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Willows Used for Phytoremediation Increased Organic Contaminant Concentrations in Soil Surface

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Featured Application: Field phytoremediation with willow shrub (*Salix* spp.).

Abstract: The *Salix* genus includes shrub species that are widely used in phytoremediation and various other phytotechnologies due to their advantageous characteristics, such as a high evapotranspiration (ET) rate, in particular when cultivated in short rotation intensive culture (SRIC). Observations made in past field studies suggest that ET and its impact on soil hydrology can also lead to increases in soil pollutant concentrations near shrubs. To investigate this, sections of a mature willow plantation (seven years old) were cut to eliminate transpiration (Cut treatment). Soil concentrations of polychlorinated biphenyls (PCBs), aliphatic compounds C10–C50, polycyclic aromatic hydrocarbons (PAHs) and five trace elements (Cd, Cr, Cu, Ni and Zn) were compared between the Cut and the uncut plots (*Salix miyabeana* 'SX61'). Over 24 months, the results clearly show that removal of the willow shrubs limited the contaminants' increase in the soil surface, as observed for C10–C50 and of 10 PAHs under the Salix treatment. This finding strongly reinforces a hypothesis that SRIC of willows may facilitate the migration of contaminants towards their roots, thus increasing their concentration in the surrounding soil. Such a "pumping effect" in a high-density willow crop is a prominent characteristic specific to field studies that can lead to counterintuitive results. Although apparent increases of contaminant concentrations contradict the purification benefits usually pursued in phytoremediation, the possibility of active phytoextraction and rhizodegradation is not excluded. Moreover, increases of pollutant concentrations under shrubs following migration suggest that decreases would consequently occur at the source points. Some reflections on interpreting field work results are provided.

Keywords: phytoremediation; polycyclic aromatic hydrocarbons (PAHs); polychlorinated biphenyls (PCBs); trace elements (TEs); petroleum hydrocarbons (PHCs); *Salix*; willow; field trials; evapotranspiration

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1. Introduction

Willow shrub cultivars are unequivocally among the most versatile environmental plant approach. These fast growing phreatophytic woody plants are frequently used in short rotation intensive culture (SRIC) for biomass production [1], often intended for bioenergy and biofuel processes [2]. Willows also show strong tolerance to several contaminants, such as nitrogen rich wastewater [3], trace elements (TEs) [4], various petroleum hydrocarbons compounds [5,6], as well as pesticides [7], making them effective riparian buffer strips in agricultural systems [8]. More recently, their utilization has been extended to treatment wetlands [9] as well as vegetation filters designed to treat landfill leachate [10]. These plants can additionally be used in phytoremediation to extract or degrade contaminants [4,11], or, as evapotranspiration (ET) covers, to contain them in the soil [12,13]. Furthermore, the use of willow for environmental purposes can generally be considered low-cost compared to conventional approaches, and also benefits from strong social acceptability [14]. However, successful soil decontamination by phytoremediation is often challenging to demonstrate clearly, especially in field studies [15], characterized by many sources of variation that can influence the concentration and distribution of contaminants in soil [16].

In past phytoremediation field experiments, our study group has observed stable, but also increased soil pollutant concentrations under a specific willow plantation (*Salix miyabeana* 'SX61' and 'SX64'), even after almost a decade of cultivation [6,17], contrary to initial hypotheses and objectives. The main findings concerning the establishment (first year) of this plantation can be found in Guidi et al. [6]. Although no scientific publication has reported the behavior of the soil contaminants on this site after the first four growing seasons, our research group was able to observe that no significant decrease in soil contaminant concentrations occurred for any of the compounds tested (several TEs, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)). These observations were hypothesized to be the result of several interacting factors, such as the heterogeneity of soil contamination, as well as the transport of contaminants from deeper to shallower soil depths, as a result of water uptake by the willows.

At the beginning of the fifth growing season (corresponding to the T0 in Fortin Faubert et al. [17]), all analyzed contaminants on the site were found in significantly higher concentration under willows than under the control treatment. We hypothesized that the absence of convincing soil decontamination under the willow plantation could be attributable to the attraction of the dissolved contaminant fraction towards the root zones, facilitated by the high evapotranspiration rate of willow fields under SRIC management.

Accordingly, removing the mature willow cover would theoretically limit the transfer of contaminants into the cut area. This study aimed to explore the effects of willow tree removal from a mature (seven-year-old) plantation on both organic and inorganic contaminant concentrations in surface soil over time (24 months) and was conducted inside the boundaries of a willow field established in 2010 on a former industrial site in southern Quebec, Canada [6].

