrient concentrations of abscising leaves collected from each varietal plot every November (mass loss during leaf senescence was assumed to be relatively small; Chapin et al., 1990) by the total leaf biomass estimates from September. The specific leaf area (i.e., cm^2 g^{-1}; Tharakan et al., 2005a) of the September leaves was determined using a leaf surface area meter (LI-3100; LI-COR Inc., Lincoln, NE) and these values were used to calculate the nutrient resorption proficiency, which is based on the absolute levels to which nutrients are reduced in abscised leaves (g nutrient m^{-2}; Killingbeck, 1996).

In a separate companion study, Stadnyk (2010) measured the fine and coarse root biomass of all varieties at each site and these values were supplemented with relative biomass proportions among stems, stool, and fine/coarse root fractions reported in the literature (Rytter, 2001) to estimate the annual below-ground biomass within each plantation throughout the rotation. The decomposition of stool and coarse root biomass was considered to be negligible throughout the rotation (Rytter, 2012), with the nutrient content of these two biomass sinks only estimated at the end of the rotation. Conversely, fine-root biomass was estimated annually. September and November leaves, stool, and root (fine and coarse fraction) tissue samples were prepared and analyzed for N, P, K, S, Ca, and Mg concentration, following the same manner as the stem samples, to estimate annual nutrient immobilization within above- and below-ground willow tissues. Adequate weed control is essential for not only supporting the successful establishment and growth of SRC willow plantations (Labrecque et al., 1994), but also for reducing nutrient uptake by non-crop vegetation (Chapter 5). Weed control throughout the rotation was adequate; therefore, nutrients immobilized in weeds were considered negligible and not quantified (Chapter 5).

4.4.5 Nutrient losses through leaching and denitrification

Nutrient fluxes beyond the effective willow rooting zone were measured throughout the rotation using suction lysimeters (60 kPa; SoilMoisture Equipment Corp., Santa Barbara, CA, USA) installed (60 cm depth) within the four SX64 plots at each site. As with TF collection, collecting leachate samples under each willow variety for comparison was cost-prohibitive and, therefore, it was assumed that SX64 was representative of all varieties. Leachate volumes were measured and sub-sampled contemporaneously with the BD and TF samples and were handled and analysed in like manner. The upper limit of water leached through the soil profile during each growing season was estimated for each site, using a basic soil water balance model (Koerner and Daniel, 1997), and this amount (kg ha^{-1}) was multiplied by the leachate nutrient concentration data to provide an approximation of maximum annual nutrient leaching losses. Meteorological input data for the water balance model were collected at each site using a weather station (Campbell Scientific, Edmonton, AB, Canada). Lysimeter samples were not collected during the establishment year and so the leachate concentrations measured during the first growing season post-coppice were applied to the water balance model output for the establishment year to estimate leaching losses. Nitrous oxide emissions were found to be negligible at the Saskatoon site (<< 1 kg N ha^{-1}; Ens, 2012) and presumably were the same for the other three sites given the negligible denitrification losses from SRC willow plantations often reported (Drewer et al., 2012; Gauder et al., 2012; Schmidt-Walter and Lamersdorf, 2012).

4.4.6 Nutrient transfers through soil organic matter mineralization, canopy exchange, leaf litter decomposition, and fine root turnover

The potential contribution of net mineralization of soil organic matter (SOM) to plant available soil N and S pools was measured using an incubation procedure (Curtin and Campbell, 2008). Field moist soils from each site were sieved (< 4 mm) and 5 g were placed in polypropylene containers and maintained at field capacity for a period of eight weeks. An incubation temperature of 15°C was used to simulate the average growing season temperature among all four sites. Subsamples of the field moist soils and incubated soil samples were then prepared and extracted for inorganic N (NH_4^+-N and NO_3^--N) and S as previously stated. The potential net mineralization contributions from organic N and S were calculated by subtracting the initial extractable nutrient levels from the final extractable levels after the incubation period.

Throughfall water nutrient enrichment due to foliar leaching, as precipitation passed through the willow canopy, was quantified for each nutrient and applied to the nutrient budget whenever applicable. Additionally, these foliar nutrient leaching data were used in estimating nutrient resorption efficiency (% of initial nutrients resorbed during leaf senescence; Yuan et al., 2005) prior to leaf abscission (i.e., $\text{Nutrients}_{\text{Resorbed}} = \text{Nutrients}_{\text{September Leaves}} - \text{Nutrients}_{\text{November Leaves}} - \text{Nutrients}_{\text{Leached}}$) reported in Chapter 3. Unlike such immediate nutrient transfer to the plant available nutrient pools from living tissue, most nutrients within fallen leaves are immobilized in the organic material and consequently, are not readily available for plant uptake until re-mineralized.

Litterbags were used to measure the rate of decomposition and nutrient release of leaf litter throughout the rotation (Chapter 3). Estimating leaf litter inputs and maximum decomposition limits should provide valuable insight into C fluxes and nutrient cycling within these production systems (Berg and Laskowski, 2005; Prescott, 2005). Using the estimated nutrient release rate constants and accumulated leaf litter biomass over the four-year rotation, the contribution of leaf litter nutrient release to the plant available soil nutrient pool were calculated (Chapter 3). The leaf litter cohorts considered in the calculations for nutrient cycling during the rotation were three years of establishment year leaf litter (i.e., pre-coppice leaf biomass), two years of nutrient release from the first year post-coppice leaf litter, and one year of nutrient release from the second year post-coppice leaf litter. Given that the willow was harvested three years after coppicing, the remaining nutrient release contributions from the first to third year post-coppice leaf litter (e.g., the third year leaf litter) was strictly counted as a nutrient sink in the nutrient budget and, therefore, associated with the second rotation.

Below-ground biomass nutrient sinks (e.g., stool, fine and coarse root nutrient contents) and annual nutrient transfers from fine-root turnover to the plant available nutrient pools at each site were estimated using relative proportion and minirhizotron data collected during the rotation (Stadnyk, 2010) and also supplemented with literature values (Rytter, 2001; Püttsepp et al., 2007; Rytter, 2012). Nutrient resorption during fine root senescence was assumed to be negligible (Nambiar, 1987; Aerts et al., 1992); therefore, all fine root nutrients were considered available for cycling back into the soil.

4.4.7 Meteorological conditions

A Campbell Scientific CR10X (Campbell Scientific Inc., Edmonton, AB, Canada) was used at each site to monitor air temperature, rainfall, relative humidity, and wind speed throughout the study. Soil temperature (0-60 cm) was also assessed. Potential evapotranspiration (Thornthwaite and Mather, 1957) and Aridity Index (UNEP, 1992) were estimated annually for each site using the measured climate data. Accumulated snow depth was measured at each site annually in February. The beginning and ending of each growing season was determined using a 5 ^{o}C baseline daily mean temperature, sustained or unsustained for at least five consecutive days, respectively, using both air and soil temperatures for comparison. Growing season length was calculated annually in this manner. The number of growing degree days each year were calculated according to Kopp et al. (2001), by summing the differences between the daily mean temperature and a 5 ^{o}C base temperature, with days having a daily mean temperature below the base level given a zero value.

4.4.8 Statistical Analyses

Means comparisons of measured variables were performed using least significant differences (LSD; Tukey-Kramer's method of multiple comparison) at a significance level of 0.05 using PROC MIXED in SAS (Littell et al., 2006; version 9.2; SAS Institute, Cary, NC, USA), with groupings performed with the pdmix800 SAS macro (Saxton, 1998). The effects of variety were considered fixed, while those of site and replicate (nested within site) were considered random. Normality of distributions (PROC UNIVARIATE) and homogeneity of variances (Bartlett's test) of all data sets were verified, and when required, the data were Log_{10} transformed prior to analysis. A principal component analysis was performed using JMP 10 (Version 10; SAS Institute, Cary, NC, USA) to investigate the relationship between harvested willow biomass and relevant soil, plant tissue, and climate characteristics measured. Correlation strength among variables is indicated by the cosine of the angle between variable vectors and variable groupings were arbitrarily based on an angle of 30^{o} (i.e., $r = 0.87$). Vectors of directly and indirectly correlated variables point in the same or opposite direction, respectively, whereas uncorrelated variables have vectors at right angles to each other.

4.5 Results

4.5.1 Comparing soil nutrient supplying power among plantations

For all nutrients except P, the soil nutrient availability, measured using either chemical extractions or *in situ* burials of PRS™-probes during the growing season, was least at Prince Albert and similar among the other sites (Tables 4.2 and 4.3). Likewise, the measured levels of pH, organic C, and organic C:N were lowest at Prince Albert, compared with the finer-textured lower bulk density soils at the other sites, which supports the lowest agriculture capability classification rating of the sandy Prince Albert soil. The relative ranking of initial extractable

Table 4.3. Mean (n = 24) cumulative nutrient supply rates, measured using *in situ* burials of PRS™-probes inserted into the A horizon from June to October during the pre-coppice establishment year, at different willow plantations in Saskatchewan, Canada.

Site	NH_4^+	NO_3^-	Total N†	P	K	S	Ca	Mg
	(μg 10cm^{-2} 16 weeks^{-1})							
Prince Albert	2.2b‡	679.2b	681.3b	96.6a	87.1c	96.7c	4285.0a	489.2c
Birch Hills	2.8ab	1828.7a	1831.5a	7.6b	170.0b	541.5a	4873.5a	700.0b
Saskatoon	3.6a	1553.5a	1557.1a	8.7b	284.9a	111.8bc	3939.5a	968.8a
Estevan	2.0b	1531.8a	1533.8a	6.3b	136.1b	168.3b	5553.8a	607.2b

† NH_4^+-N + NO_3^--N

‡ Means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

levels of soil nutrients among the four sites was Ca > Mg > K > S > N > P (Table 4.2).

4.5.2 Inputs into the plant available soil nutrient pool

The historic soil mineral weathering supply of P, K, Ca, and Mg to the plant available nutrient pool was minor in proportion to their initial extractable levels (i.e., << 1%; Tables 4.2 and 4.4). Atmospheric deposition of nutrients during the three years were 32.6, 4.3, 21.3, 27.0, 39.2, and 14.1 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively, with the majority of nutrients derived from wet deposition (Table 4.5). Canopy exchange reactions were greatest for N, resulting in direct foliar uptake of 21.0 kg N ha^{-1} from atmospheric deposition after three years, leading to negative net throughfall values and much less contribution of atmospheric N to the soil nutrient pools compared with the other nutrients having positive net throughfall balances (Table 4.5). Specifically, strong foliar absorption of atmospheric N (approximately 8× greater absorption of NH_4^+-N than NO_3^--N; data not shown) by the willow canopy contrasted with the foliar leaching of K and to a lesser extent P and Ca. Atmospheric S and Mg depositions were essentially non-reactive with the willow canopy, resulting in throughfall contributions to the plant available soil nutrient pool strictly from wet and dry deposition (Table 4.5). The combined effects of atmospheric deposition and canopy exchange on plant available nutrient pools are represented in the measured throughfall values of 11.6, 7.7, 31.9, 26, 43.1, and 13.6 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively.

4.5.3 Outputs from the plant available soil nutrient pool

The average stem biomass removed from the four sites at time of coppicing and harvest was 0.6 and 18.4 Mg ha^{-1}, respectively (Table 4.6), with the range in harvested biomass among willow varieties and sites three-years after coppicing of 2.8-41.2 Mg ha^{-1} (data not shown). The nutrients exported from each site through the removal of willow stems, during coppicing following the establishment year in addition to harvesting the willow stems at the end of the initial four-year rotation, averaged 65.6, 9.7, 47.9, 10.6, 136.1, and 18.2 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively (Table 4.6). With the exception of harvested stem P content at Estevan, the trend in nutrient removals offsite during coppicing and harvesting followed the pattern of biomass productivity, with Prince Albert and Estevan having the largest amount of stem nutrients

Table 4.4. Mean (n = 4) potential net N and S mineralization (0-20 cm; measured using an eight-week incubation) and historical mineral weathering of P, K, Ca, and Mg (0-60 cm; measured using the elemental depletion method) from soil collected at different willow plantations in Saskatchewan, Canada.

Site	N[†]	P	K	S	Ca	Mg
	$(kg\ ha^{-1}\ year^{-1})$					
Prince Albert	36.5b[‡]	0.1	2.0	15.4b	17.6	4.9
Birch Hills	47.0a	0.1	2.2	18.7b	14.7	3.3
Saskatoon	50.4a	0.1	7.7	35.0a	3.7	2.7
Estevan	47.7a	0.1	2.4	43.8a	2.2	1.4

[†] NH_4^+-N + NO_3^--N

[‡] For N and S values, means within a column followed by the same letter are not significantly different (*P* > 0.05) using LSD

Only one sample was collected for each soil horizon within a single pedon at each site for estimating historic P, K, Ca, and Mg mineral weathering supply rates, which precluded any assessment or analysis of variance

Table 4.5. Mean (n = 16) total bulk deposition (BD), throughfall (TF), net throughfall (NTF), dry deposition (DD), and canopy exchange (CE) fluxes after three years post-coppice, measured during the growing season using open-ended polypropylene containers located outside of the plantation and under the canopy of willow variety SX64, at different locations in Saskatchewan, Canada.

Flux	N[†]	P	K	S	Ca	Mg
	(kg ha^{-1})					
BD	32.6 (0.8)[‡]	2.8 (0.2)	14.0 (1.1)	16.3 (0.7)	23.6 (1.6)	8.7 (0.8)
TF	11.6 (0.9)	7.7 (0.7)	31.9 (1.8)	26.0 (1.4)	43.1 (2.3)	13.6 (1.1)
NTF	-21.0 (1.3)	4.9 (0.6)	18.0 (0.9)	9.7 (1.2)	19.5 (1.5)	4.9 (0.8)
DD	NA[§]	1.5 (0.1)	7.3 (0.6)	10.7 (1.1)	15.6 (1.4)	5.4 (0.7)
CE	-21.0 (1.3)	3.4 (0.7)	10.6 (1.1)	-0.9 (1.6)	3.8 (1.4)	-0.5 (1.0)

[†] NH_4^+-N + NO_3^--N

[‡] Overall mean (standard error) values are reported due to significant ($P < 0.05$) site × year effect

[§] Assumed to be zero; therefore, $CE_N = NTF_N$ (Bélanger et al., 2002)

Table 4.6. Mean (n = 24) biomass and nutrient content in the harvested stems (bark + wood) of several willow varieties exported offsite during an initial four-year rotation at different plantations in Saskatchewan, Canada.

Site	Biomass	N	P	K	S	Ca	Mg
	($Mg\ ha^{-1}$)	($kg\ ha^{-1}$)					
	Coppiced biomass one year after planting						
Prince Albert	1.1 (0.1)[†]	10.7 (1.1)	1.9 (0.2)	6.4 (0.9)	0.9 (0.1)	19.8 (2.2)	1.9 (0.2)
Birch Hills	0.3 (< 0.1)	3.1 (0.2)	0.4 (< 0.1)	1.4 (0.1)	0.3 (< 0.1)	5.5 (0.5)	0.8 (0.1)
Saskatoon	0.4 (< 0.1)	3.2 (0.3)	0.4 (< 0.1)	1.5 (0.2)	0.3 (< 0.1)	5.2 (0.5)	1.7 (0.2)
Estevan	0.5 (< 0.1)	3.8 (0.3)	0.5 (< 0.1)	2.3 (0.2)	0.6 (0.1)	11.5 (0.9)	2.0 (0.2)
	Harvested biomass three years after coppicing						
Prince Albert	13.7 (1.7)	42.3 (4.8)	7.8 (0.8)	31.7 (3.6)	8.3 (1.0)	115.8 (14.6)	9.6 (1.1)
Birch Hills	19.3 (0.8)	68.6 (3.8)	10.6 (0.7)	45.9 (2.8)	11.3 (0.6)	139.2 (10.9)	17.5 (0.7)
Saskatoon	13.9 (1.1)	53.4 (5.4)	9.3 (0.9)	44.5 (3.8)	9.6 (1.2)	86.6 (7.1)	17.3 (1.3)
Estevan	26.6 (1.7)	77.4 (5.0)	7.7 (0.5)	58.0 (3.8)	11.1 (0.7)	160.8 (14.0)	21.8 (1.6)

[†] Site mean (standard error) values are reported due to significant ($P < 0.05$) variety × site effect

immobilized after the establishment year and third-year after coppicing, respectively (Table 4.6).

The average amount of leaf litter produced after four years among the willow varieties and sites was 7.1 Mg ha^{-1} (Table 3.3). Although there were no significant differences ($P > 0.05$) in cumulative leaf litter biomass after four years among the six varieties, leaf litter production was lowest at Saskatoon. The average amount of nutrients contained within the leaf litter among the willow varieties and sites was 80.8, 14.6, 108.1, 24.5, 255.7, and 41.2 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively (Table 3.3). The N, P, and Mg contents of leaf litter were not significantly different ($P > 0.05$) among varieties. Generally, for the remaining nutrients and when comparing leaf litter nutrient content among sites, there was a trend of increasing nutrient content with increasing biomass. Exceptions to this were observed with the P and Mg contents of Estevan and Prince Albert leaf litter, respectively (Table 3.3).

The average amount of nutrient resorption during leaf senescence among the willow varieties and sites was 64.2, 5.7, 38.8, 7.7, 41.7, and 5.3 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively (Table 4.7). There was no significant difference ($P > 0.05$) in N, P, and S resorption prior to leaf fall among the varieties along with no discernible trend in observed differences in K, Ca, or Mg resorption among varieties. For every nutrient, there was a strong direct linear relationship between the leaf nutrient content in September with nutrient resorption during leaf senescence and leaf litter nutrient content (data not shown). The lowest September leaf nutrient content (data not shown), nutrient resorption (Table 4.7), and leaf litter nutrient content (Table 3.3) were consistently observed with willow varieties growing at Saskatoon.

Additional sinks of plant available soil nutrients included the below-ground willow biomass that consisted of stools and fine and coarse roots, which were 1.2, 10.5, 0.8 Mg ha^{-1}, respectively, after an initial four-year rotation among willow varieties and sites, with the trend in nutrient immobilization related to the relative biomass of these below-ground tissues (Table 4.8). Among the sites, the average amount of nutrients tied up in stools was 4.2, 0.7, 1.5, 1.1, 1.9, and 0.9 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively. The average amount of nutrients tied up in fine and coarse roots after an initial four-year rotation was 148.9, 40.1, 58.2, 38.2, 50.4, and 39.2 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively, greater than 92% of which were contained within the fine root fraction (Table 4.8). As previously mentioned, nitrous oxide emissions were assumed to be negligible at the study sites, so the only below-ground loss of nutrients measured

Table 4.7. Mean cumulative nutrient resorption prior to leaf fall during an initial four-year rotation for several willow varieties, measured by comparing leaf nutrient content in early September with abscising leaves in November and correcting for foliar leaching losses, at different plantations in Saskatchewan, Canada.

	N	P	K	S	Ca	Mg
Variety (n = 16)			(kg ha^{-1})			
Allegany	66.5a[†]	4.9a	32.3c	7.4a	25.0b	7.4a
Canastota	63.7a	6.8a	47.1a	7.9a	50.1a	5.0b
Fish Creek	55.0a	6.0a	31.8c	6.8a	25.6b	7.1a
Sherburne	64.2a	5.0a	41.0ab	7.9a	25.7b	4.1bc
SX61	72.9a	5.7a	35.0bc	7.9a	68.5a	3.0c
SX64	62.9a	5.8a	45.6ab	8.2a	55.0a	5.3b
Site (n = 24)			(kg ha^{-1})			
Prince Albert	52.5b[‡]	8.5a	46.6a	7.9a	44.6b	3.2c
Birch Hills	78.7a	6.0b	48.9a	10.5a	40.4b	5.3b
Saskatoon	35.9b	2.6c	17.0b	3.3b	20.8c	4.8bc
Estevan	89.8a	5.7b	42.7a	9.0a	60.9a	8.0a

[†] For each variety, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

[‡] For each site, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

Table 4.8. Mean (n = 24) biomass and nutrient content of the stool and fine and coarse root tissues of several willow varieties after an initial four-year rotation at different plantations in Saskatchewan, Canada.

Site	Biomass (Mg ha^{-1})	N (kg ha^{-1})	P	K	S	Ca	Mg
	Stool†‡						
Prince Albert	0.9 (0.1)§	3.1 (0.4)	0.5 (0.1)	1.1 (0.1)	0.8 (0.1)	1.4 (0.2)	0.6 (0.1)
Birch Hills	1.3 (0.1)	4.4 (0.2)	0.7 (< 0.1)	1.6 (0.1)	1.2 (< 0.1)	1.9 (0.1)	0.9 (< 0.1)
Saskatoon	0.9 (0.1)	3.1 (0.2)	0.5 (< 0.1)	1.1 (0.1)	0.8 (0.1)	1.4 (0.1)	0.7 (< 0.1)
Estevan	1.7 (0.1)	6.0 (0.4)	1.0 (0.1)	2.2 (0.1)	1.6 (0.1)	2.7 (0.2)	1.3 (0.1)
	Fine roots¶						
Prince Albert	10.9 (1.4)	154.2 (20.3)	36.6 (5.0)	52.8 (7.2)	34.9 (4.8)	46.0 (6.3)	35.8 (4.9)
Birch Hills	11.7 (0.7)	165.6 (9.9)	43.3 (2.7)	62.5 (3.9)	41.3 (2.6)	54.4 (3.4)	42.4 (2.6)
Saskatoon	12.1 (1.0)	170.9 (13.5)	52.8 (4.3)	76.1 (6.1)	50.3 (4.1)	66.3 (5.3)	51.6 (4.2)
Estevan	7.1 (0.8)	100.3 (11.1)	26.6 (3.3)	38.4 (4.8)	25.4 (3.1)	33.5 (4.1)	26.0 (3.2)
	Coarse roots†						
Prince Albert	0.6 (0.1)	4.2 (0.5)	0.8 (0.1)	1.5 (0.2)	0.8 (0.1)	1.1 (0.1)	0.8 (0.1)
Birch Hills	0.9 (< 0.1)	6.0 (0.2)	1.2 (< 0.1)	2.1 (0.1)	1.2 (< 0.1)	1.5 (0.1)	1.2 (< 0.1)
Saskatoon	0.6 (< 0.1)	4.3 (0.3)	0.9 (0.1)	1.5 (0.1)	0.8 (0.1)	1.1 (0.1)	0.8 (0.1)
Estevan	1.2 (0.1)	8.3 (0.5)	1.6 (0.1)	2.9 (0.2)	1.6 (0.1)	2.1 (0.1)	1.6 (0.1)

† Estimated at harvest

‡ Includes both above- and below-ground component

§ Site mean (standard error) values are reported due to significant ($P < 0.05$) variety × site effect

¶ Estimated annually and summed

in this study was leaching. Average nutrient leaching losses across sites during the four-year rotation were 6.8, 0.4, 1.0, 22.5, 45.6, and 31.6 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively (Table 4.9). Nitrate comprised 85% of the N leached (data not shown) and there were no discernible trends in nutrient leaching among sites.

4.5.4 Transfers into the plant available soil nutrient pool

The contribution of potential net N and S mineralization during the growing season averaged 45 and 28 kg ha^{-1} yr^{-1}, respectively (Table 4.4). A large degree of foliar absorption of atmospheric N precluded foliar leaching of either NH_4^+-N or NO_3^--N (89% of the inorganic N intercepted and retained by the willow canopy was NH_4^+-N; data not shown). Canopy exchange through foliar leaching contributed to soil K, P, and Ca availability during the four-year rotation, providing 3.4, 10.6, and 3.8 kg ha^{-1}, respectively, while S and Mg atmospheric depositions were essentially non-reactive with the willow canopy (Table 4.5). The contribution of nutrients from leaf litter decomposition during the rotation across varieties and sites was 21.5, 3.9, 47.3, 10.5, 112.0, and 18.5 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively, with the Estevan soil receiving 72% more nutrients from decomposing leaf litter during the rotation than other sites (Table 3.7). Decomposing fine root biomass contributed 49.9, 12.3, 17.7, 11.7, 15.4, and 12.0 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively, with 41% less nutrient transfers from fine root decomposition at Estevan compared to the other sites (Table 4.10). The average contribution of decomposing leaf and fine root litter to the plant available soil nutrient pool from during the initial four-year rotation across varieties and sites was 71.4, 16.1, 64.9, 22.2, 127.4, and 30.5 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively. The majority of the N (70%), P (76%), and S (53%) contribution came from fine root turnover, while leaf litter supplied the majority of K (73%), Ca (88%), and Mg (61%; Tables 3.7 and 4.10).

4.5.5 Soil nutrient availability changes after four years

After the initial rotation there were calculated deficits (i.e., decreased net balance relative to initial extractable level) for all nutrients except S (Table 4.11). The average deficits among varieties and sites were -17.3, -39.7, -112.8, -282.3, and -73.7 kg ha^{-1} of N, P, K, Ca, and Mg, respectively, with Estevan and Birch Hills displaying a 55% greater nutrient deficit after four years compared to Prince Albert and Saskatoon (Table 4.11). There were no significant differences ($P > 0.05$) in either N or Ca deficits among the sites, with the smallest P deficit

Table 4.9. Mean (n = 4) leached nutrients during an initial four-year rotation, measured by monitoring leachate nutrient concentrations using suction lysimeters installed at 60 cm within plots of willow variety SX64, at different plantations in Saskatchewan, Canada.

Site	N†	P	K	S	Ca	Mg
	(kg ha^{-1})					
Prince Albert	2.6 (1.0)‡	1.2 (0.5)	2.0 (0.2)	33.9 (4.9)	52.4 (3.7)	26.8 (3.2)
Birch Hills	1.7 (0.9)	0.1 (< 0.1)	0.3 (0.1)	22.8 (1.4)	97.5 (1.5)	54.5 (3.5)
Saskatoon	8.2 (0.4)	0.1 (< 0.1)	0.4 (< 0.1)	22.2 (7.4)	9.0 (0.2)	22.5 (0.9)
Estevan	14.7 (4.2)	0.2 (< 0.1)	1.4 (0.1)	11.2 (0.8)	23.5 (1.2)	22.6 (2.5)

† NH_4^+-N + NO_3^--N

‡ Site mean (standard error) values are reported due to significant ($P < 0.05$) site × year effect

Table 4.10. Mean (n = 24) nutrients released from fine root turnover during an initial four-year rotation for several willow varieties at different plantations in Saskatchewan, Canada.[†]

	N	P	K	S	Ca	Mg
Site	($kg\ ha^{-1}$)					
Prince Albert	58.5 (7.7)[‡]	12.9 (1.8)	18.6 (2.5)	12.3 (1.7)	16.2 (2.2)	12.6 (1.7)
Birch Hills	55.3 (3.5)	13.3 (0.9)	19.1 (1.2)	12.7 (0.8)	16.7 (1.1)	13.0 (0.8)
Saskatoon	52.9 (4.2)	14.8 (1.2)	21.4 (1.7)	14.1 (1.1)	18.6 (1.5)	14.5 (1.1)
Estevan	33.0 (3.7)	8.0 (1.0)	11.6 (1.4)	7.7 (0.9)	10.1 (1.2)	7.8 (1.0)

[†] Nutrient release estimated using site-specific relative proportional ratios for biomass and fine root turnover (0.96 $year^{-1}$) from (Stadnyk, 2010) and decomposition rate (20% $year^{-1}$) from (Püttsepp et al., 2007)

[‡] For each nutrient, site mean (standard error) values are reported due to significant ($P < 0.05$) variety × site effect

Table 4.11. Mean (n = 24) change in plant available soil nutrient pool after an initial four-year rotation for several willow varieties at different plantations in Saskatchewan, Canada.

Site	N	P	K	S	Ca	Mg
	($kg\ ha^{-1}$)					
Prince Albert	-23.2a[†]	-52.6b	-75.9a	28.5c	-221.9a	-41.1a
Birch Hills	-12.4a	-39.4b	-152.9b	23.8c	-335.4a	-96.7b
Saskatoon	-12.1a	-46.0b	-100.6a	95.9b	-159.7a	-73.2ab
Estevan	-21.6a	-19.4a	-120.3a	142.9a	-365.7a	-83.9ab
	(%)					
Prince Albert	-41.9b	-35.4a	-10.6b	31.0a	-1.5a	-3.2b
Birch Hills	-18.3a	-243.3b	-11.8b	2.9b	-1.6a	-2.2b
Saskatoon	-12.2a	-72.2a	-5.1a	14.5b	-0.8a	-0.8a
Estevan	-21.7a	-54.4a	-8.9b	18.7b	-1.9a	-2.7b

[†] For each nutrient, means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

observed at Estevan, the largest K deficit at Birch Hills, and the only difference in Mg deficit was between Birch Hills and Prince Albert. The measured S surpluses ranged from 23.8-142.9 kg ha^{-1}, with the largest surpluses occurring at Estevan and Saskatoon (data not shown).

When the net soil nutrient balance was expressed as a percent change from the initial extractable pool level, the average decrease in plant available nutrient pools was -24, -101, -9, -1, and -2% for N, P, K, Ca, and Mg, respectively, with a 17% increase in available soil S (Table 4.11). There was no significant effect ($P > 0.05$) of willow variety on the percent change in nutrient pools (data not shown). Plant available N decreased 24% across all sites, with the largest depletion found at Prince Albert. The largest calculated negative net balance occurred with the soil P budget depletion of 243% at Birch Hills, which was a 4.5× greater P depletion than the other sites (Table 4.11). Saskatoon had the smallest soil K and Mg depletions, while there was no significant difference ($P > 0.05$) in soil Ca depletion among sites. Available soil S increased 17% among sites, with the replenishment at Prince Albert 2.5× greater than the other sites after four years (Table 4.11).

4.5.6 Soil nutrient budget after initial rotation

During the four-year rotation, inputs to the soil nutrient budget across varieties and sites were 204, 8, 37, 135, 87, and 30 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively (Table 4.12). Inputs of N and S to available pools from net mineralization of SOM during the rotation were approximately 5× greater than inputs from atmospheric deposition. Contributions to soil P were larger from atmospheric depositional compared to soil mineral weathering, but had similar K, Ca, and Mg inputs. Irrespective of nutrient, the relative additions to the plant available soil nutrient pool by atmospheric deposition, SOM mineralization, and soil mineral weathering were 48, 27, and 25%, respectively (Table 4.12).

Average outputs from the soil nutrient budget across varieties and sites were -314, -67, -226, -97, -500, and -134 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively, with the relative nutrient sinks being: below-ground biomass (37%), leaf litter (35%), stems (18%), and leaching (9%). During the four-year rotation, the soil N, P, and S taken up by willow was primarily immobilized in below-ground tissues (50, 62, and 40%, respectively; predominantly the fine roots), with sequestered soil K, Ca, and Mg largely immobilized in leaf litter (48, 51, and 31%, respectively; Table 4.12). Larger amounts of N, P, and S were immobilized in stool and root biomass

Table 4.12. Mean (n = 96) soil nutrient budget after the initial four-year rotation for several willow varieties growing at different willow plantations in Saskatchewan, Canada.†

Budget Variable	Nitrogen‡	Phosphorus	Potassium
	(kg ha^{-1})		
Initial extractable soil nutrients (0-60 cm)	79.6 (2.1)	66.1 (5.7)	1322.1 (49.1)
	---------------------------- Inputs	----------------------------	
Soil mineral weathering (0-60 cm)	0.0	0.2 (< 0.1)	14.4 (1.1)
Soil net mineralization (0-20 cm)	181.2 (2.4)	0.0	0.0
Atmospheric deposition	22.6 (0.5)	6.5 (0.1)	25.8 (0.1)
	--------------------------- Outputs	---------------------------	
Coppiced stems	-5.4 (0.5)	-0.8 (0.1)	-3.0 (0.4)
Harvested stems	-60.2 (2.8)	-8.9 (0.4)	-44.7 (2.0)
Leaf litter	-82.7 (3.7)	-15.5 (1.7)	-115.5 (8.0)
Stool and roots§	-158.6 (7.6)	-41.8 (2.2)	-61.2 (3.1)
Leaching¶	-6.6 (0.6)	-0.4 (0.1)	-1.1 (0.1)
	-------------------------- Transfers	--------------------------	
Canopy exchange#	0.0	3.2 (0.1)	10.8 (0.1)
Leaf litter decomposition	21.1 (1.5)	3.9 (0.4)	47.5 (5.2)
Fine root turnover	50.4 (2.7)	12.3 (0.7)	17.8 (1.0)
Net soil nutrient balance††	62.3 (7.0)	26.4 (5.2)	1209.3 (48.6)
% Change in soil nutrients‡‡	-23.7 (10.2)	-104.6 (10.3)	-9.2 (0.6)

Table 4.12. (continued).

Budget Variable	Sulfur	Calcium	Magnesium
		(kg ha^{-1})	
Initial extractable soil nutrients (0-60 cm)	579.3 (32.3)	18524.0 (273.3)	4573.6 (342.7)
	----------------------------	Inputs	----------------------------
Soil mineral weathering (0-60 cm)	0.0	39.4 (3.0)	12.4 (0.6)
Soil net mineralization (0-20 cm)	110.6 (5.1)	0.0	0.0
Atmospheric deposition	31.4 (0.1)	47.6 (0.6)	17.4 (0.3)
	----------------------------	Outputs	----------------------------
Coppiced stems	-0.6 (< 0.1)	-10.9 (1.0)	-1.7 (0.1)
Harvested stems	-10.1 (0.5)	-125.4 (6.6)	-16.4 (0.8)
Leaf litter	-25.7 (1.5)	-264.9 (16.8)	-42.6 (2.4)
Stool and roots[§]	-40.4 (2.1)	-53.6 (2.7)	-41.1 (2.1)
Leaching[¶]	-20.2 (0.6)	-44.7 (3.8)	-32.2 (1.5)
	----------------------------	Transfers	----------------------------
Canopy exchange[#]	0.0	3.4 (0.4)	0.0
Leaf litter decomposition	10.5 (0.7)	110.9 (9.4)	18.4 (1.0)
Fine root turnover	11.8 (0.6)	15.5 (0.8)	12.1 (0.7)
Net soil nutrient balance[††]	639.5 (35.0)	18241.7 (270.7)	4499.9 (342.0)
% Change in soil nutrients[‡‡]	16.6 (1.7)	-1.5 (0.1)	-2.2 (0.1)

[†] Overall mean (standard error) values are reported due to significant ($P < 0.05$) variety × site effect

[‡] NH_4^+-N + NO_3^--N whenever applicable

[§] Stool and coarse root biomass estimated at harvest and fine root biomass estimated annually

[¶] Leachate collected at 60 cm depth and assumed to be lost to willow uptake

[#] Zero values indicate foliar consumption of atmospheric nutrients (i.e., negative canopy exchange values; Table 4.5). These nutrients did not originate from soil, but are a component of the biomass output values; therefore, the net soil nutrient balance value for N, S, and Mg was credited accordingly

[††] Initial extractable soil nutrients + inputs + outputs + transfers

[‡‡] ((Net soil nutrient balance - initial extractable soil nutrient level)/initial extractable soil nutrient level) × 100. Reported value is mean of all site/plot data; not calculated from table data

compared to the above-ground biomass. Conversely, the above-ground biomass contained 4× the amount of K, Ca, and Mg; of which, 65% was in the leaf litter (Table 4.12).

Nutrient transfers into the plant available pools across all varieties and sites were 71.5, 20.9, 83.4, 32.3, 145.4, and 35.3 kg ha^{-1} of N, P, K, S, Ca, and Mg, respectively. Fine root turnover was more important for augmenting soil N and P levels, leaf litter decomposition for soil K, Ca, and Mg, while the available soil S pool was supported equally among transfer mechanisms (Table 4.12). The nutrient outputs after the initial rotation were 6, 144, 100, 118, and 122% greater for N, P, K, Ca, and Mg, respectively, resulting in a net balance deficit for all nutrients except S, which had a 39% surplus after four years. The average decrease in plant available soil N, P, K, Ca, and Mg pools after four years among varieties and sites was 24, 105, 9, 2, and 2%, respectively, with an average increase in soil S of 17% (Tables 4.11 and 4.12).

4.6 Discussion

4.6.1 Baseline soil fertility at each willow plantation

The soil properties at each site were within the recommend range for supporting SRC willow growth (Jug et al., 1999a; Abrahamson et al., 2002). The observed differences in soil type and associated properties among the four sites are largely related to the varying soil parent materials, along with past management practices, and historical climate/vegetation regime (Anderson, 1988). Three of the sites were developed on moderately fine to fine textured, glacio-lacustrine (Birch Hills and Saskatoon) or alluvium (Estevan) deposits, while the Prince Albert soil was developed on coarse to moderately coarse textured glacio-fluvial deposits. Consequently, although there were measured differences in the inherent nutrient supplying power of all soils (Tables 4.2 and 4.3), the Class 5-6 soil at Prince Albert was the poorest quality soil tested. For example, SOM content (i.e., organic C and N) is often considered a key 'soil quality' measure, given its intimate association with essential physical, chemical, and biological properties controlling soil productivity (Gregorich et al., 1994; Wood et al., 2000). The lowest SOM content of the Prince Albert soil, therefore, is an important indicator of the differences in long-term soil nutrient availability, especially soil N and S. Likewise, coarse textured soils have comparatively less P, K, Ca, and Mg-bearing minerals, along with reduced sorption capacity, compared with finer textured soils. However, past management practices can also play a critical role in soil nutrient supply, as evidenced by the greater soil P availability at Prince Albert

(Tables 4.2 and 4.3), which resulted from repeated applications of high fertilizer P rates prior to establishing the willow plantation.

4.6.2 Inputs into the plant available soil nutrient pool

4.6.2.1 Soil mineral weathering

The contributions of P, K, Ca, and Mg to the plant available nutrient pool from soil mineral weathering are consistent with those reported elsewhere (Melkerud et al., 2003; Watmough and Dillon, 2003), but the relative importance of these contributions to the overall nutrient budget were negligible (i.e., < 0.1%) compared to their initial extractable levels. However, it is possible that the relative proportion of mineral weathering inputs may have been underestimated as afforested agricultural soil can have augmented mineral weathering rates, presumably due to greater root biomass favouring increased production of acidic root exudates and symbiotic mycorrhizal associations (e.g., hybrid poplar; Lafleur et al., 2013).

4.6.2.2 Atmospheric deposition

Increasing leaf surface area as the plantations aged, resulted in a significant ($P < 0.05$) site × year effect among the measured atmospheric deposition (BD + DD) and CE variables, so the mean cumulative values (and associated standard error) after the three-year post-coppice measurement period prior to harvesting were reported (Table 4.5). The measured nutrient inputs via atmospheric deposition were consistent with literature values (Bélanger et al., 2002; Greaver et al., 2012) and similar to the other inputs, atmospheric N deposition (i.e., approximately 10 kg N ha^{-1} yr^{-1}; Table 4.5) played a more important role in improving soil N availability compared to other nutrients, relative to their initial extractable levels (Table 4.2). Ammonium-N was the predominant inorganic N form in the bulk deposition samples (data not shown), which agrees with the finding of Lovett and Lindberg (1993). The fundamental difference between the willow canopy and open collectors located outside of the plantation is evidenced by the lack of significant difference ($P > 0.05$) in measured BD nutrient concentrations over the four-year rotation contrasted with the increased DD nutrient concentrations as the plantations aged and developed more leaf surface (data not shown), indicating the increased capacity of the developing willow canopy to filter the air, thereby capturing additional nitrogenous compounds in airborne particles or gases.

