

# The effect of irrigation on nitrogen uptake and use efficiency of two willow (*Salix* spp.) biomass energy varieties

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Hangs, R. D., Schoenau, J. J., Van Rees, K. C. J. and Knight, J. D. 2012. **The effect of irrigation on nitrogen uptake and use efficiency of two willow (*Salix* spp.) biomass energy varieties.** *Can. J. Plant Sci.* **92**: 563–575. Nitrogen (N) fertilizers historically have been applied to support increased productivity of purpose-grown willow (*Salix* spp.) biomass energy plantations. However, a frequently observed lack of willow growth response to added fertilizer N is often attributed to poor fertilizer use efficiency. The objective of this study was to determine the effect of irrigation on the recovery of broadcast <sup>15</sup>N-labelled fertilizer, applied during the final year of a 3-yr rotation, by two willow varieties. A split-split-plot experiment was established on a fertile heavy clay soil in Saskatoon, SK, Canada, which consisted of two willow varieties (Charlie and SV1), three irrigation treatments (no irrigation, 75%, and 100% field capacity), and two fertilization treatments (1 × and 2 × the recommended fertilizer rate of 100:30:80:20 N:P:K:S; kg ha<sup>-1</sup>). Irrigation increased fertilizer N uptake by Charlie, but had no effect on the amount taken up by SV1, which was attributed to greater N use efficiency of SV1 compared with Charlie when irrigated. Eighty-two percent of the applied fertilizer N was accounted for in the following sinks: 43% in the soil (0–60 cm), 31% in the willow tissues (i.e., stems, leaves, stump, and roots), 7% in the LFH layer, and <1% in the non-crop vegetation; the balance (approximately 18%) was presumed lost primarily through denitrification from the poorly drained soil, but possibly some may have leached below the root zone as well. Although the willow varieties accessed only a portion of the applied fertilizer N during the year of application, the majority of the residual fertilizer N was conserved within the production system and, therefore, remained available for willow uptake in subsequent years.

**Key words:** Irrigation, <sup>15</sup>N-labelled fertilizer, nitrogen use efficiency, willow

Hangs, R. D., Schoenau, J. J., Van Rees, K. C. J. et Knight, J. D. 2012. **Effet de l'irrigation sur l'absorption et l'assimilation de l'azote par deux variétés de saule (*Salix* spp.) dont la biomasse sert à la production d'énergie.** *Can. J. Plant Sci.* **92**: 563–575. On recourt depuis toujours aux engrais azotés pour accroître la productivité des plantations de saule (*Salix* spp.) destinées à la production de biomasse pour l'énergie. Le fait que la croissance du saule ne réagisse pas à l'application de tels engrais est souvent attribué à une piètre assimilation de l'amendement. La présente étude devait établir l'incidence de l'irrigation sur la récupération d'un engrais répandu à la volée et marqué au <sup>15</sup>N, appliqué la dernière année d'un assolement de trois ans comprenant deux variétés de saule. Les auteurs ont procédé à une expérience à dispositif en tiroir sur un sol lourd mais fertile d'argile à Saskatoon, en Saskatchewan (Canada). L'expérience portait sur deux variétés de saule (Charlie et SV1), sur trois méthodes d'irrigation (aucune irrigation, 75 % et 100 % de la capacité au champ) et sur deux taux de fertilisation (1 × et 2 × le taux recommandé de 100:30:80:20 kg de N:P:K:S par hectare). L'irrigation a rehaussé l'absorption du N par Charlie, mais n'a eu aucun effet sur la quantité de N absorbée par SV1, ce qu'on attribue à une assimilation plus efficace de cet élément par la seconde variété, comparativement à la première, quand il y a irrigation. On a retrouvé 82 % du N appliqué dans les puits suivants : 43 % dans le sol (0 à 60 cm de profondeur), 31 % dans les tissus du saule (à savoir, tiges, feuilles, souche et racines), 7 % dans la couche LFH et < 1 % dans la végétation sauvage, le reste (environ 18 %), présume-t-on, ayant été perdu principalement à la suite de la dénitrification résultant du mauvais drainage du sol, bien qu'une partie ait pu migrer sous la zone racinaire par lixiviation. Bien que les deux variétés de saule n'aient accédé qu'à une partie de l'engrais appliqué l'année du traitement, la majeure partie du N résiduel est demeurée dans le système de production, de sorte qu'elle était encore disponible en vue de son absorption par les arbres les années subséquentes.

**Mots clés:** Irrigation, engrais marqué au <sup>15</sup>N, assimilation de l'azote, saule

Establishing purpose-grown willow (*Salix* spp.) plantations as a renewable dedicated bioenergy and bioproduct feedstock is advantageous for numerous reasons. Favourable characteristics include its easy propagation and fast-growing nature, along with important environmental benefits like providing a cleaner energy source

relative to fossil fuels, acting as an effective vegetation filter for environmentally harmful compounds, and increasing biodiversity within the agricultural landscape

**Abbreviations:** FC, field capacity; NUE, nitrogen use efficiency

(Sage and Robertson 1994; Reddersen 2001; Volk et al. 2006; Main et al. 2007). Nitrogen (N) is considered to be the principal soil nutrient influencing willow plantation productivity (Labrecque et al. 1998; Weih and Nordh 2005). This is not surprising given that N is a critical element in numerous plant components including amino acids, chlorophyll, coenzymes, enzymes, nucleic acids, nucleotides, and proteins (Mengel and Kirkby 2001). Consequently, inorganic N fertilizers have been used extensively for several decades, in attempts to promote the successful establishment and growth of planted willow. However, the reported growth response of numerous willow varieties to added fertilizer N when grown under field conditions has been inconsistent (Table 1), thereby precluding definitive relationships (i.e., calibrated fertilizer recommendations) between applied fertilizer N rates and subsequent willow biomass yields from being developed and applied universally.

