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Intercropping *Caragana arborescens* with *Salix miyabeana* to satisfy nitrogen demand and maximize growth

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Abstract Willow shows great promise as a biomass crop and is now used worldwide. However, willow is a nutrient and water demanding plant that often requires the use of nitrogen (N) fertilizer to maximize growth on poor soils. The intercropping of *Salix miyabeana* with the atmospheric N_2-fixing *Caragana arborescens* on poor soils of the Canadian Prairies could provide a portion of the N demand of the willow. The main objectives were to: (1) determine the yield potential, N nutrition and water use efficiency (WUE) of willow and caragana grown in pure and mixed plantations across a range of soil productivity and (2) assess the extent of atmospheric N_2-fixation by the caragana within the first rotation in central Saskatchewan. We found large differences in willow yields, foliar nitrogen and WUE across the sites. The willow yields (1.24 to 15.6 t dry matter ha^{-1} over 4 years) were low compared to northeastern North American values and reflect the short and dry summers of the region. The yields were positively correlated to foliar N (ranging between 14.3 and 32.4 mg g^{-1}), whereas higher WUE (expressed as $\delta^{13}C$) were not positively correlated to water availability but to higher yields. Caragana N_2-fixation (measured using ^{15}N isotope dilution) was not active at the most productive site but up to 60% of the foliar N was of atmospheric origin at the two other sites. Willow growth increased with caragana proportions at the least productive site, which is typical of the benefits of N_2-fixing plants on the growth of other plants on poor soils. At the most productive site, caragana decreased the growth of willow early on due to competition for resources, but willow eventually shaded caragana to a point of significant canopy decline and dieback. It is therefore more

appropriate to intercrop the two species on less productive soils as caragana is more likely to add N to the system via N_2-fixation and is less likely to be shaded out by willow.

Keywords Willow · caragana · intercropping · growth · foliar N · atmospheric N_2-fixation · water use efficiency

Introduction

As energy demands increase worldwide over the next few decades, the production of woody biomass crops as a renewable energy source is expected to increase as well [17]. Willow (*Salix*) species are commonly used in temperate regions as a short rotation intensive culture (SRIC) for bioenergy, wood products and environmental benefits [1, 9, 43]. Willow clones have been selected and grown in SRIC in northern temperate regions for more than four decades and are well known for their ability to regrow after multiple rotations and produce significant biomass in a short time [26, 37].

The province of Saskatchewan, where this study was conducted, has 20.8 million ha of marginal land (classes 4, 5 and 6 under the Canadian Land Inventory) [46] that could be used for growing SRIC of willow without having to compete for productive agricultural land intended for food production [23]. The potential of SRIC of willow in the Canadian Prairies is therefore enormous but studies on SRIC of willow growing on soils characterized by low water and nutrient availability are lacking. Adegbidi et al. [3] reported annual removals of 75 to 86 kg N ha^{-1} by SRIC of willow grown in New York State. In the Federal Republic of Germany, Jug et al. [24] measured annual exports of 92 to 270 kg N ha^{-1}, whereas Labrecque et al. [25] and Aronsson and Bergström [6] showed potential removal between 100 and 200 kg N ha^{-1} in Quebec and Sweden, respectively. Such large N exports could quickly limit the long-term productivity of these systems, especially those with low nitrogen (N) availability. Willow species

are also water demanding and will likely not perform well in areas where water stress is an issue [63]. Soil productivity could be maintained using N based fertilizers and irrigation but the CO_2 footprint associated with such practice and the increased possibility of N_2O emissions from the soil would considerably depreciate the energy efficiency ratio of the system [10], making it not much more efficient than burning fossil fuels.

The idea of growing willow in the company of a common N_2-fixing shrub used for shelterbelt in the Canadian Prairies, i.e. *Caragana arborescens* Lam. (caragana), has been proposed as a means to fix atmospheric N, augment soil N availability and transfer it to the willow for increased and sustained yields on poor soils. Caragana species are known for their capacity to form root nodules, fix atmospheric N and enhance soil N availability [53]. Several authors have reported the synergistic effect associated with mixed plantations of a N_2-fixing tree/shrub species with a non N_2-fixing tree/shrub species [15]. Nitrogen availability could possibly augment in mixed SRIC and benefit N nutrition of willow and biomass production as a whole in the mid-term, especially for N poor sites, and reduce the CO_2 footprint of the system by lowering the use of N fertilizer for optimum plant growth. Mixed (multiclonal or multispecies) systems (e.g. [5] for willow; [11] for poplars) also could address concerns raised about reduced biodiversity and productivity losses from pest and disease in willow monocultures [61] or other fast growing tree/shrub species. However, the introduction of caragana in SRIC of willow could be a challenge because of its invasive nature [20] and potentially low tolerance to coppicing.

Whether the interactions between willow and caragana favour competition or facilitation for resources (e.g. transfer of atmospherically fixed N by the caragana to the willow, better water use efficiency) is therefore unknown, nor is the long-term operational feasibility of such a system elucidated. To investigate the potential of this association, pure and mixed plantations of willow

and caragana were experimentally established in 2007 at three sites in Saskatchewan with contrasting soil productivity. The main question addressed with this study was: What is the yield potential, nutrition and water use efficiency of willow and caragana grown over a full (4 years) rotation in pure and mixed plantations across a range of soil productivity? A second question addressed was: What is the extent of atmospheric N fixation by the caragana within the first (2 years) rotation?

Material and Methods

Site Description

The study was carried out in three former agricultural fields in central Saskatchewan, Canada; two of them located in the city of Saskatoon (Saskatoon 1 and 2) and a third (Harris) located 90 km south west of Saskatoon. General site characteristics and cultural history are shown in Table 1. The climate of the region is semi-arid with long cold winters and short but warm summers. The soils at Saskatoon 1 and 2 are slightly alkaline with heavy clay and sandy clay loam textures, respectively; the soil at Harris is moderately acidic with a loamy sand texture. Organic C and extractable NO_3^- are higher in Saskatoon 1 than the two other sites, whereas the variation in C:N ratios is small across the sites.

Experimental Design

Plantations were established in May 2007 in a randomized complete block design, using 25 cm willow unrooted cuttings and caragana rooted whips of about 80 cm. Every site had 3 blocks with the following 5 treatments: monoculture of willow (W; *Salix miyabeana* Seemen (SX64)), monoculture of caragana (C; *Caragana arborescens* Lam.), intercropping with a 2:1

willow:caragana ratio (2W:1C) and intercropping with a 1:1 willow:caragana ratio (1W:1C). Sites were first tilled to a depth of approximately 20 cm using a rotary tiller. Cuttings and whips were then inserted in the soil to a depth of about 20 cm with a planting dibble in a double row design with a spacing of 1.5 m between the double rows, 0.75 m between rows, and 0.60 m inter-rows, for a density of 14 818 shrubs per hectare. Each treatment/plot in a block contained 36 shrubs in a 15.75 m^2 plot. The blocks and the plots were each delineated by a double row buffer of willow. See Figure 1 for plot arrangements for the 2W:1C and 1W:1C treatments. The SX64 willow clone has been demonstrated to be productive and hardy in SRIC in northeastern North America [27, 54]. The willow cuttings came from the Woody Biomass Program at the State University of New York, whereas the caragana (Ross) was grown at the Agroforestry Development Center of Agriculture and Agri-food Canada in Indian Head, Saskatchewan. In order to limit soil disturbance, weeds were controlled initially with two applications of glyphosate in May and June of 2007 and then weed control was done by hand every two weeks until September 2007. Weed control was also required in May and June of 2008, but no weed control was required thereafter at any of the sites because of significant shading by the shrubs.