2. Materials and Methods

2.1. Experimental Site

The experimental site is located in the municipality of Varennes, south of the Island of Montreal, Quebec, Canada (45°42′02.8″ N, 73°25′53.4″ W). The site centroid lies less than 350 m from the south shore of the St. Lawrence River, approximately 3.5 m above river water level. The region has a temperate climate (annual average temperature: 6.2 °C; annual average precipitation 980 mm) [18]. Characterized by flat terrain, the site once hosted primarily petrochemical activities, as well as ethanol and titanium dioxide pigment production. Settling ponds were used between 1963 and 1975 to control liquid releases produced by the factory's refining operations. Between 1972 and 1979, sludge was spread following a land farming approach, which ultimately led to the soil contamination of the site. All industrial operations ceased in 2008, and since 2010 part of the site has hosted three successive phases of phytoremediation experiments.

2.2. Previous Studies on the Site and Present Experimental Layout

2.2.1. Soil Characterization (2010)

Soil characterizations were carried out on the experimental part of the site in early 2010. Table 1 presents physico-chemical soil properties and shows that it has a clay texture, with a pH of 7.7 and 9.6% organic matter content. According to Guidi et al. [6], at that time, the sector was mainly contaminated by a mixture of PAHs, PCBs and trace elements (TEs), found mainly in the soil surface (0–60 cm).

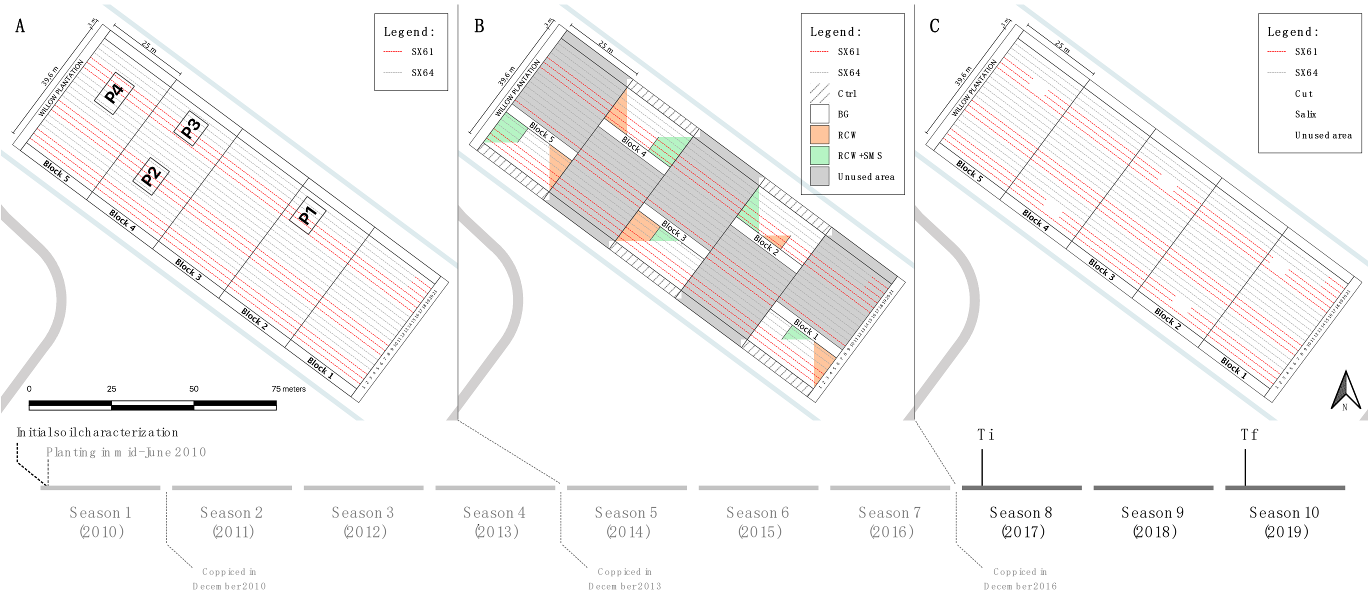

Figure 1. Evolution of the experimental design over time. (**A**) The first experimental phase (phase 1) on what is referred to as the GERLED site in Guidi et al. [6]. The 21 dotted lines inside the willow plantation refer to the rows planted with the cultivar 'SX61' (red lines) and with the cultivar 'SX64' (grey lines). P1 to P4 refer to the sampling plots in their study [6]; (**B**) the second experimental phase (phase 2) studied by Fortin Faubert et al. [17]. Colored areas refer to the experimental plots supplemented with spent mushroom substrates (SMS) and/or with ramial chipped wood (RCW), or simply left as bare ground (BG). The control sections (Ctrl) were located at the extremity of each block. Although preserved as part of the plantation, the sections in dark grey were not used in the present study (Unused area); (**C**) the present experiment (phase 3). Colored areas refer to the experimental plots where willows were cut (Cut) or left in place (Salix). Adapted from Guidi et al. [6].

Table 1. Soil characteristics of the site in 2010.