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

Studies applying stable isotope-enriched fertilizers, such as  $^{15}\text{N}$ -labelled ammonium nitrate, to trace the fate of added N are rare within the short-rotation coppice willow literature. This is surprising given the well-documented efficacy of  $^{15}\text{N}$  in tracking the fate of

applied fertilizer N within traditional forestry plantations, regardless of whether the fertilizer is side-banded (Sadanandan Nambiar and Bowen 1986), broadcast across the soil surface and/or tree canopy (Staples et al. 1999; Bryan Dail et al. 2009), or placed adjacent to the outplanted seedling root plug within a controlled-release fertilizer bag (Hangs et al. 2003). The objective of this study was to determine the effect of irrigation on the fertilizer N uptake and use efficiency of  $^{15}\text{N}$ -labelled fertilizer, by two willow varieties, within a 3-yr-old willow plantation. To our knowledge, this is one of only a few studies that have used  $^{15}\text{N}$ -labelled fertilizer to examine fertilizer N dynamics within short-rotation willow plantations (Christersson 1987; Konecsni 2010; Quaye 2011), and the first to investigate the effect of irrigation on fertilizer N uptake by willow. It is well known that irrigation promotes enhanced recovery of applied fertilizer N by target species within conventional agricultural systems (Raun and Johnson 1999). We hypothesized that irrigating the willow would increase its growth within our semi-arid environment and, therefore, improve its fertilizer N uptake. Considering the manufacture and application of inorganic fertilizer N accounts for more than half of the non-renewable fossil fuel energy inputs for producing willow, in addition to a significant portion of production cost (Heller et al. 2003; McKenney et al. 2011), insights into fertilizer N dynamics that can be used to promote increased fertilizer N recovery and use efficiency by purpose-grown willow will certainly benefit both the environmental and economic “bottom lines”, which are necessary goals in promoting willow as a viable biomass energy alternative.

## MATERIALS AND METHODS

### Study Site and Willow Varieties

The data for this study were collected in 2010 from a 3-yr-old willow plantation, within the Canadian Wood Fibre Centre willow variety trial, located on the University of Saskatchewan campus in Saskatoon, Saskatchewan, Canada (UTM coordinates: 13U 389970 5776342). The following site and willow variety information was initially reported in Hangs et al. (2011). The soil is a heavy clay Orthic Vertisol of the Sutherland Association, developed on glacial lacustrine parent material, with a pH and electrical conductivity ( $\text{dS m}^{-1}$ ) of 7.1 and 0.33, respectively. The semi-arid temperate location receives on average 350 mm of annual precipitation (70% occurring from May to September) and has a mean annual temperature of  $2^\circ\text{C}$ , with approximately 112 frost-free days. The Agriculture Capability Classification rating of the soil is Class 2, with moderately severe limitations due to a lack of precipitation [Saskatchewan Centre for Soil Research (SCSR) 1978]. Prior to establishing the variety trial in 2006, the site was continuously cropped to a mixture of barley and oats. Pre- and post-planting site preparation included both

mechanical (e.g., deep tillage, light cultivation, mowing, and hand weeding) and chemical (linuron 1.7 kg a.i. ha<sup>-1</sup> and glyphosate 2.0 kg a.i. ha<sup>-1</sup>) treatments to control non-crop vegetation. The willow were planted using a 0.6 × 0.6 m grid spacing for each 30-m-long triple-row bed, with 2.0-m spacing between the beds (approximately 15 625 stems ha<sup>-1</sup>). Two willow varieties were planted: Charlie (*Salix alba* × *Salix glauca*) and SV1 (*Salix dasycadus*), which are the standard varieties for comparison (e.g., survival, yield, pest resistance, etc.) within Canada and the United States of America, respectively (D. Sidders, Canadian Forest Service, personal communication; Volk et al. 2006).

### Experimental Design

In the spring of 2008, prior to bud break, the 2-yr-old willows were coppiced. Three rates of both irrigation and fertilizer treatments were imposed on each bed, arranged in a split-split-plot experimental design (whole plot factor: variety; subplot factor: irrigation rate; and, sub-subplot factor: fertilizer rate), and replicated three times. Each experimental unit consisted of nine willow plants arranged in a 3 × 3 array separated by a 1.8-m buffer. The three irrigation treatments consisted of either no additional water added above rainfall, or drip irrigation used to maintain soil moisture at 75 or 100% field capacity (FC), measured using Watermark<sup>®</sup> soil moisture sensors installed within each plot (Irrometer Company, Inc., Riverside, CA, USA; Spaans and Baker 1992). A Campbell Scientific CR10X was used to monitor soil moisture and control irrigation timing. The amount of water received (including growing season precipitation) by the control, 75, and 100% field capacity plots after 3 yr was approximately 834, 2060, and 3286 mm, respectively. The three fertilization treatments included no fertilizer or fertilizer broadcast applied once annually in June over the 3-yr rotation either at the recommended rate (1 ×) or twice the recommended rate (2 ×). The recommended rate consisted of a balanced fertilizer blend of 100:30:80:20 (N:P:K:S; kg ha<sup>-1</sup>), which was intended to not only match the willow growth requirements, but also replenish nutrients exported when harvesting willow with anticipated annual biomass production of 15–22 Mg ha<sup>-1</sup> (Perttu 1993; Danfors et al. 1998; Adegbidi et al. 2001). The 2 × recommended rate was intended to test the upper limit of willow growth response to added fertilizer, when grown under optimal moisture conditions. Previous work in Sweden reported negligible nitrate leaching from heavily fertilized (i.e., up to 240 kg N ha<sup>-1</sup> applied annually) willow plantations after the first growing season (Dimitriou and Aronsson 2004). Consequently, leaching was not expected to be a problem in this study with the 2 × recommended fertilizer rate treatment of 200 kg N ha<sup>-1</sup> applied annually, because of the established willow root systems and heavy clay soil at this site. The fertilizers used to develop the two blended rates were ammonium nitrate, mono-