Growth Measurements

The number of surviving shrubs and stem height of all the shrubs in each plot were measured by species at the end of the 2008 growing season. In the fall of 2008, stem diameter at 30 cm from the soil surface was measured on all the shrubs in the plots. The largest canopy diameter [7] was also measured on all the shrubs in the plots. These are small plots and the edge effects after one rotation appear to be marginal. As the plantations and root systems mature, however, it will be important to sample only centre shrubs. For an estimate of above ground

biomass after two growing seasons, 12 shrubs per species per site (i.e. 1 shrub per species per plot) were harvested in September 2008. The leaves were separated from the harvested stems. Both components were dried at 35ºC for 60 days in a drying room, checked for constant weight and then weighed. Small subsamples were then put in an oven for 16 hours at 65ºC to validate that the material was completely dry. The weights were calculated on a g shrub^{-1} basis per species and are later referred to as "individual willow" or "individual caragana" dry matter production. These are reported as leaf, stem and total (leaf+stem) dry matter. The leaf dry weight could be slightly underestimated because of few leaves had fallen. In April of 2011, after four full seasons of growth, the three plantations were harvested by hand, separated by species and weighed in the field for fresh weights at the plot level. Subsamples were then dried as indicated above for 30 days. The difference between fresh and dry weights was used to convert fresh stem biomass of willow and caragana to dry stem biomass. The plot scale dry weights were then converted to ton ha^{-1} and are later referred to as "total" dry matter production. These data were also used to calculate individual species dry matter production as a comparison with the dry matter data after two years.

Soil Sampling, Monitoring and Analyses

Three soil samples per plot were collected at a depth of 0–10 cm both before planting (early May 2007, after site preparation) and at the end of the second growing season (late September to early October 2008). This depth corresponds to where most of the willow and caragana fine roots develop. The samples were air-dried at room temperature and then passed through a 2-mm mesh.

Topsoil water and temperature were measured bimonthly 5 cm below the soil surface at the same three locations inside each plot from June to September 2008 using respectively Time Domain Reflectometry (FieldScoutTDR300, Spectrum® Technologies, Plainfield, Illinois) and a thermocouple thermometer. The three sampling sites were located between four shrubs within the double rows. At one of the locations inside each plot, a time series study of mineral N (total N, NO_3^- and NH_4^+) was also conducted. Plant Root Simulator™ probes (PRS™ probes) (Western Ag Innovations, Saskatoon, Saskatchewan) were buried vertically on May 31st at 10 cm below the soil surface using four pairs of probes per plot to assess NO_3^- and NH_4^+. During the experiment, the PRS™ probes were replaced four times (once every four weeks), which extended the sampling period to mid-September. The probes were washed with deionized water immediately following removal from the soil. The four pairs were pooled by plot, yielding three measurements of total N, NO_3^- and NH_4^+ per treatment per site. Unlike chemical extractions (e.g. KCl extracts also used in this study), which are static indices of nutrient availability at a given point in time, the data obtained from the PRS™ probes provide a proxy for nutrient supply rates as the resins continuously adsorb charged ionic N species over the burial period [40]. For clarity, the mineral N data generated with the PRS™ probes and KCl extracts are hereafter referred to as "soil available N" and "soil extractable N", respectively.

Soil particle size distribution was determined on one sample per plot using the Horiba Partica LA–950 Laser Particle Analyzer after pre-treatment with NaOCl to remove organic matter as well as $(NaPO_3)_6$ and sonication to further breakdown the aggregates and disperse the particles. All other soil analyses were performed on all the samples collected (i.e. 3 per plot). Soil pH was determined using a 1:2 (w/w) soil:water ratio. Organic C concentration was determined on ground samples (<60 µm) by dry combustion at 1100ºC [and after removal of

carbonates with HCl vapors] and infrared detection (LECO C632 Analyzer) [65]. Total N concentration also was determined on ground samples (<60 μm) by dry combustion at 1150ºC and infrared detection (LECO CNS 2000). Exchangeable cations were extracted using an unbuffered 0.1 M $BaCl_2$ solution [19]. Soil extracts were filtered (Whatman filters Nº 42) before analyzing them for Ca, Mg, K, Na, Mn, Fe and Al concentrations using atomic absorption spectrometry. Soil available P was extracted with 0.5 M $NaHCO_3$ solution [38]. The extract solution was adjusted to pH 8.5 before measuring PO_4^{3-} levels colourimetrically with a Technicon Autoanalyser. Soil mineral N, i.e. NO_3^- and NH_4^+, was extracted with a 2 M KCl solution [34], whereas washed PRS^{TM} probes for the time series mineral N study were eluted with 0.5 M HCl solution for 60 minutes. Nitrate and NH_4^+ concentrations in the extracts and eluates were measured colourimetrically with a Technicon Autoanalyzer.

Foliage Sampling and Analyses

Foliage samples from three random shrubs per species within each plot were collected in September 2008 in the upper tier of the canopy. The samples were first oven-dried at 65ºC for 24 hours and then coarsely ground prior to leaf N and C determination using the Leco CNS 2000 Analyzer. Subsamples were then further ground (<60μm) and a 1.0 ± 0.15 mg subsample was analyzed for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios using a continuous flow isotope ratio mass spectrometer interfaced with a RoboPrep Sample Converter (Europa Scientific, Crewe, UK). The working standard for $\delta^{13}C$ determination was lentil (*Lens culinaris*) straw with a $\delta^{13}C$ of –27.6‰ relative to the PeeDee belemnite standard. The $\delta^{13}C$ of the sample was calculated as followed:

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ in the sample and standard, respectively. The $\delta^{13}C$ value is a reliable integrative measure of water use efficiency (WUE) throughout the growing season in dry climates where other environmental factors vary minimally [35, 52]. Our sites were located in the semi-arid prairies in a region where potential evapotranspiration exceeds precipitation. Variability among other environmental factors were reduced through the use of common treatments—including the use of an identical willow clone and caragana—and management practices at all sites. The major factor controlling foliar $\delta^{13}C$ values should therefore be soil water availability as affected by site and soil properties and possibly the different shrub mixtures.

The working standard for $^{15}N/^{14}N$ was pea grain (atom $\%^{15}N$ content = 0.36726). The deviation of the sample $^{15}N/^{14}N$ ratio from that of the atmosphere ($\delta^{15}N$) was calculated as followed:

$$\delta^{15}N = ((R_{sample} / R_{standard}) - 1) \times 1000$$

The percentage of ^{15}N in the caragana leaves derived from the atmosphere (%Ndfa) was estimated using the ^{15}N natural abundance method [18]. This method has been used on several occasions with leguminous tree/shrub species in natural and managed systems and is said to provide more reliable estimates than the acetylene reduction assay [e.g., 16]. Non fixing trees/shrubs have positive $\delta^{15}N$ due to the soil N dominance, whereas the atmospheric N captured by the N_2-fixing trees/shrubs dilutes the $\delta^{15}N$, yielding negative values. The deviation between foliar willow and caragana $\delta^{15}N$ values was therefore used to calculate the %Ndfa in the caragana as followed:

$$\%Ndfa = \left(\frac{\delta^{15}N_0 - \delta^{15}N_t}{\delta^{15}N_0 - \delta^{15}N_a} \right) \times 1000$$

where $\delta^{15}N_0$ refers to the non fixing reference willow grown in monoculture, $\delta^{15}N_t$ refers to the N_2-fixing caragana and $\delta^{15}N_a$ refers to the N_2-fixing caragana inoculated with 5 different strains of mesorhizobium and grown for 90 days in a N-free environment [22]. The $\delta^{15}N$ of leaves collected from the inoculated caragana ranged from -2.58 to -0.66. All mesorhizobium strains also augmented the allocation of N into the leaves. We used the average of -1.66 for $\delta^{15}N_a$.

