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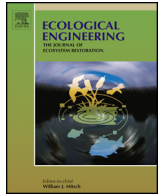
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Willows for the treatment of municipal wastewater: Performance under different irrigation rates



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ABSTRACT

Willow cropping is increasingly spreading worldwide for various purposes including vegetation filter. Willow plantations are highly nutrient-demanding and site fertilization may be required to maintain soil fertility and nutrient balance. In this context, municipal wastewater could be a valuable source of nutrients (especially N and P) and water for plant growth. The aim of this study was to assess the performance of willows to recycle municipal wastewater supplied at different rates. In particular, we sought to evaluate the quality of groundwater water collected under willow and assess the effect of wastewater supply on willow growth. Irrigation with wastewater had a positive effect on willow growth and biomass yield. It was also estimated that willows were able to remove nearly 90% of the N and 85% of the P found in the wastewater. This study shows that a willow vegetation filter is very efficient at removing nutrients found in wastewater.

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1. Introduction

Willow cropping is increasingly spreading worldwide for various purposes including bioenergy (Volk et al., 2006), phytoremediation (Wieshammer et al., 2007), erosion control (Wilkinson, 1999), rehabilitation of degraded soils (Vandenhove et al., 2001), streambank restoration (Schaff et al., 2003), and other mitigation purposes (Kuzovkina and Quigley, 2005). The use of willow for recovery/recycling of nutrients from waste is another option that has been shown to be very attractive for it may enhance the biomass yield while reducing environment pollution risks (Börjesson and Berndes, 2006).

Willow plantations are highly nutrient-demanding and in some cases to respond well to fertile sites (Mitchell et al., 1999). Thus, fertilization is very important to compensate the removal of the stem biomass at harvest and thereby maintain soil fertility and nutrient balance (Adegbi et al., 2001). Municipal wastewater, even when pre-treated, is a valuable source of nutrients (mainly nitrogen and phosphorous) and water for plants (Perttu, 1999) and does not pose much sanitary risk especially when used on

non-food, non-fodder crops (Hasselgren, 1998). Thus, when using pre-treated municipal wastewater on willows, the biomass yield of the stand, and thereby the economic value of the harvestable biomass, normally increase due to extra nutrient and water supply to plants (Rosenqvist et al., 1997; Dimitriou and Rosenqvist, 2011). Moreover, willow stands may significantly reduce the nitrogen and phosphorous concentrations in the wastewater either by direct root uptake or by other mechanisms (e.g., denitrification) (Aronsson and Perttu, 2001). For instance, it has been shown that the root systems of mature willow stands can take up 75–95% of the nitrogen and phosphorus in the wastewater (Börjesson, 1999). Therefore, irrigating willows with municipal wastewater can provide substantial yield increases without considerably increasing the risk of groundwater pollution and eutrophication of water bodies.

Despite a relatively large body of information concerning the use of willows as vegetation filter technology it mostly originates from few northern European countries (chiefly Sweden, Estonia and Denmark) and information is scarce from other temperate regions where willow is successfully grown. In general, the performance of a vegetation filter system depends on several properties of the crop (e.g., water demands and evapotranspiration rates, nutrient-use efficiency) that vary broadly among different species and within the same species among location. Eastern Canada is one of the regions in the world where willow crop shows very high levels of biomass yields both in the short- (Labrecque and Teodorescu, 2005; Volk et al., 2011) and the long-term periods (Guidi Nissim et al., 2013). Since evapotranspiration and nutrient

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uptake are linked to a great extent to biomass yield, is likely that eastern Canada willow crops would show very high performance as vegetation filters. The aim of the current study was to assess the performance of willows to recycle municipal wastewater supplied at different rates on a highly-productive site and in particular (i) to evaluate the quality of groundwater collected under willow stands and how it compares to legal limit values; (ii) to assess the impact of wastewater supply on willow aboveground biomass yield.

2. Materials and methods

2.1. Study site

The trial was carried out on a site in Saint-Roch-de-l'Achigan (45°50' 50"N – 73° 38'27"W), 55 km northeast of Montreal (Quebec), Canada. The region has a humid continental climate characterized by wide seasonal temperature variations, warm, humid summers and cold winters. According to the nearest weather station (Mascouche, Quebec; 45°45'N; 73°36'W; ~11 km), the average annual temperature for the 1981–2010 period was 6.4 °C, and average annual precipitation was 998 mm, with 40% falling during the growing season (Environment Canada 2014). The average number of degree-days (>5 °C) is 2100, and the growing season lasts approximately 180 days. The experimental field was formerly used for conventional agricultural crop (maize). At the beginning of the trial, soil texture was characterized at two depths and determined to be sandy loam in the top layer (0–20 cm) and loamy sand in the lower layer (20–40 cm) (Table 1). Both organic matter content and nutrient concentrations (including N, P, K, Ca and Mg) were higher in the top soil layer, whereas pH was slightly lower in the top layer than in the 20–40 cm layer.

2.2. Site preparation, irrigation system setup and willow planting

In spring 2008, prior to planting the site was mowed and the ground ploughed with a rotary tiller to a depth of 0.15 m. The experimental site was then planted at density of about 16,000 plants per hectare with the willow cultivar *Salix miyabeana* SX67. In order to prevent weed development, a pre-emergence residual herbicide mix (2.30 kg ha⁻¹ Devrinol and 0.37 kg ha⁻¹ Simazine) was applied. During the first growing season (2008), the field was weeded between willow rows with a vibra-shank cultivator. In autumn 2008, all willows were cut back to allow the development of a denser stand canopy.