ammonium phosphate, ammonium sulfate, and potassium chloride. During each year of the 3-yr rotation, the irrigation and fertilizer treatments were initiated in early June to avoid exacerbating potential frost damage in late May and also to ensure the willow were vigorously growing, in order to increase the fertilizer use efficiency (Abrahamson et al. 2002). Likewise, irrigation ceased at the beginning of September, to prepare the willow for a possible early frost. At the end of the third growing season, following leaf fall, all stems (including branches) within each treatment plot were cut 5 cm above ground level using a brush saw, chipped, and dried to a constant weight for biomass measurement.

### Determining the Fate of Applied Fertilizer N

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

**Table 1. Reported growth response of willow (*Salix* spp.) to fertilizer N additions under field conditions**

Location	<i>Salix</i> species (variety)	Fertilizer rate(s) (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Year(s) assessed	Relative yield <sup>2</sup> (%)	Reference
Canada	<i>S. purpurea</i> × <i>S. miyabeana</i> (Saratoga), <i>S. sachalinensis</i> × <i>S. miyabeana</i> (Marcy), <i>S. viminalis</i> × <i>S. miyabeana</i> (Tully Champion), <i>S. dasyclados</i> × ? (India)	100	2	-20 to 44	Konecni (2010)
Denmark	<i>S. viminalis</i> (78-112), <i>S. viminalis</i> (78-183)	37.5 (year 1) 75 (years 2, 3)	1-3	-20 to 41	Mortensen et al. (1998)
England	<i>S. viminalis</i> (Bowles Hybrid)	60	2 and 4	7 to 26	Mitchell et al. (1992)
Estonia	<i>S. viminalis</i> (78021, 78101, 78112, 78183, 78195, 82007), <i>S. dasyclados</i> (81090)	60 (year 1) 160 (year 2) 170 (year 3)	1-3	-31 to 340	Heinsoo et al. (2002)
Estonia	<i>S. viminalis</i> (78183), <i>S. dasyclados</i> (81090)	60 (year 1) 160 (year 2) 170 (year 3)	2 and 3	186 to 206	Heinsoo et al. (2009)
Finland	<i>S.</i> × <i>dasyclados</i> (P6011), <i>S.</i> 'Aquatika' (V769)	50, 100, 150, 200	1-3	0 to 312	Hytönen (1994)
Finland	<i>S.</i> × <i>dasyclados</i> (P6011), <i>S.</i> 'Aquatika' (V769), <i>S.</i> 'Aquatika' (E4856)	100	1-3	-20 to 270	Hytönen (1995)
Germany	<i>S. viminalis</i>	50, 100	1-5 6-10	-37 to 73	Hofmann-Schielle et al. (1999)
Germany	<i>S. schwerinii</i> × <i>S. viminalis</i> (Tora)	40, 80	1-3	-8 to 1	Boehmel et al. (2008)
Ireland	<i>S.</i> × 'Aquatika Gigantea' (Korso)	45-250	1-6	7 to 9	McElroy and Dawson (1986)
Sweden	<i>S. viminalis</i> (78021, 78101, 78112, 78183), <i>S. dasyclados</i> (81090)	45, 60, 75, 90, 150, 180	1-4	-29 to 788	Alriksson (1997)
Sweden	<i>S. schwerinii</i> E. Wolf. × <i>S. viminalis</i> L. (Björn), <i>S.</i> <i>burjatica</i> Nasarow × <i>S. dasyclados</i> Wimm. (Gudrun), <i>S. viminalis</i> (Jorr), <i>S. dasyclados</i> (Loden), <i>S. schwerinii</i> × <i>S. viminalis</i> (Tora), ( <i>S. schwerinii</i> × <i>S. viminalis</i> ) × <i>S. viminalis</i> (Tordis)	100 (2nd year) 90 (3rd year)	1-3	78 to 142	Weih and Nordh (2005)
USA	<i>S. dasyclados</i> (SV1), <i>S. alba</i> (L.) (SA22), <i>S. alba</i> var. <i>sanquinea</i> (SA2), <i>S.</i> × <i>rubra</i> (SAM3), <i>S. purpurea</i> (L.) (SH3)	336	1-5	-7 to 175	Kopp et al. (1993)
USA	<i>S. purpurea</i> L. (SP3)	336	1-5	-17 to 54	Kopp et al. (1996)
USA	<i>S. eriocephala</i> × <i>S. eriocephala</i> (S25), <i>S. exigua</i> × <i>S. eriocephala</i> (S301), <i>S. eriocephala</i> × <i>S. eriocephala</i> (S546), <i>S. alba</i> var. <i>sanquinea</i> (SA2), <i>Salix dasyclados</i> (SV1)	100, 200, 300	2	-83 to 500	Ballard et al. (2000)
USA	<i>S. dasyclados</i> (SV1), <i>S. alba</i> (L.) (SA22), <i>S. alba</i> var. <i>sanquinea</i> (SA2), <i>S.</i> × <i>rubra</i> (SAM3), <i>S. purpurea</i> (L.) (SH3)	224	1-9	2 to 43	Adegbidi et al. (2001)
USA	<i>S. dasyclados</i> (SV1), <i>S. alba</i> var. <i>sanquinea</i> (SA2), <i>S. purpurea</i> (L.) (SH3)	336	1-10	-15 to 87	Kopp et al. (2001)
USA	<i>S. dasyclados</i> (SV1)	100, 200, 300	1-3	7 to 33	Adegbidi et al. (2003)
USA	<i>S. sachalinensis</i> (SX61), <i>S. discolor</i> (S365)	90	1	-4 to 79	Arevalo et al. (2005)
USA	<i>S. dasyclados</i> (SV1)	100	1-3	-18 to 33	Quaye et al. (2011)

