

1 **Willow Production Systems for Bioenergy Feedstock and C** 2 **sequestration in Canada and northern U.S.A.: A Review**

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14 15 **Abstract**

16 Willow short rotation coppice (SRC) systems are becoming an attractive practice because they
17 are a sustainable system fulfilling multiple ecological objectives with significant environmental
18 benefits. A sustainable supply of bioenergy feedstock can be produced by willow on marginal
19 land using well-adapted or tolerant cultivars. Across Canada and northern U.S.A., there are
20 millions of hectares of available degraded land that have the potential for willow SRC biomass
21 production, with a C sequestration potential capable of offsetting appreciable amount of

1 anthropogenic green-house gas emissions. A fundamental question concerning sustainable SRC
2 willow yields was whether long-term soil productivity is maintained within a multi-rotation SRC
3 system, given the rapid growth rate and associated nutrient exports offsite when harvesting the
4 willow biomass after repeated short rotations. Based on early results from the first willow SRC
5 rotation, it was found willow systems are relatively low nutrient-demanding, with minimal
6 nutrient output other than in harvested biomass.

7 The overall aim of this manuscript is to summarize the literature and present findings and data
8 from ongoing research trials across Canada and northern U.S.A. examining willow SRC system
9 establishment and viability. The research areas of interest presented here are the crop production
10 of willow SRC systems, above- and below-ground biomass dynamics and the C budget,
11 comprehensive soil-willow system nutrient budget, and soil nutrient amendments (via
12 fertilization) in willow SRC systems. Areas of existing research gaps were also identified for the
13 Canadian context.

14

15 **Keywords:**

16 Short rotation coppice, SRC willow cultivar biomass yield, carbon (C) sequestration, root
17 development, nutrient budget, fertilization

18

19 **1. Introduction**

20 Willow (*Salix* spp.) is widespread across Canada's boreal forest and Aspen Parkland ecoregion
21 (Johnson et al., 1995) and, therefore, is the first choice of short rotation coppice (SRC) species to

1 establish in Saskatchewan (Amichev et al., 2011; 2012). Willow shrubs are ideal for SRC
2 systems (Keoleian and Volk, 2005) because they are fast-growing, can easily propagate from
3 cuttings, and have a large amount of easily exploitable genetic diversity that can be used in
4 conventional breeding and molecular biotechnology (Dickmann, 2006). Willow shrubs have the
5 ability to re-sprout after a disturbance, whether through natural breaking, grazing, or human
6 induced coppicing or pruning (Keoleian and Volk, 2005). When used in SRC systems, willows
7 are capable of growing on marginal agricultural land (Labrecque and Teodorescu, 2005) and
8 previously mined regions as reclamation and land utilization tools (Gruenewald et al., 2007).

9 Verwijst (2001) provided a comprehensive review comparing the genus *Salix* to the genus
10 *Populus*, and comparing willow to poplar cultivation for bioenergy production in order to
11 highlight differences between willow and poplar management with respect to provided services
12 and products. The review paper also addressed the common problems and challenges faced when
13 cultivating willow or poplar, such as species selection and breeding, pest and disease control, and
14 design of production systems (Verwijst, 2001). In short, compared with the genus *Populus*, *Salix*
15 has a much broader genetic base (i.e., approximately ten times larger), more extensive
16 geographical and physiognomic range, and offers a much greater variety of ecosystem services
17 and environmental applications (Verwijst, 2001).

18 A sustainable supply of fuel wood can be produced by willow on marginal land using well-
19 adapted or tolerant cultivars (Gruenewald et al., 2007). There are approximately 147 Mha of
20 degraded and abandoned agricultural lands within North America potentially available for
21 establishing SRC systems aimed at bioenergy feedstock production (Hoogwijk et al., 2005;
22 Lemus and Lal, 2005). Saskatchewan, one of the prairie provinces of Canada, with > 2 Mha of
23 available degraded land, has the potential for SRC biomass production, with a C sequestration

1 potential capable of offsetting up to 80 % of the annual anthropogenic greenhouse gas emissions
2 in the province (Amichev et al., 2012).

3 Previous efforts have been made to promote the establishment of willow SRC industry for
4 bioenergy feedstock production. The Salix Consortium is one such effort that was intended to
5 advance willow crops from the experimental trials phase into a commercial business enterprise in
6 the U.S.A., and was based on research from the U.S.A. (Volk et al., 2006), Sweden (Mola-
7 Yudego and Gonzales-Olabaria, 2010), Canada (Mosseler, 1990), and the UK (Armstrong et al.,
8 1999; Bell et al., 2006). Based in New York (U.S.A.), the Salix Consortium (formerly the
9 Empire State Biopower Consortium) was formed in 1995 to facilitate commercialization of
10 willow biomass production in the Northeastern and Midwestern regions of the U.S.A.
11 (Abrahamson et al., 1998; 2002). Similarly, in Canada, governmental programs such as the
12 Agricultural Bioproducts Innovation Program (ABIP) and the Saskatchewan Biofuels Investment
13 Opportunity (SaskBIO) have been developed to promote this industry and encourage extension
14 activities to transfer information between researchers and landowners. Future energy crop
15 production is most likely to occur on marginal agricultural land in order to avoid conflict with
16 food production and compromising food security (Aylott et al., 2010; Volk and Luzadis, 2009).
17 Willow plantations established on marginal lands can decrease fibre demands on existing natural
18 forests and provide a means to recycle organic residues, such as sewage sludge and animal
19 manures (Labrecque and Teodorescu, 2005). For example, there are an estimated 4 Mha of salt-
20 affected abandoned land across the Canadian prairies, approximately 1.6 Mha in Saskatchewan
21 alone, which is unsuitable for arable crop production, but could support SRC production of salt-
22 tolerant willow cultivars (Hangs et al., 2011). Earlier studies have highlighted the ability of
23 willow to grow well on a variety of soil types (Scholz and Ellerbrock, 2002; Stolarski et al.,

1 2011) and the recent work in Saskatchewan by Hangs (2013) was in agreement where first
2 rotation willow SRC yields were $>10 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ on sandy soil without fertilization.

3 In order to produce willow biomass, it is necessary to combine knowledge of both forestry and
4 agronomy practices (Keoleian and Volk, 2005). Throughout the growth cycle of willow SRC
5 systems, intensive management is required in site preparation, planting, weed and pest control,
6 fertilization, coppicing, and harvesting. A fundamental question concerning sustainable SRC
7 willow yields, therefore, is whether long-term soil productivity is maintained within a multi-
8 rotation SRC system, given the rapid growth rate and associated nutrient exports offsite when
9 harvesting the willow biomass after repeated short rotations (i.e., three to five years). A clear
10 understanding of soil nutrient dynamics and soil nutrient budgets during the establishment phase
11 is required to accurately forecast the sustainability of willow SRC systems and the necessity of
12 soil nutrient amendments. For example, fertilization traditionally has been used as a management
13 tool to support the establishment and growth of willow SRC crops; however, its efficacy has
14 been inconsistent (Hangs et al., 2012b; Quaye and Volk, 2013). Other SRC crop establishment
15 factors include the inherent soil fertility at a given site (Mitchell, 1995; Quaye et al., 2011),
16 genotypic variability in nutrient requirements, uptake capacity and/or utilization efficiency
17 (Adegbidi et al., 2001; Weih and Nordh, 2005), genotype x environment interactions (Ballard et
18 al., 2000a; Hofmann-Schielle et al., 1999), previous land use history, suppression of competing
19 vegetation, and mechanical site preparation (Abrahamson et al., 1998; 2002).

20 The majority of research on willow SRC systems has focused on the above-ground portion of the
21 system, and relatively few studies have been done on the morphological stages of willow fine
22 roots. The processes of fine root production and mortality in a system are difficult to examine
23 due to the high degree of spatial and temporal variability (Norby et al., 2004). However,

1 knowledge about the below-ground portion of willow SRC systems for the climate and soils of a
2 given region, and the dynamics of willow fine roots, in particular, is crucial to understanding the
3 soil-willow interaction with regard to soil C sequestration, nutrient cycling, and long-term
4 sustainability of SRC systems. For example, as a direct result of coppicing in intensively
5 managed willow SRC systems, biomass storage below-ground was increased through
6 encouraging more rapid fine root turnover and contribution to stable soil humus pools (relative to
7 undisturbed woodland sites) which promoted soil C sequestration, e.g., 0.41 Mg C ha⁻¹ yr⁻¹ and
8 0.51 Mg C ha⁻¹ yr⁻¹ to a depth of 23 and 50 cm, respectively (Grogan and Matthews, 2002), as
9 well as increased soil organic matter levels (Zan et al., 2001).

10 The aim of this manuscript is to summarize the literature and present findings and data from
11 ongoing research trials across Canada and northern U.S.A. examining willow SRC system
12 establishment and viability (Table 1). The research areas of interest presented here are as
13 follows: crop production of willow SRC systems, above- and below-ground biomass dynamics
14 and the C budget, comprehensive nutrient budgets, and nutrient amendments (via fertilization) in
15 willow SRC systems.