4.6.3 Outputs from the plant available soil nutrient pool

4.6.3.1 Stem nutrients

The average harvestable biomass of the six varieties during the initial four-year rotation across the four sites was 19 Mg ha^{-1}, with associated nutrient removals of 13.3-20.3, 2.1-2.8, 9.5-15.1, 2.3-2.9, 23.0-43.1, and 2.9-6.0 kg ha^{-1} $year^{-1}$ of N, P, K, S, Ca, and Mg, respectively (Table 4.6). When corrected for differences in annual stem biomass production, these annual nutrient removal rates are similar to other studies (Hytönen, 1995b; Labrecque et al., 1998; Jug et al., 1999a; Adegbidi et al., 2001; Park et al., 2004; Ens et al., 2013). Under conditions of adequate soil fertility, willow productivity is primarily controlled by moisture availability and temperature (Labrecque and Teodorescu, 2003); therefore, it is not surprising that the measured yields were lower than typical willow yields reported in the literatures. The relatively low willow productivity observed at the sites is typical within the province (Moukoumi et al., 2012; Ens et al., 2013) and simply reflects the less favourable growing conditions in Saskatchewan compared to many other regions. For example, including the establishment year, the average annual production was 4.8 Mg ha^{-1} $year^{-1}$, is lower than the average annual yields of 6-10 Mg ha^{-1} often reported in the literature (e.g., González-García et al., 2012; Rosenqvist et al., 2013; Serapiglia et al., 2013), but especially compared to upper end of willow productivity, > 20 Mg ha^{-1} $year^{-1}$, potential in USA (Adegbidi et al., 2001; Quaye and Volk, 2013) and Europe (Christersson, 1986; Stolarski et al., 2008). In the humid continental climate of eastern Canada, Labrecque and Teodorescu (2005) reported an average productivity of approximately 16 Mg ha^{-1} $year^{-1}$ for the varieties SX61 and SX64 after four relatively suboptimal growing seasons in southern Quebec. In a companion study, Guidi Nissim et al. (2013) measured SX61 and SX64 yields up to 21.3 and 24.3 Mg ha^{-1} $year^{-1}$, respectively, which is almost 3.5× their productivity in this study. The semi-arid temperate climate of Saskatchewan simply does not allow for the maximum genetic potential growth rates of willow to be achieved, with an average 103 frost free days, 419 mm mean annual precipitation, < 2 ^{o}C mean annual temperature and 1500 growing degree days (baseline 5 ^{o}C) among the four sites (Table 4.1). The influence of geoclimatic effects on willow productivity has been documented (Alriksson, 1997) and was apparent within this study as well. Estevan received more rainfall (40%), growing degree days (44%), air and soil temperatures (35%), and photosynthetically active radiation (45%) compared to the other three sites (data not shown), thereby explaining the greater willow productivity and nutrient removals at Estevan (Table 4.6). Moisture availability is the primary factor affecting willow growth, especially within temperate regions (Mitchell, 1992; Lindroth and Båth, 1999). Irrigation alone increased willow productivity at the Saskatoon site by more than 130% (Chapter 5), so moisture limitations within Saskatchewan can be mitigated. Additionally, extending the post-coppice rotation length to five years increased the mean annual increment greatly (Chapter 6); therefore, it appears that a longer rotation cycle is necessary within this semi-arid temperate climate to support the economic sustainability of SRC willow plantations.

The significant (P < 0.05) variety × site × year interaction effects on stem nutrient contents were primarily due to the underlying differences in willow productivity among varieties and sites (CV of 46%; Table 4.6) and to a lesser extent the differences in stem nutrient concentrations (average CV of 26% among nutrients; data not shown). The calculated NUE (kg nutrient Mg biomass^{-1}) differed between the one-year-old coppiced and three-year-old post-coppice harvested biomass among varieties and sites. Specifically, the NUE of the one-year-old coppiced biomass was 7.4-10.0 (N), 0.9-1.7 (P), 4.2-5.7 (K), 0.8-1.1(S), 15.5-23.2 (Ca), and 1.8-5.0 (Mg), while that of the harvested biomass was 2.9-3.8 (N), 0.3-0.7 (P), 2.2-3.2 (K), 0.4-0.7 (S), 6.1-9.0 (Ca), and 0.7-1.2 (Mg); representing an average increase (%) in NUE of 170 (N), 161 (P), 87 (K), 79 (S), 164 (Ca), and 253 (Mg). Such increases in NUE with age have been recognized for some time and are primarily attributed to decreasing ratio of nutrient-rich bark to wood with increasing stem biomass (Ericsson, 1984). Numerous authors over the last several decades have referred to SRC willow as a nutrient demanding crop; however, it is prudent to put the NUE characteristics of willow in perspective by noting that although the existence of large varietal differences of NUE among willow varieties (Weih and Nordh, 2002; Tharakan et al., 2005a), relative to both conventional agricultural crops and alternative biomass energy crops (e.g., *Miscanthus*, switchgrass, maize, and oilseed rape), willow can be successfully grown with a fraction of the nutrients, thereby providing a higher NER (Boehmel et al., 2008; Weih et al., 2011; Kering et al., 2012). For example, a common four-year canola (*Brassica* spp.) – wheat (*Triticum* spp.) rotation in Saskatchewan produces approximately 9.5 Mg ha^{-1} of biomass, while removing approximately 400 (N), 100 (P), 200 (K), 75 (S), 130 (Ca), and 50 (Mg) kg ha^{-1} from the soil (Canadian Fertilizer Institute, 2001). The average four-year willow yield more than doubled the biomass produced compared to a typical annual crop rotation over the same period, but more importantly, the relative reduction in soil nutrient removals by SRC willow was profound. Specifically, the NUE of willow is 12 (N), 21 (P), 8 (K), 15 (S), 2 (Ca), and 6 (Mg) times greater than a canola-wheat rotation, resulting in relative decreases (%) of 84 (N), 90 (P), 76 (K), 86 (S), 3 (Ca), and 64 (Mg) removal from the soil nutrient reserves after four years.

4.6.3.2 Leaf litter nutrients

The measured foliar biomass and nutrient concentrations of both pre-senescent and abscised leaves are within the range reported in the literature (Makeschin, 1994; Hytönen, 1995b; Labrecque et al., 1998; Jug et al., 1999a; Labrecque and Teodorescu, 2001; Kahle et al., 2010). Despite only accounting for approximately 29% of the above-ground biomass after four years (Tables 3.3 and 4.6), willow leaves were the largest above-ground sink of soil nutrients. Relative to the total amount of nutrients immobilized above-ground among varieties and sites, 69, 63, 72, 72, 64, and 69% of the N, P, K, S, Ca, and Mg, respectively, were in pre-senescent leaves (data not shown). The same trend in above-ground biomass occurred with abscising leaves collected in November, albeit with a reduced proportionality due to nutrient resorption during senescence, where the relative percentage of above-ground N, P, K, S, Ca, and Mg immobilized in leaves were 56, 53, 67, 69, 64, and 69, respectively, (Tables 3.3 and 4.6). This disproportionate nutrient content of leaf biomass relative to the above-ground woody biomass components has been noted previously (Simon et al., 1990; Hytönen, 1995a). As expected, there were strong relationships (R^2 0.64-1.00; $P < 0.05$; data not shown) among the various leaf variables measured, including biomass and nutrient content, pre-senescent leaf nutrient content and nutrient resorption, along with abscised leaf nutrient content throughout the rotation regardless of variety, site, or year (Tables 3.3 and 4.7). There was 50% greater variation in leaf biomass compared to foliar nutrient concentration among varieties and sites (data not shown), therefore biomass was a more important controller of leaf litter nutrient content. For example, as with stem nutrient immobilization, the smallest leaf nutrient sink at Saskatoon was consistent with its reduced productivity compared to the other sites, which is consistent with the expectation of increased nutrient loss to litter with increasing litter biomass (or vice versa; Weih and Nordh, 2005). Notwithstanding the measured differences in foliar K, Ca, and Mg resorption during senescence among varieties (Table 4.7), the lack of an apparent trend, presumably is due to the relatively narrow diversity among varieties tested, which were S. *miyabeana, S. sachalinensis, S. purpurea*, and hybrids thereof.

There were minimal differences in resorption efficiency (%)/resorption proficiency (g m^{-2}) among varieties during the rotation, with average values across the sites of 42.8/0.9, 36.2/0.2, 34.5/1.0, 29.7/0.3, 16.2/2.1, and 17.7/0.4 for N, P, K, S, Ca, and Mg, respectively (data not shown). Such nutrient remobilization and storage in perennial tissues is a fundamental mechanism of nutrient conservation that is advantageous for promoting early growth the following spring when root activity and nutrient uptake from cold soil is limited. These varietal differences in nutrient resorption efficiency and resorption proficiency are within the range of values found in the literature (Killingbeck, 1996; Rytter, 2001; von Fircks et al., 2001) and help explain the measured differences in willow productivity among varieties observed during the initial rotation (data not shown). Birch Hills consistently had the highest resorption efficiency throughout the rotation among the sites (an average of 30% greater for N, P, K, and S, but no difference in Ca and Mg) and this supports the assertion by del Arco et al. (1991) that resorption efficiency of woody species (including *Salix*) in semi-arid climates is highly dependent on water availability. Birch Hills had the shallowest water table and highest soil moisture content at depth each year of the rotation (Table 4.2). Furthermore, this agrees with the findings of Chapter 5 where irrigation stimulated earlier initiation of leaf senescence, resulting in more than double the nitrogen resorption efficiency compared with non-irrigated willow. The differences in resorption proficiency among the sites were invariably linked with the relative differences in soil nutrient level among sites (Tables 4.2 and 4.3). Such direct relationships between reduced leaf litter nutrient concentrations (i.e., enhanced foliar nutrient resorption) and corresponding low soil test level often is considered a nutrient conservation phenotypic response by plants under conditions of reduced soil nutrient availability (Millard, 1996; Mengel and Kirkby, 2001).

The proportion of leaf biomass relative to total above-ground biomass production decreased over the rotation, constituting 30, 27, 20, and 16% of total above-ground biomass production after the establishment year and the three years post-coppice, respectively (data not shown) and this changing pattern of C allocation has been recognized previously (Hytönen, 1995a; von Fircks et al., 2001). Though nutrient resorption during leaf senescence is an important nutrient conservation mechanism supporting subsequent willow growth (Rytter, 2001; Yuan et al., 2005), it was interesting to note that regardless of variety and site, there was decreased nutrient resorption prior to leaf fall as the plantations matured. Specifically, 20, 25, 39, 31, 1, and 60% less N, P, K, S, Ca, and Mg, respectively, was resorbed during senescence by the end of the initial four-year rotation compared to the establishment year (data not shown), which appears to contradict the common belief that SRC willow increases nutrient conservation over time. Aerts (1996) notes that evergreen species conserve nutrients by synthesizing leaves with inherently lower nutrient concentrations rather than possessing high nutrient resorption efficiency. Perhaps this is valid for willow as well for certain nutrients, namely N, S, and Ca, which had decreased its pre-senescent foliar concentrations by 37, 15, and 4%, respectively, by the final year of the rotation compared to the establishment year and this would help counterbalance the reduced nutrient resorption efficiency. Conversely, pre-senescent foliar P, K, and Mg concentrations increased annually (23, 7, and 20%, respectively) from the establishment year to the end of rotation (data not shown), so for these nutrients the mitigation mechanism for conserving these nutrients (if any) is not apparent. There was 31% greater variation in leaf litter nutrient contents compared to pre-senescent nutrient status among varieties and sites (data not shown) and this is likely due to the sensitivity of nutrient resorption dynamics to be more stochastically controlled by a variety of variables (e.g., moisture availability, leaf longevity, timing of abscission, etc.) than growing season foliar nutrient status; (Killingbeck et al., 1990; del Arco et al., 1991; Escudero et al., 1992). Notwithstanding the inherent variability in nutrient resorption, at the end of the four-year rotation, this dynamic nutrient pool accounted for 99, 59, 82, 73, 31, and 30% of the N, P, K, S, Ca, and Mg, respectively, found within the harvested biomass (Tables 4.6 and 4.7) and, therefore, represents a critical recycled nutrient pool within SRC willow plantations.

4.6.3.3 Below-ground biomass nutrients

The biomass and nutrient status estimates of willow stool, fine and coarse root tissues in this study are within the range of values reported in the literature (Hytönen, 1995a; Rytter, 1999; Matthews, 2001; Rytter, 2001; Zan et al., 2001; Püttsepp et al., 2007; Pacaldo et al., 2011; Rytter, 2012). The average total biomass productivity of the six willow varieties across the four sites at the end of the four-year rotation was 39 Mg ha^{-1}; with approximately ⅓ of the C allocated to below-ground biomass (32%) and the remaining 19 and 49% apportioned to leaves and stems, respectively (Tables 3.3, 4.6, and 4.8). Furthermore, the majority of immobilized N, P, and S were found in the below-ground biomass, while the majority of K, Ca, and Mg were present in the above-ground biomass, primarily the leaf litter. The bulk of both biomass (84%) and nutrient content (95%) dedicated to below-ground tissue were associated with fine roots (Table 4.8), which highlights the relative importance of the fine root fraction in SRC willow production systems (Rytter, 2012). Generally, the productivity trends among stool and fine and coarse root biomass follow that of both stem and leaf production, with the exception of fine root growth at Estevan (Tables 3.3, 4.6, and 4.8).

The physiology of allocating C to willow roots is dependent on many biotic and abiotic factors. Under adverse soil moisture and nutrient conditions, up to 70% of the metabolic assimilate can be directed to root tissue production; however, under ideal growing conditions that support intense stem growth, the competition for photosynthates or non-structural carbohydrates (if photosynthates are insufficient) can occur, which favours stem growth over root growth (Ericsson, 1984; Ericsson et al., 1996). As previously mentioned, in addition to having fertile soil, the superior growing season conditions at Estevan supported greater willow productivity throughout the rotation. With ample soil moisture and nutrients readily available for plant uptake, there was no need for the growing willow at Estevan to allocate valuable photoassimilates to root production for enhancing resource acquisition (Martin and Stephens, 2006b). Weih and Nordh (2005) highlight the inverse relationship between leaf area productivity (i.e., stem production per leaf area) and root growth and was confirmed in this study with the Estevan willow having the highest calculated leaf area productivity values concomitant with the least fine root productivity (data not shown).

4.6.3.4 Nutrient Leaching

The effect of site disturbance and minimal vegetation cover attendant with SRC willow establishment, especially on previously agricultural land, can often result in appreciable nutrient leaching losses (Mortensen et al., 1998; Jug et al., 1999a; Schmidt-Walter and Lamersdorf, 2012; Ens et al., 2013). Such short-term losses, however, are offset by smaller losses during the 20+ year life-span of SRC willow systems, compared to characteristic nutrient leaching losses incurred with annual crop production systems over the same time frame (Goodlass et al., 2007). Given the similarity in pre- and post-planting management among the four sites in this study, it was assumed that the site disturbance differences among sites were negligible. The estimated nutrient leaching losses during the initial four-year rotation are in agreement with other studies (Mortensen et al., 1998; Park et al., 2004; Balasus et al., 2012), albeit at the lower end of available data in the literature. The relatively minor leaching losses observed are no doubt related to the semi-arid conditions in Saskatchewan, but also the absence of any nutrient-laden amendments within this study compared to other published work. The majority of previous SRC willow leaching studies strictly examined N and P, because of their association with non-point source pollution effects on proximal water bodies. In this study, leaching of N, P, K, S, Ca, and Mg primarily occurred during the establishment year and first year-post-coppice; with the proportion of total nutrients leached decreasing each year of the rotation at all sites and more than 90% occurring during the spring period between snowmelt and bud burst (data not shown). Similar inter- and intra-seasonal trends have been reported previously (Park et al., 2004; Goodlass et al., 2007; Schmidt-Walter and Lamersdorf, 2012) and presumably are consequence of reduced soil water and nutrient demand due to a combination of an undeveloped root system during the first two years, along with delayed root activity and associated water and nutrient uptake prior to bud break each spring, when early season growth is primarily supported by nutrient supply translocated internally from perennial organs (Ericsson, 1994b; Millard, 1996; von Fircks et al., 2001). The moisture deficit during each growing season was manifest in the lack of percolating water through the soil profile during the summer months, which frequently resulted in no sample collection within lysimeters, as has been reported elsewhere (Heinsoo and Holm, 2010).

For every nutrient except Mg, the leaching losses from the plant available nutrient pool were more than compensated for by atmospheric deposition (Tables 4.5 and 4.9). Given the extensive deep-rooted fibrous perennial root system of willow and high rates of canopy interception and evapotranspiration, there is minimal percolation of precipitation through the soil profile during the growing season and, therefore, can be successfully used as a vegetation filter for wastewater management practices (Börjesson, 1999; Dimitriou et al., 2012a; Schmidt-Walter and Lamersdorf, 2012). The measured N leaching losses after four years at Estevan were 3.5× larger than the other sites, but given the more favourable soil moisture and nutrient conditions at Estevan such increased N leaching is to be expected (Dimitriou et al., 2012a). The remaining trends in nutrient leaching losses among sites are primarily attributable to their different soil types (Table 4.2). Specifically, P leaching was greatest at Prince Albert and this reflects the much larger residual soil P pool in that soil. The greater leaching losses of base cations (e.g., K, Ca, and Mg) and S observed at Prince Albert is due to the lower cation-exchange capacity (data not shown) and organic matter content of this sandy soil, which was not as capable of retaining nutrient ions within its profile. Base cation leaching is often cited to explain the relative decrease in soil pH following land conversion to SRC willow; however, give the semi-arid climate of Saskatchewan and phreatophytic nature of willow, leaching accounted for less than 10% of base cation removal from soil in this study, with the remainder sequestered in plant tissues, 53% of which was located in leaf litter (Tables 3.3, 4.7, 4.9, and 4.10).

Despite its limited nutrient retention capacity, the inherently poor N supplying soil helps to explain the minimal N leaching losses at Prince Albert (Table 4.9). Despite being the highest ranked agricultural soil in this study, the measured N leaching losses at Birch Hills were the same as the least fertile soil at Prince Albert and this is attributed to N uptake by non-crop vegetation. Although vegetation management during the rotation was adequate for all four sites (Section 4.4.1), Birch Hills received the least weed control of all sites because of logistical constraints. Consequently, during the initial two years prior to canopy closure, there was an understory component consisting of volunteer canola (previous crop; *Brassica napus* L., cv. Invigor 2733), along with Canada thistle (*Cirsium arvense* L.) that blanketed the plantation until it was eventually shaded out by the overstorey willow. In the meantime, this non-crop component probably acted as a sink for available soil N that would have otherwise been leached out of the system at Birch Hills, as observed with Estevan and Saskatoon with soils of similar N supplying power (Tables 4.2, 4.3, and 4.9). While N uptake by weeds is lost to willow uptake that year, the experience at Birch Hills raises an interesting point. When trying to manage for long-term site sustainability, maintaining a vegetation cover on a site is advantageous in minimizing N lost from the ecosystem via N loss pathways (i.e., erosion, leaching, and denitrification), especially on N-deficient sites such as Prince Albert. However, non-crop species are known to have a detrimental effect on the establishment and growth of planted willow. Perhaps a synergism can exist by introducing a non-competitive underseeded perennial cover-crop prior to plantation establishment, which will not only act as a temporary nutrient sink until the willow becomes established, but also control the more noxious annual weeds. Adiele and Volk (2011) showed that white clover (*Trifolium repens* L.) can successfully reduce both soil erosion and suppressed weed growth during the establishment year without affecting willow growth. Although leaching losses were not assessed in their study, the ability of white clover to assimilate soil inorganic N when available, despite its ability to fix atmospheric N_2, is apparent (Griffith et al., 2000) and, therefore, possibly represents an effective management tool balancing the needs of the willow with the long-term soil nutrient budgets. Underseeding the SRC willow with a non-competitive leguminous perennial is unquestionably more advantageous compared to underseeding with antagonistic competitive grasses that will decrease willow productivity (Scholz and Ellerbrock, 2002).

4.6.4 Transfers into the plant available soil nutrient pool

4.6.4.1 Soil organic matter mineralization

The pattern of soil N and S mineralization contributions to nutrient supply among soil types are primarily related to the relative differences in organic matter content. Nevertheless, the similarity in S mineralization potential between the soils at Birch Hills (highest organic matter content) and Prince Albert (lowest organic matter content) is surprising, although the indefinite relationship between S mineralization and organic matter content has been recognized for some time (Williams, 1967) and may be a function of relatively low sulfatase activity in the Birch Hills soil (Tabatabai and Bremner, 1970). The measured soil N and S mineralization rates are typical for Saskatchewan soils (Roberts, 1985), with these annual inputs relatively more important for N compared to S, representing an average of 59% of the initial extractable N across the sites, compared with only 8% for S (Tables 4.2 and 4.4).

4.6.4.2 Leaf litter decomposition and fine root turnover

Modelling efforts aimed at estimating the climate change mitigation potential of SRC willow plantations are highly dependent on reliable input parameters; in particular, litter decomposition data for predicting the magnitude of C sequestration in these plantations over the long-term (Amichev et al., 2012; Rytter, 2012). Additionally, the agronomic significance of immobilized leaf litter nutrient cycling, within the context of plantation sustainability and fertility management, has been recognized for some time (Ericsson, 1984). The decomposition of plant tissue (e.g., leaf litter or fine root turnover) is a primary mechanism for C and nutrient cycling within most terrestrial ecosystems (Berg and Laskowski, 2005; Rytter, 2012) and SRC willow plantations are certainly no exception. The estimates of leaf and root litter N cycling are at the lower end of literature values, but this is a function of not only the lower biomass productivity of these plantations, but also the values are estimates of actual nutrient additions to the plant available nutrient pools, as opposed to total N contents of leaf and root litter tissue reported elsewhere. Regardless, these findings support the contention that nutrient cycling from leaf litter decomposition and fine root turnover are important nutrient cycling mechanisms that help to satisfy the nutritional demands of SRC willow plantations (Christersson, 1986; Ericsson, 1994a; Rytter, 2001). The Saskatoon soil received 65% less nutrient contributions from leaf litter, but 26% more nutrient contributions from fine root turnover compared to the other sites (Tables 3.7 and 4.10) and this is primarily a function of the difference in biomass allocation (i.e., root growth favoured) under the drier growing season conditions at Saskatoon throughout the rotation. The significant ($P < 0.05$) variety × site interaction effect on leaf litter and fine root turnover nutrient additions were primarily due to differences in biomass instead of variation in tissue nutrient concentrations (data not shown).

4.6.4.3 Canopy exchange

When throughfall measurements are used to quantify atmospheric deposition contributions to plant available nutrient pools, it is important to distinguish between atmospheric nutrients and intrasystem nutrient transfers recycled through canopy exchange, along with quantifying foliar leaching for improving the accuracy of foliar nutrient resorption estimates (Duchesne et al., 2001; Staelens et al., 2008). The canopy budget model was useful in determining the relative importance of DD and CE contribution to NTF and their proportional influence varied depending on the nutrient. Throughout the rotation, the willow canopy acted as a sink for atmospheric N, while 69, 59, and 20% of the NTF flux of P, K, and Ca, respectively, originated from canopy leaching, and S and Mg were entirely DD with negligible canopy interactions (Table 4.5). The influence of DD and CE increased each year of the rotation at all sites for all nutrients (data not shown) and this is presumably due to increasing leaf surface area as the willow canopy matured. The CE trends observed in this study, namely foliar consumption of atmospheric N (dominated by NH_4^+-N), foliar leaching of P, K, and Ca, and strictly DD contributions of S and Mg, are commonly reported in the literature (Duchesne et al., 2001; Park et al., 2004; Meiresonne et al., 2007; Staelens et al., 2008). There were no significant differences ($P > 0.05$) among sites for any estimated canopy variable and while this is not surprising considering only one variety was examined, it does point to the lack of genotype × environment effect on canopy-atmosphere interactions for a *S. miyabeana* variety, despite large differences in total growing season precipitation and leaf biomass among sites across a broad geoclimatic area (Tables 3.3 and 4.1).

4.6.5 Changes in available soil nutrient pools

Although there were no significant differences ($P > 0.05$) in measured plant available N pool deficits among the four sites after the initial rotation, when the net soil N balance was expressed as a percent change in initial extractable N pool level, the Prince Albert soil experienced the greatest percent depletion and this is due to the relatively poor N supply power of this coarse-textured soil (Table 4.4). The smallest soil P deficit occurred at Estevan and probably is due to reduced P sequestered in leaf and fine root biomass compared to the other sites (Tables 4.7 and 4.9). The most intriguing observation in this study was the more than 200% decrease in initial extractable soil P pool calculated at Birch Hills (Table 4.12). At this site, approximately 55 kg P ha^{-1} were required to produce approximately 41 Mg ha^{-1} of above- and below-ground biomass over four years, despite an initial available soil P level of only 16 kg ha^{-1} (Tables 4.2, 4.6, 4.7, and 4.9). The Birch Hills site was the only willow plantation established immediately following an annual crop production year. Instead of previously fallowed land, the Birch Hills plantation was planted following a high-yielding canola crop that would have depleted soil P levels and contributed to the calculated soil P deficiency (approximately 10 kg P ha^{-1} yr^{-1}) during the first rotation. It seems reasonable to assume that this apparent shortfall in soil P availability observed at Birch Hills was satisfied by the well-established ability of mycorrhizae to augment soil mineral weathering under conditions of low P availability (Hoffland et al., 2004; Rosenstock, 2009), especially given the synergism between these beneficial root-associated fungi and SRC willow (Püttsepp et al., 2004; Dimitriou et al., 2011; Corredor et al., 2012).

The consistently large K content in the willow biomass growing at Birch Hills relative to other sites explains the greatest soil K deficit observed there; however, the percent depletion in soil K at Birch Hills was not significantly different ($P > 0.05$) than Estevan or Prince Albert (Table 4.12). The smallest percent soil K depletion occurred at Saskatoon and can be attributed to not only its relatively poor production, but also the abundant K-bearing clay minerals in Saskatoon, which provided the largest available soil K level of all sites (Table 4.2).

Sulfur was the only nutrient with a calculated soil supply surplus after the initial rotation, with the largest surplus occurring at Estevan primarily due to a combination of less S immobilized in fine roots, but also greater S release from SOM mineralization (Tables 4.4 and 4.9). The largest percent soil S replenishment occurred at Prince Albert and is not surprising given the relatively poor S supplying ability of this sandy soil (Tables 4.2–4.4). Although there was no significant difference ($P > 0.05$) in atmospheric S deposition among the sites (average of 8.6 kg S yr^{-1}; data not shown), the proportional contribution of this atmospheric deposition to available soil S supply was greater for the Prince Albert soil due to its smaller initial S level.

Despite having the largest calculated nutrient deficits, the lack of calculated differences in available soil Ca among the sites is indicative of the calcareous nature of Saskatchewan soils, which have abundant plant available Ca (Table 4.2). The lone dissimilarity in available soil Mg deficits among sites occurred between Birch Hills and Prince Albert and the larger deficit in Birch Hills was due to its greater productivity and associated Mg sequestered in above-and below-ground tissues (Tables 4.2, 4.6, 4.7, and 4.9). Similar with soil K, the smallest percent soil Mg depletion at Saskatoon can be attributed to its relatively poor productivity during the rotation and its soil clay mineralogy providing the largest Mg supply (Table 4.2). The only measured difference in calculated changes in plant available nutrient pools among the six willow varieties across the four sites occurred with less K and Ca removals from soil by the variety Fish Creek compared to Canastota (data not shown). Although there were significant ($P < 0.05$) variety × site effects on biomass production (Tables 4.6 and 4.9), the relative lack of observed differences in soil nutrient withdrawal among varieties, evidently must be due to the offsetting significant ($P < 0.05$) variety × site effects on above- and below-ground tissue nutrient concentrations (i.e., nutrient use efficiency) concomitant with different phenotypic responses to productivity gradients.

4.6.6 Overall nutrient budget after initial four-year rotation

4.6.6.1 Nitrogen

At the end of the initial four-year rotation, soil N outputs were seven percent greater than soil N inputs, resulting in an average removal of 17 kg N ha^{-1} across varieties and sites, representing a 24% decrease in soil N reserve (Table 4.12). The contributions of the different system inputs to willow N demand were: soil mineralization (55%), atmospheric deposition (16%; including CE), leaf litter decomposition (7%), and fine root turnover (15%). Approximately 21 kg N ha^{-1} was scavenged from atmospheric deposition by the willow leaves during the three years post-coppice (Table 4.5) and considering this atmospheric N did not originate from the soil, but was a component of the budgeted immobilized biomass N outputs, the net soil N balance value was corrected accordingly (Table 4.12). Removing approximately 25% of the soil N reserve during the initial rotation is substantial, especially when expecting a 30% increase in above- and below-ground productivity (with a concomitant increased nutrient demand) during the subsequent rotation (Pacaldo et al., 2011; Volk et al., 2011). However, it is important to recall that more than half of the leaf litter nutrients immobilized during the first rotation were not included in the budget. Specifically, the remaining leaf litter nutrient contributions (i.e., a single year of nutrient release from the first year post-coppice leaf litter decomposition, two years of nutrient release from the second year-post coppice leaf litter, and three-years of nutrient release from the harvest year leaf litter) will be released during the second rotation. Consequently, assuming a 30% increase in nutrient demand during the second rotation compared to the first, the residual leaf litter decomposition nutrients from the first rotation will provide approximately 42% of the soil N demand during the second rotation. Likewise, N will be cycled from fine root turnover immobilized during the first rotation and will provide approximately 49 kg N ha^{-1} over the next two rotations, assuming a 20% $year^{-1}$ release (Püttsepp et al., 2007) and 89% $LV_{Biomass}$ (Rytter, 2012).

Although fine roots represent a larger sink for immobilized nutrients within SRC willow plantations (Tables 3.3 and 4.8), fine roots have a slower decomposition rate compared to leaf litter and, therefore, represent a more slow-release nutrient source over time. The ability of leaf litter decomposition and nutrient cycling to satisfy a large portion of N demand during subsequent rotations has been recognized previously (Ingestad and Ågren, 1984; Christersson, 1986; Ericsson, 1994a), and when coupled with nutrient additions from fine root turnover, soil mineralization, atmospheric deposition, foliar nutrient resorption, along with its relatively low nutrient demanding nature, it is not surprising to see the lack of willow growth response to added fertilizer often reported in the literature, especially when grown on good agricultural soils (Chapter 5).

Despite the efficient internal nutrient cycle within SRC willow plantations, with minimal system losses through either denitrification (e.g., Schmidt-Walter and Lamersdorf, 2012) or leaching (e.g., Table 4.9), it is prudent to assume that a portion of recycled nutrients will be immobilized by the microbial community each year and in the case of N, the relatively unavailable recalcitrant stable organic N fraction. Additionally, considering the expected lifespan of a willow plantation is seven rotations (Heller et al., 2003) and changes will occur in above- and below-ground growth allocation patterns in later rotations as more biomass is allocated to stem growth at the expense of reduced root and leaf growth (Hytönen, 1995a; Weih and Nordh, 2005), which will reduce nutrient cycling from these tissues. Consequently, I would concur with the conventional practice of applying sufficient fertilizer to compensate for nutrient losses associated with harvesting willow stems (Ericsson, 1994b; Abrahamson et al., 2002), which would equate to approximately 25 kg N ha^{-1} yr^{-1} with the current production levels.

4.6.6.2 Phosphorus

At the end of the initial four-year rotation, soil P outputs were 144% greater than soil P inputs, resulting in an average removal of 40 kg P ha^{-1} across varieties and sites, representing a 105% decrease in soil P reserve (Table 4.12). However, the average percent change in soil P level across sites in the overall budget is affected by the disproportionate influence of the Birch Hills soil P budget, which had a calculated decrease of 242% in soil P reserves compared with 54% for the other three sites (data not shown). The contributions of the different system inputs to willow P demand were: atmospheric deposition (17%; including CE), leaf litter decomposition (6%), and fine root turnover (18%). There may also be some contribution from mineralization of soil organic P that was not measured in this study. As with the deferred N release, P immobilized during the first rotation within leaf litter will provide 36% of the soil P demand during the second rotation, while fine root turnover will supply approximately 19 kg P ha^{-1} over the next two rotations.

The largest calculated depletion in soil nutrient reserves among the sites after the first rotation occurred with soil P and maintaining adequate soil P levels is important for not only supporting long-term site productivity, but also minimizing risk of disease and herbivory (Jug et al., 1999b). In addition to conventional inorganic fertilizer amendments, returning residual wood ash back to the plantation can effectively restore soil P supply (Scholz and Ellerbrock, 2002; Park et al., 2005). Wastewater sludge and pig slurry are other alternative fertilizer amendment successfully used to improve soil P fertility within SRC willow plantations, although care must be taken in choosing appropriate application rate and soil type to prevent groundwater contamination (Labrecque et al., 1998; Cavanagh et al., 2011; Dimitriou et al., 2012a). Given its diffusion-limited nature in soil, P is relative immobile compared to other nutrients and, therefore, fertilizer P additions (inorganic or organic) will typically accumulate near the surface, which is proximally ideal in terms of availability to the majority of willow roots. The degree of soil P depletion observed among the four sites and projected need for fertilizer P going forward is surprising given the relatively low P demand of willow often reported in the literature, even on sandy soil with inherently low levels of P-bearing minerals (e.g., Boelcke and Kahle, 2008). Using data from this study, P inputs from soil mineral weathering and atmospheric additions are inadequate to meet the soil P demand of the growing willow and applications of 25 kg P ha^{-1} year^{-1} may be required to maintain the soil P supply under the expected willow growth and internal P cycling regime. Some consideration of the possible contributions from organic P mineralization and enhanced P solubilisation and uptake through beneficial fungal relationships should be considered in future studies of P cycling in SRC willow systems on the prairies.

4.6.6.3 Potassium

At the end of the initial four-year rotation, soil K outputs were 100% greater than soil K inputs, resulting in an average removal of 113 kg K ha^{-1} from the soil across varieties and sites, representing a 9% decrease in soil K reserve (Table 4.12). The contributions of the different system inputs to willow K demand were: soil mineral weathering (6%), atmospheric deposition (15%; including CE), leaf litter decomposition (21%), and fine root turnover (8%). Likewise, the first rotation leaf litter will provide 81% of the soil K demand during the second rotation, while fine root turnover will supply approximately 27 kg K ha^{-1} over the next two rotations. Despite relatively large soil K deficits over multiple rotations, a sizable soil K reserve helps to mitigate the impact, as observed elsewhere (Labrecque et al., 1998; Hofmann-Schielle et al., 1999; Kahle et al., 2010). Returning wood ash to the site can also assist in maintaining soil K fertility (Scholz and Ellerbrock, 2002; Park et al., 2005).

4.6.6.4 Sulfur

At the end of the initial four-year rotation, inputs to the soil S reserve were 62% greater than outputs, resulting in an average surplus of 60 kg S ha^{-1} across varieties and sites, representing a 17% increase in soil S reserve (Table 4.12). The contributions of the different system inputs to soil S supply were: soil organic matter mineralization (70%), atmospheric deposition (15%; including CE), leaf litter decomposition (7%), and fine root turnover (8%). Also, the first rotation leaf litter will provide 47% of the soil S demand during the second rotation, while fine root turnover will supply approximately 18 kg S ha^{-1} over the next two rotations. Given the measured soil S surplus at each site, along with the apparent low S-demanding nature of the varieties examined, I see no concern for the long-term soil S supplies limiting willow growth at any site. With limited available information on S cycling from other environments where willow is grown, presumably more investigations of S dynamics within SRC willow plantations will be forthcoming.

4.6.6.5 Calcium

At the end of the initial four-year rotation, soil Ca outputs were 130% greater than soil Ca inputs, resulting in an average removal of 282 kg Ca ha^{-1} across varieties and sites, representing a 2% decrease in the measured soil Ca reserve (Table 4.12). The contributions of the different system inputs to willow Ca demand were: soil mineral weathering (9%), atmospheric deposition (11%; including CE), leaf litter decomposition (24%), and fine root turnover (3%). Moreover, the first rotation leaf litter is predicted to provide 85% of the soil Ca demand during the second rotation, while fine root turnover will supply approximately 24 kg Ca ha^{-1} over the next two rotations. The largest measured nutrient removals from soil reserves in this study occurred with Ca, however, chronic large exports of soil Ca over multiple rotations will have minor impact on the long-term soil Ca supply for the majority of Saskatchewan soils and this is consistent with other reports (Labrecque et al., 1998; Hofmann-Schielle et al., 1999; Kahle et al., 2010). Specifically, the soil parent material within the bottom 2/3 of the province is glacial drift derived from Devonian sedimentary bedrock, which primarily consists of limestone and dolomite, resulting in abundant Ca and Mg supply (Richards and Fung, 1969; Rennie and Ellis, 1978).

4.6.6.6 Magnesium

At the end of the initial four-year rotation, soil Mg outputs were 122% greater than soil Mg inputs, resulting in an average removal of 74 kg Mg ha^{-1} across varieties and sites, representing a 2% decrease in soil Mg reserve (Table 4.12). The contributions of the different system inputs to willow Mg demand were: soil mineral weathering (12%), atmospheric deposition (17%; including CE), leaf litter decomposition (18%), and fine root turnover (12%). In addition, the first rotation leaf litter will provide 63% of the soil Mg demand during the second rotation, while fine root turnover will supply approximately 18 kg Ca ha^{-1} over the next two rotations. Depletion of soil Mg reserves were second only to soil Ca depletion, but similar to Ca, the majority of Saskatchewan soils have abundant Mg supply, which along with Ca, should support multiple SRC willow rotations. Although such ample soil Mg supply is common (Labrecque et al., 1998; Hofmann-Schielle et al., 1999), it is certainly not universal. For example Kahle et al. (2010) expressed concern regarding the effect of multiple SRC willow rotations on sustainable soil Mg supply; however, their extractable soil Mg level was approximately 350× less than the average extractable level of the soils in this study.

4.6.7 Long-term soil fertility and SRC willow sustainability

Future energy crop production is most likely to occur on marginal agricultural land in order to avoid conflict with food production and compromising food security (Volk and Luzadis, 2009; Aylott et al., 2010). Previous studies have highlighted the ability of willow to grow with minimal soil nutrient demand on a variety of soil types (Scholz and Ellerbrock, 2002; Quaye, 2011; Stolarski et al., 2011) and this study was no exception, with some varieties achieving annual production greater than 10 Mg ha^{-1} yr^{-1} on the sandy soil at Prince Albert without fertilization during the rotation (data not shown). However, these results indicate that despite the relatively low nutrient-demanding nature of willow, long-term soil productivity will need to be managed with nutrient amendments, particularly N and P, albeit at application rates that are a fraction of what is required to sustain the growth of annual crops or contemporary alternative biomass energy crops. Furthermore, unlike first generation bioenergy crop residues or second generation (e.g., *Miscanthus*, switchgrass, and reed canary grass) feedstocks requiring chronic removals of all above-ground biomass, the SRC willow plantations in this study cycled more than seven tonnes per hectare of leaf litter biomass prior to harvest (Table 3.3), which will play an important role not only in long-term nutrient cycling, but also augmenting SOM levels (Lal, 2009).

There are many biotic and abiotic factors that can have a deleterious impact on SRC willow plantation productivity, including: drought and winter kill, animal and insect herbivory, disease, and competition from non-crop vegetation. Given the many uncontrollable factors that can potentially affect plantation productivity, it is prudent to effectively manage something that can be controlled, namely preventing nutrient deficiencies through proper soil fertility management, in order to better manage the risk by producing high quality vigorous plants that are more resilient to external stressors. Assessing the soil nutrient supply prior to plantation establishment will assist in the selection of suitable afforestation sites and allowing economically and environmentally appropriate fertility amendment decisions to be made for promoting successful plantation establishment and growth. Optimizing fertilization practices by applying gained knowledge of the underlying biogeochemical cycling of soil nutrients is necessary for maximizing the economic, environmental, and societal benefits commonly associated with SRC willow production. With its relatively small population, coupled with a large areal extent of suitable afforestable land base, Saskatchewan is well-positioned to become a world-leader in developing renewable dedicated SRC willow biomass energy systems. In order to realize this potential, however, effective incentive programs, similar to those successfully implemented in other jurisdictions (El Kasmioui and Ceulemans, 2012; Buchholz and Volk, 2013), are required to facilitate the expansion of SRC willow on the millions of suitable hectares in the province.

4.6.8 Additional agronomic considerations

Characterizing the nutrient status of a plant, in particular, estimating the relative nutrient demands of a given woody crop using relative tissue nutrient contents, has been practiced for some time (Wittwer and Immel, 1980). Considering N is often the principal soil nutrient governing willow growth (Labrecque et al., 1998; Weih and Nordh, 2005), researchers often examine and report the relative proportions of willow stem nutrients normalized according to N content (i.e., N = 100%, P = P content/N content × 100, K = K content/N content × 100, etc.). Additionally, critical stem nutrient concentrations or numerous iterations of intranutrient ratios (e.g., N:P, N:K, P:K, Ca:Mg, etc.) are used as a diagnostic tool. While these approaches have been successfully used to detect existing growth-limiting nutrient limitations with SRC willow plantations, an important limitation is their inability to identify important trends in shifting nutrient demand over time. For instance, comparing the change in N-normalized stem tissue P concentration of one-year-old coppiced willow stems with three-year-old post-coppice harvested stems growing at Birch Hills (Table 4.13), indicates that relative to stem N content, the P content increased for half of the varieties, while two decreased in relative P content, and one did not change over time. When trying to manage for long-term soil productivity of plantations, it is imperative to know the relative demands of each nutrient as the plantation matures. Using the conventional tissue nutrient diagnostic approach, the land manager will naturally assume either the plant demand for N decreased or P demand increased over time. However, when each stem nutrient content is expressed in terms of total nutrient content analyzed (e.g., N : N/(N + P + K + S + Ca + Mg × 100), it is not only apparent that there are no significant differences ($P > 0.05$) in N and P status among varieties, but also, for all but one variety, both N and P demand actually decreased relative to other nutrients as the willow matured (Table 4.13). Moreover, the reduction in N demand is greater than P for the varieties Alleghany and Canastota, similar reduction for Fish Creek and Sherburne, but a greater reduction in P demand than N for SX61. For the variety SX64, N demand decreased, while P demand increased during the rotation. At Birch Hills, the relative S demand of growing willow over time increased between 19.9-65.7% relative to other nutrients (Table 4.13), while the relative demands of K and Ca increased (33.2 and 0.5%, respectively; data not shown), and Mg decreased (-12.2%; data not shown). Normalizing nutrient contents relative to total stem nutrient content broadens the context and allows relative differences among nutrients including N (which is no longer always 100 and thus rendered uninterpretable by itself) to be recognized and acted upon. In the case of this study, this approach provides only six numbers to easily compare among varieties and sites for interpreting changing nutrient status and concomitant nutrient demands of willow during the initial four-year rotation. The overall ratio (including all varieties and sites) indicating the percent change in relative nutrient content (i.e., demand) between comparing harvested stem biomass with coppiced biomass was -4 : -1 : 42 : 59: -1: -21; indicating nominal decreases in the relative contents of N, P, and Ca over time compared to significant increases in K and S and decreased Mg, which is important information for a land manager to know when deciding fertilization

Table 4.13. Mean (n = 4) nitrogen (N), phosphorus (P), and sulfur (S) status of stems (bark + wood) of several willow varieties exported offsite during an initial four-year rotation at Birch Hills, Saskatchewan, Canada.