<sup>2</sup>Measured oven-dried stem wood yield relative to willow grown in non-fertilized plots (e.g., -20 to 44 indicates the reported growth response to added fertilizer N ranged from a 20% decrease to a 44% increase in yield among treatments, relative to the control).

vegetation was sparse, due to extensive vegetation management; thus, all roots were assumed to be that of willow.

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

### Effect of Irrigation on Willow Stem Nitrogen Dynamics

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

### Statistical Analyses

The  $^{15}\text{N}$  enrichment data were subject to analysis of variance via a split-split-plot model using PROC MIXED in SAS (version 9.2; SAS Institute, Inc., Cary, NC). Willow variety was the whole plot factor, irrigation rate was the subplot factor, and fertilizer rate (nested within irrigation) was the sub-subplot factor. Means comparisons were performed using least significant differences (LSD; equivalent to Fisher's protected

LSD) at a significance level of 0.05, with groupings obtained using the pdmix800 SAS macro (Saxton 1998). Homogeneity of variances (Bartlett's test) and normality of distributions (PROC UNIVARIATE) of all data sets were checked prior to the analysis. No data transformations were necessary.

## RESULTS

### Fate of Applied Fertilizer Nitrogen

A summary of the analysis of variance, comparing the effects of fertilization rate and irrigation level on the recovery (i.e., as a percentage of total  $^{15}\text{N}$  applied) of broadcast  $^{15}\text{N}$ -labelled fertilizer from the various plant tissue and soil components, is presented in Table 2. Neither the willow variety nor the rate of fertilizer applied had any effect on the recovery of fertilizer N in this study. However, irrigation influenced the amount of fertilizer N recovered in some willow tissues and non-crop vegetation, along with recovery in the LFH layer and varying depths of the mineral soil profile (Table 2). Furthermore, the two willow varieties responded differently to irrigation. Specifically, irrigation resulted in greater fertilizer N accumulation in the stems of Charlie, but not in SV1 stems (Table 3). Likewise, there was no effect of irrigation on the amount of fertilizer N present in either the September leaves or the stump of either willow variety. The abscising leaves (November leaves) of both Charlie and SV1 growing without irrigation contained larger amounts of fertilizer N compared with the irrigated willow (Table 3). Conversely, the fine root fraction of both varieties and the coarse root fraction of Charlie within the irrigated plots accumulated more fertilizer N relative to the non-irrigated willow. The non-irrigated SV1 accumulated more fertilizer N in the coarse root fraction than the irrigated. Overall, irrigation increased the total recovery of applied fertilizer N by Charlie, but not SV1 (Table 3). The amount of fertilizer N acquired by non-crop vegetation was less in irrigated plots. Except for a larger accumulation of fertilizer N in the fine roots of SV1 within the 100% FC plots, the two irrigation rates had the same effect on fertilizer N accumulation among the plant tissues examined (Table 3).

Although there was no effect of irrigation on  $^{15}\text{N}$  fertilizer recovery from the soil to a 60-cm depth (i.e., LFH layer+mineral soil) in Charlie or SV1 plots, measured differences were apparent when examining the individual pools separately (Table 4). The LFH layer within the irrigated plots of both willow varieties contained more fertilizer N compared with the non-irrigated plots. More fertilizer N was present in the upper 15 cm of mineral soil within the Charlie plots, but no significant differences ( $P > 0.05$ ) were measured among the remaining depths (Table 4). Within the SV1 plots, fertilizer N accumulated in the 30- to 60-cm soil depth under the highest irrigation level. Otherwise, water supplied in excess of 75% had no effect on the

Table 2. Summary of analysis of variance comparing the effects of fertilization (fert) and irrigation (irrig) on the recovery of <sup>15</sup>N-labelled fertilizer, from various plant tissue and soil components, applied in plots growing the willow varieties Charlie and SV1