In spring 2009, a 0.72 ha area was delineated and laid out for the trial in a strip-plot design. A sub-irrigation system was installed between willow rows at a depth of 0.30 m. Wastewater was supplied through a 1.5 km long hose linking the Saint-Roch-de-

l'Achigan's effluent treatment facility to the willow plantation. A filtration system was installed at the pump outlet to remove coarse particles. Flowmeters, power-supplied by an electronic solar energy panel, were installed and programmed to deliver the scheduled volume of wastewater for each treatment, and to control irrigation levels throughout the growing season. The main chemical and physical characteristics of the irrigation wastewater are shown in Table 2.

2.3. Irrigation and fertilization treatments

The strip-block experimental setup comprised 4 blocks within which 2 factors were randomized: irrigation (main plot factor; 4 levels, D0, D1, D2, D3) and fertilization (sub-plot factor; 2 levels, fertilized/unfertilized), to constitute 32 plots. Each plot had a surface of 225 m² and contained 10 rows with approximately 35 plants per row.

Four treatments corresponding to four wastewater doses, D1, D2 and D3, as well as D0 (the latter with no irrigation, i.e., control), were scheduled, and each treatment was applied along ten willow rows (Table 3). During the first 2-years rotation (2009–2010), we supplied four wastewater irrigation doses as follows: 0 mm (D0), 300 mm (D1), 393 mm (D2), 584 mm (D3) in 2009 (128 days of irrigation) and 0 mm (D0), 414 mm (D1), 487 mm (D2), 794 mm (D3) in 2010 (150 days of irrigation) which corresponded in both years to an increase of wastewater supply of about 33% between treatments. During the second 2-year rotation (2011–2012) the irrigation rates were: 0 mm (D0), 185 mm (D1), 386 mm (D2), 634 mm (D3) in 2011 (134 days of irrigation) and 0 mm (D0), 302 mm (D1), 601 mm (D2), 926 mm (D3) in 2012 (135 days of irrigation) which corresponded to an increase of wastewater supply between treatments of about 33% in the first year and 50% in the second year.

In addition to the wastewater treatment, a fertilization treatment (fertilized or not) was also applied (i.e., 100 kg ha⁻¹ of N and 60 kg ha⁻¹ of P), as illustrated in Table 3. Fertilization was performed after each harvest (i.e., 2009 and 2011), in two steps, i.e., 30% of the total amount of nitrogen was applied at the end of May and the remaining 70% at the end of June.

2.4. Monitoring equipment setup

During the late spring of 2009, one groundwater sampler (Model 1900 Soil moisture Equipment Corp.) was set out in the center of each plot (on the 5th row) according to the manufacturer's specifications. Boreholes were drilled using a manual auger, and soil water samplers were inserted into the ground to an

Table 1
Soil physico-chemical properties of the study site.

Parameters	Units	Soil depth (cm)	
		0–20	20–40
Sand	wt%	76	79
Silt	wt%	15	13
Clay	wt%	9	8
Texture		Sandy loam	Loamy sand
Organic matter	wt%	2.57	1.99
Total N	wt%	0.12	0.09
pH		6.69	6.74
Available P	mg kg ⁻¹	87.5	72
Exchangeable K	mg kg ⁻¹	58.7	46.4
Exchangeable Ca	mg kg ⁻¹	1189	962
Exchangeable Mg	mg kg ⁻¹	70.3	56
Exchangeable Na	mg kg ⁻¹	5.2	4.2

Table 2
Physico-chemical properties of wastewater used for irrigation from 2009 to 2012.

Parameters	Units	2009–2010	2011–2012
pH	–	8.04	8.11
Conductivity	uS cm ⁻¹	1269	977
Total N	mg L ⁻¹	29.7	20.2
NH ₄ -N	mg L ⁻¹	20.8	13.9
NO ₃ -N	mg L ⁻¹	0.2	0.02
Organic N	mg L ⁻¹	8.7	6.3
Total reactive P	mg L ⁻¹	1.17	0.11
Soluble reactive P ¹	mg L ⁻¹	1.08	0.1
Available K	mg L ⁻¹	11.4	10.6
Available Ca	mg L ⁻¹	38.4	24.5
Available Mg	mg L ⁻¹	22.1	9.7

Mainly PO₄-P (Jones and Reynolds, 2006).

Table 3

Amount of water and N and P added through irrigation and fertilization each year from 2008 to 2012.

Year	Treatments			Water input (mm)		N loads (kg ha ⁻¹ yr ⁻¹)		P loads (kg ha ⁻¹ yr ⁻¹)	
	Irrigation (mm)	Fertilization (kg ha ⁻¹)		Rainfall	Irrigation + rainfall	Through irrigation	Total	Through irrigation	Total
		N	P						
2008	–	–	–	638	–	–	–	–	–
2009	D0 = 0	0	0	652	652	–	–	–	–
	D1 = 300				952	89	89	3.5	3.5
	D2 = 393				1045	117	117	4.6	4.6
	D3 = 584				1236	173	173	6.8	6.8
	D0 = 0	100	60	652	652	–	100	–	60.0
	D1 = 300				952	89	189	3.5	63.5
	D2 = 393				1045	117	217	4.6	64.6
	D3 = 584				1236	173	273	6.8	66.8
2010	D0 = 0	0	0	771	771	–	–	–	–
	D1 = 414				1185	123	123	4.8	4.8
	D2 = 469				1240	139	139	5.5	5.5
	D3 = 794				1565	236	236	9.3	9.3
2011	D0 = 0	100	60	829	829	–	–	–	–
	D1 = 185				1014	37	37	0.2	0.2
	D2 = 386				1215	78	78	0.4	0.4
	D3 = 634				1463	128	128	0.7	0.7
	D0 = 0	0	0	829	829	–	100	–	60.0
	D1 = 185				1014	37	137	0.2	60.2
	D2 = 386				1215	78	178	0.4	60.4
	D3 = 634				1463	128	228	0.7	60.7
2012	D0 = 0			698	698	–	–	–	–
	D1 = 302				1000	61	61	0.3	0.3
	D2 = 601				1299	121	121	0.7	0.7
	D3 = 926				1624	187	187	1.0	1.0
Annual average									
	D0	UF ^a					0		0
		F ^b					50		30
	D1	UF					78		2
		F					127		32
	D2	UF					114		3
		F					164		33
	D3	UF					181		4
		F					231		34

^a Unfertilized.^b Fertilized.

average depth of 0.80 m. In total, thirty-two water samplers were installed.