Variety	N	P	S	N	P	S
	($\%_{N\text{-}100}$)[†]			($\%_{Total\text{-}100}$)[‡]		
--------------------- Coppiced biomass one year after planting ---------------------						
Allegany	100	14.0a[§]	13.7a	30.1a	4.2a	4.1a
Canastota	100	14.9a	10.2b	25.8a	3.8a	2.6b
Fish Creek	100	14.7a	10.0b	31.0a	4.5a	3.1ab
Sherburne	100	15.4a	11.1ab	26.4a	4.0a	2.9ab
SX61	100	14.4a	10.6ab	26.5a	3.8a	2.8ab
SX64	100	14.5a	9.5b	25.5a	3.7a	2.4b
------------------- Harvested biomass three years after coppicing -------------------						
Allegany	100	14.3c	18.6a	26.1a	3.7ab	4.8a
Canastota	100	16.4ab	15.9a	21.8ab	3.6ab	3.5ab
Fish Creek	100	14.7bc	18.2a	26.2a	3.8a	4.7a
Sherburne	100	15.3abc	16.4a	22.3ab	3.4ab	3.6ab
SX61	100	13.8c	14.7a	21.2b	2.9b	3.1b
SX64	100	17.1a	16.4a	24.1ab	4.1a	3.9ab
--- Percent change in tissue nutrient status between 1-year vs. 3-year biomass ---						
Allegany	-	3.0ab[¶]	41.0a	-12.8a	-10.0a	24.9a
Canastota	-	7.5ab	57.1a	-10.2a	-4.0a	42.8a
Fish Creek	-	0.0b	81.2a	-15.0a	-14.9a	53.1a
Sherburne	-	-0.1b	39.2a	-12.9a	-12.8a	19.6a
SX61	-	-4.6b	73.3a	-14.1a	-18.0a	65.7a
SX64	-	18.3a	48.0a	-3.4a	13.6a	27.1a

[†] Normalized to N content in tissue (e.g., N content/N content × 100)

[‡] Normalized to total nutrient content in tissue (e.g., N/(N + P + K + S + Ca + Mg) × 100)

[§] Means within a column followed by the same letter are not significantly different ($P > 0.05$) using LSD

[¶] (Harvested nutrient status - coppiced nutrient status)/coppiced nutrient status) × 100 practices given soil test values. Additionally, considering commercial fertilizers are blended according to their total analysis (i.e., relative proportions among nutrients) and not relative to N content, such a diagnostic approach would have a practical advantage also.

4.7 Conclusion

Establishing SRC willow plantations as a renewable energy feedstock is advantageous for different social, economic, energetic, and environmental reasons. However, there is a fundamental need to examine the sustainability of willow production over multiple rotations. Based on the results from an initial four-year rotation, I found SRC willow plantations to be relatively low nutrient-demanding, with minimal nutrient outputs from the production system other than via harvested biomass. Despite possessing efficient nutrient cycling, observed deficits in plant available soil N and P at the end of the initial rotation suggest that sustaining harvestable yields over multiple rotations will require supplemental fertilizer amendments; albeit using application rates that are a fraction of what is required by annual agronomic crops and other alternative biomass energy species. Amendments maintaining long-term soil fertility will promote the sustainability of these purpose-grown biomass energy plantations, thus helping advance SRC willow as a viable alternative in Canada's bioenergy sector.

5. THE EFFECT OF IRRIGATION ON NITROGEN UPTAKE AND USE EFFICIENCY OF TWO WILLOW (*SALIX* SPP.) BIOMASS ENERGY VARIETIES

5.1 Preface

Chapters 3 and 4 showed that despite efficient leaf litter nutrient cycling contributing a substantial portion of willow nutrient demands, depending on the soil type, the long-term sustainability of short-rotation coppice (SRC) willow plantations will necessitate some type of nutritional amendment, particularly N and P, for supporting its adoption throughout Saskatchewan (Chapter 6). Although N is the foremost soil nutrient influencing SRC willow productivity, reported willow growth response to fertilizer N in the past several decades has been inconsistent. Considering the important role fertilization plays in the net energetic, environmental, and economic consideration for SRC willow production, insights into fertilizer N dynamics can lead to increased fertilizer N use efficiency by willow will be advantageous. Measured increases in fertilizer use efficiency following irrigation is common within the conventional agronomy literature, so it was felt that inclusion of the effects of irrigation on the fertilizer N uptake and use efficiency of ^{15}N-labelled fertilizer by two willow varieties would be desirable. Only a few studies have used ^{15}N-labelled fertilizer to examine fertilizer N dynamics within SRC willow plantations and this is the first to investigate the effect of irrigation on fertilizer N uptake by willow. Note: this paper is published in the Canadian Journal of Plant Science (92: 563-575) and received the 2013 Canadian Society of Agronomy Award for *Best 2012 Agronomy Paper* in the Canadian Journal of Plant Science. The co-author contributions to this manuscript were greatly appreciated and consisted of: J.J. Schoenau (provided financial assistance, field work support, methodological guidance, and manuscript editing); K.C.J. Van Rees (provided financial assistance, field work support, Campbell Scientific CR10X, and manuscript editing); and, J.D. Knight (provided methodological guidance, mass spectrometer analyses, and manuscript editing).

5.2 Abstract

Nitrogen fertilizers historically have been applied to support increased productivity of purpose-grown willow (*Salix* spp.) biomass energy plantations. However, a frequently observed lack of willow growth response to added fertilizer nitrogen (N) often is attributed to poor fertilizer use efficiency. The objective of this study was to determine the effect of irrigation on the recovery of broadcast ^{15}N-labelled fertilizer, applied during the final year of a three-year rotation, by two willow varieties. A split-split-plot experiment was established on a fertile heavy clay soil in Saskatoon, SK, Canada and consisted of two willow varieties (Charlie and SV1), three irrigation treatments (no irrigation, 75%, and 100% field capacity), and two fertilization treatments (1× and 2× the recommended fertilizer rate of 100:30:80:20 N:P:K:S; kg ha^{-1}). Irrigation increased fertilizer N uptake by Charlie, but had no effect on the amount taken up by SV1, which was attributed to greater nitrogen use efficiency of SV1 compared to Charlie when irrigated. Eighty-two percent of the applied fertilizer N was accounted for in the following sinks: 43% in the soil (0-60 cm), 31% in the willow tissues (i.e., stems, leaves, stump, and roots), 7% in the LFH layer, and < 1% in the non-crop vegetation; the balance (approximately 18%) was presumed lost primarily through denitrification from the poorly-drained soil, but possibly some may have leached below the root zone as well. Although the willow varieties accessed only a portion of the applied fertilizer N during the year of application, the majority of the residual fertilizer N was conserved within the production system and, therefore, remained available for willow uptake in subsequent years.

5.3 Introduction

Establishing purpose-grown willow (*Salix* spp.) plantations as a renewable dedicated bioenergy and bioproduct feedstock is advantageous for numerous reasons. Favourable characteristics include its easy propagation and fast-growing nature, along with important environmental benefits like providing a cleaner energy source relative to fossil fuels, acting as an effective vegetation filter for environmentally harmful compounds (e.g., fertilizers, herbicides, insecticides, heavy metals, etc.) and increasing biodiversity within the agricultural landscape (Sage and Robertson, 1994; Reddersen, 2001; Volk et al., 2006; Main et al., 2007). Nitrogen (N) is considered to be the principal soil nutrient influencing willow plantation productivity (Labrecque et al., 1998; Weih and Nordh, 2005). This is not surprising given that N is a critical element in numerous plant components including amino acids, chlorophyll, coenzymes, enzymes, nucleic acids, nucleotides, and proteins (Mengel and Kirkby, 2001). Consequently, inorganic nitrogen fertilizers have been used extensively for several decades, in attempts to promote the successful establishment and growth of planted willow. However, the reported growth response of numerous willow varieties to added fertilizer N when grown under field conditions has been inconsistent (Table 5.1), thereby precluding definitive relationships (i.e., calibrated fertilizer recommendations) between applied fertilizer N rates and subsequent willow biomass yields from being developed and applied universally.

The observed inconsistency in willow growth response to added fertilizer N in field studies has been attributed to a number of biotic and abiotic factors, namely: disease, insect, and herbivory damage (Cambours et al., 2006; Heiska et al., 2007; Toome et al., 2009; Konecsni, 2010); excessive soil moisture (Hofmann-Schielle et al., 1999); fertilizer toxicity(Mortensen et al., 1998); genotypic variability in N requirement, uptake capacity, and/or utilization efficiency (Good et al., 1985; Kopp et al., 1993; Adegbidi et al., 2001; Weih and Nordh, 2005); genotype × environment interactions (McElroy and Dawson, 1986; Hofmann-Schielle et al., 1999; Ballard et al., 2000); inadequate fertilizer N rate, placement and/or timing of application (Mitchell et al., 1999; Adegbidi et al., 2003; Quaye et al., 2011); interspecific competition with non-crop species (Kopp et al., 1993; Ballard et al., 2000; Weih and Nordh, 2005); intraspecific competition (Alriksson, 1997; Kopp et al., 2001; Heinsoo et al., 2009); induced soil nutrient imbalances (Nilsson and Ericsson, 1986; Kopp et al., 1996); inherent soil fertility (Good et al., 1985; Mitchell, 1995; Quaye et al., 2011); reduced cold hardiness (von Fircks, 1994; Hytönen, 1995b; Cambours et al., 2006); soil pH and texture (Kopp et al., 1996; Alriksson, 1997); and sub-optimal growing season temperatures (Alriksson et al., 1997). Given that moisture availability is the primary control influencing the growth of willow, especially within temperate regions (Mitchell, 1992; Lindroth and Båth, 1999), it is not surprising that poor willow growth response to added fertilizer N often is attributed to insufficient precipitation (Mitchell et al., 1992; Alriksson et al., 1997; Weih and Nordh, 2005; Quaye et al., 2011). Adequate soil moisture is fundamental for supporting not only plant growth, but also fertilizer nutrient ion movement (i.e., availability) within the soil.

Studies applying stable isotope enriched fertilizers, such as ^{15}N-labelled ammonium nitrate, to trace the fate of added N are rare within the short-rotation coppice willow literature.

Table 5.1. Reported growth response of willow (*Salix* spp.) to fertilizer N additions under field conditions.

Location	*Salix* Species (variety)	Fertilizer Rate(s) (kg N ha^{-1} $year^{-1}$)	Year(s) Assessed	Relative Yield† (%)	Reference
Canada	*S. purpurea* × *S. miyabeana* (Saratoga), *S. sachalinensis* × *S. miyabeana* (Marcy), *S. viminalis* × *S. miyabeana* (Tully Champion), *S. dasyclados* × *?* (India)	100	2	-20 — 44	Konecsni (2010)
Denmark	*S. viminalis* (78-112), *S. viminalis* (78-183)	37.5 (year 1) 75 (years 2, 3)	1 — 3	-20 — 41	Mortensen et al. (1998)
England	*S. viminalis* (Bowles Hybrid)	60	2 and 4	7 — 26	Mitchell et al. (1992)
Estonia	*S. viminalis* (78021, 78101, 78112, 78183, 78195, 82007), *S. dasyclados* (81090)	60 (year 1) 160 (year 2) 170 (year 3)	1 — 3	-31 — 340	Heinsoo et al. (2002)
Estonia	*S. viminalis* (78183), *S. dasyclados* (81090)	60 (year 1) 160 (year 2) 170 (year 3)	2 and 3	186 — 206	Heinsoo et al. (2009)
Finland	*S.* × *dasyclados* (P6011), *S.* 'Aquatica' (V769)	50,100,150, 200	1 — 3	0 — 312	Hytönen (1994)
Finland	*S.* × *dasyclados* (P6011), *S.* 'Aquatica' (V769), *S.* 'Aquatica' (E4856)	100	1 — 3	-20 — 270	Hytönen (1995a)

Table 5.1. (continued).

Location	*Salix* Species (variety)	Fertilizer Rate(s) (kg N ha^{-1} $year^{-1}$)	Year(s) Assessed	Relative Yield[†] (%)	Reference
Germany	*S. viminalis*	50, 100	1 — 5 6 — 10	-37 — 73	Hofmann-Schielle et al. (1999)
Germany	*S. schwerinii* × *S. viminalis* (Tora)	40, 80	1— 3	-8 — 1	Boehmel et al. (2008)
Ireland	*S.* × 'Aquatica Gigantea' (Korso)	45 — 250	1 — 6	7 — 9	McElroy and Dawson (1986)
Sweden	*S. viminalis* (78021, 78101, 78112, 78183), *S. dasyclados* (81090)	45, 60, 75, 90, 150, 180	1— 4	-29 — 788	Alriksson (1997)
Sweden	*S. schwerinii* E. Wolf. × *S. viminalis* L. (Björn), *S. burjatica* Nasarow × *S. dasyclados* Wimm. (Gudrun), *S. viminalis* (Jorr), *S. dasyclados* (Loden), *S. schwerinii* × *S. viminalis* (Tora), (*S. schwerinii* × *S. viminalis*) × *S. viminalis* (Tordis)	100 (second year) 90 (third year)	1 — 3	78 — 142	Weih and Nordh (2005)
USA	*S. dasyclados* (SV1), *S. alba* (L.) (SA22), *S. alba* var. sanquinea (SA2), *S.* × *rubra* (SAM3), *S. purpurea* (L.) (SH3)	336	1 — 5	-7 — 175	Kopp et al. (1993)

Table 5.1. (continued).

Location	*Salix* Species (variety)	Fertilizer Rate(s) (kg N ha^{-1} $year^{-1}$)	Year(s) Assessed	Relative Yield[†] (%)	Reference
USA	*S. purpurea* L. (SP3)	336	1 — 5	-17 — 54	Kopp et al. (1996)
USA	*S. eriocephala* × *S. eriocephala* (S25), *S. exigua* × *S. eriocephala* (S301), *S. eriocephala* × *S. eriocephala* (S546), *S. alba* var. sanquinea (SA2), *Salix dasyclados* (SV1)	100, 200, 300	2	-83 — 500	Ballard et al. (2000)
USA	*S. dasyclados* (SV1), *S. alba* (L.) (SA22), *S. alba* var. sanquinea (SA2), *S.* × *rubra* (SAM3), *S. purpurea* (L.) (SH3)	224	1 — 9	2 — 43	Adegbidi et al. (2001)
USA	*S. dasyclados* (SV1), *S. alba* var. sanquinea (SA2), *S. purpurea* (L.) (SH3)	336	1 — 10	-15 — 87	Kopp et al. (2001)
USA	*S. dasyclados* (SV1)	100, 200, 300	1 — 3	7 — 33	Adegbidi et al. (2003)
USA	*S. sachalinensis* (SX61), *S. discolor* (S365)	90	1	-4 — 79	Arevalo et al. (2005)
USA	*S. dasyclados* (SV1)	100	1 — 3	-18 — 33	Quaye et al. (2011)

[†]Measured oven-dried stem wood yield relative to willow grown in non-fertilized plots (e.g., -20 — 44 indicates the reported growth response to added fertilizer N ranged from a 20% decrease to a 44% increase in yield among treatments, relative to the control)

This is surprising given the well documented efficacy of ^{15}N in tracking the fate of applied fertilizer N within traditional forestry plantations─ regardless if the fertilizer is side-banded (Sadanandan Nambiar and Bowen, 1986), broadcast across the soil surface and/or tree canopy (Staples et al., 1999; Bryan Dail et al., 2009), or placed adjacent to the outplanted seedling root plug within a controlled-release fertilizer bag (Hangs et al., 2003). The objective of this study was to determine the effect of irrigation on the fertilizer N uptake and use efficiency of ^{15}N-labelled fertilizer, by two willow varieties, within a three-year-old willow plantation. To our knowledge, this is one of only a few studies that have used ^{15}N-labelled fertilizer to examine fertilizer N dynamics within short-rotation willow plantations (Christersson, 1987; Konecsni, 2010; Quaye, 2011), and the first to investigate the effect of irrigation on fertilizer N uptake by willow. Irrigation often promotes enhanced recovery of applied fertilizer N by target species within conventional agricultural systems (Raun and Johnson, 1999). We hypothesized that irrigating the willow would increase its growth within our semi-arid environment and, therefore, improve its fertilizer N uptake. Considering the manufacture and application of inorganic fertilizer N accounts for more than half of the non-renewable fossil fuel energy inputs for producing willow, in addition to a significant portion of production cost (Heller et al., 2003; McKenney et al., 2011), insights into fertilizer N dynamics that can be used to promote increased fertilizer N recovery and use efficiency by purpose-grown willow will certainly benefit both the environmental and economic 'bottom lines', which are necessary goals in promoting willow as a viable biomass energy alternative.

5.4 Materials and Methods

5.4.1 Study site and willow varieties

The data for this study were collected in 2010 from a three-year-old willow plantation, within the Canadian Wood Fibre Centre willow variety trial, located on the University of Saskatchewan campus in Saskatoon, Saskatchewan, Canada (UTM coordinates: 13U 389970 5776342). The following site and willow variety information was initially reported in Hangs et al. (2011; Appendix B). The soil is a heavy clay Orthic Vertisol of the Sutherland Association, developed on glacial lacustrine parent material, with a pH and electrical conductivity (dS m^{-1}) of 7.1 and 0.33, respectively. The semi-arid temperate location receives on average 350 mm of annual precipitation (70% occurring from May to September) and has a mean annual temperature of 2^{o}C, with approximately 112 frost-free days. The Agriculture Capability Classification rating of the soil is Class 2, with moderately severe limitations due to a lack of precipitation (SCSR, 1978). Prior to establishing the variety trial in 2006, the site was continuously cropped to a mixture of barley and oats. Pre- and post-planting site preparation included both mechanical (e.g., deep tillage, light cultivation, mowing, and hand weeding) and chemical (linuron– 1.7 kg a.i. ha^{-1} and glyphosate– 2.0 kg a.i. ha^{-1}) treatments to control non-crop vegetation. The willow were planted using a 0.6 × 0.6 m grid spacing for each 30 m long triple-row bed, with 2.0 m spacing between the beds (approximately 15,625 stems ha^{-1}). Two willow varieties were planted: 'Charlie' (*Salix alba* × *Salix glatfelteri*) and 'SV1' (*Salix dasyclados*), which are the standard varieties for comparison (e.g., survival, yield, pest resistance, etc.) within Canada and the U.S., respectively (D. Sidders, Canadian Forest Service, personal communication; Volk et al., 2006).

5.4.2 Experimental design

In the spring of 2008, prior to bud break, the two-year-old willows were coppiced. Three rates of both irrigation and fertilizer treatments were imposed on each bed, arranged in a split-split-plot experimental design (whole plot factor: variety; subplot factor: irrigation rate; and, sub-subplot factor: fertilizer rate), and replicated three times. Each experimental unit consisted of nine willow plants arranged in a 3 × 3 array and were separated by a 1.8 m buffer. The three irrigation treatments consisted of either no additional water added above rainfall, or drip irrigation used to maintain soil moisture at 75 or 100% field capacity (FC), measured using Watermark® soil moisture sensors installed within each plot (Irrometer Company, Inc., Riverside, CA, USA; Spaans and Baker, 1992). A Campbell Scientific CR10X was used to monitor soil moisture and control irrigation timing. The amount of water received (including growing season precipitation) by the control, 75, and 100% field capacity plots after three years was approximately 834, 2060, and 3286 mm, respectively. The three fertilization treatments included no fertilizer or fertilizer broadcast applied once annually in June over the three-year rotation either at the recommended rate (1×) or twice the recommended rate (2×). The recommended rate consisted of a balanced fertilizer blend of 100:30:80:20 (N:P:K:S; kg ha^{-1}), which was intended to not only match the willow growth requirements, but also replenish nutrients exported when harvesting willow with anticipated annual biomass production of 15-22 Mg ha^{-1} (Perttu, 1993; Danfors et al., 1998; Adegbidi et al., 2001). The 2× recommended rate was intended to test the upper limit of willow growth response to added fertilizer, when grown under optimal moisture conditions. Previous work in Sweden reported negligible nitrate leaching from heavily fertilized (i.e., up to 240 kg N ha^{-1} applied annually) willow plantations after the first growing season (Dimitriou and Aronsson, 2004). Consequently, leaching was not expected to be a problem in this study with the 2× recommended fertilizer rate treatment of 200 kg N ha^{-1} applied annually, because of the established willow root systems and heavy clay soil at this site. The fertilizers used to develop the two blended rates were ammonium nitrate, monoammonium phosphate, ammonium sulfate, and potassium chloride. During each year of the three-year rotation, the irrigation and fertilizer treatments were initiated in early June to avoid exacerbating potential frost damage in late May and also to ensure the willow were vigorously growing, in order to increase the fertilizer use efficiency (Abrahamson et al., 2002). Likewise, irrigation ceased at the beginning of September, to prepare the willow for a possible early frost. At the end of the third growing season, following leaf fall, all stems (including branches) within each treatment plot were cut 5 cm above ground level using a brush saw, chipped, and dried to a constant weight for biomass measurement.

5.4.3 Determining the fate of applied fertilizer N

At the beginning of June during the third growing season, 10 kg N ha^{-1} of double ^{15}N-labelled ammonium nitrate fertilizer (10% enrichment; Cambridge Isotope Laboratory, Inc., Andover, MA, USA) was broadcast as a component of the prescribed fertilizer N treatments within the 1× (i.e., 10 and 90 kg N ha^{-1} of ^{15}N-labelled and unlabelled fertilizer N, respectively) and 2× (i.e., 10 and 190 kg N ha^{-1} of ^{15}N-labelled and unlabelled fertilizer N, respectively) recommended fertilizer treatment plots. In order to quantify the rate of fertilizer N resorption from leaves of both willow varieties among the treatments, fully expanded leaves were randomly selected and harvested from throughout the canopy of each treatment plot once irrigation ceased ('September leaves'). The LFH layer within each treatment plot was also sampled at this time, which was prior to leaf fall. The LFH layer consisted of three organic soil horizons comprised of plant debris (e.g., fallen leaves, twigs, etc.) at varying stages of decomposition─ ranging from easily recognizable litter (L horizon) to a humified material with indiscernible origin (H horizon). In mid-November, subsequent to willow growth cessation, the different sinks were sampled for their ^{15}N content within each fertilized plot included the senesced leaves prior to abscission ('November leaves'; for comparing fertilizer N content with 'September leaves' and collected in the same manner), willow stems (sampling method stated previously), willow stump (the central stump within each 3 × 3 plot was extracted and sectioned at the distinct boundary between dormant axillary buds and lateral root development, which invariably corresponded with the LFH-mineral soil interface; only the upper portion of the stump was analyzed), and all non-crop vegetation (harvested at the ground level). Additionally, four root sampling cores (0-20 cm) were collected from each plot, using an 8 cm diameter bucket auger (Eijkelkamp, Agrisearch Equipment BV, Giesbeek, Netherlands), and composited. The root cores were pre-treated by shaking in 1.2*M* $NaHCO_3$ for 15 min, to separate the roots from the heavy clay soil (Hangs et al., 2012; Appendix A), and then washed and collected using a 0.5 mm mesh sieve. The roots were divided into fine (i.e., < 2 mm) and coarse-size fractions. The non-crop vegetation was sparse, due to extensive vegetation management, thus all roots were assumed to be that of willow.

All plant tissue samples were dried to a constant weight, thoroughly milled, homogenized, and then a subsample was finely ground using a rotating ball-bearing mill. Total N and ^{15}N enrichment were determined using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies; Valencia, CA, USA) coupled to a Thermo Delta V mass spectrometer with Conflo IV interface (Thermo Finnigan; Bremen, Germany). Soil cores (5 cm dia) were also collected from each fertilized plot at four depths (0-15, 15-30, 30-45, and 45-60 cm) using a hydraulic punch (Stumborg et al., 2007), dried to a constant weight, and finely ground using a rotating ball-bearing mill. All soil samples were analyzed for total N and ^{15}N enrichment in the same manner as the plant tissue samples. For each plant and soil sample, fertilizer ^{15}N content was determined by multiplying its ^{15}N enrichment concentration by its mass.

5.4.4 Effect of irrigation on willow productivity and stem N dynamics

In order to better understand the dynamics of applied fertilizer N for supporting harvestable biomass productivity within these biomass energy plantations, the stem ^{15}N enrichment data of both willow varieties were further examined using two common diagnostic approaches, namely N use efficiency assessment and vector nutrient analysis. Nitrogen use efficiency was simply calculated as the total harvested stem biomass produced per mass of N found in the stem tissue (Adegbidi et al., 2001). Relative differences in stem productivity and fertilizer N status (i.e., concentration and content) between willow grown with and without irrigation were evaluated using vector nutrient analysis (Timmer, 1991). Specifically, for each imposed treatment, stem biomass and fertilizer N status of both willow varieties were normalized relative to their respective values measured in the non-irrigated plot (i.e., common reference). This simple approach allows for reliable diagnostic interpretations (e.g., treatment-induced responses of stem nutrient status as either dilution, sufficiency, deficiency, luxury consumption, toxicity, or induced deficiency), in a single integrated figure, based on vector direction and magnitude. Excellent reviews of the vector analysis technique are presented in Timmer (1991) and Haase and Rose (1995).

5.4.5 Statistical analyses

The ^{15}N enrichment data were subject to analysis of variance via a split-split-plot model using PROC MIXED in SAS (version 9.2; SAS Institute, Cary, NC., USA). Willow variety was the whole plot factor, irrigation rate was the subplot factor, and fertilizer rate (nested within irrigation) was the sub-subplot factor. Means comparisons were performed using least significant differences (LSD; equivalent to Fisher's protected LSD) at a significance level of 0.05, with groupings obtained using the pdmix800 SAS macro (Saxton, 1998). Homogeneity of variances (Bartlett's test) and normality of distributions (PROC UNIVARIATE) of all data sets were checked prior to the analysis. No data transformations were necessary.

5.5 Results

5.5.1 Fate of applied fertilizer N

A summary of the analysis of variance, comparing the effects of fertilization rate and irrigation level on the recovery (i.e., as a percentage of total ^{15}N applied) of broadcast ^{15}N-labelled fertilizer from the various plant tissue and soil components, is presented in Table 5.2. Neither the willow variety nor the rate of fertilizer applied had any effect on the recovery of fertilizer N in this study. However, irrigation influenced the amount of fertilizer N recovered in some willow tissues and non-crop vegetation, along with recovery in the LFH layer and varying depths of the mineral soil profile (Table 5.2). Furthermore, the two willow varieties responded differently to irrigation. Specifically, irrigation resulted in greater fertilizer N accumulation in the stems of Charlie, but not in SV1 stems (Table 5.3). Likewise, there was no effect of irrigation on the amount of fertilizer N present in either the September leaves or the stump of

Table 5.2. Summary of analysis of variance comparing the effects of fertilization (Fert) and irrigation (Irrig) on the recovery of ^{15}N-labelled fertilizer, from various soil and plant tissue components, applied in plots growing the willow varieties Charlie and SV1.

Effect	Num df	Soil					
			Depth (cm)				
		LFH	0-15	15-30	30-45	45-60	Total Soil Recovery
Variety	1	0.17	0.91	0.40	0.18	0.22	0.71
Irrig	2	< 0.01[†]	0.08	0.86	0.12	0.07	0.88
Fert(Irrig)	2	0.42	0.20	0.87	0.45	0.30	0.56
Variety*Irrig	2	0.56	0.04	0.07	< 0.01	0.01	0.06
Variety*Fert	1	0.60	0.56	0.83	0.73	0.26	0.89
Variety*Irrig*Fert	2	0.71	0.69	0.48	0.53	0.66	0.63

Table 5.2. (continued).

Effect	Plant							
	Leaves				Roots			
	Sept.	Nov.	Stems	Stump	Fine (< 2mm)	Coarse (> 2mm)	Total Willow Recovery	Weeds
Variety	0.58	0.38	0.07	0.38	0.56	0.50	0.42	0.33
Irrig	0.47	0.04	0.03	0.93	< 0.01	0.87	0.69	0.01
Fert(Irrig)	0.48	0.52	0.79	0.41	0.33	0.85	0.54	0.39
Variety*Irrig	0.06	0.12	< 0.01	0.19	0.06	0.02	< 0.01	0.10
Variety*Fert	0.42	0.60	0.67	0.97	0.36	0.24	0.28	0.46
Variety*Irrig*Fert	0.76	0.33	0.62	0.06	0.43	0.83	0.11	0.47

[†] Significant ($P < 0.05$) effects are highlighted

Table 5.3. Mean (n = 6) percent recovery of broadcasted ^{15}Nitrogen-labelled fertilizer by two willow bioenergy varieties and non-crop vegetation grown in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity (FC).

		Leaves				Roots			
Variety	Irrigation Level	September	November	Stems	Stump	Fine (< 2 mm)	Coarse (> 2 mm)	Total Willow Recovery	Weeds
Charlie	None	24.8a[†]	16.6a	8.0b	0.9a	3.4c	0.6b	29.5b	1.2a
	75% FC	26.9a	13.0b	14.4a	1.2a	6.3ab	0.8a	35.9a	0.2bc
	100% FC	25.1a	11.6b	12.7a	1.2a	5.7ab	0.9a	32.1ab	0.1c
SV1	None	33.9a	18.6a	9.6ab	1.7a	3.2c	0.8a	33.9ab	0.3b
	75% FC	25.1a	13.4b	9.0ab	1.1a	4.2bc	0.5b	28.4b	0.1c
	100% FC	22.4a	9.6b	9.9ab	1.3a	6.8a	0.5b	28.1b	0.1c

[†] Within each column, values having the same letter are not significantly different ($P > 0.05$) using LSD

either willow variety. The abscising leaves (November leaves) of both Charlie and SV1 growing without irrigation contained larger amounts of fertilizer N compared to the irrigated willow (Table 5.3). Conversely, the fine-root fraction of both varieties and the coarse-root fraction of Charlie within the irrigated plots accumulated more fertilizer N relative to the non-irrigated willow. The non-irrigated SV1 accumulated more fertilizer N in the coarse-root fraction than the irrigated. Overall, irrigation increased the total recovery of applied fertilizer N by Charlie, but not SV1 (Table 5.3). The amount of fertilizer N acquired by non-crop vegetation was less in irrigated plots. Except for a larger accumulation of fertilizer N in the fine roots of SV1 within the 100% FC plots, the two irrigation rates had the same effect on fertilizer N accumulation among the plant tissues examined (Table 5.3).

Although there was no effect of irrigation on ^{15}N fertilizer recovery from the soil to a 60 cm depth (i.e., LFH layer + mineral soil) in Charlie or SV1 plots, measured differences were apparent when examining the individual pools separately (Table 5.4). The LFH layer within the irrigated plots of both willow varieties contained more fertilizer N compared to the non-irrigated plots. More fertilizer N was present in the upper 15 cm of mineral soil within the Charlie plots, but no significant differences ($P > 0.05$) were measured among the remaining depths (Table 5.4). Within the SV1 plots, fertilizer N accumulated in the 30 to 60 cm soil depth under the highest irrigation level. Otherwise, water supplied in excess of 75% had no effect on the recovery of applied fertilizer N in soil. Generally speaking, at the end of the growing season after application, 82% of the broadcast ^{15}N-labelled fertilizer was accounted for, with approximately 80% of the recovered fertilizer N present within the willow tissues, non-crop vegetation, LFH layer, and the upper 15 cm of mineral soil (Fig. 5.1).

5.5.2 Effect of irrigation on willow biomass production and stem N dynamics

For both willow varieties, irrigation increased the biomass production (Table 5.5 and Fig. 5.2), along with the N use efficiency for all plant tissues, except for the September leaves of SV1 (Table 5.6). The highest irrigation level increased the N use efficiency of the September leaves and fine roots of Charlie above that of the 75% FC level and the non-irrigated treatment; otherwise, there were no significant differences ($P > 0.05$) in N use efficiency for any tissues of either willow variety between the 75 and 100% FC irrigation level. The calculated N use efficiency for the production of stem, stump, and fine root tissues was greater for SV1 compared

Table 5.4. Mean (n = 6) percent recovery of broadcasted ^{15}Nitrogen-labelled fertilizer in soil, supporting the growth of two willow bioenergy species, in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity (FC).

			Mineral Soil Depth (cm)				
Variety	Irrigation Level	LFH	0-15	15-30	30-45	45-60	Total Soil Recovery
Charlie	None	3.8c†	32.8a	8.4ab	4.3bc	2.8bc	52.0a
	75% FC	7.2ab	23.0b	7.9ab	5.5ab	3.4ab	47.1a
	100% FC	6.8ab	25.0b	7.0b	3.5b	2.5bc	44.9a
SV1	None	5.8bc	28.1ab	7.4ab	3.8c	2.4c	47.6a
	75% FC	8.2a	25.7ab	7.9ab	4.8bc	3.1bc	49.7a
	100% FC	8.7a	25.0ab	9.4a	6.8a	4.2a	54.1a

† Within each column, values having the same letter are not significantly different (*P* > 0.05) using LSD

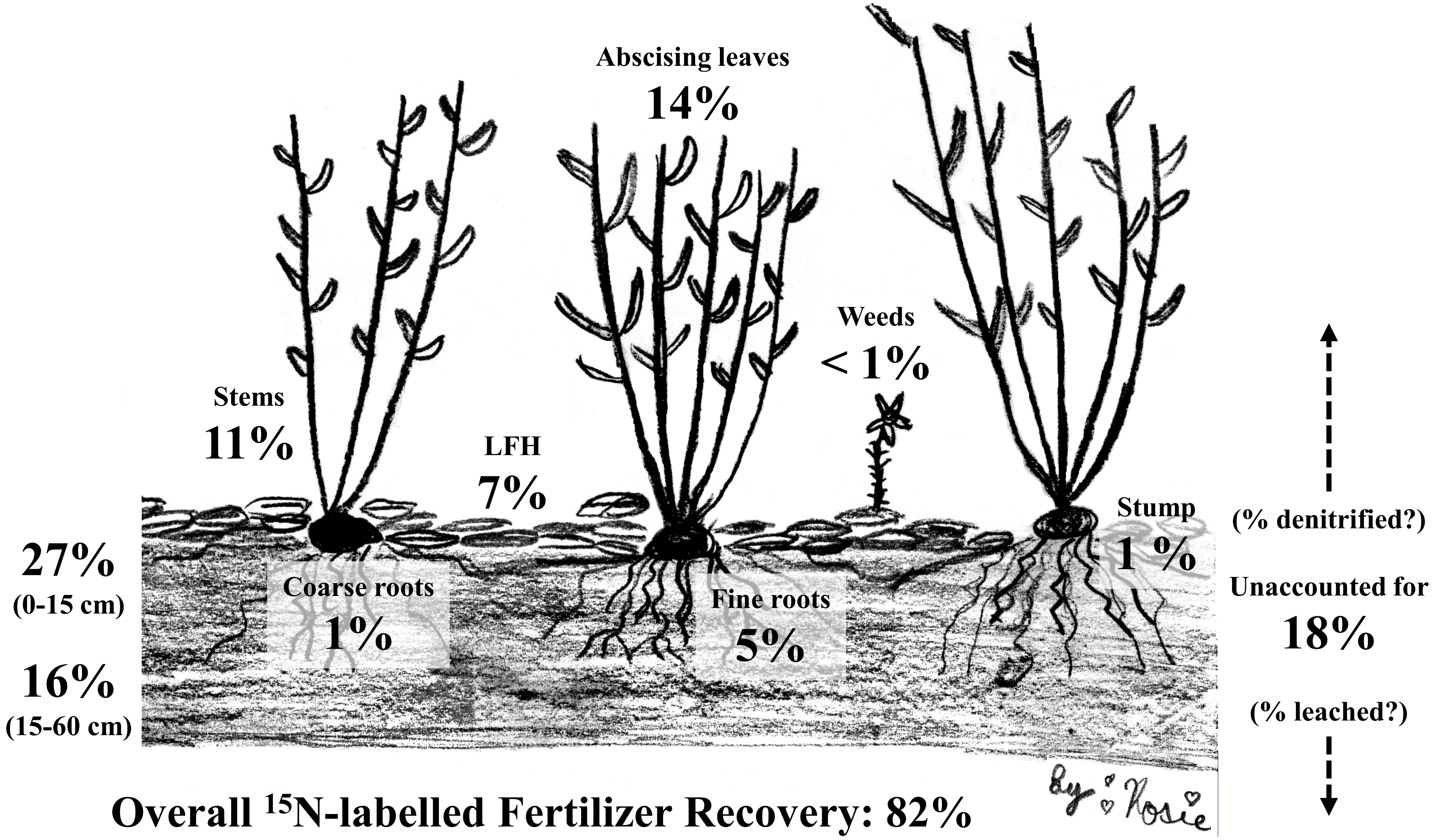

Fig. 5.1. Mean (n = 6) fate of broadcasted ^{15}N-labelled fertilizer after the growing season of application.

Table 5.5. Summary of analysis of variance comparing the effects of fertilization (Fert) and irrigation (Irrig) on the above- and below-ground biomass production from various plant tissues, applied in plots growing the willow varieties Charlie and SV1.