16

17 [Insert Table 1 here]

18

19 **2. Crop production of willow SRC systems: an overview**

20 A crucial decision made at the establishment phase of each willow SRC system is site selection.
21 Abrahamson et al. (2002) concluded that shrub willows grow best in loamy soils with a well-

1 developed soil structure and >45cm rooting depth. Although data showed slower initial willow
2 establishment on soils with high clay content, willow growth and productivity in successive
3 rotations was greater in these soils, likely due to much greater nutrient exchange capacity relative
4 to sandy soils (Abrahamson et al., 2002). Similar findings were reported by Ens et al. (2013a) in
5 regard to soil and climate variables affecting willow productivity (*S. purpurea*, "Hotel" cultivar)
6 and nutrition across a broad range of sites in Canada. In general, the greatest yields were
7 observed on sites receiving adequate moisture (especially summer precipitation in first two
8 years) with calcareous medium textured soils (Ens et al., 2013a). The lowest "Hotel" yields were
9 observed on sandy soils, sites with very low precipitation, or a combination of both (Ens et al.,
10 2013a). In addition, it has been suggested that excessively well or poorly drained soils or
11 moderately acidic or alkaline soils (pH <5.5 or >8.0) could also lead to poor willow growth
12 (Abrahamson et al., 2002).

13 In a SRC system, willows are grown from unrooted cuttings that have been harvested from one-
14 year-old shoots during the dormant winter season (Keoleian and Volk, 2005). The planting
15 density of willow SRC systems is greatly dependant on the entire production system because it
16 affects management decisions, such as weed control and harvest efficiency (Keoleian and Volk,
17 2005). For willow SRC systems, experimental planting densities of 15,300 plants ha⁻¹ (Heller et
18 al., 2004) and 10,000 to 20,000 plants ha⁻¹ (Christersson et al., 1993) have been recommended.
19 Higher densities tend to be more efficient at using resources earlier in the rotation, yet have
20 higher establishment costs (Bullard et al., 2002), while lower densities have lower costs, but lead
21 to a delayed peak of mean annual increment (Keoleian and Volk, 2005). A study carried out by
22 Adegbidi et al. (2001) observed the effects of planting density on other components of the

1 production system and found that planting densities, whether 107600, 36960, or 15000 plants ha⁻¹
2 had no significant effect on annual biomass production.

3 Willow SRC systems are generally harvested on a 3- to 4-yr cycle and are managed using a
4 coppice practice (Keoleian and Volk, 2005). Willows are coppiced (cut to <5 cm tall) after the
5 first growing season during the dormant season (winter) when carbohydrate reserves are at their
6 maximum level in the root tissues (Sennerby-Forsse, 1995). Therefore, coppicing refers to the
7 severing of all aboveground biomass in order to stimulate reinvigoration and accelerate growth
8 toward the theoretical maximum (Sennerby-Forsse, 1995). Coppicing has been found to double
9 the density of willow SRC systems by increasing the number of shoots by 3- to 4-fold (Hytönen,
10 1995). After 3 to 4 years of growth, harvesting takes place in the dormant season when woody
11 tissue nutrients are translocated to the roots and most foliage nutrients have been deposited on
12 the soil for onsite recycling (Hangs et al., 2013; Lemus and Lal, 2005). Since willow are capable
13 of vigorous re-sprouting after each harvest, 7 to 10 harvests are possible from a single planting
14 resulting in a plantation life span of more than 20 years (Keoleian and Volk, 2005).

15 One of the many advantages of growing willow as bioenergy feedstock is its large net energy
16 ratio (i.e., high energy output to input ratio). Specifically, at the end of seven three-year harvests,
17 approximately 55 units of harvested bioenergy can be created with 1 unit of fossil fuel input
18 (Keoleian and Volk, 2005) when the farm gate is considered the edge of the system. More recent
19 life cycle analysis that includes haul distances and the uncertainty associated with certain parts of
20 the system has reported energy yields of 18.3 to 43.3 for every 1 unit of fossil fuel invested
21 (Caputo et al., 2013). In any of these studies, the net energy ratio is strongly influenced by yield.
22 Yield, however, was found to be highly dependent on many factors including genetic diversity,
23 soil fertility, climate and crop management (Keoleian and Volk, 2005). Recent work also

1 suggested that many willow cultivars were tolerant of moderately to severely saline soils
2 commonly found in western Canada (Hangs et al., 2011). For example, Hangs et al. (2011)
3 reported sufficient willow growth of a number of willow cultivars developed as bioenergy
4 feedstock under moderately saline conditions (≤ 5.0 dS/m), and even severe salinity (≤ 8.0 dS/m).
5 Across the U.S.A. and Canada, weed competition and insufficient soil moisture (Ens et al.,
6 2013a) were identified as the greatest site limitations affecting willow establishment and biomass
7 production in SRC systems, followed by adverse effects by pests (Vujanovic and Labrecque,
8 2002), although there has been no evidence of pests significantly affecting willow yields
9 (Zalesny et al., 2011). Recently, Corredor et al. (2012; 2014) reported that both host genotype
10 and health status influence the composition of fungal communities in the rhizosphere of willow.
11 Irrigation has been used to ameliorate moisture regime limitations and was shown to
12 significantly increase willow growth on heavy clay soils (Hangs et al., 2012b). For example,
13 willow yields as high as $27 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ were reported in trials in New York in an irrigated and
14 fertilized plantation (Adegbidi et al., 2001).

15

16 **3. Above-ground willow biomass growth for C sequestration and bioenergy feedstock** 17 **production**

18 A willow SRC trial involving 30 willow cultivars was established in Saskatoon, Saskatchewan in
19 order to evaluate the growth and adaptability of willow SRC crops to the soils and climate of the
20 province. A prepared manuscript reporting the first-rotation yields of these 30 willow cultivars is
21 presently in the process of journal peer-review and, therefore, it is referred to as the '30-cultivar
22 study' in the current review paper. The 30-cultivar study was established using dormant willow

1 cuttings, each 25 cm long, obtained from the State University of New York - College of
2 Environmental Science and Forestry (SUNY-ESF) which were planted in 2007 at the University
3 of Saskatchewan (Latitude 52.126632; Longitude -106.608294; elevation 510 m above sea level)
4 at Saskatoon, Saskatchewan, Canada. The study site was located in the Elstow Plain ecodistrict
5 of the Moist Mixed Grassland ecoregion within the Prairies ecozone of Saskatchewan (SLC,
6 2006). The soils at this site are a heavy clay Sutherland Orthic Vertisol (Agriculture Canada,
7 1998). Mean annual precipitation at this site is 375 mm, mean annual temperature is 2°C, and the
8 average number of frost days is 253 annually (EC-NCD, 2008). All willow cultivars were
9 planted by hand in spring of 2007 in four separate replications (approximately 7 x 9 m area each)
10 with three double-rows of thirteen plants per row with 1.5 m spacing between double-rows, 0.60
11 m between rows in a double-row, and 0.60 m between plants within a row, resulting in a planting
12 density of approximately 15,873 plants ha⁻¹. Willow cultivars were coppiced at the end of the
13 first growing season and first-rotation biomass was manually harvested from each plot three
14 years later.

15 The average first 3-yr-rotation harvested biomass yield in the 30-cultivars study was 10.5 (Oven-
16 dry) Mg ha⁻¹ (annual increment = 3.5 Mg ha⁻¹ yr⁻¹), ranging from 4.0 to 17.4 Mg ha⁻¹ (1.3 to 5.8
17 Mg ha⁻¹ yr⁻¹). These observed yields were similar to other reported willow yields from sites in
18 Saskatchewan (Ens et al., 2013b; Moukoui et al., 2012) and some sites in the U.S.A. (Kiernan
19 et al., 2003). For example, harvest yields for the SX64 (*Salix miyabeana*) cultivar in the 30-
20 cultivars study, ranging from 9.0 to 12.1 Mg ha⁻¹ (3.0 to 4.0 Mg ha⁻¹ yr⁻¹) were similar to yield
21 data by Moukoui et al. (2012) across a range of site quality for three sites near Saskatoon, 1.2
22 to 15.6 Mg ha⁻¹ after 4 years (0.4 to 5.2 Mg ha⁻¹ yr⁻¹). Also, the yields of the highest producing
23 cultivars at the Saskatoon site of the 30-cultivars study overlapped the lower end of first rotation

1 willow yields reported by Kiernan et al. (2003) for older cultivars in the U.S.A. (planted between
2 1993 and 2001), 5.2 to 9.3 Mg ha⁻¹ yr⁻¹, but were lower than first rotation yields of new willow
3 genotypes (planted from 2005 to 2007), 6.1 to 12.9 Mg ha⁻¹ yr⁻¹, reported by Volk et al. (2011).
4 Based on our findings in the 30-cultivars study, we suggested that the relatively lower yields of
5 some of the cultivars studied in Saskatoon could be due to either herbicide damage (herbicide
6 used for weed control), poor willow root extension due to heavy clay soils, a notable winter
7 dieback of some cultivars, moisture regime limitations during the first rotation (Hangs et al.,
8 2012b), and low plant-available soil N levels (Moukouri et al., 2012). Our observations from
9 the 30-cultivars study agreed with previous reports that growing degree days (GDD, base 5°C)
10 (Kopp et al., 2001; Moukouri et al., 2012) was a very important growth limiting factor for
11 willow SRC systems, and the much lower yields observed in the 30-cultivar study adequately
12 corresponded to the lower GDD count at these northern latitudes under Canadian climatic
13 conditions. A similar decrease in willow SRC biomass yield resulting from GDD decrease was
14 previously reported by Kopp et al. (2001) who found that 2.4 to 4.1 % willow yield decreases
15 could occur for each 1 % decrease in GDD. In recent studies in Ontario, Canada, significantly
16 increased willow SRC yields were reported in an agroforestry tree-intercropping system (4.86
17 Mg ha⁻¹ yr⁻¹) compared to a conventional willow SRC system (3.02 Mg ha⁻¹ yr⁻¹) due to
18 complementary growth-promoting interactions as influenced by the presence of mature trees (21-
19 yr old mixed tree species) along the willow rows (Cardinael et al., 2012; Clinch et al., 2009).