Parameters	Units	Values	Parameters	Units	Values
Cation-exchange capacity	meq 100 g^{-1}	43.50	PCBs [c]	mg kg^{-1}	57.58 ± 11.70
pH [a]	-	7.70	Cadmium [c]	mg kg^{-1}	1.75 ± 0.15
pH buffer	-	>7.50	Chromium [c]	mg kg^{-1}	659.50 ± 127.22
Soil texture	-	Clay	Copper [c]	mg kg^{-1}	1380.00 ± 201.57
Clay	%	46.00	Nickel [c]	mg kg^{-1}	42.90 ± 2.22
Silt	%	33.90	Lead [c]	mg kg^{-1}	34.00 ± 8.12
Sand	%	20.10	Zinc [c]	mg kg^{-1}	386.50 ± 72.13
Organic matter	%	9.60	Acenaphthene [c]	mg kg^{-1}	0.56 ± 0.18
K + Mg + Ca saturation	%	100.00	Acenaphtylene [c]	mg kg^{-1}	1.98 ± 0.38
P (P/Al) saturation	%	16.50	Anthracene [c]	mg kg^{-1}	18.15 ± 4.90
Ca saturation	%	81.60	Benz[a]anthracene [c]	mg kg^{-1}	0.43 ± 0.09
K saturation	%	3.10	Benzo[a]pyrene [c]	mg kg^{-1}	0.28 ± 0.07
Mg saturation	%	15.30	Benzo[ghi]perylene [c]	mg kg^{-1}	0.48 ± 0.12
Parameters	**Units**	**Values**	Chrysene [c]	mg kg^{-1}	0.40 ± 0.09
Al [b]	mg kg^{-1}	48.00	Fluoranthene [c]	mg kg^{-1}	0.54 ± 0.20
B [b]	mg kg^{-1}	1.40	Fluorene [c]	mg kg^{-1}	0.94 ± 0.21
Ca [b]	mg kg^{-1}	7090.00	Indeno[1,2,3-cd]pyrene [c]	mg kg^{-1}	0.32 ± 0.09
Cu [b]	mg kg^{-1}	417.00	Naphthalene [c]	mg kg^{-1}	0.42 ± 0.13
Fe [b]	mg kg^{-1}	178.00	Phenanthrene [c]	mg kg^{-1}	2.62 ± 0.71
K [b]	mg kg^{-1}	525.00	Pyrene [c]	mg kg^{-1}	1.34 ± 0.41
Mg [b]	mg kg^{-1}	800.00	1-Methylnaphthalene [c]	mg kg^{-1}	0.42 ± 0.13
Mn [b]	mg kg^{-1}	11.00	2-Methylnaphthalene [c]	mg kg^{-1}	0.42 ± 0.12
P [b]	mg kg^{-1}	80.00	1,3-Dimethylnaphthalene [c]	mg kg^{-1}	0.55 ± 0.18
Zn [b]	mg kg^{-1}	85.60	2,3,5-Trimethylnaphthalene [c]	mg kg^{-1}	0.40 ± 0.13

Soil samples were collected at 0–30 cm below ground. [a] Water extraction. [b] Melich III method. [c] Chemical analysis were performed by AGAT Laboratories Ltd. (Montreal, QC, Canada) following the recommended provincial methods for environmental analyses [19–23]. Five soil samples were collected at 0–30 cm below ground in each plot (P1, P2, P3 and P4, see Figure 1A). Values are the averages (mean ± SD, n = 20). Table was adapted from Guidi et al. [6].

2.2.2. Phase 1 (2010–2013)

The first experimental phase involved establishment of a 5475 m^2 willow plantation (*Salix miyabeana* 'SX61' and 'SX64') under a SRIC management strategy. This experiment was conducted to investigate decontamination of shallow soil polluted by a mixture of organics and TEs and is referred to as the GERLED sector in Guidi et al. [6]. The cultivars were planted in seven randomly distributed groups of three rows, for a total of 21 rows distanced by 1.8 m between each other (Figure 1A). Planting was carried out mechanically, and cuttings were spaced by 0.3 m apart in each row, for an equivalent total density of 18,500 plants per hectare.

A first cut was performed at the end of the first season (December 2010) and a second one at the end of the fourth growing season (December 2013). An area adjacent to the plantation was kept unplanted to serve as a control plot. This plot, referred to as P5 in Guidi et al. [6], is not shown in Figure 1A.

2.2.3. Phase 2 (2014–2016)

The second experimental phase (Figure 1B) aimed to investigate the bioremediation impacts of both cultivars ('SX61' and 'SX64') supplemented or not with spent mushroom substrates (SMS) of *Pleurotus ostreatus* and ramial chipped wood (RCW) of *Salix* spp. [17]. The effect of RCW was investigated alone, as well as in combination with SMS. The initial concentration of organics and TE soil concentrations in planted plots was higher than in unplanted plots. Moreover, when comparing soil pollutant concentrations at the end of this experiment to the situation in 2010 (six years prior), a tendency towards either more important lowering or weaker increases (depending on the contaminant) was observed in unplanted plots. The plantation was coppiced again in December 2016.