Effect	Num df	Stems	Leaves	Stump	Roots: Fine (< 2mm)	Roots: Coarse (> 2mm)	LFH
		-------------	-------------	*P*-values	-------------	-------------	-------------
Variety	1	0.0185†	0.0445	0.0007	0.3200	0.3819	0.0683
Irrig	2	< 0.0001	< 0.0001	0.0003	< 0.0001	< 0.0001	0.0006
Fert(Irrig)	4	0.0543	0.3871	0.7840	0.6701	0.8809	0.6044
Variety*Irrig	2	0.0330	0.0162	0.7584	0.0612	0.0659	0.0133
Variety*Fert	2	0.8583	0.6290	0.6225	0.7251	0.7120	0.0983
Variety*Irrig*Fert	4	0.1180	0.6022	0.1161	0.4278	0.6073	0.8961

† Significant ($P < 0.05$) effects are highlighted

to Charlie when irrigated (Table 5.6). The effect of irrigation on stem biomass and fertilizer N status of Charlie and SV1 is readily apparent in the vector nomogram when using their respective growth in non-irrigated plots as the reference normalized to 100 (Fig. 5.3). Given that there were no significant differences ($P > 0.05$) between the two irrigation rates on measured stem biomass or stem tissue fertilizer N content and concentration for both willow varieties (data not shown), only the average irrigation response vector was drawn for each variety, in order to reduce clutter. The vector diagnosis reveals that when irrigated, Charlie increased stem growth and uptake of fertilizer N, but the fertilizer N concentration decreased compared to the willow grown in the non-irrigated plots (Fig. 5.3). This response vector represents a typical growth dilution response─ indicating improved growing conditions (due to irrigation) that supported greater biomass gain relative to fertilizer N uptake, where N is sufficient and non-limiting (vector shift A; Timmer, 1991). Specifically, irrigation increased stem biomass and fertilizer N uptake (i.e., ^{15}N content) by Charlie up to 133% and 80%, respectively, while decreasing the stem tissue fertilizer N concentration up to 35% (Fig. 5.3). Irrigating SV1 increased its biomass up to 124%; however, there was no change in fertilizer N uptake and up to 53% reduction in stem tissue

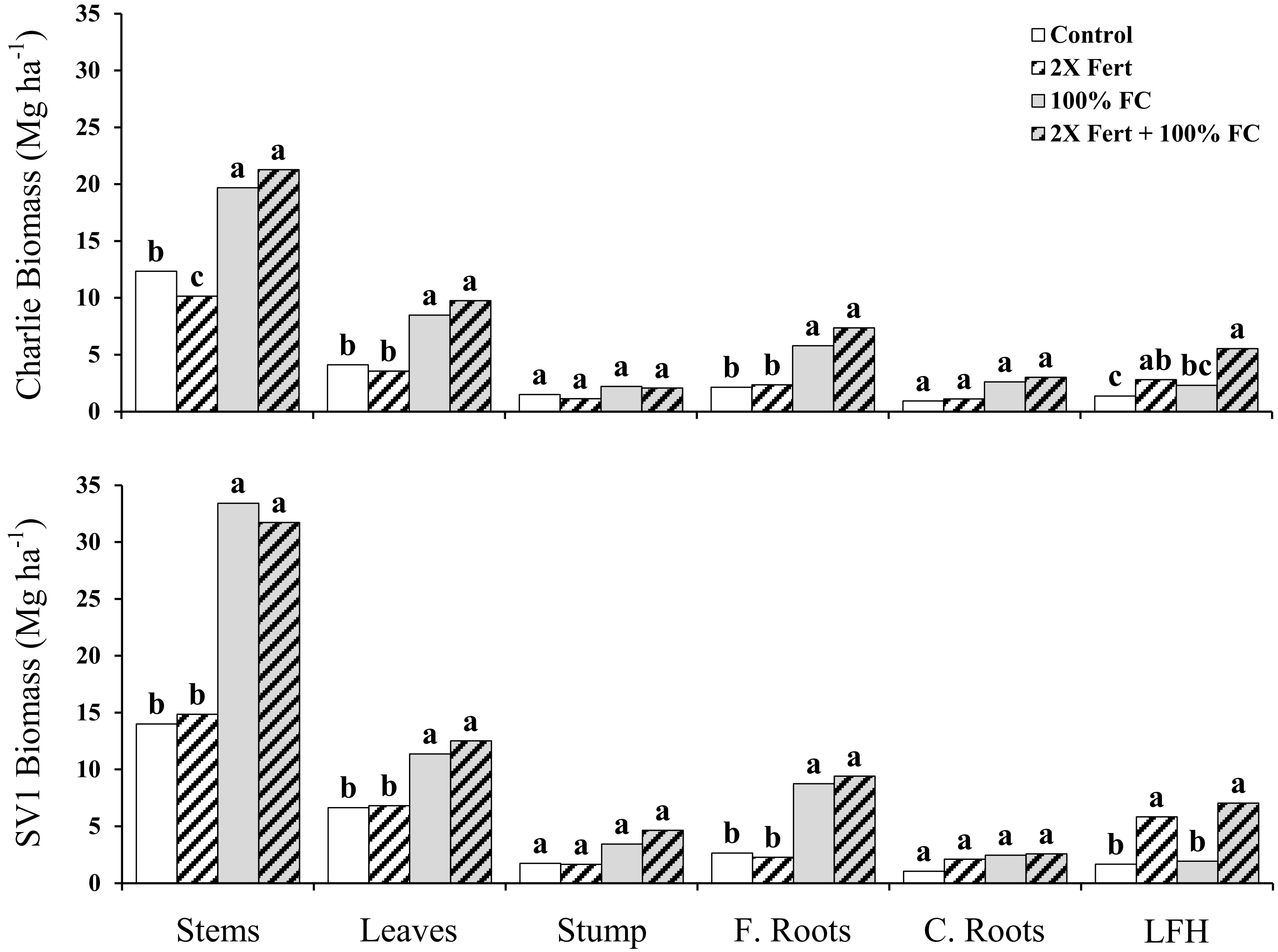

Fig. 5.2. Mean (n = 3) effect of fertilization, irrigation, and fertilization combined with irrigation on the dry biomass of selected plant tissue components of two willow varieties after three growing seasons. The treatments included either no fertilizer or additional water added (Control), fertilizer addition at 2× the recommended rate (2× Fert; 200:60:160:40 kg ha^{-1} N:P:K:S), drip irrigation used to maintain the available soil moisture at field capacity throughout the growing season (100% FC), or a combination of 2× Fert and 100% FC. For each variety and component, bars with the same letter are not significantly different (*P* > 0.05) using LSD.

Table 5.6. Mean (n = 6) nitrogen use efficiency of two willow bioenergy varieties grown in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity (FC).

					Roots	
		September Leaves	Stems	Stump	Fine (< 2 mm)	Coarse (> 2 mm)
Variety	Irrigation Level	(g oven-dry biomass g N^{-1})				
Charlie	None	51.0b[†]	228.0c	290.7d	64.3c	168.5b
	75% FC	50.5b	259.2b	336.0cd	88.5b	229.9a
	100% FC	59.8a	285.9b	370.2bc	100.3a	228.7a
SV1	None	43.1b	257.8bc	291.3d	68.4c	165.6b
	75% FC	43.4b	322.5a	399.8ab	106.8a	221.7a
	100% FC	42.7b	317.4a	414.1a	108.2a	214.5a

[†] Within each column, values having the same letter are not significantly different ($P > 0.05$) using LSD

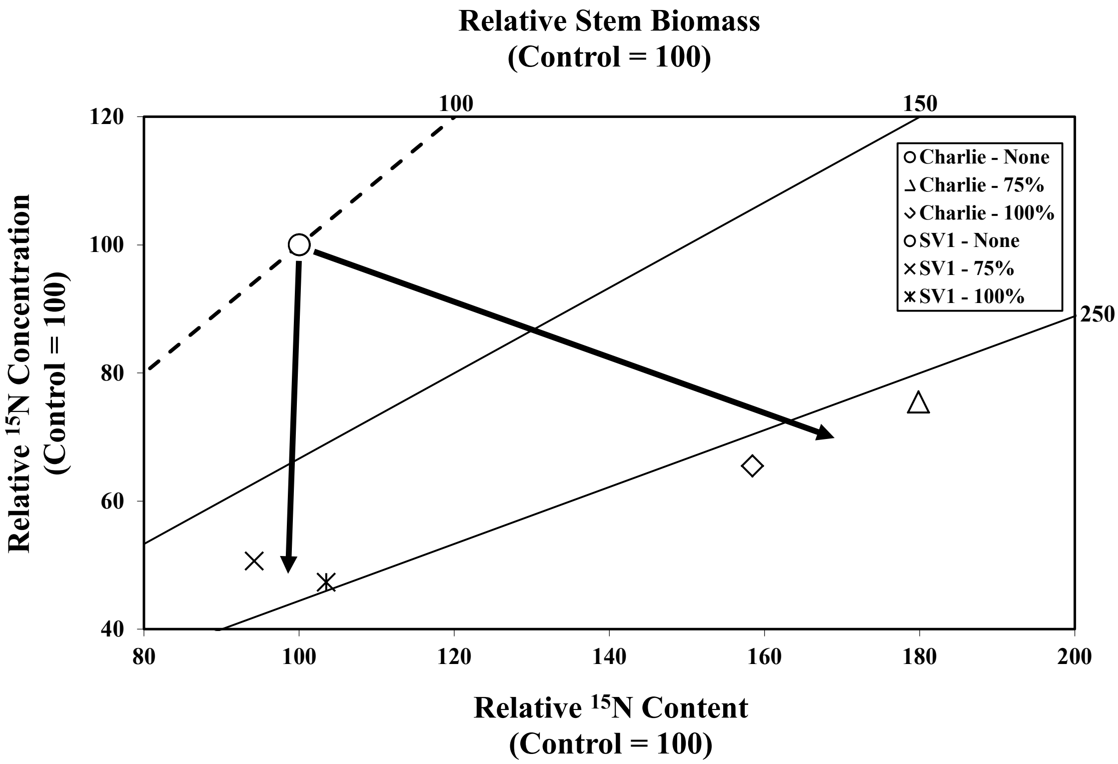

Fig. 5.3. Vector nomogram of relative leaf biomass, ^{15}N content, and ^{15}N concentration in stems of two willow bioenergy varieties grown in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity. Biomass and ^{15}N status of seedlings grown in control plots (i.e., no irrigation) served as the reference and were normalized to 100. Diagonal isolines correspond to the relative biomass. Note: there were no significant differences (*P* > 0.05) between the two irrigation rates on measured stem biomass or stem tissue fertilizer N content and concentration for both willow varieties, so the average response vector was drawn for each variety to reduce clutter.

fertilizer N concentration compared to non-irrigated plots (Fig. 5.3). Although the SV1 response vector is also classified as a growth dilution response to irrigation (where N is sufficient and non-limiting despite markedly increased growth), evidently SV1 is capable of supporting greater biomass production per unit of N when irrigated compared to Charlie.

5.6 Discussion

5.6.1 Fate of applied fertilizer N

Approximately one-third of the ^{15}N-labelled fertilizer was taken up by the target willow varieties (Table 5.3), which is not only comparable to that reported for annual field crops (≈ 30%; Raun and Johnson, 1999), but also represents a much greater uptake by willow than is reported elsewhere (0.39 to 10.6% recovery after two years; Konecsni, 2010). Unlike the

Konecsni (2010) study, where the ^{15}N-labelled fertilizer was applied during the establishment year of a willow plantation, we applied the ^{15}N-labelled fertilizer to a two-year-old plantation, with a four-year-old root system. The greater fertilizer N accumulation by willow observed in this study is likely due to the increased N requirement of older willow, along with the ability of its more extensive root system to capture greater amounts of applied fertilizer N. We hypothesized that irrigation would increase fertilizer N recovery by both willow varieties; this was true for Charlie, but not for SV1 (Table 5.3). It is interesting to note that while irrigation increased both fine and coarse root biomass for both varieties (Table 5.5), the ratio of fine roots to coarse roots only increased with SV1 (up to 132%; data not shown). This relative increase in fine root proportion would contribute to increased root surface area and, therefore, greater N assimilation potential. Additionally, SV1 had almost 30% more fine roots than Charlie in the 100% FC plots, indicating a different carbon allocation pattern between the varieties under ideal moisture conditions. Consequently, SV1 was not lacking in its capacity to sequester fertilizer N with its abundant fine roots; instead, the observed difference in fertilizer N uptake between the willow varieties could be attributed to the differences in their respective N use efficiency. Tharakan et al. (2005a) examined the N use efficiency of 30 willow varieties, after a three-year rotation in central New York, and reported SV1 to have the greatest N use efficiency of all varieties tested. Irrigation increased the N use efficiency of both willow varieties in our study, nonetheless, the ability to sustain increased biomass productivity when irrigated, without a concomitant increase in N uptake, was more prominent with SV1 (Table 5.6 and Fig. 5.3). Arguably, the magnitude of increased N use efficiency with irrigation, for both willow varieties, would likely have been more pronounced if the site did not receive an anomalously large amount of precipitation (70% more than the 100-yr average) during the third growing season.

Another possible mechanism for the different fertilizer N contents between varieties could be inherent differences in their NH_4^+-N and NO_3^--N root uptake capacity. Differences in N uptake rates among willow varieties have been reported (Ericsson, 1981), thus observed differences in fertilizer N accumulation between Charlie and SV1 could be partly explained by dissimilar root physiology. Moreover, symbiotic relationships with ectomycorrhizal fungi can increase the nutrient uptake capacity of willow, although the intensity of mycorrhizal colonization and utility for increasing nutrient uptake is variable among varieties (Jones et al., 1991; Tibbett and Sanders, 2002) and can be either enhanced or inhibited with fertilizer N additions (Baum et al., 2002). Hofmann-Schielle et al. (1999) suggest that enhanced carbon allocation to fine root development in willow is a consequence of inadequate nutrient uptake, resulting from insufficient ectomycorrhizal roots. Perhaps the increased N use efficiency observed with SV1 is an adaptation to its relatively poor NH_4^+-N and NO_3^--N root uptake capacity, in spite of its abundant fine roots, and warrants further investigation.

There was a substantial amount of fertilizer N present in both September and November leaves for both varieties (Table 5.3 and Fig. 5.1). Irrigation stimulated earlier initiation of leaf tissue N resorption prior to abscission, resulting in less N lost in fallen leaves compared with non-irrigated willow. This effect was visually apparent each fall when walking through the plots, evidenced by a marked difference in colour change and magnitude of leaf fall with increasing irrigation level. Contrariwise, irrigation was expected to delay leaf senescence and possibly even increase the susceptibility to winter damage from an early frost episode; however, each year the initiation of leaf senescence began with the 100% FC plots and ended with the non-irrigated plots. The measured nutrient resorption efficiency (i.e., % of nutrients resorbed from senescing leaves; Yuan et al., 2005) reflected this with up to 49% of the fertilizer N resorbed from the September leaves in the 100% FC plots compared to as low as 21% in the non-irrigated plots (data not shown). Important sinks for this seasonally translocated N consists of the willow stems, stump, and root system, which support stem regrowth the following year. After harvest, the willow stump and root system are often considered to be key suppliers of remobilized N supporting the initial growth of new stems (Karp et al., 2011); however only a small proportion of the accumulated fertilizer N was found in the perennial stump tissue (Table 5.3). Clearly, the previously dormant axillary buds located on the stump play a critical role in the regeneration success of these coppice systems (Sennerby-Forsse and Zsuffa, 1995). Nevertheless, given the partitioning of fertilizer N predominantly in the willow root system at the end of the growing season (Table 5.3), it appears that the reserve N stored in the root system is a primary source of remobilized N that will be translocated via the xylem to developing stem tissues (Dickmann and Pregitzer, 1992). The fine-roots accumulated up to 664% more fertilizer N than the coarse roots (Table 5.3), and are likely the greatest source of this remobilized N supply. The larger accumulation of fertilizer N by the fine-roots can be attributed to greater fertilizer N concentration and biomass (data not shown).

Woody crop fertilizer N use efficiency is reduced by losses to non-target sinks, either temporarily (e.g., non-crop vegetation and microbial immobilization) or permanently (e.g., leached and denitrified), regardless of fertilizer N form or method of application (Preston and Mead, 1994; Staples et al., 1999; Hangs et al., 2003). Adequate weed control is imperative for supporting optimal fertilizer N recovery by willow, especially in these intensively managed systems where soil moisture and nutrient conditions are optimal for non-crop plant growth. The level of weed control maintained each season was excellent, as evidenced by the small amount of applied fertilizer N lost to non-crop vegetation (Table 5.3). Incomplete canopy closure within the non-irrigated plots led to increased weed production and resulted in greater accumulation of fertilizer N by non-crop vegetation. As previously mentioned, the LFH layer within each plot was sampled prior to leaf fall. It is assumed that the recovered fertilizer N in the LFH layer was immobilized by microbes decomposing the litter during the growing season (Preston et al., 1990). Irrigation increased the fertilizer N recovered within the LFH layer (Table 5.4), reflecting elevated microbial activity under the more favourable conditions. The largest sink for applied fertilizer N was the mineral soil, accounting for approximately 43% of total recovery (Table 5.4); 93% of which was present within the upper 45 cm where willow roots predominate (Rytter and Hansson, 1996). As expected, the established root system and heavy clay soil at this site minimized leaching below this depth, although willow has been found to root as deep as 1 m even in heavy clay soils (Alriksson et al., 1997; Adegbidi et al., 2001). Irrigation reduced the fertilizer N recovered in the upper 15 cm within the Charlie plots (Table 5.4), which corresponds well with the measured increased fertilizer N uptake by Charlie when irrigated (Table 5.3). Conversely, given the 53% greater SV1 biomass compared to Charlie in plots maintained at 100% FC (Fig. 5.2), the presence of more fertilizer N at depth in the SV1 plots is likely due to its higher N use efficiency, and hence lower fertilizer N uptake by SV1 when irrigated (Table 5.6), which allowed more fertilizer N to be leached. Undoubtedly, some of the unaccounted for fertilizer N was deeper than the 60 cm depth sampled, but most was assumed to be lost to denitrification considering the imperfectly drained soil at the site. Anoxic microsites can frequently occur (even at FC) within the abundant micropores of heavy clay soil (Colbourn, 1988). van der Salm et al. (2007) suggests approximately 25% of fertilizer N applied to heavy clay agricultural soils is lost from the system, of which 90% and 10% is through denitrification and leaching, respectively.

Within the agronomic literature, the amount of ^{15}N-labelled fertilizer recovered by the target crop typically increases with increasing rates of fertilizer N addition, with or without irrigation (Pilbeam et al., 1997; Tran and Tremblay, 2000; Khelil et al., 2005). The absence of a fertilizer rate effect (or any interaction thereof) on the amount of fertilizer N recovered by the willow in this study (Table 5.2), is probably due to the fertile Class 2 agricultural soil at the study site (SCSR, 1978), which supplied sufficient N for willow growth. Additionally, one of the many advantageous characteristics of purpose-grown willow for use in different land reclamation, phytoremediation, bioengineering, and agroforestry applications is its relatively low nutrient demanding nature (Tharakan et al., 2005a; Kuzovkina and Volk, 2009). The usefulness of a low nutrient requirement is perhaps best demonstrated within a biomass energy context. In particular, notwithstanding the large varietal differences in N requirement among willow varieties (Weih and Nordh, 2002), relative to other annual and perennial bioenergy crops (e.g., *Miscanthus*, switchgrass, maize, and oilseed rape) that require fertilizer N to meet production goals, often willow can be successfully grown without added N, thereby providing a higher net energy return (Boehmel et al., 2008).

5.6.2 Long-term recovery of applied fertilizer N

After accounting for the fertilizer N lost from the system through stem harvesting, along with presumed denitrification and leaching, the remaining sinks containing approximately 71% of the applied N included the LFH layer (including abscised willow leaves and non-crop vegetation residue), mineral soil, and the willow stumps and roots (Fig. 5.1). Litterfall is a primary mechanism for nutrient cycling within most ecosystems (Hughes and Fahey, 1994) and in willow, foliar N levels in late August can account for as much as 64% of the plant N despite representing only 20% of its mass (Hytönen, 1995a). Although willow will resorb up to 50% of this N during leaf senescence (von Fircks et al., 2001), a substantial amount of fertilizer N remained in abscising leaves (Fig. 5.1). Approximately one-third of the leaf litter N will be released during the first year of decomposition, with the majority liberated after three years (Šlapokas and Granhall, 1991a; 1991b). Up to 42% of this released N is believed to enter the stabile humus pool of N after four years, thereby representing long-term retention within the system (Šlapokas, 1991b). A similar N release pattern is expected for the fertilizer N immobilized within the existing LFH layer (prior to leaf fall) and the non-crop vegetation residue given the comparable tissue N concentrations among them (data not shown). Tissue N concentration is a principal determinant of litter quality and a strong factor controlling litter decomposition rates and nutrient cycling (Weih and Nordh, 2002). Although we did not differentiate between the inorganic and organic pools of fertilizer N recovered in the soil, Aronsson (2001) found that approximately 97% of the fertilizer N applied to a loamy sand soil was immobilized within the organic N pool after one growing season. Ostensibly then, the vast majority of fertilizer N recovered in our fertile clay soil should be similarly retained within the organic N pool, with up to 8% remineralized annually (Preston and Mead, 1994). Unlike perennial willow stumps that continually regenerate stems over multiple rotations, willow fine roots have rapid turnover and decomposition rates (Rytter and Hansson, 1996; Rytter, 1999). Fine roots contribute significantly to N cycling within a plantation as they alternate between N source and sink, thus preserving N in the system (Rytter and Rytter, 1998; Püttsepp et al., 2007), which is manifested by the larger amount of fertilizer N recovered in the fine roots compared to the coarse roots (Fig. 5.1).

Conventional agricultural and forestry research have employed ^{15}N-labelled fertilizers for decades to study the long-term fate of applied nitrogenous fertilizers, but this has not been the case with short-rotation intensive culture willow research. This study is no exception, given its single season timeframe. After the first rotation, willow plantations readily achieve canopy closure. At this stage, their annual N requirements are primarily supplied by internal cycling, litter decomposition, available inorganic soil N, and remineralized organic soil N, requiring minimal fertilizer N additions to achieve desired yields (Ingestad and Ågren, 1984; Ericsson, 1994a; Alriksson et al., 1997). Prolonged (i.e., multi-rotational) investigations are required to better understand and appreciate the mechanisms controlling plant availability of applied fertilizer N accumulated within different sinks not only during the year of application, but also in subsequent years. Such insights into fertilizer N dynamics will enable the evaluation of N use efficiency from both environmental and economic perspectives. Moreover, the use of such ^{15}N-labelled studies can help to support successful management decisions for these biomass energy plantations and promote their long-term sustainability.

5.7 Conclusion

Purpose-grown willow represents a feasible bioenergy feedstock; however, there needs to be sufficient biomass production to support the environmental and economic viability of these plantations. Fertilizer N often is used to achieve this goal, but is associated with substantial cost, along with the possibility of deleterious offsite environmental effects. Consequently, efficient use of fertilizer N, in the year of application and beyond, is essential for minimizing any environmental impact and maximizing economic returns. In this study, irrigation increased fertilizer N accumulation by the willow variety Charlie, but not SV1, which was attributed to greater N use efficiency of irrigated SV1 compared to Charlie. Irrigation also stimulated increased resorption of fertilizer N in leaves prior to abscission for both willow varieties, resulting in greater N storage available for supporting new stem growth the following year. Eighty-two percent of the applied fertilizer N was recovered in the willow tissues, LFH layer, non-crop vegetation, and soil (0-60 cm), with the balance presumably lost primarily through denitrification from the poorly-drained soil. The long-term role fertilizer N plays within the N cycle of willow plantations remains to be seen and will undoubtedly require the use of ^{15}N-labelled fertilizers, to separate the contribution of remineralized fertilizer N from other N sources, along with quantifying subsequent remineralized fertilizer N losses due to denitrification and/or leaching. Further research is needed to track the fate of fertilizer N over a longer time frame (i.e., multiple rotations) to better understand fertilizer N dynamics within these intensively managed systems and to help advance willow as a viable biomass energy alternative in an evolving global bioenergy sector.

6. OVERALL SYNTHESIS AND CONCLUSIONS

6.1 Summary of Findings

The primary objective this PhD thesis was to address the biogeochemical cycling of the major plant nutrients during the initial four-year rotation of SRC willow, along with developing a comprehensive nutrient budget for each nutrient (Chapters 3 and 4). Given the production constraints identified and the desire to investigate different practical aspects of SRC willow plantation productivity, additional complementary studies were conducted: including investigating the fate of applied nitrogenous fertilizer (Chapter 5); developing a novel technique for separating willow roots from high clay content soil (Appendix A); developing and validating a novel technique for non-destructively measuring harvestable willow biomass that is independent of site or variety (Appendix B and C); and finally, identifying salt-tolerant willow varieties that are suited for afforesting abandoned saline land in Saskatchewan (Appendix D).

Specifically, this PhD research has furthered the science of SRC willow production systems in several ways, namely:

i) The estimated leaf litter decomposition ($k_{Biomass}$ and $LV_{Biomass}$) and nutrient release ($k_{Nutrient}$ and $LV_{Nutrient}$) values are the first reported values for *Salix* spp. and advances our understanding of C sequestration and nutrient cycling potentials within these biomass energy plantations (Chapter 3). The results of this study supported the hypothesis that leaf litter mass loss and nutrient release characteristics would vary among willow varieties, sites, and nutrients. Specifically, knowing the relative differences in leaf litter nutrient release characteristics among varieties is advantageous for selecting varieties possessing quicker nutrient release (e.g., Fish Creek) for use in plantations, in order to reduce fertilizer requirement, compared to using other varieties with slower nutrient release characteristics (e.g., Sherburne) in environmentally sensitive areas where enhanced nutrient immobilization would mitigate non-point source pollution. Moreover, the strong negative relationship between SOC level and $k_{Biomass}$ was most pronounced at Prince Albert; presumably due to a greater soil microbial response to added leaf litter C source in this sandy soil with inherently poor fertility and low SOC level. The accumulation of this recalcitrant portion of annual leaf litter is a valid explanation for the measured increase in SOC level following the introduction of SRC willow on sandy soils formerly in agricultural production. Unlike contemporary first generation or herbaceous second generation bioenergy crops, the SRC willow plantations in this study cycled more than seven tonnes of leaf litter per hectare during the first rotation, which will enhance SOM levels and long-term nutrient cycling. Lastly, distinguishing between leaf litter nutrients with slow (e.g., N) and quick (e.g., base cations) release characteristics provides valuable information for forecasting the nutrition requirements needed to sustain long-term soil fertility and willow productivity;

ii) The comprehensive examination of all nutrient vectors (i.e., input, output, and transfers) associated with the plant available soil nutrient pools and corresponding nutrient budgets (Chapter 4) is the first reported for SRC willow plantations. The results of this study supported the hypothesis that nutrient dynamics within various soil and plant pools would vary among willow varieties, sites, and nutrients– as related to the effect of pedoclimatic conditions on plantation productivity. Quantifying the biogeochemical cycling of N, P, K, S, Ca, and Mg within SRC willow plantations during the initial four-year rotation provided insight into the long-term productivity of these purpose-grown biomass energy systems on a variety of soil types, thus helping to predict the need of supplemental nutrient amendments over multiple rotations. In particular, SRC willow plantations are relatively low nutrient-demanding compared to annual agronomic crops and other alternative biomass energy species, with minimal nutrient outputs from the production system other than via harvested biomass. However, notwithstanding the minimal nutrient export in harvested biomass over multiple rotations and efficient nutrient cycling within plantations, observed deficits in plant available soil N and P at the end of the initial rotation suggest that sustaining harvestable yields over multiple rotations will require supplemental fertilizer amendments; albeit at rates that are a fraction of that required by other perennial crops;

iii) Tracking the fate of ^{15}N-labelled fertilizer within SRC willow plantations (Chapter 5) supported the hypothesis that alleviating water deficit through irrigation would increase fertilizer accumulation through increased willow production, however, this was only true for one of the varieties tested (Charlie). For the variety SV1, irrigation decreased fertilizer use efficiency by increasing the N use efficiency by the willow. Irrigation also promoted increased fertilizer N resorption from leaves prior to abscission for both varieties, resulting in greater N storage (primarily in the fine-roots) available for remobilization the following year to support new stem growth. Less than 20% of the applied fertilizer N was unaccounted for, primarily lost via denitrification from the poorly-drained soil, with the balance either accumulated by the target willow crop or available for willow uptake in subsequent years. The observed lack of willow growth response to fertilizer N that was observed in my study is commonly reported and is likely due to willow's low nutrient demanding nature, the fertile Class 2 agricultural soil of the study site, and efficient N cycling in these SRC willow plantations (Chapter 3 and 4);

iv) The first principles of Solonetzic soil genesis, (i.e., Na-induced dispersion of soil colloids) were used to develop a novel method of separating willow roots from a Vertisol (70% clay) by using a $NaHCO_3$ pre-treatment before conventional washing (Appendix A). This technique was needed and especially useful to provide a reliable estimate of fine root nutrient content of willow grown in the high clay content soil at Saskatoon. The results of this study confirmed the hypotheses that shaking soil core samples in a solution with abundant Na, would saturate the clay surfaces with Na, disperse clay aggregates and liberate the bound roots (especially the fine roots), resulting in reduced washing duration and water usage while increasing the fine-root recovery compared to conventional washing. Moreover, the $NaHCO_3$ pre-treatment provided fine-root biomass data that was biologically more meaningful, as evidenced by its strong correlation with above-ground willow biomass, which may be a function of increased smaller higher-order fine roots recovery. Djomo et al. (2011) synthesized data from all available SRC willow life cycle analyses and reported that net greenhouse gas emissions ranged from 0.7 to 10.6 g CO_{2eq} MJ^{-1}, but Caputo et al. (2013) calculated values of -6.9 to -2.7 g CO_{2eq} MJ^{-1} and pointed out that they included improved estimates of below-ground C sequestration in fine roots unlike the vast majority of studies examined by Djomo et al. (2011). Consequently, it is essential to include reliable measures of root biomass in order to report an accurate net GHG balance for SRC willow production systems;

v) Developing a non-destructive novel alternative mensurative technique for estimating harvestable willow biomass, with the accuracy of conventional allometric techniques, but providing reliable data more quickly, economically, and without subjective measurement errors

(Appendix B). Additionally, unlike allometry, this technique is independent of willow variety, age, or location (Appendix C). Given the highly significant relationship ($R^2 = 0.95$; $P < 0.0001$) between 'stem area index', measured using the LAI-2000 Plant Canopy Analyzer, and harvestable willow biomass, the results of these two studies supported my hypothesis that this simple elegant technique is a promising alternative for estimating harvestable biomass in SRC willow plantations. Optimal timing of harvest operations is critical for supporting favourable net GHG emissions, economics, and net energy returns within SRC willow plantations (Buchholz and Volk, 2011; McKenney et al., 2011; Faasch and Patenaude, 2012; Caputo et al., 2013). The viability of SRC willow plantations on many levels, therefore, relies on dependable yield estimates for supporting both effective management decisions (e.g., irrigation, fertilization, or pest control) to promote adequate growth throughout the rotation and optimal harvest timing; and

vi) Growth measurements of different willow varieties on soils with varying salinity under controlled environment conditions indicated that the majority of willow varieties tested tolerated moderate salinity ($EC_e \leq 5.0$ dS m^{-1}), and also identified several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) that are tolerant of severe soil salinity ($EC_e \leq 8.0$ dS m^{-1}). To my knowledge, this is the first work examining the salt tolerance of SRC willow varieties and clearly indicates the potential to successfully establish and grow SRC willow plantations on salt-affected land in western Canada (> 1 Mha in Saskatchewan alone). Establishing willow plantations on saline land should provide utility for otherwise non-productive lands that are currently abandoned or grow low return forage crops. Additionally, growing salt-tolerant willow varieties may be a reclamation tool used to revitalize these unproductive agricultural lands by mitigating the build-up of surface salts. Consequently, after the plantation lifespan (22 years) perhaps the willow would act as a remediation technique to reduce salt levels for adequate annual crop growth.

6.2 Implications and Recommendations

6.2.1 Suitable short-rotation coppice willow rotation length within Saskatchewan

Generally speaking, 10 Mg ha^{-1} yr^{-1} of harvestable biomass is the critical production threshold required for the economic success of a SRC willow plantation, with a range of 8-12 reported in the literature, depending on regional circumstances (e.g., Dimitriou and Rosenqvist, 2011; Buchholz and Volk, 2013). The average stem biomass harvested among willow varieties and sites three years after coppicing in this study was 4.8 Mg ha^{-1} $year^{-1}$ (Chapter 4), with the range in harvested biomass of 6.1-13.7 Mg ha^{-1} $year^{-1}$ (data not shown). In a companion study examining the growth of 30 different willow varieties at Saskatoon, the range in harvested biomass after three years was 0.3-7.5 Mg ha^{-1} $year^{-1}$ (data not shown). The six varieties chosen for the four sites in this study are some of the best performers within SUNY-ESF's extensive breeding program and evidently well represented the potential growth of SRC willow within Saskatchewan. As discussed in Chapter 4, the lower yields measured in this study compared to other regions likely reflects the less favourable growing conditions in Saskatchewan, particularly limited moisture availability within our semi-arid climate, compared to conditions in eastern Canada, the U.S., and Europe. Irrigation can be used to increase willow productivity as shown in Chapter 5, but likely is cost-prohibitive. Still, this study highlights the phreatophytic nature of willow and its potential to benefit from shallow water tables where these conditions exist (e.g., slough margins and riparian areas) in the semi-arid prairies.

In the absence of irrigation and until superior genetic (i.e., possessing greater water use efficiency) planting stock is available, I believe that simply extending the post-coppice rotation cycle would be the most cost-effective management strategy to increase average annual yields, thereby supporting the economic sustainability of SRC willow plantations within the province. Increasing mean annual increment with longer rotations are commonly reported (e.g., Szczukowski et al., 2005; Stolarski et al., 2008; Guidi Nissim et al., 2013) and this study was no exception. Specifically, at Estevan the annual productivity of all six varieties was greater after a five-year rotation compared to the standard three-year rotation commonly used within the US and Europe (Table 6.1). Additionally, extending the rotation length by two years allowed more time for productivity differences among the varieties to become apparent, which is advantageous for assisting in selecting the superior varieties for our growing conditions. Harvesting cost is the most important factor influencing the profitability of SRC willow production (McKenney et al., 2011; Krasuska and Rosenqvist, 2012; Buchholz and Volk, 2013; Rosenqvist et al., 2013), so it is critical for farmers to optimize harvest timing to maximize their economic return. Extending the rotations from three to five years would reduce the number of harvesting operations over the lifespan of the plantation, which would improve the economics of willow production, especially considering the increased biomass production attendant with a longer five-year rotation. Based

Table 6.1. Mean (n = 4) annual productivity of several willow varieties, with different rotation lengths, in a short-rotation coppice willow plantation at Estevan, Saskatchewan.

Variety	Three-Year	Five-Year
	($Mg\ ha^{-1}\ yr^{-1}$)	
Allegany	9.8ab,B[†‡]	23.4a,A[§]
Canastota	5.5b,B	9.6c,A
Fish Creek	11.3a,A	14.0b,A
Sherburne	8.6ab,A	11.5c,A
SX61	10.6ab,A	13.2bc,A
SX64	7.8ab,B	13.0bc,A

[†] Means within a column followed by the same small letter are not significantly different ($P > 0.05$) using LSD

[‡] Means within a row followed by the same capital letter are not significantly different ($P > 0.05$) using LSD

[§] Apparent increased separation of the variety Alleghany from others after five years is due to greater productivity, along with increased accuracy of allometric measurements.

on the results of this study, therefore, I recommend the adoption of post-coppice rotation lengths of at least four years with commercial SRC willow plantations in Saskatchewan.

6.2.2 Amending mislabelling of willow nutrient requirement

A key practical management recommendation alteration that is an outcome from this Ph.D. work is that over the last several decades numerous authors have chronically misclassified SRC willow as ‘nutrient demanding’, when this is not the case. Instead, this research clearly shows that compared to traditional agricultural crops and alternative bioenergy crops (e.g., canola, corn, *Miscanthus*, switchgrass, etc.), willow can produce equal biomass using a fraction of the nutrients. Given the relatively low nutrient demanding nature of willow, a great opportunity exists to develop SRC willow as a bioenergy feedstock on marginal land in Saskatchewan (Amichev et al., 2012). Growing the low nutrient requirement willow on millions of hectares of marginal land in Saskatchewan is an exciting prospect for many reasons, especially when used as a bioenergy feedstock displacing GHG-intensive coal in power production.

6.2.3 Utilizing short-rotation coppice willow to mitigate provincial greenhouse gas emissions

Forty percent of global energy demand is currently met using coal (IEA, 2012b) and Saskatchewan is above average with 65% of power supplied to consumers from SaskPower-owned facilities sourced from coal (SaskPower, 2011). Unlike the majority of other Canadian provinces and territories, which exhibited relatively stable GHG emissions during 1990-2010, Saskatchewan's emissions increased by 69% (due to its soaring resource-based economy; e.g., oil, natural gas, potash, and uranium industries), with per-capita GHG emissions three times the national average (Environment Canada, 2012; Government of Canada, 2012). Coal-powered electricity generation accounts for more than 95% of GHG emissions within the sector (SaskPower, 2011; Statistics Canada, Fuel Consumed for Electric Power Generation, CANSIM Table 127-0004; Government of Canada, 2012). However, new federal government regulations for the electricity sector, which apply emission performance standards to coal-based power generation throughout the country, require Saskatchewan's existing plants to meet emission standards ($\leq$ 420 kg CO_{2eq} MW_e^{-1}) by the end of their 50-year lifespan or be decommissioned. With a relatively abundant (i.e., 300-year) low-cost supply of lignite coal available in the province, SaskPower is exploring different environmentally sustainable ways to ensure coal remains part of its fuel supply for the foreseeable future (SaskPower, 2012).

Direct combustion for power generation by co-firing of biomass with coal is a commercially mature solid fuel blending practice that has been implemented within the stationary fuel global community for some time (Tillman, 2000; Baxter, 2005; van Loo and Koppejan, 2008). Co-firing purpose-grown willow biomass with coal (as the base fuel) would be a relatively rapid and inexpensive method of incorporating renewable energy sources into SaskPower's power generation portfolio, and has been validated in many countries (e.g., U.S., Sweden, Ireland, Poland; Hillring, 2003; Styles and Jones, 2007a; Hoogwijk et al., 2009; Volk and Luzadis, 2009). Co-firing blends of willow range anywhere from 5-20% (Tillman, 2000; Heller et al., 2004; Styles and Jones, 2007a). Moreover, life cycle analyses (incorporating the entire willow fuel chain) indicate electricity generation using willow biomass, either by co-firing with coal or in dedicated biomass-only power plants, has no effect on net electricity generation efficiency while significantly improving the quality of plant emissions. For example, reductions in CO_2, NO_x, SO_2, particulates, and Hg emissions occur in essentially direct proportion to the percentage of biomass blended (Heller et al., 2004; Keoleian and Volk, 2005; Tharakan et al., 2005b; Styles and Jones, 2007b; Djomo et al., 2011). The net energy return of co-fired willow biomass-derived electricity (11-13:1) is also considerably larger compared to contemporary coal- and natural gas (NG)-based power generation, with NERs of 0.31:1 and 0.40:1, respectively (Spath and Mann, 2000; Heller et al., 2004). Conversely, existing integrated carbon-capture and storage (CCS) technologies reduce plant efficiency, thereby lowering the NER of coal- and NG-generated electricity further (Spath and Mann, 2004). Other advantages of co-firing willow biomass compared with CCS-coal and CCS-NG include greater net GHG reductions per kWh of electricity output, no uncertainty regarding the long-term fate and environmental effects of sequestered CO_2, proven technology, less technical and capital investment risk due to relatively minor plant modification, lower derates (i.e., prolonged economic lifespan), and rapid implementation (Spath and Mann, 2004; Baxter, 2005; CCPC, 2011). As a result, co-firing is a preferred approach for retrofitting older coal-fired plants with an economic lifespan of less than 25 years, as is the case for the three coal-fired plants in Saskatchewan, compared to the more capital-intensive CCS approach (CCPC, 2011).

In 2012, SaskPower set a new record for annual electricity supplied (22,129 GW_e) and 2013 is on pace to surpass this (SaskPower, 2012). With its relatively high dependency on coal and enormous areal extent of suitable afforestable land per capita, Saskatchewan is in an enviable position of becoming a world-leader in commercializing willow biomass energy as a dedicated renewable feedstock for displacing a portion of its current GHG-intensive base load electricity generation. Amichev et al. (2012) also recognized the potential of co-firing willow biomass within Saskatchewan's existing coal-fired power plants as a viable GHG mitigation strategy. In fact, they identified more than 2 Mha of marginal agricultural land (i.e., Agricultural Capability Classes 4 and 5 with severe to very severe limitations for crop growth) unsuitable for annual food and feed crops, but ideal for SRC willow production. Nevertheless, as noted by the authors, the practical amount of available land for afforestation in the province will be smaller, due to economic constraints, and I would certainly concur. Specifically, their marginal lands are primarily located in the south western and south-central regions of the province and could support any new small or medium size distributed heating and power generation facilities.

However, considering the relatively centralized location of the three coal-fired power plants in south-eastern Saskatchewan, the economic viability of transporting the harvested willow biomass to these power stations will decrease the practical amount of this estimated 2 Mha considerably. Consequently, there is a geographical limit placed upon the hauling distance, which depends on the regional circumstances, but generally is reported to be within an approximately 100 km radius from the end user (Caputo et al., 2013; Rosenqvist et al., 2013; Stephen et al., 2013). Moreover, the fossil fuel inputs associated with feedstock transportation is an important controller of both NER and net GHG emissions of SRC willow production (Heller et al., 2004; Djomo et al., 2011; Caputo et al., 2013), so it is prudent to minimize feedstock hauling distance from both energetic and environmental perspectives also.

With this in mind, I asked Dr. Beyhan Amichev to refine his original estimate by identifying suitable marginal lands for SRC willow production within several radii (25-125 km) of the three existing coal-fired power plants in south-eastern Saskatchewan. For a detailed explanation of his methodology, refer to Amichev et al. (2012). The new GIS spatial analysis precluded overlapping polygons (i.e., quantified land was allocated to only one station), thereby preventing feedstock competition among stations. The new analysis identified approximately 0.5 Mha of afforestable marginal land, within an economically viable hauling distance from Saskatchewan's three coal-fired power stations, which could support SRC willow production (Fig. 6.1 and Table 6.2). According to these estimates, a 40% co-firing rate would require approximately 51,151 to 153,452 ha of plantations depending on the station, which represents 126, 50, 37% of the available land within a 125 km radius of the Boundary Dam, Poplar River, and Shand power stations, respectively (Tables 6.2 and 6.3). The apparent deficiency in available marginal land for Boundary Dam is simply due to its location between the other two power stations (Fig. 6.1) and the imposed non-overlapping polygon option used during the spatial analysis. Clearly, there would be more marginal land available to the Boundary Dam within its 125 km radius, which is currently allocated to another power station but is not required to meet their 40% willow biomass co-firing blend. In other words, co-firing willow biomass at a 40% blend with coal would require an aggregate of 312,464 ha (66% of available marginal land) of SRC willow plantations for providing the necessary feedstock for all three power stations (Table 6.3).