20 As part of the 30-cultivars study, and based on the 3PG willow growth modeling work in
21 Amichev et al. (2011), expected first 3-yr rotation yield of SV1 willow SRC systems for all
22 marginal agricultural areas across Saskatchewan averaged 13.6 and 11.8 Mg ha⁻¹ (annual
23 increments 4.5 and 3.9 Mg ha⁻¹ yr⁻¹) for farm lands in the Prairies and Boreal Plains ecozones of

1 the province, respectively. In the willow yield map of the 30-cultivars study we indicated >4.9
2 Mg ha⁻¹ (1.6 Mg ha⁻¹ yr⁻¹) increase of SV1 production, relative to that at the Saskatoon site (9.8
3 Mg ha⁻¹; annual increment 3.3 Mg ha⁻¹ yr⁻¹), on >0.3 Mha of land in Saskatchewan, which
4 emphasized the potential use of this map as a decision-making tool for the bioenergy industry.

5 Carbon sequestration in willow SRC systems occurs in two distinct forms – the ecosystem and
6 the harvested biomass transferred out of the ecosystem (Amichev et al., 2012). Due to the rapid
7 willow growth and short biomass harvesting rotations, nearly 75% of C removed from the
8 atmosphere and locked within ecosystem components was in dead organic matter (DOM) pools –
9 which included the litter layer, dead fine roots, and soil (Amichev et al., 2012). Even after
10 biomass removal at harvest time, Amichev et al. (2012) projected an increase of DOM C pools at
11 an average rate of 0.9 Mg ha⁻¹ yr⁻¹, equivalent to an increase from 205 to 292 Tg C (1 Tg=1
12 million metric tons) for 2.12 Mha of marginal agricultural land in Saskatchewan, over two full
13 cycles of seven 3-yr willow SRC rotations (i.e., 44-yr simulation period). Their projected C
14 sequestration rates were lower than rates reported in southwestern Quebec for willow SRC
15 systems (1.29 Mg C ha⁻¹ yr⁻¹) (including root and soil C stocks), and were approximately equal to
16 the rates for switchgrass (*Panicum virgatum*) SRC systems (1.09 Mg C ha⁻¹ yr⁻¹) (Lemus and
17 Lal, 2005).

18 A much more notable C removal from the atmosphere was projected within the cumulative
19 harvested biomass transferred out of the willow SRC systems every 3 years (Amichev et al.,
20 2012). For example, after two full cycles of seven 3-yr rotations (i.e., 44 years), the average
21 potential C stock in cumulative harvested willow biomass on farm land in the Prairie ecozone of
22 Saskatchewan was 244 Mg C ha⁻¹ (rate = 5.5 Mg C ha⁻¹ yr⁻¹). This rate was about 20% higher

1 than the average potential C capture in harvested biomass for farm land in the Boreal Plain
2 ecozone of Saskatchewan, 203 Mg C ha⁻¹ (rate = 4.6 Mg C ha⁻¹ yr⁻¹) (Amichev et al., 2012).

3 In sum, the total amount of C removed from the atmosphere in two full cycles of seven 3-yr
4 willow SRC rotations in the form of ecosystem C and C in harvested biomass averaged 292 and
5 215 Mg C ha⁻¹ (= 6.6 and 4.9 Mg C ha⁻¹ yr⁻¹) in the Prairie and the Boreal Plain ecozones of
6 Saskatchewan, respectively (Amichev et al., 2012). These findings showed that significant
7 amounts of atmospheric CO₂ could be removed by willow SRC systems. Although the C uptake
8 in harvested biomass for willow SRC systems is not a long-term storage of C, the amount of C
9 released to the atmosphere in the process of generating energy from willow feedstock was
10 captured from the atmosphere in the previous 3-5 years, depending on rotation length (Amichev
11 et al., 2012). This short time for willow SRC systems to achieve C neutrality, compared to
12 decades or longer periods for woody biofuels from conventional forest operations, had been
13 recognized to greatly enhance the suitability of willow SRC systems for use to off-set fossil fuels
14 (Amichev et al., 2012).

15 Due to a lack of other C studies for willow SRC systems in Saskatchewan, Amichev et al. (2012)
16 emphasized the need for additional empirical data, including willow root biomass, soil fertility
17 assessment, woody debris decay, and long-term plant mortality, all of which could be used to
18 improve and validate projected C budget of willow SRC systems in the province. The use of
19 willow SRC systems as a bioenergy crop is promising, but more research from additional trials at
20 different sites was deemed necessary regarding the long-term potential of these systems to
21 sustain a biomass industry in Saskatchewan (Amichev et al., 2012). Additional willow SRC trials
22 across the province would also serve as validation for willow SRC system yield maps, which will

1 be critically important for the next phase of biomass industry development in Saskatchewan - site
2 selection for establishment of bioenergy processing plant facilities.

3

4 **4. Below-ground willow development: what we know so far**

5 General assumptions and recent data collected across a chronosequence of willow biomass crops
6 indicate that coarse root biomass increases early in the life of the crop, reach a steady mass and
7 remain stable for the remainder of the lifetime of the crop (Pacaldo et al., 2013; Heller et al.,
8 2003), while the temporal dynamics of fine roots are more closely correlated to the surrounding
9 environment (Leuschner et al., 2004). Root turnover studies are particularly difficult due to the
10 simultaneous nature of fine root production and mortality (Rytter, 1999; Rytter and Hansson,
11 1996). In a method comparison study, including destructive methods, namely soil coring and
12 whole tree excavation (Achat et al., 2008; Rytter, 1999), and non-destructive methods such as the
13 minirhizotron method (Vargas and Allen, 2008) for *in situ* quantification of fine root turnover,
14 Rytter (1999) found that different methods of quantifying fine-root turnover produced various
15 results. Singh et al. (1984) concluded that root system studies and data could be very sensitive to
16 peaks in fine root production and mortality, and that overestimation or underestimation in
17 biomass can result from this temporal variability. Optimal sampling dates for roots determined
18 by the seasonal maxima and minima are, however, difficult to determine (Block, 2004).

19 Willow fine roots can account for approximately 30 to 50% of annual net primary production
20 (NPP) (Greer et al., 2006; Rytter, 1999) with a ratio of fine roots to above-ground production
21 ranging from 0.4 to 1.2, depending on the environment and year of growth (Rytter, 2001).

22 Willow root systems are characterized by tap and fibrous root arrangements (Puttsepp, 2004).

1 Brundrett and Kendrick (1990) described the willow root system being comprised of long
2 straight roots bearing shorter, curved roots with root hairs scattered throughout the system.
3 Willow roots typically reach an average depth of 25 to 30 cm during the first growing season,
4 extending into greater depths during the second growing season (Rytter and Hansson, 1996). The
5 rooting depth is primarily responsive to the moisture gradient in the soil profile (Volk et al.,
6 2001) and is relatively deeper when compared to annual crops, such as mustard (*Brassica*
7 *juncea*), tobacco (*Nicotiana tabacum*) and maize (*Zea mays*) (Keller et al., 2003). This vertical
8 distribution is likely a factor in the high survival rates of willow plantations established on
9 marginal sites because deeper rooting allows for greater accessibility to limited soil resources
10 (Volk et al., 2001). Some of the notable multiple roles of willow root systems include anchorage,
11 nutrient acquisition and storage, and phytoremediation (Corseuil and Moreno, 2001; Jackson et
12 al., 1997; Karrenberg et al., 2003) as well as soil C sequestration (Block et al., 2006; Lemus and
13 Lal, 2005; Zan et al., 2001).

14 Stadnyk (2010) used three different willow roots measurement and observation methods at
15 several different research trials in Saskatchewan: minirhizotron, sequential soil coring, and
16 whole-tree excavation methods. On average (across all six cultivars), fine root biomass values
17 using the soil coring method ranged from 0.02 to 0.3 Mg ha⁻¹ which were considerably smaller
18 than fine root values found using the minirhizotron method, ranging from 0.8 to 7.4 Mg ha⁻¹,
19 while average root biomass value determined using the whole-tree excavation method was 0.5
20 Mg ha⁻¹ (Stadnyk, 2010). Stadnyk (2010) noted that minirhizotron fine root biomass values may
21 be overestimated due to experimental artifacts such as soil-tube interface gaps formed in certain
22 soil types such as Vertisols. In the first growing seasons, monthly fine root NPP was similar
23 across the six willows studied and reached a peak of 3.4±1.8 Mg ha⁻¹ (Stadnyk, 2010). These fine

1 root NPP values were similar to that of a hybrid poplar plantation (Gunderson et al., 2008) and
2 were also similar to that of basket willow reported by Rytter (2001) (2.7 to 6.5 Mg ha⁻¹ yr⁻¹).
3 However, in other work in the literature, where alternative methods were used for NPP
4 measurement, such as sequential soil cores and ingrowth cores, annual fine root NPP values were
5 lower, ranging from 1.3 Mg ha⁻¹ yr⁻¹ (Steele et al., 1997) to 2.5 Mg ha⁻¹ yr⁻¹ (Ostonen et al.,
6 2005) possibly due to the limitations of these methods to account for the simultaneous nature of
7 fine root growth and mortality.