2.2.4. Phase 3 (2017–2019)

Following these previous observations regarding the absence of convincing soil decontamination after seven seasons of willow growth, the authors of this study introduced a new experimental layout in the plantation in 2017 (Figure 1C). In order to investigate the present research question focusing on the effect of the harvest of willow trees on the behavior of the contaminants in the soil below, two treatments, Cut and Salix (uncut), were replicated over five blocks and integrated in the plantation where cultivar 'SX61' was present. The plots consisted of two four-meter-long rows of cultivar 'SX61', resulting in 16 m^2 Cut plots. Trees were cut at the very base of their trunk, using a forestry brush cutter. Regular maintenance was necessary to eliminate regrowth of the trees. All experimental plots were laid where no ground cover treatment had been applied in the previous experimental phase (no soil amendments, referred as bare ground (BG) in Fortin Faubert et al. [17]).

2.3. Soil Sampling

The first soil sampling was done concomitantly with the willow cutting in June 2017 (Ti) and a second in June 2019 (Tf), which established the duration of the experiment (24 months). Soil samples were compared between similar seasons to avoid the influence of the seasonal fluctuations in the soil hydrology, as recommended by Fortin Faubert et al. [17].

Samples were collected with a manual auger at a depth of 0–30 cm in both experimental conditions. One composite soil sample (pool of three) was initially collected (Ti) for both experimental conditions in each of the five (5) blocks. To reduce the variance in data caused by the possible heterogeneous distribution of pollutants in the soil, subsequent samples were taken within a 30 cm radius of the initial ones. After two seasons (Tf), three composite samples (pool of three) were collected for both experimental conditions in each of the five (5) blocks. Samples were collected in amber glass containers (System Plus Ltd., Baden, ON, Canada) and immediately sent to an external laboratory for chemical analysis (AGAT Laboratories Ltd., Montreal, QC, Canada) to assess the soil concentrations of PCBs by GC-MS, C10-C50 by GC-FID, PAHs by GC-MS and six TEs (Cd, Cr, Cu, Ni and Zn) by ICP-

OES, following the recommended provincial methods for environmental analyses [19–23]. Each of these methods used duplicates, blanks and certified standard reference materials (CRM C-QME-01, Quebec Ministry of Environment Congener Mix, Accustandard, New Haven, CT, USA), (CRM 51165, Fuel Oil (Diesel)—50% Weathered, Absolute Standards, Inc., Hamden, CT, USA), (CRM Q-11226-O, Custom PAH MTL & QC, NSI Lab Solutions, Raleigh, NC, USA), (SRM 3108, Cd standard solution; SRM 3112a, Cr standard solution; SRM 3114, Cu standard solution; SRM 3136, Ni standard solution, SRM 3168a, Zn standard solution; National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) to assure quality control and quality assurance and followed the provincial guidelines for analytical work in chemistry DR-12-SCA-01 [24].

2.4. Data Analyses

Statistical analyses were performed using JMP® Pro V.15.0.0 (SAS Institute, Cary, NC, USA). Soil contaminant variations were submitted to a one-way analysis of variance (ANOVA) test. In order to meet the assumption of equal variance, log-transformation of data was performed according to Levene's test, or, when a non-random pattern was observed in the "residual by predicted" plot.

3. Results

3.1. Soil Contaminant Concentrations between Treatments

In the initial soil samples collected and analyzed before the experiment in June 2017 (Ti), concentrations of all targeted organic and inorganic contaminants were not significantly different between the Cut and Salix treatments (fourth column from the right in Table 2). It was hence appropriate to use raw concentration values from June 2019 to compare the treatment effect after two years of experimentation (Tf).

None of the five analyzed TEs showed different values between treatments at Tf (third column from the right in Table 2). Likewise, PCBs and C10–C50 also showed similar values between treatments after two years. Some significant differences in PAH concentrations were recorded. Five compounds (i.e., acenaphthene, acenaphthylene, chrysene, 1-methylnaphthalene and 2-methylnaphthalene) were found in significantly higher concentrations under the Salix treatment (Cut < Salix).

3.2. Soil Contaminant Variations over Time

In order to better understand what led to the absence or presence of significantly different concentrations between treatments at the end of this two-year experiment, we investigated changes in soil contaminant concentrations in each treatment individually. For each treatment (Cut and Salix), concentrations of all contaminants in the initial samples (Ti) and the final ones (Tf) were compared (last two columns of Table 2), and the significant difference noted when present.

Under the Cut treatment, no significant differences in concentrations were recorded between Ti and Tf, for any of the compounds. Conversely, statistical comparisons of soil concentrations under the Salix treatment identified 11 significant differences between the beginning (Ti) and the end (Tf) of the experiment. All of the significant differences identified a higher concentration after two years (Ti < Tf) and concerned C10–C50, acenaphthylene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[ghi]perylene, chrysene, fluorene, 1-methylnaphthalene, 2-metylnaphthalene, 1,3-dimetylnaphthalene and 2,3,5-trimetylnaphthalene.

Table 2. Comparison of soil contaminant concentrations between treatments at each sampling time and between sampling times in each treatment.