Data Analysis

Analysis of variance (ANOVA) with the Holm-Sidak test was used to test for site differences and treatment effects on growth variables as well as foliar variables. Means of the samples in each plot were used for statistical analyses. These were analyzed with treatment and site as sources of variation. No data transformation was necessary to meet the requirements of normality of the residuals and equal variance. Considering the lower degrees of freedom, statistical significance was accepted at $\alpha = 0.10$ to avoid not detecting a difference occurring in nature (type II error) [31]. We referred to as "marginally significant" when α was between 0.05 and 0.10. Site and treatment effects using a repeated-measures ANOVA were not tested on time-series soil data (i.e. temperature, soil water and available N) as normality of the residuals or equal variance could not be achieved. However, monthly effects were assessed by site individually. Statistical analyses were performed with the SigmaStat 4.0 module in SigmaPlot 12 (Systat Software Inc., San Jose, California).

Results

Site effects. Willow survival after two years increased significantly in the order: Harris (64–71%) < Saskatoon 2 (80–96%) < Saskatoon 1 (>90%) (Figure 2). The survival of caragana was greater

than 95% at all the sites, except for the 1W:1C treatment at Harris which was 88% (Figure 3). Harris had significantly lower caragana survival compared to the Saskatoon sites.

After two years of growth, willow stem height increased significantly in the order: Saskatoon 2 (76–119 cm) < Harris (120–136 cm) < Saskatoon 1 (153–178 cm) (Figure 2). Caragana stem height also increased significantly in the order: Harris (74–92 cm) < Saskatoon 2 (101–124 cm) < Saskatoon 1 (134–145 cm) (Figure 3). Identical patterns were observed for stem diameter and canopy dimension (Figures 2 and 3) and individual willow and caragana dry matter production (Table 2).

After four years, total and individual willow dry matter production of the plots increased significantly in the order: Saskatoon 2 < Harris < Saskatoon 1 (Table 3). The total and individual willow dry matter production of the pure willow plots and mixed treatments also followed this pattern across the sites. Individual caragana dry matter after four years was significantly greater at the Saskatoon sites than at Harris when comparing site averages although the pure plots exhibited similar dry matter across the sites (Table 3).

Average soil water content increased in the order: Harris < Saskatoon 2 < Saskatoon 1 and, at all sites, the average monthly soil water content decreased from June to August and then increased in September. Average soil water content at Harris, Saskatoon 2 and Saskatoon 1 was 9%, 26% and 36%, respectively in June; 8%, 25% and 28%, respectively in July; 2%, 10% and 12%, respectively in August; and 5%, 15% and 22%, respectively in September. Average soil temperatures averaged about 2.3°C higher at Harris (19.9°C) than at either Saskatoon 1 or Saskatoon 2 (17.7 and 17.5°C, respectively). As expected, average soil temperatures increased throughout the summer months (i.e., June through August), before cooling in September. Warming and cooling were more pronounced in Saskatoon 2 and Harris. Average soil

temperatures at Saskatoon 1, Saskatoon 2 and Harris was 16.2, 15.6 and 21.5°C, respectively in June; 18.6, 19.1 and 21.0°C, respectively in July; 20.5, 23.2 and 24.0°C, respectively in August; and 15.4, 11.9 and 13.1°C, respectively in September.

Available soil N measured using the PRS™ probes (Figure 4) was, on average, greater at Saskatoon 1 (246 μg 10-cm^{-2} month^{-1}) and Harris (201 μg 10-cm^{-2} month^{-1}) than at Saskatoon 2 (131 μg 10-cm^{-2} month^{-1}). Similar results were observed for the KCl-extractable N (Table 1). At both Saskatoon sites, available soil N decreased throughout the summer, before recovering somewhat during September (Figure 4). At Harris, available soil N also decreased from June to July, but then increased in August and September.

Foliar N concentrations in willow were significantly higher at Harris than at either Saskatoon sites, and were higher at Saskatoon 1 than at Saskatoon 2 (Figure 5). Indeed, at Harris, foliar N concentrations were generally >3%, whereas at Saskatoon 2 they were <2%. Foliar N levels in caragana were generally higher than those in willow and decreased in the order: Saskatoon 1 > Saskatoon 2 > Harris (Figure 5). For both willow and caragana, δ^{15}N values for the leaves varied among sites, with the δ^{15}N decreasing in the order: Saskatoon 1 > Harris > Saskatoon 2 (Figure 6). Foliar δ^{13}C values of both willow and caragana also varied among sites, with foliage at the Saskatoon 2 site exhibiting significantly lower δ^{13}C values than foliage at either the Harris or Saskatoon 1 sites (Figure 6).

Using the δ^{15}N natural abundance method, it was determined that the proportion of total N in the caragana leaves that originated from the atmosphere (i.e., N obtained from biological N_2-fixation) in the pure plots ranged from 7–28% (mean of 22%) and 8–30% (mean of 19%) at

Harris and Saskatoon 2, respectively. At Saskatoon 1, all the N in the caragana leaves of the pure plots originated from the soil (i.e. Ndfa of 0%).

Treatment effects. Within sites, the growth and survival of willow after two years was largely independent of treatment — this was especially true at the Harris site (Figure 2). Whereas some treatment differences were observed at the Saskatoon sites (i.e. stem height and canopy dimension at Saskatoon 2 and stem diameter (marginally significant differences) at Saskatoon 1), there was no consistent pattern. In fact, the two sites behaved quite differently, with willow growth variables decreasing at Saskatoon 1 and increasing at Saskatoon 2 with increasing density of caragana (Figure 2). At Saskatoon 1, individual willow dry matter production after two years also decreased significantly with increasing caragana density (results not shown).

The treatments had very little impact on caragana growth variables after two years, except for Harris (Figure 3). Indeed, at Harris, there was a (small) significant decrease in survival of the caragana in the 1W:1C treatment relative to the other two treatments. Stem height, stem diameter and canopy dimension in the 2W:1C treatment at Harris were also marginally significantly lower than those in the 1W:1C and pure caragana treatments. Individual caragana dry matter yields were also larger in the pure caragana treatment at both Harris and Saskatoon 2, but the differences were not significant from the other treatments (results not shown).

At Saskatoon 1, total dry matter production after four years was significantly greater in the pure willow and mixed treatments compared to the pure caragana plots, whereas the willow monocultures were significantly more productive than the other treatments at Harris (Table 3). The pure caragana plots yielded significantly more dry matter than the pure willow plots and 2W:1C treatment at Saskatoon 2, whereas the 1W:1C treatment produced significantly more dry matter than the pure willow plots (Table 3). At that same site, individual willow dry matter

production after four years also increased significantly with increasing caragana density (Table 3). Individual caragana dry matter after four years was significantly lower in the 2W:1C treatment than the other treatments at all sites (Table 3).

Treatment effects were detected on foliar $\delta^{15}N$ only at Harris, and then only for the caragana. Indeed, foliar $\delta^{15}N$ of caragana were significantly higher in the pure and 1W:1C treatments compared to the 2W:1C treatment. No significant treatment effects on foliar N concentrations and $\delta^{13}C$ values were detected at any of the sites.

The 2W:1C treatment at Harris and Saskatoon 2 showed the largest %Ndfa of all treatments with respective ranges of 43 to 62% (mean of 53%) and 8 to 58% (mean of 34%). The 1W:1C treatment was similar to the pure caragana plots with a range of 1 to 34% (mean of 13%) at Harris and 0 to 30% (mean of 15%) at Saskatoon 2. At Saskatoon 1, only one 2W:1C plot showed a positive %Ndfa of caragana leaves (9%). All N in the willow leaves originated from the soil at both Saskatoon sites, whereas willow leaves in the mixed plots at Harris had %Ndfa varying between 2 and 33% (means of 24% and 13% in the 2W:1C and 1W:1C treatments, respectively).