8 The minirhizotron method also allowed assessment of willow root turnover and longevity.
9 Willow fine root turnover in Saskatchewan ranged from 0.9 to 1.1 yr⁻¹ (Stadnyk, 2010) and were
10 similar to values found for a northern hardwood forest, 0.7 to 2.0 yr⁻¹ (Burke and Raynal, 1994),
11 and for a mixed stand, 0.45 to 2.19 yr⁻¹ (Nadelhoffer et al., 1985). However, fine root turnover
12 values from another willow SRC system studied in Sweden by Rytter and Rytter (1998) were
13 much higher, 4.9 to 5.8 yr⁻¹. These higher values were suggested by the authors to be due to
14 reduction of water and nutrient restrictions in fast growing plantations (Rytter and Rytter, 1998).

15 Stadnyk (2010) also carried out whole tree extractions to examine the coarse root structure and
16 relative biomass of the entire two-year-old plant within a 1-m radius from the cutting to a depth
17 of approximately 30 cm. On average (across six cultivars), total root biomass ranged from 0.5 to
18 1.0 Mg ha⁻¹, with lateral root systems reaching up to 128 cm from the cutting (Stadnyk, 2010).
19 Similar root biomass data were reported from an irrigated willow potted trial in Quebec (0.6 Mg
20 ha⁻¹) (Guidi and Labrecque, 2010). Willow biomass crop root systems that were excavated to a
21 depth of 45 cm across a chronosequence ranging from 5 to 19 years old showed that standing
22 below-ground biomass, including coarse and fine roots and below-ground stool, was about 14.1
23 Mg ha⁻¹ at age 5 and reached 31.4 Mg ha⁻¹ at age 14 and remained unchanged at age 19 (Pacaldo

1 et al., 2013). The root: shoot ratio for all cultivars studied by Stadnyk (2010) ranged from 0.09 to
2 0.51, which was similar to values found in a hybrid poplar system in India (0.12 to 0.31) (Swamy
3 et al., 2006), and was similar to values in an irrigated willow potted trial (0.54) (Guidi and
4 Labrecque, 2010).

5 The contribution of the roots to the soil CO₂ efflux is proportional to fine root production (Norby
6 et al., 2002). As the roots senesce, the less recalcitrant portions decompose and are incorporated
7 into soil organic matter, the rates of which are dependent on factors such as climate, edaphic
8 conditions and fine root diameter (Puttsepp, 2004). It is well understood that SRC systems in the
9 initial years following establishment sequester less carbon if the mineralization rate is initially
10 high, or even experience C loss due to tillage and site preparation practices (Ens et al., 2013b;
11 Girouard et al., 1999; Hansen, 1993). One growing season after coppicing, the estimated fine
12 root C content of willow in Saskatchewan ranged from 0.1 to 0.4 Mg C ha⁻¹ (Stadnyk, 2010)
13 which were much smaller than those found by Zan et al. (2001), 2.3 Mg C ha⁻¹, in a 4-yr-old
14 willow SRC system in southwestern Quebec. In Ontario, Canada, Cardinael et al. (2012)
15 reported increased below-ground C pools (1.5 Mg C ha⁻¹, relative to a control, 1.3 Mg C ha⁻¹) in
16 an agroforestry, tree-intercropping willow production system which promoted significant
17 increase (from baseline levels) of soil organic carbon by 48 % (during the first willow rotation)
18 relative to a conventional willow SRC system where soil organic carbon increased by 27 %. In
19 Pacaldo et al. (2013) chronosequence study standing fine root biomass ranged from 5.6 Mg ha⁻¹
20 at age five to a 6.7 Mg ha⁻¹ at age 19, and at one site located on different soils in a 14-year-old
21 willow stand, the standing fine root biomass was 9.9 Mg ha⁻¹. The differences in stand age and
22 site conditions among the studies, among other factors, could explain the differences in fine root
23 C stocks.

1 The present understanding about root distribution in the soil profile is that the majority of willow
2 roots grow close to the surface and substantially decrease with soil depth (Hendrick and
3 Pregitzer, 1996; Kummerow et al., 1990). However, findings from other studies diverge from
4 this current understanding indicating an increase, then a decrease in root density with depth
5 (Liedgens and Richner, 2001; Nicoullaud et al., 1994). This pattern was also observed in the first
6 growing season (post-coppice) for the willow cultivars Canastota (*Salix sachalinensis* x
7 *miyabeana*, cultivar ID 9970-036) and Sherburne (*S. sachalinensis* x *miyabeana*, cultivar ID
8 9871-31), and in the second growing season (post-coppice) for willow cultivars Canastota,
9 Sherburne, Fish Creek (*S. purpurea*, cultivar ID 9882-34), and Allegany (*S. purpurea*, cultivar
10 ID 99239-015)) in Saskatchewan (Stadnyk, 2010). Stadnyk (2010) reported a significant increase
11 of fine root biomass at all depths from 2008 to 2009, although there was no clear relationship
12 between fine root biomass and soil depth for any of the cultivars; the highest fine root biomass
13 was observed at 10-20 cm depth averaging 2.6 Mg ha⁻¹ in the first growing season.

14 Fine root systems under short rotation willow coppice appeared to be more responsive to edaphic
15 controls on the plant system than to inherent biological characteristics of the species (Stadnyk,
16 2010). In assessing the influence of soil type on early root growth habits of willow, it was
17 observed that the sandy soils provided better growth environments for willow roots than soils of
18 high clay content. In areas of insufficient moisture, such as the semi-arid prairie conditions, roots
19 were likely to extend laterally and vertically to locate sources of water (Stadnyk, 2010).

20 However, dry, fine textured soils provided mechanical restrictions to root growth, which was not
21 a concern in coarse textured soils. It is suspected that the lack of soil moisture at some sites had a
22 substantial effect on willow fine root growth, but further studies are needed in this regard
23 (Stadnyk, 2010). This is particularly true in prairie landscapes where a shallow groundwater

1 table can supply adequate amounts of moisture for growth, which can compensate for low
2 precipitation and a low soil water-holding capacity.

3

4 **5. Comprehensive nutrient budget of willow SRC systems**

5 Willows have the natural ability to cycle available soil nutrients internally and externally aiding
6 their long-term growth (Ericsson, 1994; Hangs et al., 2013). First, willows have the ability to
7 store temporarily in their perennial woody components (i.e., coarse roots, above- and below-
8 ground stool) large amounts of nutrients (Ericsson, 1994) in the form of N compounds, starch,
9 sugars, fats and hemicellulose (Bollmark et al., 1999). This internal cycling of nutrients within
10 high-yielding willow plantations was recognized as a natural means to limit nutrient export
11 offsite and to decrease fertilizer requirements, thus reducing the overall production costs of the
12 system (Ericsson, 1994). A second important nutrient cycle that has been noted in willow SRC
13 systems occurs externally at the leaf litter-soil interface. Approximately one-third of the total
14 nutrient demand could be met by mineralization of leaf litter in established bioenergy plantations
15 (Ericsson, 1994). The nutrient supply released from leaf litter was found to depend on biotic and
16 abiotic factors, such as pH, temperature, soil moisture, N:lignin ratio and microbial activity
17 (Ericsson, 1994). Bollmark et al. (1999) observed that the amount of N lost from senescing
18 leaves directly corresponded to increase of N in perennial organs, such as roots and shoots, thus
19 helping close the nutrient cycling loop once the leaves decompose and the nutrients become plant
20 available.

21 Nutrient budget studies in willow SRC systems are available in the literature (Alriksson, 1997;
22 Ericsson, 1984; Hytönen, 1996); however, there was no work available that studied

1 comprehensively the nutrient budget of willow SRC systems that included nutrient inputs,
2 outputs, and transfers. Therefore, in order to develop reliable nutrient budgets for SRC willow
3 production in Saskatchewan, trials of several willow cultivars were established at different sites
4 across a 500 km north-south pedo-climatic gradient (Hangs, 2013). Hangs (2013) quantified
5 comprehensively the nutrient budget of nitrogen (N), phosphorus (P), potassium (K), sulphur (S),
6 calcium (Ca), and magnesium (Mg) for the first 3-yr rotation of coppiced willow SRC systems
7 (i.e. initial 4-yr period). The above- and below-ground nutrient pools that were accounted for by
8 Hangs (2013) included: nutrient flux into the system (e.g., atmospheric deposition and soil
9 mineral weathering), out of the system (e.g., coppiced and harvested biomass, and leaching) and
10 nutrient transfers within the soil-willow system, which are neither input nor output into/from the
11 system (Fig. 1, 2, and 3). For the purposes of the current paper, the transfer pools were further
12 divided into two general groups in regard to expected time of nutrient release from these pools
13 back into the soil-willow system: (i) ephemeral transfer pools that include canopy exchange and
14 soil organic matter mineralization, and (ii) perennial transfer pools that include leaf litter, dead
15 fine roots, stool, standing fine and coarse root biomass, and leaf biomass (Fig. 1, 2, and 3).