Parameters ($mg\ kg^{-1}$)	June 2017 (Ti)			June 2019 (Tf)			*p*-Value			
							Cut vs. Salix		Ti vs. Tf	
	Cut		Salix	Cut		Salix	at Ti	at Tf	in Cut	in Salix
Cd	1.86 ± 0.15		1.80 ± 0.21	1.83 ± 0.61		1.05 ± 0.78	0.6657	0.2582	0.9364	0.1772
Cr	991.00 ± 134.28		962.60 ± 110.21	1018.87 ± 190.37		1074.6 ± 139.24	0.7090	0.6397	0.8163	0.2346
Cu	2714.00 ± 916.01		2552.00 ± 668.93	2484.13 ± 704.18		2688.60 ± 834.20	0.5870	0.6761	0.5310	0.7450
Ni	89.80 ± 6.65		85.00 ± 9.57	95.93 ± 23.16		75.20 ± 25.80	0.4389	0.3920	0.6527	0.5737
Zn	503.00 ± 75.41		481.20 ± 37.57	526.27 ± 61.21		579.60 ± 86.20	0.6499	0.3411	0.6755	0.1657
PCBs	103.50 ± 31.96		97.36 ± 25.93	89.84 ± 21.22		94.93 ± 19.20	0.7930	0.7043	0.4690	0.8770
C10-C50	4000.00 ± 1364.35		3640.00 ± 1670.75 ^{B}	6231.33 ± 2422.89		8191.33 ± 1818.87 ^{A}	0.7727	0.2945	0.2285	**0.0255 ***
Acenaphthene	0.66 ± 0.15		0.68 ± 0.33 ^{B}	0.52 ± 0.19	<	0.84 ± 0.29 ^{A}	0.8979	**0.0253 ***	0.2630	**0.0533**
Acenaphthylene	4.00 ± 1.74		3.72 ± 2.18 ^{B}	3.58 ± 1.32	<	6.75 ± 2.89 ^{A}	0.8127	**0.0413 ***	0.1342	**0.0023 ****
Anthracene	26.34 ± 8.88 ^{b}		20.36 ± 13.17 ^{B}	33.45 ± 9.38 ^{a}		35.55 ± 14.23 ^{A}	0.3097	0.8715	**0.0591**	**0.0907**
Benzo[a]anthracene	0.56 ± 0.19		0.46 ± 0.29 ^{B}	0.49 ± 0.17		0.66 ± 0.24 ^{A}	0.5185	0.1785	0.2361	**0.0388 ***
Benzo[a]pyrene	0.28 ± 0.13 ^{a}		0.31 ± 0.24	0.19 ± 0.10 ^{b}	<	0.39 ± 0.21	0.8180	**0.0995**	**0.0522**	0.1160
Benzo[b]fluoranthene	0.30 ± 0.16		0.32 ± 0.23 ^{B}	0.25 ± 0.13		0.43 ± 0.24 ^{A}	0.8868	0.2357	0.1300	**0.0097 ****
Benzo[ghi]perylene	0.46 ± 0.19 ^{a}		0.38 ± 0.26 ^{B}	0.35 ± 0.08 ^{b}	<	0.61 ± 0.25 ^{A}	0.5965	**0.0521**	**0.0608**	**0.0120 ***
Chrysene	0.36 ± 0.11 ^{a}		0.38 ± 0.26 ^{B}	0.28 ± 0.10 ^{b}	<	0.47 ± 0.21 ^{A}	0.8605	**0.0499 ***	**0.0731**	**0.0198 ***
Fluoranthene	0.58 ± 0.15		0.68 ± 0.29	0.64 ± 0.20		0.77 ± 0.25	0.3262	0.3354	0.7010	0.5060
Fluorene	1.06 ± 0.32		0.98 ± 0.52 ^{B}	1.05 ± 0.30	<	1.74 ± 0.59 ^{A}	0.7489	**0.0556**	0.9099	**<0.0001 ******
Indeno[1,2,3-cd]pyrene	0.32 ± 0.15 ^{a}		0.29 ± 0.20	0.19 ± 0.09 ^{b}	<	0.36 ± 0.16	0.7598	**0.0584**	**0.0514**	0.1026
Naphthalene	0.34 ± 0.09		0.32 ± 0.16	0.32 ± 0.07		0.41 ± 0.10	0.7780	0.1550	0.6645	0.1178
Phenanthrene	2.22 ± 0.75	>	1.88 ± 0.65	2.45 ± 1.02		2.91 ± 1.19	**0.0673**	0.2881	0.6652	0.1162
Pyrene	1.68 ± 0.54		1.86 ± 0.92	1.93 ± 0.56		2.31 ± 0.90	0.6911	0.5823	0.3863	0.3577
1-methylnaphthalene	0.38 ± 0.08		0.36 ± 0.11 ^{B}	0.43 ± 0.12	<	0.56 ± 0.20 ^{A}	0.7489	**0.0435 ***	0.5729	**0.0318 ***
2-methylnaphthalene	0.30 ± 0.07		0.30 ± 0.12 ^{B}	0.43 ± 0.10	<	0.59 ± 0.15 ^{A}	1.0000	**0.0244 ***	0.1154	**0.0009 *****
1,3-dimetylnaphthalene	0.50 ± 0.10		0.46 ± 0.15 ^{B}	0.67 ± 0.29	<	0.87 ± 0.41 ^{A}	0.5415	**0.0935**	0.3856	**0.0408 ***
2,3,5-trimetylnaphthalene	0.22 ± 0.04		0.20 ± 0.07 ^{B}	0.31 ± 0.15		0.39 ± 0.15 ^{A}	0.3739	0.1288	0.3101	**0.0093 ****