Discussion

Willow Growth, Nitrogen Nutrition and Water Use Efficiency

In terms of pure willow plots, Saskatoon 1 (15.6 t ha^{-1} of dry matter) and Harris (13.3 t ha^{-1}) were the most productive sites, whereas Saskatoon 2 (1.24 t ha^{-1}) was the least productive site (Table 3). The yields of the mixtures also followed this pattern across the sites. For northeastern USA, estearn Canada and Europe, willow SRIC generally produces between 5 and 15 t dry matter ha^{-1} yr^{-1} [2; 14; 27; 37]. The yields are sometimes as high as 20 t ha^{-1} yr^{-1} [26].

Since our data are for four years of growth, the yields measured in this study are either at the lower limit of this range (Saskatoon 1 and Harris) or well below (Saskatoon 2). Yet, the yields at Saskatoon 1 and Harris are about average for SRIC of willow in the Prairie Provinces [14]. This reflects in part the harsh growing conditions in the Saskatoon area with low rainfall and a shorter growing season than in eastern Canada and northeastern USA. The very low yields at Saskatoon 2 can also be linked to soil quality. Willow generally responds favourably to fertilization, particularly to N [4; 25], but responses to fertilization can be delayed by up to 10 years on fertile arable land [36]. Foliar N levels of *S. eriocephala*, *S. exigua*, and *S. Lucida* grown with a nutrient solution maintained at optimum levels for the species were respectively 28.4, 35.4 and 28.0 mg g^{-1} of dry weight [51]. Willow at Saskatoon 1 and Harris benefited from high soil N availability, which favoured high foliar N concentrations (25.0–32.4 mg g^{-1}) relative to the willow leaves at Saskatoon 2 (14.3–16.2 mg g^{-1}) (Figure 5). The foliar N concentrations measured at Harris and Saskatoon 1 are within the optimal range as proposed by Simon *et al*., which obviously benefited the shrubs in terms of dry matter production. At Saskatoon 2, however, foliar N concentrations are much lower than optimums and thus, willow growth was significantly impaired.

Fast growing willows require a steady supply of water for maximum growth. Lindroth and Båth [28] reported that *S. viminalis* produces about 6.3 g of dry mass per kg of water transpired when nutrients are not a limiting factor. Using the equation for stem dry matter production developed by Lindroth and Båth (with summer precipitation of ~250 mm and a fraction of precipitation released as transpiration estimated with the BioSim model [42] of 0.50), about 20 t ha^{-1} of dry matter should be produced in the Saskatoon area over a 4 year period if we assume similar WUE between *S. viminalis* and *S. miyabeana*. The measured willow yields at Harris and Saskatoon 1 are slightly below these estimates, but the yields at Saskatoon 2 are

largely below despite similar climatic conditions. This means that willow growth at these sites is not linked to water availability as initially assumed. It is rather restricted by degree-days at Saskatoon 1 and Harris and highly restricted by N availability at Saskatoon 2.

Plant tissue $\delta^{13}C$ is often used as a proxy for water stress of plants and WUE [30; 32]. It is determined by whether plant stomata are open or closed, with $\delta^{13}C$ rising when the more closed they are to reduce transpiration in periods of water stress. A less negative $\delta^{13}C$ value therefore generally indicates higher WUE. Large differences in WUE were found among young willow cultivars artificially exposed to water stress [63; 64]. Cultivars generally showed greater WUE under water stress. Conversely, Schifman et al. [47] showed small differences in $\delta^{13}C$ values under field conditions but the willow cultivars with the largest range in $\delta^{13}C$ had higher survival under drier conditions, suggesting that some cultivars have a greater ability to increase WUE during temporary drought. Using genotypes that combine both high productivity and WUE is considered an advantage in semi-arid areas, especially in the context of climate change.

In our study, the more productive pure willow plots at Saskatoon 1 and Harris had the least negative foliar $\delta^{13}C$ values, whereas the least productive willow plots at Saskatoon 2 had the most negative values (Figure 6). According to $\delta^{13}C$ theory, it thus seems consistent that willow had greater WUE (or less negative $\delta^{13}C$) at Harris due to the low soil water content (2–9%), but not at Saskatoon 1 as this site exhibited the greatest soil moisture (12–36%). Similarly, Schifman et al. [47] showed that $\delta^{13}C$ values in willow wood were not only negatively related to growing season precipitation but also positively related to plant canopy age and stem size. There are three main drivers likely to explain the fact that $\delta^{13}C$ values increase at the most productive sites : (1) *Leaf photosynthetic capacity* — several studies showed positive relationships between N uptake, $\delta^{13}C$, WUE and dry matter production of conifers and deciduous trees [e.g., 13;

29]. These relationships are consistent with the fact that leaf photosynthetic capacity and plant dry matter production are favoured by high N nutrition and that higher photosynthetic capacity tends to maintain lower CO_2 concentrations inside the leaves, thus yielding less negative foliar $\delta^{13}C$; (2) *Canopy irradiance regime* — because Saskatoon 1 and Harris also had much taller shrubs, these sites likely had greater leaf area. A greater irradiance regime at Saskatoon 1 and Harris could thus partly explain the greater $\delta^{13}C$ values as it controls canopy (leaf) temperature, vapour pressure deficit and thus transpiration rates [62]. If more intense radiation augments transpiration rates, then leaves are likely to close their stomata more often; and (3) *Plant age and/or size* — as plants become older and/or larger, recent literature suggests that water demand increases and this is generally accompanied with an increase in WUE (or $\delta^{13}C$) [e.g. 47; 56].

At Saskatoon 1 and Harris where plants were larger and leaf photosynthetic capacity and canopy irradiance regime likely also high, the foliar $\delta^{13}C$ values were less negative and suggest efficient WUE. At Saskatoon 2, which showed moderate soil water content, the willow plants did not nearly grow as fast and developed much smaller canopies, and thus, the more negative $\delta^{13}C$ values that developed suggest more prodigal WUE. The differences in growth rates between the sites are so large that the pattern in $\delta^{13}C$ values and WUE seemed to be controlled by intrinsic plant factors as opposed to extrinsic factors such as precipitation and/or soil water (as in other studies that induced drought artificially [63; 64]). Willow species are not generally very drought tolerant because of their shallow rooting habits [58] and Adegbidi et al. [3] showed that biomass production in irrigated willow plots in New York state was increased by about 3-fold. Our results indicate that water demand of the willow is still low because of their juvenile state and that water stress did not affect the growth over a two year period. As water demand increases with plantation age, the impacts of water stress on growth could potentially be indicated by reduced

$\delta^{13}C$ values in this semi-arid environment. Our results indicate, however, that WUE as inferred with $\delta^{13}C$ must be interpreted with care due to the possible effects of photosynthesis (rates) and shrub size.