2.5. Sampling and measurement

2.5.1. Soil quality

Soil chemical properties were assessed at the end of each rotation cycle (i.e., 2010 and 2012). Soil samples were taken with a soil auger 7 cm in diameter at depths of 0–20 cm and 20–40 cm. Each soil sample was prepared by pooling four soil subsamples at two different depth layers in each plot ($n = 32$). NO₃-N and NH₄-N were determined by extraction in KCl solution (Maynard and Kalra, 1993). Available P was determined following the Mehlich III method using a Lachat Flow Injection Analyzer (Tran and Simard, 1993) whereas Inductively Coupled Atomic Emission Spectrometry (ICP-AES) was used to determine the concentrations of exchangeable Ca, K, and Mg. Organic matter concentration was determined by the weight loss-on-ignition method (Schulte and Hopkins, 1996).

2.5.2. Water quality

At the beginning of each rotation cycle (May 2009–May 2011), wastewater samples were collected at the treatment facility to

determine nutrient concentrations, whereas groundwater was collected periodically starting in spring 2010. Since the installation of groundwater samplers may cause significant disturbance to the soil (MacDonald et al., 2008) a stabilization period of a minimum of six months is recommended before using them. For this reason, the soil solution was collected starting the second year after planting (i.e., spring 2010). Sampling was performed four times each year, covering most of the plant's growing season. Samples of both irrigation and groundwater were analysed for total N, NH₄-N, NO₃-N, total P and available P with a flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI, USA).

One objective of this study was to assess how soil solution concentrations of NO₃-N and PO₄-P following pre-treated wastewater application would compare with Canada's and Quebec's regulations in this matter. However, neither government has regulations concerning nutrient concentrations of drainage or groundwater. Hence, we decided to compare NO₃-N and PO₄-P concentrations of water samples collected from groundwater samplers with thresholds from regulations applicable to drinking or surface water. The Quebec Environmental Quality Act (Gouvernement du Québec, 2011) stipulates that NO₃-N concentrations in drinking water should not exceed 10 ppm (10 mg L⁻¹).

Similarly, the Federal-Provincial-Territorial Committee on Drinking Water (Health Canada, 2014) recommends that NO₃-N concentrations in drinking water should not exceed 10 ppm (10 mg L⁻¹). Regarding phosphates, Quebec's provincial norms indicate that PO₄-P concentrations in surface water should not exceed 0.03 ppm (30 µg L⁻¹) (MDDEFP, 2013). Thus, in our study, concentrations below 10 ppm (10 mg L⁻¹) of NO₃-N and below 0.03 ppm (30 µg L⁻¹) of PO₄-P were considered acceptable with respect to Quebec's provincial regulation. Currently, in Canada no regulations exist regarding total N and NH₄-N applicable to drinking or surface waters.

2.5.3. Growth measurement and biomass assessment

The growth of willows in response to the wastewater and fertilization treatments was estimated by measuring the height and basal diameter of the main stem as well as the number of stems per stool at the end of each rotation after leaf shedding on six living plants per plot, randomly chosen. Moreover, each selected plant was harvested and weighed in the field using an electronic scale. To evaluate dry matter of willow aboveground biomass, subsamples of aboveground fresh biomass were brought to the laboratory, where they were oven dried at 80 °C to constant weight for biomass estimation. Productivity (oven dried Mg per hectare – Mg ha⁻¹ yr⁻¹) was calculated by taking into account the plantation density re-evaluated at the end of the rotation cycle.

2.8. Data analysis

All collected data were analysed using two-way ANOVA. Post-hoc comparisons (Tukey HSD and *t*-test) were made to contrast the levels of the independent variables, and differences were deemed significant when *p* ≤ 0.05. All analyzes were performed using JMP 8.0 (SAS Institute, Cary, NC).

3. Results

3.1. Plant growth

Irrigation with wastewater had in general a positive effect on most willow growth parameters in both rotation cycles (Table 4). In particular, plants supplied with the highest wastewater rates (D2 and D3) showed greater stem diameter and height compared to the control (D0) and the D1 treatments. However, D2 and D3 willows had on average less shoots per stool, at least during the first rotation. Furthermore, biomass yield was significantly affected by the wastewater application. In the first rotation, the biomass of D3

treatment was significantly higher (i.e., 23.5 odt ha⁻¹ yr⁻¹) than other wastewater treatments and the control (22% higher than D2, 41% higher than D1 and 98% higher in D0, respectively). During the second rotation, D3 and D2 treatment had the highest biomass yield (average 18.4 odt ha⁻¹ yr⁻¹). These values were significantly higher than D1 and control (average 10.5 odt ha⁻¹ yr⁻¹). Fertilization also had a positive effect on biomass yield but only during the first rotation, after which both treatments were not significantly different.

3.2. Decontamination potential

The results regarding N concentrations in groundwater are shown in Fig. 1. The dynamic of the three main nitrogen forms was different. NO₃-N concentration (Fig. 1A) in groundwater was in general higher in comparison to its initial concentration (i.e., 0.2 mg L⁻¹) in the wastewater regardless of the irrigation and fertilization treatment. This was particularly evident during the second year of the first rotation (2010) and the first year of the second rotation (2011). However NO₃-N concentration was always low enough to meet Quebec's limit values (i.e., 10 mg L⁻¹). Ammonium (NH₄-N) concentration in groundwater was very low compared to the input wastewater levels throughout the whole trial (Fig. 1B). Finally total nitrogen concentration in groundwater was also very low in comparison to the wastewater in all treatments with very few exceptions during the first rotation and the first year of the second rotation (Fig. 1C).