Values are the average (mean ± SD, n = 5 for each treatment at Ti; n = 15 for each treatment at Tf) contaminant concentrations ($mg\ kg^{-1}$). Asterisks indicate a significant (Student's *t*-test, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$) difference in concentration between treatments at each sampling time and between sampling times under each treatment. Bold grey *p*-values are between 0.05 and 0.1. Symbols of comparison (> or <) identify the direction of the differences between treatments at each time, while lowercase letters (a or b) indicate differences between times under the Cut treatment, and uppercase letters (A or B) indicate differences between times under the Salix treatment.

4. Discussion

4.1. General Pattern

Following the 24-month experiment, five PAHs showed significantly higher concentrations under the Salix treatment than in the plots without willows (Cut) (Table 2). The changes over time in the concentration of four of the five PAHs (acenaphthylene, chrysene, 1-methylnaphthalene and 2-methylnaphthalene) suggest that increases under the Salix treatment led to their differences between treatments. Although it is less obvious for the fifth PAH (acenaphthene), an increase under the Salix treatment may have once again led to the significant difference observed between treatments after two years, since the statistical difference was marginally significant ($p = 0.0533$). This level of significance is considered fairly, rather than extremely, reliable, given the many potential contributing factors involved in a dataset gathered on a full-size contaminated former industrial site [15]. The heterogeneity of soil contamination is a common feature of post-industrial zones and systematic soil sampling will most likely produce data with substantial variance, which can make it more challenging to show significant treatment effects statistically [15]. Accordingly, it has been proposed that a value of 10% ($p \leq 0.1$) instead of 5% ($p \leq 0.05$), could be an acceptable level of significance in such circumstances [15]. Following this recommendation, the p-values between 5% and 10% are presented in Table 2. They will be used to complement the interpretation of the results and will be referred to as tendencies.

When considering these tendencies, five additional PAHs contribute to the previously mentioned pattern of higher concentrations of contaminants under the Salix treatment in the final samples (Tf). Again, increases in concentrations under the Salix treatment over time seem to have produced most of these differences, but some decreases in Cut might also have played a role. Phenanthrene also appeared to have increased over time in the Salix treatment, since it initially showed a tendency towards higher concentrations under the Cut treatment, but by the end of the experiment was similarly concentrated in both treatments. Anthracene was the only compound of importance in this study to show a tendency to increase under both treatments over time.

Overall, these results clearly show that removal of the willow trees in the mature plantation limited the contaminants' increase in the soil surface, suggesting that such increases were mainly driven by the presence of willows. This finding strongly reinforces an earlier hypothesis that SRIC of willows may facilitate the migration of contaminants towards their roots, possibly by means of their high evapotranspiration rate, thus increasing their concentration in the surrounding soil [17].

The finding that soil contaminant concentrations increase under a willow phytoremediation crop differs from the results of most phytoremediation studies that monitored changes in soil concentrations (i.e., the reduction of pollutant concentrations in soil (see Macci et al. [25] for an example). Increases in soil pollutant concentrations under willows may appear surprising and incompatible with the objective of soil phytoremediation. Nevertheless, it is essential to consider possible dynamics at play that could generate such data, as well as the implications of these results for further phytoremediation field work.

4.2. Convective Transport of Dissolved Chemicals towards the Root Zone

Plant transpiration is known to create water potential gradients from leaves to bulk soil, which can generate convective transport of dissolved chemicals from the adjacent bulk soil towards the roots [26,27]. Rhizospheric accumulations of these chemicals can then be expected, especially if the quantity transferred by water mass flow surpasses plant requirements [28]. Soil scientists generally describe this phenomenon in a macronutrient acquisition context, but it could also concern micronutrients (i.e., Cu, Mn, Zn and Co) [29] and some TEs, as observed for Pb in Klassen et al. [30]. In their controlled laboratory study, the exclusionary mechanisms of metal resistance were suspected to promote the accumulation of Pb in the rooting zone of *Betula occidentalis* after its mobilization in the rhizosphere. Moreover, the simultaneous mobilization of other chemicals like sulfate or phosphate in the rhizosphere may promote the precipitation of soluble elements, leading to their enrichment over time in a relatively less-mobile form [28,30]. Interestingly, our results showed no effect of treatments on soil TE concentrations. Although TEs may have been subject to convective transport towards the roots, the ability of willows to bioaccumulate many of these TEs in their tissues [4] may have prevented their accumulation in the surrounding soil.