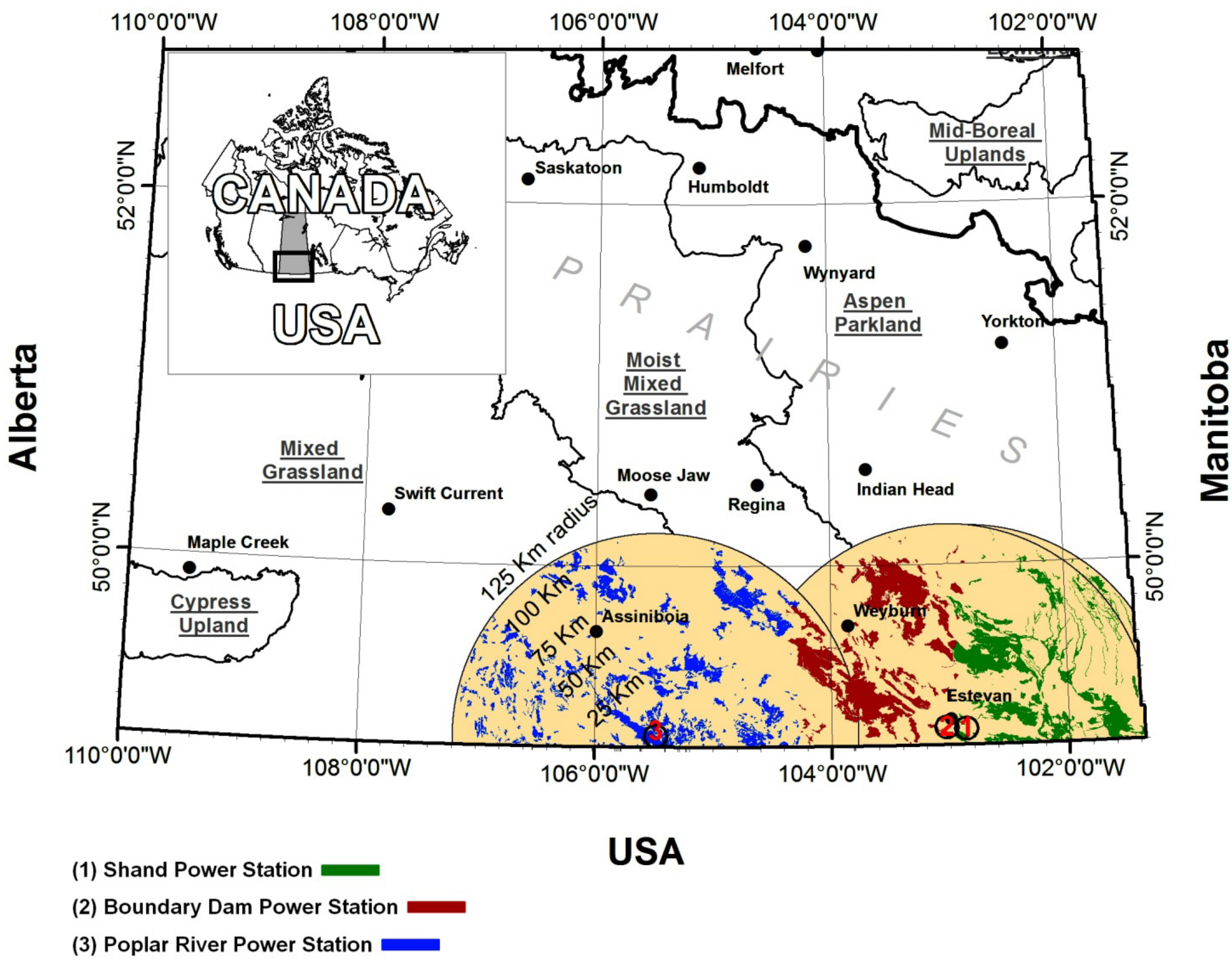

Fig. 6.1. Available marginal land (Agricultural Capability Classes 4 and 5) suitable for SRC willow production, at varying distances, surrounding the three coal-fired power stations in southern Saskatchewan. The marginal land polygons were assigned to the closest plant within a given radius (denoted by the colour-coding). ArcGIS10 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) map courtesy of Dr. Beyhan Amichev.

Table 6.2. Suitable marginal land (Agricultural Capability Classes 4 and 5) available for SRC willow production, at varying distances, surrounding the three coal-fired power stations in south-eastern Saskatchewan.

	Distance (km)				
	25	50	75	100	125
Power Plant	(ha)				
Boundary Dam	1,919[†]	12,790	37,256	78,850	122,124
Poplar River	22,040	50,607	103,336	139,674	214,140
Shand	7,970	32,780	84,550	108,513	139,505

[†] ArcGIS10 (Environmental Systems Research Institute, Inc., Redlands, CA, USA) estimates graciously provided by Dr. Beyhan Amichev, following the method of Amichev et al. (2012)

If willow biomass was co-fired at 40%, it would extend the available coal supply time-frame from 300 to 500 years. Additionally, the total GHG emission reductions from the three power stations after a 22-yr SRC willow plantation lifespan would be 15.2 Mt CO_{2eq} (Table 6.3) and according to the U.S. Environmental Protection Agency's online Greenhouse Gas Equivalencies Calculator, this equates to the emissions of: 143,939 passenger vehicles annually; combustion of 6,450,477,478 L gasoline, 35,348,837 barrels of oil, and 633,333,333 home barbeque propane cylinders; annual electricity use of 103,430 homes; and burning 105,555 railcars of coal! The reduction in GHG intensity and associated GHG emissions of a 40% blended willow feedstock, compared to the 'business as usual' scenario of using 100% coal at all three power stations was roughly 10% lower than the proportion of willow biomass blended (Table 6.3) and is in agreement with other reports (Heller et al., 2004; Styles and Jones, 2007b). However, when willow is blended at rates greater than 40%, the reduction is approximately 20% lower than the corresponding co-firing rate, which is due to the GHG emissions associated with the necessary torrefication/densification pre-treatment of the willow biomass prior to co-firing.

Despite the significant GHG emission reductions observed with a 40% substitution of willow biomass, new federal regulations require coal-fired electricity generation units to have GHG intensities of less than 420 kg CO_{2eq} MW_e^{-1} and, therefore, a considerably larger proportion of co-fired willow biomass would be required to meet this new emission performance

Table 6.3. Marginal land (Agricultural Capability Classes 4 and 5) required to provide adequate feedstock, and the associated greenhouse gas (GHG) emission reduction, associated with co-firing willow biomass at different rates within the three coal-fired power stations in south-eastern Saskatchewan over a 22-year SRC willow plantation lifespan.

	Co-firing rate (%)				
	5	10	15	20	40
Power Station[†]	Land Required (ha)[‡]				
Boundary Dam	14,904	29,808	44,712	59,616	153452[§]
Poplar River	10,476	20,952	31,428	41,904	107861
Shand	4,968	9,936	14,904	19,872	51151
	------------------ GHG Emissions Reduction (Mt CO_{2eq})[¶] ------------------				
Boundary Dam	1.1	2.1	3.2	4.2	7.5
Poplar River	0.7	1.5	2.2	3.0	5.2
Shand	0.4	0.7	1.1	1.4	2.5

[†]Net capacity of the Boundary Dam, Poplar River, and Shand power stations are 828, 582, and 276 MW_e, respectively (SaskPower, 2011)

[‡] Calculated by multiplying the percentage of coal-derived energy displaced using co-fired willow biomass by the conversion factor of 360 ha MW_e^{-1}, which was determined using a model based on co-firing trials and assumes annual productivity of 10 Mg oven-dry biomass ha^{-1} $year^{-1}$ (Dr. Tim Volk, personal communication). For example, Boundary Dam has a net capacity of 828 MW_e and if five percent (41.4 MW_e) is to come from co-fired willow biomass, then 14,904 ha (41.4 MW_e × 360 ha MW_e^{-1}) of SRC willow is required to provide the necessary feedstock

[§] Willow biomass must be torrefied and densified (i.e., pelletized) prior to co-firing when blended at rates > 20%. Torrefaction reduces willow feedstock weight by 30% and increases its energy density by 11% (from 19.8 to 22 MJ kg^{-1}; Prins et al., 2006; Bridgeman et al., 2008) and this was factored into the feedstock requirement. However, these two parameters may change depending on torrefaction conditions (e.g., temperature, heating rate, and residence time)

[¶] Calculated by: GHG $Emissions_{Coal}$ – GHG $Emissions_{Co\text{-}firing}$. The GHG emissions for each scenario were estimated using available complete fuel chain (including transportation and combustion) GHG intensity data for willow biomass (132 kg CO_{2eq} MWe^{-1}; Styles and Jones, 2007b), along with torrefied willow biomass and Saskatchewan lignite coal (265 and 1290 kg CO_{2eq} MW_e^{-1}, respectively; Dr. David Sanscartier, personal communication) standard (85%; Table 6.4). Displacing 85% of the coal would reduce GHG emissions by 32.3 Mt CO_{2eq} after 22 years, representing approximately 44% of Saskatchewan's 2011 anthropogenic GHG emissions (Environment Canada, 2012), and also extend the available coal supply from 300 to 2000 years. Additionally, this co-firing rate would require a total of 663,984 ha of SRC willow plantations, which is 40% more marginal land than is estimated to be available within 125 km of the three power stations (Table 6.3). However, there are a number of reasons why the refined 0.5 Mha estimate of available marginal land is probably underestimated, namely: i) the Prairie Farm Rehabilitation Administration (PFRA) land-use data used in the calculation is circa 1993-1995 and, therefore, may not accurately reflect post-1995 conditions; ii) only Class 4-5 marginal lands were considered, while the results of this Ph.D. work showed that some willow varieties growing on the Class 6 soil at Prince Albert were capable of producing > 10 Mg ha^{-1} yr^{-1} during the first rotation without supplemental fertility or irrigation (data not shown); iii) a soil salinity threshold of 8 mS cm^{-1} was used; however, several willow varieties have been identified that are tolerant of severe salinity (Appendix D); iv) and finally, only marginal land within the province was quantified (Figure 6.1). Presumably, there would be farmers in the bordering jurisdictions of Manitoba, Montana, and North Dakota (within the 125 km radius) who would be interested in diversifying their production system and growing willow on their abandoned land if there was a market for the harvested biomass. Consequently, it is realistic to assume that the amount of suitable marginal land around the three coal-powered plants could be much greater than the estimated 0.5 Mha, which could provide the necessary feedstock for supporting the adoption of 85% co-firing rate. However, forage crops may be grown on a proportion of these marginal lands and, therefore, expansion of the entire land base for SRC willow production could be displacing land out of feed production for energy production.

Notwithstanding the likelihood of underestimating the amount of suitable marginal land available for SRC willow production, it is important to also note that I used a conservative three-year rotation production limit of 10 Mg oven-dry biomass ha^{-1} yr^{-1}. Specifically, there was more than one variety capable of achieving this yield after a three-year rotation and all but one variety produced this after a five-year rotation (Table 6.1). Also, it is well know that willow productivity increases up to 40% with successive rotations (e.g., Guidi Nissim et al., 2013; Pacaldo et al., 2013; Quaye and Volk, 2013). As a result, even if the 0.5 Mha estimate is held constant, it is reasonable to assume that there would be adequate willow feedstock available to

Table 6.4. Greenhouse gas (GHG) intensity, GHG emission reduction, and percentage of available marginal land (Agricultural Capability Classes 4 and 5) utilized associated with co-firing willow biomass at different rates within the three coal-fired power stations in south-eastern Saskatchewan.

Co-firing rate	GHG Intensity	Reduced GHG Emissions†	Marginal Land Utilized‡
(%)	(kg CO_{2eq} MW_e^{-1})	(%)	(%)
0	1290	0	0
5	1232	4	6
10	1174	9	13
15	1116	13	19
20	1058	18	26
40	880	32	66
85	420§	68	140

† Compared to 'business as usual' scenario with 100% coal-generated power production
‡ Percentage of total available marginal land within a 125 km radius of all three power stations
§ New federal government performance standard for coal-fired electricity generation units, which must be met by the three Saskatchewan stations within the next 25 years or be decommissioned

support an 85% co-firing rate. In order to place this proposed marginal land requirement into perspective, it is important to recall that in 2005 the Saskatchewan government's mandate was to plant 1.6 Mha of short-rotation woody crops over 20 years, which was the impetus for this Ph.D. research, and the most extreme scenario presented here (i.e., 85% co-firing rate; requiring 663,984 ha) represents only 41% of the initial government directive.

The results of this study should provide utility for future analyses examining the viability of establishing commercial SRC willow plantations to deliver a reliable and environmentally friendly feedstock as a solid fuel to displace GHG-intensive fossil coal in existing coal-fired power stations in Saskatchewan. Co-firing is a simple, low-tech, inexpensive, and well-established method to reduce GHG emissions associated with electricity generation in the near-term, involving relatively minor technical adaptation (Tillman, 2000; Hillring, 2003; Baxter, 2005). Blending willow biomass with coal would help SaskPower comply with new federal

GHG regulations, thereby effectively serving to satisfy two key SaskPower mandates; namely, retaining coal in their electricity generating portfolio in the long-term and utilizing existing power stations– in order to minimize operating costs and support the economic integrity of the crown corporation (SaskPower, 2012). However, legislative change and policy support instruments (i.e., government subsidies) are critical to initiate the commercialization of SRC willow production (Sims et al., 2010). Consequently, the Saskatchewan government will need to initiate efforts to displace GHG-intensive coal with purpose-grown willow biomass in its coal-fired power stations to provide a significant market for SRC willow biomass production. Also, abundant willow feedstock will be a paramount necessity for investors supporting second generation biofuel conversion technologies and the associated biorefinery business model in the province, as the lack of sustainable supply chain for lignocellulosic feedstock is a principal barrier to their establishment (Kudakasseril Kurian et al., 2013). Furthermore, if the augmented environmental services (e.g., enhance soil quality, biodiversity, and C storage) provided by SRC willow plantations grown on these marginal lands can be valuated and corresponding subsidies returned to the farmer, this would be further incentive stimulating farmers to integrate this woody biomass crop into their production system (Dimitriou et al., 2011; Stolarski et al., 2011). Finally, blending renewable willow biomass with non-renewable fossil coal will also help satisfy increasing public demand for greener energy alternatives.

6.3 Future Research

Although coppicing woody plant species has been practiced and undoubtedly studied for thousands of years (e.g., *"For there is hope for a tree; when it is cut down, that it will sprout again, and its shoots will not fail"*; Job 14:7), this Ph.D. work has attempted to address a number of existing knowledge gaps related to SRC willow production systems, as well as identified the following areas where further research is required, which are outlined below.

6.3.1 Studying long-term fertilizer nutrient cycling using stable isotopes

While N is normally regarded as the most limiting nutrient in SRC willow production, this research has identified soil P availability as another important nutrient to manage over multiple rotations (Chapter 4). Likewise, strong correlations between willow productivity and subsequent depletion of soil Ca and Mg have been reported elsewhere (Ens et al., 2013). Though fertilizer amendments can be used to sustain soil fertility, the fate of applied fertilizers within SRC willow plantations after a prolonged time frame (i.e., throughout several rotations) is unknown. Consequently, fertilizer amendments labelled with stable isotopes other than ^{15}N (e.g., ^{31}P, ^{39}K, ^{34}S, ^{44}Ca, and ^{24}Mg) need to be used as well to examine fertilizer nutrient cycling within these plantations, to improve the understanding of fertilizer nutrient dynamics within different sinks during the year of application and beyond. Such longer-term insights into fertilizer nutrient dynamics will help to optimize fertilizer application rates and timing for achieving desired economic and environmental directives.

6.3.2 Long-term nutrient cycling and carbon dynamics of residual coarse woody debris, coarse root, and stool biomass.

Amichev et al. (2012) emphasized the need for branch decay data for validating long-term C budget models of SRC willow systems. Indeed, considerable branch biomass remains on site after harvest, but additionally, existing harvesting systems have efficiencies of around 90% (willow stools are cut 5-10 cm above-ground), which leaves substantial stem biomass behind as well. Moreover, the accumulated stool and associated coarse roots remaining post-harvest represents a significantly greater nutrient and C sink (> 25 Mg ha^{-1}; Pacaldo et al., 2013) compared to the initial rotation (2 Mg ha^{-1}; Table 4.7). With reported wide C:N values of 98, 113, and 200 for the stem, coarse root, and stool tissues, respectively (Pacaldo et al., 2013), these tissues presumably will have very slow decomposition and nutrient release rates after plantation termination, especially if left intact and not chipped up. Consequently, it is important to not only quantify the decomposition and nutrient release rates of these residual materials, but also the limit values associated with their mass loss and nutrient release for developing the most accurate C and nutrient budget models possible for SRC willow production systems.

6.3.3 Implications of using willow biochar as a soil amendment on greenhouse gas emissions

As the science evolves and knowledge increases with any new production system over time, there can be a change in understanding and this has been the case with respect to the net GHG emissions of SRC willow production. The progression of willow LCA indicated that at the end of a complete life span (i.e., seven three-year rotations), purpose grown willow was initially considered a weak C source (3.7 Mg CO_{2eq} ha^{-1}; Heller et al., 2003), then subsequently C-neutral

(Keoleian and Volk, 2005), and most recently a moderate sink for atmospheric C (-42.9 Mg CO_{2eq} ha^{-1}; Pacaldo et al., 2013). The principal difference among these inconsistent LCAs has been increased crediting of the C stored in the stool and coarse roots during the 22-year plantation lifespan. This is an excellent example of how LCA boundary conditions can have a profound influence on the subsequent conclusions, which in this case have further supported the position that SRC willow plantations are a legitimate renewable energy alternative for mitigating atmospheric GHG concentrations. Consequently, it is important to continue investigating all aspects of willow bioenergy feedstock, especially the waste streams created from biomass energy conversion technologies (e.g., recycling of nutrients in ash through land application). Specifically, although native wetland willow biomass is currently being used in Saskatchewan for space heating applications (Mirck and Schroeder, 2013) and clearly it has potential for use as a feedstock for electricity generation (Section 6.2.3), in both cases, simple biomass combustion is the least economically attractive option relative to value-added processing technologies, such as fast-pyrolysis, which is the most likely near-term end user of purpose-grown willow biomass, due to the favourable economics associated with multiple end uses of the pyrolysis oil (or bio-oil) produced.

Pyrolysis is a thermochemical process under anaerobic conditions involving rapid heating (500 ^{o}C for approximately two seconds) and subsequent cooling of lignocellulosic biomass to form secondary energy carriers, primarily consisting of bio-oil (80% of feedstock) and co-products biochar and gases (e.g., CH_4, H_2, and CO_2; Overend, 2004). Bio-oil is not only a renewable liquid fuel that can be directly substituted for fossil fuel in any application (e.g., static heating or electricity generation facility), but also can be upgraded to make platform chemicals or longer hydrocarbon liquid fuels for displacing fossil fuels (e.g., diesel, gasoline, and kerosene), which is why bio-oil is commonly called 'bio-crude' (McKendry, 2002; Bridgwater, 2012; Clark et al., 2012). Fan et al. (2011) examined the combustion of 100% willow bio-oil in existing large-scale electricity generation stations and reported LCA GHG intensity reductions from 79-86% depending on the fossil fuel displaced. Displacing coal-fired power generation resulted in the greatest GHG emission reductions and if GHG intensity values for the lower quality lignite coal used in Saskatchewan were used then calculated GHG emissions reduction would have been 89%. SRC willow is an attractive feedstock for fast pyrolysis due to its relatively low moisture content and high heating values of produced bio-oil and biochar

(Greenhalf et al., 2013). In commercial-scale pyrolysis systems, the goal is to maximize bio-oil production while using the less valuable biochar and gaseous co-products for process heat and/or drying the raw biomass feedstock prior to its pyrolysis. Using the biochar and gases as energy sources helps to maximize both the economic and environmental benefits, because it minimizes additional fossil energy used and the only waste streams are flue gas and ash.

Despite biochar being the least economically desirable pyrolysis co-product, there is increasing awareness of its utility as a soil amendment for improving both the agronomic and environmental functionality of soil (Kwapinski et al., 2010; Alotaibi and Schoenau, 2012), which is not only an economic positive for the farmer, but can also further mitigate atmospheric GHG concentrations. Matovic (2011) estimates that using willow biochar as a soil conditioner (to increase the stable soil C stock for millennia) could offset more that 300% of the target anthropogenic GHG emissions in Canada for the next two centuries given its large landbase and, is a promising abatement strategy for increasing atmospheric C levels, especially within semi-arid temperate climates like Saskatchewan with limited net primary productivity. In fact, some believe that sequestering C in soils via biochar amendments are the most efficient GHG mitigation strategy per unit of biomass, second only to biomass-displaced coal-fired power generation (Fowles, 2007). Willow feedstock pyrolysis research to date has focused primarily on evaluating the influence of variable process parameters on relative product yields and characteristics (Nowakowski et al., 2007; Greenhalf et al., 2012; 2013), however, to my knowledge no one has examined the influence of willow biochar on soil GHG emissions. Additionally, previous biochar amendment research has concentrated on tropical soils (i.e., old and highly-weathered, with acidic pH and low organic matter content and fertility; Blackwell et al., 2009), while the influence of biochar application on the relatively young and fertile soils of temperate regions is largely unknown. Future research, therefore, needs to investigate the potential of willow biochar to mitigate GHG emissions from Saskatchewan soils and is currently being investigated as a follow-up to this PhD thesis research.

6.3.4 Land-based municipal effluent treatment via short-rotation coppice willow plantations

The beneficial effects of applying municipal waste to SRC willow plantations in temperate regions have been reported by others in Europe (Rosenqvist et al., 1997; Rosenqvist and Dawson, 2005; Dimitriou and Rosenqvist, 2011; Holm and Heinsoo, 2013), U.S. (Adegbidi et al., 2003; Quaye and Volk, 2013), and eastern Canada (Labrecque et al., 1995; Labrecque et al., 1997; Labrecque et al., 1998). However, no one has examined its long-term effect on nutrient dynamics and willow growth in semi-arid environments like Saskatchewan, where the average annual precipitation is a fraction of that received during these other studies, especially when applied to marginal soils with inherently poor fertility and limited moisture holding capacity.

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APPENDIX A. A NOVEL PRE-TREATMENT FOR RAPIDLY SEPARATING WILLOW ROOTS FROM HIGH CLAY CONTENT SOIL

A.1 Preface

When trying to quantify willow root biomass, in particular the fine root fraction, it quickly became apparent how difficult and time-consuming it would be to separate the willow root biomass from the heavy clay soil matrix of the Saskatoon site. This study details the use of an efficient method to rapidly separate willow roots from a high clay content soil, allowing for reliable estimates of root biomass in this difficult to work with soil type. Collecting accurate root biomass data is essential for developing reliable nutrient budgets (Chapter 4) which form the basis of long-term fertilization prescriptions (Chapter 5) and are necessary for supporting the successful implementation of different proposed short-rotation coppice willow applications in Saskatchewan (Chapter 6). Note: this paper is published in Biomass and Bioenergy (46: 793-800). The co-author contributions to this manuscript were greatly appreciated and consisted of: J.J. Schoenau (provided financial assistance and manuscript editing); and K.C.J. Van Rees (provided financial assistance and manuscript editing).

A.2 Abstract

Numerous studies have examined the root dynamics of willow biomass energy crops growing on medium to light-textured soils, using either soil coring, minirhizotron techniques, or a combination thereof. However, neither approach is well suited for studying roots in expansive high clay content soil. Our objective was to test the efficacy of a novel inexpensive root washing pre-treatment, using baking soda ($NaHCO_3$), for facilitating the separation of willow roots from a Vertisol (70% clay). Soil cores were collected from within a willow variety trial plot of Tully Champion (*Salix viminalis* x *S. miyabeana*) and were either conventionally washed (i.e., no pre-treatment) or washed following a pre-treatment consisting of shaking the sample for 15 min with either deionized water or 1.2 mol dm^{-3} $NaHCO_3$. Measurement variables included washing duration, water usage, and recovery of fine (< 2 mm) and coarse roots. The ranking of washing duration and water usage was $NaHCO_3$ pre-treatment < deionized water pre-treatment < conventional washing. Compared to conventional washing, the $NaHCO_3$ pre-treatment reduced the washing duration and water usage by 45 and 61%, respectively, while increasing the fine-root recovery by 26%. There was no significant difference ($P > 0.05$) in the fine root recovery between the deionized water and $NaHCO_3$ pre-treatments or the coarse root recovery among the three washing methods. Developing a quicker technique of separating willow roots from high clay content soil, which conserves water and increases fine root recovery, should promote further investigations of root growth dynamics within this traditionally difficult soil type.

A.3 Introduction

Although initially proposed as a renewable dedicated bioenergy feedstock, with indirect environmental benefits through fossil fuel displacement (Christersson et al., 1993), purpose-grown shrub willow (*Salix* spp.) are increasingly employed in an array of ecotechnology applications that directly benefit the environment (Mirck et al., 2005; Kuzovkina and Volk, 2009). As with all plant species, willow depends on its root system to support its successful establishment and growth. Furthermore, understanding willow root growth dynamics is particularly important considering its coppice regeneration and concomitant dependency on its perennial root stock to provide stability for the above-ground growth, water and nutrient uptake, and also carbohydrate and nutrient storage (Volk, 2001). Additionally, willow roots can account for more than 50% of the annual net primary productivity (Rytter, 1999), representing a significant portion of the carbon sequestering ability of this fast-growing woody species. Reliable root biomass estimates are needed to: assist breeders with developing suitable genotypes with desired carbon allocation to roots *vs*. above-ground biomass (Rytter and Rytter, 1998); elucidating the effects of different biotic and abiotic stresses on willow productivity (Dickmann and Pregitzer, 1992); understanding the effect of global climate change on root dynamics and its impact on ecosystem carbon balance (Norby and Jackson, 2000); and finally, providing valuable data for the development and validation of nutrient and carbon budgets for different proposed willow applications, along with their associated models (Rytter, 1999; Amichev et al., 2011).

Notwithstanding the apparent need for reliable root biomass data, examining roots, particularly the fine-root fraction, is tedious, labour intensive, destructive, and expensive given the inaccessibility of root systems compared to the above-ground biomass component. Minirhizotrons are considered one of the best non-destructive alternatives for studying root growth dynamics *in situ* within managed or natural ecosystems (Johnson et al., 2001) and has recently been successfully used to study willow root morphology and growth dynamics (Rytter and Rytter, 2011). However, minirhizotrons alone are of limited use for estimating willow root mass or length densities in the bulk soil, unless used in conjunction with soil coring methods (Rytter and Rytter, 2011). Furthermore, within semi-arid environments like Saskatchewan, minirhizotrons are unsuitable for studying willow root dynamics in Vertisolic (i.e., high clay content) soil, consisting predominantly of 2:1 clay minerals (e.g., smectites) that exhibit tremendous shrink/swell properties with fluctuating soil moisture. Such argilliturbatic soil creates large gaps between the minirhizotron tube and bulk soil, which encourages preferential root growth and confounds fine root biomass estimates (Stadnyk, 2010). Consequently, soil coring will provide the most accurate estimate of below-ground biomass partitioning of willow growing on high clay content soil. However, separating willow roots from a soil with greater than 40% clay requires considerably more time and water compared to loam or sandy soil (Rytter, 2001), which increases the probability of fine root damage and loss (Stadnyk, 2010). Automated washing systems have been developed (Smucker et al., 1982; Oliveira et al., 2000), but are relatively expensive and best suited for use with medium to light-textured soils (Carlson and Donald, 1986), thereby necessitating longer washing cycles as clay content increases, which can lead to increased fine root damage (Kosola et al., 2007). Despite their convenience, mechanized washers are incapable of washing roots cleaner than conventional manual washing, especially when processing clay-rich soil (Böhm, 1979). Such logistical difficulties may explain the scarcity of studies reporting willow root growth dynamics in high clay content soil and indicate the need for developing a better alternative to conventional washing, which involves no pre-treatment to assist in root separation from the clay matrix.

Solonetzic or sodic soils occur worldwide and their genesis results from the presence of abundant sodium (Na)-salts within the soil profile; either inherent within the parent material or supplied by groundwater discharge (Miller and Brierley, 2011). The high exchangeable-Na content causes soil alkalization, with the Na-saturated clay minerals having thicker diffuse double layers, causing repulsion and deflocculation of clay particles, which eluviate from the A to the B horizon to form a dense hardpan layer (Pawluk, 1982). Sodium compounds have been successfully used to facilitate the separation of roots from high clay content soil, namely: sodium acetate (Azcue, 1996); sodium chloride (Huttel, 1975; Tardieu, 1986), sodium hydroxide (McQueen, 1968), and sodium phosphates (Schuurman and Goedewaagen, 1971; van Noordwijk, 1993; Cassel et al., 1995). Other chemical pre-treatments include: acetic acid (Mitchell et al., 1993; Azcue, 1996), EDTA (Azcue, 1996), hydrochloric acid (Böhm, 1979; Azcue, 1996), hydrogen peroxide (Dobrynin, 1968), $MgCl_2$ (Azcue, 1996), and oxalic acid (Heringa et al., 1980). These techniques often involve soaking samples for several hours, which can not only alter root tissue colour, thereby confounding the separation of living vs. dead roots (Böhm, 1979), but also affect subsequent nutrient analyses by either contaminating the root sample or leaching nutrients from the root tissue (Azcue, 1996; Snowdon et al., 2002). Hence, it is preferential to utilize an innocuous, environmentally benign, readily available, and inexpensive alternative root washing pre-treatment. The objective of this study was to apply the first principles of Na-induced dispersion of soil colloids to develop an improved method of separating willow roots from high clay content soil by using a $NaHCO_3$ pre-treatment before washing. We hypothesized that shaking soil core samples in a solution with abundant Na, would saturate the clay surfaces with Na, disperse clay aggregates and liberate the bound roots (especially the fine roots), resulting in more efficient root-soil separation and increased fine-root recovery compared to conventional washing. Developing a technique that rapidly separates willow roots from clay-rich soil will not only save time, but also conserve water, which is advantageous for supporting global initiatives targeting the sustainability of this vital resource (Holger, 2009). Additionally, improving fine root estimates within clay soils should facilitate further root studies involving this traditionally difficult soil type.

A.4 Materials and Methods

A.4.1 Study site and willow variety

The data for this study were collected in the fall of 2011 from a five-year-old shrub willow variety trial located on the University of Saskatchewan campus in Saskatoon, SK, Canada (UTM coordinates: 13U 389970 5776342). The site information is described in detail in Hangs et al. (2011) and a brief summary follows. The soil is a heavy clay Orthic Vertisol (Sutherland Association (SCSR, 1978); 70% clay content), developed on glacial lacustrine parent material, with a pH and electrical conductivity (dS m^{-1}) of 7.1 and 0.33, respectively. The semi-arid temperate location receives on average 350 mm of annual precipitation (70% occurring from May to September) and has a mean annual temperature of 2^{o}C, with approximately 112 frost-free days. The Agriculture Capability Classification rating of the soil is Class 2, with moderately severe limitations due to a lack of precipitation (SCSR, 1978). In 2007, thirty willow varieties, developed by the State University of New York College of Environmental Science and Forestry (SUNY-ESF) breeding program, were planted as follows: each varietal plot (6.3 x 7.8 m) consists of 78 plants (three double-rows of 13 plants row^{-1}), with spacings of 1.5 m between the double-rows, 60 cm between rows within the double-row, and 60 cm between plants within the row; resulting in a planting density of approximately 15,873 plants ha^{-1} (Fig. A.1). The above-ground willow biomass within all variety plots was harvested in the spring of 2011, so at the time of this study, each multi-stemmed willow plant (i.e., stool) consisted of one-year-old stems on five-year-old roots. The willow variety used in this study was Tully Champion (*Salix viminalis* x *S. miyabeana*); selected given its superior above- and below-ground biomass production on both arable and marginal soils (Smart and Cameron, 2008; Hangs et al., 2011a).

A.4.2 Soil core collection and washing procedures

Thirty-six soil cores were systematically collected from within a single plot of Tully Champion (Fig. A.1), using an 8 cm diameter bucket auger (Eijkelkamp Agrisearch Equipment BV, Giesbeek, GLD, The Netherlands), and sampled down to a depth of 30 cm, where the majority of willow roots occur (Rytter, 1999; Heinsoo et al., 2009). At the time of sampling, the

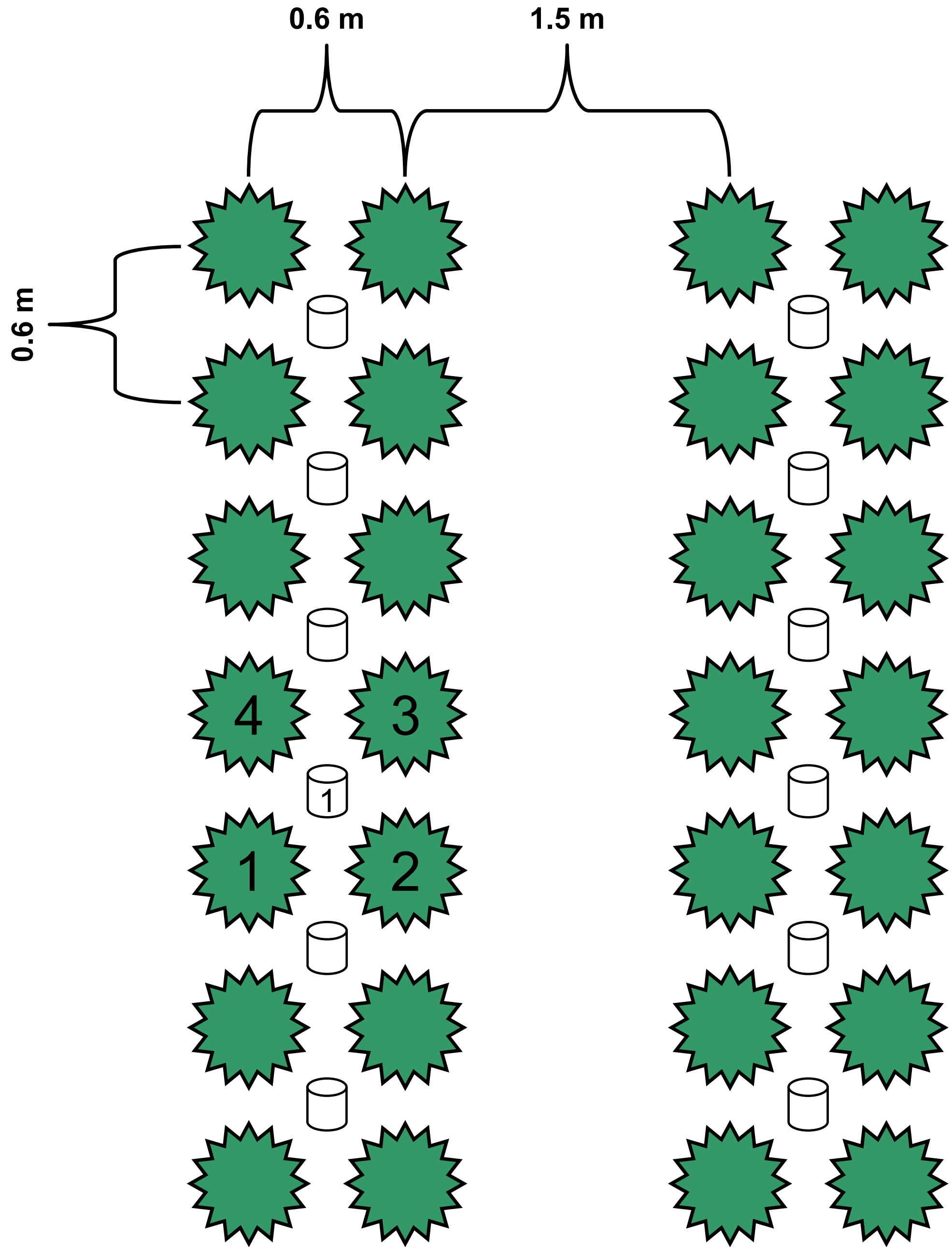

Fig. A.1. Willow variety trial plot layout and soil core sampling locations. Note: in order to examine the relationship between measured above-ground willow biomass and recovered fine and coarse root biomass following each washing treatment, the average stool biomass (e.g., 1, 2, 3, and 4) surrounding each collected soil core (e.g., 1) was related to the recovered fine and coarse root biomass of the core.

soil moisture content was near field capacity (35%; v v^{-1}). Each soil core was placed in a polyethylene bag and frozen until processed (Schuurman and Goedewaagen, 1971). The effect of freezing on root tissue biomass was assumed to be negligible (Böhm, 1979), though deep freezing should be avoided if determining nutrient contents, due to increased cell electrolyte leakage within fine root tissues (Rytter and Rytter, 2010). The frozen soil cores were thawed overnight at room temperature, randomly divided into three groups, and assigned to one of three treatments prior to washing: i) no pre-treatment, ii) shaken in deionized water for 15 min, or iii) shaken in a $NaHCO_3$ solution for 15 min. A 1.2 mol dm^{-3} $NaHCO_3$ solution was prepared by dissolving a standard 500 g box of Arm and Hammer® (Princeton, NJ, USA) baking soda into 5 L of deionized water. Each soil core was placed in a 11.3 L Rubbermaid® storage container, submerged in either deionized water or 1.2 mol dm^{-3} $NaHCO_3$, and shaken for 15 min at 144 rpm on a G10 Gyrotory Shaker (New Brunswick Scientific, Edison, NJ, USA). All soil core samples were manually washed with tap water by a single person and the roots were collected using a double-sieve system (2 mm and 0.5 mm mesh). The roots were divided into fine (< 2 mm) and coarse-size fractions, dried (65 ^{o}C) to a constant mass, and weighed. The non-crop vegetation within the plot was sparse, due to extensive vegetation management and canopy closure (2011), thus all roots were assumed to be that of willow. No attempt was made to distinguish between live and dead roots; therefore, all root biomass was measured. Fine and coarse root biomass density (g dm^{-3}) was calculated by dividing the dried root biomass of each size fraction by the total volume of augered soil (i.e., 8 cm dia x 30 cm depth; 1.5 dm^3). The% ash content of all root tissues was determined by weighing the ash residue after ignition in a Barnstead Thermolyne© FB 1400 muffle furnace (Dubuque, Iowa, USA) at 650 ^{o}C for 6 h (Oliveira et al., 2000). The root biomass data was corrected for its ash content and the values presented on an ash-free basis. The time required to wash each soil core and the amount of water used (Water Saver™, AbsolutelyNew, Inc. San Francisco, CA, USA) were both measured. The water usage data included the volume of deionized water used in the two shaking pre-treatments; however, neither the pre-treatment setup period nor the 15 min shaking time were included in the washing duration measurement.

A.4.3 Relationship between above-ground biomass and recovered root biomass fractions

A conventional allometric equation was developed to estimate above-ground willow biomass, by calibrating measured stem diameter with harvested leafless biomass from 12 stems representing the diameter range within the plot (Arevalo et al., 2007). The allometric equation was derived using a simple non-linear power regression expressed in Equation A.1:

$$HB = aD_{30}^{b} \qquad \text{(Eq A.1)}$$

where HB and D_{30} are the harvested oven-dry biomass (stem + branches) and measured stem diameter (at 30 cm height) and a and b are the allometric coefficient and exponent constants, respectively. The stems of each willow stool within the plot were likewise measured, their diameters applied to the allometric equation ($y = 0.0847x^{2.645}$; $R^2 = 0.99$; $P < 0.001$; n = 12), and summed to estimate above-ground willow biomass for each stool. In order to examine the relationship between measured above-ground willow biomass and recovered fine and coarse root biomass following each washing treatment, the average biomass of the four stools surrounding each collected soil core was compared to the recovered fine and coarse root biomass from the core (Fig. A.1).

A.4.4 Statistical analyses

Measurement variables were analysed using PROC MIXED in SAS (version 9.2; SAS Institute Inc., Cary, NC., USA). Means comparisons were performed using least significant differences (LSD; equivalent to Fisher's protected LSD) at a significance level of 0.05, with groupings obtained using the pdmix800 SAS macro (Saxton, 1998). PROC REG was used to carry out simple linear regressions with pooled data (n = 12) to quantify the relationship between measured above-ground willow biomass and recovered fine and coarse root biomass following each washing treatment. Normality of distributions (PROC UNIVARIATE) and homogeneity of variances (Bartlett's test) of all data sets were checked prior to the analysis. No data transformations were necessary.

A.5 Results and Discussion

A.5.1 Washing duration and water usage

The time required to separate the willow roots from the clay-rich soil ranged from 6.9 to 12.4 minutes per core among the treatments (Fig. A.2a). Shaking the soil cores in either deionized water or a $NaHCO_3$ solution for 15 min prior to washing, reduced the washing duration compared to conventional washing. The amount of water used per core sample varied from 76.1 to 194.7 L among the treatments (Fig. A.2b). The most efficient use of time and water occurred following the $NaHCO_3$ pre-treatment, which reduced washing duration and water usage by 45 and 61%, respectively, compared to conventional washing (Fig. A.2c). The measured differences in washing duration and water usage among the treatments are elucidated by visually comparing the soil core samples prior to washing. Specifically, unlike conventional washing, where the retention of subangular blocky aggregate structure is apparent (Fig. A.3a), shaking the core sample in deionized water dissolved some aggregates (Fig. A.3b), due to the destabilizing slaking effect of water immersion (Hillel, 1982), which then facilitated the separation of roots from the clay soil. Shaking the soil core in a $NaHCO_3$ solution, however, completely dispersed the clay particles and destroyed the aggregates, resulting in an amorphous suspension that was more conducive for root separation (Fig. A.3c). The abundant exchangeable-Na exchanged with the Ca on the clay mineral surfaces, deflocculating the soil aggregates, while the free HCO_3^- reacted with the liberated Ca^{2+} to form calcium bicarbonate, which commonly occurs within alkaline calcareous sodic soils (Pawluk, 1982), thus removing an important flocculating element from solution. No attempt was made to examine the effects of different concentrations of $NaHCO_3$ and/or shaking interval on subsequent washing duration and water usage. Both parameters may need adjusting if the clay content of the soil core increases significantly beyond the 70% tested here; however, the $NaHCO_3$ concentration of 1.2 mol dm^{-3} used in this study is close to the saturation limit of $NaHCO_3$ at room temperature, therefore, it is expected that increasing the shaking time would be the best approach to pre-treat soil cores with clay contents in excess of 70%, followed by replenishing the $NaHCO_3$ solution.