The patterns of phosphate (PO₄-P) and total phosphorous (P) concentrations are shown in Fig. 2A and B. PO₄-P concentration in groundwater was very high (although lower than in wastewater [SRP=0.11 mg L⁻¹, in 2009–2010 and 0.10 mg L⁻¹ in 2011–2012]) and in most samplings (i.e., the second year of the first rotation and the first year of the second rotation) was above Quebec's limit value (i.e., 30 µg L⁻¹). However, starting the second year of the second rotation these values dropped below the legal limits.

When comparing the decontamination efficiency of different wastewater irrigation rates to the unirrigated control, we found that the concentration levels of several pollutants in the groundwater changed accordingly (Figs. 3 and 4). Total N concentration showed a general increase in all treatments compared to the control whereas NH₄-N and NO₃-N and P forms were on average lower than the control.

Data concerning the global decontamination performance of the system are shown in Table 5. The irrigation rates significantly affected the average nitrogen concentration in groundwater, but only under fertilised condition. In such case, the highest total N was found for D2 and D3 irrigation treatments, which differed

Table 4
Mean (±standard deviation, SD) and results of two-way ANOVA tests describing the statistical significance of irrigation and fertilization on various willow growth parameters during the first and second rotation cycles.

Traitement	Levels	Aboveground biomass (Mg ha ⁻¹ yr ⁻¹)				Stem height (m)				Stem diameter (cm)				Number of shoots											
		First rotation		Second rotation		First rotation		Second rotation		First rotation		Second rotation		First rotation		Second rotation									
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD								
Irrigation	D0	11.9	2.9	d	9.9	2.9	b	4.83	0.34	a	4.21	0.60	b	28.1	3.2	b	27.0	5.9	c	6.0	1.7	a	6.1	2.4	a
	D1	16.8	2.4	c	11.4	2.5	b	5.10	0.26	a	4.31	0.50	b	34.3	3.4	ab	29.6	5.0	bc	3.8	1.2	ab	6.0	2.4	a
	D2	19.3	3.2	b	18.6	5.1	a	4.82	0.78	a	5.65	0.70	a	38.2	10.5	ab	39.4	8.5	a	3.0	1.1	b	4.9	2.0	a
	D3	23.5	4.6	a	18.2	5.4	a	5.33	0.40	a	5.27	0.70	a	43.7	7.3	a	35.9	7.9	ab	3.4	1.2	b	5.6	2.2	a
Fertilization	UF	15.8	4.2	b	13.0	4.9	a	4.84	0.60	a	4.7	0.90	a	33.3	7.9	a	29.9	6.9	b	4.1	1.7	a	6	2.3	a
	F	20.1	5.6	a	16.1	6.0	a	5.20	0.40	a	5.02	0.80	a	38.8	8.8	a	36	8.8	a	3.9	1.8	a	5.3	2.1	b
Anova <i>p</i> -value	Irrigation	<0.01			<0.01			0.21			<0.01			<0.01			<0.01			0.01			0.40		
	Fertilization	0.02			0.24			0.11			0.18			0.01			0.03			0.72			0.02		
	Irr. × Fert.	0.18			0.56			0.50			0.29			0.11			0.45			0.74			0.07		

Within columns, different letters indicate significant differences (*p* ≤ 0.05) according to Tukey HSD test for irrigation and *t*-test for fertilization.

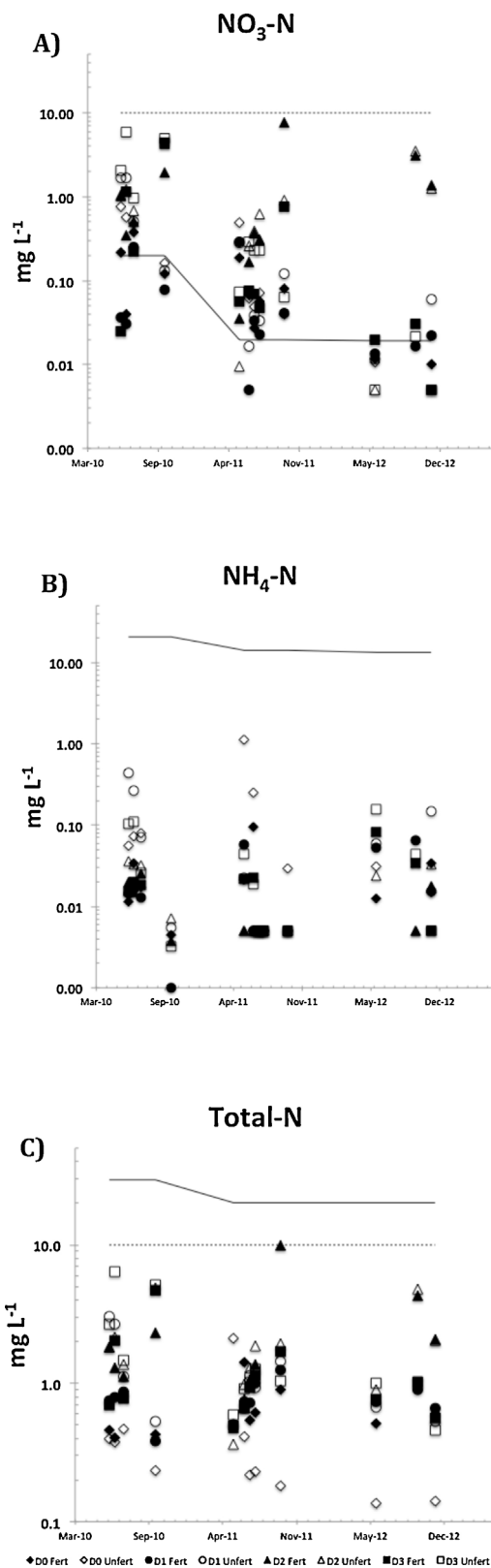


Fig. 1. Nitrogen concentration in groundwater during the first (2009–2010) and second (2011–2012) rotation cycles: (A) Nitrate (B) Ammonium (C) total N. Continuous lines show the concentration in the incoming wastewater. Dotted lines represent the concentration limit (where available) for drinkable and surface waters according to Minister of Sustainable Development, Environment, Wildlife and Parks of Quebec (MDDEFP, 2012).