Effect	df	Plant										Soil						
		Leaves					Roots					Total willow recovery	Weeds	LFH	Depth (cm)			
		Sep.	Nov.	Stems	Stump	Fine (<2 mm)	Coarse (>2 mm)	willow recovery	Coarse (>2 mm)	Fine (<2 mm)	0-15				15-30	30-45	45-60	Total soil recovery
Variety	1	0.58	0.38	0.07	0.38	0.56	0.50	0.42	0.33	0.17	0.91	0.40	0.18	0.22	0.71			
Irrig	2	0.47	<b>0.04<sup>2</sup></b>	<b>0.03</b>	0.93	< <b>0.01</b>	0.87	0.69	<b>0.01</b>	< <b>0.01</b>	0.08	0.86	0.12	0.07	0.88			
Fert(Irrig)	2	0.48	0.52	0.79	0.41	0.33	0.85	0.54	0.39	0.42	0.20	0.87	0.45	0.30	0.56			
Variety × Irrig	2	0.06	0.12	< <b>0.01</b>	0.19	0.06	<b>0.02</b>	< <b>0.01</b>	0.10	0.56	<b>0.04</b>	0.07	< <b>0.01</b>	<b>0.01</b>	0.06			
Variety × Fert	1	0.42	0.60	0.67	0.97	0.36	0.24	0.28	0.46	0.60	0.56	0.83	0.73	0.26	0.89			
Variety × Irrig × Fert	2	0.76	0.33	0.62	0.06	0.43	0.83	0.11	0.47	0.71	0.69	0.48	0.53	0.66	0.63			

<sup>2</sup>Significant ( $P < 0.05$ ) effects are shown in bold.

recovery of applied fertilizer N in soil. Generally speaking, at the end of the growing season after application, 82% of the broadcast <sup>15</sup>N-labelled fertilizer was accounted for, with approximately 80% of the recovered fertilizer N present within the willow tissues, non-crop vegetation, LFH layer, and the upper 15 cm of mineral soil (Fig. 1).

### Effect of Irrigation on Willow Stem Nitrogen Dynamics

For both willow varieties, irrigation increased the NUE for all plant tissues, except for the September leaves of SV1 (Table 5). The highest irrigation level increased the NUE of the September leaves and fine roots of Charlie above that of the 75% FC level and the non-irrigated treatment; otherwise, there were no significant differences ( $P > 0.05$ ) in NUE for any tissues of either willow variety between the 75 and 100% FC irrigation level. The calculated NUE for the production of stem, stump, and fine root tissues was greater for SV1 compared with Charlie when irrigated (Table 5). The effect of irrigation on stem biomass and fertilizer N status of Charlie and SV1 is readily apparent in the vector nomogram when using their respective growth in non-irrigated plots as the reference normalized to 100 (Fig. 2). Given that there were no significant differences ( $P > 0.05$ ) between the two irrigation rates on measured stem biomass (Hangs et al. manuscript in preparation) or stem tissue fertilizer N content and concentration (data not shown) for both willow varieties, only the average irrigation response vector was drawn for each variety, in order to reduce clutter. The vector diagnosis reveals that when irrigated, Charlie increased stem growth and uptake of fertilizer N, but the fertilizer N concentration decreased compared with the willow grown in the non-irrigated plots (Fig. 2). This response vector represents a typical growth dilution response, indicating improved growing conditions (due to irrigation) that supported greater biomass gain relative to fertilizer N uptake, where N is sufficient and non-limiting (vector shift A; Timmer 1991). Specifically, irrigation increased stem biomass and fertilizer N uptake (i.e., <sup>15</sup>N content) by Charlie up to 133% and 80%, respectively, while decreasing the stem tissue fertilizer N concentration up to 35% (Fig. 2). Irrigating SV1 increased its biomass up to 124%; however, there was no change in fertilizer N uptake and up to 53% reduction in stem tissue fertilizer N concentration compared with non-irrigated plots (Fig. 2). Although the SV1 response vector is also classified as a growth dilution response to irrigation (where N is sufficient and non-limiting despite markedly increased growth), evidently SV1 is capable of supporting greater biomass production per unit of N when irrigated compared with Charlie.

**Table 3.** Mean ( $n=6$ ) percent recovery of broadcast  $^{15}\text{N}$ -labelled fertilizer by two willow bioenergy varieties and non-crop vegetation grown in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity (FC)

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

*a-c* Within each column, values having the same letter are not significantly different ( $P > 0.05$ ) using LSD.

**DISCUSSION**

**Fate of Applied Fertilizer Nitrogen**

Approximately one-third of the  $^{15}\text{N}$ -labelled fertilizer was taken up by the target willow varieties (Table 3), which is not only comparable with that reported for annual field crops ( $\approx 30\%$ ; Raun and Johnson 1999), but also represents a much greater uptake by willow than is reported elsewhere (0.39 to 10.6% recovery after 2 yr; Konecni 2010). Unlike the Konecni (2010) study, where the  $^{15}\text{N}$ -labelled fertilizer was applied during the establishment year of a willow plantation, we applied the  $^{15}\text{N}$ -labelled fertilizer to a 2-yr-old plantation, with a 4-yr-old root system. The greater fertilizer N accumulation by willow observed in this study is likely due to the increased N requirement of older willow, along with the ability of its more extensive root system to capture greater amounts of applied fertilizer N. We hypothesized that irrigation would increase fertilizer N recovery by both willow varieties; this was true for Charlie, but not for SV1 (Table 3). It is interesting to note that while irrigation increased both fine and coarse root biomass for both varieties, the ratio of fine roots to coarse roots only increased with SV1 (up to 132%; data not shown). This relative increase in fine root proportion would contribute to increased root surface area and, therefore, greater fertilizer N assimilation potential. Additionally, SV1 had almost 30% more fine roots than Charlie in the 100% FC plots, indicating a different carbon allocation

pattern between the varieties under ideal moisture conditions. Consequently, SV1 was not lacking in its capacity to sequester fertilizer N with its abundant fine roots; instead, the observed difference in fertilizer N uptake between the willow varieties could be attributed to the differences in their respective NUE. Tharakan et al. (2005) examined the NUE of 30 willow varieties, after a three-year rotation in central New York, and reported SV1 to have the greatest NUE of all varieties tested. Irrigation increased the NUE of both willow varieties in our study; nonetheless, the ability to sustain increased biomass productivity when irrigated, without a concomitant increase in N uptake, was more prominent with SV1 (Table 5 and Fig. 2). Arguably, the magnitude of increased NUE with irrigation, for both willow varieties, would likely have been more pronounced if the site did not receive an anomalously large amount of precipitation (70% more than the 100-yr average) during the third growing season.