16 Across all study sites, the average initial pre-planting soil extractable cumulative nutrient pool
17 was 25,145 Kg ha⁻¹, with the lowest stocks represented by N (<1%; 80 Kg ha⁻¹) and P (<1%; 66
18 Kg ha⁻¹), followed by S (2%, 579 Kg ha⁻¹), K (5%, 1,322 Kg ha⁻¹), Mg (18%, 4,574 Kg ha⁻¹) and
19 dominated by Ca (74%, 18,524 Kg ha⁻¹) (Fig. 1, 2, and 3), which is typical of the calcareous
20 prairie soils within western Canada (Hangs, 2013). On average, approximately 1.6% of initial
21 cumulative soil nutrients, equal to 393 kg ha⁻¹, were removed from the willow SRC system
22 during the initial four years via harvesting (266 kg ha⁻¹), leaching (105 kg ha⁻¹), and coppicing
23 (22 kg ha⁻¹) events (Fig. 1, 2, and 3). Nutrient removal ranged from 1% (Ca, and Mg) to 91% (N)

1 relative to initial levels of the individual soil nutrients (Fig. 1, 2, and 3). However, this nutrient
2 removal off-site, at the whole soil-willow system scale, was countered in full, partially by
3 nutrient release from ephemeral nutrient transfer pools ($=309 \text{ Kg ha}^{-1}$ or 1.2% relative to initial
4 cumulative levels) mainly via organic matter mineralization (292 kg ha^{-1}), and partially by
5 nutrients added into the system from input sources, i.e., atmospheric deposition and soil mineral
6 weathering ($=218 \text{ Kg ha}^{-1}$ or 0.9% relative to initial cumulative levels) (Fig. 1, 2, and 3). Nutrient
7 release within the soil-willow system from ephemeral transfer pools ranged from 0% (Ca, Mg) to
8 228% (N), and nutrient additions from input sources ranged from $<1\%$ (Ca, Mg) to 28% (N)
9 relative to initial levels of individual soil nutrients (Fig. 1, 2, and 3).

10

11 [Insert Fig. 1 here]

12 [Insert Fig. 2 here]

13 [Insert Fig. 3 here]

14

15 As a result, the average net nutrient levels for the initial 4-yr nutrient budget of the whole soil-
16 willow system in Saskatchewan (to be clearly distinguished from plant available nutrient budget)
17 was a surplus of 0.5% relative to initial cumulative soil nutrient levels. This cumulative nutrient
18 budget change accounted for six nutrients presented in Fig. 1, 2, and 3 and was estimated using
19 Eq. 1. as follows: $0.5\% = 100 * (218 + 309 - 393 \text{ Kg ha}^{-1}) / 25,145 \text{ Kg ha}^{-1}$. Calculating a whole
20 soil-willow system nutrient budget separately for individual nutrients revealed that half were in
21 surplus (165% for N, $<1\%$ for K, 19.2% for S) and half were in negligible deficit ($<1\%$ for P, Ca,

1 Mg) at the end of the 4-yr period (Fig. 1, 2, and 3). Individual nutrient budget change was also
 2 estimated using Eq. 1. For example, N nutrient budget change was estimated as $165\% = 100 * (23$
 3 $+ 181 - 72 \text{ Kg ha}^{-1}) / 80 \text{ Kg ha}^{-1}$ (Fig. 1, 2, and 3)

$$4$$

$$5 \quad \text{Nutrient budget change (\%)} = \frac{\sum_{i=1}^n (\text{Input}_i + \text{Ephemeral}_i - \text{Output}_i)}{\sum_{i=1}^n (\text{Initial}_i)} * 100 \quad \text{Eq.1}$$

6

7 Where i (1 to n) = soil nutrient; $n = 6$ (N, P, K, S, Ca, and Mg) for the estimation of cumulative
 8 nutrient budget change (%), and $n = 1$ (i.e., individual nutrient) for the estimation of nutrient
 9 budget change (%) of an individual nutrient; Input_i = additions of nutrient i into the soil-willow
 10 system via atmospheric deposition and soil mineral weathering; Ephemeral_i = release of nutrient
 11 i into the soil-willow system from ephemeral transfer pools (canopy exchange and organic matter
 12 mineralization); Output_i = removal of nutrient i from the soil-willow system via harvesting,
 13 leaching or coppicing; Initial_i = pre-planting initial stocks of nutrient i within the soil-willow
 14 system.

15 Approximately 2.4% relative to initial cumulative nutrient levels, equal to 611 Kg ha^{-1} , were
 16 taken up by willow plants and stored in the form of foliage, stool, fine and coarse root biomass
 17 during the initial 4-yr period of willow SRC systems in Saskatchewan (i.e. perennial transfer
 18 pools; Fig. 1, 2, and 3). The nutrients in these pools would persist from one rotation to the next
 19 and remain onsite for a total of seven 3-yr rotations and, therefore, for the purposes of this paper,
 20 they were considered neither nutrient input nor output into/from the whole soil-willow system
 21 (Fig. 1, 2, and 3). The nutrient budget in our present paper focused solely on the short-term (4-

1 yr) initial period of willow SRC establishment and accounted for all processes in regard to
2 nutrient input, output and short-term transfer (ephemeral pools within the system) (Fig. 1, 2, and
3 3). The nutrient storage and release into/from perennial transfer pools were not included in the
4 estimation of percent nutrient budget flux for the whole soil-willow system as these pools largely
5 influence the long-term nutrient availability on the site, which was beyond the scope of the
6 present paper.

7 Hangs (2013) presented alternative agronomic nutrient budget estimation in willow SRC systems
8 that calculated net changes in plant available nutrient levels, which are more conducive to
9 fertility management practices suited to farmers, as opposed to the whole soil-willow system we
10 employed. In the nutrient budget estimation Hangs (2013) treated foliage, stool, fine and coarse
11 root biomass pools as nutrient outputs from the plant available nutrient stocks, while they were
12 assigned in perennial transfer pools within the soil-willow system in our present paper and were
13 not included in the nutrient budget change (Eq.1). Due to this different approach, Hangs (2013)
14 estimated negative change in plant available N (-24%), K (-9%), Ca (-2%) and Mg (-2%) levels
15 and positive change in plant available P (105%) and S (17%) levels after an initial four year
16 period relative to initial soil nutrient levels.

17 A similar study which we have done also showed negative plant available N nutrient change and
18 significant nutrient loss during the initial establishment years for willow grown on former
19 agricultural fields in central Saskatchewan, Canada. The three willow (*S. miyabeana* Seemen
20 (SX64)) plantations (Harris and Saskatoon 1 and 2) were intercropped with caragana (*Caragana*
21 *arborescens*) at different levels of intensity [i.e. monoculture of willow (W), monoculture of
22 caragana (C), mixing with a 2:1 willow:caragana ratio (2W:1C), mixing with a 2:1
23 willow:caragana ratio with irrigation (2W:1W-irr), and mixing with a 1:1 willow:caragana ratio

1 (1W:1C)]. Plantations were established (late May) in a randomized complete block design using
2 25 cm willow cuttings and 80 cm caragana whips (see Moukouri et al. (2012) for a full
3 description of the sites and experimental design). In order to limit soil disturbance, weed control
4 was done by hand every two weeks from May to September 2007 and 2008. Three soil samples
5 per plot were collected at the end of the first and second growing seasons (late September to
6 early October 2007 and 2008) at a depth of 0–10 cm. The samples were air-dried at room
7 temperature and then aggregates were gently broken down with a rolling pin to pass through a 2-
8 mm mesh. Soil mineral N, i.e. NO_3^- and NH_4^+ , was extracted with a 2 N KCl solution (Maynard
9 et al., 2007). Nitrate and NH_4^+ concentrations in the extracts were measured colorimetrically
10 with a Technicon Autoanalyzer.

11 Soil available N was mainly in the NO_3^- form and mineral N levels were higher for the Harris
12 and Saskatoon 1 site compared to Saskatoon 2 and generally declined between the end of the first
13 and second growing seasons (Table 2). The decrease was statistically significantly at all sites
14 when all treatments were combined; however, for willow monoculture plots, the only significant
15 decrease was at Saskatoon 1 where the dry matter production was the highest (Moukouri et al.,
16 2012). Willow stem height and diameter, crown dimension and dry matter production declined
17 from the N-rich to the N-poor site as follows: Saskatoon 1>Harris>Saskatoon 2. The
18 proportional decrease in soil available N was reversed: Saskatoon 2>Harris>Saskatoon 1, which
19 suggests that the potential for soil mineral N depletion is greater on more productive soils with
20 greater initial N availability. Similarly, Ens et al. (2013b) also reported an average soil [NO_3^- +
21 NH_4^+]-N reduction of 3.1 and 2.6 mg kg^{-1} for 0-20 cm and 20-40 cm soil depths, respectively, in
22 the initial three year period of nine short-rotation willow trial sites of *S. purpurea* (“Hotel”
23 cultivar) established on a variety of soil types across Canada. The sites that responded the most,

1 at least for NO_3^- at the 0-20 cm depth, were also sites which appeared to have higher initial soil
2 available N and biomass yields. This general pattern of N impoverishment indicates that the rate
3 of uptake of N by willow exceeds the maximum rate of N mineralization. Unlike the two
4 previous studies, however, Ens et al. (2013b) estimated soil nutrient change using a retrospective
5 analysis approach by sampling the soil from adjacent reference plots to use as a surrogate source
6 for the expected initial pre-planting soil nutrient levels at the willow trial sites. Also, in
7 comparison to Hangs (2013), the willow stands studied in Ens et al. (2013b) were not coppiced
8 in year 1.