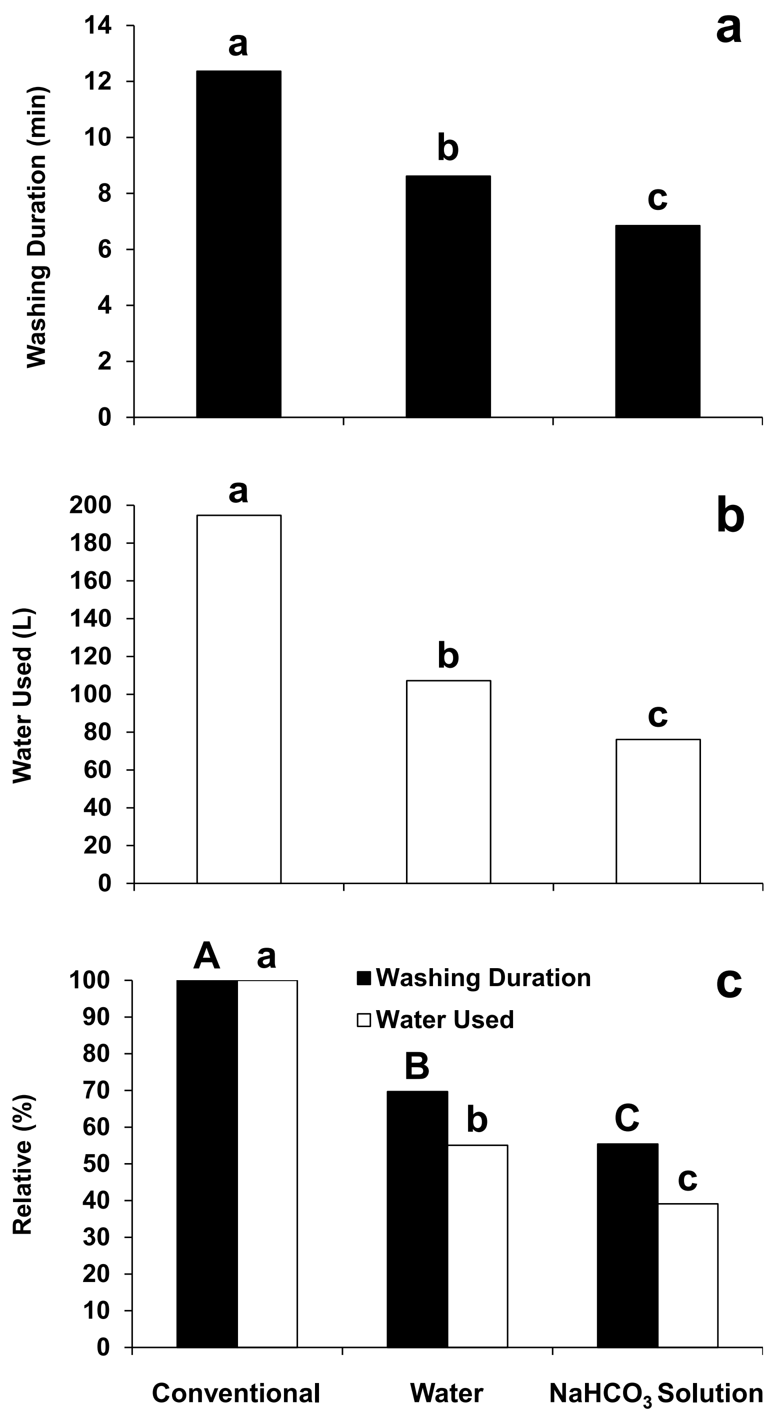

Fig. A.2. Mean (n = 12) washing duration (a) and water used (b) to separate willow roots from a clay-rich (70%) soil core, either conventionally washed or washed following a pre-treatment consisting of shaking the sample in solution for 15 min with either deionized water or 1.2 mol dm^{-3} $NaHCO_3$. Relative differences among the methods are also shown (c). Note: Treatment bars having the same letter are not significantly different ($P > 0.05$) using LSD. For (c), only means comparisons within measurement variable (i.e., similar letter case) are valid.

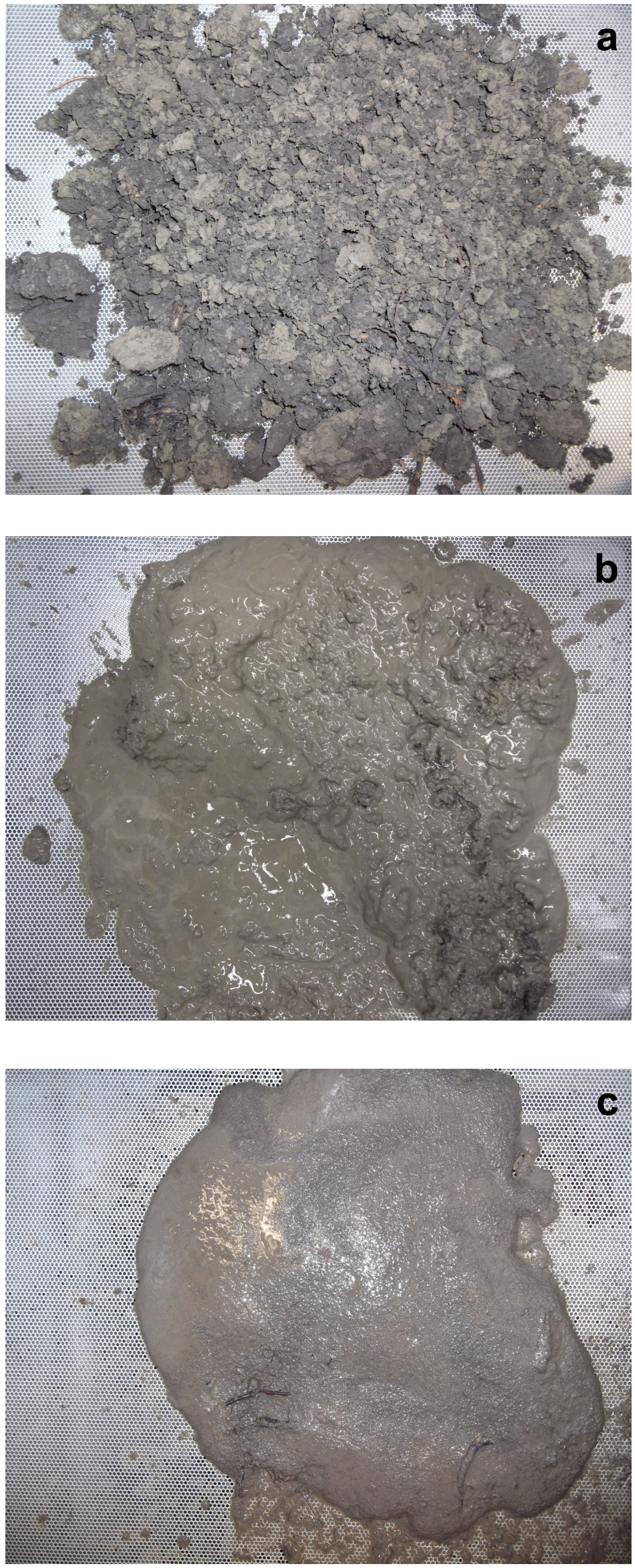

Fig. A.3. Clay-rich (70%) soil cores prior to manual washing without pre-treatment (a) or shaken in solution for 15 min with either deionized water (b) or 1.2 mol dm^{-3} $NaHCO_3$ (c).

A.5.2 Recovery of fine and coarse roots

The fine root biomass density estimates among the treatments ranged from 0.7 to 0.9 g dm^{-3} (Fig. A.4a). The corresponding stand-level fine root biomass estimates ranged from 2.1 to 2.7 Mg ha^{-1}, however, this may be an overestimation given the systematic sampling scheme used. Specifically, instead of randomly distributing the soil core sampling throughout the plantation area, we purposely avoided sampling within the larger inter-row space (Fig. A.1), to preclude the effect of site maintenance (e.g., reduced root density following tillage; (Stadnyk, 2010)) from introducing artifacts that would confound our treatment comparisons. Shaking the soil cores in a $NaHCO_3$ solution increased fine root recovery by 26% compared to conventional washing (Fig. A.4c). There were no significant differences ($P > 0.05$) in coarse root recovery (Fig. A.4b) or fine root:coarse root (data not shown) among the treatments. Willow fine roots are more susceptible to damage and loss during washing relative to coarse roots (Stadnyk, 2010); consequently, the measured increase in fine root biomass recovery after shaking in a $NaHCO_3$ solution is not surprising, given the effectiveness of this pre-treatment in separating the roots from the high clay content soil. Minimizing the washing duration undoubtedly supported a greater recovery of fine roots, especially the smaller higher-order fine root fraction (i.e., < 0.5 mm) that can comprise the majority of willow fine roots (Rytter and Rytter, 1998). Although not quantified, visually there were considerably smaller fine roots recovered following the $NaHCO_3$ pre-treatment compared to the other two treatments. Reduced washing activity not only creates less disturbance (i.e., fragmentation) of the root system, which increases the likelihood of collecting intact root branches in a 0.5 mm sieve, but also decreases leaching losses of water-soluble compounds from root tissues (Azcue, 1996; Oliveira et al., 2000; Püttsepp et al., 2007). While there was no significant difference ($P > 0.05$) in fine root recovery between samples shaken in either deionized water or $NaHCO_3$ solution prior to washing (Fig. A.4a), the $NaHCO_3$ pre-treatment is advantageous for reducing both washing duration and water usage. The average ash content of fine and coarse root subsamples was 20.2% and 8.9%, respectively, with no significant differences ($P > 0.05$) among the washing methods for either root size fraction (data not shown). The greater proportion of mineral particles associated with the fine root fraction has been acknowledge previously and can account for as much as 50% of the washed root mass (Böhm, 1979).

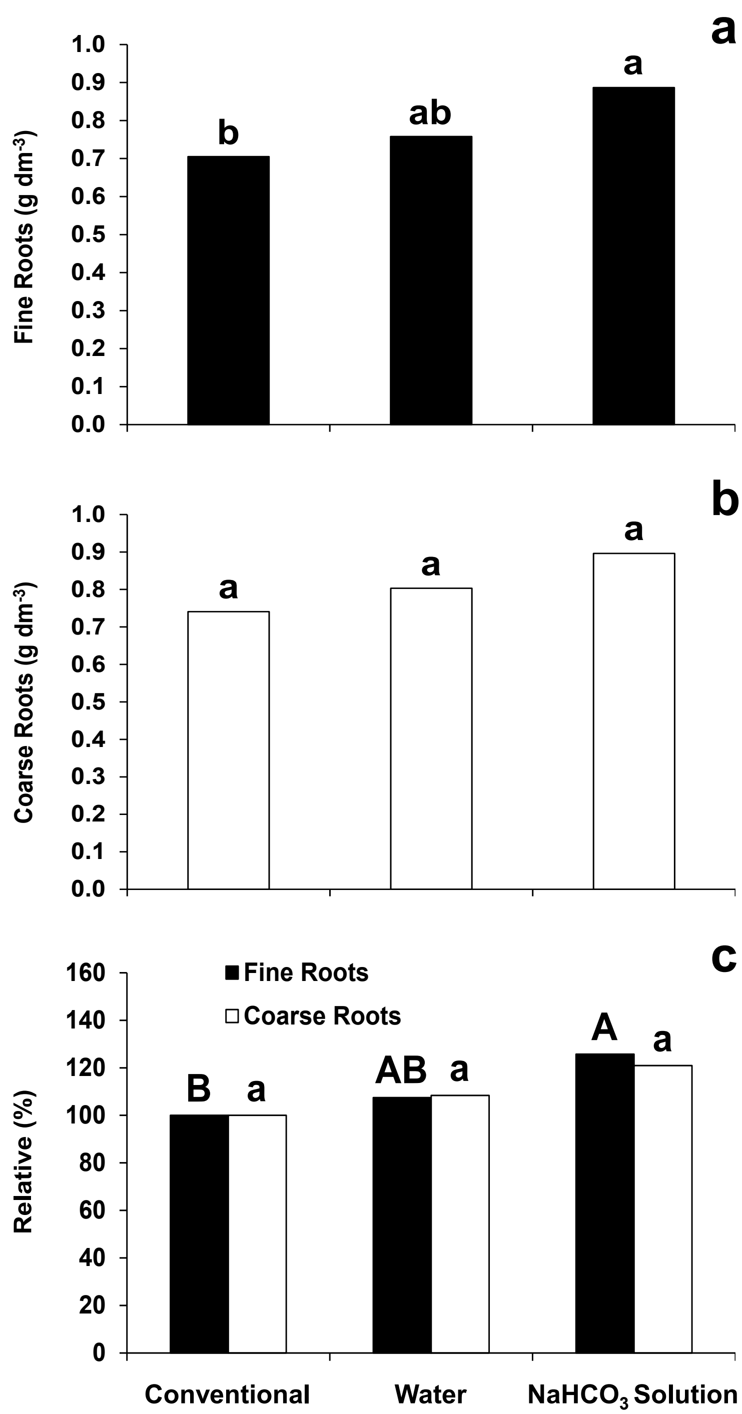

Fig. A.4. Mean (n = 12) willow fine (< 2 mm; a) and coarse root (b) biomass recovered from clay-rich (70%) soil cores, either conventionally washed or washed following a pre-treatment consisting of shaking the sample in solution for 15 min with either deionized water or 1.2 mol dm^{-3} $NaHCO_3$. Relative differences in willow root recovery among the methods are also shown (c). Note: Bars having the same letter are not significantly different (*P* > 0.05) using LSD. For (c), only means comparisons within a root size fraction (i.e., similar case) are valid.

A.5.3 Relationship between above-ground biomass and recovered root biomass fractions

The measured above-ground willow biomass was only correlated with the fine root biomass estimates following the $NaHCO_3$ pre-treatment (R^2 = 0.56, $P < 0.01$; Fig. A.5). Removal of the seemingly errant fine root observation of 1.0 g dm^{-3} (Fig. A.5c) from the regression analysis greatly improves the model (i.e., $R^2 = 0.79$, $P < 0.0003$); however, a Grubbs' test (alpha = 0.05) failed to identify it as an outlier and there was no visually apparent reason for the atypical fine root proliferation at that location to justify its removal from the data set. The $NaHCO_3$ pre-treatment appears to provide fine root biomass data that is biologically more meaningful, compared to the other two treatments, which may be a function of its ability to increase the recovery of smaller higher-order fine roots. The lack of correlation between above-ground willow biomass and coarse root biomass estimates, regardless of soil core treatment (data not shown), supports the critical role fine roots play in water and nutrient uptake for supporting willow productivity (Rytter and Hansson, 1996). Fine roots are particularly important in the semi-arid climate of Saskatchewan, where moisture availability often is considered the primary controller limiting growth for both annual and perennial plant species (Akinremi et al., 1996; Hogg and Schwarz, 1997).

A.6 Conclusion

Roots are an integral component of plant morphology and physiology, in addition to an important constituent of the plant carbon sink. Therefore, documenting root growth dynamics, in particular the fine root fraction, is essential. Minirhizotrons are the preferred method of studying willow roots, but are unsuitable for use within high clay content soil located in semi-arid environments. In these clay-rich soils, conventional soil cores will provide the most reliable estimate of below-ground biomass partitioning of willow, but separating the roots from the predominantly clay matrix is difficult using conventional washing procedures. A pre-treatment involving shaking soil core samples with a $NaHCO_3$ solution for 15 min, prior to washing, decreased the amount of time and water required to separate willow roots from the clay soil, while increasing fine-root recovery. The results of this study should facilitate and promote more investigations of root growth dynamics in high clay content soils, which may otherwise be avoided due to the inherent difficulties in liberating roots using conventional washing techniques. Further studies will advance our understanding of willow root growth dynamics, along with attendant management implications, when cultivating this fast-growing woody species on high clay content soils.

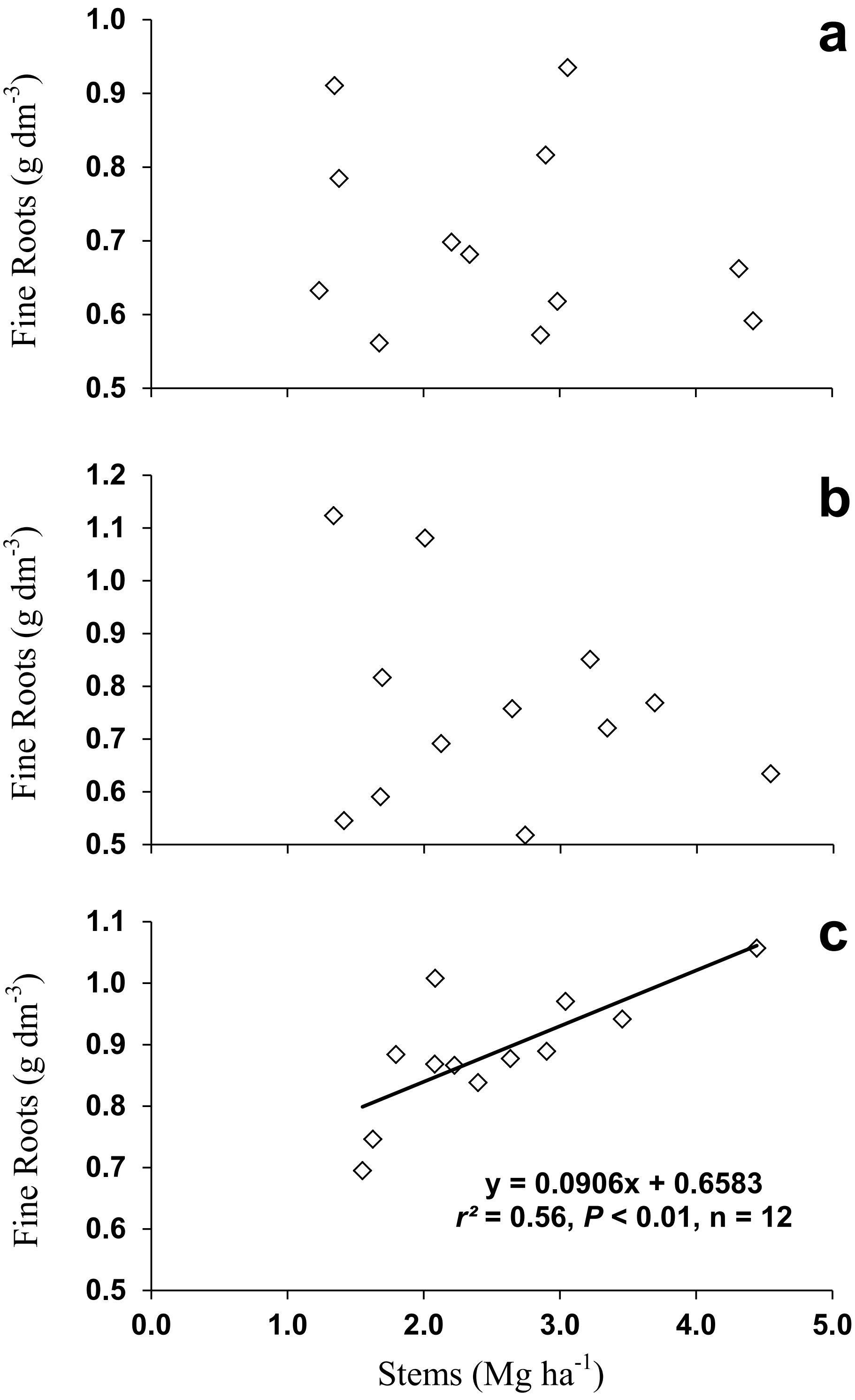

Fig. A.5. Relationship between harvested oven-dry willow biomass (stem + branches) and fine root biomass recovered from clay-rich (70%) soil cores, either conventionally washed (a) or washed following a pre-treatment consisting of shaking the sample in solution for 15 min with either deionized water (b) or 1.2 mol dm^{-3} $NaHCO_3$ (c).

APPENDIX B. A SIMPLE TECHNIQUE FOR ESTIMATING ABOVE-GROUND BIOMASS IN SHORT-ROTATION WILLOW PLANTATIONS

B.1 Preface

Assuming that short-rotation coppice willow plantations reach a commercial stage in Saskatchewan someday (Chapter 6), the necessity will exist to efficiently measure the harvestable biomass throughout the rotation in order to determine the necessity of different management practices needed to maximize the growers' return on investment. This paper detailed the use of the LAI-2000 instrument to estimate harvestable willow biomass, which is a novel method of estimating above-ground willow biomass, involving measuring light attenuation through the willow canopy, to calculate a 'stem area index' for relating to harvested willow biomass. Note: this paper is published in Biomass and Bioenergy (35: 2156-2162). The co-author contributions to this manuscript were greatly appreciated and consisted of: J.J. Schoenau (provided financial assistance and manuscript editing); K.C.J. Van Rees (provided financial assistance and manuscript editing); and, X. Guo (provided the LAI-2000 Plant Canopy Analyzer and manuscript editing).

B.2 Abstract

Successful purpose-grown willow production systems require regular monitoring of willow growth to apply timely management techniques for increased productivity and timing of harvest for maximizing profit. The objective of this study was to assess the efficacy of a novel method of estimating above-ground willow biomass, involving measuring light attenuation through the willow canopy, to calculate a 'stem area index' for relating to harvested willow biomass. Two different willow clones, with contrasting growth form, were used: single stem (Charlie) and multi-stem (SV1). Given the strong correlations ($R^2 > 0.97$; $P < 0.05$) between the measured stem area index and harvested willow biomass, regardless of growth form, it appears that this simple mensurative technique is a promising alternative for estimating above-ground biomass in short-rotation willow plantations.

B.3 Introduction

Considering that harvesting operations are the greatest single cost incurred with short-rotation willow production systems (Mitchell et al., 1999; Keoleian and Volk, 2005; Tharakan et al., 2005), it is imperative for producers to optimize the timing of harvest, based on accurate estimations of current yield, in order to support the greatest economical return on investment. Additionally, monitoring annual production rates will be invaluable for making effectual management decisions prior to harvest, such as prompting fertilization or pest control to increase productivity, for meeting both economic objectives and/or contractual obligations with industrial partners relying on feedstock commitments. Manually collecting above-ground samples for biomass estimates can be time consuming, costly, susceptible to subjective errors, and inherently destructive. As a result, it is prudent to develop a rapid, cost-effective, and non-destructive technique that yields reliable biomass estimates to support effective management decisions in a timely manner. The conventional non-destructive technique is allometry– defined by a simple empirical relationship between size and mass, which involves calibrating measured stem diameter (at a specified height) with subsequently harvested biomass (Nordh and Verwijst, 2004; Arevalo et al., 2007). However, developing reliable allometric equations specific to clone, age, and site characteristics is prohibitively time-consuming and costly in many situations (Arevalo et al., 2007). Since the mid-1980s, several alternative non-destructive remote sensing meteorological approaches have been successfully used for collecting a variety of plant mensuration data, including: light detection and ranging (LiDAR; Næsset, 1997; Hopkinson et al., 2004); radar (Imhoff et al., 1998; Axelsson et al., 1999); sonar (McKerrow and Harper, 1999); and vegetation spectral reflectance (Casanova et al., 1998; Yang et al., 2007). Notwithstanding their efficacy at providing reliable estimates, these sophisticated techniques are expensive and inherently complicated during the data collection and processing stages. As such, there remains a need to develop a mensurative technique for estimating willow biomass, having not only the accuracy of allometry, but also non-destructively and economically providing data rapidly.

This study evaluated the use of the LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, NE; Fig. B.1) to measure the 'gap fraction', which is the fraction of the sky visible from beneath the canopy, by quantifying the fraction of sky that is blocked by branches or stems (Welles and Norman, 1991; LI-COR, 1992). Briefly, the LAI-2000 measures light attenuation(i.e., reduction in amplitude and intensity) as it passes through a vegetative canopy and its utility has been reported in hundreds of articles covering a range of vegetation types, including: willow plantations (Tharakan et al., 2008); shrubs and grasses (He et al., 2007; Sonnentag et al., 2007); coniferous, deciduous, and mixedwood forests (Gower and Norman, 1991; Comeau et al., 1998); annual crops (Dobermann et al., 1995; Hicks and Lascano, 1995; Rudorff et al., 1996); vineyards (Johnson and Pierce, 2004); turfgrass (Yuen et al., 2002); and even non-crop species (Thevathasan et al., 2000). The LAI-2000 measures gap fraction by concurrently measuring the difference between the diffuse incident radiation at the top of the canopy with the diffuse transmitted radiation under the canopy– assessed at five different central angles relative to the zenith (7, 23, 38, 53, and 68^{o}), using a "fish-eye" 148° field-of-view optical sensor (LI-COR, 1992; Fig. B.1). The sensor is strictly sensitive to short-wave radiation (i.e., < 490 nm), which minimizes the effect of light scatter within the canopy, therefore, resulting in a much stronger contrast between plant material and sky elements (Leblanc et al., 2005). The LAI-2000 uses all five zenithal angle gap fraction measures to simultaneously calculate leaf area index (LAI; ratio of the canopy foliage area to ground area), using well established inversion and integration models describing radiation transfer through vegetation canopies (Welles and Norman, 1991; Jonckheere et al., 2004).

Fig. B.1. Estimating above-ground leafless willow biomass using a LAI-2000 Plant Canopy Analyzer to measure the 'gap fraction' (i.e., fraction of the sky visible from beneath the canopy) corresponding to five sensor rings centred on different zenithal angles.

Notwithstanding its popularity, a common criticism of the LAI-2000 is that its measured LAI values are not ‘true’ LAI values. Specifically, because of its 490 nm filter, it cannot distinguish between radiation intercepted by photosynthetic leaves vs. non-photosynthetic woody stems and branches within the canopy, which can lead to a LAI overestimation (Weiss et al., 2004). The intention of this study, however, was to use the LAI-2000 to measure LAI *after leaf fall*, in order to test its utility as a surrogate measure of leafless above-ground biomass within willow plantations. By measuring the gap fraction of non-photosynthetic woody material, the LAI-2000 is, therefore, essentially providing a measure of ‘stem area index’ (SAI), which can be calibrated with harvested biomass. The objective of this study was to measure the SAI of two different willow clones having contrasting growth forms, using the LAI-2000, and relate these data to harvested above-ground biomass. Given that field observations clearly indicate the effect variable above-ground willow biomass has on transmitted radiation at ground level, it was hypothesized that the LAI-2000 would provide a fast and reliable SAI measurement and, thus, serve as an effective alternative for estimating harvestable willow biomass.

B.4 Materials and Methods

B.4.1 Study site

The data for this study were collected in the spring of 2008 from a two-year-old willow plantation located on the University of Saskatchewan campus in Saskatoon, Saskatchewan, Canada (UTM coordinates: 13U 389970 5776342). The soil is a heavy clay Orthic Vertisol of the Sutherland Association, developed on glacial lacustrine parent material, with a pH and electrical conductivity (dS m^{-1}) of 7.1 and 0.33, respectively. The semi-arid temperate location receives on average 350 mm of annual precipitation (70% occurring from May-September) and has a mean annual temperature of 2 ^{o}C, with approximately 112 frost-free days. The Agriculture Capability Classification rating of the soils are Class 2-3, with moderately severe limitations due to a lack of precipitation. For a complete description (i.e., drainage, stoniness, map unit, etc.) of the soils see (SCSR, 1978). Prior to establishing the plantation in 2006, the site was continuously cropped to a mixture of barley and oats. Pre- and post-planting site preparation included both mechanical (deep tillage, light cultivation, tandem disc, mowing, and hand weeding) and chemical (linuron– 1.7 kg a.i. ha^{-1} and glyphosate– 2.0 kg a.i. ha^{-1}) treatments to control non-crop vegetation.

The willow plantation is a clonal trial arranged in a randomized complete block design that is replicated three times. The willow were planted using a 0.6 x 0.6 m grid spacing for each 30 m long triple-row bed, with 2.0 m spacing between the beds (approximate density of 15,625 stems ha^{-1}). Two willow clones, having contrasting growth forms (Figs. B.2a and B.2b), were used: Charlie (*Salix alba* x *Salix glatfelteri*; single stem) and SV1 (*Salix dasyclados*; multi-stem), which are the standard clones for comparison (i.e., survival, yield, pest resistance, etc.) within Canada and the U.S., respectively (D. Sidders, Canadian Forest Service, personal communication; Volk et al., 2006).

B.4.2 Development of stem area index as surrogate for estimating harvestable willow biomass

A LAI-2000 was used to measure the gap fraction and subsequently calculate the SAI for leafless willow within each clonal bed for correlation with harvested above-ground willow biomass (Fig. B.1). Briefly, three different sampling scales (between-bed, within-row, and single plant) were used to collect SAI measurements using the LAI-2000 (Fig. B.3). Each of these sampling scales has been successfully used to measure gap fraction for a variety of plant crops with either discontinuous or heterogeneous canopies (Welles and Cohen, 1996; Weiss et al., 2004). However, given the contrasting growth forms of the two willow clones studied, all three approaches were assessed to determine which provided the most reliable estimate of SAI for routine use within the short-rotation willow plantation context. All three sampling scales involved the following: placing the sensor near the soil surface; using both a 45^{o} and 90^{o} view cap (consisting of a 315^{o} and 270^{o} opaque mask, respectively) to restrict the azimuthal range of the sensor– necessary to prevent light not transmitted through the canopy from influencing the measurements (a common concern with discontinuous row crops), but also to obscure the operator from the sensor; one above-canopy measurement was taken for every four below-canopy measurements (in the same azimuthal direction) to allow the LAI-2000 to determine the fraction of diffuse incident radiation passing through the willow canopy– required for calculating the SAI of the triple-row clonal bed; and finally, taking measurements under diffuse sky conditions (i.e., overcast), in order to avoid direct sunlight and/or light scattering within the canopy from influencing the readings. If these were operational-scale plantations, then these sampling scales would be randomly located within the plantation; however, in view of its small research-scale plot size, each sampling scale was systematically set up to sample the entire triple-

Fig. B.2. Two willow clones, having contrasting growth forms, were used in this study: Charlie (*Salix alba* × *Salix glatfelteri*; single stem; a) and SV1 (*Salix dasyclados*; multi-stem; b). Note: white reference rods were used in a separate photogrammetry study.

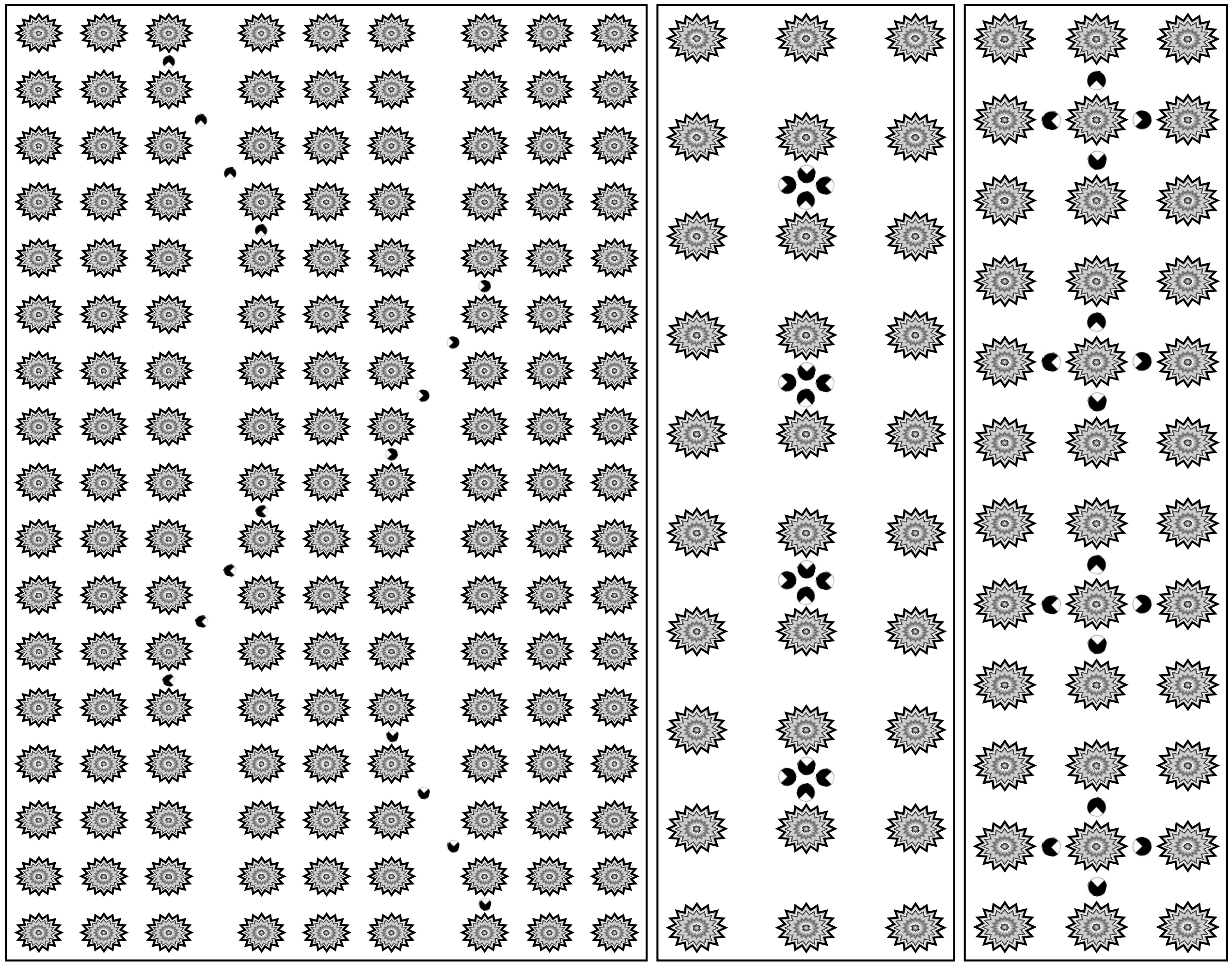

Fig. B.3. Schematic illustrating the use of the LAI-2000 Plant Canopy Analyzer (with 90^{o} view cap indicated by white fraction of circle), at varying sampling scales, to measure stem area index for correlation with harvested biomass within a willow plantation. Each stem area index measurement was based on a total of 16 below-canopy and four corresponding above-canopy (not shown) readings within each of three replicated 30 m triple-row clonal beds.

row 30 m bed, while avoiding possible edge effects (Fig. B.3).

The between-bed sampling scale involved collecting four below-canopy measurements along each of four diagonal transects within each measurement area (Fig. B.3). Along each transect, measurements were taken with the sensor oriented in one of the four cardinal azimuthal directions (i.e., north, east, south, and west), in order to integrate the gap fraction in both across- and along-row directions. The within-row sampling scale consisted of taking four measurements (one in each cardinal azimuthal direction) at four sample points within the triple-row clonal bed measurement area only (Fig. B.3). The single plant sampling scale involved selecting four plants within the measurement area, with the LAI-2000 located below the canopy drip line, oriented toward the base of each plant, and a measurement taken in each of the four cardinal directions (Fig. B.3). For each clone and sampling scale, SAI was calculated based on a total of 16 below-canopy and four corresponding above-canopy measurements within each of the three replicated 30 m beds. The LAI-2000 employs Equation B.1 to calculate SAI by numerical integration using the gap fraction measurements (*T*; below-canopy reading divided by above-canopy reading) from each of the five measured zenithal angles; where the subscript *i* refers to each of the detector rings with view angle centred at θ, using a corresponding fixed weighting factor (*w*) for each view angle (LI-COR, 1992):

$$\mathrm{SAI} = -2\sum_{i=1}^{5} \ln\left(T(\theta_i)\right)\cos\theta_i \, w(\theta_i) \qquad \text{(Eq B.1)}$$

The SAI values then were correlated with the corresponding willow biomass that was subsequently harvested from each bed, dried at 65 ^{o}C to a constant weight, and weighed. All of the leafless above-ground biomass from each bed was harvested and the oven-dry weights extrapolated to a stand level (i.e., total oven-dry tonnes of biomass per hectare) for relating to the measured SAI values.

B.4.3 Statistical analyses

Simple linear regressions were performed using the REG procedure in SAS (Version 9.1, SAS Institute Inc. Cary, NC) to quantify the relationship between SAI, measured using the LAI-2000, and harvested oven-dry biomass for both willow clones. Homogeneity of variances and normality of distributions of data sets were checked before any statistical analyses were performed. No data transformations were necessary.

B.5 Results and Discussion

B.5.1 Relationship between measured stem area index and harvested willow biomass

For both willow clones in this study, there was a strong linear correlation between SAI, measured using the LAI-2000, and harvested above-ground biomass (Figs. B.4a and B.4b). For the single-stemmed Charlie, the single plant sampling scale (using the 45^o view cap) provided the only significant (P < 0.05) model predicting willow biomass, while the within-row sampling scale (using the 90^o view cap) was the only significant (P < 0.05) model for the multi-stemmed SV1 (Table B.1). The differences in efficacy among the sampling scale/view cap combinations used to measure the SAI of the two clones was due to the effect of willow growth form on gap fraction distributions within the clonal beds and its concomitant influence on the LAI-2000 measurement (LI-COR, 1992). Specifically, when measuring the SAI of the single-stemmed Charlie, it was prudent to use the single plant sampling approach (with the narrower 45^o view cap) in order to sample more of the woody material and less interplant area; otherwise, the LAI-2000 measured a larger gap fraction and underestimated the SAI. Conversely with the multi-stemmed SV1, the within-row sampling approach (using the wider 90^o view cap) sampled a larger area of these relatively dense beds; however, using the 45^o view cap the LAI-2000 incorporated less of the gap fraction and overestimated the SAI. The relationship between measured SIA and harvested biomass, using pooled data from both willow clones, was not as strong (R^2 = 0.57; P = 0.08), and is not surprising in view of the marked differences in growth form and attendant dissimilarity in light attenuation characteristics between the two willow canopy types.