Caragana Growth and Atmospheric N_2-Fixation

Growth differences in pure plots of *Caragana arborescens* were much smaller between the sites (~9 t ha^{-1} over 4 years) compared to willow. Overall, dry matter production of caragana was 30 to 40% lower than willow at the two most productive sites but it was at least 7-fold larger at Saskatoon 2. This indicates that caragana is generally well adapted to the (long) cold winters and dry conditions of the Canadian Prairies and can produce reasonable yields on soils with low N availability (e.g. Saskatoon 2) due to its N_2-fixing ability [48]. Similar to willow, caragana growth after two years was correlated to foliar N concentrations, although the concentration range was relatively small (32 to 39 mg g^{-1}, Figure 5). Unlike willow, however, foliar N concentrations were not correlated to available soil N after 2 years — caragana at Saskatoon 2 had greater productivity and foliar N than at Harris, whereas Saskatoon 1, similar to willow, had the highest productivity and foliar N. The growth of plants is optimal when N is supplied in both NH_4^+ and NO_3^- forms [8], but NH_4^+ concentrations of soils are often 10-1000 times lower than those of NO_3^- [33]. Despite lower available and KCl-extractable soil N, Saskatoon 2 was the only site to show a large pulse (July) of NH_4^+ during the growing season, creating a mean molar NH_4^+/NO_3^- ratio of 2.3 for all 4 treatments (results not shown). This ratio was much smaller for June, August and September (0.05) but still higher than the ratio of 0.02 calculated at the two other sites. Despite that most plants preferentially take up NH_4^+ when both forms are present (as NH_4^+ requires less energy for uptake and assimilation than NO_3^-, whereas NO_3^- has to be reduced

prior to assimilation) and that this phenomena occurs even if NH_4^+ is present at lower concentrations than NO_3^- (as generally seen in our soils) [60], the higher molar NH_4^+/NO_3^- ratios at Saskatoon 2 likely modulated a higher NH_4^+ uptake and foliar N levels in the caragana. It is reasonable to think that the soil would provide a large part of the N required for growth in the early development stages of caragana, before root nodules have fully developed and significant N_2-fixation started to take place, thus explaining the correlation between foliar N and plant growth. However, after 4 years of growth, the more homogeneous caragana yields between the sites suggest a larger role of root nodules and N_2-fixation to satisfy the plant's N requirement.

When harvesting the stems to determine relative dry matter production, a few caragana roots were excavated at Saskatoon 2 and well formed root nodules were observed. Using the $\delta^{15}N$ natural abundance method to assess the percentage of total N in the leaves that was derived from the atmosphere (%Ndfa), it was determined that 0 to 58% of the N in caragana leaves at Saskatoon 2 was derived from biological N fixation. The %Ndfa at Saskatoon 2 was higher than %Ndfa at Saskatoon 1 (0%) but overlapped with %Ndfa at Harris (1–62%). The %Ndfa in caragana leaves at Saskatoon 2 and Harris was also generally higher in the 2W:1C than the pure or 1W:1C plots. In a study of the N_2-fixing ability of eight *Acacia senegal* provenances grown from seeds for 3 years, Raddad et al. [41] showed that %Ndfa was greater for provenances collected from the area of N poor sandy soils relative to those collected from a clayey soil area with higher N availability. The provenances from sandy soils started fixing atmospheric N_2 earlier than the provenances originating from the clayey area. Thomas et al. [55] investigated nodulation and N_2 fixation from a tropical legume shrub (*Gliricidia sepium*) in controlled conditions and also reported that high N availability had an inhibitory effect in both nodule production and nitrogenase activity. According to Dommergues [12], when the amount of

available N in the soil exceeds a certain threshold, soil N is preferred over atmospheric N_2 for meeting the N requirements of legume trees/shrubs. The %Ndfa results along with the average caragana yields at Saskatoon 2 validate that the N_2-fixing potential of caragana root nodules is greater at N poor sites. The fact that the %Ndfa at Saskatoon 2 and Harris was greater in the 2W:1C than in the pure caragana and 1W:1C plots also suggests that greater competition for soil N by the willow favoured nodulation of the caragana roots and augmented N_2-fixation rates.

Although a few studies assessed the N fixation potential of caragana using the acetylene reduction method [21, 59], we are unaware of literature reporting the %Ndfa of *Caragana* spp. The %Ndfa for Harris and Saskatoon 2 are generally lower than field studies conducted on mature legume shrubs (e.g., *Prosopis* spp. in Shearer et al. [50]; *Caesalpiniaceae*, *Mimosaceae*, and *Papilionaceae* spp. in Roggy et al. [44]). However, they fall within the range reported for *Acacia* species after 2 years or less of growth [41], and are similar to the values reported by Schulze et al. [49] for *Acacia* species along a moisture gradient in Namibia (i.e., 10–30 %Ndfa). Whether the N_2-fixing capacity of caragana in the intercropped system will increase in the mid term as the nodules develop [41] or will decrease in the long term as N availability in the system increases [12, 53] remains to be tested.

Interestingly, it is at the N poor Saskatoon 2 site that mixing caragana and willow had a positive effect on willow growth and yield — increasing the percentage of caragana in the plots from 33% to 50% resulted in greater willow stem height and canopy dimension (Figure 2) as well as individual willow dry matter production after four years (Table 3). The benefits of introducing caragana on available soil N were not statistically detected at Saskatoon 2 possibly because of the large variation in the data set (Figure 4), nor were the benefits on willow foliar N levels observed because of a blocking effect (Figure 5). Also, the increased willow growth

potentially had a dilution effect on foliar N concentrations as the canopy became larger [45]. As a whole, however, the total amount of N contained in the willow canopy after two years followed the same pattern as canopy size: 646 mg N shrub^{-1} in the 1W:1C treatment, 452 mg N shrub^{-1} in the 2W:1C treatment and 276 mg N shrub^{-1} in the pure willow treatment (measured from individual willow dry leaf matter after two years of growth). Our results on growth variables are thus consistent with other studies that show the benefits of introducing N_2-fixing species (i.e. *Casuarina equisetifolia* and *Leucaena leucocephala*) on the early nutrition and growth (2 years) of *Eucalyptus robusta* [39] and understory vegetation [57] on relatively poor soils. We suggest that a similar effect can be observed in the short term in SRIC of willow when mixed with the N_2-fixing *Caragana arborescens*.

On the other hand, at the most productive Saskatoon 1 site, mixing willow with caragana had a negative effect on willow growth and yield — willow stem diameter and individual willow dry matter production after two years declined when the percentage of caragana in the plots was increased from 33% to 50%. The Saskatoon 1 site may provide insights as to how willow and caragana interact and compete for N and light on productive soils. With high N uptake (based on dry matter production relative to willow and foliar N concentrations of ~4% for all treatments), together with the fact that it did not fix appreciable amounts of atmospheric N, caragana was in direct competition with willow for soil N. Again, no difference in willow foliar N concentrations was detected between treatments. But based on individual willow dry leaf matter measured after two years and foliar N levels, we estimate that willow shrubs absorbed in their canopy 43.5 g N plot^{-1} in the 1W:1C treatment, 85.5 g N plot^{-1} in the 2W1:C treatment and 132 g N plot^{-1} in the pure willow treatment. In the case of caragana, we estimate that the shrubs absorbed in their canopy 81.6 g N plot^{-1} in the 1W:1C treatment and 86.1 g N plot^{-1} in the 2W1:1C treatment.

When averaged over the whole plot, caragana took up 65% of the total leaf N in the 1W:1C treatment and 50% in the 2W:1C treatment despite fewer shrubs, which indicates the significant uptake of N by the species at this site.