The impacts of plants on the mobilization of hydrophobic organic compounds are less well documented. However, there is some evidence that plants can contribute to the accumulation of organic chemicals such as PAHs [31], which generally sorb to soil particles, decreasing their transport rate and increasing the time required for their remediation [32]. The release of organic acids with root exudates can increase their solubility and therefore their mobility [33]. Colloids, as mobile bacteria, may also enhance the transport of PAHs in the subsurface of soil [34]. Once in the rhizosphere, relatively hydrophobic compounds, such as high molecular weight (HMW) PAHs, can then adsorb and bind strongly to the roots [35]. Such adsorption to the roots can apparently increase with lipid content [36], as well as with plant age, due to a greater total root mass [36]. Although it is generally accepted that low molecular weight (LMW) PAHs are potentially more soluble and therefore more mobile than heavier compounds [37], our investigations did not reveal any statistical relationships between the behavior of the concentration (increase, stable or decrease) of individual PAHs and their molecular weight (data not shown). The mobilization of chemicals by mass flow, driven by plant transpiration, has been mostly documented on the individual plant scale (i.e., glass tubes [31]), and less is known about this phenomenon on a larger scale (i.e., field scale).

It is now relatively well documented that the ET rate of most willow species used in environmental projects is high enough to affect the hydrology of the soil below [38]. Rapidly growing willow crops are indeed able to act as a "biological pump", thus influencing groundwater flow patterns, whereby the trees are able to reach the water table [39,40]. Hydraulic control has been developed as a technique that uses trees as ET cover to remove contaminated soil water, in order to contain or control the migration of water-soluble contaminants in the subsurface [40]. We suspect that the high evapotranspiration rate of the willows in our experiment led to increases of pollutant concentrations under the Salix treatment over time through similar mechanisms. The ET of willows may have created water potential gradients from leaves to the peripheries of the plantation, or to the deeper soil layers, thus generating convective transport of dissolved chemicals from these zones towards the surface soil under the plantation, where samples were collected. Such transport of contaminants may have been inhibited by the removal of willow trees from our plantation, since all significant increases over time were only observed under the Salix treatment.

Water supply has been identified as one of the most important driving factors of ET across willow species [38,41]. The data graciously shared by PÉTROMONT INC. allowed us to establish that the water table fluctuated mainly between depths of 0.6 to 1.3 m. Moreover, the experimental site was situated at less than 300 m from the St. Lawrence River. We therefore believe it is possible that the willows on the plantation were able to interact with the potentially contaminated water table and cause a "transpirational influx" of water towards the willow roots above, leading to rising concentrations of C10–C50 and many PAHs in soil samples collected close to living willow roots. Our experimental site was situated within a larger contaminated open site, with a long history of contamination, including some years of land farming and the presence of several former decantation basins less than a hundred meters away.

4.3. Cutting Trees Did Not Remove the Roots

Since willows are known for their great potential to reduce deep percolation and leaching of contaminants in ET cover applications [13], we would have expected to find stronger evidence of decreasing contamination under the Cut treatment over time. However, as the experimental site was an open system without any physical barriers between plots, the complete removal of the aerial parts of the willows in the Cut plots may not have totally stopped the impact on soil water dynamics.

Due to the complexity associated with the excavation of complete root systems, few studies have investigated the root length of willows under SRIC conditions. Phillips et al. [42] were able to observe the belowground plant growth of willows and poplars and reported lateral root spread of 5–11 m from the stem of *Salix matsudana*, after only nine months of growth (270 days) in New Zealand. It is therefore possible to assume that each individual seven-year-old root system in the present plantation could already have extended beyond the limit of its respective experimental unit. Consequently, the willows growing outside the Cut plots could still have accessed that soil and impacted the water and chemicals mass flows, although most likely to a lesser degree. Additionally, an opening in the willow field might at the same time have allowed for increased evaporation by reducing shading, thereby influencing surface soil hydrology.

Furthermore, it has been found that the roots and stumps of dead or cut trees can be kept alive through root grafts with living residual trees [43]. When tree root systems spread laterally and intermingle, connections between mature individuals may occur by grafting [44]. Natural root grafting is a fairly common phenomenon occurring among many trees including willows [45] and which has been observed under SRIC conditions in a past study conducted at a location near the study site [46]. Willows outside the Cut plots may thus have used the former root system of the cut willows directly, to absorb water and nutrients from the soil in the Cut plots.

Overall, lateral root spreading, and root grafting may have maintained a sufficiently high transpiration rate in the Cut plots, thus limiting the expected decreases of contaminant concentrations over time. To prevent this, a much greater area could have been used for Cut plots. Also, a fine and deep cut could have been made around the Cut plots to inhibit living root activity in the Cut plots; however, this would not have stopped new roots from accessing it.