Another possible mechanism for the different fertilizer N contents between varieties could be inherent differences in their  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N root uptake capacity. Differences in N uptake rates among willow varieties have been reported (Ericsson 1981); thus, observed differences in fertilizer N accumulation between Charlie and SV1 could be partly explained by dissimilar root physiology. Moreover, symbiotic relationships with ectomycorrhizal fungi can increase the nutrient uptake capacity of willow, although the

**Table 4.** Mean ( $n=6$ ) percent recovery of broadcasted  $^{15}\text{N}$ -labelled fertilizer in soil, supporting the growth of two willow bioenergy species, in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity (FC)

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

*a-c* Within each column, values having the same letter are not significantly different ( $P > 0.05$ ) using LSD.

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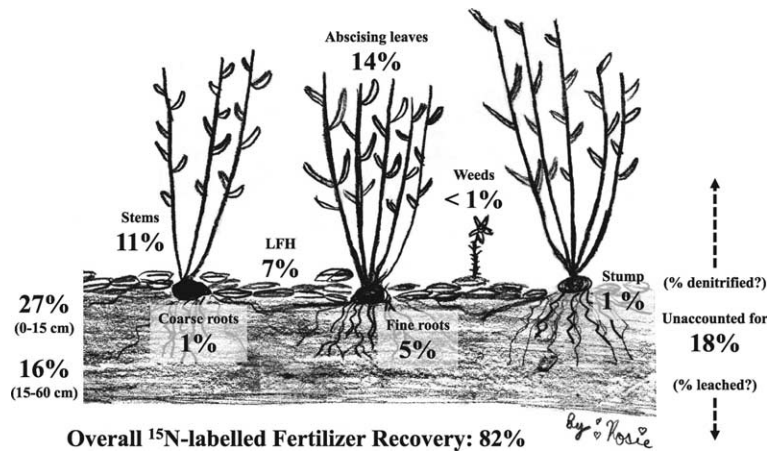


Fig. 1. Overall fate of broadcasted  $^{15}\text{N}$ -labelled fertilizer after the growing season of application.

intensity of mycorrhizal colonization and utility for increasing nutrient uptake is variable among varieties (Jones et al. 1991; Tibbett and Sanders 2002) and can be either enhanced or inhibited with fertilizer N additions (Baum et al. 2002). Hofmann-Schielle et al. (1999) suggest that enhanced carbon allocation to fine root development in willow is a consequence of inadequate nutrient uptake, resulting from insufficient ectomycorrhizal roots. Perhaps the increased NUE observed with SV1 is an adaptation to its relatively poor  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N root uptake capacity, in spite of its abundant fine roots, and warrants further investigation.

There was a substantial amount of fertilizer N present in both September and November leaves for both varieties (Table 3 and Fig. 1). Irrigation stimulated earlier initiation of leaf tissue N resorption prior to abscission, resulting in less N lost in fallen leaves compared with non-irrigated willow. This effect was visually apparent each fall when walking through the plots, evidenced by a marked difference in colour change and magnitude of leaf fall with increasing irrigation level. Contrariwise, irrigation was expected to delay leaf senescence and possibly even increase the susceptibility to winter damage from an early frost episode; however, each year the initiation of leaf senescence began with the

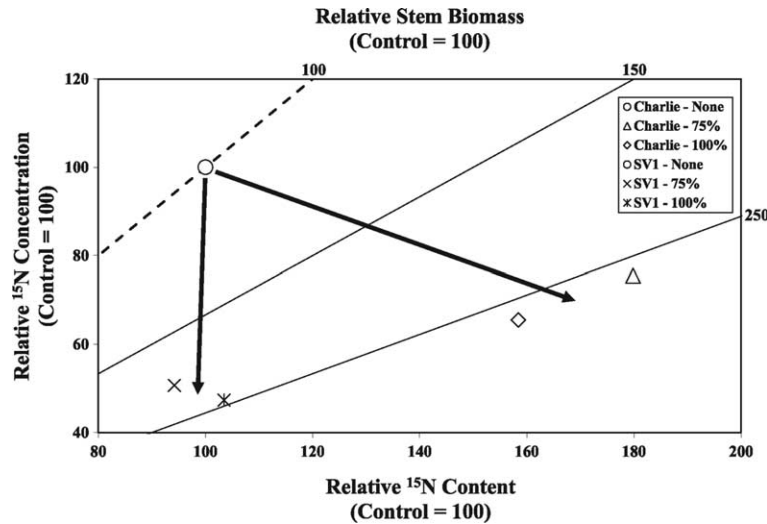
100% FC plots and ended with the non-irrigated plots. The measured nutrient resorption efficiency (i.e., percentage of nutrients resorbed from senescing leaves; Yuan et al. 2005) reflected this with up to 49% of the fertilizer N resorbed from the September leaves in the 100% FC plots compared with as low as 21% in the non-irrigated plots (data not shown). Important sinks for this seasonally translocated N consists of the willow stems, stump, and root system, which support stem regrowth the following year. After harvest, the willow stump and root system are often considered to be key suppliers of remobilized N supporting the initial growth of new stems (Karp et al. 2011); however, only a small proportion of the accumulated fertilizer N was found in the perennial stump tissue (Table 3). Clearly, the previously dormant axillary buds located on the stump play a critical role in the regeneration success of these coppice systems (Sennerby-Forsse and Zsuffa 1995). Nevertheless, given the partitioning of fertilizer N predominantly in the willow root system at the end of the growing season (Table 3), it appears that the reserve N stored in the root system is a primary source of remobilized N that will be translocated via the xylem to developing stem tissues (Dickmann and Pregitzer 1992). The fine roots accumulated up to 664% more fertilizer