B.5.2 Using a non-linear power regression to model the relationship between stem area index and harvestable willow biomass

Considering the LAI-2000 was designed to estimate two-dimensional leaf shading area, the instrument is insensitive to variations in plant morphology, in particular, stem diameter (T. Demetriades-Shah, LI-COR Inc., personal communication). For instance, a stem with a diameter of 0.5 cm and height of 100 cm will have a projected area of 50 cm^2 (i.e., 0.5 x 100 cm) and a volume of 19.6 cm^3 (i.e., Π x 0.25^2 x 100 cm), while another stem having an identical height but double the diameter, has a projected area of 100 cm^2 and volume of 78.5 cm^3. As a result, a doubling of stem diameter results in a doubling of projected stem area (i.e., SAI), but the

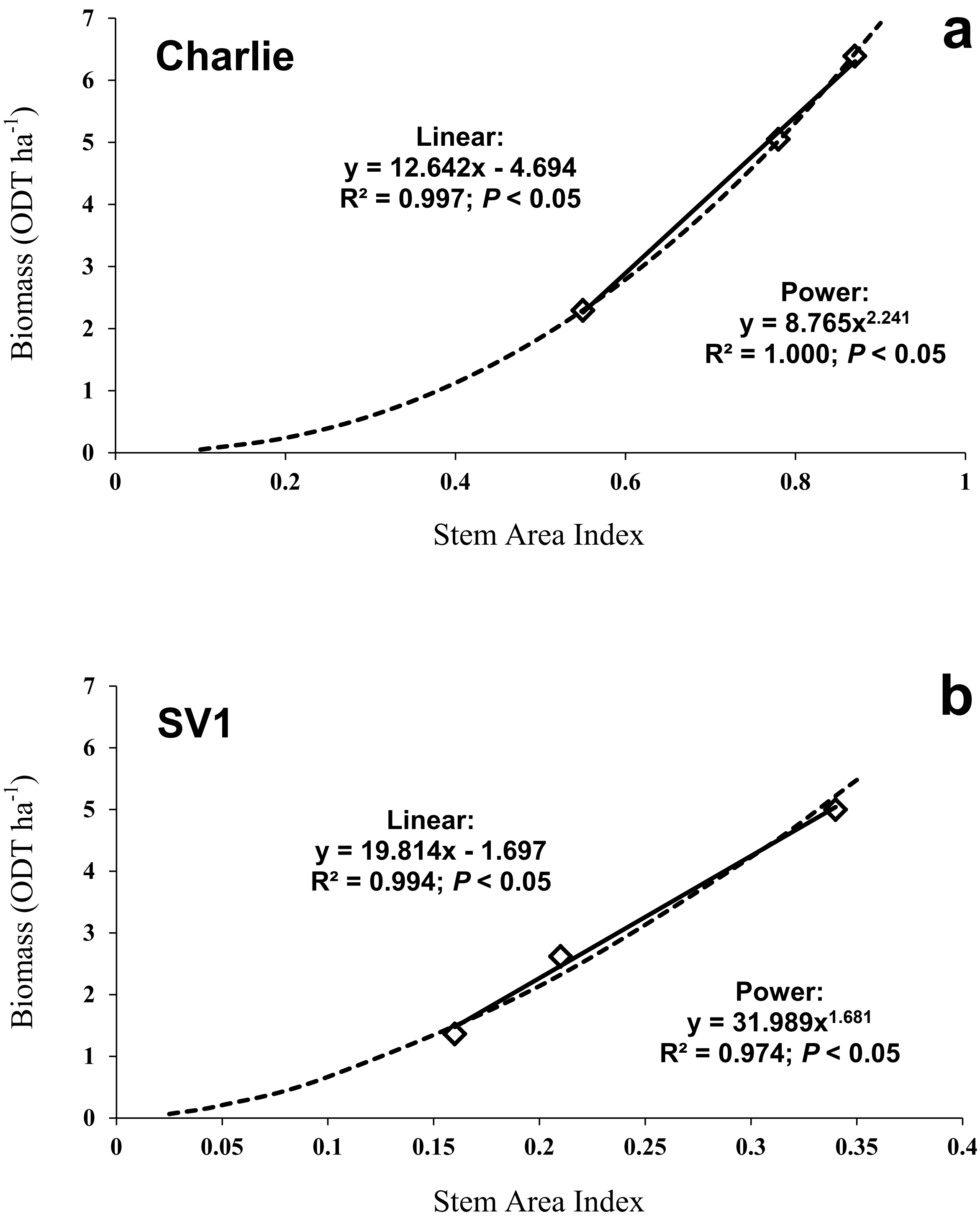

Fig. B.4. Relationship between stem area index, measured using a LAI-2000 Plant Canopy Analyzer, and harvested bed biomass of different two-year-old willow clones with either a linear (a) or non-linear (b) power regression model. Note: single plant (45^o view cap) and within-row (90^o view cap) sampling scales were used to measure the stem area index for Charlie and SV1 clones, respectively.

Table B.1. Coefficient of determination (R^2) and corresponding *P*-values for linear regression equations describing the relationship between stem area index (x), measured using the LAI-2000 with different view caps and sampling scales, and harvested above-ground dry biomass (y) for two willow clones.

View Cap	Sampling Scale	Equation	R^2	*P*-value
	---------------------- Charlie (*Salix alba* x *Salix glatfelteri*) -----------------------			
	Between-Bed	y = 23866x - 2105	0.483	0.511
45°	Within-Row	y = 15110x - 1467	0.565	0.458
	Single Plant	y = 12642x - 4694	0.997	0.032
	Between-Bed	y = 3534x + 3729	0.018	0.916
90°	Within-Row	y = -21964x + 11752	0.490	0.506
	Single Plant	y = -24221x + 17656	0.376	0.580
	------------------------------ SV1 (*Salix dasyclados*) ------------------------------			
	Between-Bed	y = 28012x - 743	0.031	0.888
45°	Within-Row	y = 16490x - 1735	0.689	0.377
	Single Plant	y = -3583x + 4282	0.048	0.860
	Between-Bed	y = 22050x - 904	0.989	0.067
90°	Within-Row	y = 19814x - 1697	0.994	0.048
	Single Plant	y = 17072x - 2130	0.522	0.486

volume (and presumably biomass too– assuming similar wood density) will be *four times* larger (Fig. B.5). Consequently, thicker stems will have a smaller SAI to biomass ratio than thinner stems of similar height. Such a relationship is inherently non-linear and, therefore, helps to explain the negative intercept observed in the linear regression models for both clones in this study (Figs. B.4a and B.4b). This apparent shortcoming is also inherent with photogrammetric methods that have been successfully used to estimate willow biomass, but like this study, evidently has a negligible effect on their resultant empirical linear or quadratic models predicting willow biomass, with reported R^2 values as high as 0.97 (P < 0.001; Ens et al., 2009). Notwithstanding the strong linear relationship observed in this study, simple non-linear power regressions were also performed on the data from both clones, using PROC REG in SAS, for comparison purposes (Figs. B.4a and B.4b). The power regression follows Equation B.2:

$$y = bx^a \quad \text{(Eq B.2)}$$

where x and y are the measured SAI values and harvested biomass and a and b are the allometric exponent and coefficient constants, respectively. There was minimal difference between the quality of linear and power regression models in this study; however, with a larger number of samples covering a greater range in paired SAI and harvested biomass values, presumably the curvilinear power model would be superior, as it allows for a more biologically-meaningful intercept– using an exponential fit compared to a linear fit (Fig. B.5). Regardless of which model is used, the obvious statistical restraint in this study is apparent when dealing with such a small number of replications (i.e., n = 3), given the experimental design of the clonal trial plots used to test the LAI-2000 methodology. Although this approach may be limited in terms of statistical power, from a practical standpoint however, an obvious advantage of the LAI-2000 is its ability to integrate much of the variability within each clonal bed into a single SAI measurement; therefore, precluding the need to sample many ‘representative’ plots, which despite increasing the degrees of freedom, will undoubtedly result in missing some inconsistencies in growth within a bed and consequently affect the accuracy of the biomass estimate.

B.5.3 Robustness of stem area index relative to traditional allometric technique

Conventional allometric equations for estimating above-ground willow biomass were also developed for each clone by calibrating measured stem diameter (at 30 cm height) with

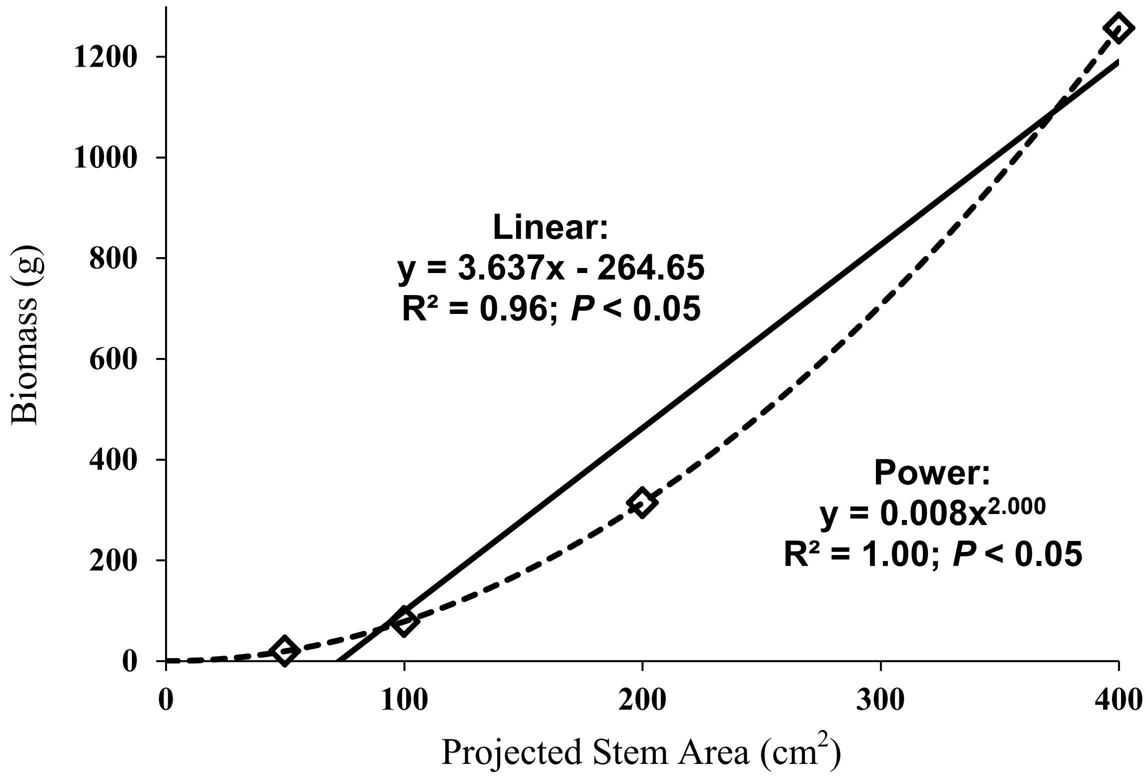

Fig. B.5. Theoretical dataset illustrating the effect of increasing willow stem diameter (0.5, 1, 2, and 4 cm) on the linear and non-linear power regression relationships between projected two-dimensional stem area and leafless stem biomass (assuming a constant height of 100 cm, cylindrical stem shape, and wood density of 1 g cm^{-3}).

harvested leafless biomass from 30 systematically sampled stools within the plantation (Arevalo et al., 2007). The observed allometric models, encompassing the stem diameter range present, were stronger for multi-stemmed SV1 (y = 21.806x - 105.46, R^2= 0.81; P < 0.05; n = 79) compared to the single-stemmed Charlie (y = 26.106x - 87.543, R^2= 0.67; P < 0.05; n = 30); however, the allometric relationships in this study were not as robust as those typically reported in the literature for willow (i.e., R^2 values ~0.95; Hytönen and Kaunisto, 1999; Bond-Lamberty et al., 2002; Arevalo et al., 2007). These relatively poor correlations are probably due to the non-coppiced management of the study plantation. Along with adopting a triple-row bed design, the Canadian Forest Service short-rotation willow production system also differs from conventional plantation protocols used in other countries, by harvesting after four years of growth without coppicing the first year after establishment. Coppicing promotes the production of larger numbers of uniformly-shaped stems (i.e., relatively homogeneous diameter to biomass ratio), which would support the development of stronger allometric models. Consequently, the influence of the relatively consistent stem morphology of the non-coppiced SV1, having greater homogeneity among its stems, is apparent when comparing the observed larger R^2 value of the SV1 allometric model relative to the single-stemmed Charlie model. Specifically, unlike the natural multi-stemmed shrub growth form of SV1, the tree growth form of Charlie was inherently more variable within the plantation due to inconsistent branching and, therefore, resulted in relatively weaker correlations between stem diameter and biomass, which commonly occurs among tree species having a relatively heterogeneous structure (Ter-Mikaelian and Korzukhin, 1997; Lambert et al., 2005). Likewise with the non-coppiced management of the multi-stemmed SV1, where the marked presence of varying degrees of sylleptic branching (i.e., branching originating from lateral buds along the stem) was evident in this plantation (Fig. B.2b), whereas this is typically uncharacteristic of coppiced SV1 plantations. Despite the uncoppiced nature of the willow in this study and associated variability in above-ground biomass form, the LAI-2000 was effective in accounting for the variability in harvestable biomass of both willow clones, regardless of whether a linear or non-linear regression model was used. Nevertheless, more work needs to be done, involving a larger sample size, in order to validate the results of this study.

B.6 Conclusion

Traditional methods of estimating short-rotation willow plantation productivity by developing and implementing allometric relationships for different species can be time consuming, costly, and susceptible to subjective errors. The LAI-2000, however, provides a relatively simple, rapid, and reliable measure of SAI that was highly correlated with harvestable willow biomass. Consequently, the use of the LAI-2000 appears to be a promising non-destructive and elegant mensurative technique for providing reliable estimates of above-ground biomass, thereby supporting effective management decisions throughout the rotation of purpose-grown willow plantations. Further research is needed, however, to determine if the observed relationships between measured SAI and harvestable willow biomass remains consistent over multiple years and rotations with different clones growing across a geoclimatic gradient, on a variety of soil types, under a coppiced management system typically used in the U.S. and Europe.

APPENDIX C. MEASURING HARVESTABLE BIOMASS IN SHORT-ROTATION WILLOW BIOENERGY PLANTATIONS USING LIGHT ATTENUATION

C.1 Preface

The rationale for this study was to validate the novel mensurative technique introduced in Appendix B throughout a complete three-year short-rotation coppice (SRC) willow rotation at different sites. Non-destructive and elegant mensurative techniques are needed for providing reliable above-ground SRC willow biomass estimates to support effective management decisions within commercial plantations in Saskatchewan (Chapter 6). Note: this paper is published in BioEnergy Research (6: 83-90). The co-author contributions to this manuscript were greatly appreciated and consisted of: F.C. Stevenson (provided statistical analyses and manuscript editing); J.J. Schoenau (provided financial assistance and manuscript editing); and, K.C.J. Van Rees (provided financial assistance and manuscript editing).

C.2 Abstract

Routine monitoring of above-ground biomass within purpose-grown willow biomass energy production systems is important for timing harvest and other operations to maximize profit and increase plantation productivity. The objective of this study was to assess the efficacy of an elegant non-destructive mensurative technique for providing reliable estimates of harvestable biomass for six willow varieties during a three-year rotation. The LAI-2000 Plant Canopy Analyser was used to measure the stem area index of growing willow and relate it to harvestable biomass at four locations within Saskatchewan, Canada over a three-year period. Given the highly significant relationship (R^2 = 0.95; P < 0.0001) between measured stem area index and harvestable willow biomass, independent of variety, age, or location, this simple mensurative technique is a promising alternative for estimating above-ground biomass in short-rotation willow plantations.

C.3 Introduction

The economic viability of willow bioenergy plantations relies on accurate monitoring of annual production rates for supporting effective management decisions (e.g., irrigation, fertilization, or pest control) to promote optimal growth throughout the rotation. Moreover, harvesting operations represent the greatest expenditure incurred with short-rotation willow production systems (Mitchell et al., 1999; Keoleian and Volk, 2005); therefore, it is imperative for producers to optimize harvesting timing, based on reliable yield estimates, to maximize the economic return on their investment. Conventional allometric models estimating willow biomass, defined by an empirical relationship between stem diameter and biomass, are typically specific to variety, age, and location, while their development and implementation can be time consuming, costly, and susceptible to subjective errors (Telenius and Verwijst, 1995; Heinsoo et al., 2002; Arevalo et al., 2007). Consequently, a need exists for an alternative non-destructive mensurative technique for estimating willow biomass, with the accuracy of allometry, but providing reliable data more quickly and economically.

With the advent of digital cameras and development of complimentary user-friendly software, non-destructive photogrammetric approaches are becoming increasingly popular for estimating above-ground biomass. Photogrammetry involves capturing digital pictures of a target object and then using software to integrate the images and count pixels. Photogrammetry has been used to provide accurate measures of spruce biomass (Ter-Mikaelian and Korzukhin, 1997; Ter-Mikaelian and Parker, 2000) and above-ground biomass in short-rotation willow plantations (Gower and Norman, 1991; Ens et al., 2009). Although providing reliable data, the widespread adoption of photogrammetry may be limited because of the labour-intensive and tedious post-processing of digital images, in addition to the subjective determination proper stem/sky contrast thresholds when estimating biomass (Engelbrecht and Herz, 2001; Jonckheere et al., 2004).

The LAI-2000 Plant Canopy Analyzer (LI-COR Inc., Lincoln, NE) has been successfully used to measure light attenuation (i.e., reduction in amplitude and intensity) as it passes through a vegetative canopy and its utility for measuring leaf area index has been reported in hundreds of articles encompassing a range of vegetation types, including: annual crops (Dobermann et al., 1995; Hicks and Lascano, 1995; Rudorff et al., 1996); coniferous, deciduous, and mixedwood forests (Gower and Norman, 1991; Comeau et al., 1998); shrubs and grasses (He et al., 2007; Sonnentag et al., 2007); turfgrass (Yuen et al., 2002); vineyards (Johnson and Pierce, 2004); willow plantations (Tharakan et al., 2008; Hangs et al., 2011b; Appendix B); and non-crop species (Thevathasan et al., 2000). The LAI-2000 measures gap fraction by concurrently measuring the difference between the diffuse transmitted radiation under the canopy with the diffuse incident radiation at the top of the canopy and its novel use in estimating stem area index (SAI) of leafless above-ground willow biomass has been reported (Hangs et al., 2011b; Appendix B). However, Hangs et al. (2011b; Appendix B) only examined the use of the LAI-2000 after one growing season at a single location. The objective of this study was to determine if the strong relationship between SAI and harvestable willow biomass remained consistent over a three-year rotation, involving six willow varieties growing on a variety of soil types at four locations across a geoclimatic gradient, under a coppiced management system typically used in the U.S. and Europe.

C.4 Materials and Methods

C.4.1 Study locations and willow variety trial

The data for this study were collected from four willow variety trial plantations located along a 500 km north-south gradient within Saskatchewan, Canada, chosen to represent the diverse soil types and climatic conditions present within the province (Tables 3.1 and 3.2). In the spring of 2007, six willow varieties, developed by the State University of New York College of Environmental Science and Forestry (SUNY-ESF) breeding program, were planted at each location in a randomized complete block design (n = 4) following the protocols of Abrahamson et al. (Abrahamson et al., 2002). The six willow varieties used were: Allegany (*Salix purpurea*), Canastota (*Salix sachalinensis* x *miyabeana*), Fish Creek (*Salix purpurea*), Sherburne (*Salix sachalinensis* x *miyabeana*), SX61 (*Salix sachalinensis*), and SX64 (*Salix miyabeana*). Each varietal plot (6.3 x 7.8 m) consists of 78 plants (three double-rows of 13 plants row^{-1}), with spacings of 1.5 m between the double-rows, 60 cm between rows within the double-row, and 60 cm between plants within the double-row; resulting in a planting density of approximately 15,873 plants ha^{-1} (Fig. C.1). In the spring of 2008, the willow plants were cut down to encourage coppicing (i.e., the production of a large number of shoots from the established root system) and were grown for an additional three years before harvesting. At the end of each growing season, for each location and willow variety plot, seven stems (representing the diameter range within each plot) were destructively sampled for developing allometric equations, by calibrating measured stem diameter (at 30 cm height) with harvested leafless biomass (Arevalo et al., 2007). These plot-specific allometric models ($R^2 > 0.98$; $P < 0.001$), were applied to the diameter and stem density measurements from each plot to estimate above-ground willow biomass, which was extrapolated to a stand level (i.e., total oven-dry tonnes of biomass per hectare), for relating to its corresponding SAI, measured using the LAI-2000. Stem heights, diameters (at 30 cm height), and counts of the central 18 stools within each varietal plot were assessed after each growing season to characterize the above-ground morphology of each willow variety through the rotation (Table C.1).

C.4.2 Measuring stem area index throughout the three-year rotation

At each location, the LAI-2000 was used to measure the SAI within all varietal plots in the spring prior to leaf flush. For operational-scale plantations, several representative sites within the plantation would be selected and measured; however, given the small research-scale plot sizes in this study, a systematic within-row sampling scheme was used to avoid edge effects (Hangs et al., 2011b; Appendix B). At each sampling point, measurements were taken with the "fish-eye" optical sensor oriented in each of the four cardinal azimuthal directions (i.e., north, east, south, and west), in order to integrate the gap fraction in both across- and along-row

Table C.1. Mean (n = 16) selected morphological properties of six willow varieties growing at four different locations throughout Saskatchewan, Canada during a three-year rotation.

Variety	Height† (cm)	Diameter (mm)	Stems per stool (#)
	---------- 2008 ----------		
Allegany	166.9 (17.2)‡	5.8 (0.4)	13.7 (1.5)
Canastota	175.0 (19.0)	6.6 (0.4)	8.2 (0.7)
Fish Creek	198.6 (12.4)	6.5 (0.3)	9.4 (0.9)
Sherburne	215.5 (16.7)	6.9 (0.3)	9.9 (1.0)
SX61	223.5 (18.0)	7.1 (0.4)	9.6 (1.0)
SX64	200.7 (10.7)	7.3 (0.3)	8.2 (0.7)
	---------- 2009 ----------		
Allegany	227.0 (22.2)	9.4 (0.6)	12.2 (1.0)
Canastota	254.5 (19.2)	11.3 (0.5)	6.5 (0.6)
Fish Creek	277.7 (18.4)	10.2 (0.6)	8.5 (0.7)
Sherburne	303.9 (20.1)	11.1 (0.5)	8.2 (0.5)
SX61	317.2 (25.1)	13.1 (0.8)	6.1 (0.3)
SX64	277.3 (14.1)	12.3 (0.7)	6.7 (0.5)
	---------- 2010 ----------		
Allegany	275.6 (18.1)	11.7 (0.7)	9.9 (0.6)
Canastota	292.8 (9.3)	12.8 (0.9)	6.9 (0.7)
Fish Creek	363.2 (17.5)	13.3 (0.6)	7.7 (0.5)
Sherburne	348.4 (14.9)	13.2 (0.6)	7.3 (0.4)
SX61	327.6 (19.7)	13.5 (1.2)	6.6 (0.6)
SX64	313.2 (6.5)	13.7 (0.9)	7.0 (0.7)

† For each property, replicate values are a mean of the central 18 stools within each varietal plot
‡ Mean (standard error)

directions (Fig. C.1). The LAI-2000 sensor was placed near the soil surface and a 270° view cap (consisting of a 90° opaque mask) was used to restrict the azimuthal range of the sensor to obscure the operator from the sensor (Fig. C.2). Open-sky 'above-canopy' measurements were simultaneously collected using a second LAI-2000 system (with a 270° view cap) placed on a tripod outside of the plantation. After data collection, the two independent sensors were cross calibrated and data sets merged according to LI-COR (LI-COR, 1992). The LAI-2000 calculates the SAI by comparing the incident radiation above the willow canopy with the transmitted radiation below the canopy, using well established inversion and integration models describing radiation transfer through a vegetation canopy (Welles and Norman, 1991; Jonckheere et al., 2004). For each varietal plot (i.e., experimental unit), SAI was calculated based on 16 above- and 16 below-canopy measurements. Measurements were taken under diffuse sky conditions (e.g., overcast or prior to sunrise), in order to avoid direct sunlight and/or light scattering within the canopy from influencing the readings.

C.4.3 Statistical analyses

Biomass data for each crop were analyzed with the PROC MIXED procedure of SAS (Littell et al., 2006). The effects of year and variety were considered fixed while those of location and replicate (nested within location) were considered random. The effect of SAI (slope coefficient) was modeled as both a fixed and random effect using a random coefficient model; a SAI x location interaction variance estimate was estimated in addition to a fixed effect linear slope coefficient. Exploratory analysis revealed that a quadratic slope coefficient did not improve model fit. The linear regression model trend lines were forced through the origin (no intercept). Trend lines commencing at the origin allowed for a more biologically meaningful intercept (i.e., the biomass should be zero when the SAI is 0; Hangs et al., 2011b; Appendix B). Best linear unbiased predictor (BLUP) deviations for the intercept and linear slope coefficients at each location from the overall intercept and linear or quadratic slope coefficients were used to further explore variability of trend lines among locations. Regression slope coefficients, corresponding variance estimates, and deviations were declared significant at $P < 0.05$.

Measurements were repeated on the same plots at each location across years. A number of covariance structures to model the repeated measurements were tested with the repeated statement for PROC MIXED. An antedependence (1st order) covariance structure, modeled

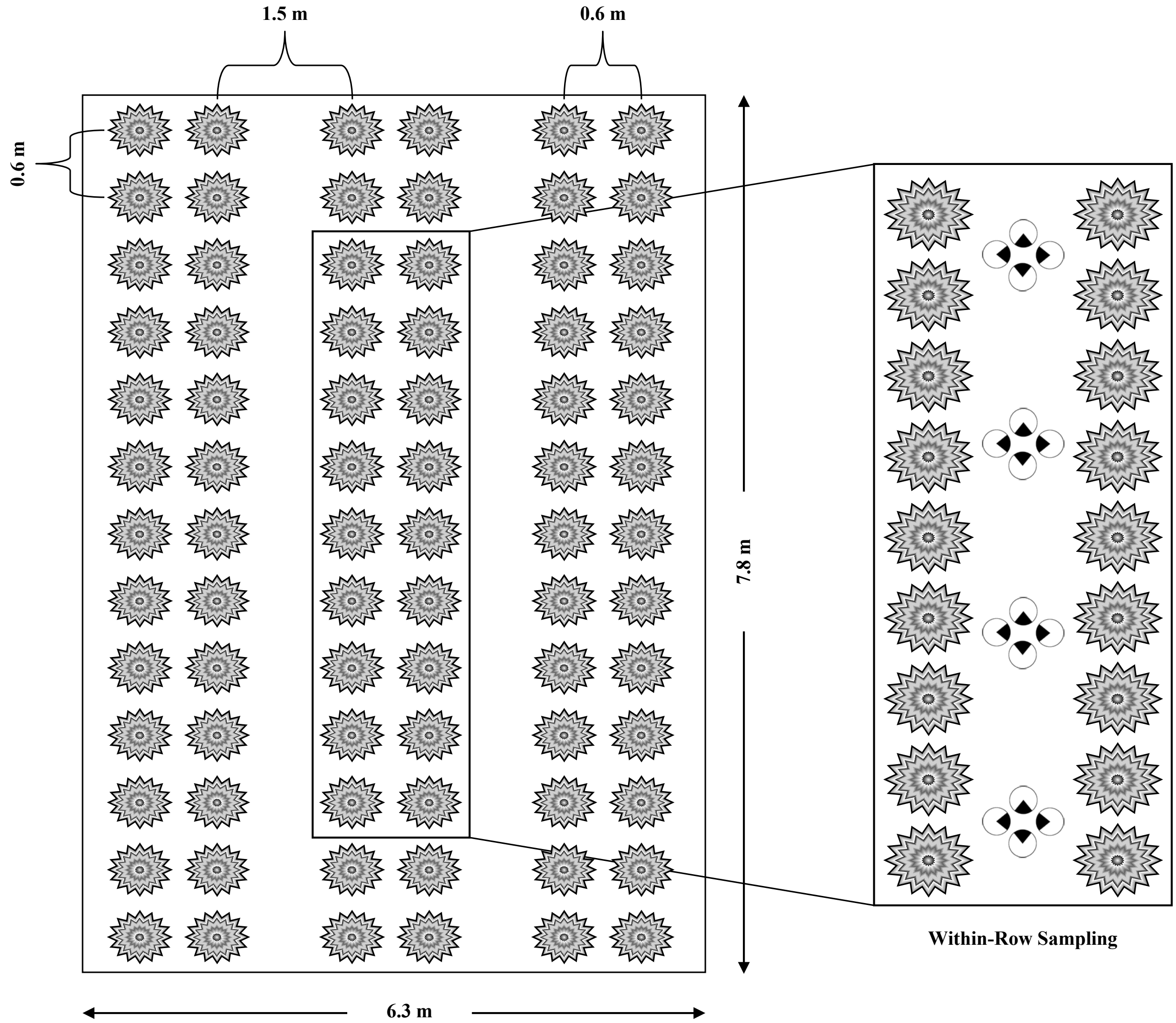

Fig. C.1. Schematic illustrating the use of the LAI-2000 plant canopy analyzer (with 270° view cap indicated by white fraction of circle) to measure stem area index for correlation with above-ground willow biomass within each varietal plot. Each stem area index measurement was based on a total of 16 below-canopy and 16 corresponding above-canopy (not shown) readings.

Fig. C.2. Estimating above-ground leafless willow biomass using a LAI-2000 Plant Canopy Analyzer to measure the 'gap fraction' (i.e., fraction of sky visible from beneath the canopy not blocked by branches or stems) corresponding to five sensor rings centred on different zenithal angles. At each sampling location, measurements were taken with the "fish-eye" optical sensor oriented in each of the four cardinal azimuthal directions to integrate the gap fraction in both across- and along-row directions.

independently for each location, provided best fit (least corrected Akaike's information model fit criterion value). This covariance structure models a separate variance estimate for each year and models correlations between immediately adjacent years and between years one year apart.

Based on the preceding analyses, the paired observations of SAI and corresponding plot biomass collected over the three years were randomly split into two parts. Fifty percent (i.e., two of the four replicates from each variety, location, and year combination; n = 144) were used to model the relationship between SAI and above-ground biomass for all six willow varieties using a simple linear regression (without an intercept) using PROC REG. The remaining 50% of the data was used to validate the model by fitting observed vs. predicted values and simultaneously comparing its slope coefficient to 1 using the TEST statement in PROC REG. This random sectioning of data was repeated three times to ensure the validity of the results. Following these analyses, an overall linear regression model (without intercept) was fitted to all paired observations of SAI and willow biomass (n = 288) along with 95% confidence and prediction intervals created using JMP® (Version 10; SAS Institute, Cary, NC). Finally, regression slope coefficients were calculated using PROC REG and means comparisons among years were performed using least significant differences (LSD; equivalent to Fisher's protected LSD) at a significance level of 0.05 using PROC MIXED, with groupings obtained using the pdmix800 SAS macro (Saxton, 1998).

C.5 Results and Discussion

C.5.1 Relationship between stem area index and harvested willow biomass

There was a strong and highly significant linear relationship between SAI, measured using the LAI-2000, and harvestable willow biomass each year of the three-year rotation for all six willow varieties tested (Table C.2; Fig. C.3). Additionally, there was no significant effect ($P > 0.05$) of either location or the interactions SAI x location, SAI x willow variety, SAI x willow variety x year, and willow variety x year on the relationship between SAI and harvestable willow biomass. A notable deviation between our results and those of Hangs et al. (2011; Appendix B) was the lack of SAI x willow variety interaction in our study, despite diverse above-ground morphologies among the six varieties we examined (Table C.1). Their study involved relatively small varietal plots, which necessitated using the two smallest view caps (45^{o} and 95^{o}) available for the LAI-2000, in order to prevent edge effects from confounding the SAI measurements.

Table C.2. Analysis of variance examining the relationship between stem area index (SAI), measured using the LAI-2000, and harvestable biomass for six willow varieties (Variety) growing at four different locations (Site) throughout Saskatchewan, Canada during a three-year (Year) rotation.

	Effect	Num df	*P*-value
Fixed	SAI	1	< 0.0001
	Variety	5	0.1051
	Year	2	0.2605
	SAI x Variety	5	0.1987
	SAI x Year	2	0.0105
	Variety x Year	10	0.1866
	SAI x Variety x Year	10	0.1032
Random	Site	3	0.2124
	SAI x Site	3	0.1847

Concomitant with a smaller field of view is increased sensitivity to sensor placement, which was manifested in their study by requiring different view caps and sampling schemes to collect accurate SAI measurements for the two willow varieties possessing dissimilar growth form. For the larger varietal research plots in our study or commercial-scale plantations where edge effects are less of a concern, using the 270^{o} is recommended, because it allows for the maximum field of view for gap fraction calculations, resulting in consistent SAI measurements regardless of growth form (i.e., no SAI x willow variety effect; Table C.2). Given the consistency in this empirical relationship among numerous willow varieties and the four willow plantations located throughout the province (consisting of different soil types and growing season conditions), it would appear that SAI is a robust means of estimating harvestable willow biomass that is independent of willow variety and location. These results are encouraging given the sensitivity to changing willow stem growth habit among locations (i.e., confounding G x E effects) reported for other optical techniques estimating willow biomass (Ens et al., 2009). The validity of the

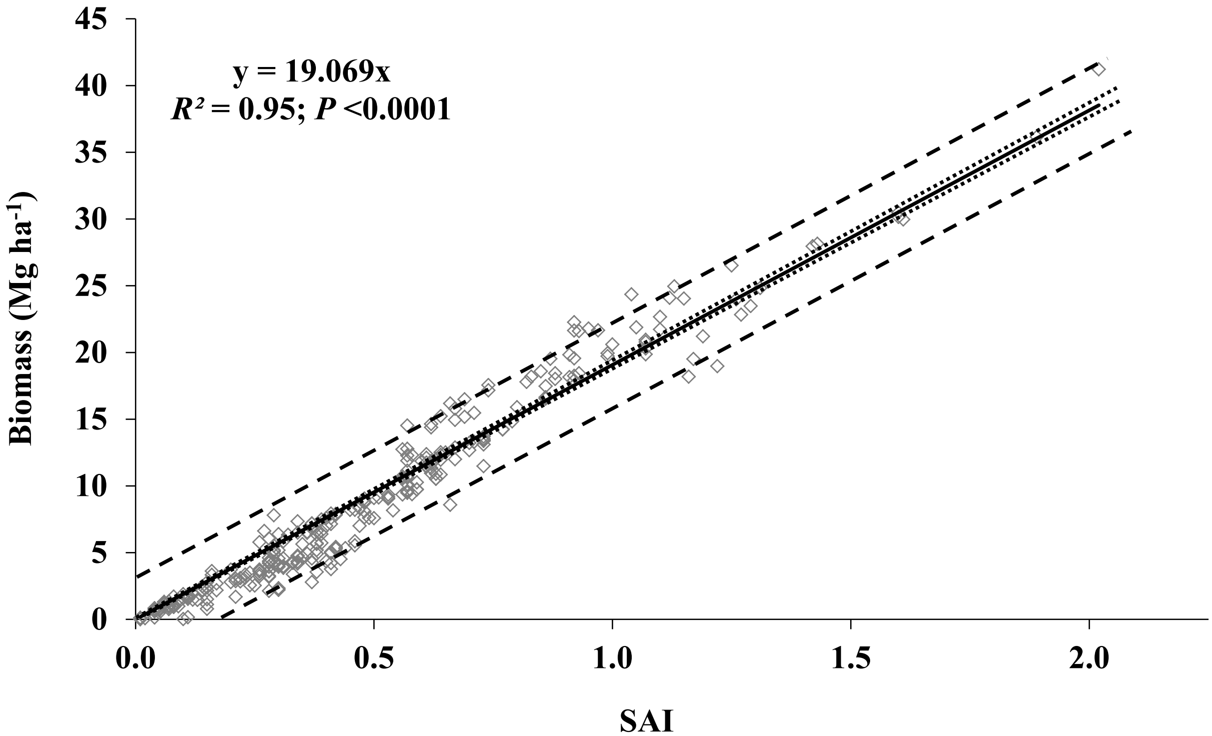

Fig. C.3. Relationship (n = 288) between estimated harvestable leafless willow biomass and stem area index (SAI), measured using a LAI-2000 Plant Canopy Analyzer, of six willow varieties growing at four locations in Saskatchewan throughout a three-year rotation. Smaller and larger dashed lines about the solid trendline are 95% confidence and prediction intervals, respectively.

relationship between SAI and harvestable willow biomass is also apparent by examining the fitted observed vs. predicted values (Fig. C.4). The calculated slope coefficient was significantly different ($P < 0.0001$) than one, however, this is not surprising considering the extreme statistical power with such large degrees of freedom (i.e., n = 144). Moreover, the slope deviation from unity was less than two percent, which is operationally irrelevant compared to the expected 60-90% variability in mechanical harvest efficiency of willow biomass using current technology (Schroeder et al., 2009; Philippe Savoie, Agriculture and Agri-Food Canada, personal communication).

C.5.2 Operational considerations when measuring stem area index using the LAI-2000

Although the conventional method of estimating willow plantation productivity by developing and implementing allometric relationships has its shortcomings, the LAI-2000 has

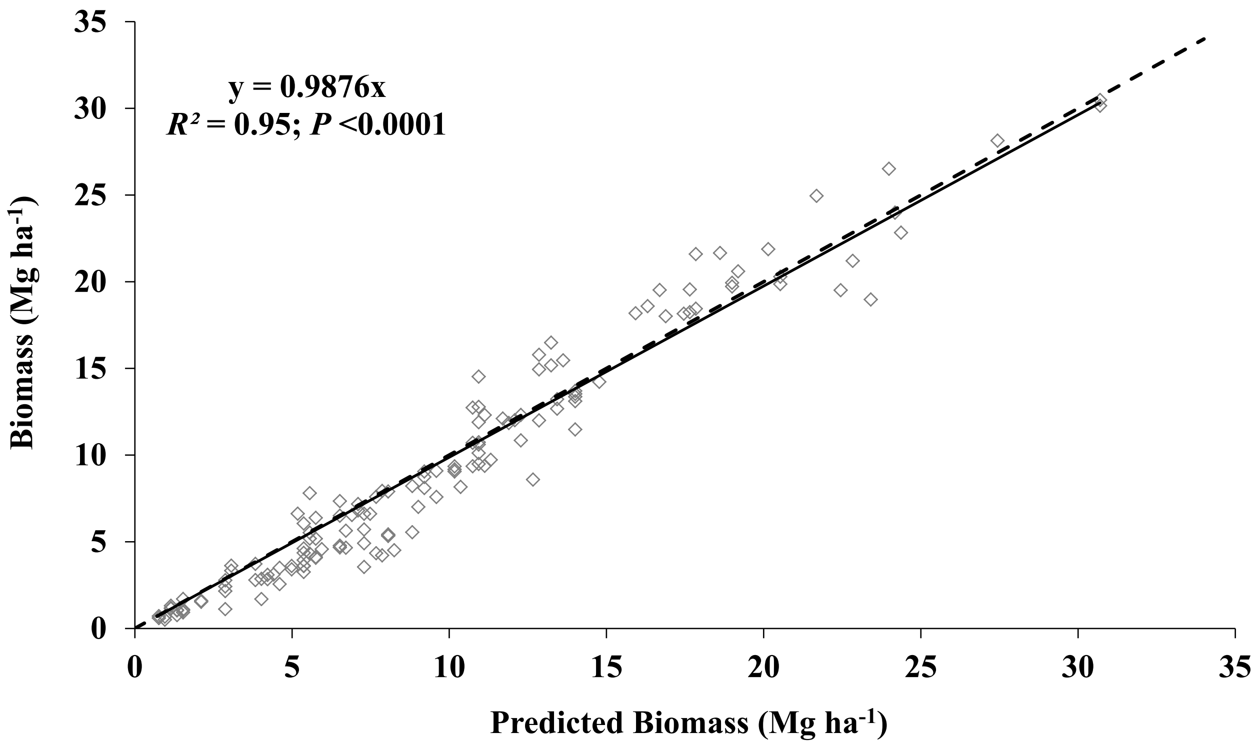

Fig. C.4. Relationship (n = 144) between estimated harvestable leafless willow biomass and predicted biomass, based on a linear regression model relating stem area index, measured using a LAI-2000 Plant Canopy Analyzer, to willow biomass of six willow varieties growing at four locations in Saskatchewan throughout a three-year rotation. Dashed line is 1:1 line.

limitations as well. Specifically, the significant SAI x year effect ($P = 0.0105$; Table C.2), was caused by interference from herbaceous understory plants within the plots while collecting SAI measurements. Similar to photogrammetry, the LAI-2000 cannot distinguish between the non-crop understory vegetation and target willow stems and branches (Fig. C.5), which underestimates the gap fraction, thus overestimates the SAI and decreases the resultant slope coefficient when relating the SAI to the surrounding willow biomass. Despite an aggressive vegetation management program, three of our locations were relatively fertile arable land with an extensive seed bank and sufficient moisture that promoted non-crop plant growth, especially during the first two years. Adequate weed control is not only critical for the successful establishment and growth of willow plantations (Abrahamson et al., 2002), but also for collecting accurate SAI measurements using the LAI-2000. In a related study, the pre- and post-planting vegetation management practices effectively controlled the non-crop species, so understory species interference was not a problem (Hangs et al., 2011b; Appendix B). For this

Fig. C.5. Comparison between gap-fraction perspective with (a) and without (b) adequate control of understory non-crop plants.

study, an attempt was made to manually remove the understory plants prior to collecting the SAI measurements, which was a nuisance and not 100% effective, as evidenced by the changing slope coefficients over time (Table C.3). The increasing slope coefficient of regression models as the plantations aged was due to decreasing non-crop species abundance (i.e., less interference with SAI measurements) as the willow stand reached canopy closure and shaded out the understory plants. Despite the weed-prone nature of these fertile agricultural fields, subsequent

Table C.3. Mean (n = 24) linear regression slope coefficients for the relationship between stem area index, measured using the LAI-2000, and harvestable biomass for six willow varieties growing at four different locations throughout Saskatchewan, Canada during a three-year rotation.

Year	Slope†
2008	15.94b
2009	16.93b
2010	19.98a

† Values having the same letter are not significantly different (*P* >0.05) using LSD

willow rotations will achieve canopy closure much quicker, as shoots regenerate from an established root system, thereby reducing the understory plant community and supporting the collection of consistent SAI data thereafter.