Apart from the abovementioned phenomenon related to water dynamics, many contaminants may have remained strongly bound to the mature root systems lying in the Cut plots, thus preventing their decrease over time. It is well known that *Salix* spp. can immobilize various TEs and organic compounds in/on their roots through absorption/adsorption [4,11,47–50]. It would have been interesting to investigate this question in the present study, but the focus was on monitoring the soil concentrations only. The presence of fine roots in the soil samples could also explain why no significant decreases of contaminants were found under the Cut treatment over time. However, a plausible decay of at least some of the roots following cutting may also have begun to release some otherwise strongly root-sorbed contaminants into the soil. Six months after willow harvest, Watson [51], reported significant increases in soil solution Pb concentrations, and interpreted that finding as an effect of root degradation and Pb release. Such increases in soil contaminant solubility could then lead to their leaching and unwanted loss in the environment [52]. Since tendencies towards contaminant decreases have been observed for some PAHs under the Cut plots, it is possible that such releases could have begun slowly, and that significant reduction of contamination levels would have begun to occur and been observed if the monitoring had lasted a few more years. The present investigation did not reveal any variation in PCB or TE concentrations under either treatment over time, suggesting that all of them would have been well stabilized on the site, probably bound to either soil particles or root material (dead or alive).

4.4. Results Interpretation and Implications for Field Trials

The challenges associated with monitoring changes in soil contamination over time are not new [15]. The results presented here demonstrate how counterintuitive results gathering can be in a field trial. However, field studies are essential for the development of phytoremediation, among other reasons, because not every aspect of an open field system can be tested under controlled conditions. For instance, the pumping effect that a high-density willow crop under SRIC management has on soil hydrology is a major field characteristic that cannot be taken into account in typical greenhouse experiments.

The numerous factors interacting in field studies are a source of variance in field-gathered data that can prevent researchers from attaining practical objectives related to environmental cleaning [15]. It can also be very challenging to identify the mechanisms responsible for the observations made. Based on the findings here, important recommendations to mitigate the effects of spatial heterogeneity at field sites are: to combine subsamples into a composite samples in each experimental plot [53], to collect samples as close as possible to the same sampling point over time, and to be flexible when establishing statistical significance thresholds [15].

To our knowledge, very few studies have reported increases in contaminant concentrations under phytoremediation, as was the case here. Since phytoremediation studies usually hypothesize soil purification as a benefit, it is possible that previous findings suggesting no effect, or even opposite effects, have just been considered as failures and regarded as unsuitable for publishing or incomplete. Studies that fail to confirm a hypothesis are generally underrepresented in the literature compared to studies that succeed in doing so, producing so-called publication bias [54]. Nevertheless, rather than remain unpublished, studies with such negative results should be available to the scientific community to help interpret other types of findings [55].

Apparent increases of concentrations over time do not exclude the possibility that active degradation [15] and extraction are occurring in the rhizosphere [30]. In this context, the remediation effect of willows (i.e., lowering soil contamination levels), most likely by rhizodegradation, may have been masked by continuous transfer of a mobile fraction of the contaminants present near the plantation, followed by their accumulation close to willow roots. The experience acquired during this study led us to the conclusion that, on this particular site, sampling the soil close to the trees, as per usual practice, might not yield an accurate estimate of the phytoremediation process in progress. Liste and Alexander [31] also pointed out that a reduction in pollutant concentration in soil is unlikely to be evident in samples from surface soil that is extensively penetrated by roots, due to the possible movement of chemicals towards them. Following the previous experimental phase, which took place on the same experimental site, our study group observed no significant effect of willow on any TE variation in soil, despite evidence that some TEs were substantially eliminated from the ground by plant uptake [17].

Finally, this experiment could be considered a case of pollutant containment, which has been proven effective elsewhere [56]. If SRIC of willows leads to such increases of pollutant concentrations under trees, it is probably also because some decreases are occurring elsewhere, which is relevant and desirable in a context of low investment risk management strategy. Containing contaminants in the root zone, even if carried out in a non-perennial time frame, still implies that these compounds do not migrate into the environment, which includes the St. Lawrence River in our case.

5. Conclusions

The present study suggests that SRIC of willows may influence the migration of contaminants into the soil and to do so in a manner that increases soil contaminant concentrations under the trees, as recorded here. This could seem to contradict the relevancy of using willows as a phytoremediation crop. The apparent movement of contaminants towards the willow roots implies that the remediation benefits could be masked or would be better observed somewhere other than close to the trees. In this context, the remediation efficacy attributed to the plantation appears strongly dependent on the spatial distribution of soil sampling. Moreover, to cope with the high variability inherent to nature, a consequent level of flexibility in data analysis and interpretation could help to identify tendencies and a general pattern in data that are relevant for understanding the system's functioning. We believe the somewhat surprising results presented here provide valuable information that will help the scientific community to better understand results obtained in the field and to improve phytoremediation implementation.

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