9 Nutrient uptake by willow plants is partially countered by nutrient release from decomposing
10 litter components within the whole soil-willow system. The average input to plant available soil
11 nutrient pool from decomposing leaf and fine root litter during the initial rotation in
12 Saskatchewan was 71, 16, 66, 22, 127 and 30 kg ha^{-1} of N, P, K, S, Ca and Mg, respectively (Fig.
13 1, 2, and 3) (Hangs, 2013). The majority of the N (70 %), P (76 %), and S (53 %) contribution
14 came from fine root turnover, while leaf litter supplied the majority of K (73 %), Ca (88 %), and
15 Mg (61 %) (Hangs, 2013). Willow leaves in SRC systems in Saskatchewan, accounting for less
16 than a third of the above-ground biomass, were the largest above-ground sink of soil nutrients,
17 containing about 69, 63, 72, 72, 64, and 69 % of the total amount of N, P, K, S, Ca and Mg,
18 respectively, stored in above-ground biomass (Hangs, 2013). These findings supported the
19 current understanding that, in the long-term, nutrient cycling from leaf litter decomposition and
20 fine root turnover are very important natural mechanisms that help to satisfy the nutritional
21 demands of SRC willow plantations (Christersson, 1986; Ericsson, 1994; Ingestad and Ågren,
22 1984; Rytter, 2001).

23

1 [Insert Table 2 here]

2

3 Another long-term nutrient storage pool in willow SRC systems is the below-ground plant
4 material. The nutrient status estimates of willow stool, fine and coarse root tissues in SRC
5 systems in Saskatchewan (Hangs, 2013) were within the range of values reported in the literature
6 (Pacaldo et al., 2011; Puttsepp et al., 2007; Rytter, 2012; Zan et al., 2001). The majority of N, P,
7 and S were stored in the below-ground biomass, while the majority of K, Ca, and Mg were in the
8 above-ground biomass, primarily the leaf litter (Fig. 1, 2, and 3) (Hangs, 2013). The bulk of both
9 biomass (84 %) and nutrient content (95 %) dedicated to below-ground tissue were associated
10 with fine roots (Hangs, 2013). Even in older willow biomass crops the standing fine root biomass
11 made up 47 to 67% of the total below-ground biomass (Pacaldo et al., 2013), which highlights
12 the relative importance of the fine root fraction in SRC willow production systems (Rytter,
13 2012).

14 In general, willow can be successfully grown with much less soil nutrient uptake relative to other
15 biomass energy crops (e.g. miscanthus (*Miscanthus x giganteus*), switchgrass, etc.) (Boehmel et
16 al., 2008; Kering et al., 2012; Weih et al., 2011). The annual nutrient removals via harvestable
17 biomass in the first 3-yr rotation of coppiced willow SRC systems of six willow cultivars were
18 13 to 20, 2 to 3, 10 to 15, 2 to 3, 23 to 43, and 3 to 6 Kg ha⁻¹ yr⁻¹ for N, P, K, S, Ca, and Mg,
19 respectively (Hangs, 2013). Although the soil nutrient demands differ among willow cultivars
20 (Tharakan et al., 2005; Weih and Nordh, 2002), an obvious reason for the better nutrient use
21 efficiency of willow SRC systems could be the fact that willow foliage, and the nutrients
22 contained in senescent leaves, stayed on site and were re-used by the willow. Unlike the

1 removals of all above-ground biomass in perennial herbaceous bioenergy plantations such as
2 miscanthus and switchgrass, the SRC willow plantations cycled >7 Mg of leaf litter biomass
3 prior to harvest (Hangs, 2013), which would influence long-term nutrient cycling and increase
4 soil organic matter levels (Lal, 2009). This capacity of leaf litter N cycling in willow SRC
5 systems has been recognized previously in regard to meeting nutrient demands in subsequent
6 rotations (Christersson, 1986; Ericsson, 1994). However, despite the efficient internal and
7 external nutrient cycle within willow SRC systems, fertilizer application was recommended to
8 compensate for long-term nutrient losses from harvesting willow stems (Abrahamson et al.,
9 2002; Ericsson, 1994), which would equate to approximately 25 kg N ha⁻¹ yr⁻¹ with current
10 production levels observed in Saskatchewan (Hangs, 2013).

11

12 **6. Nutrient amendments for willow SRC systems**

13 Producing and maintaining yields in willow SRC systems requires an adequate supply of
14 nutrients (Adegbidi et al., 2003). Similar to the observations by Ens et al. (2013b), Kowalik and
15 Randerson (1994) reported that the major limiting factor to biomass production in willow SRC
16 systems was N availability; therefore, fertilization was recommended to maintain growth rates
17 over many rotations (Lemus and Lal, 2005). Despite the extensive research on fertilizer use in
18 SRC systems, the optimal time to apply fertilizer is still unknown for SRC systems in North
19 America. Previous research has examined the effects of fertilizer application after the first three-
20 year harvest cycle (Christersson, 1987), for four consecutive years following planting (Alriksson
21 et al., 1997), annually for up to nine years following planting (Adegbidi et al., 2001), prior to
22 planting and six years following planting (Gruenewald et al., 2007), and most commonly in the

1 growing season following coppicing, which is the second growing season (Adegbidi and Briggs,
2 2003; Adegbidi et al., 2003; Ballard et al., 2000b; Hytönen and Kaunisto, 1999).

3 A recent study conducted under Canadian conditions studied N fertilization recovery and the
4 effects of fertilization in the year of planting for willow SRC systems (Konecsni, 2010).

5 Konecsni (2010) examined the effects of first year N fertilization on biomass production of five
6 willow cultivars in two different ecozones in Saskatchewan. Differences in soil type at the two
7 sites resulted in more significant differences in willow yields compared to yield response to
8 fertilizer treatments (Konecsni, 2010). For example, tree heights, soil NO₃-N and foliar-P were
9 all found to be significantly greater on one of the sites while the number of shoots per tree,
10 biomass yield, soil PO₄⁻ and foliar-N and N:P ratios were significantly greater on the other site
11 (Konecsni, 2010). The single application of N fertilizer in the first year did not have any positive
12 or negative effects on willow growth in these SRC systems; biomass was 0.6 to 1.2 and 0.7 to 0.9
13 Mg ha⁻¹ for fertilized and control plots, respectively (Konecsni, 2010). Similar lack of willow
14 yield response to N additions or organic amendments were also reported by Quaye and Volk
15 (2013). However, there were single isolated cases where foliar N and shoot diameter differed
16 significantly between fertilizer treatments (Konecsni, 2010). The majority of N recovered by the
17 willow was accumulated in the leaf components, although such recovery was very low among all
18 cultivars (Konecsni, 2010). Only a negligible fraction of the applied N (0.3 to 4.8%) was
19 recovered by the foliar tissue and this poor fertilizer recovery may explain the lack of fertilizer
20 response in their study (Konecsni, 2010). Booth (2008) reported similar findings in northern
21 Saskatchewan, where recovery of N fertilization in the year of planting was 0.8 to 2.5% by
22 poplar (*Populus spp.*) leaves.

1 The findings by Konecni (2010) suggested that in the year of planting, willow SRC system
2 management should be directed to ensure high plant survival, such as applying weed control,
3 while in the second year after planting, the focus could be shifted to fertilizer application to
4 ensure optimal willow growth of the post-coppiced willow plants with well-developed root
5 systems. For example, fertilizers applied in the year after coppicing increased biomass
6 production of willow SRC systems by 8 to 134 %, 7 to 75 % and 9 to 39 % over control
7 treatments in years one, two, and three, respectively (Adegbidi et al., 2003). Although the ability
8 of irrigation to increase willow biomass yield in semi-arid Saskatchewan was expected, Hangs et
9 al. (2012b) reported that irrigation also increased fertilizer use efficiency. In other studies
10 elsewhere, fertilizer applications, whether using organic or inorganic fertilizers, have also been
11 reported to significantly increase the biomass production of bioenergy SRC systems (Adegbidi et
12 al., 2001; Arevalo et al., 2005; Ballard et al., 2000b; Christersson, 2006; Ferm et al., 1989;
13 Gruenewald et al., 2007; Hytönen and Kaunisto, 1999). Most recently, studies in the U.S.A.
14 (Quaye and Volk, 2013) and Canada (Cardinael et al., 2012) for first rotation willow production
15 showed that, depending on the nutrient status of the sites, willow biomass can be produced
16 without fertilizer additions, most likely due to the high internal nutrient cycling in willow SRC
17 systems.