Table 5. Mean ( $n=6$ ) N use efficiency ( $\text{g oven-dry biomass g N}^{-1}$ ) of two willow bioenergy varieties grown in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity (FC)

Variety	Irrigation level	September leaves	Stems	Stump	Roots	
					Fine (< 2 mm)	Coarse (> 2 mm)
Charlie	None	51.0 <sup>b</sup>	228.0 <sup>c</sup>	290.7 <sup>d</sup>	64.3 <sup>c</sup>	168.5 <sup>b</sup>
	75% FC	50.5 <sup>b</sup>	259.2 <sup>b</sup>	336.0 <sup>cd</sup>	88.5 <sup>b</sup>	229.9 <sup>a</sup>
	100% FC	59.8 <sup>a</sup>	285.9 <sup>b</sup>	370.2 <sup>bc</sup>	100.3 <sup>a</sup>	228.7 <sup>a</sup>
SV1	None	43.1 <sup>b</sup>	257.8 <sup>bc</sup>	291.3 <sup>d</sup>	68.4 <sup>c</sup>	165.6 <sup>b</sup>
	75% FC	43.4 <sup>b</sup>	322.5 <sup>a</sup>	399.8 <sup>ab</sup>	106.8 <sup>a</sup>	221.7 <sup>a</sup>
	100% FC	42.7 <sup>b</sup>	317.4 <sup>a</sup>	414.1 <sup>a</sup>	108.2 <sup>a</sup>	214.5 <sup>a</sup>

a-d Within each column, values having the same letter are not significantly different ( $P > 0.05$ ) using LSD.





**Fig. 2.** Vector nomogram of relative leaf biomass, <sup>15</sup>N content, and <sup>15</sup>N concentration in stems of two willow bioenergy varieties grown in plots without irrigation (None) or irrigated to maintain soil at either 75 or 100% field capacity. Biomass and <sup>15</sup>N status of seedlings grown in control plots (i.e., no irrigation) served as the reference and were normalized to 100. Diagonal isolines correspond to the relative biomass. Note: there were no significant differences ( $P > 0.05$ ) between the two irrigation rates on measured stem biomass or stem tissue fertilizer N content and concentration for both willow varieties, so the average response vector was drawn for each variety to reduce clutter.

N than the coarse roots (Table 3), and are likely the greatest source of this remobilized N supply. The larger accumulation of fertilizer N by the fine roots can be attributed to greater fertilizer N concentration and biomass (data not shown).

Woody crop fertilizer N use efficiency is reduced by losses to non-target sinks, either temporarily (e.g., non-crop vegetation and microbial immobilization) or permanently (e.g., leached and denitrified), regardless of fertilizer N form or method of application (Preston and Mead 1994; Staples et al. 1999; Hangs et al. 2003). Adequate weed control is imperative for supporting optimal fertilizer N recovery by willow, especially in these intensively managed systems where soil moisture and nutrient conditions are optimal for non-crop plant growth. The level of weed control maintained each season was excellent, as evidenced by the small amount of applied fertilizer N lost to non-crop vegetation (Table 3). Incomplete canopy closure within the non-irrigated plots led to increased weed production and resulted in greater accumulation of fertilizer N by non-crop vegetation. As previously mentioned, the LFH layer within each plot was sampled prior to leaf fall. It is assumed that the recovered fertilizer N in the LFH layer was immobilized by microbes decomposing the litter during the growing season (Preston et al. 1990). Irrigation increased the fertilizer N recovered within the LFH layer (Table 4), reflecting elevated microbial activity under the more favourable conditions. The largest sink for applied fertilizer N was the mineral soil, accounting for approximately 43% of total recovery (Table 4), 93% of which was present within the upper 45 cm where willow roots

predominate (Rytter and Hansson 1996). As expected, the established root system and heavy clay soil at this site minimized leaching below this depth, although willow has been found to root as deep as 1 m even in heavy clay soils (Alriksson et al. 1997; Adegbedi et al. 2001). Irrigation reduced the fertilizer N recovered in the upper 15 cm within the Charlie plots (Table 4), which corresponds well with the measured increased fertilizer N uptake by Charlie when irrigated (Table 3). Conversely, given the 29% greater SV1 biomass compared with Charlie in plots maintained at 100% FC (Hangs et al. manuscript in preparation), the presence of more fertilizer N at depth in the SV1 plots is likely due to its higher NUE, and hence lower fertilizer N uptake by SV1 when irrigated (Table 5), which allowed more fertilizer N to be leached. Undoubtedly, some of the unaccounted for fertilizer N was deeper than the 60-cm depth sampled, but most was assumed to be lost through denitrification considering the imperfectly drained soil at the site. Anoxic microsites can frequently occur (even at FC) within the abundant micropores of heavy clay soil (Colbourn 1988). van der Salm et al. (2007) suggests approximately 25% of fertilizer N applied to heavy clay agricultural soils is lost from the system, of which 90 and 10% is through denitrification and leaching, respectively.