The decrease in willow stem diameter and individual willow dry matter after two years that accompanied an increase in caragana in the intercropped plots at Saskatoon 1 could also be indicative of some competition for light. Willow and caragana are both shade-intolerant species, but because of its N_2-fixing ability, caragana is potentially a better competitor than willow, the latter being generally considered a poor competitor for nutrients, water and light [2]. During the third year of growth, however, we began to observe signs of caragana canopy decline in the mixtures at Saskatoon 1. When these were harvested in the spring of 2011 after four full years of growth, individual willow dry matter production had become similar between treatments, whereas individual caragana dry matter was lower in the 2W:1C treatment (Table 3). There was also some evidence of caragana dieback in the mixtures, which was validated during the summer as most caragana did not regenerate after coppicing. We do not suspect that the full dieback of caragana at the start of the second rotation was associated with its low tolerance to coppice because the caragana at Harris and Saskatoon 2 regenerated strongly after the harvest. Rather, the results suggest that willow can outcompete caragana for light on productive soils as it tends to put much more apical growth than caragana later in the first rotation and early in the second rotation. In this respect, N constraints only had a marginal short term effect on willow growth at that site. At Saskatoon 2 and Harris, the 2W:1C treatment also had lower caragana dry matter (Table 3), but the shade created by the willow was not severe enough to lead to some caragana dieback after a full rotation. In conclusion, it seems more appropriate to intercrop these two

species on less productive soils as caragana is more likely to add N to the system via atmospheric N_2-fixation and is less likely to be shaded out by the willow in the next rotation.

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Table 1. General description of the three study sites.

	Latitude/ longitude	Elevation (m)	Degree days (°C per year)	Summer precipitation (mm)	Soil pH	Soil texture (%sand/%clay)	Soil organic C (%)	C:N ratio	Extractable NO_3-N (mg kg^{-1})	Cultural history (last 5 years)
Harris	51º67'N 107º66'W	541	1555(30.3)	291(134)	5.90(0.04)	Loamy sand (85.4/8.6)	1.18(0.03)	9.51(0.13)	27.9(2.72)	Wheat and canola (broadcast N and P fertilization)
Saskatoon 1	52º13'N 106º61'W	587	1570(40.9)	320(117)	7.87(0.03)	Clay (13.0/67.4)	3.14(0.04)	8.90(0.12)	34.0(2.41)	Oats and barley (broadcast N and P fertilization)
Saskatoon 2	52º09'N 106º46'W	510	1609(39.1)	288(102)	8.20(0.02)	Sandy clay loam (52.3/32.9)	1.73(0.02)	10.5(0.14)	7.53(0.78)	Hay and tree nursery (bag fertilization)

*Degree-days (base of 5°C) and summer precipitation are averages modelled from the yearly 2007 and 2010 data using the hydroclimatological model BioSIM [42]. Soil data (0-10 cm) are from all the samples collected prior to planting. Values in parentheses are standard errors of the mean.

Table 2. Leaf, stem and total (leaf + stem) individual willow and caragana dry matter (DM) production at the three sites after two growing seasons.

	Leaf DM	**Stem DM**	**Total aboveground DM**	**Leaf DM**	**Stem DM**	**Total aboveground DM**
	(g shrub^{-1})					
	Willow			**Caragana**		
Harris	42.4(5.84)b	74.9(10.7)b	117.3(16.5)b	25.9(2.94)c	64.0(8.14)c	89.9(11.0)c
Saskatoon 1	119.8(8.70)a	233.4(22.0)a	353.2(30.3)a	94.4(9.58)a	264.2(29.6)a	358.6(38.4)a
Saskatoon 2	30.2(5.62)b	49.3(11.4)b	79.5(17.0)b	54.9(6.65)b	127.8(10.2)b	182.7(16.4)b

Table footnote: W: willow, C: caragana. Values in parentheses indicate standard error. A significant difference between the sites is indicated by different letters within a column.

Table 3. Total (willow+caragana) and individual willow aboveground dry matter (DM) production at the three study sites after four growing seasons.

	Treatment	Willow aboveground DM	Caragana aboveground DM	Total aboveground DM
		—— g shrub^{-1} ——		t ha^{-1}
Harris	W	580.7(51.9)	-	13.3(1.19)a
	2W:1C	461.5(61.5)	106.9(17.9)b	8.66(0.67)b
	1W:1C	489.8(55.6)	318.4(78.4)a	9.24(0.39)b
	C	-	360.5(45.1)a	8.24(1.03)b
	Site average	510.7(35.9)B	261.9(78.4)B	9.85(0.71)B
Saskatoon 1	W	680.2(76.2)	-	15.6(1.74)a
	2W:1C	748.6(28.5)	274.4(29.4)b	15.6(0.04)a
	1W:1C	605.5(17.1)	530.6(33.2)a	13.0(0.36)a
	C	-	394.1(38.8)a	9.01(0.89)b
	Site average	678.1(41.3)A	399.7(74.0)A	13.3(0.96)A
Saskatoon 2	W	54.3(29.4)b	-	1.24(0.67)c
	2W:1C	98.2(24.3)a	154.9(29.7)b	3.86(0.56)b
	1W:1C	195.6(105.9)a	370.6(46.8)a	6.47(1.32)ab

	C	-	388.7(49.0)a	8.89(1.12)a
	Site average	116.0(41.7)C	304.7(75.1)AB	5.11(0.91)C

Table footnote: W: willow, C: caragana, irr: irrigation. Values in parentheses indicate standard error. "Site average" values are the mean (and standard error) for the site, averaged across treatments, and are compared between sites—a significant difference between the sites is indicated by different capital letters within a column. Within sites, a significant difference between treatments is indicated by different lower case letters within a column.

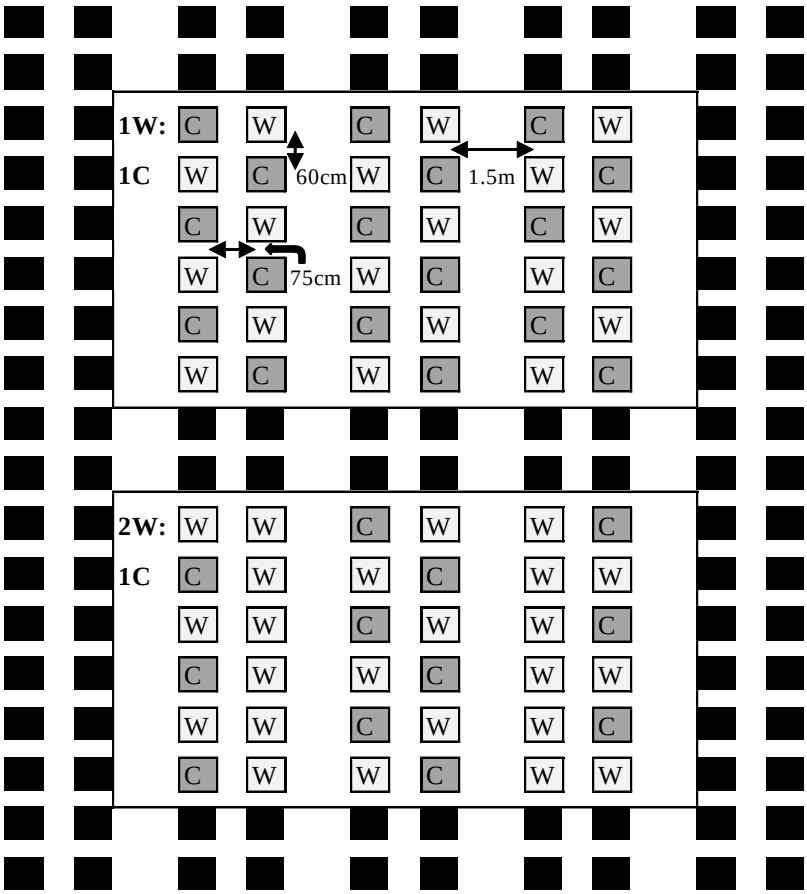

Figure 1. Experimental layout for the 2W:1C and 1W:1C treatments at the Harris, Saskatoon 1 and Saskatoon 2 sites. Note that the black squares are willow buffer trees.