18 There are a great number of fertilizers available for use in willow SRC systems. Types of
19 fertilizers that have been already studied included green manure (Arevalo et al., 2005),
20 anaerobically digested sewage sludge, composted poultry manure, composted sewage sludge
21 (Adegbidi and Briggs, 2003), waste water and landfill leachates (Hasselgren, 1998), slow-release
22 inorganic fertilizers (Adegbidi et al., 2003), and ash by-products (Hytönen and Kaunisto, 1999).
23 Slow-release fertilizers can both minimize leaching losses and maximize fertilizer effects, but

1 they are often quite expensive (Adegbidi et al., 2003) and immense amounts of energy were
2 consumed in their production accounting for 20-30 % of total bioenergy production costs
3 (Hasselgren, 1998). Fertilization with biosolids, such as sewage sludge and animal manure, was
4 favourable with SRC systems because it was a non-food crop, which decreased the risk of
5 disease transmission to humans (Keoleian and Volk, 2005). Biosolids were also an attractive
6 fertilizer option because they were energy-efficient, contained P and K that could also be utilized
7 by willow, and were much more cost-efficient relative to synthetic fertilizers (Heller et al.,
8 2003). However, the application of biosolids as fertilizers could also raise environmental
9 concerns because excess application of biosolids, exceeding the willow's uptake capacity for N
10 and P, could lead to contamination of surface and groundwater (Adegbidi and Briggs, 2003). Ash
11 by-product from the burning of biomass during bioenergy conversion (through gasification) had
12 also been used as a soil amendment to supply nutrients (Hytönen and Kaunisto, 1999). The use
13 of ash by-products as a soil amendment could provide a unique opportunity to create a nutrient
14 circling loop in which a portion of the soil nutrients transported offsite in the woody components
15 of willow SRC systems are recycled to the soil to foster future willow growth.

16

17 **7. Concluding remarks**

18 Willow SRC systems are becoming an attractive practice because they are a sustainable system
19 fulfilling multiple ecological objectives with significant environmental benefits (Rockwood et
20 al., 2004). Willow plantations can remain productive for approximately 20 to 30 years
21 (Abrahamson et al., 1998; Heller et al., 2003) with removal of biomass occurring approximately
22 seven times in 3- to 4-yr rotations (Keoleian and Volk, 2005). Willow SRC have the capability to

1 increase site (Keoleian and Volk, 2005) and soil quality (Lemus and Lal, 2005) when planted on
2 agricultural land. When compared to annual cropping systems, SRC can increase soil porosity,
3 infiltration, preferential flow and hydraulic conductivity in clayey soils (Mele et al., 2003). In
4 order to satisfy plant nutritional requirements, the deep perennial rooting systems of woody
5 species are capable of absorbing cations and other trace elements that are inaccessible to shallow
6 annual root systems. The absorbed nutrients are returned to the soil surface through litterfall,
7 thereby enhancing nutrient cycling of the site (Mele et al., 2003).

8 Willow SRC systems can provide many benefits to farmers, the most obvious being additional
9 cash flow from bioenergy feedstock production, especially when grown on marginal land
10 unsuitable for annual food crop production. However, a more comprehensive assessment of the
11 value of willow SRC systems should take into consideration non-monetary benefits as well, such
12 as increased biodiversity, C sequestration, quality of seepage water and aesthetic values
13 (Gruenewald et al., 2007). For example, it is well-established that willow SRC systems can play
14 a significant role in reducing atmospheric CO₂ levels by sequestering C for long-term storage in
15 their extensive root systems (Sanchez et al., 2007; Smith, 1995), as well as producing feedstock
16 as fossil fuel substitutes for energy production (Amichev et al., 2012).

17 Despite the decades-long study of willow SRC systems in Europe and North America, there are
18 still research gaps that must be addressed. Comprehensive life cycle assessment of willow SRC
19 systems has not been completed in Canada, especially within the semi-arid temperate regions,
20 yet they are crucially important in understanding the environmental and socio-economic benefits
21 of willow crop establishment. Furthermore, with the relatively new establishment of willow
22 plantations in Saskatchewan, obtaining accurate below-ground C sequestration values is
23 important in the development and validation of C budget models for SRC systems comprised of

1 different willow cultivars. Specifically, fine roots are often a focal point of below-ground C
2 sequestration as they represent approximately 60% of total willow root C (Grigal and Berguson,
3 1997; Hangs et al., 2012a; Zan et al., 2001). Additionally, maintaining currently established
4 willow trials in Saskatchewan should become a priority, as these sites would continue providing
5 necessary data in regard to expected willow yields, nutrient budget flux, C flux, and overall site
6 sustainability in each consecutive rotation.

7 Establishing willow SRC systems in Saskatchewan for bioenergy feedstock production is
8 advantageous for renewable energy considerations, and a fundamental question that has to be
9 answered is the sustainability of willow production over multiple rotations. Based on early
10 results from an initial four-year rotation in Saskatchewan (Hangs, 2013), it was found that
11 willow SRC systems were relatively low nutrient-demanding, with minimal nutrient output other
12 than in harvested biomass. However, even with very efficient nutrient cycling in the initial 4-yr
13 period, we would expect future deficits of plant available soil nutrients to occur, in particular N
14 and P, without nutrient amendments. Over the course of multiple rotations, continuing nutrient
15 uptake within tree biomass and repeated harvesting will likely necessitate fertilization to ensure
16 sufficient site nutrient supply. Nutrient amendments in willow SRC systems would maintain
17 long-term soil fertility, thus helping advance purpose-grown willow biomass energy crops as a
18 viable alternative in Canada's bioenergy sector.

19

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4

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- 8

1 **Figure captions**

2 Fig. 1 Mean (N=96) nitrogen (N) and phosphorus (P) whole soil-willow system budget (kg ha⁻¹)
3 for the initial 4-yr establishment period, including the coppice year and first 3-yr rotation, of
4 willow SRC plantations of several willow cultivars established at different locations across
5 Saskatchewan, Canada.

6 Fig. 2 Mean (N=96) potassium (K) and sulphur (S) whole soil-willow system budget (kg ha⁻¹)
7 for the initial 4-yr establishment period, including the coppice year and first 3-yr rotation, of
8 willow SRC plantations of several willow cultivars established at different locations across
9 Saskatchewan, Canada.

10 Fig. 3 Mean (N=96) calcium (Ca) and magnesium (Mg) whole soil-willow system budget (kg ha⁻¹)
11 for the initial 4-yr establishment period, including the coppice year and first 3-yr rotation, of
12 willow SRC plantations of several willow cultivars established at different locations across
13 Saskatchewan, Canada.

14

15

1 **Tables**

2

- 1 Table 1 A list of references, ordered by province in Canada and state in the U.S.A., with findings and data from research work in
 2 willow SRC systems that are summarized in the current manuscript.

State or Province, Country	Reference	State or Province, Country	Reference
Alberta, Canada	Ens et al. (2013a; 2013b) Corredor et al. (2012)	Delaware; Maryland; Minnesota U.S.A.	Caputo et al. (2013) Kiernan et al. (2003) Volk et al. (2006; 2011)
Saskatchewan, Canada	Amichev et al. (2011; 2012) Ens et al. (2013a; 2013b) Corredor et al. (2012; 2014) Hangs (2013) Hangs et al. (2011; 2012a; 2012b; 2013) Konecsni (2010) Moukoumi et al. (2012) Stadnyk (2010)	New York, U.S.A.	Abrahamson et al. (1998; 2002) Adegbidi and Briggs (2003) Adegbidi et al. (2001; 2003) Arevalo et al. (2005) Ballard et al. (2000a; 2000b) Caputo et al. (2013) Heller et al. (2003; 2004) Keoleian and Volk (2005) Kiernan et al. 2003 Kopp et al. (2001) Pacaldo et al. (2011; 2013) Quaye and Volk (2013) Quaye et al. (2011) Volk et al. (2001; 2006; 2009; 2011)
Manitoba, Canada	Ens et al. (2013a; 2013b)		
Ontario, Canada	Cardinael et al. (2012) Clinch et al. (2009) Ens et al. (2013a; 2013b)		
Quebec, Canada	Girouard et al. (1999) Guidi and Labrecque (2010) Labrecque and Teodorescu (2005)	Pennsylvania, U.S.A.	Kiernan et al. (2003) Volk et al. (2006)

	Vujanovic and Labrecque (2002)		
	Zan et al. (2001)	Vermont, U.S.A.	Caputo et al. (2013)
Connecticut; Wisconsin U.S.A.	Caputo et al. (2013)		Kiernan et al. (2003)
	Volk et al. (2006; 2011)		Quaye and Volk (2013)
			Volk et al. (2006; 2011)
Michigan, U.S.A.	Volk et al. (2006)		