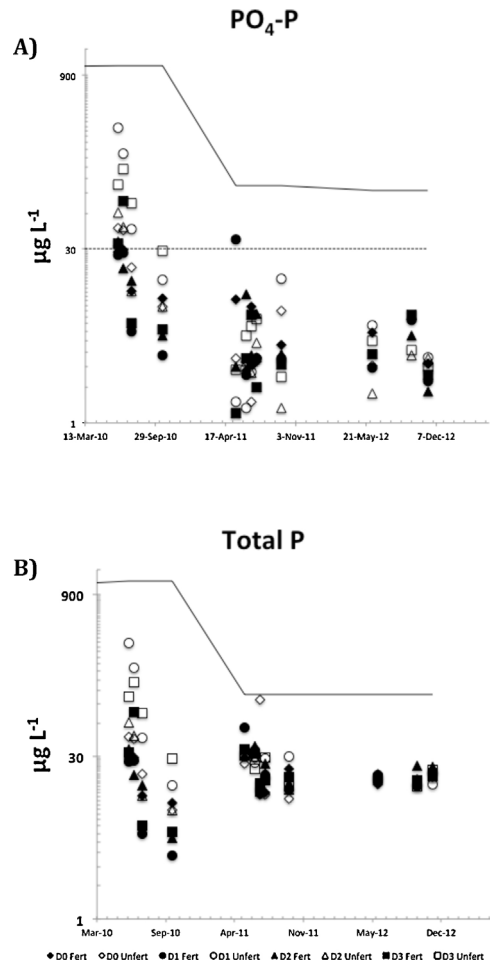


Fig. 2. Phosphorus concentration in groundwater water during the first (2009–2010) and second (2011–2012) rotation cycles: (A) phosphate (B) Total. Continuous lines show the concentration in the incoming wastewater. Dotted lines represent the concentration limit (where available) for surface water according to Minister of Sustainable Development, Environment, Wildlife and Parks of Quebec (MDDEFP, 2012).

significantly from D1 and D0. $\text{NH}_4\text{-N}$ concentration was significantly higher under unfertilised condition and did not vary among the irrigation treatments. The highest average nitrate concentration was found under D2 irrigated and fertilised willows. The average concentrations of both phosphorous forms were not affected by any treatment.

3.2.1. Soil quality

Several soil chemical properties were significantly affected by the irrigation and/or fertilisation treatments (Table 6). Irrigation with wastewater increased soil concentration of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, available P and exchangeable K in the top layer (0–20 cm) and $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, available P, exchangeable Ca and Mg, and OM in the bottom layer (20–40 cm). In particular, we found significantly less $\text{NH}_4\text{-N}$ at the end of the second rotation in the control (D0) compared to the other irrigation treatments. We also found significantly less $\text{NO}_3\text{-N}$ in the control (D0) at the end of the first (both layers) and second (top layer only) rotations. In the top layer, available P concentration was significantly higher in D2 than in D1 at the end of the first rotation, and significantly higher in D2 compared to D1 and D3 at the end of the second rotation. In the bottom layer, we also found a trend for higher concentrations of