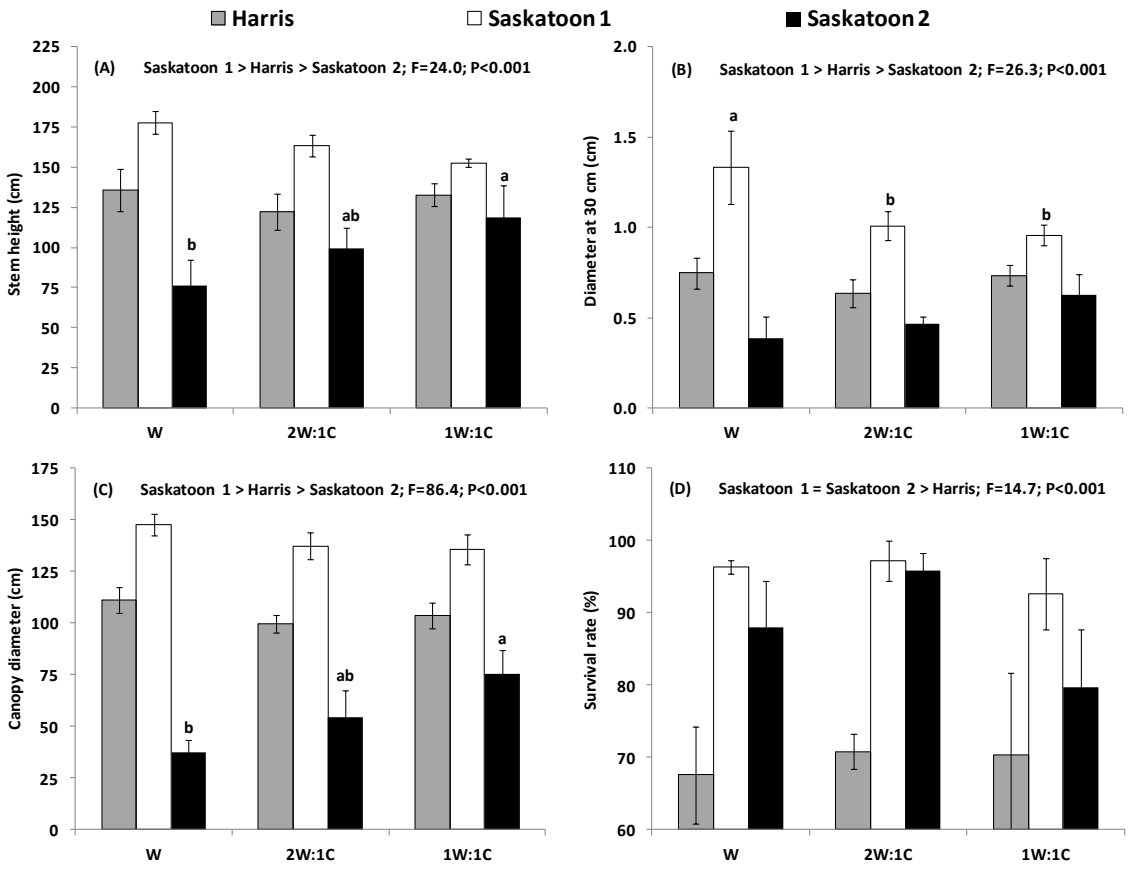

Figure 2. Stem height (A), stem diameter (B), canopy diameter (C) and survival rate (D) for willow (W) in monoculture and intercropped with caragana (C) at the Harris, Saskatoon 1 and Saskatoon 2 sites. Within sites, treatments identified with different letters are significantly different averages at the α = 0.10 level of probability. Site differences (averaged across treatments) are listed at the top of each panel.

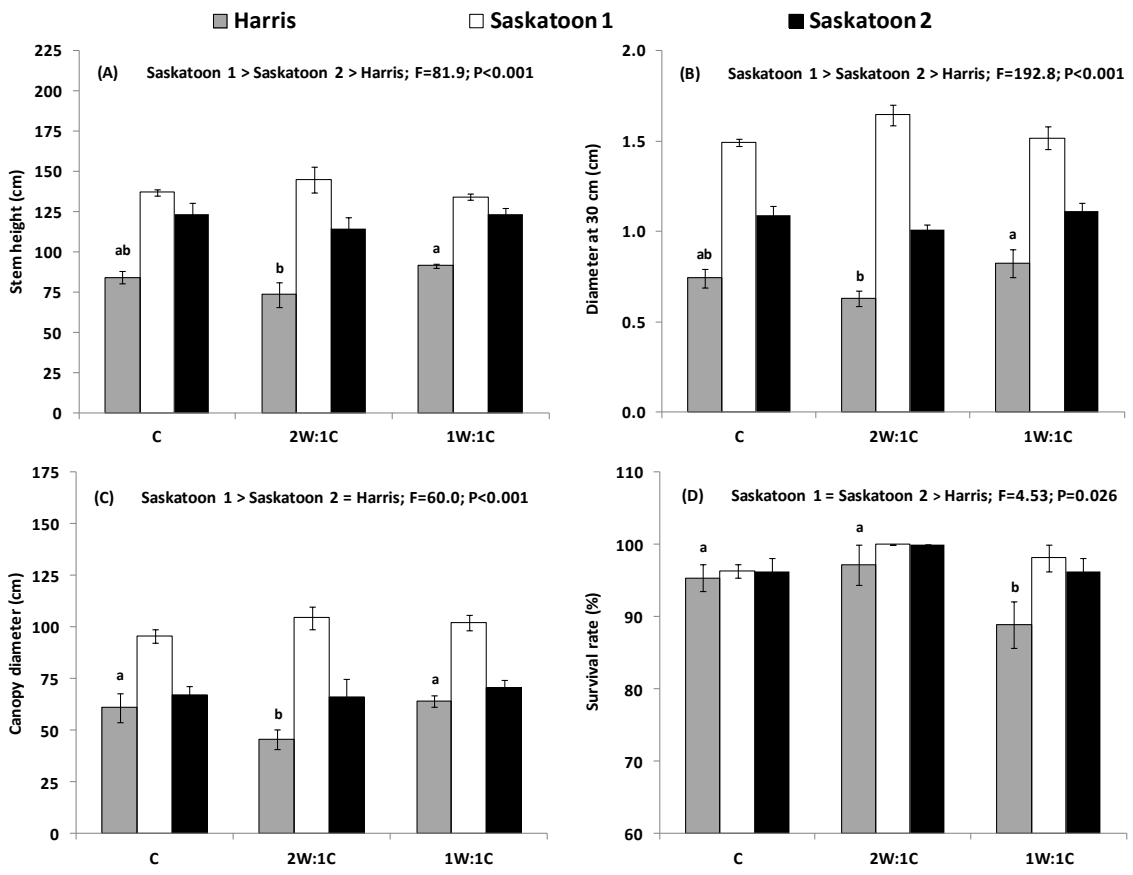

Figure 3. Stem height (A), stem diameter (B), canopy diameter (C) and survival rate (D) for caragana (C) in monoculture and intercropped with willow (W) at the Harris, Saskatoon 1 and Saskatoon 2 sites. Within sites, treatments identified with different letters are significantly different averages at the α = 0.10 level of probability. Site differences (averaged across treatments) are listed at the top of each panel.