1

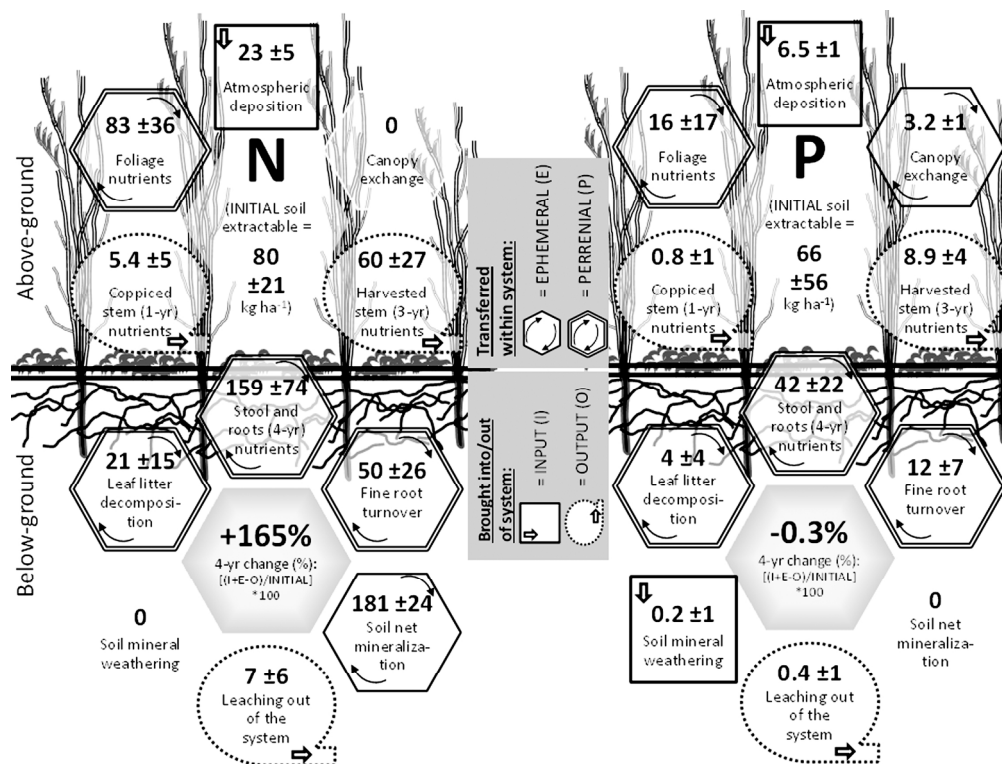
1 Table 2 Soil total N and available mineral N between the end of the first and second growing
 2 seasons in Saskatchewan, Canada.

Site	Type of analysis	† NO ₃ -N (mg kg ⁻¹)		[NO ₃ ⁻ + NH ₄ ⁺]-N (mg kg ⁻¹)	
		End of 1st season	End of 2nd season	End of 1st season	End of 2nd season
Harris	‡ All treatments combined	27.9a (2.72)	8.08b (2.71)	30.8a (2.71)	13.6b (0.92)
	‡ Willow monocultures only	23.5 (6.08)	7.09 (1.04)	27.7 (6.77)	11.6 (2.12)
Saskatoon 1	All treatments combined	34.0a (2.42)	4.27b (0.28)	40.2a (2.54)	4.55b (0.32)
	Willow monocultures only	38.5a (2.20)	4.48b (0.12)	43.7a (2.16)	4.51b (0.13)
Saskatoon 2	All treatments combined	7.53a (0.79)	3.27b (0.52)	10.1a (0.82)	3.49b (0.53)
	Willow monocultures only	5.81 (1.85)	7.37 (3.47)	9.23 (1.89)	8.16 (3.08)

3 † Values in parentheses indicate standard error.

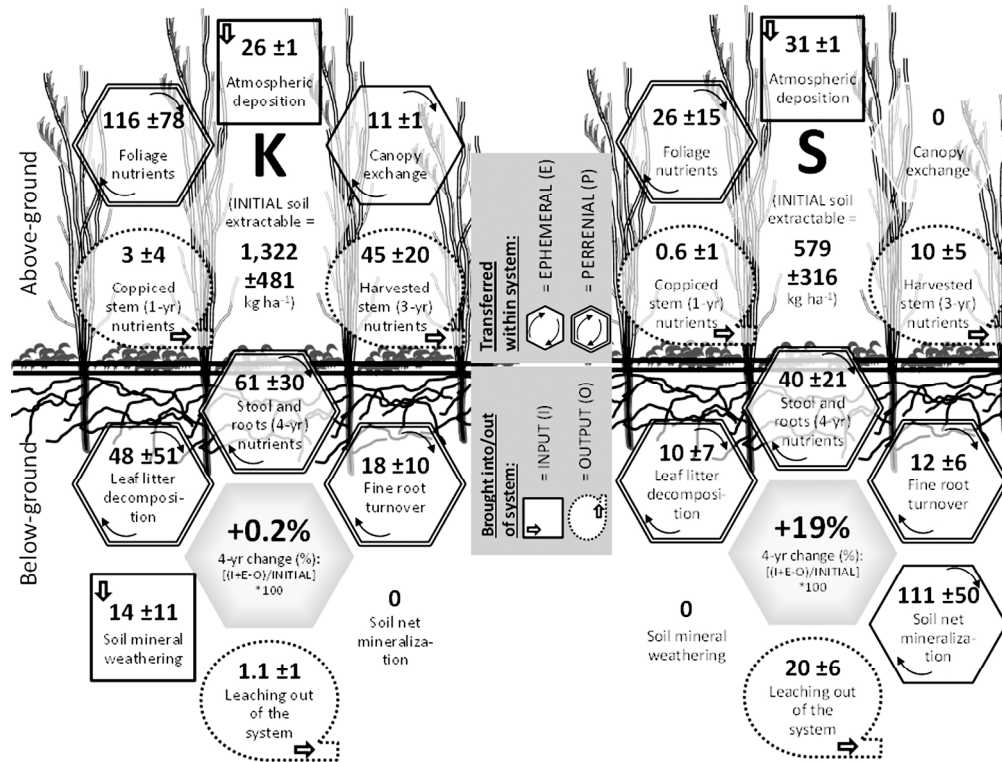
4 ‡ A significant difference between the end of the first and second growing seasons (within a site)
 5 is indicated by different letters. "All treatments combined" means that all treatment plots were
 6 included in the analysis, whereas "Willow monocultures" means that only the willow
 7 monoculture plots were tested.

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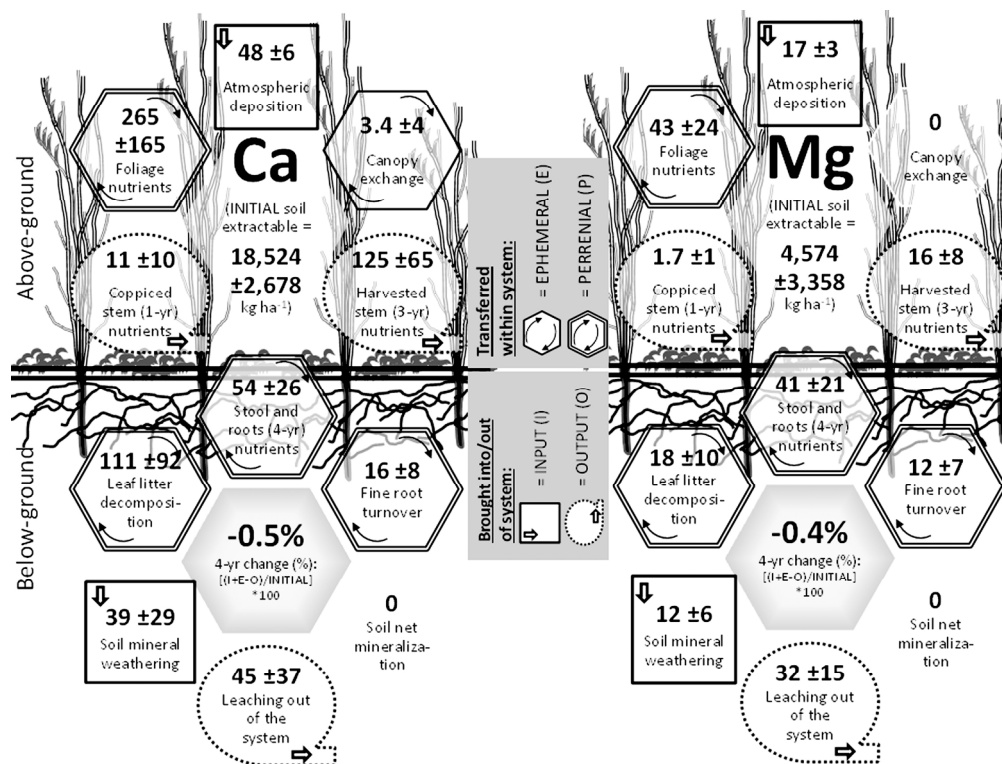


Mean (N=96) nitrogen (N) and phosphorus (P) whole soil-willow system budget (kg ha⁻¹) for the initial 4-yr establishment period, including the coppice year and first 3-yr rotation, of willow SRC plantations of several willow cultivars established at different locations across Saskatchewan, Canada.

192x145mm (300 x 300 DPI)



Mean (N=96) potassium (K) and sulphur (S) whole soil-willow system budget (kg ha⁻¹) for the initial 4-yr establishment period, including the coppice year and first 3-yr rotation, of willow SRC plantations of several willow cultivars established at different locations across Saskatchewan, Canada.
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Mean (N=96) calcium (Ca) and magnesium (Mg) whole soil-willow system budget (kg ha⁻¹) for the initial 4-yr establishment period, including the coppice year and first 3-yr rotation, of willow SRC plantations of several willow cultivars established at different locations across Saskatchewan, Canada.
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