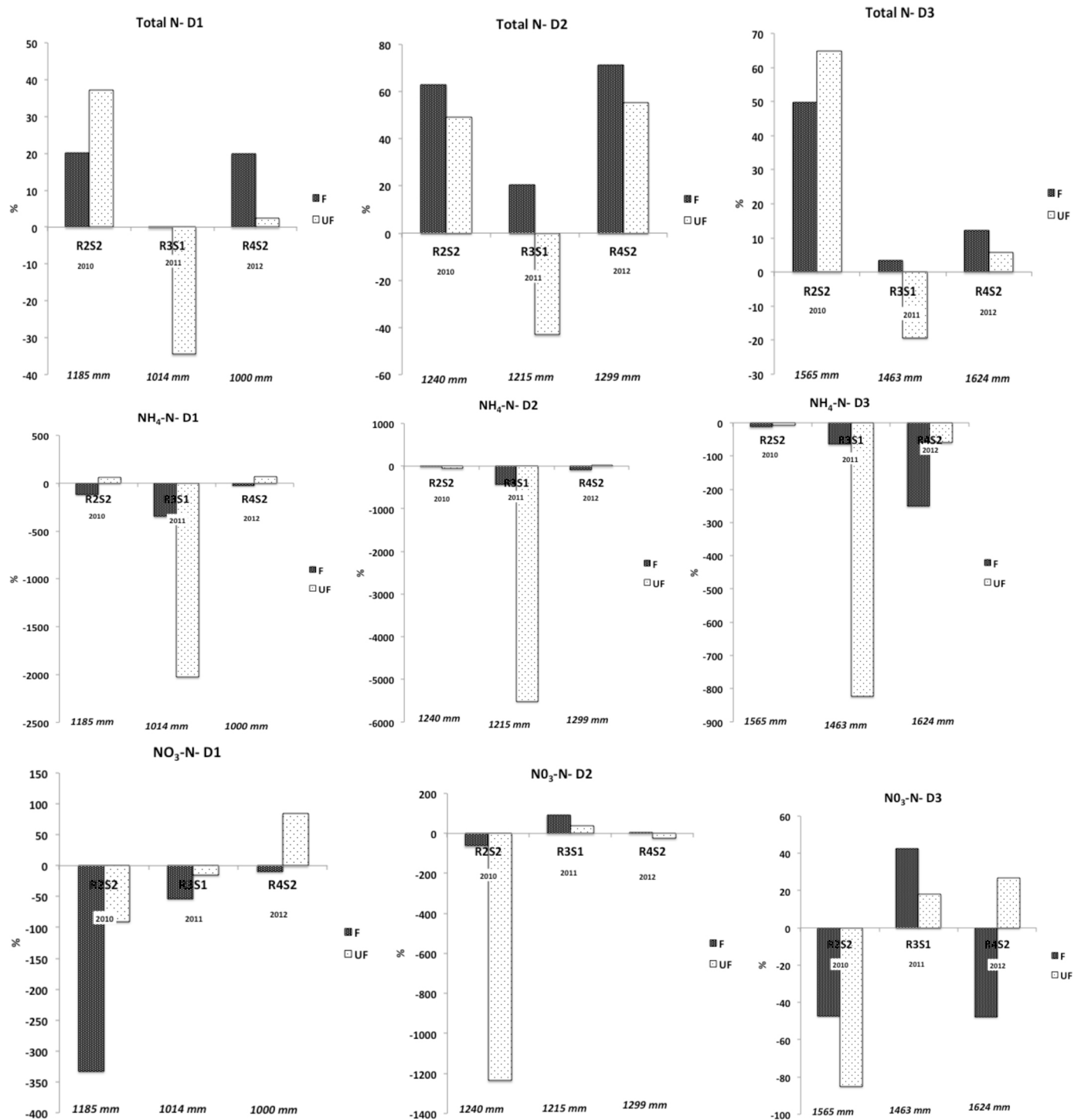


Fig. 3. Variation (%) of the concentration of the main N-compounds in groundwater following different irrigation rates (D1, D2, D3) in comparison to the control (D0). Values are calculated as $([X]_{\text{irrigated willow}} - [X]_{\text{control willow}}) / [X]_{\text{control willow}} \times 100$. Negative values represent a decrease in concentration due to the irrigation treatment. Positive values represent an increase due to the same factor. R2S2 (2-year-old roots on 2 year-old stem); R3S1 (3-year-old roots on 1-year-old stem); R4S2 (4-year-old roots on 2 year-old stem). Values in italic at the bottom show the amount of water (irrigation + rainfall) supplied to each treatment.

available P under the D2 treatment in both rotations. Finally, higher Ca, Mg and organic matter concentration were found in the D2 irrigation treatment but only for the bottom soil layer.

4. Discussion

Several studies have previously shown the high decontamination efficiency of willow vegetation filters. For instance, a recent

trial conducted in Estonia has shown that following the application of pre-treated wastewater containing $29 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ and $4 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ willow was able to retain approximately 58% and 70% of total N and P, respectively (Holm and Heinsoo, 2013). Likewise, researchers in Sweden have reported removal rates up to 90–96% for N and 94% for P in short-rotation coppice willow irrigated with untreated wastewater containing high loads of nutrients ($370 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, $30 \text{ kg P ha}^{-1} \text{ yr}^{-1}$), without notable leaching into