Another practical limitation of the LAI-2000 is the need to collect SAI measurements under diffuse sky conditions (e.g., overcast), in order to avoid direct sunlight and/or light scattering within the canopy from influencing the instrument readings. If SAI measurements are required in a timely manner, in the absence of favourable overcast conditions, then it is necessary to collect measurements in the consistent diffuse light conditions immediately preceding sunrise or after sunset. Although the light conditions are ideal during these times, logistically, the routine use of the LAI-2000 becomes impractical if the plantation is located some distance away. Notwithstanding these operational limits, an obvious advantage of the LAI-2000 is its ability to integrate much of the variability in willow biomass into a single SAI measurement; therefore, precluding the need to sample a large number of 'representative' plots within a plantation. Considering the relative ease and speed of collecting a single SAI measurement (approximately two minutes, based on 16 above- and 16 below-canopy readings used in this study), a hectare of willow could be assessed in roughly 30 minutes. The robust relationship between SAI and harvestable willow biomass observed throughout a three-year rotation of numerous willow varieties growing at several locations is encouraging. However, it would be prudent to validate the accuracy of this non-destructive technique using other willow varieties differing in morphological characteristics, in particular specific wood density, which can vary considerably among willow varieties (Sennerby-Forsse, 1985; Tharakan et al., 2005). The mean stem density of the willow varieties examined in this study was 0.41 g cm^{-3} (± 0.07), so a correction factor may be necessary if measuring the SAI of other willow varieties having substantially different stem densities.

C.6 Conclusion

The LAI-2000 provided a relatively easy, fast, and accurate measure of SAI that was highly correlated with harvestable willow biomass, over a three-year rotation, for numerous willow varieties growing on a variety of soil types across a geoclimatic gradient. Accordingly, measuring SAI, using the LAI-2000, appears to be a promising non-destructive and elegant mensurative technique for providing reliable estimates of above-ground biomass to support effective management decisions throughout the rotation of purpose-grown willow plantations. However, we recommend validating the observed relationship between measured SAI and harvestable willow biomass for other willow varieties, before adopting this method for routine use in commercial biomass energy production systems.

APPENDIX D. EXAMINING THE SALT TOLERANCE OF WILLOW (*SALIX* SPP.) BIOENERGY SPECIES FOR USE ON SALT-AFFECTED AGRICULTURAL LANDS

D.1 Preface

Chapters 4 and 5 examined the viability of growing short-rotation coppice (SRC) willow plantation at several locations throughout Saskatchewan, with and without supplemental irrigation and nutrient amendments. However, in order to minimize their risk, farmers will arguably allocate SRC willow plantations on their marginal land, in particular salt-affected land with little to no opportunity cost (Chapter 6). The four field sites utilized in this thesis research were non-saline and did not allow the opportunity to evaluate salt-tolerance effects. To identify salt-tolerant willow varieties suitable for growing on the millions of salt-affected hectares in western Canada is exciting. Note: this paper is published in the Canadian Journal of Plant Science (91: 509-517). The co-author contributions to this manuscript were greatly appreciated and consisted of: J.J. Schoenau (provided financial assistance, methodological support, and manuscript editing); and, K.C.J. Van Rees (provided financial assistance and manuscript editing); and, H. Steppuhn (provided methodological support and manuscript editing).

D.2 Abstract

Dryland salinity is a significant limitation on crop production across the Canadian prairies, with an estimated four million hectares of salt-affected land. The potential exists to make better use of saline marginal lands by developing them into willow (*Salix* spp.) plantations as a bioenergy feedstock; however, relatively little is known about the salt tolerance of willow. The objective of this study was to compare the relative salt tolerance of 37 different native and exotic willow varieties grown under controlled environment conditions on soils of varying salinity. The soils were collected from a farm field in south-central Saskatchewan along a hillslope catena influenced by saline seep salinity, containing high concentrations of sulfate salts, which commonly occurs within western Canada. Most willow varieties tested in this study were able to tolerate moderately-saline conditions ($EC_e \leq 5.0$ dS m^{-1}). In addition, several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) showed no reduction in growth with severe salinity ($EC_e \leq 8.0$ dS m^{-1}). These results indicate that some willow varieties are quite salt-tolerant and suitable for establishment on salt-affected soils in Saskatchewan and abroad.

D.3 Introduction

The use of biomass-derived energy accounts for approximately 10% of the global energy requirement (Berndes et al., 2003). However, with growing desire worldwide for secure and environmentally acceptable energy sources, there is increased interest in developing biomass production systems for use as a dedicated or 'purpose-grown' feedstock for biomass energy. Canada is no exception, with its high per capita energy consumption and the majority of its energy demand used for transportation and building utilities (Cuddihy et al., 2005). Natural Resources Canada, along with a number of Canadian provinces, declares bioenergy to be a legitimate and sustainable source of energy that will constitute a significant portion of future energy production. The establishment of purpose-grown shrub willow (*Salix* spp.) plantations represents a viable bioenergy feedstock, especially if the willow can be successfully grown on unproductive land that is marginal for annual crop production, such as saline land. With escalating public concern over the displacement of arable land from food production into bioenergy production, a great opportunity exists to realize economic and environmental benefits, through the development of non-consumable woody crops, like willow, for marginal land that is deemed unsuitable for annual crop production (Van Rees, 2008).

Dryland salinity is a significant agronomic problem across the Canadian prairies (Acton and Gregorich, 1995). According to Eilers et al. (1995), the incidence of salinity can be summarized as follows: i) the majority (62%) of arable land in the prairies contains less than 1% saline soil; ii) 36% of the arable land contains 1-15% saline soil; and iii) 2% of the arable land contains more than 15% saline soil. Generally speaking, soil salinity affects around 10% of the cultivated land within the prairies, or approximately four million hectares, translating into farm income losses of approximately $250 million annually (Dumanski et al., 1986). A number of studies have examined salinity in Saskatchewan soils (Hogg and Henry, 1984; Henry et al., 1985; Keller and Van der Kamp, 1988), but accurate estimates of saline-affected area are difficult to establish due to its large areal extent and inherent variability caused by the ephemeral nature of salts moving through the soil profile. Nevertheless, it is has been estimated that there are approximately 1.6 million ha of saline soils in Saskatchewan alone (Rennie and Ellis, 1978) and these lands are either being used to grow low return forage crops or have been abandoned altogether. The potential exists, therefore, to make better use of these saline lands by developing them into short-rotation intensive culture willow plantations, which is not only economically positive for the farmer, but also may provide environmental benefits, such as precluding the build-up of surface salts given willow's phreatophytic nature, along with promoting increased biodiversity within the agricultural landscape. To our knowledge, no empirical work has been done to examine the growth of different willow varieties on soils with varying salinity. The objective of this study was to determine the relative growth response of numerous exotic and native willow varieties grown in saline soils. Identifying salt-tolerant varieties could promote the use of willow plantations to revitalize these unproductive agricultural lands; thereby supporting agricultural diversification in Saskatchewan and abroad.

D.4 Materials and Methods

D.4.1 Collection and preparation of saline soils

The saline soils used in this study were collected from a continually-cropped field (pea-wheat-barley rotation), located approximately seven kilometers southeast of Central Butte, SK (UTM Co-ordinates: 13U 400114 5620205). The soils were predominantly Solonetzic loam soils of the Kettlehut Association, with an Agricultural Capability Classification rating of Class Four (SCSR, 1985). Soils of varying salinity were collected along a hillslope catena influenced by saline seep salinity, containing high concentrations of sulfate salts, which commonly occurs within western Canada (Wiebe et al., 2007). The development of saline seeps along such hillslopes is primarily due to the effects of a semi-arid climate and local hydrogeology on the translocation and subsequent concentration of naturally occurring salts within near-surface discharge soil layers downslope. Briefly, the soils at this site are greatly influenced by the relatively thin glacial till parent material, derived from the underlying Cretaceous marine clay-shale bedrock rich in Na, Ca, and Mg sulphate salts. Saline seeps typically develop wherever saline groundwater occurs within 1.5 m of the surface, coupled with a local recharge zone, such as upland areas with slopes of 0-2% (Miller et al., 1981; Daniels, 1987). Excess soil water (i.e., beyond evapotranspirative demand) in the upland recharge area infiltrates beyond the root zone, through thin shale-modified salt-rich parent material and contacts the impermeable shale, before moving laterally downslope as unsaturated flow (Holm and Henry, 1982; Henry et al., 1987). As the groundwater follows the local hydraulic gradient downslope, it dissolves and carries salts until concentrating them at or near the soil surface through capillary action and evaporation, particularly during the drier mid-summer months. Consequently, there is a distinct gradient of increasing soil salinity moving downslope, often with the formation of a white salt crust in the depressional area where the salt concentration is the highest.

Soils were intensively sampled along a 300 m transect, from the top of the knoll to the depression, and their electrical conductivities measured using a Accumet AP85 pH/EC meter (Fisher Scientific, Pittsburgh, PA). Four slope positions were selected to represent the range of soil salinity encountered along the hillslope. Soil was collected from the Ap horizon (approximately 0-20 cm) at each location, air-dried, and then blended to to achieve the desired salinity levels for the pot study− determined using electrical conductivity values derived from 1:2 (soil:water) extractions ($EC_{1:2}$). The four target salinity levels ($EC_{1:2}$; dS m^{-1}), classified according to (Henry et al., 1987), were: non-saline (0.1); slightly-saline (1.0); moderately-saline (2.0); and severely-saline (4.0). Logistically, the use of 1:2 extractions supported the quickest and most precise blending of the soil into the desired salinity levels; however, the salinity of the saturated paste extracts (EC_e) were also determined for each soil and will be referred to henceforth. Additionally, subsamples of each soil type were collected and submitted to a local soil testing lab (ALS Laboratory Group, Saskatoon, SK) for detailed salinity and nutrient availability assessment (Table D.1).

Table D.1. Selected properties of saline soils used to screen for salt tolerance among different native and exotic willow (*Salix* spp.) varieties.

	Nutrients†												
	NO_3-N	P	K	SO_4-S	Ca	Mg	Na	Cl	$EC_{1:2}$‡	EC_e§	SAR¶	ESP#	pH
Soil Type	(mg kg^{-1})				(mg L^{-1})								
Non-saline	7	19	423	13	74	41	44	8	0.1	0.8	1.4	0.8	7.1
Slightly-saline	8	51	649	295	410	219	239	22	1.0	3.6	3.4	3.6	7.4
Moderately-saline	17	54	657	708	494	334	404	36	2.0	5.0	4.9	5.6	7.6
Severely-saline	16	40	674	1610	486	462	900	72	4.0	8.0	9.9	11.7	7.9

† Extractable nutrients

‡ Electrical conductivity of a 1:2 (soil:water) extract

§ Electrical conductivity of a saturated paste extract

¶ Sodium adsorption ratio derived using equation from Henry et al. (1987): SAR = ([Na]) ((0.5 x ([Ca] + [Mg]))$^{-½}$; where [ ] is in mmol L^{-1}

Exchangeable sodium percentage derived using equation from Henry et al. (1987): ESP = ((1.47 x SAR)-1.26) ((0.0147 x SAR) + 0.99)$^{-1}$

D.4.2 Experimental design, willow material, growing conditions, and sampling protocols

The experimental setup was a completely randomized design with four replicates. A total of 592 pots were used (37 willow varieties x four saline soils x four replicates). Plant material of37 different willow varieties was collected from one-year-old stools in the spring of 2009 from clonal trial plots located in Saskatoon (Table D.2) and sectioned into 15 cm cuttings. Cutting diameter varied considerably among the willow varieties, which can influence establishment success (Burgess et al., 1990), so initial cutting diameters were measured and subsequently related to the willow growth variables at the end of the experiment for each saline soil. One-litre pots were filled with 1.3 kg of selected saline soil (bulk density approximately 1.3 g cm^{-3}) and watered to field capacity (28%, v v^{-1}), before inserting a single willow cutting. Pots were maintained at field capacity by watering every two days for the first two weeks and then daily for the remainder of the experiment. The surface of each pot was covered with white plastic beads to reduce evaporative losses. All pots were placed randomly in a Conviron® controlled environment chamber (Conviron Ltd., Winnipeg, MB). The willow were grown under an 18:6 h (light:dark) photoperiod, with air temperatures of 22:18 °C (day:night). Relative humidity was approximately 70%. Lighting was provided using Cool White VHO fluorescent and incandescent lamps (Sylvania, Drummondville, ON). Photon flux density was approximately 400 μmol m^{-2} s^{-1} at canopy level and was measured using a LI-COR quantum light meter (model LI-189; LI-COR Inc., Lincoln, NE.). After 60 days, plant heights (tallest shoot) were measured before each pot was harvested and separated into leaves, shoot(s), cutting, and roots. The roots were collected by washing the potted soil over a fine (0.5 mm) mesh sieve. All plant material was dried to a constant weight and the above- and below-ground plant biomass determined. Prior to drying the leaves, total leaf area for each plant was determined using a leaf surface area meter (LI-3100; LI-COR Inc., Lincoln, NE). Root mass fraction was calculated as root biomass:total biomass (Coyle and Coleman, 2005). Given the large variation in cutting size among the willow varieties, cutting biomass was not included in the total biomass value. In order to facilitate the comparison of salt tolerance among the different varieties, relative biomass assessments were made by normalizing the willow growth response to increased salinity relative to its growth under non-saline conditions (Steppuhn et al., 2008).

Table D.2. Thirty-seven selected native and exotic willow (*Salix* spp.) varieties screened for salt tolerance.

Variety	Species	Sex	Variety	Species	Sex
(1) Allegany	*S. purpurea*	F	(20) Saratoga	*S. purpurea x S. miyabeana*	F
(2) Alpha	*S. viminalis*	F	(21) Saskatoon D3	*S. discolor*	?
(3) Canastota	*S. sachalinensis x S. miyabeana*	M	(22) Saskatoon E3	*S. eriocephala*	?
(4) Charlie	*S. alba x S. glatfelteri*	?	(23) Sherburne	*S. sachalinensis x S. miyabeana*	F
(5) Cicero	*S. sachalinensis x S. miyabeana*	F	(24) SV1	*S. dasyclados*	F
(6) Fabius	*S. viminalis x S. miyabeana*	F	(25) SX61	*S. sachalinensis*	F
(7) Fish Creek	*S. purpurea*	M	(26) SX64	*S. miyabeana*	M
(8) Hotel	*S. purpurea*	?	(27) Taberg	*S. viminalis x S. miyabeana*	F
(9) India	*S. dasyclados x ?*	M	(28) Truxton	*S. viminalis x S. miyabeana*	F
(10) Juliet	*S. eriocephala*	?	(29) Tully Champion	*S. viminalis x S. miyabeana*	F
(11) Marcy	*S. sachalinensis x S. miyabeana*	F	(30) Verona	*S. viminalis x S. miyabeana*	F
(12) Millbrook	*S. purpurea x S. miyabeana*	F	(31) 94001	*S. purpurea*	M
(13) Oneida	*S. purpurea x S. miyabeana*	M	(32) 00X-026-082	*S. eriocephala*	M
(14) Oneonta	*S. purpurea x S. miyabeana*	M	(33) 00X-032-094	*S. eriocephala*	?
(15) Onondaga	*S. purpurea*	M	(34) 01X-268-015	*S. viminalis x (S. sachalinensis x S. miyabeana)*	?
(16) Otisco	*S. viminalis x S. miyabeana*	F	(35) 9837-77	*S. eriocephala*	F
(17) Owasco	*S. viminalis x S. miyabeana*	F	(36) 9882-041	*S. purpurea*	F
(18) S25	*S. eriocephala*	F	(37) 99208-038	*S. viminalis x S. miyabeana*	F
(19) S365	*S. caprea*	F			

D.4.3 Statistical analyses

Measurement variables were analysed using PROC GLM in SAS (version 9.1; SAS Institute Inc., Cary, NC.). Means comparisons were performed using least significant differences (LSD) at a significance level of 0.05. The LSD option was used to carry out pairwise t tests (equivalent to Fisher's protected LSD) of the different means, with groupings obtained using the pdmix800 SAS macro (Saxton, 1998). Homogeneity of variances and normality of distributions of all data sets were checked prior to the analysis. No data transformations were necessary.

D.5 Results and Discussion

D.5.1 Willow establishment and growth response to salinity

Although there was a marked delay in bud flush observed for all varieties as salinity increased, there were no differences in plant mortality among the four saline soil types, with 97% survival overall at the end of the experiment. The native variety Saskatoon D3 (*Salix discolor*) was the only willow variety unable to survive the highest salinity level in this study ($EC_e \leq 8.0$ dS m^{-1}). The ease of willow establishment on a variety of soil types, from non-rooted cuttings, is well established (Volk et al., 2006) and is advantageous for supporting its widespread use as a purpose-grown biomass energy crop. The apparent sensitivity of willow to increased salt level was dependent on the growth variable assessed, in that only the severely-saline conditions ($EC_e \leq$ 8.0 dS m^{-1}) significantly impacted plant height, shoot biomass, leaf biomass, and leaf surface area (Fig. D.1). Conversely, the number of shoots per plant, root biomass, and total plant biomass were more sensitive to increasing soil salinity. The largest root mass fraction (root biomass:total biomass) occurred with the highest salinity level (Fig. D.1) and is indicative of increased plant stress (Coyle and Coleman, 2005). There was no correlation between initial planted cutting diameter and any measured willow growth variable among the saline soil types (data not shown), which highlights the superseding importance of cutting quality, as opposed to size, that is key for successful willow establishment and growth.

While the majority of native and exotic willow varieties tested were sensitive to increasing soil salinity, several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) showed no reduction in growth with severe salinity ($EC_e \leq 8.0$ dS m^{-1}; Figs. D.2, D.3, and D.4). Additionally, unlike the others, these five varieties had no change in the measured root

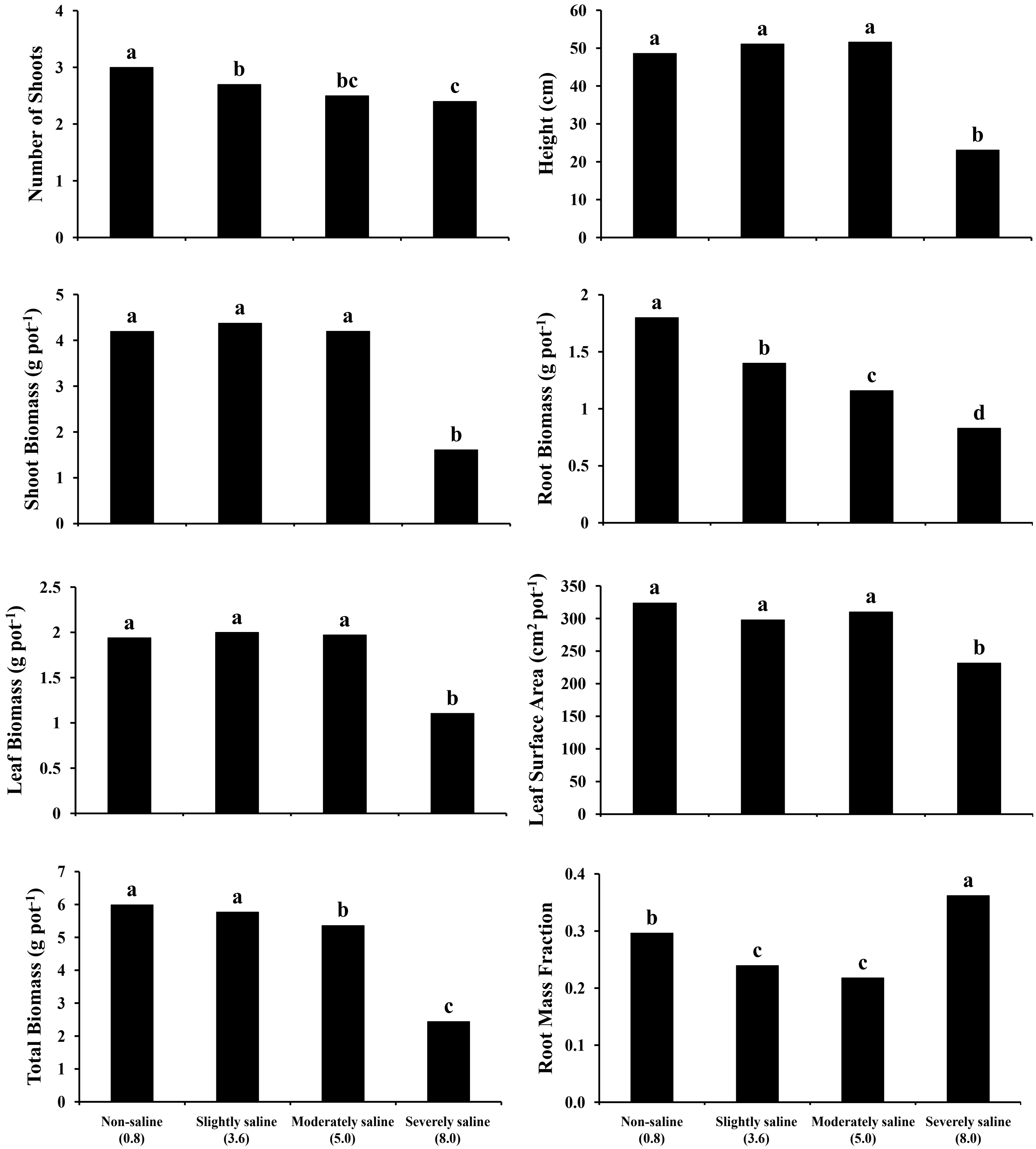

Fig. D.1. Mean (n ≥ 144) number of shoots, shoot height, shoot (including leaves) and root biomass, leaf biomass, leaf surface area, total biomass (shoot + root), and root mass fraction (root biomass:total biomass) of 37 different native and exotic willow varieties grown for 60 days in soils with increasing salinity (EC_e; dS m^{-1}). Bars having the same letter are not significantly different (*P* < 0.05) using LSD. Note: due to plant mortality, the number of replicates was not equal (i.e., 148) among soil salinity levels.

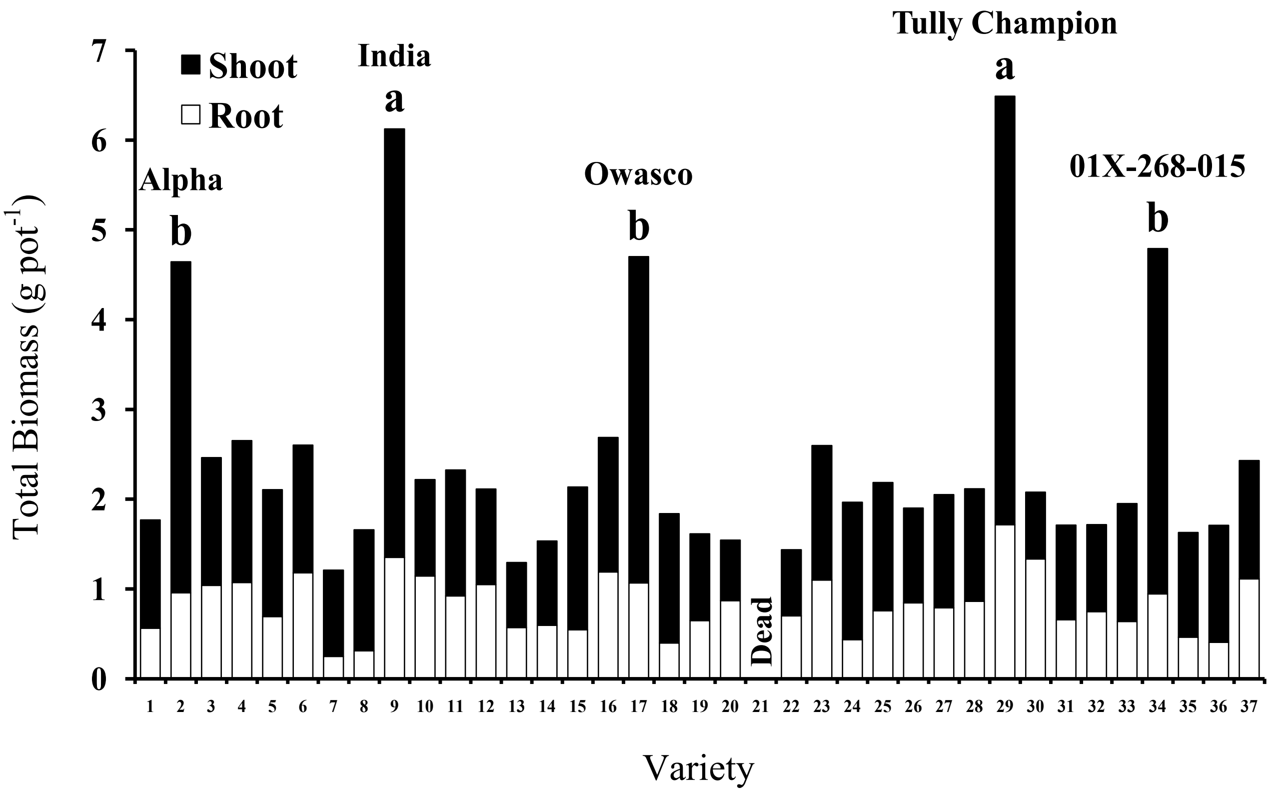

Fig. D.2. Total biomass (i.e., shoot + root; n = 4) of different native and exotic willow varieties grown for 60 days in severely-saline ($EC_e \leq 8.0$ dS m^{-1}) soil. See Table D.2 for variety identification. Bars having the same letter are not significantly different (*P* < 0.05) using LSD. Note: shoot biomass includes leaf biomass.

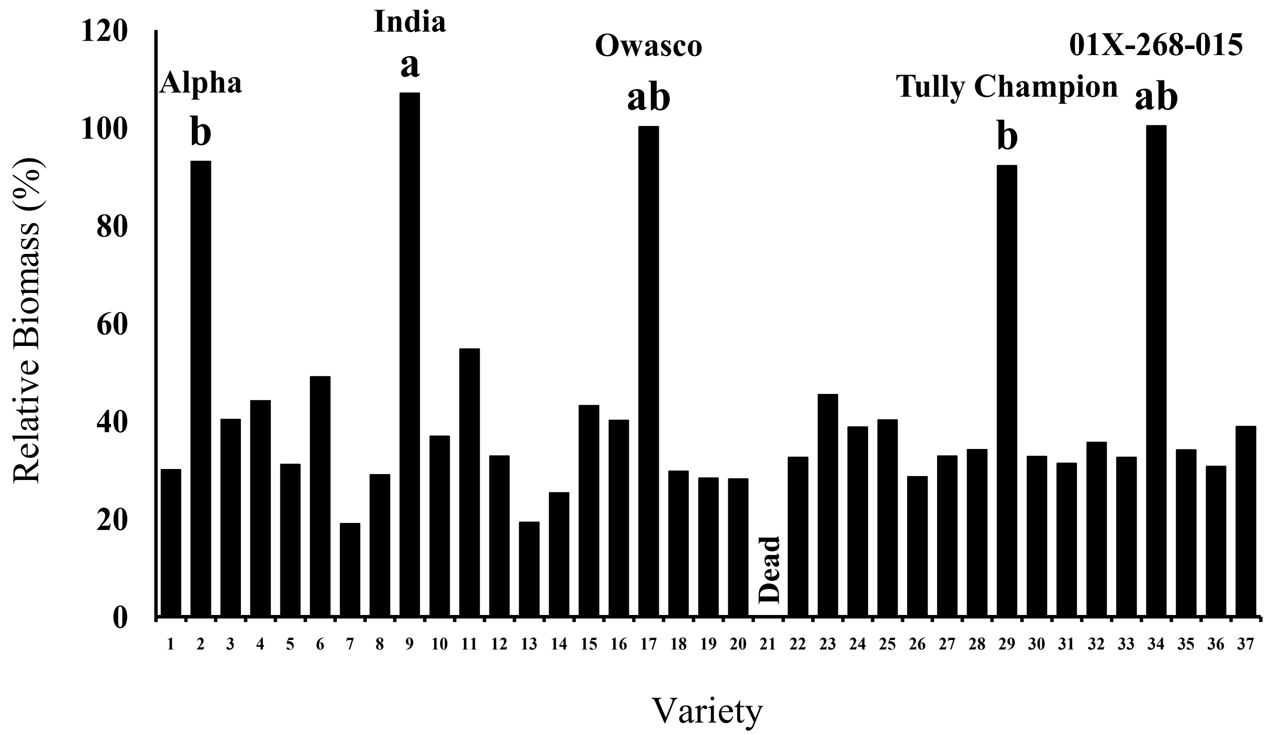

Fig. D.3. Relative total biomass (i.e., shoot + root; n = 4) of different native and exotic willow varieties grown for 60 days in severely-saline ($EC_e \leq 8.0$ dS m^{-1}) soil. Relative biomass was determined by normalizing the willow growth response to increased salinity relative to its growth on non-saline soil. See Table D.2 for variety identification. Bars having the same letter are not significantly different *(P* < 0.05) using LSD. Note: shoot biomass includes leaf biomass.

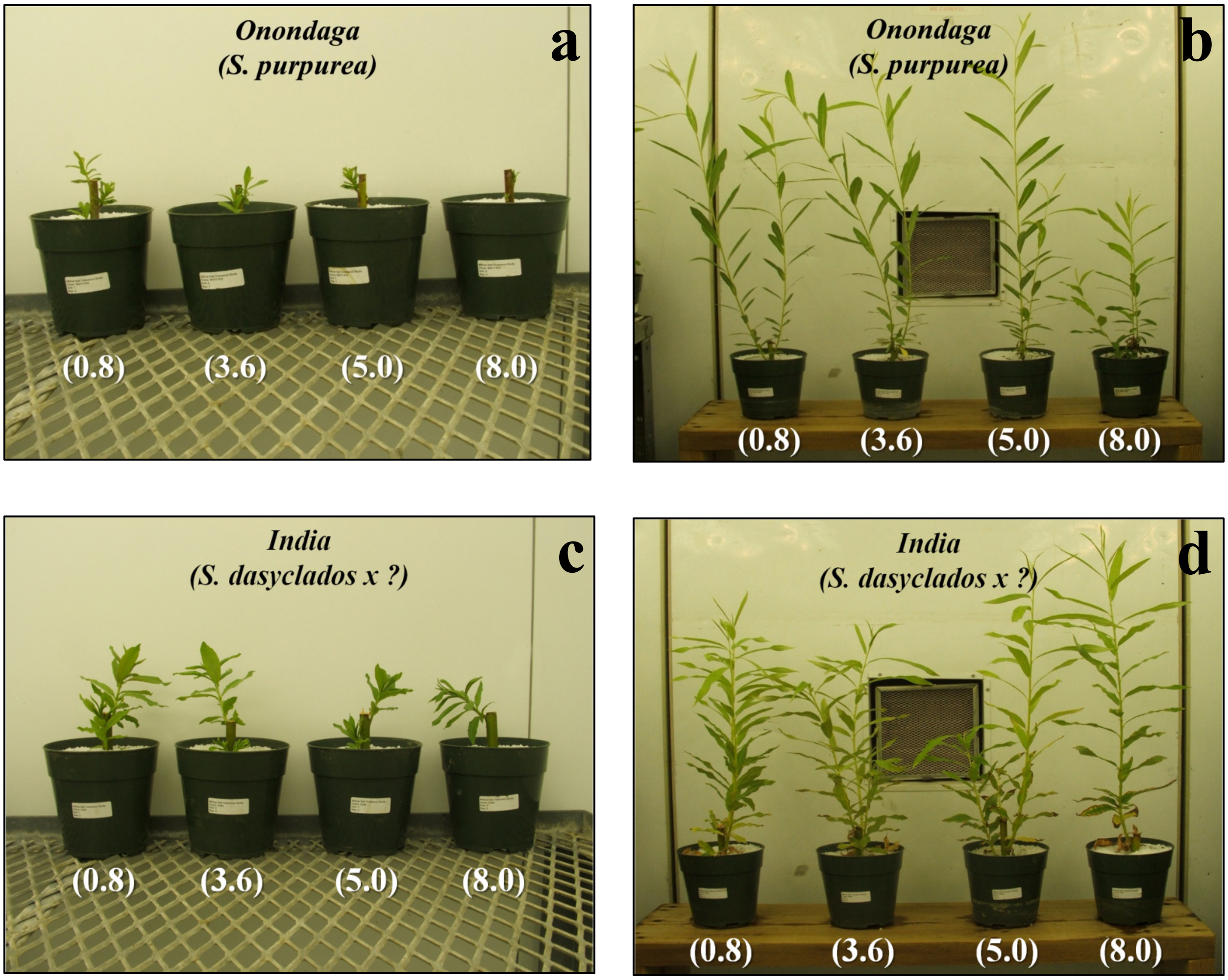

Fig. D.4. The effect of increasing soil salinity (EC_e; dS m^{-1}) on growth of relatively salt intolerant ('Onondaga'; above) and tolerant ('India'; below) willow varieties after 10 (a, c) and 60 (b, d) days.

mass fraction with increasing substrate salinity (data not shown) and, therefore, presumably were less-stressed and this was reflected in their sustained growth under increasingly saline conditions. Conversely, the occurrence of chlorotic leaves, necrotic patches, and premature leaf senescence for the remainder of willow varieties growing on the severely-saline soil, are common nutrient deficiency/ion toxicity symptoms in woody plants due to high tissue concentrations of Na^+ and Cl^- (Kozlowski, 1997; Chen et al., 2002). Moreover, the five relatively salt tolerant varieties had noticeably more lush appearance (i.e., greener and larger shoots) with increasing salinity after the 60-day growth period (Fig. D.4d) and this improved growth is probably attributable to the presence of residual fertilizer nitrogen and phosphorus in these highly saline soils. Specifically, past management practices at the site where the soils were collected involved uniform application of a consistent rate of fertilizer across all regions of the hillslope over several years. However, given the historically poorer crop growth in the salt-affected areas, reduced plant uptake and removal have resulted in higher extractable soil nutrient levels in these saline lower slope soils (i.e., Soil 3 and 4; Table D.1). For this reason, saline areas often can be the most nutrient-rich locations within a field and in this study, provided a growth advantage for the salt-tolerant varieties.

D.5.2 Relative salt tolerance among willow varieties

Anecdotally, willow is generally believed to have moderate tolerance to soil salinity (Kuzovkina and Quigley, 2005), which is confirmed by this study; however, a clear trend in effect of parentage was apparent among the five salt-tolerant willow varieties. Relatively greater salt tolerance was observed with the presence of *S. viminalis* or the hybridization of *S. viminalis* with *S. miyabeana* (Table D.2). The only exception may be the variety 'India', because its parentage is unknown− although some believe that it is a hybridized *S. viminalis* species (Cheryl Hendrickson, personal communication), which is realistic given its measured salt tolerance in this study. However, recent DNA fingerprinting work indicates that 'India' is related to *S. dasyclados* (Ngantcha, 2010). Nevertheless, it is reasonable to hypothesize that a possible introgression of the *S. viminalis species* genome into the *S. dasyclados* genome occurred, by the backcrossing of an interspecific *S. viminalis* x *S. dasyclados* hybrid with its *S. dasyclados* parent, to produce the variety 'India' (Alain Ngantcha, personal communication). Such an introgression would explain the salt tolerance observed in 'India' compared to the relatively salt-intolerant *S. dasyclados* willow variety 'SV1' tested (Fig. D.3). There was still considerable variabiltity in salt tolerance among the pure or hybrid *S. viminalis* varieties tested and this genotypic variation in salt tolerance among willow has been reported elsewhere (Stolarska, 2008; Aronsson et al., 2010; Mirck and Volk, 2010b). Identifying the specific physiological mechanism(s) responsible for the measured differences in salt tolerance among willow varieties is clearly beyond the scope of this screening trial; however, speculation regarding potential mechanisms based on the available literature is warranted.

D.5.3 Possible physiological adaptations of willow to salinity

Woody plants are known to synthesize and accumulate compatible organic solutes, such as glycine betaine, proline, and soluble carbohydrates, in the cytoplasm to regulate osmotic potential (Kozlowski, 1997). For example, proline content in leaves of *S. viminalis* was found to be an excellent indicator of salt tolerance among different varieties (Stolarska, 2008). Accumulating the endogenous phytohormone abscisic acid (ABA), which increases water-use efficiency by supporting plant morphological and physiological responses to saline stress, is another commonly known plant adaptation to salt-induced water deficit also utilized by willow (Liu et al., 2001). Measuring the response of gas exchange variables to increasing salinity, such as reduced stomatal conductance, is also an indicator of the concomitant water stress associated with salt stress in willow (Liu et al., 2001; Wrobel et al., 2006; Mirck and Volk, 2010b). As a reduction in stomatal conductance is a common plant response to osmotic stress in an effort to mitigate water deficiency, it was thought that the relative differences in adaptation to salinity among the willow varieties tested would be mirrored by their adaptation to water stress (Munns, 2002). An increasingly popular surrogate measure of water-use efficiency is the use of measured $^{13}C/^{12}C$ carbon isotope ratios within sampled plant leaves and the relationship between water-use efficiency and $\Delta^{13}C$ has been confirmed with willow (Weih and Nordh, 2002). In a separate clonal field trial, from which the plant material for this study was collected, $\Delta^{13}C$ value-based water-use efficiency rankings of the 37 clones were determined. The assumption is that the five relatively salt-tolerant varieties in this study would have been ranked within the top 10; however, their water-use efficiency rankings were scattered, with no discernible trend among the 37 willow varieties (data not shown). The lack of correlation between water-use efficiency and salt tolerance supports the concept that root exclusion mechanisms, which minimize root uptake of antagonistic cations and anions (e.g., Na^+ and Cl^-), are the principal response to soil salinity for woody plants (Allen et al., 1994; Munns, 2002).

Additionally, high soil calcium concentrations have been reported to mitigate salt stress experienced by woody plants (Rengel, 1992; Maas, 1993) and evidence suggests a buffering ability of cytosolic Ca^{2+} to maintain cell wall membrane integrity and serve as an important intracellular secondary messenger controlling plant water relations (Suhayda et al., 1992; Anil et al., 2005; Cousson, 2007). Consequently, willow varieties possessing high Ca^{2+} root uptake capacities and ability to maintain high Ca^{2+} tissue levels may be ideal for planting on these highly calcareous soils typical within the western Canadian prairies (Table D.1).

D.5.4 Reclaiming salt-affected marginal lands using salt-tolerant willow

From a land management point of view, the degree of saline seep expression is controlled by local climatic, hydrogeological, and agricultural factors. The potential to mitigate the aggravating effects of adverse climate and hydrogeological processes is limited. Consequently, implementing agricultural practices aimed at managing hillslope water dynamics is the only practical option available to help prevent, control, or reverse saline seep development. Specifically, cropping systems that adopt the use of deep-rooted, phreatophytic, and perennial species, such as willow, would greatly reduce the accumulation and deep percolation of available soil water lost below the rooting zone in the recharge area, (Miller et al., 1981; Henry et al., 1987; Wiebe et al., 2007). Furthermore, establishing salt-tolerant willow within seepage areas also would support the amelioration of this saline soil, by lowering of the water table in these shallow groundwater flow system discharge areas, thereby supporting leaching of the salts from the profile over time (Daniels, 1987; Henry, 2003). The opposite becomes apparent when willow rings around sloughs are removed, which often hastens slough-ring salinity problems by trapping less snow (i.e., reduced leaching potential) and increasing evapotranspiration-driven capillary rise and accumulation of root-zone salts (PFRA, 2000).

D.5.5 Future work and practical considerations with identifying salt-tolerant willow

Willow has a very broad genetic base, with an estimated 450 species within the genus *Salix* (Argus, 1997)− of which, 125 species are currently being investigated for use in short-rotation intensive culture plantations (Keoleian and Volk, 2005). Given that the willow varieties examined in this study were primarily hybrids among only 10 different willow species (Table D.2), the apparent differences in salt tolerance observed among the relatively small number of varieties tested is promising, considering the enormous amount of untested willow genotypes available. However, the logistics involved in screening large numbers of willow genotypes for salt tolerance using pot studies is impractical. Instead, the use of *in vitro* screening techniques (i.e., tissue culture) need to be developed, which should be a more cost-effective and rapid method to facilitate the selection process, until genes controlling salt tolerance are identified and their associated screening tools developed. After narrowing down the number of potential candidates, further refinement in selection prior to field validation should be done using Canada's Salt Tolerance Testing Facility, which has been successfully used to assess salt tolerance among hybrid poplar varieties (Steppuhn et al., 2008). Such a facility could also determine whether the salinity tolerance responses of willow observed in this study, using sulphate-dominated soils typically found in western Canada (Table D.1), are similar to chloride-dominated saline soils that are more common globally (Szabolcs, 1989).

D.6 Conclusion

Establishing purpose-grown willow plantations with salt-tolerant varieties on salt-affected soil provides utility for otherwise non-productive land, thereby avoiding the displacement of arable land from food production. The identification of salt-tolerant willow varieties is important when considering options for reclaiming salt-affected marginal lands within western Canada, such as toe-slope areas within prairie landscapes, which preclude the growth of many annual crops. Most of the willow varieties tested in this study were able to tolerate moderately-saline soils ($EC_e \leq 5$ dS m^{-1}). Furthermore, several varieties (Alpha, India, Owasco, Tully Champion, and 01X-268-015) showed no reduction in growth with a severe salinity level ($EC_e \leq 8$ dS m^{-1}). However, field trials ultimately are required to validate the differences in salt tolerance among willow varieties observed in this controlled environment study.