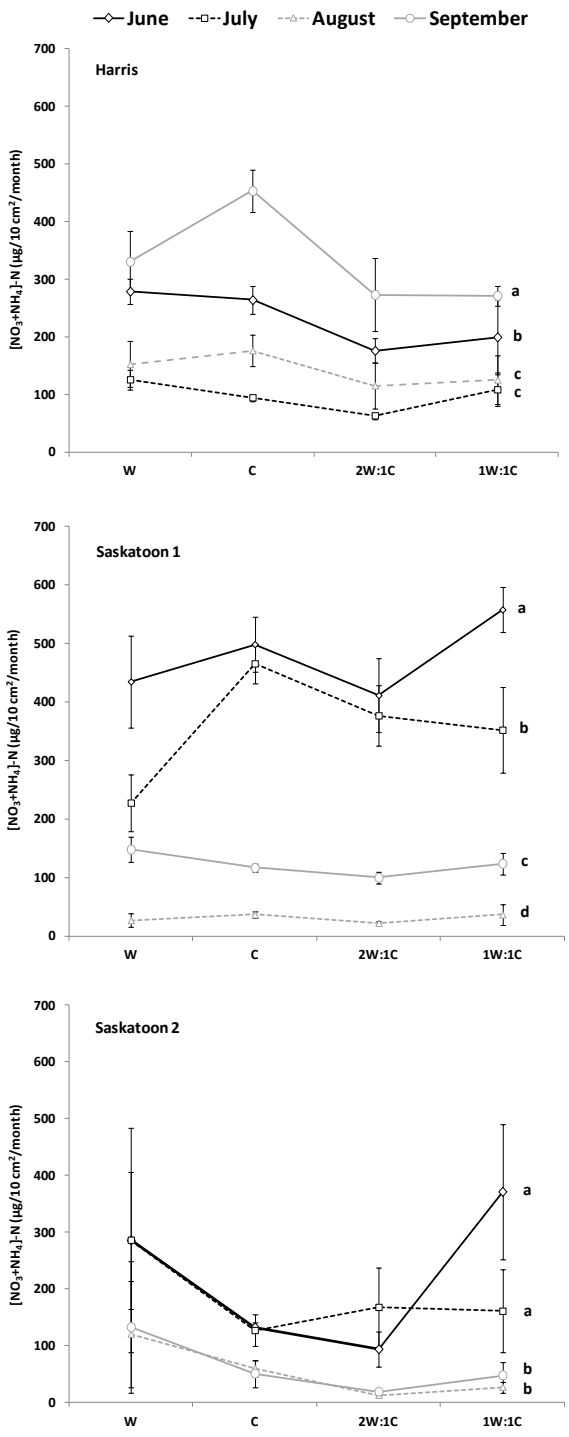

Figure 4. Available soil nitrogen ($[NO_3+NH_4]$-N) at the Harris, Saskatoon 1 and Saskatoon 2 sites measured using PRS^{TM}-probes. Within sites, months identified with different letters are significantly different at the $\alpha = 0.10$ level of probability.

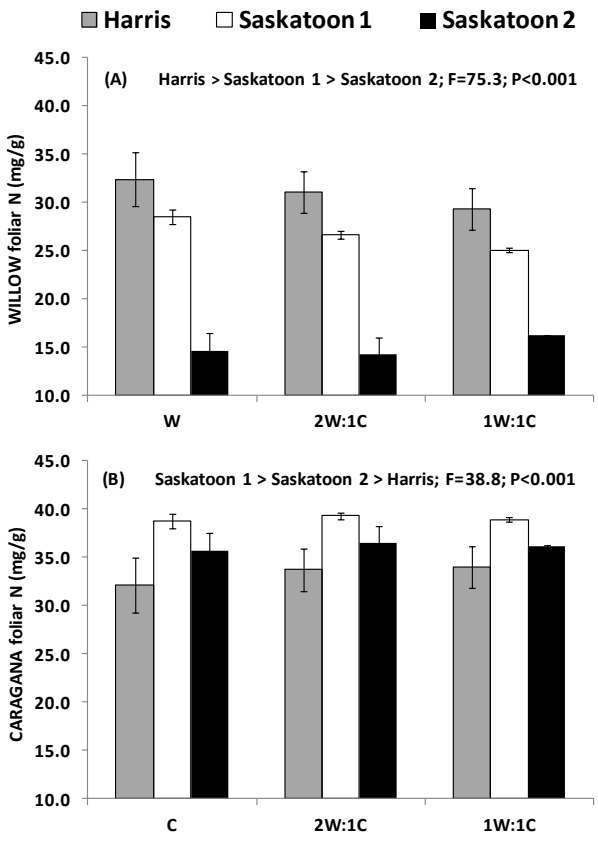

Figure 5. Foliar concentrations of nitrogen in willow (A) and caragana (B) at the Harris, Saskatoon 1 and Saskatoon 2 sites. Within sites, treatments identified with different letters are significantly different at the α = 0.10 level of probability. Site differences (averaged across treatments) are listed at the top of each panel.

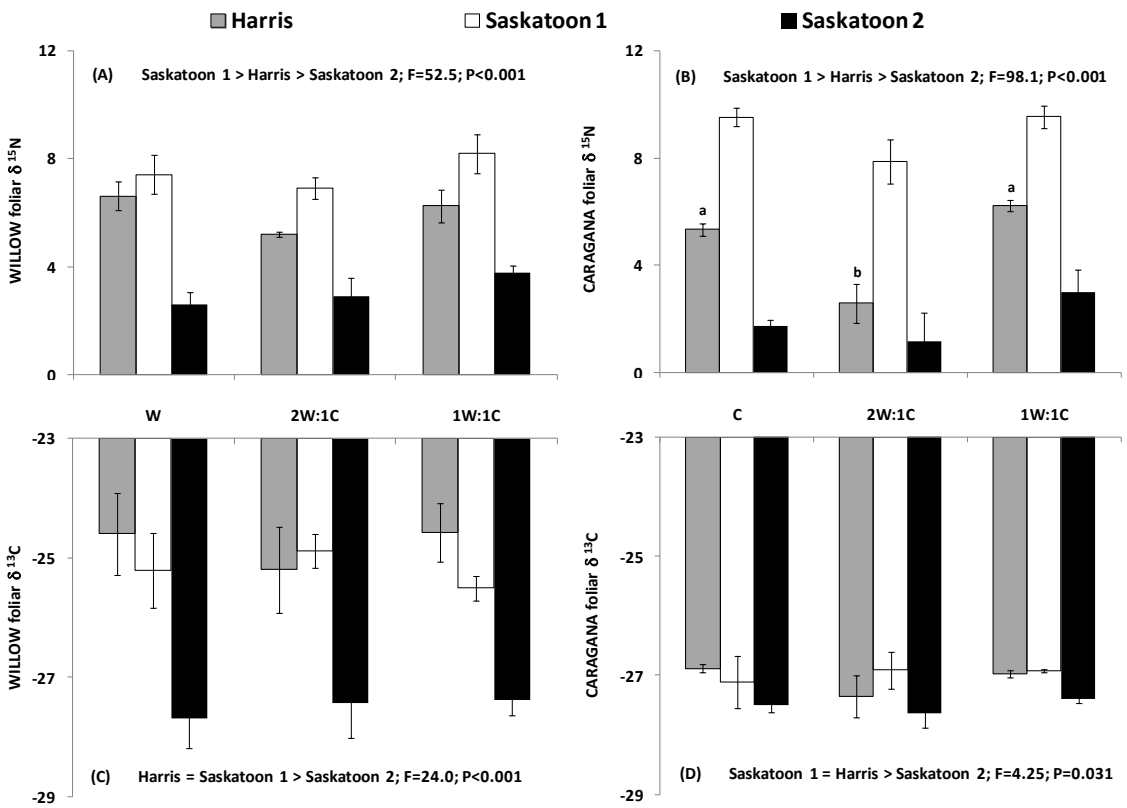

Figure 6. Foliar $\delta^{15}N$ and $\delta^{13}C$ of willow (A, C) and caragana (B, D) at the Harris, Saskatoon 1 and Saskatoon 2 sites. Within sites, treatments identified with different letters are significantly different at the α = 0.10 level of probability. Site differences (averaged across treatments) are listed at the top of each panel.