Within the agronomic literature, the amount of <sup>15</sup>N-labelled fertilizer recovered by the target crop typically increases with increasing rates of fertilizer N addition, with or without irrigation (Pilbeam et al. 1997; Tran and Tremblay 2000; Khelil et al. 2005). The absence of a fertilizer rate effect (or any interaction thereof) on the

amount of fertilizer N recovered by the willow in this study (Table 2), is probably due to the fertile Class 2 agricultural soil at the study site (SCSR 1978), which supplied sufficient N for willow growth. Additionally, one of the many advantageous characteristics of purpose-grown willow for use in different land reclamation, phytoremediation, bioengineering, and agroforestry applications is its relatively low nutrient demanding nature (Tharakan et al. 2005; Kuzovkina and Volk 2009). The usefulness of a low nutrient requirement is perhaps best demonstrated within a biomass energy context. In particular, notwithstanding the large varietal differences in N requirement among willow varieties (Weih and Nordh 2002), relative to other annual and perennial bioenergy crops (e.g., miscanthus, switchgrass, maize, and oilseed rape) that require fertilizer N to meet production goals, often willow can be successfully grown without added N, thereby providing a higher net energy return (Boehmel et al. 2008).

### Long-term Recovery of Applied Fertilizer Nitrogen

After accounting for the fertilizer N lost from the system through stem harvesting, along with presumed denitrification and leaching, the remaining sinks containing approximately 71% of the applied N included the LFH layer (including abscised willow leaves and non-crop vegetation residue), mineral soil, and the willow stumps and roots (Fig. 1). Litterfall is a primary mechanism for nutrient cycling within most ecosystems (Hughes and Fahey 1994) and in willow, foliar N levels in late August can account for as much as 64% of the plant N despite representing only 20% of its mass (Hytönen 1995). Although willow will resorb up to 50% of this N during leaf senescence (von Fircks et al. 2001), a substantial amount of fertilizer N remained in abscising leaves (Fig. 1). Approximately one-third of the leaf litter N will be released during the first year of decomposition, with the majority liberated after 3 yr (Šlapokas and Granhall 1991 a, b). Up to 42% of this released N is believed to enter the stable humus pool of N after 4 yr, thereby representing long-term retention within the system (Šlapokas 1991). A similar N release pattern is expected for the fertilizer N immobilized within the existing LFH layer (prior to leaf fall) and the non-crop vegetation residue given the comparable tissue N concentrations among them (data not shown). Tissue N concentration is a principal determinant of litter quality and a strong factor controlling litter decomposition rates and nutrient cycling (Weih and Nordh 2002). Although we did not differentiate between the inorganic and organic pools of fertilizer N recovered in the soil, Aronsson (2001) found that approximately 97% of the fertilizer N applied to a loamy sand soil was immobilized within the organic N pool after one growing season. Ostensibly then, the vast majority of fertilizer N recovered in our fertile clay soil should be similarly retained within the organic N pool, with up to 8% remineralized annually (Preston and Mead 1994). Unlike perennial willow

stumps that continually regenerate stems over multiple rotations, willow fine roots have rapid turnover and decomposition rates (Rytter and Hansson 1996; Rytter 1999). Fine roots contribute significantly to N cycling within a plantation as they alternate between N source and sink, thus preserving N in the system (Rytter and Rytter 1998; Puttsepp et al. 2007), which is manifested by the larger amount of fertilizer N recovered in the fine roots compared with the coarse roots (Fig. 1).

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

### CONCLUSION

Purpose-grown willow represents a feasible bioenergy feedstock; however, there needs to be sufficient biomass production to support the environmental and economic viability of these plantations. Fertilizer N is often used to achieve this goal, but is associated with substantial cost, along with the possibility of deleterious offsite environmental effects. Consequently, efficient use of fertilizer N, in the year of application and beyond, is essential for minimizing any environmental impact and maximizing economic returns. In this study, irrigation increased fertilizer N accumulation by the willow variety Charlie, but not SV1, which was attributed to greater NUE of irrigated SV1 compared with Charlie. Irrigation also stimulated increased resorption of fertilizer N in leaves prior to abscission for both willow varieties, resulting in greater N storage available for supporting new stem growth the following year. Eighty-two percent of the applied fertilizer N was recovered in the willow tissues, LFH layer, non-crop vegetation, and soil (0–60 cm), with the balance presumably lost primarily through denitrification from the poorly drained soil. The long-term role fertilizer N plays within the N cycle of willow plantations remains to be seen and will

undoubtedly require the use of  $^{15}\text{N}$ -labelled fertilizers to separate the contribution of remineralized fertilizer N from other N sources, along with quantifying subsequent remineralized fertilizer N losses due to denitrification and/or leaching. Further research is needed to track the fate of fertilizer N over a longer time frame (i.e., multiple rotations) to better understand fertilizer N dynamics within these intensively managed systems and to help advance willow as a viable biomass energy alternative in an evolving global bioenergy sector.

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