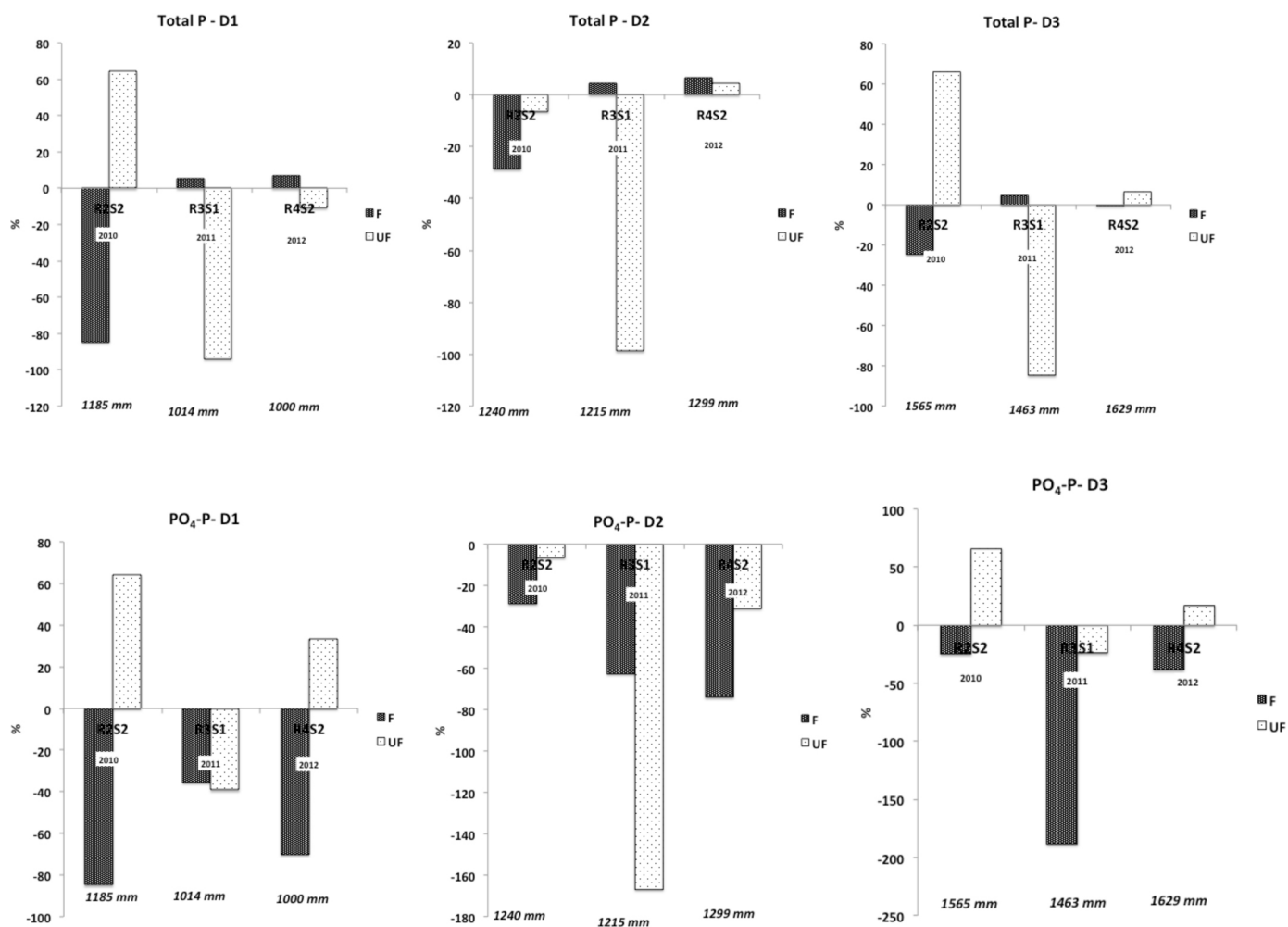


Fig. 4. Variation (%) of the concentration of the main P-compounds in groundwater following different irrigation rates (D1, D2, D3) in comparison to the control (D0). Values are calculated as $([X]_{\text{irrigated willow}} - [X]_{\text{control willow}}) / [X]_{\text{control willow}} \times 100$. Negative values represent a decrease in concentration due to the irrigation treatment. Positive values represent an increase due to the same factor. R2S2 (2-year-old roots on 2 year-old stem); R3S2 (3-year-old roots on 1-year-old stem); R4S2 (4-year-old roots on 2 year-old stem). Values in italic at the bottom show the amount of water (irrigation + rainfall) supplied to each treatment.

groundwater (Dimitriou and Aronsson, 2011). In the current study, the estimated average of removal rates over three years ranged from 86.4% for fertilized D2 (containing $164 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to 96.6% for fertilized D1 (containing $127 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) for N, and from 82.7% for fertilized D1 ($33 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) to 86.8% for fertilized D2 ($32 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) for P. The highest nutrient supply treatment (fertilized D3= $231 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, $34 \text{ kg P ha}^{-1} \text{ yr}^{-1}$) showed intermediate removal rates of 94.8% for N and 85.9% for P, both in line with the Swedish study especially concerning N. Despite the high amounts of N and P supplied to the plants during the two rotations by the D3 treatment (which greatly exceeded the current fertilization recommendations for willow in the province of Quebec [Guidi et al., 2013]), the plant-soil system seemed to be very efficient in nutrient retention. In this case, denitrification in the root zone might have also contributed to decreasing N concentration in the system, as it has been found elsewhere (Aronsson and Perttu, 2001). It should also be noted that some fractions of nutrients uptaken by the plants return to the soil through root and litter decomposition (Ericsson, 1994). *S. miyabeana* has been shown to have the slowest nutrient-release rate from litter among several species and cultivars (i.e., *S. purpurea*, *S. sachalinensis* and their crossings) (Hangs et al., 2014), thereby enabling this species to be used in environmentally sensitive projects where enhanced nutrient immobilization would be required.

At the beginning of the current trial, only total P concentration was found to be above the legal safety limits in the groundwater. This result apparently does not agree with most literature data which show high P-retention efficiency in willow. For instance, a recent study showed low phosphorus concentrations in groundwater when the willow vegetation filter was irrigated with wastewater (Werner and McCracken, 2008), whereas another reported no phosphorus leaching when wastewater was applied to willows grown in clay-soil lysimeters, and limited leaching in sand-soil lysimeters (Dimitriou and Aronsson, 2011). Our results were likely due to the relative high P concentration in the soil at the beginning of the current trial, which was above average for most agricultural lands (Dodd and Mallarino, 2005). In addition, these figures related to P concentration were also higher than those reported by other studies carried out on willow vegetation filters (Truu et al., 2009).

The current trial also shows that in southern Quebec the cultivar used in this study (SX67) is a very high water demanding willow variety. For instance in Sweden, other willow species used for sewage remediation were supplied with average irrigation rates of about 2.5 mm day^{-1} during the growing season with no environmental problems reported (Dimitriou and Aronsson, 2005), whereas during two successive rotations we were able to use higher supply rates (3.4 and 5.4 mm day^{-1} for D2 and D3, respectively) with comparable results in terms of environmental

Table 5
Mean (\pm standard deviation, SD) and results of two-way ANOVA tests describing the statistical significance of irrigation and fertilization on soil water chemistry.

Fertilization	Irrigation rate	Total N (mg L ⁻¹)		NH ₄ -N (mg L ⁻¹)		NO ₃ -N (mg L ⁻¹)		Total P (μ g L ⁻¹)		PO ₄ -P (μ g L ⁻¹)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fertilized (F)	D0	0.66	0.58	0.02	0.04	0.11	0.25	22.4	10.5	10.7	10.9
	D1	0.77	0.38	0.02	0.03	0.08	0.18	22.0	17.0	10.0	17.5
	D2	2.23	3.82	0.02	0.01	1.40	3.23	23.0	11.4	10.2	10.5
	D3	1.36	2.22	0.02	0.03	0.67	2.33	26.1	32.3	13.7	33.7
	Mean	1.26	0.72	0.02	0.00	0.56	0.62	23.4	1.9	11.1	1.7
Unfertilized (UF)	D0	0.91	0.75	0.15	0.69	0.26	0.37	31.2	53	12.4	13.9
	D1	1.27	1.04	0.10	0.19	0.5	0.78	76.9	150.3	59.7	135.1
	D2	1.98	2.41	0.02	0.02	1.13	2.36	25.6	16.9	11.3	17.1
	D3	2.02	2.59	0.04	0.06	1.35	2.7	45.7	62.3	34.0	64.6
	Mean	1.55	0.54	0.10	0.06	0.8	0.51	44.8	23.0	29.4	22.8
Anova <i>p</i> -values	Irrigation	0.02		0.15		<0.01		0.27		0.21	
	Fertilization	0.10		0.02		0.08		0.09		0.06	
	Irr. \times Fert.	0.04		0.33		<0.01		0.11		0.08	
Tukey and <i>t</i> -test groupings ^a	Irrigation	F (D2 = D3 > D1 = D0) UF (D0=D1=D2=D3)		D0 = D1 = D2 = D3		F (D2 > D3 = D1 = D0) UF (D0 = D1 = D2 = D3)		D0 = D1 = D2 = D3		D0 = D1 = D2 = D3	
	Fertilization	F = UF		F < UF		D0 (F = UF) D1 (F > UF) D2 (F = UF) D3 (F = UF)		F = UF		F = UF	

^a For total N and NO₃-N, the Tukey grouping illustrates the significant interaction effect between irrigation and fertilization.

Table 6
Mean (\pm standard deviation, SD), and results of two-way ANOVA tests describing the statistical significance of irrigation and fertilization on soil chemical properties.

Soil layer	Treatments	Levels	NH ₄ -N (mg kg ⁻¹)		NO ₃ -N (mg kg ⁻¹)		P (mg kg ⁻¹)		K (mg kg ⁻¹)		Ca (mg kg ⁻¹)		Mg (mg kg ⁻¹)		Organic matter (%)	
			2010	2012	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012
0–20 cm	Irrigation	D0	3.03	0.70b	1.74b	1.06	104ab	91ab	53	34b	1523	858	71	69	3.36	3.13
		D1	3.28	2.00b	2.43b	0.51	80c	67b	44	44b	1138	794	59	51	3.35	3.18
		D2	3.47	4.60a	4.60a	1.61	112a	99a	54	69a	1146	1093	78	70	3.41	3.50
		D3	2.37	4.75a	4.75a	1.34	81bc	71b	45	69a	991	872	61	66	3.26	3.25
Fertilization	F	2.82	2.80	3.39	1.33	94	82	50	64 a	1113	915	66	64	3.34	3.39	
	UF	3.25	2.30	3.38	0.93	94	82	48	44 b	1286	893	69	64	3.35	3.14	
Anova p-value	Irr.	0.19	<0.01	0.08	0.08	<0.01	0.01	0.27	<0.01	0.15	0.06	0.06	0.13	0.35	0.22	
	Fert.	0.40	0.68	0.96	0.07	0.81	0.92	0.58	0.04	0.24	0.52	0.44	0.96	0.85	0.20	
	Irr. × Fert.	0.51	0.39	0.96	0.63	0.35	0.36	0.33	0.27	0.52	0.32	0.38	0.30	0.66	0.95	
20–40 cm	Irrigation	D0	2.59	0.50b	1.26b	1.22ab	46ab	45 b	46	22	899	346b	41ab	37ab	2.36 ab	1.83 ab
		D1	2.51	1.30ab	1.38ab	0.45b	30b	38 b	33	17	728	397ab	37b	22b	2.18ab	1.41b
		D2	2.79	1.70a	1.80a	2.02a	72a	73 a	39	21	909	604a	60a	43a	2.84a	2.20a
Fertilization	D3	2.20	1.60a	1.54ab	0.79ab	41ab	51 b	33	31	707	529ab	43ab	48a	2.13b	1.52 b	
	UF	2.6	1.40	1.54	1.22	51	57	37	20	807	519	45	36	2.36	1.68	
Anova p-value	F	2.45	1.10	1.45	1.02	44	47	39	26	814	419	46	39	2.40	1.80	
	Irr.	0.24	0.01	0.04	0.04	0.03	<0.01	0.06	0.06	0.25	0.03	0.02	<0.01	0.04	<0.01	
	Fert.	0.08	0.15	0.47	0.81	0.48	0.27	0.76	0.11	0.92	0.40	0.86	0.58	0.85	0.69	
		Irr. × Fert.	0.36	0.69	0.49	0.13	0.06	0.19	0.18	0.32	0.06	0.54	0.17	0.14	0.20	

risk). This would support the claim that willows are not only well adapted to withstand prolonged flooded or saturated soils (Jackson and Attwood, 1996) but that some are also characterized by high water requirements (Bialowiec et al., 2007; Hartwell et al., 2010).

Finally, we also noticed a significant increase of biomass yield following wastewater supply. In this case, the abovementioned Swedish study (i.e., (Dimitriou and Aronsson, 2005) reported average biomass yield of 10 Mg ha⁻¹ yr⁻¹. The average biomass production achieved in our study over two rotation cycles was 14 Mg ha⁻¹ yr⁻¹ in the lowest (D1), 19 Mg ha⁻¹ yr⁻¹ in the intermediate (D2) and 20 Mg ha⁻¹ yr⁻¹ in the highest (D3) wastewater supply treatments. These yields were comparable to those expected for well-maintained commercial *S. miyabeana* SX67 plantations in the region, and, as previously reported, confirm southern Quebec as one of the most suitable Canadian regions to grow willow for biomass production (Labrecque and Teodorescu, 2005; Guidi Nissim et al., 2013).

5. Conclusion

This study shows that over the long-term willow vegetation filters are very efficient at uptaking nutrients found in wastewater, even when irrigation rates are relatively high. Nutrient retention, defined as the difference between nutrient input through fertigation and leaching, in willow vegetation filters was very high for both nitrogen and phosphorous. High nutrient retention was accompanied by high biomass yield of *S. miyabeana* SX67 cultivar which shows high potential for use in vegetation filters in Quebec.

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